Total Synthesis of Isoprenoid Quinone Natural Products and their Biological Examination

A thesis presented

by

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Thesis acceptance form goes here
Dedicated to Sarah,

this simply wouldn’t exist without you.
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List of abbreviations

- **Ac** acetyl
- **AIBN** azobis(isobutyronitrile)
- **Ar** aryl
- **BOM** (benzyloxy)methyl or methoxybenzyl
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tbody>
<tr>
<td>iBu</td>
<td>isobutyl</td>
</tr>
<tr>
<td>nBu</td>
<td>n-butyl</td>
</tr>
<tr>
<td>sBu</td>
<td>sec-butyl</td>
</tr>
<tr>
<td>tBu</td>
<td>t-butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>benzoyl</td>
</tr>
<tr>
<td>CAN</td>
<td>Ceric Ammonium Nitrate</td>
</tr>
<tr>
<td>CuTC</td>
<td>copper(I) thiophene-2-carboxylate</td>
</tr>
<tr>
<td>dba</td>
<td>dibenzylideneacetone</td>
</tr>
<tr>
<td>DCE</td>
<td>dichloroethane</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DMAPP</td>
<td>dimethylallyl pyrophosphate</td>
</tr>
<tr>
<td>DMDO</td>
<td>dimethyldioxirane</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>EDG</td>
<td>electron-donating group</td>
</tr>
<tr>
<td>ESI-TOF</td>
<td>electrospray ionization-time of flight</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
</tr>
<tr>
<td>EWG</td>
<td>electron-withdrawing group</td>
</tr>
<tr>
<td>HMPA</td>
<td>hexamethylphosphoramide</td>
</tr>
<tr>
<td>HRMS</td>
<td>high-resolution mass spectrometry</td>
</tr>
<tr>
<td>hv</td>
<td>light</td>
</tr>
<tr>
<td>IBX</td>
<td>2-iodoxybenzoic acid</td>
</tr>
<tr>
<td>IPP</td>
<td>isopentenyl pyrophosphate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>KHMDS</td>
<td>potassium hexamethyldisilazide</td>
</tr>
<tr>
<td>LDA</td>
<td>lithium diisopropylamide</td>
</tr>
<tr>
<td>Ln</td>
<td>ligands</td>
</tr>
<tr>
<td>LG</td>
<td>leaving group</td>
</tr>
<tr>
<td>SN2</td>
<td>substitution, nucleophilic, bimolecular</td>
</tr>
<tr>
<td>M</td>
<td>metal</td>
</tr>
<tr>
<td>m-CPBA</td>
<td>meta-chloroperbenzoic acid</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>MeCN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>Ms</td>
<td>methanesulfonyl (mesyl)</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PCC</td>
<td>pyridinium chlorochromate</td>
</tr>
<tr>
<td>Pd/C</td>
<td>palladium on charcoal</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>iPr</td>
<td>isopropyl</td>
</tr>
<tr>
<td>PTLC</td>
<td>preparative thin-layer chromatography</td>
</tr>
<tr>
<td>py</td>
<td>pyridine</td>
</tr>
<tr>
<td>RCM</td>
<td>ring-closing metathesis</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetra-n-butylammonium fluoride</td>
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Abstract

The synthetic efforts covered in this thesis were embarked upon not just for their opportunity as challenging targets but also for their relevance to other fields. Molecular biology and biosynthesis motivated the pursuit of the merochlorins and promising anti-tumor activity inspired our work towards antroquinonol A. Both of these molecules belong to or derive from the isoprenoid quinone class of natural products. The merochlorin route purposefully pursued biosynthetically inspired intermediates while the antroquinonol route attempted to be amenable to analogue synthesis. The results of both synthetic efforts lent knowledge and understanding to biology while also delivering interesting syntheses of complex molecules.
CHAPTER 1

Introduction: the chemistry of isoprenoid quinones
1.1 Total synthesis and the life sciences

“What I cannot create, I do not understand.” –Richard Feynman

Richard Feynman’s parting words to the world are an oft-used rallying cry for synthetic chemists. The synthesis of urea in 1826 set off almost two hundred years of progress that has resulted in a modern synthetic chemistry that now seems to have no limits to the complexity of its targets. However, synthetic chemistry has never been and never will be solely concerned with targets based solely on the complex challenge they offer. Thanks to organic chemistry, the library of compounds available today has been used to discover medicines, reveal enzymatic mechanisms and develop everyday materials. The work summarized in this thesis is another collaborative entry into the vast number of syntheses that seek to increase understandings of other fields through the construction of natural products.

The story of chemotherapy often begins with the work of Sidney Farber and his use of anti-folates for the treatment of leukemia.\(^1\) Farber hypothesized that, if normal white cells depend on folic acid for growth, leukemia cells—engorged, overgrown white blood cells—may be even more dependent on folic acid. And so he proposed the use of an anti-folic acid, an antagonist of folic acid, to get remissions. Of course, this was not his first hypothesis. To begin, he proposed that leukemia could be caused by a deficiency in folate and thus sought to treat it with folic acid itself. The results were horrifying, with folic acid greatly increasing the rapidity and deadliness of his patient’s leukemia. Like most of the sciences, empirical evidence still reigned supreme in 1940s medicine. Little mechanistic understanding of chemistry and disease existed. His
response to this setback was a simple one: if folate made the leukemia worse, could an “anti-folate” be successful? He turned to his friend and unsung hero Yella Subbarao. Subbarao, who was the first to isolate and purify ATP, was working towards creating synthetic versions of the biomolecules he had spent his life studying. He had successfully synthesized folic acid (1.1) in an effort to sell it as a nutritional supplement (figure 1.1). However, as with all good synthetic routes, he had access to hundreds of folate analogues and it was these divergent products that Farber was after. With a synthetic library borrowed from Subbarao, Farber would go on to discover the first known chemotherapy, aminopterin (1.2). This story would be repeated many times as libraries of compounds from the dye and textile industries would be used to discover more and more treatments.
Stories of this nature continue to be told in the last few years, stories where synthetic chemists shed light on the structure and mechanism of medicinally relevant compounds. In 2012, chemists revealed that vendors meant to be supplying the leukemia drug bosutinib (1.3) were mistakenly supplying an incorrect isomer (1.4).\(^3\) This revelation potentially invalidated a significant amount of work on the biology and medicinal properties of molecule. In addition, in 2014 the Janda group pointed out that a mistake in structural analysis of the potential anti-cancer drug, TIC10 (1.6), had allowed an incorrect isomer (1.5) to be patented and almost brought to human trails.\(^4\)

The synthetic efforts covered in this thesis were embarked upon not just for their opportunity as challenging targets but also for their relevance to other fields. Molecular biology and biosynthesis motivated the pursuit of the meroclorins and promising anti-tumor activity inspired our work towards antroquinonol A. Both of these molecules belong to or derive from a particular class of natural products: isoprenoid quinones.

1.2 Quinone-derived natural products and their bioactivity

Quinones are ubiquitous amongst living systems, being found widely distributed in the natural world within bacteria, plants and animals.\(^5\) Quinones and their derivatives play a significant role in many biological processes from phosphorylation to electron transfer.\(^6\) A large number of chemical derivatives with 1,4-benzoquinone as the basic subunit exhibit prominent pharmacological applications such as antibiotic,\(^7\) antitumor,\(^8\) antimalarial,\(^9\) antineoplastic,\(^10\) anticoagulant\(^11\) and herbicidal activity.\(^12\) The copiousness
Figure 1.2 Classes of quinone containing natural products.

of articles describing the aforementioned multi-functional aspects serves as a grand testimony to the contemporary interest in quinone chemistry. The particular class of quinones investigated here are the isoprenoid quinones.

Isoprenoid quinones are amphiphilic, in that they are composed of a polar head group, which is quinone-derived and a hydrophobic side chain, which is terpene-derived (figure 1.2). The lipid-soluble isoprenoid side chain anchors them in membrane lipid bilayers, whereas the hydrophilic quinone head group enables interaction with hydrophilic sites of proteins. It is generally accepted that long-chain, isoprenoid quinones localize in the hydrophobic mid-plane region of the lipid bilayer, whereas the polar head can oscillate between mid-plane and polar interphase of the membrane.\(^\text{13}\)
These compounds are grouped into the naphthoquinones (1.1 and 1.2) and anthracyclines (1.3), where the quinone group is fused to another ring and finally the benzoquinones (1.4-1.7). Notable amongst the isoprenoid polycyclic quinones is doxorubicin (1.3), which is a widely used as a front-line chemotherapeutic agent. Coenzyme Q10 (1.4) (CoQ10, also called ubiquinone) is a well-studied molecule of the isoprenoid benzoquinone class. Coenzyme Q10 is a nutrient found in humans and is a vital tool in the electron shuttling chain of human respiration. Finally, there is a large and structurally interesting class of isoprenoid benzoquinones with rearranged structures (1.8-1.11). All of the compounds shown in this class have received significant interest form the synthetic community due to their complex structures but also due to their bioactivity.

Quinones have a number of modes of activity in biological systems; the accumulated data about the biological activity of quinone moieties suggest redox processes and/or Michael-type addition-elimination reactions (scheme 1.1). As mentioned before, quinone reactivity is largely based on their ease and reversibility of reduction. Scheme 1.1 shows the two-electron reduction of coenzyme Q10 (1.4) and tocopherolquinone (1.5), important and representative essential nutrients to humans. NAD(P)H occupies the binding site of QR1 and transfers its hydride to FAD, the resulting NAD(P)+ leaves the site to be replaced by the quinone substrate. Hydride transfer from FADH2 (1.14) results in reduction of the quinone.

Anthracyclines, such as doxorubicin (1.3), can have a number of different mechanisms responsible for their cytotoxicity. In general, these compounds have an affinity for DNA due to their ability to intercalate based on their flat and aromatic structure. Scheme 1.1 shows a representative example of the reactivity of semi-quinone
Scheme 1.1 Quinone bioactivity mechanisms of action.

2 Electron Reduction of Quinones by Quinone Reductase 1

Coenzyme $Q_{10}$ (1.4) $\rightarrow$ ubiquinol (1.12) + 2 $\sigma^\prime$, 2 H$^+$

$\alpha$-tocopherolquinone (1.5) $\rightarrow$ vitamin E (1.13)

QR1 $\rightarrow$ FADH$_2$ (1.14)

Anti-mitotic Activity from Single Electron Reduction of doxorubicin

Doxorubicin (1.3) $\rightarrow$ P450 Reductase $\rightarrow$ semiquinone radical (1.15) $\rightarrow$ oxygen free radicals $\rightarrow$ DNA/RNA alklation (1.16) $\rightarrow$ DNA alkulation (1.17) $\rightarrow$ strong DNA intercalating agent

DNA Cross-linking via Quinone-methide Intermediate

Mitomycin (1.7) $\rightarrow$ mitomycin (1.18) $\rightarrow$ DNA alklation (1.19) $\rightarrow$ DNA alklation (1.21) $\rightarrow$ DNA alklation (1.22) $\rightarrow$ Crosslinked DNA Adduct (1.23)

Also: pleurotin (1.11)
radicals (1.15) generated by single electron reduction. These radical species can generate reactive oxygen species that can go on to damage DNA or can directly alkylate DNA themselves.

Bioreductive drugs are a range of compounds that are inactive in their administered form, but upon metabolic reduction are transformed into a cytotoxic species. Mitomycin C (1.7) is archetypal example of this class of compound. After reduction by QR1 to the hydroquinone form (1.18), reactive Michael-type electrophilic centers are generated at C-1 and C-10 leading to cross-linking of DNA.14

1.3 Quinone synthesis: natural and synthetic

The described bioreactivity, leading to medicinal applications, makes quinones an important class of compounds. In addition to this, quinones have served as valuable building blocks in organic chemistry and for this reason much research has been conducted on efficient and novel routes towards their syntheses. Interest in quinone synthesis has been observed since 1836, when the first and most common quinone, benzoquinone (1.25) was synthesized by the Liebig laboratory via oxidation of natural quinic acid (1.24).16 Scheme 1.2 summarizes a number of synthetic methodologies to access quinones from a diverse range of starting materials. The most common route taken to quinones is the oxidation of substituted benzene rings and the most frequently used are silver oxide, manganese oxide, nitric acid, salcomine/O2, chromium oxidants, selenic anhydride, ceric ammonium nitrate (CAN) and DDQ.5 Scheme 1.2 shows the production of quinones by oxidation of phenols (1.29)17, and anilines (1.32)18 via both single electron oxidant
Scheme 1.2 Synthetic methods for quinone formation.

Quinone Synthesis: Oxidation

![Chemical structures showing quinone formation through different oxidation methods.]

(Fremy’s salt) and a transition metal oxidant (Pb(OAc)$_4$). In addition to this, 4-alkyl-substituted phenols can also be oxidized to quinones with concomitant 1,2-shift of the 4-subsituted group (1.27).$^{19}$

A number of strategies for quinone formation are based around the formation of the aromatic ring from smaller precursors. The Harrity$^{20}$ group employed a Wolff-Dotz
benzannulation reaction between alkynlboronic esters and fischer carbenes $^{1.33}$ to access boron-substituted quinones $^{1.34}$ after oxidation of the resulting hydroquinone. The Liebeskind$^{21}$ and many other groups employed strategy for ring expansion of cyclobutenones. The alkyne-substituted cyclobutenones $^{1.36}$ undergo a thermal pericyclic ring opening and subsequent electrocyclic ring closing to afford a range of substituted quinones. Langer and co-workers$^{22}$ reported the synthesis of functionalized $p$-benzoquinones based on [3+3] cyclizations of 1,3-bis-silyl enol ethers with 2-acyloxy-3-(silyloxy-2-en-1-ones) $^{1.38}$. Deprotection and oxidation of the products afforded the benzoquinones $^{1.39}$. Finally, Mathur$^{23}$ reported a photochemically promoted one-step synthesis of bis-vinyl-1,4-benzoquinones. Irradiation of vinylalkyne $^{1.40}$ with CO in the presence of Fe(CO)$_5$ yielded the desired 2,5 and 2,6-substituted quinones.

The bioxyntheses of certain vital quinones are well studied and are summarized in scheme 3. CoQ$_{10}$ $^{1.52}$ and menaquinone-8 $^{1.1}$ are representative examples of quinones that are synthesized via the biomolecule shikimate $^{1.42}$. In both cases, a series of eliminations results in a lowly-substituted aromatic ring. In the case of the naphthoquinone 1.1, the second aromatic ring is accessed via an alkylation and subsequent condensation of 2-oxo-glutarate to yield the naphthohydroquinone $^{1.45}$. The most well studied enzyme of this pathway is the class of prenyltransferases that are responsible for attaching pyrophosphate-activated isoprene groups to the aromatic rings. The isoprene substituted aromatic groups $^{1.48}$ and $^{1.46}$ then undergo a series of substitutions to install the methyl and oxygen groups found in the final natural
Scheme 1.3 Proposed quinone biosynthesis.

products (1.1 and 1.52).

The proposed biosynthesis of mitomycin C (1.7) shown in scheme 3 is demonstrative of quinone natural products that incorporate sugar precursors. The quinone moiety is derived from the molecule 3-amino-5-hydroxybenzoic acid (AHBA 1.59). The condensation of AHBA with D-glucosamine results in most entire scaffold of the natural product. Subsequent oxidation of the phenol to the quinone and functional group manipulations results in mitomycin C (1.7). An observation we can make from these examples is that most of the molecule is assembled before the formation of the quinone itself, perhaps to circumvent problems arising from unpredictable quinone reactivity. The final biosynthesis shows in scheme 1.3 is demonstrative of quinones that arise from polyketide precursors, which is the case for many of the anthracycline family of quinones. Doxorubicin (1.3) is shown to arise via a series of ketone reductions and condensations that form the tetracyclic core.

