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Natural Products from New Zealand

Latrunculia Species Sponges

Tanja Grkovic

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

The University of Auckland, 2008
Abstract

In a survey of the secondary metabolite chemistry profiles of ten New Zealand, one Antarctic and one South African-sourced collections of *Latrunculia* spp. sponges, eighteen discorhabdin alkaloids have been isolated. Four of those, namely discorhabdin K, 3-dihydro discorhabdin A, 1-thiomethyl discorhabdin G*/I, and 16a,17a-dehydro discorhabdin W were fully characterized as new natural products in the series. In addition, for the first time, five enantiomeric pairs and two sets of diastereomers of the naturally-occurring discorhabdin alkaloids were identified. The absolute configuration of all of the chiral compounds isolated, including new natural products, has been established upon comparison of the observed experimental data with the results of time dependant density functional theory calculations of the electronic circular dichroism spectra.

A structure activity relationship study on discorhabdin B, the main natural product of the Wellington-sourced sponges, has identified four reactive centres on the molecule and yielded nine novel semi-synthetic derivatives. Consequently, a new discorhabdin biosynthetic tree was proposed which highlighted discorhabdin B as a crucial precursor to a number of other naturally-occurring analogues. The importance of the iminoquinone moiety and the spirodienone ring with respect to the bioactivity of the compounds in a range of naturally-occurring discorhabdins was demonstrated. A new semi-synthetic derivative, 1-discorhabdyl discorhabdin D, was identified as a potent anti-malarial agent and has opened new possibilities for the therapeutic development of the discorhabdin alkaloids.
Declaration

This is to certify that:

1) This thesis comprises only the authors original work, except where indicated below;

2) Due acknowledgment to all other material used has been made in the main text of the thesis

My overall contribution to the work presented in this thesis is approximately 90%, based on the following:

Chapter 2
95% Time dependent density functional theory calculations of the electronic circular dichroism spectra were the work of Professor Daneel Ferreira, Dr Yuanqing Ding and Dr Xing-Cong Li at Department of Pharmacognosy and National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi.

Chapter 3
95% One-electron reduction potentials are the work of Associate Professor Robert F. Anderson and Dr. Sujata S. Shinde at Department of Chemistry, The University of Auckland
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In my biased opinion not knowing what magic compound you might have dissolved in the NMR tube is the most fun a natural product chemist can ever have. However the task of obtaining good quality and an informative NMR dataset for the same can sometimes be challenging. A very big thank you goes to Mr. Michael Walker and Dr Michael Schmitz for help with NMR data acquisition, and to Mrs. Raisa Imatdieva for obtaining the mass spectral data.

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<th>Description</th>
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<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>A-549</td>
<td>Human lung adenocarcinoma</td>
</tr>
<tr>
<td>BV</td>
<td>Benzyl viologen</td>
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<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>CaN</td>
<td>Calcineurin</td>
</tr>
<tr>
<td>CD</td>
<td>Circular dichroism</td>
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<td>CN</td>
<td>Cyanoalkyl-derivatized silica</td>
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<td>calcd</td>
<td>Calculated</td>
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<td>Duroquinone</td>
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<tr>
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<td>Dithiothreitol</td>
</tr>
<tr>
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<td>Electronic circular dichroism</td>
</tr>
<tr>
<td>ED₅₀</td>
<td>50% Effective dose</td>
</tr>
<tr>
<td>EI</td>
<td>Electron impact</td>
</tr>
<tr>
<td>E₁/₂</td>
<td>Half-wave reduction potential</td>
</tr>
<tr>
<td>E₇</td>
<td>One electron reduction potential</td>
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FAB  Fast atom bombardment
GC   Gas chromatography
GI50 50% Growth Inhibition
GSH Reduced glutathione
HCT-116 Human colon tumor cell line
HIV-1 Human immunodeficiency virus type 1
HMBC Gradient heteronuclear multiple-bond correlation
HPLC High performance liquid chromatography
HR High resolution
HSQC Gradient heteronuclear single-quantum correlation
HT-29 Human colon cancer cell line
Hz Hertz
IC50 50% Inhibitory concentration
IDO Indoleamine-2,3-dioxygenase
IR Infrared
J Coupling constant
J-HMBC Long-range gradient heteronuclear multiple-bond correlation
KB Human oral epidermoid cancer cell line
L-1210 Mouse lymphatic leukemia cell line
L-1210/DX Doxorubicin resistant mouse lymphatic leukemia cell line
m Multiplet
M mol/L
MCF-7 Human breast cancer cell line
Me Methyl
MeCN Acetonitrile
MeOH Methanol
MIC Minimum inhibitory concentration
MS Mass spectrometry
MTPA α-Methoxy-α-trifluoromethylphenyl-acetic acid
Mult Multiplicity
m/z Mass to charge ratio
NADPH Nicotine adenine dinucleotide phosphate
NCI National Cancer Institute
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>NIWA</td>
<td>National Institute of Water and Atmospheric Research</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser enhancement spectroscopy</td>
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<td>Parts per million</td>
</tr>
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<tr>
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</tr>
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</tr>
<tr>
<td>$\tau_{\text{mix}}$</td>
<td>Mixing time</td>
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<tr>
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<td>$^{13}$C NMR</td>
<td>Carbon-13 nuclear magnetic resonance spectroscopy</td>
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1. Introduction
The field of marine natural products encompasses chemistry, biology, ecology and pharmacology. In very simple terms, natural products are an organism’s strategy for survival. Whereas primary metabolites are involved in the basic growth and maintenance functions of an organism, small molecules or natural products associated with the secondary metabolism assist its producer in various ecological ways such as predator deterrence, mediation of competition, facilitation of reproduction and defence from pathogens.\(^1\) In contrast to the primary biosynthetic pathways that are product oriented and face evolutionary selection towards a specific target, having a primarily ecological function the secondary metabolite pathways face an evolutionary pressure towards constant adaptation and are diversity oriented.\(^2\) Derived from the isoprenoid, acetogenin, amino acid, shikimate, nucleic acid and the carbohydrate pathways, secondary metabolites display a range of structures and add to the variety of naturally-sourced molecules.

Natural products are an important avenue of new drug discovery. In the latest review of the sources of new and approved drugs for human diseases covering the period from September 1981 to June 2006, from a total of 983 small molecules being developed as drug candidates, 5.7% were sourced from natural products, 27.6% were natural product derivatives and 17.1% were synthetic molecules that were developed based on an identified natural product pharmacophore.\(^3,4\) The marine environment has yielded a significant number of potential drug leads, including sponge-sourced metabolites halichondrin B (1),\(^5\) (+)-discodermolide (2)\(^6\) and the bryostatins, exemplified by bryostatin 1 (3),\(^7\) sourced from an endosymbiotic bacterium of a bryozoan *Bugula neritina*.\(^8\)
The most significant leads of a true marine natural product in the development as an anticancer drug are the two ascidian-sourced metabolites ecteinascidin 743 (4) and aplidin (5). Ecteinascidin 743 (4), isolated from the Mediterranean ascidian *Ecteinascidia turbinata*, was active in the picomolar range in *in vitro* models against leukemic and human solid tumor cell lines, and was found to bind to DNA and inhibit protein synthesis. Currently, marketed as Yondelis™, compound 4 is involved in phase II clinical trials for ovarian, soft tissue sarcoma, breast, endometrial, prostate and non-small cell lung cancers. Aplidin (5), a depsipeptide isolated from another Mediterranean ascidian *Aplidium albicans*, showed potent *in vitro* and *in vivo* activity against a range of human haematological and solid tumor cell lines. The compound was found to cause apoptosis in growing cancer cells. Aplidin is currently in phase II trials for melanoma, multiple melanoma, pancreatic cancer, head, neck, bladder, gastric and prostate tumors, non-Hodgkin lymphoma, non-small cell lung and small cell lung cancer and acute lymphoblastic leukaemia. Due to high selectivity and low toxicity to somatic cells that compound 5 has shown in phase I/II trials, it is also being evaluated for a range of pediatric haematological diseases.
The phylum Porifera is a major source of natural products research, it has yielded many bioactive compounds and provided a great diversity of the isolated structures. The latest marine natural product review by Blunt et al., shows that 235 new compounds, or 30% of total published in 2006 were sourced from the work on phylum Porifera. Furthermore, cytotoxicity data compiled at the National Cancer Institute, sources Poriferans as the number one source of bioactive hits, followed by Bryozoa, Chordata, Cnidaria, Rhodophyta, Echinodermata and Mollusca. High number of natural product publications and clinical leads reflects the large number of work associated with the phylum, as since the very beginnings of marine chemical bioprospecting work, sponges have by far been the most extensively researched animal group.

The coastal territory of New Zealand covers the exclusive economic zone of 200 nautical miles, corresponding to a sea area of 4.83 million km$^2$. Dominated by two very different oceanic currents, the warm East Auckland Current in the upper North Island and the cold Southland Current in the South Island, this large marine area displays a range of temperate and cold-temperate marine ecosystems. The diversity and richness of the local coastline is also reflected in a number of natural products publications sourced from the area, MarinLit lists 254 publications and 363 chemical structures containing the keyword New Zealand.

This thesis examines the natural products chemistry form New Zealand-sourced *Latrunculia* spp. sponges. The genus has a metropolitan distribution, with the local species well represented in both biomass and diversity on exposed rocky shores. The following section provides an introduction on the natural product chemistry reported from the genus *Latrunculia*, as well as other related genera in the Poriferan families Latrunculiidae and Acarnidae.
1.1 Natural Products from *Latrunculia* Species Sponges

Sponges of the genus *Latrunculia* du Bocage, 1869 (Porifera: Demospongiae: Latrunculiidae) are widely distributed across the South Pacific Ocean, inhabiting hard rocky substrates in exposed coastal environments.\(^\text{17}\) The most distinguishing morphological feature of the genus is the discorhabd microscleres, with the number and shape of the whorls taken as the morphometric variables to distinguish between different species. In the most recent taxonomic review, Kelly and coworkers identified 27 species, divided into two subgenera: *Latrunculia* (*Latrunculia*) containing 14 separate species and *Latrunculia* (*Biannulata*) with 13 species.\(^\text{17}\) The main characteristics and distribution of the two sub-genera are summarized in Table 1.1

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>Latrunculia</th>
<th>Biannulata</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphological features</strong></td>
<td>Acanthodiscorhabds with three leaf-like crenulated whorls</td>
<td>Acanthodiscorhabds with two central parallel whorls</td>
</tr>
<tr>
<td><strong>Habitat</strong></td>
<td>Deep-water</td>
<td>Coastal waters, mostly shallow-water</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>Southern Ocean, New Zealand, Australia, South Pacific Islands, Philippines, South Africa, South America, Japan, Korea, Cape Verde Islands and the Azores.</td>
<td>New Zealand, Australia, South Africa, Canary Islands, Sea of Okhotok, British Columbia</td>
</tr>
<tr>
<td><strong>New Zealand species</strong></td>
<td><em>Latrunculia oxydiscorhabda</em> Alvarez et al., 2002</td>
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<td><em>Latrunculia millerae</em> Alvarez et al., 2002</td>
<td><em>Latrunculia millerae</em> Alvarez et al., 2002</td>
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</tbody>
</table>

Table 1.1. Summary of the genus *Latrunculia* taxonomic revision, adapted from Samaai *et al.*\(^\text{17}\)

In a study of the New Zealand-sourced *Latrunculia* species, Miller *et al.* have investigated brown and green sponges collected from five different locations: Three Kings Islands, Tutukaka, Wellington, Kaikoura and Doubtful Sound (Figure 1.1).\(^\text{16}\) Genotype profiling based on alloenzyme electrophoresis revealed each collection to be genetically distinct, and together with the morphological examination of the discorhabd microscleres and secondary metabolite profiling, the authors concluded that there are at least eight different species of *Latrunculia* in New Zealand coastal waters. The sponges, even though genetically distinct between populations, showed a large number of identical genotypes
within a collection, suggesting that asexual reproduction is common. At two of the collection sites, Three Kings Islands and Kaikoura, two co-occurring phenotypes green and brown sponges were shown to be genetically, morphologically and chemically distinct, and thus represented two sympatric species. A more detailed taxonomic study followed, where Alvarez and coauthors named and outlined a key to differentiating between nine different New Zealand species. Graphical representation of the current *Latrunculia* genus distribution is shown in Figure 1.1.

**Figure 1.1.** Distribution of *Latrunculia* species sponges in New Zealand coastal waters, adapted from Samaai et al. and Alvarez et al.17,18

In an ecological study lasting over two and a half years, Duckworth and Battershill examined growth, survival and recruitment of *Latrunculia* sp. in the Wellington Harbor. The authors found that sponges reproduce year round with new recruits showing moderate survival, there were no signs of predation on the species and the mortality of the population as a whole was low and unaffected by seasonal cycles. The study found no
allelophatic interactions with co-occurring organisms, with the exception of an occasional fouling ascidian *Didemnum candidum*. Production of secondary metabolites, taken as a bi-monthly IC₅₀ value against a P388 cell line, was shown to correlate with sea-water temperature, being lowest in the early spring months and highest in late summer. Extensive work on the aquaculture of the species has shown that the sponges can be successfully grown on mesh arrays to give high biomass yield and produce the same levels of secondary metabolites as wild populations.²⁰,²¹

1.1.1 Review of the Published Literature on *Latrunculia* spp. and Other Related Natural Products

*Latrunculia* spp. sponges produce a range of bioactive alkaloid natural products named after the genus characteristic microscaleres, the discorhabdins.²² The term is synonymous with prianosins and epinardins, isolated from the Japanese collection of the sponge *Prianos melanos* and an unidentified green sponge from the South Indian Ocean respectively.²³,²⁴ The compounds contain a core a pyrido[2,3-h]pyrrolo[4,3,2-de]quinoline tetracyclic skeleton bound to various spiro-substituents at the C-6 position. The following is a short review on the natural products published in the literature with the common pyrroloiminoquinone moiety, and their reported biological activities.

![Figure 1.2. Structure of a generalized discorhabdin molecule, showing atom numbering.](image-url)
1.1.1.1 Discorhabdins, Prianosins and Epinardins

The first published compound in the series, discorhabdin C (6), was isolated by bioassay guided fractionation from the New Zealand sponge *Latrunculia* sp. du Bocage (later re-examined and identified as *Latrunculia* (*Biannulata*) *kaikoura*)\(^1\)\(^\text{17,18}\) by Blunt, Munro and coworkers in 1986.\(^2\)\(^\text{22}\) The structure was fully assigned by a single-crystal X-ray diffraction study on the trifluoroacetate salt of 6. At the time this was the first example of a natural product with a pyrido[2,3-\(h\)]pyrrolo[4,3,2-de]quinoline tetracyclic skeleton. The following year, Kobayashi *et al.* published the structure of prianosin A (7) isolated from an Okinawan sponge *Prianos melanos*\(^2\)\(^\text{23}\) (recently re-identified as *Strongylodesma* sp.).\(^2\)\(^\text{25}\) Compound 7 was different from 6 in that it had a different spiro-ring substitution and an extra thioether bridge between C-5 and C-8, thus having three stereocenters at C-5, C-6 and C-8. The absolute stereochemistry of prianosin A was resolved by an X-ray diffraction study of the free base of 7 and established as (5\(R\),6\(S\),8\(S\)). It is very important to note that of all the chiral discorhabdins known, compound 7 is the only one with defined absolute stereochemistry, and the stereochemistry of all other hereafter reviewed compounds will be denoted with an asterix, indicating relative assignments only.

![Chemical structures of 6, 7, and 8](image)

Publication of discorhabdins A (7) and B (8) together with a complete set of spectral data for 6 from a New Zealand-sourced *Latrunculia* collection (*L. (*Biannulata*) *kaikoura*)\(^1\)\(^\text{17,18}\) followed.\(^2\)\(^\text{26}\) The structure of discorhabdin A was identical to that of prianosin A, while discorhabdin B was the spiro-dienone analogue of 7. Relative stereochemistry of 8 was established as (6\(S^*\),8\(S^*\)) by NOE difference NMR experiments and upon comparison with 7.\(^2\)\(^\text{26}\) Compounds 6, 7 and 8 were strongly cytotoxic to murine leukemia P388 cells with ED\(_{50}\) values of 0.03, 0.05 and 0.1 \(\mu\)g/mL respectively. Compound 7 induced Ca\(^{2+}\) release from sarcoplasmic reticulum ten times more potent then caffeine in the assay.\(^2\)\(^\text{23}\) Furthermore, New Zealand-sourced discorhabdins C (6)\(^2\)\(^\text{22}\) and B (8)\(^2\)\(^\text{26}\) have been evaluated
at the US National Cancer Institute (NCI) \textit{in vitro} disease-oriented primary anti tumor screen, with the average GI$_{50}$ over sixty human tumor cell lines of 0.14 and 0.71 $\mu$M respectively.$^{27}$ Compound 6 was selective for the colon and leukemia subpanels and met the criteria for \textit{in vivo} testing, but unfortunately due to insufficient activity in follow-up tests, discorhabdin C was abandoned for future drug development.$^{27}$

Further three metabolites isolated from \textit{P. melanos} were prianosins B (9), C (10) and D (11).$^{28}$ Compound 9 is the 16,17 dehydrogenated analog of 7, while compounds 10 and 11 posses an extra heterocyclic ring between the N-18 imine and C-2 on the \textit{spiro}-ring. The absolute stereochemistry of 9 was assigned as (5$R$,6$S$,8$S$) by virtue of exhibiting an identical circular dichroism spectra to that observed for prianosin A (7). The relative stereochemistry of 10 and 11 was assigned as (6$R^*$,8$S^*$) by interpolation of the circular dichroism spectra and assuming a common biosynthetic pathway of the clearly related set of compounds. The authors assigned the C-2 stereocentre as $S^*$ for 10 and $R^*$ for 11, using molecular modeling where a substituent on the upper $\beta$ face of the molecule was impossible, and an NOE enhancement of the H-2 signal upon irradiation of the $\alpha$ (lower-face) methylene pair on C-17. However, Kobayashi and coworkers isolated prianosins as free bases using normal-phase silica and Sephadex LH-20 chromatography. As free bases, 10 and 11 had very poor solubility, and all spectral studies on the compounds were carried out with their acetylated derivatives. The actual structures, initially published as the phenols on C-11 were later corrected by the authors to the iminoquinone form.$^{29}$ The first correct structure for 10 and 11 were shown by Perry \textit{et al.}$^{30}$ Discorhabdin D (11), isolated as a hydrochloride salt from \textit{L. brevis (L. (Bianululata) kaikoura)}$^{17,18}$ was freely soluble in methanol and a full set of spectral data was provided in the publication. The authors proposed an identical stereochemistry on all three stereocenters (2$S^*$,6$R^*$,8$S^*$): 6$R^*$,8$S^*$ based on a zig-zag long-range coupling between H-4 and H-7$\alpha$ protons, and 2$S^*$ based on
an NOE enhancement of the H-2 signal upon irradiation of the α lower-face hydrogen at C-17, (Figure 1.3).

![Figure 1.3](image)

**Figure 1.3.** Crucial NMR correlations used to assign the relative stereochemistry of 11, adapted from Perry *et al.*

Compounds 9, 10, and 11 were cytotoxic against murine lymphoma L-1210 cells with IC<sub>50</sub> values of 2.0, 0.15 and 0.18 μg/mL; against murine lymphoma L-5178Y cells with IC<sub>50</sub> values of 1.8, 0.024 and 0.048 μg/mL; and against human epidermoid carcinoma KB cells with IC<sub>50</sub> values of >5.0, 0.57 and 0.46 μg/mL respectively. Although at an ED<sub>50</sub> of 6.0 μg/mL somewhat less potent then discorhabdins A-C in the P388 assay, 11 was shown to have significant *in vivo* activity with T/C 132% at 20 mg/kg. New Zealand-sourced 11 has also gone through the NCI 60 cell line tumor panel screen with a mean panel average GI<sub>50</sub> value of 2.87 μM, but due to lack of selectivity was not further pursued as an anti-cancer drug candidate.

Discorhabdins E (12) and F (13) were isolated as minor metabolites from the New Zealand discorhabdin C (6)-sourced sponge *L. cf. bocagei.* (The species has recently tentatively been assigned as *L. (Latrunculia) triverticillata*.) Although initially no spectral data for the compounds was supplied, compound 12 was fully characterized in a later publication by Copp *et al.* When examined for an optical rotation at various wavelengths from 250 to 700 nm, 12 was found to be racemic, suggesting the compound is a mono
debromination product of 6, known to have two enantiomeric conformations in the solid state. Discorhabdin E (12) was found moderately cytotoxic against murine leukemia P388 cells with an IC₅₀ of 206 μg/mL. Discorhabdin G (14), a 7,8-dehydro derivative, was isolated together with discorhabdin C (6) from Antarctic L. apicalis (re-identified as L. (Latrunculia) biformis). The compound was optically active and the authors assigned the (6R*) relative stereochemistry in the publication. There has been some ecological work done with 14, Baker and coworkers have shown that discorhabdin G is concentrated mainly in the first two mm of the sponge tissue, and at natural concentrations the compound is a potent feeding deterrent against a common Antarctic starfish predator Perknaster fuscus.

A significant amount of confusion is associated with discorhabdins G*-O (15-21). The metabolites were originally isolated from New Zealand Latrunculia spp. and published with no spectral data as a poster presentation at the 37th Annual Meeting of the American Society of Pharmacognosy in 1996. The late 1990s and early 2000s saw seven new publications on discorhabdin alkaloids from sponges collected around the world, and inevitably some of the New Zealand-sourced compounds were re-isolated and renamed or published multiple times with different names. In an attempt to clarify the situation, Blunt, Munro and coworkers, in a review of bioactive alkaloid compounds, presented a summary of all of the New Zealand isolated discorhabdins. Unfortunately, some of the compounds were identified incorrectly due to an error in the structure in one of the summary schemes. The following section on discorhabdins 15–21 takes account of the original spectral data that was kindly provided by Professors John Blunt and Murray Munro.
Compound 15, 1-debromo discorhabdin B (8), is known as: discorhabdin G published in a poster presentation by Blunt, Munro and coworkers\textsuperscript{35} and later renamed discorhabdin I by the same authors in Urban \textit{et al.}\textsuperscript{36} (with no thioether linkage between C-5 and C-8, which upon repeat examination of the original spectra is apparently present)\textsuperscript{37}; discorhabdin G* by Davies-Coleman and coworkers isolated from South African \textit{L. bellae}\textsuperscript{38} (re-identified as \textit{Cyclacanthia bellae})\textsuperscript{39} and finally discorhabdin I isolated from a South American collection of \textit{L. (Latrunculia) brevis}\textsuperscript{40}. The relative stereochemistry, depicted by all authors but Davies-Coleman and coworkers, was shown as (6\text{S}*,8\text{S}*). Compound 15 was strongly cytotoxic against colon tumor cell lines HCT-116 and HT-29 with IC\textsubscript{50} values of 0.33 and 0.35 \(\mu\)M respectively.\textsuperscript{38,40} New Zealand-sourced 15 showed an NCI mean panel average GI\textsubscript{50} of 3.38 \(\mu\)M with no differential cytotoxicity profile.\textsuperscript{27}

Discorhabdin H (16) was first presented by Blunt, Munro and coworkers\textsuperscript{35} but later renamed discorhabdin J in Urban \textit{et al.}\textsuperscript{36} However, the incorrect structure given in the paper is now confirmed to have actually been \(\Delta^4\)\textsuperscript{37} Compound 16 was eventually fully characterized in a publication by Antunes \textit{et al.}, isolated from the South African sponge \textit{Strongylodesma algoaensis}.\textsuperscript{38} The authors assigned relative stereochemistry as (1\text{R}*\text{*},2\text{R}*,6\text{R}*,8\text{S}*,7'S) relying on the assumption of (2\text{R}*,6\text{R}*,8\text{S}*) of a 1-thiohistidine substituted discorhabdin D (11)-type compound and ROESY correlations observed from H-2 and H7\text{\textalpha} positioned on the lower face of the molecule to H-1. The absolute stereochemistry of the thiohistidine residue was established by an ozonolysis of the histidine moiety and breakdown to aspartic acid which was analyzed on a chiral gas chromatography (GC) column and found to have (7'S) configuration. New Zealand-sourced compound 16 showed an NCI mean panel average GI\textsubscript{50} of 13.3 \(\mu\)M with no differential cytotoxicity profile.\textsuperscript{27}

Discorhabdin L (17), isolated by Blunt, Munro and coworkers\textsuperscript{35} and incorrectly identified under the same name as the 4,5-dihydro analog by Urban \textit{et al.}\textsuperscript{36} was eventually fully characterized by Reyes \textit{et al.}, from a South American collection of \textit{L. (Latrunculia) brevis}.\textsuperscript{40} Relative stereochemistry (6\text{R}*,8\text{S}*) about the discorhabdin-core of the molecule was assigned based on a long-range zig-zag COSY correlation from H-4 to H-7\text{\textalpha} as in 11, and the stereochemistry at C-1 and C-2 were assigned (1\text{R}*,2\text{S}*) based on an NOE difference experiment where upon irradiation of the H-1 resonance the authors observed a
1% enhancement of the H-7α resonance and a 2.7% enhancement of the H-2 resonance. Compound 17 was active in the sub-micromolar range against a panel of 14 tumor cell lines, with most effective potency against the HT-29 colon cell line showing a GI50 value of 0.12 μM.40

Discorhabdin N (18), again, originally isolated from New Zealand-sourced Latrunculia sp. by Blunt, Munro and coworkers35 and incorrectly identified under the same name as the 4,5-dihydro analog by Urban et al.,36 was fully characterized by Antunes et al. sourced from South African L. bella,38 (re-identified as Cyclacanthia bella).39 In addition to the 1-glycyl metabolite, Antunes et al. also identified and characterized the amino and methoxy analogues, simply named 1-amino discorhabdin D (19) and 1-methoxy discorhabdin D (20). No stereochemistry was assigned to the three compounds in the publication. Compounds 18, 19 and 20 were cytotoxic against human colon tumor cell line HCT-116 with GI50 values of 2.24, 0.19 and 0.23 μM respectively.

