ABSTRACT

PERRY, NICHOLAS. Chemistry Inspired by Synoxazolidinone C: Synthesis and Biological Evaluation of Analogs and Synthetic Progress towards the Natural Product. (Under the direction of Dr. Joshua Pierce).

The first chapter of this thesis begins with an introduction to natural product chemistry and the therapeutic benefits of compounds isolated from nature. No discussion of this topic is complete without an assessment of how natural products have contributed to the drug discovery process. The small but growing role of marine natural products in this realm is discussed. From there, the discussion moves on to the attempted synthetic access of compounds derived from natural sources, including contributions natural product synthesis has made to the fundamental understandings of organic reactivity and synthesis. Next, the reader will be introduced to the biologically active oxazolidinone class of molecules, as well as the synoxazolidinone family of marine natural products. Finally, an argument for the synthetic pursuit of the synoxazolidinones and more general bicyclic 4-oxazolidinones is made.

The second chapter of this thesis describes our efforts towards the synthesis of synoxazolidinone C. Previous work in the Pierce group towards the related marine natural products synoxazolidinones A and B is presented. However, the different heterocyclic core necessitates a different synthetic approach. Synthesis of the building blocks was achieved, but construction of the 4-oxazolidinone core with the requisite substituents for the natural product has remained elusive. Syntheses of analogous 4-oxazolidinone cores were achieved, however.

The final chapter of this thesis describes a blend of a methods development and medicinal chemistry investigation into the synthesis and biological activity screening of

bicyclic 4-oxazolidinones. Different ways of constructing the core heterocycle, as well as appending different aromatic groups on these structural motifs were investigated. Antimicrobial and biofilm assays were performed to evaluate the potency of these newly-synthesized compounds as part of a broader aim to expand the scientific community's knowledge of the potential medicinal merit of oxazolidinones.

Chemistry inspired by synoxazolidinone C: synthesis and biological evaluation of analogs and synthetic progress towards the natural product

by
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Master of Science

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BIOGRAPHY

Nicholas “Nick” Perry was born on September 5, 1992 in Long Beach, California, and lived much of his childhood in Torrance, California. His first memory is of the 1994 Northridge earthquake, and his second is attending a Los Angeles Dodgers game in 1996 that went into 18 innings. He has eaten In-n-Out Burger and Mexican food since as long as he can remember. With these Southern California credentials in hand, he and his mother Christine moved with family to Maryland in 2000. In 2005, Nick’s sister Claire was born in California to his father Bill and stepmother Libby. It was in Maryland where Nick met his future wife, Kayla Belote, in 2006. Graduating from Calvert High School in Prince Frederick, Maryland in 2010 as an active member of the Wind Ensemble and Jazz Band programs, Nick enrolled at Salisbury University in Salisbury, Maryland. He studied chemistry, conducting research under the guidance of Professors Seth Friese and Stephen Habay. He investigated intramolecular cyclization reactions as a means of natural product synthesis, and embarked on a route to the synthetic target pestacin before graduating in 2014 with his Bachelor of Science degree. After a year outside academia, including a stint as an analytical chemist for the Maryland Department of Agriculture, Nick was lured to Raleigh, North Carolina by the promise of craft beer and fascinating chemistry. Enrolling in the graduate program at NCSU, he joined the research group of Professor Joshua Pierce, investigating the synoxazolidinone class of marine natural products. Nick and Kayla were married at the JC Raulston Arboretum, part of the NCSU campus, on April 8, 2017. Nick’s research endeavors culminate with this master’s thesis.

ACKNOWLEDGMENTS

There are many people who deserve thanks, and without whom, I would not have been successful in this pursuit. Outside of NCSU Chemistry, I must thank my family for providing me with educational opportunities, pushing me to excel as a student, and encouraging my interest in science, particularly my parents Bill and Christine and my maternal grandfather Fredric Dellinger; my past teachers and college professors for giving me the education which has made me who I am today and encouraging me to pursue grad school, particularly Mrs. Patricia Fraley, Mrs. Suzanne Kistler, Mr. David Spain, and Mr. Karl Sturge (Calvert High School), Dr. Alison Dewald, Dr. Seth Friese, Dr. Stephen Habay, Mrs. Mindy Howard, and Dr. Frederick Kundell (Salisbury University); my wife Kayla, for emotional support and providing a rock of stability in my grad school career; and my friends from childhood through the present that have given me someone to talk to and take my mind off of chemistry when I need to, particularly Steven Cenname, Christian Nicholes, and Jay Reyes. Inside NCSU Chemistry, I need to thank Dr. Pierce, for I likely would not have been accepted into, would not have enrolled in, and would not still be at NCSU without him. I also need to thank my group members for being personally supportive as well as immensely helpful to my project. I was honored to join their ranks, and while I was the weakest student, they all helped me with something along the lines, and helped me attain this success. Particularly vital to the progress of my research was Grant Edwards, who has a wealth of laboratory knowledge and project ideas, and served as a project mentor. He also performed some of the biological activity screening of my compounds. Yasamin Moazami was also the first grad student I really got to know upon arriving in the summer of 2015. She helped me adjust to

the big leagues of grad school at a large, competitive research university. During my time in the Pierce group I became close friends with Alain Valery, who introduced me to new things, was always willing to engage in a philosophical or political discussion, and forced me to think introspectively about my scientific career and my own culture.

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LIST OF ABBREVIATIONS

ABDA: 4-aminobutyraldehyde dimethyl acetal

Ac_2O: acetic anhydride

$AlMe_3$: trimethylaluminum

ATCC: American Type Culture Collection

Bn: benzyl

^{13}C NMR: carbon nuclear magnetic resonance

Cat. #: catalog number

$CDCl_3$: deuterated chloroform

CFU: colony forming unit

$CHCl_3$: chloroform

CH_2Cl_2: dichloromethane, aka methylene chloride

CSA: camphorsulfonic acid

CV: crystal violet

Cy: cyclohexyl

Da: Daltons

DCE: 1,2-dichloroethane

DIBAL-H: diisobutylaluminum hydride

DIPEA: diisopropylethyl amine

DMAP: 4-dimethylaminopyridine

DMBA: 2,4-dimethoxybenzyl amine

DMF: N,N-dimethylformamide

DMSO: dimethyl sulfoxide

EDCHCl: 1-ethyl-3-(3-diethylaminopropyl)carbodiimide-hydrochloride

Et_2O: diethyl ether

Et_3N: triethylamine

EtOAc: ethyl acetate

EtOH: ethanol

equiv: equivalents

FT-IR: Fourier-transform infrared

Grubbs I: Grubbs 1st generation catalyst

Grubbs II: Grubbs 2nd generation catalyst

^{1}H NMR: proton nuclear magnetic resonance

h: hours

HESI: heated electrospray ionization

HOAc: acetic acid

HRMS: high-resolution mass spectrometry

IC_{50}: half maximal inhibitory concentration

IR: infrared

MeI: methyl iodide

Mes: mesyl

M: Molar

MeCN: acetonitrile

MeOH: methanol

MHB: Mueller-Hinton broth

MIC: minimum inhibitory concentration

MoA: mechanism of action

min: minutes

mM: milliMolar

mmol: millimoles

MRSA: Methicillin-resistant *Staphylococcus aureus*

N-acetyl-gly: *N*-acetyl-glycine

$NaHCO_3$: sodium bicarbonate

NaOAc: sodium acetate

NCS: N-chlorosuccinimide

NRX: no reaction observed

OD_{540}: optical density at 540 nm wavelength

Ph: phenyl

PMB: para-methoxybenzyl

PMBCl: para-methoxybenzyl chloride

ppm: parts-per-million

*p*TsOH: para-toluenesulfonic acid

PVC: polyvinyl chloride

quant.: quantitative yield

rt: room temperature

SAR: structure-activity relationship

s.m.: starting material

TBDPS: tert-butyldiphenylsilyl

TBDPSCl: tert-butyldiphenylsilyl chloride

TBS: tert-butyldimethylsilyl

TBSCl: tert-butyldimethylsilyl chloride

TFA: trifluoroacetic acid

THF: tetrahydrofuran

TIPS: triisopropylsilyl

TIPSCl: triisopropylsilyl chloride

TLC: thin-layer chromatography

TSA: tryptic soy agar

TSBG: tryptic soy broth with 0.5% glucose supplement

UV: ultraviolet

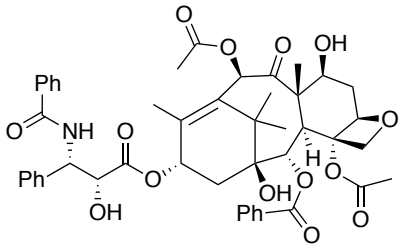
CHAPTER 1

General Introduction

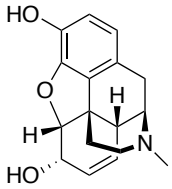
Abstract: Natural products are a fruitful source of therapeutic compounds, whether the biologically active compound is a natural product itself or is inspired by one. The study of natural products has contributed significantly to modern drug discovery and the fundamental understanding of organic synthesis. Marine natural products, while historically underrepresented in this field due to shortcomings in technology, are emerging as an exciting source of lead molecules. The synoxazolidinone family of marine natural products contains the oxazolidinone heterocyclic scaffold, which has shown activity against a large range of Gram-positive bacteria.

1.1 Natural Products and Drug Discovery

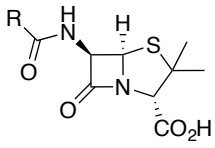
Nature has provided humankind with a rich source of therapeutic compounds for centuries. In more modern times, pharmaceutical companies have derived many drug molecules or molecular architectures from natural products.[1] Professor Alan Harvey, of the University of Strathclyde, UK, asserts, "Natural products have been the single most productive source of leads for the development of drugs."[2] In fact, several well-known pharmaceuticals are isolated from nature, or were inspired by natural products (Figure 1.1).[3]



Paclitaxel (Taxol ©), anti-cancer, isolated from the tree *Taxus brevifolia*



Morphine, opiate analgesic



Penicillin, antibiotic class isolated from *Penicillium* fungi

Figure 1.1: Examples of pharmaceutical agents isolated from nature

The use of natural products as therapeutic treatments dates back very early in human civilization.[4] Evidence of the use of the flower *Colchicum autumnale* to treat pain associated with gout dates back 2,000 years. The biologically active component of this flower, which is native to Great Britain and Ireland, and subsequently spread throughout continental Europe, is (-)-colchicine (Figure 1.2).[5] (-)-Colchicine is approved in the United States for the treatment of several other ailments, including familial Mediterranean fever, scleroderma, and amyloidosis.[6]

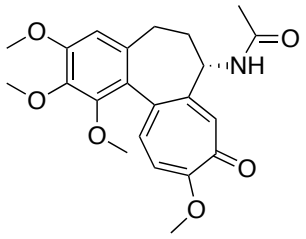


Figure 1.2: (-)-Colchicine, an apoptosis inducing natural product

Pestacin (Figure 1.3) is a 1,3-dihydroisobenzofuran which has been isolated from the endophytic fungus *Pestalotiopsis microspora*, found in the rainforests of Papua New Guinea. The leaves of the *Terminalia morobensis* tree infected with this fungus have been known in traditional medicine across Southeast Asia for centuries for its antiviral and antifungal characteristics.[7] Pestacin has recently been shown to possess anti-cancer activity in some studies, and its synthesis has been pursued unsuccessfully by chemists over the years.[8]

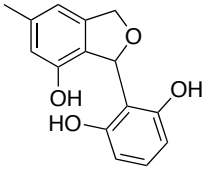


Figure 1.3: Pestacin, a biologically active natural product

An important natural product with a more successful story is the anti-cancer agent paclitaxel, marketed under the trade name Taxol© (Figure 1.1). Paclitaxel was originally isolated in 1962 from the bark of the plant *Taxus brevifolia*, or Pacific yew. Bristol-Myers Squibb received approval to sell it in the United States in 1992.[3] Its first laboratory synthesis was reported by Professor Robert Holton's group at Florida State University in 1994,[9] with Professor K.C. Nicolaou's group at The Scripps Research Institute in California achieving another total synthesis later that year.[10] It is currently a multi-billion dollar drug used to treat patients with breast, lung, ovarian, and pancreatic cancer. Its mechanism of action was elucidated in murine models. Paclitaxel's astonishing success has induced a worldwide search for similar molecular scaffolds expressing similar biological activity, along with improved features. As a result, analogs of paclitaxel have been produced.[3]

The development of natural products into commercialized drugs contains inherent challenges, however. Many of these compounds' sources are difficult to locate in nature, difficult to extract from their source organisms, or are produced by their source organisms in minute concentrations. Environmental consequences and ethical questions also arise with exploiting those resources. Consequently, alternative approaches and sources of natural products have become of interest to the scientific community. The ability to access these molecules synthetically and expand the synthesis to an economical and reliable industrial scale is a process undertaken by scientists and engineers.[11]

An important notion in the field of drug discovery are Lipinski's Rules, which govern what is a "drug-like" molecule. Developed by Christopher Lipinski and co-workers at Pfizer,

a molecule must violate no more than one of the following rules in order to be considered a promising drug candidate:[12]

1. There can be no more than 5 hydrogen bond donors
2. There can be no more than 10 hydrogen bond acceptors
3. The molecular weight must be less than 500 Da
4. The octanol-water partition coefficient (log*P*) must not exceed 5

Natural products, however, are said to be exceptions to Lipinski's Rules, operating in a "parallel universe" of chemical space. Nature operates with a parsimonious set of building blocks and maintains low hydrophobicity and hydrogen bonding potential in order to make compounds with molecular weights exceeding 500 Da. The synthetic chemist has tens of thousands of starting blocks at their disposal, and once a reliable pathway is established, can modify the input to achieve a different outcome. Nature, however, partitions its limited building blocks into many different pathways. Natural products are also amenable to modification; as there is some evolutionary reason for their existence, nature has had to modify them over time.[13]

Moreover, Lipinski's Rules do not apply to antimicrobial agents. These compounds need to penetrate bacterial cells, which are prokaryotic, meaning they do not contain a membrane-bound nucleus. However, Lipinski's Rules were formulated to concern compounds which need to penetrate eukaryotic cells.[14] Not needing to following Lipinski's Rules gives more flexibility in the drug development process of antibiotics. As will be discussed later in this document, the class of molecules in this study have demonstrated antimicrobial activity.

David Newman and Gordon Cragg, of the National Cancer Institute, conducted a thorough review on the prevalence of natural products in the clinic. Of the 1,355 drugs that

entered the clinic between 1981 and 2010, 50% were either a natural product, made from a natural product, or inspired by a natural product. Many of these had antibacterial, anti-cancer, antihypertensive, and hormone regulatory functions. Only 29% were purely synthetic (Figure 1.4).[15]

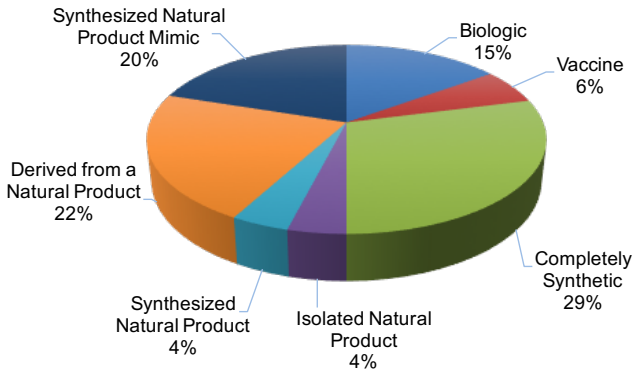


Figure 1.4: Sources of new drugs approved worldwide, 1981-2010

Natural product synthesis has contributed immensely to the field of organic chemistry. Achieving stereocontrol in chemical synthesis, particularly that of sp^3 hybridized carbons, was a vexing problem for synthetic chemists. Landmark total syntheses of cantharidin (Figure 1.5) by Professor Gilbert Stork's group at Columbia University in 1951 and reserpine (Figure 1.5) by Professor R.B. Woodward's group Harvard University in 1956 demonstrated remarkable advances in stereocontrol.[16] Professor Woodward was awarded the Nobel Prize in Chemistry in 1965 "for his outstanding achievements in the art of organic synthesis," and his presentation speech by the Nobel Committee of Chemistry specifically mentioned reserpine.[17] Another crucial innovation to come out of the pursuit of natural

products was retrosynthetic analysis, or retrosynthesis. The formalized process of transforming a target molecule into a set of simpler precursors in order to pursue the most optimal synthesis was developed by Professor E.J. Corey of Harvard University. His synthesis of longifolene (Figure 1.5) in 1961 was a pivotal point for retrosynthesis.[16] Professor Corey was awarded the 1990 Nobel Prize in Chemistry for this contribution, among others.[17]

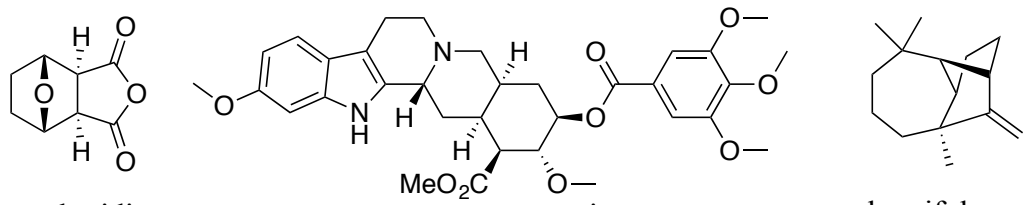


Figure 1.5: Seminal examples in natural product synthesis

Despite this promise and importance, there still exists a perception that natural products are too complex and their syntheses are too difficult to access, resulting in a declining interest among large pharmaceutical companies. Natural product chemistry has been taken up by academia and smaller companies, and has undergone a renaissance since the early 2000's, chiefly due to advances in analytical technology, spectroscopy, and high-throughput screening.[1]

1.2 Marine Natural Products

The world's oceans are teeming with biodiversity. Organisms in this environment have faced tremendous evolutionary pressure, and have produced a stunning supply of intricate molecules for their survival.[1] While natural products serving as drugs or drug leads

overwhelmingly tend to be of terrestrial origin, marine invertebrates have been a rich source of structurally diverse bioactive secondary metabolites.[18] The first marine natural product to enter the clinic was Ziconotide©, a synthetic peptide derived from the sea snail *Conus magus*, used to ameliorate pain in patients afflicted with a spinal cord injury. The Irish firm Élan Pharmaceuticals received approval to sell it in the United States in 2004 and in the European Union the following year.[1] Four additional marine natural products have since been approved in the United States, along with a plethora of other compounds in different phases of preclinical and clinical trials.[19] Professor Tadeusz Molinski, a leading marine natural products chemist at the University of California, San Diego, notes the high hit rate for marine natural products in screening for drug leads, and describes marine natural products chemistry as building a "legacy of discovery of biomedical probes." Professor Molinski also evokes the "wine dark sea" of Homer's *Iliad* and *Odyssey* in describing the fathomless complexities and promise of marine natural products.[1]

1.3 Oxazolidinones

Oxazolidinones are a class of molecules consisting of a nitrogen- and oxygen-containing heterocycle and a carbonyl. Oxazolidinones are classified as 2- and 4-oxazolidinones based on the relative locations of the heteroatoms (Figure 1.6).

