Antarctic Tunicates and Endophytic Fungi: Chemical Investigation and Synthesis

by

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Dedication

I dedicate this dissertation to my family, particularly my mother and father who have supported me in all ways possible. I appreciate their encouragement and assistance when it was time to leave Colorado and continue my education in Hawaii. I am grateful for the monetary and, especially, the emotional support provided in times of need. I am thankful for the possibilities afforded me by my parents love, support and encouragement.

I dedicate this dissertation to the past and to the future: my grandparents, who brightened my childhood, and the newest member of our family, Grace Kelli (b. 2010.09.08), who will, no doubt, be intelligent and hilarious like the rest of the family. The sky's the limit, kiddo!

I thank my girlfriend, Lauren, for all the great moments that kept me sane the past few years. Be it a trip to the grocery store or a trip through Europe, I enjoy and relish every moment we spend together.

Lastly, I would like to dedicate this dissertation to my friends, past and present, for sharing enough GT’s and IPA’s to drown lesser men (and women). Mahalo!

“Call on God, but row away from the rocks.”

Dr. Hunter S. Thompson (1937-2005)
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<table>
<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>5-HT</td>
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<td>Heteronuclear Single Quantum Coherence</td>
</tr>
<tr>
<td>HTS</td>
<td>high throughput screening</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------------------------------------------</td>
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<tr>
<td>HWE</td>
<td>Horner-Wadsworth-Emmons</td>
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<tr>
<td>'Pr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>'PrOH</td>
<td>isopropanol</td>
</tr>
<tr>
<td>ITDL</td>
<td>in vitro drug luminescence</td>
</tr>
<tr>
<td>KHMDS</td>
<td>Potassium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>LAH</td>
<td>lithium aluminum hydride</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LRMS</td>
<td>low resolution mass spectrometry</td>
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<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
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<td>MMV</td>
<td>Medicines for Malaria Venture</td>
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<td>trifluoromethylsulfonyl (trifyl)</td>
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<td>trimethylsily</td>
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<tr>
<td>Troc</td>
<td>Trichloroethoxycarbonyl</td>
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<tr>
<td>Ts</td>
<td>4-toluenesulfonyl (tosyl)</td>
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<tr>
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<td>University of South Florida</td>
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Abstract

Drug discovery is reliant on new developments in natural product chemistry as well as advances in chemical synthesis. The interconnectivity and interdependence of natural and synthetic investigation in drug discovery is evident. The chemical exploration reported herein elaborates the relationship between natural product chemistry and chemical synthesis. Of particular interest are chemicals from organisms residing in less accessible environments, particularly Antarctica and endophytic microbial communities. Degradation via reductive ozonolysis of palmerolide A, a macrocyclic polyketide isolated from the Antarctic tunicate *Synoicum adareanum*, and subsequent synthetic preparation of the resulting polyols (1,2,6-hexanetriol and 1,2,3,6-hexanetetraol) led to a revision in the absolute configuration of the bioactive natural product (7R, 10R, 11R to 7S, 10S, 11S). A partial synthesis of palmerolide A (C3-14) was completed using Grubb’s 2nd generation catalyst to couple fragments formed using the previously developed methodology from the degradation study. Isolation of indole-pyrimidine containing alkaloids meridianins A, B, C, and E from the Antarctic tunicate *Synoicum* sp. prompted a synthetic investigation of psammopemmin A, a related alkaloid from the Antarctic sponge *Psammopemma* sp. resulting in reassignment of the structure of psammopemmin A to that of meridianin A. Both meridianin A and psammopemmin A were synthesized through a Suzuki coupling of the same 4-indolol nucleophile to the apposite pyrimidine electrophile. Several synthetic 3-pyrimidylindole analogs were also prepared and investigated for central nervous system, antimalarial, and cytotoxic activity. Chemical investigation of extracts from mangrove fungal endophytes that displayed antimalarial properties *in vitro* resulted in the isolation of several potent but cytotoxic and cytostatic compounds: cytochalasin D, roridin E, and 12,13-deoxyroridin E.
Chapter 1. Drugs, nature, and synthesis

1.1 Natural products as sources for drugs

Compounds derived from natural sources are essential for new drug discovery. According to analysis of all drugs approved world-wide from 1981-2006, seventy percent of the new chemical entities reported are natural products, derived from natural products, or inspired by natural products.\(^1\) It is not surprising that organisms produce a multitude of bioactive molecules. Organisms survive based on their ability to generate and retain chemical diversity at low cost.\(^2\) The inherent diversity, selectivity, and potency of natural products ensure their utility in the drug discovery and development process. Both marine\(^3\) and microbial\(^4\) environments are sources for a wealth of natural products. At the interface of macro- and microenvironment are microbial symbionts, thought to be responsible for producing a number of compounds isolated from marine macroorganisms.\(^5\) The unfathomable diversity intrinsic to marine and microbial environments ensures unlimited drug discovery potential.

1.2 Drugs from the sea

The antecedent search for biologically active compounds from marine sources can be traced, in part, to the laboratory of Paul Scheuer, who methodically investigated the chemistry of marine invertebrates beginning in the 1950’s.\(^6\) Since those initial explorations, bioactive drugs derived from marine organisms have proven their therapeutic utility.\(^7\) The potential for marine natural products as pharmaceuticals is impressive; many compounds isolated from the marine environment have progressed to clinical trials.\(^8\) To date, one non-peptide drug originating from the marine environment has made it to the commercial sector.
Ecteinascidin 743 (= ET-743, trabectedin, Yondelis®, 1.1), a tris(tetrahydroisoquinoline), was the first marine natural product anticancer drug to be sold commercially.\(^9\) Originally described by the Rinehart group,\(^{10,11}\) with simultaneous publication by Wright et al.\(^{12}\) from an antiproliferative extract of the Caribbean tunicate *Ecteinascidia turbinata*, ET-743 was isolated in low yields (0.0001%) along with several other analogs (ET-729: 1.2; ET-745: 1.3; ET-759A: 1.4; ET-759B: 1.5; ET-770: 1.6). ET-743 was found to target DNA transcription by a complex mechanism of action involving DNA binding\(^{13}\) with an unusual requirement: the cell must possess a proficient nucleotide excision repair (NER) system.\(^{14}\) This differs from all other known DNA interacting agents which require deficient NER systems to exert cytotoxic effects. Supply problems of ET-743 were addressed initially with large-scale aquaculture then later alleviated using an efficient semisynthetic procedure (see section 1.4.3). Under the trade name Yondelis®, ET-743 is marketed by PharmaMar in partnership with Johnson and Johnson in the European Union as treatment for advanced soft tissue sarcoma and is currently in development for ovarian, prostate, lung, breast and pediatric cancers.

Discodermolide (1.7), a polypropionate-derived polyhydroxy-δ-lactone isolated from the rare Bahaman deep-water (300 m) sponge *Discodermia dissoluta*, is a potent microtubule stabilizing agent (MSA).\(^{15,16}\) MSA’s impede the cellular cycle by stabilizing microtubules in conditions which would normally be destabilizing, effectively disrupting microtubule dynamics.\(^{17}\) Discodermolide
was found to stabilize microtubules more potently than taxol\textsuperscript{18} generating immense interest in the compound. Problems with obtaining quantities suitable for preclinical evaluation led to the development of efficient gram scale syntheses of discodermolide (see section 1.4.3). Although an extremely promising lead, Phase 1 clinical trials were halted on discodermolide due to lack of efficacy at tolerated doses.

![Discodermolide](image)

\textbf{1.7: discodermolide}

Initial investigation of the shallow water Japanese sponge \textit{Halichondria okadai} revealed a cytotoxic polyether C\textsubscript{38} fatty acid derivative designated okadaic acid (1.8).\textsuperscript{19} Due to remarkable antitumor activity demonstrated by \textit{H. okadai} extracts \textit{in vivo}, subsequent reinvestigation of sponge revealed small quantities of several cytotoxic polyether macrolides, including halichondrin B (1.9) which was shown to potently (nanomolar concentrations) inhibit cell growth.\textsuperscript{20} The low yields of halichondrins (halichondrin B: 20 \(\mu\)g/kg; total halichondrins: \(\sim\)0.14 mg/kg) from \textit{H. okadai} presented a barrier for preclinical trials. Fortuitously, a new species of deep-water sponge, \textit{Lissodendoryx} n. sp. 1 from the South Island of New Zealand, was discovered to contain the compounds in higher concentrations (halichondrin B: \(\sim\)0.4 mg/kg; total halichondrins: \(\sim\)1.5 mg/kg).\textsuperscript{21} One metric ton of \textit{Lissodendoryx} n. sp. 1 was harvested via trawling (80 – 100 m) which resulted in isolation of halichondrin B (310 mg), quantities sufficient for preclinical trials. As aquaculture techniques\textsuperscript{22} were in development to provide the gram scale quantities of halichondrin B necessary for clinical trials, truncated synthetic halichondrin analogs were found to retain the natural product’s potent activity. Even though halichondrin D had advanced to Phase I clinical trials in 2002, focus was directed to the most promising of the truncated synthetic analogs (see section 1.4.4).
The abovementioned compounds represent a small fraction of marine-derived chemistry exhibiting intriguing chemical structure and exciting bioactivity, a majority of which are isolated from temperate and tropical waters. Because marine natural product discovery programs have traditionally focused on organisms in tropical and temperate waters, a wealth of cold-water organisms remain largely unexplored. Although understudied, marine invertebrates residing in cold water have elaborated many interesting and biologically relevant compounds. Chapters 2 and 3 of this dissertation examine some remarkable chemistry isolated from cold-water marine invertebrates.

1.3 Drugs from microorganisms

Microbe-derived drug discovery began in 1928 in the laboratory of Alexander Fleming when a *Penicillium* mold was observed to kill *Staphylococcus aureus*, leading to the discovery of the antibiotic β-lactam, penicillin (1.10). An abundance of useful antibiotics derived from microbes have been isolated since that initial discovery including streptomycin (1.11), an aminoglycoside from *Streptomyces griseus*, the dichloroacetamide chloramphenicol (1.12) from *Streptomyces*...
venezuelae and the tetracyclines from Streptomyces spp., the first of which was named chlortetracycline (1.13). As well as antibiotic activity, microbial secondary metabolites display a broad range of biological utility including, but not limited to, immunosuppressant, antitumor, insecticidal, heribicidal, and antiparasitic properties. Calicheamicins, salinosporamides and epothilones are notable microbial natural products currently in clinical development possessing both interesting structural features in addition to compelling antitumor properties.

Calicheamicins, extraordinarily potent antitumor compounds, were isolated from the bacterium Micromonaspora echinospora. Calicheamicin $\gamma_1^B$ (1.14) was initially isolated from culture but upon refermentation in the presence of sodium iodide, a similar compound differing only in halogenation of the benzene ring was discovered (calicheamicin $\gamma_1^I$, 1.15). The remarkable and complex structure of calicheamicin $\gamma_1^I$, including four carbohydrate moieties, a hexasubstituted benzene ring, an $N$-$O$ glycosidic bond, a trisulfide, and a bicyclo-enediyne system, was determined via spectroscopic techniques in conjunction with noteworthy chemical degradation studies (see section 1.4.1). Antitumor properties of calicheamicin $\gamma_1^I$ arise from its ability to cleave double-stranded DNA. Although calicheamicin $\gamma_1^I$ is generally toxic, a prodrug was developed by attaching, via calicheamicin’s sulfide linkage, an antibody (anti-CD33 monoclonal-) which directs the molecule selectively to certain cancer cells types. The resulting drug,
gemtuzumab ozogamicin, is approved by the Food and Drug Administration (FDA) for the treatment of relapsing patients suffering from acute myeloid lymphoma.\textsuperscript{28}

![Chemical structure of calicheamicin](image1)

\textbf{1.14: calicheamicin }\gamma_1^\text{Br}, X=\text{Br} \\
\textbf{1.15: calicheamicin }\gamma_1^\text{I}, X=\text{I}

Exploration of microbial constituents isolated from ocean sediment resulted in the identification of a new genus of actinomycetes (\textit{Salinospora}) which was found to yield a potent proteosome inhibitor, salinosporamide A (from \textit{S. tropica} CNB476, shake flask: 4 mg/L culture media, \textbf{1.16}).\textsuperscript{29}

Nereus Pharmaceuticals licensed the compound and began industrial scale fermentation after modifying the culture media to meet current Good Manufacturing Practice (cGMP) guidelines.\textsuperscript{30}

The company isolated an individual colony of \textit{S. tropica} CNB476 from the original strain that produced more salinoporamide A concomitant with less undesirable analogs. Researchers at Nereus discovered that addition of solid resin (XAD-16) to the culture considerably increased quantities of salinosporamide A due to the inherent instability of the $\beta$-lactone in aqueous solution. The fermentation was ultimately optimized to obtain quantities of material suitable for clinical trials resulting in about a hundred-fold increase in production of salinosporamide A (fermentor: 360 mg/L, shake flask: 450 mg/L). Salinosporamide A completed Phase 1 clinical trials for the treatment of multiple myeloma in 2010, just seven years since its discovery.
Epothilones, microtubule-stabilizing polyketide macrolides, were isolated from myxobacterium *Sorangium cellulosum*.\(^\text{31}\) Epothilones A (1.17) and B (1.18) were the first non-taxane-based MSA’s to be discovered.\(^\text{32,33}\) Both compounds are at least as potent as taxol in stabilizing microtubules. Gram-scale isolation (4.8 g 1.17; 2.1 g 1.18 from 230 L culture broth) and crystal structure of epothilones A and B were subsequently reported.\(^\text{34}\) Due to the excellent potency and efficacy of the naturally occurring epothilones many promising, clinically-significant synthetic analogs have been also been prepared (see section 1.4.4). Natural epothilone B as well as five synthetic epothilones have completed Phase I clinical trials leading to investigation of several (epothilone B and two synthetic derivatives) in Phase II studies to assess efficacy in a variety of tumor types.\(^\text{35}\) Phase III clinical trials of epothilone B are currently underway for treatment of patients with taxane- and platinum-resistant disease.\(^\text{36}\)

![Chemical structure of epothilones A and B](image)

1.17: epothilone A, R = H  
1.18: epothilone B, R = Me

1.4 Relevance of synthesis in natural product chemistry

The essential role of synthesis in natural product drug discovery is undeniable.\(^\text{37}\) The structural complexity present in many naturally occurring compounds necessitates advanced spectroscopic techniques, often times in conjunction with wet chemical methods, to fully elucidate absolute configuration. Derivatization and degradation are common synthetic methods utilized to elucidate intricate chemical structures. Total synthesis, considered to be the ultimate verification of structure, is especially useful for validating complex natural products isolated in scarce amounts. Because many natural products are found in low abundance, total- and semisyntheses have been utilized to alleviate supply problems of clinically promising drugs. Inspired chemists have constructed analogs of biologically active natural products to probe and tailor bioactivity, often
times resulting in more potent and/or less toxic derivatives. The symbiotic relationship between natural product chemistry and organic synthesis cannot be overstated. The value of chemical synthesis to identify, supply, and modify compounds originating from natural sources will be emphasized in the remainder of this chapter.

1.4.1 Structure elucidation and verification via degradation studies

Degradation studies of naturally occurring compounds have proved instrumental in the structure elucidation and verification of complex molecules. Degradation studies to determine chemical structure were commonplace before the advent of X-ray and nuclear magnetic resonance (NMR) spectroscopic techniques (circa 1950’s). Although not as common as it once was, chemical degradation for structure elucidation and verification is still an important part of natural products chemistry, evidenced by an extraordinary study in which the structure of calicheamicin \( \gamma_1 \) was determined.

Spectroscopic analysis of calicheamicin \( \gamma_1 \) (1.15), a compound displaying activity in biochemical induction assays (BIA; used to identify DNA damaging antitumor compounds), revealed four glycosides and one aglycone. Difficulty further interpreting NMR data due to signal overlap prompted degradation studies (Scheme 1.1). \( N \)-acetylated calicheamicin \( \gamma_1 \) (1.19) was subjected to acidic methanolysis yielding, among other products, the methyl glycoside of a 6-deoxyhexopyranose (1.20; both \( \alpha \) and \( \beta \) anomers), the methyl glycoside of an \( N \)-acytethyl-\( N \)-ethylaminoxylopyranose (1.21; 7/3 mixture of anomers confirmed by synthesis) and hexasubstituted benzene 1.22. To find degradation products retaining BIA activity, methanolysis was performed on a strong cation exchange resin (Dowex) column resulting in thioesters 1.23 and 1.24 (as well as their anomers) along with the BIA active calicheamicin pseudoaglycone 1.25. Structures of 1.23 and 1.24 were determined with NMR analysis. Mass data indicated that fragment 1.25 was the natural product minus ring D and E. Based on careful fast atom bombardment mass spectroscopy (FABMS) and NMR examination of 1.25, ring A and B were determined to be linked through an unusual \( N \)-O glycosidic bond.
The arduous task of determining the aglycone structure of 1.25a was completed, in part, with thoughtful X-ray, NMR, and FABMS analysis of reaction product 1.26 and deuterated 1.26-\textit{d}_2 which were recovered from mixtures of PPh_3/CH_2Cl_2/CH_3OH and PPh_3/CD_2Cl_2/CD_3OD, respectively (Scheme 1.2). The deuterated benzene product (1.26-\textit{d}_2) could only be explained by a enediyne biradical cycloaromatization (Bergman cyclization) initiated by the destabilizing effect of thiol adding in Michael fashion to the \(\alpha,\beta\)-unsaturated ketone (1.25b→1.27). Because only natural and degradation products retaining the endiyn e system displayed BIA activity, the endiyne moiety was determined to be responsible for the observed DNA-damaging effects. The degradation study revealed that nature has found an elegant way to retain - and discriminately liberate - the quite reactive endiyne functionality. Nicolaou et. al\textit{et} synthesized calicheamicin \(\gamma_1\).
and confirmed the proposed structure, further validating the effectiveness of degradation to elucidate complex natural products.

1.4.2 Structure verification via total synthesis

Spectroscopic techniques [NMR, high resolution mass spectroscopy (HRMS)] and chemical degradation are quite powerful and allow for structure elucidation of elaborate molecules. Structural misassignment in the literature is not uncommon, however, due to investigator error by data misinterpretation. Simple bookkeeping errors\(^{42}\) are often responsible for misassignment, although structural data from exceptionally complex molecules can lead the most careful scientist to the wrong conclusion. Total synthesis provides an unambiguous means to verify proposed structures. Chemical literature abounds with structures of natural products that have been
corrected through total synthesis. The total synthesis of diazonamide A highlights the structure reassignment of an exceedingly complex and bioactive marine natural product.

In 1991, diazonamide A (proposed: 1.28; revised: 1.29), an unusual halogenated cyclic peptide with potent antitumor properties, was isolated by Clardy and Fenical from the colonial ascidian *Diazona annulata* collected in the Philippines. The compound’s highly intricate structure and compelling bioactivity captured the interest of dozens of synthetic research groups which culminated, after ten years of investigation - and frustration - in structural reassignment. When first isolated, the structure of diazonamide A could not be elucidated with NMR and HRFABMS alone due to a large number of unprotonated carbons and heteroatoms. Several subunits were revealed but their connectivity remained elusive. Because the compound would not crystallize, X-ray analysis of diazonamide A was not possible. Due to similar NMR, ultraviolet (UV), and infrared (IR) data between diazonamide A and diazonamide B, X-ray diffraction of the *p*-bromobenzamide derivative of diazonamide B (1.30, initially assigned) afforded connectivity of the compounds’ subunits. The conversion of proposed hemiacetal 1.28 to the acetal found in 1.30 was postulated to occur during the acylation reaction. The proposed structure for diazonamide A (1.28) was synthesized in 2001 by Harran et. al.
Synthesis of the proposed structure of diazonamide A (1.28) began with formation of modified dipeptide 1.30 from 1.31, 1.32, and 1.33 (Scheme 1.3). A pivotal Heck cyclization of 1.30 formed 1.34 which, after phenol-protection, was dihydroxylated to 1.35. The diazonamide core (1.36) was formed from 1.35 through an acid catalyzed pinacol rearrangement in a near perfect stereoselective manner. After reduction, protecting group manipulation, and selective bromination, 1.36 was converted to lactone 1.37 upon treatment with acid. Lactone opening with N-dimethylaluminumtryptamine followed by oxidation furnished 1.38 which was briefly photolyzed (350 nm) to afford 1.39 after acetylation. Oxidation/cyclodehydration of 1.39 formed bis(oxazoyl)indole 1.40. Surprisingly, exposing 1.40 to UV (300 nm) light resulted in the formation of 1.41 with the loss of HBr. With the critical framework synthesized, 1.41 was converted to the proposed structure of diazonamide A (1.28) with facile dichlorination and removal of protecting groups. Unfortunately, however, the synthesized compound was quite unstable and differed in physical properties with the natural product.
Scheme 1.3. Synthesis of originally proposed diazonamide A led to structural reassessment and ultimately the correct structure.
Harran et al. re-evaluated the natural product’s physical and experimental data and discovered that the natural product did not release valine upon acid digestion nor did spectroscopic data support the valine residue. They theorized the C37 substituent was actually an OH and not an NH₂. This change necessitated an alteration elsewhere in the molecule to rectify the molecular mass observed. Reexamination of mass data of natural diazonamide A and crystal structure of the p-bromobenzamide derivative of diazonamide B (1.30) indicated the acetal proposed for 1.30 was likely retained in natural diazonamide A as opposed to the proposed hemiacetal. X-ray and HRMS suggested the acetal structure was actually a hemiaminal ether in which O3 should be an NH leading Harran et al. to propose revised structure 1.29 for diazonamide A. A year later, Nicolaou et al. completed the total synthesis of 1.29 using strategies developed by Harran as well as another method differing in order of macrocycle formation finally verifying the revised structure as that of natural diazonamide A.

1.4.3 Drug supply by total synthesis and semisynthesis

Culturable microbes and cultivatable plants are excellent sources for drugs. However, reliable drug supply from natural sources is often hampered by rarity or inaccessibility of the producing organism. Particularly susceptible are drugs from the sea. Although marine organisms afford a wealth of interesting bioactive chemistry, sustainable supply of marine-derived drugs is limited. Not only are most marine macroorganisms and their microbial fauna largely unculturable, but many marine natural products are found in low natural abundance. Consequently, large scale aquaculture would likely be too inefficient as a viable source of drugs. Pharmacological investigation of discodermolide and ecteinascidin, two promising marine-derived antitumor agents, was hindered by lack of adequate supply. Supply demands were alleviated through total synthesis and semisynthesis, respectively.

Several highly efficient syntheses of discodermolide were developed allowing preparation of gram quantities sufficient for initial clinical testing. Schreiber et al. completed the total synthesis of ent-discodermolide (ent-1.7) establishing the absolute configuration of the molecule.
Surprisingly, ent-discodermolide was found to have similar cytotoxic effects as the natural product. Schreiber et al. used the same methodology to synthesize the natural enantiomer.\textsuperscript{51}

The synthesis relied upon the absolute configuration of Roche ester starting material (1.43) which was converted to two diastereomers via Roush crotylation (1.44, 1.45) further elaborated to three fragments of discodermolide (1.46, 1.47, 1.48) that were then stitched together to form the natural product, effectively completing the divergent/convergent synthesis (Scheme 1.4). Key reactions include Stille-Gennari HWE olefination in the formation of 1.46, Negishi coupling in the formation of 1.48, a Nozaki-Kishi coupling of 1.46 and 1.47, and finally enolate alkylations to join 1.48 and 1.49. The synthesis was completed in an overall yield of 4.3% (longest linear sequence: 24 steps).

\begin{center}
\textbf{Scheme 1.4}. Schreiber’s synthesis of discodermolide starting with Roche ester.
\end{center}

Smith et al. reported the synthesis of discodermolide in 6% overall yield (24 linear steps) which resulted in an impressive 1.0 gram of the natural product using a Roche ester-derived aldehyde to form fragments which were coupled to generate the natural product, analogous to Schreiber’s synthesis.\textsuperscript{52} Paterson et al. employed two synthetic methods to produce discodermolide. The relatively high-yielding (10.3% over 23 steps, longest linear sequence) first generation synthesis employed chiral auxiliary groups and reagents to direct stereochemistry.\textsuperscript{53} A second generation
synthesis was lower yielding (5.1% over 24 linear steps) but required fewer total steps (35) and no chiral auxiliary groups or reagents, depending instead on substrate to control stereochemistry.\textsuperscript{54} Novartis Pharma AG, utilizing key steps from syntheses reported by Smith and Paterson, completed large scale synthesis of discodermolide in apposite quantities (60 g!) for Phase I clinical trials.\textsuperscript{55}

Aquaculture provided suitable quantities of eceinascidin-743 (ET-743, 1.1) for preclinical and early Phase clinical trials but yields were variable. Supply issues plagued development until scientists at PharmaMar developed a semisynthesis of ET-743 from cyanosafracin B (1.50, Scheme 1.5), a readily available (kilogram scale!) antibiotic from the optimized fermentation broth of \textit{Pseudomonas fluorescens}.\textsuperscript{56}

\textbf{Scheme 1.5. ET-743 could be derived from the antibiotic cyanosafracin B.}

Cyanosafracin B (1.50), after protecting group manipulation, was converted to 1.51 though an unstable hydroquinone intermediate (Scheme 1.6). More protecting group manipulation and cleavage of the amide by Edman degradation formed amine 1.52. A key step in the semisynthesis was the conversion of amine 1.52 to alcohol 1.53 upon treatment with sodium nitrite/acetic acid (NaNO\textsubscript{2}/AcOH), effectively completing a formal total synthesis by generating an intermediate in the total synthesis of ET-743 previously reported by Corey et al.\textsuperscript{57} The remaining conversion of 1.53 to ET-743 followed Corey’s protocol. EDC promoted esterification of alcohol 1.53, deallylation, and subsequent oxidation of the phenol with (PhSeO)\textsubscript{2}O resulted in
hydroxylated product 1.54. Quinone methide formation via Swern protocol then liberation of the thioether to generate thiolate ion resulted in nucleophilic attack of quinone forming 1.55 after acetylation. Removal of the Troc group, oxidation of the resulting α-amino lactone to the α-keto lactone then treatment with 2-[3-hydroxy-4-methoxyphenyl]ethyamine (1.56) afforded stereospecific formation of the spiro tetrahydroisoquinoline 1.57. Protecting group removal and conversion of cyano-group to alcohol with silver nitrate (AgNO₃) resulted in the semisynthesis of ET-743 (1.1) from cyanosafracin B (1.50). The initial synthetic work of Corey et al. in combination with the ingenuity of Pharma Mar scientists led to the development of gram-scale semisynthesis of a rare marine natural product, enabling production of commercial quantities.