Discorhabdins K (21), M (22) and O (23) isolated from New Zealand-sourced Latrunculia sp. were presented, but to date have never been fully characterized.35 Shown as the dethio analog in Urban et al.,36 examination of the original NMR spectra for 21, confirmed the presence of a thioether bridge between C-5 and C-8.37 Compound 21 was submitted to the
NCI 60 human tumor cell line anticancer drug screen and showed a mean panel average GI$_{50}$ value of 8.81 μM with no differential cytotoxicity profile.$^{27}$

Discorhabdin P (24), the first N13–methylated analogue, was isolated as a free base from a deep water Batzella sp. sponge collected off the Bahamas coast and characterized by NMR spectroscopy and single crystal X-ray diffraction.$^{41}$ The original voucher specimen was later re-identified as a Strongylodesma species.$^{25}$ Compound 24 was found to be a potent inhibitor of the phosphatase activity of calcineurin and the peptidase activity of CPP32, whereas related compound discorhabdin C (6) was inactive in the same assay. Discorhabdin Q (25), the 16,17-dehydro analogue of discorhabdin B (8), was isolated as the major metabolite of sponges L. (Biannulata) purpurea, Zyzzya massalis, Z. fuliginosa and Zyzzya sp. collected from Australia and Fiji, and assigned (6$^*$S,8$^*$S) relative stereochemistry based on structural homology with 8.$^{42}$ Compound 25 was moderately cytotoxic with an NCI mean panel average IC$_{50}$ of 0.5 μg/mL (1.21 μM).$^{42}$ The authors hypothesized that the observed drop in bioactivity is due to the full aromatization of the iminoquinone moiety and furthermore commented that this might have hindered the compound from being discovered earlier in the classical bioassay-guided isolation procedures. Discorhabdin R (26), 1-debromo-1,2-epoxy discorhabdin B (8) analogue, was isolated from an Australian collection of Negombata sp, and an Antarctic Latrunculia sp.$^{43}$ (the latter is possibly L. (Biannulata) purpurea).$^{25}$ Relative stereochemistry about the discorhabdin core was assigned (6$^*$R,8$^*$S) based on the structural similarities with compound 8, while the orientation of the epoxide at C-1 and C-2 was not resolved due to a lack of conclusive data given by NOESY and NOE difference NMR experiments. Compound 26 was found active against Gram-positive and -negative bacteria.
Discorhabdins S (27), T (28) and U (29) were isolated as free bases from a deep sea Batzella sp. sponge (re-identified as a Strongylodesma sp.) collected in the Bahamas. The authors assigned (6S*) stereochemistry for all three compounds, based on structural similarities with discorhabdins B (8) and Q (25). The compounds were assessed against three mammalian cancer cell lines with, P388 murine leukemia IC_{50} values of 3.08, >5 and 0.17 μM; A-549 human lung adenocarcinoma IC_{50} values of >5, >5 and 0.17 μM; and PANC-1 human pancreatic cells IC_{50} values of 2.6, 0.7 and 0.069 μM for 27, 28 and 29 respectively. The apparent drop in bioactivity with the 16,17-dehydro analogues 27 and 28, parallels that observed for discorhabdin Q (25).

Discorhabdin V (30) and the related 1-hydroxy,14-bromo discorhabdin V (31) were isolated from a South African collection of Tsitsikamma pedunculata. The compounds were moderately active against the HCT-116 human colon tumor cell line with IC_{50} values of 1.27 and 12.50 μM for 30 and 31 respectively. Stereochemistry was not shown in the publication. Two tribrominated discorhabdin analogues, 14-bromo discorhabdin C (32) and 3-dihydro,14-bromo discorhabdin C (33), were isolated by Davies-Coleman and coworkers from the sponge Tsitsikamma favus collected from South Africa. The orientation of the hydroxy substituent on the C-3 of the spirodienol ring in 33 was not resolved by the authors. Three other spirodienol discorhabdins are known, 34, 35 and 36.
were isolated as natural products from South African T. pedunculata by Antunes et al.\textsuperscript{38} Compound 34 is also a known sodium borohydride reduction product of discorhabdin C (6).\textsuperscript{32} Orientation of the hydroxy substituent on C-3 was not shown by either authors. Naturally-occurring spirodienol derivatives 34, 35 and 36 were active against HCT-166 cell line with IC\textsubscript{50} values of 0.32, 0.20 and 0.22 μM respectively\textsuperscript{38}, while semi-synthetic 34 showed an NCI mean panel IC\textsubscript{50} of 3.09 μM and was selective for small cell lung and colon cancer subpanels.\textsuperscript{32}

![Diagram of compounds 34, 35, and 36](image)

Recently, the first dimeric analogue, discorhabdin W (37) was reported from a New Zealand-sourced collection of Latrunculia sp.\textsuperscript{46} Compound 37 was strongly cytotoxic against P388 cell line, with an IC\textsubscript{50} value of 0.08 μM, comparable to that of discorhabdin B (8), listed in the same publication as 0.09 μM. The authors proposed a biosynthetic origin of 37 to go through precursor 8, as when discorhabdin B (8) was irradiated with UV/vis, trace amounts of discorhabdin W (37) were detected by analytical HPLC. Furthermore, on an analytical scale, 37 could be reduced back to 8 with a common disulfide reducing reagent dithiothreitol (DTT), (Scheme 1.1).

![Diagram of biosynthetic origin of discorhabdin W (37)](image)

\textbf{Scheme 1.1.} Biosynthetic origin of discorhabdin W (37), adapted from Lang et al.\textsuperscript{46}
Epinardins A, B, C and D (38, 39, 40 and 41) were isolated from an unidentified deep-water green sponge collected from the Indian Ocean. Compounds 39, 40 and 41 are closely related to discorhabdin C (6) with an allylic alcohol functionality about the spiro-ring. Based on structural similarities with 2-hydroxy discorhabdin D (10) and NOESY data not shown in the publication, the authors proposed (2$R^*$,3$R^*$,6$R^*$) relative stereochemistry for epinardin A (38). The relative stereochemistry at C-2 in compounds 39, 40 and 41 was established via a NOESY correlation from H-2 to H-7α positioned on the lower face of the molecule. Relative stereochemistry at C-8 in 41 was established via NOESY data (not elaborated on in the publication) and a prediction that the axial position for the methoxy substituent may be a consequence of the anomeric effect. The authors noted that when 41 was left standing in an acetone solution it converted to 40, whereas the reverse was not observed for 40 left in a solution of methanol and Amberlyst 15. Epinardins A (38) and C (40) were tested against L-1210 and doxorubicin-resistant L-1210/DX murine lymphocytic leukemia cells, 38 was moderately active with an IC$_{50}$ value of 1.7 μg/mL and resistance index of 4, while 40 was strongly active with an IC$_{50}$ value of 0.324 μg/mL but showed a poor resistance index of 1.
Makaluvic acids A (42) and B (43) were isolated from a Micronesia collection of the sponge *Z. fuliginosa* \(^{47}\) (*fulginiosa*, name was incorrectly given in the paper). \(^{25}\) The structure of 42 was confirmed by a single crystal X-ray diffraction study. Compound 42 was found inactive against the murine leukemia P388 cell line suggesting that the iminoquinone chromophore is crucial for the bioactivity of the series. Recently, makaluvic acid C (44) and N-1-β-D-ribofuranosylmakaluvic acid C (45) were isolated from a South African sponge *Strongyloidesma aliwaliensis*. \(^{48}\) Stereochemistry of the ribofuranose was confirmed by chiral GC analysis of the hydrolyzed sugar moiety, while the β orientation of the glycosidic bond was established with a NOESY experiment showing correlations from H-1' to H-4'. Compounds 44 and 45 were evaluated against human oesophageal cancer WHCO-1 cell line, with 44 showing no activity at an IC\(_{50}\) value of >150 μM, and 45 being moderately active with an IC\(_{50}\) value of 61.0 μM.

Damirones A (46) and B (47) having a pyrroloorthoquinone moiety, were isolated from a Palauan sponge *Damiria* sp \(^{49}\) (re-identified as *Z. fuliginosa*). \(^{25}\) Another Micronesia collection of *Z. fuliginosa* yielded damirone C (48). \(^{50}\) N-1-β-D-ribofuranosyldamirone C (49) was isolated from the South African sponge *S. aliwaliensis*. \(^{48}\) The stereochemistry of
the ribofuranose and orientation about the glycosidic bond was established in an identical manner to that for 45. Compounds 48 and 49 were moderately active against human oesophageal cancer WHCO-1 cell line, with IC\textsubscript{50} values of 56.0 and 38.0 \(\mu\text{M}\) respectively.\textsuperscript{48}

Batzellines A (50), B (51) and C (52) were isolated from a Bahamas-sourced collection of a \textit{Batzella} sp. sponge\textsuperscript{51} (re-identified as \textit{Strongylodesma} sp.).\textsuperscript{25} Highly substituted with a chlorine, methyl and thiomethyl substituents on the pyrrolo\textit{ortho}aminoquinone core, the compounds have small hydrogen to carbon ratios, and their structures were resolved with a combination of X-ray crystallography, NMR, HRMS and semi-synthetic methods. Compound 50 was evaluated the NCI 60 human tumor cell line anticancer drug screen and showed selective toxicity in several of the melanoma cancer cell lines.\textsuperscript{52} Batzelline A (50) was also very potent against the human lung cancer A-549 cell line, and was selected for \textit{in vivo} studies, however due to lack of efficacy was abandoned for future drug development.\textsuperscript{52} A recent Australian collection of \textit{Z. massalis} yielded 6-dechlorobatzelline C (53).\textsuperscript{53} Batzelline D (54) was isolated from an Indopacific sponge \textit{Z. fuliginosa}.\textsuperscript{54} Tested together with a range of other related iminoquinone analogues for the ability to inhibit HIV-1 envelope-mediated fusion \textit{in vitro}, compound 54 was found inactive.
Isobatzellines A (55), B (56), C (57) and D (58) isolated from a deep-water Batzella sp. sponge, (re-identified as Strongyloidesma sp.)\textsuperscript{25} collected in the Bahamas are closely related to the batzellines with an iminoquinone chromophore instead of the aminoquinone.\textsuperscript{55} The authors found compound 55 converts to the fully aromatized 58 on a TLC plate or upon treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The isobatzellines were moderately toxic against murine leukemia P388 cell line and showed moderate anti-fungal activity against Candida albicans, whereas the batzellines were inactive in the same assays. Isobatzelline E (59) was isolated from an Indopacific collection of the sponge Z. fuliginosa.\textsuperscript{54} Compound 59 was found inactive for the ability to inhibit HIV-1 envelope-mediated fusion in vitro, whereas the 3,4-dihydro analogue 57 was active in the same assay.\textsuperscript{54}

![Chemical structure](attachment:image.png)

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<th>Compound</th>
<th>Structure</th>
<th>Activity</th>
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<td>60</td>
<td>( R_1=\text{NH}, R_2=R_3=\text{H} )</td>
<td>Inhibited CaN and CPP32 with IC\textsubscript{50} values of 0.55 and 0.02 μg/mL respectively, inhibited CPP32 with an IC\textsubscript{50} value of 2.21 μg/mL.</td>
</tr>
<tr>
<td>61</td>
<td>( R_1=\text{O}, R_2=R_3=\text{H} )</td>
<td>Inhibited CaN with an IC\textsubscript{50} value of 2.21 μg/mL.</td>
</tr>
<tr>
<td>62</td>
<td>( R_1=\text{NH}, R_2=\text{Ac} )</td>
<td>Inhibited CPP32 with an IC\textsubscript{50} value of 0.8 μg/mL, while 63 was not a CPP32 inhibitor with an IC\textsubscript{50} value of 8.80 μg/mL.</td>
</tr>
<tr>
<td>63</td>
<td>( R_1=\text{O}, R_2=R_3=\text{Ac} )</td>
<td>Not a CPP32 inhibitor with an IC\textsubscript{50} value of 8.80 μg/mL.</td>
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</table>

Secobatzellines A (60) and B (61) were isolated by bioassay guided fractionation looking for new protein phosphatase inhibitors from a deep-water Batzella sp. sponge collected in the Bahamas\textsuperscript{56} (re-identified as Strongyloidesma sp.)\textsuperscript{25} The structures were determined by a combination of NMR, HRMS and semi-synthetic methods, with the absolute stereochemistry of the secondary alcohol functionality in both compounds not resolved. The authors showed that compound 60 could be hydrolyzed to the quinone 61 in aqueous ethanol, suggesting it could be an isolation procedure artifact. The two secobatzellines and their diacetates 62 and 63 were tested for the phosphatase activity of calcineurin and the peptidase activity of CPP32, with 60 inhibiting CaN and CPP32 with IC\textsubscript{50} values of 0.55 and 0.02 μg/mL respectively, and 61 inhibiting CaN with an IC\textsubscript{50} value of 2.21 μg/mL. The diacetate 62 inhibited CPP32 with an IC\textsubscript{50} value of 0.8 μg/mL, while 63 was not a CPP32 inhibitor with an IC\textsubscript{50} value of 8.80 μg/mL. The four compounds were also assessed against murine leukemia P388 cell line with IC\textsubscript{50} values of 0.06, 1.22, 0.01 and 3.83 μg/mL, and human lung carcinoma A-549 cell line IC\textsubscript{50} values of 0.04, 2.86, 0.12 and...
4.68 μg/mL for 60, 61, 62 and 63 respectively. The assays clearly demonstrated that the iminoquinone moiety is crucial for the bioactivity of the compounds.

Makaluvamines represent a diverse array of pyrroloiminoquinone structures with substitutions about all three nitrogen atoms. Makaluvamines A-F (64-69) were isolated as topoisomerase II inhibitors from the Fijian sponge Z. marsailis (re-identified as Z. fuliginosa) and showed potent activity against human colon tumor HCT-116 and xrs-6 Chinese hamster ovary cell lines. Makaluvamine F (69), one of the most biologically active compounds in the series, was strongly cytotoxic against P388 and xrs-6 cell lines with IC\textsubscript{50} values of 0.17 and 0.08 μM respectively; it prevented topoisomerase II-mediated DNA strand passage with a decatenation inhibition of 25 μM; was a DNA intercalator with a K\textsubscript{s} value of 0.021 μM and showed reductive cleavage of DNA at a concentration of 1.1 μM. In the publication, the authors also investigated reduction potentials of compounds 64-69 at a constant pH of 7.5 and found that most exhibit negative E\textsubscript{1/2} ranging between -285 and -180 mV. Radisky et al. hypothesized that due to the fused-ring, planar, aromatic and positively charged makaluvamine structure, the molecules are able to intercalate into the double helix of DNA. Furthermore, the iminoquinone moiety could be reduced to a reactive aminophenol intermediate which then could lead to the production of
DNA single-stranded breaks. Both of the proposed mechanisms of action were thought to important for the observed bioactivity of the makaluvamine alkaloids. Interestingly, makaluvamine B (65) 3,4-dehydro analogue, was the only compound that could not be reduced under experimental conditions. Compound 65 was a DNA intercalator but showed no activity in the HCT-116, xrs-6, decatenation inhibition and reductive cleavage assays. The apparent inactivity of compound 65 is consistent with similar fully aromatized structures of isobatzellines C (58) and D (59), and discorhabdins Q (25), S (27) and T (28). Makaluvone (80), an orthoquinone isolated together with makaluvamines A-F, was a DNA intercalator but showed no activity towards HCT-116 and xrs-6 cell lines and was not a topoisomerase II inhibitor, showing the importance of the iminoquinone functionality for the activity of the series.57

Makaluvamine G (70) was isolated as the major compound form a Micronesian collection of the sponge Histodermella sp.58 (re-identified as Z. fuliginosa).25 The compound was moderately cytotoxic to P388, A-549, HT-29 and human breast cancer MCF-7 cell lines with an IC50 value of 0.5 μg/mL, KB cell line with an IC50 value of 0.35 μg/mL and did not inhibit topoisomerase II. Interestingly, the authors noted that even at the highest in vivo dose tested at 210 mg/kg, 70 was not toxic to mice. Makaluvamines H-M (71-76) were isolated from another Micronesian collection of the sponge Z. fuliginosa.50 Compounds 71-76 were moderately cytotoxic against HCT-116 cell line and showed mild activity against Bacillus subtilis. Makaluvamine N (77) was isolated from Z. fuliginosa collected from the Philippines.59 The compound was cytotoxic against HCT-116 cell line with an LC50 of 0.6 μg/mL and inhibited topoisomerase II unwinding of pBR322 at 5.0 μg/mL. Makaluvamine O (78), together with three sesquiterpene-phenols and eight other indole alkaloids was isolated from a Jamaican collection of the sponge Smenospongia aurea.60 Compound 78 was moderately cytotoxic to Plasmodium falciparum with an IC50 of 0.94 μg/mL and was inactive against a non-malignant mammalian Vero cell line. Cautious of the fact that thus far most pyrroloiminoquinone alkaloids have been reported from closely related sponges of the order Poecilosclerida, the authors pointed out that cross contamination of sample with co-occurring Zyzzya and Strongylodesma spp. sponges was possible. Makaluvamine P (79) isolated from Z. fuliginosa collected from the Vanuatu Islands, was moderately cytotoxic against KB cells (64% inhibition at 3.2 μg/mL), strongly inhibited xanthine oxidase with an IC50 of 16.5 μM, and had moderate antioxidant
activity. The structure of makaluvamine V (81) has been identified in a US patent describing the secondary metabolites and structurally related compounds sourced from a Fijian collection of the sponge *Zyzya Marsalis*. Compound 81 in an essay with seven other makaluvamine analogues, A (64), C–E (66-68), H (71), I (72) and N (77) showed the lowest topoisomerase II inhibition and the weakest affinity for DNA intercalation. Recently, *N*-1-β-D-ribofuranosylmakaluvamine I (82) has been reported from a South African-sourced *Strongylodesma aliwaliensis* sponge. The compound was moderately cytotoxic to the WHCO-1 cell line with an IC₅₀ value of 1.6 μM.

Utkina and coworkers have isolated makaluvamines C (66), E (68), G (70), H (71) and damirones A (46) and B (47) from an Australian collection of the sponge *Z. fuliginosa* and examined the metabolite profile differences between ethanolic extracts of fresh and freeze-dried sponges. Compounds 66 and 71 were the major metabolites in freshly extracted sponges, while in the freeze-dried sponges 46 and 47 were the dominant compounds present. The authors showed that makaluvamines C (66) and H (71) convert in high yield to the orthoquinones damirones B (47) and A (46) respectively upon treatment with sodium hydroxide, and cautioned that the two damirones are possible isolation procedure artifacts.

Pentacyclic bispyrroloiminoquinones tsitsikammamines A (83) and B (84) were originally isolated together with several discorhabdin C-type (6) analogues from the sponge *Tsitsikamma favus* collected from South Africa. Compounds 83 and 84 were cytotoxic, showed moderate anti-bacterial and anti-fungal activity and did not inhibit topoisomerase I and II enzymes. The two compounds, together with tsitsikammamine A N-oxime (85) and tsitsikammamine B N-oxime (86) were re-isolated from South African sponges *T. favus* and *T. pedunculata* by Davies-Coleman and coworkers. Cautiously, the authors noted that the isolation procedure involving reversed-phase chromatography and acidic solvents
may have hydrolyzed some of the real N-18-oxide natural products to the N-18-oxime derivatives 85 and 86. Attempts to convert 83 to the N-oxide analogue with hydrogen peroxide and meta-chloroperbenzoic acid were unsuccessful. While compounds 83 and 84 were moderately cytotoxic to HCT-116 cell line with IC$_{50}$ values of 1.41 and 2.38 μM, the N-oxide analogues 85 and 86 were inactive with IC$_{50}$ values of 128.21 and 16.54 μM respectively. A related compound, veiutamine (87) was isolated from a Fijian sponge Z. fuliginosa. Veiutamine was biologically active showing a mean IC$_{50}$ of 0.12 μg/mL against a panel of 25 cell lines and with an IC$_{50}$ of 0.3 μg/mL against HCT-116, seven times more active than makaluvamine D (67) at 2.0 μg/mL.

Seco-derivatives of the tsitsikammamines, the bispyrroloquinones zyzzyanones A–D (88-91) were isolated from an Australian collection of the sponge Z. fuliginosae. In addition to the new structures, the authors showed a semi-synthetic route from makaluvamines G (70) and L (75) into 88 and 89 in the presence of ammonia (Scheme 1.2), and cautioned that the compounds may be isolation procedure artifacts. Compounds 88–91 showed moderate cytotoxicity to mouse Ehrlich carcinoma cells with IC$_{50}$ values of 25 μg/mL, again stressing the importance of the iminoquinone chromophore for the activity of the series.

Scheme 1.2. Semi-synthetic route to zyzzyanones A (88) and B (89) from makaluvamines G (70) and L (75).
A study examining the ability of several damirones, makaluvamines and zyzzyanones to protect cell membranes of the sea urchin *Strongylocentrotus nudus* eggs from UV-radiation has identified makaluvamine H (71), damirone A (46) and zyzzyanone A (88) to be photolysis products of makaluvamine G (70); and makaluvamine C (66), damirone B (47) and zyzzyanone B (89) to be photolysis products of makaluvamine L (75) (Scheme 1.3).

The authors proposed that initially a homolytic cleavage of the *N*-para-hydroxystyryl bond converts 70 and 75 into the tricyclic 66 and 71 respectively, which then convert to the corresponding orthoquinones 46 and 47, the major products of the reaction. Zyzzyanones A (88) and B (89) are thought to be formed through a radical intramolecular cyclization reaction of parent makaluvamines 70 and 75, and consequent hydrolysis of the imine bond.
The pyrroloiminoquinones and related compounds are not only restricted to the marine phylum Porifera. Wakayin (92) has been isolated from an ascidian Clavelina sp. collected in Fiji.\textsuperscript{69} Compound 92 was cytotoxic to the HCT-116 cell line with an IC\textsubscript{50} of 0.5 \(\mu\)g/mL, inhibited topoisomerase II activity and showed activity against Gram-positive bacterium Bacillus subtilis with an MIC of 0.3 \(\mu\)g/mL. Terrestrial slime moulds are also a recorded source of makaluvamine and damirone alkaloids. Makaluvamine A (64) was isolated from a mass culture of Didymium bahiense,\textsuperscript{70} and recently a culture of Didymium iridis plasmodial cells yielded makaluvamine I (72) and damirone C (48).\textsuperscript{71}

Haematopodin (93), a pyrroloquinoline derivative, was isolated from fruiting bodies of the fungus Mycena haematopus.\textsuperscript{72} The absolute stereochemistry about the chiral centre at C-6a was assigned \(R\) by a single crystal X-ray diffraction study. However the authors noted that compound 93 was an isolation procedure artifact, and the structure of the true natural product was not resolved in the original publication. Mycenarubins A (94) and B (96) were recently reported from fruiting bodies of Mycena rosea.\textsuperscript{73} The authors tentatively assigned the stereochemistry at C-4 as \(S\) for 94, based on comparison of NMR and circular dichroism spectra with that of compound 93. Proof came with synthesis of the core structure 95, which had an identical circular dichroism spectrum to that of 94. The minor metabolite, mycenarubin B (96) is a dimer of compound 94 with the two monomeric units connected through an imine moiety. The absolute stereochemistry of 96
was assigned (4$S$,4$'$S) based on similarities of CD spectra of compounds 94 and 96. Compound 94 did not show any antimicrobial or antifungal activity, however the authors noted a possible ecological function of the metabolites as the brightly colored fruiting bodies of *M. rosea* are rarely attacked by predators in the field. This publication was closely followed with another by the same authors re-examining the natural products chemistry of *Mycena haemapoptus*, the original source organism of haematopodin (93). The experimental procedure that involved short extraction times and immediate metabolite purification with semi-preparative HPLC identified a new compound haematopodin B (97) as the main natural product present in *M. haemapoptus* as well as three minor metabolites mycenarubins D (98), E (99) and F (100). The absolute stereochemistry of the four new compounds was established upon comparison of the CD spectra with that of 93 and 94. Spiteller and co-workers have shown that intact fungal fruiting bodies contain the iminoquinone 97, while in the mechanically injured fruiting bodies left to stand in methanol over several hours, the quinone 93 was the main natural product present.

The genus *Mycena* has recently been a source of three other related compounds. Sanguinones A (101), B (102) and sanguinolentaquinone (103) were isolated from the fruiting bodies of *M. sanguinolenta*. The absolute stereochemistry of 101 and 102 was established as (4$S$,12$S$) and (4$S$,12$S$,14$S$) respectively, from the NMR coupling constants and NOE experiments and upon comparison of CD spectra with that of mycenarubin A (94). Both 101 and 102 were found to be unstable in solution, and could not be tested for biological activity. The decomposition product of 101 was fully characterized as decarboxydehydrosanguinone A (104).
A pyrroloquinone analogue, exiguamine A (105) has recently been reported from a Papua New Guinean sponge *Neopetrosia exigua*. The structure of the compound was determined by a combination of NMR and single crystal X-ray diffraction study, and 105 was found as an optically inactive racemate about the C-19 stereocentre. Exiguamine A was a very potent indoleamine-2,3-dioxygenase (IDO) inhibitor, with a Ki of 210 nM.