Figure 1.6: 2-oxazolidinone (left) and 4-oxazolidinone (right)

DuPont first developed oxazolidinones in 1978 for use against certain plant pathogens. In 1987, DuPont researched two oxazolidinone compounds for antibacterial activity, but ceased investigation when they were found to be toxic to mammalian cells. Upjohn Pharmaceuticals, which through a series of acquisitions fell under the control of Pfizer, began studying oxazolidinones in 1996 and developed nontoxic variants of the DuPont compounds. They received approval to sell one of them, linezolid (Figure 1.7), as an antibiotic in the United States in 2000 under the trade name Zyvox©. This drug is used to treat skin infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium*, and penicillin-resistant *Staphylococcus pneumoniae*. It has been shown to have a 73.3% clinical cure rate. Often, patients receive the aforementioned bacterial infections from surgical procedures, or from complications brought on by respiratory tract infections or tuberculosis.[20]

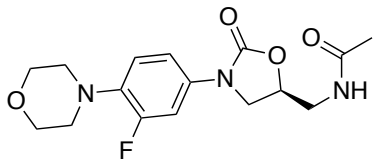


Figure 1.7: Linezolid

Antibiotics function by binding to a target and interfering with its activity. Oxazolidinone antibiotics interfere with ribosomal function and inhibit protein synthesis. A 2006 study from the Indiana University School of Medicine suggests that oxazolidinones specifically inhibit mitochondrial protein synthesis by binding to the P site on the 50S ribosomal subunit.[21] Elucidating a target for this class of antibiotics was a key development

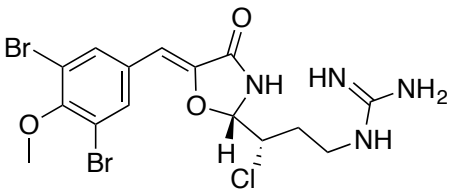
for understanding its mechanism of action, conducting resistance mechanism studies, and further refinement of synthetic targets.

Resistance has been observed to almost every antibiotic deployed in the clinic. The growing need for new classes of antibiotics has dramatically outpaced the development thereof. By virtue of linezolid, the oxazolidinones represent the first new class of antibiotics introduced in the clinic since the 1970s. According to The Infectious Disease Society of America, 70% of infections contracted in hospitals in the United States are resistant to at least one antibiotic.[22] To this vein, the UK Chief Medical Officer released a report in 2013 calling antimicrobial resistance a serious world threat on the same level as terrorism. The report claims our modern health system will soon be similar to that of the early 1800s, where a small cut or a minor medical procedure becomes life-threatening due to the risk of infection. Antimicrobial resistance will disproportionately affect poverty-stricken countries in Africa and Asia, where public health systems are inadequate to handle it.[23] The oxazolidinone scaffold is present in a relatively new family of marine natural products, the synoxazolidinones, discussed in the next section.

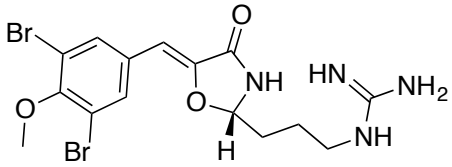
1.4 The synoxazolidinone family of marine natural products

A group led by Professor Margey Tadesse of the University of Tromsø isolated synoxazolidinones A and B in 2010 from the ascidian *Synoicum pulmonaria* off the coast of Norway.[24] They isolated synoxazolidinone C from the same organism in the same location the following year.[25] Synoxazolidinones are a 4-oxazolidinone, which are significantly rarer than 2-oxazolidinones. Still rarer, synoxazolidinone C features the unprecedented bicyclic

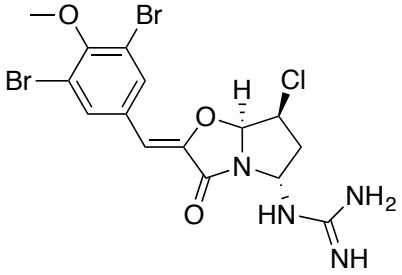
oxazolidinone scaffold. **Figure 1.8** illustrates these structures as well as relevant biological activity.[26]



Synoxazolidinone A
Coryneacterium glutamicum: 6.25
S. aureus: 10
MRSA: 10
Saccharomyces cereisiae: 12.5



Synoxazolidinone B
S. aureus: 30
MRSA: 30



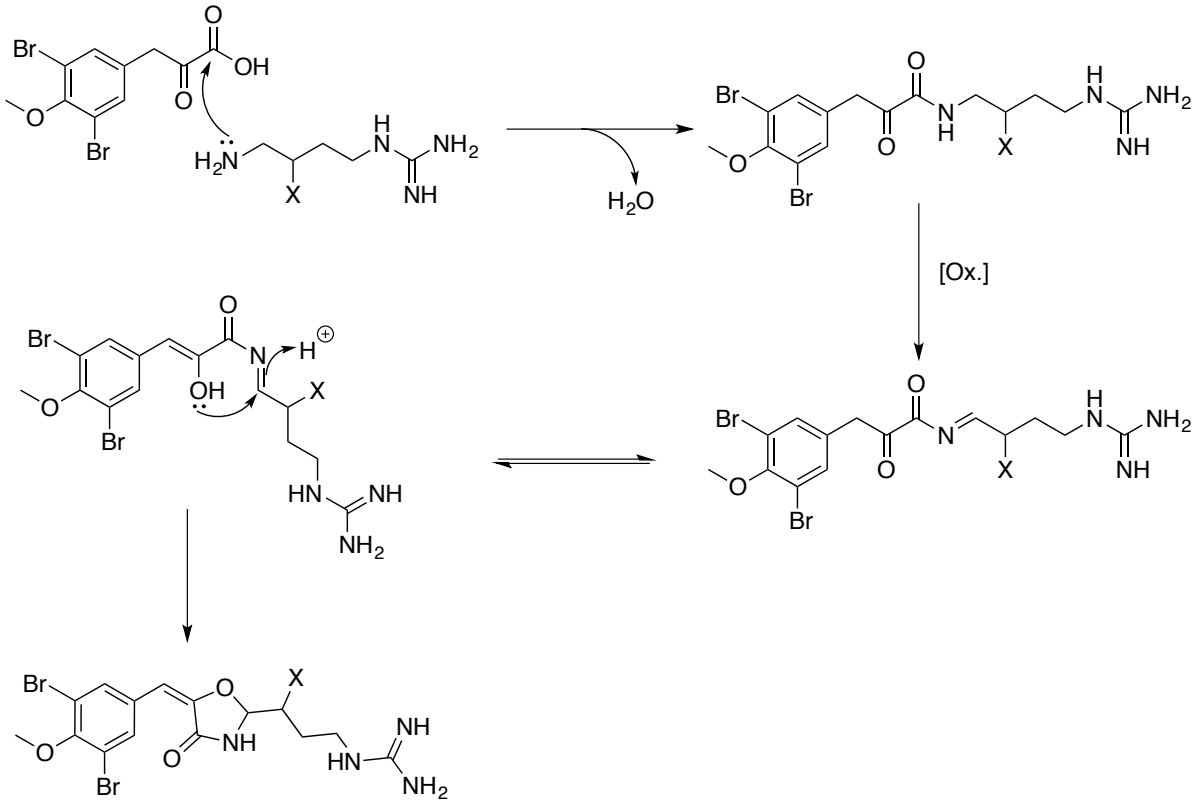
Synoxazolidinone C
S. aureus: 10
MRSA: 10
Escherichia coli: 30
E. faecalis: 20

IC_{50}: 30.5
A2058 (melanoma), HT-29 (colon carcinoma), MCF-7 (breast adenocarcinoma)

Figure 1.8: Synoxazolidinones and their MIC values (μg/mL) against certain bacteria. IC_{50} values (μM) for synoxazolidinone C against various cancer cell lines are also presented.

The synoxazolidinones have also shown antifouling capabilities. Fouling hinders the passage of ships through waterways as sessile benthic marine organisms cling to the hull and create drag. This discovery also has implications in the field of biofilm inhibition, a major public health concern.[26] A biofilm is a group of microorganisms which stick to each other and adhere to a surface.[27] Biofilms are implicated in many bacterial infections, causing up to 80% of them according to one study by the NIH, and are a growing health concern as well.[28]

The isolation chemists propose a biosynthetic route to synoxazolidinones A and B (Figure 1.9) in which a brominated tyrosine metabolite condenses with an arginine derivative to form a dipeptide. Formation of an imine, tautomerization, and cyclization of the appended oxygen nucleophile result in the natural product.[24] This acylation/cyclization cascade was leveraged in Shymanska's synthesis of synoxazolidinones A and B.[26] It was also employed as the strategy to construct the 4-oxazolidinone ring in our efforts towards synoxazolidinone C.



Scheme 1.1: Proposed biosynthesis of synoxazolidinones A (X=Cl) and B (X=H). The isolation chemists are uncertain how Cl is incorporated into the molecule in the case of synoxazolidinone A. Furthermore, this mechanism does not account for diastereoselectivity.

1.5 Conclusion

Despite the rarity of 4-oxazolidinone scaffold, oxazolidinones are a promising class of marine natural products established in medicinal chemistry. As discussed earlier, natural product synthesis has the capability of answering biological questions while simultaneously advancing the fundamental understanding of organic synthesis. The 4-oxazolidinone thus represents an enticing synthetic target.

1.6 References

(1) Molinski, T.F.; Dalisay, D.S.; Lievens, S.L.; Saludes, J.P. *Nature Rev.* **2009**, *8*, 69.

(2) Harvey, A.L. *Drug Discovery Today* **2008**, *13*, 894

(3) (a) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M. *J. Med. Chem.* **1995**, *38*, 1666. (b) Oberlies, N. H.; Kroll, D. J. *J. Nat. Prod.* **2004**, *67*, 129. (c) Huey, R. B.; Ward, P. D. *Science* **2005**, *308*, 395. (d) Ishikawa, H.; Colby, D. A.; Seto, S.; Va, P.; Tam, A.; Kakei, H.; Rayl, T. J.; Hwang, I.; Boger, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 4904.

(4) (a) Ji, H.; Li, X.; Zhang, H. *EMBO rep.* **2009**, *10*, 194. (b) Wender, P. A.; Baryza, J. L.; Brenner, S. E.; DeChristopher, B. A.; Loy, B. A.; Schrier, A. J. *J. Proc. Natl. Acad. Sci. USA,* **2011**, *108*, 6721. (c) Wender, P. A.; Loy, B. A.; Schrier, A. J. *Isr. J. Chem.* **2011**, *51*, 453. (d) Wender, P. A.; Verma, V. A. *Org. Lett.* **2008**, *10*, 3331. (e) Wender, P. A.; DeChristopher, B. A.; Schrier, A. J. *J. Am. Chem. Soc.* **2008**, *130*, 6658.

(5) Graening, T.; Schmalz, H. G. *Angew. Chem. Int. Ed*. **2004**, *43*, 3230.

(6) (a) Peters, R. S.; Lehman, T. J.; Schwabe, A. D. *West. J. Med*. **1983**, *138*, 43. (b) Livneh, A.; Zemer, D.; Langevitz, P.; Sohar, E.; Laor, A.; Pras, M. *Arthritis Rheum.* **1994**, *37*, 1804.

(7) Harper, J.K.; Arif, A.M.; Ford, E.J.; Strobel, G.A.; Porco, J.A.; Tomer, D.P.; Oneill, K.L.; Grant, D.M. *Tetrahedron* **2003** *59*, 2471.

(8) (a) Karmakar, R.; Pahari, P.; Mal, D. *Tetra. Lett.* **2009**, *50*, 4042. (b) Perry, N.W.; Friese, S.J.; Habay, S.A.; Jenkins, T. Unpublished results, Salisbury Univ.

(9) Holton, R.A.; Somoza, C.; Kim, H.B.; Liang, F.; Biediger, R.J.; Boatman, P.D.; Shindo, M.; Smith, C.C.; Soekchan, K. *J. Am. Chem. Soc.* **1994**, *116*, 1597.

(10) Nicolaou, K.C.; Yang, Z.; Liu. J..; Ueno, H.; Nantemert, P.G.; Guy, R.K.; Claiborne, C.F.; Renaud, J.; Couladouros, E.A.; Paulvannan, K.; Sorensen, E.J. *Nature* **1994**, *367*, 630.

(11) Gangadevi, V.; Muthumary, J. *Mycol. Balc.* **2008**, *5*, 1.

(12) Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3.

(13) Ganesan, A. *Curr. Opin. Chem. Biol.* **2008**, *12*, 306.

(14) Lewis, K. *Mar. Drugs Rev.* **2013**, *52*, 371.

(15) Newman, D.J. Cragg, D.M.; *J. Nat. Prod.* **2012**, *75*, 311.

(16) Wilson, M.A.; Danishefsky, S.J. *J. Org. Chem*. **2006**, *71*, 8329.

(17) *Nobel Lectures, Chemistry, 1963-1970*. Amsterdam: Elsevier, 1972.

(18) Melander, R.J.; Liu, H.; Stephens, M.D.; Bewley, C.A; Melander, C. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5863.

(19) Lei, J.; Zhou, J. *J. Chem. Inf. Comput. Sci*. **2002**, *42*, 742.

(20) Bozdogan, B.; Appelbaum, P.C. *Int. J. Antimicrob. Agents* **2004**, *23*, 113.

(21) McKee, E.; Feguson, M.; Bentley, A.T.; Marks, T.A. *Antimicrob. Agents Chemother.* **2006**, *50*, 2042.

(22) Clatworthy, A.E.; Pierson, E.; Hung, D.T. *Nature Chem. Biol.* **2007**, *3*, 541.

(23) UK Antimicrobial Resistance Strategy and Action Plan. UK Chief Medical Officer, London, 11 March 2013.

(24) Tadesse, M.; Strøm, M.B.; Svenson, J.; Jaspars, M.; Milne, B.F.; Tørfoss, V.; Andersen, J.H.; Hansen, E.; Stensvåg, K.; Haug, T. *Org. Lett.* **2010**, *12*, 4752.

(25) Tadesse, M.; Svenson, J.; Jaspars, M.; Strøm, M.B.; Abdelrahman, M.H.; Andersen, J.H.; Hansen, E.; Kristiansen, P.E.; Stensvåg, K.; Haug, T. *Tetra. Lett.* **2011**, *52*, 1804.

(26) Shymanska, N.V.; Ph.D. dissertation, North Carolina State Univ., 2016.

(27) Trepos, R.; Cervin, G.; Hellio, C.; Pavia, H.; Stensen, W.; Stensvåg, K.; Svendsen, J.S.; Haug, T.; Svenson, J. *J. Nat. Prod.* **2014**, *77*, 2105.

(28) Vert, M.; Doi, Y.; Hellwich, K.; Hess, M.; Hodge, P.; Kubisa, P.; Rinaudo, M.; Suché, F. *Pure Appl. Chem.* **2012**, *84*, 377.

(29) "Research on microbial biofilms (PA-03-047)." National Heart, Lung, and Blood Institute. NIH, Bethesda, MD, 2012.

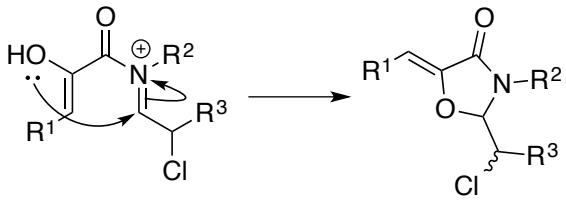
CHAPTER 2

Studies Towards the Synthesis of Synoxazolidinone C

Abstract: Progress towards the synthesis of synoxazolidinone C is presented. The synthetic route centers around a different strategy than the one employed for synoxazolidinones A and B. For synoxazolidinone C, it was attempted to build the bicyclic 4-oxazolidinone scaffold one ring at a time. While building blocks for this scaffold were successfully prepared, synthesis of the required skeleton for the natural product has remained elusive. Undaunted, we were inspired to look more broadly at constructing mono- and bicyclic 4-oxazolidinonees.