1.4 Isoprenoid quinone reactivity explored through total synthesis

The three most relevant quinone reactivities to this thesis are demonstrated in scheme 1.4: prenylation, substitution and Diels-Alder reactivity. First discussed is the synthesis of the previously mentioned molecule CoQ₁₀ (1.4). This synthesis by Lipshultz emphasizes the challenges that prenylation affords a chemist. Biosynthetic prenylations occur via electrophilic activation at the carbon that will form the new bond. However, alkylation with a nucleophilic aromatic group can result in a multitude of side reactions, including Sn2’ substitution and E2 elimination at either of the other two allylic carbons.
To circumvent this, Lipshultz and coworkers inverted the nucleophile/electrophile relationship, synthesizing a terpene-chain precursor containing a nucleophilic vinylaluminum species (1.69) and an electrophilic benzylic chloride (1.66). The combination of these two reactants was high yielding and efficient, leading to a route towards 1.4 that could produce significant amounts of the natural product. The synthesis

**Scheme 1.4** Quinone reactivity explored through total synthesis.
of ilimaquinone by Theodorakis\textsuperscript{27} and coworkers highlights the nature of quinone substitution chemistry. Simple 1,4-benzoquinone is appended to the terpene side chain via a photo-mediated Barton decarboxylation and radical addition across the quinone double bond. This proclivity of quinones to react with radicals has been well documented.\textsuperscript{28} Next, NaOMe addition and reoxidation produced the regiochemistry found in 1.72. This regiochemistry arises from the greater electrophilicity of the carbon opposite of the sulfur group. To understand this, it is helpful to imagine the presence of a heteroatom (sulfur in this case) $\beta$ to a ketone-like carbon of a quinone resulting in reactivity that is more akin to a vinylogous ester (this functionality is highlighted in red for clarity). Vinylogous esters are less electronegative than simple enones and thusly; the NaOMe reacts with this moiety. Finally, transesterification of the resulting vinylogous esters results in the two natural products shown (1.73 and 1.74).

The multitude of rearranged isoprenoid quinones offer great insight into the reactivity quinones, the most common strategy of which is that of their proclivity for electrocyclization reactions. This is because, apart from their electron-deficient nature, which makes them apt partners for electron-rich or electron-neutral dienes, quinones also contain useful chemical functionality that can form the basis for further transformations after the Diels–Alder reaction.\textsuperscript{29} We can see an example of this in the synthesis of colombiasin A (1.10) by Rychnovsky\textsuperscript{30}. Bicycle 1.77 is furnished via a [4+2] cycloaddition that occurs selectively on the unsubstituted side of the starting quinone 1.78. Subsequent manipulations restore the quinone moiety, which is used in another [4+2] cycloaddition, this time in an intramolecular fashion, to furnish the natural product colombiasin A (1.10). In a synthesis by Harrowven and coworkers\textsuperscript{31}, the same intermediate is accessed in a
shorter sequence by using the ring expansion of the cyclobutenone \(1.76\) that was highlighted in scheme 2. While thermal conditions also yielded colombiasin A, Harrowven found that he could access the interesting 3.2.1 bicyclic structure of elisapterosin A (1.9) via a Lewis-acid promoted cyclization.

Taken together, these syntheses paint the picture of both the history and state-of-the-art chemistry employed to synthesize isoprenoid quinone natural products. The work in this thesis rests entirely on the significant progress achieved by these synthetic chemists.

1.5 References


CHAPTER 2

Syntheses of the merochlorins and their biological study
2.1 Introduction

Polyhalogenated benzo- and naphthoquinones natural products have been found to be potent inhibitors of plant and bacterial ureases.\(^1\) As Ashiralieva and Kleiner have shown, the halogen itself is at least in part responsible for inhibitory activity as the activity decreased considerably when halogens were replaced with other functional groups.\(^2\) The polyhalogenated quinones are often members of the meroterpenoid natural product class. Many members of this family have shown promising preclinical biological activity. Four new members of this class were isolated in 2012 by the groups of Prof. Bradley Moore and Prof. Gerwick.\(^3\) These new molecules were painstakingly isolated from a new species of bacteria isolated from the ocean floor in La Jolla, California and were named merochlorin A-D (2.1-2.4).

**Figure 2.1** Isolation and bioactivity of the merochlorins.
The structures, found in figure 2.1, demonstrate an interesting mix of complexity and biological activity. Each natural product is comprised of a sesquiterpene side chain and an aromatic, bicyclic core. In each case however, significant rearrangement has occurred either via cyclizations, as in the case of merochlorins A (2.1) and B (2.2), or through alkylations and oxidations as is the case for merochlorins C (2.3) and D (2.4). In addition, the sesquiterpene side chain in each of these molecules contains a rare isoprene connectivity where the C2 carbon of isoprene is attached to the head of another isoprene.4 We have dubbed this torso-to-head connectivity as opposed to the more traditional head-to-tail connectivity. The monoterpenes lavandulol and lavandulyl acetate (both C10), isolated from the eponymous lavender oil, exhibit a similar C(1′)–C(2) branching as that observed in the merochlorin terpene moieties, albeit with different double-bond arrangements.4c These small and complex molecules, densely functionalized with chlorines and oxygens, make for interesting synthetic targets in their own right. However, our work towards the syntheses of these molecules did not begin due to their structures but instead due to their biosynthetic proposal and promising activity. Most exhilarating perhaps was the fact that merochlorin A possessed potent antibiotic activity against a number of drug resistant strains of bacteria3a.

Before we began our synthetic work, the Moore group had already begun investigating the biosynthetic hypothesis for the merochlorins from a gene mining approach.3b They identified a gene cluster containing a number of enzymes believed were responsible for assembling the merochlorins. Most interesting of which was a new class of vanadium-dependent haloperoxidase that they believed was responsible for both the introduction of chlorine atoms and also for the interesting rearranged structures of A and
B, which they named Mcl24. However, they were unable to isolate significant amounts of their proposed intermediates from the bacteria. Because of this, they sought a collaboration with our group so that we might apply our knowledge of synthetic chemistry to assist in elucidating their molecular biology proposals.

The Moore group’s original biosynthetic proposal is shown in scheme 1. Starting with the melanin precursor tetrahydroxynaphthalene (THN, 2.5), they proposed that a prenyltransferase, Mcl23, appended the sesquiterpene side chain to produce a planar, highly unsaturated molecule we have named pre-merochlorin (2.6). From this single precursor, the vanadium-dependent haloperoxidase enacts a series of chlorinations leading to the highly unusual proposed intermediates 2.7 and 2.8. SN2 alkylations then deliver both merochlorins A and B. Merochlorins C and D are proposed to arrive via a

Scheme 2.1 Original merochlorin biosynthetic hypothesis.
initial oxidation of the THN starting material to deliver the pentahydroxy intermediate 2.9. This new oxidation pattern results in the prenyltransferase delivering the terpene side chain at a different carbon atom than before. A series of oxidations and chlorinations then deliver the remaining two natural products of this family.

While our work on the merochlorins was still progressing, a number of syntheses from other groups were published while another was published shortly after.8-10 These routes and the conditions used were similar to our own and therefore a publication was not pursued for the later half of this work.

2.2 Meroterpenoid synthetic precedence and retrosynthesis

The literature on isoprenoid quinones is rich with outstanding syntheses.11 Of particular inspiration for our work was Scott Snyder’s synthesis of napyradiomycin A1.12 This natural product is another chlorinated meroterpenoid and it shares many of the same structural characteristics as the merochlorins. Namely, it shares a THN (2.5) derived aromatic core with appended and subsequently rearranged chlorinated terpene side chain.

The synthesis begins with the oxidation of THN (2.5) to the molecule flaviolin (2.11). This simple aromatic species already contains every oxygen atom in the final natural product.13 Flaviolin was then reacted with methyl crotonaldehyde under acid catalysis to give the ABC-ring (2.12) system via a tandem Knoevenagel/6-π-electrocyclization. They then developed one of the first highly enantioselective chlorination procedure by using a chiral binol derivative ligand and borane, producing the chlorohydrin 2.13 in an enantiomeric excess of 93%.14 They installed the remaining two isoprene groups in a multi-step process by first appending a two carbon aldehyde-containing unit through a Johnson-
Claisen and subsequent reduction (2.14) followed by a Wittig reaction with the phosphonium salt 2.15. A chlorination and pair of deprotections completes the synthesis of \((-\)-napyradiomycin (2.16).

**Scheme 2.2** Snyder’s total synthesis of \((-\)-napyradiomycin A1.

We drew much inspiration from the use of the highly oxidized flaviolin molecule as a starting point. By exploiting the reactivity of this naphthoquinone, Snyder and coworkers could quickly get to the key chlorination steps of their synthesis instead of wasting time on complex oxidations of complex intermediates. However, the presence of these oxygens required the use of multiple protecting groups and resulted in four additional steps used to install and remove these. In our own oxygen rich molecules, merochlorin A-D, we were inspired to seek a route that would use chemistry that is tolerant of unprotected phenol groups.\(^{15}\) In addition, the installation of the geranyl sidechain required 5 steps to complete. Their intermediate was not conducive to a simple alkylation with the readily available farnesylbromide and therefore required a significant investment of time for a work around. This inspired us to seek a route that would simplify the installation of the terpene sidechain by using their readily accessible electrophilic variants.\(^{16}\)
With previous syntheses of related natural products and the biosynthesis in mind, we began to form our synthetic plan in the form of the retrosynthesis shown in scheme 2.3. Our synthetic goal was collaborative in nature. Not only did we seek an efficient synthesis that could prepare the natural products and analogs for bioactivity testing, we also sought to go through biomimetic intermediates that could be used to further elucidate the biosynthesis of the merochlorins.

With this in mind, we proposed that both merochlorins A and B (2.1 and 2.2) could be accessed directly through an oxidative chlorination of the proposed putative intermediate pre-merochlorin (2.6). Echoing a vast amount of work on biomimetic syntheses,17 we sought to take advantage of the potential natural proclivity of the intermediate to react under oxidative conditions to produce the natural products. In proposing this, we thought we could escape the need for any protecting groups in the final stages due to the fact that nature had prepared the molecule for this particular transformation without protecting groups. However, our proposed mechanism for this transformation differed significantly from the Moore’s group proposal. Instead of a stepwise process involving the chlorination of the olefins of the terpene side chain, we thought the natural products would arise from oxidative [5+2] cycloadditions, involving the C2 carbon atom in the case of 2.1 and the C3 oxygen atom in the case of 2.2. Similar reactions had been used to form 3.2.1 bicycles in the past.18,19

Another departure from the proposed biosynthesis was the idea that it may be possible to access the carbon skeleton of merochlorins C and D (2.3 and 2.4) through the same intermediate: pre-merochlorin (2.6). This idea was inspired by the oxidative 1,2-alkyl shift methodologies detailed in chapter 120 and also from other biosynthetic proposals.21
Thus, the peroxide intermediate \textbf{2.7} would subsequently undergo the 1,2-alkylshift, delivering the terpene side chain to the C3 position and elimination of the C3 oxygen atom would restore aromaticity.

**Scheme 2.3** A biomimetic retrosynthesis of the merochlorin family.

With routes planned to merochlorins A-D all arriving through the proposed intermediate \textbf{2.6}, it became even more important for an efficient plan for its synthesis. With lessons learned from other syntheses, we sought to ensure that our terpene side chain would be appended to our aromatic core as a readily accessible allylic electrophile instead of any of the complicated means for producing a nucleophilic allylic center. Luckily for us, we reasoned that THN (\textbf{2.5}) would be most nucleophilic at the C4 position and thusly could be reacted directly with an appropriate electrophile to produce the carbon-carbon bond at the desired location. Without protecting groups, we knew there would be potential
challenges of O-alkylation but enough methods existed to favor C-alkylation that we decided to move forward with a protecting group free synthetic plan.\textsuperscript{16}

Finally, we were left with the unusual torso-to-head connected sesquiterpene \textit{2.8}. To limit the number of steps we planned to access this molecule from the commercially available \textit{trans,trans}-farnesol (\textit{2.9}). This would allow us to focus on the key torso-to-head connectivity and give us our desired alkene stereochemistries. The methods we pursued for producing this uncommon terpene connectivity are explored in the next section.

\subsection*{2.3 Synthesis of the terpene side chain}

All the routes pursued towards the terpene side chain involved the alkylation of farnesyl bromide. However, each route varies in the method by which the \textit{gem}-dimethyl tetrasubstituted olefin was produced. Our initial efforts focused on using a Horner-Wadsworth-Edmonds (HWE) reaction with acetone to form the \textit{gem}-dimethyl moiety. The sodium hydride promoted alkylation between farnesyl bromide (\textit{2.11}) and the two-carbon unit phosphonate (\textit{2.10}) proceeded smoothly to afford the HWE precursor (\textit{2.12}) in 89\% yield. The HWE reaction, conducted with sodium ethoxide and acetone, was more problematic. It resulted in the desired enoate (\textit{2.13}) in a unoptimized 32\% yield but with difficulties in purifying the non-polar starting material, impurities and products leading to decreased yields. We were able to conduct the subsequent DIBAl-H reduction of the enoate to produce our desired triene, isosesquilavandulyl alcohol (\textit{2.14}), but we did not pursue the scale
Scheme 2.4 Synthesis of the terpene side chain: isosesquilavandulyl alcohol.

up and optimization of this route due to the low yield and difficulty in purification.

Next we pursued a method of installing the gem-dimethyl group through a cross coupling of a methyl group and an enol phosphate.\(^{22}\) We prepared our substrate for this conjugate addition by again alkylating farnesyl bromide (2.11) but this time using ethyl acetoacetate (2.15). Deprotonation of the β-ketoester (2.16) and subsequent trapping of the enolate with ethyl chlorophosphate resulted in our desired enoate (2.17). To our disappointment, we were unable to form the gem-dimethyl group through this coupling reaction strategy in more than 10% yield. Rather than continue optimizing this route, we were inspired to pursue a shorter and more efficient strategy.
Based on work done on the alkylation of allenoates,\textsuperscript{23,24} we imagined the gem-dimethyl group arising from the methyl cuprate conjugate addition to a methyl-substituted alkyanoate (2.18). The most attractive part of this idea was that we could perhaps trap farnesyl bromide with the resulting allenoate, giving us the entire terpene carbon skeleton in a single step. We were pleased to see that the transformation went cleanly and produced the desired carbon skeleton in a fantastic 91% yield with no discernible by products to complicate purification. After reduction with DIBAl-H, we began preparing a series of leaving group activated versions of our side chain. The alcohol (2.14), bromide (2.20), acetate (2.21) and carbonate (2.22) would all be prepared without complications and used to investigate the alkylation of THN (2.5).

### 2.4 The synthesis and study of pre-merochlorin

With the terpene side chain in hand, we looked towards combining the naphthalene aromatic core to complete pre-merochlorin. We used the procedure developed by Snyder and coworkers\textsuperscript{12} to produce multi-gram quantities of THN (2.5) via an alkali fusion reaction of the sulfonate salt (2.24) and hydroxide salts at 275 C. Scheme 5 shows this reaction and provides a picture of the eutectic salt bath we used to achieve these high temperatures in the lab.

With multi-gram quantities of both THN and the side chain available to us, we began pursuing their union. Multiple strategies were pursued using one of the multiple activated side chains (2.14-2.22). Simple Friedel-Crafts reaction of the allylic bromide (2.20) and THN lead to a complicated mixture from which no desired pre-merochlorin could be isolated. Simple activation of the allylic alcohol (2.14) with BF\textsubscript{3} Et\textsubscript{2}O gave trace
Scheme 2.5 Chemical synthesis of premerochlorin.

amounts of the intermediate but was never brought to an acceptably high yield. Next we considered using Tsuji-Trost chemistry in order to form our desired carbon-carbon bond. Tamaru and coworkers had developed a method for directly alkylation naphthalenes with allylic alcohol using a palladium catalyst and in situ activation of the alcohol with a Lewis acid.\(^\text{25}\) This method delivered pre-merochlorin (2.6) in a respectable yield. However, using the carbonate (2.22) in place of the free alcohol, we were able to produce pre-merochlorin in a 44-66% yield using the same conditions.

We found pre-merochlorin to be somewhat unstable to air, as it would slowly decompose over the course of a day if left exposed to air. In addition, purification was difficult as the compound seemed to react in the presence silica gel if care was not taken to purify the compound quickly. These complications, while surmountable, likely lead to our relatively modest yield that seemed to vary with each reaction. Nonetheless, with pre-merochlorin in hand, we began our efforts to use our synthetic intermediate to gain greater understanding of the merochlorin biosynthesis.
Thusly, we delivered over 100 mg of pre-merochlorin to our collaborators in the Moore lab and with this compound they were able to shed significant light on their biosynthetic hypothesis.26 First, they developed a mutated version of the meroclorin producing actinomycete bacteria (Strain CNH189) that was a knockout for the proposed prenyltransferase enzyme Mcl23. This strain did not endogenously produce meroclorins A and B due to the fact that it could not synthesize pre-merochlorin (2.7) by attaching the terpene side chain to THN. However, when CNH189 was exposed to synthetic pre-merochlorin (2.7), the synthesis of both A and B was rescued. This led us to believe that our biosynthetic hypothesis, where pre-merochlorin was the key intermediate, was correct.