Dendrine A (106), together with makaluvamine O (78) and a terpene hydroquinone aureol were isolated from a Japanese sponge *Dictyodendrilla* sp. Although not directly related with the iminoquinone compounds reviewed here, the structure of this dimeric hydroxyl indole alkaloid resembles that of pyrrolohydroquinone. Compound 106 was mildly cytotoxic against a P388 cell-line with an IC$_{50}$ value of 32.5 μg/mL, and showed inhibitory activity against Gram-positive bacteria *B. subtilis* and *Micrococcus luteus* with an MIC of 8.3 and 4.2 μg/mL respectively, and a fungus *Cryptococcus neoformans* with an MIC of 8.3 μg/mL.
1.2 Summary and Aims

There are over seventy reported pyrroloiminoquinone and pyrroloquinone alkaloids found mainly in two sponge families Latrunculidae and Acarnidae. The almost exclusive occurrence of discorhabdins, prianosins, makaluvic acids, damirones, batzellines, isobatzellines, secobatzellines, makaluvamines, tsitsikammamines, zyzzyanones, and veiutamine in four closely related sponge genera *Latrunculia*, *Strongylodesma*, *Tsitsikamma* and *Zyzzya* provides the possibility to call the pyrroloiminoquinone alkaloids taxonomic markers for the two Porifera families. However, taxonomy of this group of sponges has gone through considerable changes and revisions in the last decade.16-18,25,39

In New Zealand the genus *Latrunculia*, initially thought to consist of a single species *Latrunculia* sp. du Bocage, is now known as at least eight separate species falling in two subgenera. These taxonomic changes have a profound effect on the natural product chemistry literature. For example, sponge *Prianos melanos*, the original source and name inspiration of the prianosins, is now placed in the genus *Strongylodesma*. Unless the taxonomic revision involves re-examination of the original material worked on by a particular natural products group, classification down to a species level presents a difficult challenge. Recently Blunt, Munro and coworkers reported a new natural product discorhabdin W (37) from a Milford Sound collection of *Latrunculia* sp.46 The sponge material was described as “…having anisodiscorhabd microscleres and anisostyle megascleres indicative of *Latrunculia* species, with shape and conformation of the whorls different from those found in a previously investigated New Zealand *Latrunculia*…” However, current taxonomic review of the New Zealand-sourced *Latrunculia* recognizes two different species co-occurring in the Milford Sound, *L. (Latrunculia) fiordensis* and *L. (Biannulata) millerae*, with the shape of the discorhabd recognized by the authors as the only differing feature between the two.18 Thus, unfortunately a repeat re-examination of the original material is warranted before any definitive conclusions on the taxonomy of this sample can be made. Therefore, even though the argument that the compounds are appropriate taxonomic markers is valid, the continuing changes in the taxonomy of the species coupled with a large number of natural product chemistry publications in the last decade make this a difficult task.
Reports of wakayin from an ascidian (phylum Chordata), damirones and makaluvamines from slime-moulds (kingdom Protista) and haematopodins, mycenarubins and sanguinones from fruiting bodies of a mushroom (kingdom Fungi) show that the production of pyrroloiminoquinone secondary metabolites is not restricted to the phylum Porifera, the marine environment or even the kingdom Animalia. Occurrence of a secondary metabolite across different phyla suggests the possibility that a symbiotic organism might to be the true producer of the compound. Sponges are known to form close symbiotic associations with bacteria, and many natural products have been isolated from sponge-cultured marine microbes. A single biosynthetic study has examined the possibility of pyrroloiminoquinone production by an endosymbiont. In an experiment showing tyramine involvement in the biogenesis of the discorhabdins, Blunt, Munro and coworkers have grown discorhabdin B producing sponges with and without the presence of antibiotics. The authors found similar amounts of radiolabelled discorhabdin B in both treatments, indicating that discorhabdin production is a function of sponge cells, rather than a bacterial symbiont.

While the discorhabdins and related pyrroloiminoquinones and pyrroloorthoquinones present a novel group of chemical structures, they are also of considerable biomedical interest. Active in the micro- and nano-molar range against a standard set of mammalian cancer cell lines like murine leukemia P388, human epidermoid carcinoma KB and human colon carcinoma HCT-116, some like discorhabdin C and dienol discorhabdin C also showed differential cytotoxicity profiles at the NCI’s 60 cell human tumor cell line anticancer drug screen. However progress towards development of the compounds as viable drug leads was abandoned due to their high cytotoxicity to somatic cells.

Little is known about the discorhabdin and related compounds mode and mechanisms of action. Discorhabdin A induces Ca release from sarcoplasmic reticulum. Makaluvamines and damirones are topoisomerase II inhibitors, and can intercalate into the DNA double helix. Discorhabdin P and secobatzellines A and B are inhibitors of two protein phosphatase enzymes involved in the immune system. Isobatzelline C inhibits HIV-1 envelope-mediated fusion in vitro, and makaluvamine O is moderately cytotoxic to Plasmodium falciparum. From a large number of biological activity data available in the literature, several trends on structure/activity of the
pyrroloiminoquinone and pyrroloorthoquinone alkaloids are evident. Quinones and orthoquinones like the tsitsikammamines and batzellines are inactive or at best only moderately active, while the iminoquinone structures all display potent biological activities. Unsaturation about ring B reduces cytotoxicity, as evident in discorhabdins Q (25), S (27), T (28), isobatzelline E (59) and makaluvamine B (65), as does an additional ring between the imine and C-2 exemplified by discorhabdins D (11), H (16), L (17) and N (18). However, since discorhabdin D is currently the only known compound with significant *in vivo* activity, these generally less-cytotoxic compounds can potentially be appropriate drug candidates, given that selectivity towards a certain target is identified.

The absolute stereochemistry of all chiral pyrroloiminoquinones, except the two prianosins A (7) and B (9), still remains unresolved. The majority of the compounds are isolated as trifluoroacetate or hydrochloride salts which have so far not yielded good quality crystals. For all discorhabdin D (11)-type molecules, most of the stereochemical assignments are based upon a long-range planar zig-zag correlation from H-4 on the spiro-ring to one of the methylene pairs on C-7 (Figure 1.3). The theory behind this five-bond correlation was established in 1969 as a structure determination tool for molecules showing “…long-range coupling constants with absolute magnitudes of 0–2.5 Hz, which leads to experimental problems associated with accurate measurements of small line separations in NMR spectra.”80 Today, almost forty years later, due to poor and inconclusive NOE data about the spiro-ring, most authors still rely on this correlation to establish hydrogens on the lower face of the discorhabdin molecule. Furthermore, based on structural similarities and an assumption of common biosynthetic origin, the relative stereochemistry at the C-6 spiro-centre and C-8 is assumed by most authors to be identical to that of prianosin/discorhabdin A (7).

This thesis aims to identify the natural products chemistry of twelve *Latrunculia* spp. collections, ten sourced from around New Zealand, one from Antarctica and one from South Africa. Four new structures, as well as several enantiomeric pairs of known discorhabdin natural products are presented. In chapter two, the optical and chiroptical properties of the isolated metabolites are investigated by optical rotation data and circular dichroism spectroscopy, and their absolute stereochemistry established upon comparison of the observed experimental data with the results of time dependant density functional
theory (TDDFT) calculations of electronic circular dichroism spectra. Chapter three presents the results of a structure-activity relationship study of the main natural product of the Wellington-sourced *Latrunculia* sp. sponges, discorhabdin B (8).
2. Natural Products Isolation and Structure Elucidation
A comparative study of the natural products chemistry of twelve *Latrunculia* spp. specimens was undertaken. Sponge samples were sourced from the following locations: Three Kings Islands, Tutukaka, Wellington, Kaikoura, Doubtful Sound and Milford Sound in New Zealand; a single Antarctic specimen collected in the Ross Sea and a South African specimen of *Cyclacanthia bellae*. Nine New Zealand collections were chosen to represent the current known taxonomy of the genus, as well as the original collection sites of the five local *Latrunculia* natural products chemistry publications. New Zealand collection sites, species assignments according to the two latest taxonomic revisions of the genus and the collection locations of the previous publications are shown in Figure 2.1.

**Figure 2.1.** Collection sites and species assignments of the *Latrunculia* spp. used in the current study (in blue) and previously published work (in red).

This study did not make any attempts towards a taxonomic identification of the sponge specimens used. The New Zealand collections, sourced from the National Institute of Water and Atmospheric Research’s (NIWA) MNP collection, were the source material for some of the earlier taxonomic and ecological studies. However, none of the
sample-specific codes match with those of the original sponges examined in the taxonomic study by Alvarez et al.\textsuperscript{18} Thus, in Figure 2.1, the species names have been tentatively assigned based on descriptions of the chemistry and phenotypic characteristics only, but unfortunately three of the current study collections: Doubtful Sound, Milford Sound and Three Kings Islands, could not be identified following this methodology and would require a more detailed examination of the discorhabd microscleres. The South African sample of \textit{C. bellae}, a kind gift from Professor Davies-Coleman, represents the source material worked on for the natural products study of the Latrunculid sponges outlined in Antunes et al.\textsuperscript{38}

2.1 Metabolite Isolation and Identification

Isolation and purification work has identified eighteen discorhabdin natural products, six new structures, four of which are new natural products \textsuperscript{21, 107, 108 and 109} and two isolation procedure artifacts \textsuperscript{110 and 111}. Enantiomeric pairs of known discorhabdins B (8), G*/I (15), L (17) and W (37) as well as two diastereomers of discorhabdin H (16), were also identified (Figure 2.2)
Figure 2.2. Natural products isolated from New Zealand \textit{Latrunculia} spp. sponges in the current study.
2.1.1 Discorhabdin-Specific General Methodology

To prevent inter-species contamination, all extraction and isolation work was performed on single sponge specimens. Preliminary work had identified discorhabdin alkaloids as the only metabolites of interest present in *Latrunculia* spp., and subsequently a customized extraction and isolation procedure was developed. In order to avoid any artifacts and optimize the time-consuming chromatography work, the following set of conditions were applied:

- Short but repetitive extraction times. The sponge was extracted repeatedly with methanol, but was left to soak in the solvent in 20 minute increments only. All of the initial chromatography work was undertaken within a week of the extraction.
- During the extraction and initial chromatography work, care was taken not to contaminate the crude extract with acid.
- Since most of the discorhabdins have similar polarity under strongly acidic conditions, a system was developed where the crude extract was run down a Sephadex LH-20 column with methanol and no trifluoroacetic acid. This allowed for a more efficient initial fractionation and less time-consuming subsequent chromatography work.

The first sponge specimen worked on, Wellington (Barret’s Reef), was fractionated by reversed-phase flash chromatography using a steep gradient from water (0.05% TFA) to methanol, with each fraction analyzed on an analytical HPLC C₈ rocket column. The isolation work took a minimum of three columns per purified compound and three months to complete. Since a total of six discorhabdins, A (7), B (8), G*/I (15), H (16), Q (25) and 1-thiomethyl G*/I (108), had very similar polarities under strongly acidic conditions, it became obvious that the current analytical HPLC conditions and the flash chromatography work designed to mimic the same, were inadequate and time consuming for the particular set of compounds. Extraction and compound isolation procedure for the Wellington-sourced *L. (Biannulata) wellingtonesis* (BR) sponge is presented below.
Figure 2.3. Analytical HPLC trace (C₈ rocket column) of the MeOH extract of the Wellington-sourced L. (Biannulata) wellingtonesis (BR).

A single specimen of wet L. (Biannulata) wellingtonesis (BR) was extracted with MeOH. The solvent was filtered and then removed in vacuo to give a dark brown crude extract (4.43 g), which was subjected to C₁₈, C₈ and CN flash (MeOH, H₂O-TFA (0.05%)) and Sephadex LH-20 (MeOH-TFA (0.05%)) chromatography. The following discorhabdin alkaloids were isolated: discorhabdin A (7) trifluoroacetate salt (6.6 mg, 0.15% wet weight, Rₜ 6.1 min), discorhabdin B (8) trifluoroacetate salt (80.6 mg, 1.82% wet weight, Rₜ 6.1 min), discorhabdin D (11) trifluoroacetate salt (12.7 mg, 0.29% wet weight, Rₜ 5.8 min), discorhabdin G*/I (15) trifluoroacetate salt (24.6 mg, 0.56% wet weight, Rₜ 6.1 min), discorhabdin H (16) trifluoroacetate salt (52.9 mg, 1.19% wet weight, Rₜ 6.1 min), discorhabdin L (17) trifluoroacetate salt (39.0 mg, 0.88% wet weight, Rₜ 5.3 min), discorhabdin N (18) trifluoroacetate salt (20.0 mg, 0.45% wet weight, Rₜ 5.3 min), discorhabdin Q (25) trifluoroacetate salt (4.8 mg, 0.11% wet weight, Rₜ 6.1 min), 1-thiomethyl discorhabdin G*/I (108) trifluoroacetate salt (3.2 mg, 0.072% wet weight, Rₜ 6.1 min) and 1-discorhabdyl discorhabdin D trifluoroacetate salt (111) (5.8 mg, 0.13% wet weight, Rₜ 7.4 min). Metabolite isolation procedure is outlined in Scheme 2.1.
Scheme 2.1. Wellington-sourced L. (Biannulata) wellingtonesis (BR) metabolite isolation scheme.
In contrast, according to the modified method, when the crude extract was chromatographed on a Sephadex LH-20 resin, several of the metabolites including the dimers 37 and 109 were separated in the very first column, with many others requiring only a few subsequent purification steps. Compounds such as discorhabdin B (8), Q (25), W (37) and 16a,17a-dehydro discorhabdin W (109) were isolated as free bases, while others due to their low solubility as free bases, especially the N-18 imine, C-2 bridged compounds such as discorhabdin L (17), required acidification before any subsequent chromatography work. An example of the modified method is given below (Scheme 2.2).

![Image](image.png)

**Figure 2.4.** Analytical HPLC trace (C₈ rocket column) of the MeOH extract of the Wellington-sourced *L. (Biannulata) wellingtonesis* (SEAS-LAT-BR-15-1).

A single specimen of freeze dried *L. (Biannulata) wellingtonesis* (SEAS-LAT-BR-15-1) (12.472 g) was extracted with MeOH. The solvent was filtered and then removed in vacuo to give a dark brown crude extract (3.84 g). A portion of the crude extract (582 mg) was subjected to Sephadex LH-20 (MeOH), C₁₈, and C₈ flash (MeOH, H₂O-TFA (0.05%)) chromatography, yielding: discorhabdin B (8) as a free base (15.8 mg, 0.83% dry weight, Rₜ 5.5 min), discorhabdin G*/I (15) trifluoroacetate salt (11.8 mg, 0.62% dry weight, Rₜ 5.5 min), discorhabdin L (17) trifluoroacetate salt (20.4 mg, 1.08% dry weight, Rₜ 4.5 min), discorhabdin Q (25) as a free base (3.0 mg, 0.16% dry weight, Rₜ 5.5 min),
discorhabdin W (37) as a free base (9.1 mg, 0.48% dry weight, R_T 6.9 min), 3-dihydro discorhabdin A (107) trifluoroacetate salt (3.7 mg, 0.20% dry weight, R_T 5.5 min), and 16a,17a-dehydro discorhabdin W (109) as a free base (2.4 mg, 0.12% dry weight, R_T not visible on the HPLC trace). Metabolite isolation procedure is outlined in Scheme 2.2.

Scheme 2.2. Wellington-sourced *L. (Biannulata) wellingtonesis* (SEAS-LAT-BR-15-1) metabolite isolation scheme.

This modified methodology was applied to the consequent nine extractions. All of the discorhabdin alkaloids isolated were kept separate and fully characterized with a set of NMR spectroscopy, mass spectrometry, optical rotation and circular dichroism data.
2.2 Definition of Absolute Configuration of the Discorhabdin Alkaloids

Upon comparison of the optical rotation and circular dichroism spectra of the purified discorhabdins, it became apparent that (+) and (-) enantiomers of five known compounds, discorhabdins B (8), G*/I (15), H (16), L (17) and W (37) had been isolated. Each pair of compounds 8, 15, 17 and 37 exhibited identical NMR and mass spectral data, and differed only in the sign of their \([\alpha]_D\) values and circular dichroism spectra. Compound 16 showed subtle differences in the \(^1\)H and \(^{13}\)C NMR spectra and also displayed opposite \([\alpha]_D\) values and circular dichroism spectra. A short summary on the enantiomeric pairs and location of sponge samples that they have been isolated from is presented below. The (+) and (-) designations are based on the sign of optical rotation at the sodium-D line (589 nm) or alternatively at a wavelength of 578 nm on a mercury lamp.

- (-)-Discorhabdin B (8) was isolated from Milford Sound (95 MS-1-(1-10)) and Doubtful Sound (95 DS (1-10)) collections.
- (+)-Discorhabdin B (8) was found in all three Wellington collections (BR), (SEAS-LAT-2-BR-(15-1)) and (LAT-SCA-L-2) and an Antarctic-sourced sponge (MNP 7829).
• (-)-Discorhabdin G*/I (15) was isolated from Milford Sound (95 MS-1-(1-10)), Doubtful Sound (95 DS (1-10)), and the South African C. bellae sponge.

• (+)-Discorhabdin G*/I (15) was isolated from two Wellington collections (BR) and (SEAS-LAT-2-BR-(15-1)) and a Kaikoura-sourced sponge (97 KK 1-17).

• (-)-Discorhabdin H (16) was isolated from a Wellington-sourced sponge (BR).

• (+)-Discorhabdin H (16) was isolated from a Milford Sound-sourced sponge (95 MS-1-(1-10)).

• (-)578-Discorhabdin L (17) was isolated from Wellington (BR) and (SEAS-LAT-2-BR-(15-1)), Kaikoura (97 KK 1-17) and Tutukaka (97 TUT 2-10) sourced collections.

• (+)578-Discorhabdin L (17) was isolated from Milford Sound (95 MS-1-(1-10)), Doubtful Sound (95 DS (1-10)) and South African C. bellae collections.

• (-)-Discorhabdin W (37) was isolated from two Wellington (SEAS-LAT-2-BR-(15-1)) and (LAT-SCA-L-2) and Antarctic (MNP 7829) sourced sponges.

• (+)-Discorhabdin W (37) was isolated from Milford Sound (95 MS-1-(1-10)) and Doubtful Sound (95 DS (1-10)) collections.

A comparison of the optical rotation data for compounds 8, 15, 17 and 37 given in previous publications (Figure 2.5), has revealed that even though some antipodal discorhabdins were previously isolated, without the benefit of having both enantiomers at hand most authors assumed the absolute stereochemistry of the isolated natural products to be the same as that of discorhabdin A (7). In all of the thirteen publications describing the chemistry of chiral discorhabdin alkaloids, including one by the author of this thesis, and numerous other reviews and related natural product publications, the absolute stereochemistry about the spiro-centre and C-8 was assumed to be the same as that of discorhabdin A (7).
Figure 2.5. Summary of published and experimental optical rotation data for compounds 8, 15, 17 and 37 (the units of optical rotation are deg cm$^3$ g$^{-1}$ dm$^{-1}$).

Figure 2.5 shows that there is a large amount of variation in the magnitude of the experimental optical rotation data reported for alkaloids 8, 15, 17 and 37. For example, Wellington-sourced discorhabdin G*I (15) had an $[\alpha]_D$ of +540 deg cm$^3$ g$^{-1}$ dm$^{-1}$, while 15 from Doubtful Sound Latrunculia sp. showed an $[\alpha]_D$ of -720 deg cm$^3$ g$^{-1}$ dm$^{-1}$. Since discorhabdin alkaloids are brightly colored and have very strong chromophores, optical rotations that give satisfactory instrument energies are made at very low concentrations. At a concentration ($c$ 0.05) or 0.5 mg/mL, the smallest increment on the polarimeter used in this study 0.001°, represents 20 deg cm$^3$ g$^{-1}$ dm$^{-1}$, and thus the measurement error on the values is estimated between ±40 and 80 deg cm$^3$ g$^{-1}$ dm$^{-1}$. Furthermore, the alkaloid protonation state has shown to be important as there were significant differences observed between the optical rotation values of compounds 8 and 37 as trifluoroacetate salts and free bases. As a trifluoroacetate salt, (+) and (-)-discorhabdin W (37) exhibited an $[\alpha]_D$ of +80 and -80 deg cm$^3$ g$^{-1}$ dm$^{-1}$ respectively, while the free base forms showed an $[\alpha]_D$ of +240 and -260 deg cm$^3$ g$^{-1}$ dm$^{-1}$ respectively. This acid-base differential was also apparent in the ECD spectra of (+) and (-)-discorhabdins B (8), (Figure 2.6), and (+) and (-)-W (37), (Figure 2.7).
Although uncommon, racemic, scalemic and antipodal marine natural products have been reported from several sponges,\textsuperscript{82-89} ascidians\textsuperscript{90-92} and marine microorganisms.\textsuperscript{93,94}

12-Hydroxyambilofuran (112) and 12-acetoxyambilofuran (113) were isolated as 3:1 mixtures or predominantly (12S) enantiomers from a Western Australian sponge \textit{Spongia} sp.\textsuperscript{82} The term scalemic was used in the publication to describe non-racemic mixtures of
enantiomers of the two isolated compounds. In this study, while discorhabdins 8, 15, 17 and 37 had equal and opposite circular dichroism spectra, optical purity could not be established with optical rotation data alone and other methods towards enantiomeric purity determination were needed to determine whether the compounds were optically pure or scalemic mixtures.

2.2.1 Enantiomeric Purity of the Isolated Discorhabdins

Determination of enantiomeric excess and assignment of the absolute configuration of chiral molecules is commonly examined by the Mosher method. Secondary alcohol or amine functionality is reacted with R or S-α-methoxy-α-trifluoromethylphenyl-acetic acid (MTPA), and based on the anisotropic effect of the resulting diastereomeric esters or amides, the products give a specific set of 1H NMR shifts. Since the initial publication forty years ago, there are now a range of different chiral-shift reagents available for addition onto various functionalities.

The ketone functionality on the C-3 position of the spiro-ring of discorhabdin C (6) was previously successfully and in high yield reduced with sodium borohydride to the spirodienol 34. With the intention to functionalize the secondary alcohol at C-3, identical experimental conditions were applied to discorhabdins B (8), G*/I (15), and W (37). Unfortunately, at a molar excess of sodium borohydride no reaction was observed on all three substrates.

As an alternative, cyclodextrins were chosen as chiral shift reagents. Cyclodextrins are cyclic oligosaccharides with glucose units linked by α-1,4 bonds to form a truncated cone-like structure (Figure 2.8). The number of the monomeric glucose can vary from 5, 6 and 7 units to give α-, β-, or γ-cyclodextrin respectively, and this determines the size of the cavity.
The spatial orientation of the hydroxyl groups in the cyclodextrin molecule creates a polarity differential between internal and external surfaces.\textsuperscript{96} In the structure of $\beta$-cyclodextrin (Figure 2.9) the internal surface of the cavity is hydrophobic as it is lined with glycosidic linkages and H-3' and H-5' methine groups, while the outside surface with primary and secondary hydroxyl groups is hydrophilic. This differential accounts for the high solubility of cyclodextrins in aqueous media, and their ability to form complexes with less polar substrates. Given that the guest molecule fits into the cavity, cyclodextrins can complex with a range of neutral and ionic organic and inorganic molecules, and even noble gases.\textsuperscript{97} This is largely due to the hydrophobic internal surface which can facilitate complex formation through van der Waals interactions, hydrogen bonding and displacement of water molecules.\textsuperscript{97}
Before the onset of chiral HPLC columns, cyclodextrins were widely used in the pharmaceutical industry as means of enantiomeric purity determination. The $^1$H NMR detection method is based upon diastereomeric complex formation between cyclodextrins and chiral substrates and subsequent integration of resonances of each diastereomer in the spectrum. Recently, this method has been applied to resolve enantiomeric purity of two antipodal forms of marine natural product trans-5-hydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-4-(2''-imidazolyl)-1,2,3-trithiane ($^{114}$). The (+) and (-) enantiomers of $^{114}$ were isolated from New Zealand-sourced collections of ascidians *Aplidium* sp. and *Hypistiozoa fasmeriana* respectively, and were equal in all spectral aspects except the optical rotation. Pearce *et al.* have shown that while single (+) or (-) enantiomers of $^{114}$ and $\beta$-cyclodextrin ($\beta$-CD) gave a single set of resonances in the $^1$H NMR spectrum, racemic mixture of the two with $\beta$-CD showed a spectrum with all resonances but one for $^{114}$ doubled.
2.2.1.1 Enantiomeric Purity of Discorhabdin B (8)

Following the methodology outlined in Pearce et al. an attempt was made to complex discorhabdins B (8), G*/I (15), L (17) and W (37) with β-CD. Racemic mixtures of 8, 15, 17 and 37 were prepared by mixing the (+) and (-) enantiomers until \([\alpha]_D = 0\) and the ECD spectrum showed a flat line. Titrations of racemates of all four compounds with β-CD did not show any discorhabdin resonance doubling or shifts in the β-CD spectra. Repeat titrations of 8 and 37 with α- and γ-cyclodextrin respectively, also failed to induce any 1H NMR shifts. Finally, an attempt was made to complex (+) and (-)-discorhabdin U (29), accessed as a semi-synthetic derivative of discorhabdin B (8), with β-CD. Compound 29 was prepared by reacting 8 with an excess of methyl iodide or trimethylphosphate in basic media (Scheme 2.3).

\[
\begin{align*}
\text{CH}_3\text{I, K}_2\text{CO}_3, \text{acetone, } 80^\circ\text{C, 3hrs} & \quad \text{39\%} \\
\text{or} \\
\text{PO(OCH}_3)_3, \text{K}_2\text{CO}_3, \text{90}^\circ\text{C, 3hrs} & \quad 70-90\%
\end{align*}
\]

\[
\begin{align*}
\text{8} & \quad \text{Br} \\
\text{or} \\
\text{29}
\end{align*}
\]

**Scheme 2.3.** Synthetic route towards discorhabdin U (29).

β-CD titrations of racemic mixture of discorhabdin U (29), prepared by mixing the (+) and (-) enantiomers until \([\alpha]_D = 0\) and the ECD spectrum showed a flat line, resulted in significant changes in the 1H NMR spectrum of 29. Single (+) and (-) enantiomers of 29 with β-CD did not show any doubling of resonances, while racemic 29 with β-CD showed doubling of H-1, H-4, H-16 and H-23 signals, consistent with formation of a diastereomeric complex (Figure 2.10).
In order to determine the host-guest stoichiometry that gave maximal observed shifts, a titration study was undertaken where the molar amount of discorhabdin U (29) was kept constant and varying amounts of β-CD were added. A ratio of 1:3 of discorhabdin U:β-CD was found to give the greatest induced chemical shift changes. Structural information on host-guest binding was obtained through a 2D ROESY NMR experiment ($\tau_{\text{mix}}$ 800 ms). Intramolecular correlations between H-3' and H-5' of β-CD and H-1, H-4, H-16 (weak) and H-23 of 29 were observed, suggesting partial inclusion of the discorhabdin molecule into the cyclodextrin cavity (Figure 2.11). Resonances for H-7, H-17 and H-22 of 29 were obscured by the residual HOD peak and H-5' of β-CD, and no information on binding or shift changes could be reported.
To ensure that enantiomeric purity can be established by the integration of the doubled resonances, scalemic mixtures of enantiomers of 29 were prepared. The H-1 resonance was used for the integration and calculation of the enantiomeric excess. Figure 2.12 shows that enantiomeric excess as low as nine to one can be detected by this method, and thus (+) and (-) enantiomers of discorhabdin B (8) isolated from sponge samples were considered >90% enantiopure.

Figure 2.11. Partial contour plot of the 2D ROESY NMR spectrum of (+) discorhabdin U (29) and β-CD at a molar ratio of 1:3, in D2O.