2.1 Introduction and Background

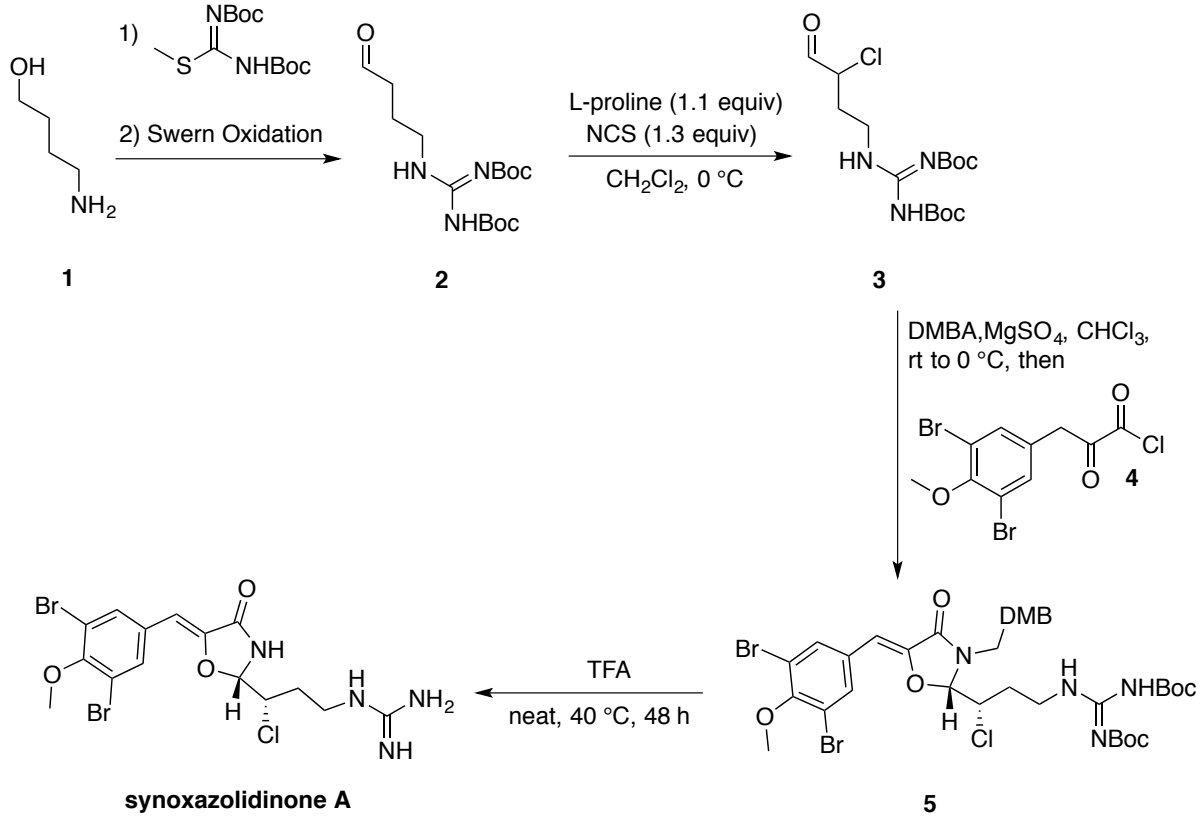
Given the enticing synthetic targets posed by the synoxazolidinones, the Pierce group has made it a goal to access these compounds. Nataliia Shymanska, a 2016 Ph.D. graduate of the Pierce group, achieved syntheses of synoxazolidinones A and B.[1] An imine acylation/cyclization cascade to construct the 4-oxazolidinone ring central to the molecule was envisioned (Scheme 2.1). During this process, alkene geometry would be dictated by stereoelectronic effects concerning R^1 and the oxygen atoms.



Scheme 2.1: Acylation/cyclization cascade to construct 4-oxazolidinone skeleton

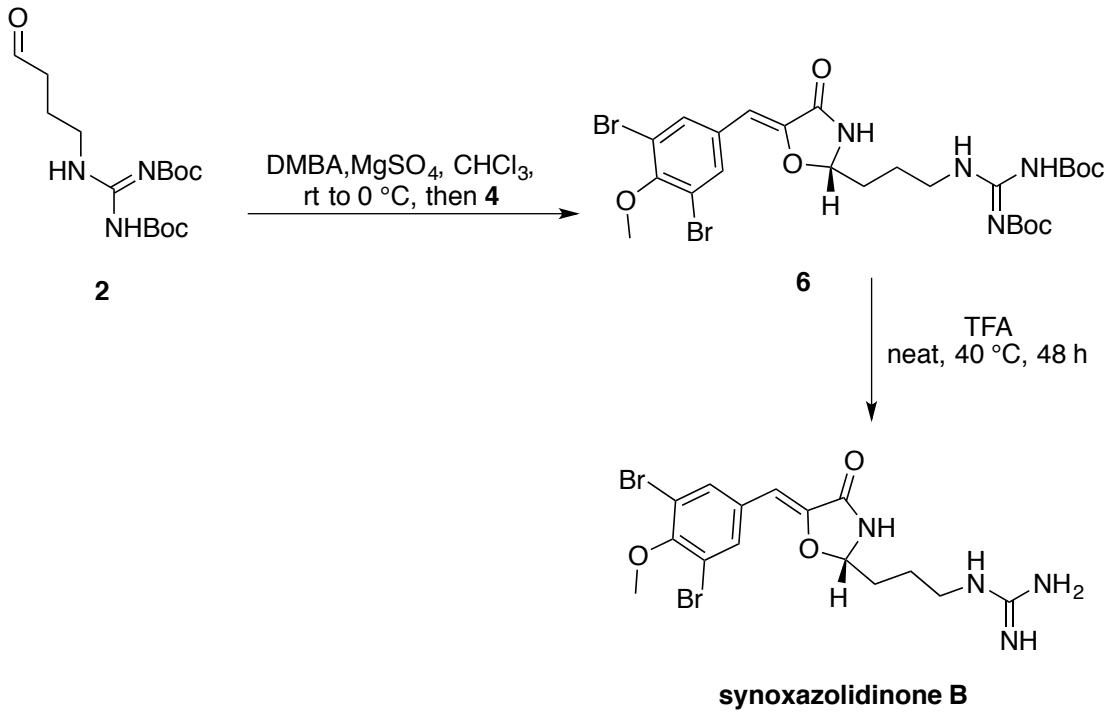
Shymanska's syntheses both began by protecting the amine of 4-aminobutanol **1** with a Boc-guanidine group (Scheme 2.2). The alcohol was then oxidized under Swern conditions to arrive at **2**. At this point the syntheses of the natural products diverged. Synthesis of synoxazolidinone A continued with an attempt at asymmetric alpha chlorination of **2**.[1] All

attempts at catalytic chlorination were unsuccessful, despite this being a well-established approach on other substrates.[2] It was reasoned that the the Boc-protected guanidine moiety complicated the reaction. Stoichiometric L-proline was employed to chlorinate, producing **3** in 60% yield, but a racemic product. **3** was then reacted with DMBA and acid chloride **4**, inducing acylation/cyclization cascade to produce the Boc-protected natural product skeleton **5** with 4:1 diastereoselectivity, where the major diastereomer is that of the natural product. Deprotection with trifluoroacetic acid produced synoxazolidinone A in 19% overall yield, with no erosion of the diasteromeric ratio.[1]



Scheme 2.2: Shymanska's synthesis of synoxazolidinone A

Synoxazolidinone B was synthesized in a more facile manner (Scheme 2.3), since installation of the secondary chloride moiety was not required. **2** was reacted directly with DMBA and **4** to produce **6** from the same cascade. Subsequent deprotection yielded the natural product in a 20% overall yield.[1]

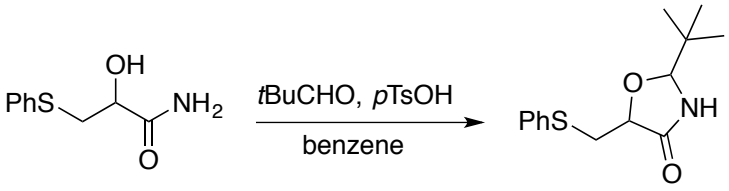


Scheme 2.3: Shymanska's synthesis of synoxazolidinone B

Shymanska also attempted to develop a structure-activity relationship (SAR) for these compounds by synthesizing analogs and testing them against various strains of bacteria. Testing analogs of synoxazolidinones A & B against various bacteria, she in general found that some substitution is required on the aromatic ring, having Cl improves the activity of the compound, and that the guanidine portion of the molecule is not required for activity against Gram-positive bacteria. She also found that both enantiomers of synoxazolidinone B have the

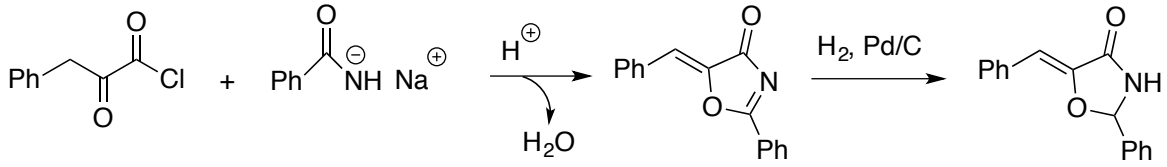
same activity. Beyond these findings, it is unclear, however, exactly what about the synoxazolidinones structurally makes them active.[1]

Syntheses of 4-oxazolidinones are much less common in the literature than their 2-oxazolidinone counterparts. In addition to Shymanska's valuable contributions, Professor William Roush's group at Indiana University synthesized a 4-oxazolidinone from the addition of an amine to an aldehyde, followed by intramolecular cyclization closing the ring in 86% yield and a 2:1 *cis:trans* ratio (Scheme 2.4).[3]



Scheme 2.4: Roush's 4-oxazolidinone synthesis

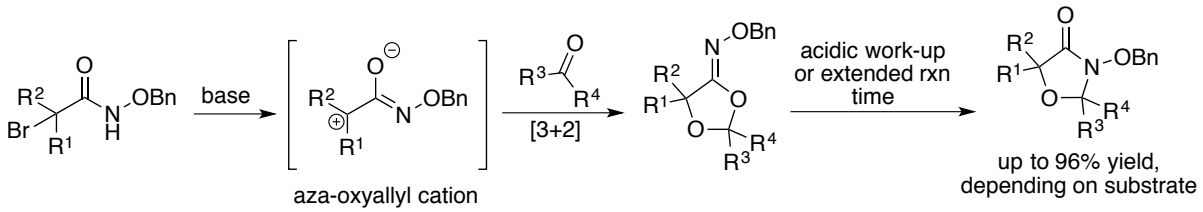
Additionally, Professor Divi Iyengar's group at the Indian Institute of Chemical Technology prepared a 4-oxazolone from the condensation of an oxopropanoyl chloride and a benzoylphenylpropenamide. Catalytic hydrogenation of the 4-oxazolone produced a 4-oxazolidinone in a 90% yield without disturbing the exocyclic double bond (Scheme 2.5).[4]



Scheme 2.5: Iyengar's 4-oxazolidinone synthesis

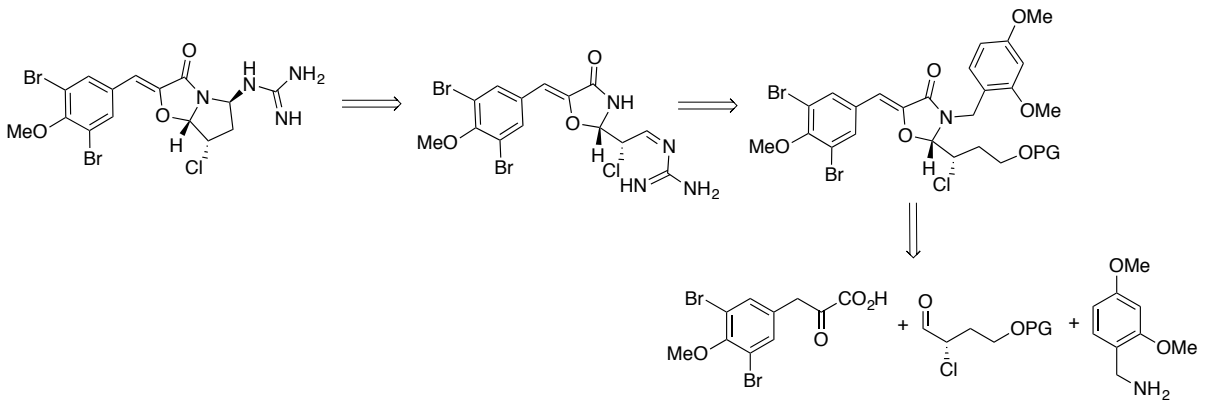
Finally, the research group of Professor Christopher Jeffrey at University of Nevada, Reno reported the *in situ* generation of an azaoxyallyl cation intermediate from an α-

halohydroxamate. Exposure of the cation intermediate to a variety of carbonyl compounds generated 4-oxazolidinones in up to 96% yield (Scheme 2.6), although their R_1 and R_2 substrate scope was not explored and their R_3 substrate scope included nothing similar to our needs for synoxazolidinone C. Nevertheless, their proposed mechanistic pathway, involving an aza-[3+2] cycloaddition, marks an interesting contribution to this burgeoning field. They note the isolation of imidate products, and only observe rearrangement to the 4-oxazolidinone upon acidic work-up or "extended reaction time."[5]



Scheme 2.6: Jeffrey's synthesis of 4-oxazolidinones

As shown in **Figure 1.8**, however, synoxazolidinone C features a bicyclic oxazolidinone scaffold. To the best of our knowledge, it has never been observed in another molecule in nature, nor has it been synthesized in a laboratory prior to our efforts. A new strategy to construct such a scaffold is required, and to date, no synthesis of synoxazolidinone C has been published. Our retrosynthetic analysis (Scheme 2.7) involved constructing the second ring by cyclizing the chlorinated guanidine, which would come from the chlorinated aldehyde, linked by an alkyl chain to a protected alcohol. The first oxazolidinone ring would come from methods employed by Shymanska towards synoxazolidinones A and B, combining the aryl-oxopropanoic acid, the chlorinated aldehyde, and commercially available DMBA.

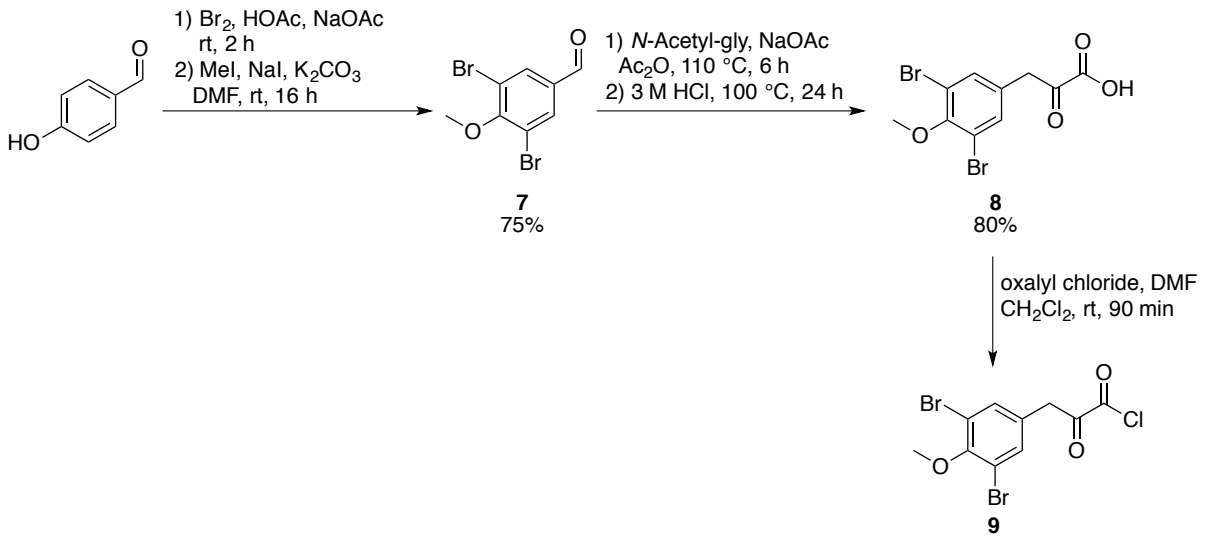


Scheme 2.7: Retrosynthetic analysis of synoxazolidinone C

2.2 Synthetic Progress Towards Synoxazolidinone C

2.2.1 Synthesis of the aromatic moiety

The synthetic route to synoxazolidinone C currently under study involves first constructing the monocyclic 4-oxazolidinone scaffold, and the initial steps mirror those of Shymanska's work (Scheme 2.8). Commercially obtained 4-hydroxybenzaldehyde was treated with Br_2 and HOAc to achieve bromination at both ortho positions with respect to the hydroxyl group. The hydroxyl group was then converted to a methoxy group by treatment with MeI to obtain **7**. Employing Erlenmeyer-Plöchl azalactone synthesis, treatment with NaOAc and *N*-acetyl-glycine produced the azalactone intermediate, which was then hydrolyzed under reflux to produce the oxopropanoic acid **8**.

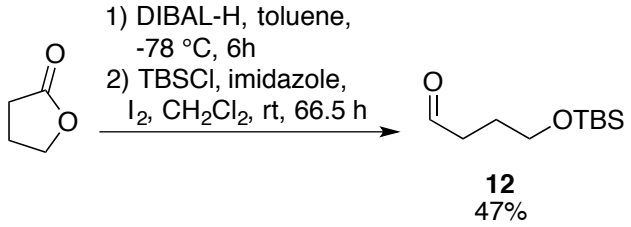


Scheme 2.8: Generation of the oxopropanoyl chloride **9** in synoxazolidinone C synthesis

Purification of **8** presented a minor challenge. Given that phenylpyruvic acids are difficult to purify on normal phase silica gel, the usual process of flash chromatography was avoided. Purification of **2** was finally achieved by dissolving the crude brown product in a minimal amount of hexane, and then recrystallizing from Et_2O. The best recrystallization yield was achieved by placing the flask in a -30 °C freezer for 48 h. A clear yellow liquid was found on top of a brown oil. LC-MS analysis of both phases revealed the liquid to be pure **8**, while the oil was a mixture of **8**, starting material, and other impurities. Repeating the recrystallization procedure on the leftover oil did not significantly improve yield. With **8** in hand, the conversion to **9** was achieved with oxalyl chloride and a drop of DMF. Acid chlorides tend to be unstable, so it was desirable to perform the conversion from **8** to **9** in the same solvent as the subsequent reaction, and only prepare **9** immediately before the subsequent reaction was performed.

