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Morinaka, Brandon Isamu

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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Marine Natural Products: Integrated Spectroscopic Solutions for Structure Elucidation

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Chemistry

by

Brandon Isamu Morinaka

Committee in charge:

Professor Tadeusz F. Molinski, Chair
Professor Michael D. Burkart
Professor Judy E. Kim
Professor Bradley S. Moore
Professor Yitzhak Tor

2011
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Chair

University of California, San Diego

2011
DEDICATION

For my family who have always supported me.
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<tbody>
<tr>
<td>Ac</td>
<td>acetyl</td>
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<tr>
<td>Aq</td>
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</tr>
<tr>
<td>Boc</td>
<td>t-butoxycarbonyl</td>
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<tr>
<td>CD</td>
<td>circular dichroism</td>
</tr>
<tr>
<td>CDA</td>
<td>chiral derivatizing agents</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DIBAL</td>
<td>diisobutylaluminum hydride</td>
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<tr>
<td>DMAP</td>
<td>(N,N)-dimethylaminopyridine</td>
</tr>
<tr>
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<td>(N,N)-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
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<td>ECCD</td>
<td>exciton coupled circular dichroism</td>
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<td>infrared</td>
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<td>MTPA</td>
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VITA

Education:

2003 University of California, Santa Cruz
Bachelor of Science, Chemistry

2011 University of California, San Diego
Doctor of Philosophy, Chemistry

Publications:


7. Dalisay, D. S.; Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. “A Tetrachloro Polyketide Hexahydro-1H-isoindolone, Muironolide A, from


ABSTRACT OF THE DISSERTATION

Marine Natural Products:
Integrated Spectroscopic Solutions for Structure Elucidation

by

Brandon Isamu Morinaka

Doctor of Philosophy

University of California, San Diego, 2009

Professor Tadeusz F. Molinski, Chair

This dissertation describes the structure determination and biological activity of four groups of sponge derived secondary metabolites by integrated approaches using synthesis and circular dichroism.

Chapter 2 describes the isolation and structure determination of brominated ene–yne tetrahydrofuran fatty acids, mutafurans A–G from the
marine sponge *Xestospongia muta*. Two optically active ene–yne tetrahydrofuran model compounds were synthesized for chiroptical comparison to the natural product. Ene–yne and diyne alcohols and their corresponding naphthoate derivatives were synthesized for chiroptical studies. The antifungal activity of mutafurans A–G is reported.

Chapter 3 describes the identification of the feeding deterrent chemotype in the marine sponge *Phorbas amaranthus* against the common bluehead wrasse, *Thalassoma bifasciatum*. Two types of sulfated sterols were isolated from the highly deterrent polar extracts: amaranzoles A–F and amaroxocanes A and B. The structure determination of amaranzoles A and B was assisted by synthesis of optically active hydroxy-phenyl imidazole model compounds for comparison of NMR and CD to the natural products. Amaraoxocane B showed feeding deterrent activity against *Thalassoma bifasciatum* at higher than natural concentrations.

Chapter 4 describes the identification of *bis*–piperidine alkaloids, xestoproxamines A–C from the marine sponge *Neopetrosia proxima*. The stereochemistry of the C23 methyl group in xestoproxamine C was elucidated by a Hoffman degradation/cross metathesis protocol and comparison to an optically active model. A method was established to assign the absolute configuration of the *bis*–piperidine core in xestoproxamines A–C by chemical conversion to *bis*–*p*–bromophenacyl quaternary ammonium salts, and
interpretation of the resulting ECCD spectra. The cytotoxic activity for xestoproxamines A–C is reported.

Chapter 5 describes the isolation and structure determination of mollenyne A from *Spirastrella mollis*. The complete stereostructure was determined by a combination of NMR, CD, and chemical conversion. The biological activity for mollenyne A is reported.
CHAPTER 1

INTEGRATED APPROACHES TO THE CONFIGURATIONAL ASSIGNMENT OF MARINE NATURAL PRODUCTS

1.1 Marine Natural Products as a Source of Chemical Diversity and Drug Leads

The marine environment is among the most diverse and prolific source of natural products. The unmatched chemical diversity of secondary metabolites from invertebrates (sponges, tunicates, nudibranchs, etc.) and marine microorganisms have led to the discovery of promising pharmacologically bioprobes and exciting drug candidates.¹ Presently, three marine derived compounds are approved for use as therapeutic agents; ziconotide (Prialt, 1.1), a ‘cysteine knot’ peptide isolated by Baldomera and coworkers² from the cone snail Conus magus with potent analgesic properties for neuropathic pain in patients who no longer tolerate morphine. Yondelis (Trabectidin, ET-743, 1.2), a complex hydroisoquinoline alkaloid reported by the Rinehart group in 1984 from the Caribbean tunicate Ecteinascidia turbinata,³ was approved in Europe in 2007 for soft tissue sarcoma. The polyether toxin, halichondrin B (1.3) reported by Uemura from a marine sponge Halichondria okadai,⁴ provided a scaffold for synthetic refinements by truncation to eribulin mesylate (Halaven, 1.4) a molecule half its size but retaining most of its potency. Halaven was approved in the USA late 2010 for treatment of solid tumors.
Figure 1.1. Currently used marine-derived pharmaceuticals: ω-conotoxin VIIA (1.1), Et-743 (1.2), halichondrin B (1.3), and Eribulin mesylate (1.4).

These three examples illustrate an underappreciated fact: the structure of therapeutic natural products with stereochemical complexity have been solved mostly with integrated chemical approaches requiring detailed, painstakingly acquired data, sometimes over the course of several years, often
with limited sample size. Modern natural product discovery efforts are now focused on rare or scarce materials from niche sources. The available amounts of many of these 'nanomole-scale' natural products make their structures impossible to solve using historical approaches. The issues of procurement for biological evaluation and scale-up production are another matter (e.g. the commercial Yondelis is produced by ~14 steps from cyanosafacin, a compound readily available from another natural product\textsuperscript{5}).

In natural product drug discovery, the structure is fundamental and primal. Fundamentally, the information content in a natural product – whether it be for structure-activity relationship, or informing retrosynthetic design for procurement by total synthesis – is revealed by structure elucidation. Finally, the holy grail of intellectual property is the New Chemical Entity (NCE) or New Active Substance (NAS)\textsuperscript{1b} – a bioactive molecule of an unprecedented structural type.

Although modern developments in spectroscopic techniques enable the identification of the structures of microgram-samples of complex small molecules, there is no general solution to the absolute configuration of a compound, those even containing a few stereocenters. The absolute stereostructure is solved on a case-by-case basis.

The structure of this review will cover briefly the current state of instrumentation for the determination of relative and absolute configuration. Instrumental developments have been covered extensively in previous
reviews and mentioned here only in passing. Second, case studies will be presented where chemical approaches to structure elucidation have been integrated with spectroscopy to solve stereochemical problems. To serve the dual purposes of economy of pages and contemporary interests, the review is restricted to cases since about 2000; the time during which NMR technologies (cryoprobe) have undergone major evolution, The majority of the efforts in each case study were completed only after the planar structure – a 2D structure lacking stereochemical assignments – had been proposed. In all cases, the natural product is a rare entity, generally procured from uncommon marine organisms rather than sustainable fermentation methods. It may be of interest to readers to be informed of the scale of the chemical operations. Most chemical transformations were carried out on the microscale (if not nanomole-scale), and the corresponding quantities are given in some of the Figures. We have tried to select a variety of molecules of different structural types that often dictate a unique approach to a particular problem, but they do not necessarily constitute the ‘most difficult’ cases. The examples for this review subscribe to one of the following criteria: (a) push the limit to spectroscopic and chemical methods, (b) demonstrate applicability and limitations of new techniques, (c) resolve configurational assignment of particularly difficult or marine natural products with potent biological activity. Finally, we hope the selected case studies provide some inspiration to a readership with broad interests: natural products chemists and synthetic organic chemists with
passionate interests in the art of structure determination and synthesis of marine natural products.

1.2 NMR Based Methods for Structure Determination and Stereochemical assignment.

1.2.1 Current State of NMR.

Nuclear magnetic resonance spectroscopy is still the most important tool for modern structure determination. Over the past 20 years, NMR-sensitivity has steadily increased to provide a refined tool to ‘view’ unexplored regimes of natural products. Around 1992, 500 MHz 3 mm inverse-detect probes were introduced reducing the sample volume from ~600 µL (5 mm tube) to ~140 µL and effectively doubling the S/N.7 The applicability of the 500 MHz 3 mm inverse detect probe was demonstrated by measurements of sample of brevetoxin-C (800 µg, 0.95 µmole) and complete 1H and 13C assignments based on homonuclear and heteronuclear 2D experiments.8 A substantial advancement came with the advent of commercial 500 MHz 3 mm cryogenic probes that appeared around 2000 and showed an increase of S/N of ~3.5 times compared to a room temperature 500 MHz 3 mm probe.9 Currently, 600 MHz 10 and 1.7 mm microcryoprobe NMR spectrometers allow the acquisition of standard 2D NMR data only a few nanomoles of a sample. The least sensitive standard 2D experiment (gHMBC) can be acquired on 15 nmol (5.4 µg) of strychnine over a weekend (43 h).11 These contemporary
developments in NMR are having profound effects on the conduct of natural products chemistry.

![Chemical Structures](image)

**Figure 1.2.** Structures of marine natural products characterized at the microgram scale.

The complete structure elucidation of complex natural products on micrograms of material have been typically carried out with assistance of a parent compound. Pteriatoxins A–C (1.5–1.7)\textsuperscript{12} reported by Uemura from the Okinawan bivalve *Pteria penguin* are derivatives of pinnatoxin A (1.8)\textsuperscript{13} and the full structures (except the configuration of the sidechains) of 1.5–1.7 were...
established by homonuclear based (\(^1\)H, COSY, and HOHAHA) experiments. Phorbaside F (1.9)\(^{14}\) and hemi-phorboxazole (1.10)\(^{15}\) from the marine sponge Phorbas sp. are derivatives of phorbaside A (1.11)\(^{16}\) and phorboxazole A (1.12),\(^{17}\) respectively. The structure determination of these two metabolites were assisted with NMR data acquired on the corresponding parent compound, and also made use of modern 1.7 mm microcryoprobe NMR which for acquisition of HSQC and HMBC data.

These impressive case studies above also illustrate the limit of what NMR alone can provide. New chemical entities and derivatives with isolated stereochemical elements pose the most difficult challenges for modern structure determination. Exclusive reliance on NMR for structure elucidation imposes a limitation: the acquisition for heteronuclear (\(^1\)H, \(^{13}\)C) based 2-dimentional NMR data (most importantly HMBC). Microcryoprobe NMR, allows acquisition of HMBC data on only a few micrograms,\(^{11}\) and allows elucidation of a constitutional formula and planar structure. The relative configuration can then be solved by inherently less sensitive indirect detected \(J\)-resolved experiments (i.e. HETLOC, HSQC-HECADE, HSQMB, etc.) as well as NOESY and ROESY experiments.

Often, the relative and/or absolute configuration is not obtainable by NMR alone, and alternate strategies must be employed. Chemical conversion and degradation of natural products is an integral part of structure elucidation. In most cases of nontrivial stereochemical assignments, empirical chemical
manipulations become essential. These case-by-case scenarios test the expertise of the natural product chemist with synthetic manipulations down to a few micrograms of material. Several examples are presented in this review which are testament, of the practicing chemist, not just instrumental proficiency but of skill and tenacity.

1.2.2 Nuclear Overhauser Effect (NOESY) and J coupling (\(^1\text{H}-\)^1\text{H})

Measurement of dipolar coupling (NOESY and ROESY) and interpretation of \(^1\text{H}-\)^1\text{H} coupling constants are the most widely used tools for determining the relative configuration of small molecules. NOESY experiments are also useful in acyclic systems where constraints placed by \(^1\text{H}-\)^1\text{H} and \(^1\text{H}-\)^13\text{C} scalar couplings lead to unique solutions. Complications arise when rotamers contribute to conformational mobility, however judicious application of molecular modeling or ab initio calculations often resolves ambiguous solution.

1.2.3 The \(^{13}\text{C} \text{NMR Acetonide Method (Rychnovsky)}\)

First described in 1990, the \(^{13}\text{C} \text{acetonide method developed by Rychnovsky}\) answered a fundamental configurational question in polyketides – how to assign the relative configuration of \textit{syn}- and \textit{anti}-1,3-diols? Diols are converted to the corresponding acetonides, and \(^{13}\text{C} \text{chemical shifts of the geminal methyl groups can be used to differentiate the two diastereomers. The method was based on symmetry principles and conformational preferences.}
The meso-like syn-1,3-diol acetonide (SDA), adopts a chair 1,3-dioxolane conformation with axial and equatorial methyl groups. The anti-1,3-diol acetonide (ADA) with C2-like symmetry, prefers the twist-boat conformation to minimize 1,3-diaxial interactions; and the methyl groups are almost equivalent. In the SDA, the axial and equatorial methyl groups resonate at ~δ 30 ppm and ~δ 20 ppm, respectively. In the ADA case, the methyl group chemical shifts are almost identical to each other (~δ 25 ppm). As the size difference of substituents at C4 and C6 increase, the 1,3- dioxane ring tends to adopt a chair conformation. It should be noted that the symmetry elements in 1,2-diol acetonides are also useful for assignment purposes.

![Conformations, chemical shifts, and nOes for (a) syn-1,3-diol acetonide (SDA) and (b) anti-1,3-diol acetonide (ADA).](image)

Figure 1.3. Conformations, chemical shifts, and nOes for (a) syn-1,3-diol acetonide (SDA) and (b) anti-1,3-diol acetonide (ADA).

Evans extended the $^{13}$C acetonide method to polypropionate chains containing branched methyl groups at the C5. The acetonide quaternary carbon is also diagnostic for configuration (SDA, ~98.1 ppm; ADA, ~100.6 ppm).

Three standard 2D NMR experiments: the NOESY/ROESY, HSQC/HMQC, and HMBC experiments allow extension of the method to more
complex polyacetonide systems. In the SDA, the axial methyl group shows nOe correlations to H4 and H6 axial protons. In the ADA, one acetonide methyl shows an nOe correlation to H4, and the other methyl shows an nOe to H6. Sensitivity is also improved by using the simple experiment of using 13C–labeled acetone for acetonide preparation.23

1.2.4 Chiral Derivatizing Agents (Mosher’s Method)

Originally proposed by Mosher in 197324, and refined by Ohtani and coworkers in 199125, the Mosher’s and modified Mosher’s method (MMM) represent the most widely used tool for determining the absolute configuration of secondary alcohols. Optically pure 2-methoxy-2-phenyl-2-trifluoromethyl acetic acid (MTPA) or the corresponding acid chloride (MTPA-Cl) are the most commonly used chiral derivatizing agents (CDAs). Differential chemical shifts are aligned for each group L1 and L2, and fitted to the configurational models (Figure 1.4).

The generally accepted model, conformer a (Figure 1.4), suggests that the carbinol proton, ester carbonyl, and trifluoromethyl group in the same plane. However, conformational studies carried out by Riguera et. al. demonstrate three major conformers (a-c) are present in MTPA esters.26 In conformer b, the phenyl ring is twisted and deshields L2. Conformer c has the trifluoromethyl group antiperiplanar to the carbinol proton and aryl group twisted which deshields L1. These opposing shielding and deshielding effects contribute to the relatively small net magnitudes of Δδ values and may even
give anomalous alternation in sign.\textsuperscript{27} In any case, the MMM has a built in ‘self-examination mechanism’ where ambiguous results point to the need for an alternate approach to assignment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Conformers for (R)-MTPA esters (a) major conformer leading to reliable configurational assignment, other conformers (b and c) also present.}
\end{figure}

The methoxyphenylacetic acid (MPA) may be a more reliable CDA because only two major ester conformers (a and b, Figure 1.5.) are present leading to $\Delta \delta$ values of greater magnitude. The model for MPA places the methoxy group, ester carbonyl, and carbinol proton in the same plane (a, Figure 1.5). L$_1$ is shielded in the (R)-MPA ester and L$_2$ is unaffected, and the opposite effects are observed with the (S)-MPA esters. Note that the phenyl group lies to the opposite side with respect to the MTPA esters. For this reason, $\Delta \delta$ values are defined by a different formula ($\Delta \delta = \delta_R - \delta_S$).
Figure 1.5. Conformers for (R)-MPA esters. (a) Major conformer used for configurational assignment. (b) Minor conformer.

The anisotropic differences between MTPA and MPA esters in addition to derivatives of other CDAs have been recently quantified by Hoye’s group. (−)-Menthol was converted to both (R) and (S) diastereomeric esters and Δδ values = δR − δS were acquired for each derivative. The absolute average for all the values, reflects the discriminating power of the CDA, were calculated for each pair. These comparative absolute values of Δδ may be useful when choosing a CDA to assign the configuration of a secondary hydroxyl group flanked by one or more CH₂ chains. 2-NMA gives rise to particularly large anisotropy. Successful configurational assignments have been achieved for challenging compounds such as ginnol²⁸ and shishidemdiol using 2-NMA (2-naphthylmethoxyacetic acid).
Figure 1.6. Discriminating power of various CDAs. Mean $\Delta \delta$ values of menthyl esters. ($\Delta \delta = \delta R - \delta S$).

MPA esters are also used to ‘fingerprint’ diastereomers from degradation or chemical conversion for comparison with optically pure standards synthesized for comparison by NMR. The properties of CDAs and applications have been the subject of extensive reviews by Riguera,29 Kusumi and Ohtani30, and Wenzel.31

1.2.4 J-Based Configurational Analysis (Murata’s Method)

In 1990 Murata and Matsumori published a seminal paper on32 ‘J-based configurational analysis’ (JBCA). This method takes advantage of $^1$H-$^1$H and $^1$H-$^{13}$C coupling constants in order to assign anti or gauche relationships of vicinally substituted groups. JBCA, an integrated technique that combines information from homonuclear and heteronuclear coupling with NOE, is frequently used for the determination of the relative configuration of ‘contiguous’ or 1,3-skipped stereogenic centers in acyclic molecules.
Table 1.1. $^1$H-$^1$H and $^1$H-$^{13}$C coupling constants for the assignment of relative configuration for vicinally-disubstituted chains using JBCA.

<table>
<thead>
<tr>
<th>Rotamer (magnitude of coupling)</th>
<th>$^3J$ $^1$H-$^1$H coupling constants</th>
<th>$^3J$ $^1$H-$^{13}$C coupling constants</th>
<th>$^2J$ $^1$H-$^{13}$C coupling constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti (large)</td>
<td>9 - 12 Hz</td>
<td>6 - 8 Hz</td>
<td>-</td>
</tr>
<tr>
<td>gauche (small)</td>
<td>2 - 4 Hz</td>
<td>1 - 3 Hz</td>
<td>-</td>
</tr>
<tr>
<td>anti (large)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gauche (small)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti (small)</td>
<td></td>
<td></td>
<td>0 - -2 Hz</td>
</tr>
<tr>
<td>gauche (large)</td>
<td></td>
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<td>-5 - -7 Hz</td>
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<td>CH₃</td>
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<td>OH</td>
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<td>OH</td>
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<td>5 - 7 Hz</td>
<td>0 - 2 Hz</td>
</tr>
<tr>
<td>OH</td>
<td>0 - 4 Hz</td>
<td>1 - 3 Hz</td>
<td>-4 - -6 Hz</td>
</tr>
<tr>
<td>Cl</td>
<td>7.5 - 10.5 Hz</td>
<td>4 - 5 Hz</td>
<td>-0.5 - 4 Hz</td>
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<tr>
<td>Cl</td>
<td>1 - 3.5 Hz</td>
<td>0 - 3 Hz</td>
<td>-3.5 - -6.5 Hz</td>
</tr>
</tbody>
</table>

The $^3J_{HH}$ and $^2,^3J_{HC}$ coupling constants are measured indirectly through a combination of NMR experiments. $^1$H-$^1$H coupling constants can be extracted from 1D-$^1$H NMR spectra, 1D-TOCSY, or absolute value cross–peaks in E-COSY type experiments. $^1$H-$^{13}$C coupling constants are typically measured from HETLOC, HSQC-HECADE, PS HMBC, J-resolved HMBC, or HSQMBC experiments. The HETLOC and HSQC-HECADE experiments are the most sensitive and easily interpretable, but limited to spin systems with
contiguous TOCSY coherence transfer. For subunits that contain quaternary centers or small $^1$H-$^1$H couplings, PS-HMBC, J-HMBC or HSQMBC experiments are used. The advantages and disadvantages of each of these experiments as well as interpretation of data are outlined in a detailed review by Williamson.\textsuperscript{33} The extracted coupling constants are ordered into either 'small' or 'large' ranges and fit to an empirical model that reports the relative configuration of the attached substituents (Table 1.1).

Recently, Carreira and coworkers\textsuperscript{34} disclosed a detailed report on the coupling constant values for synthetic polychlorinated contiguous stereogenic systems commonly encountered in chlorinated sulfolipids\textsuperscript{35}. The study verified the JBCA method is applicable to polychlorinated natural products, however subtle differences in coupling constant values should be taken into account.

Prior to the JBCA method, the only reliable way to elucidate the relative configuration of contiguous stereogenic centers was by a combination of the $^{13}$C acetonide method, and multi-step partial or total synthesis followed by spectroscopic comparison with the natural product. Extensive surveys on the use of NMR for the assignment of relative configuration have been reviewed by Riccio and coworkers.\textsuperscript{36}

The task of assignment of molecules with isolated 'stereosegments' where lack of NMR correlations prevent the relay of configurational information remains a challenge, even with integrated techniques. The magnitude of the problem can be stated simply: for a molecule with $n$ isolated defined stereo-
segments, the maximum possible number of stereoisomers is $2^n$. Methods for connecting isolated 'islands' of stereochemistry within complex molecules is one of the outstanding problems in natural product structure elucidation, but one that has inspired innovative and imaginative solutions.

1.2.5 Universal NMR Database (Kishi)

Kishi observed, through observation of numerous examples of configurational assignments in complex polyketides prepared by synthesis, that small systematic patterns of $^1$H NMR and $^{13}$C NMR chemical shift differences are associated with different diastereomers. Expanding on this observation, Kishi’s group compiled NMR data that came to be known as as the ‘universal database’ (UDB) to assign the relative configuration of contiguous stereogenic units. The UDB is also useful for the relative and absolute configurational assignment of complex polyketides.

The UDB works under the assumption that: “(1) the structural properties of these clusters are inherent to the specific stereochemical arrangement of the (small) substituents on the carbon backbone and (2) the structural properties of these clusters are independent from the rest of molecule, when they are sufficiently separated from each other.” In practice, stereogenic subunits need only be separated by two or more methylene groups so that they may be treated independently. The $^1$H or $^{13}$C NMR chemical shifts of the carbon framework in the molecule are averaged, and these values are
subtracted from the respective chemical shifts of the molecule under examination. For a given diastereomer, these aggregated deviations ($\pm \Delta \delta$) are characteristic of the relative configuration of each diastereomer and can be compared to the deviations of other diastereomers.

**Figure 1.7.** Synthetic databases included in the Universal Database (UDB) by Kishi.
Not surprisingly, the most straightforward analysis is comparison from a database where the side chains closely resemble that of the compound in question. However, it may be possible to adjust the chemical shifts appropriately of a given database to apply it to a substrate not identical to the database. The UDB has also been extended to absolute configuration assignment with the use of chiral anisotropic NMR reagents.\textsuperscript{39}

An extension of UDB\textsuperscript{40} matches overlapping contiguous triads of \textsuperscript{1}H-\textsuperscript{1}H coupling constants with those of synthetic diastereomers of defined configuration for the purpose of assignment in polyol and polyacetoxy compounds. This is advantageous over chemical shift comparison because it is less influenced by solvent and substituents on side chain and mainly influenced by the local conformation. This method has been successfully applied to the configurational assignment of sagittamide A and is reviewed in Section 1.7.12.\textsuperscript{41}

The relative configurational assignment of the 1,3,5-triol segment of caylobolide A (\textbf{1.224}) employed the UDB approach.\textsuperscript{42} Database C\textsuperscript{40} (Figure 1.7) encompassing four diastereomers: syn/anti (\textbf{c.1}), syn/syn (\textbf{c.2}), anti/syn (\textbf{c.3}), and anti/anti (\textbf{c.4}) were compared with caylobolide A and exhibited a pattern that was consistent with the \textit{syn/anti} model. Comparative analysis also revealed that the chemical shift of the central carbinol carbon is also reflective of relative configuration.
1.3 Chiroptical methods

1.3.1 Polarimetry

Optical rotation $[\alpha]_D$ is at the most widely used method for chiroptical characterization and determination of optical purity of organic compounds through calculation of enantiomeric excess ($\%$ ee). Modern commercial digital polarimeters employing high light throughput, short-path microcells (as low as 0.1 dm, 100 $\mu$L) and accurate polarizers are capable of measuring optical rotations on less than a milligram of substances with a relatively large $[\alpha]_D$ value (>20). Assignments are often made by chiroptical comparisons with synthetic compounds. The limitation is that degradative approaches often require sacrifice of a large amount of the parent compound, a luxury not always granted in cases where the sample is rare and mass-limited. It is now possible, as shown by the Wipf group, that accurate ab initio calculation of molar rotations by time-dependent DFT methods by van Hoff’s superposition principle can discriminate between diastereomers. This approach was used in the absolute stereoassignment of the bistramides.\textsuperscript{113} Calculated molar rotations complements the UDB approach, by avoiding degradative approaches but has another limitation. In absence of independent measurements of the $[\alpha]_D$ of the natural product, the method largely rests on trust and reliance that the reported literature values were measured accurately on very pure samples; a condition that, regrettably, does not always prevail. Current literature shows that the ab initio molar rotation approach to
stereochemistry has not been largely adopted yet. Degradative routes relying on NMR analysis are still more popular.

### 1.3.2 UV-Vis and Electronic Circular Dichroism

Electronic circular dichroism (ECD) arises from differential absorption of left and right circularly polarized light; it represents one of the most useful techniques available for stereochemical and conformational analysis of chiral molecules. Molar CD like UV-vis is inherently more sensitive than most types of spectroscopic techniques (i.e. [α]D, IR, NMR, etc.) and obeys the Beer-Lambert law. amenable to very small samples (i.e. μg). ECD analysis is applicable only to compounds with chromophores in the UV-vis region (λ_max >200 nm), or those where suitable chromophores can be synthetically appended. Rather than exhaustively reviewing sector rules, two examples pertinent to natural products – the octant rule for cyclohexane and the Mo(OAc)4 method for vicinal diols – will be illustrated.

### 1.3.3 The Octant Rule

Pioneering work by Moskiewitz, Crabbé, Djerassi, Lightner and others lead to development of a series of empirical 'sector rules' for assignment of configuration, the best known of which is the 'octant rule' for cyclic ketones. For example, the qualitative contributions to the Cotton effect due to the forbidden n-π* transition of the C=O group (λ_max 284 nm) in 3R or 3S-methylcyclohexanone can be predicted by considering contributions of
atoms within each of the eight sectors (Figure 1.9) formed by three intersecting planes: the nodal plane at the C=O group and two orthogonal planes bisecting the C=O bond. The success of the octant rule was supported by demonstrating that many numerous ORD and CD of cyclohexanones conform to this empirical rule.

![Diagram of octant rule](image)

**Figure 1.8.** Octant rule for saturated ketones and origin of the positive Cotton effect observed for (3R) methylcyclohexanone.

### 1.3.4 Snatzke’s Method

Snatzke’s method employing dimolybdenum tetraacetate is a generally useful technique for the assignment of 1,2-diois. The methodology is based upon chelation of the diol to the molybdenum, giving a restricted conformer and induced CD (ICD). The resulting Cotton effect can be correlated to the configuration of the diol. The conformation of the complex is influenced by the size of the substituents (R_L and R_S). The bulkier substituents (R_L and R’_L) will orient in the pseudo-equatorial position to avoid steric interactions to the
complex. The Cotton effect at $\lambda \approx 305$ nm correlates with the sign of the O–C–C–O dihedral angle. The simplicity of this empirical method which requires simply mixing the reagent with the diol (10 $\mu$mol) in DMSO, and acquisition of CD spectra at room temperature.$^{49}$

\[
\begin{align*}
\text{Scheme 1.0. Preferred conformations of Mo(OAc)$_4$–dil complexes. } R_L, R'_L & = \text{larger substituent. } R_S, R'_S = \text{smaller substituent.}
\end{align*}
\]

1.3.5 The Exciton Chirality Method (Nakanishi)

The exciton chirality CD method (ECCD), is a non-empirical configurational assignment by CD, largely developed and popularized by Nakanishi and Harada.$^{50}$ Degenerate or near-degenerate chromophores undergo exciton coupling, with resultant Davydov splitting of the transition, that is dependent upon oscillator strength, and both the distance and angle, $q$ between the respective electronic transition dipole moments. in ECD, chiral molecules exhibit strong biphasic Cotton effects whose signs are directly correlated with the sign of $\theta$. For example, the dibenzoate method is used to assign diols by measurement of the split Cotton effects of their corresponding dibenzoate diesters.
Figure 1.9. Applications of the exciton chirality method for the assignment of configuration. Signs of helicity angles, $\theta$, correlate with the sign of the split Cotton effect.

Three selected examples demonstrating the versatility of the exciton chirality method are shown in Figure 1.8. The bis-dimethylamino benzoate of 5α-cholestan-2β,3β shows a positive split Cotton effect between the C2 axial and C3 equatorial benzoate chromophores. The configuration of allylic alcohols (cyclic and acyclic) are readily assigned by the allylic benzoate method.\textsuperscript{51} (R)-Non-1-en-3-ol (1.14) was converted to the corresponding $p$-bromobenzoate (1.15), which is assigned the $R$ configuration based on observation of a positive split Cotton effect resulting from exciton coupling between the benzoate ($\lambda \sim 230$ nm) and ene chromophores.

The method is not limited to dibenzoates of diols; ECD has been exploited in more complex multichromophoric systems of triols, higher polyols, and aminopolyols.\textsuperscript{52} The sensitivity of ECD lends a great advantage to assignments of chromophoric derivatives of complex molecules. A limitation to
simple interpretations of ECD include interference from other interactive chromophores. More recently, prediction of the ECD has been made possible by ab initio calculations using time-dependent DFT methods, although these have been largely applied to molecules with rigidly oriented chromophores, or more flexible molecules where the orientations of the transition dipole moments in each conformer, along with their Boltzmann distributions, can be also reliably calculated.\textsuperscript{53}

1.3.6 Infrared and Vibrational Circular Dichroism

Infrared spectroscopy (IR) has been the traditional tool for functional group identification in unknown organic compounds. Sadle, reporting of IR of natural products has declined in recent years; it is often relegated to \textit{pro forma} characterization without interpretation, although modern Fourier transform IR (FTIR) with attenuated total reflectance (ATR) makes it easy to obtain spectra of samples of than 50 $\mu$g with full sample recovery.

Vibrational circular dichroism (VCD), which arises from differential absorption of left and right circularly polarized IR, can be used for assignment of absolute configuration. The advantages of VCD include richly detailed bands, even from the 'fingerprint region', and ease of ab initio calculation of VCD spectra. Several assignments of natural products by VCD have been reported.\textsuperscript{54} The barrier of wider usage may be due to it's relative insensitivity compared to other spectroscopic methods (samples of several milligrams and
hours of acquisition time are required), and the need for relatively expensive, uncommon instrumentation.

1.4 Inferences from Biosynthesis or Bioinformatics

Stereochemically defined natural products are useful for the configurational assignment of derivatives and structurally related metabolites. The construction of similar compounds are often carried out by analogous enzymes in a conserved stereospecific manner. Biosynthetic inferences were successfully used in combination with spectroscopic methods for the stereochemical assignments of psymberin (1.100, Section 1.7.8) and dictyostatin (1.98, Section 1.7.7). Genetic and bioinformatics–based approaches are increasingly used for structure and configurational assignment.\textsuperscript{55}

1.5 X-Ray Crystallography

X-ray crystallography is the ultimate method for structural determination,\textsuperscript{56} however it is singularly dependent upon good quality diffracting crystals that exceed critical minimum dimensions (10-100 \(\mu\)m). While this is routinely achieved for overexpressed biomacromolecules using high-throughput crystal optimization techniques, it is quite a different story for a natural product that is only available in microgram amounts – one has limited options for making saturated solutions with only \(\mu\)L amounts of solvent! The power of X-ray crystallography becomes most apparent with highly
functionalized alkaloids where NMR fails; when the formula is so depauperate in hydrogen (H/C ratio <2, the so-called Crews rule), that 2D NMR experiments provide few useful cross-peaks. This review will only cover some representative examples of X-ray structures of optically active compounds where NMR-based structure determination failed. See determination of spirastrellolide B by microscale two-step chemical degradation leading to an X-ray quality crystal – all from a 100 µg sample!

1.6 Chemical Synthesis

The ideal solution to a stereochemical problem would be to synthesize all possible stereoisomers for comparison of spectroscopic data. From a practical standpoint, this task is neither tractable nor necessary. The most favorable option is to carry out chemical degradation to simpler compounds that are more amenable to synthesis and or spectroscopic analysis. Often, synthesis of ‘key’ segments for comparison suffice. All cases require expert and refined skills that are the traits of well–trained – and fearless! – natural product chemists.
1.7 Selected Examples

1.7.1 Amphidinols

Marine dinoflagellates are the source of some of the most biologically potent and structurally complex metabolites isolated to date. The amphidinols, first disclosed in 1991 by the Yasumoto group are antifungal polyhydroxy polyene metabolites from the marine dinoflagellate *Amphidinium klebsii*. The stereochemical determination of amphidinol 3 (1.18) was one of the initial reports that demonstrated the effectiveness of the JBCA method applied to a complex natural product.

![Diagram of amphidinol 3 (1.18)](image)

**Figure 1.10.** Murata’s strategy for configurational assignment of amphidinol 3 (1.18).

The configurational assignment was preceded by a division of the molecule into three sterochemical subunits: (a) C2-C14, (b) C20-C27, and (c)
C32-C52. The relative configuration of subunits (b) and (c) were assigned using JBCA, and relevant coupling constants ($^{2,3}J_{HC}$ and $^{2}J_{HH}$) were extracted from HETLOC, PS-HMBC, and E-COSY experiments. The relative configuration between C39 and C44 was assigned from on coupling constant and NOE data. In addition, stereoassignments of the diastereotopic methylene protons (CH$_2$-22 and CH$_2$-26) allowed complete assignment of the relative configuration of the C20-C27 segment.

Scheme 1.1. Degradation of ampidinol 3.

The absolute configurations at C6, C10, C14, C23 and C39 were assigned from degradation products. Amphenol 3 (1.18) was oxidized (HIO$_4$), reduced (NaBH$_4$), esterified (S- or R-MTPA-Cl), and purified (RP-HPLC) which gave esters (1.19–1.21, Scheme 1.1). The MMM was used for both sets of
MTPA esters 1.19ab and 1.21ab to assign the 6R, 10R, 14R, and 39R configurations. The ¹H chemical shift differences for C11 (±0.01, 0.0) were small and approach the limits of mutual interactions between the two MTPA groups compromising interpretation. The (R)-MTPA ester 1.20a was compared to both (R)- and (S)- MTPA esters of authentic (R)-methyl-1,4-butanediol which revealed the 23S configuration.

To assign the C2 configuration, a degradation approach reminiscent of the configurational assignment of C2 in ciguatoxin was adopted.⁶⁰ Amphidinol 3 (1.18) was subjected to protection (BOM-Cl), dihydroxylation (OsO₄), oxidation (NaIO₄), reduction (NaBH₄), and acylation (p-BrBz-Cl) to give the protected ester 1.24. Comparison of the latter product with optically pure standards by chiral HPLC (Chiralpak AD) and showed a 2S configuration.

The reported configurational assignment of amphidinol 3 (1.18) was one of the first reports using JBCA on a complex natural product. The full stereochemical assignment was made with only 3 mg of material for degradation experiments and 8 mg of ¹³C enriched material for NMR experiments. It should be recalled that previous studies to gain this type of stereochemical information would have required much more material (> 100 mg) for chemical conversion or degradations studies; for example configurational assignments of complex natural products included mycoticins by Schreiber’s group,⁶¹ nystatin by Beau’s group,⁶² and roflamycoin by Rychnovsky’s group.⁶³
Scheme 1.2. Degradation of amphidinol 3 for the configurational assignment of C2, and synthetic models (1.25–1.28) used for comparison of NMR data.

In 2008, Oishi and coworkers synthesized all diastereomeric models of amphidinol 3 (1.18) encompassing C2, C6, and C10 (1.25–1.28) and compared $^{13}$C chemical shifts with 1.18, and showed protected pentaol 1.26 was the closest match and that the C2 stereocenter had been assigned incorrectly.$^{64}$ To verify the C2 configuration, vinyl alcohol 1.29 was cleaved from amphidinol 3 (1.18, 50 µg) by cross metathesis using Grubbs’ second generation catalyst in the presence of excess ethylene (Scheme 1.2). The cross metathesis product was compared with authentic standards, which verified the C2 configuration should be revised to the R configuration. It was uncertain how the misassignment was made, but an HPLC peak corresponding to the protected ester 1.24 may have contaminantted the degradation product. Amphidinol 3 (1.18) has been the subject of intense
synthetic efforts by research groups led by Cossy,65 Rychnovsky,66 Roush,67 Paquette,68 Oishi,69 Crimmins,70 and Marko.71

Scheme 1.3. Structure and configurational assignment of karlotoxin 2 (1.30) by Hamann.

Since the initial reports of the amphidinols, additional families of related metabolites have been isolated: the luteophanols72, lingshuiols,73 and karatungiol,74 from Amphisminium sp. and most recently the karlotoxins from Karlodinium veneficum.75 Blooms of K. veneficum have been implicated in massive fish kill events. In 2010, the Hamann group reported the full structure for karlotoxin 2 (1.30) by a similar approach applied to amphidinol 3 (Scheme 1.3).76 The JBCA method secured C14-C18, C21-C24, and C28-C49. The absolute configurations of C6, C10, C14, C21, and C28 were derived from NMR analysis of degradation (HIO₄/NaBH₄/(R)- or (S)-MPA) products 1.31–
1.33. The $\Delta \delta$ values ($\delta R-\delta S$) were used for 1.31ab, and 1.32–1.33 in comparison with authentic standards. It is worth noting that although the $\Delta \delta$ values for 1.31ab are larger in magnitude than those observed for similar segments derived from amphidinol 3 (1.19ab), overlapping anisotropies of the MPA esters are observed and the assignment at C6 becomes equivocal. Interestingly, amphidinol 3 (1.18) and karlotoxin 2 (1.30) share almost identical structural features in the C30-C52 region (amphidinol numbering), but the reported absolute configuration was antipodal. The stereochemical fidelity of these assignments should be revealed by total synthesis.

1.7.2 Mycalolides

![Diagram of mycalolides]

1.35 $R=\text{OMe}$ Mycalolide B  
1.36 $R=\text{H}$ Mycalolide C  
1.37 $R=\text{OH}$ 38-Hydroxymycalolide B

1.38 30-Hydroxymycalolide A  
1.39 32-Hydroxymycalolide A

Figure 1.11. Structures of mycalolides (1.34–1.39).
Trisoxazole macrolides belong to a structurally unique group of sponge and nudibranch derived natural products that have received attention from the chemists and biologists. The ulapualides A and B\textsuperscript{77} and kabiramide C\textsuperscript{78}, published simultaneously in 1986 by the Scheuer and Fusetani groups, respectively were the first reported members of the trisoxazole class. Additional related trisoxazole macrolides including the halichondramides,\textsuperscript{79} mycalolides,\textsuperscript{80} and the jaspisamides\textsuperscript{81} appeared in the following decade.

**Figure 1.12.** Fusetani/Panek strategy for configurational assignment of the mycalolides.

The trisoxazole macrolides exhibit potent antifungal and cytotoxic activities due to their property of binding tightly to G-actin, and inducing depolymerization of F-actin, and disruption of actin filament formation and organization.\textsuperscript{82} Because of their structural complexity, the trisoxazole macrolides eluded complete stereochemical assignment until the report of an elegant chemical correlation study of various mycalolide congeners. For the first time, the stereochemistry of a trisoxazole was defined by the Fusetani and

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**C30 Absolute: modified Mosher's method of 1.38**

**C22–C33 Relative: degradation, comparison to synthetic models**

**C24 Absolute: modified Mosher's method**

**C32 Absolute: modified Mosher's method of 1.39**

**C37 Absolute: hydrolysis authentic standards**

**C3 Absolute: modified Mosher's method**

**C8/C9 Absolute: degradation, chiral HPLC**

1.35 Mycalolide B
Panek groups in 1999.\(^{83}\)

Mycalolide B (1.35), like other trisoxazoles exhibit conformational isomerism in the NMR spectra due to slow interconversion of the N-formyl amide. Consequently, NMR experiments were hampered and stereochemical assignments were secured through chemical conversion to compounds more suitable for spectroscopic analysis. In an effective reaction sequence, mycalolide B (1.35) was subjected to oxidation (RuO\(_4\)),\(^{84}\) methanolysis and lactonization to provide bislactone 1.40 (Scheme 1.4) in sufficient yield for 2D NMR experiments. Extensive NMR analysis of 134 suggested both lactone rings were in the boat conformation.\(^{85}\) NOESY and coupling constant data were used to assign the relative configuration of both lactone rings, and the anti orientation between H24 and H26, however the relative configuration between C26/C27, and C27/C30 remained ambiguous.

Scheme. 1.4. Degradation and MMM on mycalolide B (1.35), 30-hydroxymycalolide A (1.38), and 32-hydroxymycalolide A (1.39).
The secondary hydroxyl groups located throughout the mycalolidc structures were useful for assignment by the MMM. The configuration at these positions as well as other segments were likely conserved among all mycalolidc congeners based on a common biogenesis. Therefore, the MMM was independently applied to C3, C30 and C32 in mycalolidc B (1.35), 30-hydroxymycalolidc A (1.38), and 32-hydroxymycalolidc A (1.39), respectively (Scheme 1.4). Oxidative degradation of mycalolidc C (1.36) and protection of the product gave alcohol (1.45), and the C24 center assigned by MMM (Scheme 1.5).

**Scheme 1.5.** Degradation of mycalolidc C (1.36).

The side chain relative configuration was confirmed after perruthenate–catalyzed oxidative degradation of 38-hydroxymycalolidc B (1.37) to acetate 1.47. The NMR data for the degradation product (1.47) was indistinguishable from synthetic 1.47, but different from that of epimeric 1.48 (Scheme 1.6). This comparison unambiguously allowed the assignment of the relative and absolute configuration of the C22-C33 of the mycalolides.
**Scheme 1.6.** Oxidative degradation of 38-hydroxymycalolide B (1.37).

The 8R and 9S absolute configurations of 1.37 were assigned by perruthenate degradation and conversion to bis-p-bromophenacyl derivative 1.49 (Scheme 1.6) and comparison to authentic standards by chiral HPLC (Chiralcel OD). Finally, the configuration at C37 of the natural products 1.35-1.37 were assigned by saponification, derivatization (p-bromophenacylbromide) to 1.50-1.52 (Scheme 1.7), and comparison with authentic standards by chiral HPLC (Chiralcel OJ).

**Scheme 1.7.** Hydrolysis of the side chain in mycalolides B (1.35), C (1.36), and 38-hydroxymycalolide B (1.37).
Figure 1.13. Actin-binding marine macrolides.

The mycalolide assignments were the first reported stereochemical assignments of any trisoxazole macrolide. Shortly after, total synthesis of (−)-mycalolide A (1.34) by the Panek group, verified the stereochemical assignment. In 2004, Rayment and coworkers acquired an X-ray crystal structure of structurally related ulapualide A bound to an actin complex, which showed the stereochemistry is conserved between the mycalolides and the
ulapualides. The side chain of the trisoxazole macrolides bears structural resemblance to other actin-binding marine macrolides including reidispongiolide A (1.53), sphinxiolide (1.54), aplyronine (1.55), and scytophycin (1.56) (Figure 1.13). Rayment and coworkers have reported X-ray crystal structures of a number of these macrolides demonstrating a powerful benefit of stereocomplex small molecules bound to proteins: total stereochemical assignment. This is significant for trisoxazoles as none produce X-ray quality crystals.

### 1.7.3 Oceanapiside

![Oceanapiside](image)

- **1.57 Oceanapiside**
- **1.58 Rhizochalin**
- **1.59 Leucettamol A**

*Figure 1.14. Structures of dimeric α,ω-functionalized sphingolipids from sponges.*

α-ω-Functionalized sphingolipids (Figure 1.14) from marine sponges are C_{28}–C_{30} long chain lipids that are terminated as a 2-amino-3-alkanol or 2-amino-1,3-alkanediol. These lipids represented an interesting challenge for
configurational assignment because stereosegments are separated by a long hydrocarbon chain and effectively insulated, making NMR correlations of the chain termini impossible. The first configurational assignment of this family was carried out on oceanapiside (1.57a)\textsuperscript{93} from Oceanapia sp. collected from Port Phillip Bay, Victoria. Oceanapiside showed good antifungal activity against fluconazole resistant Candida glabrata (MIC = 10 µg/mL); the aglycone (oceanin; 1.57b) is more active (MIC = 3 µg/mL) presumably due to improved cell permeability.

The initial report by Molinski established a planar structure for oceanapiside (1.57a) and the configuration of the D–glucose residue. The position of the carbonyl group relied on MALDI-MS-MS measurements of the C10, C12-d\textsubscript{4} isotopomer obtained upon standing in MeOH-d\textsubscript{4} (23°C, 2 months) (however this was later revised). Acidic methanolysis (HCl/MeOH, 80°C, 2h) of 1.57a gave a mixture of α,β anomers 1-O-methyl-D-glucopyranosides, identical by high-performance TLC, and the aglycone, oceanin (1.57b) with authentic material.

\textbf{Figure 1.15.} Molinski’s strategy for configurational assignment of oceanapiside.
A follow-up report solved the C2, C3, C26, C27 configurations of oceanin by fitting ‘hybrid’ ECCD spectra from perbenzoylated synthetic aminoalcohols to that of perbenzoyl–oceanin. This general approach to α,ω–functionalized sphingolipids was reliant upon the assumption that the CD spectra can be treated as a superposition of exciton couplets. Local vicinal benzoyl groups give rise to ECCD but not between separate terminal benzoyl groups.

**Scheme 1.8.** Synthetic models (1.60–1.61) used to generate hybrid CD spectra, and conversion of oceanapisde to perbenzoylated aglycon 1.62. CD spectra for 1.62 (dotted line), and 1.60b+1.61a (solid line) (taken from ref. 84b).
The advantage of this approach is only four model compounds (1.60–1.61) were necessary and sufficient to create all 16 stereopermutations by simple linear combinations of model CD spectra. Perbenzoyl–oceanin (1.62) showed an ECCD spectrum uniquely superimposable upon the combination erythro-160b + threo-1.61a leading to the 2S, 3R, 26R, 27R configuration (Scheme 1.8).

The successful configurational assignment of oceanapiside represents a relatively simple and concise solution to a difficult stereochemical problem, one that was unlikely to solved by total synthesis since the optical rotary strengths of chiral amino alcohols are generally weak and NMR would not discriminate the diastereomers. The hybrid ECCD method has also been successfully applied to rhizochalin (1.58) from Rhizochalina incrustata, leucettamol A (1.59) from Leucetta microrhaphis, and additional $\alpha$-$\omega$–functionalized sphingolipids. It is interesting to note that leucettamol A (1.59) was assumed to be racemic because the $[\alpha]_D$ was $\sim$0. However, ECCD studies by Molinski showed leucettamol A (1.59) is optically active and has pseudo–C$_2$ configuration.

1.7.4 Amphidinolide E

The Kobayashi group have revealed dinoflagellates of the genus Amphidinium separated from flatworm Amphiscolops spp. collected in Okinawa, to be a highly productive source of cytotoxic polyketide marine macrolides. Collectively known as the amphidinolides, over 34 macrolides
have been described to date. Structurally, the macrolide varies in ring size and frequently embody tetrahydrofuran or pyran rings, with variable levels of unsaturation, hydroxylation, and methylation as typically observed in small molecules constructed by Type I PKS biosynthetic machinery. As most of these macrolides are often functionally– and stereochemically–rich, they have provided challenges for structural assignment by integrated methods. At the same time, the potent cytotoxicity of these molecules have been the subject of many synthetic efforts.\textsuperscript{98} A full account of all the meticulous assignments, and reassignments by total synthesis is beyond the scope of this review, but we will consider amphidinolide E as a technically challenging, and representative contribution to the structure determination of amphidinolides.

![Diagram of amphidinolide E](image)

**Figure 1.16.** Kobayashi’s strategy for configurational assignment of amphidinolide E.

The isolation (0.9 mg) and planar structure of amphidinolide E was first reported in 1990. Amphidinolide E (1.63) showed mild cytotoxicity against murine leukemia cell lines: L1210 (IC\textsubscript{50} 2.0 µg/mL) and L5178Y (IC\textsubscript{50} 4.8
\( \mu \text{g/mL}. \)

By 2002, a larger amount of 1.63 (2 mg) was secured by repetitive cultivation from hundreds of liters, which allowed the full stereochemical assignment to be made. The strategy for configurational assignment integrated the use of NMR, CD, chemical conversion, and synthesis of optically active model compounds and standards.

Scheme 1.9. Chemical conversion of amphinolide E (1.63).

The relative configuration of 1.63 was assigned by a combination of JBCA, NOESY and chemical conversion. The cis orientation of the tetrahydrofuran ring system was identified by NOESY correlations between H13/H16. Formation of the 7,8-isopropylidene analog 1.64 (Scheme 1.9) allowed the threee assignment between H7 and H8. The C16 to C19 relative configuration was assigned by JBCA and NOESY data in addition to chemical
conversion. The orientation between H17 and H18 was confirmed from interpretation of NMR of bis-acetonide derivative. Amphidinolide E was hydrogenated (H2, Rh/Al2O3), further reduced (LiAlH4), and the resultant alcohol (1.65) converted to bis-isopropylidene 1.66 (Scheme 1.9).

**Scheme 1.10** Preparation of MTPA esters (1.69) and p-methoxycinnamate derivatives (1.70).

The absolute configuration of amphidinolide E (1.63) was assigned by a combination of the MMM, exciton chirality method, and degradation. The threeo orientation of the H7/H8 diol allowed for application of the di-benzoate method.50 Compound 1.63 was converted to the p-methoxycinnamate derivative (1.70) to avoid overlap with lower wavelength chromophores (Scheme 1.10). The resulting ECCD spectra showed a negative split–Cotton effect, consistent with the 7R,8R configuration. Application of the MMM to the tri-MTPA esters (1.69ab) gave the 17R configuration (Scheme 1.10). The remote C2 methyl group was assigned by periodate cleavage–borohydride reduction (Scheme 1.9) to attain diols 1.67ab. 1H NMR signals of diastereotopic C1 methylene group are diagnostic for the configuration of the
methyl group, and was assigned the R configuration. Finally, tetrahydrofuran degradation products (1.68ab) were compared to authentic standards prepared by synthesis.