Figure 2.12. An expansion of the 1H NMR spectra of racemic and scalemic mixtures of (+) and (-)-discorhabdin U (29), showing the doubled H-1 resonance.
2.2.1.2 Enantiomeric Purity of Discorhabdin W (37)

Enantiomeric purity of discorhabdin W (37) could also be established through the semi-synthetic derivative 29. Previously reported analytical-scale reduction and photolysis reactions of discorhabdins W (37) and B (8) (Scheme 1.1)\(^{46}\) were made on a larger scale with all products fully characterized by NMR spectroscopy, mass spectrometry and chiroptical data. Addition of dithiothreitol (DTT) to a free base solution of 37 in acetonitrile gave the monomer 8, however in very low isolated yield of about 5%. An alternative disulfide reducing reagent tris (2-carboxyethyl)phosphine (TCEP) was chosen, due to the reagents high solubility in water, stability and ability to react under acidic conditions.\(^{99,100}\) Stirring in water, at room temperature over five minutes, TCEP reduced (-) and (+)-37 to (+) and (-)-8 respectively, in high yields of 70–90%. The products, (+) and (-)-semi-synthetic discorhabdins B (8), were identical in all aspects to the naturally-occurring compounds. The two semi-synthetic enantiomers of 8 were then converted to discorhabdin U (29) and titrated with β-cyclodextrin. Both (+)-29 and β-CD and (-)-29 and β-CD mixtures gave a single set of resonances, showing that naturally-occurring (+) and (-)-discorhabdin W (37) was >90% enantiopure.

2.2.1.3 Diastereomeric Purity of Discorhabdin H (16)

Discorhabdin H (16) has a chiral amino acid thiohistidine substituent on C-1 of the discorhabdin core, the absolute stereochemistry of which was established as (7'S) by Antunes et al.\(^{38}\) The (+) and (-) diastereomers of compound 16 isolated from New Zealand sponges, showed differences in the \(^1\)H NMR (Figure 2.13) and \(^{13}\)C NMR spectra localized mainly on the resonances of the thiohistidine and the discorhabdin spiro-ring. Since there
was no doubling of (+) and (-) 16 specific signals in the NMR spectra, the two antipodes of compound 16 were assumed to be diastereopure.

**Figure 2.13.** $^1$H NMR spectra of a) Wellington-sourced (-) and b) Milford Sound-sourced (+)-diastereomers of discorhabdin H (16) with the most significant changes in resonances highlighted in red.

Attempts towards semi-synthetic derivatives of discorhabdins G*/I (15) and L (17) were unsuccessful, as 15 is unstable in basic solutions and 17 has very limited solubility as a free base. Thus, enantiomeric purity of compounds 15 and 17 was not determined.
### 2.2.2 Absolute Stereochemistry Calculations

Absolute stereochemistry of prianosin/discorhabdin A (7) has been secured by means of single crystal X-ray diffraction, and later confirmed by total synthesis.\(^{23,101}\) As trifluoroacetate salts, synthetic enantiomers \((5R,6S,8S)\) and \((5S,6R,8R)\) of compound 7 gave equal and opposite optical rotation values at \([\alpha]_D = +388\ deg\ cm^3\ g^{-1}\ dm^{-1}\) and \(-390\ deg\ cm^3\ g^{-1}\ dm^{-1}\) respectively, and showed similar potency against the HCT-116 cell line with IC\(_{50}\) values of 0.03 and \(<0.03\ \mu g/mL\) respectively.\(^{101}\) While the absolute stereochemistry of three other prianosins B (9), C (10), and D (11) was established through comparison of the circular dichroism spectra with that of 7,\(^{29}\) the stereochemistry of all other chiral discorhabdins/epinardins stands as relative assignments only.

![Structures of (+)-(5R,6S,8S)-7 and (-)-(5S,6R,8R)-7](image)

In this study, compound 7 was isolated from a Wellington-sourced sponge (BR) and as a trifluoroacetate salt \([\alpha]_D = +310\ deg\ cm^3\ g^{-1}\ dm^{-1}\) and free base \([\alpha]_D = +200\ deg\ cm^3\ g^{-1}\ dm^{-1}\) was in good agreement with the optical rotation data for the published \((5R,6S,8S)\) Japanese \(P.\ melanos\)-sourced compound.\(^{23,101}\) Thus, the stereochemistry of New Zealand-sourced (+)-discorhabdin/prianosin A (7) was also \((5R,6S,8S)\). Since enantiopure antipodal pairs of five other known discorhabdin alkaloids were isolated from New Zealand sponges a detailed stereochemical study was warranted.

Comparison of Wellington-sourced (+)-discorhabdin/prianosin A (7) optical rotation data (TFA salt \([\alpha]_D = +310\ deg\ cm^3\ g^{-1}\ dm^{-1}\))\(^{101}\) with that of the Wellington sourced (+)-discorhabdin B (8) (TFA salt \([\alpha]_D = +330\ deg\ cm^3\ g^{-1}\ dm^{-1}\)), taken with the structural similarities between the two compounds, all suggested that (+)-8 was likely to have the same absolute stereochemistry at C-6 and C-8 to that of (+)-7. However, there is evidence that the absolute stereochemistry of closely related natural products cannot be determined or assumed based upon the sign of \([\alpha]_D\) alone. Three terrestrial iridoid natural products,
plumericin (115), oruwacin (116) and prismatomerin (117) have the same absolute stereochemistry about all five chiral centers, but the sign and magnitude of their optical rotation values, $[\alpha]_D = +204$ deg cm$^3$ g$^{-1}$ dm$^{-1}$, $+193$ deg cm$^3$ g$^{-1}$ dm$^{-1}$ and $-136$ deg cm$^3$ g$^{-1}$ dm$^{-1}$ respectively, vary markedly. In the publication, the authors cautioned against stereochemical predictions based on optical rotation data, as compound 117 was initially assumed to be an enantiomer or a diastereomer about the core structure of 115 and 116, but calculations of the vibrational circular dichroism (VCD) spectra of 117 secured the absolute stereochemistry to be $(1R,5S,8S,9S,10S)$.102

Methods of determining the absolute stereochemistry of a chiral compound include X-ray crystallography,23 total synthesis,101 Mosher ester NMR analysis,103 and quantum chemical calculations of chiroptical properties such as electronic circular dichroism (ECD) and vibrational circular dichroism (VCD).104,105 Circular dichroism is a property of chiral molecules based on differential absorption of left and right circulatory polarized light.106 As light absorption is associated with electronic transitions and chromophores, all molecules having stereogenic centers, chiral axes and helical structures close to a conjugated π-system or a metal complex will show an induced circular dichroism.106 Since these are structural features of a molecule, quantum chemical calculations of ECD and VCD can be used to determine the absolute stereochemistry.104,105

Discorhabdin alkaloids have rigid and conformationally restrained structures with chromophores adjacent to chiral centers. The molecules, both as free bases and trifluoroacetate salts give strong optical rotary and ECD spectra. In order to establish the absolute stereochemistry of discorhabdins B (8), G*/*I (15), L (17) and W (37), time
dependant density functional theory (TDDFT) calculations, using Gaussian 03, of the ECD of the four compounds were undertaken.

All of the theoretical calculations of the ECD spectra presented in this thesis were the work of Professor Daneel Ferreira, Dr Yuanqing Ding and Dr Xing-Cong Li at Department of Pharmacognosy and National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi. With respect to the material presented in sections 2.2.2.1, 2.2.2.2, 2.2.2.3 and 2.2.2.4 the author of this thesis isolated and purified the natural products in question and recorded their experimental ECD spectra. TDDFT calculations are presented with permission of the three collaborators.
2.2.2.1 Absolute Stereochemistry of Discorhabdin B (8)

TDDFT calculations of the ECD spectrum expected for (6S,8S)-8 were in excellent agreement with the measured ECD for both the free base and TFA salt forms of the Wellington-sourced (+)-8 (Figure 2.14).

![Figure 2.14](image)

Figure 2.14. ECD spectra for (6S,8S)-discorhabdin B (8) in gas phase (black), methanol solvation model (red) and experimental (blue) for a) free base form and b) TFA salt form.

The absolute stereochemistry of the (+)-enantiomer of discorhabdin B (8) isolated from Wellington and the Antarctic *Latrunculia* spp. is thus (6S,8S). For the (-)-enantiomer with equal and opposite ECD spectra isolated from Milford and Doubtful Sound sponges, the absolute stereochemistry is concluded to be (6R,8R). The ECD spectrum of (+)-8 isolated from the South American *L. brevis*\textsuperscript{107} was found to be identical to that of the Wellington (+)-enantiomer, establishing the absolute stereochemistry of this sample as (6S,8S). Comparison of the published $[\alpha]_{D}$ value from the Kaikoura New Zealand-sourced original publication of 8,\textsuperscript{26} is in good agreement with that measured for the Wellington
(+)-enantiomer (Figure 2.5), therefore securing the stereochemistry as (6S,8S) and confirming that the assignments on the stereochemistry of this compound by Perry et al. were correct.26

Both (+)-8 and (-)-8 were equally potent against the P388 murine leukemia cell line with IC$_{50}$ values of 0.20 and 0.17 μM respectively, indicating that the observed biological activity of discorhabdin B is independent of the stereochemistry of the compounds.

2.2.2.2 Absolute Stereochemistry of Discorhabdin G*/I (15)

TDDFT calculations, of the ECD spectrum expected for the (6S,8S) discorhabdin G*/I (15) were in excellent agreement with the experimentally measured ECD of the Wellington-sourced (+)-enantiomer of 15 (Figure 2.15). Thus, the absolute stereochemistry of (+)-15 is (6S,8S) and the (-)-15 enantiomer is (6R,8R).

![ECD spectra for the TFA salt form of (6S,8S)-discorhabdin G*/I (15) in gas phase (black), methanol solvation model (red) and experimental (blue).](image)

**Figure 2.15.** ECD spectra for the TFA salt form of (6S,8S)-discorhabdin G*/I (15) in gas phase (black), methanol solvation model (red) and experimental (blue).

![Structures of (+)-(6S,8S)-15 and (-)-(6R,8R)-15](image)
In this study the $(6S,8S)$ (+)-enantiomer of 15 was isolated from Wellington and Kaikoura sponge populations, while $(6R,8R)$ (-)-15 was found in Doubtful and Milford Sound *Latrunculia* spp. and the South African crude extract of *C. bellae*. A comparison of the $[\alpha]_D$ value reported for 15 isolated from a South American collection of *L. brevis* is in good agreement with that measured for the (-)-enantiomer (Figure 2.5), securing the stereochemistry as $(6R,8R)$. Therefore, of the two antipodes, $(6R,8R)$ discorhabdin G*/*I is the known natural product from both South African$^{38}$ and South American$^{40}$ collections. The absolute stereochemistry of (-)-15 depicted by Reyes *et al.*$^{40}$ therefore is incorrect. The two enantiomers of discorhabdin G*/*I, (+)-15 and (-)-15 were equally potent against a P388 cell line with IC$_{50}$ values of 0.60 and 0.53 μM respectively.

**2.2.2.3 Absolute Stereochemistry of Discorhabdin W (37)**

Unlike any other compound in the series, discorhabdin W (37) has a disulfide bridge connecting two identical monomeric subunits. Due to the free rotation of this structural feature and complications in the number of possible conformers and solvation models, the TDDFT ECD spectrum of 37 was calculated for the free base form in gas phase only. Calculations of the ECD spectrum expected for the $(6S,6'S)$ discorhabdin W (37) were in good agreement with the experimentally measured ECD of the Wellington-sourced (-)-enantiomer of 37 (Figure 2.16). The stereochemistry of the (+) enantiomer with equal and opposite ECD spectra was therefore $(6R,6'R)$.

![Figure 2.16](image)

*Figure 2.16. ECD spectra for the free base of $(6S,6'S)$-discorhabdin W (37) in gas phase (red), and experimental (blue).*
Semi-synthetic reduction of 37 to the monomer discorhabdin B (8) and the oxidation of 8 to 37, gave further evidence that the orientation of the spirodienone ring in (-)-(6S,6'S)-37 is identical to that of (+)-(6S,8S)-8. All of the semi-synthetic products outlined in Scheme 2.4 were identical in all aspects, including chiroptical properties, to the naturally-occurring compounds.

Scheme 2.4. Semi-synthetic reductions and oxidations of discorhabdins W (37) and B (8).

Compound 37 has previously been reported from a Milford Sound collection of *Latrunculia* sp. A comparison of the of the published [α]₀ value is in good agreement with that measured for the (+)-enantiomer (Figure 2.5), establishing the stereochemistry of the known natural product as (6R,6'R). Therefore, the original publication depicts the absolute stereochemistry incorrectly.
Both (-)-37 and (+)-37 were equally potent against a P388 cell line with IC$_{50}$ values of 0.13 and 0.10 μM respectively.

2.2.2.4 Absolute Stereochemistry of Discorhabdin L (17)

In the original publication of 17, Reyes et al. established the relative stereochemistry of the compound as (1$^R$*,2$^S$*,6$^R$*,8$^S$*), relying on a “zig-zag” COSY correlation from H-4 to H-7α to resolve the orientation of the spiro-ring and an NOEDIFF NMR experiment to resolve the relative stereochemistry at C-1 and C-2.\(^{40}\) The only chiroptical data given in the publication was an observed $[\alpha]_D = 0$. Both enantiomeric pairs of compound 17 isolated from New Zealand sponges exhibited identical NMR spectroscopic and mass spectrometric data to those previously published, with an $[\alpha]_D = 0$, but significant optical rotary dispersion values at 578 and 546 nm (Figure 2.5).

For the chiral thioether-bridged discorhabdins, the five-bond “zig-zag” correlation (Figure 1.3) has been the only reliable method of determining the upper and lower-face orientation of the methylene protons on C-7. In this study, the orientation of the two C-7 hydrogens on compound 17 was determined through a heteronuclear long-range coupling $J$-HMBC NMR experiment which examined the $^3J(H, C)$ coupling between H-7A, H-7B and C-20.\(^{108}\) An energy minimized Chem3D model of (1$^R$,2$^S$,6$^R$,8$^S$) 17, showed predicted dihedral angles of 58° and 178° for (H7α, C-20) and (H-7β, C-20) respectively, which would qualitatively correspond to a Karplus predicted small coupling constant for $^3J$(H-7α, C-20) and large coupling for $^3J$(H-7β, C-20), (Figure 2.17).
Figure 2.17. Chem3D energy minimized model of \((1R,2S,6R,8S)\) discorhabdin L (17). The model is positioned with C-6-C-7 bond lined up to show H-7\(\alpha\), H-7\(\beta\)-C-20 dihedral angles.

A heteronuclear long-range coupling \(J\)-HMBC experiment (scaling factor = 20) determined the \(\sp{3}J\)(H, C) coupling constants as \(\sp{3}J\)(H-7A, C-20) = 6.9 Hz and \(\sp{3}J\)(H-7B, C-20) = 3.5 Hz. Thus, it was concluded that H-7A (\(\delta\) 2.76 ppm, dd) was positioned on the upper \(\beta\)-face of the molecule while H-7B (\(\delta\) 2.49 ppm, d) was on the lower \(\alpha\)-face (Figure 2.18).
An attempt to determine the stereochemistry at C-1 via a 2D NOESY experiment was unsuccessful, as for (+)578 and (-)578 enantiomers, the H-1 resonance exhibited correlations to both H-7α and H-7β. The observed effect was not a factor of long exchange associated with 2D NOESY experiments, as NOE correlations from H-1 to both H-7α and H-7β were seen for a range of mixing times from 800 ms down to 200 ms.

An NOEDIFF experiment, as previously used by Reyes et al., secured the stereochemistry of (-)578-17 C-1 position as (1R).

TDDFT calculations of the ECD spectrum expected for (1R,2S,6R,8S)-17 were in good agreement with the experimentally measured ECD of the Wellington sourced (-)578-17 (Figure 2.19). However, for the same compound, a calculation assuming the (1S,2S,6R,8S) absolute stereochemistry (Figure 2.20) gave virtually identical spectra. Thus, while TDDFT calculations were effective in securing the stereochemistry of C-2, C-6 and C-8
chiral centers, the orientation of the hydroxyl substituent could not be determined in this manner.

Figure 2.19. ECD spectra for the TFA salt of (1R,2S,6R,8S)-discorhabdin L (17) in gas phase (black), methanol solvation model (red) and experimental (blue).

Figure 2.20. ECD spectra for the TFA salt of (1S,2S,6R,8S)-discorhabdin L (17) in gas phase (black), methanol solvation model (red) and experimental (blue).

In the study, discorhabdin L (17) was isolated from seven out of twelve specimens worked on, with (1R,2S,6R,8S) (-)-17 found in Wellington, Tutukaka, and Kaikoura populations and the enantiomer (1S,2R,6S,8R) (+)-17 restricted to the Milford and Doubtful Sound sponges. The South African C. bellae crude extract examined also contained the (1S,2R,6S,8R) (+)-enantiomer. Unfortunately, since the original publication of 17 by Reyes et al.\textsuperscript{107} did not give any useful chiroptical data ([α]D = 0), the stereochemistry of the South American-sourced compound remains unknown.
Both (-)_{578}-17 and (-)_{578}-17 showed similar potency against a P388 cell line with IC\textsubscript{50} values of 1.08 and 0.78 μM respectively.
2.2.3 Absolute Stereochemistry Definition of Other Known Discorhabdin Alkaloids Isolated from New Zealand *Latrunculia* spp. Sponges

Absolute stereochemistry of five other known chiral discorhabdins D (11), 2-OH D (10), H (16), N (18) and Q (25) isolated from New Zealand *Latrunculia* spp. sponges was defined by comparison of experimental ECD spectra with those observed for discorhabdins L (17) and B (8).

2.2.3.1 Discorhabdin D (11)-Type Compounds

Four known discorhabdin D-type compounds 10, 11, 16 and 18 were isolated from local *Latrunculia* spp. sponges. A short summary of their structures and known chiroptical data is presented in Figure 2.21.

![Discorhabdin D (11)](image1)

Discorhabdin D (11)
*Perry et al.*

![2-OH Discorhabdin D (10)](image2)

2-OH Discorhabdin D (10)
*Cheng et al.*

![Discorhabdin H (16)](image3)

Discorhabdin H (16)
*Antunes et al.*

![Discorhabdin N (18)](image4)

Discorhabdin N (18)
*Antunes et al.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Location</th>
<th>Optical Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Free base</td>
<td>[α]D = +358 (c 0.01)</td>
</tr>
<tr>
<td></td>
<td>Kaikoura TFA salt</td>
<td>[α]D = +80 (c 0.05)</td>
</tr>
<tr>
<td></td>
<td>Wellington TFA salt</td>
<td>[α]D = 0 (c 0.05)</td>
</tr>
<tr>
<td>11</td>
<td>Published HCl salt</td>
<td>[α]D = 0 (c 0.15)</td>
</tr>
<tr>
<td></td>
<td>Wellington TFA salt</td>
<td>[α]D = -160 (c 0.15)</td>
</tr>
<tr>
<td>16</td>
<td>Published Wellington ECD data</td>
<td>[α]D = 0 (c 0.05)</td>
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<tr>
<td></td>
<td>Wellington TFA salt</td>
<td>[α]D = -60 (c 0.05)</td>
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<tr>
<td>18</td>
<td>Published Wellington ECD data</td>
<td>[α]D = +40 (c 0.05)</td>
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<tr>
<td></td>
<td>Wellington TFA salt</td>
<td>[α]D = +180 (c 0.05)</td>
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</table>

Figure 2.21. Summary of published and experimental chiroptical data for compounds 10, 11, 16 and 18 (the units of optical rotation are deg cm$^2$ g$^{-1}$ dm$^{-1}$).
The ECD spectrum of Wellington-sourced \((-\)_546-discorhabdin D (11)\) was consistent in both sign and magnitude with that of Wellington-sourced \((1R,2S,6R,8S)\) \((-\)_578-discorhabdin L (17)\) (Figure 2.22), thereby establishing the absolute stereochemistry of this compound as \((2S,6R,8S)\).

![Figure 2.22. Experimental ECD spectra of the TFA salt forms of \((-\)_578-(1R,2S,6R,8S)-discorhabdin L (17)\) in blue, the Wellington-sourced \((-\)_546-discorhabdin D (11)\) in red.](image)

The experimental optical rotation data at \([\alpha]_{578}\) and \([\alpha]_{546}\) for the Wellington-sourced trifluoroacetate salt of \((2S,6R,8S)\) 11 is not in good agreement with that of the hydrochloride salt for the published Kaikoura, New Zealand-sourced 11 (Figure 2.21). However, the measurements were taken at different concentrations, and the more dilute value in this study may have given smaller optical rotation data than those expected. Based on the same sign of the optical rotation at 546 nm, the absolute stereochemistry of discorhabdin D presented in Perry \textit{et al.}\textsuperscript{30} is proposed to be \((2S,6R,8S)\).

The ECD spectrum of the Kaikoura-sourced \((+)-2\)-hydroxy discorhabdin D (10) was consistent in sign with that of the Wellington-sourced \((-\)_578-(1R,2S,6R,8S)-discorhabdin L (17), (Figure 2.23) thereby establishing the absolute stereochemistry of this compound as \((2S,6R,8S)\).
Figure 2.23. Experimental ECD spectra of the TFA salt forms of \((-)_{578}(1R,2S,6R,8S)\)-discorhabdin L (17) in blue, and the Kaikoura-sourced \((+)\)-2-hydroxy discorhabdin D (10) in red.

A comparison of the ECD data for the free base form of the published Japanese P. melanos-sourced \((2S,6R,8S)\) 10, with its major Cotton effects at \(\lambda_{\text{max}} (\Delta \varepsilon)\) 352 (+41.1), 308 (-17.3) and 258 (-43.3), is by sign only, in good agreement with the experimentally observed data for the trifluoroacetate salt of the Kaikoura-sourced \((2S,6R,8S)\) \((+)\)-10 at \(\lambda_{\text{max}} (\Delta \varepsilon)\) 354 (+8.6), 303 (-4.9) and 255 (-11.3). The magnitude for the three Cotton effects is drastically different. However, since the two spectra were measured for different protonation states of 10 and many of the compounds in the discorhabdin series were shown to have different chiroptical properties between free base and trifluoroacetate salt forms, a direct comparison of the two ECD spectra should not be made.

Isolated as a pair of diastereomers, the ECD spectra of \((+)\) and \((-)\) discorhabdin H (16) were equal and opposite. The ECD spectrum of the Wellington-sourced \((-)\)-16 was identical to that of the Wellington-sourced \((-)_{546}\)-discorhabdin D (11) indicating that the induced circular dichroism properties of this molecule were due to the core spirodienone moiety only. Based on the similarities of the ECD spectra of the Wellington-sourced \((-)_{578}(1R,2S,6R,8S)\)-discorhabdin L (17) with that of the Wellington-sourced \((-)\)-diastereomer of 16, (Figure 2.24), the absolute stereochemistry of C-2, C-6 and C-8 stereocenters of the \((-)\)-diastereomer were assigned as \((2R,6R,8S)\) and the Milford Sound-sourced \((+)\)-diastereomer as \((2S,6S,8R)\). The stereochemistry of C-1 was secured via a 2D
NOESY experiment. In the NOESY spectrum of the (-)-16 diastereomer, correlations from H-1 to H-7α and H-2 were observed, positioning H-1 on the lower face of the molecule. Thus, the absolute stereochemistry of the discorhabdin core of (-)-16 is (1R,2R,6R,8S) and (+)-16 is (1S,2S,6S,8R).

Figure 2.24. Experimental ECD spectra of the TFA salt forms of (-)-(1R,2S,6R,8S)-discorhabdin L (17) in blue, and the Wellington-sourced (-)-diastereomer of discorhabdin H (16) in red.

In this study, no attempt was made to confirm the (7'S) absolute stereochemistry of the thiohistidine residue as found by Antunes et al.38 and the assignments stand as relative stereochemistries only.
Table 2.1. Comparison of the ECD Cotton effect maxima of the two New Zealand and a South African-sourced compound 16.

<table>
<thead>
<tr>
<th>Wellington (-)-(1R,2R,6R,8S,7'S*)-16</th>
<th>Milford Sound (+)-(1S,2S,6S,8R,7'S*)-16</th>
<th>South Africa Antunes et al.38</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{max}}) ((\Delta\varepsilon)) (nm (M(^{-1})cm(^{-1})))</td>
<td>255 (-23.6)</td>
<td>256 (+22.2)</td>
</tr>
<tr>
<td>283 (-2.9)</td>
<td>282 (+4.1)</td>
<td>270 (-2.7)</td>
</tr>
<tr>
<td>305 (-8.11)</td>
<td>305 (+9.2)</td>
<td>300 (-3.99)</td>
</tr>
<tr>
<td>366 (+14.9)</td>
<td>365 (-13.1)</td>
<td>358 (+8.59)</td>
</tr>
</tbody>
</table>

Table 2.1 shows a comparison of the ECD Cotton effect maxima values for the published South African-sourced 16 with that of the two experimentally found for the Wellington (1R,2R,6R,8S,7'S*) and the Milford Sound (1S,2S,6S,8R,7'S*) sourced diastereomers. Apart from the complete absence of the strongest Cotton effect recorded for this compound at 255-256 nm, the South African sample of 16 shows a fairly good match with that of the Wellington-sourced (-)-diastereomer, thereby establishing the absolute stereochemistry of the known compound as (1R,2R,6R,8S,7'S). The stereochemistry of the discorhabdin core of the molecule was thus correctly represented in the original publication.

The two diastereomers of discorhabdin H were shown inactive against the murine leukemia P388 cell line with IC\(_{50}\) values of >8.2 \(\mu\)M for both (+)-16 and (-)-16.

The ECD spectrum of the Wellington-sourced (-)-discorhabdin N (18) was consistent in both sign and magnitude with that of the Wellington-sourced (-)\(_{578}\)-(1R,2S,6R,8S)-discorhabdin L (17), (Figure 2.25), thereby establishing the absolute stereochemistry of the C-2, C-6 and C-8 chiral centers as (2S,6R,8S).
The absolute stereochemistry about the C-1 stereocentre was established via $J$-HMBC and NOESY NMR experiments. The H-7B proton resonance ($\delta_{H} 2.47$) was shown to be on the $\alpha$-face of the molecule by a heteronuclear long-range coupling $J$-HMBC experiment looking at $^3J(H, C)$ coupling between the two H-7 protons and C-20, as well as H-7 and C-5 [$^3J(H-7A, C-20 = 7.0 \text{ Hz}; H-7B, C-20 = 3.4 \text{ Hz}), (^3J(H-7A, C-5 = 3.2 \text{ Hz}; H-7B, C-5 = 10.5 \text{ Hz})$]. In a NOESY NMR experiment, the H-1 ($\delta_{H} 3.60$) resonance showed strong correlations to both H-2 ($\delta_{H} 4.33$) and H-7B ($\delta_{H} 2.47$) resonances. Correlations from H-1 to both H-2 and H-7$\alpha$ secured the absolute stereochemistry of the C-1 stereocentre as (1$R$) (Figure 2.26). Therefore, the absolute stereochemistry of the Wellington-sourced (-)-18 was concluded to be (1$R$,2$R$,6$R$,8$S$).