2.2.2 Synthesis of butanal

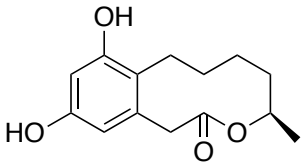
Meanwhile, attempts to access the butanal necessary to construct the requisite 4-oxazolidinone scaffold were made. It was envisioned that gamma-butyrolactone could be opened by treatment with DIBAL-H and the alcohol subsequently protected with a silyl protecting group (Scheme 2.9). Yields were modest and the reactions featured low conversion, despite following literature procedure.[6]



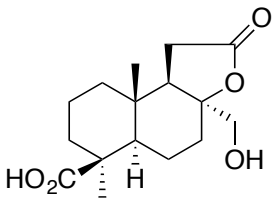
12
47%

Scheme 2.9: Opening of lactone and protection of alcohol to furnish necessary butanal **12**

The intermediacy of a Weinreb amide was then investigated. *N*-methoxy-*N*-methyl amides were synthesized in the early 1980's by Professor Steven Weinreb at Pennsylvania State University and investigated as effective acylating agents. Weinreb amides are known for their facile preparation and ease with which they can be reduced to furnish aldehydes.[7] Weinreb amides have been employed in total syntheses, such as those of xestodecalactone A (Figure 2.1) by Professor Samuel Danishefsky's group at Columbia University[8] and (+)-asperolide C (Figure 2.2) by Professor Erick Carreira's group at ETH Zürich.[9]



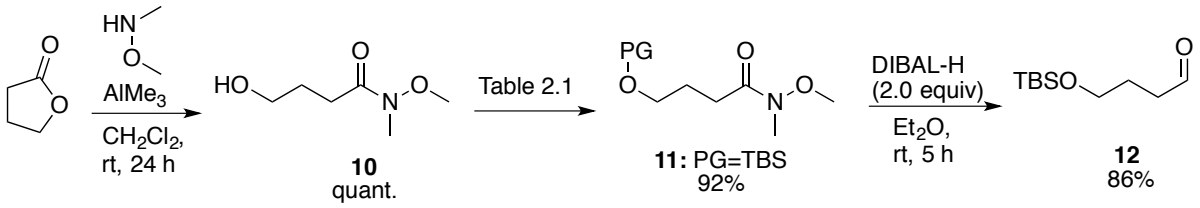
xestodecalactone A



(+)-asperolide C

Figure 2.1: Examples of natural products synthesized using a Weinreb amide as an intermediate.

A Weinreb amide **10** was produced by opening gamma-butyrolactone with *N*-methoxymethylamine and $AlMe_3$ on gram scale and in quantitative yield (Scheme 2.10). Various protecting group strategies for the alcohol were investigated (Table 2.1), and the best protecting group was found to be a tert-butyldimethylsilyl ether, producing **11** in 92% yield. The next step in this Weinreb amide approach was to reduce the amide to an aldehyde. DIBAL-H efficiently performs this transformation, and conditions vary between literature protocols. At first, the reaction was attempted at -78 °C for a reaction time of 1.5 h.[10] This method generated only a 51% yield of **12**, with an impurity detected. Next, the reaction was attempted at room temperature for a reaction time of 5 h.[11] This method generated **12** in an 86% crude yield.



Scheme 2.10: Weinreb amide approach to butanal

Table 2.1: Protecting group strategies in synthesis of **11**

Protecting Group	Conditions	% Yield
PMB	PMBCl (3.2 equiv), NaI (0.2 equiv), K_2CO_3 (2.2 equiv), DMF, 100 °C	11
PMB	PMBCl (3.2 equiv), 5 mol% CSA, DMF, 100 °C, 45 min	NRX
PMB	PMBCl (3.2 equiv), NaH (3.0 equiv), THF, rt, 3 h	NRX
TBDPS	TBDPSCl (1.1 equiv), Et_3N (1.3 equiv) DMAP (0.1 equiv), CH_2Cl_2, rt, 18 h	30
TIPS	TIPSCl (1.3 equiv), imidazole (1.5 equiv), CH_2Cl_2, rt, 17 h	39
TBS	TBSCl (1.1 equiv), imidazole (1.3 equiv), DMAP (0.1 equiv), CH_2Cl_2, rt, 17 h	92

While the Weinreb amide approach appeared viable, it seemed somewhat inefficient, since it required 3 steps to reach the desired butanal. A shorter process to the butanal was devised involving the mono-protection of 1,4-butanediol, followed by a Swern oxidation (Scheme 2.10). This method of oxidizing primary or secondary alcohols was developed in the late 1970's by Professor Daniel Swern and co-workers at Temple University. Activation of DMSO with TFAA or oxalyl chloride, followed by addition of an organic base such as Et_3N was shown to be a very efficient method employing mild conditions and non-toxic reagents (in contrast to chromium-based oxidations which were previously the norm). A notable downside of this reaction is the production of foul-smelling dimethyl sulfide, although the odor can be mitigated with proper planning and careful laboratory technique. The Swern oxidation has been employed in many total syntheses, including the first total synthesis of the marine natural product (+)-deoxyneodolabelline (Figure 2.3) by Professor David Williams and co-workers at Indiana University.[12]

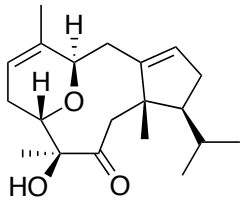
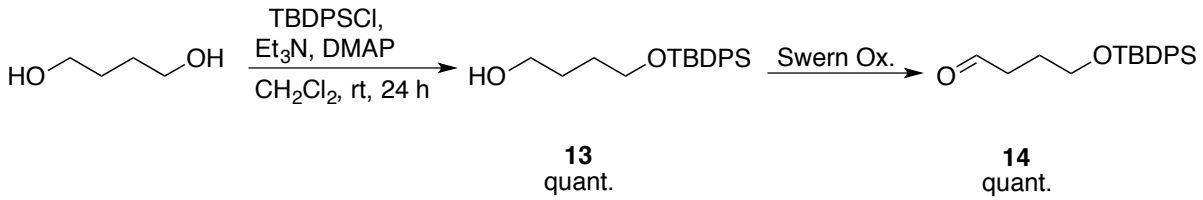


Figure 2.2: (+)-deoxyneodolabelline



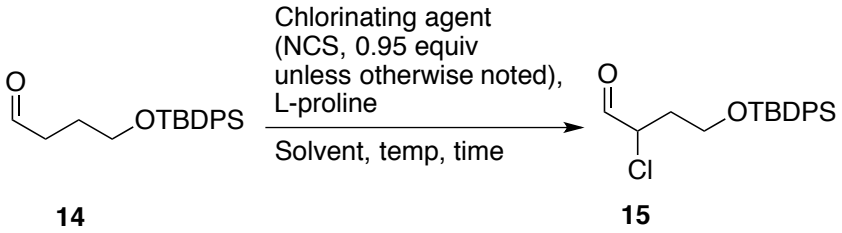
Scheme 2.11: Synthesis of butanal **14**

Following literature procedure[13], commercially obtained 1,4-butanediol was mono-protected with a TBDPS group. The unprotected alcohol was oxidized under Swern conditions to afford the butanal **14**, with both steps affording quantitative yields.

2.2.3 Alpha-chlorination of butanal

With **14** in hand, efforts turned towards chlorination of the alpha carbon. Since the early 2000's, alpha chlorination of aldehydes has garnered a lot of attention from the synthetic community. During his time as a post-doctoral researcher at the California Institute of Technology, David MacMillan and co-workers published a report of alpha- chlorination of aldehydes in enantioselective fashion using organocatalysts. The catalysts used by MacMillan were often very expensive and complicated purification techniques were used to achieve results. Our efforts used several other catalysts but found L-proline to achieve the best yields.[2a]

Proline is the only natural amino acid with secondary amine functionality. The amino proton is less acidic than other amino acids, thus enhancing the nitrogen's nucleophilicity. Having nucleophilic (via the nitrogen) and electrophilic (via the carboxylic acid) sites affords proline bifunctional reactivity. It can also go through enamine, imine, and metal catalysis modes.[14] However, L-proline is known to give poor enantioselectivity in this chemistry,[2b] so a racemic pursuit of synoxazolidinone C was envisioned, under the premise that we could return and achieve an enantioselective chlorination at a later time. **Scheme 2.12** depicts the general approach toward alpha-chlorination, with **Table 2.2** detailing our results thereof.



14 **15**

Scheme 2.12: Alpha-chlorination of butanal **14**

Table 2.2: Attempts at alpha-chlorination of butanal 14

Entry	Solvent	Catalyst	Conditions	% Yield	Product Remarks
1	CH_2Cl_2	L-proline (0.05 equiv)	rt, 16 h	95	Mix of s.m. & product
2	CH_2Cl_2	L-proline (1.0 equiv)	reflux, 5 h	63	Dichlorinated
3	CH_2Cl_2	L-proline (1.0 equiv)	reflux, 16 h	42	Dichlorinated
4	DCE	L-proline (1.0 equiv)	reflux, 16 h	44	Aldehyde signal barely observed
5	$CHCl_3$	L-proline (1.0 equiv)	reflux, 16 h	23	Mix of s.m. & product
6	CH_2Cl_2	Pyrrolidine (1.0 equiv)	reflux, 16 h	78	Chlorination of aldehyde carbon
7	CH_2Cl_2	Catalyst 1 (1.0 equiv)	reflux, 3 h	>100	Very complex product mixture
8[a]	CH_2Cl_2	L-proline (1.0 equiv)	reflux, 22 h	41	Dichlorinated
9	CH_2Cl_2	L-proline (1.0 equiv)	reflux, 15 h	38	Mix of mono and dichlorinated products. SiO_2 purification unsuccessful.
10[b]	CH_2Cl_2	L-proline (0.1 equiv)	rt, 4 h	62	Mixture of s.m., mono, and dichlorinated. Pentane extraction removed impurities but didn't separate aldehydes.
11[c]	CH_2Cl_2	L-proline (0.1 equiv)	reflux, 17 h	12	Same as above.
12[b]	CH_2Cl_2	L-proline (0.1 equiv)	rt, 5 h	76	Same as above. Kugelrohr distillation also unsuccessful.

Notes: a: Used 2,3,4,5,6,6-hexachloro-2,4-cyclohexadien-1-one as chlorinating agent instead of NCS. b: 1.1 equiv of NCS were used instead of 0.95. c: Used trichloromethanesulfonyl chloride (1.1 equiv) as chlorinating agent instead of NCS. Catalyst 1: (2S)-2-[diphenyl[(trimethylsilyl)oxy]methyl]pyrrolidine

Several attempts to alpha-chlorinate were taken using a slight deficiency (0.95 equiv) of the chlorinating agent, which was typically NCS. This method usually required a stoichiometric amount (1.0 equiv) of L-proline, which defeats the purpose of using an organocatalysts, and forceful reaction conditions (usually refluxing overnight). Yields were generally not good and reaction products were complex mixtures.

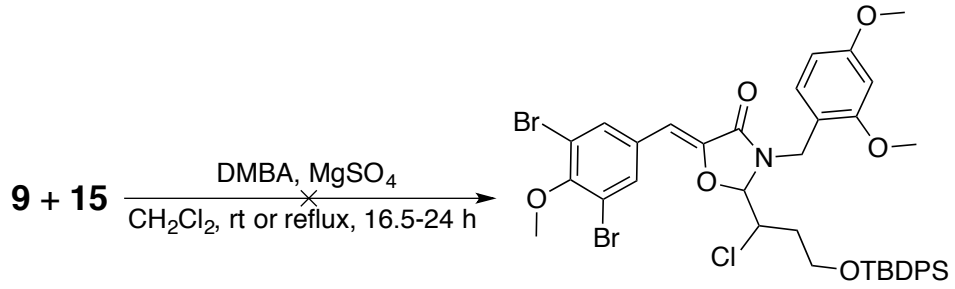
Professor Karl Anker Jørgensen's group at Aarhus University in Denmark also published work on alpha-chlorination of aldehydes.[2b] Jørgensen detailed using a slight excess of chlorinating agent and a truly catalytic amount of the organocatalysts. Furthermore, reactions proceeded in approximately one hour and could be achieved at room temperature. Our investigation quickly adopted this new approach. The crude ^{1}H NMR spectrum suggested a mixture of starting material, desired monochlorinated product, as well as dichlorinated product, evidenced by three different aldehyde signals: a triplet, doublet, and singlet. Jørgensen made no mention of a similar observation, although their group detailed a pentane extraction to purify their product. This pentane extraction was replicated in our study, and while 3 aldehyde signals were still observed, the ^{1}H NMR spectrum appeared much cleaner.

A Kugelrohr distillation was performed at 90 °C. Kugelrohr distillation is usually an effective method of distilling high-boiling liquids, and has been demonstrated to distill aldehydes.[15] A clear, colorless oil was collected, but the ^{1}H NMR spectrum of this oil appeared the same as that of the oil following the pentane extracted. It was thus concluded that the pentane extraction rid the mixture of some impurities, but the aldehyde mixture could not be separated. This mixture of aldehydes was carried on to the oxazolidinone formation

step, under the premise that a mixture of differently chlorinated oxazolidinones could be separated later; moreover, Shymanska demonstrated that both enantiomers of synoxazolidinones A and B have equal activity,[1] so an enantioselective synthesis of synoxazolidinone C the first time was not viewed as crucial.

2.2.4 Construction of 4-oxazolidinone skeleton

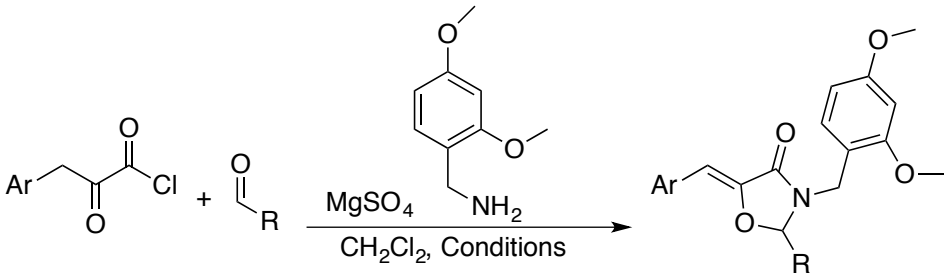
With **9** and **15** in hand (**15** existing as a mixture of aldehydes as described in the previous section) we attempted to synthesize the 4-oxazolidinone skeleton found in synoxazolidinone C by following Shymanska's acylation/cyclization cascade.[1] The compounds were reacted with commercially obtained DMBA as shown in **Scheme 2.13**. Lamentably, our several attempts always resulted in a crude mixture of starting material and impurities being observed in the ^{1}H NMR spectrum; no oxazolidinone signal (we would expect a doublet around 5.3-5.6 ppm) was ever observed, even if the reaction were performed under reflux conditions.



Scheme 2.13: Failed attempts at constructing 4-oxazolidinone skeleton found in synoxazolidinone C

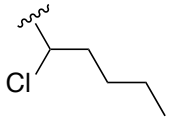
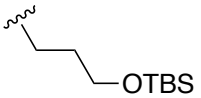
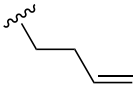
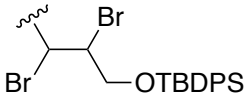
We thought it prudent to explore the construction of such a heterocycle a little more in-depth, so attempts to synthesize analogous compounds were undertaken (Scheme 2.14).

Table 2.3 details these results. In general, alkyl chains terminating in a silyl ether-protected alcohol were unsuccessful in this reactivity. 2-chlorohexanal, as well as chlorinated and non-chlorinated pentenals were successful, however. These observations lead to the conclusion that steric hindrance is a significant factor in the formation of these 4-oxazolidinones, and that bulky TBDPS- or TBS-protected alcohols prevent reactivity. Whether or not the chain resulting from the reacted aldehyde is substituted with halogens seems to have no effect.



Scheme 2.14: Attempts at constructing analogous 4-oxazolidinone scaffolds

Table 2.3: Attempts at constructing analogous 4-oxazolidinone scaffolds

Entry	R=	Conditions	Result
1		rt, 18 h	32% yield
2		rt, 18 h	S.m. consumed but no oxazolidinone signal observed in ^{1}H NMR spectrum
3		rt, 19 h	22% yield
4		rt, 16 h	S.m. consumed but no oxazolidinone signal observed in ^{1}H NMR spectrum

Note: Aromatic group was dibromomethoxy for entries 1 and 2, and phenyl for entries 3 and 4.

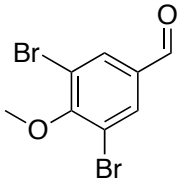
2.3 Conclusions

As discussed earlier, synoxazolidinone C provides an enticing synthetic target and a valuable addition to our group's ongoing research into the synoxazolidinone class of marine natural products. Its bicyclic 4-oxazolidinone scaffold requires a different synthetic approach than the one undertaken by Shymanska for synoxazolidinones A and B. Our efforts in this chapter were based off of the strategy of constructing one ring at a time. While the building blocks were successfully prepared, piecing them together to form the oxzolidinone ring has proven to be a roadblock so far. This setback has sparked an interest in studying how such a structure can be formed, as shown in **Table 2.3**. It has also inspired a broader look at constructing the novel bicyclic 4-oxazolidinone scaffold, including some strategies for constructing both rings in the same step, as well as an analysis of the scaffold's biological activities. These investigations are presented in the following chapter.

2.4 Experimental Section

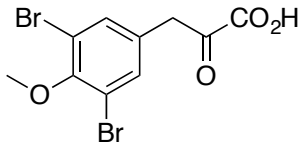
General considerations: THF and CH_2Cl_2 were purified using an alumina filtration system. Reagents and other solvents were commercially obtained and used without further purification. Reactions were monitored by TLC analysis (EM Science pre-coated silica gel 60 F_{254} plates, 250 μm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a $KMnO_4$ solution (1.5 g of $KMnO_4$, 10 g of K_2CO_3, and 1.25 mL of a 10% NaOH solution in 200 mL of H_2O). Reactions were also monitored by LC-MS (Shimadzu LC-MS 2020 with Kinetex 2.6 μm C18 50 x 2.10 mm column). Flash chromatography on SiO_2 used to purify the crude reaction mixtures was performed on a

Biotage Isolera system utilizing Biotage cartridges. IR spectra were determined on a Jasco FT-IR-4100 spectrometer, with sample loaded onto a KBr plate. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Mercury-VX 300 MHz, a Varian Mercury-VX 400 MHz, or a Varian Mercury-Plus 300 MHz instrument in $CDCl_3$ unless otherwise noted. Chemical shifts were reported in ppm with the residual solvent peak used as an internal standard ($CDCl_3$ = 7.26 ppm for ^{1}H and 77.23 ppm for ^{13}C). ^{1}H NMR spectra were run at 300 or 400 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet), integration (number of protons), coupling constant for splitting patterns besides singlet or multiplet. ^{13}C NMR spectra were run at 400 MHz using a proton-decoupled pulse sequence with a d_1 of 1 second unless otherwise noted, and are tabulated by observed peak. HRMS data were obtained on a Thermo Fisher Scientific Exactive Plus mass spectrometer using HESI by the NCSU Mass Spectrometry Facility.