Scheme 1.6. PharmaMar’s semisynthesis of ET-743 from cyanosafracin B.
1.4.4 Synthetic derivatives of natural products as drugs

The first examples of marine natural product-inspired, clinically useful synthetic derivatives were antiviral ara-A (vidarabine, 1.58) and chemotherapy agent ara-C (cytarabine, 1.59). Both compounds owe their structure to sponge-derived bioactive arabinose-containing nucleosides isolated by Bergmann in the 1950's. More recently it was found that twenty-seven percent of the 1184 drugs approved worldwide from 1981-2006 are derived from natural products, including semisynthetic derivatives and totally synthetic compounds containing a pharmacophore that is natural in origin. The number jumps to thirty-seven percent if synthetic natural product mimics - such as peptidic isosteres - are included in the analysis. Medicinal chemists, through synthetic procedures, tweak already biologically active natural products to identify pharmacophores responsible for desired bioactivity, eliminate unneeded or detrimental structural features, and, ultimately, optimize pharmacological properties. Eribulin mesylate and several epothilone analogs are examples of clinically successful synthetic derivatives of natural origin.

Using synthetic methods developed by Kishi, several truncated analogs of halichondrin B (1.9) were found to be equipotent to the natural product in antitumor assays. Because obtaining halichondrin B from natural sources proved problematic, the truncated analogs, with about 70% the molecular mass of the natural compound, were more easily synthesized and thus, an attractive alternative. Further screening by Eisai Company revealed that the truncated halichondrin B ketone analog E7389 (ER-086526), later developed as eribulin mesylate (1.60), possessed highly potent in vitro and in vivo anticancer activities with a wide therapeutic window in vivo. Eribulin mesylate completed Phase II studies as a monotherapy for refractory breast cancer and showed promising results in patients with advanced breast cancer. Currently,
eribulin mesylate is undergoing Phase III trials as a late-stage treatment for locally recurrent or metastatic breast cancer. After completing total synthesis of epothilones A (1.17) and B (1.18), Danishefsky et al. proceeded to assemble close to fifty epothilone analogs. Danishefsky discovered that synthetic desoxyepothilone B, (KOS-862, epothilone D, 1.61) displayed low in vivo toxicity in contrast with epothilone B (highly toxic in mice) while retaining antitumor effects. Desoxyepothilone B has completed Phase II clinical trials for colorectal, gastric, ovary, renal and other cancers. The production and evaluation of over 300 semisynthetic epothilone analogs by researchers at Bristol-Meyers Squibb led to ixabepilone (BMS-247550, aza-epothilone B, 1.62) the lactam derivative of epothilone B which displayed antitumor properties toward a wide range of cancers in Phase II trials. Recently, Phase III clinical trials of combination ixabepilone therapy in the treatment of patients resistant to taxol and anthracyclines showed a statistically significant improvement in progression-free survival although toxicity was a concern.
1.5 Research objectives

The interconnectivity and interdependence of natural product chemistry and chemical synthesis in drug discovery is demonstrated through the chemical exploration reported herein. Investigation of less accessible and therefore less studied environments, particularly Antarctica and endophytic microbial communities, has afforded exciting chemistry and promising biologically active leads. Synthetic analogs inspired by Antarctic-derived natural products have displayed promising bioactivity. The chemical investigation, degradation, and synthetic studies presented herein disclose a number of remarkable compounds derived from natural sources as well as synthetic operations utilized to study their interesting structure and biological activity.

1.6 References Cited


Chapter 2. Synthetic studies of palmerolide A

2.1 Isolation, structure elucidation, and bioactivity of palmerolide A

The palmerolides are a family of macroyclic polyketides found in the abundant Antarctic tunicate *Synoicum adareanum* collected at the National Science Foundation’s Palmer Station, on the Antarctic Peninsula. The major metabolite, palmerolide A (2.1), displays 18 nM inhibition of UACC-66 melanoma and includes among its biochemical targets the pH regulatory vacuolar-ATPase (V-ATPase), for which it is a potent inhibitor (IC$_{50}$ = 2 nM). V-ATPases are largely responsible for cellular and organellular pH regulation but have been implicated in cancer treatment due in part to the low pH requirement and concomitant overexpression of V-ATPases of some cancer cell types. In ongoing studies at the National Cancer Institute at Frederick, palmerolide A induced markers of autophagy and the transcription factor Hypoxia Induction Factor-1α (HIF-1α), but the mechanism underlying palmerolide A-induced cell death in human tumor cells remains unclear. Palmerolide A remains of interest for development due, in contrast to other V-ATPase inhibitors such as bafilomycin (2.2), to its lack of neurotoxicity at therapeutic levels. The microbial community of *S. adareanum* has been investigated for genes coding for polyketide synthases (PKS) in an ongoing effort to characterize bacteria responsible for producing polyketide-derived palmerolide A.


The planar structure of palmerolide A was established on the basis of extensive 2D NMR experimentation while the stereochemical assignment required a combination of spectroscopic and derivatization techniques. The two secondary alcohols, C7 and C10, were amenable to stereochemical analyses by Mosher’s method. The remaining stereocenters were determined relative to C10 using through-space NMR techniques such as Rotating frame Overhauser Effect Spectroscopy (ROESY) along with \(^nJ_{CH}\)-based analysis. Configuration of C19 and 20 relative to C7, 10 and 11 was proposed based on NOE data suggesting the macrolide conformed to a teardrop-like structure. The originally proposed structure (1a) was determined to have an absolute configuration of 7R, 10R, 11R, 19R, 20R. The unique and potent bioactivity as well as the intriguing structure of palmerolide A has generated considerable interest in developing synthetic access to the compound (see section 2.2 for a review). The molecule’s complexity prompted us to perform degradative studies using a chiral pool based strategy to verify the initial stereochemical assignments (see section 2.3) which then led us to synthesize the C3-14 fragment (see section 2.4).
2.2 Palmerolide A as a synthetic target

To date, three total syntheses and one structure activity relationship study of palmerolide A have been published. Several partial syntheses as well as a formal total synthesis have also been reported.

2.2.1 Total syntheses of palmerolide A

2.2.1.1 Total synthesis by De Brabander

De Brabander et al. reported the first total synthesis of ent-palmerolide A in 2007. His group began by synthesizing the originally proposed structure 2.1a. De Brabander envisioned constructing the C1-24 portion (2.3) of palmerolide A with a convergent approach (Scheme 2.1) utilizing three main fragments: 2.4 (C9-15), 2.5 (C16-24), and 2.6 (C1-8).

Scheme 2.1. De Brabander's retrosynthetic strategy for formation of palmerolide A.

Vinyl pinacol borate ester 2.4 was formed from D-arabitol (2.7) through intermediate 2.8 (Scheme 2.2). A key step in the formation of fragment 2.5 was the diastereoselective Mukaiyama aldol condensation of dienol silyl ether 2.9a and vinyl iodide 2.10 to form 2.11. Phosphonate ester 2.6 was synthesized from δ-valerolactone (2.12). Coupling fragments 2.4 and 2.5 via Suzuki methodology yielded an intermediate that was esterified with 2.6, setting up a ring closing Horner-Wadsworth-Emmons (HWE) olefination to form macrolactone 2.3.
Stereoselective reduction of 2.3 gave the preferred C7 epimer. Curtius rearrangement followed by trapping the isocyanate intermediate (2.12a) with Grignard reagent 2.13 completed the enamide pendant 2.14 (Scheme 2.3). Installing the carbamate at C11 followed by protecting group removal afforded initially proposed palmerolide A (2.1a).

Differences in the NMR data of synthesized 2.1a and that of natural palmerolide A led De Brabander et al. to reevaluate the assignments. The group was confident in their stereochemical assignment of synthetic 2.1a. Mosher analysis of C7 confirmed the center’s absolute configuration. Stereochemistry at C10 and 11 was derived from D-arabitol. X-ray analysis of a
crystal intermediate containing C19 and 20 corroborated the assignments at those centers. De Brabander then examined the natural product data and found assignments for C7, 10, and 11 were made on sound evidence. He also felt that the relative configuration between C19 and 20 was justifiable but the relative configuration of C19 and 20 to C7, 10, and 11 (found on ROESY analysis) was suspect. The researchers decided to repeat the synthesis with 2.4, ent-2.5, and 2.6. The resulting compound (ent-2.1b) possessed the same absolute configuration at C7, 10, and 11 (all R) but opposite configuration at C19 and 20 (now both S) when compared to 2.1a. NMR data, as well as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) behavior of ent-2.1b coincided with that of natural palmerolide A. Circular dichroism (CD) spectra of the two compounds were mirror images, however, suggesting that De Brabander et al. had synthesized the enantiomer of the natural product. The absolute configuration of naturally occurring palmerolide A was revised to 7S, 10S, 11S, 19R, 20R (2.1b).

2.2.1.2 Total synthesis by Nicolaou

Shortly after De Brabander reported synthesis of revised palmerolide A (ent-2.1b), Nicolaou et al. reported a total synthesis of the natural enantiomer (2.1b) along with several stereoisomers including the originally proposed structure. Nicolaou’s group also used a convergent, three fragment approach to achieve synthesis of the originally proposed structure which could be easily amended to synthesize other stereoisomers of palmerolide A. Using this methodology the group was first to synthesize the revised structure, 2.1b, from fragments 2.15, 2.16 and 2.17 (Scheme 2.4). By varying the order in which the fragments were combined, Nicolaou was able to determine the most efficient method to form the macrocycle was via olefin ring closing metathesis (RCM) from 2.18 or Yamaguchi macrolactonization from 2.19.
Analogous to De Brabander’s synthesis of fragment 2.5 via stereoselective Mukaiyama aldol methodology, Nicolaou synthesized 2.15 using 2.9b and 2.10 (Scheme 2.2) resulting in comparable yields and better diastereomeric ratios. Configuration at C10 and 11 was selectively formed by crotylation of 2.20 with diisopinocamphenylborane 2.21 resulting in 2.22 which was further elaborated to afford vinyl stannane 2.16 (Scheme 2.5). Epoxidation of 2.23 then hydrolytic kinetic resolution with Jacobsen’s catalyst (2.24) resulted in diol 2.25 and epoxide 2.26 en route to 2.17 and ent-2.17, respectively.
With 2.15, 2.16 and 2.17 in hand, Nicolaou et al. proceeded to assemble the fragments with particular focus on efficiency (Scheme 2.4). Stille coupling of vinyl iodide 2.15 and vinyl stannane 2.16 then Yamaguchi-type esterification with acid 2.17 furnished 2.18. Alternatively, esterifying 2.15 with 2.17 followed by coupling to 2.16 improved yield of 2.18. Protecting group removal and oxidation of 2.18 set up chain elongation via Takai olefination resulting in vinyl iodide 2.26 (Scheme 2.6). RCM with Grubbs II catalyst (2.27) proved to be an efficient method to form the macrolide which was then coupled to primary enamide 2.28 using Buchwald methodology affording the revised structure of palmerolide A (2.1b).
Nicolaou et al. found that Yamaguchi macrolactonization was also an effective method to form the macrolide portion of palmerolide A (Scheme 2.4). Less efficient ring closing methods were also investigated, including Mitsunobu cyclization forming the macrolide at C1-19, intermolecular HWE olefination at C2-3, and intermolecular Stille coupling at C15-16.

![Scheme 2.6. Nicolaou's revised palmerolide A (2.1b) endgame.](image)

Nicolaou et al. found that Yamaguchi macrolactonization was also an effective method to form the macrolide portion of palmerolide A (Scheme 2.4). Less efficient ring closing methods were also investigated, including Mitsunobu cyclization forming the macrolide at C1-19, intermolecular HWE olefination at C2-3, and intermolecular Stille coupling at C15-16.

In 2008, Nicolaou and co-workers reported the synthesis and bioactivity of a multitude of palmerolide A stereoisomers and analogs using strategies developed en route to palmerolide A. Along with ent-2.1b, nine diastereomers of palmerolide A, were prepared as were six analogs differing only in their amide linkages at C24. Several deoxygenated derivatives of palmerolide A were also synthesized. From this structure activity relationship (SAR) study, Nicolaou inferred the anticancer properties of palmerolide A are most dependent on the enamide moiety as changes to the substituents on the ring had less effect on activity. It was also noted that the benzoylamide analog was more potent than 2.1b while removing the C7-hydroxyl group had no effect on potency. Less complex analogs, either equipotent or more so, would be more easily accessible and thus more attractive drug leads.

2.2.1.3 Total synthesis by Hall

In 2009, Hall et al. reported a third total synthesis of palmerolide A (2.1b) utilizing catalytic asymmetric organoboron methodologies developed in their lab. Using a less modular, but still
convergent, approach compared to those employed by De Brabander and Nicolaou, Hall envisioned constructing macrolide 2.29 (Scheme 2.7) from two fragments, the “left” comprising C14-24 (2.30) and the “right” (C1-13, 2.31).

Scheme 2.7. Hall’s convergent synthesis of 2.1b from 2.30 and 2.31.

Hall began synthesis of 2.30 with an enantioselective E-crotylboration of aldehyde 2.10 with 2.32 using catalyst 2.33 resulting in alcohol 2.34 in excellent yield, diastereoselectivity, and enantiomeric excess (Scheme 2.8). Successive Wittig olefinations furnished 2.35 which underwent Sonogashira coupling and alkyne hydrozirconation yielding fragment 2.30, the “left” portion of the macrolide.

Scheme 2.8. Hall’s synthesis of fragment 2.30.

Synthesis of fragment 2.31 commenced (Scheme 2.9) with an enantioselective hetero [4+2] cycloaddition/allylboration of 2.36 and 2.37 by way of Jacobsen designed (Schiff base)chromium
(III) catalyst (2.38). Intermediate 2.39 reacted with another equivalent of 2.36 resulting in alcohol 2.40a which upon acylation gave 2.40b. An unprecedented Claisen-Ireland [3,3] rearrangement followed (through transition state 2.41) giving 2.42a and finally, after oxidation, 2.42b. With configuration at C7, 10, and 11 set, 2.42b was then elaborated to fragment 2.31, the “right” portion of the macrolide.

![Diagram of the reaction sequence](image)

**Scheme 2.9.** Hall’s stereoselective route to fragment 2.31.

Hall et al. then coupled fragments 2.30 and 2.31 with an sp²-sp³ B-alkyl Suzuki coupling. Yamaguchi macrolactonization completed 2.29. Hall’s endgame was identical to De Brabander’s (Scheme 2.3) converting 2.29 to isocyanate 2.12b and finally enamide 2.14b. Hall then installed the carbamate at C11. Protecting group removal afforded palmerolide A (2.1b).

### 2.2.2 Partial syntheses of palmerolide A

Several approaches to various fragments of palmerolide A have been reported [18-22] (Scheme 2.10). Shortly before DeBrabander published the revised structure of palmerolide A, Kaliappan and Gowrisankar reported synthesizing the “northern hemisphere” of originally proposed 2.1a comprising C1-9 and C15-21 (2.43). [18] Notable transformations include using an Evans chiral auxiliary to set configuration at C7, a palladium (II) (PdII) catalyzed allylic rearrangement to furnish E16 selectively, and olefin cross metathesis (OCM) with Grubb’s 2nd generation catalyst to form E2. Maier et al. constructed the linear fragment C3-23 (2.44) en route to 2.1a. [19] Key steps
include forming C10 and 11 stereocenters via Sharpless dihydroxylation, Red-Al mediated reduction of an alkyne to form E8, and Stille coupling at C15-16. Chandrasekar et al. synthesized the C1-14 fragment (2.45a) of revised palmerolide A (2.1b). The researchers relied on deoxygenative rearrangement of an alkynol, an asymmetric dihydroxylation of the resulting diene ester which installed C11 and 12 hydroxyls, and CBS (Corey-Bakshi-Shibata) reduction to selectively afford the C7 hydroxyl. Cantagrel et al. reported the C3-15 (2.46) and C16-23 (2.47) fragments of 2.1b. Significant transformations included an Evan’s chiral auxiliary aldol condensation forming C19 and 20 centers, an enatoselective reduction with Noyori’s catalyst to set C7 configuration, and a diastereoselective acetylenic Grignard addition forming C10 configuration. Dudley et al. synthesized the C1-15 region (2.45b) of revised palmerolide A using an optimized Claisen-type condensation of vinyllogous acyl triflates to form the C1-8 subunit and appending it to C9-15 via a convergent HWE olefination. Stereocenters C10 and 11 were formed via asymmetric dihydroxylation (AD-mix-α). Configuration at C7 was installed using a CBS reduction.

**Scheme 2.10.** Partial syntheses of palmerolide A (2.1).
2.2.3 Formal total synthesis of palmerolide A

Formation of an advanced intermediate from Nicolaou’s total synthesis$^{14,15}$ (2.47, Scheme 2.11) by Maier and Jagel$^{23}$ was reported culminating in the formal total synthesis of palmerolide A (2.1b). Using methods they had previously reported,$^{19}$ the researchers were able to synthesize 2.48 which could then be coupled to 2.49 via HWE olefination yielding 2.50. The macrolide was formed via a highly stereospecific Heck cyclization. Takai olefination then cleavage of silyl protecting groups yielded 2.47, completing the formal total synthesis.

Scheme 2.11. Formal total synthesis of palmerolide A by Maier and Jagel.

2.3 Degradation of palmerolide A to confirm absolute configuration

Naturally occurring palmerolide A was subjected to degradative studies$^{11}$ to verify the stereochemical assignments determined by spectroscopic and derivatization techniques.$^1$ Reductive ozonolysis of palmerolide A could be used to cleave the molecule into several polyol
fragments. Comparison of the naturally derived fragments to those synthesized from chiral pool starting material would verify the proposed structure.

2.3.1 Ozonolysis of palmerolide A

Subjecting palmerolide A to ozonolysis followed by reduction with sodium borohydride (Scheme 2.12) resulted in quantitative yields of hexane-1,2,6-triol (2.51) and hexane-1,2,3,6-tetraol (2.52). Specific rotations of -9.0 and -8.1 were recorded for triol 2.51 and tetraol 2.52, respectively. Degradation of 2.1a should afford triol R-2.51 as well as (2R,3R)-2.52. Hexane-1,2,6-triol is available commercially but only in racemic form. (S)-Hexane-1,2,6-triol has been reported,\(^24\) having a specific rotation of -3.4. This was at odds with the rotation of degradation product 2.51 (i.e. R-2.51 should bear a rotation of +3.4) in sign as well as magnitude prompting us to develop chiral pool based syntheses of polyols hexane-1,2,6-triol and hexane-1,2,3,6-tetraol.

![Scheme 2.12](image)

2.1a: palmerolide A (originally proposed)

**Scheme 2.12.** Reductive ozonolysis of palmerolide A isolated from Synoicum adareanum.

2.3.2 Synthesis of hexane-1,2,6-triol

(R)-Hexane-1,2,6-triol (R-2.53) was prepared from the (R)-acetonide of glycerol (2.54, Scheme 13). Acetonide 2.54 was oxidized via Swern\(^25\) protocol. The resulting aldehyde underwent Wittig olefination forming 2.55.\(^26\) In the course of this preparation, the Wittig product 2.55 was reductively ozonolyzed back to 2.54 to confirm that epimerization had not occurred. Recovered 2.54, however, was found to have significantly epimerized (ee 56% of original).
A homologue of 2.54, the acetonide of (R)-butane-1,2,4-triol (R-2.56), after oxidation, could be subjected to Wittig olefination to R-2.57 while retaining its optical purity based on a similar reductive ozonolysis back to R-2.56 (Scheme 2.14). A 1,4-reduction of the conjugated ester with lithium aluminium hydride (LAH) produced the terminal alcohol R-2.58 in moderate yields. Hydrolysis afforded the desired triol R-2.53 under mildly acidic conditions. Spectral and chromatographic data (^1H NMR, ^13C NMR, GC/MS, ESI-MS) of (R)-hexane-1,2,6-triol (R-2.53) matched that of commercially available (±)-hexane-1,2,6-triol.

The specific rotation of synthetic (R)-hexane-1,2,6-triol (R-2.53, +11.1) was then compared to degradation product (2.51, -9.0). We were satisfied to find the magnitude of the specific rotation from the synthetic product more closely matched the degradation product, but were disappointed to find the sign of the rotation to be opposite that of the degradation product. Final verification of the C7 configuration was achieved by preparation of (S)-hexane-1,2,6-triol (S-2.53), starting from the acetonide of (S)-butane-1,2,4-triol (S-2.56, Scheme 2.14). Oxidation of S-2.56 to the requisite aldehyde was achieved via Swern protocol, avoiding use of Dess-Martin periodinane, a costly reagent. Horner-Wadsworth-Emmons olefination was utilized to form S-2.57 in a more efficient manner than the Wittig reaction used to form the R-enantiomer. As an alternative to the modest yielding reduction of R-2.57 with LAH, a two step reduction with diisobutylaluminum hydride (DIBALH) to the allylic alcohol then catalytic hydrogenation afforded S-2.58 in better overall yield. The specific rotation of the (S)-triol (S-2.53, -11.6) matched the degradation product (2.51, -9.0)
obtained from ozonolysis of palmerolide, suggesting the configuration of palmerolide A’s C7 stereocenter as bearing the S configuration, rather than the originally published 7R.

2.3.3 Synthesis of hexane-1,2,3,6-tetraol

Stereocenters at C10 and C11 of palmerolide A were analyzed by comparing the degradation product tetraol 2.52 to analogs synthesized from the chiral pool. The configuration at C10 and C11 could be explored by synthesis of both (2R,3R)- and (2S,3S)-hexane-1,2,3,6-tetraol (R,R-2.59 and S,S-2.59, respectively). Synthesis from either L(+)- or D(-)-2,3-O-Isopropylidene-threitol (2.60) yielded the desired tetraol in six steps. Preparation of (2R,3R)-hexane-1,2,3,6-tetraol (Scheme 2.15) began with the monoacetylation of diol (R,R)-2.60 followed by oxidation of the unprotected alcohol via Dess-Martian periodinate. Immediate Wittig olefination of the subsequent aldehyde produced an inseparable mixture of E/Z isomers (2.61). The desired tetraol (R,R)-2.59 was obtained from 2.61 after catalytic hydrogenation, acid hydrolysis, then reduction. The specific rotation of (R,R)-2.59 (+9.9) was at odds with the corresponding ozonolysis product 2.52 (-8.1).
Preparation of tetraol (S,S)-2.59 was achieved using a slightly different synthetic route starting with commercially available (S,S)-2.60 (Scheme 2.16). Selective benzylation [silver oxide (Ag₂O) and benzyl bromide]²⁷ of (S,S)-2.60 produced the mono-benzylated alchohol which was subjected to Dess-Martin oxidation and Wittig olefination to afford alkene 2.62 as a mixture of E/Z isomers. E-2.62 could be obtained exclusively using HWE conditions. Catalytic hydrogenation of 2.62 yielded the unsaturated and deprotected alcohol 2.63. Tetraol (S,S)-2.59 was realized by reduction with LAH then acid hydrolysis of 2.63. (S,S)-2.59 displayed a specific rotation of -10.0 which agreed with that of the degradation product obtained from ozonolysis (-8.1) in sign and magnitude inferring the natural product was actually 10S, 11S.

2.3.4 Re-evaluation of absolute configuration

The results of the degradation study compelled us to re-examine the previously reported¹² data from the Mosher’s analysis to establish whether our procedures were in error or the method itself failed. Lab notebooks indicated the correct conversion of the acid chloride stereochemistry to the corresponding ester (ie. (R)-acid chloride to (S)-ester). The esterification of palmerolide A with
(R)-methoxytrifluoromethylphenylacetoyl chloride was repeated forming the (S)-MTPA ester which was found to bear 1H NMR shifts that matched the data originally assigned to the (R)-MTPA ester. Thus, the samples or data sets derived from the original MTPA esters were found to be mislabelled necessitating a revision to the absolute configuration of palmerolide A at C7 from R to S. The C10 configurational assignment was made from the same MTPA products, necessitating that we re-evaluate the C7/C10 MTPA diester. We found that C10 had been subject to the same transposition. Because the C11 configuration is based on $^2J_{CH}$ and $^3J_{CH}$ conformational analysis of the C10/C11 spin system, both C10 and 11 must be revised to the S configuration. In summary, the absolute configuration of palmerolide A should be revised to 7S, 10S, 11S based on this degradation study and re-evaluation of the Moshers analysis.

2.4 Synthesis of the C3-14 fragment of palmerolide A

With the correct absolute configuration at C7, 10, and 11 determined, efforts were directed to the reconstruction of palmerolide A using similar synthetic routes as the ones described above employed to generate the chiral polyols. The syntheses of the fragments 2.53 and 2.59 were modified for coupling using one of the many olefination reactions described in literature.

2.4.1 Julia-Kocienski olefination

To form the C3-14 segment of the macrolide, we decided that E8-alkene could be formed from an aldehyde derived from triol 2.51 coupled to a sulfonyl derived from tetraol 2.52 based on Julia-Kocienski protocol. Compostella et al. demonstrated that Julia–Kocienski olefination is useful in constructing trans-olefins with an α-alkoxy aldehyde and an aliphatic sulfonyl or a β-alkoxy sulfonyl (no β-elimination observed) and aliphatic aldehyde, but to our knowledge no examples exist in which both coupling components contain alkoxy substituents. Using this methodology, we envisioned fragment C3-14 (2.64) could be formed from sulfonyl 2.65 derived from triol 2.53, coupled with aldehyde 2.66, derived from tetraol 2.59 (Scheme 2.17).
The synthesis of triol 2.53 was modified to generate sulfone 2.65 (C3–8) from alcohol S-2.58 (Scheme 2.18). Anticipating an olefin ring closing metathesis reaction to form the macrolide portion of palmerolide A, intermediate terminal alkene 2.67 was required. Dess-Martin oxidation of S-2.58 followed by Wittig olefination and hydrolysis yielded the desired alkene 2.67. Monotosylation followed by silylation of the free secondary alcohol led to sulfone precursor 2.68. Treating tosylate 2.68 with 1-phenyl-1H-tetrazole-5-thiol and potassium carbonate under refluxing conditions\(^\text{31}\) yielded a thioether intermediate, which was then oxidized with catalytic amounts of sodium tungstate, phenylphosphonic acid, methyltrioctylammonium hydrogen sulfate and an excess of 30% hydrogen peroxide\(^\text{32}\) to generate sulfone 2.65. Aldehyde 2.66 was realized by Dess-Martin oxidation of 2.63 (Scheme 2.18), an intermediate in the synthesis of (2S,3S)-hexane-1,2,3,6-tetraol [(S,S)-2.59].
Attempts at coupling 2.65 and 2.66 using Julia-Kocienski methodology were not successful in our hands, yielding low recoveries of unreacted starting material with no evidence of β-elimination of the tert-butyldimethylsilyl- (TBS-) ether in 2.65.