The biosynthesis of the terpene side chain was also investigated. The proposal, shown in figure 2.2, was a direct attack of the C2 carbon of dimethylallyl pyrophosphate (DMAPP 2.26) on the pyrophosphate containing carbon of geranyl pyrophosphate (GPP

Figure 2.2 Biosynthetic mechanism studies of premerochlorin.
promoted by the enzyme mcl22. To explore the role of mcl22 in merochlorin biosynthesis, our collaborators deleted the gene in a heterologous Streptomyces host strain expressing the mcl gene cluster. The mutation completely abolished the production of merochlorin derivatives. We then tested Mcl22 activity in enzymatic assays using GPP and DMAPP as substrates, followed by RP-HPLC analysis (figure 2A). Gratifyingly, a single product was formed (2.28) and confirmed by comparison to our synthetic version. This intriguing reaction may either involve stabilization of a transiently formed carbocation at C3 of DMAPP, or alternatively, could be initiated at the same position by attack of a nucleophilic amino acid residue (e.g., a cysteine thiolate).

Kinetic studies on mcl22 point towards it being a magnesium dependent prenyl synthase. Figure 2.3B shows that the addition of MgCl₂ to the mcl22 reaction shown in scheme 6 boosted the enzymatic activity nearly fourfold to (850 ± 20) mU·mg⁻¹ (kcat of 20.3 min⁻¹), whereas the metal-chelating agent ethylenediaminetetraacetate (EDTA) completely abolished product formation. These results are in line with the common Mg²⁺ dependency of prenyl diphosphate synthases.

Figure 2.3 Studies on the biosynthesis of the terpene side chain.
The synthetic intermediates produced on our path towards meroclorin successfully lent clarity to the biosynthetic origin of the meroclorin family. Moving forward, we sought a simple chemical means to convert the now confirmed biosynthetic intermediate premerochlorin \( (2.6) \) into each of the four meroclorins.

### 2.5 Oxidations and chlorinations of premerochlorin

With premerochlorin in hand, we sought to develop the key \([5+2]\) oxidative cyclization reaction of pre-merochlorin. If our hypothesis was correct, and the cyclizations were not promoted by an alkene-chloronium interaction, as was proposed by the Moore group, non-chlorine containing oxidants should be able to form des-chloro versions of the meroclorins.

A representative table of oxidants and conditions is shown in table 2.1. It was quickly realized the pre-merochlorin was very sensitive to oxidation and that even exposure to air and silica gel would produce complex mixtures of oxidized products. Most oxidants were unsuccessful due to the fact that they produced a complex mixture of products out of which nothing could be isolated (entries 4-9, Table 2.1). Optimized conditions were developed that yielded des-chloromeroclorin A \( (2.29) \) in moderate yields based on the work of Jason Green and Tom Pettus.\(^{18}\) Thusly, Pb(OAc)\(_4\) yielded both \( 2.29 \) and \( 2.30 \) in a ratio of 8:1 favoring the 3.2.1 bicyclic structure of meroclorin A. Interestingly, conditions using PhI(OAc)\(_2\) yielded predominately \( 2.30 \) however in significantly lower yields. There was no discernible pattern or explanation for these selectivities. A concurrent study to our work would show that protecting group changes on premerochlorin could control the path of cyclizatoin.\(^9\)
Table 2.1 Oxidative cyclizations of premerochlorin

In no case was the 1,2-alkyl shift product (2.31) observed. This seems to agree with the knockout studies shown in scheme 2.6 where premerochlorin only was able to rescue the synthesis of merochlorins A and B, rather than C and D. Therefore, our proposal that all four merochlorins could arise from a the premerochlorin molecule seems refuted. However, other unpredicted oxidative cyclizations did occur. Most notably, we isolated the structure 2.32 where the [5+2] cyclization has occurred on the ring not containing the terpene side chain forming a very unusual 7-memebered ring. This structure was confirmed by 1D and 2D NMR analysis.
With the help of the lab of Floyd Romesberg, we tested the bioactivity of the des-chloromerochlorin A (2.29). If this more simple structure could still exhibit the promising antibiotic activity against medically resistant strains of bacteria, it could be of significant interest to the medical field. We were gratified to learn that des-chloromerochlorin A (2.29) had similar minimum inhibitory concentrations (MIC) against *S. aureus* NCTC 8325 and MRSA N315 strains of 4 μg/mL when compared to merochlorin A (2.1).

Although we had a comparably bioactive compound in 2.29, we still sought the completion of our chemical synthesis of the merochlorins. Our investigations into the chlorination of premerochlorin are detailed in table 2.2. It was quickly found that most chloronium containing conditions proved only to yield complex mixtures out of which nothing could be isolated (entries 1-4 and 9-15). However, with authentic samples of the merochlorins in hand, our collaborator Dr. Stefan Dielhelm in the Moore lab was able to identify a large number of the molecules present in some of these mixtures. He found that when premerochlorin was exposed to NCS in the presence of a base (entries 6 and 7), a number of structures with the desired mass could be isolated which included the desired natural products merochlorins A and B. Specifically with the additive i-Pr₂NH, a mixture of 8 different compounds were identified and isolated in a cumulative yield of 30%.
Table 2.2 Chlorination of premerochlorin.

<table>
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<th>Entry</th>
<th>Chlorinating Agent</th>
<th>Additive</th>
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<th>Products Detected</th>
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<td>CDCl₃</td>
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<td></td>
<td>THF</td>
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<tr>
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<td>K₂CO₃ (100 equiv)</td>
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<tr>
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<tr>
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</table>
mixture included the deschloro and natural merochlorin A and B structures, with the natural products unfortunately being only a small percent of the mixture. In addition to this, chlorine isomers (2.30 and 2.32) and over chlorination products (2.31 and 2.32) were also identified.

Chemical mimics of the vanadium catalyzed enzymatic process were unsuccessful in producing any isolatable merochlorin-like products (entry 15). However, when similar conditions were used but the enzyme itself was included, it was found that significant amounts of both merochlorin A and B were synthesized and were able to be isolated. While, we were unable to complete a robust chemical synthesis of these natural products, we were able to complete an enzymatic synthesis.

Interestingly, when the complex mixture from entry 6 was compared to the natural extract of the merochlorin producing bacteria, it was found that each and every one of the 8 synthetic structures identified also existed in the natural extract. This leads us to believe that the vanadium-dependent haloperoxidase chlorinates the premerochlorins unselectively and that each of the isomers is also a natural product.

Therefore our work with the merochlorins was concluded with 10 total natural products synthesized, a revised biosynthetic hypothesis and the identification of the bioactivity of a simplified natural product.

2.6 References


2.7 Experimental

**General Experimental.** All reactions were carried out under an inert argon atmosphere with dry solvents under anhydrous conditions unless otherwise stated. Dry dichloromethane (DCM), tetrahydrofuran (THF), toluene (PhMe) and were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (¹H-NMR) homogeneous material, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica plates (60F-254), using UV light as the visualising agent and an acidic solution of p-anisaldehyde and heat, or KMnO₄ and heat as developing agents. Flash silica gel chromatography was performed using E. Merck silica gel (60, particle size 0.043–0.063 mm). Chiral HPLC was performed using a Hitachi LaChrom Elite HPLC system. NMR spectra were recorded on Bruker DRX-600 and AMX-400 instruments and were calibrated using residual undeuterated solvent as an internal reference (CHCl₃ @ 7.26 ppm ¹H-NMR, 77.16 ppm ¹³C-NMR). The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d =
doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionisation time-of-flight (ESI-TOF) reflectron experiments. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. Melting points were recorded on a Fisher-Johns 12-144 melting point apparatus and are uncorrected.

Analytical and semipreparative HPLC (Figs 2.2 & 2.3). Analytical reversed phase HPLC (Agilent 1200 series with a photodiode array detector) was conducted with a Luna 5 μm C18(2) column (150 mm x 4.6 mm, Phenomenex, USA) using 10% (v/v) MeCN (containing 0.1 % trifluoroacetic acid) as liquid phase. The buffer was gradually exchanged for MeCN in a linear gradient from 10 to 100% (v/v) MeCN over 16 min at a flow rate of 0.7 mL min⁻¹ (Figure 2, SI Figs 1, 6-9). For isoprene diphosphate separation (Figure 1, SI Figure 3), a 3.5 μm ZORBAX Extend-C18 column (150 mm x 4.6 mm, Agilent Technologies, USA) was employed using water buffered with 25 mM NH₄HCO₃ (pH 8.0) as liquid phase at a flow rate of 0.7 mL min⁻¹. After 3 min, the buffer was gradually exchanged for MeCN using a linear gradient from 0 to 100% (v/v) MeCN over 15 min. Products were quantified based on their absorption at 254 nm or 214 nm using a standard curve. Semi-preparative reversed phase HPLC for the purification of enzymatically produced 5 (for NMR analyses) was performed using an Agilent 1260 Infinity HPLC with a 5 μM C18(2) column (250 mm x 10.00 mm, Phenomenex, USA) and a similar NH₄HCO₃-buffered MeCN gradient as described above for the analytical HPLC.

Feeding experiment with pre-merochlorin 4. Over the course of 5 days, 0.5 mg of 4
(dissolved in 20 μL DMSO) was added daily to densely grown 50 mL cultures of the Δmcl23 deletion mutant of S. sp. strain 1152 in R5-media (103 g/L sucrose, 0.25 g/L K₂SO₄, 10.12 g/L MgCl₂·6H₂O, 10 g/L glucose, 0.1 g/L Casaminoacids, 5 g/L yeast extract, 5.73 g/L TES (N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid), 80 μg/L ZnCl₂, 400 μg/L FeCl₃·6H₂O, 20 μg/L CuCl₂·2H₂O, 20 μg/L MnCl₂·4H₂O, 20 μg/L Na₂B₄O₇·10H₂O, 20 μg/L (NH₄)₆Mo₇O₂₄·4H₂O, 50 mg/L KH₂PO₄, 3 g/L L-proline, 2.94 g/L CaCl₂, and 280 μg/L NaOH). The same volume of DMSO (without 4) was added to a control culture of the same strain. As an additional control, the native S. sp. strain 1152 lacking the mcl gene cluster was fed with DMSO + 4 or DMSO only. On day 6, the liquid cultures were extracted with 50 mL of EtOAc. The extracts were subsequently dried with a rotary evaporator, redissolved in 250 μL MeCN and analyzed by ESI-HRMS (negative mode).

**Chemical synthesis of terpene side chain**

![Chemical synthesis diagram](attachment:image.png)

**Scheme S2.1** synthesis of isosesquilavandulyl phospphate
2.13 (S7): A suspension of CuBr·SMe₂ (1.9 g, 9.4 mmol) in THF (60 mL) was cooled to 0 °C and methyl lithium (12 mL, 18.8 mmol; 1.6 M in Et₂O) was added slowly. The resulting clear solution was cooled to -78 °C and ethyl 2-butynoate (1.1 mL, 9.4 mmol) was added. The mixture was stirred at -78 °C for 1 h before geranyl bromide² (4.1 g, 18.8 mmol) was added. The reaction was warmed to 0 °C and stirred for another 2 h. The reaction was quenched by addition of sat. NH₄Cl and diluted with EtOAc. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine and dried over MgSO₄. The solvent was evaporated and the residue was purified by flash column chromatography (hexanes/EtOAc 40:1 → 9:1) to give 7 as a colorless liquid (2.28 g, 8.6 mmol, 91%).

Rf 0.62 (9:1, hexanes/EtOAc); ¹H-NMR (500 MHz, CDCl₃): 5 0.99-5.02 (m, 2H), 4.17 (qd, J = 7.1, 1.6 Hz, 2H), 3.00 (d, J = 6.9 Hz, 2H), 2.05 (dt, J = 13.5, 3.9 Hz, 2H), 2.01-1.94 (m, 5H), 1.80 (s, 3H), 1.69-1.63 (m, 6H), 1.59 (s, 3H), 1.28 (td, J = 7.1, 1.5 Hz, 3H);
¹³C-NMR (125 MHz, CDCl₃): 29.169.3, 141.5, 135.4, 131.0, 127.1, 124.1, 121.5, 59.8, 39.5, 28.6, 26.5, 25.5, 22.7, 21.6, 17.5, 15.9, 14.1; IR νmax (film)/cm⁻¹: 2977, 2920, 1713, 1446, 1376, 1280, 1207, 1172, 1070; HRMS (ESI) m/z calculated for C₁₇H₂₉O₂ ([M+H]⁺) 265.2162, found 265.2157.

isosesquilavandulyl alcohol 2.14 (S8): To a solution of ester S7 (2.28 g, 8.6 mmol) in CH₂Cl₂ (50 mL) at -78 °C was added DIBAL-H (26 mL, 26 mmol; 1 M in hexanes). The
reaction was stirred for 2 h at -78°C and then quenched by addition of EtOAc and sat. 
NH₄Cl. The phases were separated and the aqueous phase was extracted three times with 
EtOAc. The combined organic phases were washed with brine and dried over MgSO₄. The 
solvent was evaporated and the residue was purified by flash column chromatography 
(hexanes/EtOAc 9:1) to give alcohol 8 as a colorless liquid (1.44 g, 6.5 mmol, 75%).

Rₜ 0.50 (4:1, hexanes/EtOAc); ¹H-NMR (500 MHz, CDCl₃): δ 5.06 (dtt, J = 8.5, 5.6, 
1.4 Hz, 2H), 4.09 (s, 2H), 2.86 (d, J = 7.1 Hz, 2H), 2.09-2.04 (m, 2H), 2.01-1.97 (m, 2H), 
1.75 (s, 3H), 1.71 (s, 3H), 1.68-1.65 (m, 6H), 1.59 (s, 3H); ¹³C-NMR (125 MHz, CDCl₃): 
δ 135.6, 131.5, 129.9, 124.3, 123.4, 122.8, 62.1, 39.8, 29.6, 26.6, 25.7, 20.5, 20.2, 17.7, 
16.0; IR ν max (film)/cm⁻¹: 3335 (br), 2968, 2920, 1446, 1379, 998; HRMS (ESI) m/z 
calculated for C₁₅H₂₆O ([M+H]+) 223.2056, found 223.2051.

**isosesquilavandulyl pyrophosphate 2.28 (S5):** To a solution of alcohol S8 (50 mg, 0.22 
mmol) in THF (2 mL) at 0 °C was added PBr₃ (25 μL, 0.27 mmol). The reaction was stirred 
at 0 °C for 45 min and then quenched by addition of ice water. The aqueous phase was 
extracted three times with hexanes. The combined organic phases were washed with brine 
and dried over MgSO₄. The solvent was evaporated and the crude residue was dissolved 
in MeCN (2 mL). Tris(tetrabutylammonium) hydrogen pyrophosphate (400 mg, 0.44 
mmol) was added and the mixture was stirred over night. The solvent was removed and the 
crude product was purified by flash column chromatography (i-PrOH/aq. NH₃/H₂O 6:2:0
(→ 6:3:1) and preparative HPLC (xxx, eluent: 25mM NH₄HCO₃/MeCN 95:5 → 5:95) to give diphosphate 5 as a white solid (35 mg, 0.09 mmol, 42%).

Rₚ 0.58 (6:3:1, i-PrOH/aq. NH₃/H₂O); ¹H-NMR (500 MHz, D₂O): 5.18-5.12 (m, 2H), 4.47-4.41 (m, 2H), 2.86 (d, J = 7.4 Hz, 2H), 2.10 (q, J = 7.6 Hz, 2H), 2.02 (t, J = 7.3 Hz, 2H), 1.77 (s, 3H), 1.71 (s, 3H), 1.69 (s, 3H), 1.66 (s, 3H), 1.59 (s, 3H); ¹³C-NMR (150 MHz, D₂O): 136.3, 133.8, 133.2, 128.0, 124.4, 122.4, 65.1, 38.9, 28.8, 25.8, 24.8, 20.0, 19.7, 16.9, 15.5.

**Synthesis of pre-merochlorin 2.6**

![Chemical structure](Image)

isosesquilavandulyl carbonate 2.22 (S9): To a solution of alcohol 8 (300 mg, 1.35 mmol) in Et₂O (10 mL) at 0 °C was added pyridine (0.2 mL, 2.7 mmol) and ethyl chloroformate (130 μL, 1.35 mmol). The reaction was stirred at ambient temperature over night and then quenched by addition of 1 M HCl and diluted with EtOAc. The phases were separated and the aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine and dried over MgSO₄. The solvent was evaporated and the
residue was purified by flash column chromatography (hexanes/EtOAc 9:1) to give carbonate 9 as a colorless liquid (322 mg, 1.1 mmol, 81%).