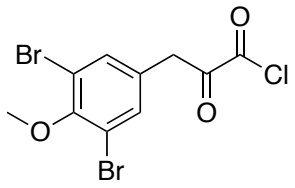


Synthesis of 3,5-dibromo-4-methoxybenzaldehyde (7): 2.00 g (16.1 mmol) 4-hydroxybenzalehyde and 4.03 g (48.3 mmol) NaOAc were dissolved in 30 mL glacial HOAc in a round-bottom flask. Meanwhile, 1.75 mL (33.8 mmol) Br_2 was dissolved in 15 mL HOAc acid in a beaker. The Br_2/HOAc solution was added to the flask, and the mixture developed a cloudy orange appearance. The solution was stirred for 2 h at rt exposed to air. Product was isolated via vacuum filtration and dissolved in 25 mL anhydrous DMF in a flame dried round bottom flask. 5.0 g (36 mmol) K_2CO_3, 2.5 g (16 mmol) NaI, and 1.54 mL

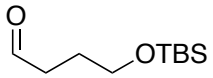
(24.5 mmol) MeI were added. The solution developed a cloudy brown appearance. Reaction was stirred for 16 h at rt under N_2 before being quenched with H_2O. The aqueous layer was extracted with Et_2O. Organic layers were combined and washed successively with 5% aqueous LiCl, H_2O, and brine before being dried with $MgSO_4$ and concentrated en vacuo. Product was a white solid. A yield of 2.17 g (45%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match literature.[1]



Synthesis of 3-(3,5-dibromo-4-methoxyphenyl)-2-oxopropanoic acid (8): 0.14 g (1.70 mmol) NaOAc, 0.20 g (1.70 mmol) *N*-acetyl-gly, and 3.0 mL Ac_2O were added to a round-bottom flask and placed in a hot oil bath. 0.500 g (1.70 mmol) 3,5-dibromo-4-methoxybenzaldehyde were added. The solution turned yellow within 10 min of adding starting material, and a brown-yellow after 30 min. The reaction was stirred for 6 h under reflux. The flask was removed from the oil bath and the product congealed to a yellow paste. Congealed product was dissolved in 10 mL THF. 20 mL 3 M HCl was added, and reaction was stirred for 24 h under reflux. Organic layers were combined, dried ($MgSO_4$) and concentrated en vacuo. Crude product was dissolved in Et_2O and hexane was added to this solution, which was placed in -30 °C freezer for 48 h to precipitate impurities. A yellow liquid was isolated and concentrated en vacuo to obtain an orange solid. A yield of 0.60 g (80%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match literature.[1] This procedure was followed for preparation of oxopropanoic acids in Section 3.3.

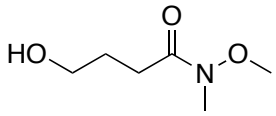


Synthesis of 3-(3,5-dibromo-4-methoxyphenyl)-2-oxopropanoyl chloride (9): 0.046 g (0.131 mmol) 3-(3,5-dibromo-4-methoxyphenyl)-2-oxopropanoic acid was dissolved in 10 mL dry CH_2Cl_2 in a flame-dried round-bottom flask. The mixture was cooled to 0 °C in an ice bath. 0.02 mL (0.235 mmol) oxalyl chloride was added via syringe. The ice bath was removed and reaction was stirred for 90 min at rt under N_2. The mixture was added directly to next step without characterization or purification due to the unstable nature of acid chlorides. This procedure was also followed for the preparation of the corresponding 3-phenyl-2-oxopropanoyl chloride.

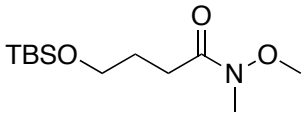


Attempted synthesis of 4-((*tert*-butyldimethylsilyl)oxy)butanal (12): 0.0508 mL (0.6667 mmol) gamma-butyrolactone and 1.0 mL (0.67 mmol) DIBAL-H were added to 1.3 mL dry toluene in a flame-dried round-bottom flask. The reaction was stirred for 6 h at -78 °C under N_2 before being quenched with 0.08 mL MeOH and warmed to 0 °C in an ice bath. 0.5 mL dry CH_2Cl_2, 0.076 g (2.00 mmol) imidazole, 0.192 g (1.33 mmol) I_2, and 0.063 g (0.73 mmol) TBSCl were added to the flask. The reaction was stirred for 66.5 h at rt under N_2. A brown-orange residue was left in the flask. This residue was dissolved with EtOAc. 10 mL saturated $Na_2S_2O_3$ was added and the flask was shaken vigorously. The appearance of the reaction mixture changed to colorless. The organic layer was separated and concentrated en

vacuo. Product was a white crystalline solid. A yield of 0.0639 g (47.4%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data inconsistent with pure desired product.

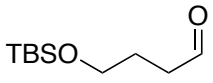


Synthesis of 4-hydroxy-*N*-methoxy-*N*-methylbutanamide (10): 2.8 g (29 mmol) *N,O*-dimethylhydroxylamine was dissolved in 70 mL dry CH_2Cl_2 in a flame-dried round-bottom flask. The mixture was cooled to 0 °C in an ice bath. 15 mL (29mmol) $AlMe_3$ was added dropwise via syringe. The solution was stirred for 15 min at 0 °C after all of the $AlMe_3$ was added. Meanwhile, 2.00 mL (26.3 mmol) gamma-butyrolactone was dissolved in 74 mL dry CH_2Cl_2 in a second flame-dried round-bottom flask. This solution was then transferred to the first flask. The ice bath was removed and the reaction was stirred for 24 h at rt. The reaction was quenched with 20 mL H_2O. Product was extracted with CH_2Cl_2 (3 x 50 mL). Organic layers were combined, dried with $MgSO_4$, and concentrated en vacuo. Product was a colorless oil. A yield of 1.8397 g (47.62%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match literature.[9] **ESIMS** ($[M+H]^+$) calculated for $C_6H_{14}NO_3$: 148.1, observed: 148.2.

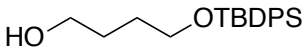


Synthesis of 4-((*tert*-butyldimethylsilyl)oxy)-*N*-methoxy-*N*-methylbutanamide (11): The entire quantity of (**10**) was dissolved in 45 mL dry CH_2Cl_2 in a flame-dried round-bottom flask. 1.12 g (16.3 mmol) imidazole, 0.15 g (1.3 mmol) DMAP, and 2.11 g (13.8 mmol) TBSCl were added. The reaction mixture turned cloudy immediately upon addition of TBSCl. The reaction was stirred for 17 h at rt under N_2. The reaction was quenched with 15

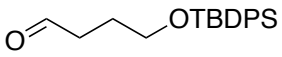
mL saturated NH_4Cl and 25 mL deionized H_2O. Product was extracted with CH_2Cl_2 (3 x 20 mL). Organic layers were combined, dried with $MgSO_4$, and concentrated en vacuo. Product was a clear, pale yellow oil. A yield of 3.03 g (92.9%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match literature.[16] **ESIMS** ($[M+H]^+$): calculated ($C_{12}H_{28}NO_3Si$): 262.2, observed: 262.2.



Synthesis of 4-((*tert*-butyldimethylsilyl)oxy)butanal (12): 0.0990 g of **11** was dissolved in 4.2 mL Et_2O in a round-bottom flask. The solution was cooled to 0 °C in an ice bath. 0.13 mL (0.76 mmol) DIBAL-H was added dropwise via syringe. The ice bath was removed and reaction was stirred at rt. After 3 h, TLC (20% EtOAc in hexane) showed 2 spots, R_f = 0.13 and 0.51. S.M. had R_f = 0.13 in the same solvent system, indicating a mix of S.M. and product existed in the reaction flask. No further progress was observed after an additional 2 h. The reaction mixture was poured into a solution of aqueous tartaric acid and Et_2O (3 g tartaric acid dissolved in 40 mL deionized H_2O, then 15 mL Et_2O added). Layers were separated. The aqueous layer was washed with Et_2O (3 x 10 mL). Organic layers were combined, dried, and concentrated en vacuo. Crude product was a pale white oil. A yield of 0.0657 g (85.73%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match literature.[17]

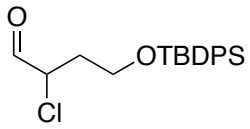


Synthesis of 4-((*tert*-butyldiphenylsilyl)oxy)butan-1-ol (13): 0.09 g (0.71 mmol) DMAP, 1.10 mL (7.84 mmol) Et_3N, 1.89 mL (7.13 mmol) TBDPSCl, and 2.00 mL (22.0 mmol) 1,4-butanediol were dissolved in 55.0 mL dry CH_2Cl_2 in a flame-dried round-bottom flask. The reaction was stirred for 24 h at rt under N_2 before being quenched with saturated NH_4Cl. Product was extracted with CH_2Cl_2. Product was purified via flash chromatography (20-40% EtOAc in hexane) and spotted on TLC to show an R_f = 0.21 (20:80 EtOAc:hexane). Product was a colorless oil. A yield of 2.34 g (100%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match literature.[18]

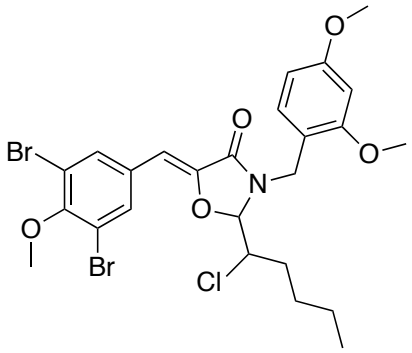


Synthesis of 4-((*tert*-butyldiphenylsilyl)oxy)butanal (14) via Swern oxidation: 35.4 mL dry CH_2Cl_2, 0.59 mL (6.84 mmol) oxalyl chloride, and 0.98 mL (13.69 mmol) dry DMSO were added to a flame-dried round-bottom flask at -78 °C. This solution was stirred for 30 min. Meanwhile, 2.34 g 4-((*tert*-butyldiphenylsilyl)oxy)butan-1-ol was dissolved in 10 mL dry CH_2Cl_2 in another flame-dried round-bottom flask. This second solution was transferred to the first solution dropwise via syringe. The new solution was stirred for 1 h at -78 °C. 4.98 mL (35.65 mmol) Et_3N was added. The reaction was stirred for 20 min at -78 °C, then for 40 min at 0 °C. The reaction was quenched with H_2O and extracted with CH_2Cl_2. Organic layers were combined, dried with $MgSO_4$, and concentrated en vacuo. 10 mL Et_2O were added and flask was placed in 0 °C freezer overnight. Precipitate was isolated via filtration, and the liquid filtrate was azeotropically co-evaporated with benzene (3 x 6 mL). Product was a

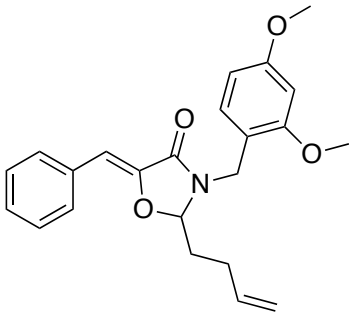
yellow oil. A yield of 2.24 g (96.2%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match literature.[19]



Synthesis of 4-((*tert*-butyldiphenylsilyl)oxy)-2-chlorobutanal (15): 0.2158 g (0.6610 mmol) of **14** was dissolved in 4 mL dry CH_2Cl_2. The solution was cooled to 0 °C in an ice bath. 0.10 g (0.73 mmol) NCS and 7.61 mg (0.066 mmol) L-proline were added. The ice bath was removed and reaction was allowed to warm to rt. The reaction was stirred under N_2 and was monitored via ^{1}H NMR spectroscopy, looking for the appearance of an aldehyde doublet. After 5.5 h, reaction progress seemed to stop. 10 mL of pentane was added to the reaction mixture and a cloudy white precipitate formed. The reaction mixture was allowed to sit for 1 h to maximize precipitation, then gravity filtered through cotton 3 times. The filtrate was concentrated en vacuo. Product was a clear, colorless oil. A yield of 0.1822 g (76.37%) was obtained. ^{1}H NMR still showed 3 aldehyde signals, but in general, spectrum appeared cleaner. A Kugelrohr distillation (90 °C, 10 mbar) was performed and a colorless oil was collected. ^{1}H NMR spectrum matched the previous one. Material was carried on to oxazolidinone formation. **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): 9.81 (t, 1H, J=1.63), 9.63 (d, 1H, J=1.94), 9.33 (s, 1H), 7.69 (m, 4H), 7.42 (m, 6H), 4.56 (m, 1H), 4.43 (m, 1H), 4.32 (m, 1H), 3.85 (m, 1H), 3.72 (m, 1H), 2.73 (m, 1H), 2.69 (m, 1H), 2.54 (m, 1H), 2.28 (m, 1H), 2.19 (s, 1H), 2.08 (m, 1H), 1.92 (m, 1H), 1.65 (m, 1H), 1.08 (s, 9H).



Synthesis of (*Z*)-2-(1-chloropentyl)-5-(3,5-dibromo-4-methoxybenzylidene)-3-(2,4-dimethoxybenzyl)oxazolidin-4-one (Table 2.3, Entry1): 0.01 g (0.07 mmol) 2-chlorohexanal prepared according to literature procedure[20] was dissolved in 0.60 mL dry CH_2Cl_2 in a flame-dried round-bottom flask. 0.038 g (0.31 mmol) MgSO4 and 0.014 mL (0.016 g, 0.093 mmol) DMBA were added. The reaction was stirred for 20 min under N_2 before being cooled to 0 °C in an ice bath. 0.031 g (0.085 mmol) of **9** dissolved in 0.34 mL CH_2Cl_2 were added to the solution. The reaction mixture bubbled and quickly developed a red color upon addition of **9**. The ice bath was removed and the reaction was stirred for 18 h at rt under N_2. $MgSO_4$ was removed via vacuum filtration and solvent was removed en vacuo. Product was purified via flash chromatography (1-20% EtOAc in hexane). Product was a red residue. A yield of 0.0148 g (32.3%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): 7.67 (d, 2H, J=7.39), 7.36 (t, 4H, J=7.61), 6.46 (s, 2H), 6.26 (s, 1H), 5.55 (d, 1H, J=1.76), 4.88 (d, 1H, J=14.52), 4.66 (s, 1H), 4.34 (d, 1H, J=14.52), 4.18 (s, 2H), 3.85 (s, 3H), 3.80 (s, 6H), 2.17 (s, 3H).



Synthesis of (*Z*)-5-benzylidene-2-(but-3-en-1-yl)-3-(2,4-dimethoxybenzyl)oxazolidin-4-one (Table 2.3, Entry 3): Prepared following the procedure for **Table 2.3, Entry 1**, using 0.35 mL (0.30 g, 3.4 mmol) commercially obtained 4-pentenal as the aldehyde and 2-oxo-3-phenylpropanoyl chloride, which was prepared following the procedure for **9** starting from commercially obtained phenylpyruvic acid, as the acid chloride. Product was purified via flash chromatography (20-50% EtOAc in hexane). Product was a yellow oil. A yield of 0.251 g (22.3%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) 7.65 (d, 2H, J=8.73), 7.34 (m, 4H), 6.47 (s, 1H), 6.45 (s, 1H), 6.26 (s, 1H), 5.80 (m, 1H), 5.38 (d, 1H, J=4.80), 5.01 (m, 2H), 4.24 (d, 1H, J=14.75), 4.11 (d, 1H, J=7.13), 3.82 (s, 3H), 3.80 (s, 3H), 2.18 (m, 3H), 1.81 (m, 1H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm) 162.2, 161.0, 158.3, 144.2, 136.8, 134.1, 131.6, 129.0, 128.4, 127.2, 115.9, 155.8, 155.5, 104.6, 102.8, 98.5, 90.3, 55.4, 55.3, 52.8, 38.4, 32.5, 26.6. **IR** (FT-IR, salt plate, cm^{-1}) 2934, 1706, 1412, 1291, 1214, 1032, 915, 761, 694. **HRMS** (ESI, $[M+H]^+$) calculated for $C_{23}H_{26}NO_4$: 380.18563, observed: 380.18504.

2.5 References

(1) Shymanska, N.V.; An, I.H.; Pierce, J.G. *Angew. Chem. Int. Ed.* **2014**, *53*, 5401.

(2) (a) Brochu, M.P.; Brown, S.P.; MacMillan, D.W.C. *J. Am. Chem. Soc.* **2004**, *126*, 4108. (b) Halland, N.; Braunton, A.; Bachmann, S.; Marigo, M.; Jorgensen, K.A. *J. Am. Chem. Soc.* **2004**, *126*, 4790. (c) Review: France, S.; Weatherwax, A.; Lectka, T. *Eur. J. Org. Chem.* **2005**, 475.

(3) Roush, W.R.; Essenfeld, A.P.; Warmus, J.S.; Brown B.B. *Tetra. Lett.* **1989**, *30*, 7305.

(4) Laxmi, Y.S.; Iyengar, D.S. *J. Chem. Soc. Perkin Trans. 1* **1995**, 3043.

(5) Acharya, A.; Montes, K.; Jeffrey, C.S. *Org. Lett.* **2016**, *18*, 6082.

(6) (a) Stephens, B.E.; Liu, F. *J. Org. Chem.* **2009**, *74*, 254. (b) Opalka, S.M.; Steinbacher, J.L.; Lambris, B.A.; McQuade, D.T. *J. Org. Chem.* **2011**, *76*, 6503.

(7) Nahm, S.; Weinreb, S.M. *Tetra. Lett.* **1981**, *22*, 3815.

(8) Yoshino, T.; Ng, F.; Danishefsky, S.J. *J. Am. Chem. Soc.* **2006**, *128*, 14185.