This work represents a tour de force modern structure determination where the full configurational assignment was completed on a sample of less than 2 mg. Total syntheses of amphidinolide E (1.63) have been completed by the Roush101 and Lee102 groups, and confirmed the configurational assignment proposed by Kobayashi.

1.7.5 Bistramides

In 1988, bistramide A (1.71) the first member of a family of cytotoxic spiroketals, was reported from the tunicate Lissoclinum bistratum by the Verbist group in New Caledonia,103 The complete planar structure was disclosed by the Ireland and coworkers by in 1992.104 Later, a number of analogs with similar structures were reported by the same group105b and independently by the Hawkins group in Australia. The bistramides have attracted interest due to their broad, potent antiproliferative effects arising from inhibition of actin polymerization through tight covalent binding to G-actin, and disruption of the microfilament cytoskeleton.106 A high resolution X-ray crystal structure of an actin-bistramide A complex revealed that the binding site of bistramide A (1.71) has little overlap with other small molecule actin inhibitors (e.g. swinholide A,107 kabiramide A,108 and other structurally related
macrolides\textsuperscript{109,110}. Rationally designed analogs and a fluorescent probe showed the enone functionality participates in covalent modification of the protein target.\textsuperscript{111}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure17.png}
\caption{Structures for Bistramides A (1.71) and C (1.72).}
\end{figure}

Although the bistramides were reported in 1988, the first partial relative configuration did not appear for more than a decade until the proposal by the Sollidie group in 2000. NOESY data acquired on the acetate derivative (1.72) of bistramide A established the relative configurations of both the tetrahydropyran (C6-C11) and spiroketal segments (C22-C31) (Figure 1.19).\textsuperscript{112}

With a proposed relative configuration of these two fragments, the Wipf group initiated efforts toward defining the relative and absolute configuration of bistramide C (1.73).\textsuperscript{113} Wipf’s strategy synthesized the three stereosegments of bistramide C and analysis of their contributions to the molar rotation of the natural product.
**Figure 1.18.** Wipf's strategy for the configurational assignment of bistramide C (1.73).

The stereochemically less–complex bistramide C (1.73) with one less stereocenter (C37) relative to bistramide A (1.71), was chosen as the target. Two models, anti–1.74 and syn–1.75 were prepared to model the C15/C16 stereocenters of the amide. Comparison of $^1$H NMR to bistramide A, showed a closer match to anti–1.74. The synthetic strategy narrowed the number of possible diastereomers to 16 based on Sollide's relative configuration for the tetrahydropyran (C6-C11), C15,C16 stereocenters, and spiroketal subsections (C22-C31). Despite the arbitrary choice of the target diastereomer 1.76, with only a 1 in 16 probability being correct, the convergent strategy provided a representative stereo-fragment along with chiroptical data (molar rotations) that, collectively, informed their proposal for the complete absolute configurational of 1.73.
Figure 1.19. Relative configuration of bistramide A (1.71).

Bistramide diastereomer 1.73 was synthesized by a two-segment (1.77 and 1.78) coupling strategy. The NMR data for 1.77 matched that reported for bistramide C (1.73), except for the $^{13}$C chemical shift of C34. Since the NMR data for the C1-C15 portion of 1.77 closely matched those of bistramide C (1.73), the relative configuration between the tetrahydropyran and the amide linkage was correctly assigned in the synthetic diastereomer. Therefore, the discrepancy in $^{13}$C NMR shifts was a result of mismatch between configurations of the amide and spiroketal, the spiroketal and C34, or a combination of the two. With several synthetic intermediates comprising all stereochemical elements of bistramide C, a synthetic diastereomer, and molar rotations for bistramide C, the chiroptical analysis could be completed.
Table 1.2. Molar rotation values of bistramide diastereomers.

<table>
<thead>
<tr>
<th>Stereogenic segments composing bistramide</th>
<th>C22-C31 Spiroketal</th>
<th>C34 ketone</th>
<th>[M]_D</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6-C11 THP/amide</td>
<td>(22S, 23R, 27R, 31R)</td>
<td>(34R) [M]_D = -51</td>
<td>-88</td>
</tr>
<tr>
<td></td>
<td>[M]_D = -156</td>
<td>(34S) [M]_D = +51^d</td>
<td>+14</td>
</tr>
<tr>
<td>[M]_D = +119^a</td>
<td>[M]_D = +156^b</td>
<td>(34S) [M]_D = +51^d</td>
<td>+326</td>
</tr>
<tr>
<td>[M]_D = -119</td>
<td>[M]_D = -156</td>
<td>(34S) [M]_D = +51^d</td>
<td>-224</td>
</tr>
<tr>
<td></td>
<td>(22R, 23S, 27S, 31S)</td>
<td>(34R) [M]_D = -51</td>
<td>-14</td>
</tr>
<tr>
<td></td>
<td>[M]_D = +156^b</td>
<td>(34S) [M]_D = +51^d</td>
<td>+88</td>
</tr>
</tbody>
</table>

^a synthetic ^b derived from synthetic 1.78. ^c from normanicone (+)-1.79.

The molar rotations of each subunit were summed according to Van’t Hoff’s principle of optical superposition.\textsuperscript{114} Molar rotations of the synthetic model compounds were calculated from measured optical rotations and summed to give molar rotation data for the completely assembled bistramide C diastereomer (Table 1.2 and Figure 1.20). The tetrahydropyran/amide (1.77) containing the 6S,9R,11R,15S,16R configuration constituted a diastereomer with [M]_D +119. The C34–containing fragment was represented by (+)-normanicone (1.79) previously reported by Bestmann.\textsuperscript{115} The molar rotation contribution for the spiroketal (C22-C31) portion was calculated by subtracting the molar rotation of (−)-normanicone ([M]_D −51) from synthetic 1.78 ([M]_D +105) to give a value of [M]_D +224. These values were summed to return the calculated value of [M]_D +224 for bistramide diastereomer 1.76 that
closely matched the experimental measurement of [M]<sub>D</sub> +211. The same analysis was applied to bistramide C (Figure 1.21). Combinations of all permutations of chiral subunits showed that natural bistramide C (1.73) with an experimental molar rotation of [M]<sub>D</sub> +70 should have the 6R,9S,11S,15R,16R,22R,23S, 27S,31S,34S configuration (calculated [M]<sub>D</sub> +88).

![Figure 1.20. Assignment of absolute configuration of bistramide diastereomer (1.76) by Van’t Hoff’s principle of optical superposition.](image)

In a subsequent report, the Wipf group calculated [M]<sub>D</sub> values for each subunit from Boltzmann weighted energy minimized conformations, and summed as before to obtain reasonable values for each diastereomer and natural product. The benefits of the superpositions of molar rotations are obvious: none of the original material was required for chemical conversions for the assignment of configuration. The disadvantage is the necessity of synthesis of fairly advanced intermediates and, of course, accurately reported [α]<sub>D</sub> for natural product free of strongly rotating contaminants. Fortunately, all
subunit contribution to the \([M]_D\) were relatively large (> 50) giving combinations with significantly different \([M]_D\) values. A cautionary note is appropriate: the superposition method is probably less suitable for molecules whose synthetic fragments show only weak rotatory power (low \([M]_D\)) or similar \([M]_D\) where the combinations may not be sufficiently discriminated.

**Figure 1.21.** Van't Hoff principles to assign absolute configuration of natural bistramide C (1.73).

Subsequently, the Kozmin group successfully synthesized bistramide A (1.71), including both 37R and 37S diastereomers, the latter showed \(^{13}\)C NMR data identical to the natural product and confirmed the stereochemical predictions by the Wipf group were correct.\(^{117}\) In 2005, the Wipf group successfully synthesized natural bistramide C, identical to the proposed configuration.\(^{118}\) Since then, several total syntheses of the bistramide class have been reported.\(^{119}\)
1.7.6 Schulzeines

The Fusetani group reported schulzeines A-C (1.80–1.82), α-glucosidase inhibitors from the marine sponge *Penares schulzei* from Hachijo-island, Japan. The schulzeines are characterized by a 9,11-dihydroxytetrahydroisoquinoline unit connected to a sulfated fatty acid amide. The enzyme inhibitory activity of schulzeines A-C (1.80–1.82) range from 48-170 nM.

![Chemical structures of Schulzeines A-C](image)

**Figure 1.22.** Schulzeines A-C (1.80–1.82).
Methanolysis of **1.80** provided four cleavage products (**1.83–1.86**, Scheme 1.11). Analysis of FAB-MS/MS fragmentation data of the ring–C opened product (**1.83**) located the sulfate groups and, consequently, the full planar structure of Schulzeine A (**1.80**).

![Image of Schulzeine A](image)

**Figure 1.23.** Fusetani’s strategy for configurational assignment of Schulzeine A (**1.80**).

Methanolysis product **1.84** was used to assign the configuration of C3 and C11 in the dihydroisoquinoline ring system. NOE correlations verified stereochemical fidelity of **1.84** a configuration unchanged from the parent compound. The free amine was reacted with **(R)**- or **(S)**-MTPA-Cl (pyr, 1h) followed by saponification to amides **1.90ab** (Scheme 1.12). The Δδ chemical shift differences were fairly small, however the 3S,11R configuration could be assigned based on Mosher type analysis.¹²¹
Scheme 1.11. Acid hydrolysis of schulzeine A (1.80).

Conversion of the triol 1.85 (Scheme 1.12) to the isopropylidene protected alcohol and esterification with (S)- and (R)-MTPA-Cl gave MTPA esters 1.89ab. leading to the 14’S configuration. The trans-relationship between H17’ and H18’ was evident from ROESY crosspeaks observed between H17’ and one methyl group (δ 1.34 ppm) and H18’ and the methyl group on the opposite face (δ 1.32 ppm).

The relative configuration of the tetrahydrofuran 1.86 from the methanolysis was assigned by comparison of \(^{13}\)C chemical shifts to synthetic derivative 1.87 (Scheme 1.11)\(^{122}\). Conversion to both (R)- and (S)-MTPA esters 1.90ab, and analysis of \(^1\)H NMR gave the C18’S configuration. The stereochemical outcome upon formation of the tetrahydrofuran 1.86 is
explained by initial hydrolysis of the C17' sulfate followed by $S_N^2$ displacement of the C14' sulfate.

**Scheme 1.12.** MTPA esters from hydrolysis products of schlizzone A (1.80).

The final stereocenter (C20') was be assigned by degradation and comparison to optically pure standards (Scheme 1.13). Schlizzone A (1.80) was subjected to desulfuration under mild acidic conditions (TsOH)$_{123}$ oxidation–reduction (NaIO$_4$, NaBH$_4$) to give the primary alcohol 1.91, which was converted to the corresponding (R)-MTPA ester (1.91). The $^1$H NMR data of 1.91 was compared with both (S)- and (R)-MTPA esters (1.93ab) of (S)-3-methyl-1-pentanol which was consistent with the 20'S configuration. Thus, completion of the full stereochemical assignment of schlizzone A.
Scheme 1.13. Degradation of Schulzeine A (1.80) for configurational assignment of C19.

Schulzeine B (1.81) was subjected to a similar analysis as 1.80 (Scheme 1.14). NMR and FABMS data of the desulfation product showed absence of the methyl branch. Correlations from a ROESY experiment placed H3 and H11b were on the same face of the fused ring system. The absolute configuration of 1.81 was assigned from the methanolysis products (1.94–1.96), and showed Schulzeine B to be epimeric to sschulzeine A at C11b.

Scheme 1.14. Acid hydrolysis of Schulzeine B (1.81).

It is also worth mentioning that this work is reminiscent of previous studies carried out on α-glucodisase inhibitors penarolide sulfates A1 and A2 (proline containing sulfated macrolides)\textsuperscript{124} and penasulfate A (sulfated lipids).\textsuperscript{125} Total synthesis of Schulzeines A and B has been reported by the Gurjar\textsuperscript{126} and Romo\textsuperscript{127} groups. The Wardrop group synthesized Schulzeines A-
C$^{128}$, and showed the configurations at C20' in 1.80 should be inverted. The reason for the anomaly in assignments is uncertain.

### 1.7.7 Dictyostatin

In 1994, Pettit and coworkers disclosed the structure of a highly potent antimitotic marine macrolide, dictyostatin from *Spongia* sp. collected in Maldives.$^{129}$ The yield of dictyostatin was extremely low (1.35 mg from 400 kg wet. wt of sponge), and only a partial assignment (1.98a) of the relative configuration was disclosed.

**Figure 1.24.** Pettit’s proposed structure and Paterson/Wright revised structure for dictyostatin.

A decade later, following reisolation of (−)-dictyostatin from a North Jamaican lithistid sponge, a collaborative effort between the Paterson and Wright groups lead to an assignment (1.98b) for the absolute configuration (which differed considerably from 1.98a proposed by Pettit) based on high-field (700 and 800 MHz) NMR experiments, molecular modeling, and biosynthetic considerations.$^{130}$ Detailed analysis of NMR data (*J* coupling and
NOESY), showed the C1–C16 to be relatively rigid, adopting only one preferred conformation (Figure 1.25).

![Figure 1.25](image)

**Figure 1.25.** Configurational assignments made for (a) C6 to C12 and (b) C9 to C16 by NOESY and JBCA.

On the other hand, NMR data (NOESY and J coupling) for the C16–C26 segment were consistent with two rapidly interconverting conformations (Figure 1.26). Evidence for this phenomenon was provided by intermediate coupling constants observed between: H-19 to H-18a, H-19 to H-20, H-19 to Me-20 and H-20 to H-21. The two conformers differed in the orientation of C1/C2 (s-cis or s-trans), but were formulated as (a) and (b) to satisfy NOESY data. Molecular modeling (Macromodel, MM2, MonteCarlo search) gave a low energy conformation consistent with the s-trans configuration and fully consistent with NMR data.

![Figure 1.26](image)

**Figure 1.26.** Two conformations consistent with NMR data: (a) C1/C2 s-cis and (b) C1/C2 s-trans.
The structural features of dictyostatin bear strong resemblance to discodermolide (1.99, Figure 1.27)\textsuperscript{131} another highly potent anti–cancer polyketide\textsuperscript{132} reported by Gunasekera and coworkers from the deep water marine sponge *Discodermia dissolute*. Although the two natural products derive from different sponges, the relative configuration of 1.98 mapped to the corresponding segments in discodermolide (1.99). Therefore, it was assumed that the absolute configuration of dictyostatin was likely the same based on a similar biogenesis to discodermolide (1.99) the absolute configuration of which was defined by total synthesis of ent–discodermolide by Schreiber.\textsuperscript{133}

This represents a prime example of the power of high field NMR and biosynthetic inferences to arrive at the complete stereostructure for a complex natural product. Shortly after the proposed configurational assignment for dictyostatin (1.98), total syntheses by the Paterson\textsuperscript{134} and Curran\textsuperscript{135} groups reported that the proposed stereostructure was correct. The material provided by total synthesis is the only viable source of this rare natural product for biological testing. Currently, efforts are driven towards SAR studies of synthetic derivatives, further biological testing, and optimization of a total synthesis to provide material for pre–clinical trials.
1.7.8 Psymberrin

Psymberrin\(^{136}\) (also known as irciniastatin A,\(^{137}\) 1.100) is a highly cytotoxic polyketide from the marine sponges *Psammocinia* reported independently by the Crews and Pettit groups, respectively. In the NCI 60-cell line screen, psymberrin showed selectivity for several melanoma, breast, and colon cancer cell lines (LC\(_{50} < 2.5\) nM) over leukemia cell lines (LC\(_{50} > 25\) mM). The structure composition of psymberrin was intriguing because it resembled the compound pederin (1.101)\(^{138}\) isolated from the beetle *Paederus* sp. and other sponge metabolites (e.g. onnamide A (1.102)\(^{139}\) mycalamide,\(^{140}\) and theopederin\(^{141}\) from *Theonella* sp). Metagenomic analysis of whole sponge DNA by Piel and coworkers have identified the putative genes responsible for the production of psymberrin.\(^{142}\)
Figure 1.28. Structures for psymberin (1.100), pederin (1.101), and onnamide (1.102).

NMR spectroscopic analysis of 1.100 by Crews was more detailed and is discussed below. The planar structure was assigned by a combination of MS, IR, NMR and chemical conversions (methylation and acetylation), and stereochemical analysis proceeded by independent consideration of three subunits a–c. JBCA for the C4 and C5 stereocenters (subunit a) was unsuccessful due to complications from intermediate $^2J_{HC}$ couplings (3 Hz) between H4/C5 and H5/C4.143

The tetrahydropyran ring system (subunit b) was deduced by coupling constant and NOESY correlations (Figure 1.29). The C8/C9 configurational assignment was made based on large $^1H^-^1H$ coupling between H8/H9 ($J = 8.0$ Hz) which orients these protons anti. NOESY correlation between OMe-8/H10$_{eq}$ and H8/H13$_{ax}$ established the rotamer depicted in Figure 1.29.
NOESY correlations of the C15-C17 stereotriad (c) established two rotamers in both C15/C16 and C16/C17 (Figure 1.27c).

**Figure 1.29.** Configurational analysis of psymberin (1.100): (a) C5 assigned by analogy to pederin (1.101) and onnamide (1.102). (b) Preferred conformation of the tetrahydopyran and relevant nOes, and (c) two conformational rotamers about C15/C16 and C16/C17.

The absolute configuration of the C17 stereocenter was assigned by comparative CD analysis. The CD spectra for psymberin showed a strong positive Cotton effect at λ 280 nm, and that of dihydrocoumarin 1.103 (R-configuration) shows a negative Cotton effect at λ 275 nm assigned to the “n→π*” transition. Therefore psymberin (1.100) was assigned the opposite configuration (C17R) to that of 1.103 (Figure 1.29). The remainder of the molecule was assigned from assumptions of a similar biogenesis to pederin and related sponge metabolites (i.e. onnamide).
Scheme 1.15. Stereochemical determination of the amide side chain in psymberin (1.100).

Following the initial report of psymberin, two synthetic approaches by the Williams\textsuperscript{145} and Floreancig\textsuperscript{146} groups to the diastereomeric models of psymberin established the relative and absolute configurations of the amide side chain. The Floreancig group stereospecifically synthesized the four diastereomers (1.105–1.108, Scheme 1.15). PMB ether 1.104 was deprotected with ceric ammonium nitrate and, under acidic conditions (MeOH, H$_2$SO$_4$, 60°C), gave tetrahydrofuran 1.105 without racemization. The other three diastereomers (1.106–1.108) were prepared by similar procedures to give the four tetrahydrofuran derivatives needed for analysis. Separation of the four diastereomers was achieved by chiral GC (Chiralallex G–TA). Methanolic hydrolysis of psymberin (1.100) under identical conditions provided tetrahydrofuran 1.105 that was identical to the compound derived from 1.104 (Scheme 1.15). Therefore, the side chain of psymberin has the 4S,5S configuration. Psymberin has been the subject of many total and fragment syntheses, which have verified the stereo–assignment.\textsuperscript{147}
1.7.9 Caminoside A

Caminoside A (1.109),\textsuperscript{148} a glycolipid from the marine sponge \textit{Caminus spareoconia} from Dominica was the first active natural product identified in a screening effort to identify small molecule inhibitors of a type III bacterial secretory pathway. Compound \textbf{1.109} exhibits an IC\textsubscript{50} = 20\textmu M in the assay.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{caminoside_a}
\caption{Strategy for configurational assignment of caminoside A (1.109).}
\end{figure}

Structurally, caminoside A (1.109) contains an oligosaccharide glycone comprised of four sugars (two equivalents of D−glucose, D−6-deoxytalose, and L−quinovose) appended to an oxygenated lipid aglycone that were identified by NMR and MS data. Compound \textbf{1.109} was subjected to methanolic acid hydrolysis (Scheme 1.16), to yield the aglycone (1.110), as well as a mixture of the mono−methyl glycosides, which were separated. The glycosidic fraction was acetylated and purified by HPLC to give D−α-1-methoxy-2,3,4,6-O-tetraacetylglucose (1.111) and D−β-1-methoxy-2,3,4-O-
triacetyl-6-deoxyglucose (1.112), both of which matched authentic samples by specific rotation. The third product, 1.113 (1-methoxy-2,3,4-O-triacetyl-6-deoxytalose) was assigned by NMR, and then subjected to acid hydrolysis before comparison with authentic standards.

**Scheme 1.16.** Configurational assignment of sugars in caminoside A (Andersen).\(^{148}\)

The configuration of the aglycone portion was assigned by Molinski and coworkers after conversion of 1.110 to its corresponding bis-TPP ester (1.118), measurement of liposomal exciton coupled circular dichroism (LECCD), and comparison to optically active models (Scheme 1.17).\(^ {149}\) Previous CD studies on meso 1,5-, 1,7-, and 1,9-glycol bis-TPP esters showed that the absolute configuration of these systems are readily assigned by interpretation of the resulting positive or negative bisignate ECCD spectra obtained in liposomally–ordered media.\(^ {150}\) In isotropic media, the latter compounds show only baseline spectra. Caminoside aglycone (1.110) was converted to the naphthoate derivative, and the ketone was reduced to the corresponding alcohol (1.115) as a C2 epimeric mixture (1:1). The mixture of diastereomers was subjected to kinetic resolution (Novozym 435, vinyl
acetate), to give pure 2R–acetate diastereomer 1.116a. Removal of acyl groups by Ammoniolyis of 1.116a gave the diol 1.117, which was converted to the bis–TPP ester 1.118 (TPP-piv, Et₃N).

![Scheme 1.17 Molinski’s configurational assignment of caminoside A aglycon. Conversion of aglycon 1.110 to bis-TPP ester 1.118.](image)

The CD spectrum for 1.118 (MeOH) showed no significant Cotton effects. However, when CD spectra of 1.118 was acquired in DSPC liposomes, a bisignate positive ECD spectra was observed. Therefore caminososide A (1.109) has the 10R configuration. LECCD methodology should be useful for assignment of other natural products containing 1,n– diols (n= odd; 3, 5, 7, 9, etc.).
1.7.10 Polytheonamides

The highly cytotoxic polytheonamides A (1.119) and B (1.120) show a remarkable array of alternating L- and D-tert-alkyl glycine residues. They were first reported in 1992\textsuperscript{151} by Fusetani and coworkers, and revised in 2005\textsuperscript{152} The amino acid composition was established by amino acid analysis, and NMR analysis of the whole acid–hydrolysate. The N-terminus of polytheonamide B was originally assigned as a carbamoyl group, but later revised to 5,5-dimethyl-2-oxo-hexanoyl group based on reduction of 1.120 with sodium borohydride (Scheme 1.18) to give secondary alcohol epimers 1.121. Subsequently, the 44\textsuperscript{th} residue, first formulated as a $\gamma$-hydroxy-t-leucine was revised to a $\beta,\beta$-dimethylmethionine sulfoxide based careful interpretation of MS and NMR data.

The sequence assignment of amino acids was carried out by NOESY (n to n+1 crosspeaks of NH to H$\alpha$). In DMSO-$d_6$, polytheonamide B (1.120) is in a random-coil conformation which facilitated sequential assignment.

The absolute configuration of the constituent amino acids were secured by chiral GCMS and Marfey’s analysis\textsuperscript{153} of the total acid hydrosylate. Initial analysis showed the L-amino acids were: Thr, Ile, Glu, Val, and $\beta$Melle, and D-amino acids consisted of: HO–Asp, Ser, and $\alpha$Thr. The remaining amino acids: Ala, t-Leu, Asp, HO–Val were mixed D and L configurations and a sequence specific stereochemical method was used to address this problem.
Scheme 1.18. Chemical conversions of polytheonamide B.

Partial hydrolysis of polytheonamide B (1.120) (HCl/EtOH, 70°C, 30 min) afforded a complex mixture of peptide fragments. The composition of several fragments were identified by FABMS, and the N-terminal amino acids of each fragment were identified by dansylation, partial hydrolysis, and chiral GC or Marfey’s analysis, followed by Edman degradation. Exhaustive analysis of the peptide fragments gave the complete configuration of all amino acid residues except for the sulfoxide containing amino acid. The β,β-dimethylmethionine sulfoxide did not survive acidic hydrolysis, therefore 1.120 was reduced to the corresponding β,β-dimethyl methionine analog (1.122) and cleaved with cyanogens bromide (Scheme 1.18) to the corresponding L-β,β-
dimethylhomoserine lactone (1.123) which was compared with authentic samples by GC analysis.

Finally, the structure of polytheonamide B (1.120) showed the same gross structure as polytheonamide A (1.119). This led to the proposal that the difference between the two compounds was either a change in configuration of one or more amino acids or the sulfoxide stereocenter. Serendipitously, autooxidation of 1.122 obtained from either polytheonamides A or B gave a 1:1 mixture of polytheonamides A (1.119) and B (1.120). In addition, separate oxidation of polytheonamides A (1.119) or B (1.120) (oxone, Scheme 1.18), provided the same sulfones (1.124) confirming that the two peptides were epimers at S.

The heroic effort of structure elucidation of polytheonamides A and B, has now been followed by the first total synthesis of these peptides by the Inoue group.154 In addition, a solution conformation based on NMR and molecular modeling has also been reported which shows that the potent cytotoxicity associated with these peptides is attributed to their pore forming abilities through a unique β-helix motif.155 A single molecule of polytheonamide B spans 45Å, or about three times longer than gramicidin A, another pore forming peptide. It is interesting to note that the polytheonamides are more effective pore-forming peptides than other synthetic non-natural alternating (D- and L-form) peptides.
1.7.11 Citrinadin A

Citrinadin A (1.125) obtained by fermentation of Penicillin citrinadin, separated from a red alga is a pentacyclic spirooxindole alkaloid containing 7 stereogenic centers.\(^{156}\) It exhibits modest activity against murine leukemia L1210 and human epidermoid carcinoma KB cells (IC\(_{50}\) 6.2 and 10 mg/mL, respectively). The planar structure was assembled by 2D NMR data.

![Figure 1.31. Kobayashi’s strategy for stereochemical assignment of citrinadin A (1.125).](image)

The relative configuration of the pentacyclic core of citrinadin A was established by ROESY data and \(^1\)H-\(^1\)H coupling constants (Figure 1.32a). The relative orientation of the spirooxindole system was secured from ROESY correlations from H4 to both NMe26 and Me29.

The absolute configuration L-\(N,N\)-dimethyl valine residue was assigned by acid hydrolysis (1N HCl) and chiral HPLC (Sumichiral OA-5000, 1 mM CuSO\(_4\) aq).\(^{157}\) In a subsequent report, the Kobayashi group assigned the C14 absolute configuration of the pentacyclic core by ROESY correlations relayed from the 2S-\(N,N\)-dimethylvaline residue (Figure 1.32b).\(^{158}\) The chlorohydrin
derivative (1.126) obtained by treatment of citrinadin A with HCl (50 mM in MeOH).

**Figure 1.32.** NOESY correlations for (a) citrinadin A (1.125) and (b) citrinadin A chlorohydrin (1.126).

No standard methods to assign the isolated C21 stereocenter in the epoxide ring were available. Consequently, vibrational circular dichroism (VCD) spectra of the natural product was measured and compared to those of enantiomeric aryl keto-epoxides, 2R-(+)-1.127 and 2S-(−)-1.127 that were synthesized in five steps from benzaldehyde (Figure 1.33). Although the VCD spectra for the model compounds did not show exact mirror images as expected, the Cotton effect at 1230 cm\(^{-1}\), was attributed to symmetrical stretching of the epoxide ring, and the Cotton effects of the model spectra were opposite. This band was used to assign the configuration in the natural product. Therefore the complete assignment of citrinadin A was assigned as 3S,8S,2R,14R,16S,18R,21S. The use of VCD by comparative methods should
gain popularity as instrumentation becomes more available and sensitivity improves.

![Chemical Structure](image)

**Figure 1.33.** Synthetic epoxides (−)-1.127 and (+)-1.127 used for comparison of VCD to citrinadin A (1.125).

### 1.7.12 Sagittamide A

The sagittamides,\(^{159}\) reported by Lievens and Molinski from an unidentified tunicate collected in Micronesia are polyacetoxy long–chain \(\alpha,\omega\)-dicarboxylic amides of ornithine and valine.

![Chemical Structures](image)

**Figure 1.34.** Strategies by Molinski and Kishi for the configurational assignment of sagittamide A (1.128).
L-ornithine and L-valine were identified by Marfey’s analysis\textsuperscript{153} of the acid hydrolysate of 1.128. Two different configurational assignments by the Molinski\textsuperscript{160} and Kishi\textsuperscript{161} groups were proposed for the contiguous hexa-acetoxy segment, however the Kishi configuration was verified by total synthesis as described.

![Diagram](image)

**Figure 1.35.** $^3J_{HH}$ profiles for tetraol peracetates. (A = anti, S = syn). Taken from ref. 162.

The UDB approach was advanced beyond $^{13}$C chemical shift profiles to $^3J_{HH}$ coupling constant profiles derived from synthetic compounds of known configuration reported in the literature.\textsuperscript{162} Profiles were generated for all the diastereomers of a contiguous tetracetate for a total of eight subgroup profiles (SSS, AAA, ASA, SAS, SSA, ASS, SAA, and AAS) (Figure 1.35). Polycetates containing greater than four contiguous stereocenters can be assigned by overlap of adjacent coupling constant profiles.
**Figure 1.36.** UDB approach for assigning the hexahydroxy acetate portion of sagittamide A. (A = anti, S = syn). Taken from ref. 161.

The hexa-acetoxy profile (A) of 1.128 was divided into three subgroups: (D) C5–C8, (C) C6–C9, and (B) C7–C10. The profile for each subgroup was compared to the subgroup profiles generated for the tetraol peracetates. The closest match for subgroups D, C, and B were SAA, ASA,
and SAS, respectively. The relative configuration of the hexa–acetoxy segment to be defined as $5S^*, 6S^*, 7S^*, 8S^*, 9S^*, 10R^*$.

![Image of chemical structures](Image)

**Figure 1.37.** Diastereomers synthesized by Kishi.

The Kishi group proceeded to synthesize two diastereomers 1.128 and the antipode of 1.129 (Figure 1.37). $^1$H NMR mixing experiments showed antipode I was identical to natural sagittamide, while the antipode of 1.129 showed slight differences in the acetoxy $^1$H signals.

Griesinger and coworkers reported the use of residual dipolar couplings (RDCs) for the assignment of the relative configuration of the hexa–acetoxy segment of sagittamide A. $^{163}$ A combination of JBCA and NOE data narrowed the relative configuration to four diastereomers. Measurement of RDCs and comparison to Boltzman–weighted calculated values gave only one diastereomer (with the same relative configuration as 1.128) with data consistent with the measured values. This work demonstrated the use of RDCs as an additional parameter for resolving inconclusive results from JBCA.
1.7.13 Gymnocins

Gymnocin A (1.130), a highly cytotoxic polyether toxin was isolated from the red tide dinoflagellate Karenia (formerly Gymnodinium) mikimotoi by Satake and coworkers in 2002.\textsuperscript{164} The structure of 1.130 was assembled through analysis of 2D NMR and detailed analysis by FAB collision–induced dissociation (CID) MS. The relative configuration of the polyether system (all rings oriented in the trans cisoid fashion) was assigned from NOE and coupling constant data. The C50S absolute configuration of gymnocin A was addressed by application of the MMM to esters 1.131ab. Soon after the isolation, asymmetric synthesis of (+)-gymnocin A (1.130) was completed by Sasaki, verifying the absolute stereostructure.\textsuperscript{165}

In 2005 Satake and coworkers reported another polyether derivative related to 1.132 they named gymnocin B (1.132).\textsuperscript{166} Gymnocin B is the largest contiguous polyether compound reported to date. The structure determination (planar and relative conformation) of 1.132 was carried out in a similar fashion as gymnocin A. Unfortunately, the secondary hydroxyl groups were unreactive towards MTPA-Cl, and the MMM was not applicable. That same year, Berova and coworkers reported a tour de force effort to assign the absolute configuration of gymnocin B by chemical chiroptical methods.\textsuperscript{167}
Figure 1.38. Berova’s strategy for configurational assignment of gymnocin B (1.132).

Long range exciton coupled CD between chromophores separated by 50Å was described by Nakanishi using $p$-(meso-triphenylporphyrin)-carboxylic acid (−TPP) esters of brevetoxin B as chromophores.$^{168}$ The brevetoxin polyether system adopts a preferred conformation in polar solvents (MeOH/H$_2$O) that leads to a strong bisignate exciton coupled CD spectrum. These principles were applied to the assignment of gymnocin B (1.132).

TPP chromophores were attached to the less reactive −OH groups in relatively high yield by a combination esterification/ cross metathesis protocol (Scheme 1.19). This method was perhaps inspired by previous successes in attaching styrene chromophores using cross metathesis$^{169}$ to allylic alcohols and allylic amines.$^{170}$ Gymnocin B (1.132, 300 µg) was converted to the acetonide (2,2 dimethoxypropane, PPTS), and acylated with acryloyl chloride which gave ester 1.133. The acryloyl ester (1.133) was subjected to cross metathesis with vinyl−TPP 1.135 in the presence of Grubbs' second generation catalyst (1.134) which gave bis-TPP-cinnamate ester 1.136 (∼10
μg). A positive exciton coupled split Cotton effect ($\lambda$ (MeOH) 419 nm ($\Delta\varepsilon$ +11), 414 nm ($\Delta\varepsilon$ -15)) was observed in the CD spectra for 1.136, which confirmed both TPP groups were attached.

**Scheme 1.19.** Conversion of gymnocin A (1.130) to MTPA esters (1.131ab), and conversion of gymnocin B (1.132) to bis-TPP ester (1.136).
Figure 1.39. NOESY correlations establishing the relative configuration of the BC and JK ring systems of gymnocin B (1.132).

The relative orientation of the hydroxyl groups (pseudoaxial or pseudoequatorial) in 1.136 were critical for defining the direction of electronic transition dipoles. Evaluation of coupling constants and NOE data for gymnocin B (1.132) established that both hydroxyl groups (C10 and C37) are oriented in the pseudoaxial position (Figure 1.39). Conformational analysis (MMFF94) on a series of truncated monocinnamate (rings systems including: A-C, I-K, H-K, G-K, and F-K) and biscinnamate models (A-O) verified that the cinnamate esters orient in the axial position and with an s-cis conformation of the double bond. Conformational analysis of the complete gymnocin B TPP cinnamate system showed that while the C10 ester remains in the axial position, the C37 ester adopted an equatorial position. The Boltzman distribution of the lowest energy conformations (< 7 kcal/mol), showed the projection angle is positive in sign and should give rise to a positive split Cotton effect if gymnocin B (1.132) contained the 10S and 37S configuration. Finally, quantitative calculated CD spectra of the three lowest energy
conformers showed that the magnitude of the Cotton effects were were of substantial magnitude.

1.7.14 Spirastrellolides

The think–red encrusting sponge, Spirastrella coccinea from Dominica has been the source of a group of highly antimitotic spiroketal macrolides named the spirastrellolides, isolated by the Andersen group.\textsuperscript{171} Spirastrellolide A is the most abundant with high anti-mitotic activity against MCF7 human cancer cells (80 nM)\textsuperscript{172}. To ease purification the spirastrellolides were isolated as their methyl esters by treatment of either the crude extract or partially purified fractions with TMS-diazomethane. The planar structure and partial relative configuration for spirastrellolide A was depicted as 1.137 (Figure 1.40) in the initial report based on HRCIMS and 2D NMR analysis. The relative configuration of the C13-C21 spiroketal and C27-C29 portion of the second spiroketal were assigned by coupling constant and ROESY correlations.

The following year, larger amounts of spirastrellolide A methyl ester (45.1 mg) were purified which allowed chemical conversions to be carried out. ESIFTMS of peracetylated spirastrellolide A suggested that the originally assigned structure was incorrect due to an error in the original molecular formula; the correct molecular formula should be C\textsubscript{53}H\textsubscript{83}O\textsubscript{17}Cl.\textsuperscript{173} Further NMR analysis showed spirastrellolide A is the structure depicted 1.138. Following revision of the planar structure, a thorough investigation to assign the relative configuration was carried out by extensive analysis of J coupling and ROESY
data of the diacetonide derivative 1.140 (Figure 1.40). This analysis led to stereochemical assignments of the three isolated stereogenic units (C3-C7, C9-C21, and C27-C38) which reduced the number of possible diastereomers for 1.133 to 16.

**Figure 1.40.** Originally proposed structure for spirastrellolide A (1.137), and corrected structure for spirastrellolide A (1.138).

The configurations, relative and absolute except C46, was solved by X-ray diffraction by chemical conversion of spirastrellolide B (1.141), a dihydro, dechloro derivative of 1.138. Detailed NMR analysis of spirastrellolide B methyl ester (1.142) and comparison to spirastrellolide A methyl ester (1.139) revealed that the relative configuration was conserved between both macrolides. The bisacetonide derivative (1.143) of spirastrellolide B was subjected to perruthenate cleavage (RuCl₃/NaClO₄) and alkylation with p-bromophenacylbromide to provide phenacyl ester 1.144 (Scheme 1.20). The
C22/C23 acetonide had been cleaved and the C23 hydroxyl oxidized to the ketone.

Scheme 1.20. Structure for spirastrellolide B (1.142) and conversion to p-bromophenacyl derivative (1.144).

Slow evaporation of 1.144 from CH₃CN gave crystals suitable for X-ray diffraction studies. X-ray data showed the relative configurations for the three subunits in spirastrellolide A were identical to those in spirostrellolide B, and the presence of a heavy atom (bromine) provided the anomalous dispersion required to solve the absolute stereostructure. The unique structure of spirastrellolide and its potent biological activity has attracted significant attention in the synthetic community. Several fragment syntheses of 1.141 have been reported by De Brabander, Forsyth, Paterson, Hsing, Furstner, Smith, and Phillips. Total syntheses of spirastrellolide A methyl ester and spirastrellolide F methyl ester have been accomplished by the Paterson and Furstner groups, respectively.
1.7.15 Shishididemiols

![Chemical structures of Shishididemiols A and B, Didemniserinolipid B, and Cyclodidemniserinol sulfate.](image)

**Figure 1.41.** Structures of Shishididemiols A (1.145) and B (1.146), didemniserinolipid B, and cyclodidemniserinol sulfate.

In 2007, Matsunaga and coworkers disclosed the complete structures of long chain lipids, shishididemiols A (1.145) and B (1.146) from a tunicate of the family Didemnidae. Compounds 1.145 and 1.146 showed antibacterial activity against the fish pathogenic bacterium *Vibrio anguillarum* (20 μg/6.5 mm φ disk zone of inhibition; 8 and 7 mm for 1.145 and 1.146, respectively).
The shishidemiols resemble serinolipids previously reported from Didemnid tunicates (e.g. didemniserinolipid B (1.160)\textsuperscript{184} and cycloidemniserinolipid A (1.159)\textsuperscript{185}).

The planar structures of the shishidemiols were established by 2D NMR and MS. The location of the C16 hydroxyl was assigned by analysis of fragmentation ions observed by FAB-MS/MS data. The structures of the shishidemiols contain several isolated stereochemical elements, and presented several challenges for configurational assignment. Each isolated stereo segment was independently assigned by a combination of chemical conversions, and several different uses of CDAs.

**Figure 1.42.** Matsunaga’s strategy for configurational assignment of shishidemiol A (1.145).

The *cis* relative configuration of the epoxide was assigned from observation of large vicinal coupling (*J* = 4.1 Hz). The C6/C7 relative configuration was assigned by conversion of 1.145 to the C6/C7 acetonide 1.149 (Scheme 1.21). CBz-protected shishidemiol A (1.147), was subjected
to epoxide ring opening (MgBr$_2$• Et$_2$O) which gave bromohydrin (1.148), that was converted to the acetonide 1.149.

![Diagram of chemical reactions and structures]

**Scheme 1.21.** Conversion of shishididemniol A (1.145) to acetonide (1.149), ∆δ’s (ppm) from application of the MMM to C2, C6 and C30.
NOESY crosspeaks for acetonide (1.149) revealed the *trans* orientation. The 2S, 6S, and 30S stereochemical assignments were assigned by the MMM from MTPA esters 1.151ab. Acetonide (1.149) was also converted to its MTPA esters (1.150ab), which showed the configurational assignment at C2 was consistent with the MTPA esters (1.151ab) derived from 1.145.

**Scheme 1.22.** Conversion of shishisidemniol A (1.145) to tyramine MTPA esters (1.153ab), and selected $^1$H NMR chemical shifts for 1.153ab, and synthetic models 1.154ab and 1.155ab.

The absolute configuration of the tyramine portion of the molecule was addressed by cleavage of the amide bond, conversion to the per-MTPA esters
and comparison to model compounds (Scheme 1.22). Acidic hydrolysis of
shishididemniol A (1.145) gave a mixture of epimers at the C2’ position,
however hydrazinolysis gave only one isomer which was treated with both (R)-
and (S)- MTPA-Cl and gave esters 1.153\textsubscript{ab}. For \textsuperscript{1}H-NMR comparisons, two
sets of diatereomeric esters were synthesized: 1.154\textsubscript{ab} for comparison of H9’
to H11’ and 1.155\textsubscript{ab} for comparison to H1’ and H2’. Comparative NMR
analysis showed the tyramine portion to contain the 2’R and 10’S absolute
configurations.

Chiral derivatizing agent, 2-naphthylmethoxyacetic acid (2-NMA) was
used to assign the remaining isolated C16 stereocenter (Scheme 1.23).
Anisotropic studies have shown that 2-NMA shows favorable anisotropic
effects over long distances, and 2-NMA was been used previously for the
assignment of remote stereocenter in ginnol.\textsuperscript{28} Bromohydrin 1.148 was
subjected to periodate cleavage followed by reduction (NaBH\textsubscript{4}) to the primary
diol 1.156, which was protected as the TBDPS ether 1.157. Both (R)- and (S)-
2-NMA was coupled to the secondary alcohol 1.157. Differential \textsuperscript{1}H NMR
analysis of 1.158\textsubscript{ab} showed C16 was consistent with the R configuration.
Scheme 1.23. Conversion of bromohydrin 1.148 to (R)- and (S)-2-NMA esters (1.158ab) and Δδ values.

The complete stereochemical assignment of shishidimieniol A included a number of chemical conversions, and variations incorporations of MTPA esters to assign the absolute configuration of the six isolated stereocenters. The C16 stereocenter was assigned by $^1$H NMR of the (S)– and (R)–2-NMA esters, which showed differential $^1$H chemical shifts ten carbons removed from the stereocenter. The methodology used in this investigation should be useful for other serinol lipids of this class.
1.7.16 Phorbasides

![Chemical structures of phorbasides](image)

**Figure 1.43.** Structures and activities for auriside A (1.161), callipeltoside A (1.163), lyngbouilloside (1.164), dolastatin 19 (1.162), and phorbaside A (1.11).

The phorbasides A-E, and F (1.11) from the marine sponge *Phorbas* sp. are the most recent additions to a group of cytotoxic glycosidic macrolides from various marine organisms which include: aurisides A (1.161) and B and dolastatin 19 (1.162) from the sea slug *Dolabella auricularia*, callipeltoside A (1.163) from the sponge *Callipelta* sp., and lyngbyabouilloside (1.164) from the cyanobacteria *Lyngbya bouillonii*.
Configurational assignment of auriside A (1.161) by combined $J$ coupling and NOESY analysis which established the complete relative configuration. The absolute configuration was assigned by degradation, and comparison with authentic standards of 1.165 and ent-1.165. The configurational assignment of the aurisides was verified by total synthesis by Paterson.\textsuperscript{190}

The callipeltosides and phorbasides contain 3'-O-methyl evalose, a C–methyl sugar, and an additional chromophoric stereochemical element, a diene-yne chlorocyclopropane and ene-yne chlorocyclopropane, respectively. Although the relative configuration of the cyclopropane has been assigned by coupling constant and NOE data, no general method has been presented to establish the relative and absolute configuration between the macrolide ring and chlorocyclopropane. Prior to the report of the phorbasides, configurational assignment of the callipeltosides was only possible through total synthesis reported by Trost\textsuperscript{191} and Evans.\textsuperscript{192}

![Diagram](image)

**Figure 1.44.** Molinski’s strategy for configurational assignment of phorbaside A (1.11).
To circumvent the need for total synthesis, the Molinski group developed a quantitative approach to simultaneously solve the absolute configuration of both the configuration of the side chain and C13 of the macrolide ring using circular dichroism (Figure 1.45). Both UV and CD spectra for phobasides A and B were dominated by the ene-yne chlorocyclopropane chromophore, and displayed a moderately intense positive CE [$\lambda_{\text{max}}$ 232 nm ($\Delta \varepsilon$ +9.1), 241 (+8.1)]. The contribution of the ene-yne chlorocyclopropane lacking the C13 stereocenter was observed by synthesis of two stereospecific model compounds 1.166 and ent-1.166. The sign of the CEs for model compounds 1.166 and ent-1.166 was informative of the configuration of the cyclopropane, but the magnitude was less than expected. The additional contributions to the Cotton effects were attributed to the chiral C13 stereocenter and confirmed by comparison with two additional synthetic diastereomers 1.167 and 1.168. The CD spectra for 1.167 was almost identical to phorbaside A (1.11) and accounted for both the sign and magnitude of the CEs observed for the natural product. Surprisingly, the absolute configuration of the chlorocyclopropane in phorbaside A (1.11) was assigned opposite to that of callipeltoside A (1.163), while the configuration of the macrocycle was conserved.
Figure 1.45. NOESY correlations used to establish the relative conformation in phorbaside A, and synthetic models (1.166–1.168) used for quantative CD comparison. (a) CD spectra for phorbaside A (1.11), 1.116, and ent–1.116. (b) CD spectra for phorbaside A (1.11), 1.167, and 1.168. Taken from ref. 16b.

Next, a method for the assignment of the absolute configuration of the sugar in phorbaside A was developed. The assignment of sugar configuration using exciton coupled CD was developed by Nakanishi, however they were not practical for the tertiary OH in the C–methyl sugar, O–methyl evalose, so a more reactive aryl-isocyanate was chosen for derivatization. Phorbaside A (1.11) was subjected to a three step degradation sequence: methanolysis, naphthoylation, and reaction with 6-methoxynaphthylisocyanate to give 1.170 (Scheme 1.24). The CD spectrum of 1.170, derived from L-rhamnose gave a weak positive bisignate Cotton effect
\[ \lambda_{\text{max}} 241 \text{ nm (}\Delta \varepsilon + 2.6), 226, (-1.5); A = 4.1 \] identical in sign and magnitude to that of authentic \textbf{1.170} prepared from L-rhamnose.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image}
\caption{Conversion of phorbaside A (\textbf{1.11}) to dichromophoric (\textbf{1.170}). CD spectra for (a) synthetic-\textbf{1.70}, and (b) \textbf{1.70} derived from phorbaside A.}
\end{figure}

Total synthesis of phorbaside A (\textbf{1.11}) by Paterson has confirmed the configurational assignment by Molinski.\textsuperscript{194}

\subsection{1.7.17 Goniodomin}

Goniodomin was first reported by Burkholder and coworkers in 1968 from the marine dinoflagellate \textit{Goniodoma} sp. collected in La Parguera, Puerto Rico.\textsuperscript{195} At this time the information was summed as follows, “The molecular formula, \( \text{C}_{43}\text{H}_{58}\text{O}_{11} \), has been established for this compound. Physical and chemical data indicate the presence of five hydroxyl groups, a lactone ring, four ether linkages and a dihydrogeranyl side chain in this molecule”. The complete planar structure was not revealed until 1988, when
Murakami and coworkers isolated goniodomin A from *Goniodoma pseudogoniaulax* and conducted extensive NMR experiments (COSY, COLOC, PSNOESY).\textsuperscript{196}

![Diagram of goniodomin A](image)

**Figure 1.46.** Strategies by Fujiwara and Sasaki for the configurational assignment of goniodomin A (1.171).

Almost two decades passed before the Fujiwara group disclosed the synthesis of model compounds to establish the relative configuration of goniodomin A (1.171, Figure 1.47).\textsuperscript{197}Synthesis of A-ring models 1.172 and 1.174, and comparison of NMR data revealed consistency with goniodomin A. In particular, the C32/C33 and C33/C34 relative configurations are assigned to the *cis* and *trans* configurations, respectively. Macroyclic model compounds 1.176 and 1.177 were synthesized to represent the D/E rings of goniodomin A (1.171). 1.176 with a *trans* C21/C22 orientation was more consistent with the coupling constant observed for goniodomin A.
**Figure 1.47.** Model compounds synthesized by Fujiwara.

In 2008, just prior to Fujiwara’s report on the relative configuration of the DE ring system, the Sasaki group proposed the relative and absolute configuration of goniodomin A (1.171) based on high-field NMR (500, 600, and 900 MHz) experiments in different solvents, chemical conversion of the natural product, and comparison with synthetic model compounds. High-field NMR experiments, J-coupling and NOESY correlations were used to secure the relative configuration of the relatively rigid macrolide ring containing A through E (Figure 1.48(a and b)). The three orientation of the C26/C27 diol was assigned by conversion to the corresponding acetonide (1.178, Scheme 1.25) and NOESY correlations from one methyl group to H26 and the other methyl
group to H27. The relative configuration of the macrolide portion was relayed to the C31 sterocenter by NOESY correlations (Figure 1.48(c)).

![Diagram](image)

**Figure 1.48.** Selected NOESY correlations for the Assignment of relative configuration of the macrolide ring and sidechain (ring F) for goniodomin A (1.171).

The relative configuration between C31 and C32 as well as the absolute configuration were assigned by chemical methods (Scheme 1.25). After considerable experimentation, it was found that cleavage of the C26,C27 diol of 1.171 with Pb(OAc)_4 gave labile di-aldehyde 1.179, of sufficient stabiliity for immediate NMR analysis before spontaneous conversion to aldehyde 1.180. Synthesis of model compounds 1.181 and 1.182 provided chemical shift data that was indicative of the relative configuration between H31 and H32. The NMR data for di-aldehyde 1.179 showed a closer match to 1.181.
Scheme 1.25. Conversion of goniodomin A to acetonide 1.178 and di-aldehyde 1.179, and synthetic model aldehydes 1.181 and 1.182.

The absolute configuration of goniodomin (1.171) was secured from conjugated ketone 1.183a, prepared by treatment of 1.180 with (S)-MTPA-Cl (Scheme 1.26). Authentic diastereomeric MTPA esters 1.183ab were formed from 1.181, which showed goniodomin (1.171) contained 33R and 34S configurations.
Scheme 1.26. Conversion of model ketone (S)- or (R)-MTPA-Cl.

1.7.18 Plakinic Acids I – L

Figure 1.49 Structures for plakinic acids I – J (1.184–1.187).