Rf 0.66 (4:1, hexanes/EtOAc); \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 5.07 (tt, J = 7.1, 1.5 Hz, 1H), 5.01 (t, J = 7.3 Hz, 1H), 4.62 (s, 2H), 4.18 (q, J = 7.2 Hz, 2H), 2.83 (d, J = 7.1 Hz, 2H), 2.09-2.03 (m, 2H), 2.00-1.96 (m, 2H), 1.78 (s, 3H), 1.73 (s, 3H), 1.67 (s, 3H), 1.65 (s, 3H), 1.59 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H); \(^{13}\)C-NMR (75 MHz, CDCl\(_3\)): \(\delta\) 155.2, 135.6, 133.2, 131.0, 126.4, 124.1, 121.8, 66.7, 63.5, 39.6, 29.3, 26.5, 25.5, 20.4, 20.3, 17.5, 15.8, 14.1; IR \(\nu\)\(\text{max}\) (film)/cm\(^{-1}\): 2980, 2918, 1744, 1448, 1377, 1250, 993; HRMS (ESI) m/z calculated for C\(_{18}\)H\(_{30}\)O\(_3\)Na (\([\text{M+Na}]^+\)) 317.2087, found 317.2088.

Pre-merochlorin 2.6 (S4): A screw cap vial was charged with carbonate 9 (160 mg, 54 mmol), tetrahydroxynaphthalene (THN)\(^3\) (162 mg, 0.82 mmol) and Pd(PPh\(_3\))\(_4\) (31 mg, 0.03 mmol, 5 mol%). THF (2 mL) was added and the mixture was degassed by freeze/pump/thaw (3 times). A solution of triethylborane (0.82 mL, 0.82 mmol; 1 M in THF) was added and the vial was sealed carefully. The mixture was heated to 50 °C and stirred for 2 days. The reaction was poured into a separatory funnel containing sat. NH\(_4\)Cl and hexanes. The aqueous phase was extracted twice with hexanes and the combined organic phases were washed with brine and dried over MgSO\(_4\). The solvent was removed and the residue was taken up in hexanes and filtered through a plug of silica gel (eluent: hexanes/Et\(_2\)O 1:1). The resulting yellow solution was concentrated and trituration of the residue with cold hexanes gave 4 (95 mg, 0.24 mmol, 44%), which was sufficiently pure
for further use. Although 4 is highly air sensitive, the compound could be stored under N\textsubscript{2} at -80 °C for several weeks.

R\textsubscript{f} 0.00 (4:1, hexanes/EtOAc; decomposition); \textsuperscript{1}H-NMR (500 MHz, CDCl\textsubscript{3}): \textsuperscript{6}H 6.68 (d, J = 2.2 Hz, 1H), 6.38-6.16 (m, 2H), 5.13-5.09 (m, 1H), 4.97-4.92 (m, 1H), 3.57 (s, 2H), 2.63 (d, J = 6.8 Hz, 1H), 2.05-1.97 (m, 5H), 1.93 (q, J = 8.2, 7.5 Hz, 1H), 1.81 (s, 3H), 1.70 (s, 3H), 1.61 (s, 3H), 1.26 (s, 3H); \textsuperscript{13}C-NMR (150 MHz, d\textsubscript{6}-DMSO): \textsuperscript{13}C 157.5, 155.2, 154.1, 151.8, 137.3, 132.2, 131.9, 130.9, 124.8, 124.3, 123.6, 107.1, 106.5, 97.2, 97.1, 96.8, 39.7, 29.5, 27.8, 26.5, 26.0, 21.2, 21.0, 18.0, 16.1; IR \nu_{\text{max}} (film)/\text{cm}^{-1}: 3485 (br), 3286 (br), 2969, 2928, 1616, 1424, 1376, 1279, 1218, 1121; HRMS (ESI) m/z calculated for C\textsubscript{25}H\textsubscript{33}O\textsubscript{4} ([M+H]\textsuperscript{+}) 397.2373, found 397.2364.

**Chemical chlorinations of per-merochlorin (2.6)**

Various chlorinating reagents were tested to effect oxidative cyclization/chlorination of substrate 4 (Table S1). The reactions were analyzed by analytical HPLC and HR-LCMS. The UV spectra of the HPLC peaks were compared with the UV data of merochlorin A and B.
Table S1 screened conditions for chlorination of pre-merochlorin.

A screw cap vial was charged with a solution of substrate 2.6 (1 mg, 2.5 μmol) in the indicated solvent (0.2 mL) under nitrogen gas. Chlorinating agent (5 μmol) and additives, if applicable, were added to the solution. The reactions were analyzed by analytical HPLC after 15 min, 60 min, 2 h and 18 h (for representative HPLC chromatographs see Figure S10). Promising reaction mixtures were further analyzed by HR-LCMS.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Chlorinating Agent</th>
<th>Solvent</th>
<th>Product formation</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>HCDO</td>
<td>EtOH</td>
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</tr>
<tr>
<td>2</td>
<td>HCDO</td>
<td>MeCN</td>
<td>no</td>
</tr>
<tr>
<td>3</td>
<td>HCDO</td>
<td>CCl4/NEt3 (100:1)10</td>
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</tr>
<tr>
<td>4</td>
<td>HCDO</td>
<td>CCl4/DMF (9:1)</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>TCCA</td>
<td>MeOH</td>
<td>no</td>
</tr>
<tr>
<td>6</td>
<td>TCCA</td>
<td>MeCN</td>
<td>no</td>
</tr>
<tr>
<td>7</td>
<td>TCCA</td>
<td>CH2Cl2</td>
<td>no</td>
</tr>
<tr>
<td>8</td>
<td>TCCA</td>
<td>AcOH</td>
<td>no</td>
</tr>
<tr>
<td>9</td>
<td>NCS</td>
<td>MeOH</td>
<td>trace</td>
</tr>
<tr>
<td>10</td>
<td>NCS</td>
<td>MeCN</td>
<td>trace</td>
</tr>
<tr>
<td>11</td>
<td>NCS</td>
<td>CH2Cl2</td>
<td>trace</td>
</tr>
<tr>
<td>12</td>
<td>NCS</td>
<td>AcOH</td>
<td>no</td>
</tr>
<tr>
<td>13</td>
<td>t-BuOCl</td>
<td>CH2Cl2</td>
<td>no</td>
</tr>
<tr>
<td>14</td>
<td>t-BuOCl</td>
<td>MeCN</td>
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<tr>
<td>15</td>
<td>HOCl11</td>
<td>acetone</td>
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<tr>
<td>16</td>
<td>HCl/H2O2/AcOH12</td>
<td>petroleum ether</td>
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</tr>
<tr>
<td>17</td>
<td>SOCl2/i-Pr2NH13</td>
<td>toluene</td>
<td>no</td>
</tr>
<tr>
<td>18</td>
<td>CuCl2/LiCl/O214</td>
<td>AcOH</td>
<td>no</td>
</tr>
<tr>
<td>19</td>
<td>CBMG (Palau’chlor)</td>
<td>CHCl3</td>
<td>no</td>
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</tbody>
</table>

HCDO = 2,3,4,5,6,6-hexachloro-2,4-cyclohexadiene-1-one; TCCA = trichloroisocyanuric acid; NCS = N-chlorosuccinimide; CBMG = chlorobis(methoxycarbonyl)guanidine.
The reaction conditions were further studied by screening various base additives and chlorinating agents (Table S2). The most promising conditions were repeated on a 5 mg scale and the product mixture was isolated for quantification (yields not optimized). The combined yield of cyclized products was determined after silica gel purification (eluent: hexanes/EtOAc 4:1).

A negative control reaction was performed with base only (entry 13). No cyclization products were observed conducting the reaction under a nitrogen atmosphere or open to air. The same result was obtained using NaH as base (data not shown).

<table>
<thead>
<tr>
<th>Entry[a]</th>
<th>Chlorinating Agent</th>
<th>Temperature</th>
<th>Additive</th>
<th>Approx. Convn.[b]</th>
<th>Combined Yield[c]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HCDO</td>
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<td>NEt₃ (6 equiv.)</td>
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<td>n.d.</td>
</tr>
<tr>
<td>2</td>
<td>HCDO</td>
<td>0 °C</td>
<td>i-Pr₂NH (6 equiv.)</td>
<td>20-30%</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>TCCA</td>
<td>rt</td>
<td>i-Pr₂NH (2 equiv.)</td>
<td>decomp.</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>NCS</td>
<td>0°C</td>
<td>i-Pr₂NH (2 equiv.)</td>
<td>30-40%</td>
<td>30%</td>
</tr>
<tr>
<td>5</td>
<td>NCS</td>
<td>rt</td>
<td>lutidine (2 eqv.)[^6]</td>
<td>trace</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>NCS</td>
<td>rt</td>
<td>t-BuNH₂ (2 equiv.)</td>
<td>20-30%</td>
<td>n.d.</td>
</tr>
<tr>
<td>7</td>
<td>t-BuNCI₂[^7]</td>
<td>rt</td>
<td>-</td>
<td>10%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>8</td>
<td>NCS</td>
<td>-78 °C</td>
<td>i-Pr₂NH (2 equiv.)</td>
<td>40-50%</td>
<td>28%[^6]</td>
</tr>
<tr>
<td>9[^d]</td>
<td>NCS</td>
<td>60 °C</td>
<td>t-BuNH₂ (2 equiv.)</td>
<td>decomp.</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>NCS</td>
<td>-78 °C</td>
<td>KOr-Bu (2 equiv.)</td>
<td>trace</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>NCS</td>
<td>-78 °C</td>
<td>NaH (2 equiv.)</td>
<td>30-40%</td>
<td>22%[^f]</td>
</tr>
<tr>
<td>12</td>
<td>NCS</td>
<td>rt</td>
<td>NaH (2 equiv.)</td>
<td>decomp.</td>
<td>-</td>
</tr>
<tr>
<td>13[^e]</td>
<td>-</td>
<td>rt</td>
<td>i-Pr₂NH (2 equiv.)</td>
<td>decomp.</td>
<td>-</td>
</tr>
</tbody>
</table>

[a] Reaction conditions: substrate 4 (1 mg, 2.5 μmol), CH₂Cl₂ (0.2 mL), chlorinating agent (2.0 equiv.), additive, 1 h. [b] determined by approximate comparison of HPLC product peaks and oxidation side products. [c] Reaction repeated with 5 mg of 4, product mixture isolated by silica gel chromatography (eluent: hexanes/EtOAc 4:1). [d] CHCl₃ as solvent. [e] Control experiment with base only, no formation of cyclized products under nitrogen or air. [f] Only unchlorinated cyclization products isolated. HCDO = 2,3,4,5,6,6-hexachloro-2,4-cyclohexadiene-1-one; TCCA = trichloroisocyanuric acid; NCS = N-chlorosuccinimide; decomp. = decomposition; n.d. = not determined.
Isolation and identification of the cyclization products

The enzymatic cyclization of 4 was compared with the products obtained by chemical chlorination of 4 by HR-LCMS analysis (Figure S11). Both UV and MS data (Figures S12, S13) indicated the formation of similar but not identical products.
Figure S11. Comparison of the enzymatic cyclization of 4 (top) with the NCS/i-Pr$_2$NH chlorination (bottom) by HR-LCMS (Phenomenex Luna C18(2) 5µ 100 x 4.6 mm column; MeCN/H$_2$O 5% → 95%; UV trace at 254 nm). a: deschloro-merochlorin B (6); b: merochlorin B (2); c: isochloro-merochlorin B (7); d: descloro-merochlorin A (8); e: merochlorin A (1). For MS data see Figure S13 and S13, respectively.

Figure S12. Major MS-peaks found for Mcl24 products (UV peaks a-e from Figure S11, top).
Figure S13. Major MS-peaks found for chemical chlorination products (UV peaks a-e from Figure S11, bottom).

The chemical chlorination/cyclization of 4 was repeated several times on a 5-10 mg scale and the three major product peaks were isolated by semi-preparative HPLC (Phenomenex Synergi 4 μ Polar-RP 250 x 10 mm column, MeCN/H₂O; 70% MeCN → 95% MeCN) (Figure S14). Peak 3 in Figure S14 contained minor amounts of dichlorinated compound 9, which was separated from major component 8 by a second round of HPLC purification (MeCN/H₂O; 75% MeCN → 85% MeCN). NMR characterization led to the identification of compounds 6-9 (see following pages).

Figure S14. Semi-prepHPLC trace (254 nm) of the chemical cyclization of 4. Peak 1: deschloro-merochlorin B (6); peak 2: isochloro-merochlorin B (7); peak 3: mixture of deschloro-merochlorin A (8) (major; ~80%) and dichloro-merochlorin B (9) (~20%).
Table S3. NMR spectroscopic data for deschloro-merochlorin B (6) (in d$_6$-DMSO).

<table>
<thead>
<tr>
<th>No.</th>
<th>δC</th>
<th>δH</th>
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<td>1</td>
<td>182.1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>106.0</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>163.2</td>
<td>-</td>
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<td>4</td>
<td>100.5</td>
<td>6.12 (d, $J = 2.2$ Hz, 1H)</td>
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<td>5</td>
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<td>-</td>
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<td>6</td>
<td>102.9</td>
<td>6.14 (d, $J = 2.2$ Hz, 1H)</td>
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<td>58.3</td>
<td>-</td>
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<td>9</td>
<td>51.2</td>
<td>2.86 (s, 1H)</td>
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<td>10</td>
<td>95.6</td>
<td>-</td>
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<tr>
<td>11</td>
<td>182.1</td>
<td>-</td>
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<tr>
<td>12</td>
<td>96.6</td>
<td>5.48 (s, 1H)</td>
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<td>13</td>
<td>48.9</td>
<td>2.76 (d, $J = 17.9$ Hz, 1H), 2.43 (d, $J = 16.6$ Hz, 1H)</td>
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<tr>
<td>14</td>
<td>130.9</td>
<td>-</td>
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<tr>
<td>15</td>
<td>34.2</td>
<td>2.87 (d, $J = 16.7$ Hz, 1H), 2.71 (d, $J = 14.4$ Hz, 1H)</td>
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<td>16</td>
<td>43.2</td>
<td>1.69-1.64 (m, 2H)</td>
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<td>17</td>
<td>21.6</td>
<td>1.98 (p, $J = 7.6$, 7.2 Hz, 2H)</td>
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<td>18</td>
<td>123.2</td>
<td>5.05 (t, $J = 7.0$ Hz, 1H)</td>
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<td>19</td>
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<td>-</td>
</tr>
<tr>
<td>20</td>
<td>21.0</td>
<td>1.51 (s, 3H)</td>
</tr>
<tr>
<td>21</td>
<td>25.1</td>
<td>1.60 (s, 3H)</td>
</tr>
<tr>
<td>22</td>
<td>17.2</td>
<td>1.49 (s, 3H)</td>
</tr>
<tr>
<td>23</td>
<td>124.9</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>21.0</td>
<td>1.70 (s, 3H)</td>
</tr>
<tr>
<td>25</td>
<td>21.6</td>
<td>1.35 (s, 3H)</td>
</tr>
<tr>
<td>5-OH</td>
<td>-</td>
<td>10.35 (s, 1H)</td>
</tr>
</tbody>
</table>

HR-LCMS analysis of pure 6 confirmed the molecular formula: C$_{25}$H$_{31}$O$_4$ ([M+H]$^+$); calc.: 395.2217; found: 395.2197.
Table S4. NMR spectroscopic data for isochloro-merochlorin B (7) (in d$_6$-DMSO).