(9) Jeker, O.F.; Kravina, A.G.; Carreira, E.M. *Angew. Chem. Int. Ed.* **2013**, *52*, 12166.

(10) Labarre-Laine, J.; Beniazza, R.; Desvergnes, V.; Landais, Y. *Org. Lett.* **2013**, *15*, 4706.

(11) Calter, M.A.; Liao, W.; Struss, J.A. *J. Org. Chem.* **2001**, *66*, 7500.

(12) (a) Sharma, A.K.; Swern, D. *Tetra. Lett.* **1974**, *15*, 1503. (b) Omura, K.; Sharma, A.K.; Swern, D. *J. Org. Chem*. **1976**, *41*, 957. (c) Huang, S.L.; Omura, K.; Swern, D. *Synthesis* **1978**, 297. (d) Kürti L.; Czakó, B. *Strategic Applications of Named Reactions in Organic Synthesis*; Amsterdam: Elsevier, 2005; pp 450-451. (e) Williams, D.R.; Heidebrecht, R.W. *J. Am. Chem. Soc.* **2003**, *125*, 1843.

(13) (a) TBDPS: Logan, A.W.J.; Parker, J.S.; Hallside, Michal, S.; Burton, J.W. *Org. Lett*. **2012**, *14*, 2940. (b) Swern: Commandeur, M.; Commandeur, S.; Cossy, J. *Org. Lett*. **2011**, *13*, 6018.

(14) (a) Bharadwaj, A. Scheidt Group Meeting, Northwestern Univ., 26 October 2004. Web. Accessed 16 April 2017. (b) Dalko, P.I.; Moison, L.; *Angew. Chem. Int. Ed.* **2004**, *43*, 5138. (c) Notz, W.; Tanaka, F.; Barbas, C.F.; *Acc. Chem. Res.* **2004**, *37*, 580. (d) List, B. *Tetrahedron* **2002**, *58*, 5573.

(15) Katagiri, T.; Kutose, K.; Shimokawa, N.; Kusunoki, N.; Uneyama, K. *Tetrahedron* **1999**, *55*, 9163.

(16) Kerr, W.J.; Morrison, A.J.; Paterson L.C. *Tetrahedron* **2015**, *71*, 5356.

(17) Kwan, E.E.; Scheerer, J.R.; Evans, D.A.; *J. Org. Chem.* **2013**, *78*, 175.

(18) Qian, P.; Yao, W.; Huang, L.; Meng, X.; Li, Z.; *Tetra. Lett.* **2015**, *56*, 5238.

(19) Ning, X.; Wang, M.; Yao, C.; Chen, X.; Kang, Y.; *Org. Lett.* **2016**, *18*, 2700.

(20) Snyder, S.A.; Treitler, D.S.; Brucks, A.P.; Sattler, W. *J. Am. Chem. Soc.* **2011**, *133*, 15898.

CHAPTER 3

Synthesis and biological activity screening of bicyclic 4-oaxazolidinones

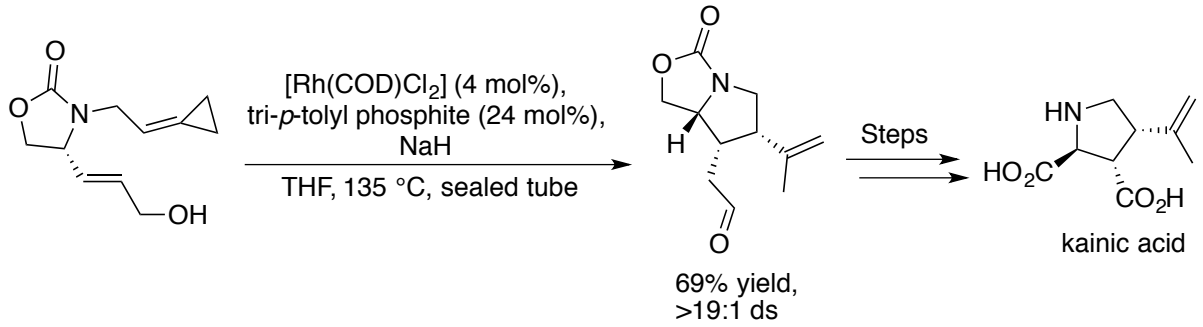
Abstract: The bicyclic 4-oxazolidinone scaffold observed in synoxazolidinone C has been synthesized using several methods. The des-chloro, des-guanidine analog of synoxazolidinone C has been synthesized. Biological activity data for these, and analogous compounds, are presented as a contribution to endeavors of the Pierce group.

3.1 Introduction and Background

In their paper on new synthetic technology for the rapid construction of novel heterocycles, Professor K.C. Nicolaou and co-workers describe a formidable "challenge of developing simple methods for the rapid construction of complex, biologically relevant compounds" facing contemporary organic synthesis, and that total synthesis endeavors are responding to this task by providing "novel forums and opportunities for exploration and for the discovery and development of new chemistry."[1] To our knowledge, no bicyclic 4-oxazolidinone scaffold such as the one found in synoxazolidinone C has ever been discovered in another molecule in nature or synthesized in a laboratory before our efforts. As a goal of the Pierce group is to not only synthesize compounds, but answer questions about chemistry and biology inspired by natural products and the syntheses thereof, this endeavor transitioned from a total synthesis attempt to more of a synthetic methods and medicinal chemistry project. First, we wanted to learn if such a bicyclic scaffold could be synthesized. This question was investigated via three different synthetic methods: RCM, an acid-promoted cyclization involving a butyl amine, and an acid-promoted cyclization of an arylpropanamide. We also wanted to explore the biological activity of these structures. As discussed earlier in this document, the synoxazolidinones show antimicrobial and anticancer

activity, and could serve as promising leads for biofilm inhibition. We wanted to refine scientific knowledge of the biological activity of these structures. More specific to synoxazolidinone C, we wanted know what part of the natural product gives it is biological efficacy, in hopes of developing an SAR.

Interestingly, a remarkably similar bicyclic 2-oxazolidinone scaffold has been prepared as an intermediate in the total synthesis of another marine natural product. The research group of Professor P. Andrew Evans, then at the University of Liverpool, UK, and subsequently at Queen's University, Canada, synthesized (-)-α-kainic acid using a rhodium-catalyzed ene-cycloisomerization of an alkenyldienecyclopropane as the key step to furnish this bicyclic structure (Scheme 3.1). (-)-α-kainic acid is a kainoid natural product first isolated in 1953 from the cold-water marine algae *Digena simplex* off the coast of Japan. It exhibits potent anthelmintic and neuroexcitatory activity.[2]



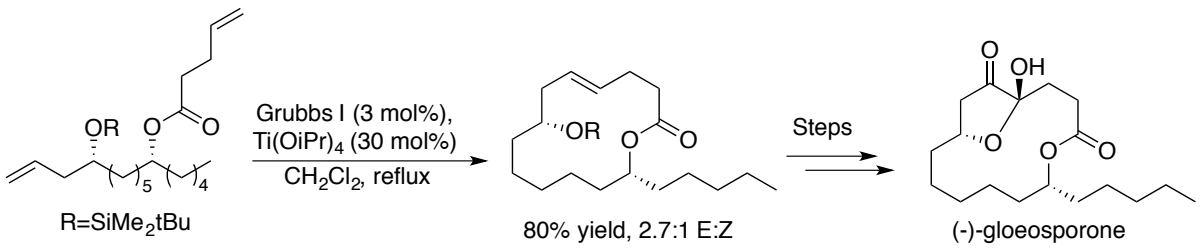
Scheme 3.1: Evans rhodium-catalyzed cyclization to form bicyclic 2-oxazolidinone in the total synthesis of (-)-α-kainic acid

3.2 Ring-closing metathesis strategy

3.2.1 Overview

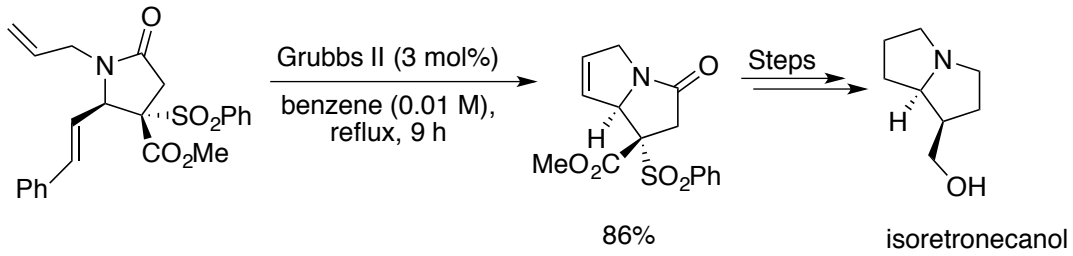
Ring-closing metathesis (RCM) is a powerful, atom-economical synthetic technique featuring the intramolecular metathesis of two terminal alkenes. The resulting cycloalkene is usually five-, six-, or seven-membered, although larger rings are known. These reactions tend to be high-yielding, as there are limited alternate pathways the reaction can take. Most steps are reversible, and the overall reaction is driven by the loss of ethylene gas, which is the only irreversible step. These reactions usually involve a ruthenium-based catalyst, very dilute (c. 0.1 M) reaction conditions, and are often performed under reflux. Investigations into RCM were carried out by many researchers throughout the 1980s and 90s. For their pioneering work in this field, the 2005 Nobel Prize in Chemistry was awarded to Yves Chauvin (French Petroleum Institute), Robert Grubbs (California Institute of Technology), and Richard Schrock (Massachusetts Institute of Technology).[3]

RCM-based strategies in natural product synthesis have literature precedent. The research group of Professor Alois Füstner at the Max Planck Institute, Germany reported RCM-based macrocyclizations in the pursuit of two natural products, (+)-ricinelaidic acid lactone and (-)-gloeosporone (the later depicted in Scheme 3.2). Such a macrocycle had never been synthesized via an RCM strategy before. In line with a discussion in Chapter 1 of this document, this natural product synthesis work expanded the substrate scope of RCM, advancing the fundamental understanding of organic reactivity.[4]



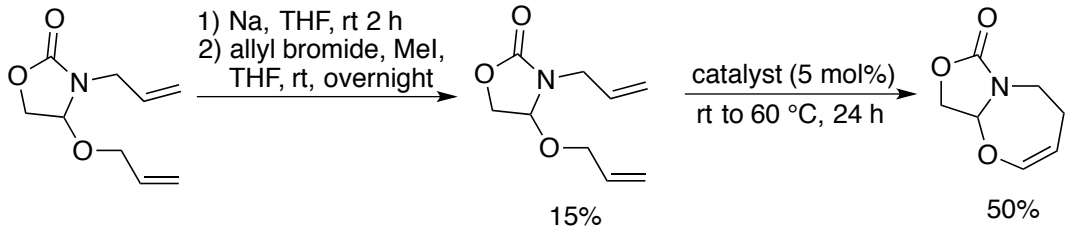
Scheme 3.2: Füstner's application of RCM to natural product synthesis

More recently, Professor Jared Shaw's research group at University of California, Davis synthesized a pyrrolizidine core, analgous to a synoxazolidinone without the cyclic oxygen, in the synthesis of isoretronecanol (Scheme 3.3).[5]



Scheme 3.3: Shaw's application of RCM to natural product synthesis

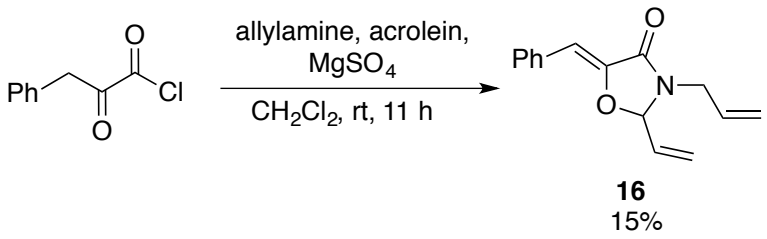
Finally, the research group of Professor Jürgen Martens at Carl von Ossietzky Universität Oldenburg, Germany synthesized a bicyclic 2-oxazolidinone in an RCM strategy (Scheme 3.4). In the synthesis of their RCM precursor, they describe a 15% yield after three purifications on silica gel. Dismal yields have been a problem plaguing our research in oxazolidinone synthesis as well. In the case of analogous thaizolidinethiones, Martens reports the formation of by-products, thus diminishing the yield.[6]



Scheme 3.4: Martens synthesis of bicyclic 2-oxazolidinone using RCM

3.2.2 Results and Discussion

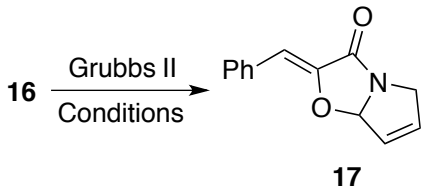
To explore the viability of an RCM strategy, it was first necessary to construct the RCM precursor featuring two terminal alkenes. A 4-oxazolidinone was synthesized according to the general method employed in the previous chapter. As an aside, this reaction furthered our knowledge of the synthesis of these 4-oxazolidinones, as smaller amines and aldehydes worked very well in constructing this skeleton. The requisite RCM precursor (**16**) was synthesized from allylamine, acrolein, and 2-oxo-3-phenylpropanoyl chloride, which was prepared following the procedure for **9** starting from commercially obtained phenylpyruvic acid, and purified to provide a 15% yield (Scheme 3.5).



Scheme 3.5: Preparation of RCM precursor

With **16** in hand, an RCM investigation to generate **17** was launched, depicted in **Scheme 3.6**, with the results summarized in **Table 3.1**. Conducting the reaction at reflux in the solvent with the lowest boiling point saw the highest yielding reaction, and the shortest

required reaction time, although there was an impurity present which was difficult to remove. The purest product was obtained in benzene. Since it was the most pure, **17** synthesized in benzene was carried on for further chemical and biological studies. Performing the reaction in toluene, the highest-boiling solvent, saw the lowest yield and a crude mixture. It was thought that at this high of a boiling point (111 °C), the allyl could have isomerized; however, the ^{1}H NMR spectrum showed no evidence of this side reaction, as no methyl signal was observed.



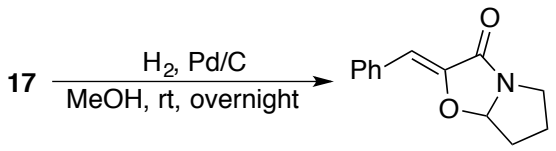
Scheme 3.6: RCM to construct unsaturated bicyclic 4-oxazolidinone scaffold **17**

Table 3.1: Summary of RCM results

Entry	Conditions	Result
1	CH_2Cl_2, rt, 24 h	NRX
2	CH_2Cl_2, reflux, 3.5 h	20% yield (crude)
3	benzene, reflux, 6.5 h	11% yield
4	toluene, reflux, 6.25 h	5% yield (crude)

A hydrogenation reaction was attempted (Scheme 3.7) to see if the cyclic alkene could be reduced selectively over the acyclic one. The reaction was set up following a standard procedure. LC-MS showed a mass peak corresponding to the hydrogenated product. The reaction was worked up, but given the small scale of the reaction, work-up was difficult,

and nothing was observed in the ^{1}H NMR spectrum. Since the masses of the two possible hydrogenated products are equal, we cannot conclusively say whether we hydrogenated the cyclic alkene selectively over the acyclic one. To determine this would be a goal of future study.



Scheme 3.7: Attempted hydrogenation of **17**

3.2.3 Conclusion

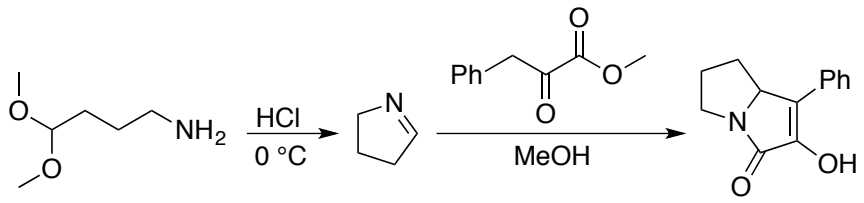
RCM showed to be a viable way of constructing a bicyclic 4-oxazolidinone. Purification of products, however, was somewhat problematic, and low yields hindered the study of hydrogenating the cyclic alkene. The RCM strategy still followed the paradigm from the synoxazolidinone C total synthesis attempt of constructing the bicycle one ring at a time. A more efficient approach to arrive at the bicyclic scaffold was desired, and is the focus of sections 3.3. and 3.4.

3.3 Strategy Involving Cyclization with 4-aminobutyraldehyde Dimethyl Acetal

3.3.1 Overview

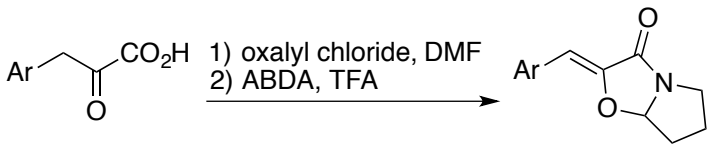
Similar bicyclic scaffolds exist in the realm β-lactam antibiotics, and their syntheses have been investigated for decades. One method is to perform an imine addition to diethyl oxaloacetate.[7] This chemistry has been investigated in the Pierce group by Alex Cusumano.

The imine used is 1-pyrroline, prepared from the acid-promoted cyclization of 4-aminobutyraldehyde dimethyl acetal (ABDA) (Scheme 3.8).[8]



Scheme 3.8: Synthesis of bicyclic lactams in the Pierce Group

For this project, we envisioned the analogous oxygen-addition beginning from oxopropanoyl chloride in an acid-promoted cyclization with ABDA (Scheme 3.9).