Relatively few methods exist for solving the absolute stereochemistry of remote or isolated methyl branched stereocenters. The Molinski group used liposomal CD of naphthamides to assign remote methyl branched stereocenters in plakinic acids I (1.184) and J (1.185)\textsuperscript{199}, endoperoxides from
the marine sponge *Plakortis halichondroides* from the Bahamas. ω-Phenyl polyketide peroxides have received interest due to their sub-micromolar activity against parasites, including the Malaria vector *Plasmodium falciparum*.\(^{200}\)

**Figure 1.50.** Molinski’s strategy for the configurational assignment of the plakinic acids.

The relative and and absolute configuration of the six-membered ring peroxide was solved by conventional methods (NOESY measurements and the MMM). Plakinic acid I (1.184) was methylated (CH\(_2\)N\(_2\), ether), and subjected to hydrogenolysis to the ring opened diol (1.188), which was transformed into both MTPA esters (1.189ab). Standard analysis led the 3S, 4S, 6R configurations (Scheme 1.27).

**Scheme 1.27.** Configurational assignment at C3 of plakinic acid I.

The stereochemistry at C8 in the acyclic chain of plakinic acids I (1.184) or J (1.185) was not readily assignable by standard NMR methods that typically relay stereo–information to the nearest chiral center of known
configuration (C6). Plakinic acid I (1.184) was reduced with Fe(II)Cl₂ which gave three products, 1.190, 1.191, and 1.192 (Scheme 1.28) by intermolecular radical fission. Chloride (1.190) was converted to the azide (1.193) and the product reduced to the free amine (1.194), which was derivatized with 6-methoxy–2–naphthoyl chloride to furnish naphthamide 1.195.

Scheme 1.28. Conversion of plakinic acid I (1.184) to naphthamide 1.195.

The CD spectra of 1.195 in MeOH showed no significant Cotton effects due to conformational averaging, however CD spectra were acquired in DSPC readily revealed strong Cotton effects of the opposite sign and magnitude as synthetic (S)-1.195 (Figure 1.51). Therefore the configuration at C8 in plakinic acid I was assigned as R. The same approach was used for plakinic acid J (1.185), which showed the same configuration. In addition, plakinic acids K (1.186) and L (1.187) containing two methyl branches were assigned by liposomal CD by a similar degradation protocol gave dimethyl naphthamide 1.196, and comparison to optically active synthetic models (1.197–1.199).²⁰¹ The liposomal CD spectrum of 1.196 showed similar sign, but subtle
differences in magnitude and presence of additional bands attributed to diastereomeric differences of extended chromophores in the liposomal bilayer.

![CD spectra for napthamides derived from plaknic acids](image)

**Figure 1.51.** CD spectra for napthamides derived from plaknic acids: (a) L–CD of d) (S)-1.195, (e) (±)-1.195, (f) (R)-1.195; (b) L-CD of (2R, 6S)-1.196 from 1.186 (solid line), (2S,6R)-1.197 (dotted line); (c) L–CD of (2R,6R)-1.199 (solid line), (2S,6S)-1.198 (dotted line). Taken from references 199 and 201.

### 1.7.19 Biselyngbyaside

Biselyngbyaside (1.200), a glycosidic polyketide macrolide from the marine cyanobacteria *Lyngbya* sp. from Okinawa that was reported by Suenaga and coworkers in 2009. Biselyngbyaside showed a mean GI$_{50}$ of 0.60 μM against 39 human cancer cell lines. The 2D structure was
established in a straightforward manner by MS and 2D NMR, and the relative configuration of the 3-O-methyl glucoside was assigned by NOESY and $^1$H-$^1$H couplings, all protons showed axial orientation.

**Figure 1.52.** Suenaga’s strategy for configurational assignment of Biselyngbyaside (1.200).

The remote stereocenters insulated by sp$^2$ carbons precluded assignment by $J$-based methods. The configurations at C3 and C7 were addressed by chemical degradation, and the MMM. Biselyngbyaside was subjected to hydrolysis under basic conditions to give methyl ester 1.201, and was converted to both (S)- and (R)-MTPA esters (1.202ab, Scheme 1.29). $\Delta$$\delta$ chemical shift differences were consistent with the 18R configuration.

**Scheme 1.29.** Hydrolysis of biselyngbyaside, and configurational assignment
of C16 by the MMM.

Since the methanolysis induced elimination of the C3 oxygen, an approach was chosen to reduce this problem. Hydrogenolysis under acidic conditions provided methyl ester 1.203 and an anomic mixture of O-methylglucosides 1.204 (Scheme 1.30). The MMM was applied to 1.205ab derived from 1.203 to give the R configuration for C3. The absolute configuration of the glucoside 1.204 was assigned by the CD spectra after conversion to both tri-p-bromo-benzoate derivatives 1.206 and 1.207 which were separable by HPLC. The tribromobenzoate derivative 1.207 showed identical CD spectra (λmax 238 nm (Δε +7.2), 254 nm (Δε -17.2) to the tribromobenzoate derived from 1-O-methyl-D-glucose.

Scheme 1.30. Hydrogenolysis of biselyngbyaside.

The final two stereocenters in biselyngbyaside (1.200) were identified by a three step degradation, followed by NMR and chromatographic comparison to optically pure standards (Scheme 1.31). Ozonolysis of 1.200 followed by reduction gave the corresponding diol, which was immediately derivatized with p-bromophenylisocyanate gave 1.208 in good yield. Optically
enriched standards 1.208 and 1.209 were prepared for comparison of NMR data to differentiate diastereomers. Synthetic and naturally derived 1.208 were identical by NMR. To identify the absolute configuration of natural 1.208, its corresponding enantiomer was synthesized for comparison by HPLC analysis. Chiral HPLC (Chiralpak IA) showed natural and synthetic 1.208 to have identical retention times ($t_R = 6.5$ min) when compared to ent-126 (8.1 min), which corroborated the 7S and 10S configuration for biselyngbyaside (1.200).

![Scheme 1.31. Oxidative degradation of biselyngbyside (1.200), and synthetic models (1.208–1.209).](image)

1.7.20 Muironolide

Muironolide A (1.210) is the third class of macrolides along with phorboxazoles and phorbasides co-occurring in the marine sponge Phorbas sp from Western Australia. Three structural features set muironolide A (1.210) apart from other marine macrolides: a hexahydro-1H-isoindolinone-triketide ring, a trans-2-chlorocyclopropyl ketide (CCK), and trichloromethylcarbinol ester. The entire structure determination and preliminary biological testing was
carried out on 90 µg of isolated material.

![Diagram](image)

**Figure 1.53.** Molinski’s strategy for configurational assignment of muironolide A (1.210).

The planar structure was established by MS and NMR data. The trichlorocarbinol ester was confirmed by comparison of \(^1\)H and \(^{13}\)C spectra to model compound **1.211**. The relative configuration of the isoindolone ring and macrocycle were addressed by coupling constant and NOESY data (Figure 1.54).

![Diagram](image)

**Figure 1.54.** Muironolide A and NOESY correlations.

Attempted assignment of the relative configuration by NOESY or \(J\)-based methods were unsuccessful in relaying the stereochemistry of the CCK element to the other macrolide ring stereocenters. Consequently, a microscale degradative approach was used for **1.211**, and correlation of the products with
standards of known configuration (Scheme 1.32). Muironolide A (112, 30 μg) was subjected to base hydrolysis, derivatization with bromo-2-acetylnaphthalene to give 1.212 that showed an identical retention time (Chiralpak AD) to authentic material.

Scheme 1.32. Degradation of Muironolide A (1.210) for configurational assignment of the CCK unit.

The CD spectra for 1.210 [λmax 186 (Δε +58.5), 225 (-37.2)] revealed a negative exciton split Cotton effect due to coupled π→π* transitions of two unsaturated carbonyl chromophores: the enamide C7-C9 and the enoate C1-C3. This was interpreted as arising from the negative torsional angle of −116° (obtained from NOESY correlations and molecular modeling) for the π→π* transition dipole and the 4R, 5R, 11S, 14R, and 17R configuration of muironolide.
1.7.21 Enigmazole

Enigmazoles A (1.214) and B (1.215) were isolated from extracts of a marine sponge *Cinachyrella enigmata* from Papua New Guinea, that showed differential activity against a c-Kit mutant of murine mast cells. They were identified using standard bioassay guided fractionation of the cytotoxic n-butanol extract. Several stereochemical assignment problems of these structurally unique macrolides were successfully solved through an elegant combination of microscale chemical conversions and capillary NMR measurements.

![Structure of enigmazoles A (1.214) and B (1.215).](image)

**Figure 1.55.** Structure of enigmazoles A (1.214) and B (1.215).

The planar structure for enigmazole A (1.214) was assigned by 2D NMR. Unique features included a phosphate ester and a 2,4-disubstituted oxazole side chain. Enigmazole A (1.214) was divided into four sections for stereochemical analysis: (a) C1-C5, (b) THP: C7-C11, (c) C15-C17, and (d) C23. Each subunit was addressed independently, and then relayed together. A trans-disubstituted tetrahydropyran (THP) ring was assigned from syn-diaxial
protons with mutual NOESY crosspeaks and coupling constant data. The absolute configuration of 15S was determined by the MMM after methylation of the phosphate group (TMS-CH$_2$N$_2$) and conversion to both (R)- or (S)-MTPA-esters 1.216ab.

Figure 1.56. Gustafson's strategy for the configurational assignment of enigmazoles A (1.214).

Compound 1.214 was converted to 1.217 by phosphoramidation (CH$_3$NH$_2$, HOBT), base hydrolysis (KOH/MeOH), and methylation (Scheme 1.34). The relative configuration of the syn-1,3-diol (C15/C17) was established by the $^{13}$C acetonide method on 1.218.$^{19}$

Phenylglycine methyl ester (PGME) was chosen as a suitable CDA for the assignment of C2 (Scheme 1.34). Compound 1.217 was subjected to methanolysis and coupled to each of (R)- and (S)- 2-phenylglycine methyl ester (PGME, HOBT) to give 1.219ab.$^{204}$ Analysis of the $^1$H NMR anisotropic shifts revealed the 2S configuration.
Scheme 1.33. Configurational assignment at C15 of enigmazole A (1.214).

The phosphate group was resistant to enzymatic hydrolysis, but succumbed to mild basic conditions (wet DMSO, potassium acetate) to give lactone 1.220 (Scheme 1.35). The C4S,C5S configurations established by NOESY correlations. Only the 7R,11R diastereomer was consistent with J coupling, NOESY data, and molecular modeling.

Scheme 1.34. Ring opening to methyl ester 1.217, conversion to acetonide 1.218, and conversion to (R)- and (S)-PGME esters 1.219ab.
The final stereocenter in enigmazole A was assigned by chemical conversion and comparison with optically pure (R)-acetoin (Scheme 1.35). Perruthenate cleavage of \textbf{1.214} (RuCl$_3$-NaIO$_4$) gave 3-methoxy-2-butane, which was converted to the 2,4-dinitrophenylhydrazone (\textbf{1.221}),\textsuperscript{208} and compared to that of an authentic sample obtained from (R)-acetoin.\textsuperscript{209} The Cotton effects in the CD spectra of naturally-derived and synthetic enigmazole were identical, therefore enigmazole A (\textbf{1.214}) has the 23\textit{R} configuration.

\textbf{Scheme 1.35.} Formation of δ-lactone \textbf{1.220}, and conversion of side chain to dinitrophenylhydrazone derivative \textbf{1.221}.

The complete structure elucidation of enigmazole A represents an exceptional example of modern structure elucidation by chemical methods. In total, 13 chemical manipulations were successfully executed on \textsim{4.5 mg of enigmazole A or subsequent derivatives. The stereochemical assignment relied solely on chemical conversions, in contrast to JBCA analysis typically used for polyketide type compounds. The assigned stereostructure of \textbf{1.214} was identical to the compound prepared by total synthesis reported by Skepper and Molinski in 2010.\textsuperscript{210}
1.8 Current Challenges to Configurational Assignment

The above examples demonstrate complete solutions to a complex stereochemical problems can be addressed using modern NMR/CD instrumentation with only a few milligrams or even micrograms of a natural product. Nevertheless, outstanding problems remain. Several complex marine natural products are only partially solved.

The structures of the Nigricanosides A (1.222) and B, unusual ether-linked gly coglycerolipids from the green alga Avrainv ill ea nigrans collected in Dominica were recently reported by the Andersen group.211 The compounds were available in small amounts (A, 800 µg; B, 400 µg), but sufficient for determination of the 2D structure and initial biological evaluation. Nigricanosides A and B methyl esters showed potent activity against MCF-7 and HCT-116 cells (~ 3 nM), and their acid forms are suspected to have greater activity. The limited amount of material prevented further experiments for stereocemical determination, and either additional material must be procured or synthesis of appropriate diastereomeric models must be undertaken. The potent activity and unique structure prioritizes the nigricanosides as a prime target for both complete stereochemical determination and total synthesis.
Figure 1.57. Current challenges to configurational assignment.
Caylobolide (1.224), a macrolide from the cyanobacterium Lyngbya majuscula, contains a series of 1,5-diols that are not readily assignable by standard methods. The Molinski group has developed a method using liposomal CD for the assignment of 1,n-diols (n = 5, 7, 9) system, however, their application to polyols requires additional understanding of the fundamental photophysics of liposomal exciton coupled CD.

Symbiodinolide (1.225), a 62-membered polyol macrolide from the dinoflagellate Symbiodinium sp. is a representative of super carbon chain molecules along with palytoxin and maitotoxin. At nanomolar concentrations, symbiodinolide has been shown to induce significant increase in [Ca^{2+}] in human neuroblastoma cells. The complete configurational assignment of symbiodinolide is ongoing, and the subject of intense stereochemical efforts by the Uemura group.

The oxazole containing polyketide macrolide theonezolide A was isolated by Kobayashi in 1995 from the marine sponge Theonella swinhoei. Although ozonolysis of 1.223 successfully yielded a number of degradation products, the products contain multiple isolated stereogenic portions that are not readily assignable and are the subject of ongoing investigations.

Marine sponges have been a significant source of polycyclic amine alkaloids. The relative configurations of these compounds are often easily addressed by NMR (NOESY experiments), however the absolute configuration of these molecules remain a challenge. Upenamide (1.226) reported by
Scheuer is one member of these alkaloids and the configuration of the secondary hydroxyl is readily assigned by the MMM, but the relative configuration between the two rings is a significant challenge. Madangamine (1.227) is another polycyclic alkaloid that does not contain functional groups for chemical conversion, and awaits complete configurational assignment.

1.9 Conclusions

Natural products (NPs) are a significant and important source for new small-molecule drug leads. Stereocomplexity often distinguishes natural products from synthetic medicinal drugs. The foregoing examples illustrate the power of integrated structure analysis by spectroscopic techniques and chemical methods augmented by rational asymmetric synthesis.
1.10 References


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2435–2440.


CHAPTER 2

MUTAFURANS A–G, ENE–YNE TETRAHYDROFURAN FATTY ACIDS
FROM THE MARINE SPONGE XESTOSPONGIA MUTA

2.1 Introduction

The secondary metabolites from sponges of the genus Xestospongia, family Petrosidae are of interest because of a well-documented association of bacterial flora that coexist within various species of this sponge, and these microorganisms may be responsible for production of the natural products that have been isolated. Nucleic acid analysis showed that as much as 56% of rRNA in Xestospongia sp. from Western Australia is constituted by eubacterial 16S rRNA.¹ Sponges containing a high population of microorganisms have been termed ‘bacteriosponges’ of ‘high–microbial–abundance’ (HMA) sponges.² Hentschel and coworkers have reported Xestospongia muta (figure 2.1) from the Caribbean as a HMA sponge and holds a bacterial population density of 8 x 10⁸ microorganisms per gram of wet wt.³ Evaluation of sponge–microbial metabolite processes by genetic techniques have shown that a large number of genes found in various sponges belong to an unusual type of small polyketide synthase (PKS) termed “sponge symbiont ubiquitous pks” (sup), present in a cosmopolitan baceteria of the candidate phylum “Poribacteria”.⁴ Recently, Piel and coworkers have suggested that “Poribacteria” associated with HMA sponges including Xestospongia muta are responsible for the production of methyl-branched fatty acids.⁵
Alkylated sterols and brominated polyunsaturated fatty acids (BPUFAs) are hallmark metabolites of *X. muta*, the common “barrel” sponge found throughout the Caribbean, and *X. testudinaria* which is found in the Indo-Pacific. BPUFAs have been characterized largely as their methyl esters. The first example, 2.1, was described by Schmitz and coworkers. from *X. muta* in 1978, and subsequently, the dehydro-free fatty acid (2.2) and triene 2.3 were characterized by the Scheuer group (Figure 2.2).

![Image of two sponges, one labeled *Xestospongia muta* and the other *Agelas sp.*, with text below.](image_url)

**Figure 2.1.** Two HMA sponges, *Xestospongia muta* (back) and *Agelas sp.* (front) in the Bahamas. (photograph taken by Joe Pawlik, University of North Carolina, Wilmington)

Subsequent reports by us and other investigators described additional ene-yne and polyene-yne fatty acids of common carbon chain lengths C₁₆ to C₂₀ containing complex combinations of brominated 1,3-dienes and ene-ynes,
diynes, \( \omega \)-brominated acetylenes, and even \( \text{C}_{14} \) \( \omega,\omega \)-dibromovinylidenes, some of which are represented in Figure 2.2. Here, we describe our recent investigations into antifungal compounds from \textit{Xestospongia muta} that uncovered a new family of chiral antifungal brominated ene-yne 2,5-disubstituted tetrahydrofurans which we have named mutafurans A–G (2.7–2.13, figure 2.3). Compounds 2.1, 2.1, and 2.7–2.10 were found to be active against \textit{Cryptococcus neoformans}, an opportunistic fungus commonly linked to the pathologies of HIV patients.

![Diagram of Mutafurans A-G](image)

**Figure 2.2.** BPUFAs isolated from sponges of the Genus \textit{Xestopongia}.

### 2.2 Isolation and Planar Structure for Mutafurans A-G

Four collections of the sponge \textit{Xestospongia muta} from different sites in the Bahamas in 2004 were immediately frozen and kept at \(-20\, ^\circ\text{C}\) until extraction. The CH\(_2\)Cl\(_2\)–soluble fraction from a MeOH–CH\(_2\)Cl\(_2\) (2:1) extract of
one specimen (04-15-042) was treated with TMS–diazomethane, and the products purified of the mixture by filtration through silica followed by reversed-phase HPLC to give methyl esters of the known carboxylic acids 2.1 along with 2.2, a new optically active compound mutafuran A (3, Figure 1), $[\alpha]_{D}^{23} = -19.4 (c 0.248, \text{MeOH}),$ and related mutafurans B–G (2.7–2.13).

![Diagram of mutafuran structures](image)

**Figure 2.3.** Structures for mutafurans A–G (2.7–2.13)

The molecular formula of 2.7 was established as C$_{21}$H$_{29}$O$_3$Br by HREIMS ($m/z$ 408.1262, M$^+$, $\Delta$ –3.2 mmu). Six of the seven double bond equivalents (DBEs) in the structure of 2.7 could be accounted for by three C=C double bonds, one COOMe group ($\delta$ 173.2, s), and a CC triple bond from interpretation of the $^1$H and $^{13}$C NMR (C$_6$D$_6$), DEPT gHSQC, gHMBC, and gCOSY experiments (Table 2.1). A strong UV band ($\lambda_{\text{max}}$ 238 nm, log $\varepsilon$ = 4.56) suggested conjugation; interpretation of the COSY data assigned the chromophore to a terminal Z,Z-20-bromo-17,19-diene ($\delta$ 5.37, dt, $J = 9.9, 7.7$ Hz, H17; 6.46, t, $J = 10.6$ Hz, H18; 6.51, dd, $J = 10.6, 6.6$ Hz, H19; 5.87, d, $J = 6.2$ Hz, H20) and an ene-yne: interpretation of $^{13}$C NMR data revealed a triple
bond (δ 89.6, s, C9; 83.2, s, C10) conjugated to the remaining E-double bond (δ 5.52, d, J = 15.8 Hz, H11; 6.06, dt, J = 15.8, 7 Hz, H12). Two oxymethine signals were observed, CH–O (δ 3.99, quint, J = 6.2 Hz, H5) and a low-field propargylic ether CH signal (δ 4.78, t, J = 5.5 Hz, H8), which were mutually correlated through an HMBC cross-peak (H8 to C5).

**Table 2.1. NMR data for mutafuran A (2.7)**

<table>
<thead>
<tr>
<th>no.</th>
<th>δ_C</th>
<th>δ_H (mult., J in Hz.)</th>
<th>HMBC</th>
<th>COSY</th>
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</thead>
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<tr>
<td>2</td>
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* 400 MHz in benzene-d₆.  
** 100 MHz, multiplicities assigned from DEPT and HSQC.  
*** 600 MHz, optimized for J = 8 Hz, correlations from proton(s) stated to the indicated carbon.

Additional HMBC cross-peaks were observed from H8 to diastereotropic CH₂ groups at C6 and C7 (Table 2.1) and the sp hybridized carbons C9 and
C10 and the sp² carbon C11. Because the FTIR spectrum of 2.7 lacked OH stretching bands, the remaining oxygen was placed within a tetrahydrofuran ring, which also accounted for the seventh DBE. HMBC correlations from H5 to C3 and H2 to C3 and C4 supported the 2,5-disubstituted tetrahydrofuran ring located four carbons removed from the COOMe terminus (Table 2.1). Placement of the remaining CH₂ groups in a chain between C12 and C17 completed the structure of 2.7.

Mutafurans B (2.8), E (2.11), and F (2.12) are isomeric with 2.7 (HRMS) and are shown by COSY analysis to be double bond isomers at the 20-bromodiene terminus (Table 2.2). Compounds 2.9 and 2.10 are the 19-E- and 19-Z-dihydro derivatives of 2.7 corresponding to reduction of the penultimate double bond C17–C18 of 2.7. Mutafuran G (2.13) was isolated in very small amounts (60 μg), sufficient only for HRMS and ¹H NMR. The HRESI-TOFMS mass spectrum of 2.13 showed an isotope pattern consistent with the presence of two Br atoms and provided a formula of C₂₁H₂₉O₃Br₂ (m/z 489.0616 [M + H]⁺, Δ -1.8 mmu) which required substitution of an H atom for Br in the formula of 2.9 or 2.10. The ¹H NMR spectrum showed the signals of H2–H12 found in 2.9 and replacement of the vinyl proton signals with a single olefinic triplet (δ 6.04, t, J = 7.6 Hz, H19). The chemical shift of this signal was identical with that of a terminal ω,ω-dibromovinylidene group in a synthetic long-chain analogue,¹⁰ consequently the structure 2.13 was assigned to mutafuran G as shown in figure 2.3.
Table 2.2. Selected \(^1\)H NMR data for mutafurans B-G (2.8-2.13).

<table>
<thead>
<tr>
<th>no.</th>
<th>2.8</th>
<th>2.9</th>
<th>2.10</th>
<th>2.11</th>
<th>2.12</th>
<th>2.13</th>
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<tbody>
<tr>
<td>17</td>
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<td>0.95 (m)</td>
<td>1.10 (m)</td>
<td>5.25 (dt, 16.2, 6.0)</td>
<td>5.15 (dt, 10.8, 8.4)</td>
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<td>18</td>
<td>6.50 (ddd, 15.2, 7.6, 1.6)</td>
<td>1.57 (qd, 7.2, 1.6)</td>
<td>2.05 (qd, 7.0, 1.6)</td>
<td>5.60 (dd, 16.2, 10.4)</td>
<td>5.60 (t, 10.8)</td>
<td>1.73 (q, 7.4)</td>
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<tr>
<td>19</td>
<td>6.23 (10.4, 7.6)</td>
<td>5.96 (dt, 13.6, 7.2)</td>
<td>5.65 (q, 7.0)</td>
<td>6.95 (ddd, 13.6, 10.8, 0.8)</td>
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<td>20</td>
<td>5.71 (d, 7.6)</td>
<td>5.70 (dt, 13.6, 1.6)</td>
<td>5.86 (dt, 7.0, 1.6)</td>
<td>5.91 (d, 13.6)</td>
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</tbody>
</table>

\(^{a}\) Acquired at 400 MHz in Benzene-\(d_6\)

2.3 Configurational Assignment of the Mutafurans

The relative configuration of the tetrahydrofuran ring in 2.7 and 2.9 were assigned by 1D NOESY (Figure 2.4). Selective spin inversion of the H8 signal gave a weak transannular NOE to the methylene proton signal at C4 but not H5. This strongly suggested a trans-disubstituted tetrahydrofuran ring. Confirmation of the assignment was secured by comparison of 2.7 with model compounds of defined configuration prepared through synthesis (vide infra).

Figure 2.4. (a) 1D nOe by irradiation at H8 in mutafuran A (2.7) and C (2.9). Two possible configurational assignments for mutafurans, (a) (5R, 8S) and (b) (5S, 8R).
Figure 2.5 CD spectra of (a) 2.7, (b) 2.10, (c) 2.9, (d) (−)-2.23, (e) (−)-2.24.

The CD spectra of 2.7 (Figure 2.5) and congeners 2.9 and 2.10 were similar; as each displayed a negative Cotton effect (CE) with weak double minima at short wavelengths and a maximum at higher wavelengths (e.g., 3: \( \lambda_{\text{max}} \) 227 (\( \Delta \varepsilon \) −0.46), 237 (\( \Delta \varepsilon \) −0.5), 252 (\( \Delta \varepsilon \) +0.26)). The absolute configuration of 2.7 was secured from analysis of the CE associated with the ene-yne tetrahydrofuran moiety as follows. Although compound 2.7 contains two conjugated systems, only the 9-ene-11-yne, and not the terminal diene, is expected to be active in its CD spectrum because of its proximity to the chiral element. No empirical sector rules have been established for determinations of
this system. In principle, qualitative MO methods may apply,\textsuperscript{11} or the CD spectrum of 2.7 could be calculated from density functional theory (DFT) methods; however, difficulties were anticipated from these approaches due to the low barrier of rotation about the C8–C9 bond.

The C8–C9 bond links the axially symmetric triple bond to the tetrahydrofuran ring (first sphere of asymmetry); however, the former is essentially a “free rotor” with a very small barrier to rotation ($\Delta E < 0.5$ kcal mol$^{-1}$, MM2). Free rotation is expected to average contributions to the CD from all rotamers and give a small but nonzero CE at room temperature; however, accurate calculation of the CD spectrum (e.g., DFT) would be made difficult by uncertainties in predicting accurate Boltzmann distributions of C8–C9 rotamers.

To avoid errors associated with calculating CD spectra for ene-yne tetrahydrofurans, we chose to make an empirical comparison with suitable model compounds. The synthesis of optically pure standards, 2.14 and 2.15 model compounds. The synthesis of optically pure standards, 2.14 and 2.15 for chiroptical comparison to the natural products was carried out as shown in Scheme 2.1. Preparation of model ene-yne tetrahydrofurans started from known lactone 2.18 derived from L–glutamic acid.\textsuperscript{12} Addition of TMS-acetylide to 2.18 in the presence of Et$_2$AlCl$_2$ and BF$_3$Et$_2$O gave a 4:1 mixture of diastereomers 2.19 and 2.20, which were separable by silica chromatography.\textsuperscript{13} The relative configurations of 2.19 and 2.20 were
confirmed as \textit{trans} and \textit{cis}, respectively based on NOESY data. Each acetylene \textbf{2.19} and \textbf{2.20} was subjected to deprotection to \textbf{2.21} and \textbf{2.22}, then Sonagashira coupling to (\textit{E})-iododecane to give \textbf{2.14} and \textbf{2.15}.\textsuperscript{14}

\begin{center}
\textbf{Scheme 2.1.} Synthesis of model ene-yne tetrahydrofurans (\textit{\textendash})\textbf{2.14} and (\textit{\textendash})\textbf{2.15}.
\end{center}

The ene-yne CES (but not $[\alpha]_D$) appear to be generally correlated with the configuration of the propargylic carbon and relatively independent of the substituents at C5 as shown by (\textit{\textendash})\textbf{2.14} and (\textit{\textendash})\textbf{2.15} (Figure 2.5). Although \textbf{2.14} and \textbf{2.15} were both levorotatory, the CD spectra of (5S,8S)-(\textit{\textendash})\textbf{2.14} and (5S,8R)-(\textit{\textendash})\textbf{2.15} were almost equal and opposite with the CE, dictated solely by the configuration of the propargylic carbon in the tetrahydrofuran ring. The similar magnitudes of CES in (\textit{\textendash})\textbf{2.14}, \textbf{2.7}, \textbf{2.9}, and \textbf{2.10} also suggest that the natural products are optically pure, at least within the experimental error of CD measurement (estimated as $\pm20\%$). This would suggest that \textbf{2.7}–\textbf{2.13} are likely the products of biosynthetic enzyme-mediated transformations rather than artifacts that could arise from autoxidation and spontaneous intramolecular cyclization. The biosynthesis of BPUFAs, particularly the origin of the $\omega$-brominated terminus, is presently unknown.
2.4 Chiroptical analysis of ene–yne and diyne alcohols.

During the course of our chiroptical investigations of the ene-yne-tetrahydrofuran moiety of the mutafurans, we were encouraged that the Cotton effects used to assign the C5 configuration of 2.7 could also be used to assign the configuration of ene-yne or diyne alcohols. Because only weak Cotton effects were observed for mutafurans and model compounds 2.23 and 2.24, additional optically enriched model compounds were synthesized.

\[
\text{Scheme 2.2. Synthesis of ene-yne alcohol (}2.23\text{), acetate (}2.26\text{), and naphthoate (}2.28\text{).}
\]

A simple ene-yne alcohol 2.23 was synthesized from known acetylenic alcohol 2.24\textsuperscript{15} as shown in Scheme 2.2. Desilylation of (S)-2.24, followed by protection as the acetate ester (2.25) and Sonogashira coupling with (E)-iododecene in the presence of bis(triphenylphosphine)palladium (II) dichloride and Cul to give ene-yne acetate ester 2.26. Treatment of 2.26 with ammonia gave the ene-yne alcohol 2.23. Conversion of 2.23 to the corresponding naphthoate (2.27) was carried out to provide an additional ene-yne derivative for analysis.
CD spectra for the three ene-yne derivatives 2.23, 2.26, and 2.28 showed Cotton effects of varying intensity and sign (Figure 2.6). Alcohol 2.23 showed three weak Cotton effects at λ_{max} 222 (Δε +0.50), 235 (Δε +0.77), 244 (Δε +0.56)) similar in magnitude to the ene-yne tetrahydrofurans. Acetate 2.26 showed a medium Cotton effect of opposite sign at λ_{max} 228 (Δε −3.0). The naphthoyl derivative 2.28 showed a bisignate Cotton effect spectrum that arises from exciton coupling between the ene-yne chromophore and the naphthoate chromophore. The Cotton effects observed for ene-yne alcohols,
acetate, and naphthoate derivatives can be used for configurational assignment. The latter derivatives would be preferred because of the significantly larger extinction coefficient for the charge transfer band 'L_2'. These methods will be applicable to synthetic compounds and natural products of unknown configuration such as 2.28 and 2.29 isolated from the seeds of *Acanthosyris spinescens*.\(^{16}\)

Noting that a large split Cotton effect was observed for ene-yne naphthoate 2.28, we decided to synthesize a diyne naphthoate (2.30) to evaluate applicability of diyne–naphthoate ECCD for configurational assignment of sponge metabolites such as diplyne C (2.31) (Scheme 2.3).\(^{17}\) Alcohol (S)-2.24 was acylated to give naphthoate ester 2.32, which was desilylated (TBAF, 86%) to propargylic O-napthoate 2.33 and transformed into the diyne naphthoate (S)-2.30 (CuCl, NH_2OH-HCl, n-BuNH_2, 1-bromoheptyne).

![Scheme 2.3. Synthesis of diyne naphthoate 2.30 and structure of diplyne A (2.31).](image_url)
Figure 2.7. CD spectra for (a) 2.33 in CH$_3$CN and 2.30 in (b) CH$_3$CN, (c) hexane, and (d) MeOH at 23°C.

The CD spectra of (S)-2.30 (CH$_3$CN) also showed strong bisignate CEs [$\lambda$ 225 ($\Delta\varepsilon$ -24.6), 242 ($\Delta\varepsilon$ +35.3)] (Figure 2.7). Because the $\pi\rightarrow\pi^*$ transition of the conjugated diyne occurs at lower wavelengths $\lambda$ 214 nm ($\varepsilon$ 37,300), the short-wavelength component of the exciton couplet of (S)-2.30 [$\lambda$ 188 nm ($\Delta\varepsilon$ -37.8)] is at the edge of instrument detection limits; however, in (S)-2.27 this high energy component reveals itself readily [$\lambda$ 225 nm ($\Delta\varepsilon$ -24.6)] due to the presence of a red-shifted extended en-ynene chromophore. Nevertheless, as noted by Nakanishi and co-workers$^{18}$ only the sign of the long-wavelength component is necessary and sufficient to assign allylic benzoates; this also
applies for propargylic naphthoates.\textsuperscript{19} Due to the stronger oscillator strength of the $^1B_b$ transition, even acyclic nonconjugated propargylic O-naphthoates should be amenable to chiroptical analysis, unlike the corresponding O-benzoates that show only weak or nondetectable ECCD from the shorter wavelength acetylene chromophore ($\lambda \lesssim 190$ nm).\textsuperscript{19} This is demonstrated in the CD spectrum of \textbf{2.33} (Figure 2.7), which displays a weaker yet still prominent positive component [$\lambda = 240$ nm ($\Delta \varepsilon = 4.6$)] of the biphasic Cotton effect.

\textbf{2.5 Antifungal Activity of the Mutafurans}

\begin{table}[h]
\centering
\begin{tabular}{lcccccccc}
\hline
\textbf{Compound} & \textbf{C. albicans ATCC 14503} & \textbf{C. albicans 96-489}\textsuperscript{b} & \textbf{C. albicans UCD-FR1}\textsuperscript{b} & \textbf{C.\textit{ glabrata}}\textsuperscript{b} & \textbf{C.\textit{ krusei}} & \textbf{C.\textit{ grubii}} \\
\hline
\textbf{2.1 Me ester} & 32 & 32 & 64 & 64 & 32 & 8 \\
\textbf{2.2 Me ester} & 32 & 32 & 64 & 64 & 32 & 16 \\
\textbf{mutafuran A (2.7)} & 32 & 32 & NT & 32 & NT & 8 \\
\textbf{mutafuran B (2.8)} & 16 & 32 & 64 & 64 & 16 & 8 \\
\textbf{mutafuran C (2.9)} & 16 & 32 & 64 & 64 & 16 & 4 \\
\textbf{mutafuran D (2.10)} & 16 & 32 & 64 & 64 & 16 & 8 \\
\hline
\end{tabular}
\caption{Antifungal activity (MIC\textsubscript{50}, \textmu{g}/mL) for BPUFAs from \textit{X. muta}.\textsuperscript{a}}
\end{table}

\textsuperscript{a} The \textit{in Vitro} susceptibility of each compound was determined by the broth micro dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS).\textsuperscript{20} \textsuperscript{b} Fluconazole-resistant (MIC $> 64$ \textmu{g}/mL). Performed by Dr. Doralyn S. Dalisay.

Compounds \textbf{2.1}, \textbf{2.2}, and \textbf{2.7–2.10} showed moderate antifungal activity against the pathogenic fungus \textit{Cryptococcus neoformans} var. \textit{grubii} (Table 2.3). The MICs for compounds \textbf{2.1}, \textbf{2.2}, and \textbf{2.7–2.10} were 8, 16, 8, 8, 8, and
4 \mu g/mL, respectively. Compounds 2.1, 2.2, and 2.7–2.10 showed no or weak activity against *Candida albicans* ATCC14503 and the fluconazole-resistant strains *Candida albicans* 96-489 and *Candida glabrata*.

### 2.6 Conclusions

We have characterized a new family of chiral oxygenated BPUFAs named mutafurans A–G (2.7–2.13). Variability in the BPUFAs isolated from *X. muta* is worth mentioning. In our investigations of *X. muta* from the Bahamas we have consistently observed 2.1 as the major BPUFA and its coexistence with the mutafurans in the crude extracts of *X. muta* from little San Salvador, San Salvador, and Plana Cay islands in the Bahamas. Patil and coworkers reported several C_{16} and C_{18} BPUFAs but neither 2.1 nor the mutafurans were detected in samples of *X. muta* collected at Columbus Island, Bahamas. Finally, we have obtained samples of *X. muta* from Conch Wall, Florida Keys and again measured 2.1 as the major BPUFA, consistent with the Schmitz report of an *X. muta* sample from Summerland Key, Florida. In addition, mutafurans were not detected in samples from Key Largo.

The mutafurans have recently been used as ‘markers’ contained in healthy sponge tissue in the investigation of the ‘sponge-orange-band’ (SOB) disease currently infecting *X. muta* sponges in the Caribbean. The origin of the mutafurans is unknown at this time, but there is little presence of secondary metabolite production in sponges affected by SOB.
Our chiroptical studies of the ene-yne tetrahydrofuran moiety has validated the circular dichroic method for ene-yne THFs, ene-yne and di-yne alcohols and their naphthoate derivizatives. We have observed very large ECCD spectra for O-propargylic naphthoate derivatives of ene-yne alcohols and di-yne alcohols that make this method ideal for microscale analysis of similar natural products. This method has recently been successfully used in combination with another ECCD method for the configurational assignment of the recently reported faulknerynes A-C.22

Chapter 2 is, in part, a reproduction of the material as it appears in the following publication: Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. “Ene-yne Tetrahydrofurans from the Sponge Xestospongia muta Exploiting a Weak CD Effect for Assignment of Configuration” Org. Lett. 2007, 9, 1975-1978. The dissertation author was the primary researcher/author on this paper.

2.7 Experimental Section

General Experimental Procedures. NMR spectra were acquired using a 400 MHz Varian Mercury or a 600 MHz Bruker Avance spectrometer equipped with a cryoprobe. 1H and 13C NMR spectra were referenced to δ 7.24 and 77.0 ppm, (CDCl3) or δ 7.16 and 128.39 ppm (benzene-d6). HREI spectra were acquired on a MAT900XL (ThermoFinnigan) FAB double focusing mass spectrometer. IR spectra were obtained on a Thermo Nicolet Avatar 360 IR spectrometer. UV spectra were recorded on a Hewlett Packard 8452A Diode Array Spectrometer. Optical rotations were measured using a Jasco P-1010
polarimeter. Circular dichroism (CD) spectra were measured on an Aviv 215 or a Jasco 810 spectropolarimeter. Dry solvents (CH$_2$Cl$_2$, Et$_2$O, THF) were prepared by passage through commercial activated alumina cartridges under an inert atmosphere. Semi-preparative HPLC was carried out using dual Dynamax SD-200 pumps, a Dynamax UV-1 UV detector operating at 250 nm, and a Phenomenex Luna phenyl-hexyl column (5μ particle, 10x250mm). Analytical HPLC was carried out using dual Rainin HPXL pumps, a Hewlett Packard 1040M Series II PDA detector operating at 272 and 225 nm, and an Alltech Econosil Si column (4.6mm x250mm).

**Extraction and Isolation.** Collections of *Xestospongia muta* were made at depths from −10 to −20 m (0418051, lat 24° 03.029’ N. long 074° 32.191’ W; 0415043, lat 22° 36.756’ N. long. 73° 39.497’ W; 0415042 lat 22° 36.756’ N. long. 73° 39.497’ W; 0409029, lat 24° 35.160’. long 075° 58.477’) were made in the Bahamas in July, 2004 and kept frozen at −20 °C until extracted. The frozen specimen (0415042, wet wt.= 214.2g) was extracted at room temperature with MeOH-CH$_2$Cl$_2$ (2:1) overnight (2x). The MeOH-CH$_2$Cl$_2$ solution was evaporated and partitioned between 20% H$_2$O in MeOH and hexanes. The hexane (‘A’) layer was removed and the aqueous MeOH adjusted to 60% H$_2$O (v/v) in MeOH and partitioned against CH$_2$Cl$_2$. The CH$_2$Cl$_2$ layer was removed and the solvent removed to give a brown oil (‘B’, 1.18 g). The resulting aqueous layer (H$_2$O) was partitioned against n-BuOH to give the fourth partition (C). A portion (723.3 mg) of ‘B’ was dissolved in 8 mL
of MeOH:ether (1:7) and treated with TMS diazomethane (1.5 mL, 2.0 M in diethyl ether) and stirred at room temperature for 30 min. The solvent was removed under reduced pressure and the mixture subjected to chromatography (SiO₂) using a stepwise gradient from 100% hexanes to 100% ethyl acetate and finally flushed with 100% MeOH to furnish seven fractions. A portion (10.7 mg) of the second fraction was subject reverse phase HPLC (9:91 H₂O-MeOH) to yield compound 2.1 (2.0 mg) and compound 2.2 (3.7 mg, c.f. the corresponding free acid) with spectroscopic properties (¹H NMR, ¹³C NMR, and UV) in agreement with literature values.⁷ ⁸

The third fraction from SPE was subject to reversed phase (Phenylhexyl) HPLC (14:86 H₂O-MeOH) to yield eight fractions. Fraction 8 contained mutafuran C (2.9, 1.5 mg). Fractions 2, 3, and 5 were individually separated by RP HPLC (14:86 H₂O-MeOH) to yield mutafurans-B (2.8, 0.9 mg), -A (2.7, 1.6 mg), and –D (2.10, 1.4 mg). Fractions 6 and 7 were subjected to another round of HPLC (14:86 H₂O-MeOH, followed by 3:1:1 MeOH: i-PrOH: H₂O) to yield mutafurans E (2.11, 0.14 mg) and –F (2.12, 0.12 mg). Fraction 4 from SPE was purified by RP HPLC (16:4:1 MeOH/i-PrOH:H₂O), followed by 28:72 H₂O-CH₃CN) to provide mutafuran-G (2.13, ~0.06 mg).

**Mutafuran A (2.7):** colorless oil; [α]₂⁰ ³²⁻19.4 (c 0.248, MeOH); IR (KBr) νmax 2926, 2855, 1736, 1327, 1297, 1247, 1180, 1075 cm⁻¹ UV (MeOH) λmax 238 nm (log ε 4.56); HREIMS m/z 408.1262 [M]+ (calcd for C₂₁H₂₉O₃Br, 408.1295); ¹H NMR, ¹³C NMR; See Table 2.1. CD; see Fig. 2.5
**Mutafuran B (2.8):** colorless oil; [α]$_D^{23}$ –57.5 (c 0.168, hex) UV (hexane) $\lambda_{\text{max}}$ 229 nm (log $\varepsilon$ 3.64) HRFABMS $m/z$ 409.1365 [M+H]$^+$ (calcd for C$_{21}$H$_{30}$O$_3$Br, 409.1373); $^1$H NMR see Table 2.2.

**Mutafuran C (2.9):** colorless oil; [α]$_D^{23}$ –12.9 (c 0.224, MeOH); IR (KBr) $\nu_{\text{max}}$ 2926, 2854, 1736, 1436, 1180, 1141, 1075 cm$^{-1}$; UV (hexane) $\lambda_{\text{max}}$ 229 nm (log $\varepsilon$ 4.11); HRFABMS $m/z$ 411.1539 [M+H]$^+$ (calcd for C$_{21}$H$_{32}$O$_3$Br, 411.1529); $^1$H NMR see Table 2.2; $^{13}$C NMR (100 MHz, C$_6$D$_6$): $\delta$ 173.6 (C1), 34.3 (C2), 22.4 (C3), 35.5 (C4), 78.9 (C5), 31.8 (C6), 34.2 (C7), 69.0 (C8), 89.9 (C9), 83.7 (C10), 110.5 (C11), 144.9 (C12), 33.5 (C13), 28.9-29.2 (C14-C17), 33.3 (C18), 138.6 (C19), 104.9 (C20), 51.2 (OMe); CD: see Figure 2.5.

**Mutafuran D (2.10):** colorless oil; [α]$_D^{23}$ –18.7 (c 1.07, MeOH); IR (KBr) $\nu_{\text{max}}$ 2926, 2855, 1737, 1436, 1248, 1194, 1172, 1053, 1020 cm$^{-1}$; UV (hex) $\lambda_{\text{max}}$ 229 nm (log $\varepsilon$ 4.02); HRFABMS $m/z$ 411.1520 [M+H]$^+$ (calcd for C$_{21}$H$_{32}$O$_3$Br, 411.1529); For $^1$H NMR see Table 2.2. CD: see Figure 2.5.

**Mutafuran (2.11):** colorless oil; HREIMS $m/z$ 408.1294 [M]$^+$ (calcd for C$_{21}$H$_{29}$O$_3$Br, 408.1295); For $^1$H NMR see Table 2.2.

**Mutafuran F (2.12):** colorless oil; HREIMS $m/z$ 408.1294 [M]$^+$ (calcd for C$_{21}$H$_{29}$O$_3$Br, 408.1295); For $^1$H NMR see Table 2.2.

**Mutafuran G (2.13):** colorless oil; HRESI-TOFMS $m/z$ 489.0616 [M+H]$^+$ (calcd for C$_{21}$H$_{30}$O$_3$Br$_2$, 489.0634); $^1$H NMR see Table 2.2.

**Synthesis of ene-yne tetrahydrofuran model compounds:**

**Ene-yne tetrahydrofuran (2.14).** 1-Iodododecene (123 mg, 0.42 mmol) in 5
mL of triethylamine was added to Pd(PPh₃)₂Cl₂ (20 mg, 28 µmol) and Cul (16 mg, 0.09 mmol). The mixture was stirred at room temp. for 30 min. The alkyne 2.21 (67 mg, 0.28 mmol) in 2.5 mL of triethylamine was then added dropwise. The resulting mixture was stirred for another 30 min. The triethylamine was removed under reduced pressure and the resulting mixture was purified by chromatography (SiO₂ SPE cartridge, 2% ether in hexane) followed by normal phase HPLC (1.5% ether in hexane) to obtain 39.8 mg (35%) of compound 2.14. [α]D²⁴ −22.6 (c 0.53, hexane); IR (KBr) νmax 2954, 2926, 2855, 1462, 1253, 1180, 1139, 1074, 1005, 954, 837, 777; UV (hexane) λmax 229 nm (log ε 4.20); ¹H NMR (400 MHz, C₆D₆) δ 0.05 (s, 2 x CH₃, TBS), 0.93 (t, J = 7.2 Hz, H19), 0.96 (s, 3 x CH₃, TBS), 1.11-1.32 (m, H11-H18), 1.58-1.67 (m, H3), 1.84 (qd, J = 7.2, 1.6 Hz, H10), 1.87-1.96 (m, H3', H4), 3.54 (ddd, J = 15.2,10.8, 4.4 Hz, H1), 4.19-4.24 (m, H2), 4.86-4.89 (m, H5), 5.55 (dq, J = 16.0, 1.6 Hz, H8), 6.14 (dt, J = 16.0, 6.8 Hz, H9); ¹³C NMR (100 MHz, C₆D₆) δ -4.8 (2 x CH₃, TBS), 14.7 (CH₃, C19), 18.9 (C, TBS), 23.5 (CH₂, C18), 26.5 (3 x CH₃, TBS), 28.0 (CH₂, C3), 29.4-32.7 (CH₂, C11-C17), 33.6 (CH₂, C10), 34.2 (CH₂, C4), 66.1 (CH₂, C1), 69.8 (CH, C5), 79.8 (CH, C2), 83.9 (C, C7), 89.4 (C, C6), 110.4 (CH, C8), 145.2 (CH, C9); HRFABMS m/z 406.3269 [M]⁺ (calcd for C₂₅H₄₅O₂Si, 406.3262)

**Ene-ylene tetrohydrofuran (2.15).** Compound 2.22 (15 mg, 0.063 mmol) was subjected to the same conditions described above, except the reaction time after addition of the alkyne was 1 hr. The crude product was purified by
chromatography (SiO₂, SPE cartridge, 2% ether in hexane), and subjected to normal phase HPLC (2% ether in hexane) to obtain 8.2 mg (32%) of compound 2.15. [α]D 24° = 10.4 (c 0.56, hexane); IR (KBr) νmax 2954, 2926, 2855, 2223, 1784, 1463, 1388, 1254, 1180, 1129, 1090, 1006, 955, 837, 778 cm⁻¹; UV (hexane) λmax 229 nm (log ε 4.03) ¹H NMR (400 MHz, C₆D₆) δ 0.12 (s, CH₃, TBS), 0.13 (s, CH₃, TBS), 0.93 (t, J = 7.6 Hz, H19), 0.10 (s, 3 x CH₃, TBS), 1.58-1.65 (m, H3), 1.72-1.81 (m, H3’ and H4), 1.84 (q, J = 6.8 Hz, H10), 1.89-1.96 (m, H4’), 3.65 (dd, J = 10.4, 5.6 Hz, H1), 3.77 (dd, J = 10.0, 4.8 Hz, H1’), 3.96 (p, J = 6.4 Hz, H2), 4.65 (t, J = 6.0 Hz, H5), 5.55 (dd, J = 16.0, 1.6 Hz, H8), 6.15 (dt, J = 15.6, 7.6 Hz, H9); ¹³C NMR (100 MHz, C₆D₆) δ -4.7 (2 x CH₃, TBS), 14.7 (CH₃, C19), 19.0 (C, TBS), 23.5 (CH₂, C18), 26.5 (3 x CH₃, TBS), 28.7 (CH₂, C3), 29.4-32.7 (CH₂, C11-C17), 33.6 (CH₂, C4), 34.0 (CH₂, C10), 67.0 (CH₂, C1), 69.7 (CH, C5), 80.8 (CH, C2), 83.9 (C, C7), 89.4 (C, C6), 110.4 (CH, C8), 145.2 (CH, C9); HRFABMS m/z 406.3259 [M]⁺ (calcd for C₂₅H₄₅O₂Si, 406.3262)

**TMS-Alkynes (2.19 and 2.20).** TMS acetylene (62 μL, 0.44 mmol) was dissolved in 1.4 mL of ether. The mixture was cooled to 0 °C, and n-BuLi (163 μL, 0.41 mmol) was added dropwise. The solution was stirred at 0 °C for 30 min. Et₂AlCl (1.8 M in tol, 0.42 mmol) was added dropwise. The mixture was stirred for 35 min then cooled to -78 °C. BF₃•Et₂O (0.38 mmol) was added slowly, and the mixture was stirred for 15 min. A solution of acetoxyfuran 2.18 (prepared from L-glutamic acid by literature methods)¹² in CH₂Cl₂ was added,
and solution was allowed to warm to 0 °C and stirred for an additional 14 hrs. The reaction was quenched with 1.4 mL sat NaHCO₃. The mixture was then extracted with CH₂Cl₂ (3x). The combined organic layers were dried with Na₂SO₄, washed with brine, and evaporated to give a crude oil which was purified by chromatograph (SiO₂ SPE, 2% ether in hexane) to give trans-2.19 (31.5 mg) cis-2.20 (3.9 mg). The relative configurations of the tetrahydrofuran rings were assigned from NOE measurements. trans-2.19: [α]D²⁴ –23.8 (c 0.5, hexane); IR (KBr) νmax 2956, 2929, 2857, 2171, 1472, 1251, 1074, 841, 777, 760 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.05 (s, 9H), 0.14 (s, 6H), 0.86 (s, 9H), 1.75-1.84 (m, 1H), 1.90-1.98 (m, 1H), 2.02-2.11 (m, 1H), 2.13-2.21 (m, 1H), 3.59 (ddd, J = 6.4, 4.4, 2.0 Hz, 2H), 4.13-4.19 (m, 1H), 4.63 (t, J = 6.8, 1H); ¹³C NMR (100 MHz, CDCl₃) δ -5.4 (2 x CH₃), -0.1 (3 x CH₃), 18.3 (C), 25.9 (3 x CH₃), 27.3 (CH₂), 33.5 (CH₂), 65.3 (CH₂), 69.0 (CH), 79.3 (CH), 88.8 (C), 105.5 (C); HRFABMS m/z 311.1846 [M]+ (calcd for C₁₆H₃₁O₂Si₂, 311.1857).
cis-2.20: [α]D²⁴ – 8.0 (c 0.5, hexane); IR (KBr) νmax 2956, 2929, 2857, 1251, 1134, 1084, 842, 777 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.05 (s, 9H), 0.13 (s, 6H), 0.87 (s, 9H), ), 1.82-1.90 (m, 1H), 1.92-2.02 (m, 2H), 2.05-2.11 (m, 1H), 3.58 (dd, J = 10.0, 5.6 Hz, 1H), 3.72 (dd, J = 10.8, 5.2 Hz, 1H), 3.98 (p, J = 6 Hz, 1H), 4.53 (t, J = 6 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ -5.3 (2 x CH₃), -0.2 (3 x CH₃), 18.4 (C), 26.0 (3 x CH₃), 28.1 (CH₂), 33.2 (CH₂), 66.1 (CH₂), 68.9 (CH), 80.4 (CH), 88.9 (C), 105.8 (C); HRFABMS m/z 311.1862 [M]+ (calcd for C₁₆H₃₁O₂Si₂, 311.1857).
**Alkyne (2.21).** * trans-Alkyne 2.19* (39 mg, 0.12 mmol) was dissolved in 0.9 mL of MeOH. K$_2$CO$_3$ (70 mg, 0.51 mmol) was added and the mixture was stirred for 2 hrs. The mixture was poured into 5 mL water and extracted with CH$_2$Cl$_2$ (3 x 5mL). The organic layers were combined washed with brine (10 mL), dried with Na$_2$SO$_4$, and the solvent evaporated to give the deprotected alkyne 2.21 as a slightly yellow oil (30.1 mg, 96%) of: [α]$_D$ $^{24}$ –20.5 (c 0.61, hexane); IR (KBr) $\nu_{\text{max}}$ 3312, 2954, 2929, 2857, 1471, 1463, 1253, 1115, 1075, 1005, 837, 777 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.02 (s, 6H), 0.86 (s, 9H), 1.77-1.85 (m, 1H), 1.93-2.00 (m, 1H), 2.04-2.12 (m, 1H), 2.14-2.22 (m, 1H), 2.40 (d, J = 2 Hz, 1H), 3.60 (d, J = 4.4 Hz, 2H), 4.14-4.20 (m, 1H), 4.63-4.67 (m, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ -5.4 (2 x CH$_3$), 18.3 (C), 25.9 (3 x CH$_3$), 27.1 (CH$_2$), 33.3 (CH$_2$), 65.2 (CH$_2$), 68.3 (CH), 79.4 (CH), 83.9 (CH); HRFABMS m/z 240.1540 [M]$^+$ (calcd for C$_{13}$H$_{24}$O$_2$Si, 240.1540).