2.4.2 Grubb’s olefin cross metathesis

A new route to join fragments derived from 2.53 and 2.59 was devised utilizing olefin cross metathesis (OCM). We chose Grubbs second generation catalyst because Type II/Type III cross couplings are predicted to produce moderate to high yields with little to no homodimerization. The new route required that each fragment terminate in an olefin. The synthesis of a fragment bearing the palmerolide A (2.1b) C3-8 centers (e.g., 2.65) was modified to produce olefin-metathesis substrate 2.69 (Scheme 2.19). Benzylation followed by acid hydrolysis of intermediate S-2.58 resulted in the formation of diol 2.70. A one pot protecting group manipulation produced secondary acetate 2.71. Dess-Martin oxidation and Wittig olefination resulted in the formation of terminal olefin 2.69.

The fragment bearing the palmerolide A (2.1b) C9–14 segment, 2.72 (Scheme 2.18), was derived from aldehyde 2.66 by a Wittig reaction. Combination of olefins 2.69 and 2.72 (Scheme 2.20) using Grubbs second generation catalyst (2.27) proceeded smoothly to generate the desired E-isomer, as predicted, in moderate yields (2.73, C3–14 of palmerolide A) with no recovery of homodimers nor unreacted starting material. Steric bulk at both allylic positions in the product may explain why Z-2.73 was not observed. One cannot rule out the possibility of E/Z isomerization via secondary metathesis of 2.73, which could also explain selective E-isomer.
formation. The fact that no homodimers were found and only the \( E \)-isomer of 2.73 was isolated suggests 2.69 and 2.72 may be reacting in a selective type II/type III fashion as postulated by Grubbs et al.\(^3\) However, due to the moderate yield of 2.73 and no evidence of either homodimer, it is unclear of which olefin type (II or III) 2.69 and 2.72 should be considered.

In summary, 2.73, the C3-14 portion of palmerolide A, was constructed from commercially available chiral building blocks \( S \)-2.56 and \( (S,S) \)-2.60. The total synthesis of palmerolide A based on this chiral pool approach is ongoing and when completed should offer a facile route to a multitude of derivatives for use in structure activity relationship (SAR) and structure property relationship (SPR) studies.

2.5 References Cited

5. Anne Monks, personal communication.


Chapter 3. Meridianin A and psammopemmin A: structure investigation

3.1 Indole and pyrimidine containing natural products

Nitrogen-containing heterocyclic compounds derived from natural sources are abundant in structural complexity and bioactivity. Of particular interest to this study are indole- and pyrimidine-containing metabolites derived from marine invertebrates. Sponges (Porifera) and tunicates (Urochordata) have afforded a diverse assortment of biologically promising indole\(^1\)- and pyrimidine\(^2\)-containing alkaloids.

Indole alkaloids that bear a nitrogen containing heterocycle at the 3-position have displayed a rich array of bioactivity. Dragmacidin (3.1), a piperazine-linked bisindole alkaloid with cytotoxic properties, was isolated from the deep water marine sponge *Dragmacidon* sp.\(^3\) The compound displayed low *in vitro* IC\(_{50}\) values toward P-388 (leukemia), A-549 (human lung), HCT-8 (human colon), and MDAMB (human mammary) cancer cell lines. Dragmacidin D (3.2), another piperazine-linked bisindole was isolated from the sponge *Spongosorites* sp.\(^4\) Dragmacidin D exhibited antiviral properties versus feline leukemia virus (FeLV), antitumor properties versus P-388 and A-549 cancer cells, and also inhibited the growth of the fungal pathogens *Candida albicans*, and *Cryptococcus neoformans*.

![3.1: dragmacidin](image1.png)  
![3.2: dragmacidin D](image2.png)
Topsentin (3.3), bromotopsentin (3.4) and nortopsentsins A-C (3.5-3.7), bis(indolyl)imidazole alkaloids isolated from the deep-sea Caribbean sponge Spongosorites ruetzleri exhibit antitumor and antiviral activity toward several important targets. Topsentin displayed activity toward P-338, HCT-8, A-549, and T47D (breast) cancer cells in vitro as well as in vivo activity toward P-388 and B16 (melanoma) cell lines. Topsentin and bromotopsentin exhibited in vitro antiviral activity toward herpes simplex (HSV-1), Vesivular stomatitis virus (VSV), and coronavirus A-59. Nortopsentsins A-C (3.5-3.7) displayed inhibitory activity toward P-388 cancer cells. Semisynthetic nortopsentsins, dimethylated at the imidazole nitrogens, showed a significant increase in activity toward P-388.

![Chemical structures](image)

A multitude of aplysinopsins, tryptophan derivatives bearing an imidazoline moiety, have been isolated from sponge genera worldwide. Aplysinopsins have also been found in corals (predominately from the genus Tubastrea) as well as a Tubastrea predator, the mollusk Phestilla melanobrachia. Aplysinopisin (3.8), initially found in eight Indo-Pacific sponge species, has shown anticancer and antimicrobial properties. Several aplysinopsins and synthetic analogs have also shown activity in serotonin (5-HT) receptor assays.

![Chemical structure](image)
3.1.1 Indoles and pyrimidines from Southern cold water sponges

A plethora of discorhabdins, cytotoxic pyrroloiminoquinone alkaloids, have been isolated from both temperate and tropical sponges of the family Latrunculiidae.\(^\text{11}\) The cold water sponge *Latrunculia apicalis*, found in McMurdo Sound, Antarctica, has afforded discorhabdin G (3.9)\(^\text{12}\) as well as discorhabdin C (3.10, previously reported from New Zealand collected *Latrunculia* sp.).\(^\text{13}\) Discorhabdin C is a particularly potent deterrent, defending *L. apicalis* from predation by the spongivorous sea star *Perknaster fuscus*. Discorhabdin R (3.11), found to contain a sulfide as well as an epoxide, has been isolated from two Antarctic sponges in the Latrunculiidae family (*Negombata* and *Latrunculia* sp.).\(^\text{14}\)

![Discorhabdins](image)

The bright red Antarctic sponge *Kirkpatrickia variolosa* elaborates colorful pigments named variolins A (3.12) and B (3.13),\(^\text{15,16}\) aminopyrimidine containing fused tricyclic heteroaromatic alkaloids.\(^\text{17}\) N(3')-methyl tetrahydrovariolin B (3.14) was also isolated from *K. variolosa*. Variolin B was shown to be a very potent inhibitor of P-388 cancer cells. Variolin B was later found to inhibit cyclin dependent kinase-2 (CDK2) and CDK3 selectively over CDK4.\(^\text{18}\) Variolin B and a synthetic derivative, deoxyvariolin B (3.15), were found to inhibit phosphorylation of histone H1, mediated by several CDKs, effectively interrupting the normal progression of the cell cycle.\(^\text{19}\) These promising findings have prompted PharmaMar, a company specializing in marketing drugs derived from marine sources, to assess the variolins in preclinical trials.
Psammopemmins A-C (3.16-3.18), 4-hydroxyindole alkaloids, were isolated from the Antarctic sponge *Psammopemma* sp.\textsuperscript{20} The 5-(2'-bromo-4-aminopyrimidine) moiety reported in psammopemmins is unprecedented in both terrestrial and marine natural products. No bioactivity data was reported for psammopemmins A-C.

3.12: variolin A  
3.13: variolin B, R=OH  
3.14: N(3')-methyl tetrahydrovariolin B

3.15: deoxyvariolin B, R=H

3.16: psammopemmin A  
3.17: psammopemmin B  
3.18: psammopemmin C

3.1.2 Indoles and pyrimidines from Southern cold water tunicates

Aplicyanins A-F (3.19-3.24), tetrahydropyrimidine substituted indoles isolated from the Antarctic tunicate *Aplidium cyaneum* were shown to be cytotoxic and antimitotic.\textsuperscript{21} The absolute configuration of the aplicyanins is not known. Aplicyanin E, when subjected to chiral HPLC, was shown to be enantiomerically pure. Aplicyanins C-F bear a rather uncommon 1N-methoxyindole subunit. Aplicyanins B, D, and F displayed GI\textsubscript{50} values in the submicromolar range versus HT-29 (colon), A-549, and MDAMB cancer cells lines while aplicyanins A, C, and E were far less active. The *N*-acetyl group is clearly crucial for the biological activity observed.
The green tunicate *Aplidium meridianum*, collected in the South Georgia Islands, elaborates seven aminopyrimidine containing indole alkaloids, meridianins A-G (3.24-3.30). Meridianins A-E were initially screened against murine mamarian adenocarcinoma cells (LMM3). Meridianins B-E displayed micromolar IC$_{50}$ values against LMM3 cells. Further biological investigation revealed that meridianins A-F were low micromolar inhibitors of various cyclin-dependent kinases (CDKs), glycogen synthase kinase-3 (GSK-3), protein kinase A (PKA), as well as other protein kinases. The most potent inhibitors, meridianins B and E, are 4-hydroxyindole indole alkaloids differing in bromination pattern. Meridianins B-F exhibited cytotoxic activity at low micromolar ranges. Meridianin A, however, displayed no cytotoxicity toward HEp-2 (laryngeal carcinoma), HT-29, and LMM3 cells at the highest concentration examined.
3.2 Review of meridianin syntheses

The meridianins have attracted significant synthetic interest due to their biological activity as well as their unique and relatively simple structure. Many of the naturally occurring meridianins have been synthesized. Particular attention has been focused on meridianin analogs as well as methods to efficiently construct a variety of derivatives for further biological examination.

3.2.1 Synthesis of natural meridianins

Meridianin D (3.27) and its debromo- analog meridianin G (3.30) were synthesized via coupling of 2-amino-4-chloropyrimidine (3.31) and the appropriate protected indole boronic acid (3.32) using a Suzuki-Miyaura protocol (Scheme 3.1).25

Along with the tricyclic core of variolin, the synthesis of meridianins C (3.26), D (3.27), and E (3.28) was reported.26 A follow up publication described the synthesis of meridianin A (3.24) using the same methods.27 Heating the suitably functionalized N-tosyl-3-acylindole (3.33) with dimethylformamide dimethylacetal (DMF-DMA) afforded enaminone 3.34 (Bredereck protocol,28 Scheme 3.2). Condensation of 3.34 with guanidine chlorohydrate in the presence of sodium carbonate resulted in concomitant detosylation forming meridianins C, D, and intermediate 3.35. Debenzylation and debromination of 3.35 with H2 and palladium (Pd) yielded meridianin A. Meridian E was formed via selective debenzylation of 3.35 with trifluoroacetic acid (TFA) and thioanisole. Hydrogenation in different solvents formed either meridianin A or E. In the initial report (ref. 26), meridianin E was produced from intermediate 3.35 via hydrogenation (H2, Pd/C, EtOH, 88%). In the follow up report (ref. 27), meridianin E was formed from 3.35 by treatment.
with TFA and thioanisole (65%) while hydrogenation of 3.35 (H₂, Pd/C, EtOAc) resulted in the debrominated compound, meridianin A, in 83% yield.

\[
\begin{align*}
\text{Scheme 3.2. Enaminone condensation to form meridianins A, C, D, and E.}
\end{align*}
\]

Meridianins C, D, and G were synthesized efficiently using a carbonylative alkynylation followed by subsequent cyclocondensation.²⁹ Carbonylative Sonogashira coupling of trimethylsilylacetylene and appropriately functionalized 3-iodoindole 3.36 using Pd(PPh₃)₂Cl₂ catalyst resulted in trimethylsilylalkyne 3.37 (Scheme 3.3). Condensation of 3.37 with guanidine in the presence of sodium carbonate with concurrent deprotection resulted in meridianins C, D, and G. Absent from the report is any discussion of the reactivity of 4-hydroxyindoles (or protected forms thereof) toward carbonylative alkynylation.

\[
\begin{align*}
\text{Scheme 3.3. Sonagashira carbonylation to form meridianins C, D, and G.}
\end{align*}
\]

Meridianin G (3.30), referred to in this report as 6-debromomeridianin D, was produced from 3-cyanoacetylindole (3.38) in four steps (Scheme 3.4).³⁰ Treatment of 3.38 with DMF-DMA...
afforded enaminonitrile **3.39** which was then condensed with guanidine resulting in 5'-cyanomeridianin G (**3.40**). Base hydrolysis then decarboxylation yielded the desired meridianin G.

![Scheme 3.4. Formation of meridianin G from 3-cyanoacetylindole.](image)

3.2.2 Synthesis and biological activity of meridianin analogs

Several 3-(5'-(2'amino)pyrimidyl)indole alkaloids were synthesized prior to the discovery of the meridianins in 1998. The first report, in 1967, describes the synthesis of two compounds very similar in structure to that of the meridians. Alkaloids **3.41a** and **3.41b** were formed by reacting 3-methylthio-3-(3-indolyl)acrylic acid derivatives (**3.42a,b**) with guanidine (Scheme 3.5). In 1993, the synthesis of over 200 pyrimidine derivatives and their utility in asthma therapy was reported. Enaminone **3.43**, 3-methylaniline (**3.44**), and cyanamide were condensed under acidic conditions to form **3.45**, in effect, 2'-methylaniline substituted meridianin G (Scheme 3.5). Pyrimidylindole **3.45** was found to inhibit histamine release from basophil cells. Compounds that block immunological release of mediators like histamine could be developed into antiasthma agents.
Several synthetic (bis)indole and pyrazine meridianin analogs constructed using Suzuki methodology were found to inhibit growth of a variety of cancer cell lines. \(^{33}\) (Bis)indole alkaloid \(3.46\) displayed strong activity against IGROV1 (ovarian carcinoma). The pyrazine-substituted indole alkaloids displayed varying amounts of cytotoxicity and selectivity. \(N\)-tosyl indole \(3.47\) showed across the board cytotoxicity, inhibiting the growth of every cell line tested. Alternatively, methoxy substituted pyrazine \(3.48\) specifically and effectively inhibited HOP-92 (non-small lung cancer) cells.

Iso-meridianins C \(3.49\) and G \(3.50\) were prepared by microwave-assisted Fischer indole synthesis (Scheme 3.6). \(^{34}\) Suitably substituted phenylhydrazine \(3.51\) (from pyrimidine \(3.52\)) was
heated in a conventional microwave oven with zinc chloride (ZnCl₂) in a small amount of dimethylformamide (DMF) resulting in N-Boc-deprotection and indole formation. The resulting iso-meridianins C and G were devoid of biological activity.

Scheme 3.6. Fischer indole synthesis of iso-meridianins C and G.

An environmentally benign method to construct the 2'-piperidinyl meridianin analog 3.53a and related trisubstituted pyrimidines has been described using basic alumina as a solid support as well as a catalyst. Chalcone 3.54, S-benzylthiuronium chloride (3.55) and piperidine dissolved in ethanol, concentrated onto basic aluminum and heated intermittently with microwave resulted in 3.53a (Scheme 3.7). Biological activity of 3.53a and other trisubstituted pyrimidines synthesized in this study was not reported. A reaction of the apposite chalcone (3.54) with guanidine derivative 56 afforded several similar trisubstituted pyrimidines (3.53b-d, Scheme 3.7) that displayed antimalarial properties, inhibiting *Plasmodium falciparum* in vitro.

Scheme 3.7. Synthesis of a meridianin related trisubstituted pyrimidines.
Reaction of suitably substituted chalcone 3.57 with guanidine afforded 6'-alkyl substituted meridianin analogs 3.58a-c.\textsuperscript{37} When screened for \textit{in vivo} pregnancy interceptive activity in hamsters, 3.58c was 50% effective, whereas 3.58a and 3.58b were devoid of activity.

![Scheme 3.8. Synthesis of 6'-alkyl substituted meridianin analogs.](image)

Indoyluracils 3.59 resembling meridians have been synthesized from condensation of ureidopropenoate 3.60 (Scheme 3.9).\textsuperscript{38} Using DMF-DMA and a substituted guanidine, 3.60 was formed in similar fashion as 3.34 (Scheme 3.2) and 3.39 (Scheme 3.4). Perplexingly, no mention of biological activity, or lack thereof, was reported for these compounds. The authors note that the pyrimidyl N1 could be coupled to a sugar to form novel nucleosides.

![Scheme 3.9. Uracil meridianin derivatives.](image)

Using Bredereck methods described in Scheme 3.2,\textsuperscript{26,27} meridianins C (3.26), G (3.30), and seven monohalogenated meridianin analogs (3.61a-g) were synthesized from the suitably
substituted enaminone 3.34. In a similar fashion, numerous monohalogenated, N1-alkyl-substituted meridianin derivatives were constructed from alkylated enaminone 3.62. The crystal structure of N1-methylmeridianin G was obtained to assist in molecular modeling of the interaction between meridianin analogs and the adenonsine triphosphate (ATP) pocket of kinases. Structure activity relationship studies were ongoing at the time of publication. No bioactivity data is available to date. Following a Bredereck protocol and bromination at 5', a Suzuki coupling was used to construct 5'-aryl substituted meridianin G derivatives (3.63a-g). The N1-methyl derivatives of 3.63a-g were also synthesized. The anticancer utility of 3.63a-g was reported in a follow up publication. Screening the compounds against various kinases as well as MCF-7 (breast) and PA 1 (ovarian) cancer cell lines revealed that N1-methyl 3.63c, N1-methyl 3.63b, 3.63f, and 3.63g were selectively cytotoxic toward PA 1 cells. Interestingly, none of the active compounds displayed any significant kinase inhibition.

Several 6'-substituted meridianin C and G analogs were synthesized (Scheme 3.10) via metal-free alkynylation of indole 3.64 with various α-oxo ketene dithioacetals (3.65). Refluxing 3.64 and 3.65 in trifluoroacetic acid:dichloromethane (TFA:DCM) resulted in the formation of functionalized indole 3.66. Condensation with guanidine nitrate yielded N1-alkylated-6'-substituted meridianin derivatives 3.67a-g. Oxidative debenzylation of 3.67a-d resulted in 6'-substituted meridianins (3.68a-d). Biological activity was not reported.
Meridianin analogs 3.69a-c as well as a handful of 2'-chloromeridianin derivatives were constructed by thermal annulation of nitrosoarenes with 4-ethynylpyrimidines.\(^4\) Heating a 1:1 ratio of substituted nitrosoarene 3.70 and 2-amino-4-ethynylpyrimidine (3.71) in toluene resulted in the formation of meridianin derivatives 3.69a-c (Scheme 3.11). At time of publication, investigation of the mechanism of the reaction as well as the bioactivity of the compounds synthesized was ongoing.

**Scheme 3.10.** Direct metal free alkynylation of indoles to form meridianin analogs.

**Scheme 3.11.** Indolization of nitroarenes to form meridianin analogs.
3.2.3 Synthesis and biological activity of meriolins

The remarkable biological activity of the meridianins and the variolins as well as the structural commonalities between the two alkaloids prompted interest in synthesizing 3-(2'-aminopyrimidin-4-yl)-7-azaindole, a structural hybrid later dubbed meriolin 1 (3.70, Fig. 3.1).

![Fig. 3.1. Meriolin, a structural hybrid of meridianin and variolin](image)

The first synthesis of 3-(2'-aminopyrimidin-4-yl)-7-azaindole (3.70) was reported along with the synthesis of meridianins A, C, D, and E and similarly relied on Bredereck methodology. Analogous to the synthesis of meridianins in Scheme 3.2, meriolin 1 (3.70) was formed through condensation of guanidine and azaindolylamidine 3.71 (Scheme 3.12). Meriolin 1 was also synthesized via condensation of guanidine and trimethylsilylalkynone 3.72 (Scheme 3.12) reported in conjunction with the synthesis of meridianins C, D, and G (see Scheme 3.3). Initial screening demonstrated that meriolin 1 inhibits protein kinases hSGK1 and Tie-2 at low micromolar levels.

![Scheme 3.12. Condensation with guanidine to form meriolin 1.](image)
The synthesis and biological characterization of fourteen meriolin analogs (meriolins 1-14, 3.70-3.83) revealed the compounds to be potent CDK inhibitors.\textsuperscript{44,45} When screened for selectivity against thirty-two kinases, meriolins displayed enhanced specificity toward CDKs over variolins, especially CDK2 and CDK9. Upon crystallization of variolin B and meriolin 3 with pCDK2/cyclin A, both compounds were found to bind in the ATP binding site of the kinase but in different orientations. Meriolin 3 was found to be forty times more efficient at inhibiting Ewing’s sarcoma than the control, roscovitine (a purine analog, 3.84), in xenograft assays. Meriolin 3 also showed nanomolar IC\textsubscript{50} values against spheroid and monolayer HCT-116 (colon) cancer cell lines. SAR studies revealed that the 2'-amino group was necessary for CDK inhibition as 2'-methylthiomeriolin (meriolin 12, 3.81) as well as 2'-H-meriolin (meriolin 13, 3.82) showed a significant loss of activity. Changing the substituent at C4 (R\textsuperscript{1}) to a more aliphatic group led to increased activity, theorized to be due to favorable interactions with the glycine-rich loop of the kinase. Bulky substitutes at C6 (R\textsuperscript{3}) led to less inhibition, perhaps by shielding hydrogen bonding interactions between N2, N7 and the kinase. Meriolins are potent CDK inhibitors as well as strong antiproliferatives and warrant further investigation.
In our ongoing search for bioactive metabolites, we investigated the chemical composition of the yellow top tunicate, *Synoicum* sp.,\(^46\) collected in the benthos surrounding Palmer Station, Antarctica. The lyophilized tunicate was extracted with polar and nonpolar solvents. The nonpolar extract was subjected to successive medium and high performance liquid (Scheme 3.13) to yield meridianins A (3.24), B (3.25), C (3.26), and E (3.28). NMR data of meridianins isolated from *Synoicum* sp. were compared to that of the previously reported meridianins isolated from *Aplidium meridianum*\(^{22}\) and found to be congruous.

**Scheme 3.13.** Isolation of meridianins A, B, C, and E
In the process of verifying the structure of the meridianins, it was noted that the physical data reported for meridianin A, B, and E bore a striking resemblance to psammopemmin A (3.16), C (3.18), and B (3.17), respectively, indole alkaloids isolated from the Antarctic sponge Psammopemma sp. Little difference exists between the reported NMR data of meridianin A and psammopemmin A (Table 3.1). To verify the structure originally proposed for psammopemmin A as well as investigate biological activity of the meridianins and analogs thereof, synthetic studies were initiated.

![Diagram of meridianin A and psammopemmin A](image)

**Table 3.1.** $^1$H NMR and $^{13}$C shifts of meridianin A (3.24) and psammopemmin A (3.16).

<table>
<thead>
<tr>
<th>Position</th>
<th>3.24 $\delta_H$</th>
<th>3.24 $\delta_C$</th>
<th>3.16 $\delta_H$</th>
<th>3.16 $\delta_C$</th>
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<td>13.57 (s)</td>
<td>13.55 (s)</td>
<td>11.75 (s)</td>
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<td>11.75 (brs)</td>
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<td>113.7</td>
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</tr>
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<td>7.12 (d)</td>
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<tr>
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<td>8.10 (d)</td>
<td>160.7</td>
<td>160.7</td>
</tr>
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<td>6.72 (s, 2H)</td>
<td>6.68 (brs, 2H)</td>
<td>158.3$^e$ (C5')</td>
</tr>
</tbody>
</table>

$^a$ in ppm in $d_6$-DMSO.  
$^b$ isolated from Aplidium meridianum.  
$^c$ isolated from Synoicum sp.  
$^d$ isolated from Psammopemma sp.  
$^e$ assignments may be interchanged.
3.4 Synthesis and biological activity of 3-pyrimidylindoles

A strategy to couple indoles to amino substituted pyrimidines was developed using Suzuki-Miyaura protocol to synthesize both meridianin A and psammopemmin A, as well as 3-pyrimidylindole analogs. The compounds were evaluated for biological activity.

3.4.1 Synthesis of meridianin A, 4-methoxymeridianin A, and 5-bromomeridinanin E

Meridianin A (3.24) was formed in six steps from indolone 3.85 in 17% overall yield (Scheme 3.14). The 4-indolol moiety of meridinain A was synthesized by the dehydrogenation of commercially available tetrahydroindolone 3.85 using Pd/C in refluxing diisobutyl ketone, a modest yielding yet economic reaction. The phenol of 4-indolol (3.86) was then masked as a silyl ether (3.87). A two-step, one-pot N1 silylation and bromination reaction resulted in the suitably substituted indole 3.88. Transmetallation with t-butyllithium then addition of borate 3.89 generated vinyl borate 3.90 which could be coupled to 2-amino-4-chloropyrimidine using a Suzuki protocol resulting in an intermediate (3.91) bearing the 4-hydroxymeridianin skeleton. Desilylation with tetra-n-butylammonium fluoride (TBAF) afforded meridianin A (3.24) in good yields.

The 4-methoxy analog of meridianin A was synthesized using a route analogous to that producing meridianin A (Scheme 3.14). 4-Indolol (3.86) was refluxed with methyl iodide under basic conditions to form 4-methoxyindole (3.92). After silylation and bromination resulting in the formation of 3.93, transmetallation followed by addition of 3.89 gave vinyl borate 3.94. Coupling 3.94 to 2-amino-4-chloropyrimidine with Pd0 resulted in 3-pyrimidylindole 3.95. Upon treatment with TBAF, 4-methoxymeridianin A (3.96) was obtained.

Dibromination of meridianin A (3.24) with pyridinium tribromide resulted in the formation of 5-bromomeridinanin E (3.97, Scheme 14).
3.4.2 Synthesis of psammopemmin A and 2'-chloropsammopemmin A

Psammopemmin A (3.16) differs from meridianin A (3.24) in connectivity and substitution of the 3-pyrimidyl-moiety. Using the Suzuki methodology developed to synthesize meridianin A, we needed only to change the pyrimidine coupling partner to afford psammopemmin A. Synthesis of the pyrimidine moiety (Scheme 3.15) began with the amination of 2,4-dichloropyrimidine (3.98) followed by iodination to yield 4-amino-2-chloro-5-iodopyrimidine (3.99). Substitution of the 2'-chloro group using hydrogen bromide in acetic acid (HBr/AcOH) resulted in 4-amino-2-bromo-5-
iodopyrimidine (3.100). As a general rule, oxidative addition of Pd$^0$ to halopyrimidines takes place at C4>C2>>C5.$^{53}$ We expected compound 3.100 to couple at C5 rather than C4, however, due to the more reactive iodo- substituent.

Vinyl borate 3.90 was then coupled to pyrimidine 3.100 with tetrakistriphenylphospine palladium resulting in an intermediate (3.101) bearing the psammopemmin skeleton. Desilylation of the resulting protected 3-(5'-pyrimidyl)-indole using TBAF resulted in very low yields of the psammopemmin A free base, 3.102, which was quite unstable under basic conditions. However, deprotection of 3.101 with hydrogen fluoride/pyridine (HF-pyridine)$^{54}$ produced the psammopemmin A free base cleanly as a white precipitate from the reaction solution. Forming the reported$^{20}$ HX salt of psammopemmin A was problematic and led to decomposition. Psammopemmin A hydrochloride (3.103) could be prepared by bubbling HCl gas through a $d_6$-DMSO solution of 3.102. Immediately analyzing the sample via NMR was necessary as the compound began to decompose after a few hours.