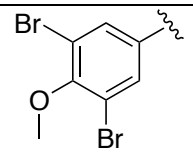
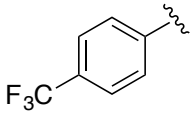
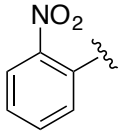
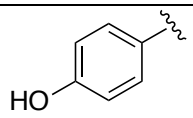
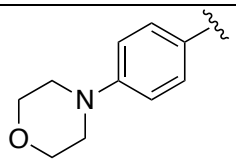
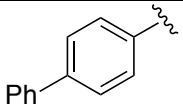
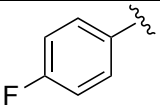
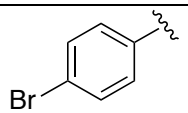


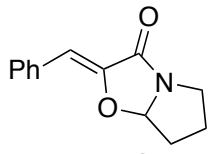
Scheme 3.9: Acid-promoted cyclization involving ABDA to furnish bicyclic oxazolidinone

3.3.2: Results and Discussion

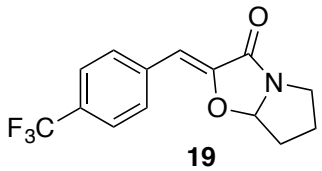
These studies began with the requisite oxopropanoic acid, and **Table 3.2** summarizes the results. In general, this method did not enjoy wide substrate scope, and its products were challenging to purify. Experimentation with different solvent gradients for flash chromatography, recrystallization strategies, and Prep TLC proved fruitless in some cases. However, two compounds came out of this method that signified a key advance in this project (Figure 3.1), as they were to our knowledge the first bicyclic 4-oxazolidinones synthesized, purified, and characterized. They were also used for biological activity screening, elaborated on in Section 3.5.

Table 3.2: Summary of results from the acid-promoted cyclization involving ABDA to furnish bicyclic oxazolidinone scaffold

Entry	Ar =	Time	Result
1	Ph	16 h	18% yield
2		16 h	NRX
3		16 h	13% yield
4		16.5 h	36% crude yield, unable to purify
5		15 h	NRX
6		22 h	NRX
7		17 h	29% crude yield, unable to purify
8		20 h	11% crude yield, unable to purify
9		21 h	6% crude yield, unable to purify



18



19

Figure 3.1: Lead compounds for biological activity screening to come out of acid-promoted cyclization involving ABDA dimethyl acetal

Entries 1 and 3 produced compounds **18** and **19**, respectively, which were the best examples to come out of this method. After several attempts to purify these products, purification was finally achieved using reverse phase chromatography. They were used in an MIC assay and a biofilm inhibition study, discussed in Section 3.5. Unfortunately, the dibromomethoxy aromatic moiety could not be synthesized in this manner (Entry 2). Entries 6 and 7 were attempted as biphenyls and morpholine substituents are common medicinal chemistry targets, and the morpholine scaffold is present in linezolid. Entry 9 was attempted in the hopes of employing cross-coupling chemistry, such as a Suzuki reaction, to produce further analogs. Regrettably, these reactions either failed outright, or had impurities which were impossible to separate after several attempts.

3.3.3 Conclusion

The method described in this section provided access to two compounds, shown in **Figure 3.1**, which supported the biological activity studies goals of this project and the Pierce group. Our goal of accessing the bicyclic 4-oxazolidinone scaffold was realized. This method, however, was unable to reliably produce other compounds of synthetic or medicinal interest. Continuing along these lines, we sought another method of accessing the bicyclic 4-oaxazolidinone scaffold, which is discussed in Section 3.4.

3.4 Acid-Promoted Cyclization of Arylpropanamide Strategy

3.4.1 Overview

Another route to the bicyclic 4-oxazolidinone route we envisioned was the acid-promoted cyclization of an arylpropanamide, which would be synthesized using a peptide coupling reagent. The arylpropanamide would come directly from an oxopropanoic acid, eliminating the inconvenient need to convert to the acid chloride. EDC coupling is a one-pot procedure driven by the formation of a urea by-product, which can be removed with acidic aqueous washings. EDC couplings, and the broader realm of carbodiimide couplings, have been extensively used to form amide bonds, in both academic research laboratories and on industrial scales, and several reviews have been published.[9] The research group of Professor Dale Boger at The Scripps Research Institute in California twice employed EDC coupling in their total synthesis of vancomycin aglycon (Figure 3.2).[10]

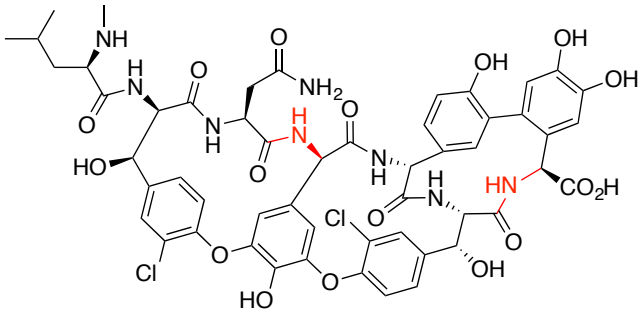
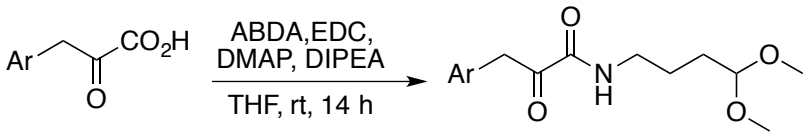


Figure 3.2: Vancomycin aglycon, EDC couplings from Boger's synthesis highlighted in red

3.4.2 Results and Discussion

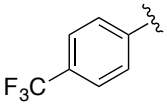
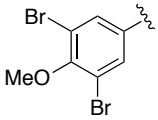
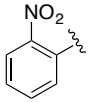
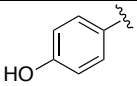
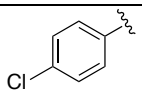
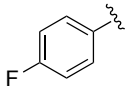
As shown in **Scheme 3.9**, with the results summarized in **Table 3.3**, a variety of arylpropanamides were prepared by performing an EDC coupling with the corresponding oxopropanoic acids and ABDA. As with the method described in Section 3.3, we had success

with phenyl and para-CF_3 aryl groups, and to our delight, the dibromomethoxy version was synthesized successfully as well. Reactions with a few other aryl moieties from the commercially obtained oxopropanoic acids were attempted without satisfactory result.

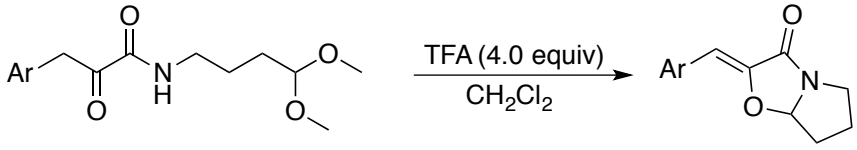


Scheme 3.10: Preparation of arylpropanamides via EDC coupling

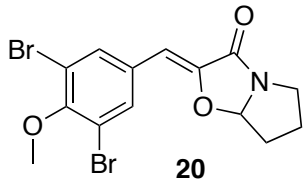
Table 3.3: Summary of results of preparation of arylpropanamides via EDC coupling

Entry	Ar =	Result
1	Ph	51% yield
2		34% yield
3		19% yield
4		NRX
5		NRX
6		Observed consumption of s.m. but no product in LC-MS
7		Observed consumption of s.m. but no product in LC-MS

With three arylpropanamides in hand, we performed a cyclization using TFA, as shown in **Scheme 3.10**, with results summarized in **Table 3.4**. In all three cases, cyclization was readily achieved, with the dibromomethoxy version requiring reflux conditions to proceed. Gratifyingly, this method produced **20**, the des-chloro, des-guanidine analog of synoxazolidinone C (Figure 3.2), and in a relatively high yield for this chemistry. **20** marks the closest compound to synoxazolidinone C synthesized in this work, and was used for biological activity screening, as discussed in Section 3.5. This method also provided another route to **18** and **19**, although in comparable yields to the method described in Section 3.3.



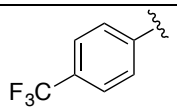
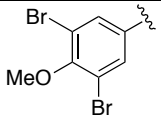
Scheme 3.11: Cyclization of arylpropanamides to furnish the bicyclic 4-oxazolidinone scaffold



20

Figure 3.3: Des-chloro, des-guanidine synoxazolidinone C

Table 3.4: Cyclization of arylpropanamides to furnish the bicyclic 4-oxazolidinone scaffold

Entry	Ar =	Conditions	% Yield
1	Ph	rt, 4 h, 40 min	20
2		rt, 4 h, 40 min	12
3		reflux, 4 h	43

3.4.3 Conclusion

The cyclization of arylpropanamides gave us access to a novel compound **20**, which is the compound most closely approximating synoxazolidinone C we have been able to synthesize to date. It also provided by far the best yield we have achieved for constructing any kind of oxazolidinone skeleton in this work. While we were unable to expand this method to other aromatic moieties, we demonstrated access to compounds **18** and **19**, which had already been prepared in this work. Future studies will include more attempts to diversify the aromatic moieties we can use with this method, as well as attempting to apply substitution to the butyl amine (or some variant of one) to see if functionality can be installed on the right-side ring of the bicycle. Such results would be of great interest to our group, as functionality on that ring is the only barrier between our current position and synoxazolidinone C.

3.5 Biological Activity Studies

3.5.1 Overview

As discussed in Section 1.3, the synoxazolidinone class of marine natural products have shown potent biological activity, particularly as antimicrobial agents. The worldwide spread of antibiotic-resistant bacteria poses a major crisis for public health systems and the healthcare industry. The scientific and medical communities have taken a two-pronged approach combating the growing threat of antimicrobial resistance. The first way consists of conserving the efficacy of existing antibiotics by replacing them with analogous compounds that show better activity. The second way involves using such compounds as chemical probes

to elucidate resistance pathways.[11] Biologically active small molecules such as natural products and their analogs are very suitable for these roles, as discussed in Chapter 1.

The mechanism of action (MoA) of a drug details how it achieves its biological function. The MoA of many antibiotics involves enzyme inhibition. Enzymes are large biomolecules, consisting of a series of amino acids folded into a three-dimensional structure. They catalyze biochemical reactions by providing an alternate, lower energy transition state.[12] Inhibition of enzymes thus inhibits the progress of biochemical reactions necessary for the bacteria's survival. Many antibiotics, such as penicillin and vancomycin, function by inhibiting the transpeptidases, enzymes responsible for producing and cross-linking strands of peptidoglycan, a net-like polymer which constitutes the bacterial cell wall. Preventing this process weakens the integrity of the cell wall, inducing cell death.[13] Mutations in gene codings for transpeptidases leads to diminished interaction with antibiotics, contributing to antimicrobial resistance.[14] As mentioned in Chapter 1, however, oxazolidinones work by inhibiting mitochondrial protein synthesis. This different MoA means oxazolidinones may be effective against bacteria to which other classes of antibiotics have been rendered useless. While linezolid-resistant *Staphylococcus aureus* has been observed,[15] resistance to linezolid is still generally low.[16]

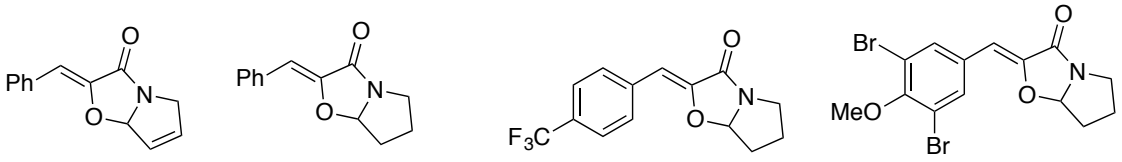
Oxazolidinones have also shown activity against biofilms, another major growing public health threat. Biofilms form as bacteria stick to each other to form an aggregate, and then adhere to a surface. There are several causes for bacteria to undergo this process, but it is often a response to nutritional cues, recognition of a suitable site on which to adhere, or even exposure to sub-inhibitory concentrations of antibiotics.[17] When bacteria switch from

free-floating to existing in biofilms, they undergo a phenotypic shift, resulting in a difference in gene regulation, affecting a drug's ability to function.[18] An estimated 60-70% of hospital-acquired infections are associated with the implantation of a medical device, either from the device itself or contaminated surgical equipment. This problem costs the world's healthcare industry $5 billion per year. If the infection turns into a biofilm, it can become very difficult to treat, as it becomes resistant to antibiotics and the host's immune system.[19]

While Shymanska attempted to develop an SAR with analogs of the synoxazolidinones she synthesized, she was unable to elucidate a conclusive SAR.[20] With several bicyclic 4-oxazolidinones prepared in this work, we thought it an important contribution to this project and the Pierce group's endeavors to perform an analysis of the biological activity of these compounds.

3.5.2 Results and Discussion

MIC and biofilm inhibition assays were performed on four of the compounds synthesized in this work, and the data are shown in **Figure 3.4**. Disappointingly, the bicyclic oxazolidinones with phenyl, para-CF_3, and dibromomethoxy aromatic moieties displayed no activity against MSSA, and no dose-dependent response against MRSA.



17
MIC >128 μg/mL
(MSSA ATCC 29213)

18
MIC >128 μg/mL
(MSSA ATCC 29212)

$IC_{50} > 80$ μM
(MRSA ATCC 33591)

19
MIC >128 μg/mL
(MSSA ATCC 29212)

$IC_{50} > 80$ μM
(MRSA ATCC 33591)

20
MIC >128 μg/mL
(MSSA ATCC 29212)

$IC_{50} > 80$ μM
(MRSA ATCC 33591)

Figure 3.4: Biological activity data of selected compounds synthesized

3.5.3 Conclusion

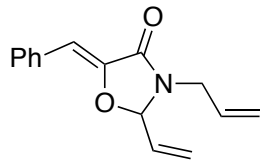
The data that all of the synoxazolidinone C analogs with the unfunctionalized bicyclic scaffold reveal that some substitution on the bicycle is required. Simply having the correct aromatic moiety is insufficient for activity. This adds information to the enigma that is trying to elucidate an SAR for the synoxazolidinones. While perplexing, having this data is vital for understanding what makes the synoxazolidinones so active.

3.6 Conclusion

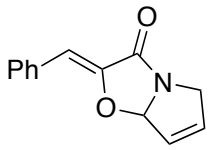
While the natural product synoxazolidinone C has not yet been synthesized, in this chapter we have demonstrated three methods for constructing the key bicyclic 4-oxazolidinone scaffold. Rapid access of this necessary heterocyclic core can inspire further synthetic attempts to the natural product. A survey of the biological activity of several of these unfunctionalized bicyclic analogs of the natural product revealed no activity, and while disappointing, furthers our knowledge of these fascinating compounds, as we know that these substitution patterns alone are insufficient for biological activity, and therefore as drug leads.

3.7 Experimental Section

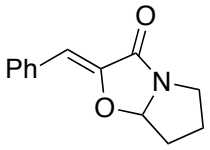
See Section 2.4 for general considerations.



Synthesis of (*Z*)-3-allyl-5-benzylidene-2-vinyloxazolidin-4-one (16): 0.10 mL (0.08 g, 1.4 mmol) allylamine, 0.09 mL (0.08 g, 1.3 mmol) acrolein, and 0.56 g (4.6 mmol) $MgSO_4$ were dissolved in 10 mL dry CH_2Cl_2 and stirred for 20 min at rt under N_2 before the flask was cooled to 0 °C in an ice bath. 0.20 g 2-oxo-3-phenylpropanoyl chloride (prepared following the procedure for preparing **9**, starting from commercially obtained phenylpyruvic acid) in 30 mL dry CH_2Cl_2 was added to the flask. The ice bath was removed and the reaction was stirred for 11 h at rt under N_2. $MgSO_4$ was removed via vacuum filtration and solvent was removed en vacuo. Product was purified via flash chromatography (20-50% EtOAc in hexane). Product was a red residue. A yield of 0.039 g (14.6%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): 7.79 (d, 2H, J=7.11), 7.40 (m, 2H), 7.32 (m, 1H), 6.71 (s, 1H), 6.29 (s, 1H), 5.84 (m, 1H), 5.40 (m, 1H), 5.21 (m, 1H), 3.96 (m, 1H), 3.75 (m, 1H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm): 170.0, 138.23, 133.7, 130.3, 128.6, 121.7, 117.5, 113.7, 84.2, 44.4, 42.1, 40.9, 37.67, 36.5, 31.1, 29.8. **HRMS** (ESI, $[M+H]^+$) calculated for $C_{15}H_{16}NO_2$: 242.11756, observed: 242.11706.

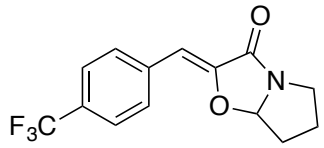


Synthesis of (*Z*)-2-benzylidene-5,7a-dihydropyrrolo[2,1-*b*]oxazol-3(2*H*)-one (17): 0.01 g Grubbs 2nd generation catalyst was added to a flame-dried round-bottom flask under argon. 12.4 mL dry benzene and 0.03 g **16** were added. The reaction was stirred for 3 h under reflux conditions in an oil bath, when LC-MS showed reaction progress had stopped. An additional 0.01 g catalyst were added. After an additional 3.5 h, LC-MS again showed no reaction progress. The reaction mixture was also spotted on TLC (20% EtOAc in hexane) and one spot (R_f = 0.17) was observed. The reaction flask was removed from the oil bath and solvent was removed en vacuo. Product was purified via flash chromatography (10-30% EtOAc in hexane). Product was a clear, colorless oil. A yield of 0.0028 g (10.6%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$, ppm): 7.66 (d, 1H), 7.59 (d, 2H), 7.31 (m, 10H (expect 3H, overintegration likely due to presence of residual benzene, 7.36 ppm), 6.32 (s, 1H), 6.22 (s, 1H), 5.33 (m, 1H), 4.38 (m, 1H), 3.84 (m, 1H), 3.34 (m, 2H).