**Alkyne (2.22).** Compound 2.20 (30 mg, 0.096 mmol) was subject to the same conditions described above to yield 2.22 (18 mg, 76%); [α]$_D$ $^{24}$ –23.8 (c 0.58, hexane); IR (KBr) $\nu_{\text{max}}$ 3357, 2955, 2924, 2853, 2360, 1659, 1632, 1467, 1249, 1098, 838 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.04 (s, 3H), 0.5 (s, 3H), 0.87, (s, 9H), 1.84-1.93 (m, 1H), 1.93-2.04 (m, 2H), 2.08-2.17 (m, 1H), 2.39 (d, J = 1.6 Hz, 1H), 3.58 (dd, J = 10.0, 6.0 Hz, 1H), 3.72 (dd, J = 10.4, 5.2 Hz), 3.99 (p, J = 6.2 Hz), 4.55 (ddd, J = 7.2, 5.6, 2.0 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ -5.3 (2 x CH$_3$), 18.4 (C), 25.9 (3 x CH$_3$), 28.1 (CH$_2$), 33.2 (CH$_2$), 65.9 (CH$_2$), 68.3 (CH), 72.5 (CH), 80.6 (CH), 84.1 (C); HREIMS m/z 239.1465 [M-
\[ \text{H}^- \text{ (calcd for C}_{13}\text{H}_{25}\text{O}_2\text{Si, 239.1462).} \]

**Synthesis of ene-yne alcohol models:**

**Ene-yn-ol (2.23).** A solution of 2.26 in MeOH (100 \( \mu \)L) was treated with anhydrous \( \text{NH}_3 \) (2M in MeOH, 300 \( \mu \)L), stirred overnight, and then concentrated under a stream of \( \text{N}_2 \). The crude mixture was separated by chromatography (silica cartridge, 95:5 hexanes/EtOAc) to yield alcohol 2.23 as an oil (4.8 mg, 91%). \( [\alpha]_D^{24} \) +5.3 (c 0.02, CHCl\(_3\)); \( ^1\text{H} \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 0.86 (t, \( J = 6.8 \) Hz, 3H), 1.02−1.38 (m, 21H), 1.49−1.86 (m, 7H), 2.07 (dq, \( J = 1.2, 7.2 \) Hz, 5H), 4.22 (m, 1H), 5.47 (dq, \( J = 15.6, 1.6 \) Hz, 1H), 6.13 (dt, \( J = 15.6, 7.2 \) Hz, 1H); \( ^{13}\text{C} \) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 14.1 (CH\(_3\)), 22.7 (CH\(_3\)), 25.9 (CH\(_2\)), 26.3 (CH\(_2\)), 28.2 (CH\(_2\)), 28.5 (CH\(_2\)), 28.6, 29.1 (CH\(_2\)), 29.3 (CH\(_2\)), 29.4 (CH\(_2\)), 29.5 (CH\(_2\)), 29.6 (CH\(_2\)), 29.7 (CH\(_2\)), 31.9 (CH\(_2\)), 33.1 (CH\(_2\)), 44.3 (CH), 67.7 (CH), 84.5 (C), 87.5 (C), 108.8 (CH), 145.4 (CH). The configuration of the carbinol center in 14 was confirmed by the modified Mosher’s method.\(^{23}\) **(R)-MTPA ester of compound 14:** Compound 14 (0.8 mg, 2.6 \( \mu \)mol) was dissolved CH\(_2\)Cl\(_2\)−pyridine (1:1 200 \( \mu \)L). (S)-MTPA-Cl (5 \( \mu \)L, 0.27 mmol) was added and the mixture stirred for 1.5 h. The volatiles were removed and the crude product separated by chromatography (silica, 95:5 hexanes−EtOAc) to yield the (R)-MTPA ester of compound 2.23 (1.3 mg, 95%). \( ^1\text{H} \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 0.86 (t, \( J = 6.8 \) Hz, 3H), 1.05−1.36 (m, 21H), 1.63−1.74 (m, 5H), 1.81 (brd, \( J = 12.4 \) Hz, 1H), 2.08 (qd, \( J = 6.8, 1.2 \) Hz, 2H), 3.54 (s, 3H), 5.42 (d, \( J = 5.6 \) Hz, 1H), 5.43 (dq, \( J = 15.6, 1.6 \) Hz, 1H), 6.12 (dt, \( J = 15.6, 6.8 \) Hz, 1H).
Hz, 1H). **(S)-MTPA ester of compound 14:** The same procedure was applied to compound 14 (1.0 mg, 3.3 µmol) with (R)-MTPA-Cl (5 µL, 0.27 mmol) to yield the corresponding (S)-MTPA ester (1.4 mg, 99%) yield. 1H NMR (400 MHz, CDCl₃) δ 0.86 (t, J = 6.8 Hz, 3H), 0.99–1.38 (m, 21H), 1.60–1.71 (m, 5H), 1.71 (m, 1H), 2.09 (qd, J = 7.2, 1.6 Hz, 2H), 3.57 (d, J = 1.2 Hz, 3H), 5.42 (d, J = 5.6 Hz, 1H), 5.46 (dq, J = 16.0, 1.6 Hz, 1H), 6.15 (dt, J = 16.0, 6.8 Hz, 1H).

**(S)-1-Cyclohexylprop-2-ynyl Acetate (2.25).** Compound (S)-2.24 (52.4 mg, 0.21 mmol) was dissolved in THF (2 mL), and TBAF (0.25 mL, 1 M in THF) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 30 min, and the solvent was removed under reduced pressure to give a crude product that was immediately subject to acetylation (Ac₂O, 30 µL) in anhydrous pyridine (500 µL) at 25 °C for 48 h. The volatiles were removed and the mixture separated (silica cartridge, 3:7 hexane–CH₂Cl₂) to provide (−)-2.25 (14.1 mg, 38%). [α]_{D}^{24} = −66.6 (c 0.011, CHCl₃) [lit. [α]_{D}^{24} = −65.5 (c 1.5, CHCl₃)]. ¹⁵ MS and NMR data were identical with literature values.

**Ene-yne acetate (2.26).** A mixture of 1-iododecane (43 mg, 0.12 mmol), (Ph₃P)₂PdCl₂ (5.5 mg, 7.8 µmol), Cul (6.0 mg, 0.032 mmol) and Et₃N (2.5 mL) were stirred at rt for 30 min. This solution was then added dropwise to a solution of alkyne 2.25 in Et₃N (1 mL) and the mixture reaction stirred at rt for 1.5 h. The volatiles were removed and the residue separated by flash chromatography (silica cartridge, 3:97 EtOAc/hexanes) to yield acetoxy en-yne
2.26 as an oil (21.5 mg, 79%). $[\alpha]_D^{24}$ -88.0 (c 0.004, CHCl$_3$); IR (KBr) $\nu_{max}$ 2925, 2854, 1743, 1451, 1369, 1229, 1017, 977, 955 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.86 (t, $J = 7.2$ Hz, 3H), 1.04–1.36 (m, 21H), 1.61–1.84 (m, 6H), 2.06 (s, 3H), 2.06 (qd, $J = 7.2$, 1.6 Hz, 5H), 5.30 (dd, $J = 6.4$, 2.0 Hz, 1H), 5.45 (dq, $J = 15.6$, 2.0 Hz, 1H), 6.15 (dt, $J = 15.6$, 7.2 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1 (CH$_3$), 21.1 (CH$_3$), 22.7 (CH$_2$), 25.7 (CH$_2$), 25.8 (CH$_2$), 26.2 (CH$_2$), 28.1 (CH$_2$), 28.6 (CH$_2$), 29.1 (CH$_2$), 29.3 (CH$_2$), 29.4 (CH$_2$), 29.5 (CH$_2$), 29.6 (CH$_2$), 31.9 (CH$_2$), 33.1 (CH$_2$), 42.0 (CH), 68.9 (CH), 74.0 (C), 83.9 (C), 84.7 (C), 108.6 (CH), 146.0 (CH), 170.2 (C). HREIMS m/z 346.2869 [M]$^+$ (calcd for C$_{23}$H$_{38}$O$_2$: 346.2866).

**Naphthoate Ester (2.27).** A solution of enyn-ol 2.23 (2 mg, 6.57 $\mu$mol) in CH$_2$Cl$_2$ (200 $\mu$L) was treated with 2-naphthoic acid (2.8 mg, 16.4 $\mu$mol), EDC (3.0 mg, 19.7 $\mu$mol), and a small crystal of DMAP. The resulting mixture was stirred for 48 h, concentrated, and separated by flash chromatography (SiO$_2$) to afford naphthoate ester (S)-2.27 (2.4 mg, 82%). $[\alpha]_D^{24}$ +20.2 (c 0.94, CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.61 (bs, 1H), 8.07 (dd, 8.4, 1.2 Hz, 1H), 7.95 (d, 8.0 Hz, 1H), 7.86 (d, 8.4 Hz, 2H), 7.55 (m, 2H), 6.17 (dt, 16.0, 7.2 Hz, 1H), 5.62 (dd, 5.6, 1.2 Hz, 1H), 5.49 (dq, 16.0, 0.8 Hz, 1H), 2.07 (qd, 6.8, 1.6 Hz, 2H), 1.66–1.98 (m, 4H), 1.22 (m, 20H); HREIMS m/z 458.3176 [M]$^+$ (calcd for C$_{32}$H$_{42}$O$_2$: 458.3179).

**Diyne naphthoate (2.30).** A mixture of 2.33 (7.8 mg, 27 $\mu$mol), CuCl (0.5 mg, 5.3 $\mu$mol), and NH$_2$OH·HCl (2.8 mg, 40 $\mu$mol) was suspended in MeOH (150
μL) at 0 °C under N₂ and treated dropwise with neat n-butylamine (250 μL). The mixture was stirred for 10 min and treated dropwise with a solution of 1-bromoheptyne (4.6 mg, 26.7 μmol) in MeOH (100 μL). The mixture was stirred at 0 °C for 1 h and then poured into ice-water (2 mL) before acidification with 5% H₂SO₄ and extraction with ether (3 × 4 mL). The combined ether extracts were washed with brine, dried with anhydrous MgSO₄, and separated by flash chromatography (silica cartridge, 8:1, hexanes/diethyl ether) to give diyne (S)-2.30 as an oil (8.2 mg, 80%). [α]دوا²³ +58.8 (c 1.24, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.60 (bs, 1H), 8.05 (dd, J = 8.4, 1.2 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.55 (m, 2H), 5.55 (d, J = 6 Hz, 1H), 2.25 (t, J = 6.8 Hz, 2H), 1.67–1.99 (m, 6H), 1.50 (p, J = 7.6 Hz, 2H), 1.17–1.37 (m, 4H), 0.87 (t, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 165.6 (C), 135.6 (C), 132.4 (C), 131.3 (CH), 129.4 (CH), 128.4 (CH), 128.2 (CH), 127.8 (CH), 127.1 (C), 126.7 (CH), 125.3 (CH), 81.8 (C), 72.0 (C), 71.2 (C), 69.2 (CH), 64.5 (C), 42.2 (CH), 31.0 (CH₂), 28.6 (CH₂), 28.3 (CH₂), 27.8 (CH₂), 26.1 (CH₂), 25.8 (CH₂), 25.7 (CH₂), 22.1 (CH₂), 19.2 (CH₂), 13.9 (CH₃). HREIMS m/z 386.2238 [M]+ (calcd for C₃₇H₅₀O₂, 386.2240).

**Triethyilsilyl alkynyl ester (2.32).** Alkynol (S)-2.24 (10.4 mg, 0.041 mmol) was converted into naphthoate ester 2.32 (13.5 mg, 82%) using the procedure described above. [α]دوا²₄ +12.6 (c 1.93, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.61 (bs, 1H), 8.07 (dd, J = 8.4, 1.2 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.55 (m, 2H), 5.56 (d, J = 6.0 Hz, 1H), 1.67–1.99 (m, 6H),
1.13–1.32 (m, 4H), 0.98 (t, J = 8.0 Hz, 9H), 0.59 (q, J = 8.0 Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.7 (C), 135.5 (C), 132.4(C), 131.2 (CH), 129.4 (CH), 128.3 (CH), 128.1 (CH), 127.7 (CH), 127.4 (C), 126.6 (CH), 125.4 (CH), 103.0 (C), 88.6 (C), 69.3 (CH), 42.1 (CH), 28.7 (CH$_2$), 28.1 (CH$_2$), 26.3 (CH$_2$), 25.8 (CH$_2$), 25.7 (CH$_2$), 7.8 (CH$_3$), 4.2 (CH$_2$); HREIMS $m/z$ 406.2330 [M]$^+$ (calcd for C$_{26}$H$_{34}$O$_2$Si, 406.2323).

**Alkynyl ester (S)-(2.33).** Naphthoate ester 2.32 (13.5 mg, 0.033 mmol) was dissolved in THF (1 mL), and the solution was cooled to 0 °C. TBAF (1 M in THF, 33.2 µL) was added dropwise, and the mixture was stirred for 10 min before removal of the volatiles. The residue was purified by flash chromatography (SiO$_2$, 9:1 hexanes/ether) to afford essentially pure propargyl O-naphthoate 2.33 (8.1 mg, 86%). [α]$_D^{23}$ $-$12.8 (c 1.19, CHCl$_3$); FTIR (KBr) $\nu_{\max}$ 3298, 2929, 2853, 1719, 1281, 1225, 1195, 1129, 1088, 973, 777, 761 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 8.61 (bs, 1H), 8.07 (dd, J = 8.4, 1.2 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.55 (m, 2H), 5.51 (dd, J = 6.0, 2.4 Hz, 1H), 2.48 (d, J = 2.4 Hz, 1H), 1.68–2.00 (m, 6H), 1.18–1.32 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.7 (C), 135.6 (C), 132.4 (C), 131.3 (CH), 129.4 (CH), 128.4 (CH), 128.2 (CH), 127.8 (CH), 127.1 (C), 126.7 (CH), 125.3 (CH), 80.3 (C), 74.3 (CH), 68.6 (CH), 41.8 (CH), 28.5 (CH$_2$), 28.2 (CH$_2$), 26.2 (CH$_2$), 25.8 (CH$_2$), 25.7 (CH$_2$).
2.8 References


(10) Cf. 1,1-dibromonon-1-ene (\(^1\)H NMR, benzene-\(d_6\), \(\delta\) 6.06, t, \(J = 7.6\) Hz, H8), prepared from octanal (CBr\(_4\), PPh\(_3\), CH\(_2\)Cl\(_2\)).


Figure 2.8. $^1$H NMR spectrum of mutafuran A (2.7) (400 MHz, benzene–$d_6$).
Figure 2.9. $^{13}$C NMR spectrum of mutafuran A (2.7) (100 MHz, benzene–$d_6$).
Figure 2.10. gHSQC spectrum of mutuarian A (2.7) (600 MHz, benzene-d$_6$).
Figure 2.11. gHMBC spectrum mutafuran A (2.7) (600 MHz, benzene-\( d_6 \), \( ^{2.3}J = 8 \) Hz).
Figure 2.12. COSY spectrum of mutafuran A (2.7) (400 MHz, benzene–d$_6$).
Figure 2.13. $^1$H NMR spectrum of mutafuran B (2.8) (400 MHz, benzene–$d_6$).
Figure 2.14. $^1$H NMR spectrum of mutafuran C (2.9) (400 MHz, benzene–$d_6$).
Figure 2.15. $^{13}$C NMR spectrum of mutafuran C (2.9) (100 MHz, benzene–$d_6$).
Figure 2.16. gHSQC spectrum of mutafuran C (2.9) (600 MHz, benzene–$d_6$).
Figure 2.17. gHMBC spectrum of mutafuran C (2.9) (600 MHz, benzene–$d_6$). $J_{HC} = 8$ Hz.
Figure 2.18. gCOSY spectrum of mutafuran C (2.9) (600 MHz, benzene–d₆).
Figure 2.19. $^1$H NMR spectrum of mutafuran D (2.10) (400 MHz, benzene–$d_6$).
Figure 2.20. $^1$H NMR spectrum of mutafuran E (2.11) (400 MHz, benzene–d$_6$).
Figure 2.21. H NMR spectrum of mulgaluran F (2.12) (400 MHz, benzene–d$_6$).
Figure 2.22. $^1$H NMR spectrum of mutafuran G (2.13) (400 MHz, benzene–$d_6$).
Figure 2.23. $^1$H NMR spectrum of compound 2.20 (400 MHz, CDCl$_3$).
Figure 2.24. $^{13}$C NMR spectrum of compound 2.20 (100 MHz, CDCl$_3$).
Figure 2.25. $^1$H NMR spectrum of compound 2.19 (400 MHz, CDCl$_3$).
Figure 2.26. $^{13}$C NMR spectrum of compound 2.19 (100 MHz, CDCl$_3$).
**Figure 2.27.** $^1$H NMR spectrum of compound 2.22 (400 MHz, CDCl$_3$).
Figure 2.28. $^{13}$C NMR spectrum of compound 2.22 (100 MHz, CDCl$_3$).
Figure 2.29. $^1$H NMR spectrum of compound 2.21 (400 MHz, CDCl$_3$).
Figure 2.30. $^{13}$C NMR spectrum of compound 2.21 (100 MHz, CDCl$_3$).
Figure 2.31. $^1$H NMR spectrum of compound 2.15 (400 MHz, benzene–$d_6$).
Figure 2.32. $^{13}$C NMR spectrum of compound 2.15 (100 MHz, benzene–$d_6$).
Figure 2.33. $^1$H NMR spectrum of compound 2.14 (400 MHz, benzene–d$_6$).
**Figure 2.34.** $^{13}$C NMR spectrum of compound 2.14 (100 MHz, benzene–$d_6$).
Figure 2.35. $^1$H NMR spectrum of 2.27 (400 MHz, CDCl$_3$).
Figure 2.36. $^1$H NMR spectrum of 2.30 (400 MHz, CDCl$_3$).
Figure 2.37. $^{13}$C NMR spectrum of 2.30 (100 MHz, CDCl$_3$).
Figure 2.38. $^1$H NMR spectrum of 2.26 (400 MHz, CDCl$_3$).
Figure 2.39. $^{13}$C NMR spectrum of 2.26 (100 MHz, CDCl$_3$).
Figure 2.40. $^1$H NMR spectrum of 2.23 (400 MHz, CDCl$_3$).
Figure 2.41. $^{13}$C NMR spectrum of 2.23 (100 MHz, CDCl$_3$).
Figure 2.42. $^1$H NMR spectrum of 2.32 (400 MHz, CDCl$_3$).
Figure 2.43. $^{13}$C NMR spectrum of 2.32 (100 MHz, CDCl$_3$).
CHAPTER 3

AMARANZOLES AND AMAROXOCANES, SULFATED STEROLS THAT CHEMICALLY DEFEND THE SPONGE PHORBAS AMARANTHUS

3.1 Caribbean Marine Sponges are Chemically Defended from Predatory Reef Fishes

Many Caribbean marine sponges are chemically defended against reef predators by secondary metabolites. Sponges are the dominant soft-bodied benthic invertebrates on Caribbean coral reefs and play important roles in carbon and nutrient cycling that shape the reef community. In 1995, Pawlik and Fenical conducted a systematic survey of Caribbean sponge chemical

![Chemical Structures]

**Figure 3.1.** Previously identified feeding deterrent compounds from Caribbean marine sponges.
defenses. Of 71 crude extracts obtained from different sponge species, 45 (69%) deterred feeding by the bluehead wrasse, *Thalassoma bifasciatum*, a common omnivore on Caribbean reefs.¹

In selected follow-up studies, the secondary metabolites responsible for feeding deterrence were identified, including oroidin (3.1)² and stevensine (3.2)³ (2-amino imidazole alkaloids) from *Agelas clathroides* and *Axinella corrugata*, respectively, the formosides (3.3)⁴ (triterpene glycosides) from *Erylus formosus*, and amphitoxin (3.4)⁵ (a polymeric pyridinium alkaloid) from *Amphimedon compressa* (Figure 3.1).

![Image](image_url)

**Figure 3.2.** *Phorbas amaranthus* in the Florida Keys (Photograph by Joe Pawlik).

*Phorbas amaranthus* Duchassaing & Michelotti 1864 a velvet–textured sponge that is common in parts of the Florida Keys with less common distribution in the Bahamas (Figure 3.2). Crude extracts from *P. amaranthus* displayed strong feeding deterrent activity, which led us to investigate the
chemical consituents of this sponge. Previous investigations led to the isolation of nonpolar steroids from *P. amaranthus*: from hexane extracts, ring-A contracted nonpolar steroids phorbasterones A-D (3.7-3.12, Figure 3.3)\(^6\) along with the known anthosterones A (3.5) and B (3.6).\(^7\) The nonpolar steroids were not responsible for deterrence in fish feeding assays, and attempts to isolate the active components from the first two collections of *P. amaranthus* were unsuccessful.

![Figure 3.3. Anthosterones A (3.5) and B (3.6), and Phorbasterones A-D (3.7-3.12).](image)

**3.2 Second Attempt to Isolate Feeding Deterrent Compounds and Isolation of amaranzole A.**

Following initial attempts to isolate feeding deterrent metabolites from a 1995 collection (95-021) of *P. amaranthus*, and the report of phorbasterones A–D from non-polar fractions of a 2002 collection (02-13-054) of *P. amaranthus*, attention was focused on the more polar fractions. The aqueous MeOH extract was subjected to a series of fractionation steps (including HP-20, HW-40, and LH-20), in which all active fractions were combined before the
next purification step (Figure 3.4). The first fraction derived from the LH-20 fractionation was active, however HPLC analysis showed the fraction to contain >20 compounds. Insufficient sample remained for purification and retesting, therefore a different fraction containing a metabolite that may partially account for the deterrent activity, was pursued guided by NMR and previous structural similarities to a compound previously partially characterized from the 1995 collection.⁸

**Figure 3.4.** Isolation tree for 2002 collection of *P. amaranthus* (02-13-054). (HP-20, HW-40, and LH-20 fractionation steps carried out by Dr. Makoto Masuno, Assays carried out by Prof. Joe Pawlik and Jonathon Cowart).
The partial structure determination carried out by T. Hong\textsuperscript{8} showed a sulfated steroidal skeleton containing a para–di–substituted benzene ring. The identification of sulfate moieties was extremely important for choosing a neutral buffered purification strategy that reduced unfavorable interactions with underivatized silica and prevented cleavage of these groups. The fourth fraction from the LH-20 column was subjected to reversed-phase HPLC (MeOH/H\textsubscript{2}O + 0.1 M NaClO\textsubscript{4}) to give amaranzole A (3.13).

3.3 Structure Determination of Amaranzole A (3.13).

The structure of Amaranzole A represents a new chemotype, a steroid bearing a C24-N-imidazolyl group that appears to derive from a confluence of isoprenoid biosynthesis and aromatic amino acid metabolism (cf. polymastiamide 3.14)(Figure 3.5).\textsuperscript{9} The structure of 3.13 was deduced by interpretation of spectroscopic data, including a unique exciton-coupled circular dichroism (ECCD) spectrum associated with an N–imidazolyl allylic system, that informed us of the C24 configuration.

The \textsuperscript{1}H NMR spectrum of 3.13 showed signals typical of a sterol (e.g., high-field singlets at $\delta$ 0.59, s; 1.11, s due to angular Me groups), and also aryl proton signals (see below). The negative ion HR MALDI MS spectrum ($m/z$ 859.2177, $M - \text{Na}^+$) gave a molecular formula of C$_{36}$H$_{48}$N$_2$Na$_3$O$_{13}$S$_3$, while ESIMS showed fragment ions from characteristic losses of SO$_3$ and Na$^+$ (see Experimental Section). The presence of sulfate esters was confirmed by FTIR which showed a strong band at $\nu$ 1235 cm$^{-1}$. 
Figure 3.5. Structure of amaranzole A (3.13) and polymastiamide A (3.14).

Interpretation of the $^1$H NMR, COSY, NOESY, HSQC, and HMBC spectra revealed oxymethines at C2 ($\delta$ 4.90, m; 76.8, d), C3 ($\delta$ 4.27, dt, $J = 12.0, 4.2$ Hz; 78.9, d), and C6 ($\delta$ 4.21, td, $J = 11.4, 4.8$ Hz; 78.1, d) of a 5α,10β-cholest-25-ene carbon skeleton (Table 3.1). The $^{13}$C chemical shifts of C2, C3, and C6 were shifted downfield by 7-9 ppm compared to those of a corresponding steroidal triol model, which was consistent with placement of O-sulfate groups at these positions. Differences in the side chain of 3.13 compared to the saturated side chain of cholesterol were revealed by $^1$H NMR and COSY. Geminal proton signals on an sp$^2$ carbon ($\delta$ 4.70, br s; 4.99, br s) and an olefinic methyl group ($\delta$ 1.72, br s) were assigned to a terminal isopropylidene group. Vicinal coupling constant data ($J$) as well as cross-peaks from NOESY (Figure 3.6) allowed the relative stereochemistry of the sterol nucleus to be assigned as depicted.
Table 3.1. NMR Data for amaranzole A (3.13).

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<tr>
<th>No.</th>
<th>D_1^a</th>
<th>D_3^a</th>
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<td>34</td>
<td>159.5</td>
<td></td>
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<td></td>
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</tbody>
</table>

^a 100 MHz. multiplicities from gHSQC. ^b 600 MHz. ^c Peaks may be interchanged. 
^d Assigned by gHSQC. ^e Assigned by gHMBC (J_2= 8 Hz).
Figure 3.6. NOESY correlations for amaranzole A.

The remaining elements included two aromatic groups, not typically found in steroids, and were assigned as follows. Distinctive downfield aromatic $^1$H signals included an AA'BB' pattern corresponding to a para-substituted phenol ($\delta$ 6.87, d, $J = 8.4$ Hz, 2H; $\delta$ 7.17, d, $J = 8.4$ Hz, 2H) and two broad singlets ($\delta$ 7.20, br s; 8.02, br s) that sharpened upon addition of CF$_3$COOD to the NMR sample. The phenol was confirmed by observation of pH-dependent UV bands (Table 3.2). The UV spectrum of 3.13 showed two reversible pH dependent changes: a red shift ($\Delta \lambda = +8$ nm) at pH 10, characteristic of a phenoxide ion, and a blue shift in the presence of acid pH 2 ($\Delta \lambda = -13$ nm) that appeared to be associated with a nitrogenous heterocycle. The above data accounted for all the atoms in the formula except the balance of C$_3$N$_2$H$_2$.

Table 3.2. UV data for pH dependence of amaranzole A (3.13) and 3.15.

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_{\text{max}}$/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.13</td>
</tr>
<tr>
<td>MeOH</td>
<td>247</td>
</tr>
<tr>
<td>+ 3M HCl (pH~2)</td>
<td>234</td>
</tr>
<tr>
<td>+ 3M NaOH (pH~10)</td>
<td>255</td>
</tr>
</tbody>
</table>
The remaining three degrees of unsaturation required that the latter elements be assembled into a heterocycle, either a disubstituted pyrazole or imidazole. Careful examination of the HMBC data for 3.13 revealed no correlations between the broad H28 and H29 singlets of the putative heterocycle and the sterol side chain of 3.13, nor were any significant NOEs observed. Nevertheless, a disubstituted imidazole structure was favored due to the presence of the low-field $^1$H NMR signals (δ 8.02 s), consistent with H2 of imidazole rather than H3 or H5 of a 1,4-disubstituted pyrazole.\(^\text{11}\)

In order to verify both the nature of the heterocycle and its substitution pattern, we synthesized 1,5- and 1,4-disubstituted imidazoles, 3.15 and 3.16, respectively (Scheme 3.1). The imine obtained by condensation of $p$-hydroxybenzaldehyde with iso-propylamine was treated with $p$-toluenesulfonyl methylisocyanide (TosMIC) under Schöllkopf conditions\(^\text{12}\) to obtain the 1,5-disubstituted imidazole 3.15 after thermal elimination of $p$-toluenesulfinic acid in fair yield.

![Scheme 3.1. Synthesis of 1,5- and 1,4-disubstituted imidazoles.](image-url)
Alternatively, reversal of the order of the reactions, starting this time with \( p \)-anisaldehyde, gave the complementary 1,4–disubstituted isomer.\(^{13}\) Addition of isocyanomethyl-\( p \)-toluenesulfonate to \( p \)-anisaldehyde in the presence of NaCN gave oxazoline \( 3.17 \), which was immediately heated with iso-propylamine in xylene at reflux to generate \( 3.18 \). Demethylation of \( 3.18 \) gave 1,4- disubstituted imidazole \( 3.16 \).

\[\text{Figure 3.7. Comparison of } ^1\text{H NMR (400 MHz, CD}_3\text{OD + 0.1\% TFA-d) for amaranzole A (3.13) with 1,5- and 1,4-disubsstituted models, 3.15 and 3.16, respectively.}\]

Comparison of the \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR spectra of the natural product and models revealed a close similarity of amaranzole A (3.13) with 3.15 (Figure 3.7), but not 3.16. Compound 3.15 also exhibited the same pH-dependent UV spectra observed in 3.13 (Table 3.2). Having assigned the constitution of the substituted imidazole, we turned our attention to the absolute configuration of 3.13. While there can be little doubt of the 5\( \alpha \),10\( \beta \) configuration of the steroid
nucleus from biosynthetic precedents, the configuration of the remote allylic C24 stereocenter of the side chain posed a problem. We expected that the CD spectrum of 3.13 would be influenced solely by asymmetric perturbation of the p-hydroxyphenyl imidazole that, in turn, is dictated by the angular disposition of the two aromatic rings with respect to C24 and the C25-C26 double bond.

On the other hand, if the barrier to rotation about the C24-N bond were low, Cotton effects would be averaged out by rapid rotation and interpretation of the CD spectrum would be equivocal.

\[
\text{Scheme 3.2. Synthesis of hydroxy-phenyl imidazolyl model (+)-3.19.}
\]

To avoid these complications, a CD comparison of 3.13 with an optically active synthetic model compound, \((R)-(+)-3.19\), was undertaken. \((R)-(+)-3.19\) was synthesized as shown in Scheme 3.2 starting with commercially available \((R)-(-)-2\text{-amino-1-butanol, which was transformed through aldehyde 3.20, alcohol 3.21, and ketone 3.22 to the N-Boc-protected allylamine 3.23.}
Conversion of \(3.23\) to (+)-3.19 was completed under Schöllkopf-type conditions as before (Scheme 3.2).

The CD spectra (MeOH, Figure 3.8) of 3.13 and (+)-3.19 showed Cotton effects of the same sign and similar magnitudes. Thus, the C24 configuration of 3.13 is \(R\). The strong Cotton effects at \(\sim200\) nm in 3.13 and (+)-3.19 appeared to be solely associated with exciton coupling between the isolated terminal olefinic bond (\(\lambda < 200\) nm) and the imidazole ring, but not the weakly conjugated \(\rho\)-hydroxyphenyl group (\(\lambda = 250\) nm). Because the end absorption of MeOH (~205 nm) interfered with observation of Cotton effects at lower wavelength, the CD spectra of 3.13 and (+)-3.19 were acquired in CH\(_3\)CN/H\(_2\)O (3:7). Both compounds clearly showed the anticipated ECCD spectra; compound 3.19 displayed a weak positive CE at \(\lambda\) 256 nm (\(\Delta\varepsilon < +0.2\)), a negative CE at 218 nm (\(\Delta\varepsilon = -0.9\)) and stronger positive effect (\(\lambda\) 201 nm, \(\Delta\varepsilon = +8.4\)) followed by a strong negative CE (\(\lambda\) 189 nm, \(\Delta\varepsilon = -6.4\)). Identical CEs were seen in amaranzole A (3.19), except the intensity of the CE at \(\lambda\) 201 nm was decreased (\(\Delta\varepsilon = +4.5\)). Consequently, the (24\(R\))-configuration is assigned to amaranzole A. Additional evidence for exciton coupling was obtained by hydrogenation of (+)-3.19 (H\(_2\), Pd/C, Scheme 3.2). Saturation of the terminal double bond in 3.19 eliminated the more intense band at 215 nm, present in (+)-3.19 and (−)-3.24, but retained the weaker long wavelength band (\(\lambda\) 250 nm), albeit with inverted sign.
Figure 3.8. CD spectra for amaranzole A, (+)-3.19, and (−)-3.24 in MeOH at 23°C (a, b, c) and CH₃CN/H₂O (3:7) (d, e, f).

Analysis of the origin of the ECCD in (+)-3.19, and (−)-3.24 is made difficult by conformational mobility of the N1-side chain on the imidazole ring, uncertainties of the conformer populations about several rotors, including the C3–N, C2–C3, and C3–C4 bonds, and direction of the transition dipole polarization. However, early studies of allylic alcohol and amine derivatives provide some guidance. Nakanishi and coworkers demonstrated that the major conformation of benzoates esters of secondary allylic alcohols is well-defined and consistent for all members; the lowest energy conformation is that
in which the C=C bond eclipses the allylic methine H$_a$. The transition dipole moment of the benzoate chromophore is oriented roughly along the C–O bond and subtends a negative helicity in (R)-benzoates of allylic secondary alcohols that gives rise to a negative ECCD. In (S)-enantiomers, the sign of the ECCD is reversed. Skowronek and Gawronski showed that the phthalimides derived from simple (R)-allylic amines also give rise to a negative ECCD that arises from essentially the similar conformation (Fig. 3.9). Similar trends were observed for naphthimidines of allylic amines. In contrast, the sign of the ECCD in (+)-3.19 is reversed; (R)-allylic imidazoles 3.13 and 3.19 show a positive ECCD effect. The reversal of sign implies either the transition dipole moment vector of the imidazole has changed direction with respect to the $\pi$-$\pi^*$ transition of the C=C double bond, or the dominant conformation of the molecules are different, or both.

The conformation of (+)-3.19 was defined by nOe measurements and calculations. Irradiation of the vinyl methyl group in (+)-3.19 gave rise to a significant nOe at H3, but no nOe was observed between the vinyl proton signals and H3, suggesting the major conformation is that depicted in Figure 3.9. NOe was also observed from H3 to the ortho protons (H1’) on the phenyl ring of 3.19, consistent with a conformation in which the H3 methine is syn to the phenyl ring (Fig. 3.9). The lowest energy conformation of 3.19 was also calculated using molecular mechanics (Spartan, PM3), and found to be entirely consistent with the nOe results, although, other low-lying rotamers
may contribute to the global conformer distribution in 3.13 and (+)-3.19.

**Figure 3.9.** Dominant conformation of (3R)-N-allyc heterocycles (a) (R)-phthalimide showing directions of \( \pi-\pi^* \) electronic transition dipole moments and helicity of chromophores (after Skowronek and Gawronski\textsuperscript{15}) (b) (R)-N-allyl imidazole (+)-3.19 and nOe

To further characterize the ECCD of allylic imidazoles, we carried out measurements of pH-dependent CD spectrum of (+)-3.19 (Fig. 3.10). At neutral or alkaline pH, the CD spectrum of (+)-3.19 (30% CF\(_3\)CH\(_2\)OH: aqueous phosphate buffer) were essentially the same, however, lowering the pH lead to a progressive diminution of the positive CE at \( \lambda \sim 200 \text{ nm} \) until, at pH 5.3, the sign of the long-wavelength CE was inverted (\( \Delta \varepsilon \sim -8 \)). Within the limits of experimental error, it appeared that all CD spectra passed through an isobestic point at \( \lambda \sim 210 \text{ nm} \) suggesting the pH-dependency of the CD spectrum was associated with reversible protonation of the imidazole ring. Grebow and Hooker showed, using Hückel MO calculations, that the orientation of the transition dipole moment of the neutral form of imidazole and protonated imidazole are significantly different.\textsuperscript{17} A corresponding change in transition dipole orientation in (+)-3.19 upon protonation of the imidazole nitrogen may be responsible for the observed pH-dependent CE spectra.
factors, such as small changes in the Boltzman-weighted populations of conformations upon protonation of the imidazole N, cannot be excluded although this seems unlikely, and the former explanation would seem to to be upheld.

Figure 3.10. pH-dependent CD spectra for compound 3.19 (30% CF₃CH₂OH in H₂O, 20 mM Na₂HPO₄/NaH₂PO₄), T = 23°C.
3.4 Identification of Feeding Deterrent Chemotype: Isolation of Amaroxocanes A and B and Amaranzoles B-F.

**Phorbas amaranthus**
Key Largo, Florida (06-04-04b)
1005 mL eq
131.2g dry wt.

- Extract, Partition (DCM/MeOH aq.)
  - 06PA2D
    - 980 mL eq
    - 3.28g
  - 06PA2M
    - 980 mL eq
    - 48.6g
  - C18 (stepwise 9:1 H2O/MeOH, 1:9 H2O/MeOH, iPrOH)
  - 06PA2M,1
    - 680 mL eq
    - 28.5g
  - 06PA2M,2
    - 680 mL eq
    - 5.83g
  - 06PA2M,3
    - 680 mL eq
    - 1.38g
  - C18 Prep HPLC (73:27 to 23:77 H2O/CH3CN + 1.5 M NaClO3)

- RCM12
  - 135 mL eq
  - 68.3mg
  - deterrent at 16x natural concentration
- RCM3
  - 135 mL eq
  - 61.6mg
- RCM4
  - 135 mL eq
  - 23.8mg
  - deterrent at 4x natural concentration
- RCM5
  - 135 mL eq
  - 126.5mg
- RCM6
  - 135 mL eq
  - 403.3mg
  - deterrent at 2x natural concentration
- RCM7
  - 135 mL eq
  - 187.0mg
- RCM8
  - 135 mL eq
  - 52.2mg

**Step 4**

- RP HPLC
  - AmA (3.13)
  - AmC (3.28)
  - AmD (3.29)
- RP HPLC
  - AmB (3.27)
  - AmE (3.30)
  - AmF (3.31)

**Am = Amaranzole**

**Ax = Amaroxocane**

**Figure 3.11.** Isolation tree and identification of feeding deterrent compounds against Thalassoma bifasciatum (Assays carried out by Prof. Joe Pawlik, Wai Leong, and Tse-Lynn Loh).

Two previous attempts to identify the feeding deterrent compounds from *P. amaranthus* (95-021 and 02-054) led to the deduction that the metabolite was likely a sulfated sterol. The tendency of sulfate groups to hamper purification using standard chromatographic methods, partially
explained why activity was repeatedly lost during purification and previously active fractions became complex mixtures. Using a simplified three–step purification with C18 HPLC employing a neutral buffered mobile phase, we retained activity that partitioned into a number of semi-pure HPLC fractions (Figure 3.11). Further HPLC of the most active fraction (RCM6) that inhibited feeding of *Thalassoma bifasciatum* gave amaroxocanes A (3.25) and B (3.26). In addition, amaranzoles B-F; 3.27-3.31 structurally related to amaranzole A were identified.

### 3.5 Structure Determination of Amaroxocanes A and B

Amaroxcane A (3.25) was isolated as a colorless glass with a molecular formula C_{55}H_{85}Na_{3}O_{17}S_{3} based on HRESIMS (*m/z* 1159.4764 [M – Na+] , ∆ = +1.4 mmu). The steroidal nature of 3.25 was evident in the ¹H NMR spectrum, which showed two shielded methyl singlets (δ 0.64, s, C-18; 0.86, s, C-18') and two methyl doublets (δ 0.95, d, 3H, J = 6.7 Hz; 1.00, d, 3H, J = 6.6 Hz) with chemical shifts corresponding to methyl groups H3-18 and H3-21, respectively. The presence of OSO₃Na and alcohol groups was supported by strong bands in the IR spectra at ν 1216 and 3453 cm⁻¹, respectively, and losses of SO₃Na (103 amu) in the negative ion ESIIMS spectra. The molecular formula, with approximately twice the expected number of carbon atoms (C55, cf. cholesterol C27), implied that 3.25 was a dimeric sulfated sterol.
Figure 3.12. Structures for amaroxocanes A (3.25) and B (3.26).

The eastern and western hemisphere for 3.25 were assembled in a straightforward manner by analysis of COSY, TOCSY, and HMBC correlations (Figure 3.13). Structure determination of the western hemisphere began with an HMBC correlation from Me-19 to C-1, C-5, C-9, and C-10. COSY and TOCSY correlations delineated the spin system H-1 through H-7, which included a methyl doublet at C4 (δ 1.28, d, J = 6.6 Hz). The $^{13}$C chemical shifts for C-2 (δ 72.8 ppm) and C-3 (δ 73.4) implied OH substitution, while the $^{13}$C chemical shift for C-6 (δ 75.1) was more consistent with a sulfate half-ester O(SO$_3$)Na. Proton signals due to H-7 (δ 2.33 and 2.91), H-11 (δ 2.10 and 2.20), and H-14 (δ 1.94) showed chemical shifts consistent with allylic hydrogens and a double bond at the C-8/C-9 position that was further supported by an HMBC cross-peak from Me-19 to C-9. The H-14 to H-21 spin
system was established from COSY and TOCSY correlations. HMBC correlations from Me-18 and Me-21 completed the ABCD ring system in the western hemisphere.

![Chemical structure of amaroxocane A](image)

**Figure 3.13.** Selected 2D NMR correlations for amaroxocane A (3.25).

The eastern hemisphere was assembled in a similar manner; however a few differences were observed. The methyl doublet at C-4’ (δ 28.5) was absent from the eastern hemisphere. A double bond was placed at the C-8’/C-14’ position on the basis of $^1$H chemical shifts consistent with allylic hydrogens H-7’ (δ 1.92, m; 3.02, m), H-9’ (δ 1.87, m), and H-15’ (δ 2.26, m; 2.35, m). HMBC cross-peaks from H-7’ to C-8’ and C-14’ were also observed.

The $^{13}$C NMR chemical shift for C-3’ (δ 71.8, d) and a downfield shift (Δ δ +0.14 ppm, Figure 3.14) upon measurement of the $^{13}$C NMR spectrum in deuterated solvent supported substitution by a free hydroxyl substituent, while downfield shifts observed for C-2’ (δ 79.4, d) and C-6’ (δ 78.7, d) and negligible $^{13}$C NMR deuterium isotope shifts were consistent with O-sulfate esters (Figure 3.14).\textsuperscript{18} The two halves of 3.25 were shown to be linked through an
oxabicycle formed through oxidative fusion of the side chains. The gem-dimethyl signals for Me-26 and Me-27 showed HMBC correlations to the quaternary carbon C-25 (δ 41.5, s) and also showed correlations to C-24 and C-27'. COSY and TOCSY correlations connected H-24 to the former spin system containing H-22 to H-26', establishing substructure A (Figure 3.13). Reconciliation of the strong HMBC correlations from H-26' and H-27' to the oxygenated quaternary carbon C-25' required a cyclopentane ring. C-25' was connected to the eastern hemisphere by HMBC correlations from H-26' and H-27' to C-24'. Finally, C-22 was connected to C24' via an ether linkage supported by an HMBC correlation from H-24' to C-22. The complete planar
structure for amaroxocane A (3.25) was now secured, and attention was
turned to addressing the relative and absolute configurations.

![Newman projections along the C20-C22 bond of 3.25 depicting the two possible conformers for the (20S,22R) diastereomer with H20-C20-
C22-H22 dihedral angles of (a) $\theta = +90^\circ$ and (b) $\theta = -90^\circ$, and for the
(20S,22S) diastereomer with H20-C20-C22-H22 dihedral angles of (c) $\theta = +90^\circ$ and (d) $\theta = -90^\circ$. Ring C residues and the angular methyl group are
removed for clarity. Only (a) accommodates both the observed NOESY cross
peaks (double headed arrows) and $^3J$ (H20-H22) $\sim 0$ Hz observed for 3.25.

The relative configuration for 3.25 could be assigned by NOESY and
analysis of coupling constant data. The equatorial disposition of H-2 in the
western hemisphere was evident from the small $^3J_{HH}$ vicinal coupling to H-3,
and H-3 was axial on the basis of NOESY correlations to axial protons H-1
and H-5. An equatorial orientation was assigned for H-4, which showed small
vicinal couplings and NOESY correlations to H-3 ($\delta$ 3.96, dd, $J = 6.1$, 3.5 Hz)
and H-5 ($\delta$ 2.02, dd, $J = 12$, 4.1 Hz), and H-6 ($\delta$ 4.87, dt, $J = 12$, 7.6 Hz)
showed large coupling ($J = 12$ Hz) to H-5. A trans fused ring system was
established for the C/D ring system on the basis of axial orientation of Me-18
and H-14. Assignment of the eastern hemisphere was carried out in a similar
manner. H-2’ ($\delta$ 4.72, brq, $J = 2.6$ Hz) showed small coupling ($J = 2.6$ Hz) to H-
3’ (δ 3.80, m), which showed NOESY correlations to H-5’ (δ 1.61 m). H-6’ was assigned as axial on the basis of large coupling to H-5’. Me-18’ showed NOESY correlations to both axial H-11’ and H-16’. The oxygenated methine H-22 (δ 3.63, dd, J = 11.8, 4.6 Hz) was informative of the relative configuration and conformation of the oxabicycle and showed NOESY correlations to H-16ax/eq, H-23ax, H-23’ax/eq, and H-26’ (δ 2.05, m).

The absolute configuration of the different heterocyclic rings in 3.25 and 3.26 was addressed by relaying the configuration at C-20 to the heterocyclic linker. The natural R configuration for C-20 of both hemispheres can be safely assumed on the basis of biogenic principles, and the absolute configuration of the oxabicycle could be established through assignment of the relative configuration between the C-20 and C-22 bond. A dihedral angle of θ = ±90° was evident for H20-C20-C22-H22 from lack of scalar coupling (J ≈ 0 Hz) between the H-20 and H-22 protons. Of the two possible signs for the dihedral angle, only θ = +90° was consistent with the ensemble of NOEs observed in 3.25, and 22R was deduced as the most likely configuration based on NOESY correlations observed between H-17/H-23eq and H-16ax/eq/H-22 (see Figure 3.15). Therefore the complete stereochemical assignment for 3.25 is as depicted.

The molecular formula for the second major constituent, amaroxocane B (3.26), was derived as C_{54}H_{82}Na_3O_{20}S_4 on the basis of negative ion HRESIMS (m/z 1247.3965 [M–Na]–, Δ = −1.0 mmu). The ¹H NMR and mass
spectral data suggested 3.26 was also a sulfated sterol dimer. Most of the signals in the $^1$H NMR appeared to be doubled, leading us to believe the constitution and relative configuration of the sterol core in each hemisphere was the same. This was confirmed by analysis of 2D NMR (COSY, HSQC, and HMBC). The bridged bicyclic linker showed substantial differences compared to that of amaroxocane A (3.25). The gem-dimethyl groups Me-26 (δ 1.32, s, 3H) and Me-27 (δ 1.23, s, 3H) were now attached to an oxygenated quaternary carbon C-25 (δ 85.5, s). The H-24 signal (δ 2.15, m) was again assigned to the spin system H-22-H-25, similar to that found in compound 3.25. An ether linkage was evident between C-22 (δ 71.7, d) and C-24’ (δ 86.2, d) by an HMBC correlation from H-22 to C-24’. A second oxygenated quaternary carbon attached to Me-27’ was inserted between C-24’ and C-26’, supported by HMBC correlations from the methyl singlet (Me-27’) to oxygenated quaternary carbon (C-25’), the bridgehead methylene (C-26’), and the oxygenated methine (H-24’). Finally, the molecular formula required an additional degree of unsaturation, which was satisfied by a cyclic ether between C-25 and C-25’ forming a dioxabicyclo[4,2,1]nonane identical to that found in the crellastatins$^{19}$ and hamigerols.$^{20}$ The absolute and relative configuration of 3.26 was established upon similar principles used for 3.25.
Table 3.3. NMR data (CD$_3$OD) for amaroxocane A (3.25).