<table>
<thead>
<tr>
<th>No.</th>
<th>δC</th>
<th>δH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>182.8</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>106.4</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>158.1</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>104.9</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>158.1</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>102.8</td>
<td>6.40 (s, 1H)</td>
</tr>
<tr>
<td>7</td>
<td>145.9</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>58.2</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>51.3</td>
<td>2.88-2.85 (m, 1H)</td>
</tr>
<tr>
<td>10</td>
<td>96.1</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>182.8</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>96.7</td>
<td>5.55 (s, 1H)</td>
</tr>
<tr>
<td>13</td>
<td>49.9</td>
<td>2.73 (d, $J = 16.8$ Hz, 1H); 2.45 (d, $J = 16.9$ Hz, 1H)</td>
</tr>
<tr>
<td>14</td>
<td>130.6</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>34.4</td>
<td>2.85-2.75 (m, 2H)</td>
</tr>
<tr>
<td>16</td>
<td>43.3</td>
<td>1.67 (dd, $J = 16.9, 8.8$ Hz, 2H)</td>
</tr>
<tr>
<td>17</td>
<td>21.6</td>
<td>1.97 (tt, $J = 14.1, 6.8$ Hz, 1H)</td>
</tr>
<tr>
<td>18</td>
<td>123.3</td>
<td>5.04 (t, $J = 7.2$ Hz, 1H)</td>
</tr>
<tr>
<td>19</td>
<td>131.0</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>20.9</td>
<td>1.50 (s, 3H)</td>
</tr>
<tr>
<td>21</td>
<td>25.4</td>
<td>1.59 (s, 3H)</td>
</tr>
<tr>
<td>22</td>
<td>17.2</td>
<td>1.48 (s, 3H)</td>
</tr>
<tr>
<td>23</td>
<td>125.3</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>21.0</td>
<td>1.70 (s, 3H)</td>
</tr>
<tr>
<td>25</td>
<td>21.6</td>
<td>1.35 (s, 3H)</td>
</tr>
</tbody>
</table>

LC-MS analysis of pure 7 confirmed the molecular formula: C$_{25}$H$_{30}$ClO$_4$ ([M+H]$^+$); calc.: 429.1827; found: 429.1834.
The enzymatic reaction was scaled up according to the following procedure: Synthetic pre-merochlorin (4) (25 mg, 0.06 mmol) was dissolved in DMSO (1 mL). The substrate was partitioned into 70 eppendorf tubes containing pH 6.0 MES buffer (1 mL: 50 mM MES, 50 mM KCl, 100 μM Na3VO4, 1 mM H2O2, 50 μg/mL Mcl24). Each eppendorf tube was put under nitrogen gas and closely sealed. The reaction was incubated at 30 °C for 18 h. The aqueous layer was then extracted three times with EtOAc (50 mL). The combined organic phases were washed with brine and dried over MgSO4. The solvent was removed and the residue was passed through a short silica gel column (eluent: hexanes/EtOAc 4:1). After concentration, the residue was purified by preparative HPLC (Phenomenex Synergi 10μ Hydro- RP 250 x 21 mm column, MeCN/H2O; 75% MeCN → 85% MeCN) to give 6 (1.0 mg), 2 (0.9 mg), 7 (0.4 mg), 8 (1.4 mg) and 1 (1.2 mg) (Figure S15). The 1H-NMR spectra of the individual compounds were compared to the synthetic material or to the isolated natural products merochlorin A and B respectively (see following pages). The optical rotation of the enzymatic products was measured and compared to literature values, if applicable:18 deschloro-merochlorin B (6) [α]D25.5° = +3 (c = 0.20, MeOH); merochlorin B (2) [α]D25.5° = +4.5 (c = 0.18, MeOH), literature:2 [α]D21° = +23 (c = 0.27, MeOH); isochloro-merochlorin B (7) [α]D25.5° = +8.8 (c = 0.08, MeOH); deschloro-merochlorin A (8) [α]D25.5° = +2.5 (c = 0.28, MeOH); merochlorin A (1) [α]D25.5° = +2.5 (c = 0.24, MeOH), literature:19 [α]D21° = +3 (MeOH).
Figure S15. Preparative HPLC chromatogram (254 nm) of the enzymatic reaction (25 mg scale).
Figure S16. $^1$H-NMR comparison between enzymatic (top) and synthetic (bottom) 6. (600 MHz in d$_6$-DMSO).
Figure S17. $^1$H-NMR comparison between enzymatic and isolated (bottom) merochlorin B (2). (600 MHz in d$_6$-DMSO).
Figure S18. $^1$H-NMR comparison between enzymatic (top) and synthetic (bottom) 7. (600 MHz in d$_6$-DMSO).
Figure S19. $^1$H-NMR comparison between enzymatic (top) and synthetic (bottom) 8. (600 MHz in d$_6$-DMSO).
Figure S20. $^1$H-NMR comparison between enzymatic (top) and isolated (bottom) 1. (600 MHz in d$_6$-DMSO).
2.8 Spectra

EtO₂C-

500 MHz ¹H-NMR in CDCl₃

125 MHz ¹³C-NMR in CDCl₃
500 MHz $^1$H-NMR in D$_2$O

500 MHz $^{13}$C-NMR in D$_2$O
500 MHz $^1$H-NMR in CDCl$_3$

150 MHz $^{13}$C-NMR in d$_6$-DMSO
deschloro-macrochlorin B
(synthetic)
600 MHz 1H
in d6-DMSO
Isochloro-merochlorin B (T)
(synthetic)
600 MHz $^1$H
in $d_2$-DMSO

spectroscopy graphs
deschloro-merochlorin A (8)
(synthetic)
600 MHz $^1$H
in d$_6$-DMSO
dichloro-merocillin B (9)
(synthetic)
600 MHz \(^1\)H
in DMSO (DMSO-d_6)
HR-LCMS analysis of pure 9 confirmed the molecular formula: C_{32}H_{30}Cl_{2}O_{4} ([M+H]^+); calc.: 463.1437; found: 463.1436.
NMR spectra.

**500 MHz $^1$H-NMR in CDCl$_3$**
126 MHz $^{13}$C-NMR
in CDCl$_3$
2.9 Distribution of credit

Synthetic planning was a collaborative process between Dr. Yoshi Ishihara, Prof. Phil S. Baran and myself. All synthetic work, up through the synthesis of the deschloromeroclorins including pre-merochlorin, was conducted solely by myself. From there, Dr. Stefan Dielhelm conducted much of the work on the chlorination of pre-merochlorin and the story told in this theis is completed using some of his findings which were published in a separate paper.27 Entries 6-8 and 16 of table 2.2 were conducted by Dr. Dielhelm. The identification of many of the merochlorin isomers (which we both produced independently) as natural products was solely the work of Dr. Dielhelm.

All investigations into the enzymatic mechanisms of merochlorin biosynthesis were conducted by Dr. Robin Teufel and Dr. Leonard Kaysser of Prof. Bradley Moore’s laboratory with material supplied by myself. The antibacterial studies on deschloromeroclorin A were conducted by Dr. Barrios Steed of Prof. Floyd Romesberg’s laboratory.
CHAPTER 3

Antroquinonol A: Scalable Synthesis and Preclinical Biology of a Phase 2 Drug Candidate
3.1 Introduction

Antroquinonol A (3.1, Figure 1A) is a quinone-derived natural product reported to have remarkable medicinal potential in the areas of oncology, immunology, and even diabetes.\textsuperscript{1} More than ten publications have delineated the exciting activities of 3.1 and as a consequence, an investigational new drug (IND) application was filed by Golden Biotech Corp. in 2010.\textsuperscript{2} It is currently in Phase II clinical trials (in the USA and Taiwan)\textsuperscript{3} for the treatment of non-small-cell lung cancer (NSCLC; Phase II began in January 2014)\textsuperscript{3} and has been granted orphan drug status by the FDA for the treatment of pancreatic cancer, and acute myeloid leukemia. Given the excitement surrounding this natural product\textsuperscript{4} coupled with its relative structural simplicity, an effort was launched to pursue its synthesis and biological evaluation as part of the academic-industrial symbiosis between Scripps and Bristol-Myers Squibb (BMS).\textsuperscript{5} In this Communication, an enantioselective, scalable, and modular synthesis of 3.1 is reported. Access to copious amounts of pure 3.1 enabled a detailed biological reevaluation that is in contrast to the reported preclinical efficacy of the compound in a nude mouse model harboring human hepatoma xenografts and may temper the enthusiasm surrounding this natural product.

3.2 Initial strategies towards antroquinonol A

At the outset of this project, no synthesis of 1 existed and its isolation from the rare Taiwanese fungus Antrodia camphorata was not a practical means for studying pure antroquinonol at BMS.\textsuperscript{6} A set of plans, ranging in level of ambition and precedent were evaluated (Figure 1) before settling on the substituted quinone 3.10 as our final starting
point. Six representative blueprints are illustrated and can be divided conceptually into approaches that build the hydroxy enone of \(3.1\) via cyclization strategies or via the semi-reduction of a quinone system. Among the ring-building approaches, Danheiser-type annulation (\(3.2\))^7, stepwise conjugate addition/annulation (\(3.3 + 3.4\))^8 and Diels-Alder

\[\text{Figure 3.1} \text{ Summary of retrosynthetic analyses explored for the synthesis of 3.1.} \]

(\(3.5 + 3.6\))^9 strategies were all pursued, leading either to lengthy sequences, low-yielding key steps, or unstable starting materials (\(3.4\) and \(3.6\) rapidly isomerize). Inspired by the related structure of Coenzyme Q3 (\(3.7\)), extensive efforts to controllably reduce such systems were explored to no avail.\(^{10}\) Attempted allylic alcohol isomerization\(^{11}\) by utilizing 1,4-diol \(3.8\) was not possible due to the inherent instability of such systems (spontaneous aromatization). Finally, efforts centering on a tandem 1,2-/1,4-addition\(^{12}\) to silyl-dienone
3.9\textsuperscript{13} failed due to the tendency of silyl lithium reagents to reduce quinones rather than add to them. Collectively, these failures led us to the simplest possible approach: a conjugate addition to a substituted quinone.

3.3 Total synthesis of antroquinonol A

Re-imagining this quinone as quinone-monoketal 3.10 gives the starting material “directionality”, allowing selective introduction of the nucleophile and electrophile through a Michael addition and subsequent 1,2-alkylation.\textsuperscript{14} This strategy inherently makes the synthesis modular, allowing a host of nucleophiles and electrophiles to be quickly appended to any desired quinone starting material.

Scheme 1 depicts the fully optimized six-step, enantioselective, scalable synthesis of antroquinonol A (3.1). Each step was meticulously studied (see Supporting Information for tables of screened conditions) and as a result, gram quantities of 3.1 were generated for biological screening. The synthesis commences with the formation of quinone-monoacetal 3.12 from commercial benzaldehyde 3.11. Baeyer-Villiger and dearomative oxidations followed by a trans-ketalization gave the desired quinone-monoacetal in an overall yield of 64%.\textsuperscript{15} Extensive screening of both the identity of the ketal protecting group (see Scheme 1B) and also of enantioselective conjugate addition

**Scheme 3.1** Total synthesis of (+)-antroquinonol A (3.1).
conditions\textsuperscript{16} led to a one-pot procedure that produced the vicinally difunctionalized product 3.14 with moderate yield and high stereoselectivity. Conjugate addition product 3.13 was also prepared in order to confirm absolute stereochemistry \textit{via} X-ray crystallography. L-Selectride proved best in producing the 4,5-\textit{cis} stereochemistry of the natural product with a d.r. of 3:1. Hydrolysis turned out to be a significant challenge, with most acidic conditions leading to a complex mixture of elimination products. Montmorillonite K10 clay was a sufficiently mild proton source for hydrolysis of ketal 3.14, producing enantioenriched (+)-antroquinonol A (3.1) in an overall yield of 13% and with 96% ee. Over one gram of the natural product has been prepared to date.

3.4 Preclinical investigation of antroquinonol A

With synthetic (+)-3.1 in hand, we began testing the compound against a panel of human tumor cell lines \textit{in vitro}, previously reported in the literature for the natural product. As outlined in Table 1, natural antroquinonol (3.1) is reported to have low micromolar activity against the MDA-MB-231 breast, HepG2 hepatocellular and LNCaP prostate tumor cell lines and low nanomolar activity versus the Hep 3B hepatocellular carcinoma (HCC) cell line.\textsuperscript{1,17} The reported IC\textsubscript{50} value in the lung tumor cell line (A549) for the natural product is 25 \textgreek{M}.\textsuperscript{2c} In our hands, 3.1 is \textasciitilde 3–70 fold less cytotoxic against these cell lines except for the A549 cell line where it is \textasciitilde 4 fold more potent than previously reported. The activity of 3.1 in the Hep 3B cell line was particularly striking, where it is significantly less potent when compared to the natural product. To be thorough, the enantiomer of 3.1, (-)-antroquinonol A was tested in a similar panel much like the natural product, high micromolar activity was seen for the Hep 3B and A549 cell lines (Supporting
Table S6). The observed and reported high micromolar activity of 3.1 against a NSCLC cell line (A549) is somewhat puzzling since the natural product is in Phase II trials for the treatment of this tumor type. It was indicated in the package submitted to the FDA for Phase I studies that “In vivo study in NOD/SCID mice with A549 subcutaneous xenografts consistently showed tumor growth suppression after 2

weeks of oral 30 and 60 mg/kg antroquinonol treatment.”\(^{18}\) In addition, the natural product is reported to have in vivo activity in a Hep 3B tumor model (\textit{vide infra}).\(^{19}\) Because of the promising efficacy in this hepatocellular model and the limited in vitro activity against the A549 cell line, we reasoned that an active metabolite(s) could potentially be contributing to the observed \textit{in vivo} activity seen with 3.1.

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
\textbf{entry} & \textbf{cell line} & \textbf{IC\textsubscript{50} \textbf{\textmu M reported in the lit.}} & \textbf{IC\textsubscript{50} \textbf{\textmu M for 1\textsuperscript{b} (72 h)}} & \textbf{IC\textsubscript{50} \textbf{\textmu M for 15 (72 h)}} \\
\hline
1 & MDA-MB-231 & 2.6 ± 0.05\textsuperscript{f} & 19 ± 1.6 & >25 \\
2 & HepG2 & 4.3 ± 0.03\textsuperscript{f} & >25 & >25 \\
3 & LNCaP & 6.1 ± 0.07\textsuperscript{f} & 22 ± 5.4 & >25 \\
4 & Hep 3B & 0.13 ± 0.02\textsuperscript{f} & 8.9 ± 2.1 & >25 \\
5 & PANC-1 (48 h)\textsuperscript{e} & 19\textsuperscript{g,h} & >25 & >25 \\
6 & AsPC-1 (48 h)\textsuperscript{e} & 20\textsuperscript{g,h} & >25 & >25 \\
7 & A549 (12 h)\textsuperscript{e} & 25\textsuperscript{i} & 6.7 ± 2.5 & 10.8 ± 5.8 \\
8 & H441 & 25\textsuperscript{i} & >25\textsuperscript{e} & >25 \\
\hline
\end{tabular}
\caption{Oncology panel \textit{in vitro} data.}
\end{table}

3.5 Identification of a new metabolite and its synthesis
When 1 was subjected to metabolite identification/biotransformation studies using human, rat and mouse liver microsomes, it was rapidly converted to a major metabolite across all three species (Figure 2), the structure of which was tentatively assigned as the acid 3.15 based on MS/MS and ¹H NMR data.

In order to confirm this structure and produce enough material for biological evaluation, a synthesis of 3.15 was undertaken. As a testament to the modularity of the synthetic route to 3.1, acid 3.15 was easily accessed through an identical strategy. Simply
replacing farnesyl bromide with an oxidized derivative, followed by alkylation, reduction, and hydrolysis conditions furnished antroquinonol analogue 3.17. Not surprisingly, chemoselective oxidation of the primary alcohol in the presence of the partially reduced quinone core of antroquinonol proved challenging. Employing TEMPO as a catalytic oxidant proved successful.\textsuperscript{21} Subsequent oxidation of the sensitive aldehyde under Pinnick conditions gave the desired acid metabolite 3.15, which spectroscopically matched the isolated material from liver microsomes (\textit{vide supra}).

Compound 3.15 was subjected to the same tumor cell line panel as 3.1 (Table 3.1), however, little to no cytotoxicity was observed.\textsuperscript{22} Further metabolism studies where 3.1 was incubated with human, rat and mouse hepatocytes showed that compound 3.15 is most likely rapidly oxidized and degraded to a known inactive metabolite (Met2) most likely \textit{via} mitochondrial β-oxidation (Supporting Figure S3.2).\textsuperscript{23a,b,c}

\textbf{3.6 In vivo experiments on antroquinonol A}

Although the source of the Hep 3B tumor cell lines used in the literature is different than the one described herein (see Table 3.1 footnote), we decided to conduct an \textit{in vivo} study with synthetic antroquinonol (3.1) based on the reported efficacy of the

\begin{table}[h]
\centering
\small
\begin{tabular}{|l|c|c|}
\hline
PK parameters & ip dosing\textsuperscript{a} & po dosing\textsuperscript{a} \\
\hline
$C_{\text{max}}$ (nM) & 457 ± 45 & 79 ± 52 \\
$T_{\text{max}}$ (h) & 4 ± 3 & 2 ± 1 \\
AUC$_{\text{last}}$ (nM·h) & 2290 ± 142 & 266 ± 88 \\
\hline
\end{tabular}
\caption{Mouse Pharmacokinetic Data}
\end{table}

\textsuperscript{a}Corn oil was used as the dosing vehicle.