Figure 2.26. Chem3D energy minimized model of (-)-(1$R$,2$S$,6$R$,8$S$)-discorhabdin N (18).
A comparison of the ECD Cotton effect maxima values for the published South African-sourced 18\textsuperscript{38} with those observed for (1\textit{R},2\textit{S},6\textit{R},8\textit{S}) (-)-18 shows a complete mismatch of values (Table 2.2). Thus, unfortunately the absolute stereochemistry of the South African-sourced discorhabdin N (18) remains unknown.

<table>
<thead>
<tr>
<th>Wellington (-)-(1\textit{R},2\textit{R},6\textit{R},8\textit{S})-18</th>
<th>South Africa Antunes \textit{et al.}\textsuperscript{38}</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} (\Delta \varepsilon) ) nm (M\textsuperscript{-1} cm\textsuperscript{-1})</td>
<td>258 (-21.9) 284 (-5.4) 299 (-8.7) 360 (+20.0)</td>
</tr>
</tbody>
</table>

Table 2.2. Comparison of the ECD Cotton effect maxima of the New Zealand and South African-sourced compound 18.

\textbf{2.2.3.2 Discorhabdin B (8)-Type Compounds}

The ECD spectrum of the free base form of the Wellington sourced (+)-discorhabdin Q (25) showed similarities of the sign of the Cotton effect maxima with that of the free base form of the Wellington-sourced (+)-(6\textit{S},8\textit{S})-discorhabdin B (8), (Figure 2.27), thereby establishing the absolute stereochemistry of (+)-25 as (6\textit{S},8\textit{S}).

![Figure 2.27. Experimental ECD spectra of the free base forms of (+)-(6\textit{S},8\textit{S})-discorhabdin B (8) in blue, and the Wellington-sourced (+)-discorhabdin Q (25) in red.](image)
Compound 25 was originally isolated from an Australian/South Pacific collection of Zyzzya spp. and reported with an $\left[\alpha\right]_D$ value of -904 deg cm$^3$ g$^{-1}$ dm$^{-1}$. The $\left[\alpha\right]_D$ value for the (6$S$,8$S$) Wellington sample at +720 deg cm$^3$ g$^{-1}$ dm$^{-1}$ was opposite in sign and similar in magnitude, suggesting the known compound is the enantiomer with (6$R$,8$R$) absolute stereochemistry. Thus, the stereochemistry of 25 was incorrectly depicted in the original publication.

Discorhabdin U (29) used in this study was accessed as a semi-synthetic derivative of discorhabdin B (8) (Scheme 2.3), therefore the stereochemistry at the C-6 stereocentre of 29 was identical to that of the starting material, 8. Therefore, (+(6$S$,8$S$))-8-sourced (+)-29 had the (6$S$) stereochemistry and the (-(6$R$,8$R$))-8-sourced (-)-29 had the (6$R$) stereochemistry.

Discorhabdin U (29) displays a different chromophore to that of discorhabdin B (8), and the experimental ECD spectra of the two compounds with the same stereochemical orientation about the C-6 stereocentre were significantly different (Figure 2.28).

![Figure 2.28](image-url)

Figure 2.28. Experimental ECD spectra of the TFA salt forms of (+)-(6$S$,8$S$)-discorhabdin B (8) in blue, and (+)-(6$S$)-discorhabdin U (29) in red.
The observed difference in the ECD spectra of the two related analogues is possibly due to two different resonance states for compound 29, where in 29a the iminoquinone moiety is \textit{para} substituted, and 29b \textit{ortho} substituted. This change in chromophore close to a chiral centre is likely to have induced the observed divergence of the ECD spectra. A similar effect was observed for another 7,8-dehydro analogue, discorhabdin W (37), where the experimental ECD spectra for the trifluoroacetate salt and free base form were drastically different (Figure 2.7).

Since no chiroptical data was assigned to discorhabdins S (27), T (28) and U (29) in the original publication,\textsuperscript{44} the absolute stereochemistry of naturally-occurring Bahamas-sourced 29 remains unknown.
2.3 New Natural Products

Four new discorhabdin natural products (-)578-107, (+)-108, (-)578-109 and (+)578-109 were isolated from New Zealand *Latrunculia* spp. sponges. Discorhabdin K (21) has previously been presented as a new analogue in the discorhabdin series but no spectral data have ever been published for the compound. In this study, both (+) and (-) diastereomers of 21 were isolated.

The isolation of (-)-discorhabdin K (21) form a Doubtful Sound-sourced sponge and structure elucidation of the compound is the authors original work. Comparison of the spectral data for (-)-21 with that of discorhabdin K that was isolated from a Kaikoura-sourced sponge by Blunt, Munro and coworkers revealed all of the compound 21 specific resonances were present but with slight differences in chemical shifts. Kindly, Professors Blunt and Munro have sent a specimen of the original Kaikoura-sourced sponge material from which (+)-21 diastereomer was isolated. This thesis describes (-)-discorhabdin K as a new natural product and presents a complete set of NMR spectroscopy, mass spectrometry and chiroptical data on the known natural product (+)-discorhabdin K (21).
2.3.1 3-Dihydro Discorhabdin A (107)

(-)578-3-Dihydro discorhabdin A (107) trifluoroacetate salt was isolated as a dark green oil from Wellington-sourced *L. (Biannulata) wellingtonesis* sponge. High resolution FABMS established the molecular formula C_{18}H_{17}BrN_{3}O_{2}S. The UV chromophore of compound 107 with maxima at 204, 249, 347 and 402 nm was identical to that observed for discorhabdin A (7). The \(^1\text{H}\) NMR spectrum of 107 in DMSO-\(d_6\) revealed thirteen non-exchangeable and three exchangeable resonances. The \(^{13}\text{C}\) NMR spectrum contained eighteen resonances, nine quaternary (one carbonyl, one imine, six \(\text{sp}^2\) hybridized and one \(\text{sp}^3\)), five methines (three \(\text{sp}^3\) and two \(\text{sp}^2\) hybridized) and four \(\text{sp}^3\) hybridized methylenes. A comparison of the \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectroscopic data for discorhabdin A (7) with those observed for 107, suggested a common penta-cyclic pyrido-pyrrolo-iminoquinone structure from C-7 to C-21 (Table 2.3). The remaining atoms, C_{6}H_{6}BrO as required by the molecular formula, were one quaternary \(\text{sp}^2\) (\(\delta_C\) 126.5), one olefinic methine (\(\delta_C\) 132.6), one quaternary \(\text{sp}^3\) (\(\delta_C\) 50.0), two \(\text{sp}^3\) methines (\(\delta_C\) 69.4, 48.9) and one methylene (\(\delta_C\) 38.3) \(^{13}\text{C}\) resonances, with one olefinic singlet (\(\delta_H\) 6.42), one broad methine singlet (\(\delta_H\) 4.16), one methine doublet of doublets (\(\delta_H\) 4.28, \(J = 13.2, 4.9\) Hz) and a methylene pair (\(\delta_H\) 2.11, \(J = 13.6, 3.0\) Hz; \(\delta_H\) 1.90, \(J = 13.6, 4.8, 3.1\) Hz) observed in the \(^1\text{H}\) NMR spectrum. Interpretation of the \(^1\text{H}\)-\(^1\text{H}\) COSY and \(^1\text{H}\)-\(^{13}\text{C}\) HMBC spectra suggested the fragment was a *spiro*-hexaenol. The olefinic methine resonance at 6.42 ppm was assigned to C-1 on the *spiro*-ring based on the \(^1\text{H}\)-\(^{13}\text{C}\) HMBC correlations to C-6 (\(\delta_C\) 50.0), C-7 (\(\delta_C\) 40.7), C-8 (\(\delta_C\) 58.8) and C-20 (\(\delta_C\) 103.3). Since this proton resonance was observed as a singlet, the bromine group was placed on the neighboring C-2 olefinic \(\text{sp}^2\) carbon at \(\delta_C\) 126.5. The methine resonance at 4.28 ppm was placed on C-5 based on the observed \(^1\text{H}\)-\(^{13}\text{C}\) HMBC correlations to C-6 (\(\delta_C\) 50.0), C-7 (\(\delta_C\) 40.7), C-8 (\(\delta_C\) 58.8) and C-20 (\(\delta_C\) 103.3), and a long-range \(^1\text{H}\)-\(^1\text{H}\) COSY correlation to H-1. The resonance at H-5 was also part of a CH-CH\(_2\)-CH (\(\delta_H\) 4.28, \(J = 13.2, 4.9\) Hz; \(\delta_H\) 1.90, \(J = 13.6, 4.8, 3.1\) Hz) spin system. The
methine resonance observed at 4.16 and 69.4 ppm in the $^1$H and $^{13}$C NMR spectra suggested the presence of a secondary alcohol functionality. This hydroxy group was placed on C-3 based on the observations of a COSY correlation from the resonance at 4.16 ppm to H-1 ($\delta_H$ 6.42) and H-5 ($\delta_H$ 4.28), and the $^1$H-$^{13}$C HMBC correlations to C-1 ($\delta_C$ 132.6) and C-2 ($\delta_C$ 126.5) of the spiro-ring. The broad H-3 resonance showed vicinal coupling to the methylene pair at $\delta_H$ 2.11 and $\delta_H$ 1.90, which in turn also showed a vicinal couple to the H-5 resonance at 4.28 ppm. Thus the methylene pair had to be in between H-3 and H-5 and was positioned on C-4. The assignment was confirmed by the observed $^1$H-$^{13}$C HMBC correlations from H-4B ($\delta_H$ 1.90) to C-2 ($\delta_C$ 126.5), C-3 ($\delta_C$ 132.6) and C-6 ($\delta_C$ 50.0).

<table>
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<tr>
<th>no.</th>
<th>$^{13}$C $\delta$</th>
<th>$^1$H $\delta$ [m, $J$ (Hz)]</th>
<th>COSY</th>
<th>HMBC</th>
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<tbody>
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<td>1</td>
<td>132.6</td>
<td>6.42 (s)</td>
<td>H-3</td>
<td>C-2, C-3, C-6, C-7, C-20</td>
</tr>
<tr>
<td>2</td>
<td>126.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>69.4</td>
<td>4.16 (br s)</td>
<td>H-1, H-4A, H-4B</td>
<td>C-1, C-2</td>
</tr>
<tr>
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<td>38.3</td>
<td>2.11 (td, 13.6, 3.0)</td>
<td>H-3, H-4B, H-5</td>
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<tr>
<td>4B ($\alpha$)</td>
<td>1.90</td>
<td>(ddd, 13.6, 4.8, 3.1)</td>
<td>H-3, H-4A, H-5</td>
<td>C-2, C-3, C-6</td>
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<tr>
<td>5</td>
<td>48.9</td>
<td>4.28 (dd, 13.2, 4.9)</td>
<td>H4A, H-4B</td>
<td>C-3, C-4, C-6, C-7, C-8, C-20</td>
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<tr>
<td>6</td>
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<td>2.50 (under solvent)</td>
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<td>C-1, C-5, C-6, C-8, C-19, C-20</td>
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<td>7B ($\alpha$)</td>
<td>2.40</td>
<td>(d, 12.0)</td>
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<td>C-1, C-5, C-6, C-8, C-20</td>
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<tr>
<td>8</td>
<td>58.8</td>
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<td>H-7A, H-7B, NH-9</td>
<td>C-7, C-10</td>
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<td>10.51 (br s)</td>
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<td>H-8</td>
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<td>12</td>
<td>123.2</td>
<td></td>
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<td>127.3</td>
<td>7.37 (s)</td>
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<td>C-11, C-12, C-15, C-19, C-21</td>
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<tr>
<td>16</td>
<td>18.1</td>
<td>2.88 (m)</td>
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<td>C-14, C-15, C-17, C-19, C-21</td>
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<tr>
<td>17A</td>
<td>43.7</td>
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<td>H-16, H-17B, NH-18</td>
<td>C-15, C-16, C-19</td>
</tr>
<tr>
<td>17B</td>
<td>3.83 (m)</td>
<td></td>
<td>H-16, H-17B, NH-18</td>
<td>C-15, C-16, C-19</td>
</tr>
<tr>
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<td>7.94 (br s)</td>
<td></td>
<td>H-17A, H-17B</td>
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<td>20</td>
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<td></td>
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</tr>
<tr>
<td>21</td>
<td>123.0</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table 2.3. NMR data for (-)-378-3-dihydro discorhabdin A (107) TFA salt in DMSO-$d_6$.

The orientation of the hydroxy substituent on the C-3 stereocentre was determined via a 2D NOESY experiment. (Table 2.4). The NOESY spectrum of 107 showed a crucial correlation from H-1 ($\delta_H$ 6.55) to H-3 ($\delta_H$ 4.16), positioning this resonance on the same face of the molecule. The H-3 resonance ($\delta_H$ 4.37) also showed correlations to both H-4A ($\delta_H$ 2.11) and H-4B ($\delta_H$ 1.90). In addition, the H-1 resonance showed NOESY correlations
to H-7B ($\delta_{HH} 2.40$) and H-5 ($\delta_{HH} 4.28$) positioning these three resonances on the same face of the molecule.

<table>
<thead>
<tr>
<th>no.</th>
<th>$^1$H $\delta$ [m, J (Hz)]</th>
<th>NOESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.42 (s)</td>
<td>H-3, H-5, H-7B</td>
</tr>
<tr>
<td>3</td>
<td>4.16 (br s)</td>
<td>H-1, H-4A, H-4B</td>
</tr>
<tr>
<td>4A ($\beta$)</td>
<td>2.11 (td, 13.6, 3.0)</td>
<td>H-3, H-4B</td>
</tr>
<tr>
<td>4B ($\alpha$)</td>
<td>1.90 (ddd, 13.6, 4.8, 3.1)</td>
<td>H-3, H-4A, H-5</td>
</tr>
<tr>
<td>5</td>
<td>4.28 (dd, 13.2, 4.9)</td>
<td>H-1, H-4B</td>
</tr>
<tr>
<td>7A ($\beta$)</td>
<td>2.50 (under solvent)</td>
<td>H-7B, H-8</td>
</tr>
<tr>
<td>7B ($\alpha$)</td>
<td>2.40 (d, 12.0)</td>
<td>H-1, H-7A, H-8</td>
</tr>
<tr>
<td>8</td>
<td>5.21 (d, 2.1)</td>
<td>H-7A, H-7B, NH-9</td>
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<td>N-9</td>
<td>10.51 (br s)</td>
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<td>13.18 (br s)</td>
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Table 2.4. 2D NOESY NMR data for (-)$_{578}$-3-dihydro discorhabdin A (107) TFA salt in DMSO-$d_6$.

The absolute stereochemistry of the new natural product (-)$_{578}$-107 was determined upon comparison of the ECD spectra with that of the Wellington-sourced (+)-discorhabdin A (7) (Figure 2.29). The stereochemistry of (+)-7 has previously in section 2.2.2 been established as (5$R$,6$S$,8$S$) via comparison of optical rotation values. The ECD spectrum of compound 107 was consistent in both sign and magnitude to that of the (+)-(5$R$,6$S$,8$S$)-discorhabdin A (7), thereby establishing the absolute stereochemistry of the C-5, C-6 and C-8 chiral centers as (5$R$,6$S$,8$S$).

Figure 2.29. Experimental ECD spectra of the TFA salt forms of (+)-(5$R$,6$S$,8$S$)-discorhabdin A (7) in blue, and the Wellington-sourced (-)$_{578}$-107 in red.
Minimized energy Chem3D molecular models for the 3R and 3S epimers of (5R,6S,8S) 107 are presented in Figure 2.30. In the naturally-occurring 107, the H-3 resonance showed NOESY correlations to H-1 and both H-4α and H-4β. The model-predicted dihedral angles between H-3 and H-4α and H-4β were 62° and 56° respectively for the 3R epimer, and 165° and 49° respectively for the 3S epimer. Thus the 3R theoretical model was in agreement with the observed experimental findings of NOE correlations from H-1 to both of the H-4 protons, securing the absolute stereochemistry of Wellington-sourced (-)578-3-dihydro discorhabdin A as (3R,5R,6S,8S).

![Chem3D Energy minimized models for the (3R,5R,6S,8S) and (3S,5R,6S,8S) epimers of 3-dihydro discorhabdin A (107).](image)

(-)578-3-Dihydro discorhabdin A (107) was found moderately cytotoxic against the P388 murine leukemia cell line with an IC₅₀ of 1.83 μM, several orders of magnitude less so than the keto-analogue discorhabdin A (7) at 0.11 μM.²³
2.3.2 1-Thiomethyl Discorhabdin G*/I (108)

(+)-1-Thiomethyl discorhabdin G*/I (108) trifluoroacetate salt was isolated as a dark purple/brown oil from the Wellington-sourced *L. (Biannulata) wellingtonesis* sponge. High resolution FABMS established a molecular formula of C_{19}H_{16}N_{3}O_{2}S_{2} for 108, which required fourteen double bond equivalents. Compound 108 showed three exchangeable proton signals in the DMSO-**d**_{6} ^{1}H NMR spectrum, at 13.28, 10.83 and 8.02 ppm, corresponding to NH-13, NH-9 and NH-18 positions in a discorhabdin B-type compound. A comparison of the ^{1}H and ^{13}C NMR spectroscopic data for discorhabdins B (8) and G*/I (15) with those observed for 108 in CD_{3}OD, suggested a common penta-cyclic pyrido-pyrrolo-iminoquinone structure from C-7 to C-21 (Table 2.5). The remaining atoms, C_{6}H_{5}OS as required by the molecular formula, comprised a carbonyl (δ_{C} 181.6), two quaternary sp^{2} (δ_{C} 170.7 and 164.8), one quaternary sp^{3} (δ_{C} 52.9), two olefinic methines (δ_{C} 123.3 and 119.2) and an sp^{3} methyl (δ_{C} 14.6) ^{13}C resonances, with two olefinic doublets (δ_{H} 6.40, J = 1.1 Hz; 6.12, J = 0.6 Hz) and a three proton singlet (δ_{H} 2.56) observed in the ^{1}H NMR spectrum. Interpretation of the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC NMR spectra suggested the fragment was a spirodienone ring bearing an S-methyl substituent. The two methine resonances at 6.40 and 6.12 ppm were assigned to the spirodienone ring positions H-2 and H-4 respectively, based on their long-range ^{1}H-^{1}H COSY correlations to each other and ^{1}H-^{13}C HMBC correlations from H-2 to C-4 (δ_{C} 119.2) and C-6 (δ_{C} 52.9), and from H-4 to C-2 (δ_{C} 123.3) and C-6 (δ_{C} 52.9). The relative assignments of protons at positions C-2 and C-4 were confirmed by a long-range planar zig-zag COSY correlation between H-4 and H-7α. Interestingly, in contrast to discorhabdin A (7) and D (11)-type molecules where the lower-face 7α methylene proton is found at a lower chemical shift than the 7β resonance (Figure 2.20, Table 2.4), for compound 108 the reverse was observed. The higher shift H-7 resonance at 2.96 ppm in the ^{1}H NMR spectrum of 108 was shown to be on the α-face of the molecule by a
heteronuclear long-range coupling $J$-HMBC NMR experiment looking at $^3J(H, C)$ coupling between the two H-7 protons and C-20, as well as H-7 and C-5 [$^3J(H-7A, C-20 = 3.7 \text{ Hz}; H-7B, C-20 = 9.7 \text{ Hz}), (^3J(H-7A, C-5 = 7.3 \text{ Hz}; H-7B, C-5 = 3.8 \text{ Hz})]$. The $S$-methyl resonance ($\delta_H 2.65$) was placed at C-1 ($\delta_C 164.8$) based on $^1H-^{13}C$ HMBC correlations from the methyl protons to C-1 and C-2 on the spirodienone ring and a long-range COSY and NOESY correlation between H-2 and H-22. There were no observed NOESY correlations between the $S$-methyl and H-7$\alpha$ and H-7$\beta$ protons, indicating that the rotation around the C-2-S-C-22 bond in 1-thiomethyl discorhabdin G*/I is limited.

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Table 2.5. NMR data for 1-thiomethyl discorhabdin G*/I (108) TFA salt in CD$_3$OD.

The absolute stereochemistry of compound (+)-108 was determined upon comparison of the ECD spectra with that of the Wellington-sourced (+)-discorhabdin G*/I (15) (Figure 2.31). Near equivalence of the spectra indicated that (+)-108 contained the same skeleton absolute configuration of the (+)-(6S,8S)-discorhabdin G*/I (15).
Figure 2.31. Experimental ECD spectra of the TFA salt forms of (+)-(6S,8S)-discorhabdin G*/I (15) in blue, and the Wellington-sourced (+)-108 in red.

Compound 108 showed potent activity against the murine leukemia P388 cell line with an IC$_{50}$ of 0.28 μM.
(-)-1-Thiohistidine discorhabdin G*/I (21) trifluoroacetate salt was isolated as a dark purple/brown oil from a Doubtful Sound-sourced *Latrunculia* sp. sponge. High resolution FABMS established a molecular formula of C$_{25}$H$_{23}$N$_{6}$O$_{4}$S$_{2}$ for 21. Compound 21 showed similarities in the $^1$H and $^{13}$C NMR spectra to that of discorhabdin H (17) with the only major difference being a quaternary sp$^2$ (δ$_C$ 163.3) and a methine sp$^2$ (δ$_C$ 119.2) $^{13}$C resonances and an olefinic methine (δ$_H$ 5.94, d, $J$ = 1.3 Hz) resonance observed in the $^1$H NMR spectrum (Table 2.6). The three resonances were assigned to *spiro*-ring positions C-1 and C-2 based on the interpretation of the $^1$H-$^1$H COSY and $^1$H-$^{13}$C HMBC NMR spectra. The doublet $^1$H NMR resonance at δ$_H$ 5.94, and the $^{13}$C NMR resonance at δ$_C$ 119.2 were positioned on C-2 as the proton resonance showed a long-range $^1$H-$^1$H COSY correlations to H-4 and $^1$H-$^{13}$C HMBC correlations to C-4 (δ$_C$ 119.2) and C-6 (δ$_C$ 52.8). The H-2 resonance also showed an $^1$H-$^{13}$C HMBC correlation to the quaternary sp$^2$ resonance at δ$_C$ 163.2 which was positioned on C-1. Thus the *spiro* ring in 21 was a C-1 substituted spiroadienone as in 108. Two carbonyl resonances, C-3 and C-11, in (-)-21 could not be assigned as there were no peaks and correlations observed in the $^{13}$C and $^1$H-$^{13}$C HMBC NMR spectra.
Table 2.6. NMR data for Doubtful Sound-sourced (-)-discorhabdin K (21) TFA salt in CD$_3$OD. HMBC denotes values were taken from correlations observed on a $^1$H-$^1$C HMBC NMR experiment.

Discorhabdin K (21a) is a known natural product, the structure of which was shown in a review by Urban et al.$^{36}$

In the publication, compound 21a was shown with no thioether linkage between C-5 and C-8. However an examination of the original $^1$H NMR spectrum, kindly provided by Professors Blunt and Munro, showed that the resonances associated with H-7 and H-8 for
discorhabdin K (21a) were consistent with a thioether-bridged structure of 21.\textsuperscript{37} In order to resolve the structural inconsistency associated with this discorhabdin analogue, the authors that published discorhabdin K have kindly provided a sample of the Kaikoura-sourced \textit{Latrunculia} sp. sponge used in the original isolation and structure elucidation work. Following a similar methodology that yielded the Doubtful Sound-sourced metabolite, compound 21 was isolated from the Kaikoura-sourced \textit{Latrunculia} sp. and shown to indeed have a thioether bridge moiety between C-5 and C-8. A complete set of NMR spectral data for the Kaikoura-sourced 21 is given in Table 2.7.

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</tr>
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\textbf{Table 2.7.} NMR data for Kaikoura-sourced (+)-discorhabdin K (21) TFA salt in CD$_3$OD.

Doubtful Sound-sourced and the Kaikoura-sourced discorhabdin K (21) exhibited subtle differences in the $^1$H and $^{13}$C NMR spectra centered on the resonances of the \textit{spiro}-ring and the thiohistidine moiety (Tables 2.6 and 2.7), suggesting the alkaloids were diastereomeric rather than enantiomeric. The two compounds exhibited opposite optical
rotation values at -260 deg cm$^3$ g$^{-1}$ dm$^{-1}$ and +340 deg cm$^3$ g$^{-1}$ dm$^{-1}$ for Doubtful Sound and Kaikoura-sourced 21 respectively. The ECD spectra of (+)-21 and (-)-21 were equal and opposite (Figure 2.32), indicating that just as for discorhabdin H (16), the induced circular dichroism properties of this molecule were due to the core discorhabdin structure only.

Figure 2.32. Experimental ECD spectra of the TFA salt forms of the Kaikoura-sourced (+)-21 in blue, and the Doubtful Sound-sourced (-)-21 in red.

The absolute stereochemistry of the discorhabdin chromophore of (+)-21 and (-)-21 were determined upon comparison of the ECD spectra with that of discorhabdin G*/I (15). The ECD spectrum of (+)-21 diastereomer was consistent with that of (+)-(6$S$,8$S$)-discorhabdin G*/I (15), thereby establishing the absolute stereochemistry of the C-6 and C-8 chiral centers as (6$S$,8$S$) (Figure 2.33).

Figure 2.33. Experimental ECD spectra of the TFA salt forms of (+)-(6$S$,8$S$)-discorhabdin G*/I (15) in blue and the Kaikoura-sourced (+)-diastereomer of discorhabdin K (21) in red.
The absolute stereochemistry of the Doubtful Sound-sourced (-)-21, with equal and opposite ECD spectra was assigned \( (6R,8R) \). With enantiomeric cores established for both (+)-21 and (-)-21 the only remaining stereocentre with undefined stereochemistry was C-7' on the thiohistidine substituent. In Antunes et al., the absolute stereochemistry of the thiohistidine moiety of discorhabdin H (16) has been established as \((7'S)\) by degradative chemistry and chiral GC analysis.\(^{38}\) In this work no attempt was made to confirm the assignment made by Antunes et al., and the stereochemistry at C-7' of 21 stands as relative only.