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<th>HMBC$^{c}$ (H$\rightarrow$C)</th>
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<th>$\Delta_{J}$ [mult., J (Hz), ax/eq]$^{a}$</th>
<th>DQF-COSY$^{b}$</th>
<th>HMBC$^{c}$ (H$\rightarrow$C)</th>
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### Table 3.4. NMR data (CD$_3$OD) for amaroxocane B (3.26).

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<td>26' 36.2 (CH$_3$) 2.05 (m) 2.26 (m)</td>
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$^a$Assigned from HSQC and HMBC. $^b$600 MHz. $^c$Correlations to C8 were not observed.
3.6 Previously isolated steroidal dimers and biosynthetic considerations.

There have been a number of steroidal dimers isolated from marine sources including the cephalostatins\textsuperscript{21} and ritterazines\textsuperscript{22} (highly cytotoxic pyrazine-fused steroidal alkaloids from the worm \textit{Cephalodiscus gilchristi} and the tunicate \textit{Ritterella tokioka}), bistheonellasterone\textsuperscript{23} and bisconicasterone\textsuperscript{24} (Diels–Alder adducts from the sponge \textit{Theonella swinhoei}), and crellastatins\textsuperscript{25} and hamigerols\textsuperscript{20} (dioxabicyclic sulfated sterols from the sponges \textit{Crella} sp. and \textit{Hamigera hamigera}). The crellastatins and hamigerols are unique among the dimeric steroids possessing a 3,8-dioxabicyclo[4.2.1]nonane system formed by oxidative fusion of unsaturated sterol side chains. Amaroxocane B (3.26) contains the same heterocycle found in the crellastatins and hamigerols, and amaroxocane A (3.25) is similar to crellastatin M (3.32) in that the dimer arises from variant oxidative cyclization of the side chains to generate substituted bridged cyclopentanes (based on 3-oxabicyclo[4.2.1]nonane).

The biosynthesis of the oxabicyclic systems present in the crellastatins, hamigerols, and now the amaroxocanes poses interesting questions regarding mechanism. Dimerization reactions in sterol biosynthesis are rare, but have been observed between A rings in the cephalastatin–ritterazine family and others (see above). The most relevant to the present discussion are the side-chain-cyclized crellastatins and hamigerols. The key theme in the biosynthesis of the latter compounds is tightly coupled dimerization of steroidal carbon
skeletons through oxidation and electrophilic substitutions and additions at their side chains (Figure 3.16). Formation of the heterocyclic bridge appears to be initiated by stereoselective oxidation, most likely by hydroperoxidation or hydroxylation mediated by one or more cytochrome $P_{450}$-dependent enzymes, as typical for sterol side chain oxidation.

Figure 3.16. Structures of crellastatin M (3.32), crellastatin A (3.33), and hamigerol B (3.34).
A plausible precursor that unifies biosynthesis for all the compounds in this class appears to be either lanosterol or desmosterol (Δ^{24,25} olefin). We are in agreement with D’Auria and co-workers\textsuperscript{25c} that the C-22–O–C-24’ ether bond is probably formed first to give linked dimer \textit{i} by a substitution of the electrophilic oxygen of C-22 hydroperoxide on the CC double bond of a second molecule of sterol to position the new CC bond at the C-25/C-26 terminus (Figure 3.14). Stereoselective epoxidation of one or the other CC double bond in \textit{i} would give \textit{ii} or \textit{iii} followed by electrophilic substitution of the C25–C26 double bond (\textit{path a}) to provide the tertiary alcohol (cf. crellastatin K). Protonation of the tertiary OH group followed by elimination of H\textsubscript{2}O could initiate C–C bond formation between C-25/C-27’ and closure of the cyclopentane ring leading to \textbf{3.25}. Alternative carbocation-initiated reactions of \textit{ii} (\textit{path b}, \textit{path c}) would lead to an oxygen-bridged oxocane (3,8-dioxabicyclo[4.2.1]octane) present in \textbf{3.27}, crellastatins A–J and L, and hamigerols A and B (7). The carbocycle in crellastatin M (\textbf{3.32}) may be formed by an alternate cascade (\textit{path d}) initiated by simple \textit{endo-trig} electrophilic substitution of the C-24/C-25 double bond by a tertiary carbocation derived from \textit{iv}, giving a cyclopentene with a Δ^{25,27′} olefin.
3.7 Feeding Deterrent Activity for Amaroxocanes A and B.

Amaroxocanes A (3.25) and B (3.26) were obtained from a semi-pure fraction (06PA2M.2.RCM6) that retained activity at 2x natural concentration. HPLC and NMR analysis showed that this fraction contained two major components, amaroxocanes A and B (each ~25% of the sample), and a number of minor congeners (Figure 3.18). Assay of pure samples of 3.25 and 3.26 was carried out to test whether these compounds were responsible for the feeding deterrent activity. We believed that the amaroxocane type compounds (sulfated sterol dimers) were acting additively. Therefore testing of a pure amaroxocane at a high enough concentration to represent the mixture
(06PA2M.2.RCM6) would likely retain activity. Due to the limited amount of each pure compound isolated we tested at one concentration (8x natural concentration) that represented the amount necessary to account for the other congeners in the mixture (06PA2M.2.RCM6). Surprisingly, amaroxocane A (3.25) showed little deterrent activity (8 pellets eaten of ten), while amaroxocane B (3.26) was deterrent (3 pellets eaten of 10). In terms of structure, the difference in activity is either due to the additional sulfate group on 3.26 or the difference in the heterocyclic bridge portion, or both. Due to the weak activity also observed in the polar fraction eluting before 06PA2M.2.RCM6, which is also mainly composed of a complex mixture of tetrasubstituted sulfated sterol dimers, we speculate that the sulfate component may be more important for activity.

![HPLC chromatogram](image)

**Figure 3.18.** HPLC chromatogram for fraction 06PA2M.2.RCM6 containing amaroxocanes A (3.25) and B (3.26).
3.8 Structure Determination of Amaranzoles B-F.

Amaranzole B (3.27) was assigned the molecular formula $C_{37}H_{49}N_2Na_3O_{15}S_3$ (m/z 903.2108, $[M - Na]^-$, $\Delta$=+1.8mmu) based on negative-ion HRESIMS that corresponded the addition of the elements of CO$_2$ to 3.27, consistent with a carboxy derivative of amaranzole A (3.13). $^1$H NMR, COSY, and HSQC data of 3.27 revealed $^1$H and $^{13}$C chemical shifts for the sterol core that were essentially identical with those of 3.13; however, significant differences were observed for the side chain in the vicinity of the C24 substituent compared to 3.13. The H24 methine signal ($\delta$ 5.39, dd, $J$ = 8.0, 5.6 Hz) of 3.27 was significantly deshielded from that of 1 ($\delta$ 4.48, m) indicating a change in the electronic environment. Compound 3.27 showed only one sp2 heteroaromatic $^1$H signal ($\delta$ 7.41 ppm, Table 3.5) suggesting substitution or oxidation at C28 or C29 of the imidazole ring. In addition, the H24 methine signal in 3.27 was shifted downfield compared to that of 3.13 ($\delta$ 4.48, m). Coupled HSQC of 3.27 showed the $^1$J$_{CH}$ of the single heteroaromatic $^1$H-$^{13}$C couplet ($\delta$ 7.41 ppm, $^1J$ = 187.8 Hz) was smaller than that characteristically associated with H2-C2 of five-membered ringazole heteroaromatics ($^1J$ ~200 Hz). For example, N-methylimidazole shows coupling constants of $^1J$ = 205.7 Hz and $^1J$ = 187.9 Hz for the H2-C2 and H4-C4 spin pairs, respectively.$^{26}$ Thus, the heteroaromatic singlet ($\delta$ 7.41, s) was assigned to C29, and the (C=O)O group was placed at C28 (the depicted imidazole tautomer is arbitrary).
Table 3.5. $^1$H NMR data for amaranzoles B-F (3.27-3.31) [CD$_3$OD, 600 MHz. $\delta$ (mult., $J$ Hz)]

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$^a$obscured by solvent peak.
**Figure 3.19.** Structures for amaranzoles A-F.

Although direct observation of the C=O signal (δ 158.8, s) in the $^{13}$C NMR was not possible due to insufficient signal-to-noise of the mass-limited sample, the chemical shift was observed indirectly by HMBC (Figure 3.20).

Two constitutional isomers (Figure 3.21) are possible for the proposed 4-(p-hydroxyphenyl)imidazole-2-carboxyl substituted sterol: a, a carboxylic acid with a C24-$N$ linkage in which the imidazole is bonded to the side-chain through a nitrogen atom, and carboxylic ester b with a C24-$O$ linkage. Both chemical and spectroscopic evidence pointed to b as follows. The deshielded $^1$H NMR signal for H24 in 3.27 (δ 5.39, dd, $J = 8.0$, 5.6 Hz, 1H) is more
consistent with an esterified C24 carbinol than the CH-N bond of H24 in 3.13 (δ 4.48, m, 1H).

\[ \text{Figure 3.20. Selected gHMBC correlations and } ^{13}\text{C NMR chemical shifts for amaranzole B (3.27)}. \]

Imidazole-2-carboxylic acids are prone to spontaneous decarboxylation under neutral or acidic conditions\textsuperscript{,}\textsuperscript{27} even at room temperature, however, prolonged heating of 3.27 in pyridine or DMSO (>120° C) failed to expel CO\textsubscript{2} and returned only starting material or small amounts of de-sulfated analogs of 3.27 (\textsuperscript{1}H NMR). This evidence suggested that the structure of amaranzole B could not contain substructure a, and, by a process of elimination, must be the latter substructure b. Evidence for the latter was provided by a weak HMBC (Figure 3.20) which showed a cross peak between H24 and the carbonyl signal (δ 159, s, (C=O)O). The latter is consistent with the carboxyl chemical shifts of known 2-carboxyimidazoles (e.g, ethyl 1-methylimidazole-2-
carboxylate, δ 159.3 ppm). The structure 3.27 was confirmed in the present work by direct comparisons of the natural product with model compounds of defined constitution and absolute configuration prepared by asymmetric synthesis (vide infra).

\[ \text{Figure 3.21. Two constitutional isomers of steroidal imidazole-2-carboxylate, 3.27.} \]

The molecular formula of amaranth C (3.28), C_{36}H_{47}N_{2}Na_{3}O_{13}S_{3} – derived from negative ion HRESIMS data \( m/z 857.2036 [M–Na]^- \Delta mmu +0.6 \text{ amu} \) – is 2 amu less than the molecular formula of 3.13 suggesting a dehydro analog. This was confirmed by analysis of the \(^1\text{H} \) and 2D NMR of 3.28 which revealed a tetrasubstituted C8,9 C=C double bond in the sterol core. \(^1\text{H} \) chemical shifts and coupling constants of 3.28, showed the substitution as well as relative stereochemistry were identical to 3.13 at positions H1–H6, however the \(^1\text{H} \) NMR signals for H7 (δ 2.10 and 2.73), H11 (δ 2.12), and H14 (δ 2.07) in amaranth C (3.28) were shifted downfield with respect to those of 3.13 (H7; δ 1.01 and 2.36 ppm; H11: δ 1.31 and 1.51 ppm; H14: δ 1.06). The location of the double bond at C9, C14 in 3.28 was secured by a gHMBC correlation from
Me-19 to C9. Comparison of the $^1$H NMR signals for allylic protons of the $\Delta^{8(14)}$ sterol trisulfate ester, hamigerol B gave a good match with 3.28. The side-chain linkage of 3.28 was shown to be of the C24-N type found in 3.13 by similarity of $^1$H and $^{13}$C NMR signals for H24 and C24.

![CD Spectra](image)

**Figure 3.22.** CD Spectra for (a) amaranzole A (3.13), (b) amaranzole C (3.28), and (c) amaranzole D (3.29) in H$_2$O/CH$_3$CN (7:3) at 23°C.

Amaranzole D (3.29), C$_{36}$H$_{47}$N$_2$N$_3$O$_{13}$S$_3$, was isomeric with amaranzole C (3). $^1$H and 2D NMR evidence showed that the C=C double bond in 3.29 was isomerized to C8,C14. Allylic proton signals H9 ($\delta$ 1.71, m, 1H) and H15 ($\delta$ 2.22, m; 2.29, m) were cross-correlated (gHMBC) to sp$^2$ carbons C8 ($\delta$ 125.0, d), and C14 ($\delta$ 146.3, d). Additional gHMBC cross peaks were
observed from H7 and H9 to C8, and from C18 methyl group to C14. The absolute configuration of both amaranzole C and amaranzole D appear to be consistent that assigned for amaranzole A (3.13), based on Cotton effects with the same sign, but of different magnitude (Figure 3.22).

Amaranzole E (3.30) was the 8,14-dehydro derivative of amaranzole B (3.27) based on HRESITOFMS (m/z 901.1934, [M–Na]⁻ Δmmu = 0). Nearly identical ¹H and ¹³C chemical shifts were observed for the sterols of 3.29 and 3.30. Similarly, amaranzole F (3.31) (m/z 901.1948 [M–Na]⁻ Δmmu = +1.4) was assigned as the 8,9-dehydro derivative of amaranzole B (3.27) and comparison of ¹H, ¹³C NMR data with amaranzole C (3.28).

Since there were no precedents for the C24-N and C24-O variants that constitute the two sub-families of amaranzoles, we elected to determine the stereochemistry of 3.27 and ascertain the relationship to 3.13. The absolute configuration of stereocenters within the sterol cores in 3.13 and 3.27-3.31 are known with certainty from firm principles of sterol biosynthesis, but the C24 stereocenter in 3.13 was more difficult to assign because it is located three bonds removed from the nearest stereogenic center, C21. To solve the C24 stereocenter in 3.13, we exploited Cotton effects observable in the CD spectrum that arise from exciton coupling of π-π* transitions of the 4-(p-hydroxyphenyl)imidazolyl group and the terminal vinylidene group. However, the significant bond reorganizations in the side-chains of 3.27, 3.30 and 3.31 with respect to 3.13 required an independent solution to the configuration of
C24 in the new compounds. We turned to chiroptical comparisons of 3.27 with a different synthetic analog of defined configuration.

Scheme 3.3. Attempted synthesis of model compound 3.35.

Initially, we chose to synthesize 3.35, for direct comparison of spectroscopic data to 3.27. Synthesis of 3.35 commenced with methylation of ethyl thiooxamate (3.36)\(^{29,30}\) with Meerwein's salt\(^{31}\) (Scheme 3.3) followed by condensation with 2-amino-1-(4-methoxyphenyl)ethanone (3.37)\(^{32}\) gave imidazolecarboxylate ester 3.38. Protection of imidazole with BOM-Cl gave 3.39, which was subjected to base hydrolysis and immediate coupling (EDCI, DMAP) with allylic alcohol (3.40) to give ester 3.41. Unfortunately, neither the BOM nor the –OMe on 3.41 could be removed without hydrolysis of the ester and subsequent decarboxylation under various conditions. Consequently, an alternate strategy was taken for model comparison.

A mixture of amaranzoles B, E, and F was treated with CH\(_2\)N\(_2\) followed
by HPLC purification to give \( N,O \)-dimethyl amaranzole B (3.42) (Scheme 3.4). The position of the newly added methyl groups were assigned by HMBC correlations. \( N,O \)-dimethyl 3.42 provided a target for straightforward comparison which was amenable to the synthesis of model esters 4.43 and 4.44.

\[
\begin{align*}
\text{Scheme 3.4. Methylation of amaranzole B (3.27) to } N,O \text{-dimethyl amaranzole B (3.42).}
\end{align*}
\]

Methylation of 3.38 (\( \text{CH}_2\text{N}_2, \text{Et}_2\text{O}-\text{MeOH} \)) proceeded with concomitant transesterification, to afford a mixture of methyl esters (Scheme 3.5). The major product 3.45 (64\%) was separated from the minor regioisomer 3.46 (32\%) by silica chromatography. Base hydrolysis of 3.45 provided imidazolecarboxylic acid 3.47. The latter compound was prone to spontaneous decarboxylation at acidic pHs, even at room temperature, and required careful
handling. Synthetic \(N,O\)-dimethyl analogs \((\pm)-3.43\) and \((\pm)-R-3.44\) were prepared by coupling (EDCI, DMAP) of the acid-sensitive imidazole-2-carboxylic acid \(3.47\) with the corresponding allylic alcohols \((\pm)-3.40\) and \((\pm)-3.48\) which were made available as follows.

\[
\text{Scheme 3.5. Synthesis of model compounds \((\pm)-3.43\) and \((\pm)-R-3.44).}
\]

Addition of \(n\)-propylmagnesium bromide to methacrolein gave racemic allylic alcohol \((\pm)-3.40\), while the optically-enriched homolog, \((\pm)-R-3.48\), was obtained by asymmetric addition of diethylzinc to methacrolein in the presence of \((2R)-(\pm)-3\text{-exo-}N\)-morpholinoisoborneol \([(\pm)-\text{MIB}, \text{ Scheme 3.5}]\).\(^{33}\) The optical purity and assignment of configuration of \((\pm)-R-3.48\) (93 %ee) were secured by the modified Mosher's method\(^{34}\) after conversion of \((\pm)-R-3.48\) to the corresponding \((R)\)- and \((S)\)-MTPA esters.
Racemic (+)-3.43 and (+)-R-3.44 displayed $^1$H NMR chemical shifts (Figure 3.23), close to those of 3.27 and 3.42, particularly H3 (H24 steroid numbering, $\delta$ 5.45, dd, $J = 8.0, 5.6$ Hz, 1H and 5.36, t, $J = 6.8$ Hz, 1H, respectively), but significantly different from those of 3.13 ($\delta$ 4.48, m). In addition, the chemical shifts of the protons in the aromatic region are in agreement with the assigned regioisomer.

**Figure 3.23.** $^1$H NMR (CD$_3$OD, 600 MHz) of (a) N,O-dimethyl amaranzole B (3.42), (b) (+)-3.43, and (c) (−)-3.44
As reported earlier, the dominant feature in the CD spectrum of 3.13, and by extension, 3.27 and its dimethyl homolog 3.42, is associated with a $\pi$-$\pi^*$ transition of imidazole chromophore; the sign and magnitude of this Cotton effect is dependent upon the configuration at C24. The CD spectra of 3.42 and (−)-R-3.44 (Figure 3.24) showed the same weak Cotton effect at $\lambda$ 221 nm ($\Delta \varepsilon$ −2.8 and $\Delta \varepsilon$ −2.9, respectively); therefore 3.27 and 3.42 share the 24R configuration.

Figure 3.24. CD spectra of (a) N,O-dimethylamaranzole B (3.42) and (b) model compound (−)-R-3.44 (MeOH, 23°C).
3.9 Biosynthetic Considerations of the Amaranzole Side Chain.

The structures of amaranzoles A-F (3.13, 3.27-3.31) suggest the involvement of a common intermediate in their biosynthesis. Formation of the $p$-hydroxyphenyl-imidazole side chain may commence by coupling of an allylic alcohol (steroid side chain) and a suitable imidazole precursor, for example, hamigeramine (3.45) isolated from the marine sponge *Hamigera hamigera.* Oxidative modification at C24 of the side chain is evident in the two structural subfamilies represented by amaranzoles A–F, however, the biosynthesis of A (3.13), C (3.28), and D (3.29) clearly diverges from that of B (3.27), E (3.30) and F (3.31). Because all six natural products occur together in the sponge consistently, it is most likely their biosynthesis links the C24-N and C24-O subfamilies through a common pathway. One possibility (Figure 3.25) invokes two allylic rearrangements of imidazole 3.27; first to give the primary allylic ester $i$ that further rearranges to the transient imidazolinium-2-carboxylic acid $ii$ with formation of a C-N bond. Facile loss of CO$_2$ from $ii$ would give a relatively stable carbene $iii$ (4-substituted imidazole-2-ylidene) which undergoes 1,2-hydride migration$^{36}$ to restore the aromatic imidazole ring of 3.13.$^{37}$ The enthalpic cost of replacing the stronger C-O bond with a C-N bond would be paid in part by the energetically favorable loss of CO$_2$.

Finally, it is noteworthy that the 24R configuration is retained in all amaranzoles. If the allylic alcohol precursor to 3.27 (Figure 3.25) is formed with high stereochemical fidelity, the subsequent interconversion of the two
sub-families of amaranzoles must proceed through a tightly orchestrated, enantiospecific pericyclic mechanism with retention of configuration.

![Diagram of biosynthetic pathway](image_url)

**Figure 3.25.** A possible biosynthetic pathway linking amaranzoles A (3.13) and B (3.27).

### 3.10 Feeding Deterrent and Other Bioactivity for the Aamaranzoles.

Amaranzoles A-F, derive from a polar fraction that exhibits significant feeding deterrence. We chose to test whether amaranzoles were responsible for this activity. Two mixtures of amaranzoles – one containing 3.13, 3.28, and 3.29 (RCM3, Figure 3.11 and 3.26), and the other containing 3.27, 3.30, and 3.31 (RCM4, Figure 3.11) – were tested in fish feeding assays using a protocol we reported earlier. The mixture containing 3.13, 3.28, and 3.29 elicited deterrent activity only when tested at concentrations much higher than natural (16x). We conclude that the amaranzoles are minor contributors to the chemical defense of *Phorbas amaranthus* against *T. bifasciatum*. 
Figure 3.26. HPLC chromatogram for RCM3 containing amaranzoles A (3.13), C (3.28), and D (3.29).

Although insufficient amounts of amaranzoles B–F (3.27–3.31) were available for assay of biological activity, a brief evaluation cytotoxicity of amaranzole A (3.13) and the less-polar analogue 3.45 (obtained by acid hydrolysis of 3.13; 3 M HCl–MeOH, 75 °C) toward human colon tumor cells (HCT-116) was carried out. Compound 3.45 exhibited significant cytotoxicity ($IC_{50} = 4.4 \mu g/mL$); however, the natural product 3.13 was essentially inactive ($IC_{50} > 32 \mu g/mL$). Presumably, cell permeability of 3.13 is restricted by the highly charged sulfate groups, which is relaxed in the more lipophilic 3.45. Amaranzole A (3.13) did not show in vitro antifungal activity against a panel of fungi (Candida albicans, C. glabrata, C. krusei, and two serotypes of
Cryptococcus neoformans).

3.11 Conclusions

Our studies suggest an ecological role for amaroxocene B (3.26) as chemical defenses against predatory fish. Amaroxocene A (3.25) may also exhibit additive or synergistic chemical defense (see above), or it may be an inert byproduct of the biosynthesis of B (3.26). Amaroxocanes are water soluble, comparable to the feeding deterrent saponins found in Erylus formosus, and would diffuse readily from the sponge upon tissue injury. Although ritterazines, cephalostatins, and crellastatins exhibit cytotoxic activity against cultured cancer cells, no ecological function for these compounds has been assigned. Antifeedant activity has also been reported from crude extracts of Hamigera hamigera, however the compounds responsible for this activity were not identified.

Amaranzoles A-F, derive from an HPLC fraction that elicited deterrent activity only when tested at 16x natural concentration. Although the amaranzoles may only be minor contributors to the deterrent activity observed in the crude extracts, it is worth noting that they are present at a much lower molar concentration than the amaroxocanes. We can only speculate that - given the amaroxocanes are the major deterrent secondary metabolites and their activity appears to be higher on a per molecule basis - the amaranzoles are either products of vestigial or archaic pathways or biosynthesized for alternate purposes.
The hydroxy-imidazole side chain of the amaranzoles presented a challenge to structure determination by standard NMR methods. We successfully used chemical synthesis and CD to assign both the constitution and stereochemistry of the side chain. Both the amaranzole A and amaranzole B side chains appear to show an ECCD spectra by coupling between the imidazole and double bond. The CD spectra in each case are diagnostic of the configuration of C24 and should be generally applicable to other members of this family.

3.12 Experimental Section

**General Experimental Procedures:** NMR spectra were acquired using a 400 MHz Varian Mercury spectrometer, a 600 MHz Bruker Avance III spectrometer with a 1.7mm TCI MicroCryoProbe or 500 MHz Jeol spectrometer. $^1$H and $^{13}$C NMR spectra were referenced to δ 7.24 and 77.0 ppm, (CDCl$_3$) or δ 3.31 and 49.0 ppm (MeOH- $d_4$). UV spectra were recorded on a double-beam spectrophotometer. Optical rotations were measured using a digital polarimeter. CD spectra were acquired using a Jasco J-810 spectropolarimeter. IR spectra were obtained using an JASC 4100 FTIR spectrometer equipped with a ZnSe ATR plate. HPLC was carried out using a dual-pump preparative instrument equipped with a high-dynamic range UV-vis detector set to λ 260 nm. For semipreparative HPLC, an RP column (5 μm C18-bonded silica, 10 × 250 mm) was used. Preparative HPLC was carried on radial compression cartridges (6 μm C18-bonded silica 25 × 100 mm) and commercial HPLC grade solvents were used for liquid chromatography. THF, CH$_2$Cl$_2$, and DMF were dried by passage through dual-alumina cartridges under an atmosphere of Ar, and reagent-grade chemicals were used as purchased.

**Isolation of amaranzole A (3.13):**

**Animal Material.** Phorbas amaranthus (02-13-054) was collected by hand using scuba at -3 to -10 m at North Dry Rocks, Key Largo, FL (25°07.850¢ N, 080°17.521¢ W) and identified by one of the authors, J.R.P. The sponge was
stored for 2 months at –20 °C before extraction. A voucher specimen for # 02-13-054 is archived in our collection at the Department of Chemistry and Biochemistry, UCSD.

**Extraction and isolation.** The lyophilized tissue (339 g) was gently agitated in MeOH (800 mL) and H₂O (200 mL) using an overhead stirrer (5 °C for 24h). After filtration, extraction of the tissue was repeated twice with fresh MeOH (900 mL) and H₂O (100 mL). The aqueous methanolic extracts were combined, dried and partitioned between 15% H₂O in MeOH and hexane. The aqueous MeOH partition (83.7g) was subject to HP20 Diaion chromatography using a H₂O/MeOH gradient (100% H₂O to 100% MeOH) to give six fractions. Fractions four, five, and six were combined (14.1 g, 5.4 g further purified) and separated by gel filtration (HW40 (50% H₂O in MeOH to 100% MeOH) to give eight fractions. Fractions three and four were combined (2.39 g) and subject to chromatography on Sephadex LH20 (MeOH) to give four fractions. The fourth fraction (536 mg, 163 mg further purified) was subject to two rounds of reversed phase HPLC (49% H₂O in MeOH containing 0.5M NaClO₄) to give of amaranzole A (3.13, 5.4 mg, 0.0015% dry weight).

**Amaranzole A (3.13):** colorless glass; [α]₀²³ₒ +8.70 (c 0.39, MeOH) IR (ZnSe)
νₘₐₓ 3491, 2935, 1235, 1065, 1001 cm⁻¹; UV (MeOH) λₘₐₓ 250 nm (ε 10,000); CD (MeOH) 255 nm (Δε +0.7), 216 nm (Δε –2.5); HRMS MALDI (negative ion) m/z 859.2177 [M-Na]⁻ (calcd for C₃₈H₄₉N₂Na₂O₁₃S₃, 859.2192); ESIMS (negative ion) m/z 859 [M–Na⁺]⁻, 815 [M–3Na⁺+2H⁺]⁻, 757 [M–SO₃Na–
Na\(^{+}\)\(\text{H}^{+}\)^\(^{-}\), 735 [M–SO\(_3\)Na–Na\(^{+}\)\(\text{H}^{+}\)]\(^{-}\), and 655 [M–2SO\(_3\)Na–Na\(^{+}\)+2\(\text{H}^{+}\)]\(^{-}\); \(^{1}\)H NMR and \(^{13}\)C NMR see Table 3.1, text).

**Desulfation of Amaranzole A (3.13):** A solution of amaranzole A (3.13, 1 mg) was heated in 3 M HCl (MeOH–H\(_2\)O, 2 mL) at 75 °C for 45 min. The cooled solution was concentrated and passed through a SiO\(_2\) cartridge, which was subsequently eluted with MeOH–CH\(_2\)Cl\(_2\) to give the desulfated compound 3.45 (quant): \(^{1}\)H NMR (500 MHz, CD\(_3\)OD) (selected) \(\delta\) 3.93 (brq, \(J = 4.0\) Hz, H2), 3.50 (dt, \(J = 10.9, 4.0\) Hz, H3), 3.39 (td, \(J = 10.9, 4.6\) Hz, H6), 1.72 (s, H27), 1.00 (s, H19), 0.92 (d, \(J = 6.9\) Hz, H21), 0.63 (s, H18). The remainder of the \(^{1}\)H NMR signals were essentially identical to those of 3.13. HRESITOFMS: \(m/z\) 577.3996 [M + H\(^{+}\)] (calcd for C\(_{36}\)H\(_{53}\)N\(_2\)O\(_4\), 577.4005).

**Synthesis of model compounds 3.15 and 3.16:**

**4-(1-isopropyl-1H-imidazol-5-yl)phenol (3.15):** Isopropylamine (272 \(\mu\)L, 3.41 mmol) was stirred with a mixture of MgSO\(_4\) (1.02 g, 8.53 mmol) in triethylamine (1.5 mL) at room temperature for 1 hour. 4-hydroxybenzaldehyde (414 mg, 3.40 mmol) was added and the mixture was refluxed for 4 hours. The mixture was filtered and concentrated. The concentrated TosMIC (ptoluenesulfonyl) methyl isocyanide (860 mg, 4.43 mmol) and K\(_2\)CO\(_3\) (1.41 g, 10.2 mmol). The mixture was heated at reflux for 2 hours, then concentrated and partitioned between EtOAc and water. The aqueous layer was extracted two times with EtOAc. The organic layers were washed with brine and dried (Na\(_2\)SO\(_4\)). The crude mixture was recrystallized from MeOH/H\(_2\)O to give 207
mg (30% yield) of compound 3.15 as a slightly yellow solid. IR (KBr) \( \nu_{\text{max}} \)
3117, 2978, 2934, 2882, 2785, 2663, 2593, 1615, 1562, 1483, 1457, 1370,
1274, 1248, 1221, 933, 840 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CD\(_3\)OD) \( \delta \) 7.82 (d, 1.1
Hz, 1H), 7.17 (d, 8.4 Hz, 2H), 6.88 (d, 8.4 Hz, 2H), 6.83 (d, 1.1 Hz, 1H), 4.38
(sep, 6.8 Hz, 1H), 1.41 (d, 6.8 Hz, 6H); \(^13\)C NMR (100 MHz, CD\(_3\)OD) d 159.1
(C), 135.3 (CH), 134.4 (C), 131.9 (CH), 126.7 (CH), 122.0 (C), 116.6 (CH),
48.3 (CH), 23.9 (CH\(_3\)). HREIMS m/z 202.1102 [M]^+ (calcd for C\(_{12}\)H\(_{14}\)N\(_2\)O,
202.1101).

4-(1-isopropyl-1H-imidazol-4-yl)phenol (3.16): Methyl ether 3.18 (12.4 mg,
57.4 \( \mu \)mol) was dissolved in CH\(_2\)Cl\(_2\) (200 \( \mu \)L) and BBr\(_3\) (172.2 \( \mu \)mol, 1M in
CH\(_2\)Cl\(_2\)) was added at 0 °C. The reaction was stirred at 0 °C for 30 minutes,
then 1 hour at room temperature. The reaction was quenched with saturated
NaHCO\(_3\) (1 mL) and extracted three times with EtOAc. The combined organic
layers were washed with brine, dried (Na\(_2\)SO\(_4\)), and concentrated. The crude
mixture was purified by chromatography (SiO\(_2\), 7:93 MeOH/CH\(_2\)Cl\(_2\)) to give
compound 3.16 (10.2 mg, 88% yield). Slightly yellow glass; IR (KBr) \( \nu_{\text{max}} \)
3108, 2978, 2930, 2683, 2607, 1613, 1562, 1494, 1262, 1248, 1103, 1069, 840, 765
\( \text{cm}^{-1} \); \(^1\)H NMR (400 MHz, CD\(_3\)OD) \( \delta \) 7.69 (d, 1.4 Hz, 1H), 7.53 (d, 8.4 Hz, 2H),
7.40 (d, 1.4 Hz, 1H), 6.78 (d, 8.4 Hz, 2H), 4.44 (sep, 6.8 Hz, 1H), 1.51 (d, 6.8
Hz, 6H); 13C NMR (100 MHz, CD\(_3\)OD) \( \delta \) 157.6 (C), 143.0 (C), 136.6 (CH),
127.2 (CH), 127.1 (C), 116.3 (CH), 113.1 (CH), 50.9 (CH), 23.9 (CH\(_3\));
1-isopropyl-4-(4-methoxyphenyl)-1H-imidazole (3.18): In a re-sealable pressure tube, a mixture of oxazoline 3.17 (97 mg, 0.31 mmol), isopropylamine (125 µL, 1.26 mmol) and xylenes (1.5 mL) were heated at 135 °C for 13 hours. The residue was concentrated and partitioned between EtOAc and water. The aqueous phase was extracted two times with EtOAc, and the combined organic layers were washed with brine, dried (Na₂SO₄), and concentrated. The residue was purified by chromatography (SiO₂, 2:5 EtOAc/hexane) to give 3.18 (10 mg, 15% yield) as a yellowish-orange glass; IR (KBr) ν max 3117, 2981, 2836, 1672, 1613, 1562, 1511, 1248, 1180, 950, 830, 643 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.70 (d, 8.5 Hz, 2H), 7.65, (s, 1H), 7.16 (s, 1H), 6.92 (d, 8.5 Hz, 2H), 4.36 (sep, 6.8 Hz, 1H), 3.82 (s, 3H), 1.53 (d, 6.8 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 158.7 (C) 141.3 (C), 134.9 (CH), 126.5 (C) 126.0 (CH), 114.0 (CH), 111.5 (CH), 55.3 (CH₃), 49.6 (CH), 23.7 (CH₃); HREIMS m/z 216.1253 [M]+ (calcd for C₁₃H₁₈N₂O, 216.1257).

Synthesis of Model Compound (+)-3.19:

(R)-(tert-butyl 1-hydroxybutan-2-yl)-carbamate: (R)-2-amino-butanol (13.4 g, 0.14 mol) was dissolved in CH₂Cl₂ (200 mL). The mixture was cooled to 0 °C and di-tertbutylpyrocarbonate (29.5 g, 0.135 mol) was added. The mixture was stirred for 4 hours at room temperature. The solvent was evaporated and the residue redissolved in ether (200 mL), washed with 20% phosphoric acid (100 mL), brine (100 mL), 5% NaHCO₃ (100 mL), brine (100 mL), dried (MgSO₄), and evaporated to give the N-Boc protected alcohol (24.5 g, 96%
yield). white solid; [α]D25 +27.3 (c 2.33, CHCl3); IR (KBr) νmax 3336, 2969, 2934, 2882, 1684, 1527, 1370, 1178, 1082 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 4.61 (m, 1H), 3.67 (dd, 12.8 Hz, 5.6 Hz, 1H), 3.55 (dd, 12.8, 5.6 Hz, 2H), 2.10 (m, 1H), 1.40-1.60 (m, 2H), 1.45 (s, 9H), 0.95 (t, 7.6 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 156.6 (C), 79.5 (C), 65.2 (CH2), 54.3 (CH), 28.3 (3 x CH3), 24.5 (CH2), 10.4 (CH3); HREIMS m/z 189.1366 [M]+ (calcd for C9H19NO3, 189.1359)

(R)-(tert-butyl 1-oxobutan-2-yl)-carbamate (3.20): This procedure was similar to those reported by Krysan and coworkers.38 A solution of dry DMSO (4.97 mL, 69.9 mmol) in CH2Cl2 (5 mL) was added dropwise to a solution of oxalyl chloride (2.96 mL, 35.0 mmol) in CH2Cl2 (70 mL) at −30 °C (at a rate such that the temperature was maintained below −20 °C). After stirring for 30 minutes, the mixture was treated dropwise with a solution of N-Boc protected alcohol (5.10 g, 26.9 mmol) in CH2Cl2 (20 mL) over 2 hours. After stirring for 1 hour at −25 °C, a solution of diisopropylethylamine (15.5 mL, 88.8 mmol) in CH2Cl2 (40 mL) was added dropwise over 2 hours. Stirring was continued for an additional 1.5 hours, then the mixture was poured into an ice-cold solution of 20% aqueous citric acid and stirred for 30 minutes. The organic layer was removed and the aqueous layer was extracted once with CH2Cl2. The combined organic layers were washed with water, dried (Na2SO4) and concentrated to give the crude aldehyde (3.20, 4.96 g, 98% yield) as a light yellow oil which was used immediately in the next reaction. IR (KBr) νmax 3353, 2978, 2934, 1702, 1518, 1370, 1241, 1169, 1073 cm⁻¹; 1H NMR (400 MHz,
CDCl$_3$) δ 9.55 (s, 1H), 5.13 (m, 1H), 4.17 (q, 6.0 Hz, 1H), 1.88-1.95 (m, 1H), 1.60-1.67 (m, 1H), 1.42 (s, 9H), 0.94 (t, 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 199.9 (CH), 155.5 (C), 79.9 (C), 60.8 (CH), 28.2 (CH$_3$), 22.3 (CH$_2$), 9.4 (CH$_3$); HRTOFMS m/z 210.1096 [M+H]$^+$ (calcd for C$_9$H$_{18}$NO$_3$, 210.1101).

dert-butyl (3R)-2-hydroxypentan-3-yl)carbamate (3.21): A solution of aldehyde 3.20 (4.96 g, 26.5 mmol) in ether (250 mL) was cooled to –78 °C and treated with MeMgBr (37.8 mL, 1.4M in 3:1 toluene/THF). The slurry was stirred at 0 °C for 1 hour, brought to room temperature and stirred for an additional hour before quenching with saturated aqueous NH$_4$Cl (100 mL). The organic layer was separated, and the aqueous layer extracted three times with ether (3 x 100 mL). The combined organic layers were washed with brine, dried (MgSO$_4$), and concentrated to a crude oil which was purified by chromatography (SiO$_2$, 1:9 EtOAc: hexane) to give alcohol 8 (3.70 g, 69% yield) as a colorless oil; IR (KBr) $\nu_{\text{max}}$ 3363, 2972, 2938, 2879, 1689, 1511, 1392, 1290, 1248, 1171, 1103, 1061 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 4.74 (major, m, 1H), 4.64 (minor, m, 1H), 3.75-3.85 (m, 1H), 3.49 (minor, m, 1H), 3.31 (major, m, 1H), 2.53 (m, 1H), 1.20-1.59 (m, 2H), 1.42 (s, 9H), 1.18 (major, d, 6.4 Hz, 3H), 1.11 (minor, d, 6.4 Hz, 3H), 0.94 (minor, t, 6.8 Hz, 3H), 0.92 (major, t, 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 156.7 (C), 79.2 (C), 70.3 (minor, CH), 69.0 (major, CH), 57.8 (minor, CH), 57.4 (minor, CH), 28.3 (CH$_3$), 25.3 (CH$_2$), 23.0 (minor, CH$_3$), 20.4 (major, CH$_3$), 10.8 (minor, CH$_3$), 10.6 (major, CH$_3$); HREIMS m/z 203.1512 [M]$^+$ (calcd for C$_{10}$H$_{21}$NO$_3$, 203.1512).
(R)-3-aminopentan-2-one (3.22): A cooled solution (−78 °C) of oxalyl chloride (0.88 mL, 10.4 mmol) in CH₂Cl₂ (11 mL) was treated dropwise with dry DMSO (1.19 mL, 16.7 mmol) in CH₂Cl₂ (1.20 mL) at a rate that maintained the temperature below −65 °C, then stirred for 30 minutes. A solution of alcohol 3.21 (1.06 g, 5.22 mmol) in CH₂Cl₂ (6 mL) was then added dropwise over 30 minutes (solution maintained below −65 °C), and the mixture stirred for 1 hour. Diisopropylethylamine (5.92 mL, 33.9 mmol) in CH₂Cl₂ (16 mL) was then added dropwise over 1 hour (solution maintained below −65 °C), and the mixture stirred for 30 minutes, warmed to −40 °C, and stirred an additional 1.5 hours. The mixture was poured into a solution of 10% aqueous citric acid (75 mL) and stirred for 30 minutes. The organic layer was removed and the aqueous layer was extracted two times with CH₂Cl₂. The combined organic layers were washed with water, dried (MgSO₄) and concentrated to give the crude ketone 3.22 (1.00 g, 95% yield), which was used immediately in the next reaction. light yellow oil; IR (KBr) νmax 3353, 2978, 2943, 2882, 1711, 1518, 1370, 1256, 1178, 1082 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.24 (d, 5.0 Hz, 1H), 4.26 (dt, 5.0, 7.2 Hz, 1H), 2.15 (s, 3H), 1.85-1.95 (m, 1H), 1.54-1.61 (m, 1H), 1.40 (s, 9H), 0.85 (t, 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 207.1 (C), 155.4 (C), 79.5 (C), 60.7 (CH), 28.2 (CH₃), 26.9 (CH₃), 24.4 (CH₂), 9.1 (CH₃).

(R)-tert-butyl 2-methylpent-1-en-3-ylcarbamate (3.23): A solution of KOT-Bu (7.45 mL, 1M in THF) was added to a cooled solution (0 °C) of CH₃PPh₃Br (2.66 g, 7.45 mmol) in THF (15 mL). After stirring the mixture for 10 minutes at
room temperature, ketone 3.22 (1.00 g, 4.97 mmol) in THF (7 mL) was added and stirring continued for 10 minutes before quenching with brine. The mixture was extracted two times with diethyl ether and the combined organic layers were dried (MgSO₄), and concentrated at 0 °C. The mixture was purified by chromatography (SiO₂, 1:19 ether/pentane) to give amine 3.23 (0.82 g, 79% yield) as a colorless oil. [α]²²D +4.9 (c 0.23, CHCl₃); IR (KBr) νmax 3345, 2969, 2934, 2882, 1702, 1650, 1518, 1457, 1370, 1248, 1178, 1082, 1055, 898 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.84 (s, 1H), 4.82 (s, 1H), 4.53 (m, 1H), 3.90 (m, 1H), 1.68 (s, 3H), 1.54-1.59 (m, 1H), 1.45-1.50 (m, 1H), 1.42 (s, 9H) 0.87 (t, 7.2 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 155.4 (C), 144.9 (C), 111.1 (CH₂), 79.1 (C), 57.2 (CH), 28.4 (CH₃), 26.3 (CH₂), 18.9 (CH₃), 10.3 (CH₃); HRTOFMS m/z 222.1465 [M+Na]⁺ (calcd for C₁₁H₂₁NO₂Na, 222.1464).

**Determination of %ee for Compound 3.23.** Acetyl chloride (1 mL) was added to a solution of 3.23 (4.7 mg, 23.6 μmol) in MeOH (1 mL) at 0 °C, and the solution was stirred for 1 hour at room temperature. The solvent was removed under reduced pressure and the resulting residue was dissolved in CH₂Cl₂ (0.5 mL) and cooled to 0 °C. Triethylamine (12.0 μL, 86.1 μmol) was added followed by (S)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (11.0 μL, 59.0 μmol). The mixture was stirred for 2 hours at room temperature then diluted with CH₂Cl₂ (1 mL), and the mixture was extracted with 1N HCl, saturated NaHCO₃, brine, dried (MgSO₄), and concentrated. The product was purified by SiO₂ chromatography (1:9 ether/hexane) to give the (R)-MTPA-
amide derivative (6.4 mg, 86% yield) as a colorless glass. $^1$H NMR (500 MHz, CDCl$_3$) major isomer, δ 7.55 (m, 2H), 7.40 (m, 3H), 6.72 (d, 8 Hz, 1H), 4.92 (s, 1H), 4.90 (s, 1H), 4.32 (q, 8.5 Hz, 1H), 3.43 (d, 1.5 Hz, 3H), 1.74 (s, 3H), 1.64-1.70 (m, 1H), 1.50-1.57 (m, 1H), 0.84 (t, 7.5 Hz, 3H); HRTOFMS m/z 316.1525 [M+H]$^+$ (calcd for C$_{16}$H$_{21}$F$_3$NO$_2$, 316.1519).

The same procedure as above was repeated with 3.23 and (R)-(−)-α-methoxy-α-trifluoromethylphenylacetyl chloride to obtain 6.8 mg (91% yield) of the corresponding (S)- MTPA amide derivative. Colorless glass; $^1$H NMR (500 MHz, CDCl$_3$) major isomer, δ 7.51-7.53 (m, 2H), 7.38-7.40 (m, 3H), 6.71 (d, 7.5 Hz, 1H), 4.85 (s, 1H), 4.82 (s, 1H), 4.33 (dd, 8.0, 14.5 Hz, 1H), 3.45 (d, 1.5 Hz, 3H), 1.63-1.74 (m, 1H), 1.65 (s, 3H), 1.50-1.61 (m, 1H), 0.93 (t, 8.0 Hz, 3H); HRTOFMS m/z 316.1525 [M+H]$^+$ (calcd for C$_{16}$H$_{21}$F$_3$NO$_2$, 316.1519)

Analysis of the amide derivatives by HPLC (silica 4.6x250 mm, 1.0 mL/min, 1:9 ether/hexane, UV 254 nm) gave separations for the (R,S)-MTPA amide ($t_R$ =9.7 min) and the (R,R)-MTPA amide ($t_R$=10.4 min). %ee was determined to be 86% based on peak integrations.

(−)-(R)-2-methylpent-1-en-3-amine (3.23a): Allyl amine 3.23 (310 mg, 1.55 mmol) was dissolved in CH$_2$Cl$_2$ (1 mL) and cooled to 0 °C. TFA (1 mL) was added and the solution was stirred at 0 °C for 1 hour. The mixture was concentrated to give the TFA salt of the allyl amine 3.23a (300 mg, 91%). Slightly yellow oil; [α]$^2$$_D$ −12.6° (c 3.12, CHCl$_3$); IR (KBr) ν$_{max}$ 3423, 2978, 2078, 1676, 1536, 1431, 1387, 1204, 1146, 924, 840, 805, 728 cm$^{-1}$; $^1$H NMR
(500 MHz, CDCl₃) δ 7.31 (m, 3H), 5.16 (s, 1H), 5.08 (s, 1H), 3.68 (m, 1H), 1.75-1.82 (m, 2H), 1.77 (s, 3H), 0.93 (t, 7.5 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 139.3 (C), 116.8 (CH₂), 58.7 (CH), 24.4 (CH₂), 17.4 (CH₃), 9.5 (CH₃); HRTOFMS m/z 100.1124 [M+H]⁺ (calcd for C₆H₁₄N, 100.1126).

(R)-4-(+)-(1-(2-methylpent-1-en-3-yl)-1H-imidazol-5-yl)phenol ((+)-3.19): A solution of the above allyl amine TFA salt (300 mg, 1.41 mmol) in CH₂Cl₂ (1 mL) was treated with Et₃N (216 µL, 1.55 mmol), then MgSO₄ (422 mg, 3.53 mmol), and stirred for 1 hour at room temperature. 4-Hydroxybenzaldehyde (172 mg, 1.41 mmol) was added to the mixture which was stirred an additional 2.5 hours before heating at reflux for an additional 2 hours. The mixture was cooled, filtered, concentrated under reduced pressure. The residue was dissolved in anhydrous MeOH (2.5 mL) and treated with TosMIC (275 mg, 1.41 mmol) and K₂CO₃ (585 mg, 4.23 mmol) then heated to reflux for 2 hours. The solvent was removed under reduced pressure and the residue partitioned between EtOAc and water. The aqueous layer was extracted twice more with EtOAc and the combined organic layers were washed with brine, dried (Na₂SO₄) and volatiles removed reduced pressure. The crude product was purified by chromatography (SiO₂, 1:19 MeOH/CH₂Cl₂) to give the imidazole (+)-3.19 (122 mg, 36%). The enantiomeric purity of the sample of (+)-3.19 was determined to be 74%ee using chiral HPLC (Chiralpak AD, 250 x 4.6 mm, flow rate = 1.2 mL/min, λ = 260 nm, mobile phase = 3.5% i-PrOH in hexane + 0.2% diethylamine, tᵣ = major; 66.3 min., minor; 71.2 min.). slightly yellow glass;
$[\alpha]^{22}_D +7.76^\circ$ (c 0.59 MeOH); IR (KBr) $\nu_{\text{max}}$ 3083, 2969, 2934, 2882, 2794, 2672, 2602, 1615, 1562, 1510, 1483, 1457, 1274, 1123, 933, 845 cm$^{-1}$; UV (MeOH) $\lambda_{\text{max}}$ 248 nm ($\varepsilon$ 9,600); CD (MeOH) 257 nm ($\Delta\varepsilon$ +0.6), 218 nm ($\Delta\varepsilon$ -2.1); $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.75 (d, 1.0 Hz, 1H), 7.14 (d, 8.8 Hz, 2H), 6.89 (d, 1.0 Hz, 1H), 6.85 (d, 8.8 Hz, 2H), 4.91 (s, 1H), 4.64 (s, 1H), 4.41 (dd, 6.0, 9.6 Hz, 1H), 1.93-2.10 (m, 2H), 1.63 (s, 3H), 0.81 (t, 7.6 Hz, 3H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 159.2 (C), 145.6 (C), 136.5 (CH), 135.7 (C), 132.2 (CH), 126.8 (CH), 121.7 (C), 116.5 (CH), 113.5 (CH$_2$), 63.4 (CH), 27.2 (CH$_2$), 19.7 (CH$_3$), 11.4 (CH$_3$); HREIMS m/z 242.1416 [M]$^+$ (calcd for C$_{15}$H$_{18}$N$_2$O, 242.1414).

(R)-(−)-4-(1-(2-methylpentan-3-yl)-1H-imidazol-5-yl)phenol ((−)-3.24): A mixture of (+)-3.19 (11.8 mg, 0.049 mmol) and Pd/C (1.9 mg, 10 %) in MeOH (0.5 mL) were placed under H$_2$ (1 atm) and stirred for 24 hours. The mixture was filtered through a 0.2 µm syringe filter and concentrated to give the dihydro-compound (−)-3.24 (10.6 mg, 89%). $[\alpha]^{23}_D -13.7$ (c 1.29 CHCl$_3$); IR (KBr) $\nu_{\text{max}}$ 3117, 2969, 2934, 2882, 2794, 2672, 2602, 1615, 1562, 1483, 1387, 1283, 1169, 1108, 844 cm$^{-1}$; CD (MeOH) 245 nm ($\Delta\varepsilon$ -1.8); $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 7.75 (s, 1H), 7.14 (d, 8.0 Hz, 2H), 6.87 (d, 8.0 Hz, 2H), 6.85 (s, 1H), 3.65 (dd, 4.0, 8.8, 10.8 Hz, 1H), 1.91-2.01 (m, 2H), 1.74-1.84 (m, 1H), 0.89 (d, 7.2 Hz, 3H), 0.78 (t, 7.6 Hz, 3H), 0.71 (d, 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 159.2 (C), 136.3 (C), 136.1 (CH), 132.8 (CH), 126.7 (CH), 122.1 (C), 116.4 (CH), 64.3 (CH), 35.5 (CH), 27.2 (CH$_2$), 20.1 (CH$_3$),
20.0 (CH₃), 11.1 (CH₃); HRTOFMS m/z 245.1651 [M+H]⁺ (calcd for C₁₅H₂₁N₂O, 245.1648).