\textbf{Table 3.2} Mouse pharmacokinetic data for antroquinonol A.
natural product. Before embarking on such a study, a pharmacokinetic (PK) study of 1 in mouse was performed to determine the exposure of the compound when dosed either orally (PO) or intraperitoneally (IP). A single 50 mg/kg dose of 3.1 provided the PK profile depicted in Table 3.2, in which exposures following oral administration were significantly lower compared to those from IP dosing.

**Figure 3.3 In vitro antitumor activity of synthetic antroquinonol A.**

An *in vivo* efficacy study was conducted with 3.1 using Hep 3B HCC tumor xenografts implanted subcutaneously in female NSG mice. Despite literature reports of statistically significant antitumor activity observed with the natural product,\(^2\) compound 3.1 was inactive (<70% Tumor Growth Inhibition, TGI) in our hands when administered daily IP for 14 consecutive days. Paclitaxel served as a positive control in this study and was clearly efficacious (79% TGI) when administered on its optimal preclinical dosing regimen (24 mg/kg, qdx5, i.v.). Compound 3.1 was generally well-tolerated with no overt signs of toxicity and minimal weight loss (~4 %).
In summary, a concise, six-step, scalable, enantioselective synthesis of (+)-antroquinonol (3.1) has enabled an extensive reinvestigation of some of its reported preclinical biological properties. As outlined in this manuscript, compound 3.1 demonstrated only micromolar activity in a panel of select tumor cell lines. While the reported Hep 3B cytotoxicity data was intriguing, it was significantly less potent in our panel, therefore it was not unexpected that the compound was inactive in the xenograft model derived from this cell line. The modular synthetic route enabled us to prepare and evaluate a major metabolite identified from biotransformation studies; this compound was inactive and therefore unlikely contributing to the reported in vivo activity. Despite differences in the cell lines used for the in vitro assays\textsuperscript{24} and the manner in which the in vivo studies were conducted (e.g., the different initial tumor sizes), the lack of efficacy in an in vivo preclinical model would be a cause for concern if the compound were to be advanced into the clinic for treating hepatocellular carcinoma. As indicated earlier, antroquinonol is currently in Phase II clinical trials for the treatment of NSCLC. However, the reported in vitro IC\textsubscript{50} for antroquinonol A (3.1) as well as the data with synthetic antroquinonol (Table 3.1) suggest that the activity of the parent compound 3.1 for this tumor cell line is in the high micromolar range. Based on the in vitro cytotoxicity data for the A549 cell line, metabolic stability data in microsomes and mouse PK profile, very high exposures\textsuperscript{25} or the presence of a yet unknown active metabolite may be necessary for 3.1 to show efficacy in treating NSCLC in the clinic.
3.7 References


(3) ClinicalTrials.gov Identifier: NCT02047344


Three total syntheses of 1 were reported while this manuscript was in preparation:


The IC_{50} values for the natural product against the Hep3B and HepG2 carcinoma cell lines are reported as 0.13 ± 0.02 μM and 4.3 ± 0.03 μM respectively in ref. 1, as indicated in Table 1. The GI_{50} values for 1 against the same cell lines are listed as >30 μM and 0.22 μM respectively in ref. 2a. The cytotoxicity assay protocol used in this paper and ref. 1 employ 10% FBS and a 72 h incubation time with compound (antroquinonol) using MTS or MTT stain. The protocol outlined in ref. 2a employs 5% FBS, 48 h incubation time and a SRB stain. It is clear from the discussion (vide supra) that the protocol employed in this paper to determine cytotoxicity, closely mirrors the conditions used in ref. 1.

ClinicalTrials.gov Identifier: NCT01134016.

For discussion regarding the in vivo activity see ref. 2a, page 170, left column, 3rd paragraph. See supplementary material of ref. 2a for in vivo data. Initial tumor sizes were not identical: 50-70 mm3 (lit.)2a vs. 75-100 mm3 (BMS).

Data shown for mouse liver microsomes.


The Caco-2 value for 15 (Pc A→B, 62 nm/sec and B→A, 63 nm/sec) suggests that cell permeability may not be an issue with 15.

(a) Met2 (supplementary Figure S2; structure based on MS-MS data), is a reported metabolite of antroquinonol (b) Met2 is inactive in the H838 tumor cell line (IC_{50} > 100 μM vs. antroquinonol IC_{50} of ~ 3 μM). Ho, C-L.; Wang, J.-L.; Lee, C-C.; Cheng, H-Y, Wen, W-C.; Cheng, H. H-Y.; Chen, M. C-M. Biomedicine & Pharmacotherapy, 2014, 68, 1007. (c) Synthetic antroquinonol appears to have a
relatively better in vitro metabolic stability in human hepatocytes compared to human liver microsomes.

(24) A thorough investigation of the antiproliferative activity against a large panel of human tumor cell lines was not performed.

(25) Antroquinonol is being administered to patients at 200 mg t.i.d. in the ongoing Phase II clinical trial (ref. 3).

3.8 Experimental Procedures

**General Experimental.** All reactions were carried out under an inert argon atmosphere with dry solvents under anhydrous conditions unless otherwise stated. Dry dichloromethane (DCM), tetrahydrofuran (THF), toluene (PhMe) and were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically (\(^{1}\)H-NMR) homogeneous material, unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm E. Merck silica plates (60F-254), using UV light as the visualising agent and an acidic solution of \(p\)-anisaldehyde and heat, or KMnO\(_4\) and heat as developing agents. Flash silica gel chromatography was performed using E. Merck silica gel (60, particle size 0.043–0.063 mm). Chiral HPLC was performed using a Hitachi LaChrom Elite HPLC system. NMR spectra were recorded on Bruker DRX-600 and AMX-400 instruments and were calibrated using residual undeuterated solvent as an internal reference (\(\text{CHCl}_3\) @ 7.26 ppm \(^{1}\)H-NMR, 77.16 ppm \(^{13}\)C-NMR). The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d =
doublet, t = triplet, q = quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionisation time-of-flight (ESI-TOF) reflectron experiments. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter. Melting points were recorded on a Fisher-Johns 12-144 melting point apparatus and are uncorrected.

**2,3,4,4-tetramethoxycyclohexa-2,5-dien-1-one SI1.** To a flame-dried 250 mL round-bottomed flask equipped with a stir bar were added 2,3,4-trimethoxybenzaldehyde 11 (10.00 g, 50.97 mmol, 1.00 equiv), MeOH (100 mL), and H$_2$SO$_4$ (1.0 mL, 18 mmol, 0.35 equiv). The reaction flask was cooled to 0 ºC, upon which H$_2$O$_2$ (35% in H$_2$O; 5.67 mL, 65.85 mmol, 1.3 equiv) was added. The reaction flask was removed from the ice-water bath and the mixture was stirred at room temperature for 2 h. The mixture was diluted with EtOAc (300 mL) and was washed with NaHCO$_3$ (sat. aq.: 200 mL) followed by brine (200 mL). The organic layer was dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo to yield a red liquid.
Column chromatography (SiO₂, 1:5 to 1:3 Et₂O/hexanes) provided an orange, slightly viscous liquid (7.97 g, 73%).

**Appearance:** Orange liquid

All spectroscopic data matched the reported lit.¹

**TLC:** \( R_f = 0.25 \) (1:3 Et₂O/hexanes, UV active, stains orange upon vanillin staining).

\[
\begin{align*}
\text{7,8-dimethoxy-3,3-dimethyl-1,5-dioxaspiro[5.5]undeca-7,10-dien-9-one 12.} & \\
\text{To a flame-dried 250 mL round bottom flask equipped with stir bar were added SI1 (5.0 g, 23.34 mmol, 1.0 equiv), toluene (150 mL), 2,2-dimethyl-1,3-propanediol (4.86 g, 46.68 mmol, 2.0 equiv) and finally pyridinium p-toluenesulfonate (586.0 mg, 2.334 mmol, 0.1 equiv). This mixture was then heated to 60 ºC and stirred for one hour during which time the reaction turned an orange-red color. The vessel was then allowed to cool to room temperature, diluted with EtOAc (100 mL) and washed with NaHCO₃ (200 mL) followed with brine. The organic phase was dried over anhydrous MgSO₄, filtered, and evaporated in vacuo to give a crude solid. The product was purified by recrystallization from 20% Et₂O/Hex (12 mL per 1 g of crude) which provided white needles (5.16 g, 87%).}
\end{align*}
\]

**Appearance:** White needles (MP = 33–35 ºC)

**TLC:** \( R_f = 0.30 \) (1:3 Et₂O/hexanes, UV active, stains dark green upon vanillin staining).

**¹H NMR** (400 MHz, CDCl₃): \( \square \) 7.20 (d, \( J = 10.4 \) Hz, 1 H), 6.10 (d, \( J = 10.4 \) Hz, 1 H), 4.17 (s, 3H), 3.82 (d, \( J = 11.4 \) Hz, 2 H), 3.74 (s, 3 H), 3.70 (d, \( J = 11.4 \) Hz, 2 H), 1.28 (s, 3 H), 0.89 (s, 3H) ppm.
\(^{13}\text{C NMR}\) (150 MHz, CDCl\(_3\)): \(\delta\) 183.3, 157.6, 137.4, 135.4, 126.8, 92.3, 72.0, 61.5, 61.1, 30.3, 22.8, 22.5 ppm.

\textbf{HRMS (ESI-TOF):} calc’d for C\(_{13}\)H\(_{18}\)O\(_5\) [M+H\(^{+}\)] 254.1232, found 255.1229.

\((11R)-7,8\text{-dimethoxy-3,3,11-trimethyl-10-}-(2E,6E)-3,7,11\text{-trimethyldodeca-2,6,10-trien-1-yl)-1,5-dioxaspiro}[5.5]\text{-undec-7-en-9-one}(+)\text{-14.}\) To a flame-dried 100 mL round-bottomed flask equipped with a stir bar were added copper(II) trifluoromethanesulfonate (42.6 mg, 0.118 mmol, 0.03 equiv), (S,R,R) phosphoramidite ligand SI\(_2\)^2 (127.6 mg, 0.236 mmol, 0.06 equiv) and finally toluene (12 mL). This solution was stirred for one hour at room temperature under argon atmosphere before being cooled to \(-25^\circ\text{C}.\) To this cooled solution was added spiroketal 12 (1.000 g, 3.93 mmol, 1.00 equiv) as a solution in 5 mL toluene and then dimethylzinc (1.2 M in toluene, 8.19 mL, 9.83 mmol, 2.50 equiv) dropwise over 15 minutes during which time the reaction turns a bright yellow color. This reaction was stored in a \(-20^\circ\text{C}\) freezer for 18 hours. The reaction vessel was then placed in an ice water bath before lithium bis(trimethylsilyl)amide (1.0 M in THF, 5.90 mL, 5.90 mmol, 1.50 equiv), hexamethylphosphoramidate (1.71 mL, 9.83 mmol, 2.50 equiv), and farnesyl bromide (2.13 mL, 7.87 mmol, 2.00 equiv) were added sequentially. The reaction was stirred at 0 \(^\circ\text{C}\) for 3 hours before being diluted with Et\(_2\)O (60 mL) and quenched with NH\(_4\)Cl (sat. aq.; 100 mL). The layers were separated and the aqueous layer was extracted two times with Et\(_2\)O (100 mL). All the organic layers were combined and then washed with
water twice (100 mL) followed by brine (100 mL), and dried over MgSO₄. Evaporation in vacuo resulted in a clear yellow, non-viscous liquid. Column chromatography (SiO₂, 1:5 to 1:4 Et₂O/hexanes) provided a colorless, slightly viscous liquid (951.4 mg, 51 %).

**Appearance:** colorless oil.

**TLC:** $R_f = 0.65$ (1:3 Et₂O/hexanes, UV active, stains dark blue upon vanillin staining).

**$^1H$ NMR** (400 MHz, CDCl₃): $\delta$ 5.09–5.05 (m, 2 H), 5.00 (t, $J = 7.1$ Hz, 1 H), 4.10–4.06 (m, 4 H), 3.67–3.64 (m, 4 H), 3.55 (dd, $J = 10.8$, 1.2 Hz, 1 H), 3.43 (dd, $J = 10.8$, 1.2 Hz, 1 H), 2.69 (m, 1 H), 2.37–2.27 (m, 3 H), 2.07–1.94 (m, 8 H), 1.68 (s, 3 H), 1.62 (s, 3 H), 1.60 (s, 3 H), 1.58 (s, 3 H), 1.11–1.09 (m, 6 H), 0.88 (s, 3 H) ppm.

**$^{13}C$ NMR** (151 MHz, CDCl₃): $\delta$ 196.7, 163.2, 137.6, 137.5, 135.2, 131.4, 124.5, 124.2, 120.7, 97.1, 73.6, 71.3, 60.9, 60.5, 49.9, 40.0, 39.9, 38.6, 29.7, 27.8, 26.9, 26.8, 25.9, 23.4, 22.8, 17.8, 16.5, 16.1, 13.8 ppm.

**HRMS** (ESI-TOF): calc’d for C_{29}H_{46}O_{5} [M⁺] 474.3345, found 474.3345.

**Optical rotation:** $\left[\alpha\right]_{D}^{20} (c = 0.088, \text{MeOH}) = +51.1^\circ$.

(9R,10R,11R)-7,8-dimethoxy-3,3,11-trimethyl-10-((2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-1,5-dioxaspiro[5.5]undec-7-en-9-ol (+)-SI3. To a flame-dried 50 mL round-bottomed flask equipped with a stir bar were added (+)-14 (1.04 g, 2.190 mmol, 1.00 equiv) and toluene (12 mL). The resulting solution was cooled to -78 ºC before L-selectride (1.0 M in THF, 4.38 mL, 4.38 mmol, 2.00 equiv) was added dropwise. This reaction was
stirred for 2 hours at –78 °C before being allowed to warm to –20 °C over 4 hours. The mixture was then diluted with Et₂O (60 mL) and was filtered on a silica plug. The plug was washed with 30% EtOAc/Hex (100 mL). The solvents were removed in vacuo and column chromatography (SiO₂, 1:4 to 1:2 Et₂O/hexanes) provided the product as a colorless oil (783 mg, 1.643 mmol, 75 %).

**Appearance:** colorless oil

**TLC:** $R_f = 0.30$ (1:3 Et₂O/hexanes, UV active, stains dark blue upon *anisaldehyde* staining).

For the undesired diastereomer, $R_f = 0.35$

**$^1$H NMR** (400 MHz, CDCl₃): δ 5.19 (t, $J = 7.2$ Hz, 1H), 5.09 (m, 2H), 4.25 (d, $J = 10.4$ Hz, 1H), 4.19 (t, $J = 5.6$, 3.4 Hz, 1H), 3.94 (d, $J = 10.8$ Hz, 1H), 3.74 (s, 3H), 3.71 (s, 3H), 3.49 (dd, $J = 10.4$, 2.0 Hz, 1H), 3.36 (dd, $J = 10.8$, 2.0 Hz, 1H), 2.23-1.92 (m, 10H), 1.83-1.74 (m, 2H), 1.68 (s, 3H), 1.66 (s, 3H), 1.59 (s, 6H), 1.16 (s, 3H), 1.05 (d, $J = 6.8$ Hz, 3H), 0.79 (s, 3H) ppm.

**$^{13}$C NMR** (150 MHz, CDCl₃): δ 146.4, 144.3, 137.0, 135.3, 131.4, 124.5, 124.2, 122.6, 96.9, 73.8, 71.7, 66.5, 66.0, 59.9, 57.6, 40.6, 40.0, 39.9, 39.5, 29.5, 26.9, 26.8, 25.9, 23.9, 22.9, 17.8, 16.3, 16.2, 12.1 ppm.

**HRMS** (ESI-TOF): calc’d for C₂⁹H₄⁸O₅ [M⁺] 476.3501, found 476.3502.

**Optical rotation:** $[\alpha]_{D}^{20}$ (c=0.00323, MeOH) = +80.2°.
To a round-bottomed flask equipped with a stir bar were added (+)-SI3 (783 mg, 1.64 mmol, 1.00 equiv) and dichloromethane (12 mL). The mixture was rapidly stirred at room temperature while K10 Clay Montmorillonite (228 mg, 30 wt%) was added. The heterogeneous mixture was stirred for 1 hour at room temperature. The mixture was then directly purified using column chromatography (SiO₂, 1:3 to 1:2 Et₂O/hexanes) provided a colorless, viscous liquid (333.6 mg, 52%).