The two diastereomers of discorhabdin K were shown inactive against the murine leukemia P388 cell line with IC\(_{50}\) values of >8.2 \( \mu \)M for both (+)-21 and (-)-21.
3.3.4 16a,17a-Dehydro Discorhabdin W (109)

(-)578-16a,17a-Dehydro discorhabdin W (109) trifluoroacetate salt was isolated as a dark brown oil from the Wellington-sourced *L. (Biannulata) wellingtonesis* sponge. High-resolution ESI of compound 109 showed a pseudomolecular ion [M+H]$^+$ at m/z 824, with the doubly protonated ion [M+2H]$^{2+}$ also present at m/z 413. The isotope pattern of the molecular ion showed peaks in the 1:2:1 ratio indicating the presence of two bromine atoms in the molecule. The $^{13}$C NMR spectrum of 109 in CD$_3$OD showed 40 resonances, indicating that the structure of this large molecule was not symmetrical as in discorhabdin W (37). The $^1$H NMR spectrum of 109 in CD$_3$OD showed 16 resonances. Interpretation of the $^1$H-$^1$H COSY and $^1$H-$^{13}$C HMBC spectra suggested that nine proton resonances were consistent with a discorhabdin W (37)-type subunit, and the remaining 7 showed similarities with a discorhabdin T (28)-type molecule (Figure 2.34).

Figure 2.34. $^1$H NMR spectrum of (-)-578-109 TFA salt in CD$_3$OD showing discorhabdin T (28)-type in red and W (37)-type in green consistent resonances.
With the structure of the two subunits identified, a connection through the C-5 disulfide was made to give the irregular dimer, 16a,17a-dehydro discorhabdin W (109).

The absolute stereochemistry of the Wellington-sourced compound (-)\textsubscript{578-109} was determined upon comparison of the ECD spectra of the free base form with that of the free
base of (-)-(6S,6'S)-discorhabdin W (37) (Figure 2.35). The ECD spectrum of compound (-)\textsubscript{578-109} was essentially identical to that of (-)-(6S,6'S)-discorhabdin W (37), thereby establishing the absolute stereochemistry of Wellington-sourced (-)\textsubscript{578-109} as (6S,6aS).

![Experimental ECD spectra of the free base forms of (-)-(6S,6'S)-discorhabdin W (37) in blue and the Wellington-sourced (-)\textsubscript{578-109} in red.](image)

**Figure 2.35.** Experimental ECD spectra of the free base forms of (-)-(6S,6'S)-discorhabdin W (37) in blue and the Wellington-sourced (-)\textsubscript{578-109} in red.

The antipode of the Wellington-sourced metabolite, compound (+)\textsubscript{578-109} with identical \textsuperscript{1}H NMR (Table 2.9) and equal and opposite ECD spectrum (Figure 2.36) to that of (-)\textsubscript{578-109} was isolated from a Doubtful Sound-sourced *Latrunculia* sp. sponge. Since (+)\textsubscript{578-109} had equal and opposite ECD spectra to that of (-)\textsubscript{578-(6S,6aS)-109}, the absolute stereochemistry of the Doubtful-Sound sourced compound was concluded to be (6R,6aR). Due to a small quantity of the (+)\textsubscript{578-109} metabolite recovered (0.6 mg), \textsuperscript{13}C NMR data is not reported for the compound.

![Structures of (+)\textsubscript{578-(6R,6aR)-109} and (-)\textsubscript{578-(6S,6aS)-109}](image)
Table 2.9. $^1$H NMR data for (+)$_{578}$-16a,17a-dehydro discorhabdin W (109) TFA salt in CD$_3$OD.

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<td>8.44 (d, 5.9)</td>
</tr>
</tbody>
</table>

**Figure 2.36.** Experimental ECD spectra of the free base forms of (-)$_{578}$-(6S,6aS)-16a,17a-dehydro discorhabdin W (109) in blue and (+)$_{578}$-(6R,6aR)-16a,17a-dehydro discorhabdin W (109) in red.

Both (+)$_{578}$ and (-)$_{578}$-enantiomers of the new natural product 16a,17a-dehydro discorhabdin W (109) were equally potent against the P388 murine leukemia cell line with IC$_{50}$ values of 0.40 and 0.45 μM respectively.
2.4 Summary

This study identified eighteen discorhabdin alkaloids isolated from *Latrunculia* species sponges (Figure 2.2). Twelve of those were known and fully characterized natural products and six, 21, 107, 108, 109, 110 and 111 were new structures. Two, 110 and 111 were shown to be isolation procedure artifacts and will be discussed in the following chapter. The remaining four naturally-occurring discorhabdins, 3-dihydro discorhabdin A (107), 1-thiomethyl discorhabdin G*/I (108), discorhabdin K (21) and 16a,17a-dehydro discorhabdin W (109) were fully characterized in this work. In addition, antipodal pairs of discorhabdins B (8), G*/I (15), L (17) W (37) and 16a,17a-dehydro W (109) as well as diastereomeric pairs of discorhabdins H (16) and K (21) were isolated. The enantio- and diastereomeric pairs exhibited similar potencies against the P388 cell line, indicating that the observed biological activity was independent of the stereochemistry of the discorhabdin alkaloids.

Circular dichroism spectra, rather then optical rotation data prone to large measurement errors, were crucial in obtaining information on the stereochemistry of the series. The absolute stereochemistry of all of the naturally-occurring chiral discorhabdin alkaloids was resolved using theoretical calculations of electronic circular dichroism spectra. Based on a basic chiral dataset of the ECD spectra calculated for four compounds, discorhabdins B (8), G*/I (15), L (17) and W (37), the absolute stereochemistry of all new natural products and other known analogues in the series were determined. A summary of all of the chiral naturally-occurring discorhabdins isolated from New Zealand *Latrunculia* spp. and their absolute stereochemistry is shown in Figure 2.37.
Figure 2.37. Chiral discorhabdin alkaloids isolated from *Latruncula* spp. sponges.
Figure 2.38. Source-specific distribution of chiral discorhabdin alkaloids isolated from *Latrunculia* spp. sponges.

Figure 2.38 shows the chiral discorhabdin distribution according to the collection site of the source sponge material. No single locale exhibited the presence of both the (+) and (-) enantiomers of the same compound. In fact, all of the co-occurring metabolites in a single collection had the same spirodienone and thioether absolute configurations. The two populations at Milford and Doubtful Sound region of New Zealand were the only collections that yielded anti-discorhabin (-)-8, (-)-15, (+)-16, (+)-578-17, (+)-37 and (+)-578-109, with the stereochemistry opposite of that secured for the original publication of discorhabdin/prianosin A (7). The South African specimen of *C. bellae* also yielded (-)-15 and (+)-578-17 with the opposite stereochemistry to that of (+)-7. Comparison of the published chiroptical data for discorhabdins G*/I (15) from a South American collection and Q (25) from Australia/South Pacific-sourced collection with that of the Wellington-sourced compounds of defined stereochemistry, suggests that both of the known compounds have the opposite stereochemistry to that of (+)-7 and their absolute stereochemistry was incorrectly depicted in the original publications.
Interestingly, while all of the discorhabdins isolated in this study were enantiopure and stereospecific towards only one absolute configuration of the spirodienone ring, the South American scaffold, isolated by Reyes et al., falls outside the observed pattern. Discorhabdin G*/I (15) showed equal and opposite optical rotation data to that of the Wellington-sourced metabolite, however discorhabdins A (7) and B (8) from the same collection showed identical \([\alpha]_D\) values and ECD spectra to that of the Wellington-sourced compounds. All of the sponge material worked on in this study originates from a single sponge specimen or piece only. The South American-sourced *L. brevis* was collected by bottom trawling and is very likely to have contained multiple individual sponges. The South American *L. brevis* metabolites isolated by Reyes et al.\textsuperscript{40} are therefore not likely to represent single-sponge biosynthetic efforts, but rather population-wide.

However, it is unclear whether all these generalizations represent a genuine geographical distribution pattern or simply reflect a limited number of collections of *Latrunculia* species sponges that the current knowledge on the stereochemistry of discorhabdin alkaloids is generated from. Clearly, *Latrunculia* and related species sponges are able to biosynthesize both antipodal forms of the discorhabdin alkaloids and in future, careful account of the absolute stereochemistry determination, rather than assumptions based on a common biosynthetic origin of the alkaloids is warranted.
3. Structure-Activity Relationship Study on Natural Products from *Latrunculia* spp. Sponges
3.1 Introduction

The following chapter reviews the synthetic, semi-synthetic and biosynthetic work previously made on the discorhabdin alkaloids and outlines the results of a current structure-activity relationship study undertaken on the main natural product on the New Zealand *Latrunculia* spp., (+)-(6S,8S)-discorhabdin B (8).

3.1.1. Previous Semi-Synthetic Reactions on Discorhabdin Alkaloids

A single semi-synthetic structure/activity relationship study has been reported on the discorhabdin alkaloids. Copp *et al.* generated a series of discorhabdin C analogues by modifications on the *spiro*-cyclohehadienone ring (Scheme 3.1). Discorhabdin C (6) was reduced with sodium borohydride to the dienol 34 in high yield. The authors noted two sets of signals in the $^{13}$C NMR spectrum of 34, suggesting two possible spatial approaches to the hydride attack on 6, but did not separate the two diastereomers or resolve the stereochemistry at C-3. At 530 ng/mL (0.91 μM) against the P388 cell line, dienol derivative 34 was less cytotoxic than discorhabdin C (6) at 40 ng/mL (0.07 μM), suggesting the loss of the keto moiety at C-3 had an effect on the activity of the series. 1H-Azepine derivatives of 6 and 34 were accessed through a dienone-phenol and dienol-benzene rearrangements respectively to yield the phenol 118 and benzene 119. Both compounds 118 and 119 exhibiting P388 IC$_{50}$ values of 3050 ng/mL (5.29 μM) and 1700 ng/mL (3.03 μM) respectively, were considerably less cytotoxic than 6 and 34. Compound 118 was methylated with diazomethane to yield the derivative 120, and nitrated with fuming nitric acid in glacial acetic acid to yield the hydroxydienone 121 and debromo-nitrophenol 122. At IC$_{50}$ values of 4000 ng/mL (6.77 μM) and 8500 ng/mL (15.65 μM) compounds 120 and 122 were not strongly cytotoxic, whereas compound 121, at 385 ng/mL (0.65 μM) showed moderate cytotoxicity, again emphasizing the importance of the α-bromo-enone moiety.
Scheme 3.1. Semi-synthetic modifications of discorhabdin C (6) and analogues, adapted from Copp et al.\(^3\)

Three other discorhabdin semi-synthetic derivatives have been prepared, primarily as tools for structure elucidation. Prianosin/discorhabdin A (7) was methylated with diazomethane to yield the N-13-methyl prianosin A (123).\(^2\) No NMR spectral or cytotoxicity data have ever been reported for the compound.
Due to their low solubilities as free bases, all of the initial NMR experiments for prianosins C (10) and D (11) were performed on their acetylated derivatives 124 and 125 respectively. Acetylation reactions of prianosin C (10) and prianosin/discorhabdin D (11) under standard pyridine/acetic acid conditions yielded not only the acetate derivatives of the N-13 pyrrole and N-8 pyridine nitrogens, but also reduced the iminoquinone moiety to an aminophenol. Kobayashi and coworkers proposed this reduction took place due to severe steric hindrance of the two neighboring acetylated N-9 and N-13 positions, while Blunt, Munro and coworkers hypothesized that the observed reduction was driven by a decrease of strain at the imine end of the molecule in going from sp² to sp³ hybridization of the N-18 atom. In the Copp research group, semi-synthetic discorhabdin P (24) was generated from naturally-occurring New Zealand-sourced discorhabdin C (6) upon reaction with methyl iodide and an excess of K₂CO₃ in dry acetone. Semi-synthetic 24 was found moderately cytotoxic against the P388 murine leukemia cell line with an IC₅₀ of 0.31 μM. Furthermore, the compound has been evaluated at the NCI in vitro disease-oriented primary anti-tumor screen, with an average GI₅₀ over sixty human tumor cell lines of 0.4 μM. Showing an activity profile similar to that of discorhabdin C (6), 24 was selective for the colon and leukemia subpanels and is currently undergoing in vivo hollow fibre assay evaluation. During the course of this project, 40 mg of semi-synthetic discorhabdin P has been synthesized and delivered to the NCI.
3.1.2. Total Syntheses of the Discorhabdin Alkaloids

Synthetic efforts towards building the pyrroloiminoquinone core of the discorhabdin-like molecule involve two different approaches, as outlined in Scheme 3.2.

![Scheme 3.2. Synthetic strategies towards the iminoquinone core of the discorhabdin molecule, adapted from Urban et al.](image)

The indole approach proceeds via a tryptamine-type precursor and involves condensation of a tryptamine quinone or cyclization of 4-aminoindole. The quinoline method involves the insertion of a nitrogen atom to generate a pyrrole ring. Both approaches have been used to synthesize a range of naturally-occurring damirones, makaluvamines, batzellines and isobatzelline natural products and semi-synthetic analogues. The generation of a discorhabdin-type skeleton requires further oxidation steps to form the spiro-ring. To date, total syntheses of discorhabdins A (7), C (6), E (12) and dethiadiscorhabdin D (137) have been accomplished.

Kita and coworkers published the first total synthesis of discorhabdin C (6). The crucial step in the generation of the spiro-ring was an oxidative coupling reaction of the silyl ether 127 with the hypervalent iodine reagent PIFA in 2,2,2-trifluoroethanol to yield the desired product 6 (Scheme 3.3).

![Scheme 3.3. Spirodienone formation towards the total synthesis of discorhabdin C (6) by Kita et al.](image)
In another synthesis of discorhabdin C (6), Yamamura and coworkers utilized an electrochemical oxidation of the bromophenol 128 to yield a mixture of discorhabdin C (6) and the azepine phenol derivative 118 (Scheme 3.4).\textsuperscript{111}

Scheme 3.4. Spirodieneone formation towards the total synthesis of discorhabdin C (6) by Tao et al.\textsuperscript{111}

The authors proposed that the reaction mechanism goes via two electron oxidation of an enamine-type intermediate 129 to yield an iminium compound 130 which undergoes a proton abstraction towards discorhabdin C (6), Scheme 3.5.

Scheme 3.5. Reaction mechanism of the spirodieneone formation of discorhabdin C (6) via an electrochemical oxidation.\textsuperscript{111}

Heathcock and Marshall Aubart developed a method which utilizes Cu\textsuperscript{2+} catalyzed oxidation of the phenols 131 and 132 to the spirodieneone structures 133 and 134, towards total synthesis of discorhabdins C (6) and E (12) respectively (Scheme 3.6).\textsuperscript{112}

Scheme 3.6. Spirodieneone formation towards the total synthesis of discorhabdins C (6) and E (12) by Heathcock and Marshall Aubart.\textsuperscript{112}
In the publication the authors also showed a successful synthetic strategy towards formation of the core structure of a discorhabdin D-type molecule 137 (Scheme 3.7). The enone 135 was treated with phenyltrimethylammonium tribromide to yield the bromide 136 which upon treatment with basic alumina converted to dethiadiscorhabdin D (137). The authors proposed that the new N-18-C-2 bond formation was facilitated by the axial lower-face placement of the bromine substituent on 136 which enabled the N-18 nucleophilic attack.

Scheme 3.7. N-18-C-2 bond formation in a discorhabdin D-type molecule by Heathcock and Marshall Aubart.112

Discorhabdin A (7) represents the only thioether containing analog in the series with a completed total synthesis.101,113 Synthetic approach involved a diastereoselective step in the spirodienone formation via a hypervalent iodine reagent, followed by the introduction of a thioether bridge. The final step in the synthesis of 7 is shown in Scheme 3.8. The acetal 138 was treated with p-methoxybenzylthiol to yield the N-S acetal 139 which in situ cyclized to the thioether derivative 140. Removal of the protecting group yielded discorhabdin A (7). Starting from L- and D-tyrosine methyl ester, both naturally-occurring (+)-(6S,8S)-7 and the unnatural (-)-(6R,8R)-7 enantiomers respectively were synthesized using this strategy.101
3.1.3 Aims

Although there has been a considerable amount of synthetic work undertaken on pyrrolo-iminoquinone alkaloids, little is known about the bioactivity or the mechanism of action of the compounds. A single structure-activity relationship study by Copp et al. identified the importance of the spirodienone moiety in the activity of discorhabdin C (6).

The main natural products of the New Zealand Latrunculia spp. sponges are discorhabdins A (7), B (8) and C (6) in reported yields of up to 25-35 mg/g of dry sponge weight. In this work, the major metabolite isolated from most samples was discorhabdin B (8), with Wellington-sourced sponges yielding an average of 8 mg of (+)-8 per gram of sponge dry weight. Having the benefit of >100 mg of compound (+)-8 at hand as well as seventeen other purified discorhabdin analogues, a structure-activity relationship study on discorhabdin B was undertaken. This chapter aims to identify all of the reactive sites on the molecule and gain insight into the mechanisms of action of the discorhabdin alkaloids. Consequently a new discorhabdin biosynthetic scheme is proposed which suggests a central role for discorhabdin B in the biosynthesis of a number of other naturally-occurring analogues.
3.2. Chemical Modifications of Discorhabdin B (8)

During the course of the structure-activity relationship study on discorhabdin B (8) several reactive parts of the molecule were identified, as shown in Figure 3.1. The pyrrole ring was susceptible to a range of nucleophilic substitution reactions, the hydrogen pairs on C-7, C-16 and C-17 were acidic and reacted with an excess of base to yield the Δ7,8- and Δ16,17-dehydro analogues and finally, Michael-type addition reactions were observed on C-1 of the spirodienone ring. In total, nine semi-synthetic analogues of discorhabdin B were generated.

![Figure 3.1. Reactive sites on the discorhabdin B (8) molecule.](image)

3.2.1 N-Alkyl Analogues of discorhabdin B (8)

In the discorhabdin series N-pyrrole analogues are uncommon, with only four N-13 methylated metabolites, discorhabdins P (24), S (27), T (28) and U (29), reported from deep-sea *Strongylodesma* spp. sponges.\(^{41,44}\) The N-alkyl compounds displayed interesting biological activity: for example, discorhabdin P (24), 13-N-methyl discorhabdin C, was found to be an inhibitor of calcineurin and caspase CPP32 enzymes, whereas discorhabdin C (6) was inactive in the same assays.\(^{41}\) Discorhabdin U (29), an 13-N-methyl-5S-methyl discorhabdin B analogue, exhibited strong *in vitro* cytotoxicity against PANC-1, P388 and A-549 cell lines, comparable to that of some of the most potent of the discorhabdins.\(^{44}\)

Discorhabdin C (6) with an excess of base and methyl iodide was shown to react exclusively on the N-13 pyrrole position to yield the semi-synthetic discorhabdin P
(24), discorhabdin A (7) was reported to yield the N-13-methyl analogue (23) upon reaction with diazomethane. In an effort to expand the structure-activity relationship insight of the thioether-bridged analogues, a N-13 methylation of discorhabdin B (8) was attempted. Discorhabdin B was reacted with CH₃I and an excess of base in dry acetone to yield two products; 141 and 29. Products of this reaction were dependant upon the molar equivalents of CH₃I used, with a large excess (10 to 25 equiv.) yielding predominantly dimethyl 29, while two molar equivalents of CH₃I yielded exclusively the mono-methyl (Scheme 3.9).

Scheme 3.9. Semi-synthetic route towards discorhabdins U (29) and N-13 demethyl U (141).

The order of discorhabdin B methylation favored the thio group preferentially and only reacted at the N-13 pyrrole position in the presence of a large excess of CH₃I. Interestingly the expected mono-N-13-methyl derivative was never detected as a product and neither have any of the N-9 methylated analogues. Compounds 141 and 29 were purified by a combination of Sephadex LH-20, C₁₈ and C₈ flash chromatography and fully characterized as their trifluoroacetate salts.
3.2.1.1 N-13 Demethyl Discorhabdin U (141)

The (6S) stereoisomer of 141 was synthesized by reaction of known (6S,8S) configuration (+)-discorhabdin B (8). The new compound was characterized with a full set of NMR (Table 3.2) and chiroptical data.

Scheme 3.10 Semi-synthetic route towards N-13 demethyl discorhabdin U (141).

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<th>HMBC</th>
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</table>

Table 3.1. NMR data for the semi-synthetic N-13 demethyl discorhabdin U (141) TFA salt in CD$_3$OD.
3.2.1.2 Discorhabdin U (29)

Initial attempts at preparation of discorhabdin U (29) from discorhabdin B (8) used excess CH\textsubscript{3}I and K\textsubscript{2}CO\textsubscript{3} and afforded 29 in 39% yield (Scheme 3.9). The yield of 29 was improved significantly with the use of trimethylphosphate as an alternative methylating reagent. Heating at 90 °C in PO(CH\textsubscript{3}O)\textsubscript{3} and an excess of K\textsubscript{2}CO\textsubscript{3}, discorhabdin B (8) was converted exclusively to discorhabdin U (29) in yields of up to 90%. A small portion of (+)-discorhabdin B-sourced (+)-discorhabdin U (29) was converted to the free base and yielded \textsuperscript{1}H NMR spectroscopic data in full agreement with that published for the natural product (Table 3.1).\textsuperscript{44}

<table>
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Table 3.2. \textsuperscript{1}H NMR chemical shifts recorded for the free base form of discorhabdin U (29) in CDCl\textsubscript{3}.

Both (+)- and (-)-enantiomers of discorhabdin U (29) were synthesized and fully characterized as trifluoroacetate salts. The optical rotation values of the (+) and (-)-29 were equal and opposite at +220 deg cm\textsuperscript{3} g\textsuperscript{-1} dm\textsuperscript{-1} and -220 deg cm\textsuperscript{3} g\textsuperscript{-1} dm\textsuperscript{-1} respectively, as were the ECD spectra. Since the two compounds were semi-synthetic derivatives of discorhabdin B (8) of known stereochemistry, the absolute stereochemistry about the spiro-centre was assumed to be identical to that of the starting material.
3.2.1.3 Dibenzyl Discorhabdin U (142)

To assess the ability to introduce other alkyl groups, discorhabdin B was reacted with an excess of benzyl bromide under dry conditions in the presence of base to yield the dibenzyl derivative 142 (Scheme 3.11). (-)-Dibenzyl discorhabdin U (142) was fully characterized as a trifluoroacetate salt (Table 3.3).

Scheme 3.11. Semi-synthetic route towards dibenzyl discorhabdin U (142).

The ECD spectra of all three 7,8-dehydro (+)-discorhabdin B derivatives (+)-29, (+)-141 and (-)-142 were identical as illustrated in Figure 3.2, further supporting the stereochemical assignments of the semi-synthetic analogues.

Figure 3.2. Experimental ECD spectra of the TFA salt forms of (+)-(6S)-discorhabdin U (29) in red, (+)-(6S)-N-13 demethyl discorhabdin U (141) in green and (-)-(6S)-dibenzyl discorhabdin U (142) in blue.
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<td>159.6</td>
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<tr>
<td>20</td>
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<td></td>
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<tr>
<td>21</td>
<td>123.7</td>
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<td></td>
</tr>
<tr>
<td>22A</td>
<td>53.6</td>
<td>5.53 (d, 14.7)</td>
<td>H-24/26</td>
<td>C-12, C-14, C-23</td>
</tr>
<tr>
<td>22B</td>
<td>5.48 (d, 14.7)</td>
<td>H-24/26</td>
<td>C-12, C-14, C-23</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>137.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ArylCH 24-26</td>
<td>129.0 - 130.1</td>
<td>7.26–7.40 (m)</td>
<td>H-22</td>
<td>C-22</td>
</tr>
<tr>
<td>27</td>
<td>37.1</td>
<td>4.25 (s)</td>
<td>H-4, H-29/31</td>
<td>C-5, C-28</td>
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<td>28</td>
<td>136.4</td>
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<td></td>
</tr>
<tr>
<td>ArylCH 29-31</td>
<td>129.0 - 130.1</td>
<td>7.26–7.40 (m)</td>
<td>H-27</td>
<td>C-27</td>
</tr>
</tbody>
</table>

Table 3.3. NMR data for the semi-synthetic (-)-dibenzyl discorhabdin U (142) TFA salt in CD$_3$OD.
3.2.1.4 Biological Activity of Discorhabdin U-Type Semi-Synthetic Derivatives (+)-29, (+)-141 and (-)-142

Compounds (+)-8, (+)-29, (+)-141 and (-)-142 were evaluated for biological activity against the murine leukemia P388 cell line as well as activity against Gram-positive and -negative bacteria. The results, summarized in Table 3.4, indicate that both of the methylated semi-synthetic analogues (+)-29 and (+)-141 exhibit potent anti-proliferative activity, comparable in magnitude with the natural product discorhabdin B (8). Dibenzylation ((-)-142) led to attenuation of bioactivity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>P388 IC₅₀ (μM)</th>
<th>E. coli</th>
<th>B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-8</td>
<td>0.08</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>(+)-29</td>
<td>0.16</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>(+)-141</td>
<td>0.15</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>(-)-142</td>
<td>1.30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3.4. In vitro biological activities of compounds (+)-8, (+)-29, (+)-141 and (-)-142.

The three semi-synthetic derivatives, (+)-29, (+)-141 and (-)-142 were submitted to the National Cancer Institute in vitro primary anti tumor screen. Averaged over 60 human tumor cell lines, compounds (+)-29, (+)-141 and (-)-142 showed mean panel average GI₅₀ values of 18.8 nM, 48.8 nM and 0.255 μM respectively. Although active in nano-molar ranges, discorhabdins 29 and 141 did not show sufficient selectivity and were thus not chosen for further evaluation.