**Isolation of amaroxocanes A and B and amaranzoles B-F:**

**Extraction and Isolation**

**Animal Material.** *Phorbas amaranthus* Duchassaing & Michelotti 1864 was collected in November 2006 using scuba from Dry Rocks Reef, Key Largo, Florida (25°7.850' N, 80°17.521' W), at a depth of 20-25'. Specimens were identified by J.R.P and stored at −20 °C until required. Vouchers samples are archived at UCSD. Two specimens (06-04-004a and 06-04-004b) of *Phorbas amaranthus* collected at the same site were analyzed separately.

**Extraction and Isolation.** The first sample (06-04-004a, 88.3 g) was lyophilized, extracted with water (1L), followed by MeOH (1L), and finally CH₂Cl₂ (1L). The organic extracts were combined, dried, and partitioned between hexane and H₂O/MeOH 1:9. The hexane layer (‘A’) was separated, and the MeOH layer was adjusted to 1:1 by addition of water, then partitioned against CH₂Cl₂. The CH₂Cl₂ layer (‘B’) was separated from the aqueous MeOH layer (‘C’) and the solvents were removed under reduced pressure. The crude MeOH extract C (33.5 g) was subjected to filtration through reversed phase silica (C₁₈ cartridge, conditioned with H₂O/MeOH 19:1) eluting with water, then MeOH/CH₃CN 1:1 to give two fractions. The second fraction (7.03 g, 1.46 g further purified) was then subject to reversed phase chromatography (C₁₈ cartridge) using a stepwise gradient (H₂O/MeOH 4:1, 7:3, 3:2, 1:1, 2:3, 3:7,
1:4, 1:9, then MeOH) to attain nine fractions. A portion (42.5 mg) of the fraction (127.3 mg) eluting with H₂O/MeOH (3:2) was subject to gradient semi-preparative reversed phase HPLC (flow rate: 2.5 mL/min; mobile phase: H₂O/CH₃CN containing 0.5M NaClO₄; gradient: 7:3 isocratic 15 minutes to 2:3 over 30 minutes; λ = 254 nm) to give five fractions. The third fraction contained amaranzole A (4.2 mg, 3.13). The fourth fraction was subjected to gradient semi-preparative reversed phase HPLC (same conditions as above, except mobile phase: H₂O/CH₃CN containing 0.75M NaClO₄) to give amaranzole B (1.2 mg, 3.27).

A second lyophilized sample of Phorbas amaranthus (06-04-004b, 131.2 g) was extracted with 1L of H₂O/MeOH 1:1 (23°C, overnight). The aqueous-MeOH phase was removed and extraction was repeated twice; first with 1L H₂O/MeOH 1:1, then 1L MeOH. The sponge tissue was then blended at high speed and extracted once more with 1L MeOH (23°C, overnight) and the combined extracts concentrated under reduced pressure. The remaining sponge tissue was extracted with CH₂Cl₂ (2 x 1L). The CH₂Cl₂ extract was dried and partitioned between hexane and H₂O/MeOH 1:9. The aqueous MeOH layer was dried and combined with the previous MeOH extracts to give a crude aqueous MeOH extract (48.6 g). A portion (33.9 g) of the aqueous MeOH crude extract was filtered through a reversed phase cartridge (C₁₈, conditioned with H₂O/MeOH 19:1) eluting with H₂O/MeOH 9:1, H₂O/MeOH 1:9, and i-PrOH to give three fractions. A portion (1.16 g) of the second
eluting fraction (5.82 g) was subjected to gradient preparative reversed phase HPLC (flow rate: 25 mL/min; mobile phase: H₂O/CH₃CN containing 1.5M NaClO₄; gradient: 73:27 isocratic 10 minutes to 23:77 over 30 minutes; λ = 240 nm) to give seven fractions. A portion (4.6 mg) of the third fraction (61.6 mg) was subjected to a repeated gradient reversed phase HPLC (flow rate: 2.5 mL/min; mobile phase: H₂O/CH₃CN containing 0.5M NaClO₄; gradient: 7:3 isocratic 15 minutes to 2:3 over 30 minutes; λ = 254 nm) to give amaranzole C (0.19 mg, 3.28), amaranzole D (0.32 mg, 3.29), and amaranzole A (0.35 mg, 3.13). A portion (1.4 mg) of the fourth fraction (8.4 mg) was subjected to repeated gradient reversed phase C₁₈ HPLC (H₂O/CH₃CN containing 1.5M NaClO₄, flow rate: 2.5 mL/min; gradient: 61:39 isocratic 15 minutes to 31:69 over 30 minutes; λ = 260 nm) to give amaranzole F (0.10 mg, 3.31), amaranzole E (0.19 mg, 3.30), and amaranzole B (0.15 mg, 3.27). The sixth fraction (337.3 mg, 72.5 mg further purified) was subjected to gradient reversed phase HPLC (flow rate: 2.5 mL/min; mobile phase: water/CH₃CN containing 1.5M NaClO₄; gradient: 11:9 isocratic 15 minutes to 1:3 over 30 minutes; λ = 220 nm) to give amaroxocane A (3.25, 17.9 mg, 0.46 % dry wt.) and amaroxocane B (3.26, 6.5 mg, 0.17 % dry wt.).

**Amaroxocane A (3.25):** colorless glass; [α]²¹吸入 +51.2 (c 1.0, MeOH); IR (ATR, ZnSe) νₘₐₓ 3453, 2949, 1637, 1457, 1383, 1216, 1061, 959, 914 cm⁻¹; UV (MeOH) λₘₐₓ 218 nm (ε 6700). ¹H NMR, ¹³C NMR, see Table 3.3.
HRESITOFMS \( m/z \) 1159.4764 [M–Na]− (calcd for \( C_{55}H_{85}Na_{2}O_{17}S_{3} \), 1159.4744).

**Amaroxocane B (3.26):** colorless glass; \([\alpha]_{D}^{21} +49.8\) (c 0.92, MeOH); IR (ATR, ZnSe) \( v_{\text{max}} \) 3480, 2945, 1652, 1456, 1376, 1220, 1062, 968, 913 \( \text{cm}^{-1} \); UV (MeOH) \( \lambda_{\text{max}} \) 218 nm (\( \varepsilon \) 6900). \(^1\)H NMR, \(^{13}\)C NMR, see Table 3.4.

HRESITOFMS \( m/z \) 1247.3965 [M–Na]− (calcd for \( C_{54}H_{82}Na_{3}O_{20}S_{4} \), 1247.3975).

**Amaranzole B (3.27):** colorless glass; \([\alpha]_{D}^{21} +21.8\) (c 0.52, MeOH); FTIR (ATR, ZnSe) \( v_{\text{max}} \) 3487, 2945, 1652, 1449, 1232, 1065, 1001, 915, 839 \( \text{cm}^{-1} \); UV (\( H_2O/CH_3CN \) 7:3) \( \lambda_{\text{max}} \) 256 nm (\( \varepsilon \) 7800), 289 (\( \varepsilon \) 9300), 310 (\( \varepsilon \) 8000); CD (\( H_2O/CH_3CN \) 7:3) 218 nm (\( \Delta\varepsilon \) –1.0); \(^1\)H NMR, see Table 3.5; \(^{13}\)C NMR (from 600 MHz, HSQC/gHMBC data, \( CD_{3}OD \)) \( \delta \) 160.2 (C34), 158.8 (C35), 144.3 (C25), 142.2 (C30), 138.4 (C28), 127.5 (C32), 122.5 (C31), 120.6 (C29), 116.9 (C33), 113.3 (C26), 79.6 (C24), 78.5 (C3), 77.9 (C6), 76.2 (C2), 56.9 (C17), 55.2 (C9), 51.6 (C5), 43.4 (C13), 42.2 (C1), 40.6 (C12), 39.6 (C7), 37.8 (C10), 36.1 (C20), 34.8 (C8), 32.3 (C22), 29.9 (C14), 29.9 (C23), 28.5 (C16), 25.4 (C4), 24.3 (C15), 21.8 (C11), 18.6 (C21), 17.9 (C27), 15.6 (C19), 11.8 (C18).

HRESITOFMS \( m/z \) 903.2108 [M–Na]− (calcd for \( C_{37}H_{49}N_{2}Na_{2}O_{15}S_{3} \), 903.2090).

**Amaranzole C (3.28):** colorless glass; FTIR (ATR, ZnSe) \( v_{\text{max}} \) 3454, 2947, 1631, 1230, 1110, 1002, 949 \( \text{cm}^{-1} \); UV (\( H_2O/CH_3CN \) 7:3) \( \lambda_{\text{max}} \) 250nm (\( \varepsilon \) 8300);
CD (H₂O-CH₃CN 7:3) 201nm (Δε +3.5), 217 (Δε −8.0); ^1H NMR, see Table 3.5; HRESITOFMS m/z 857.2036 [M−Na]^− (calcd for C₃₆H₄₇N₂Na₂O₁₃S₃, 857.2030).

Amaranzole D (3.29): colorless glass; FTIR (ATR, ZnSe) νₘₐₓ 3487, 2951, 1651, 1450, 1327, 1230, 1072, 1001, 964, 916, 840 cm⁻¹; UV (H₂O-CH₃CN 7:3) λₘₐₓ 250 nm (ε 8300); CD (H₂O-CH₃CN 7:3) λ 201 nm (Δε +4.7), 215 (Δε −1.9); ^1H NMR, see Table 3.5; HRESITOFMS m/z 857.2051 [M−Na]^− (calcd for C₃₆H₄₇N₂Na₂O₁₃S₃, 857.2030).

Amaranzole E (3.30): colorless glass; FTIR (ATR, ZnSe) νₘₐₓ 3559, 2952, 1651, 1453, 1386, 1232, 1113, 1001, 918, 843 cm⁻¹; ^1H NMR, see Table 3.5; HRESITOFMS m/z 901.1941 [M−Na]^− (calcd for C₃₇H₄₇N₂Na₂O₁₅S₃, 901.1934).

Amaranzole F (3.31): colorless glass; FTIR (ATR, ZnSe) νₘₐₓ 3523, 2948, 1651, 1451, 1233, 1069, 1002, 915, 841 cm⁻¹; HRESITOFMS m/z 901.1948 [M−Na]^− (calcd for C₃₇H₄₇N₂Na₂O₁₅S₃, 901.1934).

Fish Feeding Deterrent Assays. Fish feeding assays employing the bluehead wrasse, Thalassoma bifasciatum, were carried out in paired tests using artificial food pellets (treated and control) as previously described.¹ Briefly, artificial food pellets were formulated with test fractions or pure compounds at natural concentrations found in the sponge (1X) or higher concentrations (2-16X). Groups of 3-5 fish maintained in separate “cells” in flow-through natural seawater aquaria were offered, alternately, a control
pellet (solvent treatment) followed by a treated pellet followed by another control pellet. The assay was repeated for 10 different groups of fish. Assays were conducted at UNCW’s Center for Marine Science or onboard the R/V Seward Johnson (Bahamas). Results were scored as the number of treated pellets, N, eaten, out of a total of 10. A score of 0/10 corresponded to complete deterrence and 10/10 was no different from the control. Treatments were considered ‘deterrent’ if N ≤ 6 per Fisher’s Exact Test. A summary of all fractions tested from *P. amaranthus* (06-04-004b) are summarized in Table 3.6.

**Table 3.6.** Feeding deterrence of *P. amaranthus* fractions against *Thalassoma bifasciatum*.a

<table>
<thead>
<tr>
<th>Fractionation Step</th>
<th>Sample</th>
<th>Concentration Testedb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1c</td>
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<td>10</td>
</tr>
<tr>
<td></td>
<td>06PA2M</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>06PA2M.1</td>
<td>10</td>
</tr>
<tr>
<td>Step 2d</td>
<td>06PA2M.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>06PA2M.3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>06PA2M.2.RCM12</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>06PA2M.2.RCM3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>06PA2M.2.RCM4</td>
<td>9</td>
</tr>
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</tr>
<tr>
<td></td>
<td>06PA2M.2.RCM7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>06PA2M.2.RCM8</td>
<td>10</td>
</tr>
<tr>
<td>Step 4f</td>
<td>amaroxocane A</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>amaroxocane B</td>
<td>3</td>
</tr>
</tbody>
</table>

*a* Activity given by number of pellets eaten of 10. b Multiple of natural concentration. c Partition. d Gravity column. e Prep HPLC. f Semi-prep HPLC. (Assays carried out by Dr. Joe Pawlik, Wai Leong, and Tse-Lynn Loh).
**N,O-Dimethylamaranzole B (3.42):** Excess ethereal diazomethane was added dropwise to a mixture of amaranzoles B, E, and F (≈1:1:1, 1.5 mg) in 0.5 mL MeOH. The mixture was stirred at room temperature for 1.5 h. The solvent was evaporated under a stream of N₂, and the residue was subject to three rounds of reversed phase HPLC; firstly with a gradient of 10-100% (CH₃CN/H₂O + 1M NaClO₄, over 40 min) followed by two passages with 50-100% (CH₃CN/H₂O + 1M NaClO₄ over 40 min) to give 3.42 (ca. 30 μg, based on comparison of UV to (−)-9): UV (MeOH) λₑ_max 261 nm, 286 nm, 309 nm; CD (MeOH) 221 nm (Δε −2.8); ¹H and ¹³C NMR chemical shifts for the steroidal ABCD ring system are nearly identical to those of amaranzole B (3.27). ¹H NMR (600 MHz, CD₃OD) δ 7.73 (d, 2H, 8.8 Hz), 7.61 (s, 1H), 6.95 (d, J = 8.8 Hz, 2H), 5.41 (dd, J = 8.0, 5.4 Hz, 1H), 5.08 (m, 1H), 4.90 (m, 1H), 4.05 (s, 3H), 3.82 (s, 3H), 1.84 (s, 3H); ¹³C NMR (150 MHz, CD₃OD, partial data) δ 160.2 (C34), 144.2 (C25), 142.6 (C30), 137.2 (C28), 127.4 (C32), 126.6 (C31), 123.4 (C29), 114.7 (C33), 113.6 (C26), 79.9 (C24), 55.4 (OMe), 36.5 (NMe).

**Synthesis of ester 3.41:**

**Imidazole-2-carboxylate ethyl ester (3.38):** Trimethylxlonium tetrafluoroborate (3.31 g, 22.4 mmol) was added to a stirred solution of ethyl thiooxamate²⁹ (2.59 g, 19.4 mmol) in CH₂Cl₂ (60 mL) in three portions over 1 h at room temperature. After the mixture was stirred for an additional 30 min, the solvent was evaporated and the crude product used immediately in the next step. Sodium acetate (3.18 g, 38.8 mmol), 2-amino-1-(4-
methoxyphenyl)ethanone\textsuperscript{32} (3.37, 6.5 g, 19.3 mmol), and acetic acid (60 mL) were added, and the mixture was stirred for 3 h at 100 °C. The mixture was allowed to cool to room temperature, and the solvent was removed under reduced pressure. Water (400 mL) was added, and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried (MgSO\textsubscript{4}), and the solvent was evaporated. The crude product was recrystallized from MeOH/H\textsubscript{2}O to give pure 3.38 (4.18 g, 87% yield): mp 132–134 °C; FTIR (ATR, ZnSe) \(\nu_{\text{max}}\) 2981, 2938, 2837, 1715, 1615, 1479, 1458, 1441, 1379, 1288, 1248, 1178, 1150, 1131, 1027, 795 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\textsubscript{3} + 0.1% TFA-d) \(\delta\) 7.67 (d, \(J = 8.4\) Hz, 2H), 7.42 (s, 1H), 6.92 (d, \(J = 8.4\) Hz, 2H), 4.42 (q, \(J = 7.2\) Hz, 2H), 1.38 (t, \(J = 7.2\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\textsubscript{3}+0.1% TFA-d) \(\delta\) 159.6 (C), 158.6 (C), 139.5 (C), 136.8 (C), 126.9 (CH), 122.9 (C), 120.4 (CH), 114.2 (CH), 62.2 (CH\textsubscript{2}), 55.2 (CH\textsubscript{3}), 14.0 (CH\textsubscript{3}); HRESITOFMS \textit{m/z} 247.1079 [M+H]\textsuperscript{+} (calcd for C\textsubscript{13}H\textsubscript{15}N\textsubscript{2}O\textsubscript{3}, 247.1077).

**Imidazole-2-carboxylate ethyl ester (3.39):** Benzyl chloromethyl ether (46 mL, 0.31 mmol) was added dropwise to a stirred solution of 3.38 (52 mg, 0.21 mmol) and potassium carbonate (395 mg, 2.1 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 5 hours, and then poured into water (30 mL). The aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 × 20 mL). The combined organic extracts were evaporated under reduced, and subject to silica flash chromatography (15% EtOAc/Hex) to give 3.39 (66 mg, 86% yield). IR (ATR, ZnSe) \(\nu_{\text{max}}\) 1710, 1615, 1504, 1461, 1433, 1294, 1246, 1175,
1122, 1091, 1029, 837, 743 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.54 (d, J = 8.4 Hz, 2H), 7.44 (s, 1H), 7.34-7.29 (m, 5H), 6.93 (d, J = 8.4 Hz, 2H), 5.89 (s, 2H), 4.58 (s, 2H), 4.46 (q, J = 7.6 Hz, 2H), 1.45 (t, J = 7.6 Hz, 3H); ¹³C NMR (100MHz, CDCl₃) δ 159.3 (C), 159.2 (C), 142.4 (C), 136.5 (C), 136.2 (C), 128.5 (CH), 128.1 (CH), 127.8 (CH), 126.7 (CH), 125.5 (C), 119.2 (CH), 113.9 (CH), 76.3 (CH₂), 71.1 (CH₂), 61.8 (CH₂), 55.2 (CH₃), 14.3 (CH₃); HRESITOFMS m/z 367.1650 [M+H]⁺ (calcd for C₂₁H₂₃N₂O₄, 367.1658).

**Allylic imidazole ester (3.41):** LiOH (8.9 mg, 0.213 mmol) was added to a stirred solution of compound 6 in THF/H₂O (5:3, 3.2 mL). The mixture was stirred overnight at room temperature. CH₂Cl₂ (2mL) was added, and the aqueous solution neutralized (pH~4-5) with 0.1 M HCl. The aqueous layer was extracted once more and the organic layers were dried (MgSO₄), and the solvent evaporated under reduced pressure. CH₂Cl₂ (2.5 mL) was added, the solution cooled to 0°C, followed by addition of 3.40 (9.4 mg, 0.082 mmol) and DMAP (2 mg, 0.016 mmol) in CH₂Cl₂ (2mL), and EDCI (15.7 mg, 0.082 mmol) in CH₂Cl₂ (0.4 mL). The mixture was stirred at 0°C for 2 hours then overnight at room temperature. The solvent was evaporated under reduced pressure and subjected to silica flash chromatography (4 % EtOAc/Hex) to give 15.7 mg of 3.41 (44 % yield, over three steps). IR (ATR, ZnSe) ν max 2957, 2918, 2849, 1710, 1458, 1432, 1291, 1247, 1175, 1090 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 7.75 (s, 1H), 7.73 (d, J = 8.8 Hz, 2H), 7.31-7.25 (m, 5H), 6.96 (d, J = 8.8 Hz, 2H), 5.90 (s, 2H), 5.46 (dd, J = 8.0, 5.6 Hz, 1H), 5.09 (m,
1H), 4.61 (s, 2H), 3.82 (s, 3H), 1.92-1.84 (m, 1H), 1.82 (brs, 3H), 1.79-1.70 (m, 1H), 1.49-1.34 (m, 2H), 0.76 (t, J = 7.6 Hz, 3H); HRESITOFMS m/z 435.2278 [M+H]+ (calcd for C_{26}H_{31}N_{2}O_{4}, 435.2278).

**Synthesis of Model Compounds (±)-3.43 and (+)-R-3.44:**

**N,O-dimethyl imidazole methyl ester (3.45):** A solution of ethereal diazomethane (0.2M) was added dropwise to a stirred solution of 3.38 (100 mg, 0.406 mmol) in MeOH/Et₂O (8:3, 5.5 mL) at room temperature. When a yellow color persisted, excess diazomethane (10 equiv) was added, and the reaction was stirred for 24 h. The solvent was evaporated under a stream of nitrogen, and the mixture was subjected to silica flash chromatography (1:4 EtOAc/hexanes) to give 3.45 (64 mg, 64% yield) and 3.46 (34 mg, 34% yield).

(3.45): FTIR (ATR, ZnSe) νₘₐₓ 2952, 2838, 1709, 1613, 1443, 1419, 1248, 1179, 1151, 1116, 1047, 1024, 930, 832 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.72 (d, 2H, 8.8 Hz), 7.23 (s, 1H), 6.91 (d, 2H, 8.8 Hz), 4.04 (s, 3H), 3.96 (s, 3H), 3.82 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 161.6 (C), 161.2 (C), 143.9 (C), 138.0 (C), 128.5 (CH), 127.7 (C), 123.4 (CH), 115.9 (CH), 57.2 (CH₃), 54.3 (CH₃), 38.0 (CH₃); HRESITOFMS m/z 247.1080 [M+H]+ (calcd for C_{13}H_{15}N_{2}O_{3}, 247.1077). (3.46): FTIR (ATR, ZnSe) νₘₐₓ 2952, 1711, 1453, 1290, 1252, 1201, 1129, 1029, 949, 839, 789 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.30 (d, J = 8.0 Hz, 2H), 7.14 (s, 1H), 6.98 (d, J = 8.0 Hz, 2H), 3.94 (s, 3H), 3.91 (s, 3H), 3.85 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 160.1 (C), 159.9 (C), 138.5 (C), 136.7 (C), 130.6 (CH), 128.5 (CH), 120.8 (C), 114.3
(CH), 55.3 (CH₃), 52.1 (CH₃), 33.7 (CH₃); HRESITOFMS m/z 247.1076 [M+H]+ (calcd for C₁₃H₁₅N₂O₃, 247.1077).

**Imidazole-2-carboxylic Acid (3.47):** A solution of LiOH (10.4 mg, 0.248 mmol) in THF/H₂O (8:3, 3.2 mL) was added to ester 3.45 (43 mg, 0.165 mmol), and the mixture was stirred overnight at room temperature. The THF was evaporated under reduced pressure, and the residual aqueous solution was cooled to 0 °C. Aqueous HCl (0.1M) was added dropwise to a pH=4, at which point the free acid precipitated as a white solid. The mixture was centrifuged, the supernatant removed, and the solid washed with H₂O (2 mL) and dried under high vacuum to give the free acid 10 (30.3 mg, 79%) as a colorless solid. 3.47: FTIR (ATR, ZnSe) νmax 2909, 2834, 1659, 1327, 1270, 1254, 1023, 910, 811, 796 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆) δ 7.81 (s), 7.72 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 3.95 (s, 3H), 3.77 (s, 3H); ¹³C NMR (100 MHz, DMSO- d₆) δ 159.3 (C), 158.7 (C), 138.3 (C), 137.5 (C), 126.0 (CH), 125.1 (C), 121.8 (CH), 114.1 (CH), 55.1 (CH₃), 35.7 (CH₃); HRESITOFMS m/z 233.0923 [M+H]+ (calcd for C₁₂H₁₃N₂O₃, 233.0921).

**Allylic imidazole ester (3.43):** A solution of EDCI (30.0 mg, 0.156 mmol) in CH₂Cl₂ (3 mL) was added dropwise to a stirred solution of the 3.47 (21.1 mg, 0.091 mmol), (±)-2-methylhex-1-en-3-ol₃⁹ (26 mg, 0.228 mmol), and DMAP (2.4 mg, 0.020 mmol) in CH₂Cl₂ (2 mL) at 0 °C. The mixture was stirred for an additional 2 h and then at room temperature for 18 h before removal of solvent under reduced pressure and purification of the residue by silica flash
chromatography (15:85 EtOAc/hexanes) to give the ester (±)-3.43 (22.1 mg, 74% yield): FTIR (ATR, ZnSe) νmax 2958, 1707, 1450, 1281, 1262, 1123, 1105, 836, 786 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.73 (d, J = 8.8 Hz, 2H), 7.20 (s, 1H), 6.91 (d, J = 8.8 Hz, 2H), 5.43 (t, 1H, 6.8 Hz), 5.09 (m, 1H), 4.95 (m, 1H), 4.00 (s, 3H), 3.82 (s, 3H), 1.93–1.85 (m, 1H), 1.83 (s, 3H), 1.79–1.70 (m, 1H), 1.50–1.34 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); ¹H NMR (400 MHz, CD₃OD) δ 7.70 (d, J = 8.8 Hz, 2H), 7.55 (s, 1H), 6.93 (d, J = 8.8 Hz, 2H), 5.45 (dd, J = 8.0, 5.6 Hz, 1H), 5.08 (m, 1H), 4.95 (m, 1H), 4.00 (s, 3H), 3.80 (s, 3H), 1.93–1.85 (m, 1H), 1.83 (s, 3H), 1.80–1.71 (m, 1H), 1.50–1.32 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 160.8 (C), 159.4 (C), 144.7 (C), 143.0 (C), 137.7 (C), 127.7 (CH), 127.0 (C), 123.7 (CH), 115.0 (CH), 113.8 (CH₂), 79.8 (CH), 55.7 (CH₃), 36.7 (CH₃), 35.9 (CH₂), 19.9 (CH₂), 18.4 (CH₃), 14.1 (CH₃); HRESITOFMS m/z 329.1866 [M+H]⁺ (calcd for C₁₉H₂₅N₂O₃, 329.1860).

**Allylic imidazole ester (−)-3.44:** A solution of EDCI (32.0 mg, 0.166 mmol) in CH₂Cl₂ (4 mL) was added dropwise to a mixture of 3.47 (32.0 mg, 0.138 mmol), (R)-2-methylpent-1-en-3-ol (27.6 mg, 0.276 mmol), and DMAP (1.7 mg, 0.014 mmol) in CH₂Cl₂ (1 mL) at 0 °C with stirring. The mixture was stirred at 0 °C for 2 h and then at room temperature for 18 h. The solvent was removed under reduced pressure and the residue subjected to silica flash chromatography (15:85 EtOAc/hexanes) then reversed-phase HPLC (C₁₈, 3:1 CH₃CN/H₂O) to give the ester (−)-3.44 (32.5 mg, 75% yield): [α]²⁴_D −61.9 (c
1.14, CHCl₃); FTIR (ATR, ZnSe) νₘₐₓ 2966, 1705, 1505, 1448, 1400, 1246, 1120, 1089, 1030, 949, 903, 835, 794 cm⁻¹; UV (MeOH) λₘₐₓ 261 nm (ε 16200), 286 nm (ε 15200), 309 nm (ε 8600); CD (MeOH) λ 222 nm (Δε -2.9), 287 (Δε -1.9), 306 (Δε -1.6); ¹H NMR (400 MHz, CD₃OD) δ 7.71 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 6.94 (d, J = 8.8 Hz, 2H), 5.36 (t, J = 6.8 Hz, 1H), 5.09 (m, 1H), 4.97 (m, 1H), 4.03 (s, 3H), 3.81 (s, 3H), 1.95–1.80 (m, 2H), 1.83 (s, 3H), 0.98 (t, J = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 160.8 (C), 159.4 (C), 144.3 (C), 143.0 (C), 137.7 (C), 127.7 (CH), 127.0 (C), 123.7 (CH), 115.0 (CH), 114.0 (CH₂), 81.5 (CH), 55.7 (CH₃), 36.7 (CH₃), 26.7 (CH₂), 18.4 (CH₃), 10.4 (CH₃); HRESITOFMS m/z 315.1703 [M+H]⁺ (calcd for C₁₉H₂₃N₂O₃, 315.1703).

(R)-2-Methylpent-1-en-3-ol (+)-3.48: Diethylzinc (8 mmol, 1M in hexanes) was added to a stirred solution of (+)-MIB (48.5 mg, 0.2 mmol) in hexanes (20 mL) at 0 °C. Freshly distilled methacrolein (280 mg, 4 mmol) was added dropwise to the reaction and stirred for 8 h at 0 °C. The reaction was quenched with saturated ammonium chloride (40 mL) and extracted with pentane (3 × 100 mL). The combined organic layers were dried by filtration through MgSO₄, and filtered, and the solvent evaporated under reduced pressure at 0 °C. The mixture was subjected to silica flash chromatography (1:9 diethyl ether/pentane) to give (+)-3.48 (220 mg, 55% yield, 93% ee): [α]²⁴D +4.2 (c 1.0, CHCl₃) [lit. +4.1 (CH₂Cl₂, 90% ee)]³⁹b; (-)-3.48, [α]²⁴D = -5.6 (c 1.0, CHCl₃, >98% ee).³⁹a The % ee was determined by ¹H NMR analysis of the
both (+) and (−)-MTPA esters. \(^1\)H and \(^{13}\)C NMR data were identical to
literature values.\(^{39}\)

### 3.13 References


Figure 3.29. $^{13}$C NMR spectrum of amaranth A (3.13) (100 MHz, MeOH-$d_4$)
Figure 3.30. gCOSY spectrum for amaranzole A (3.13) (600 MHz, MeOH-$d_4$).
Figure 3.31. gHSQC spectrum for amaranzole A (3.13) (600 MHz, MeOH-d₄).
Figure 3.32. gHMBC spectrum for amaranzole A (3.13) (600 MHz, MeOH-d₄, J_H-H = 8 Hz).
Figure 3.33: ROESY spectrum for amaranzole A (3.13) (800 MHz, MeOH-d4).
Figure 3.34. $^1$H NMR spectrum of compound 3.15 (400 MHz, MeOH-$d_4$).
Figure 3.35. $^{13}$C NMR spectrum of compound 3.15 (100 MHz, MeOH-d$_4$).
**Figure S336.** $^1$H NMR spectrum of compound **3.18** (400 MHz, MeOH-$d_4$).
$^{13}$C NMR spectrum of compound 3.18 (100 MHz, MeOH-$d_4$).
Figure 3.38. $^1$H NMR spectrum of compound 3.16 (400 MHz, MeOH-$d_4$).
Figure 3.39. $^{13}$C NMR spectrum of compound 3.16 (100 MHz, MeOH-$d_4$).
Figure 3.40. $^1$H NMR spectrum for compound N-Boc-(R)-2-amino-1-butanol (400 MHz, CDCl$_3$).
Figure 3.41. $^{13}$C NMR spectrum for N-Boc-(R)-2-amino-1-butanol (100 MHz, CDCl$_3$).
Figure 3.42. $^1$H NMR spectrum of compound 3.20 (400 MHz, CDCl$_3$).
Figure 3.43. $^{13}$C NMR spectrum of compound 3.20 (100 MHz, CDCl$_3$).
Figure 3.44. $^1$H NMR spectrum of compound 3.21 (400 MHz, CDCl$_3$).
Figure 3.45. $^{13}$C NMR spectrum of compound 3.21 (100 MHz, CDCl$_3$)
Figure 3.46. $^1$H NMR spectrum of compound 3.22 (400 MHz, CDCl$_3$).
Figure 3.47. $^{13}$C NMR spectrum of compound 3.22 (100 MHz, CDCl$_3$).
Figure 3.48. $^1$H NMR spectrum of compound 3.23 (400 MHz, CDCl$_3$).
Figure 3.49. $^{13}$C NMR spectrum of compound 3.23 (100 MHz, CDCl$_3$).
Figure 3.50. $^1$H NMR spectrum of compound 3.23a (400 MHz, CDCl$_3$).
Figure 3.51. $^{13}$C NMR spectrum of compound 3.23a (100 MHz, CDCl$_3$).
Figure 3.52. $^1$H NMR spectrum of compound 3.19 (400 MHz, MeOH-$d_4$).
Figure 3.53. $^{13}$C NMR spectrum of compound 3.19 (100 MHz, MeOH-$d_4$).
Figure 3.54. $^1$H NMR spectrum of compound 3.24 (400 MHz, MeOH-$d_4$).
Figure 3.55. $^{13}$C NMR spectrum of compound 3.24 (100 MHz, MeOH-$d_4$).
Figure 3.56. $^1$H NMR spectrum of amaroxcane A (3.25) (600 MHz, MeOH-$d_4$, 1.7 mm MicroCryoProbe).
Figure 3.57. $^{13}$C NMR spectrum of amaroxocane A (3.25) (100 MHz, MeOH-$d_4$).
Figure 3.60. gHMBC spectrum of amaroxocane A (3.25) (600 MHz, MeOH-$d_4$, $^nJ_{CH} = 8$ Hz).
Figure 3.61. NOESY spectrum of amaroxocane A (3.25) (600 MHz, MeOH-$d_4$, $t_m = 300$ ms).
Figure 3.62. $^1$H NMR spectrum of amaroxocane B (3.26) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 3.63. gCOSY spectrum of amaroxocane B (3.26) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 3.64. gHSQC spectrum of amaroxocane B (3.26) (600 MHz, MeOH-\textit{d}_4, 1.7 mm MicroCryoProbe).
Figure 3.65. $^1$H NMR spectrum of amaranzole B (3.27) (600 MHz, MeOH-$d_4$, 1.7mm MicroProbe).
Figure 3.66. DQF–COSY spectrum of amaranzole B (3.27) (600 MHz, MeOH-\textit{d}_4, 1.7mm MicroProbe).
Figure 3.67. gHSQC spectrum of amaranzole B (3.27) (600 MHz, MeOH-d₄, 1.7 nm MicroProbe).
Figure 3.68. gHMBC spectrum of amaranzole B (3.27) (600 MHz, MeOH-$d_4$, $^{2,3}J_{HC} = 8$ Hz, 1.7mm MicroProbe).
Figure 3.69. $^1$H NMR spectrum of amaranzole C (3.28) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 3.70. $^1$H NMR spectrum of amaranzole D (3.29) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 3.71. $^1$H NMR spectrum of amaranzole E (3.30) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 3.72. $^1H$ NMR spectrum of amaranzole F (3.31) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
**Figure 3.73.** $^1$H NMR spectrum of $N,O$-dimethyl amaranzole B (3.42) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 3.74. $^1$H NMR spectrum of compound 3.38 (400 MHz, CDCl$_3$+0.1% TFA-d).
Figure 3.75. $^{13}$C NMR spectrum of compound 3.38 (400 MHz, CDCl$_3$+0.1% TFA-d).
Figure 3.76. $^1$H NMR spectrum of compound 3.39 (400 MHz, CDCl$_3$).
Figure 3.77. $^{13}$C NMR spectrum of compound 3.39 (100 MHz, CDCl$_3$).
**Figure 3.78.** $^1$H NMR spectrum of compound **3.41** (300 MHz, CDCl$_3$).
Figure 3.79. $^{13}$C NMR spectrum of compound 3.41 (100 MHz, CDCl$_3$).
Figure 3.80. $^1$H NMR spectrum of compound 3.45 (400 MHz, CDCl$_3$).
Figure 3.81. $^{13}$C NMR spectrum of compound 3.45 (100 MHz, CDCl$_3$).
Figure 3.82. $^1$H NMR spectrum of compound 3.46 (400 MHz, CDCl$_3$).
Figure 3.83. $^{13}$C NMR spectrum of compound 3.46 (100 MHz, CDCl$_3$).
Figure 3.84. $^1$H NMR spectrum of compound 3.47 (400 MHz, DMSO-d$_6$).
Figure 3.85. $^{13}$C NMR spectrum of compound 3.47 (100 MHz, DMSO-$d_6$).
Figure 3.86. $^1$H NMR spectrum of compound 3.43 (400 MHz, MeOH-$d_4$).
Figure 3.87. $^{13}$C NMR spectrum of compound 3.43 (100 MHz, MeOH-$d_4$).
Figure 3.88. $^1$H NMR spectrum of compound (−)-(R)-3.44 (400 MHz, MeOH- $d_4$).
Figure 3.89. $^{13}$C NMR spectrum of compound (−)-(R)-3.44 (100 MHz, MeOH-$d_4$).
CHAPTER 4

XESTOPROXAMINES A-C FROM NEOPETROSIA PROXIMA.
ASSIGNMENT OF ABSOLUTE STEREOSTRUCTURE BY INTEGRATED
DEGRADATION/CHEMICAL CONVERSION-CD ANALYSIS

4.1 Di-Tertiary Amine Alkaloids from Marine Sponges

Polycyclic diamine alkaloids comprise a family of biologically active
macrocycles from sponges of the genera Haliclona, Xestospongia, Petrosia,
Neopetrosia and Reniera, among others. Each structure contains two or
more nitrogen atoms woven into a network of fused heterocyclic and
carbocyclic rings based on piperidine or cyclohexane. From the standpoint of
characterization, polycyclic amines present difficulties in their purification due
to their amphiphilic physical properties while structure elucidation is hampered
by both limited proton chemical shift dispersion in their \(^1\)H NMR spectra and
stereochemical heterogeneity. In fact, the structures of several alkaloids in
this class have succumbed only to X-ray crystallography.

Petrosin (4.1) was the first example of a naturally occurring bis-
quinolizadine macrocycle isolated from Petrosia seriata. The structure, a
racemate, was assigned by NMR and X-ray crystallography. Chemical
conversion by Kitagawa and total synthesis by Heathcock confirmed that the
natural material is a racemic (or of low optical purity). The stereochemical
interpretation of the xestospongins/araguspongine class of macrocyclic bis-1-
oxaquinolizidine alkaloids (represented by xestospongin A (4.2)\textsuperscript{6a} and (+)-7S-hydroxyxestospongin A (4.3)\textsuperscript{5}) from *Xestospongia exigua* and *Xestospongia* sp. is complex but have been addressed by chromatographic, chiroptical, and chemical methods.\textsuperscript{6} The configuration of (+)-xestospongin A [= (+)-aragusponge D (4.2)] was assigned by total synthesis,\textsuperscript{7} and (+)-7S-hydroxyxestospongin A (4.3)\textsuperscript{5} was assigned by X–ray and the modified Mosher method. The sarain alkaloids\textsuperscript{8} from *Reniera sarai* contain an unusual tertiary amine – aldehyde interaction which hampered isolation and characterization. The structure for sarain A (4.4) was ultimately solved by a combination of X–ray studies of acetylated material, and Mosher’s type analysis of the *bis*-MTPA esters.\textsuperscript{8} Ingenamine (4.5)\textsuperscript{9} from *Xestospongia ingens*, was assigned by a combination of NMR and the modified Mosher method. Recently, X–ray analysis by Garson and coworkers reported that bis-piperidine alkaloids, haliclonacyclamines A (4.7) and B (4.8)\textsuperscript{10} from *Haliclona* sp. share the same absolute configurations although the sign of the [\(\alpha\)]\textsubscript{D} is opposite.\textsuperscript{10} Manzamine A (4.9)\textsuperscript{11} was the first of the 3-alkylpiperidine alkaloids to be reported, and suitable crystals were acquired which allowed the relative and absolute configuration to be established. Haliclonin (4.10),\textsuperscript{12} the most recent addition to the diamine family contains a 3-azabicyclononane-[3.3.1] nonane system previously unreported in this class of alkaloids. The relative configuration of 4.10 was assigned by NOESY experiments, and the absolute
configuration was addressed by degradation and comparison to authentic dimethyl malate.

![Chemical Structures](image)

**Figure 4.1.** Representative tertiary diamine alkaloids from marine sponges.

The examples above illustrate the difficulties of stereochemical analysis for diamino polycyclic hydrocarbons; there are no general methods for configurational assignment. Diamine alkaloids are densely functionalized and classical Hofmann degradation experiments cannot provide useful optically
active material. Therefore, configurational assignments have been carried out by X–ray crystallography, total synthesis, or the modified Mosher method. The latter is only effective if the hydroxyl group is located within the ring system (i.e. 4.3, 4.4, and 4.6) or if its stereochemical information can be relayed into the ring system (e.g. Haliclonin (4.10)). Mixtures of enantiomers and diastereomers in natural material have even more attendant complications. Stereochemical assignments to unfunctionalized alkaloids (e.g. keramaphidin (4.11), madangamine A (4.12), haliclonacyclamines, etc.) remain the most difficult.

In this work, three new bis-piperidine alkaloids, xestoproxamines A-C (4.13-4.15) were isolated from Neopetrosia proxima, and their complete absolute stereostructure assigned using an integrated approach based on NMR and circular dichroism (CD). A notable achievement is chiroptical analysis by CD, following double quaternization of the bis-piperidine, to give N,N'-bis-bromophenacyl derivatives which display characteristic split Cotton effects that reliably report the absolute configuration of the bis-piperidine core.

4.2 Structure Determination of Xestoproxamines A-C.

The molecular formula for 4.13 was established as C_{30}H_{52}N_{2} based on HRESIMS data (m/z 441.4207, [M+H]^+). The presence of two 1,2-disubstituted carbon-carbon double bonds was supported by two coupled pairs of CH=CH spin systems in the ^1H COSY spectrum (Table 4.1, δ 5.49 m;
5.37 m; 5.31, m, and 5.28, m) and four CH sp² carbons in the ¹³C DEPT spectrum (δ 131.74, d; 131.69, d; 130.1, d, and 129.1, d). Because all sp² carbons were accounted for and no C=N bonds were present, the remaining N atoms must be sp³ hybridized; therefore, 4.13 is a di-tertiary amine related to the known alkaloids halicyclamine A (4.16),¹⁶ B (4.17),¹⁷ halicionacyclamines A (4.7), B (4.8),¹⁸ E (4.18), arenosclerin A (4.19),¹⁹ and neopetrosiamine (4.20).²⁰ The remaining four degrees of unsaturation were accommodated by four rings.

**Figure 4.2.** Xestoproxamines A–C (4.13–4.15) and representative bispiperidine alkaloids.
Interpretation of $^{13}$C, DEPT, and HSQC data showed six $N$-substituted CH$_2$ groups ($\delta$ 58.5, 56.9, 56.4, 55.3, 48.9, and 47.4) that were linked to the remaining ring and chain elements (substructures a-c, Figure 4.3), including two substituted piperidine rings, from 2D NMR evidence (DQF-COSY, TOCSY, gHSQC, and gHMBC), in particular, HMBC cross-peaks at of H3/C7, H4/C7, and H10/C3. Contiguous spin systems identified by TOCSY cross-peaks of H13b/H16b and H11b/H14b secured two linking hydrocarbon chains between the CH$_2$-N and CH-N groups. Substructure c was joined to a by HMBC cross-peaks from H2 to both C29 and C30. The final ring was closed by connecting substructure b to c through HMBC correlations from H22 to C24.

![Figure 4.3. Substructures a-c of xestoproxamine A (4.13). Points of attachment of linking chains are indicated by •.](image)

The relative configuration of the conjoined bis-piperidine rings in 4.13 (designated rings A and B, Figure 4.4a) was be established from $J$ coupling constants and NOESY data. The relative orientation of H2$_{ax}$ and H3$_{ax}$ was defined by $J$-coupling analysis using data from a 1D-TOCSY experiment; irradiation of H5$_{eq}$ ($\delta$ 3.48, brd) showed that the two large coupling constants of H1$_{ax}$ (dd, $J = 12.0, 12.0$ Hz) were paired with H1$_{eq}$ (δ 3.18, m) and H2$_{ax}$ (δ
2.29, m), respectively. In addition, H3\textsubscript{ax} shared two large scalar couplings \((J = 11.0 \text{ Hz})\) to vicinal protons H2\textsubscript{ax} and H4\textsubscript{ax}. Additional large couplings \((J = 12.0\) and \(12.0 \text{ Hz}, \text{ respectively})\) from H7\textsubscript{ax} and H9\textsubscript{ax} to H8\textsubscript{ax} \((\delta 1.06, \text{ ddd}, J = 12.0, 12.0, 12.0 \text{ Hz})\) showed the three protons to be axially disposed. The relative configuration of the remaining stereocenters in 4.13 was assigned by interpretation of NOESY correlations. The dihedral angle of \(\sim 90 ^\circ\) C between H3 and H9 was supported by lack of vicinal coupling \((^3J \sim 0 \text{ Hz})\). Conformational constraints placed on the rings by NOE and J data, in particular, mutual dipolar coupling between the pairs H2\textsubscript{ax} and H8\textsubscript{ax} and H10\textsubscript{eq} and H3\textsubscript{ax}, lead to the depicted relative configuration and a conformer that places the averaged planes of ring A and B orthogonal to each other (Figure 4.4a).

![Figure 4.4](image-url)

**Figure 4.4.** Selected NOESY correlations between the piperidine rings of (a) xestoproxamine A (4.13) and B (4.14) (b) xestoproxamine C (4.15). Points of attachment of linking chains are indicated by ⋄. Note, ring 'A' has undergone ring-flip in 4.15 with respect to 4.13.
Xestoproxamine B (4.14) has a molecular formula of C₃₀H₅₄N₂, with one degree of unsaturation less than xestoproxamine A (4.13). The ¹H NMR signals for 4.14 (Table 4.2) were similar to those of 4.13 except for the presence of two vinyl protons (δ 5.48, m and 5.33, m) indicating only one C=C double bond. 2D NMR data confirmed both 4.13 and 4.14 share the same bis-piperidine ring system, including relative configuration (J_HH and NOESY). The double bond was positioned between C17/C18 by COSY and TOCSY correlations to H19. Therefore xestoproxamine B (4.14) is 27,28–dihydro xestoproxamine A (4.13).

The third new bis-piperidine alkaloid, xestoproxamine C (4.15) C₃₁H₅₆N₂, is a higher homolog of 4.13 and 4.14 and showed a characteristic signal for a secondary methyl branch in the ¹H NMR spectrum (Table 4.3, δ 0.87, d, J = 6.7 Hz) that was absent in the latter compounds. 2D NMR and NOESY data indicated the configuration of 4.15 in the core bis-piperidine heterocycle differed from 4.13 and 4.14 but corresponded to the relative configuration found in haliclonacyclamine A (4.9)⁵. While ring B resides in the same conformation as 4.13 and 4.14, ring A in 4.15 has undergone ring–flip to the opposite chair conformation, apparently without inversion at the ring A sp³ nitrogen (NOESY, Figure 4.4b). This conformational change is a consequence of the inversion of the C2 configuration, yet in the new torsional arrangement a dihedral angle of ~90° for H3-C3-C9-H9 (³J_H3-H9 ~0 Hz) was retained.
Table 4.1. $^1$H (600 MHz) and $^{13}$C NMR (125 MHz) for xestoproxamine A (4.13) (CD$_3$OD).

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<th>No.</th>
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<th>$\delta_h$, mult (J in Hz, ax/eq)</th>
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<th>HMBC$^b$</th>
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$^a$ Determined from DEPT and HSQC. $^b$ HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon. $^c$ Observed by HSQC. $^d$ May be interchanged. $^e$ Coupling constant assigned by 1-D TOCSY (irradiation of H5$_{eq}$).
Table 4.2. $^1$H (600 MHz) and $^{13}$C NMR (125 MHz) for xestoproxamine B (4.14) (CD$_3$OD).

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<td>30</td>
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<td></td>
<td></td>
<td>1.44, m</td>
<td></td>
<td>1, 2, 3</td>
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</tbody>
</table>

$^a$ Determined from DEPT and HSQC. $^b$ HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon. $^c$ Observed by HSQC. $^d$ May be interchanged.
**Table 4.3.** $^1$H (600 MHz) and $^{13}$C NMR (150 MHz) NMR Data for xestoproxamine C (4.15) (CDCl$_3$).

<table>
<thead>
<tr>
<th>No.</th>
<th>$\delta$C, mult.$^a$</th>
<th>$\delta$H, mult (J in Hz, ax/eq)</th>
<th>DQF-COSY</th>
<th>HMBC$^b$</th>
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<tbody>
<tr>
<td>1</td>
<td>53.1, CH$_2$</td>
<td>2.93, dd (12.0, 12.0, ax)</td>
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<td>2.55, brd (12.0, eq)</td>
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<td>2.03, m (ax)</td>
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<td>3</td>
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<td>1.96, m (eq)</td>
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<td>2.12, m (ax)</td>
<td>3, 5</td>
<td>2</td>
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<td></td>
<td>1.80, m (eq)</td>
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<td></td>
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<td></td>
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<td></td>
<td>1.00, ddd (12.0, 12.0, 12.0, ax)</td>
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<td>5.44, m</td>
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<td></td>
<td>0.93, m</td>
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<td>2.48, m</td>
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<td>1.48, m</td>
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<td>1.51, m</td>
<td>22, 24, 31</td>
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<td>1.09, m</td>
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<td>25-30</td>
<td>26.8-27.7</td>
<td>1.50-1.20, m</td>
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<td></td>
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<tr>
<td>31</td>
<td>21.2, CH$_3$</td>
<td>0.87, d (6.7)</td>
<td>23</td>
<td>22, 23, 24</td>
</tr>
</tbody>
</table>

$^a$ Assigned from HSQC/HMBC. $^b$ HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.
4.3 Absolute Configuration of C23 in Xestoproxamine C.

Although xestoproxamine C (4.15) crystallizes, their quality was unsuitable for X-ray diffraction analysis. Stereoanalysis by spectroscopic means would require separate assignments of the two stereoelements in 4.15; the C23 stereocenter and the bis-piperidine core. We devised the following degradative approach for assignment of the lone C23 stereocenter exo to the heterocyclic core. Hofmann elimination of suitably quaternized nitrogen atoms in 4.15 would provide a terminal olefin (Δ21), which could then be subjected to cross metathesis with a substituted styrene to insert a chromophore next to the secondary Me branch (first sphere of asymmetry), rendering the molecule amenable to analysis by CD. Due to the scarcity of 4.15, the method was first piloted with more abundant xestoproxamine A (4.13).