**Appearance:** clear oil

**TLC:** $R_f = 0.25$ (1:2 Et₂O/hexanes, UV active, stains dark blue upon anisaldehyde staining).

**¹H NMR** (600 MHz, CDCl₃): δ 5.16 (t, $J = 7.2$ Hz, 1H), 5.09 (t, $J = 6.6$ Hz, 2H), 4.35 (t, $J = 3.6$ Hz, 1H), 4.06 (s, 3H), 3.66 (s, 3H), 2.53 (m, 1H), 2.25 (t, $J = 7.8$ Hz, 2H), 2.13-2.02 (m, 6H), 2.00-1.95 (m, 2H), 1.75 (m, 1H), 1.68 (s, 3H), 1.66 (s, 3H), 1.60 (s, 6H), 1.17 (d, $J = 6.6$ Hz, 3H) ppm.

**¹³C NMR** (150 MHz, CDCl₃): δ 197.3, 160.6, 138.2, 136.1, 135.5, 131.5, 124.5, 124.0, 121.1, 68.1, 60.8, 59.4, 43.5, 40.4, 40.0, 39.9, 27.2, 26.9, 26.6, 25.9, 17.8, 16.3, 16.2, 12.5 ppm.


**Optical rotation:** $\left[\alpha\right]_D^{\text{P}0} (c = 0.364, \text{MeOH}) = +{52.0}^\circ$.

*The enantiomer of 1, (-)-antroquinonol A, was prepared from (-)-Enone 13 and tested for its oncological activity.

**Optical rotation:** $\left[\alpha\right]_D^{\text{P}0} (c = 0.364, \text{MeOH}) = -{57.1}^\circ.$
(R)-7,8-dimethoxy-3,3,11-trimethyl-1,5-dioxaspiro[5.5]undec-7-en-9-one (+)-13. To a flame-dried 100 mL round-bottomed flask equipped with a stir bar were added copper(II) trifluoromethanesulfonate (42.6 mg, 0.118 mmol, 0.03 equiv), phosphoramidite ligand SI2 (127.6 mg, 0.236 mmol, 0.06 equiv) and finally toluene (12 mL). This solution was stirred for one hour at room temperature under argon atmosphere before being cooled to –25 ºC. To this cooled solution was added spiroketal 12 (1.000 g, 3.93 mmol, 1.00 equiv) as a solution in 5 mL toluene and then dimethylzinc (1.2 M in toluene, 8.19 mL, 9.83 mmol, 2.50 equiv) dropwise over 15 minutes during which time the reaction turns a bright yellow color. This reaction was stored in a –20 ºC freezer for 18 h. The mixture was then diluted with Et₂O (100 mL) and quenched with NH₄Cl (sat. aq.; 100 mL). The layers were separated and the aqueous layer was extracted two times with Et₂O (2x50 mL). The organic layers were combined and washed with NaOH (1M, 100 mL) and brine (100 mL), and then dried over anhydrous MgSO₄. Evaporation in vacuo resulted in a clear yellow, viscous liquid. Column chromatography (SiO₂, 1:5 to 1:4 Et₂O/hexanes) provided a white crystalline solid (584.2 mg, 55 %). *(R)-7,8-dimethoxy-3,3,11-trimethyl-1,5-dioxaspiro[5.5]undec-7-en-9-one (-)-13 was prepared with an identical procedure but the opposite ligand enantiomer (R, S, S).

Appearance: White crystalline solid

TLC: Rf = 0.35 (1:3 Et₂O/hexanes, UV active, stains orange upon vanillin staining).

¹H NMR (400 MHz, CDCl₃): δ 4.11 (s, 3 H), 3.95 (d, J = 10.8 Hz, 1 H), 3.76 (d, J = 10.8 Hz, 1H), 3.67 (s, 3H), 3.58 (dd, J = 10.8, 1.2 Hz, 1 H), 3.51 (dd, J = 10.8, 1.2 Hz, 1 H),
2.62–2.49 (m, 2 H), 2.38–2.34 (m, 1 H), 1.07 (d, J = 6.8 Hz, 3H), 1.06 (s, 3H), 0.95 (s, 3H) ppm.

$^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$ 194.7, 162.9, 137.8, 97.4, 73.1, 71.5, 60.9, 60.8, 41.1, 35.5, 29.8, 23.2, 22.8, 14.5 ppm.

HRMS (ESI-TOF): calc’d for C$_{14}$H$_{22}$O$_5$ [M$^+$] 270.1467, found 270.1467.

Optical rotation: $[\alpha]_{D}^{20}$ (c = 0.011, MeOH) = $+98.2^\circ$.

(10R,11R)-10-((2Z,6E,10E)-12-((tert-butyldimethylsilyl)oxy)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)-7,8-dimethoxy-3,3,11-trimethyl-1,5-dioxa[5.5]undec-7-en-9-one (+)-16. To a flame-dried 100 mL round-bottomed flask equipped with a stir bar was added 13 (180 mg, 0.66 mmol, 1.00 equiv), dry THF (2 mL) and hexamethylphosphoramide (0.3 mL). The mixture was cooled to -30°C and lithium bis(trimethylsilyl)amide (1M in THF, 1.33 mL, 1.33 mmol, 2.00 equiv) was added dropwise. The reaction mixture was stirred at this temperature for 15 min before bromide SI4 (554 mg, 1.33 mmol, 2.00 equiv) in dry THF (1 mL) was added dropwise. SI4 was prepared by mesylation/bromination of the corresponding allylic alcohol$^3$ and was used without isolation or purification. The mixture was warmed to 0°C and stirred for 4 hours and then quenched with NH$_4$Cl (sat. aq.: 30 mL) and extracted twice with Et$_2$O (2x30 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO$_4$, and evaporated to give the desired product.
filtered, and concentrated \textit{in vacuo}. The product was purified using column chromatography (15\% EtOAc/Hex) to yield (+)-16 (181.3 mg, 45 \%) as a colorless oil.

\textbf{Appearance:} clear oil.

\textbf{TLC:} \(R_f = 0.65\) (1:3 Et\(_2\)O/hexanes, UV active, stains dark blue upon \textit{vanillin} staining).

\textbf{\(^1H\) NMR (400 MHz, CDCl\(_3\)):} \(\delta 5.36\) (t, \(J = 6.3\) Hz, 1H), 5.08 (t, \(J = 6.3\) Hz, 1H), 4.99 (t, \(J = 7.3\) Hz, 1H), 4.09 (s, 3H), 4.07 (d, \(J = 10.7\) Hz, 1H), 3.99 (s, 2H), 3.65 (d, \(J = 10.7\) Hz, 1H), 3.63 (s, 3H), 3.55 (d, \(J = 10.7\) Hz, 1H), 3.43 (d, \(J = 10.7\) Hz, 1H), 2.70-2.66 (m, 1H), 2.39-2.34 (m, 1H), 2.33-2.25 (m, 2H), 2.15-1.92 (m, 8H), 1.61 (s, 3H), 1.60 (s, 3H), 1.59 (s, 3H), 1.09 (m, 6H), 0.90 (s, 9H), 0.88 (s, 3H), 0.05 (s, 6H)ppm.

\textbf{\(^{13}C\) NMR (150 MHz, CDCl\(_3\)):} \(\delta 196.7, 163.2, 137.5, 137.4, 135.0, 134.4, 124.5, 124.4, 120.7, 97.1, 73.6, 71.3, 68.8, 60.9, 60.5, 49.9, 40.0, 39.5, 38.5, 29.7, 27.8, 26.8, 26.3, 26.1, 23.4, 22.8, 18.6, 16.5, 16.1, 13.8, 13.6, -5.1 ppm.

\textbf{HRMS (ESI-TOF):} calc’d for C\(_{35}\)H\(_{60}\)O\(_6\)Si [M\(^+\)] 604.4158, found 604.4159.

\textbf{Optical rotation:} \(\left[\alpha\right]_D^{10} (c = 0.123, \text{MeOH}) = +25.9^\circ\)

\[
(9R,10R,11R)-10-((2Z,6E,10E)-12-((\text{tert-butyldimethylsilyl})oxy)-3,7,11-trimethylododeca-2,6,10-trien-1-yl)-7,8-dimethoxy-3,3,11-trimethyl-1,5-dioxaspiro[5.5]undec-7-en-9-ol (+)-SI5. \] To a flame-dried 50 mL round-bottomed flask equipped with a stir bar were added (+)-16 (100.0 mg, 0.165 mmol, 1.00 equiv) and toluene
(1 mL). The resulting solution was cooled to -78 °C before L-selectride (1.0 M in THF, 0.33 mL, 0.33 mmol, 2.00 equiv) was added dropwise. This reaction was stirred for 2 hours at –78 °C before being allowed to warm to –20 °C over 4 hours. The mixture was then diluted with Et₂O (3 mL) and was filtered on a silica plug. The plug was washed with 30% EtOAc/Hex (10 mL). The solvents were removed \textit{in vacuo} and column chromatography (SiO₂, 1:4 to 1:2 Et₂O/hexanes) provided the product as a colorless, slightly viscous liquid (74.2 mg, 74 %).

\textbf{Appearance:} clear liquid

\textbf{TLC:} \( R_f = 0.30 \) (1:3 Et₂O/hexanes, UV active, stains dark blue upon \textit{anisaldehyde} staining).

\textbf{\textsuperscript{1}H NMR} (400 MHz, CDCl₃): \( \delta \) 5.36 (dd, \( J = 6.8, 1.4 \) Hz, 1H), 5.19 (t, \( J = 6.8 \) Hz, 1H), 5.11 (t, \( J = 6.8 \) Hz, 1H), 4.25 (d, \( J = 10.4 \) Hz, 1H), 4.18 (t, \( J = 4.9 \) Hz, 1H), 3.99 (s, 2H), 3.94 (d, \( J = 10.4 \) Hz, 1H), 3.74 (s, 3H), 3.71 (s, 3H), 3.49 (dd, \( J = 10.4, 2.0 \) Hz, 1H), 3.36 (dd, \( J = 10.4, 2.0 \) Hz, 1H), 2.22-1.92 (m, 10H), 1.78 (m, 2H), 1.65 (s, 3H), 1.59 (s, 6H), 1.15 (s, 3H), 1.04 (d, \( J = 6.8 \) Hz, 3H), 0.90 (s, 9H), 0.79 (s, 3H), 0.05 (s, 6H) ppm.

\textbf{\textsuperscript{13}C NMR} (150 MHz, CDCl₃): \( \delta \) 146.3, 144.3, 136.9, 135.1, 134.4, 124.5, 124.4, 122.5, 96.9, 73.7, 71.7, 68.8, 66.5, 59.9, 57.6, 40.6, 40.0, 39.6, 39.5, 29.5, 26.9, 26.8, 26.3, 26.1, 23.9, 22.9, 18.6, 16.4, 16.1, 13.6, 12.1, -5.1 ppm.

\textbf{HRMS} (ESI-TOF): calc’d for C\textsubscript{35}H\textsubscript{62}O\textsubscript{6}Si [M+H\textsuperscript{+}] 607.4388, found 607.4389.

\textbf{Optical rotation:} \([\alpha]_D^{\text{10}}\) (c=0.0034, MeOH) = +61.76°.
(4R,5R,6R)-4-hydroxy-5-((2Z,6E,10E)-12-hydroxy-3,7,11-trimethyltrideca-2,6,10-trien-1-yl)-2,3-dimethoxy-6-methylcyclohex-2-en-1-one (+)-17. To a round-bottomed flask equipped with a stir bar were added (+)-SI5 (50 mg, 0.08 mmol, 1.00 equiv) and dichloromethane (1 mL). The mixture was rapidly stirred at room temperature while K10 Clay Montmorillonite (15 mg, 30 wt%) was added. The heterogeneous mixture was stirred for 1 hour at room temperature. The mixture was then filtered through a glass-frit funnel and the resulting solution was concentrated in vacuo to give the crude mixture as a clear oil. The oil was dissolved in dry THF (1 mL) and TBAF (1M in THF, 0.24 mL, 0.24 mmol, 3.00 equiv) was added. The mixture was stirred for 1 h at room temperature before being directly purified by column chromatography (SiO₂, 1:9 to 3:5 EtOAc/Hex) to give the product as an oil (16.1 mg, 48%).

Appearance: clear oil

TLC: \( R_f = 0.25 \) (1:2 Et₂O/hexanes, UV active, stains dark blue upon anisaldehyde staining).

\(^1\)H NMR (600 MHz, CDCl₃): \( \delta \) 3.41 (dd, \( J = 7.2, 1.3 \) Hz, 1H), 5.19 (dd, \( J = 7.2, 1.3 \) Hz, 1H), 4.38 (d, \( J = 3.5 \) Hz, 1H), 4.09 (s, 3H), 4.02 (s, 2H), 3.69 (s, 3H), 2.56 (m, 1H), 2.27 (t, \( J = 7.5 \) Hz, 2H), 2.20-2.00 (m, 8H), 1.77 (m, 1H), 1.68 (s, 3H), 1.63 (s, 3H), 1.58 (s, 3H), 1.20 (d, \( J = 6.9 \) Hz, 3H) ppm.
\(^{13}\)C NMR (150 MHz, CDCl\(_3\)): \(\delta\) 197.3, 160.6, 138.1, 136.1, 135.0, 134.8, 126.0, 124.4, 121.2, 69.1, 68.1, 60.8, 59.5, 43.6, 40.5, 40.0, 39.4, 27.1, 26.5, 26.2, 16.3, 16.1, 13.9, 12.5 ppm.

HRMS (ESI-TOF): calc’d for C\(_{24}\)H\(_{38}\)O\(_5\) [M+H\(^+\)] \(407.2792\), found 407.2793.

Optical rotation: \(\left[\alpha\right]_{D}^{20} (c=0.0027, \text{MeOH}) = +58.5^\circ\).

\[(2E,6E,10Z)-12-((1R,2R,6R)-2-hydroxy-3,4-dimethoxy-6-methyl-5-oxocyclohex-3-en-1-yl)-2,6,10-trimethyldodeca-2,6,10-trienoic acid (\text{+})-15.\]

To a round-bottomed flask equipped with a stir bar were added \((\text{+})-17\) (7 mg, 0.017 mmol, 1.00 equiv), DCM (0.7 mL), TEMPO (0.5 mg, 0.0003 mmol, 0.20 equiv) and (bisacetoxyiodo)benzene (5.6 mg, 0.019 mmol, 1.10 equiv) at room temperature. The mixture was stirred until complete conversion of the alcohol (4 h). The mixture was then filtered on a silica plug and the plug was washed with 1:1 EtOAc/Hex (20 mL). The solvents were removed \textit{in vacuo} and the crude mixture was dissolved in \(^7\)BuOH/H\(_2\)O (4:1, 1 mL). 2-methyl-2-butene (0.1 mL, excess) was added followed by NaH\(_2\)PO\(_4\) (14.3 mg, 0.119 mmol, 7.00 equiv) and finally NaClO\(_2\) (10.8 mg, 0.119 mmol, 7.00 equiv). The reaction mixture was stirred overnight and then diluted with EtOAc and washed with brine. The organic layer was separated, dried over anhydrous MgSO\(_4\), filtered, and evaporated \textit{in vacuo}. The crude product was purified using preparative TLC (SiO\(_2\), 7:3 EtOAc/Hex) to give the product as an oil (4.1 mg, 57 %).

**Appearance:** clear oil
**TLC:** \( R_f = 0.25 \) (1:2 Et\(_2\)O/hexanes, UV active, stains dark blue upon *anisaldehyde* staining).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 6.85 (t, \( J = 7.3 \) Hz, 1H), 5.18 (t, \( J = 7.3 \) Hz, 1H), 5.11 (m, 1H), 4.37 (d, \( J = 3.4 \) Hz, 1H), 4.06 (s, 3H), 3.66 (s, 3H), 2.58-2.48 (m, 1H), 2.34-2.17 (m, 4H), 2.15-1.98 (m, 7H), 1.83 (s, 3H), 1.64 (s, 3H), 1.61 (s, 3H), 1.17 (d, \( J = 6.7 \) Hz, 3H) ppm.

\(^{13}\)C NMR (150 MHz, CDCl\(_3\)): \( \delta \) 197.3, 171.4, 160.5, 144.8, 137.8, 136.1, 134.0, 126.9, 125.2, 121.4, 68.1, 60.8, 59.5, 43.7, 40.5, 39.9, 38.2, 27.3, 27.0, 26.3, 16.2, 16.0, 12.6, 12.3 ppm.