Vinylborate 3.90 was coupled to 4-amino-2-chloro-5-iodopyrimidine (3.99) to form 3.104 which, upon deprotection with TBAF in low yields or HF-pyr in higher yields, afforded 2'-chloropsammopemmin A (3.105, Scheme 3.15).
3.4.3 Structural reassessment of psammopemmin A

NMR data (Table 3.2) for synthetic psammopemmin A HCl (3.103) and its free base (3.102) were then compared to the natural product psammopemmin A (3.16, Table 3.1). The phenolic 4-OH signals differed significantly (3.16: δ 13.55, s; 3.102: δ 9.30, s; 3.103: δ 9.47, vbrs) suggesting these protons were in dissimilar electronic environments. However, the 4-OH signal in meridianin A (3.24) was identical to that reported for psammopemmin A (3.16, Table 3.1). The signal for H2 also differs between reported (3.16: δ 8.22, d) and synthetic (3.102: δ 7.27 d; 3.103: δ 7.33) psammopemmin A. Both meridianin A (3.24) and natural psammopemmin A (3.16) show coupling between protons on the pyrimidine ring (3.24: 5', 6', d; 3.16: 6', N1', d), while there is no obvious evidence of N1'-protonation in synthetic psammopemmin A hydrochloride (3.103). It is not clear from the 1H NMR spectrum of 3.103 which pyrimidyl-nitrogen accepts a proton (N1' or N3'). The 4'-NH2 signal of 3.102 exists as a very broad signal from δ 6.2-7.6 (centered around δ
7.00) under H2, 5, 6, and 7 signals and making integration difficult. The 4'-NH$_2$ signal of 3.103 appears even more broad (δ 6.1-8.5, centered around δ 7.45). The rather sharp 4-OH singlet in the $^1$H NMR spectrum of 3.102 (δ 9.30) significantly broadens as to be almost imperceptible in 3.103 (δ 9.47). N1' should be preferentially protonated as proposed in natural product 3.16. It has been demonstrated that monoprotonation of simple 4-aminopyrimidines occurs almost completely on N1, para to the amino group. The $^{13}$C NMR data for 3.103, however, lacks signals for C3, 2', and 6', perhaps explained by the rapid proton exchange between N1 and N3 of pyridine. This phenomenon has been reported to occur in 2,4-diaminopyrimidine resulting in two carbon signals for C2, 4, 5, and 6. Unfortunately, 3.103 slowly degrades in d$_6$-DMSO so the doubling of those carbons due to proton exchange, if occurring, could not be detected.

From comparison of the $^1$H and $^{13}$C NMR data of 3.16, 3.24, 3.102, and 3.103 we infer that the correct structure of psammopemmin A isolated from Psammopemma sp. is the same as that of meridianin A (3.24). The structure of psammopemmin C (3.18) should also be revised to that of

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**Table 3.2.** $^1$H and $^{13}$C NMR shifts$^a$ of synthetic psammopemmin A (3.102) and synthetic psammopemmin A-HCl (3.103)

<table>
<thead>
<tr>
<th>Position</th>
<th>3.102 δ$_{^1}$H</th>
<th>3.102 δ$_{^{13}}$C</th>
<th>3.102 HMBC</th>
<th>3.103 δ$_{^1}$H</th>
<th>3.103 δ$_{^{13}}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-OH</td>
<td>9.30 (s)</td>
<td>9.30 (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1-H</td>
<td>11.29 (brs)</td>
<td>11.29 (brs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.27 (d)</td>
<td>7.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>105.6</td>
<td>H2, 6', N1H</td>
<td>not observed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>115.4</td>
<td>H2, 5, 7, N1H, 4-OH</td>
<td>115.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>151.2</td>
<td>H5, 6, 4-OH</td>
<td>151.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.38 (d)</td>
<td>103.8</td>
<td>H7, 4-OH</td>
<td>6.48</td>
<td>104.0</td>
</tr>
<tr>
<td>6</td>
<td>6.93 (dd)</td>
<td>122.6</td>
<td></td>
<td>6.90$^b$</td>
<td>122.8</td>
</tr>
<tr>
<td>7</td>
<td>6.88 (d)</td>
<td>103.2</td>
<td>H5</td>
<td>6.90$^b$</td>
<td>103.2</td>
</tr>
<tr>
<td>7a</td>
<td>138.6</td>
<td>H2, 6</td>
<td>138.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>148.9</td>
<td></td>
<td></td>
<td>not observed</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>163.6</td>
<td>H6'</td>
<td>163.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>113.0</td>
<td>H6'</td>
<td>113.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>7.79 (s)</td>
<td>156.4</td>
<td></td>
<td>7.88</td>
<td>not observed</td>
</tr>
<tr>
<td>4'-NH$_2$</td>
<td>7.00 (vbrs)</td>
<td>7.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1'-H</td>
<td>not observed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$δ in ppm in d$_6$-DMSO.

$^b$Signals overlap.
meridianin B (3.25) due to the nearly identical $^1$H and $^{13}$C NMR signals reported.\textsuperscript{20,22} It is also likely that the structure of psammopemmin B (3.17) is that of meridianin E (3.28), although the NMR data for the two were obtained in different solvents so comparison is difficult.

3.4.4 Synthesis of meridoquin

Inspired by the meriolins (3.70-3.83), synthetic hybrids of meridianins and variolins (Fig. 3.1), we envisioned a meridianin-chloroquine hybrid dubbed meridoquin (3.106, Fig. 3.2). By combining the bioactive meridianin skeleton with characteristics from the widely used antimalarial chloroquine (see section 4.2), we hoped to build a new bioactive scaffold as a starting point for future studies.

Meridoquin (3.106) was achieved using Suzuki methodology to couple a suitably substituted indole moiety to the desired pyrimidine (Scheme 3.16). A low yielding but quick reaction of neat diethylamine and 2,4-dichloropyrimidine (3.98) resulted in the formation of 2-chloro-4-$N,N$-diethylaminopyrimidine (3.107) as well as the desired 4-chloro-2-$N,N$-diethylaminopyrimidine (3.108). Construction of the indole coupling partner began with the one pot $N_1$ silylation, 3-bromination of commercially available 6-chloroindole (3.109) resulting in dihaloindole 3.110. Transmetallation of 3.110 with $t$-butyllithium then addition of 3.89 resulted in intermediate 3.111 which was immediately coupled to pyrimidine 3.108 using the previously developed Suzuki protocol. Due to the electron withdrawing effects of the ring nitrogens, 4-halopyrimidines are exceptionally good electrophiles for use in Suzuki coupling reactions,\textsuperscript{53} explaining the absence of
any bisindoles in the reaction mixture. The indole-pyrimidine coupling product (3.112) was observed exclusively. Desilylation of 3.112 with TBAF afforded meridoquin (3.106).

Scheme 3.16. Synthesis of meridoquin. a. Et₂NH, neat, 1 min., 59% 3.107; 13% 3.108. b. n-BuLi, TIPSCI, THF, -79 °C to -10 °C then NBS, -78 °C, 89%. c. t-BuLi then 3.89, -78 °C. d. 3.108, Pd(PPh₃)₄, 2M Na₂CO₃, MeOH, benzene, reflux, 62% from 3.110. e. TBAF, THF, 86%.

3.4.5 Biological evaluation of 3-pyrimidylindoles

Serotonin (3.113, 5-hydroxytryptamine, 5-HT) transmission is thought to play a role in central nervous system (CNS) disorders. Compounds that bind to specific serotonin receptor subtypes could lead to treatment of CNS diseases. Selective antagonists of 5-HT₂C helped to establish the receptor's role in behaviors such as feeding and anxiety. Neuropsychiatric disorders such as major depression, anxiety, and migraine are currently being treated with 5-HT selective receptor ligands while drugs that target 5-HT₁A, 5-HT₂A, and 5-HT₂C receptors are under clinical investigation for the treatment of depression, schizophrenia, and anxiety, respectively. We were prompted to examine the binding affinity of several of our 3-pyrimidylindoles to various serotonin
receptors due to the structural features (amine-containing indole) common to our compounds and serotonin.

The binding affinity of meridianin A (3.24), 4-methoxymeridianin A (3.96), synthetic psammopemmin A (3.102), and 2'-chloropsammopemmin A (3.105) toward eleven 5-HT receptor subtypes was evaluated. Primary screening was conducted in vitro by measuring the percent inhibition of radioligand bound to the receptor in question (% inhibition = 100% - % radioactively bound). Primary assay results are summarized in Table 3.3. Secondary screening was performed in vitro on compounds showing >50% inhibition (highlighted in Table 3.3).

Table 3.3. Primary screening of 3-pyrimidylindoles for 5-HT binding inhibition.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>5-HT(_{1A})</th>
<th>5-HT(_{1B})</th>
<th>5-HT(_{1D})</th>
<th>5-HT(_{1E})</th>
<th>5-HT(_{2A})</th>
<th>5-HT(_{2B})</th>
<th>5-HT(_{2C})</th>
<th>5-HT(_{3})</th>
<th>5-HT(_{5A})</th>
<th>5-HT(_{6})</th>
<th>5-HT(_{7})</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.24</td>
<td>81.3</td>
<td>-0.6</td>
<td>57.1</td>
<td>5.1</td>
<td>11.7</td>
<td>103.7</td>
<td>84.9</td>
<td>12.5</td>
<td>21.1</td>
<td>36</td>
<td>6.9</td>
</tr>
<tr>
<td>3.96</td>
<td>83.7</td>
<td>43.8</td>
<td>76</td>
<td>1.6</td>
<td>8</td>
<td>100.3</td>
<td>91.4</td>
<td>-7.5</td>
<td>79.9</td>
<td>12.7</td>
<td>69.8</td>
</tr>
<tr>
<td>3.102</td>
<td>20.3</td>
<td>41.7</td>
<td>58.2</td>
<td>2.2</td>
<td>-11.4</td>
<td>1.4</td>
<td>-13.3</td>
<td>5.2</td>
<td>0.8</td>
<td>10.8</td>
<td>10</td>
</tr>
<tr>
<td>3.105</td>
<td>10.6</td>
<td>44.1</td>
<td>27.9</td>
<td>-2</td>
<td>4.1</td>
<td>-3.7</td>
<td>-7.2</td>
<td>5.7</td>
<td>8.7</td>
<td>17</td>
<td>12.7</td>
</tr>
</tbody>
</table>

\(^a\)\% inhibition = 100% - % radioactively bound

Meridianin A significantly inhibited binding of the radioligand to 5-HT\(_{1A}\), 5-HT\(_{1D}\), 5-HT\(_{2B}\) and 5-HT\(_{2C}\) in primary screening. To date, secondary screening using radioligand competition binding assays has been carried out on 5-HT\(_{1D}\), 5-HT\(_{2B}\) and 5-HT\(_{2C}\). Secondary screening revealed meridianin A did not inhibit binding of 5-HT\(_{1D}\) and 5-HT\(_{2C}\) with \[^{3}\text{H}\]5-carboximidotryptamine and \[^{3}\text{H}\]mesulergine, respectively. Meridainin A did, however, inhibit the binding of radioligand \[^{3}\text{H}\]lysergic acid diethylamide (\[^{3}\text{H}\]LSD) with 5-HT\(_{2B}\) (Kᵦ = 150 nM, Fig. 3.3).
Primary screening of 4-methoxymeridianin A showed the compound inhibited radioligand binding to 5-HT_{1A}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_{5A}, and 5-HT_{7}. Secondary screening toward 5-HT_{1D} and 5-HT_{2C} showed 4-methoxymeridianin A did not inhibit binding of the radiolabeled compounds. Like meridianin A, the 4-methoxy- analog significantly inhibited binding of radioligand $[^3H]$LSD with 5-HT_{2B} ($K_i = 88$ nM, Fig. 3.4). 4-Methoxymeridianin A also inhibited, to a lesser extent, binding of $[^3H]$LSD with 5-HT_{5A}, and 5-HT_{7} ($K_i = 1525$ nM, 1985 nM; Fig. 3.5 and Fig. 3.6, respectively). Secondary screening showed psammopemmin A did not inhibit binding of $[^3H]$LSD to 5-HT_{1D}. These initial findings show meridianin A is a more selective inhibitor than other compounds tested while methylation of the 4-OH group leads to more potent inhibition of radioligand binding to 5-HT_{2B} as well as broader but less potent activity versus other receptor subtypes.
In addition to 5-HT receptors, the binding effects of 3-pyrimidylindoles on other CNS receptors and transporters were investigated. The dopamine active transporter (DAT) was screened for radioligand ([3H]WIN35428, a synthetic tropane analogous to cocaine) inhibition. Meridianin A was the only compound to display activity in primary and secondary DAT screens ($K_i = 2.35 \mu M$, Figure 3.7). One compound was also found to inhibit radioligand binding ([3H]3-quinuclidinyl benzilate, [3H]QNB) of the muscarinic acetylcholine receptor ($M_5$): 2'-chloropsammopemmin A ($K_i = 7.04 \mu M$, Figure 3.8).

Malaria is a devastating disease affecting disadvantaged populations worldwide (see Chapter 4). Because many current treatments for malaria are losing efficacy due to drug-resistant parasites, new drugs are required to overcome resistance. Toward this end, the potential antimalarial activity and cytotoxicity of meridianin A (3.24), 4-methoxymeridianin A (3.96), synthetic psammopemmin A (3.102), 2'-chloropsammopemmin A (3.105) and meridoquin (3.106) were investigated. Meridianin A, 4-methoxymeridianin A, and meridoquin were active against the malaria parasite Plasmodium falciparum in initial screening. Secondary screening was conducted to determine IC\textsubscript{50} values toward P. falciparum. Meridianin A was the most potent (IC\textsubscript{50} = 12 \mu M) but 4-methoxymeridianin A (IC\textsubscript{50} = 40 \mu M) and meridoquin (IC\textsubscript{50} = 200 \mu M) also displayed some antimalarial activity. Meridianin A was cytotoxic toward A-549 lung cancer cells (IC\textsubscript{50} = 15 \mu M) but, fortuitously, both 4-methoxymeridianin A and meridoquin displayed no toxic effects at the highest concentration examined (IC\textsubscript{50} > 420 \mu M and > 333 \mu M, respectively). Psammopemmin A
and its 2’-chloro- analog showed no activity against *P. falciparum* at the highest concentration tested. The results of this screen are interesting in that 4-OH methylation of meridianin A significantly decreases the cytotoxicity of the compound while retaining antiparasitic activity.

In summary, several meridianin and psammopemmin analogs were synthesized and examined for biological activity (Figure 3.9). Meridianin A inhibited binding of [3H]LSD to 5-HT<sub>2B</sub>, inhibited radioligand binding to DAT, and inhibited growth of *P. falciparum* but was, unfortunately, found to be cytotoxic. 4-Methoxymeridianin A inhibited binding of [3H]LSD with 5-HT<sub>2B</sub>, 5-HT<sub>5A</sub>, and 5-HT<sub>7</sub>, inhibited growth of *P. falciparum*, and displayed no cytotoxic effects. Meridoquin had moderate antimalarial activity and was found to be non-toxic. Psammopemmin A was devoid of activity but its 2’-chloro- analog inhibited radioligand binding of M<sub>5</sub>.

![Figure 3.9. Summary of CNS, antimalarial, and cytotoxic activity of 3-pyrimidylindoles.](image-url)
3.5 References Cited


46. The tunicate was identified as *Synoicum* (family Polyclinidae) by Dr. Linda Cole, Smithsonian Institution, Washington, D.C. A voucher specimen is held at USF (PSC08–17).


58. *K* determinations were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda MD, USA.

59. *IC*<sub>50</sub> determinations toward *Plasmodium falciparum* were provided by Dennis E. Kyle at the Department of Global Health, College of Public Health, Tampa FL, USA.

60. *IC*<sub>50</sub> determinations toward A-549 (human lung cancer) were provided by Alberto van Olphen at the Department of Global Health, College of Public Health, Tampa FL, USA.
Chapter 4. Antimalarial natural products

4.1 The malaria dilemma

Malaria, a devastating infectious disease, was responsible for almost a million deaths and 247 million reported clinical episodes in 2008. Malaria is caused by *Plasmodium* parasites transmitted through infected *Anopheles* mosquitoes. Many *Plasmodium* species exist worldwide and can infect a variety of vertebrates. *P. falciparum* is the most deadly to humans, accounting for the majority of malaria-related deaths. *P. vivax* causes the most morbidity of the human parasites. *P. ovale*, and *P. malariae* are also human pathogens of concern.

The malaria parasite life cycle is complex, involving discreet stages in two hosts. The bite from a malaria infected female *Anopheles* mosquito transmits sporozoites into a human host which infect hepatocytes (liver cells). The sporozoites mature and release merozoites into the blood circulation, invading erythrocytes (red blood cells, RBC). After developing through ring, trophozoite, and schizont stages and asexually multiplying within the RBC’s, mature schizonts again rupture, releasing more merozoites that infect additional RBC’s. Some parasites in the RBC’s develop into male and female gametocytes which are passed to a female *Anopheles* mosquito during blood meal. Sexual reproduction in the mosquito gut forms non-motile zygotes which develop into motile ookinetes that move to the midgut wall. Ookinetes transform into oocysts which rupture releasing sporozoites that traverse to the salivary gland of the mosquito. The parasite life cycle repeats in a new host when the mosquito takes a human blood meal.

Malaria persists in 108 countries and territories, particularly affecting disadvantaged populations in Africa and, to a lesser extent, South East Asia and Latin America. The most at-risk are young children residing in Africa where malaria is responsible for 20% of childhood deaths. Other at-risk
groups include non-immune pregnant women, individuals with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), international travelers, and families returning to endemic areas after prolonged absence. International funding for the treatment, prevention and eradication of malaria increased to $1.7 billion in 2009 from $300 million in 2003 fueling noticeable reductions in the malaria burden. However, current funding falls short of the $5 billion required annually to fully combat the disease globally. Although devastating, malaria is treatable and preventable.

4.2 Current malaria treatment
4.2.1 Quinolines, antifolates, and artemisinins

Europeans learned of the antimalarial properties of South American Cinchona bark (Family: Rubiaceae) in the seventeenth century. The antimalarial constituent of the bark, a quinoline-containing alkaloid named quinine (4.1), is thought to prevent the parasite from polymerizing heme. As the parasite develops in the RBC, it cannibalizes hemoglobin subsequently releasing heme which the parasite converts to hemozoin, a non-toxic crystalline heme polymer. Inhibition of heme polymerization results in toxic levels of heme and death of the parasite. The mechanisms by which the parasite polymerizes heme and quinoline-based drugs inhibit the polymerization process is not fully understood. It is clear, though, that quinoline based therapies have been quite effective in the treatment of malaria evidenced by the success of chloroquine (CQ, 4.2), a synthetic 4-aminoquinoline derivative. CQ was used extensively, beginning in the 1950’s, to cure billions of clinical cases of malaria. Excellent clinical efficacy, economical syntheses, ease of use, and prophylactic properties contributed to the success of CQ and other synthetic quinoline derivatives including amodiaquine (4.3) and mefloquine (MQ, 4.4). The “wonder drug” was overused, however, leading to the emergence of chloroquine-resistant parasites. Other drugs targeting different metabolic processes in the parasite were developed to combat resistant strains.
Drugs that target folate metabolism eradicate *Plasmodium* parasites by inhibiting the parasite’s ability to produce folic acid and thus its ability to biosynthesize pyrimidine, purine, and amino acids. A combination of the antifolate pyrimidine-containing drugs sulfadoxine (4.5) and pyrimethamine (4.6) was found to be efficacious in treating uncomplicated CQ-resistant malaria. While both compounds show modest antiparasitic properties individually, a synergistic effect is observed when combination therapy is administered. Unfortunately, the switch from CQ to sulfadoxine/pyrimethamine (SP) to treat CQ-resistant strains, while initially effective, quickly led to the appearance of SP resistant parasites.

Artemisinin (4.7), dubbed qinghaosu by the Chinese, is the powerful antimalarial component of the Chinese medicinal herb *Artemisia annua*. Artemisinin, a unique sequiterpene 1,2,4-trioxane lactone, rapidly clears all blood stages of *Plasmodium* parasitemia through a highly-contested
mechanism of action which could involve the peroxide moiety forming radical or ionic intermediates. Poor solubility of the natural product led to semisynthetic derivatives including more lipophilic artemether (4.8) and more hydrophilic artesunate (4.9). In an intriguing effort to utilize microbes as an inexpensive producer of artemisinin, the yeast *Saccharomyces cerevisiae* was genetically modified, using genes from *A. annua*, to produce the artemisinin precursor artemisinic acid (4.10). The precursor can be easily converted to artemisinin and derivatives using through economical, well-studied synthetic procedures. Although artemisinin and its derivatives are effective single-drug treatments, artemisinin combination therapy (ACT) is recommended by the World Health Organization (WHO) to delay development of parasite drug resistance. ACT has been reported as 95% effective in curing malaria. Most malaria-endemic countries now use ACT as a first-line treatment for uncomplicated *P. falciparum* infection. Unfortunately, parasites displaying artemisinin resistance have been recently documented on the Cambodia-Thailand border.

![Chemical structures](image)

**4.7: artemisinin**  **4.8: artemether**  **4.9: artesunate**  **4.10: artemisinic acid**

### 4.2.2 Drug resistant parasites

Widespread drug resistant malaria is of enormous concern. Parasites resistant to quinoline-based drugs and sulfadoxine/pyrimethamine combination therapy have been extensively observed. The more recent emergence of artemisinin combination therapy resistance underscores an urgent need for the development of a new class of compounds. Success of future antimalarial drug discovery depends on the ability to identify parasites displaying drug resistance and subsequently screen diverse chemotypes for the ability to inhibit drug resistant parasitemia. Gratifyingly, worldwide awareness of the malaria burden has led to collaborations between academic institutions, non-profit organizations, and the pharmaceutical industry.
the support of international funding from public and private sources, these collaborators are working to combat the global malaria burden.

4.3 Natural products as antimalarial leads

Recent screening of vast synthetic chemical libraries provided by Glaxo Smith-Kline has afforded hundreds of potential antimalarial leads.\textsuperscript{18,19} Particularly exciting was the identification of nineteen new inhibitors of four validated drug targets as well as fifteen novel malarial protein binders. Researchers hunting for new antimalarial chemotypes commonly depend on synthetic combinatorial libraries to generate large numbers of compounds, which supply high throughput screening (HTS) operations.\textsuperscript{20} Synthetic chemical libraries, however, simply cannot match the diversity and vast "chemical space" found in nature. One just needs to look in the right place! Given that the most widely used and most efficacious malaria therapies were derived from natural products, further chemical investigation of natural sources for antimalarial constituents is fully warranted.

Plants have proven to be an excellent source of antimalarial alkaloids and terpenes.\textsuperscript{21} Flora used in traditional medicine to treat malaria-like symptoms, like \emph{Cinchona} and \emph{Artemisia}, are particularly well studied. Less studied but no less promising sources for potential antimalarial chemistry are microorganisms and marine macroorganisms. A number of antimalarial agents have been recently identified from marine macroorganisms.\textsuperscript{22} Crambescidin 800 (4.11), previously isolated from the Mediterranean red encrusting sponge \emph{Crambe crambe},\textsuperscript{23} displayed nanomolar activity against CQ-resistant \emph{P. falciparum} FCR3 in-vitro.\textsuperscript{24} Crambescidins bear a complex molecular structure comprised of a pentacyclic guanidine linked by a linear \(\omega\)-hydroxy fatty acid to a hydroxyspermidine moiety. Several crambescidin analogs have been prepared that show even more promising antimalarial activity.
Manzamine A (4.12), initially isolated from an Okinawan sponge *Haliclona* sp., is an antimalarial β-carboline alkaloid containing a complicated array of 5-, 6-, 8-, and 13-membered rings. More than sixty related compounds have been isolated from various sponge species. A single intraperitoneal injection of manzamine A was found to clear parasitemia in mice infected with *P. berghei*. Natural manzamines display toxicity but the semisynthetic derivative, peracylated 8-hydroxymanzamine A (4.13), has no cytotoxicity at the highest dose tested while retaining antiparasitic properties. Manzamines are purported to have been isolated from a symbiotic bacterium suggesting the molecules produced in the sponge are of microbial origin. If large scale fermentation of this microbe is feasible, ample quantities of manzamines would be available for further experimentation.

Bacteria and fungi produce a wide range of biologically active compounds that possess great diversity in chemical structure. Screening microbial extracts has resulted in the discovery of many compounds possessing antimalarial activity. Several microbe-derived natural products
display potent and promising antiparasitic activity. Nostocarboline (4.14), isolated from the cyanobacterium Nostoc sp., and synthetic dimers thereof, inhibited P. falciparum in nanomolar concentrations.32 The compounds are only weakly cytotoxic, the most encouraging possessing a selectivity index (SI) of >2,500. The screening of eighty crude marine-derived bacterial extracts led to the identification of salinosporamide A (4.15 see also section 1.3) as a nanomolar in vitro inhibitor of human Plasmodium parasites.33 The compound also significantly reduced P. yoelii parasitemia in mice. Isolated from the yeast Candida lipolytica,34 the indoloquinazoline tryptanthrin (4.16) along with several synthetic analogs displayed potent in vitro activity against CQ- and MQ-resistant Plasmodium strains.35

Microbes are an ideal source for novel antimalarial compounds. Not only do microbes produce profound chemical diversity, they are also amendable to large-scale fermentation resulting in facile access to compounds with complicated structures. Complex chemicals are undesirable mostly because the malaria burden in underdeveloped nations requires inexpensive treatment. However, an abundantly producing microbe could yield complex natural products very cost-effectively. Our lab, with international collaboration, has examined a multitude of microorganisms for antimalarial constituents with the goal of identifying lead compounds possessing potent activity against Plasmodium falciparum.

4.4 Medicines for Malaria Venture project
4.4.1 MMV project overview

With funding from Medicines for Malaria Venture (MMV),36 we endeavored to examine niche environment microbiota for new antimalarial chemotypes. The planned project would seek to
analyze ~70,000 microbes from niche environments worldwide including our own microbial libraries at the University of South Florida (USF) as well as the libraries of our academic and commercial collaborators. Our lab supplied 1,000+ Antarctic marine, Florida marine, and Florida mangrove microbial endophytes. Bacterial and fungal endophytes were isolated from samples collected on various expeditions to McMurdo Station and Palmer Station, Antarctica as well as collecting trips to several locations in Florida waters, including the Florida Keys, the Gulf of Mexico, and the Florida Everglades. Eubacteria (~5,000) isolated from Caribbean marine cave-dwelling invertebrates were supplied by Magellan BioScience (Todd Daviau and John Cronin). City University of Hong Kong (CY, Lilian Vrijmoed) and National Taiwan Ocean University (NTOU, Ka-Lai Pang) provided 5,000+ isolates of mangrove endophytes from the coastal region of China. The majority (~55,000) of the microbes analyzed were filamentous fungi isolated from various sources, largely plant in origin, collected around the world and housed at Mycosynthetix (MSX, Cedric Pearce). Biological activity screening (section 4.4.2.1) was performed at USF, College of Public Health (Dennis Kyle). Processes involving preparation of extracts, fractionation of active samples, as well as analyzing and identifying active components were carried out in our lab using the procedures described in section 4.4.2.2.