Catalytic hydrogenation of 4.13 (Pd-C, MeOH, H2), purification, and conversion of the product to the free base 4.21 by HPLC with buffered solvent (Phenomenex Lux-cellulose; CH3CN/i-PrOH/Et2NH), and immediate exhaustive alkylation with CH3I cleanly provided the bis-methiodide salt 4.22 (Scheme 1). The iodide counter ion was exchanged with hydroxide by treatment of 4.22 with either Ag2O or strong anion exchange resin (Amberlite-IRA 400, HO- form). The neat quaternary ammonium hydroxide double salt was subjected to microwave-promoted Hofmann elimination (10 min, 300 W, 140°C) to afford one major alkene product, 4.23. The structure of 4.23 was confirmed by 2D NMR (COSY, TOCSY, HSQC, and HMBC), which showed
piperidine ring B was intact: exo cleavage of the C5−N bond was supported by the $^1$H NMR multiplicity of H4 (δ 5.55, dt, $J = 17.0$, 10.3 Hz). In contrast, ring A had undergone endo cleavage of the C21–N bond; and the vinyl H22 methine signal exhibited the more complex pattern associated with a terminal allyl group (δ 5.80, dddd, $J = 17.0$, 10.3, 6.8, 6.8 Hz, 1H). Alkene 4.23 was subjected to cross metathesis with excess 2-methoxy-6-vinylnaphthalene$^{21}$ (4.24, ~10 eq) in the presence of Grubbs’ second generation catalyst (4.25)$^{22}$ which selectively engaged only the less hindered vinyl group derived from ring B to give the conjugated naphthalene 4.26.

Scheme 4.1. Hofmann degradation of xestoproxamine A (4.13) and cross metathesis with 6-MeO-2-vinylnaphthalene (4.26).
The UV spectrum of 4.29 ($\lambda_{\text{max}}$ (CH$_2$CN) 246 and 292 nm) showed characteristic bands for the conjugated naphthalene chromophore, however – as expected – this styrenyl system was CD silent due to the remoteness of the chromophore from the distal sphere of asymmetry near C2 (xestoproxamine numbering, Figure 4.5).

![Chemical Structures]

**Scheme 4.2.** Synthesis of model compound 4.32.

In order to assign the observed Cotton effect induced by asymmetry near the C23 center in 4.15, an appropriate methyl-branched model was required. Because the Cotton effect anticipated for the degradation/cross metathesis product of 4.15 would arise from asymmetric perturbation of the 2–alk–1–enyl 6-methoxynaphthalene chromophore, the model could be simple and need only place the chromophore adjacent to an allylic methyl branch of defined configuration, and appended to a short chain.
Preparation of the model is depicted in Scheme 4.2. The α-branched propionamide 4.29, obtained by diastereoselective Myers alkylation of (R,R)-(−)-N-propionylpseudoephedrine23 (4.27) with 1-iodosilyloxy ether 4.28 (n-BuLi, LiCl, (iPr)₂NEt),24 was selectively reduced (LiBH₃N[(CH₂)₄], rt) to the alcohol 4.3025 (%ee >94% by modified Mosher method).26 Swern oxidation of 4.30 to the corresponding aldehyde, followed by Wittig olefination (Ph₃PC=CH₂) gave terminal olefin 4.31. Finally, cross metathesis of 4.31 (Grubbs’ II 4.25), 6–methoxy–2–vinlynaphthalene (4.24) provided model alkene (S)-4.32.

Scheme 4.3. Hofmann degradation of xestoproxamine C (4.15) and cross metathesis with 6–methoxyl–2–vinlynaphthalene (4.25).

Xestoproxamine C (4.15) was converted to 4.33, via the intermediates 4.34-4.36 (Scheme 4.3), by a similar sequence of reactions used to transform
4.13 into 4.26. The CD spectra (Figure 4.5 and Table 4.5) of synthetic (S)-4.32 [λ 256 nm (Δε +3.9)] and 4.33 [λ 255 nm (Δε +3.8)] were of the same sign and magnitude; therefore, 4.33 and xestoproxamine C (4.15) have the 23S configuration.

![CD spectra graph](image)

**Figure 4.5.** CD spectra for (a) 4.33, (b) 4.32, (c) 4.26 (MeOH at 23°C).
4.4 Nakanishi’s Approach to Configurational Assignment of Tertiary Amines.

No simple spectroscopic method was available for defining the absolute configuration of the bis-piperidine ring system in 4.13-4.15. Since this is an outstanding problem of configurational analysis in this class of alkaloids, we turned to refinement of a general CD method for assignment of cyclic tertiary amines first published by Nakanishi and coworkers.27

Scheme 4.4. Nakanishi’s configurational assignment of quinuclidinols by the Exciton Chirality Method.

Nakanishi’s assignment of the absolute configuration of quinuclidinols27 – bicyclic tertiary amines – followed a two-step esterification of the pendant secondary OH group with p-methoxy-cinnamoyl chloride, and alkylation of the tertiary N with phenylbenzylchloride, and gave quaternary ammonium salt 4.37.
(Scheme 4.4). Interpretation of the resultant split CD spectrum arising from exciton coupling of the two arene chromophores led to the absolute configuration. The conformation of the p-methoxycinnamate group follows from other esters where the ester carbonyl aligns syn with the carbinol methine proton, and the transition dipole lies roughly parallel to the C–O bond. On the other hand, the phenylbenzyl group is a free rotor with three major gauche conformers all with small energy differences (<0.3 kcal/mol). The resultant CD spectra results from averaging of the three conformers. The conformer depicted as 4.37a has the highest contribution to the CD spectra because of the angle subtended between the chromophores is ~60-65°, whereas the other two conformers 4.37b and 4.37c have small contributions to the CD spectra because the angles are ~3° (negligible ECCD) and ~120-125° (weak positive ECCD), respectively.

4.5 Quaternization of Xestoproxamines and configurational assignment by the exciton chirality method.

Although the xestoproxamines differ considerably from the quinuclidinols the same principle could be applied: quaternization of both N atoms with a suitable chromophore and interpretation of exciton coupling CD (ECCD) would be informative of the absolute configuration of the heterocyclic bis-piperidine core. However, three possible problems were anticipated. First, the distance between chromophores attached to the N atoms in 4.13-4.15 is considerably larger than those in derivatized quinuclidinols with an expectedly
weaker ECCD. Second, it was anticipated that possible differences in conformations of the natural products and quaternized derivatives, along with the introduction of two new stereocenters at N would complicate non-empirical interpretation of ECCD effects. On the other hand, the stereochemical outcome of N-quaternization should be predictable. The alkylating reagent should approach both N atoms in 4.13-4.15 along a trajectory in line with the lone pair without inversion of the N-configuration. Third, we expected complications due to rotational freedom of the C–N bond that may give weak ECCD or one that would be not easily interpretable. In either case, the resultant configuration and conformation of the derivatives would be revealed by CD and could be interpreted as a 'fingerprint' spectrum that relates the handedness of the heterocyclic core.

The foregoing CD method would confer an important advantage to chiroptical analysis of antipodal bis-piperidines by CD over comparisons using $\alpha_0$. Optical rotations of alkanamines are typically weak in magnitude and comparisons of specific rotation alone are notoriously unreliable due to changes in sign resulting from even slight structural variants (unsaturation, regioisomers). By comparing a fingerprint Cotton effect with that of a bis-piperidine phenacyl derivative of known absolute configuration, the configuration of any member of the series could be assigned.

We initiated our chiroptical investigations by using the same phenylbenzyl chromophore Nakanishi had used for quinuclidinols.
Hydrogenation of xestoproxamine A, conversion of the product to the free base (KOH), alkylation (phenylbenzylbromide), and HPLC purification gave bisphenylbenzyl quaternary ammonium salt 4.38. CD revealed a weak bisignate ECCD spectrum (λ 248 nm (Δε +2.2), 267 (Δε –2.1)). A similar reaction sequence carried out on an alkaloid of known configuration (–)-perhaliclonacyclamine (4.39) (a gift from Prof. Mary Garson) derived from tetrahydrohaliclonacyclamine (4.40) gave 4.41, but showed no significant ECCD spectra or characteristic Cotton effects.

\[
\begin{align*}
\text{4.13} \quad \text{xestoproxamine A} & \quad \text{1) H}_2, \text{Pd/C} \\
& \quad \text{AcOH/MeOH} \\
& \quad \text{2) KOH, MeOH} \\
& \quad \text{3) PhBnBr, Tol, 100°C} \\
\text{4.38} \\
\text{4.39} \quad \text{(–)-perhaliclonacyclamine} & \quad \text{1) KOH, MeOH} \\
& \quad \text{2) PhBnBr, Tol, 100°C} \\
& \quad 2\text{CF}_3\text{COO}^- \\
\end{align*}
\]

**Scheme 4.5.** Preparation of phenylbenzyl derivatives 4.38 and 4.41.

At the onset of these chiroptical studies, encountering difficulties were foreseen in assigning the orientation of the chromophores and their transition dipoles. In Nakanishi’s quinuclidinol example (4.37), only three conformers
about the N–CH$_2$–(biphenyl) need to be considered because the
cinnamoyl group is in a fixed orientation. However, in the case of the
bispiperidine alkaloids, double quaternized derivatives $N,N'$-bis-phenylbenzyl
chromophores, nine possible gauche conformers are possible which give (+)
and (−) chirality depending on the prevailing conformation. This approach is
complicated because the torsion imposed by the benzylic methylene group
introduces variability in both the sign of the angle and distance between
phenylbenzyl groups.

![Graph showing CD spectra](image)

**Figure 4.6.** CD spectra for phenylbenzyl derivatives 4.38 and 4.41.
Table 4.4. Preparation of bis-p-bromophenacyl derivatives.

<table>
<thead>
<tr>
<th>starting material</th>
<th>C2 config</th>
<th>step 1</th>
<th>step 2</th>
<th>product</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.21 tetrahydroxestoproxamine</td>
<td>2R</td>
<td>a</td>
<td>c</td>
<td>4.42</td>
</tr>
<tr>
<td>4.39 (−)-perhalicycloncyclamine A</td>
<td>2S</td>
<td>a</td>
<td>c</td>
<td>4.43</td>
</tr>
<tr>
<td>4.44 perhalicycloncyclamine E</td>
<td>2R</td>
<td>b</td>
<td>c</td>
<td>4.45</td>
</tr>
<tr>
<td>4.34 dihydroxestoproxamine C</td>
<td>2S</td>
<td>b</td>
<td>d</td>
<td>4.46</td>
</tr>
</tbody>
</table>

conditions: a. KOH, MeOH. b. HPLC, Lux-cellulose, CH₃CN/i-PrOH/Et₂N. c. p-bromophenacylbromide, toluene, 90°C, 12h. d. p-bromophenacylbromide, toluene, μwave, 80°C.

Since the phenylbenzyl perhydrohalicycloncyclamine 4.41 lacked an EC spectra useful for configurational assignment, we chose a different chromophore that would result in more tractable interactions. An N-(4-bromophenacyl) chromophore (λmax 265 nm, ε ~12,000) was introduced by exhaustive alkylation of the alkaloids to their corresponding quaternary ammonium salts. The bis-TFA salt of tetrahydroxestoproxamine (4.21) was converted to the free base (KOH/MeOH) followed by alkylation with p-bromophenacylbromide (toluene, 90 °C) to give 4.42 after HPLC purification (Table 4.4). The CD spectrum (Table 4.5) of 4.42 showed a characteristic
negative split Cotton effect (λ = 273 nm, Δε = -6.2; 253 (+2.5)) arising from exciton coupling of equatorial and axial \( N \)-\( p \)-bromophenacetyl chromophores disposed with a negative helicity (see Figure 4.7a).

![chemical structures](image)

**Figure 4.7.** Selected NOESY correlations for bis-\( p \)-bromophenacetyl tetrahydroxestoproxamine A (4.42).

The relative conformations of the conjoined bis-piperidines in 4.42 and 4.13 maintain the same conformations present in the starting materials (analysis of \( J \) and NOESY data, Figure 4.7a). The \( p \)-bromophenacetyl group overcomes some of the complications associated with the phenylbenzyl group. Once the quaternary nitrogen is formed, an \textit{s-trans} conformation about the \( N−CH_2−(C=O)−phenyl \) is adopted. Conformational analysis (MMFF) of \( p \)-bromophenacetyl trimethyl quaternary ammonium salt (4.49), shows the \textit{s-trans} conformation to be the only relevant conformer (Figure 4.7c), presumably due to favorable dipole charge stabilazation. Assuming the bromophenacetyl groups align similarly in the bis-piperidine alkaloids, the transition dipoles align roughly...
parallel to the C–N bond, and provides easier interpretation of the resulting ECCD spectra. To verify that the predicted ECCD spectra were consistent with an assigned configuration, we chose to make empirical chiroptical comparisons with p-Br-phenacyl derivatives of a compound of known configuration.

The relative configuration of (−)-'perhaliclonacyclamine' (4.39), the hydrogenation product of (+)-tetrahydrohaliclonacyclamine A (4.40) obtained from an Indonesian sponge, *Halichondria* sp., differs from xestoproxamine A (4.13) in the relative configuration at C2 and the presence of two additional CH₂ groups in the lower linking chain. A sample of (−)-4.39 was alkylationed in the same manner (see above) to give derivative 4.43. The CD spectrum of 4.43 (λ, 273 nm (Δε –6.2); 254, (Δε +3.3); Figure 4.8 and Table 4.5) is almost identical to that of 4.42. Therefore, despite differences in relative configuration in the two piperidine rings in 4.42 and 4.43, both conform to similar chromophore alignments (a negative CH₂-N-N-CH₂ angle)–imposed largely by the constraint of the C3-C9 torsional angle–and CD reveals they share the same absolute stereostructure form with the exception of the epimeric C2 position.
A sample of haliclonacyclamine E (4.18), isolated from a Brazilian sample of *Arenosclera braziliensis*, was hydrogenated (H₂, Pd-C) to the tetrahydro–derivative 4.44 and exhaustively alkylated (p-bromophenacylbromide, 90 °C) as before to the bis-p-Br-phenacylated compound 4.45 that showed a CD spectrum (λ 273 nm, Δε = −5.8; 255 (Δε +3.1), Table 4.5) almost identical with those of 4.42 and 4.43.

The CD spectrum of the bis-p-bromophenacyl derivative (4.46, λ 273 nm, Δε = −5.8; 253 (Δε +2.4)), obtained from dihydroxestoproxamine C (4.34)
was identical to those of 4.42, 4.43 and 4.45. Conformational analysis by NMR (J coupling and NOESY, figure 4.7b) showed the A and B rings in 4.15 and 4.46 adopt the same conformations before and after quaternization.

Table 4.5. CD Data (23 °C) for Vinyl-naphthalene, Phenylbenzyl, and bis-p-Bromophenacyl Derivatives.

<table>
<thead>
<tr>
<th>No.</th>
<th>Solvent</th>
<th>( \lambda ) / nm</th>
<th>( \Delta \epsilon )^b</th>
<th>( \lambda ) / nm</th>
<th>( \Delta \epsilon )^b</th>
</tr>
</thead>
<tbody>
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<td>4.26^g</td>
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<td>–</td>
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<td>–</td>
<td>a</td>
</tr>
<tr>
<td>4.32^d</td>
<td>CH(_3)CN</td>
<td>251</td>
<td>+3.8</td>
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<td>CH(_3)CN</td>
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<td>+3.9</td>
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<td>248</td>
<td>+2.2</td>
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<td>4.42^g</td>
<td>CH(_3)OH</td>
<td>273</td>
<td>–6.2</td>
<td>253</td>
<td>+2.5</td>
</tr>
<tr>
<td>4.43^g</td>
<td>CH(_3)OH</td>
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<td>–6.9</td>
<td>254</td>
<td>+3.3</td>
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<tr>
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<td>CH(_3)OH</td>
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<td>–5.8</td>
<td>255</td>
<td>+3.1</td>
</tr>
<tr>
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<td>CH(_3)OH</td>
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<td>–5.8</td>
<td>253</td>
<td>+2.4</td>
</tr>
<tr>
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<td>273</td>
<td>+8.0</td>
<td>253</td>
<td>–4.7</td>
</tr>
</tbody>
</table>

a, only noise. b, mol\(^{-1}\)dm\(^3\)cm\(^{-1}\). c, Scheme 4.1. d, Scheme 4.2. e, Scheme 4.3. f, Scheme 4.4. g, Table 4.4

Therefore the absolute configuration of the heterocyclic cores in xestoproxamine A (4.13), haliclonacyclamine E (4.18), (−)-perhaliclonacyclamine (4.39) and xestoproxamine C (4.15) are the same, with the exception of C2 as noted above. Finally, a sample of (+)-haliclonacyclamine B (4.8) was hydrogenated to 4.47 and alkylated to give the bis \( p \)-bromophenacyl derivative 4.48, which showed the opposite ECCD spectra of 4.43 as expected. Since the absolute configurations of (−)-4.39 and (+)-4.8 were established earlier by X-ray crystallography,\(^{28}\) the stereostructures of 4.13-4.15, and 4.19 are now completely assigned. Bis-
piperidine 4.18 co-occurs with 4.19 and it is likely both of these natural products share the same heterocyclic core configuration.

Table 4.6. Specific rotations and configurational assignment of bis-piperidine Alkaloids.

<table>
<thead>
<tr>
<th>compd.</th>
<th>[α]D</th>
<th>conc. g/100 mL, solvent</th>
<th>linker chain Cn</th>
<th>absolute confign.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>top</td>
<td>bottom</td>
</tr>
<tr>
<td>4.13^b</td>
<td>+4.4</td>
<td>2.0 CH₃OH</td>
<td>C₁₀</td>
<td>C₁₀</td>
</tr>
<tr>
<td>4.14^b</td>
<td>+2.7</td>
<td>2.4 CH₃OH</td>
<td>C₁₀</td>
<td>C₁₀</td>
</tr>
<tr>
<td>4.15^b</td>
<td>−18.5</td>
<td>2.0 CHCl₃</td>
<td>C₁₀</td>
<td>C₁₀</td>
</tr>
<tr>
<td>4.16^c</td>
<td>−7.3</td>
<td>0.73 CH₂Cl₂</td>
<td>C₈</td>
<td>C₁₂</td>
</tr>
<tr>
<td>4.17^d</td>
<td>−143.5</td>
<td>0.65 ?</td>
<td>C₁₀</td>
<td>C₈</td>
</tr>
<tr>
<td>4.7^g</td>
<td>−3.4</td>
<td>1.21 CH₂Cl₂</td>
<td>C₁₀</td>
<td>C₁₂</td>
</tr>
<tr>
<td>4.8^e</td>
<td>+3.4</td>
<td>0.55 CH₂Cl₂</td>
<td>C₁₀</td>
<td>C₁₂</td>
</tr>
<tr>
<td>4.19^f</td>
<td>−3</td>
<td>0.015 MeOH</td>
<td>C₁₀</td>
<td>C₁₂</td>
</tr>
<tr>
<td>4.18^f</td>
<td>+14</td>
<td>0.02 MeOH</td>
<td>C₁₀</td>
<td>C₁₂</td>
</tr>
<tr>
<td>4.39^g</td>
<td>−20.9</td>
<td>0.205 CHCl₃</td>
<td>C₁₀</td>
<td>C₁₂</td>
</tr>
<tr>
<td>4.40^g</td>
<td>+19.4</td>
<td>0.515 CHCl₃</td>
<td>C₁₀</td>
<td>C₁₂</td>
</tr>
<tr>
<td>4.20^i</td>
<td>−10</td>
<td>1.0 CHCl₃</td>
<td>C₁₀</td>
<td>C₁₀</td>
</tr>
</tbody>
</table>

a, Core locants follow the numbering used for 4.13-4.15. Unless indicated by *, depicted CIP assignments are absolute. Configuration of the N atoms not given. b, this work. c, ref.16. d, X-ray, ref.17. e, X-ray, ref.18. f, ref.19. g, X-ray, ref.28. h, relative configuration only. g, ref.32.

A summary of the configurational analysis of 4.13-4.15 and the CIP descriptors for the core stereocenters of related bis-piperidine alkaloids is given in Table 4.6, along with a comparison of reported [α]D values. No clear trend can be seen except that the compounds have high stereochemical heterogeneity that may reflect their geographic origins. The specific rotations vary in sign and magnitude with a strong dependence upon the presence and position of unsaturation in the top and bottom linking chains and possibly the chain lengths. Haliclonaclamamines A (4.7) and B (4.8), from Haliclonas sp.
from the Great Barrier Reef, differ in the linking chains but the cores are antipodal to those of (+)-4.40 from *Halichondria* sp. collected in Indonesia. We note that the recently reported (−)-neopetrosiamine (4.20)\(^ {32}\) from the sponge *Neopetrosia proxima*,\(^ {33}\) collected in the Caribbean sea south of the Bahamas, is very similar to 4.13-4.15, but the configuration was not defined.

4.6 Bioactivity of Bispiperidine Alkaloids

Bis-piperidine alkaloids have shown modest activity against various cancer cell lines. Compounds 4.13-4.15 were assayed in vitro against human colon tumor cells (HCT-116) and showed IC\(_ {50}\) values of 21.2, 6.3, and 5.4 μM respectively.

4.7 Conclusions

In conclusion, three new bis-piperidine alkaloids, xestoproxamines A-C (4.13-4.15) are reported and their complete structures assigned by integrated MS, NMR, and chiroptical analysis. We assigned the absolute configuration of the remote methyl group in xestoproxamine C (4.15) by CD following a Hoffman degradation/cross metathesis protocol, a sensitive technique that can be extended to other natural products containing acyclic allylic methyl branches. Finally, we have shown that ECCD of *N,N*-di-p-bromophenacyl bis-piperidine alkaloids, obtained by quaternization of the tertiary amines, reliably reflects the absolute configurations of the heterocyclic cores in 4.13-4.15, independent of the relative configuration at C2. This observation was exploited to show the two groups of structures, 4.13, 4.14, 4.18, 4.19, and
4.15, 4.39, have the same absolute configuration in their core heterocyclic rings and should be applicable to other bis-piperidine alkaloids in this class.

Chapter 4 is, in part, a reproduction of the material as it appears in the following publication: Morinaka, B. I.; Molinski, T. F. “Xestoproxamines A-C from Neopetrosia proxima. Configurational Assignment of Absolute Stereostructure of Bis-piperidine Alkaloids by Integrated Degradation-CD Analysis” *J. Nat. Prod.* 2011, 74, 430-440. The dissertation author was the primary researcher/author on this paper.

4.8 Experimental Section

**General Experimental Procedures.** General procedures are described in sections 2.7 and 3.12. Yields were determined gravimetrically except for those of masses of less than \(\sim\)100 \(\mu\)g, which are estimated from UV extinction coefficients. HPLC was carried out using either a Gilson model 302 pump equipped with tandem detectors—UV-visible (ISCO model UA-5, \(\lambda\) 254 nm) and refractive index (Waters R401)—or a Rainin HPXL dual-pump equipped with an ASI QuickSplit flow splitter (7:1) between two detectors—a Jasco CD-2095 UV-CD and an ESA model 301 evaporative light scattering detector (ELSD).

**Animal Material:** The sponge *Xestospongia proxima* (now known as *Neopetrosia proxima*)\(^3^3\) was collected in June 2008 at Stirrup Cay, the Bahamas (25° 49.511N 77° 53.924' W) at a depth of 8.5 m and identified by Sven Zea (Universidad Nacional de Colombia, InvaMar). The surface of the
tissue was dark brown and free of epiphytic zoanthids. A voucher sample of
the sponge (08-13-073) is stored at UC San Diego.

**Extraction and Isolation:** A sample of *X. proxima* (252.6 g wet wt.) was
extracted with MeOH (3 x 2.5 L, 25 °C, overnight) then CH₂Cl₂/MeOH (2 x 2.5
L, 25 °C, overnight). The resulting extract was partitioned between hexanes (3
x 500 mL) and 9:1 MeOH/H₂O (1 L). The aqueous MeOH layer was removed
and the H₂O content adjusted to 1:1 MeOH/H₂O before extraction with CH₂Cl₂
(3 x 1 L). The aqueous MeOH layer was concentrated under reduced pressure
and then applied directly onto a reversed-phase (C₁₈) silica column (~ 400 g)
successively eluting with 1:9, 3:7, 1:1, 9:1 CH₃CN/H₂O + 0.2% TFA, and i-
PrOH + 0.2% TFA to give five fractions. A portion (305 mg) of the third
fraction (1.22 g) was subjected to preparative HPLC (reversed-phase, C₁₈, 10
mL min⁻¹, gradient elution with 3:7 to 2:5 CH₃CN/H₂O + 0.3% TFA over 40
min.) to give 12 fractions. The third preparative HPLC fraction (27.2 mg) was
subjected to semi-preparative HPLC (reversed-phase, Synergi-HydroRP, 4µ,
2.5 mL min⁻¹, mobile phase: 2:2:6:0.05 CH₃CN/i-PrOH/H₂O/TFA) to give
xestoproxamine A (4.13, 14.0 mg). A portion (4 mg) of the fourth preparative
HPLC fraction (24.0 mg) was subjected to semi-preparative HPLC over the
same column (2.5 mL min⁻¹, 22.5:22.5:55:0.5 CH₃CN/i-PrOH/H₂O/TFA) to give
xestoproxamine B (4.14, 2.9 mg). The entire sixth HPLC fraction was
subjected to HPLC (Phenomenex Lux-cellulose, 1.5 mL min⁻¹, mobile phase:
9:1:0.02 CH₃CN/i-PrOH/Et₂NH) to give xestoproxamine C (4.15, 1.5 mg).
**Xestoproxamine A (4.13):** Colorless glass; $[\alpha]_D^{24} +4.4$ (c 2.0, MeOH); FTIR (ATR): $\nu$ 1676, 1437, 1203, 1133, 841, 801, 723 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 4.1. HRESIMS $m/z$ 441.4207 [M+H]$^+$ (calcd for C$_{30}$H$_{53}$N$_2$, 441.4203)

**Xestoproxamine B (4.14):** Colorless glass; $[\alpha]_D^{24} +2.7$ (c 2.4, MeOH); FTIR (ATR): $\nu$ 2934, 2862, 1674, 1463, 1199, 1131, 833, 799, 721 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 4.2; HRESIMS $m/z$ 443.4362 [M+H]$^+$ (calcd for C$_{30}$H$_{55}$N$_2$, 443.4358)

**Xestoproxamine C (4.15):** Colorless solid; $[\alpha]_D^{24} -18.5$ (c 0.67, CHCl$_3$); FTIR (ATR): $\nu$ 2933, 2862, 1673, 1470, 1199, 1128, 829, 797, 721 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 4.3; HRESIMS $m/z$ 457.4513 [M+H]$^+$ (calcd for C$_{31}$H$_{57}$N$_2$, 457.4516).

**Degradation of xestoproxamine A:**

**Tetrahydroxestoproxamine A Free Base (4.21):** A solution of xestoproxamine A (4.13) TFA salt (1.0 mg) and Pd/C (10%, 0.2 mg) in MeOH + 2% AcOH (0.5 mL) was vigorously stirred under 1 atmosphere of H$_2$ overnight. The mixture was passed through a membrane filter (0.45 $\mu$). The solvent was removed from the filtrate under reduced pressure, and the residue subjected to HPLC (Phenomenex Lux-cellulose, 1.5 mL min$^{-1}$, mobile phase: 9:1:0.02 CH$_3$CN/i-PrOH/Et$_2$NH) to give tetrahydroxestoproxamine A free base (4.21, 0.55 mg) which was used immediately after characterization. Colorless solid; $[\alpha]_D^{23} -12.1$ (c 0.7, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 2.90 (dt, 1H, $J$
= 12.0, 5.5 Hz), 2.80 (dd, 1H, J = 12.5, 4.0 Hz), 2.73 (brd, 1H, J = 10.0 Hz),
2.70-2.61 (m, 5H), 2.54 (brp, 1H, J = 5.5 Hz), 2.41 (brt, 1H, J = 9.8 Hz), 2.24
(t, 1H, 11 Hz), 2.04 (d, 1H, J = 12.5 Hz), 1.87 (t, 1H, J = 11 Hz), 1.82 (m, 1H),
1.64-1.21 (m, 40H), 0.97 (m, 1H), 0.72 (q, 1H, J = 12 Hz); HRESIMS m/z

**Tetrahydroxestropaxamine A bis-Methiodide Salt (4.22):** Free base 4.21
(0.55 mg) was dissolved in CH₂Cl₂ (0.8 mL), excess CH₃I (0.2 mL) was added,
and the mixture stirred overnight in the dark. Volatiles were removed under a
stream of N₂ to give the bis-methiodide salt (4.22, 0.9 mg) as an off-white
solid. ¹H NMR (500 MHz, CD₃OD) δ 3.84 (d, J = 10.5 Hz, 1H), 3.54-3.49 (m,
3H), 3.41-3.15 (m, 8H) 3.17 (s, 3H), 3.12 (s, 3H), 2.20 – 1.22 (m, 44H), 1.19
(q, J = 12.5 Hz, 1H); ¹³C (125 MHz, CD₃OD) δ 69.4 (CH₂), 66.5 (CH₂), 64.6
(CH₂), 63.6 (CH₂), 60.4 (CH₂), 57.9 (CH₂), 53.3 (CH₃), 47.6 (CH₃), 37.0 (CH),
35.2 (CH), 32.2 (CH), 32.1 (CH₂), 31.8 (CH₂), 31.7 (CH), 29.2 (CH₂),
29.1(CH₂), 29.0 (CH₂), 28.8 (CH₂), 28.6 (2 x CH₂), 28.3 (CH₂), 28.2 (CH₂),
28.1 (CH₂), 27.4 (CH₂), 27.3 (CH₂), 26.8 (CH₂), 26.6 (CH₂), 25.7 (CH₂), 25.3
(CH₂), 22.3 (CH₂), 20.7 (CH₂), 20.2 (CH₂); HRESIMS m/z 237.2453 [M]²⁺
(calcd for [C₃₂H₆₂N₂]²⁺, 237.2454).

**Hofmann degradation of Tetrahydroxestropaxamine A bis-Methiodide
salt:** A solution of the bis-methiodide (4.22, 0.9 mg) was eluted with MeOH
through a short column of strong anion exchange resin (Amberlite IRA-400,
HO⁻ form, prepared from the Cl⁻ form, immediately before use by elution with aqueous 1N NaOH, followed by four washes; distilled H₂O until the eluate was neutral, then MeOH). The eluate was concentrated under reduced pressure to remove the volatiles, and solvent-free methohydroxide was heated (microwave, 300 W, 140 °C, 10 min). The crude product was taken up in MeOH and passed through a reversed-phase silica (C₁₈) cartridge (200 mg) by elution with MeOH + 0.1 % TFA. The solvent was removed under a stream of N₂, and the residue subjected to HPLC (reversed-phase, C₁₈ Phenomenex Luna, 5µ, 10 x 250 mm, gradient: 40-100% CH₃CN/H₂O +0.1 % TFA over 40 min) to give a single major elimination product 4.23 (0.45 mg).

4.23: ¹H NMR (600 MHz, CD₃OD, representative signals) δ 5.80 (dddd, J = 17.0, 10.3, 6.8, 6.8 Hz, 1H), 5.55 (dt, J = 17.0, 10.3 Hz, 1H), 5.31 (dd, J = 10.3, 1.4 Hz, 1H), 5.18 (dd, J = 17.0, 1.4 Hz), 4.98 (dq, J = 17.0, 1.9 Hz, 1H), 4.92 (1H, under solvent), 3.44 (brd, J = 12.5 Hz, 1H), 3.40 (brd, J = 12.5 Hz, 1H), 3.20 (td, J = 12.5, 4.7 Hz, 1H), 3.10 (td, J = 12.5, 5.2 Hz, 1H), 2.92 (s, 3H), 2.85 (s, 3H), 2.54 (t, J = 12 Hz, 1H), 2.53 (t, J = 12 Hz, 1H), 2.18 (t, J = 10.4 Hz, 1H), 2.05 (q, 7.3 Hz, 2H), 0.88 (q, J = 12 Hz, 1H); HRESIMS m/z 473.4828 [M+H]⁺ (calcd for C₃₂H₆₁N₂, 473.4829).

Cross Metathesis of 4.23 with 2-Methoxy-6-vinylNaphthalene (4.26): To a solution of alkene 4.23 (450 µg, 0.642 µmol) and 6-methoxy-2-vinylNaphthalene²¹ (4.24, 1.2 mg, 6.42 µmol) in CH₂Cl₂ (0.5 mL) was added
Grubbs' 2nd generation catalyst\textsuperscript{22} (226 \( \mu \)g, 0.265 \( \mu \)mol) in CH\(_2\)Cl\(_2\) (1 mL) in three portions over a period of 11 h at 80 °C. The solvent was evaporated under a stream of N\(_2\). The crude reaction mixture was passed through a reversed-phase C\(_{18}\) cartridge by elution with MeOH + 0.1% TFA, then subjected to HPLC (Luna, C\(_{18}\), 250 x 10 mm, 40-100% CH\(_3\)CN/H\(_2\)O + 0.1 % TFA over 40 min) to give the 6-methoxy-2-naphthyl-ethenyl derivative 4.26 (240 \( \mu \)g). UV (CH\(_3\)CN) \( \lambda_{\text{max}} \) 246, 292 nm; CD: See Table 4; \(^1\)H NMR (600 MHz, CD\(_3\)OD, representative signals) \( \delta \) 7.685 (d, \( J = 9.0 \) Hz, 1H), 7.681 (d, \( J = 8.6 \) Hz, 1H), 7.60 (brs, 1H), 7.55 (dd, \( J = 8.6, 2.0 \) Hz, 1H), 7.18 (d, \( J = 2.0 \) Hz, 1H), 7.09 (dd, \( J = 9.0, 2.0 \) Hz, 1H), 6.51 (d, \( J = 16.0 \) Hz, 1H), 6.32 (dt, \( J = 16.0, 7.0 \) Hz, 1H), 5.55 (dt, \( J = 17.0, 10.3 \) Hz, 1H), 5.31 (dd, \( J = 10.3, 1.4 \) Hz, 1H), 5.18 (dd, \( J = 17.0, 1.4 \) Hz), 3.90 (s, 3H), 2.91 (s, 3H), 2.83 (s, 3H), 2.50 (t, \( J = 12 \) Hz, 1H), 2.49 (t, \( J = 12 \) Hz, 1H), 2.26 (q, \( J = 7.0 \) Hz, 2H), 0.86 (q, \( J = 12 \) Hz, 1H); HRESIMS m/z 629.5403 [M]\(^+\) (calcd for C\(_{43}\)H\(_{69}\)N\(_2\)O, 629.5404).

**Degradation of Xestoproxamine C**

**Dihydroxestoproxamine C TFA salt (22):** A solution of xestoproxamine C (3) (0.8 mg) and Pd/C (10%, 0.2 mg) in MeOH + 2% AcOH (0.5 mL) was vigorously stirred overnight under H\(_2\) (1 atm). The solution was passed through a membrane filter (0.45 \( \mu \)m) and the volatiles removed under a stream of N\(_2\). The residue was purified by HPLC (reversed-phase, Phenomenex, Synergi-HydroRP, 4 \( \mu \), 10 x 250 mm; 3 mL min\(^{-1}\); mobile phase: 25:25:50:0.5 CH\(_3\)CN/i-PrOH/H\(_2\)O/TFA) to give dihydroxestoproxamine C (4.34). Colorless
glass; $^1$H NMR (500 MHz, CD$_3$OD, representative signals) δ 3.68 (brd, $J = 11.2$ Hz, 1H), 3.48 (td, $J = 13.0$, 4.0 Hz, 1H), 2.91 (t, $J = 12.0$ Hz, 1H), 2.84 (t, $J = 12.0$ Hz, 1H), 2.33 (brd, $J = 12.0$ Hz, 1H), 0.95 (d, $J = 7.0$ Hz, 1H); $^{13}$C NMR (125 MHz, CD$_3$OD) δ 58.0 (CH$_2$), 57.5 (CH$_2$), 56.6 (CH$_2$), 54.9 (CH$_2$), 53.1 (CH$_2$), 43.3 (CH), 40.3 (CH), 37.1 (CH), 35.74 (CH$_2$), 35.67 (CH$_2$), 33.7 (CH$_2$), 33.6 (CH$_2$), 33.3 (CH), 32.9 (CH$_2$), 31.5 (CH), 28.8 (CH$_2$), 25.56 (CH$_2$), 28.53 (CH$_2$), 27.8 (3 x CH$_2$), 27.7 (CH$_2$), 26.8 (CH$_2$), 26.6 (CH$_2$), 26.3 (CH$_2$), 26.2 (CH$_2$), 26.0 (CH$_2$), 25.6 (CH$_2$), 21.9 (CH$_2$), 21.0 (CH$_3$); HRESIMS m/z 460.4748 [M+H]$^+$ (calcd for C$_{31}$H$_{60}$N$_2$, 460.4751).

**Tetrahydroxesteproxamine C bis-Methiodide Salt (4.35):** The TFA salt 4.34 was subjected to HPLC (chiral column, Lux-cellulose, 1.5 mL min$^{-1}$, mobile phase: 9:1:0.02 CH$_3$CN/i-PrOH/DEA) to give the free base (0.5 mg). Immediately after removal of the volatiles, the free base (0.5 mg) was dissolved in CH$_2$Cl$_2$ (0.8 mL), treated with excess CH$_3$I (0.2 mL), and the mixture stirred overnight in the dark. The solvent was evaporated under a stream of N$_2$ to give the methiodide (4.35, 0.8 mg) as an off-white solid. $^1$H NMR (500 MHz, CD$_3$OD, representative signals) δ 3.87 (brd, $J = 11.4$ Hz, 1H), 3.77 (td, $J = 13.8$, 3.0 Hz, 1H), 3.21 (s, 3H), 3.12 (s, 3H), 3.04 (t, $J = 12.0$ Hz, 1H), 1.03 (d, $J = 7.0$ Hz, 3H); $^{13}$C (125 MHz, CD$_3$OD) δ 69.5 (CH$_2$), 69.0 (CH$_2$), 67.4 (CH$_2$), 65.3 (CH$_2$), 61.7 (CH$_2$), 57.2 (CH$_2$) [obscured by solvent] (CH$_3$), 45.9 (CH$_3$), 39.3 (CH), 36.4 (CH$_2$), 36.2 (CH), 35.2 (CH$_2$), 34.0 (CH$_2$), 33.3 (CH), 33.1 (CH), 32.6 (CH$_2$), 31.2 (CH), 30.2 (CH$_2$), 29.5 (CH$_2$), 29.3 (CH$_2$),
28.7 (CH₂), 28.4 (CH₂), 28.2 (CH₂), 27.94 (CH₂), 27.86 (2 x CH₂), 27.5 (CH₂), 26.9 (CH₂), 26.1 (CH₂), 25.3 (CH₂), 25.2 (CH₂), 22.1 (CH₃), 22.0 (CH₂); HRESIMS m/z 244.2531 [M]^{2+} (calcd for [C₃₃H₆₄N₂]^{2+}, 244.2532).

**Hofmann Degradation of Tetrahydroxestoproxamine C bis-Methiodide Salt (4.36):** A solution of the methiodide 4.35 in MeOH was passed through a short column of strong anion exchange resin (Amberlite IRA-400 (Cl⁻), converted to the HO⁻ form with 1N NaOH). After removal of solvent under reduced pressure, the methohydroxide was transferred to a 10 mL microwave vessel, dried, and irradiated (μwave, 300W, 140 °C over 7.5 min, then an additional 4.5 min). The crude product obtained was taken up in MeOH passed through a reversed-phase silica cartridge (C₁₈, 200 mg) eluting with MeOH + 0.1 % TFA. Solvent was removed under a stream of N₂, and the residue purified by HPLC (reversed-phase, C₁₈ Phenomenex Luna, 5 μ, 10 x 250 mm, gradient: 40-100% CH₃CN/H₂O +0.1 % TFA over 40 min) to give the double-elimination product 4.36 (~140 μg).

**4.36:** ¹H NMR (600 MHz, CD₃OD, representative signals) δ 5.67 (ddd, J = 17.2, 10.2, 7.8 Hz, 1H), 5.62 (dt, J = 17.2, 10.0 Hz, 1H), 5.35 (d, J = 10.0 Hz, 1H), 5.29 (d, J = 17.2 Hz, 1H), 4.94 (brd, J = 17.2 Hz, 1H), 2.93 (s, 3H), 2.86 (s, 3H), 2.58 (t, J = 12.2 Hz, 1H), 2.50 (t, J = 12.2 Hz, 1H), 0.98 (d, J = 7 Hz, 3H); HRESIMS m/z 487.4983 [M+H]⁺ (calcd for C₃₃H₆₃N₂, 487.4886).

**Cross Metathesis of 4.36 with 2-Methoxy-6-vinylNaphthalene (4.33):** Grubbs’ 2nd generation catalyst (95 μg, 0.112 μmol) in CH₂Cl₂ (0.8 mL) was
added in three portions over a period of 11 h to alkene 4.36 (140 µg, 0.199 µmol) and 2-methoxy-6-vinylnaphthalene21 (0.8 mg, 4.20 µmol) and the mixture stirred vigorously in a sealed vial at 80 °C. The solvent was removed under a stream of N₂ and the residue passed through a reversed-phase silica cartridge (C₁₈, 200 mg) eluting eluting with MeOH + 0.1% TFA, and finally purified by HPLC (reversed-phase, C₁₈ Phenomenex Luna, 5 µ, 10 x 250 mm, gradient: 40-100% CH₃CN/H₂O +0.1 % TFA over 40 min) to give the 6-methoxy-2-naphthyl-ethenyl derivative 4.33 (6 µg). UV (CH₃CN) λ 246, 292 nm; CD (CH₃CN) λ 251 (Δε +3.9); ¹H NMR (600 MHz, CD₃OD, representative signals) δ 7.69 (d, J = 8.6 Hz, 2H), 7.62 (brs, 1H), 7.55 (dd, J = 8.6, 2.0 Hz, 1H), 7.19 (brs, J = 2.0 Hz, 1H), 7.10 (dd, J = 9.0, 2.0 Hz, 1H), 6.48 (d, J = 16.2 Hz, 1H), 6.16 (dd, J = 16.2, 8.0 Hz, 1H), 5.58 (dt, J = 17.2, 10.0 Hz, 1H), 5.32 (d, J = 10.0 Hz, 1H), 5.27 (d, J = 17.2 Hz, 1H), 3.90 (s, 3H), 2.89 (s, 3H), 2.81 (s, 3H), 1.12 (d, J = 7 Hz, 3H); HRESIMS m/z 643.5563 [M]⁺, calcd 643.5561 for C₄₄H₇₁N₂O.

**Preparation of phenylbenzyl derivatives:**

**Bis-phenylbenzyl tetrahydroxestoproxamine A:** The acetate salt of tetrahydroxestoproxamine A (0.5 mg, 1.06 µmol) was converted to the free base by addition of KOH (200 µg, 3.57 µmol) in MeOH (0.25 mL). After a few minutes the solvent was evaporated under a stream of N₂ then under high vacuum. Toluene (0.5 mL) and phenylbenzyl bromide (17.6 mg, 71.4 µmol) were added and the mixture was stirred at 100°C for 18 h. The solvent was
evaporated under a stream of N₂, then under high vacuum, and the mixture was subjected to HPLC (reversed-phase Phenomenex, Luna, C₁₈, 10 x 250 mm; 2 mL min⁻¹ 40-100 % CH₃CN/H₂O + 0.1% TFA over 40 min) to give the bis-phenylbenzyl TFA salt 4.38 (0.6 mg). UV (MeOH) λmax 256 nm; CD (MeOH) λ 248 (Δε +2.2), 267 (Δε −2.1) nm; ¹H NMR (600 MHz, CD₃OD, representative signals) δ 7.83 (d, J = 8.2 Hz, 2H), 7.81 (d, J = 8.2 Hz, 2H), 7.68 (m, 4H), 7.63 (d, J = 8.2 Hz, 2H), 7.57 (d, J = 8.2 Hz, 2H), 7.49 (t, J = 7.6 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.41 (dd, J = 7.6, 3.5 Hz, 1H), 7.40 (dd, J = 7.6, 3.5 Hz, 1H), 4.92 (d, J = 13.8 Hz, 2H), 4.62 (m, 3H), 3.61 (brd, J = 11.7 Hz, 1H), 3.55 (brd, J = 12.0 Hz, 1H), 3.40–3.46 (m, 3H), 3.35–3.21 (m, 4H), 3.18 (brd, J = 12.5 Hz, 1H), 3.09 (ddd, J = 13.2, 10.4, 3.2 Hz, 1H), 3.04 (t, J = 12.7 Hz, 1H), 2.49 (m, 2H), 2.18 (m, 2H), 2.08 (m, 3H), 1.93–1.79 (m, 5H), 1.74–1.62 (4H), 1.53–1.16 (m, 27H); HRESIMS m/z 389.3080 [M]²⁺ (calcd for [C₅₆H₇₈N₂]²⁺ 389.3077).

**Bis-phenylbenzyl perhaliclonacyclamine:** (−)-perhaliclonacyclamine (0.3 mg, 0.634 μmol) was stirred with KOH (143 μg, 2.54 μmol) in MeOH (0.5 mL) for a few minutes, and the solvent was evaporated under a stream of N₂, then on high vacuum. Phenylbenzylbromide (3.1 mg, 12.7 μmol) and toluene (0.5 mL) was added and the mixture was stirred at 100°C for 12 hours. The solvent was evaporated under a stream of N₂, and the mixture was subjected to HPLC (reversed-phase Phenomenex, Luna, C₁₈, 10 x 250 mm; 2 mL min⁻¹ 40-100 % CH₃CN/H₂O + 0.1% TFA over 40 min) to give the bis-phenylbenzyl TFA salt
4.41 (130 μg). UV (MeOH) λ_max 255 nm; CD (MeOH) λ 260 (Δε +0.5);
HRESIMS m/z 403.3235 [M]^{2+} (calcd for [C_{56}H_{82}N_{2}]^{2+} 403.3234).

**Preparation of p-bromophenacyl derivatives:**

**Bis-p-Bromophenacyl Tetrahydroxestoproxamine A (4.42):** The acetate salt of tetrahydroxestoproxamine A (0.6 mg, 1.06 μmol) was converted to the free base by addition of KOH (238 μg, 4.25 μmol) in MeOH (0.5 mL). After a few minutes the solvent was evaporated under a stream of N₂ then on high vacuum. Toluene (0.5 mL) and p-bromophenacyl bromide (5.9 mg, 21.2 μmol) were added and the mixture was stirred at 90°C for 12 h. The solvent was evaporated under a stream of N₂, and the mixture was subjected to HPLC (reversed-phase Phenomenx, Luna, C₁₈, 10 x 250 mm; 2 mL min⁻¹ 40-100 % CH₃CN/H₂O + 0.1% TFA over 40 min) to give the bis-p-bromophenacyl TFA salt 4.42 (0.7 mg). UV (MeOH) λ_max 265 (ε 24,000) nm; CD (MeOH) λ 254 (Δε +2.6), 276 (Δε -6.0) nm; ^1H NMR (500 MHz, CD₃OD, representative signals) δ 7.99 (d, J = 8.8 Hz, 2H), 7.94 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 5.32 (d, J = 18.2 Hz, 1H), 5.17 (d, J = 18.2 Hz, 1H), 5.17 (d, J = 18.2 Hz, 1H), 5.14 (s, 2H), 4.18 (brd, J = 11.3 Hz, 1H), 4.11 (brd, J = 12.7 Hz, 1H); 3.99 (m, 1H), 3.98 (m, 1H), 3.81 (m, 2H), 3.77 (m, 1H), 3.58 (td, J = 13.1, 3.3 Hz, 1H), 3.56 (m, 1H), 3.54 (t, J = 12.8 Hz, 1H), 3.31 (under solvent), 3.24 (t, J = 12.8 Hz, 1H), 1.21 (q, J = 12.6 Hz, 1H); HRESIMS m/z 419.1868 [M]^{2+} (calcd for [C_{46}H_{68}Br_{2}N_{2}O_{2}]^{2+}, 419.1818).
**Bis-p-Bromophenacyl Perhaliclonacyclamine (4.42):** (-)-perhaliclonacyclamine$^{28}$ (4.39, 150 µg, 0.317 µmol) was stirred with KOH (71 µg, 1.27 µmol) in MeOH (0.5 mL). After a few minutes the solvent was evaporated under a stream of N₂ then on high vacuum. Toluene (0.5 mL) and p-bromophenacylbromide (1.8 mg, 6.34 µmol) were added and the mixture was stirred at 90 °C for 12 h. The solvent was evaporated under a stream of N₂, and the mixture was subjected to HPLC (reversed-phase Phenomenex, Luna, C₁₈, 10 x 250 mm; 2 mL min⁻¹; mobile phase/gradient: 40-100 % CH₃CN/H₂O + 0.1% TFA over 40 min) to give the bis-p-bromophenacyl TFA salt 4.42 (ca 44 µg). UV (MeOH) λ 265 nm; CD (MeOH) λ 254 (Δε +3.2), 272 (−6.1) nm; HRESIMS m/z 433.1973 [M]$^{2+}$ (calcd for [C₄₈H₇₂Br₂N₂O₂]$^{2+}$, 433.1975).

**Bis-p-Bromophenacyl Perhydrohaliclonacyclamine E (4.45):** A solution of (+)-haliclonacyclamine E$^{19}$ (4.18, TFA salt, 0.8 mg, 1.15 mmol) in MeOH/AcOH (49:1, 0.25 mL) was stirred with Pd/C (10%, 0.1 mg) at room temperature under H₂ (1 atm) for 48 h. The mixture was filtered through a nylon syringe filter (0.45 µm) and the solvent removed from the filtrate under a stream of N₂. The residue was subjected to HPLC (Phenomenex, Synergi Hydro-RP, 4μ, 10 x 250 mm; 2.5 mL min⁻¹, 25:25:50:0.5 CH₃CN/i-PrOH/H₂O/TFA) to give perhydrohaliclonacyclamine E TFA salt (4.44, 0.3 mg). The TFA salt was subjected to HPLC (Phenomenex Lux-cellulose, 1.5 mL min⁻¹, 9:1:0.02 CH₃CN/i-PrOH/Et₂NH) and the solvent removed under a
stream of N₂ and further dried under high vacuum. Toluene (0.5 mL) and p-
bromophenacylbromide (1.2 mg, 4.28 µmol) were added and the mixture was
stirred at 90 °C for 12 h. The solvent was removed under a stream of N₂, and
the mixture was subjected to HPLC (reversed-phase, Phenomenx, Luna, C₁₈,
10 x 250 mm; 2 mL min⁻¹, 40-100 % CH₃CN/H₂O + 0.1% TFA over 40 min) to
give the bis-p-bromophenacyl TFA salt **4.45** (ca 7.0 µg). UV (MeOH) λmax 265
nm; CD (MeOH) λ 254 (Δε +2.6), 272 (−6.0); HRESIMS m/z 433.1970 [M]²⁺

**Bis-p-bromophenacyl dihydroxestroproxamine C (4.46):** Xestoproxamine C
free base (4.15, 0.4 mg, 0.876 µmol) was stirred with Pd/C (10%, 0.1 mg) in
0.25 mL MeOH/AcOH (49:1) at room temperature under H₂ (1 atm) for 48 h.
The mixture was filtered through a membrane filter (0.45 µm) and the solvent
was evaporated under a stream of N₂ then under high vacuum. The
dihydroxestroproxamine C acetate salt was subjected to HPLC (Phenomenex
Lux-cellulose, 1.5 mL min⁻¹, mobile phase: 9:1:0.02 CH₃CN/i-PrOH/Et₂NH)
and the solvent concentrated under a stream of N₂ then dried under reduced
pressure. Dihydroxestroproxamine C free base was stirred with p-
bromophenacylbromide (3.0 mg, 10.8 µmol) in toluene at 80 °C for 2 h in a
microwave reactor (300W). The solvent was removed under a stream of N₂
and the mixture subjected to RPHPLC (column: Phenomenx, Luna, C₁₈(2), 10
x 250 mm; 2 mL min⁻¹, 40-100 % CH₃CN/H₂O + 0.1% TFA over 40 min) to
give the bis-p-bromophenacyl TFA salt derivative **4.46** (137 µg). UV (MeOH)
\( \lambda_{\text{max}} \) 265 nm; CD (MeOH) \( \lambda \) 254 (\( \Delta\varepsilon \) +3.5), 276 (\( \Delta\varepsilon \) −7.1) nm; HRESIMS m/z 426.1896 [M]^{2+} (calcd for \([C_{47}H_{70}Br_{2}N_{2}O_{2}]^{2+}\), 426.1900).