The results of the structure-activity relationship study suggested that thioether bridge moiety on discorhabdin B (8) was the most reactive position on the molecule. A bulky benzyl substituent on both the 5-S and N-13 positions significantly reduced the cytotoxic potency of the series. In order to further examine the reactivity of discorhabdin B, a semi-synthetic route towards N-13 mono-alkylated analogues was developed. It was hypothesized that in the dimeric structure of discorhabdin W (37), the reactive thioether is protected by a disulfide bond and alkylation was expected to occur exclusively on the N-13 pyrrole position.
3.2.2 Discorhabdin W (37) as a Source of N-13 Alkyl Analogues of Discorhabdin B (8)

The reactive nature of the C-5-C-8 thioether bridge and the C-7 methylene pair in discorhabdin B (8) were examined by reacting compound 8 with an excess of base. Discorhabdin B as a free base was left to stand in dark, at room temperature in deuterated acetone and an excess of K$_2$CO$_3$. In one week, the $^1$H NMR spectrum of the crude reaction mixture indicated that a significant amount of starting material had converted to a compound that was consistent with the structure of discorhabdin W (37), (Figure 3.3). On a quantitative scale, heating in DMF and an excess of K$_2$CO$_3$, (+)-discorhabdin B (8) formed the dimer (-)-discorhabdin W (37) in 32% yield. The photolysis reaction, as shown on HPLC-scale by Blunt, Munro and coworkers in the original publication of 37 also yielded the dimer (-)-37 in 40% yield (Scheme 3.12).$^{46}$ Semi-synthetic (-)-discorhabdin W was identical in all aspects including circular dichroism spectrum to the naturally-occurring (-)-(6S,6'S)-37.

![Scheme 3.12. Semi-synthetic conversion of (+)-(6S,8S)-discorhabdin B (8) into (-)-(6S,6'S)-discorhabdin W (37).](image)

As previously shown in Scheme 2.4, both naturally-occurring and semi-synthetic (-)-37 were reduced with TCEP to yield semi-synthetic (+)-discorhabdin B (8) that was consistent in all aspects including circular dichroism spectrum to the naturally-occurring (+)-8. Thus, if discorhabdin W (37) could be functionalized on the N-13 position, a semi-synthetic route towards N-13-discorhabdin B (8) analogues was possible.
Figure 3.3. Semi-synthetic conversion of (+)-(6S,8S)-discorhabdin B (8) into (-)-(6S,6'S)-discorhabdin W (37) in deuterated acetone and an excess of base.

a) (+)-discorhabdin B (8) at the start of the experiment.
b) mixture of (+)-discorhabdin B (8) and (-)-discorhabdin W (37) at day seven of the experiment.
3.2.2.1 N-13-Methyl Discorhabdin B (144)

Semi-synthetic (+)-N-13-methyl discorhabdin B (144) was synthesized from Wellington-sourced naturally-occurring (-)-(37) in two steps and an overall yield of 30% (Scheme 3.13). (-)-Discorhabdin W (37) was reacted with trimethylphosphate and an excess of base to yield (+)-discorhabdin U (29) and N-13-dimethyl discorhabdin W (143). Compound 143 was then reduced with TCEP to yield (+)-N-13 methyl discorhabdin B (144). The new semi-synthetic derivative (+)-144 was fully characterized as a trifluoroacetate salt (Table 3.5).

Table 3.5. NMR data for (+)-N-13-methyl discorhabdin B (144) TFA salt in CD₃OD.

<table>
<thead>
<tr>
<th>no.</th>
<th>¹³C δ</th>
<th>¹H δ [m, J (Hz)]</th>
<th>COSY</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>147.7</td>
<td>7.82 (s)</td>
<td></td>
<td>C-2, C-5, C-6, C-7, C-20</td>
</tr>
<tr>
<td>2</td>
<td>129.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>175.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>119.6</td>
<td>6.24 (s)</td>
<td></td>
<td>C-2, C-5, C-6</td>
</tr>
<tr>
<td>5</td>
<td>172.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>53.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7A</td>
<td>43.6</td>
<td>2.77 (d, 12.0)</td>
<td>H-7B</td>
<td>C-1, C-5, C-6, C-8, C-20</td>
</tr>
<tr>
<td>7B</td>
<td></td>
<td>2.54 (dd, 11.4, 3.6)</td>
<td>H-7A</td>
<td>C-5, C-6, C-8, C-20</td>
</tr>
<tr>
<td>8</td>
<td>62.6</td>
<td>5.70 (dd, 3.6, 1.2)</td>
<td>H-7A, H-7B</td>
<td>C-5, C-6, C-10</td>
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<tr>
<td>10</td>
<td>153.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>125.0</td>
<td></td>
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<tr>
<td>14</td>
<td>132.5</td>
<td>7.17 (s)</td>
<td>H-16, H-22</td>
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<tr>
<td>15</td>
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<tr>
<td>17B</td>
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<td></td>
<td>H-16, H-17B</td>
<td>C-15, C-16, C-19</td>
</tr>
<tr>
<td>19</td>
<td>156.3</td>
<td></td>
<td></td>
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<tr>
<td>20</td>
<td>98.7</td>
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<tr>
<td>21</td>
<td>124.2</td>
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</tr>
<tr>
<td>22</td>
<td>36.7</td>
<td>3.96 (s)</td>
<td>H-14</td>
<td>C-12, C-14</td>
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</tbody>
</table>

Since trimethylphosphosphate not only methylated the N-13 pyrrole position on 37 but also reduced the disulfide bond and dimethylated the resulting monomer to yield (+)-29, as an alternative, the reaction was attempted with methyl iodide. However, even in large molar excess of >100 equivalent CH₃I, no product was ever detected by the analytical HPLC.

Absolute stereochemistry of the new semi-synthetic discorhabdin analogue (+)-144 was established as (6S,8S). Previously in Scheme 2.4 it was shown that semi-synthetic (+)-(6S,8S)-discorhabdin B (8), prepared by TCEP reduction of naturally-occurring (-)-(6S,6'S) discorhabdin W (37) was in all aspects including ECD identical to the naturally-occurring (+)-(6S,8S)-8. This reaction involved disulfide bond reduction in
(-)-37 to the intermediate 145, in which the thiol moiety undergoes intramolecular nucleophilic attack on C-8 to create the new stereocentre. The stereochemistry about the C-6 stereocentre in 145 is identical to that of the starting material (-)-37. Thus, with the stereochemical orientation of the spiro-centre in 145 set, the thiol attack on C-8 is possible from the upper Si face only, giving the (+)-(6S,8S)-discorhabdin B (8) product (Scheme 3.14).

Following the same argument, since (+)-144 was a semi-synthetic derivative of the (-)-(6S,6'S)-discorhabdin W (37), the C-6 stereocentre had to have an identical orientation in both the starting material and the product. With the orientation of the spiro-centre set, the thiol C-8 ring closure was possible from the upper-face only, thus securing the stereochemistry of the new stereocentre as (8S). The absolute stereochemistry assignment was confirmed upon comparison of the ECD spectrum of (+)-144 with that of the Wellington-sourced (+)-(6S,8S)-discorhabdin B (8) (Figure 3.4).
Figure 3.4. Experimental ECD spectra of the TFA salt forms of (+)-(6S,8S)-discorhabdin B (8) in blue and (+)-(6S,8S)-N-13-methyl discorhabdin B (144) in red.
3.2.2.2 N-13-Benzyl Discorhabdin B (148)

Semi-synthetic (+)-N-13-benzyl discorhabdin B (148) was synthesized from the Wellington-sourced naturally-occurring (-)-discorhabdin W (37) in two steps and an overall yield of 17% (Scheme 3.15). (-)-Discorhabdin W (37) was reacted with benzyl bromide and an excess of base to yield N-13-dibenzyl discorhabdin W (147). Compound 147 was then reduced with TCEP to yield (+)-N-13 benzyl discorhabdin B (148). The new semi-synthetic derivative (+)-148 was fully characterized as a trifluoroacetate salt (Table 3.6).

![Scheme 3.15. Semi-synthetic route towards N-13-benzyl discorhabdin B (148).](image-url)
### Table 3.6.

<table>
<thead>
<tr>
<th>no.</th>
<th>$^{13}$C δ</th>
<th>$^1$H δ [m, J (Hz)]</th>
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<th>HMBC</th>
</tr>
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<td>129.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>175.8</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>119.7</td>
<td>6.24 (s)</td>
<td></td>
<td>C-2, C-5, C-6</td>
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<tr>
<td>5</td>
<td>172.6</td>
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<td>6</td>
<td>53.0 HMBC</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>7A</td>
<td>43.6</td>
<td>2.77 (d, 11.6)</td>
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<td>62.6</td>
<td>5.69 (dd, 4.0, 1.2)</td>
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<tr>
<td>11</td>
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<td></td>
<td></td>
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<tr>
<td>14</td>
<td>131.8</td>
<td>7.34 (s)</td>
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<td>C-12, C-15, C-21</td>
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<td>2.89 (dd, 8.8, 6.0)</td>
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<td>17A</td>
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<td>3.91 (m)</td>
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<td>21</td>
<td>125.0 HMBC</td>
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<td></td>
</tr>
<tr>
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<td>5.51 (d, 14.8)</td>
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<tr>
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<td>C-12, C-14, C-23, C-Aryl</td>
</tr>
<tr>
<td>23</td>
<td>137.7 HMBC</td>
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</tr>
<tr>
<td>ArylCH</td>
<td>129.0-130.0</td>
<td>7.32–7.34 (m)</td>
<td></td>
<td>C-22</td>
</tr>
<tr>
<td>24-26</td>
<td>129.0-130.0</td>
<td>7.32–7.34 (m)</td>
<td></td>
<td>C-22</td>
</tr>
</tbody>
</table>

Sourced from (-)-(6S,6'S)-37 of known stereochemistry, the absolute stereochemistry of the new semi-synthetic analogue (+)-148 was established as (6S,8S). The ECD spectrum of (+)-148 was identical to that of (+)-discorhabdin B and (+)-N-13-methyl discorhabdin B (144) (Figure 3.5).
Figure 3.5. Experimental ECD spectra of the TFA salt forms of (+)-(6S,8S)-discorhabdin B (8) in red, (+)-(6S,8S)-N-13-methyl discorhabdin B (144) in green and (+)-(6S,8S)-N-13-benzyl discorhabdin B (148) in blue.
3.2.2.3 Summary

Five methylated and benzylated semi-synthetic analogues of discorhabdin B (8) were prepared. The study identified C-5-C-8 thioether moiety and the C-7 methylene hydrogens as the most reactive features of a discorhabdin B-type molecule. Base-mediated thioether bridge opening in the presence of an electrophile yielded the N-13-demethyl discorhabdin U analogue (+)-141, and the N-13,5-S derivatives (+)-29 and (-)-142. Cytotoxic activity was correlated to the size of the introduced substituent, with the dibenzylated analogue (-)-142 showing a dramatic decrease in potency (Table 3.7). A semi-synthetic route utilizing discorhabdin W (37) was developed towards two exclusively N-13 functionalized discorhabdin B analogues (+)-144 and (+)-148. With P388 IC$_{50}$ values of 0.09 and 0.10 μM for (+)-144 and (+)-148 respectively, both of the N-13 analogues retained the same potency as that observed for discorhabdin B (8) (Table 3.7). The N-13 methylated analogue (+)-144 showed a similar activity profile to that of discorhabdin B at the NCI 60 human tumor cell line screen with a mean panel average of 0.75 μM compared to that of 0.71 μM observed for discorhabdin B.$^{27}$ However, N-13-methyl discorhabdin B (144) did not show sufficient selectivity in the assay and was not chosen for further evaluation at the NCI.
### Table 3.7. *In vitro* biological activities of compounds (+)-8, (+)-29, (-)-142, (+)-144 and (+)-148.

<table>
<thead>
<tr>
<th>Compound</th>
<th>P388 IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-8</td>
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</tr>
<tr>
<td>(+)-29</td>
<td>0.16</td>
</tr>
<tr>
<td>(-)-142</td>
<td>1.30</td>
</tr>
<tr>
<td>(+)-144</td>
<td>0.09</td>
</tr>
<tr>
<td>(+)-148</td>
<td>0.10</td>
</tr>
</tbody>
</table>

This structure-activity relationship study identified the importance of the thioether moiety in the biological activity of discorhabdin B. Analogues that retained the thioether functionality or had small methyl substituents on the 5S position were equipotent to discorhabdin B (8). The synthesis of the strongly cytotoxic (+)-N-13-benzyl discorhabdin B (148) opens the possibility for fluorescently labeled and biotinylated discorhabdin B derivatives for future cell localization and protein target identification studies.

![Figure 3.6. A simplified fluorescently labeled discorhabdin B (8) analogue.](image)
3.2.3 Semi-Synthetic 16,17-Dehydro Discorhabdin B (8) and U (29) Analogues

![Figure 3.7. Reactive sites on the discorhabdin B (8) molecule with emphasis on the 16,17-methylene pair.](image)

The reactivity of the C-16,17 methylene pair on discorhabdins B (8) and U (29) with an excess of base was examined. In an alkaline solution, discorhabdin B (8) yielded a known natural product discorhabdin Q (25), while discorhabdin U (29) yielded another known natural product, discorhabdin T (28).

3.2.3.1 Semi-Synthetic Discorhabdin Q (25)

Wellington-sourced (+)-discorhabdin B (8) refluxing in aqueous acetone and an excess of K₂CO₃ was oxidized to the 16,17-dehydro derivative discorhabdin Q (25) (Scheme 3.16). Since the absolute stereochemistry of the starting material was known, the absolute stereochemistry of the semi-synthetic derivative was concluded to be (6S,8S).

![Scheme 3.16. Semi-synthetic route towards (+)-discorhabdin Q (25).](image)
Optical rotation value of the semi-synthetic 25 at +400 deg cm$^3$ g$^{-1}$ dm$^{-1}$, was equal in sign and similar in magnitude to that of Wellington-sourced naturally-occurring 25 at +720 deg cm$^3$ g$^{-1}$ dm$^{-1}$. The two compounds also exhibited identical ECD spectra. Semi-synthetic conversion of (+)-8 to (+)-25 and the resulting match in chiroptical and $^1$H NMR spectral data for the semi-synthetic (+)-25 and naturally-occurring (+)-25 thus unequivocally confirmed the absolute stereochemistry assumption for (+)-(6$S$,8$S$)-25 made in section 2.2.3.2.

The C-16,17 oxidation reaction in aqueous acetone yielded many other unidentified decomposition products and was difficult to reproduce. As an alternative, (+)-8 was refluxed in DMF and an excess of K$_2$CO$_3$. However since the C-7 methylene pair and the thioether moiety in discorhabdin B (8) were also base-sensitive, the main product recovered from this reaction was (-)-discorhabdin W (37). Thus, to further demonstrate the reactivity of the C-16,17 methylene pairs, discorhabdin U, a 5$S$-methyl protected form of discorhabdin B, was used as a substrate.

### 3.2.3.1 Semi-Synthetic Discorhabdin T (28)

Semi-synthetic (+)-discorhabdin U (29) was heated in DMF and an excess of K$_2$CO$_3$ to yield the 16,17-dehydro discorhabdin U derivative, (+)-discorhabdin T (28) (Scheme 3.17).

![Scheme 3.17. Semi-synthetic route towards (+)-discorhabdin T (28).](image)

In the structure of discorhabdin U (29) the thioether is confined to the 5$S$-methyl moiety and the $\alpha$-acidic hydrogen on C-7 is replaced by an olefin. With the primary reactive centre of discorhabdin B now protected, the base reacted exclusively on the C-16,17
methylene pair to generate a single 16,17-dehydro product in good yield. Since the stereogenic centre of the starting material was presumed to be retained in the product structure, the absolute stereochemistry of (+)-28 was established as (6S). The product, originally isolated as a trifluoroacetate salt, in 1:1 solution of methanol and dichloromethane was found to spontaneously convert to the free base form. \(^1\)H NMR data for the free base form of (+)-28 was in good agreement with that published for the natural product (Table 3.8).

Table 3.8. \(^1\)H NMR chemical shifts recorded for the free base form of (+)-discorhabdin T (28) in CDCl\(_3\) (10% CD\(_3\)OD).

<table>
<thead>
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<th>Experimental (+)-28</th>
<th>Published(^{44})</th>
</tr>
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<td>(\delta [\text{m, J (Hz)}])</td>
</tr>
<tr>
<td>1 7.68 (s)</td>
<td>7.62 (s)</td>
</tr>
<tr>
<td>4 5.98 (s)</td>
<td>5.91 (s)</td>
</tr>
<tr>
<td>7 4.16 (d, 7.5)</td>
<td>4.10 (d, 7.5)</td>
</tr>
<tr>
<td>8 6.48 (d, 7.5)</td>
<td>6.43 (d, 7.5)</td>
</tr>
<tr>
<td>14 7.64 (s)</td>
<td>7.64 (s)</td>
</tr>
<tr>
<td>16 7.34 (d, 5.8)</td>
<td>7.30 (d, 5.8)</td>
</tr>
<tr>
<td>17 8.23 (d, 5.7)</td>
<td>8.17 (d, 5.8)</td>
</tr>
<tr>
<td>22 4.31 (s)</td>
<td>4.25 (s)</td>
</tr>
<tr>
<td>23 2.21 (s)</td>
<td>2.18 (s)</td>
</tr>
</tbody>
</table>

The (-)-(6R)-28 enantiomer was accessed as a byproduct in the methylation reaction of (-)-discorhabdin B (8) towards (-)-discorhabdin U (29) in trimethylphosphate and an excess of K\(_2\)CO\(_3\). Optical rotation \([\alpha]_D\) value of the free base form of (+)-28 at +280 deg cm\(^3\) g\(^{-1}\) dm\(^{-1}\) was similar in magnitude and opposite in sign to that of free base form of (-)-28 at -320 deg cm\(^3\) g\(^{-1}\) dm\(^{-1}\). Since discorhabdin T was only sparingly soluble in methanol, the ECD spectra of the two enantiomers were not recorded. In the original publication of 28,\(^{44}\) no chiroptical data were assigned to the naturally-occurring discorhabdin T, thus the absolute stereochemistry of the Strongylodesma sp.-sourced metabolite remains unknown.
3.2.4 Semi-Synthetic Discorhabdin D (11)-Type Analogues

Three semi-synthetic discorhabdin D-type analogues were generated from Wellington-sourced (+)-discorhabdin B (8), namely (-)-2-bromo discorhabdin D (110), (+)-1-discorhabdyl discorhabdin D (111), and 1-epi-discorhabdin L (150). Compounds 110 and 111 were isolated as natural products from Latrunculia spp. sponges used in this work, however during the course of the SAR study they were found to be artifacts. In the following two sections 110 and 111 are presented as new discorhabdin analogues, with their structure elucidated, stereochemistry established and the semi-synthetic route from (+)-discorhabdin B identified.

3.2.4.1 2-Bromo Discorhabdin D (110)

(-)-2-Bromo discorhabdin D (110) trifluoroacetate salt was isolated as dark green oil from Wellington-sourced L. (Biannulata) wellingtonesis sponge. High resolution FABMS established the molecular formula of C_{18}H_{13}BrN_{3}SO_{2}, indicating compound 110 was a constitutional isomer of discorhabdin B (8). The UV chromophore of 110 with maxima at
250, 285, 319 and 403 nm was consistent with a discorhabdin D (II)-type molecule. The $^1$H NMR spectrum of 110 in CD$_3$OD revealed ten resonances. The $^{13}$C NMR spectrum contained eighteen resonances, eleven quaternary (two carbonyl, one imine, six sp$^2$ hybridized and two sp$^3$ hybridized), three methines (two sp$^2$ hybridized and one sp$^3$ hybridized), three sp$^3$ hybridized methylenes and one sp$^3$ hybridized methine. A comparison of the $^1$H and $^{13}$C NMR data of (-)-110 with that published for discorhabdin D (II) revealed a common pentacyclic pyrido-pyrrolo-iminoquinone structure from C-7 to C-21 (Table 3.9). The remaining atoms C$_6$H$_3$BrO as required by the molecular formula were one carbonyl resonance ($\delta_C$ 187.1), one sp$^2$ hybridized quaternary resonance ($\delta_C$ 174.4), one olefinic methine resonance ($\delta_C$ 112.3), two sp$^3$ hybridized quaternary resonances ($\delta_C$ 79.7 and 45.9) and one methylene resonance ($\delta_C$ 43.9) observed in the $^{13}$C NMR spectrum; and one olefinic methine resonance ($\delta_H$ 6.12, s) and a methylene pair ($\delta_H$ 3.56, d, $J = 13.3$ Hz; 3.22, d, $J = 13.3$ Hz) observed in the $^1$H NMR spectrum. Based on similarities of chemical shifts with that reported for discorhabdin D (II) and 2-hydroxy discorhabdin D (10), the carbonyl resonance ($\delta_C$ 187.1) was placed at C-3, the sp$^2$ hybridized quaternary resonance ($\delta_C$ 174.4) at C-5 and the methine resonances ($\delta_H$ 6.12, s; $\delta_C$ 112.3) at C-4 positions of the spiro-ring. The methylene pair was placed at C-1 based on the observed $^1$H$-^{13}$C HMBC correlations from H-1A ($\delta_H$ 3.56, d, $J = 13.3$ Hz) and H-1B ($\delta_H$ 3.22, d, $J = 13.3$ Hz) to C-3 ($\delta_C$ 178.1), C-5 ($\delta_C$ 174.4) and C-6 ($\delta_C$ 45.9) on the spiro-ring, C-7 ($\delta_C$ 40.2), C-10 ($\delta_C$ 149.8) and C-20 ($\delta_C$ 101.9) on the adjoining ring and a long range correlation to C-17 ($\delta_C$ 51.7). The quaternary carbon resonance at $\delta_C$ 79.7 ppm was assigned to the spiro-ring position C-2. The methylene pair at H-1 ($\delta_H$ 3.56, 3.22), olefinic methine proton resonance at H-4 ($\delta_H$ 6.12) and H-17A resonance ($\delta_H$ 4.60) all exhibited $^1$H$-^{13}$C HMBC correlations to the quaternary carbon at $\delta_C$ 79.7 confirming placement of the resonance at C-2. Finally, the N-18 ring closure and the bromine atom as required by the molecular formula were assigned to the C-2 position to complete the structure for 2-bromo discorhabdin D (110).
The ECD spectrum of (-)-110 was nearly identical to that of (-)-546-(2S,6R,8S)-discorhabdin D (11) (Figure 3.9), thereby establishing the absolute stereochemistry of (-)-110 as (2R,6S,8S).
During the extraction and purification procedure previously outlined in Scheme 2.2, which involved no trifluoroacetic acid addition in solvents and isolation of discorhabdin B (8) as a free base, it became apparent that 2-bromo discorhabdin D (110) was an isolation procedure artifact. Discorhabdin B (8) free base, shown to be pure by $^1$H NMR spectroscopy, when dissolved in methanol (0.05% TFA), converted to a mixture of 8 trifluoroacetate salt and 110 trifluoroacetate salt. The proposed mechanism for the formation of (+)-110 from (+)-discorhabdin B free base is shown in Scheme 3.18. Compound 110 displays an unusual structure in that $N$-18 lone electron pair nucleophilic attack on C-2 did not facilitate expulsion of bromine from the intermediate 110a.

![Scheme 3.18. Proposed mechanism of formation of (-)-110 from (+)-discorhabdin B free base.](image)

Standing at room temperature in an acidic (0.05% TFA) solution of methanol (-)-110 was found to decompose into a single product. The decomposition product was characterized with a full set of NMR spectral data presented in Table 3.10. Low resolution ESIMS of the decomposition product showed a molecular ion at $m/z$ 444/446, which at 31 mass units difference from 110 suggested the new compound is a methanol adduct of 2-bromo discorhabdin D. Comparison of the $^1$H, $^{13}$C and 2D NMR data for the methanol adduct with that of (-)-110 revealed a common structure from C-1 to C-6 and from C-10 to C-21, 149a. Since the only two unassigned positions on the new discorhabdin molecule were C-7 and C-8, this was the most likely site for the methanol addition to occur. The three unidentified fragments were two methine proton resonances ($\delta_H$ 4.75, br s) and ($\delta_H$ 5.11, d, $J = 1.7$ Hz) on $sp^3$ hybridised carbons ($\delta_C$ 47.5) and ($\delta_C$ 80.5) respectively, and a methoxy resonance at ($\delta_H$ 3.32) and ($\delta_C$ 56.4). Crucial $^1$H-$^{13}$C HMBC correlations suggested the methoxy fragment F-3 is connected to F-2 as the HF-3 methyl proton resonance ($\delta_H$ 3.32) showed a strong correlation to the CF-2 carbon resonance ($\delta_C$ 80.5), and the HF-2 methine
proton resonance ($\delta_H 5.11$) also strongly correlated to the methoxy carbon resonance CF-3 ($\delta_C 56.4$).

![149a](image)

<table>
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<th>no.</th>
<th>$^{13}$C $\delta$</th>
<th>$^1$H $\delta$ [m, J (Hz)]</th>
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<th>HMBC</th>
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<td>C-2, C-4, C-5, C-6, C-20</td>
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<tr>
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<td>H-1A</td>
<td>C-2, C-3, C-5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>80.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>174.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>113.3</td>
<td>6.15 (s)</td>
<td>C-1, C-2, C-3, C-6</td>
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</tr>
<tr>
<td>5</td>
<td>177.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>48.3</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>F-1</td>
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<td>4.75 (br s)</td>
<td>HF-2</td>
<td>C-1, C-6, C-20</td>
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<tr>
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<td>5.11 (d, 1.7)</td>
<td>HF-1, NH-9</td>
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</tr>
<tr>
<td>10</td>
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<td>7.35 (s)</td>
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<td>C-12, C-15, C-21</td>
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<td>C-15, C-17</td>
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<td>4.24 (m)</td>
<td>H-16, H-17B</td>
<td>C-15, C-16, C-19</td>
</tr>
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<td></td>
<td>H-16, H-17A</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>151.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
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</tr>
<tr>
<td>21</td>
<td>121.5</td>
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</tr>
<tr>
<td>F-3</td>
<td>56.4</td>
<td>3.32 (s)</td>
<td>CF-2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.10. NMR data for the decomposition product of (-)-2-bromo discorhabdin D (110) TFA salt in DMSO-$d_6$.

Two possible structures that support the formula suggested by the molecular ion and NMR data recorded for the methanol adduct were proposed, **149c** and **149d**.

![149c](image)

![149d](image)
The methanol adduct 149c is consistent with the structure of a known compound, discorhabdin M (22) isolated from a New Zealand-sourced *Latrunculia* sp. sponge, where the methoxy substituent was placed at C-7.\(^6\) However the proposed structure 149c with F-1 placed on position C-8 and F-2 on C-7, was not in good agreement with the experimental \(^1\)H-\(^{13}\)C HMBC data, as shown in Figure 3.10. Correlations from HF-1 were observed to positions C-1 and C-6 on the *spiro*-ring and C-20. Furthermore, the HF-2 resonance showed a strong correlation to C-10. Thus, the 2D NMR data suggested the F-1 resonance had to be on C-7 and the F-2 on C-8 positions of the discorhabdin molecule as in 149d.