4.4.2 MMV project methodology

4.4.2.1 Antimalaria and cytotoxicity assays, prioritization of active extracts

Primary screening employed a novel luciferase reporter assay that utilizes transgenic blood-stage parasites to rapidly identify antimalarial extracts from a 96-well plate format. The in vitro drug luminescence (ITDL) assay\textsuperscript{37} uses Plasmodium falciparum (3D7), transfected with a luciferase construct, allowing for an assay with signal to noise ratio better than many currently in use (Sybr Green, DAPI, Pico Green). The assay was designed to reveal activity at “high” concentrations (50 \( \mu \text{g} \) crude extract/mL) and “low” concentrations (5 \( \mu \text{g} \) crude extract/mL). Extracts that displayed >67% parasite growth inhibition at both high and low concentrations were designated “active.” Extracts that showed >67% inhibition at high concentration but <67% inhibition at low concentration were labeled “partially active.”
In vitro cytotoxicity of the active extracts toward A-549 human cancer cells allowed for further prioritization of antimalarial extracts. Cytotoxicity was measured at the same “high” and “low” concentrations previously described. Both high and low concentrations were divided into three categories: cytotoxic (<75% cell viability), moderately cytotoxic (75-95% cell viability), and non-toxic (>95% cell viability). Those extracts that were found “active” in the antimalarial screen and “non-toxic” in the cytotoxicity screen were given the highest priority. Current hit rates are summarized in Table 4.1.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Extracts screened</th>
<th>Active extracts (% of screened)</th>
<th>Partially active extracts (% of screened)</th>
<th>Non-toxic active extracts (% of actives)</th>
<th>Non-toxic partially active extracts (% of p.a.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSX</td>
<td>24,780a</td>
<td>278 (0.9%)</td>
<td>2214 (9%)</td>
<td>108 (39%)</td>
<td>872 (39%)</td>
</tr>
<tr>
<td>CY/NTOU</td>
<td>5192</td>
<td>35 (0.7%)</td>
<td>269 (5%)</td>
<td>10 (29%)</td>
<td>186 (36%)</td>
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<tr>
<td>USF</td>
<td>1056</td>
<td>3 (0.3%)</td>
<td>40 (4%)</td>
<td>2 (67%)</td>
<td>14 (35%)</td>
</tr>
</tbody>
</table>

*Includes only extracts for which both antimalarial and cytotoxicity data was available.

4.4.2.2 Antarctic microbe cultivation, extraction, and processing

Tissue from sessile marine invertebrates (i.e. sponges, tunicates, soft corals, etc.) collected in the waters surrounding McMurdo Station and Palmer Station, Antarctica were preserved in glycerol solution and kept at -80 °C during shipment from Antarctica to USF. Using sterile technique, the warmed tissue (5 °C) was sectioned into fractions and immediately plated on a Difco marine agar dish which was then sealed with parafilm. The microbes were allowed to grow at 5 °C for about two weeks. Sterile loops were used to isolate various microbes growing from the tissue. The agar plates streaked with the new isolates were allowed to grow for an additional two weeks. This process was repeated until a pure isolate was obtained. Each isolate was archived in glycerol stock and kept in our microbe library at -80 °C.

The isolates were cultured on a small scale to obtain enough biomass for biological analyses. To limit error and speed processing time, all procedures were conducted in a 96-well format. A
custom built media shaker rack containing 96 “wells” of 160 mL capacity each in a 12 column X 8 row grid was used to culture 88 isolates concurrently (the last column was reserved for controls). Difco marine broth (100 mL) was added to each well. After sterilization, each isolate, either directly from the isolation plate or from regrown stock, was transferred via sterile loop to a designated well in the shaker rack. The culture media was allowed to ferment under aeration at 5 °C for three weeks. Acrylic ester resin (XAD-7, 3 g) was then added to each well to collect small non-polar and semi-polar organic metabolites. Cells were lysed with three successive freeze-thaw cycles. The resin was then filtered along with any cell mass and washed with water. The filtrate was sterilized and discarded. The resin and cell mass were then extracted with methanol (10 mL) for 24 h and removed via filtration from the methanolic extract. Concentration of the methanolic extract yielded a crude extract for each of the 88 samples cultured. The extract was redissolved in dimethylsulfoxide (DMSO) at 30 mg/mL. A portion of this solution (150 μL) was transferred to a 96 well plate and submitted for antimalarial screening. The remaining DMSO solution was transferred to a deep well plate and kept frozen for further testing.

4.4.2.3 Fungi extraction and processing

Lyophilized fungal cultures were received from City University of Hong Kong (CY) and National Taiwan Ocean University (NTOU) in 30 mL falcon tubes. Each culture was extracted with methanol (~15 mL) for at least 24 h. The methanolic extract was carefully transferred to a clean 20 mL scintillation vial which was placed in a compartmentalized tray keeping the extractions in a 96-well format (88 per tray). Concentration of the methanol extracts under a stream of air afforded the dried extract. Mycosynthetix (MSX) samples were received as solid culture material (~5 mL) in 20 mL scintillation vials. After each sample was extracted with methanol (24 h, 15 mL), the methanolic extract was subjected to the same concentration protocol used for the CY and NTOU samples. All dried extracts were processed and submitted (30 mg/ mL, DMSO) in 96-well plates analogous to the procedure developed for the Antarctic microbial extracts.
4.4.2.4 Scale up fermentation and fractionation
Antarctic microbe extracts found active in the antimalarial assay were regrown in 6X1 L cultures (Difco marine broth). After 3 weeks of aerated fermentation, XAD-7 (30 g/L) resin was added to extract the small organic metabolites from the media. Cells were then lysed in three successive freeze-thaw cycles. The resin and cell mass was separated from the broth by filtration, rinsed with water, and extracted with methanol. The methanolic extract was concentrated \textit{in vacuo} to afford the crude scale up extract. Scaled up fermentations (100X-1000X) were received from CY, NTOU, and MSX and extracted exhaustively with methanol. The methanolic extracts were concentrated to afford crude scale up extracts.

Fractionation of the scale up extracts was achieved with normal phase silica gel medium pressure liquid chromatography (MPLC). A portion (400 mg or 2 g) of the extract was fractionated through a silica gel cartridge (4g and 40g, respectively) with a gradient of hexane→ethyl acetate→methanol generating about ten fractions of increasing polarity. After concentration, a portion of each fraction was redissolved in DMSO (2 mg/ mL) and submitted for biological evaluation.

4.4.2.5 Active fractions to pure compounds
Fractions that remained active in the antimalarial assay were further investigated via nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS). Proton (\( ^1 \text{H} \)) NMR spectra were obtained for each active fraction. Those fractions with interesting spectra (i.e. those with downfield signals indicative of heteroatoms and, consequently, functional groups) were further analyzed by thin layer chromatography (TLC) and finally purified with high performance liquid chromatography (HPLC). Pure compounds were identified by obtaining physical data (\( ^1 \text{H} \) and \( ^{13} \text{C} \) NMR, MS) and searching databases such as AntiMarin for compounds with similar or identical data. Alternatively, partial structures could be elucidated via various 2-D NMR techniques [Correlation spectroscopy (COSY), Heteronuclear Single Quantum Coherence...
(HSQC, Heteronuclear Multiple Bond Correlation (HMBC), etc.) which would be used to narrow down potential structures.

4.4.2.6 Protocol validation
To validate our sample preparation procedures as well as the antimalarial assay, a crude *Artemisia annua* extract was subjected to our fractionation protocol. The concentrated fractions were then added to a plate containing MSX fractions and submitted for biological analysis. The *Artemisia* extract fractions were analyzed in an identical manner alongside the fungal fractions. Bioassay results indicated one active *Artemisia* fraction which, upon NMR and LC-MS analyses, was revealed to contain artemisinin.

4.4.3 Antimalarial compounds from endophytic mangrove fungi
Preliminary results led to isolation of several fungi-derived antimalarial natural products. Extracts from endophytic fungi isolated from mangroves inhabiting coastal China were the first to be analyzed. Scale up fermentation and extraction followed by fractionation of several fungal samples afforded two known and, unfortunately, cytotoxic and cytostatic classes of metabolites, cytochalasins and trichothecenes.

4.4.3.1 Cytochalasins
Scale up fermentation of CY-4202 was found to contain cytochalasin D (cytD, 4.17), a polyketide mycotoxin originally isolated from cultures of *Metarrhizium anisopliae* and *Hypoxylon terreina*. Cytochalasin D is a reversible potent inhibitor of actin polymerization and thus is used to probe the role of actin in various cell processes. Physical data (Table 4.2. \(^1\)H and \(^{13}\)C NMR data) of cytD isolated from CY-4202 was identical to that previously reported. An LC-MS method was developed to quickly screen fractions for the presence of cytD leading to the identification in another cytD-producing coastal China mangrove endopyte, CY-4204, the presence of which was further confirmed with \(^1\)H NMR data. Several other CY and MSX samples
appear to contain cytochalasins as their $^1$H NMR spectra are similar to cytD but LC-MS traces differ from cytD.

Cytochalasin D was previously observed to affect development of *P. berghei* but have had no inhibitory effect on *P. yoelli*. We found that many of the cytD containing fractions inhibit *P. falciparum* (in vitro) perhaps enhanced by synergistic effects from other compounds in the mixture. Pure cytD also displayed *in vitro* inhibition of *P. falciparum* with activity at nanomolar concentrations ($IC_{50} = 26$ nM). However, because the compound inhibits actin polymerization resulting in cytostasis, cytD is a poor candidate for malaria therapy.

4.17: cytochalasin D
### Table 4.2. \(^1\)H and \(^{13}\)C NMR shift\(^a\) comparison of isolated cytochalasin D (4.17) with literature values.

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<th>ref. 42 (\delta_C)</th>
<th>4.17 (\delta_C)</th>
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<td>2.15, t</td>
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\(^a\)\(\delta\) in ppm in CDCl\(_3\).

#### 4.4.3.2 Trichothecenes

Investigation of scale up fermentation CY-3923 revealed two known trichotheccene mycotoxins that were in vitro inhibitors of the malaria parasite. Trichothecenes are tetracyclic sesquiterpenes usually containing an epoxide ring. We found that roridin E (4.18) potently inhibited \(P. falciparum\) (IC\(_{50}\) < 190 nM) while its deoxy-analog, 12,13-deoxyroridin E (4.19), displayed less, but still significant, inhibition (IC\(_{50}\) = 765 nM). The compounds were cytotoxic, however, in our initial toxicity screen at the lowest dose examined (5 \(\mu\)g/mL). The structures of 4.18 and 4.19 were
confirmed upon comparison of $^1$H and $^{13}$C data with literature values (4.18: Table 4.3, 4.19: Table 4.4).  

The antimalarial properties of roridin E and related trichothecenes have been previously reported.  Roridin E showed extremely potent activity against *P. falciparum* (K1, EC$_{50}$ = 0.3 nM) but, unfortunately, similar cytotoxicity (KB, BC1, vero cells: EC$_{50}$ = 0.8 to 1.4 nM). Interestingly, roridin E acetate (4.20) more potently inhibited *P. falciparum* (K1, EC$_{50}$ = 0.1 nM) while possessing ten- to twenty-fold less cytotoxicity (KB, BC1, vero cells: EC$_{50}$ = 8.0 to 28.0 nM) than roridin E. This simple addition of an acetate moiety had a profound effect on activity. Although most tricothecenes are cytostatic or cytotoxic, it is possible that a derivative will be uncovered or semisynthetically derived that possesses the excellent antiparasitic properties currently observed as well as low or no cytotoxicity.
Table 4.3. $^1$H and $^{13}$C NMR shift$^a$ comparison of isolated roridin E (4.18) with literature values.

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$^a$$\delta$ in ppm in CDCl$_3$. 
**Table 4.4.** $^1$H and $^{13}$C NMR shift<sup>a</sup> comparison of isolated 12,13-deoxyrordin E (4.19) with literature values.

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<sup>a</sup>δ in ppm in CDCl<sub>3</sub>.

### 4.4.4 MMV project outlook

With preliminary extract screening nearing completion<sup>48</sup> and fractionation of scale up fermentation extracts well underway, we expect to discover many more antimalarial constituents in our 70,000-strong microbial extract library. The identification of artemisinin combined with the isolation of cytochalsin D and roridin E contributes to the validity of both the isolation procedure as well as the antimalarial assay. Cytotoxicity data for those extracts displaying activity in the malaria assay should steer us away from active but toxic extracts resembling the ones discussed herein. Further development of LC-MS techniques to identify common toxic compounds like
cytochalasins and roridins would speed up identification of compounds we do not want. The time saved would allow us to analyze a greater quantity of promising active extracts increasing the chance of drug lead discovery. A microbe-derived lead compound would then be investigated synthetically to identify the pharmacophore, as well as to tailor bioactivity and physical properties, analogous to operations that led to the development of clinically successful quinoline and artemisinin derivatives.

4.5 References Cited

36. Medicines for Malaria Ventures grant: MMV08/0105.


38. *Artemisia annua* extract was generously provided by Jim McChesney, Chromadex, Inc.


48. We are indebted to numerous researchers (>50!) who contributed to processing the seemingly never ending multitude of samples in both the labs of Bill Baker and Dennis Kyle.
Chapter 5. Experimental

5.1 General Procedures

Unless otherwise stated, all experiments were performed under inert atmosphere (nitrogen or argon) in oven or flame-dried glassware equipped with a magnetic stir bar and a rubber septum. All solvents used were reagent grade. Anhydrous dichloromethane (DCM) was obtained by distillation from calcium hydride (CaH). Anhydrous tetrahydrofuran (THF) was obtained by distillation from sodium/benzophenone. Dry methanol (MeOH) was obtained by distillation from magnesium and iodine (Mg, I₂). All other chemicals were purchased from Sigma-Aldrich and were used as received. Low-temperature baths of -78 °C, -60 °C, and -40 °C were obtained with an immersion cooler bath using acetone, chloroform, or acetonitrile, respectively, with dry ice (CO₂). Thin layer chromatography (TLC) was carried out using Whatman normal phase Silica gel 60Å Partisil®. TLC plates were visualized with 5% phosphomolybdic acid in ethanol (EtOH) and heating and/or UV (254 nm). Products were chromatographed on a Teledyne Isco CombiFlash Companion medium pressure liquid chromatography (MPLC) instrument using normal phase silica gel cartridges purchased from Teledyne Isco. Melting points were recorded on an Electrothermal Mel-Temp 3.0 instrument. Specific rotations were measured on an Autopol IV automatic polarimeter using Na lamp corrected to 20°C. IR spectra were recorded on a Nicolet Avatar 320 spectrometer with a Smart Miracle accessory. Low-resolution mass spectrometry (LRMS) data were recorded on an Agilent LC/MSD VL electrospray ionization mass spectrometer. High resolution mass spectrometry (HRMS) data were obtained on an Agilent LC/MSD TOF electrospray ionization mass spectrometer. ¹H and ¹³C NMR spectra were recorded on a Varian 400 MHz or 500 MHz instrument using residual protonated solvent as ¹H internal standard or ¹³C absorption lines of solvents for ¹³C internal standard. NMR data were obtained in CDCl₃ (Sigma-Aldrich), CD₃OD, or d₆-DMSO (both from Cambridge Isotope Labs).
5.2 Experimental supporting Chapter 2

5.2.1 Ozonolysis of palmerolide A forming hexane-1,2,3-triol (2.51) and hexane-1,2,3,6-tetraol (2.52)

To a solution of palmerolide A (2.1, 8.5 mg, 0.0145 mmol, 1 eq) dissolved in dry methanol, ozone was bubbled through at -78°C under stirring. A solution of methanol (1.5 mL) and sodium borohydride (10 mg, 0.264 mmol, 18.2 eq.) was added to the solution. The mixture was warmed to room temperature (rt) and stirred for 25 min. After solvent removal under reduced pressure, the resulting residue was chromatographed by silica gel MPLC to yield fragment 2.51 (eluting at 15% MeOH in chloroform, ~2 mg, 0.0149 mmol, quantitative) and fragment 2.52 (eluting at 20% MeOH in chloroform, ~2.5 mg, 0.0165 mmol, quantitative) as colorless oils.

\[
\begin{align*}
2.1 & \rightarrow \text{HO-} \quad \text{OH} \quad + \quad \text{HO-} \quad \text{OH} \\
2.51 & \quad 2.52 
\end{align*}
\]

\[
\begin{align*}
\text{2.51: } [\alpha]_D^{20} & \ -9.0 \ (c 0.100, \text{MeOH}) \; ; \; ^1\text{H NMR (500 MHz, CD}_3\text{OD}} \ \delta \ \text{(multiplicity, integration)}: \ 1.41 \ (m, 2H) ; \ 1.56 \ (m, 4H) ; \ 3.47 \ (m, 2H) ; \ 3.58 \ (m, 2H) ; \ 3.60 \ (m) ; ^{13}\text{C NMR (125 MHz, CD}_3\text{OD}} \ \delta \ 23.1, \ 33.8, \ 34.3, \ 63.0, \ 67.5, \ 73.3 ; \ ESI-MS (m/z) [M+H] = 135.1. \\
\text{2.52: } [\alpha]_D^{20} & \ -8.1 \ (c 0.200, \text{MeOH}) \; ; \; ^1\text{H NMR (500 MHz, CD}_3\text{OD}} \ \delta \ \text{(multiplicity, integration)}: \ 1.62 \ (m, 4H) ; \ 3.53 \ (m, 4H) ; \ 3.64 \ (m, 2H) ; ^{13}\text{C NMR (125 MHz, CD}_3\text{OD}} \ \delta : \ 30.3, \ 30.9, \ 63.2, \ 64.7, \ 72.7, \ 75.8 ; \ ESI-MS (m/z) [M+H] = 151.1.
\end{align*}
\]

5.2.2 Synthesis hexane-1,2,6-triol (2.53)

\[
\begin{align*}
\text{R-2.53} & \quad \text{OH-} \quad \text{OH} \\
\text{S-2.53} & \quad \text{HO-} \quad \text{OH} 
\end{align*}
\]

\[
\begin{align*}
\text{R-2.53} \ (19 \ mg, \ 0.109 \ mmol) \ & \text{was dissolved in 50% acetic acid:H}_2\text{O} \ (3 \ mL). \ \text{The solution stirred for 20 min. at rt after which the aqueous acid was evaporated under a stream of air. The resulting residue was chromatographed by silica gel MPLC (eluting at 15% MeOH in chloroform) to yield R-2.53 as a viscous colorless oil (13.6 mg, 0.101 mmol, 93%). A similar procedure applied to 40}
\end{align*}
\]
mg (0.230 mmol) of S-2.58 yielded (27.5 mg, 0.205 mmol, 89%) of (S)-hexane-1,2,6-triol (S-2.53). R-2.53: [α]_D^{20} +11.1 (c 0.100, MeOH); S-2.53: [α]_D^{20} -11.6 (c 0.100, MeOH); ¹H NMR (500 MHz, CD₃OD) δ 1.41 (m, 2H); 1.56 (m, 4H), 3.47 (m, 2H), 3.58 (m, 2H), 3.60 (m, 1H); ¹³C NMR (125 MHz, CD₃OD) δ 23.1, 33.8, 34.3, 63.0, 67.5, 73.3; ESI-MS (m/z) [M+H] = 135.1.

5.2.3 Synthesis of (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (2.54)

A stream of ozone was bubbled through a stirring solution of 2.55 (24 mg, 0.112 mmol) in dry methanol (3 mL) at -78 °C. A solution of methanol (1.5 mL) and sodium borohydride (20 mg, 0.518 mmol) was added to the solution. The mixture was warmed to rt and stirred for 25 min. After solvent removal under reduced pressure, the resulting residue was chromatographed by silica gel MPLC to yield 2.54 (eluting at 38% ethylacetate (EtOAc):Hexane, 6.5 mg, 0.492 mmol, 44% yield, 58% ee). (R)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol purchased from Sigma-Aldrich: [α]_D^{20} -13.7, (c=0.1, MeOH); 2.54: [α]_D^{20} -7.0, (c=0.1, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 1.39 (s, 3H), 1.46 (s, 3H), 3.60 (m), 3.75 (m), 3.81 (m), 4.05 (m), 4.25 (m); ¹³C NMR (100 MHz, CDCl₃) δ 25.3, 26.7, 63.0, 65.7, 76.1, 109.4; ESI-MS (m/z) [M+H] = 133.1.

5.2.4 Synthesis of (R)-4-(3-(1,3-dioxolan-2-yl)prop-1-enyl)-2,2-dimethyl-1,3-dioxolane (2.55)

To 20 mL dry DCM at 60 °C was added oxalyl chloride (540 mg, 4.16 mmol, 0.36 mL). After stirring for 5 min, DMSO (592 mg, 7.57 mmol, 0.54 mL) was added. After stirring 2 min, R-2.56 (500 mg, 3.78 mmol) dissolved in 3 mL dry DCM was added over a 5 min period. After stirring for
an additional 10 min at 60 °C, triethylamine (TEA, 1.91 g, 18.9 mmol, 2.7 mL) was added. The mixture was then warmed to rt and partitioned between EtOAc and water. The organic layer was collected. The aqueous layer was washed 2X with aliquots of EtOAc. The organic layers were combined, dried over anhydrous magnesium sulfate (anhyd. MgSO₄), and concentrated to afford (S)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (285 mg, 2.19 mmol, 58%).

To a solution of 2-(1,3-Dioxolan-2-yl)ethyltriphenylphosphonium bromide (26 mg, 0.15 mmol, dried with heat under vacuum) in dry THF (7 mL) at -78 °C was added n-BuLi dropwise until the solution turned a dark yellow (1.4 M, 0.22 mL, 0.30 mmol). After stirring for 15 min., (S)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (dissolved in 1.5 mL dry THF) was added to the stirring solution. The solution stirred at -78 °C for 10 additional minutes then was allowed to warm to rt. The mixture was partitioned between diethyl ether (Et₂O) and water. The aqueous layer was washed 2X with aliquots of Et₂O. The combined organic layers were dried over anhyd. MgSO₄, concentrated and chromatographed on silica gel MPLC affording 2.55 as a mixture of E/Z isomers as a colorless oil (eluting at 15% EtOAc:hexanes, 24 mg, 0.11 mmol, 75%).

5.2.5 Syntheses of 2-(2,2-dimethyl-1,3-dioxolan-4-yl)ethanol (2.56)

To a stirring solution of R-2.57 (47 mg, 0.219 mmol) in dry MeOH (5 mL) was added ozone at -78 °C. Sodium borohydride (40 mg, 1.06 mmol), dissolved in 1 mL MeOH was added to the solution. The mixture was warmed to rt and stirred for 25 min. After solvent removal under reduced pressure, the resulting residue was chromatographed by silica gel MPLC (eluting at 45% EtOAc in hexanes) to yield R-2.56 as a colorless oil (17.5 mg, 0.120 mmol, 55%). A similar procedure applied to 55 mg of S-2.57 (0.258 mmol) yielded 22.6 mg (0.155 mmol, 60%) of S-2.56. [α]D²⁰R-2.56: -2.4°, 89% ee; S-2.56: +2.4°, 97% ee (c 0.100, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ
(multiplicity, $J$ (Hz), integration): 1.36 (s, 3H), 1.42 (s, 3H), 1.82 (dt, 5.8, 5.4, 2H), 2.31 (bbrs), 3.59 (dd, 7.8, 7.4), 3.80 (t, 5.4, 2H), 4.09 (dd, 8.1, 6.0), 4.27 (m); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 25.7, 26.9, 35.6, 60.6, 69.4, 75.1, 109.1; ESI-MS (m/z) [M+H] = 147.1.

5.2.6 Synthesis of (E)-ethyl 4-(2,2-dimethyl-1,3-dioxolan-4-yl)but-2-enoate (2.57)

To 100 mL dry DCM at 60 °C was added oxalyl chloride (866 mg, 6.82 mmol, 0.6 mL). After stirring for 5 min, DMSO (668 mg, 8.55 mmol, 0.6 mL) was added. After stirring 2 min, $R$-2.56 (500 mg, 3.42 mmol) dissolved in 3 mL dry DCM was added over a 5 min period. After stirring for an additional 10 min at 60 °C, TEA (2.08 g, 20.5 mmol, 2.9 mL) was added. The mixture was then warmed to rt and partitioned between EtOAc and water. The organic layer was collected. The aqueous layer was washed 2X with aliquots of EtOAc. The organic layers were combined, dried over anhydrous MgSO$_4$, and concentrated to afford the aldehyde (R)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)acetaldehyde (458 mg, 3.17 mmol, 93%) as a colorless oil. A similar procedure was applied to S-2.56 (500 mg, 3.42 mmol) resulting in (S)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)acetaldehyde (480 mg, 3.38 mmol, 99%).

5.2.6.1 Synthesis of $R$-2.57

To a mixture of (ethoxycarbonylmethyl)triphenylphosphonium bromide (4.855 g, 11.31 mmol) in dry DCM (100 mL) was added triethylamine (TEA, 1.716 g, 2.354 mL, 16.96 mmol) at rt. After mixture stirred under nitrogen for 10 min, (R)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)acetaldehyde (815 mg, 5.65 mmol) dissolved in 2.5 mL dry DCM was added. The mixture stirred for 17 h at rt then was partitioned between 250 mL diethyl ether: 250 mL H$_2$O. The organic layer was collected. The aqueous layer was extracted with 250 mL diethyl ether. The organic layers were combined and dried over anhydrous MgSO$_4$. After solvent removal under reduced pressure, the resulting
Residue was chromatographed by silica gel MPLC (eluting at 20-25% EtOAc in hexanes) to yield R-2.57 as a colorless oil (544 mg, 2.54 mmol, 45%).

5.2.6.2 Synthesis of S-2.57
To a slurry of sodium hydride (NaH, 196 mg 60% in mineral oil, 4.91 mmol) in dry THF (12 mL) at 0 °C and inert atmosphere was added triethyl phosphonoacetate (1.1 g, 4.91 mmol, 0.98 mL). After stirring 10 min., (S)-2-(2,2-dimethyl-1,3-dioxolan-4-yl)acetaldehyde (544 mg, 3.77 mmol) dissolved in 2.5 mL dry THF was added. The mixture stirred an additional 10 min. and was partitioned between EtOAc:H2O. The aqueous layer was washed 2X with aliquots of EtOAc. The combined organic layers were dried over anhyd. MgSO4, concentrated and purified via MPLC (silica, elution at 25% EtOAc:hexane) to afford 573 mg of S-2.57 (2.68 mmol, 71%).