**HRMS** (ESI-TOF): calc’d for C\(_{24}\)H\(_{36}\)O\(_6\) [M\(^+\)] 420.2511, found 420.2512.

**Optical rotation:** \([\alpha]_{D}^{20} \) (c=0.0029, MeOH) = +55.1°.

**References:**


Chiral HPLC for (+)-13:

ADH Column, 20% Ethyl Acetate in Hexanes, 0.8 mL/minute flow rate
### DAD-CH1 250 nm Results

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| Totals        | 7851108| 100.00 | 548073  | 100.00   |

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X-ray crystallographic data for (+)-**13**:

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**Table S1.** Crystal data and structure refinement for Baran480.

Report date 2014-10-23
Identification code
Empirical formula
Molecular formula
Formula weight
Temperature
Wavelength
Crystal system
Space group
Unit cell dimensions
Volume
Z
Density (calculated)
Absorption coefficient
F(000)
Crystal size
Crystal color, habit
Theta range for data collection
Index ranges
Reflections collected
Independent reflections
Completeness to theta = 68.000°
Absorption correction
Max. and min. transmission
Refinement method
Data / restraints / parameters
Goodness-of-fit on F^2
Final R indices [I>2sigma(I)]
R indices (all data)
Absolute structure parameter
Extinction coefficient
Largest diff. peak and hole

Table S2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for Baran480. U(eq) is defined as one third of the trace of the orthogonalized U_ij tensor.
Table S3. Bond lengths [Å] and angles [°] for Baran480.
O(1)-C(1) 1.414(2) C(13)-H(13A) 0.9800
O(1)-C(10) 1.446(2) C(13)-H(13B) 0.9800
O(2)-C(1) 1.420(2) C(13)-H(13C) 0.9800
O(2)-C(12) 1.440(2) C(14)-H(14A) 0.9800
O(3)-C(2) 1.343(2) C(14)-H(14B) 0.9800
O(3)-C(8) 1.432(2) C(14)-H(14C) 0.9800
O(4)-C(3) 1.385(2)
O(4)-C(9) 1.438(2) C(1)-O(1)-C(10) 118.32(13)
O(5)-C(4) 1.226(2) C(1)-O(2)-C(12) 114.81(14)
C(1)-C(2) 1.543(3) C(2)-O(3)-C(8) 123.95(16)
C(1)-C(6) 1.534(3) C(3)-O(4)-C(9) 112.57(15)
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C(3)-C(4) 1.473(3) O(1)-C(1)-C(2) 111.67(15)
C(4)-C(5) 1.501(3) O(1)-C(1)-C(6) 105.12(15)
C(5)-H(5A) 0.9900 O(2)-C(1)-C(2) 110.01(15)
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C(6)-H(6) 1.0000 O(3)-C(2)-C(1) 110.28(15)
C(6)-C(7) 1.521(3) O(3)-C(2)-C(3) 127.40(18)
C(7)-H(7A) 0.9800 C(3)-C(2)-C(1) 122.32(17)
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C(8)-H(8B) 0.9800 O(5)-C(4)-C(3) 120.99(19)
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C(10)-H(10A) 0.9900 C(4)-C(5)-H(6) 112.46(16)
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C(11)-C(14) 1.529(3) C(5)-C(6)-C(1) 109.64(15)
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Table S5. Hydrogen coordinates ($x \times 10^4$) and isotropic displacement parameters ($Å^2 \times 10^{-3}$) for Baran480.

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in vitro/in vivo assays

**in vitro cytotoxicity assay - Materials and Methods**

All cell lines (A549, AsPC-1, HepG2, Hep3B, NCI-H441, LNCaP, MDA-MB-231 and PANC-1) were obtained from American Type Culture Collection (Manassas, VA). Cell lines were grown in RPMI 1640 media (Gibco) with 10% fetal bovine serum, heat inactivated (Sigma), 10mM HEPES (Gibco), and 1% penicillin/streptomycin (Gibco). All cell lines were maintained under routine maintenance conditions (37 °C, 5% CO2, high humidity).

An aliquot of each cell line was seeded to each well of a 384-well plate (Greiner) using the same growth media as outlined above. Cells were allowed to attach 24 hours before compound addition. Compounds were solubilized and serially diluted immediately prior to compound addition to cells. Following a 72 hour incubation with compound, cells were stained with CellTiter 96 Aqueous Non-Radioactive Cell Proliferation MTS stain (Promega) to determine in vitro cytotoxicity. Plates were incubated at 37 °C for 3hrs, and absorbance then measured at 492nm.

**Assessment of In Vivo Antitumor Activity in the Subcutaneously Implanted Hep3B Hepatocellular Carcinoma Xenograft Model in NSG Mice.**

Female NSG (NOD.Cg-Prkdc<sup>-scid</sup> Il2rgtm<sup>Iwjl</sup>/SzJ) mice, 6–8 weeks old, were obtained from the Jackson Laboratory. Animals were provided with food and water ad libitum and housed five per cage. Mice were maintained in accordance with Bristol-Myers Squibb’s Institutional Animal Care and Use Committee in accordance with the American
Association for Accreditation of Laboratory Animal Care (AAALAC) guidelines for the humane treatment and care of laboratory mice.

Hep3B tumor fragments maintained by serial passage *in vivo* were implanted subcutaneously in the hind flank using an 18 g trocar. Compound or vehicle dosing was initiated approximately two weeks post implant, when tumor sizes reached 75-100 mm³. Compound 1 in corn oil was administered intraperitoneally (i.p.) at 50 mg/kg on a QD 14 schedule, whereas paclitaxel in Cremophor/EtOH (50:50 diluted 1:4 in saline prior to injection) was administered intravenously (i.v.) at its optimed (MTD) preclinical regimen of 24 mg/kg using a Q2D 5 schedule. The vehicle control group was given corn oil. Tumor growth was assessed twice weekly by vernier caliper measurement. Group sizes were n = 6 with double sided tumor implants.

Antitumor activity was determined by calculating the maximum percent tumor growth inhibition (TGI) of treated animals using the formula: 

\[ \% \text{TGI} = \left\{ \frac{(C_t - T_t)}{(C_t - C_0)} \right\} \times 100 \]

where \( C_t \) = the median tumor volume (mm³) of vehicle treated control (C) mice at time t. \( T_t \) = median tumor volume of treated (T) mice at time t. \( C_0 \) is the median tumor volume of control mice at time 0. Activity is defined as a continuous \( \% \text{TGI} > 70\% \) for at least one tumor volume doubling time after the start of drug treatment. Standard error of the mean was calculated using IDBS EWorkBook built in software formula in Quantrix spreadsheets.
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<th>Cell Line</th>
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<td>MDA-MB-231</td>
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<tr>
<td>HepG2</td>
<td>&gt;25</td>
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<tr>
<td>LNCaP</td>
<td>&gt;25</td>
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<tr>
<td>Hep 3B</td>
<td>8 ± 1.2</td>
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<tr>
<td>PANC-1</td>
<td>&gt;25</td>
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<td>A549</td>
<td>14.5 ± 1.1</td>
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<td>H441</td>
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Table S6. Oncology panel in vitro data for (−)-Antroquinonol A (ent-1)

* incubation time. Source of cell lines: ATCC.

IC50 Curves for (1), (15) and enantiomer of antroquinonol (ent-1):
Experimental protocol for metabolite identification using mouse liver microsomes

MsLM incubation was carried out in 1 mg/ml microsomal proteins with 5 μM drug and 1 mM NADPH at 37 °C. LC/UV/MS analysis was done with Shimadzu LC-30AD LC pumps.
coupled with a SPD-M20A PDA detector and a Thermo LTQ Orbitrap mass spectrometer. The LC was run on a Water Acuity UPLC HSS T3 column (2.1x150 mm) in water/acetonitrile solvents and 0.1% formic acid at a flow rate of 0.5 ml/min. The LC gradient was a linear gradient of 25% - 98% acetonitrile in 9-min, after an initial ramp from 10% to 25% acetonitrile in 0.2-min. The UV traces exhibited in Fig. 2A were extracted at a wavelength range of 265 - 270 nm. A similar protocol was used for human and rat liver microsomes and for hepatocytes.

**Metabolism of 1 in rat hepatocytes**

![Figure S1. Major metabolite in Rat Hepatocytes was Met2](image)

x = sample matrix peaks, not parent related; 5-µM drug, ~2 million cells/ml, UV 260-270nm
Metabolism of 1 in human hepatocytes

**Figure S2.** Major metabolite in Human Hepatocytes was Met2.

x = sample matrix peaks, not parent related; 5-µM drug, ~2 million cells/ml, UV 260-270nm.
Metabolism of 1 in mouse hepatocytes

**Figure S3.** Major metabolite in Mouse Hepatocytes was Met2.

x = sample matrix peaks, not parent related; 5-µM drug, ~2 million cells/ml, UV 260-270nm.
Metabolism of 1 in human liver microsomes

Figure S4. Major Metabolite in Human Liver Microsome was 15.

x = sample matrix peaks, not parent related; 5-µM drug, 1-mg/ml microsomal protein,
UV 265-270nm.
**Mouse PK protocol**: Compound 1 (50 mg/kg) was administered to male Balb/C mice (n = 3 per group, 20–25 g each) as an intraperitoneal injection (i.p) and oral (p.o) dose formulated in 100% Corn oil (10mL/kg). Blood samples (~15 µL) were obtained by retro-orbital bleeding at 0.5, 1, 2, 4, and 7 hours post dose. Aliquots of blood samples (15-µL) were spotted directly onto Ahlstrom 226 DBS cards (PerkinElmer, Waltham, MA, USA). Samples were dried in ambient air for at least 2 hours and stored at room temperature in sealed bags with desiccant until analysis. A 6-mm disc from the center of the dried spot (equivalent to 12.5 µL of blood) was punched with a BSD600 Duet Automated Punch Instrument (Luminex, Austin, TX, USA), and the sample was delivered into a 96 format filter plate. A 35 µL aliquot of water was added and vortexed for 5 minutes, followed by 85 µL of acetonitrile containing the internal standard. The filter plate was then vortexed for an additional 30 minutes, centrifuged and supernatant was analyzed via LC-MS/MS.

**Raw data**

**Table 1**: Whole Blood Concentrations (nM) of compound 1 in Mice after a 50-mg/kg intraperitoneal (i.p.) injection

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<td>7</td>
<td>434.1</td>
<td>256.4</td>
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**Table 2**: Whole Blood Concentrations (nM) of compound 1 in Mice after a 50-mg/kg Oral (PO) Administration

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<th>100.0</th>
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<td>7</td>
<td>26.6</td>
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Figure S5. Screened conditions for reduction of 14.

- = yield of mixture of diastereomers.

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<td>1.3 eq. DiBzAl-H, THF, -78 °C, 6 h</td>
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<td>2.0 L-Selectride, toluene, -78 °C, 6 h</td>
<td>94</td>
<td>3:1</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>2.5 eq. L-Selectride, 3.0 eq. ZnCl₂, toluene, -78 °C, 3 h</td>
<td>64</td>
<td>&lt;1:20</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>20 mol% (R)-2-amino-CBZ, 1.2 eq. BH₃, THF, rt, 24 h</td>
<td>0</td>
<td>n.d.</td>
<td>~5% conversion</td>
</tr>
<tr>
<td>19</td>
<td>20 mol% (S)-2-amino-CBZ, 1.2 eq. BH₃, THF, rt, 24 h</td>
<td>0</td>
<td>n.d.</td>
<td>~5% conversion</td>
</tr>
<tr>
<td>20</td>
<td>20 mol% [(PPh₃)₃RhCl]₂, 2.0 eq. Et₃SiH, benzene, 50 °C, 18 h</td>
<td>n.d.</td>
<td>n.d.</td>
<td>~5% conversion</td>
</tr>
<tr>
<td>21</td>
<td>20 mol% [tricyclooctene]C₂Cl₂, 2.0 eq. Ph₂SiH₂, benzene, 50 °C, 24 h</td>
<td>n.d.</td>
<td>n.d.</td>
<td>~5% conversion</td>
</tr>
<tr>
<td>22</td>
<td>Ph₂BH, THF, -78 to 0 °C, 12 h</td>
<td>n.d.</td>
<td>&lt;1:20</td>
<td>~10% conversion</td>
</tr>
</tbody>
</table>

Figure S6. Screened ketal identity for conjugate additions.
3.9 Spectra

\[ ^1H \text{NMR} (400 \text{ MHz}, \text{CDCl}_3) \]
$^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (151 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (151 MHz, CDCl$_3$)
$^{1}H$ NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (151 MHz, CDCl$_3$)
$^{13}$C NMR (151 MHz, CDCl$_3$)
3.10 Distribution of credit

Synthetic planning was mostly conducted by Prof. Phil S. Baran and myself. The aromatic ring reduction routes and allylic alcohol isomerization ideas were Prof. Baran’s while most ring synthesis routes and the final route were ideas of mine. Dr. Eran Sella contributed a number of routes that were not covered in this thesis, including Cu-H reduction of a tetra-substituted quinone monoketal.

Synthetic chemistry experiments were run by Garrett Saul, Dr. Eran Sella and myself. Garrett Saul did early work on the scale-up of the antroquinonol A synthesis and also investigated the direct conversion of antroquinonol A to antroquinonol D. Dr. Eran Sella worked full time for around 6 months on the synthesis of antroquinonol A. He discovered and optimized the conditions for the 1,4-additions of the lithiated furan ring into enoates shown in this section. In addition, he lead and finished the scale-up of the final synthesis to produce over 1 gram of the natural product.

All pre-clinical/biological investigations of antroquinonol A, including identification of the acid metabolite, were conducted by the Bristol Myers Squibb team lead by Dr. T. G. Murali Dhar. This team included Robert M. Borzilleri, Joseph Fargnoli, Kathy A. Johnston, Haiying Zhang and Mark P. Fereshteh.

A large portion of this chapter is described in an article that I co-authored with the aforementioned names. This manuscript was co-written by Phil and I with assistance from Dr. Eran Sella.
Appendix

Curriculum vitae
Matthew Tomas Villaume  
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E-mail: villaume@scripps.edu  

Education:  
2012–Present  The Scripps Research Institute, (La Jolla, CA), Ph.D. anticipated in Jun 2016  
2008–2012  Boston College (Chestnut Hill, MA), B.Sc.  

Research Experience:  
Sep 2015 – Present  Visiting Doctoral Research Student (Biology)  
Supervisor: Prof. Kristin Baldwin  
Department of Neuroscience, The Scripps Research Institute  
Jun 2012 – Sep 2015  Doctoral Research in Chemistry (Organic Chemistry)  
Supervisor: Prof. Phil S. Baran  
Department of Chemical and Biological Sciences, The Scripps Research Institute  
Dec 2009 – June 2012  Undergraduate Honors Thesis Project in Chemistry (Organometallic Chemistry)  
Supervisor: Prof. Amir Hoveyda  
Department of Chemistry, Boston College  

Publications:  

Teaching Experience:  
Mar 2015 – June 2015  Heterocyclic Chemistry Graduate Teaching Assistant  
Supervisor: Prof. Phil S. Baran  
The Scripps Research Institute, La Jolla, CA, USA
Sep 2014 – Undergraduate Research Mentor (Garrett Saul, University of San Diego)
Jan 2015 Supplemental Instructor: Phil S. Baran
The Scripps Research Institute, La Jolla, CA, USA

Jan 2006 – General Chemistry Laboratory Teach Assistant
Apr 2006 Supervisor: Dr. Lynne O’Connel
Boston College, Chestnut Hill, MA, USA

Sep 2004 – Undergraduate Tutor in Chemistry, Math and Physics
Dec 2004 Supervisor: Dr. Kathy Duggan
Connors Family Learning Center, Chestnut Hill, MA

Awards:

Oct 2013 NSF Graduate Research Fellowship
Aug 2012 The Scripps Research Institute Dean’s Fellowship
Jun 2012 Phi Beta Kappa, Boston College Chapter
Jun 2012 Excellence in Chemistry Award, Boston College
Jun 2012 Scholar of the College Honors Thesis, Boston College
Jun 2012 Alpha Sigma Nu, Boston College Chapter
2008-2012 Dean’s Honors List, Boston College
2008-2012 Boston College Honors Program

Other Work and Volunteering Experience:

Jun 2015 – Dog Rescue Foster Home
Present The Animal Pad

Sep 2010 – Health Educator; Mental Health Workshop Leader
May 2012 Peer Health Exchange, Boston, MA

Jan 2009 – Volunteer Emergency Medical Technician (EMT)
Jan 2012 Armstrong Ambulance, Boston, MA and Eagle EMS, Boston College