**Bis-\( p \)-bromophenacyl (\(+\)-perhaliclonacyclamine B (4.46):** (\(+\)-haliclonacyclamine B TFA salt (4.8, 0.2 mg, 0.287 mmol) was stirred with Pd/C (10\%, 50 mg) in 0.25 mL MeOH/AcOH (49:1) at room temperature under \( H_2 \) (1 atm) for 48 h. The mixture was filtered through a membrane filter (0.45 \( \mu \)) and the solvent was evaporated under a stream of \( N_2 \) then subjected to HPLC (Phenomenex, Synergi Hydro-RP, 4\( \mu \), 10 x 250 mm; 2.5 mL min\(^{-1}\), 25:25:50:0.5 CH\(_3\)CN/i-PrOH/H\(_2\)O/TFA) to give tetrahydrohaliclonacyclamine E TFA salt that was converted to the free base by HPLC (Phenomenex Lux-cellulose, 1.5 mL min\(^{-1}\), mobile phase: 9:1:0.02 CH\(_3\)CN/i-PrOH/Et\(_2\)NH) and the solvent concentrated under a stream of \( N_2 \) then dried under reduced pressure. The free base was stirred with \( p \)-bromophenacylbromide (0.8 mg, 2.9 \( \mu \)mol) in toluene at 80 \( ^\circ \)C for 1 hour in a microwave reactor (300W). The solvent was removed under a stream of \( N_2 \) and the mixture subjected to RPHPLC (column: Phenomenex, Luna, C18(2), 10 x 250 mm; 2 mL min\(^{-1}\); 40-100 % CH\(_3\)CN/H\(_2\)O + 0.1% TFA over 40 min) to give the bis-\( p \)-bromophenacyl TFA salt derivative **4.46** (71 \( \mu \)g). UV (MeOH) \( \lambda_{\text{max}} \) 266 nm; CD (MeOH) \( \lambda \) 255 (\( \Delta\varepsilon \) −4.7), 273 (\( \Delta\varepsilon \) +8.0) nm; HRESIMS m/z 433.1973 [M]^{2+} (calcd for \([C_{48}H_{72}Br_{2}N_{2}O_{2}]^{2+}\), 433.1975).
Synthesis of Model Compound 4.32:

**Amide (4.29):** n-BuLi (2.21 M in hexanes, 3.0 mL, 6.64 mmol) was added to a stirred solution of anhydrous lithium chloride (0.84 g, 19.8 mmol) and diisopropylamine (0.94 mL, 6.64 mmol) in THF (20 mL) at −78 °C. The mixture was kept at −78 °C for 20 min, then placed in an ice bath for 30 min, then cooled back to −78 °C. Amide **4.27** (0.70 g, 3.16 mmol, prepared by acylation of pseudoephedrine with propionyl chloride) was added over 10 min, and the solution slowly warmed to room temperature over 1.5 h, and stirred an additional 15 min, then cooled to −40 °C. The 1-(tert-butyldimethylsilyloxy)-9-iododecane **4.29** (1.75 g, 4.39 mmol) was added dropwise and the mixture allowed to warm to room temperature overnight with stirring. The mixture was poured into saturated NH₄Cl solution (75 mL), and the organic layer separated. The aqueous layer was extracted with EtOAc (4 x 50 mL) and the combined organic layers were dried (MgSO₄), the solvent removed under reduced pressure and the crude product purified by flash chromatography (SiO₂, 1:3 EtOAc/hexanes) to give the amide **4.29** (1.20 g, 77% yield) [α]D²² = 36.7 (c 3.1, CHCl₃); FTIR (ATR): ν 3376, 2926, 2854, 1620, 1464, 1408, 1254, 1099, 1052, 835, 775, 701 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.23 (m, 5H), 4.61 (t, J = 7.2 Hz, 1H, major), 4.59 (m, 1H, minor), 4.39 (brs, 1H, major), 4.08 (p, J = 8.0 Hz, 1H, minor), 3.59 (t, J = 6.4 Hz, 2H, major), 3.58 (t, J = 6.4 Hz, 2H, minor), 2.91 (s, 3H, minor), 2.84 (s, 3H, major), 2.78 (t, J = 6.4 Hz, 1H, minor), 2.58 (sex, J = 7.2 Hz, 1H, major), 2.29 (brs, 1H, minor), 1.71
Alcohol (4.30): BH₃•THF complex (1 M in THF, 3.0 mL, 3 mmol) was added to pyrrolidine (246 μL, 3 mmol) at 0 °C, and the solution was warmed to room temperature and stirred for 45 min. The solution was cooled back to 0 °C and treated, dropwise, with n-BuLi (2.21 M in hexanes, 1.36 mL, 3 mmol) with stirring for an additional 30 min. The amide 4.29 (500 mg, 1 mmol) in THF (10 mL) was added and stirred at room temperature overnight. 3M HCl (5 mL) was added to quench the excess hydride and the layers were separated. Ether (5 mL) was added to the aqueous layer, and the mixture cooled to 0 °C before being made basic (pH 9-10) by addition of 2 N NaOH. The aqueous mixture was extracted with ether (3 x 5 mL) and the combined organic extracts washed with 1:1 brine/1 N NaOH (2 x 10 mL), dried (Na₂SO₄), the solvent evaporated, and subjected to flash chromatography (SiO₂, 12.5% EtOAc/hexanes) to give the alcohol 4.30 (135 mg, 41% yield) [α]D²³ –5.5 (c 2.1, CHCl₃); FTIR (ATR): ν 3340, 2926, 2855, 1464, 1254, 1102, 1041, 835, 775 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 3.59 (t, J = 6.4 Hz, 2H), 3.50 (dd, J =
10.0, 5.6 Hz, 1H), 3.41 (dd, J = 10.0, 6.8 Hz, 1H), 1.59 (m, 1H), 1.50 (p, J = 6.8 Hz, 2H), 1.41-1.26 (m, 15H), 1.09 (m, 1H) 0.91 (d, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.04 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 68.4 (CH$_2$), 63.3 (CH$_2$), 35.7 (CH), 33.2 (CH$_2$), 32.9 (CH$_2$), 29.9-29.4 (5 x CH$_2$), 27.0 (CH$_2$), 26.0 (3 x CH$_3$), 25.8 (CH$_2$), 18.4 (C), 16.6 (CH$_3$), $-5.3$ (2 x CH$_3$); HRESIMS m/z 353.2849 [M+Na]$^+$ (calcd for C$_{19}$H$_{42}$O$_2$SiNa, 353.2846).

**Aldehyde (4.30a):** DMSO (64 $\mu$L, 0.91 mmol) was added dropwise to a stirred solution of oxalyl chloride (51 $\mu$L, 0.60 mmol) in CH$_2$Cl$_2$ (3 mL) at $-78^\circ$C, and stirred for 10 min. A solution of the alcohol 4.30 (100 mg, 0.302 mmol) in CH$_2$Cl$_2$ was added, and the mixture stirred for 30 min at $-50^\circ$C. Et$_3$N (168 $\mu$L, 1.21 mmol) was added and the mixture brought to $-30^\circ$C followed by stirring an additional 30 min. Saturated NH$_4$Cl solution (5 mL) was added, and the layers were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (2 x 3 mL) and the combined organic extracts were dried (MgSO$_4$), concentrated and the residue purified by flash chromatography (SiO$_2$, 3% EtOAc/hexanes) to give the aldehyde 4.30a (91 mg, 92% yield) [$\alpha$]$^{{\circ}}_{D}^{24}$ +12.8 (c 3.4, CHCl$_3$); FTIR (ATR): $\nu$ 2926, 2854, 1730, 1463, 1254, 1099, 835, 774 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 9.61 (d, J = 2.4 Hz, 1H), 3.59 (t, J = 6.4 Hz, 2H), 2.32 (sd, J = 7.2, 1.6 Hz, 1H), 1.69 (m, 1H), 1.50 (p, J = 7.2 Hz, 2H) 1.25 (m, 15H), 1.08 (d, J = 7.2 Hz, 3H), 0.89 (s, 9H), 0.04 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 205.5 (CH), 63.3 (CH$_2$), 46.3 (CH), 32.9 (CH$_2$), 30.5 (CH$_2$), 29.6-29.4 (5 x
CH<sub>2</sub>), 26.9 (CH<sub>2</sub>), 26.0 (3 x CH<sub>3</sub>), 25.8 (CH<sub>2</sub>), 18.4 (C), 13.3 (CH<sub>3</sub>), −5.3 (2 x CH<sub>3</sub>); HRESIMS m/z 351.2691 [M+Na]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>40</sub>O<sub>2</sub>SiNa, 351.2690).

**Olefin (4.31):** n-BuLi (1.2 M in hexanes, 143 μL, 0.171 mmol) was added to a slurry of CH<sub>3</sub>PPh<sub>3</sub>Br (65 mg, 0.183 mmol) in THF (0.5 mL) at 0 °C, then the mixture stirred at room temperature for 5 min. The aldehyde 4.30a (40 mg, 0.122 mmol) in THF (0.5 mL) was added and stirring continued for 2 h at room temperature. Saturated NH<sub>4</sub>Cl solution (1 mL) was added followed by ether (2 mL) and the layers were separated. The aqueous layer extracted with ether (2 x 2 mL) and the combined organic extracts dried (MgSO<sub>4</sub>), concentrated, and the residue purified by flash chromatography (SiO<sub>2</sub>, hexanes) to give the chiral olefin 4.31 (35 mg, 88% yield) [α]<sub>D</sub><sup>23</sup> +6.5 (c 2.8, CHCl<sub>3</sub>) FTIR (ATR): ν 2925, 2854, 1463, 1254, 1099, 994, 909, 834, 773 cm<sup>−1</sup>; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 5.69 (ddd, J = 17.2, 10.0, 7.2 Hz, 1H), 4.94 (ddd, J = 17.2, 2.0, 1.2 Hz, 1H), 4.89 (ddd, J = 10.0, 2.4, 1.2 Hz, 1H), 3.51 (t, J = 6.8 Hz, 2H), 2.10 (m, 1H), 1.50 (p, J = 7.2 Hz, 1H), 1.25 (m, 16H), 0.97 (d, J = 6.8 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 145.1 (CH), 112.2 (CH<sub>2</sub>), 63.3 (CH<sub>2</sub>), 37.8 (CH), 36.7 (CH<sub>2</sub>), 32.9 (CH<sub>2</sub>), 29.8-29.4 (5 x CH<sub>2</sub>), 27.2 (CH<sub>2</sub>), 26.0 (3 x CH<sub>3</sub>), 25.8 (CH<sub>2</sub>), 20.2 (CH<sub>3</sub>), 18.4 (C), −5.3 (2 x CH<sub>3</sub>); HRESIMS m/z 327.3081 [M+H]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>43</sub>O<sub>2</sub>SiNa, 327.3078).

**Alkene (4.32):** A mixture of alkene 4.31 (2 mg, 6.12 μmol) and 2-methoxy-6-vinylnaphthalene (11.3 mg, 61.2 μmol) was treated with Grubbs’ 2nd generation catalyst (1 mg, 1.22 μmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) at 70 °C in a sealed
vial for 12 h. Additional catalyst (1 mg, 1.22 μmol) was added and the mixture stirred an additional 6 h at 80 °C. The solvent was removed under a stream of N₂, and the mixture passed through a reversed-phase (C₁₈) silica cartridge (CH₃CN then MeOH), followed by HPLC (reversed-phase, C₈, 85% CH₃CN/H₂O) to give alkene 4.32 (1.3 mg, 42%) [α]D²² +31.1 (c 1.0, CHCl₃); UV (CH₃CN) 246 nm (ε 41,900), 292 (ε 15,700); CD (CH₃CN) λ 255 nm (Δε +3.8). FTIR (ATR): ν 2925, 2853, 1256, 1100, 1035, 837, 777 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 7.68 (d, J = 9.0 Hz, 1H), 7.66 (d, J = 9.0, 1H), 7.62 (brs, 1H), 7.55 (dd, J = 9.0, 2.0 Hz, 1H), 7.11 (dd, J = 9.0, 2.0 Hz, 1H), 7.10 (brs, 1H), 6.46 (d, J = 16 Hz, 1H), 6.16 (dd, J = 16.0, 8.5 Hz, 1H), 3.91 (s, 3H), 3.59 (t, J = 7.0 Hz, 2H), 2.32 (sep, J = 6.5 Hz, 1H), 1.50 (m, 2H), 1.38 (m, 2H), 1.27 (m, 14H), 1.10 (d, J = 6.5 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 6H); ¹³C NMR (125 MHz, CDCl₃): δ 157.4 (C), 136.6 (CH), 133.7 (C), 133.4 (C), 129.3 (CH), 129.1 (C), 128.0 (CH), 126.9 (CH), 125.1 (CH), 124.2 (CH), 118.8 (CH), 105.8 (CH), 63.3 (CH₂), 55.3 (CH₃), 37.4 (CH), 37.2 (CH₂), 32.9 (CH₂), 29.8-29.4 (5 x CH₂), 27.4 (CH₂), 26.0 (3 x CH₃), 25.8 (CH₂), 20.8 (CH₃), 18.4 (C), −5.3 (2 x CH₃); HRESIMS m/z 482.3573 [M]⁺ (calcd for C₃₁H₅₀O₂Si, 482.3575).

4.9 References

(1) (a) Andersen, R. J.; Van Soest, R. W. M.; Kong, F. In Alkaloids: Chemical and Biological Perspectives, Pelletier, S. W., Ed.; Pergamon:


2906.


(29) It is worth recalling that it is not possible, strictly, to assign absolute configuration to chiral compounds by comparison of signs of [α]D, alone, except for enantiomers, even those as similar as 4.13-4.15 and 4.39. Two counter example are prescient and sufficient to illustrate this often ignored tenet in stereochemistry. The paired compounds (−)-perhydrohaliconacyclamine (4.39) and the parent (+)-tetradehydrohaliconacyclamine A (4.40), and (−)-haliconacyclamine A (4.7) ([α]D= -3.4) and (+)-haliconacyclamine B (4.8) ([α]D= +3.4), differ only in position of a single double bond in the lower linking chain, yet the pairs show opposite signs of specific rotation. Also, the absolute configuration of (−)-4.39 ([α]D= -20.9) was shown to be antipodal to that of (−)-4.7 despite the same sign of [α]D. Lastly, the signs of [α]D of alkaloid salts and their parent free bases may also differ.
(30) We are grateful to Professor Mary Garson, University of Queensland, for the generous gift of 4.39.

(31) We are grateful to Professor Roberto Berlinck, University of São Paulo, for the generous gift of 4.18.


(33) *Xestospongia proxima* and *Neopetrosia proxima* are synonymous; the latter name now replaces the former. Campos, M.; Mothes, B.; Eckert, R.; Van Soest, R. W. M. *Zootaxa* **2005**, *963*, 1-22.
Figure 4.9. $^1$H NMR spectrum of xestoperoxamine A (4.13) (600 MHz, MeOH-$d_4$, 1.7 mm MicroProbe).
Figure 4.10. $^{13}$C NMR spectrum of xestoproxamine A (4.13) (125 MHz, MeOH-$d_4$).
Figure 4.11. DQF–COSY spectrum of xestoproxamine A (4.13) (600 MHz, MeOH-$d_4$, 1.7mm MicroProbe).
Figure 4.12. TOCSY spectrum of xestoproxamine A (4.13) (600 MHz, MeOH-$d_4$, 1.7mm MicroProbe).
Figure 4.13. gHSQC spectrum of xestoproxamine A (4.13) (600 MHz, MeOH-d₄, 1.7mm MicroProbe).
Figure 4.14. gHMBC spectrum of xestoproxamine A (4.13) (600 MHz, MeOH-\textit{d}_4, 1.7mm MicroProbe).
Figure 4.15. HMQC–TOCSY spectrum of xestoproxamine A (4.13) ((600 MHz, MeOH-$d_4$, 1.7mm MicroProbe).
Figure 4.16. NOESY spectrum of xestopaxamine A (4.13) (600 MHz, MeOH-d$_4$, $t_{\text{mix}} = 300$ ms, 1.7 mm MicroCryoProbe).
Figure 4.17. $^1$H NMR spectrum of xestoproxamine B (4.14) (600 MHz, MeOH-$d_4$, 1.7 mm MicroProbe).
Figure 4.18. $^{13}$C NMR spectrum of xestoproxamine B (4.14) (125 MHz, MeOH-$d_4$).
Figure 4.19: gCOSY spectrum of xestoprosamine B (4.14) (600 MHz, MeOH-d₄, 1.7 mm MicroProbe).
Figure 4.20. TOCSY spectrum of xestoproxamine B (4.14) (600 MHz, MeOH-d₄, 1.7mm MicroProbe).
Figure 4.21. gHSQC spectrum of xestoproxamine B (4.14) (600 MHz, MeOH-d$_4$, 1.7mm MicroProbe).
Figure 4.22. gHMBC spectrum of xestoproxamine B \( (4.14) \) (600 MHz, MeOH-\( d_4 \), 1.7mm MicroProbe).
Figure 4.23. NOESY spectrum of xestoproxamine B (4.14) (600 MHz, MeOH-d₄, tₘᵡ = 300 ms, 1.7 mm MicroCryoProbe).
Figure 4.24. $^1$H NMR spectrum of xestoproxamine C (4.15) (600 MHz, CDCl$_3$, 1.7mm MicroCryoProbe).
Figure 4.25. DQF–COSY spectrum of xestoproxamine C (4.15) (600 MHz, CDCl$_3$, 1.7MM MicroCryoProbe).
Figure 4.26. TOCSY spectrum of xestoproxamine C (4.15) (600 MHz, CDCl₃, 1.7mm MicroCryoProbe).
Figure 4.27. gHSQC spectrum of xestoproxamine C (4.15) ((600 MHz, CDCl₃, 1.7mm MicroCryoProbe).
Figure 4.28. gHMBC spectrum of xestoproxamine C (4.15) (600 MHz, CDCl₃, 1.7mm MicroCryoProbe).
Figure 4.29. NOESY spectrum of xestoproxamine C (4.15) (600 MHz, CDCl$_3$, $t_{\text{mix}} = 300$ ms)
Figure 4.30. $^1$H NMR spectrum of tetrahydroxestoproxamine A (4.21) (500 MHz, CDCl$_3$).
Figure 4.31. $^{13}$C NMR spectrum of tetrahydroxestropropane A (4.21) (125 MHz, CDCl$_3$).
Figure 4.32. $^1$H NMR spectrum of tetrahydroxestoperoxamine A bismethiodide (4.22) (500 MHz, MeOH–d$_4$).
Figure 4.33. $^{13}$C NMR spectrum of tetrahydroxestoproxamine A bismethiodide (4.22) (125 MHz, MeOH–$d_4$).
Figure 4.34. $^1$H NMR spectrum of Hoffman degradation product 4.23 (600 MHz, MeOH–$d_4$, 1.7mm MicroCryoProbe).
Figure 4.35. $^1$H NMR spectrum of cross metathesis product 4.26 (600 MHz, MeOH–$d_4$, 1.7mm MicroCryoProbe).
Figure 4.36. $^1$H NMR spectrum of compound 4.29 (400 MHz, CDCl$_3$).
Figure 4.37. $^{13}$C NMR spectrum of compound 4.29 (100 MHz, CDCl$_3$).
Figure 4.38. $^1$H NMR spectrum of compound 4.30 (400 MHz, CDCl$_3$).
Figure 4.39. $^{13}$C NMR spectrum of compound 4.30 (100 MHz, CDCl$_3$).
Figure 4.40. $^1$H NMR spectrum of compound 4.30a (400 MHz, CDCl$_3$).
**Figure 4.41.** $^{13}$C NMR spectrum of compound 4.30a (100 MHz, CDCl$_3$).
Figure 4.42. $^1$H NMR spectrum of compound 4.31 (400 MHz, CDCl$_3$).
Figure 4.43. $^{13}$C NMR spectrum of compound 4.31 (100 MHz, CDCl$_3$).
Figure 4.44. $^1$H NMR spectrum of compound 4.32 (500 MHz, CDCl$_3$).
Figure 4.45. $^{13}$C NMR spectrum of compound 4.32 (125 MHz, CDCl$_3$).
Figure 4.46. $^1$H NMR spectrum of dihydroxestoproxamine C TFA salt (4.34) (500 MHz, MeOH–$d_4$).
Figure 4.47. $^{13}$C NMR spectrum of dihydroxestoxamine C TFA salt (4.34) (125 MHz, MeOH–$d_4$).
Figure 4.48. $^1$H NMR spectrum of dihydroestroproxamine C bismethiodide (4.35) (500 MHz, MeOH–d$_4$).
Figure 4.49. $^{13}$C NMR spectrum of dihydroxestoproxamine C bismethiodide (4.35) (125 MHz, MeOH–$d_4$).
Figure 4.50. $^1$H NMR spectrum of Hoffman degradation product 4.36 (600 MHz, MeOH–d$_4$), 1.7mm MicroCryoProbe).
Figure 4.51. $^1$H NMR spectrum of cross metathesis product 4.33 (600 MHz, MeOH–$d_4$, 1.7mm MicroCryoProbe).
Figure 4.52. $^1$H NMR spectrum of bis–phenylbenzyl tetrahydroxestoproxamine A (4.38) (600 MHz, MeOH–$d_4$).
Figure 4.53. $^1$H NMR spectrum of bis-phenylbenzyl (-)-perhaliconacyclamine (4.41) (500 MHz, MeOH–d$_4$).
Figure 4.54. $^1$H NMR spectrum of bis–$p$–bromophenacyl tetrahydroxestoproxamine A (4.42)
(600 MHz, MeOH–$d_4$, 1.7mm MicroCryoProbe).
Figure 4.55. $^1$H NMR spectrum of hexahydrohaliclonacyclamine E (4.44) (600 MHz, MeOH–$d_4$, 1.7mm microCryoProbe).
Figure 4.56. $^1$H NMR spectrum of (+)-perhydroalcyclamine B (4.47) (600 MHz, MeOH–d$_4$, 1.7 mm MicroCryoProbe).
Figure 4.57. $^1$H NMR spectrum of bis–p–bromophenacyl dihydroxestoproxamine C (4.46) (600 MHz, MeOH–$d_4$, 1.7 mm MicroCryoProbe).
CHAPTER 5
MOLLENYNE A, A BROMOCHLORO ACETYLENIC AMIDE FROM THE
MARINE SPONGE SPIRASTRELLA MOLLIS

5.1 Bromochloro Natural Products from Marine Invertebrates

Halogenated small molecules from marine sponges usually contain either one of the elements of bromine or chlorine, but rarely both.¹ Surprisingly, bromochloro metabolites from sponges are uncommon, perhaps a consequence of limitations of oxidation potentials the haloperoxidases responsible for introduction of each halogen. Two examples of bromochloro sponge natural products include the pyrrole imidazole alkaloids (axinellamine A² (5.1) from Axinella sp., tetrabromostyloguanadine (5.2)³ from Stylissa caribica, and massadine chloride (5.3)⁴ from Stylissa aff. massa, Figure 5.1) and long chain terminal vinyl bromochloro azirines (5.4 and 5.5, Figure 5.2)⁵ from Dysidea fragilis.

Figure 5.1. Bromochloro pyrrole imidazole alkaloids.
Figure 5.2. Terminal bromochloro vinylidenes from *Dysidea fragilis*.

5.2 Isolation and Planar Structure of Mollenyne A.

![Image of molecules](image)

Figure 5.3. Photograph of *Spirastrella mollis* in Plana Cay, Bahamas. Photograph by Tadeusz F. Molinski.

Proliferation assays against human colon tumor cells (HCT-116) of crude extracts from various Bahamian sponges and tunicates (n = 180) showed the extracts from the red thick encrusting sponge *Spirastrella mollis* (Figure 5.3) to be among the most active samples tested (IC50 = <0.1 µg/mL). Further purification of the CH$_2$Cl$_2$ partition from *S. mollis* by C18 flash
chromatography and reversed phase HPLC gave a new halogenated lipid we have named mollenyne A (5.6).

Mollenyne A (5.6) showed a molecular formula of C_{26}H_{36}O_{2}N_{4}Br_{2}Cl based on HRESIMS (m/z 629.0888 [M+H]^+). Six exchangeable hydrogens were present based on LRESIMS in CD_{3}OD which showed a m/z at 635 [M+H]^+. One and two dimensional NMR allowed the assignment of five substructures (A-E, Figure 5.4). Substructure A contained a spin system of three contiguous methylene groups H2 through H4 (δ 2.23, t, J = 7.4 Hz, δ 1.79, and δ 2.23, respectively) and terminated with vinyl proton H5 (δ 6.10, t, J = 7.7 Hz). C5 (δ 137.3) was attached to a quaternary Sp^2 carbon C6 (δ 128.5) by HMBC from H5 to C6. COSY and TOCSY correlations showed the oxygenated methine H7 (δ 4.73, d, J = 9.7 Hz) was coupled to methine H8 (δ 4.57, m), and H9 (δ 4.57, m) was coupled to H10 (δ, 2.97, m) which gave substructure B. Substructure C is a *trans* disubstituted alkene comprising H13 (δ 5.91, dt, J = 16.1 and 1.9 Hz) and H14 (δ 5.97, dt, J = 16.1 and 2.0 Hz). The terminus of 5.6 was identified in substructure D, which contained two methylene groups H17 (δ 2.54, td, J = 7.2 and 2.0 Hz) and H18 (δ 2.39, td, J = 7.2 and 2.6 Hz) units attached to a terminal acetylene. The final substructure E was assigned as a 1,5 diamine unit. Substructures A-E were assembled by further analysis of HMBC spectra (Figure 5.5).
**Figure 5.4.** Substructures A-E assigned from 2D NMR.

Substructures A and E were connected by an amide carbonyl group (δ 175.6) which showed HMBC correlations to both H2 and H1'. Oxygenated methine H7 showed a correlation to C5 which established the connection between substructures A and B. The C5/C6 double bond was assigned the E stereochemistry based on NOESY correlation between H4 and H7. Both H10 and H13 showed correlations to an alkyne containing C11 (δ 89.9) and C12 (δ 82.6). Substructure D was attached to C by a third alkyne consisting of C15 (δ 80.4) and C16 (δ 94.6). The left portion of 5.6 was terminated with a guanidine group based on the balance of the molecular formula and HMBC correlation from H5' to C6' (δ 158.6).

**Figure 5.5.** Assembly of substructures A-E by HMBC correlations.
Table 5.1. $^1$H (600 MHz) and $^{13}$C NMR (125 MHz) for mollenyne A (5.6) (CD$_3$OD).

<table>
<thead>
<tr>
<th>No.</th>
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<th>$\delta_C$</th>
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<th>HMBC$^b$</th>
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<td>158.6, C</td>
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$^a$ Determined from DEPT and HSQC. $^b$ HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon. $^c$ May be interchanged. $^d$ Coupling constants obscured because overlapping multiplets.
Table 5.2. $^1$H (600 MHz) and $^{13}$C NMR (150 MHz) for mollenyne A (5.6) (CD$_3$CN).

<table>
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<th>No.</th>
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<th>HMBC$^a$</th>
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<td>18, 18</td>
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</table>

$^a$ Determined from HSQC. $^b$ HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.
With the carbon backbone of 5.6 established, the placement of halogens (2 bromine and 1 chlorine) was assigned by NMR and chemical conversion. A vinyl bromide was favored over a vinyl chloride based on comparison of $^{13}$C chemical shifts to synthetic models reported in the literature. The quaternary Sp$^2$ carbon bearing a bromine ($\delta \approx 125$ ppm) is typically observed upfield of the protonated Sp$^2$ carbon ($\delta \approx 133$ ppm) in vinyl bromides. In vinyl chlorides, the opposite trend is observed and the quaternary Sp$^2$ carbon ($\delta \approx 135$ ppm) is observed downfield of the protonated Sp$^2$ carbon ($\delta \approx 125$ ppm). Based on $^{13}$C chemical shift and chemical transformation the remaining bromine and chlorine were be assigned to positions 8 and 9, respectively.

5.3 Assignment of Relative Configuration by $J$-based Configurational Analysis and Chemical Conversion

To assign the relative configuration of mollenyne A (5.6) we turned to $J$-based analysis configurational analysis (JBCA) reported by Murata for 1,2 and 1,3 methines of polyhydroxylated or methylated stereogenic subunits using homonuclear ($^3J_{HH}$) and heteronuclear ($^{2,3}J_{HC}$) coupling constants. The stereotriad of mollenyne (5.6) resembles the stereogenic ensembles found in chlorosulfolipids isolated from Adriatic shellfish and freshwater algae. Recently, the Carreira group carried out a meticulous investigation of the coupling constants derived from a series of optically active model
polychlorinated alcohols and demonstrated that subtle differences should be considered when analyzing a polychlorinated versus a polyhydroxylated system, leading to reliable basis sets.\(^6\)

The relative configuration between C8 and C9 was assigned as *threo* by the JBCA in a straightforward manner (Figure 5.6, conformer C). H8 and H9 were oriented gauche based on a small coupling \((J = 1.6 \text{ Hz})\). Because of small coupling between H8 and H9 we were unable to observe \(^2J_{HC}\) and \(^3J_{HC}\) coupling constants by HETLOC and HSQC-HECADE experiments. We used a \(J\)-resolved HMBC experiment which revealed that all heteronuclear couplings were small in magnitude. Both bromine and chlorine atoms were oriented anti to H9 and H8, respectively. C7 and C10 were assigned gauche to H9 and H8, respectively. We next attempted to assign the relative configuration of C7 and C8 by JBCA. H7 and H8 were oriented anti based on large coupling \((J = 9.0 \text{ Hz})\). HETLOC revealed that both \(^2J_{HC}\) H7/C8 and H8/C7 have large couplings which can be accommodated by two possible diastereomers (Figure 5.6, conformers a and b). These two diastereomers are typically resolved by NOESY correlations between C6 and H9 or –OH and C9. Because C6 is part of the vinyl bromide and the \(^1\text{H}\) NMR signal corresponding to the hydroxyl group was not observed, we were unable to resolve these two diastereomers, and turned to chemical conversion instead.
Vinyl chlorides and bromides are readily converted to the saturated hydrocarbon by catalytic hydrogenation. We expected that upon conversion to the vinyl bromide to a CH$_2$ group NOESY correlations would be observed between C6 and C9 if the relative configuration was *threo* (Figure 5.6, conformer a). Mollenyne A (5.6) was converted to the fully saturated 5.7 under heterogeneous catalytic hydrogenation conditions (H$_2$, Pd/C, MeOH, 3 days, Scheme 5.1). Unfortunately, no useful NOESY correlations were observed. Alternatively, bromohydrin 5.7 was exposed to basic conditions (K$_2$CO$_3$, MeOH) to give epoxide 5.8 after neutralization (AcOH), and HPLC purification. The coupling constant between H7 and H8 ($J = 2$ Hz) was consistent with an anti epoxide. Taking into account the inversion of configuration at C8 in 5.8,
the relative configuration between H7/H8 in 5.6 was therefore erythro (Figure 5.6, conformer b). The stepwise conversion of 5.6 to the saturated chloroepoxide 5.8, with consecutive losses of HBR, independently confirmed the placement of the halogens based on HRESIMS of products 5.7 and 5.8.

Scheme 5.1. Conversion of mollenyne A (5.6) to saturated chloroepoxide 5.8.

5.4 Assignment of the Absolute Stereochimetry by the Exciton Chirality Method

The complete stereostructure of 5.6 was elucidated by conversion to a benzoate derivative and a p-methoxy-cinnamate derivative and application of the exciton chirality method. Nakanishi reported that both cyclic and acyclic
allylic alcohols can be assigned by conversion to an appropriate benzoate derivative and interpretation of ECCD spectra that arises from exciton coupling between $\pi-\pi^*$ transitions of the benzoate and double bond chromophores.\textsuperscript{9} The corresponding O–benzoate for mollenyne A was expected to give rise to an ECCD spectra which would lead to assignment of the C7 stereocenter. However, some complications were anticipated from contributions by the $\pi-\pi^*$ transition of the conjugated yne-ene-yne chromophore.

Mollenyne A was converted to its benzoate derivative \textbf{5.9} under standard conditions (Bz-Cl, pyridine), and the product was purified by reversed-phase HPLC, and CD spectrum acquired in MeOH (Scheme 5.2 and Figure 5.7). Two positive Cotton effects associated with the benzoate chromophore $\lambda$ 227 nm ($\Delta\varepsilon$ +6.1), and yne-ene-yne chromophore\textsuperscript{10} ($\lambda$ 260 nm ($\Delta\varepsilon$ +7.5), 275 nm ($\Delta\varepsilon$ +6.5)) were observed and attributed to two different exciton coupled interactions as follows. The positive Cotton effect at $\lambda$ 227 nm was assigned as an exciton couplet due to positive helicity between the double bond ($\lambda$ \textasciitilde190 nm) and the benzoate chromophore ($\lambda$ = 227 nm). The negative Cotton effect expected from the C=C $\pi-\pi^*$ contribution, but was obscured by solvent (MeOH, cutoff \textasciitilde200 nm). A positive split Cotton effect between the yne-ene-yne chromophore and the benzoate was assigned to a positive helicity. The expected negative Cotton effect at $\lambda$ 227 nm is canceled by the positive couplet from the benzoate-double bond exciton couplet.
Scheme 5.2. Conversion of mollenyne (5.6) to corresponding benzoate (5.9) and ρ-methoxycinnamate (5.10) derivatives, and major conformer accounting for observed ECCD spectra.

As the foregoing interpretation of the benzoate derivative was complicated by the observation of overlapping positive and negative couplets, mollenyene A was derivatized with a red-shifted chromophore to validate our interpretation above. It was expected that a positive exciton split Cotton effect would be observed if similar chromophoric interactions were taking place. Mollenyene A (5.6) was derivatized with para-methoxycinnamoyl chloride to give 5.10 (Scheme 5.2 and Figure 5.7). As expected, a negative Cotton effect at λ 262 nm (Δε −6.6) was observed from the yne-ene-yne chromophore in addition to a broad positive Cotton effect at λ ~296 nm (Δε +10.8).
Figure 5.7. UV (lower) and CD (upper) spectra for (a) 5.6, (b) 5.9, and (c) 5.10 in MeOH at 23°C. 5.6 and 5.9 were normalized for the ene–yne–ene chromophore (see ref. 10). 5.10 was normalized to the \( p \)-methoxycinnamate chromophore (see ref. 11).

Molecular modeling (Spartan, MMFF) of a truncated benzoate model (C4 to C12) showed that that lowest energy conformer (65% of Boltzmann weighted population) was consistent with the solution structure predicted from NMR and CD studies. This integrated NMR, CD and molecular modeling analysis fully support the 7S, 8R, and 9R configurational assignment for mollenyne A (5.6).
5.5 Bioactivity of mollenyne A

![Diagram of isolation and bioactivity data for fractions derived from *Spirastrella mollis* (08–125).](image)

**Figure 5.8.** Isolation tree and bioactivity data for fractions derived from *Spirastrella mollis* (08–125).

Mollenyne A was derived from the highly cytotoxic CH<sub>2</sub>Cl<sub>2</sub> partion by several rounds of C18 chromatography (Figure 5.8). Bioassay of mollenyne A showed significant cytotoxicity against human colon tumor cells (HCT–116; IC<sub>50</sub> = 1.3 µg/mL; etoposide = 0.55 µg/mL). However, the activity observed for 5.6, does not account for the activity observed in the crude extract. The most active fraction (F5.F4) is the subject of ongoing investigation.
5.5 Conclusions

In conclusion we have isolated an unusual optically active halogenated lipid from the marine sponge *Spirastrella mollis*. The 1,5-aminoguanidine portion has been observed previously in bromotyrosine metabolites (such as clavatadine 5.11) characteristic of sponges in the genus *Suberea*.\(^{12}\) The guanidine sidechain in mollenyne A (5.6) and 5.11 could be derived by decarboxylation of homoaarginine. Several marine derived lipid amides have been reported that appear to derive from decarboxylation of corresponding amino acids (Figure 5.9). The phenethyl amine in Callyspongamide A (5.12)\(^{13}\) from *Callyspongia fistularis* and hermitamide A (5.13)\(^{14}\) from *Lyngbya majuscula* appear to derive from phenylalanine, while the tryptophan moiety in hermitamide B (5.14)\(^{14}\) may derive from tryptophan.

![Chemical structures](image-url)

**Figure 5.9.** Structures of marine–derived 1,5–aminoguanidine 5.11 and lipid amides (5.12–5.14).

The lipid portion is rather unique among sponge metabolites for two reasons. Compound 5.6 contains both the elements of bromine and chlorine which is rare among sponge metabolites, but more common in natural
products from marine algae of the genus *Laurencia*.\(^{15}\) It is plausible that the chlorine in 5.6 derives from nucleophilic addition of Cl\(^{-}\) to an enzymatically generated bromonium ion. In addition, most brominated unsaturated fatty acids reported to date lack chirality, while the formation of 5.6 appears to proceed through a series of stepwise stereospecific enzymatic transformations. The complete structure of 5.6 using a combination of chemical conversions, NMR and CD analysis. Notably, The native yne-ene-yne chromophore was exploited and through conformational analysis and interpretation of CD Cotton effects, leading to the complete stereostructure for mollenyne A.

Chapter 5 is, in part, being prepared for submission for publication of the material: Morinaka, B. I.; Molinski, T. F. “Mollenyne A, A Bromochloro Acetylenic Lipid Amide from the Marine Sponge *Spirastrella mollis*” The dissertation author was the primary researcher/author on this paper.

### 5.6 Experimental Section

**General Experimental Procedures.** UV spectra were measured on a Jasco J600 double-beam UV-vis spectrometer using spectroscopic grade solvents (Fluka) and a 1 mm quartz cell with 50 nm/min scan rate and 1 nm slit. LR ESI mass spectra were obtained on a ThermoElectron MSQ single quad mass spectrometer coupled to an Accela UPLC. \(^1\)H, \(^{13}\)C, and 2D NMR spectra were recorded on a Bruker Avance III DRX-600 (600 MHz, \(^1\)H, 1.7 mm CPTCI probe) or Varian Xsense (500 MHz) spectrometers in CD\(_3\)OD or CD\(_3\)CN using
solvent signals \([\delta_H \text{CHD}_2\text{OH} \ 3.31 \text{ ppm}; \ \delta_C \ 49.00 \text{ ppm and } \delta_H \text{CHD}_2\text{CN} \ 1.94 \text{ ppm}; \ \delta_C \ 1.32 \text{ ppm as internal standards}].\) Preparative HPLC was carried out using dual Dynamax Model SD-200 pumps, with UV–detection (Pharmacia LKB UV-1 detector operating at 254 nm). Semi–preparative HPLC was carried out using either a Gilson model 302 pump equipped with tandem detectors—UV–visible (ISCO model UA-5, \(\lambda \ 254 \text{ nm}\)) and refractive index (Waters R401)—or a Rainin HPXL dual-pump with split flow (7:1) between two detectors—a Jasco CD-2095 UV-CD and an ESA model 301 evaporative light scattering detector (ELSD). HPLC grade solvents were used for HPLC (EMD Chemicals). TLC was performed on silica gel coated 0.25 mm aluminum backed plates (Whatman AL SIL G/UV) with visualized by heating with vanillin-
\(\text{H}_2\text{SO}_4\)-EtOH.

**Animal Material.** *Spirastrella mollis* was collected in November 2008 at Plana Cay, Bahamas (22° 36.459’ N, 73° 33.755’ W) at a depth of 100 feet using scuba, and kept in EtOH at \(-20 ^\circ\text{C}\) until extraction. A voucher sample is archived at UCSD.

**Extraction and Isolation.** A frozen sample of *Spirastrella mollis* (wet wt. 712 g, dry extracted wt. 192 g) was cut into pieces and extracted with 2:1 MeOH/\(\text{CH}_2\text{Cl}_2\) (4 x 1.5 L, 23°C, overnight). These extracts were combined and the solvent evaporated. The crude extract was partitioned between EtOAc (4 x 1 L) and water (3 L). The organic layer was dried and partitioned between hexane (3 x 0.5 L) and 9:1 MeOH/\(\text{H}_2\text{O}\) (1.5 L). The hexane layers
were combined and back extracted with 9:1 MeOH/H₂O (2 x 0.5 L). The combined aqueous MeOH layers were adjusted to 1:1 MeOH/H₂O and extracted with CH₂Cl₂ (4 x 1 L). The CH₂Cl₂ layer was separated and the solvent evaporated to give a brown gum (1.084 g) which was subjected to C₁₈ flash chromatography (1:9 to 9:1 MeOH/H₂O, then MeOH) to give ten fractions. The sixth fraction (219.3 mg) was subjected to preparative reversed phase HPLC (1:9 CH₃CN/H₂O to CH₃CN gradient over 40 min) to give four fractions. The second prep HPLC fraction (12.2 mg) was subjected to two rounds of semipreparative reversed phase HPLC (23:27 CH₃CN/H₂O + 0.025 % TFA) to give 0.5 mg of 1 (2.6 x 10⁻⁶ % based on wet wt.).

**Mollenyne A (5.6):** colorless glass; UV (MeOH) λ<sub>max</sub> 261 nm, 275 nm; CD (MeOH) λ 276 nm (Δ ε −2.7); <sup>1</sup>H NMR, <sup>13</sup>C NMR, see Tables 5.1 and 5.2. HRESIMS m/z 629.0888 [M+H]<sup>+</sup> (calcd for C₂₆H₃₆O₂N₄Br₂Cl 629.0888).

**Hydrogenation of 5.6:** Mollenyne A (5.6, 250 µg) in MeOH (0.25 mL) was treated with Pd/C (10%, 30 µg) under an H₂ (1 atm) for 3 days until most of the starting material had been consumed. The solution was filtered thru a PTFE membrane filter (0.45 µ), and the solvent was evaporated under a stream of N₂. The material was purified by HPLC (column: Phenomenex, Luna, C₁₈ (2), 5µ, 10 x 250 mm; flow rate: 2 mLmin⁻¹ mobile phase: 10-100% CH₃CN/H₂O+0.1% TFA over 45 minutes) to give the fully saturated bromochloro compound 5.7 (200 µg). <sup>1</sup>H NMR (600 MHz, CD₃OD/CDCl₃): δ 4.42 (ddd, J = 8.8, 5.3, 1.7 Hz, 1H), 3.87 (dd, J = 9.4, 1.7 Hz, 1H), 3.79 (td, J =
9.4, 2.2 Hz, 1H), 3.15 (t, J = 7.1 Hz, 2H), 3.12 (t, J = 7.1 Hz, 2H), 2.17 (t, J = 7.6 Hz, 2H), 2.05–2.00 (m, 1H), 1.94–1.88 (m, 1H), 1.76–1.70 (m, 1H), 1.64–1.55 (m, 4H), 1.54–1.46 (m, 4H), 1.42–1.20 (m, 23H), 0.85 (t, J = 7.0 Hz, 3H); HRESIMS m/z 567.3031 [M+H]^+ (calcd for C_{26}H_{53}BrClN_4O_2 567.3035).

**Formation of chloroepoxide (5.8) from 5.7** (50 µg) was dissolved in MeOH (0.5 mL), and potassium carbonate (150 µg) was added and the mixture was stirred at room temperature for 1 hour. The reaction was neutralized with a solution of 95:5 MeOH/AcOH, and the solvent was evaporated under a stream of N$_2$. The mixture was subjected to HPLC (column: Phenomenex, Luna, C18 (2), 5µ, 10 x 250 mm; flow rate: 2 mLmin$^{-1}$ mobile phase: 10-100% CH$_3$CN/H$_2$O+0.1% TFA over 45 minutes) to give the chloroepoxide 5.8 (~30 µg). $^1$H NMR (600 MHz, CD$_3$OD/CDC$_3$): δ 3.54 (ddd, J = 8.0, 8.0, 4.0 Hz, 1H), 3.18 (t, J = 7.7 Hz, 2H), 3.16 (t, J = 7.7 Hz, 2H), 2.90 (ddd, J = 5.6, 5.6, 2.0 Hz, 1H), 2.86 (dd, J = 8.0, 2.0 Hz, 1H), 2.18 (t, J = 7.7 Hz, 2H), 1.93–1.88 (m, 1H), 1.79–1.72 (m, 1H), 1.65–1.27 (m, 32H), 0.90 (t, J = 7.1 Hz, 3H) HRESIMS m/z 487.3776 [M+H]^+ (calcd for C$_{26}$H$_{52}$ClN$_4$O$_2$ 487.3773).

**Preparation of mollenyne A benzoate derivative (5.9):** Mollenyne A (5.6, 50 µg) was stirred with excess benzylic chloride (5 µL) in pyridine for 2 hours at room temperature. The solvent was evaporated under a stream of N$_2$, then by high vacuum. The material was purified by gradient HPLC (column:
Phenomenex, Luna, C18 (2), 5μ, 10 x 250 mm; flow rate: 2 mLmin⁻¹ mobile phase: 10-100% CH₃CN/H₂O+0.1% TFA over 45 minutes), then by isocratic HPLC (column: Phenomenex, Synergi Hydro-RP, 4μ, 10 x 250 mm; flow rate: 2 mLmin⁻¹ mobile phase: 17:8 CH₃CN/H₂O+0.1%) to give mollenyne A benzoate 5.9 (18 μg). UV (MeOH) λ_max 235 nm, 261 nm, 275 nm; CD (MeOH) λ 227 nm (Δε +6.1), 260 nm (Δε +7.5), 275 nm (Δε +6.5); HRESIMS m/z 733.1146 [M+H]^+ (calcd for C₃₃H₄₀Br₂ClN₄O₃ 733.1150).

Preparation of mollenyne A cinnamate derivative (5.10): Mollenyne A (5.6, 50 mg) was stirred with excess p-methoxycinnamoyl chloride (1 mg) in pyridine (250 mL) for 4 hours at room temperature. The solvent was evaporated under a stream of N₂, then by high vacuum. The material was purified by gradient HPLC (column: Phenomenex, Luna, C18 (2), 5μ, 10 x 250 mm; flow rate: 2 mLmin⁻¹ mobile phase: 10-100% CH₃CN/H₂O+0.1% TFA over 45 minutes), then by isocratic HPLC (column: Phenomenex, Synergi Hydro-RP, 4μ, 10 x 250 mm; flow rate: 2 mLmin⁻¹ mobile phase: 17:8 CH₃CN/H₂O+0.1%) to give mollenyne A cinnamate ester 5.10 (17 μg). UV (MeOH) λ_max 262 nm, 275, 313; CD (MeOH) λ 262 nm (Δε −6.6), 290 nm (Δε +10.8), 296 nm (Δε +10.8), 307 nm (Δε +10.7), 312 nm (Δε +10.7); HRESIMS m/z 789.1401 [M+H]^+ (calcd for C₃₆H₄₄Br₂ClN₄O₄ 789.1412).
5.8 References


(10) CD and UV spectra were normalized using ε values reported in the literature for an ene–yne–ene chromophore. \( \lambda_{\text{max}} \) 261 nm (ε 32,000), 276 nm (ε 30,500). Crombie, L.; Jacklin, A. G. *J. Chem. Soc.* **1957**, *1632–1646.*
(11) CD and UV spectra were normalized using ε values reported in the literature for an \( p \)-methoxycinnamate chromophore. \( \lambda_{\text{max}} \) 311 nm (\( \epsilon \) 24,000). Wiesler, W. T.; Nakanishi, K. J. Am. Chem. Soc. 1990, 112, 5574–5583.


5.8 Appendix

Figure 5.10. $^1$H NMR spectrum of molleneyne A (5.6) (600 MHz, MeOH-d$_4$, 1.7mm MicroCryoProbe).
Figure 5.11. $^{13}$C NMR spectrum of molleneyne A (5.6) (125 MHz, MeOH-$d_4$).
Figure 5.12. DQF–COSY spectrum of nolloyne A (5,6) (600 MHz, MeOH–d₄, 1.7 mm MicroCryoProbe).
Figure 5.13. TOCSY spectrum of molleneyne A (5,6) (600 MHz, MeOH-d4, 1.7 mm MicroCryoProbe).
Figure 5.14. gHSQC spectrum of molleneyne A (5.6) (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 5.15. gHMBC spectrum of molleneyne A (5.6) (600 MHz, MeOH-\textit{d}_4, ^nJ_{HC}=8\text{Hz}, 1.7\text{mm MicroCryoProbe}).
Figure 5.16. $^1$H NMR spectrum of molleneyne A (5.6) (600 MHz, CD$_3$CN, 1.7mm MicroCryoProbe).
Figure 5.17. gCOSY spectrum of molleneyne A (5.6) (600 MHz, CD$_3$CN, 1.7mm MicroCryoProbe).
Figure 5.18. gHMBC spectrum of molleneyne A (5,6) (600 MHz, CD$_3$CN, $^nJ_{HC} = 8$Hz).
Figure 5.19. $J$–HMBC spectrum of molleneyne A (5.6) (600 MHz, CD$_3$CN, $^9J_{HC} = 3$Hz, $J_{scale} = 37$).
Figure 5.20. HSQC–HECADE spectrum of molleneyne A (5.6) (600 MHz, CD$_3$CN, $t_{\text{mix}} = 60$ ms).
Figure 5.21. $^1$H NMR spectrum of hydrogenation product 5.7 (600 MHz, 1:1 MeOH-\textit{d}_4/CDCl$_3$, 1.7mm MicroCryoProbe).
Figure 5.22. gCOSY spectrum of hydrogenation product 5.7 (600 MHz, 1:1 MeOH-\textit{d}_6/CDCl\textsubscript{3}).
Figure 5.23. $^1$H NMR spectrum of epoxide 5.8 (600 MHz, MeOH-$d_4$, 1.7mm MicroCryoProbe).
Figure 5.24. $^1$H NMR spectrum of benzoate 5.9 (600 MHz, MeOH- $d_4$, 1.7mm MicroCryoProbe).
Figure 5.25. $^1$H NMR spectrum of $p$-methoxycinnamate 5.10 (600 MHz, MeOH-$d_4$, 1.7 mm MicroCryoProbe).