5.2.6.3 Physical data common to R-2.57 and S-2.57
1H NMR (400 MHz, CDCl3) δ (multiplicity, J (Hz), integration) 1.29 (t, 7.1, 3H), 1.36 (s, 3H), 2.48 (m, 2H), 3.60 (dd, 8.0, 6.5), 4.06 (dd, 8.0, 6.2), 4.19 (q, 7.1, 2H), 4.20 (m), 5.91 (dt, 15.6, 1.4), 6.93 (dt, 15.6, 7.1); 13C NMR (100 MHz, CDCl3) δ 14.2, 25.5, 26.8, 36.4, 60.3, 68.8, 74.2, 109.3, 123.9, 143.7, 166.2; ESI-MS (m/z) [M+H] = 215.1.

5.2.7 Synthesis of 4-(2,2-dimethyl-1,3-dioxolan-4-yl)butan-1-ol (2.58)

To a mixture of R-2.57 (66 mg, 0.308 mmol) in anhydrous THF (7 mL) was added LiAlH4 (35 mg, 0.924 mmol) under an atmosphere of nitrogen. The mixture stirred at rt for 2 h. Potassium hydroxide solution (0.1 mL, 1 M) was carefully added to quench the reaction. After stirring for 10 min., the mixture was partitioned between DCM:H2O. The organic layer was collected. The aqueous layer was extracted 2X with DCM. The organic layers were combined and dried over
anhydrous MgSO₄. After solvent removal under reduced pressure, the resulting residue was chromatographed by silica gel MPLC (eluting at 38-42% EtOAc in hexanes) to yield \( \text{R-2.58} \) as a colorless oil (24.1 mg, 0.138 mmol, 45%). A similar procedure applied to 465 mg (2.17 mmol) of \( \text{S-2.57} \) yielded 140 mg (0.803 mmol, 37%) of \( \text{S-2.58} \). ¹H NMR (400 MHz, CDCl₃) \( \delta \) (multiplicity, integration): 1.36 (s, 3H), 1.42 (s, 3H), 1.49 (m, 2H), 1.53 (m, 2H), 1.62 (m, 2H), 3.52 (t, 7.3), 3.67 (t, 6.4, 2H), 4.06 (m), 4.12 (m); ¹³C NMR (100 MHz, CDCl₃) \( \delta \) 22.0, 25.7, 26.9, 32.6, 33.2, 62.7, 69.4, 76.0, 108.7; ESI-MS (m/z) [M+H] = 175.1

5.2.8 Synthesis of hexane-1,2,3,6-tetraol (2.59)

5.2.8.1 Synthesis of \((2R,3R)\)-hexane-1,2,3,6-tetraol \((R,R-2.59)\)

To a flask containing activated 10% Pd/C (10 mg) and a stir bar was added 2.61 (40 mg, 0.147 mmol) dissolved in 5 mL EtOH. A balloon containing H₂ gas was affixed to the flask. The mixture stirred for 4 h at rt then was filtered through Ce-Lite. The filtrate was concentrated to afford the intermediate saturated acetal (38 mg, 0.138 mmol, 94%). The intermediate saturated acetal (37 mg, 0.135 mmol) was stirred in a solution of 50% acetic acid in water for 2h at 60°C. The solvent was then evaporated under a stream of air to yield the diolal (24 mg, 0.126 mmol, 94%). To a solution of the diolal (24 mg, 0.126 mmol, 1.0 eq.) in 4 mL MeOH was added sodium borohydride (7.6 mg, 0.202 mmol, 1.6 eq.). The solution stirred for 2h then was concentrated onto silica and chromatographed on MPLC to afford \((R,R-2.59)\) as a colorless oil (11 mg, 0.073 mmol, 58% yield, eluting at 20% MeOH in chloroform). \([\alpha]_D^{20} +9.9 \text{ (c 0.100, MeOH)}\).

5.2.8.2 Synthesis of \((2S,3S)\)-hexane-1,2,3,6-tetraol \((S,S-2.59)\)

To a mixture of lithium aluminum hydride (LAH, 8 mg, 0.187 mmol) in dry THF (10 mL) at rt was added 2.63 (58 mg, 0.250 mmol) dissolved in 1 mL dry THF. The mixture stirred for 2h. TLC showed some starting material remained so additional LAH (6 mg, 0.158 mmol) was added. After
20 min., no more starting material remained. KOH solution (1 M, 0.5 mL) was cautiously added. The mixture was diluted with EtOAc, dried over anhydrous MgSO₄ and concentrated on to silica. After MPLC purification the intermediate diol (eluting at 100% EtOAc, 40 mg, 0.210 mmol, 84% yield) was obtained. The intermediate diol was added to a 50% acetic acid in water solution. The mixture stirred for 3h. The solvent was removed under a stream of air to afford (S,S)-2.59 (11 mg, 0.073 mmol, 41%) as a viscous colorless oil. \([\alpha]_D^{20} -10.0 \text{ (c 0.100, MeOH)}\).

5.2.8.3 Physical data common to \(R,R\)-2.59 and \(S,S\)-2.59

\(^1\)H NMR (400 MHz, CD₃OD) \(\delta\) (multiplicity, \(J\) (Hz), integration): 1.62 (m, 4H), 3.53 (m, 4H), 3.64 (dd, 11.1, 4.9, 2H); \(^{13}\)C NMR (100 MHz, CD₃OD) \(\delta\): 30.3, 30.9, 63.2, 64.7, 72.7, 75.8; ESI-MS (m/z) [M+H] = 151.1.

5.2.9 Synthesis of ((4\(R\),5\(R\))-5-(2-(1,3-dioxolan-2-yl)vinyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl acetate (2.61)

![2.61]

To a stirring solution of (−)-2,3-O-Isopropylidene-D-threitol (\(R,R\)-2.60, 255 mg, 1.54 mmol) in 10 mL dry DCM at rt under inert atmosphere was added 4-dimethylaminopyridine (DMAP,18 mg, 0.15 mmol), TEA (155mg, 0.214 mL, 1.54 mmol), and acetic anhydride (158 mg, 0.147 mL, 1.54 mmol). The solution stirred for 30 min then was concentrated and subjected to chromatographic purification on silica MPLC. The monoacetate (176 mg, 0.862 mmol, 57%) eluted at 25-30% EtOAc:Hex. To a stirring slurry of Dess Martin periodinane (287 mg, 0.676 mmol) in 10 mL dry DCM was added the monoacetate (92 mg, 0.451 mmol) dissolved in 2 mL dry DCM followed by pyridine (180 mg, 2.28 mmol). After 2h at rt, the mixture was quenched by addition of saturated sodium bicarbonate (sat. NaHCO₃, 5 mL) and 1 M sodium thiosulfate (Na₂S₂O₃) solution (5 mL).
This mixture was allowed to stir until both layers were clear. The mixture was partitioned between EtOAc and water. The organic layer was collected. The aqueous layer was extracted X2 with aliquots of EtOAc. The combined organic layers were dried over anhydrous MgSO₄ and concentrated to yield crude aldehyde (84 mg, 0.415 mmol, 92% yield). A slurry of (1,3-Dioxolan-2-ylmethyl)triphenylphosphonium bromide (308 mg, 0.714 mmol) and potassium tert-butoxide (80 mg, 0.714 mmol) in dry THF stirred at rt for 25 min under inert atmosphere. To the resulting dark yellow mixture was added the intermediate aldehyde (84 mg, 0.446 mmol) dissolved in 2 mL dry THF. After stirring for 2.5 h, the dark orange mixture was concentrated then partitioned between Et₂O and H₂O. The organic layer was collected. The aqueous layer was extracted 2X with aliquots of Et₂O. The combined organic layers were dried over anhydrous MgSO₄, concentrated, and chromatographed on silica MPLC (eluting at 30% EtOAc:Hex) to yield a mixture of E/Z-2.61 (51 mg, 0.187 mmol, 42%) as a colorless oil.

5.2.10 Synthesis of ethyl 3-((4S,5S)-5-(benzyloxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate (2.62)

A mixture of (+)-2,3-O-isopropylidene-L-threitol [(S,S)-2.60, 500 mg, 3.08 mmol], benzyl bromide (580 mg, 3.39 mmol, 1.1 equiv), and silver oxide (Ag2O, 1.07 g, 4.62 mmol) in dry toluene was stirred at rt for 8 h. The mixture was filtered through a plug of silica and concentrated. The resulting residue was chromatographed on silica (eluting at 35–42% EtOAc in hexanes) to yield ((4S,5S)-5-(benzyloxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (598 mg, 2.38 mmol, 77%) as a colorless oil.

To a stirring slurry of Dess–Martin periodinane (630 mg, 1.49 mmol) in 100 mL dry DCM was added ((4S,5S)-5- (benzyloxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (342 mg, 1.35
mmol) dissolved in 5 mL dry DCM followed by pyridine (534 mg, 0.54 mL, 6.75 mmol). After 30 min at rt, the mixture was quenched by addition of satd NaHCO$_3$ (50 mL) and 1 M Na$_2$S$_2$O$_3$ solution (50 mL). This mixture was allowed to stir until both layers were clear and was then partitioned between EtOAc and water. The aqueous layer was extracted 2X with aliquots of EtOAc. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated to yield the crude aldehyde (360 mg). A slurry of (ethoxycarbonylmethyl)triphenyl phosphonium bromide (683 mg, 1.59 mmol, 1.1 equiv) and NaH (38 mg, 1.59 mmol, 1.1 equiv) in 50 mL dry THF at rt stirred for 4 h. The crude aldehyde (360 mg, ~1.44 mmol) dissolved in 5 mL dry THF was then added. The mixture stirred for 6 h and was then partitioned with Et$_2$O/H$_2$O. The organic layer was collected. The aqueous layer was extracted 2X with aliquots of Et$_2$O. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated under reduced pressure. The concentrate was chromatographed by silica gel MPLC (eluting at 15% EtOAc:hexane) to afford a mixture of $E$/Z isomers of conjugate ester 2.62 (298 mg, 0.93 mmol, 69%, two steps) as a colorless oil. The $E$-isomer could be formed exclusively by substituting (ethoxycarbonylmethyl)triphenyl phosphonium bromide with triethylphosphonoacetate (60%, two steps). 

\textbf{\textit{E-2.62}:} \[
\begin{align*}
\{\alpha\}_D^{20} &- 23.4 (c 1.0, \text{CHCl}_3); \text{IR (neat) } \nu (\text{cm}^{-1}): 2988, 1722, 1654, 1090; \text{\ 1H NMR (400 MHz, CDCl}_3) \delta (\text{multiplicity, } J (\text{Hz}), \text{integration}): 1.29 (t, 6.9, 3H), 1.44 (s, 3H), 1.45 (s, 3H), 3.63 (d, 4.7, 2H), 3.96 (dt, 8.4, 4.7, 1H), 4.20 (q, 6.9, 2H), 4.43 (ddd, 8.4, 5.6, 1.7, 1H), 4.60 (s, 2H), 6.09 (dd, 15.7, 1.7, 1H), 6.89 (dd, 15.7, 5.6, 1H), 7.34 (m, 5H). \\
\text{\text{13C NMR (100 MHz, CDCl}_3) \delta: 14.3, 26.8, 27.1, 60.7, 69.5, 73.8, 77.6, 79.7, 110.3, 122.7, 127.8 (2C), 127.9, 128.6 (2C), 137.9, 144.2, 166.1. ESI HRMS [M+Na]$^+$ calcd for [C$_{18}$H$_{24}$O$_5$Na]$^+$: 343.1516, found 343.1510.
\end{align*}
\]

5.2.11 ethyl 3-((4S,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propanoate (2.63)
To activated 10% Pd/C (50 mg) was added 2.62 (278 mg, 0.87 mmol) dissolved in 10 mL EtOH. A balloon containing H₂ gas was affixed to the flask. The mixture stirred for 12 h at rt, was diluted with EtOAc, and filtered through Celite. The filtrate was concentrated to afford 2.63 as a colorless oil (194 mg, 0.84 mmol, 96%). 

^1^H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 1.23 (t, 7.2, 3H), 1.37 (s, 3H), 1.38 (s, 3H), 1.60 (br s), 1.83, (m), 1.94 (m), 2.46 (m, 2H), 3.61 (m), 3.75 (m), 3.78 (m), 3.89 (dt, 7.7, 3.6), 4.12 (q, 7.2, 2H); 

^1^3^C NMR (100 MHz, CDCl₃) δ: 14.4, 27.2, 27.5, 28.2, 30.9, 60.6, 62.0, 76.3, 81.2, 109.2, 173.4; 

ESI-MS (m/z) [M+H] = 233.1.

5.2.12 (S)-5-(2-(tert-butyldimethylsilyloxy)hept-6-enylsulfonyl)-1-phenyl-1H-tetrazole (2.65)

To 1-phenyl-1H-tetrazole-5-thiol (288 mg, 1.62 mmol) and potassium carbonate (K₂CO₃, 372 mg, 2.60 mmol) was added 2.68 (215 mg, 0.54 mmol) dissolved in 5 mL dry acetone. The stirring mixture refluxed for 20 h and was cooled to rt, and partitioned between Et₂O/H₂O. The aqueous layer was extracted 2X with aliquots of Et₂O. The combined organic extracts were dried over anhydrous MgSO₄, concentrated under reduced pressure, and subjected to silica gel MPLC to afford the thioether intermediate as white needles (mp 35–36 °C, 174 mg, 0.430 mmol, 80%). To the thioether intermediate (103 mg, 0.254 mmol) in 2 mL EtOAc was added H₂O₂ (86 μL 30% solution, 26 mg H₂O₂, 0.762 mmol), sodium tungstate (Na₂WO₄·2H₂O, 170 μL of a 5 mg/mL solution in EtOAc, 0.85 mg, 0.00254 mmol), phenylphosphonic acid (80 μL of a 5 mg/mL solution in EtOAc, 0.4 mg, 0.00254 mmol), and methyltrioctylammonium hydrogensulfate (Oct₃MeNHSO₄, 240 μL of a 5 mg/mL solution, 1.2 mg, 0.00254 mmol). After 40 h the reaction was not yet complete via TLC so another aliquot of sodium tungstate (0.00254 mmol), phenylphosphonic acid (0.00254 mmol), Oct₃MeNHSO₄ (0.00254 mmol), and (0.762 mmol) was added. This mixture stirred another 60 h and was partitioned between EtOAc/ H₂O. The organic layer was dried over
anhydrous MgSO₄, concentrated and chromatographed via silica gel MPLC afford a mixture of diastereomers of the partially oxidized sulfoxide (20 mg, 0.05 mmol, 20%) as well as desired sulfone 2.65 as a white solid (mp 62–64 °C, 48 mg, 0.110 mmol, 43%). [α]₂⁰D +15.6 (c 0.4, CHCl₃); IR (neat) ν (cm⁻¹): 3073, 2950, 2934, 2858, 1345, 1254, 1157;¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 0.03 (s, 3H), 0.06 (s, 3H), 0.84 (s, 9H), 1.48 (m, 2H), 1.67 (m, 2H), 2.10 (dt, 7.0, 7.0, 2H), 3.86 (dd, 14.9, 4.6, 1H), 4.00 (dd, 14.9, 6.6z, 1H), 4.48 (m, 1H), 5.00 (m, 2H), 5.77 (ddt, 16.9, 6.9, 3.9, 1H), 7.64 (m, 5H);¹³C NMR (100 MHz, CDCl₃) δ: -4.7, -4.1, 18.1, 23.6, 25.8 (3C), 33.6, 37.1, 62.1, 66.6, 115.4, 125.3 (2C), 129.9 (2C), 131.6, 133.3, 138.1, 154.4; ESI HRMS [M+H]⁺ calcd for [C₂₀H₃₃N₄O₃SSi]⁺: 437.2037, found 437.2024.

5.2.13 ethyl 3-(((S,S,R)-5-formyl-2,2-dimethyl-1,3-dioxolan-4-yl)propanoate (2.66)

To a stirring solution of Dess–Martin periodinane (424 mg, 1.02 mmol) in 10 mL dry DCM at rt was added 2.63 (198 mg, 0.85 mmol) then pyridine (336 mg, 0.35 mL). The solution stirred for 1 h and was then quenched with 5 mL 1 M Na₂S₂O₃ and 5 mL sat. NaHCO₃ solution. The mixture stirred until both layers were no longer cloudy. The organic layer was concentrated then repartitioned in EtOAc/H₂O. The organic layer was collected, dried over anhydrous MgSO₄, and concentrated to yield 2.66 as a colorless oil (150 mg, 0.65 mmol, 76%). [α]₂⁰D -7.4 (c 1.0, CHCl₃); IR (neat) ν (cm⁻¹): 2985, 2938, 1703, 1731;¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 1.19 (t, 7.3, 3H), 1.34 (s, 6H), 1.95 (m, 2H), 2.42 (m, 2H), 3.91 (dt), 4.04 (m), 4.07 (q, 7.3, 2H), 9.67 (s);¹³C NMR (100 MHz, CDCl₃) δ: 14.3, 26.4, 27.2, 28.7, 30.4, 60.7, 76.1, 84.7, 110.1, 172.9, 201.1; ESI HRMS [M+H]⁺ calcd for [C₁₁H₁₉O₅]⁺: 231.1227, found 231.1221.
5.2.14 (S)-hept-6-ene-1,2-diol (2.67)

To 10 mL dry DCM at -60 °C was added oxalyl chloride (787 mg, 6.20 mmol, 0.525 mL). After stirring for 5 min, DMSO (605 mg, 7.75 mmol, 0.55 mL) was added. After stirring 2 min, S-2.58 (540 mg, 3.10 mmol) dissolved in 3 mL dry DCM was added over a 5 min period. After stirring for an additional 10 min at -60 °C, TEA (1.88 g, 18.6 mmol, 2.6 mL) was added. The mixture was then warmed to rt and partitioned between EtOAc/H$_2$O. The organic layer was collected. The aqueous layer was washed 2X with aliquots of EtOAc. The organic layers were combined, dried over anhydrous MgSO$_4$, and concentrated to afford the aldehyde (S)-4-(2,2-dimethyl-1,3-dioxolan-4-yl)butanal (488 mg, 2.83 mmol, 91%) as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$) δ (multiplicity, J (Hz), integration): 1.28 (s, 3H), 1.34 (s, 3H), 1.58 (m, 4H), 2.44 (dt, 7.3, 1.6, 2H), 3.45 (t, 7.1), 3.97 (t, 7.1), 4.0 (m), 9.71 (t, 1.6); $^{13}$C NMR (100 MHz, CDCl$_3$) δ: 18.4, 25.9, 27.1, 33.1, 43.8, 69.5, 76.2, 109.1, 202.4.

To a stirring slurry of Ph$_3$PCH$_3$Br (1.95 g, 5.46 mmol) in dry THF (50 mL) at 0 °C was added potassium bis(trimethylsilyl)amide (KHMDS, 0.5 M in toluene, 10.92 mL, 5.46 mmol) over a 10 min period. The mixture was warmed to rt and stirred for 30 min. The mixture then was cooled to 0 °C and (S)-4-(2,2-dimethyl-1,3-dioxolan-4-yl)butanal (470 mg, 2.72 mmol, 1.0 equiv) dissolved in 10 mL dry THF was added over a 10 min period. The mixture stirred at 0 °C for 1.5 h. Methanol (0.6 mL) was added to quench the reaction. The mixture was diluted with Et$_2$O and filtered through Celite. The filtrate was concentrated and chromatographed by silica gel MPLC (eluting at 10-12% EtOAc: hexanes) to afford (S)-2,2-dimethyl-4-(pent-4-enyl)-1,3-dioxolane as a colorless oil (260 mg, 1.53 mmol, 56%). $^1$H NMR (400 MHz, CDCl$_3$) δ (multiplicity, J (Hz), integration): 1.29 (s, 3H), 1.34 (s, 3H), 1.45 (m, 2H), 1.55 (m, 2H), 2.02 (dt, 6.8, 1.4, 2H), 3.44 (t,
(S)-2,2-dimethyl-4-(pent-4-enyl)-1,3-dioxolane (233 mg, 1.37 mmol) was stirred in 5 mL 50% AcOH:H₂O in a flask open to air for 2 h at rt. The solvent was then removed under a stream of air to yield diol 2.67 as a colorless oil (164 mg, 1.26 mmol, 92%). [α]D²⁰ -6.5 (c 1.0, CHCl₃); IR (neat) ν (cm⁻¹): 3365, 3079, 2935, 1070, 1039; ¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 1.44 (m, 4H), 1.68 (br s), 2.03 (m, 2H), 2.07 (br s), 3.37 (dd, 10.8, 7.7), 3.59 (dd, 10.8, 3.0), 3.65 (m), 4.93 (m, 2H), 5.74 (ddt, 17.0, 6.6, 3.5); ¹³C NMR (100 MHz, CDCl₃) δ: 24.9, 32.7, 33.7, 67.0, 72.3, 115.0, 138.7; ESI HRMS [M+NH₄]⁺ calcd for [C₇H₁₈O₂N]⁺: 148.1332, found 148.1328.

5.2.15 (S)-2-((tert-butyldimethylsilyloxy)hept-6-enyl 4-methylbenzenesulfonate (2.68)

To a solution of tosyl chloride (265 mg, 1.39 mmol, 1.1 equiv) and DMAP (15 mg, 0.13 mmol) in 9 mL dry DCM and stirring at 0 °C was added 2.67 (164 mg, 1.26 mmol) dissolved in 1 mL dry DCM. The solution stirred for 5 min, then TEA (141 mg, 0.2 mL, 1.39 mmol) was added. The solution stirred at 0 °C for 4 h and then rt for 4 h. The solution was poured into a flask containing 20 mL ice, 20 mL H₂O and 10 mL 2 N HCl. The resulting mixture was extracted 2X with 50 mL aliquots of DCM. The combined organic layers were dried over anhydrous MgSO₄ and concentrated under reduced pressure. The crude concentrate was chromatographed by silica gel MPLC to afford the monotosylated alcohol as a colorless oil (315 mg, 1.11 mmol, 88%). To the monotosylated alcohol (150 mg, 0.53 mmol) in 5 mL dry DCM under stirring at 0 °C was added 2,6-lutidine (169 mg, 0.18 mL, 1.58 mmol) then tert-butyldimethylsilyl trifluoromethanesulfonate (TBS-OTf, 348 mg, 0.30 mL, 1.32 mmol). The mixture stirred for 2 h and was partitioned between
DCM/H₂O. The organic layer was collected. The aqueous layer was extracted 2X with aliquots of DCM. The combined organic extracts were dried over anhydrous MgSO₄, concentrated under reduced pressure, and subjected to silica gel MPLC to yield 2.68 as a colorless oil (215 mg, 0.53 mmol, quantitative). [α]D²⁰ -6.0 (c 0.4, CHCl₃); IR (neat) ν (cm⁻¹): 3079, 2952, 2857, 1170; ¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 0.01 (s, 3H), 0.02 (s, 3H), 0.83 (s, 9H), 1.39 (m, 4H), 2.00 (dt, 6.6, 6.6, 2H), 2.45 (s, 3H), 3.85 (m, 2H), 3.85 (m), 4.96 (m), 5.74 (ddt, 17.0, 6.6, 3.2) 7.34 (d, 8.0, 2H), 7.79 (d, 8.0, 2H); ¹³C NMR (100 MHz, CDCl₃) δ: -4.8, -4.6, 21.6, 24.0, 25.7 (3C), 26.9, 33.4, 33.6, 69.8, 73.1, 114.7, 127.9 (2C), 129.8 (2C), 133.0, 138.3, 144.7; ESI HRMS [M+H]⁺ calcd for [C₂₀H₃₅O₄SSi]⁺: 399.2020, found 399.2032.

5.2.16 (S)-7-(benzyloxy)hept-1-en-3-yl acetate (2.69)

To a stirring solution of 2.71 (66 mg, 0.248 mmol) in 5mL dry DCM at 0 °C was added Dess–Martin periodinane (158 mg, 0.372 mmol) in one portion. The mixture was warmed to rt and stirred for 45 min. Saturated NaHCO₃ (5 mL) and 1.0 M Na₂S₂O₃ (5 mL) was added. The mixture stirred for 5 min then was partitioned between Et₂O/H₂O. The organic layer was collected, dried over anhydrous MgSO₄ and concentrated under reduced pressure to yield the aldehyde intermediate as a colorless oil (65 mg, 0.248 mmol, quantitative). To a mixture of ethyltriphenylphosphonium bromide (89 mg, 0.25 mmol) in 5 mL dry THF at 0 °C was added KHMDS (0.5 M in toluene, 0.5 mL, 0.25 mmol) dropwise. After 10 min stirring at 0 °C, the aldehyde intermediate (60 mg, 0.227 mmol) dissolved in 2 mL dry THF was added dropwise. After 5 min the reaction was partitioned between Et₂O/H₂O. The organic layer was collected. The aqueous layer was extracted 2X with aliquots of Et₂O. The combined organic layers were dried over anhydrous MgSO₄ and concentrated. The crude residue was chromatographed by silica gel MPLC (eluting at 10–13% EtOAc:hexanes) to afford 2.69 as a colorless oil (37 mg, 0.141 mmol,
5.2.17 (S)-6-(benzyloxy)hexane-1,2-diol (2.70)

To a stirring solution of S-2.58 (215 mg, 1.25 mmol) in 5 mL dry THF at rt was added NaH (33 mg, 1.37 mmol) in one portion. The mixture bubbled vigorously for 5 min. After gas evolution had subsided, benzyl bromide (257 mg, 1.5 mmol) was added. The mixture stirred for 20 h and was partitioned between Et<sub>2</sub>O/H<sub>2</sub>O. The organic layer was collected. The aqueous layer was extracted 2X with aliquots of Et<sub>2</sub>O. The combined organic layers were dried over anhydrous MgSO<sub>4</sub> and concentrated. The crude residue was chromatographed by silica gel MPLC (eluting at 12-14% EtOAc/hexane) to afford the benzylated intermediate as a colorless oil (142 mg, 0.54 mmol, 43%). The benzylated intermediate was stirred in 2 mL 50% AcOH:H<sub>2</sub>O in a flask opened to air for 3 h. The mixture was then concentrated to afford 2.70 as a colorless oil (125 mg, 0.47 mmol, 94%). [α]<sub>D</sub><sup>20</sup> -3.3 (c 1.0, CHCl<sub>3</sub>); IR (neat) ν (cm<sup>-1</sup>): 3382, 2938, 2867, 1456, 1096, 1029; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (multiplicity, J (Hz), integration): 1.44 (m, 2H), 1.59 (m, 4H), 1.91 (br s), 2.14 (br s), 3.41 (dd, 11.1, 7.6), 3.47 (t, 6.4, 2H), 3.62 (dd, 11.1, 3.0), 3.69 (m), 4.49 (s, 2H), 7.27 (m), 7.32 (m, 4H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 22.4, 29.7, 33.0, 67.0, 70.3, 72.4, 73.1, 127.8, 127.9 (2C), 128.6 (2C), 138.8; ESI HRMS [M+H]<sup>+</sup> calcd for [C<sub>13</sub>H<sub>21</sub>O<sub>3</sub>]+: 225.1485, found 225.1481.
To a stirring solution of 2.70 (100 mg, 0.45 mmol) in 3 mL dry THF at rt was added TEA (54 mg, 71 mL, 0.54 mmol) followed by chlorotrimethylsilane (53 mg, 63 mL, 0.49 mmol) dropwise. After stirring 25 min, the mixture was diluted with 3 mL dry DCM. Additional TEA (162 mg, 213 mL, 0.16 mmol) followed by DMAP (5 mg, 0.045 mmol) was added. Acetic anhydride (137 mg, 1.34 mmol) was added to the stirring solution dropwise. The solution stirred for 1 h then was partitioned between Et$_2$O/2 N HCl. The organic layer was collected. The aqueous layer was extracted 2X with aliquots of Et$_2$O. The combined organic layers were dried over anhydrous MgSO$_4$ and concentrated. The crude residue was chromatographed by silica gel MPLC (eluting at 45–65% EtOAc:hexanes) to afford 2.71 as a colorless oil (66 mg, 0.249 mmol, 56%). Starting material (24%) was also recovered. 2.71: $[\alpha]_D^{20}$ -1.0 (c 1.0, CHCl$_3$); IR (neat) $\nu$ (cm$^{-1}$): 3452, 2941, 2864, 1735, 1241, 1096; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (multiplicity, $J$ (Hz), integration): 1.42 (m, 2H), 1.60 (m, 4H), 2.07 (s, 3H), 3.45 (t, 6.3, 2H), 3.61 (dd, 11.5, 6.3), 3.70 (dd, 11.5, 2.5), 4.48 (s, 2H), 4.89 (m), 7.27 (m), 7.32 (m, 5H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 21.3, 22.3, 29.7, 30.4, 65.0, 70.2, 73.2, 75.7, 127.7, 127.8 (2C), 128.6 (2C), 138.7, 171.6; ESI HRMS [M+H]$^+$ calcd for [C$_{15}$H$_{23}$O$_4$]$^+$: 267.1591, found 267.1594.