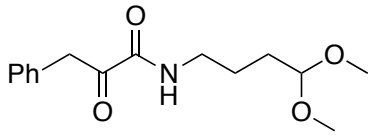
Synthesis of (*Z*)-2-benzylidenetetrahydropyrrolo[2,1-*b*]oxazol-3(2*H*)-one (18):
0.100 g (0.607 mmol) of phenylpyruvic acid was dissolved in 5 mL dry CH_2Cl_2. The solution was cooled to 0 °C in an ice bath. 0.10 mL (1.09 mmol) oxalyl chloride and 1 drop of DMF were added via syringe. The reaction was allowed to warm to rt and stirred for 90 min under a N_2. The reaction was cooled again to 0 °C in an ice bath. 0.09 mL (0.61 mmol) ABDA and

0.23 mL (3.0 mmol) TFA were added via syringe. The reaction was allowed to come to rt and stirred for 14 h under N_2. The reaction mixture was concentrated en vacuo and purified via reverse-phase flash chromatography (10-80% MeCN in a solution of 0.1% TFA in H_2O). Product was a yellow oil. A yield of 0.023 g (17.9%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$, ppm) 7.66 (s, 1H), 7.36 (s, 1H), 7.28 (s, 1H), 5.80 (dd, 1H, J=7.48, 5.40), 3.92 (m, 1H), 3.34 (m, 1H), 2.34 (m, 1H), 2.19 (m, 1H), 2.08 (m, 1H), 1.68 (m, 1H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm) 166.4, 145.8, 129.3, 128.48, 122.7, 104.8, 95.1, 42.8, 31.2, 23.4. **IR** (FT-IR, salt plate, cm^{-1}) 3354, 3060, 1712, 696. **HRMS** (ESI, $[M+H]^+$) calculated for $C_{13}H_{14}NO_2$: 216.10191, observed: 216.10207.



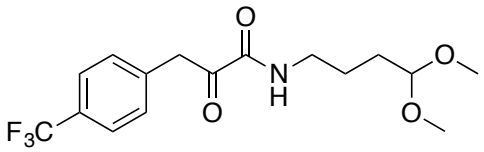
Synthesis of (*Z*)-2-(4-(trifluoromethyl)benzylidene)tetrahydropyrrolo[2,1-*b*]oxazol-3(2*H*)-one (19): 0.101 g (0.435 mmol) of 2-oxo-3-(4-(trifluoromethyl)phenyl)propanoic acid was dissolved in 5 mL dry CH_2Cl_2. The solution was cooled to 0 °C in an ice bath. 0.06 mL (0.78 mmol) oxalyl chloride and 1 drop of *N*-*N*-dimethylformamide were added via syringe. The reaction was allowed to warm to rt and stirred for 90 min under N_2. The reaction was cooled again to 0 °C in an ice bath. 0.06 mL (0.43 mmol) ABDA and 0.16 mL (2.2 mmol) TFA were added via syringe. The reaction was allowed to warm to rt and stirred for 14 h under N_2. The reaction mixture was concentrated en vacuo to produce a yellow oil. The reaction mixture was concentrated en vacuo and purified via reverse-phase flash chromatography (10-80% MeCN in a solution of 0.1% TFA in H_2O). Product was a white

residue. A yield of 0.016 g (12.8%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$, ppm) 7.74 (d, 2H, J=8.14), 7.57 (d, 2H, J=8.27), 6.27 (s, 1H), 5.83 (t, 1H, J=12.71), 3.83 (m, 1H), 3.35 (m, 1H), 2.36 (m, 1H), 2.21 (m, 1H), 2.10 (m, 1H), 1.68 (m, 1H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm) 157.4, 153.7, 130.0, 129.2, 126.0, 125.4, 102.7, 95.3, 42.9, 31.2, 23.6. **IR** (FT-IR, salt plate, cm^{-1}) 2960, 1716, 854. **HRMS** (ESI, $[M+H]^+$) calculated for $C_{14}H_{13}F_3NO_2$: 284.08929, observed: 284.08928.

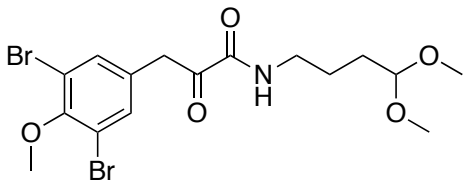


Synthesis of *N*-(4,4-dimethoxybutyl)-2-oxo-3-phenylpropanamide (Table 3.3, Entry 1): 0.5007 g (2.989 mmol) phenylpyruvic acid was dissolved in 50 mL dry THF in a flame-dried round-bottom flask, which was then cooled to 0 °C in an ice bath. 0.6946 g (3.587 mmol) EDCHCl, 1.33 mL (0.985 g, 7.47 mmol) DIPEA, 0.0738 g (0.5978 mmol) DMAP, and 0.63 mL (0.60 g, 4.5 mmol) ABDA were added. The ice bath was removed and the reaction was stirred for 14 h at rt. The reaction was quenched with $NaHCO_3$ and extracted with EtOAc. The organic layer was then washed successively with H_2O, 0.1 M HCl, and brine. The organic layer was dried with $MgSO_4$ and concentrated en vacuo. Product was purified via flash chromatography (40-60% EtOAc in hexane). Product was a yellow oil. A yield of 0.5437 g (65.12%) was obtained. **TLC** R_f = 0.35 (40% EtOAc in hexane). **^{1}H NMR** (400 MHz, $CDCl_3$, ppm) 7.33 (m, 5H), 4.36 (s, 1H), 4.22 (s, 2H), 3.32 (s, 6H), 1.62 (bs, 4H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm) 196.0, 160.0, 132.7, 129.8, 128.6, 127.1, 104.1, 53.0, 43.1, 39.2, 29.9, 24.1. **IR** (FT-IR, salt plate, cm^{-1}) 3318, 2953, 2830, 2345, 1683, 1531, 1458,

1264, 1047. **HRMS** (ESI, $[M+Na]^+$) calculated for $C_{15}H_{21}NO_4$: 302.13628, observed: 302.13608.

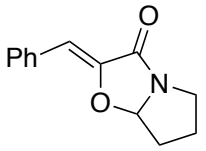


Synthesis of *N*-(4,4-dimethoxybutyl)-2-oxo-3-(4-(trifluoromethyl)phenyl)propanamide (Table 3.3, Entry 2): Prepared following the same method as **Table 3.3, Entry 1**, using **8** as the starting oxopropanoic acid, and using 40-65% EtOAc in hexane as the flash chromatography gradient. Product was a light-yellow solid. A yield of 0.1500 g (34.2%) was obtained. **TLC** R_f = 0.31 (40% EtOAc in hexane). **^{1}H NMR** (400 MHz, $CDCl_3$, ppm) 7.66 (m, 2H), 7.44 (m, 2H), 4.43 (s, 1H), 4.35 (m, 2H), 3.39 (s, 6H), 1.72 (bs, 6H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm) 195.3, 159.7, 130.2, 125.6, 125.5, 104.1, 53.2, 42.9, 39.3, 29.9, 24.1. **IR** (FT-IR, salt plate, cm^{-1}) 3314, 2946, 2834, 1683, 1527, 1326, 1164, 1120, 1066, 1020. **HRMS** (ESI, $[M+H-H_2O]^+$) calculated for $C_{16}H_{20}F_3NO_4$: 330.13115, observed: 330.13025.

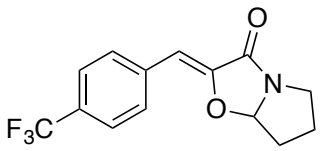


Synthesis of 3-(3,5-dibromo-4-methoxyphenyl)-*N*-(4,4-dimethoxybutyl)-2-oxopropanamide (Table 3.3, Entry 3): Prepared following the same method as **Table 3.3, Entry 1**, using **8** as the starting oxopropanoic acid, and using 40-70% EtOAc in hexane as the flash chromatography gradient. Product was a brown-yellow residue. A yield of 0.024 g (18.9%) was obtained. **TLC** R_f = 0.53 (60% EtOAc in hexane). **^{1}H NMR** (400 MHz, $CDCl_3$,

ppm) 8.28 (s, 1H), 7.37 (s, 1H), 4.34 (t, 1H, J=5.02), 3.85 (s, 3H), 3.31 (s, 6H), 1.62 (bs, 4H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm) 195.0, 159.5, 153.3, 133.9, 131.4, 118.1, 104.1, 53.2, 41.6, 39.3, 29.9, 24.1. **IR** (FT-IR, salt plate, cm^{-1}) 3326, 2930, 2830, 1721, 1683, 1531, 1473, 1427, 1264, 1191, 1128, 1070, 997, 741. **HRMS** (ESI, $[M+H]^+$) calculated for $C_{16}H_{21}Br_2NO_5$: 465.98592, observed: 465.98530.

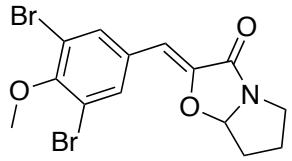


Synthesis of (*Z*)-2-benzylidenetetrahydropyrrolo[2,1-*b*]oxazol-3(2*H*)-one (Table 3.4, Entry 1): 0.14 g (0.50 mmol) *N*-(4,4-dimethoxybutyl)-2-oxo-3-phenylpropanamide was dissolved in 8.0 mL dry CH_2Cl_2 in a flame-dried round-bottom flask. 0.14 mL (0.23 g, 2.0 mmol) TFA was added. Purple color developed upon addition of TFA but quickly disappeared. The reaction was stirred for 4 h at rt under N_2, by which time a clear yellow appearance had developed. Solvent was removed en vacuo. The product was purified via reverse-phase flash chromatography (10-80% MeCN in a solution of 0.1% TFA in H_2O). Product was a brown-yellow residue. A yield of 0.0230 g (20.5%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match that of **18**.



Synthesis of (*Z*)-2-(4-(trifluoromethyl)benzylidene)tetrahydropyrrolo[2,1-*b*]oxazol-3(2*H*)-one (Table 3.4, Entry 2): Prepared following the same method as **Table 3.4, Entry 1**, using *N*-(4,4-dimethoxybutyl)-2-oxo-3-(4-(trifluoromethyl)phenyl)propanamide as the

starting material. Product was a light-yellow residue. A yield of 0.0027 g (11.9%) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$) data match that of **19**.



Synthesis of (*Z*)-2-(3,5-dibromo-4-methoxybenzylidene)tetrahydropyrrolo[2,1-*b*]oxazol-3(2*H*)-one (Table 3.4, Entry 3) (20): 0.0241 g (0.052 mmol) 3-(3,5-dibromo-4-methoxyphenyl)-*N*-(4,4-dimethoxybutyl)-2-oxopropanamide weas dissolved in 2 mL dry CH_2Cl_2 in a flame-dried round-bottom flask. 0.015 mL (0.024 g, 0.206 mmol) TFA was added. The reaction was stirred for 4 h under reflux. Product was isolated and purified following the method for Table 3.4, Entry 1. Product was a yellow-brown residue. A yield of 0.0090 g (43.3% yield) was obtained. **^{1}H NMR** (400 MHz, $CDCl_3$, ppm) 7.79 (s, 2H), 6.07 (s, 1H), 5.81 (m, 1H), 3.89 (s, 3H), 3.34 (m, 1H), 2.36 (m, 1H), 2.21 (m, 2H), 1.67 (m, 2H). **^{13}C NMR** (400 MHz, $CDCl_3$, ppm) 169.0, 153.4, 146.9, 132.9, 132.5, 118.1, 110.0, 101.0, 95.3, 60.7, 42.9, 31.2, 23.6. **IR** (FT-IR, salt plate, cm^{-1}) 2926, 1717, 1473, 1365, 1268, 997, 733. **HRMS** (ESI, $[M+H]^+$) calculated for $C_{14}H_{14}Br_2NO_3$: 401.93350, observed: 401.93329.

Biological data, bacterial strains and assay protocols

General information

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were obtained from the ATCC (33591, BAA-44) and colonies were grown on solid media as instructed. MHB (211443-BD), TSB (Remel: R455052) and D-glucose (CAS: 492-62-6) were purchased from Fisher Scientific. TSA (cat. # 22091) and linezolid (cat. # P70014) were purchased from Sigma-

Aldrich. Bacteria for biofilm inhibition and dispersion assays were cultured overnight in TSBG. All assays were run in duplicate and repeated at least two separate times for MIC assays and at least four separate times for biofilm inhibition and dispersion assays. All compounds were dissolved molecular biology grade DMSO as 100 mM stock solutions and further diluted to 10 and 1 mM stock solutions as needed. Optical densities were measured using a Thermo Scientific Genesys 20 spectrophotometer. Data for biofilm inhibition and dispersion assays was collected using a BioTek ELx808 Microplate Reader. All graphs were generated and analyzed using GraphPad Prism 7.

Broth microdilution method for determination of minimum inhibitory concentrations

MRSA (ATCC 33591 or BAA-44) was grown in MHB for 6-8 h; this culture was used to inoculate fresh MHB (5×10^5 CFU/mL). The resulting bacterial suspension was aliquoted (1 mL) into 1.5 mL tubes and compound was added from a 100 mM or 10 mM DMSO stock to achieve the desired initial starting concentration (typically 128 μg/mL). Linezolid (from a 10 mM DMSO stock) was used as a positive control. Inoculated media not treated with compound served as the negative control. Rows 2-12 of a 96-well microtiter plate were filled at 100 μL/well from the remaining inoculated media, allowing the concentration of compound to be kept uniform throughout the dilution procedure. The samples containing test compounds and linezolid were then aliquoted (200 μL) into the corresponding first row wells of the microtiter plate (two wells for each compound and two negative controls). Row 1 wells were mixed 6 to 8 times, then 100 μL was transferred to row 2. Row 2 wells were mixed 6 to 8 times, followed by a 100 μL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate. The plate was then

covered and sealed with Glad Press 'n' Seal® and incubated under stationary conditions at 37 °C. After 16 h, MIC values were recorded as the lowest concentration of compound at which no visible growth of bacteria was observed. The same protocol was used to investigate the effects of various media on activity, differing only in the use of TSB or TSBG rather than MHB.

Determination of the inhibitory effect of test compounds on MRSA biofilm formation

Inhibition assays were performed by subculturing an overnight culture of MRSA (ATCC BAA-44) to an OD_{600} of 0.01 in TSBG. Stock solutions of predetermined concentrations of the test compound were then made using the inoculated TSBG. These stock solutions were aliquoted (100 μL) into the wells of the 96-well PVC microtiter plate. Sample plates were then wrapped in Glad Press'n Seal® and incubated under stationary conditions for 24 h at 37 °C. After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were then stained with 110 μL of 0.1% solution of CV and then incubated at rt for 30 min. Plates were washed with H_2O again and the remaining stain was solubilized with 200 μL of 95% EtOH. A sample of 125 μL of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantified by measuring the OD_{540} of each well and calculated as a percentage of the control (no compound); a negative control lane wherein no biofilm was formed served as a background and was subtracted out.

3.8 References

(1) Nicolaou, K.C.; Zhong, Y.-L.; Baran, P.S. *Angew. Chem. Int. Ed.* **2000**, *39*, 622.

(2) a: Evans, P.A.; Inglesby, P.A. *J. Am. Chem. Soc.* **2012**, *134*, 3635. (b): Murakami, S.; Takemoto, T.; Shimizu, Z. *J. Pharm. Soc. Jpn.* **1953**, *73*, 1026. (c): Lebar, M.D.; Heimbegner, J.L.; Baker, B.J. *Nat. Prod. Rep.* **2007**, *24*, 774.

(3) Clayden, J.; Greeves, N.; Warren, S. *Organic Chemistry*, 2nd ed.; Oxford: Oxford U.P., 2012, pp 1023-1025.

(4) Füstner, A.; Langemann, K. *J. Am. Chem. Soc.* **1997**, *119*, 9130.

(5) Sorto, N.A.; Di Maso, M.J.; Muñoz, M.A.; Dougherty, R.J.; Fettinger, J.C.; Shaw, J.T. *J. Org. Chem.* **2014**, *79*, 2601.

(6) Stalling, T.; Saak W.; Martens, J. *Eur. J. Org. Chem.* **2013**, 8022.

(7) Bender, D.R.; Bjeldanes, L.F.; Knapp, D.R.; Rapoport, H. *J. Org. Chem.* **1975**, 40, 1264.

(8) Cusumano, A.; Pierce, J.G. Unpublished results, North Carolina State Univ.

(9) (a) Humphrey, J.M.; Chamberlin, R. *Chem. Rev.* **1997**, *97*, 2243. (b) Han, S.; Kim, Y. *Tetrahedron* **2004**, *60*, 2447. (c) Montalbetti, C.; Falque, V. *Tetrahedron* **2005**, *61*, 10827. (d) Al-Warhi, T.I.; Al-Hazimi, H.M.A.; El-Faham, A. *J. Saudi Chem. Soc.* **2012**, *16*, 97.

(10) Boger, D.L.; Miyazaki, S.; Kim, S.H.; Wu, J.H.; Castle, S.L.; Loiseleur, O.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 10004.

(11) Shymanska, N.V.; Ph.D. dissertation, North Carolina State Univ., 2016.

(12) McKee, T.; McKee, J.R. *Biochemistry: The Molecular Basis of Life*, 6th ed. Oxford: Oxford U.P., 2015, pp 190-237.

(13) a) Salton, M.R.; Kim, K.S.; "Structure." *Baron's Medical Microbiology*, 4th ed.; Galveston: U. Texas Medical Branch, 1996. b) Bauman, R.; *Microbiology with Diseases by Taxonomy*, 2nd ed.; San Francisco: Cummings, 2007. c) Katz, A.H.; Caufiled, C.E.; *Curr. Pharma. Design* **2003**, *9*, 857.

(14) Spratt, B.G. *Science* **1994**, *264*, 388.

(15) Tsiodras, S.; Gold, H.S.; Sakoulas, G.; Eliopoulos, G.M.; Wennersten, C.; Venkataraman, L.; Moellering, R.C.; Ferraro, M.J. *The Lancet* **2001**, *358*, 207.

(16) Mendes, R.E.; Deshpande, L.M.; Jones, R.N. *Drug Resistance Updates* **2014**, *17*, 1.

(17) (a) Karatan, E.; Watnick, P. *Microbiol. Molec. Biol. Rev.* **2009**, *73*, 310. (b) Hoffman, L.R.; D'Argenio, D.A.; MacCoss, M.J.; Zhang, Z.; Jones, R.A.; Miller, S.I. *Nature* **2005**, *436*, 1171.

(18) An, D.; Parsek, M.R. *Curr. Opin. Microbiol.* **2007**, *10*, 292.

(19) Bryers, J.D. *Biotechnoloy and Bioengineering* **2008**, *100*, 1.

(20) Shymanska, N.V.; An, I.H.; Pierce, J.G. *Angew. Chem. Int. Ed.* **2014**, *53*, 5401.