To a stirring slurry of Ph$_3$PMeBr (414 mg, 1.16 mmol) in 10 mL dry THF at 0 °C was added dropwise KHMDS (0.5 M in toluene, 2.32 mL, 1.16 mmol). The mixture was warmed to rt and
stirred for 30 min. The mixture was then cooled back down to 0 °C. Aldehyde 2.66 (150 mg, 0.65 mmol) dissolved in 3 mL dry THF was then added dropwise. The reaction was then partitioned between Et₂O/ H₂O. The aqueous layer was extracted 2X with aliquots of Et₂O. The combined organic layers were dried over anhydrous MgSO₄ and concentrated. The crude residue was chromatographed by silica gel MPLC (eluting at 12–16% EtOAc/hexanes) to afford 2.72 as a colorless oil (91 mg, 0.40 mmol, 62%). \([\alpha]_{D}^{20} -2.3 (c 0.7, \text{CHCl}_3)\); IR (neat) ν (cm⁻¹): 3082, 2985, 1741, 1167, 1073; \(^1\)H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 1.25 (t, 7.3, 3H), 1.39 (s, 3H), 1.40 (s, 3H), 1.83 (m),1.96 (m), 2.45 (m, 2H), 3.69 (dt, 8.3, 3.7), 4.00 (dd, 8.3, 1.4), 4.13 (q, 7.3, 2H), 5.26 (dd, 10.2, 1.4), 5.38 (dd, 17.4, 1.4), 5.80 (ddd, 17.4, 10.2, 7.0); \(^{13}\)C NMR (100 MHz, CDCl₃) δ: 14.4, 27.0, 27.1, 27.4, 30.9, 60.6, 79.8, 82.6, 109.0, 119.3, 135.2, 173.3; ESI HRMS [M+Na]⁺ calcd for \([C_{12}H_{20}O_4Na]⁺\): 251.1254, found 251.1256.

5.2.20 ethyl 3-((4S,5S)-5-((S,E)-3-acetoxy-7-(benzyloxy)hept-1-enyl)-2,2-dimethyl-1,3-dioxolan-4-yl) propanoate (2.73)

To a solution of 2.69 (20 mg, 0.076 mmol) and 2.72 (17 mg, 0.076 mmol) in dry DCM under stirring at rt was added Grubbs second generation catalyst (7 mg, 0.008 mmol). The solution stirred for 48 h then was concentrated and chromatographed by silica gel MPLC (eluting at 25-30% EtOAc:hexanes) to afford the E isomer 2.73 as a colorless oil, which solidified when placed in freezer (14 mg, 0.03 mmol, 40%). \([\alpha]_{D}^{20} -15.6 (c 0.36, \text{CHCl}_3)\); IR (neat) ν (cm⁻¹): 3032, 2988, 2931, 2867, 1738, 1372, 1241, 1093, 1079, 1022; \(^1\)H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 1.22 (t, 7.1, 3H), 1.36 (s, 6H), 1.38 (m, 2H), 1.59 (m, 4H), 1.84 (m, 2H), 2.02 (s, 3H), 2.41 (m, 2H), 3.43 (t, 6.5, 2H), 3.64 (dt, 7.7, 4.0), 3.97 (dt, 7.7, 7.2), 4.10 (q, 7.1, 2H), 4.47, (s, 2H), 5.24 (d, 6.7), 5.60 (dd, 15.6, 7.2), 5.68 (dd, 15.6, 6.7), 7.25 (m), 7.31 (m, 4H); \(^{13}\)C NMR
Lyophilized *Synoicum* sp. (192 g), collected at Palmer Station, Antarctica, was extracted 3x24 h with 1:1 DCM:MeOH followed by extraction with 1:1 MeOH:H₂O (3x24 h). Concentration of the organic filtrate under reduced pressure yielded 21.0 g of lipophilic extract. A portion of this extract (4.6 g) was fractionated on MPLC with a gradient of hexane to EtOAc to MeOH. Fractions eluting at 20–50% MeOH:EtOAc were combined (25 mg) and chromatographed via analytical reverse phase HPLC (55% MeOH:H₂O) to yield meridianins A, B, C, and E. NMR data matched that of the previously reported meridianins (Chapter 3, ref. 22).

Meridianin A (3.24), 5 mg, 


\[ \text{H NMR (400 MHz, } d_6\text{-DMSO)} \delta \text{ (multiplicity, } J \text{ (Hz), integration, position): } 6.36 \text{ (dd, 7.3, 0.5, H5), 6.27 \text{ (s, 2H, 2'}'-\text{NH}_2\text{), 6.79 \text{ (dd, 7.5, 0.5, H7), 6.96 \text{ (dd, 7.5, 7.3, H6), 7.10 \text{ (d, 5.4, H5'), 8.10 \text{ (d, 5.4, H6'), 8.22 \text{ (s, H2), 11.76 \text{ (brs, N1-H), 13.57 \text{ (s, 4-OH)})}}}} \]

\[ \text{C NMR (100 MHz, } d_6\text{-DMSO)} \delta: 102.3, 104.4, 105.4, 114.0, 114.7, 124.4, 128.5, 139.4, 152.0, 158.4, 160.5, 161.7. \]
Meridianin B (3.25), 5 mg, $^1$H NMR (400 MHz, $d_6$-DMSO) $\delta$ (multiplicity, $J$ (Hz), integration, position): 6.51 (d, 1.5, H5), 6.85 (s, 2H, 2'-NH$_2$), 7.00 (d, 1.5, H7), 7.15 (d, 5.4, H5'), 8.18 (d, 5.4, H6'), 8.28 (s, H2), 11.90 (brs, N1-H), 14.21 (s, 4-OH).

Meridianin C (3.26), <1 mg, $^1$H NMR (400 MHz, $d_6$-DMSO) $\delta$ (multiplicity, $J$ (Hz), integration, position): 6.49 (s, 2H, 2'-NH$_2$), 7.00 (d, 5.3, H5'), 7.29 (dd, 8.5, 1.9, H6), 7.39 (d, 8.4, H7), 8.10 (d, 5.3, H6'), 8.24 (s, H2), 8.75 (d, 1.9, H4), 11.85 (brs, N1-H).

Meridianin E (3.28), 3 mg, $^1$H NMR (400 MHz, $d_6$-DMSO) $\delta$ (multiplicity, $J$ (Hz), integration, position): 6.37 (d, 8.3, H5), 6.85 (2H, 2'-NH$_2$), 7.19 (d, 8.3, H6), 7.24 (d, 5.4, H5'), 8.18 (d, 5.4, H6'), 8.31 (s, H2), 11.92 (brs, N1-H), 13.93 (s, 4-OH).

5.3.2 synthetic meridianin A (3.24)

To a stirring solution of 3.91 (75 mg, 0.15 mmol) in dry THF (2 mL) at rt was added dropwise tetra-n-butylammonium fluoride (TBAF, 1.0 M, 0.3 mL, 0.3 mmol). After 5 min. aqueous sodium carbonate ($\text{Na}_2\text{CO}_3$, 2 M, 2 mL) was added. The mixture was then partitioned between sat. ammonium chloride ($\text{NH}_4\text{Cl}$)/10% MeOH in EtOAc. The aqueous layer was extracted with another aliquot of 10% MeOH in EtOAc. The combined organic layers were dried over anhyd. MgSO$_4$, concentrated, and purified via MPLC (silica, eluting at 10% MeOH:DCM) to afford meridianin A. The compound was recrystallized from MeOH/H$_2$O to yield meridianin A as yellow needles (3.24, 30 mg, 0.13 mmol, 87% yield). $\text{Mp} = 168 \, ^\circ\text{C}$; $^1$H NMR (400 MHz, $d_6$-DMSO) $\delta$ (multiplicity, $J$ (Hz), integration): 6.36 (d, 7.6), 6.73 (br s, 2H), 6.79 (d, 7.9), 6.96 (dd, 7.6, 7.9),
7.10 (d, 5.4), 8.10 (d, 5.4), 8.21 (d, 2.6), 11.73 (br s), 13.58 (s); \(^{13}\)C NMR (100 MHz, \(d_6\)-DMSO) \(\delta\): 102.4, 104.4, 105.5, 113.8, 114.4, 124.4, 128.5, 139.3, 152.0, 158.5, 160.5, 161.8; ESI HRMS [M + H]^+ calculated for \([\text{C}_{12}\text{H}_{11}\text{N}_4\text{O}]^+\): 227.0927, found: 227.0929.

5.3.3 4-indolol (3.86)

![Image of 4-indolol](image)

A stirring mixture of 1,5,6,7-tetrahydro-4H-indol-4-one (3.85, 2.50 g, 18.4 mmol) and 10% Pd/C (625 mg) in 2,6-dimethyl-4-heptanone (25 mL) was refluxed (190°C) under N\(_2\) for 48 h. The mixture was cooled to rt and filtered through Celite. The filter cake was washed with DCM. The filtrate was concentrated in vacuo. Kugel-rohr distillation of the concentrate afforded discolored 4-indolol (150°C, 4 torr) which was subjected to MPLC (silica; eluting at 30-35% EtOAc:Hex) to yield 3.86 (1.19 g, 9.0 mmol, 49% yield) as a white crystalline solid. \(^1\)H NMR (400 MHz, \(d_6\)-DMSO, not stable in CDCl\(_3\)) \(\delta\) (multiplicity, \(J\) (Hz), position): 6.31 (d, 6.1, H3), 6.43 (m, H5), 6.81 (m, H6), 6.82 (m, H7), 7.10 (d, 2.4, H2), 9.21 (s, OH), 10.86 (brs, NH); \(^{13}\)C NMR (100 MHz, \(d_6\)-DMSO) \(\delta\): 98.5, 102.8 (2C), 117.8, 121.8, 122.9, 137.9, 150.5; ESI HRMS [M + H]^+ calculated for \([\text{C}_8\text{H}_8\text{NO}]^+\): 134.0600, found: 134.0600.

5.3.4 4-(tert-butyldimethylsilyloxy)-1H-indole (3.87)

![Image of 4-(tert-butyldimethylsilyloxy)-1H-indole](image)

A mixture of 4-indolol (3.86, 1.05 g, 7.89 mmol), tert-butyldimethylsilyl chloride (1.43 g, 9.47 mmol) and imidazole (1.74 g, 25.3 mmol) was stirred in 25 mL dry DMF at rt under N\(_2\) for 1 h. The mixture was partitioned (EtOAc:water). The organic layer was washed with water then dried (anhyd. MgSO\(_4\)), filtered and concentrated under reduced pressure onto silica. Purification using
MPLC (silica; eluting at 18% EtOAC:hexane) afforded **3.87** as a white solid (1.76 g, 7.14 mmol, 91% yield). Mp=79-80 °C; IR (neat) ν (cm⁻¹): 3399, 3056, 2961, 2928, 1583; ¹H NMR (400 MHz, d₆-DMSO, not stable in CDCl₃) δ (multiplicity, J (Hz), integration, position): 0.19 (s, 6H, Si(CH₃)₂), 1.02 (s, 9H, SiC(CH₃)₃), 6.35 (m, H3), 6.41 (d, 7.5, H5), 6.93 (dd, 7.5, 8.0, H6), 7.02 (d, 8.0, H7), 7.21 (s, H2), 11.0 (brs, NH); ¹³C NMR (100 MHz, d₆-DMSO) δ: -4.4 (2C), 18.0, 25.7 (3C), 98.3, 105.4, 107.6, 121.2, 121.5, 123.8, 137.9, 148.1; ESI HRMS [M + H]⁺ calculated for [C₁₄H₂₂NOSi]⁺: 248.1465, found: 248.1468.

5.3.5 3-bromo-4-(tert-butyldimethylsilyloxy)-1-(triisopropylsilyl)-1H-indole (**3.88**)

To **3.87** (1.77 g, 7.14 mmol) in 35 mL dry THF under stirring and N₂ was added dropwise n-butyllithium (5.43 mL, 1.6 M in hexanes, 8.76 mmol) at -78 °C. The mixture was warmed to -10 °C and stirred for 10 min. before being cooled back to -78 °C. Triisopropylsilyl chloride (1.93 g, 10.0 mmol, 2.14 mL) was added dropwise. The mixture was then warmed to 0 °C and stirred until reaction was complete (via TLC). The mixture was cooled to -78 °C. N-bromosuccinimide (1.72 g, 9.64 mmol) dissolved in dry THF (10 mL) was added to the cooled mixture. After stirring for 2 h at -78 °C, the mixture was warmed to rt, diluted with 1% pyridine in hexane and filtered through Celite. Any 3-bromo-4-(tert-butyldimethylsilyloxy)-1H-indole in the mixture will lead to decomposition of the desired product upon concentration. The filtrate was concentrated onto neutral alumina and chromatographed on neutral alumina (eluting in hexanes). Brown impurities were removed by further purification via MPLC (silica; eluting at 5% EtOAc:Hex) to afford white crystalline solid **3.88** (2.69 g, 5.59 mmol, 78% yield). Mp =107-109 °C; ¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration, position): 0.35 (s, 6H, Si(CH₃)₂), 1.08 (s, 9H, Si(CH₃)₃), 1.14 (d, 18H, Si(CH₃)₂), 1.65 (sept., 3H, SiCH), 6.52 (d, 7.7, H5), 6.98 (dd, 7.7, 8.1, H6), 7.07 (d, 8.1, H7), 7.10 (s, H2); ¹³C NMR (100 MHz, CDCl₃) δ: -3.7 (2C), 13.0 (3C), 18.2 (6C), 18.7, 26.2

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(3C), 90.6, 107.7, 109.1, 121.0, 122.8, 129.7, 142.7, 149.5; ESI HRMS [M + H]^+ calculated for [C_{23}H_{41}BrNOSi_2]^+: 482.1905, found: 482.1905.

5.3.6 4-(4-(tert-butyldimethylsilyloxy)-1-(triisopropylsilyl)-1H-indol-3-yl)pyrimidin-2-amine (3.91)

To a stirring solution of 3.88 (500 mg, 1.04 mmol) in dry THF (5 mL) at -78°C under N_2 was added dropwise tert-butyl lithium (1.6 M in pentane) until the solution remained yellow then and additional aliquot (1.4 mL, 2.29 mmol) was added dropwise. The solution stirred for 15 min. Neat 2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.89, 324 mg, 1.74 mmol, 0.35 mL) was added dropwise. The mixture stirred for 1 h at -78°C and was quenched with sat. NH_4Cl. The mixture warmed to rt and was partitioned between Et_2O/sat. NH_4Cl. The aqueous layer was extracted 2X with EtO_2. The combined organic layers were dried (anhdy. MgSO_4), concentrated to afford 4-(tert-butyldimethylsilyloxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaboran-2-yl)-1-(triisopropylsilyl)-1H-indole (3.90) which was used immediately in the next reaction without further purification.

A stirring mixture of crude 3.90 (1.04 mmol), tetrakis(triphenylphosphine)palladium (100 mg, 0.09 mmol), and 2-amino-4-chloropyrimidine (113 mg, 0.87 mmol), benzene (25 mL, degassed by sparging with N_2), methanol (5 mL, degassed), and aqueous sodium carbonate (1.25 mL, 2 M, degassed) was refluxed under N_2 for 24 h. The mixture was allowed to cool to rt, diluted with EtOAc and dried with anhyd. MgSO_4. The filtrate was concentrated onto silica and purified via MPLC (silica, eluting at 40% EtOAc:hexane) to afford 3.91 (200 mg, 0.40 mmol, 46 % yield). Mp=83-85 °C; ^1H NMR (500 MHz, CDCl_3) δ (multiplicity, J (Hz), integration, position): 0.10 (s, 6H, Si(CH_3)_2), 0.90 (s, 9H, SiC(CH_3)_3), 1.17 (d, 18H, 7.6, SiCH(CH_3)_2), 1.73 (sept., 7.6, 3H, SiCH),
5.01 (brs 2'NH), 5.04 (brs, 2'NH), 6.63 (d, 7.8, H5), 7.05 (dd, 7.8, 8.1, H6), 7.15 (d, 8.1, H7), 7.22 (d, 5.3, H5'), 7.69 (s, H2), 8.19 (d, 5.3, H6'); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: -0.4 (2C), 12.8 (3C), 18.2 (6C), 18.6, 26.0 (3C), 107.9, 110.9, 113.4, 118.4, 120.7, 122.5, 133.7, 144.2, 149.5, 156.6, 162.5, 163.1; ESI HRMS [M + H]$^+$ calculated for [C$_{27}$H$_{45}$N$_4$OSi$_2$]$^+$: 497.3126, found: 497.3116.

5.3.7 4-methoxy-1H-indole (3.92)

A stirring mixture of K$_2$CO$_3$ (3.9g, 28.5 mmol), 3.86 (380 mg, 2.85 mmol), and iodomethane (4.05g, 28.5 mmol, 1.8 mL) in dry acetone (10 mL) under N$_2$ was refluxed for 6 h. The mixture was then cooled to rt, and partitioned between EtOAc/H$_2$O. The aqueous layer was extracted 2X with aliquots of EtOAc. The combined organic layers were dried over anhyd. MgSO$_4$, concentrated, and purified via MPLC (silica, eluting at 22% EtOAc:Hex) to afford 3.92 (326 mg, 2.22 mmol, 78% yield). Mp=66 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (multiplicity, J (Hz), integration): 3.98 (s, 3H), 6.55 (d, 7.7, 1H), 6.68 (m, 1H), 7.05 (d, 8.2), 7.12 (m, 2H), 8.16 (br s); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 55.3, 99.5, 99.8, 104.4, 118.5, 122.61, 122.72, 137.18, 153.33; ESI HRMS [M + H]$^+$ calculated for [C$_9$H$_{10}$NO]$^+$: 148.0757, found: 148.0757.

5.3.8 3-bromo-4-methoxy-1-(triisopropylsilyl)-1H-indole (3.93)

To 3.92 (275 mg, 1.87 mmol) in 7.5 mL dry THF under stirring and N$_2$ was added dropwise n-butyllithium (1.58 mL, 1.6 M in hexanes, 2.52 mmol) at -78 °C. The mixture was warmed to -10 °C and stirred for 10 min. before being cooled back to -78 °C. Triisopropylsilyl chloride (0.5 g,
2.62 mmol, 0.56 mL) was added dropwise. The mixture was then warmed to 0 °C and stirred until reaction was complete (via TLC). The mixture was cooled to -78 °C. N-bromosuccinimide (0.45 g, 2.52 mmol) dissolved in dry THF (10 mL) was added to the cooled mixture. After stirring for 2 h at -78 °C, the mixture was warmed to rt, diluted with 1% pyridine in hexane and filtered through Celite. Any 3-bromo-4-methoxy-1H-indole in the mixture will lead to decomposition of the desired product upon concentration. The filtrate was concentrated onto neutral alumina and chromatographed on neutral alumina (eluting in hexanes). Brown impurities were removed by further purification via MPLC (silica; eluting at 5% EtOAc:Hex) to afford white crystalline solid 3.93 (610 mg, 1.60 mmol, 85% yield). Mp =65 °C; 1H NMR (400 MHz, d6-DMSO) δ (multiplicity, J (Hz), integration): 1.06 (d, 7.4, 18H), 1.71 (sept., 7.6, 3H), 3.84 (s, 3H), 6.61 (d, 7.6,), 7.10 (d, 8.2,), 7.13 (dd, 8.2, 7.6), 7.26 (s); 13C NMR (100 MHz, d6-DMSO) δ: 11.9 (3C), 17.76 (6C), 55.24, 89.5, 101.1, 107.3, 118.0, 123.4, 129.4, 141.4, 153.0; ESI HRMS [M + H]+ calculated for [C18H28BrNOSiNa]+: 404.1016, found: 404.1025.

5.3.9 4-(4-methoxy-1-(triisopropylsilyl)-1H-indol-3-yl)pyrimidin-2-amine (3.95)

![3.95](image)

To a stirring solution of 3.93 (350 mg, 0.91 mmol) in dry THF (4.5 mL) at -78°C under N2 was added dropwise tert-butyl lithium (1.6 M in pentane) until the solution remained yellow then and additional aliquot (1.2 mL, 2.0 mmol) was added dropwise. The solution stirred for 15 min. Neat 2-Isoproxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.89, 340 mg, 1.82 mmol, 0.37 mL) was added dropwise. The mixture stirred for 1 h at -78°C and was quenched with sat. NH4Cl. The mixture warmed to rt and was partitioned between Et2O/sat. NH4Cl. The aqueous layer was extracted 2X with EtO2. The combined organic layers were dried (anhyd. MgSO4), concentrated
to afford 4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(triisopropylsilyl)-1H-indole (3.94) which was used immediately in the next reaction without further purification.

A stirring mixture of crude 3.94 (0.91 mmol), tetrakis(triphenylphosphine)palladium (104 mg, 0.09 mmol), and 2-amino-4-chloropyrimidine (118 mg, 0.91 mmol), benzene (25 mL, degassed by sparging with N₂), methanol (5 mL, degassed), and aqueous sodium carbonate (1.25 mL, 2 M, degassed) was refluxed under N₂ for 24 h. The mixture was allowed to cool to rt, diluted with EtOAc and dried with anhyd. MgSO₄. The filtrate was concentrated onto silica and purified via MPLC (silica, eluting at 40% EtOAc:hexane) to afford 3.95 (205 mg, 0.52 mmol, 57% yield). Mp =106 °C; ¹H (400 MHz, d₆-DMSO) δ (multiplicity, J (Hz), integration): 1.11 (d, 7.5, 18H), 1.74 (sept., 7.5, 3H), 3.86 (s, 3H), 6.33 (br s, 2H), 6.71 (d, 7.7), 7.16 (m, 3H), 7.77 (s), 8.16 (d, 5.2); ¹³C NMR (100 MHz, d₆-DMSO) δ: 12.0 (3C), 17.9 (6C), 55.0, 102.0, 107.5, 110.2, 117.4, 118.5, 123.1, 133.0, 143.1, 153.3, 157.0, 161.14, 163.2; ESI HRMS [M + H]⁺ calculated for [C₂₂H₃₃N₄OSi]⁺: 397.2418, found: 397.2418.

5.3.10 4-methoxymeridianin A (3.96)

To a stirring solution of 3.95 (80 mg, 0.2 mmol) in dry THF (2 mL) at rt was added dropwise TBAF (1.0 M, 0.2 mL, 0.2 mmol). After 5 min. aqueous Na₂CO₃ (2 M, 2 mL) was added. The mixture was then partitioned between sat. NH₄Cl and EtOAc. The aqueous layer was extracted 2X with aliquots of EtOAc. The combined organic layers were dried over anhyd. MgSO₄, concentrated, and purified via MPLC (silica, eluting at 10% MeOH:DCM) to afford 4-methoxymeridianin A (3.96, 30 mg, 0.13 mmol, 87% yield). Mp =213-215 °C; ¹H NMR (400 MHz, d₆-DMSO) δ (multiplicity, J (Hz), integration): 3.87 (s, 3H), 6.27 (brs, 2H), 6.64 (d, 7.2), 7.08 (m), 7.09 (m), 7.25 (d, 5.3), 7.84
(d, 2.6), 8.15 (d, 5.3), 11.64 (b.r.s); \(^{13}\)C NMR (100 MHz, \(d_6\)-DMSO) \(\delta\): 55.0, 101.2, 105.6, 109.6, 114.5, 115.4, 122.7, 127.5, 138.8, 153.3, 157.0, 161.8, 163.1. M; ESI HRMS [M + H]\(^+\) calculated for \([C_{13}H_{13}N_4O]\)^+: 241.1084, found: 241.1084.

5.3.11 5,7-dibromomeridianin A (3.97)

To a stirring solution of meridianin A (3.24, 5 mg, 0.022 mmol) in 2.0 mL dry DCM was added AcOH (0.2 mL) and pyridinium tribromide (14 mg, 0.044 mmol). The mixture stirred for 5.5 h. Na\(_2\)S\(_2\)O\(_3\) (5% aqueous, 10 mL) and 10 mL EtOAc was added. The aqueous layer was washed 2X with aliquots of EtOAc. The combined organic layers were dried over anhyd. MgSO\(_4\), concentrated to afford crude 3.96 (5 mg, 0.013 mmol, 58% yield). \(^1\)H NMR (400 MHz, \(d_6\)-DMSO) \(\delta\) (multiplicity, \(J\) (Hz)): 6.97 (s, 2H), 7.27 (d, 5.4), 7.46 (s), 8.21 (d, 5.4), 8.38 (s), 11.24 (b.r.s), 15.10 (s); ESI-MS (m/z) [M+H] = 382.9 (50%), 384.9 (100%), 386.9 (45%).

5.3.12 4-amino-2-chloro-5-iodopyrimidine (3.99)

In a flask sealed with a rubber stopper, 2,4-dichloropyrimidine (3.98, 1 g, 6.76 mmol) was stirred in 29% NH\(_4\)OH (20 mL) for 12 h. The solvent was removed in vacuo. The crude product was chromatographed via MPLC (silica) to afford 2-amino-4-chloropyrimidine (400 mg, 3.0 mmol, eluting at 6% MeOH:CHCl\(_3\), 44% yield) and 4-amino-2-chloropyrimidine (454 mg, 3.5 mmol, eluting at 10% MeOH:CHCl\(_3\), 52% yield).
N-iodosuccinimide (2.7 g, 12.0 mmol) and 4-amino-2-chloropyrimidine (1.3 g, 10.0 mmol) were stirred in AcOH (30 mL) at 60-75 °C for 3 h. After solvent removal in vacuo, the residue was partitioned between 5% Na2S2O3 and CHCl3. The organic layer was washed with sat. NH4Cl, dried over anhyd. MgSO4 and chromatographed via MPLC (silica, eluting at 45% EtOAc:hexane) to afford 3.99 (1.45 g, 5.67 mmol, 57% yield). 1H NMR (400 MHz, CDCl3) δ (multiplicity, integration): 8.38 (s, 1H), 5.65 (br s, 2H); 13C NMR (100 MHz, CDCl3) δ: 74.4, 160.5, 163.1, 163.9. The NMR data are consistent with literature values (Chapter 3, ref. 51).

5.3.13 4-amino-2-bromo-5-iodopyrimidine (3.100)

4-amino-2-chloro-5-iodopyrimidine (3.99, 0.73 g, 2.86 mmol) in 33% HBr:AcOH was stirred for 1 h then refluxed for an additional hour. The mixture cooled to rt. Solvent was removed in vacuo. Water was added to the concentrate. The solid was collected via filtration and dried under vacuum affording 3.100 (0.74 g, 2.46 mmol, 86% yield). Mp= 189-190°C; IR (neat) ν (cm⁻¹): 3330, 3168, 3009, 1633, 1549; 1H NMR (400 MHz, CDCl3) δ (multiplicity, integration): 5.78 (2H, brs, NH2), 8.31 (s, H6); 13C NMR (100 MHz, CDCl3) δ: 75.4, 152.1, 162.8, 163.9; ESI HRMS [M+H]+ calculated for [C4H4BrIN3]+: 299.8629, found: 299.8626.

5.3.14 2-bromo-5-(4-(tert-butyldimethylsilyloxy)-1-(triisopropylsilyl)-1H-indol-3-yl)pyrimidin-4-amine (3.101)
To a stirring solution of 3.88 (470 mg, 0.98 mmol) in dry THF (5 mL) at -78°C under N₂ was added dropwise tert-butyllithium (1.6 M in pentane) until the solution remained yellow then and additional aliquot (1.3 mL, 2.15 mmol) was added dropwise. The solution stirred for 15 min. Neat 2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.89, 364 mg, 1.96 mmol, 0.4 mL) was added dropwise. The mixture stirred for 1 h at -78°C and was quenched with sat. NH₄Cl. The mixture warmed to rt and was partitioned between Et₂O:sat. NH₄Cl. The aqueous layer was extracted 2X with EtO₂. The combined organic layers were dried (anhyd. MgSO₄), concentrated to afford crude 3.90 which was used immediately in the next reaction without further purification.

A stirring mixture of crude 3.90 (0.98 mmol), tetrakis(triphenylphosphine)palladium (104 mg, 0.09 mmol), and 4-amino-2-bromo-5-iodopyrimidine (3.100, 283 mg, 0.98 mmol), benzene (25 mL, degassed by sparging with N₂), methanol (5 mL, degassed), and aqueous sodium carbonate (1.25 mL, 2 M, degassed) was refluxed under N₂ for 24 h. The mixture was allowed to cool to rt, diluted with EtOAc and dried with anhyd. MgSO₄. The filtrate was concentrated onto silica and purified via MPLC (silica, eluting at 18% EtOAc:hexane) to afford 3.101 (252 mg, 0.44 mmol, 45% yield from 3.88). Mp = 131-133°C; IR (neat) ν (cm⁻¹): 3351, 3130, 2951; ¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration, position): 0.09 (s, 6H, Si(CH₃)₂), 0.83 (s, 9H, SiC(CH₃)₃), 1.16 (d, 18H, SiCH(CH₃)₂), 1.69 (sept, 3H, SiCH), 5.25 (brs, 2H, NH₂), 6.34 (d, 7.1, H5), 7.04 (dd, 7.9, 8.1, H6), 7.07 (s, H2), 7.15, (d, 8.1, H7), 7.95 (s, H6'); ¹³C NMR (100 MHz, CDCl₃) δ: -4.1 (2C), 13.0 (3C), 18.3 (6C), 18.4, 25.8 (3C), 107.9, 109.0, 109.3, 114.1, 121.7, 123.2, 130.3, 143.9, 149.6, 150.3, 156.2, 163.9; ESI HRMS [M + H]+ calculated for [C₂₇H₄₃N₄OSi₂]⁺: 575.2232, found: 575.2248.
To 3.101 (50 mg, 0.087 mmol) in 1.0 mL acetonitrile (dried over 4Å molecular sieves) and 0.1 mL pyridine (dried over 4Å molecular sieves) was added 50 µL hydrogen fluoride-pyridine (70% as HF, 30% as pyridine). After 30 min., the starting material had completely dissolved. After 1 h, a precipitate had formed. The mixture stirred for 11 additional h at rt. The precipitate was collected via filtration and washed with acetonitrile. Drying the precipitate under high vacuum resulted in 3.102 as a tan powder (15 mg, 0.049 mmol, 56%). IR (neat) ν (cm\(^{-1}\)): 3429, 3313, 3163, 3015; \(^1\)H NMR (400 MHz, \(d_6\)-DMSO) δ (multiplicity, J (Hz), integration, position): 6.38 (d, 7.1 Hz), 6.88 (d, 8.3, H7), 6.93, (dd, 8.3, 7.1, H6), 7.27 (s, H2), 7.79 (s, H6'), 9.30 (s, OH), 11.29 (brs, NH); \(^{13}\)C NMR (100 MHz, \(d_6\)-DMSO) δ: 103.2, 103.8, 105.6, 113.0, 115.4, 122.6, 123.6, 138.6, 148.9, 151.2, 156.4, 163.6; ESI HRMS [M + H]\(^+\) calculated for \([C_{12}H_{10}BrN_4O]\)^+: 305.0033, found: 305.0033.

In a 3mm NMR tube, HCl gas was bubble through 3.102 (5 mg) in \(d_6\)-DMSO (200 µL) for 1 min to form 3.103. Because 3.103 is not stable, \(^1\)H and \(^{13}\)C NMR data were obtained immediately (see Table 3.2 for NMR data).
5.3.17 5-(5-(4-(tert-butyldimethylsilyloxy)-1-(triisopropylsilyl)-1H-indol-3-yl)-2-chloropyrimidin-4-yl)amine (3.104)

![Chemical Structure](image)

To a stirring solution of 3.88 (200 mg, 0.42 mmol) in dry THF (5 mL) at -78°C under N₂ was added dropwise tert-butyllithium (1.6 M in pentane) until the solution remained yellow then and additional aliquot (0.57 mL, 0.92 mmol) was added dropwise. The solution stirred for 15 min. Neat 2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.89, 155 mg, 0.83 mmol, 0.17 mL) was added dropwise. The mixture stirred for 1 h at -78°C and was quenched with sat. NH₄Cl. The mixture warmed to rt and was partitioned between Et₂O/sat. NH₄Cl. The aqueous layer was extracted 2X with EtO₂. The combined organic layers were dried (anhyd. MgSO₄), concentrated to afford crude 3.90 which was used immediately in the next reaction without further purification.

A stirring mixture of crude 3.90 (0.42 mmol), tetrakis(triphenylphosphine)palladium (49 mg, 0.04 mmol), and 4-amino-2-chloro-5-iodopyrimidine (3.99, 106 mg, 0.42 mmol), benzene (10 mL, degassed by sparging with N₂), methanol (2.0 mL, degassed), and aqueous sodium carbonate (0.5 mL, 2 M, degassed) was refluxed under N₂ for 24 h. The mixture was allowed to cool to rt, diluted with EtOAc and dried with anhyd. MgSO₄. The filtrate was concentrated onto silica and purified via MPLC (silica, eluting at 18% EtOAc:hexane) to afford 3.104 (123 mg, 0.23 mmol, 56% yield). Mp = 83-85 °C; ¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration, position): 0.10 (s, 6H, Si(CH₃)₂), 0.83 (s, 9H, SiC(CH₃)₃), 1.16 (d, 7.3, 18H, SCH(CH₃)₂), 1.71 (sept, 7.3, 3H, SiCH), 5.40 (bres, 2H, H2), 6.55 (d, 7.8, H5), 7.05 (dd, 7.8, 8.2, H6), 7.09 (s, H2), 7.16, (d, 8.2, H7), 8.02 (s, H6'); ¹³C NMR (100 MHz, CDCl₃) δ: -4.3 (2C), 12.8 (3C), 18.1 (6C), 18.2, 25.6 (3C), 107.7, 108.9, 109.1, 113.5, 121.6, 123.0, 130.2, 143.7, 149.4, 156.1, 158.6, 164.1; ESI HRMS [M + H]⁺ calculated for [C₂₇H₄₃ClN₂OSi₂Na]⁺: 553.2556, found: 553.2540.
To a stirring solution of 3.104 (35 mg, 0.066 mmol) in dry THF (1 mL) was added dropwise TBAF (123 µL, 1.0 M, 0.132 mmol). After 8 min. the solution turned brown and 2 M Na₂CO₃ (1 mL) was added. The mixture was then partitioned between EtOAc:H₂O. The organic layer was collected, dried over anhyd. MgSO₄ and chromatographed on MPLC (silica, eluting at 10% MeOH:DCM) to afford 3.105 (5 mg, 0.019 mmol, 29% yield). Alternative procedure: To 3.104 (80 mg, 0.15 mmol) in 1.0 mL acetonitrile (dried over 4Å molecular sieves) and 0.1 mL pyridine (dried over 4Å molecular sieves) was added 125 µL hydrogen fluoride-pyridine (70% as HF, 30% as pyridine). After 30 min. the starting material had completely dissolved. After 1 h, a precipitate had formed. The mixture stirred for 11 additional h at rt. The precipitate was collected via filtration and washed with acetonitrile. Drying the precipitate under high vacuum resulted in 3.105 as a tan powder (20 mg, 0.048 mmol, 32%). ¹H NMR (400 MHz, d₆-DMSO) δ (multiplicity, J (Hz), integration, position): 6.39 (d, 7.3, H5), 6.89 (d, 8.3, H7), 6.90 (vbrs, 2H, 4’NH₂), 6.92, (dd, 7.3, 7.8, H6), 7.27 (s, H2), 7.87 (s, H6’), 9.30 (s, OH), 11.28 (brs, NH); ¹³C NMR (100 MHz, d₆-DMSO) δ: 103.1, 103.8, 105.6, 112.6, 115.5, 122.6, 123.7, 138.6, 151.2, 156.4, 157.0, 164.0; ESI HRMS [M + H]⁺ calculated for [C₁₂H₁₀ClN₄O]⁺: 261.0538, found:261.0536.
5.3.19 meridoquin (3.106)

![Chemical Structure](image)

To **3.112** (62 mg, 0.136 mmol) in 1 mL dry THF under Ar and stirring was added TBAF (0.14 mL, 0.14 mmol, 1.0 M in THF). After 5 min., the reaction was complete. Sodium carbonate (1 mL, 2.0 M) was added and the mixture was partitioned between EtOAc/H₂O. The aqueous layer was washed 2X with EtOAc aliquots. The combined EtOAc extracts were dried over anhyd. MgSO₄ and purified via MPLC (silica, eluting at 35% EtOAc:hexane) to afford **3.106** (35 mg, 0.116 mmol, 86%). Mp = 166 °C; ^1H NMR (500 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 1.29 (t, 6.9, 6H), 3.75 (q, 6.9, 4H), 6.78 (dd, 5.3, 2.1), 7.24 (d, 8.6), 7.43 (dd, 2.1, 2.1), 7.83 (s), 8.27 (dd, 5.3, 2.1), 8.45 (d, 8.6), 8.60 (br s). ^13C NMR (125 MHz, CDCl₃) δ: 13.2 (2C), 42.1 (2C), 104.3, 111.2, 116.5, 121.9, 123.1, 124.2, 126.5, 128.7, 137.2, 156.9, 161.1, 161.7; ESI HRMS [M + H]^+ calculated for [C₁₆H₁₈ClN₄]⁺: 301.1215, found: 310.1220.

5.3.20 2-chloro-4-N,N-diethylaminopyrimidine (3.107) and 4-chloro-2-N,N-diethylaminopyrimidine (3.108)

![Chemical Structures](image)

At rt, neat diethylamine (2 mL) was slowly added to 2,4-dichloropyrimidine (3.98, 500 mg, 3.38 mmol). The mixture stirred for 1 min., was diluted with EtOAc and concentrated onto silica. Purification via MPLC (silica, gradient from 0 to 35% EtOAc:hexane) afforded 4-chloro-2-N,N-diethylaminopyrimidine (3.108, eluting at 12% EtOAc:Hex, 54 mg, 0.44 mmol, 13% yield) and 2-chloro-4-N,N-diethylaminopyrimidine (3.107, eluting at 26% EtOAc:hexane, 372 mg, 2.0 mmol, 59% yield).
4-(N,N-diethyl)-2-chloroaminopyrimidine (3.107): viscous colorless liquid, solidifies in freezer; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (multiplicity, J (Hz), integration): 1.18 (t, 7.0, 6H), 3.48 (br s, 4H), 6.26 (d, 5.9), 7.96 (d, 5.9); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 12.5, 42.4, 100.9, 156.5, 160.7, 161.7; ESI HRMS [M + H]$^+$ calculated for [C$_8$H$_{13}$ClN$_3$]$^+$: 186.0793, found: 186.0794.

2-(N,N-diethyl)amino -4-chloropyrimidine (3.108): viscous colorless liquid; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (multiplicity, J (Hz), integration): 1.19 (t, 7.1, 6H), 3.61 (q, 7.1, 4H), 6.44 (d, 4.7), 8.14 (d, 4.7); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$: 12.9, 42.0, 108.0, 158.7, 160.9, 161.0; ESI HRMS [M + H]$^+$ calculated for [C$_8$H$_{13}$ClN$_3$]$^+$: 186.0793, found: 186.0791.

5.3.21 3-bromo-6-chloro-1-(triisopropylsilyl)-1H-indole (3.110)

![Chemical Structure](image)

To 6-chloroindole (3.109, 1.0 g, 6.6 mmol) in 20 mL dry THF under stirring and N$_2$ was added dropwise $n$-butyllithium (5.6 mL, 1.6 M in hexanes, 8.91 mmol) at -78 °C. The mixture was warmed to -10 °C and stirred for 10 min. before being cooled back to -78 °C. Triisopropylsilyl chloride (1.78 g, 9.24 mmol, 1.96 mL) was added dropwise. The mixture was then warmed to 0 °C and stirred until reaction was complete (via TLC). The mixture was cooled to -78 °C. N-bromosuccinimide (1.59 g, 8.91 mmol) was added to the cooled mixture in one portion. After stirring for 2 h at -78 °C, the mixture was warmed to rt, diluted with 1% pyridine in hexane and filtered through Celite. The concentrate filtrate was purified via MPLC (silica; eluting in hexane) to afford white crystalline solid 3.110 (2.26 g, 5.87 mmol, 89% yield). $\text{Mp} = 52 ^\circ \text{C}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (multiplicity, J (Hz), integration): 1.15 (d, 7.6, 18H), 1.67 (q, 7.6, 3H), 7.18 (dd, 8.3, 1.4), 7.22 (s), 7.46 (d, 1.4), 7.48 (d, 8.3). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 12.7 (3C), 18.0 (6C), 93.6, 113.8, 119.9, 121.3, 128.6, 128.7 130.4, 140.4; ESI HRMS [M + H]$^+$ calculated for [C$_{17}$H$_{26}$BrCINSi]$^+$: 386.0701, found: 386.0698.
5.3.22 4-(6-chloro-1-(triisopropylsilyl)-1H-indol-3-yl)-N,N-diethylpyrimidin-2-amine (3.112)

To a stirring solution of 3.110 (200 mg, 0.52 mmol) in dry THF (5 mL) at -78 °C under inert atmosphere was added dropwise tert-butyllithium (1.6 M in pentane) until the solution remained yellow then and additional aliquot (0.350 mL, 0.57 mmol) was added dropwise. The solution stirred for 15 min. Neat 2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (194 mg, 1.04 mmol, 0.21 mL) was added dropwise. The mixture stirred for 1 h at -78 °C and was quenched with sat. NH₄Cl. The mixture warmed to rt and was partitioned between Et₂O:sat. NH₄Cl. The aqueous layer was extracted 2X with EtO₂. The combined organic layers were dried (anhyd. MgSO₄), concentrated to afford crude 6-chloro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(triisopropylsilyl)-1H-indole which was used immediately in the next reaction without further purification.

A stirring mixture of crude 6-chloro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(triisopropylsilyl)-1H-indole (0.52 mmol), tetrakis(triphenylphosphine)palladium (35 mg, 0.03 mmol), and 2-(N,N-diethyl)amino-4-chloropyrimidine (283 mg, 0.29 mmol), benzene (5 mL, degassed by sparging with N₂), methanol (1 mL, degassed), and aqueous sodium carbonate (0.25 mL, 2 M, degassed) was refluxed under N₂ for 2 h. The mixture was allowed to cool to rt, diluted with EtOAc and dried with anhyd. MgSO₄. The filtrate was concentrated onto silica and purified via MPLC (silica, eluting at 18% EtOAc:hexane) to afford 3.112 (82 mg, 0.179 mmol, 62%). Mp = 126 °C; ¹H NMR (400 MHz, CDCl₃) δ (multiplicity, J (Hz), integration): 1.18 (d, 7.4, 18H), 1.29 (t, 7, 6H), 1.72 (sept., 7.3, 3H), 3.76 (q, 7.3, 4H), 6.80 (d, 5.5), 7.22 (dd, 8.7, 1.8), 7.49 (d, 1.8), 7.88 (s), 8.26 (dd, 5.4), 8.46 (d, 8.7); ¹³C NMR (100 MHz, CDCl₃) δ: 12.7 (3C), 13.3
(2C), 18.06 (6C), 42.04 (2C), 104.3, 113.8, 118.0, 121.7, 122.8, 127.4, 128.1, 133.8, 142.4, 157.1, 161.2, 161.6; ESI HRMS [M + H]^+ calculated for \([C_{25}H_{38}ClN_4Si]^+\): 457.2549, found: 457.2569.

5.4 Experimental supporting Chapter 4

5.4.1 Isolation of cytochalasin D (4.17)

Lyophilized 2L scale up fermentation of CY 4202 was exhaustively extracted with methanol (3 X 24h). The combined, filtered methanolic extracts were concentrated under reduced pressure. A portion of this extract (2 g) was fractionated on silica MPLC (40g column, gradient: hexane→ethyl acetate→methanol) to afford fractions A-F, eluting in increasing polarity. A portion (50 mg) of antimalarial fraction C (250 mg) was further purified on HPLC (Sunfire Prep OBO, 10X250 mm) with an isocratic solvent system (1% MeOH:CHCl₃). A large UV active peak eluting at 4.4 min. was found to be cytochalasin D (18 mg). ESI-MS (m/z) [M+H] = 508.3; \(^1\)H and \(^{13}\)C NMR data are presented in comparison with literature values in Table 4.2.
5.4.2 Isolation of roridin E (4.18) and 12,13-deoxyroridin E (4.19).

Lyophilized 2L scale up fermentation of CY 3923 was exhaustively extracted with methanol (3 X 24h). The combined, filtered methanolic extracts were concentrated under reduced pressure. A portion of this extract (2 g) was fractionated on silica MPLC (40g column, gradient: hexane→ethyl acetate→methanol) to afford fractions A-F, eluting in increasing polarity. The antimalarial fraction D (52 mg) was further purified on HPLC (Sunfire Prep C-18, 10X250 mm) with an isocratic solvent system (30% water:MeOH, 0.1% TFA). A UV (254 nm) active peak eluting at 30 min. was found to 12,13-deoxyroridin E (4.19, 3.3 mg) but the major component of the mixture was not isolated and was thought to have degraded in the acidic solution. Another portion of the original extract (2g) was carefully fractionated on silica MPLC (40g column) with a gradual gradient (50% EtOAc:hexane→100%EtOAc). Fractions of a strong UV active (254 nm) peak that eluted at 90% EtOAc:hexane were analyzed via $^1$H NMR which revealed pure roridin E (4.18, 40 mg). $^1$H and $^{13}$C NMR data are presented in comparison with literature values in Table 4.3 (4.18) and Table 4.4 (4.19).
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$^{13}$C NMR spectrum (100 MHz, CD$_3$OD) of **2.51**
$^1$H NMR spectrum (400 MHz, CD$_3$OD) of **2.52**

$^{13}$C NMR spectrum (100 MHz, CD$_3$OD) of **2.52**
$^1$H NMR spectrum (400 MHz, CD$_3$OD) of **R-2.53**

$^{13}$C NMR spectrum (100 MHz, CD$_3$OD) of **R-2.53**
$^1$H NMR spectrum (400 MHz, CD$_3$OD) of $\text{S-2.53}$

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commercially available racemic 1,2,6-hexanetriol
(R)-1,2,6-hexanetriol (R-2.53)
(S)-1,2,6-hexanetriol (S-2.53)
palmerolide A ozonysis product 2.51

$^{13}$C NMR spectra (100 MHz, CD$_3$OD) of 1,2,6-hexanetriol
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(S)-1,2,6-hexanetriol (S-2.53)
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\(^{13}\)C NMR spectrum (100 MHz, CDCl\(_3\)) of 2.65
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$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 2.67
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$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 2.70
$^1$H NMR spectrum (400 MHz, CDCl$_3$) of 2.71

$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 2.71
$^1$H NMR spectrum (400 MHz, CDCl$_3$) of **2.72**

$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of **2.72**
$^1$H NMR spectrum (400 MHz, CDCl$_3$) of 2.73

$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 2.73
Coupling simulation of H8 and H9 reveals E-configuration.
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$^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of 3.86

$^{13}$C NMR spectrum (100 MHz, $d_6$-DMSO) of 3.86
$^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of 3.87

$^{13}$C NMR spectrum (100 MHz, $d_6$-DMSO) of 3.87
$^1$H NMR spectrum (400 MHz, CDCl$_3$) of **3.88**

$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of **3.88**
\( ^1H \) NMR spectrum (400 MHz, CDCl\(_3\)) of 3.91

\( ^{13}C \) NMR spectrum (100 MHz, CDCl\(_3\)) of 3.91
\textbf{1H NMR spectrum (400 MHz, CDCl$_3$) of 3.92}

\textbf{13C NMR spectrum (100 MHz, CDCl$_3$) of 3.92}
\(^1\)H NMR spectrum (400 MHz, \(d_6\)-DMSO) of 3.93

\(^{13}\)C NMR spectrum (100 MHz, \(d_6\)-DMSO) of 3.93
$^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of 3.95

$^{13}$C NMR spectrum (100 MHz, $d_6$-DMSO) of 3.95
$^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of 4-methoxymeridianin A (3.96)

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$^1$H NMR spectrum (400 MHz, CDCl$_3$) of 3.100

$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 3.100
$^1$H NMR spectrum (400 MHz, CDCl$_3$) of **3.101**

$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of **3.101**
$^1$H NMR spectrum (400 MHz, $d_6$-DMSO) of synthetic psammopemmin A (3.102)

$^{13}$C NMR spectrum (100 MHz, $d_6$-DMSO) of synthetic psammopemmin A (3.102)
gHSQC spectrum (400 MHz, d$_6$-DMSO) of synthetic psammopemmin A (3.102)
gHMBC spectrum (400 MHz, d$_6$-DMSO) of synthetic psammopemmin A (3.102)

(expanded)
$^1$H NMR spectra (400 MHz, d$_6$-DMSO) of
synthetic psammopemmin A (102) and
synthetic psammopemmin A·HCl (103)

$^1$H NMR spectra (400 MHz, d$_6$-DMSO, expansion) of
synthetic psammopemmin A (102)
synthetic psammopemmin A·HCl (103)
$^{13}$C NMR spectra (100 MHz, d$_6$-DMSO) of synthetic psammopemmin A (102) and synthetic psammopemmin A-HCl (103)

$^{13}$C NMR spectra (100 MHz, d$_6$-DMSO, expansion) of synthetic psammopemmin A (102)
synthetic psammopemmin A-HCl (103)
$^1$H NMR spectra (400 MHz, d$_6$-DMSO, expansion) of meridianin A from Synoicum sp. (3.24) and synthetic psammopemmin A·HCl (102)

$^{13}$C NMR spectra (100 MHz, d$_6$-DMSO, expansion) of meridianin A from Synoicum sp. (3.24) and synthetic psammopemmin A·HCl (102)
$^1$H NMR Comparison of meridianin A (24) and natural psammopemmin A (3.16)

$^{13}$C NMR Comparison of meridianin A (3.24) and natural psammopemmin A (3.16)
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$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 3.104
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$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 3.105
$^1$H NMR spectrum (400 MHz, CDCl$_3$) of 3.106

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$^{13}$C NMR spectrum (100 MHz, CDCl$_3$) of 3.107
$^1$H NMR spectrum (500 MHz, CDCl$_3$) of 3.108

$^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 3.108
$^1$H NMR spectrum (500 MHz, CDCl$_3$) of \textbf{3.110}

$^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of \textbf{3.110}
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$^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of roridin E (4.18)
$^1$H NMR spectrum (500 MHz, CDCl$_3$) of 12,13-deoxyroridin E (4.19)

$^{13}$C NMR spectrum (125 MHz, CDCl$_3$) of 12,13-deoxyroridin E (4.19)
About the author

Matthew Dennis Lebar, raised in Lakewood, Colorado, briefly attended the University of Northern Colorado before deciding it was time to get out of the state. A eye-opening vacation with family in high school to Hawaii combined with the Western Undergraduate Exchange program was all it took for Matt to realize he would continue his studies at the University of Hawaii, Manoa. With inspiration and a newly minted Bachelor of Science in Chemistry from UH, Matt decided it was again time to move on. Friends wanted to move to Florida and Matt had acclimated to warmer climates. Luckily, the University of South Florida Chemistry Department gave Matt a warm welcome and accepted him into the graduate program. The rest is history.

Matt conducted all research characterized in Chapters 2.3, 2.4, 3.3, and 3.4 of this dissertation with the exception of the biological activity screening. He was also instrumental in developing extraction, fractionation and isolation methods and protocols described in Chapter 4 and was responsible for all work described in section 4.4.3. All experiments described in the experimental section (Chapter 5) were carried out by Matt.

Outside the lab, Matt enjoys (in no particular order) baseball, hiking, family, snowboarding, his girlfriend, traveling, swimming, diving, cacti, reading, computers, islands (tropical), video games, friendships, brewery tours, and gin + tonics. He also fosters two cats, presently.