![Figure 3.10](image)

**Figure 3.10.** Crucial \(^1\)H-\(^{13}\)C HMBC correlations for fragments F-1, F-2 and F-3 on the proposed structures of 149c and 149d.

In the proposed structure 149d the placement of the three fragments was in good agreement with the experimental 2D data (Figure 3.10). Since the F-1 fragment had to be an sp\(^3\) hybridised methine, the thioether ring closure was placed on C-7. However, this conformationally restricted *spiro*-thiethane ring moiety had no precedence in the natural products literature, and consequently the proposed structure 149d was also discarded. The structure of 2-bromo discorhabdin D methanol adduct remains unknown. Two different fragments 149a and 149b were identified. Stored dry in the freezer over several months the compound decomposed into an insoluble dark brown solid and no further work on structure elucidation was undertaken.

(-)-2-Bromo discorhabdin D (110) was found to be strongly cytotoxic against the murine leukemia cell line with an IC\(_{50}\) of 0.14 \(\mu\)M, comparable to that of discorhabdin B. The methanol adduct was not tested.
3.2.4.3 1-Discorhabdyl Discorhabdin D (111)

(-)-1-Discorhabdyl discorhabdin D (111) di-trifluoroacetate salt was isolated as dark green oil from the Wellington-sourced \textit{L. (Biannulata) wellingtonesis} sponge. High resolution ESIMS of compound 111 showed a pseudomolecular ion \([\text{M+H}]^+\) at \(m/z\) 747/749, with the doubly protonated ion \([\text{M+2H}]^{2+}\) also present at \(m/z\) 374/375. The molecular formula established for 111, \(\text{C}_{36}\text{H}_{24}\text{BrN}_{6}\text{O}_{4}\text{S}_{2}\), suggested the compound was a pseudo-dimer. The \(^1\text{H}\) NMR spectrum of 111 in CD\(_3\)OD showed 16 resonances consistent with 1-substituted-discorhabdin D (11)-type and discorhabdin W (37)-type monomeric units (Figure 3.11).

\textbf{Figure 3.11.} \(^1\text{H}\) NMR spectrum of 111 TFA salt in CD\(_3\)OD showing 1-substituted-discorhabdin D (11)-type in blue and discorhabdin W (37)-type in red consistent resonances.
The $^1$H NMR spectrum showed that in the discorhabdin D-type subunit, the thioether moiety was still present, thus the dimeric connection could not be established through a disulfide bond as in discorhabdins W (37) and 16a,17a-dehydro W (109). Instead, the two monomeric units were connected through a C-1 on a discorhabdin D-type through to 5S on a discorhabdin W-type bond to give the structure of 1-discorhabdyl discorhabdin D (111) (Table 3.11). The proposed C-1-S-C-26 connection was confirmed via a $^1$H–$^{13}$C HMBC experiment with a correlation from H-1 ($\delta_\text{H}$ 4.73, d, $J$ = 3.0 Hz) to C-26 ($\delta_\text{C}$ 163.5), as shown in Figure 3.12.

![Figure 3.12. $^1$H–$^{13}$C HMBC spectrum of 1-discorhabdyl discorhabdin D (111) TFA salt showing a crucial correlation from H-1 to C-26 to establish connectivity of the two monomeric units.](image-url)
Table 3.11. NMR data for (-)-1-discorhabdyl discorhabdin D (111) TFA salt in CD$_3$OD.

The ECD spectrum of Wellington-sourced (-)-1-discorhabdyl discorhabdin D (111) was consistent in both sign and magnitude with that of Wellington-sourced (-)$_{578}$-(1$R$,2$S$,6$R$,8$S$)-discorhabdin L (17) (Figure 3.13), thereby establishing the absolute stereochemistry of the C-2, C-6 and C-8 stereocenters on the discorhabdin D-type part of
the molecule in (-)-111 as (2R,6R,8S). The stereochemistry at the C-1 stereocentre was unknown as previously, in section 2.2.2.4, it was shown that the ECD spectrum was not effective in determining the stereochemistry of C-1 substituted discorhabdin D derivatives. Since the ECD spectrum of (-)-111 did not show any similarities to that of discorhabdins U (29) or W (37), the stereochemistry of the discorhabdin W-type part of the molecule could also not be deduced.

![Figure 3.13. Experimental ECD spectra of the TFA salt forms of (-)-111](image)

Figure 3.13. Experimental ECD spectra of the TFA salt forms of (-)-111 in blue and the Wellington-sourced (-)-1-discorhabdyl discorhabdin D (111) in red.

An attempt to determine the stereochemistry at C-1 via a NOESY 2D NMR experiment was unsuccessful. The H-1 ($\delta_H$ 4.73) resonance did not show any correlations to H-7A ($\delta_H$ 2.89) or H-7B ($\delta_H$ 2.72) (Table 3.12). Correlations were observed from H-1 to H-2 ($\delta_H$ 4.45) and to H-25 ($\delta_H$ 6.66) on the discorhabdin W-type part of the molecule. However, the Chem3D program was not able to calculate the thermodynamic properties of this large molecule, and none of the Chem3D models of four possible diastereomers (1R,27S), (1S,27S), (1S,27R) and (1R,27R) of the (2R,6R,8S) discorhabdin D-type core of 111 could successfully be energy minimized. Thus, the stereochemistry at C-1 and C-27 stereocenters in (-)-1-discorhabdyl discorhabdin D remained unidentified.
Table 3.12. 2D NOESY NMR data for (-)-1-discorhabdyl discorhabdin D (111) TFA salt in CD$_3$OD.

<table>
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<td>4.45 (d, 3.0)</td>
<td>H-1, H-17A, H-25</td>
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<td>4</td>
<td>6.15 (s)</td>
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</tr>
<tr>
<td>7A</td>
<td>2.89 (under impurity)</td>
<td>H-7B, H-8</td>
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<tr>
<td>7B</td>
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<td>H-7A, H-8</td>
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</tr>
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<td>H-35</td>
</tr>
<tr>
<td>38</td>
<td>3.90 (m)</td>
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</table>

Doubtful Sound-sourced collection of *Latrunculia* spp. yielded a small amount (0.5 mg) of (+)-1-discorhabdyl discorhabdin D with identical $^1$H NMR spectra and equal (with an exception of the magnitude of the Cotton effect at 365 nm) and opposite ECD spectra to that of (-)-111 (Figure 3.14). Thus Doubtful Sound-sourced (+)-111 had to be enantiomeric with respect to the discorhabdin D-type part of the molecule to the Wellington-sourced (-)-111. 2D NOESY data for the (+)-111 enantiomer were not recorded, and the absolute stereochemistry of C-1 and C-27 stereocenters in (+)-111 were unknown.
Figure 3.14. Experimental ECD spectra of the TFA salt forms of the Wellington-sourced (-)-111 in blue, and the Doubtful Sound-sourced (+)-111 in red.

During the course of the SAR study on discorhabdin B (8), 1-discorhabdyl discorhabdin D was found to be an artifact. Freshly extracted free base (+)-discorhabdin B (8), shown to be pure by $^1$H NMR, kept dry in the freezer for a period of three weeks converted into a mixture of decomposition products as indicated by analytical HPLC. The major decomposition product was identified as 1-discorhabdyl discorhabdin D (111). (+)-Discorhabdin B-sourced semi-synthetic 111 was identical in all aspects including NMR spectral data, optical rotation values and ECD spectra to that recorded for the L. (Biaannulata) wellingtonesis-sourced (--)-111. Proposed mechanism of formation of (--)-111 from Wellington-sourced (+)-(6S,8S)-discorhabdin B (8) is outlined in Scheme 3.19. In the proposed mechanism the initial step involves the imine lone electron pair of one discorhabdin B molecule attacking the acidic C-7 hydrogen on another discorhabdin B molecule to give the thioether opened derivative 8a and the protonated imine derivative.
8b. In the following step, the thioether 8a adds in a Michael-type manner onto the spiroidienone C-1 position of 8b to give the enolate 111a. The enolate then abstracts a proton from the nearby protonated imine to give 111b. Finally, the lone pair of electrons on the iminoquinone attack the electrophilic centre at C-2 and the resulting expulsion of bromine gives 1-discorhabdyl discorhabdin D (111).

Scheme 3.19. Proposed mechanism for the formation of the semi-synthetic (-)-111 from (+)-discorhabdin B (8).

Because semi-synthetic (-)-111 was identical in all aspects to the sponge-sourced (-)-111 this new discorhabdin analogue is considered to be an isolation procedure artifact. Discorhabdin B free base is present in the sponge tissue and the semi-synthetic conversion of (+)-8 to (-)-111 was just as likely to have occurred inside the frozen sponge as it did in
a sealed glass vial. Since this study did not work on freshly collected material, all of the (-)-111 and (+)-111 collected are considered to be semi-synthetic isolation procedure products. An attempt was made to improve the yield of the reaction by heating discorhabdin B free base in DMF, refluxing in acetone and heating in DMF with an excess of K₂CO₃. In all of the attempted alternative reactions, the major products as indicated by analytical HPLC were discorhabdins W (37) and Q (25). Currently the only method of accessing (-)-111 in yields of 10-15% is storing pure, dry, free base form of discorhabdin B in the freezer for a period of a couple of weeks.

Through semi-synthesis it was shown that both of the monomeric units in (-)-1-discorhabdyl-discorhabdin D molecule were sourced from (+)-discorhabdin B of known (6S,8S) stereochemistry. The absolute configuration of the spirodienone moiety in the semi-synthetic product is therefore identical to discorhabdin B, giving Wellington (+)-discorhabdin B-sourced (2R,6R,8S,27S) (-)-111 and Doubtful Sound (-)-discorhabdin B-sourced (2S,6S,8R,27R) (+)-111. The stereochemistry at C-1 in both enantiomers of 1-discorhabdyl discorhabdin D remains unknown.

(-)-1-Discorhabdyl discorhabdin D (111) was found to be moderately cytotoxic against the murine leukemia cell line with an IC₅₀ of 2.85 μM, many orders of magnitude less so than discorhabdin B at 0.08 μM, but comparable for that of the other reported C-1 functionalized discorhabdin D-type analogues.
3.2.4.3 1-Epi-Discorhabdin L (150)

Semi-synthetic discorhabdin L (17) was prepared from naturally-occurring Wellington-sourced (+)-discorhabdin B (8). Compound 8 was kept sealed at room temperature in a solution of dry acetone and an excess of K$_2$CO$_3$ for three days, at which time analytical HPLC indicated complete consumption of starting material. The crude reaction mixture was loaded on a C$_8$ column in MilliQ H$_2$O (0.05% TFA) from which two products were recovered, semi-synthetic discorhabdin W (37) and semi-synthetic discorhabdin L (17) (Scheme 3.20).

Scheme 3.20. Semi-synthetic route towards discorhabdin L (17).

Semi-synthetic discorhabdin L (17) was fully characterized as a trifluoroacetate salt. A comparison of the $^1$H and $^{13}$C NMR spectra of the semi-synthetic and Wellington-sourced naturally-occurring discorhabdin L samples revealed major differences between the two, centered mainly on the proton and carbon resonances at C-1 and C-2 (Table 3.13).
Table 3.13. \textsuperscript{1}H and \textsuperscript{13}C NMR chemical shifts for the TFA salt forms of naturally-occurring and semi-synthetic discorhabdin L (17) in DMSO-\textit{d}_6.

Interpretation of 2D NMR data for the semi-synthetic product was consistent with the structure of discorhabdin L (17). With major differences centered on the \textit{spiro}-ring and C-1 and C-2 \textsuperscript{1}H and \textsuperscript{13}C NMR resonances, the structure of the (+)-discorhabdin B (8)-sourced semi-synthetic discorhabdin L was thus proposed to be the epimer of the Wellington-sourced naturally-occurring compound at C-1, (1\textit{S},2\textit{S},6\textit{R},8\textit{S}) 150.
A proposed mechanism of formation of \(150\) from Wellington-sourced \((+)-(6S,8S)\) discorhabdin B \((8)\) is outlined in Scheme 3.21.

\[
(+)-(6S,8S)-8 \xrightarrow{\text{base}} 8a \xrightarrow{\text{H}_2\text{O}(0.05\% \text{TFA})} 150a \Rightarrow (-)-(6S,6'S)-37
\]

\[
150a \xrightarrow{\text{H}_2\text{O}(0.05\% \text{TFA})} (1S,2S,6R,8S)-150 \Rightarrow 150b
\]

**Scheme 3.21.** Proposed mechanism of formation of \(150\) from \((+)-\text{discorhabdin B (8).}\)

In the proposed semi-synthetic mechanism for the formation of \(150\), the initial step involves base mediated thioether bridge opening. Under the set experimental conditions a significant amount of the thiol intermediate \(8a\) was expected to form the disulfide structure discorhabdin W \((37)\). However at room temperature in a limited oxygen environment this reaction was previously in section 3.2.2 in deuterated acetone and an excess of \(\text{K}_2\text{CO}_3\) shown to be slow, requiring 7 days for approximately 80% completion. Upon the addition of water (0.05% TFA) to an alkaline solution of discorhabdin B, nucleophilic attack was observed on the C-1 position. The resulting enolate water adduct
150a abstracted a proton from the neighboring iminoquinone to form an enone 150b. Finally, the lone electron pair on the N-18 imine attacked the C-2 position on the spiro-ring and with the expulsion of bromine the structure for 150 was complete. Since compounds (+)-578-17 and 150 are epimers, in the semi-synthetic mechanism the water nucleophile attacks C-1 from the opposite side to that in the biosynthesis of the naturally-occurring discorhabdin L. Currently it is unclear why the semi-synthetic attack occurs from the opposite face of the molecule and yields a single (1S,2S,6R,8S) product. Interestingly, naturally-occurring (1R) discorhabdin L was found to be unstable in base, while the semi-synthetic (1S) epimer was unstable as a trifluoroacetate salt and decomposed over several weeks. The stability of the two discorhabdin L C-1 epimers, rather than selectivity of the water nucleophilic attack may explain for the recovery of a single product.

Since the semi-synthetic discorhabdin L was found unstable, no chiroptical of bioactivity data for 150 were recorded.
3.3 Biogenesis of the Discorhabdin Alkaloids

The following section reviews the current opinion on the biogenesis of the discorhabdin alkaloids, and based upon the semi-synthetic derivatives of discorhabdin B and analogues presented earlier, proposes biosynthetic routes to the new natural products and enantiomeric pairs.

3.3.1 Introduction

Biosynthesis of the pyrroloiminoquinone group of alkaloids as proposed by Blunt, Munro and co-workers proceeds via amino acids tryptophan and phenylalanine precursors.\(^{36,79}\) Starting from tryptamine through a series of oxidations outlined in Scheme 3.22, pyrroloiminoquinone 151 and pyrroloorthoquinone 152 core molecules are generated. Compounds 151 and 152 were hypothesised to be crucial precursors to all damirone, batzelline, isobatzelline, tsitsikammamine, makaluvamine and discorhabdin-type alkaloids.

**Scheme 3.22.** Biosynthetic proposal for the generation of the core molecules 151 and 152, adapted from Lill et al.\(^{79}\) and Urban et al.\(^{36}\)
Discorhabdin biogenesis was proposed to follow from a Michael-type addition of a phenylalanine-derived tyramine-like unit onto the core structure 151 to give a makaluvamine D-type structure 153, outlined in Scheme 3.22.79

Scheme 3.23. Precursors in the biosynthesis of the makaluvamine D-type structure 153.

The mechanism for the crucial oxidation step in the biosynthesis from a makaluvamine to a discorhabdin-type molecule was proposed by Marshall Aubart and Heathcock to go through intramolecular Michael-type phenolic coupling, shown as pathway A in Scheme 3.24.112 Pathway A represents the current biogenic proposal for all of the dithiadiscorhabdin and makaluvamine alkaloids. Thioether-bridged discorhabdins were proposed to originate from either a makaluvamine F-type precursor (pathway B) or sulphur insertion on a discorhabdin C-type molecule (pathway C).25,36 The mechanism of formation of discorhabdin D-type analogues as shown by Marshall Aubart and Heathcock is thought to occur through a reduction of a discorhabdin B-type molecule, protonation of the resulting enamine and displacement of the C-2 bromide by the lone pair of electrons on the iminoquinone (pathway D).112
Tyramine involvement in the biogenesis of the discorhabdins has been verified in a single biosynthetic study that examined $[^{14}C]$-labelled L-phenylalanine incorporation into discorhabdin B (8). Using transplants of New Zealand-sourced sponges that were known to produce discorhabdin B as a major metabolite in high yield, Blunt, Munro and co-workers incubated live sponge slices with the radiolabelled phenylalanine precursor for 24 hours, purified discorhabdin B and then quantified the radiochemical incorporation into the metabolite. The study confirmed $[^{14}C]$-labelled L-phenylalanine incorporation into discorhabdin B at levels ranging from 0.18 to 0.32% compared to that of <0.02% of the controls. Furthermore, the authors examined microbial involvement in discorhabdin B biosynthesis by incubating the sponges with and without the presence of antibiotics. The antibiotic treatment experiment yielded $[^{14}C]$-labelled discorhabdin B, strongly suggesting that discorhabdin biosynthesis is a property of the sponge cells rather than that of an endobacterial symbiont.
3.3.2 Proposed Central Role of Discorhabdin B in the Biosynthesis of a Number of Naturally-Occurring Analogues

Work on discorhabdin B (8) presented in section 3.1 has identified semi-synthetic routes to known, naturally-occurring analogues, discorhabdins Q (25), U (29), W (37) and an epimer of discorhabdin L (150) (Scheme 3.25). In addition, through semi-synthetic discorhabdin U (29) another known natural product discorhabdin T (28) was accessed.

Through a single compound, discorhabdin B (8), four major classes of sulphur containing analogues: 1-substituted discorhabdin D-type, discorhabdin Q-type, discorhabdin U-type and dimer discorhabdin W-type have been generated. A new biosynthetic tree is proposed
which highlights the role of discorhabdin B in the biosynthesis of a number of other naturally-occurring compounds (Scheme 3.26).

3.3.4 Biosynthesis of New Natural Products

3.3.4.1 3-Dihydro Discorhabdin A (107)

The new natural product, 3-dihydro discorhabdin A (107) is clearly a C-3 reduced analogue of the enone structure of discorhabdin A (7). In order to experimentally establish a biosynthetic link from discorhabdin A to compound 107, a C-3 ketone reduction was attempted on Wellington-sourced (+)-7. Previously Copp et al. have reported a successful reduction of discorhabdin C (6) to the dienol 34 in good yield,32 and in this work, attempts at reducing discorhabdins B (8) and W (37) with sodium borohydride in methanol under nitrogen recovered starting material, while discorhabdin G*/I (15) under identical conditions decomposed. Reduction of the Wellington-sourced (+)-(5R,6S,8S)-discorhabdin A (7) resulted in a single product in high yield (Scheme 3.27). The new semi-synthetic derivative was fully characterized as a trifluoroacetate salt (Table 3.14).

Scheme 3.27. Semi-synthetic route towards 3-dihydro discorhabdin A (107).
Table 3.14. NMR data for the semi-synthetic 3-dihydro discorhabdin A TFA salt in DMSO-$d_6$.

<table>
<thead>
<tr>
<th>no.</th>
<th>$^{13}$C δ</th>
<th>$^1$H δ [m, J (Hz)]</th>
<th>COSY</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>130.0</td>
<td>6.21 (s)</td>
<td>H-3, H-3OH</td>
<td>C-2, C-3, C-5, C-6, C-7, C-20</td>
</tr>
<tr>
<td>2</td>
<td>133.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>66.8</td>
<td>4.49 (br s)</td>
<td>H-1, H-4A, H-4B</td>
<td></td>
</tr>
<tr>
<td>3-OH</td>
<td>5.56 (d, 7.8)</td>
<td></td>
<td>H-1, H-3</td>
<td>C-2, C-3, C-4</td>
</tr>
<tr>
<td>4A ($\beta$)</td>
<td>40.5</td>
<td>2.11 (m)</td>
<td>H-3, H-4B, H-5</td>
<td>C-2, C-3, C-5, C-6</td>
</tr>
<tr>
<td>4B ($\alpha$)</td>
<td>1.96 (m)</td>
<td></td>
<td>H-3, H-4A, H-5</td>
<td>C-2, C-3, C-5, C-6</td>
</tr>
<tr>
<td>5</td>
<td>53.5</td>
<td>4.10 (dd, 12.8, 4.8)</td>
<td>H4A, H-4B</td>
<td>C-3, C-4, C-7, C-8, C-20</td>
</tr>
<tr>
<td>6</td>
<td>49.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7A ($\beta$)</td>
<td>41.4</td>
<td>2.56 (dd, 12.1, 2.9)</td>
<td>H-7B, H-8</td>
<td>C-5, C-6, C-8, C-20</td>
</tr>
<tr>
<td>7B ($\alpha$)</td>
<td>2.36 (d, 12.1)</td>
<td></td>
<td>H-7A, H-8</td>
<td>C-1, C-5, C-6, C-8, C-20</td>
</tr>
<tr>
<td>8</td>
<td>58.5</td>
<td>5.20 (d, 2.6)</td>
<td>H-7A, H-7B, NH-9</td>
<td>C-5, C-6, C-7, C-10</td>
</tr>
<tr>
<td>N-9</td>
<td>10.36 (br s)</td>
<td></td>
<td>H-8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>150.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>165.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>123.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-13</td>
<td>13.14 (br s)</td>
<td></td>
<td>H-14</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>127.2</td>
<td>7.36 (s)</td>
<td>NH-13</td>
<td>C-12, C-15, C-21</td>
</tr>
<tr>
<td>15</td>
<td>119.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>18.1</td>
<td>2.50 (m)</td>
<td>H-17A, H-17B</td>
<td>C-15</td>
</tr>
<tr>
<td>17A</td>
<td>44.4</td>
<td>3.89 (m)</td>
<td>H-16, H-17B, NH-18</td>
<td>C-15, C-19</td>
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<tr>
<td>17B</td>
<td>3.76 (td, 12.4, 7.2)</td>
<td></td>
<td>H-16, H-17A</td>
<td>C-15, C-16, C-19</td>
</tr>
<tr>
<td>N-18</td>
<td>8.50 (br s)</td>
<td></td>
<td>H-17A</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>153.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>103.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>123.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2D NMR data for the reduction product of (+)-discorhabdin A (7) were consistent with the structure proposed for 3-dihydro discorhabdin A (107). However a comparison of the $^1$H and $^{13}$C NMR shifts for the Wellington-sourced naturally-occurring (Table 2.3) and the semi-synthetic (Table 3.14) 3-dihydro discorhabdin A, revealed major differences centered mainly on the resonances of the spiro-ring.

Naturally-occurring (107) and semi-synthetic 3-dihydro discorhabdin A (154) exhibited different optical rotation values, at $\left[\alpha\right]_D +40$ and $+200$ deg cm$^3$ g$^{-1}$ dm$^{-1}$; $\left[\alpha\right]_{578} -160$ and -40 deg cm$^3$ g$^{-1}$ dm$^{-1}$ and $\left[\alpha\right]_{546} -480$ and -400 deg cm$^3$ g$^{-1}$ dm$^{-1}$ for 107 and 154 respectively. Experimental ECD spectra for (-)$_{578}$-107 and (+)-154 were also different, with the semi-synthetic 154 lacking a negative Cotton effect at 255 nm (Figure 3.15). Based on the difference observed in the NMR spectra, optical rotation values and ECD spectra of the two compounds, semi-synthetic 154 was proposed to be the C-3 epimer of the naturally-occurring (-)$_{578}$-(3R,5R,6S,8S)-3-dihydro discorhabdin A (107).
Figure 3.15. Experimental ECD spectra of the TFA salt forms of the Wellington-sourced naturally-occurring \((-\)3\(R\),5\(R\),6\(S\),8\(S\))-3-dihydro discorhabdin A (107) in blue and the (+)-discorhabdin A-sourced semi-synthetic (+)-3-dihydro discorhabdin A (154) in red.

Table 3.15 presents \(^1\)H NMR data and NOESY correlations for the naturally-occurring 107 and the semi-synthetic product 154.

<table>
<thead>
<tr>
<th>Naturally-occurring</th>
<th>Semi-synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>no.</strong></td>
<td>(^1)H δ [m, J (Hz)]</td>
</tr>
<tr>
<td>1</td>
<td>6.42 (s)</td>
</tr>
<tr>
<td>3</td>
<td>4.16 (br s)</td>
</tr>
<tr>
<td>3-OH</td>
<td>2.11 (td, 13.6, 3.0)</td>
</tr>
<tr>
<td>4A (β)</td>
<td>1.90 (ddd, 13.6, 4.8, 3.1)</td>
</tr>
<tr>
<td>4B (α)</td>
<td>4.28 (dd, 13.2, 4.9)</td>
</tr>
<tr>
<td>5</td>
<td>2.50 (under solvent)</td>
</tr>
<tr>
<td>7A (β)</td>
<td>2.40 (d, 12.0)</td>
</tr>
<tr>
<td>7B (α)</td>
<td>5.21 (d, 2.1)</td>
</tr>
<tr>
<td>8</td>
<td>10.51 (br s)</td>
</tr>
<tr>
<td>N-9</td>
<td>13.18 (br s)</td>
</tr>
<tr>
<td>N-13</td>
<td>7.37 (s)</td>
</tr>
<tr>
<td>N-18</td>
<td>7.94 (br s)</td>
</tr>
</tbody>
</table>

Table 3.15. Comparison of the \(^1\)H and NOESY NMR data for the TFA salt forms of naturally-occurring 107 on the left, and semi-synthetic 154 on the right in DMSO-\(d_6\).

The stereochemistry at the C-3 stereocentre for the naturally-occurring 107 was established as 3\(R\) based on the observed NOE correlations from H-3 to H-1 and both of H-
4A and H-4B which was in agreement with the Chem3D model (Figure 2.31). In the case of 154 a NOESY correlation was only observed between H-3 and H-4A consistent with a 3S configuration, thus the absolute stereochemistry of the (+)-discorhabdin A-sourced semi-synthetic 154 was concluded to be (3S,5R,6S,8S). The two epimers were equally potent against the P388 murine leukaemia cell line with IC50 values of 1.83 μM and 2.06 μM for 107 and 154 respectively.

The logical biosynthetic precursor of the naturally-occurring (-)578-(3R,5R,6S,8S)-3-dihydro discorhabdin A (107) is (+)-(5R,6S,8S)-discorhabdin A. In the biosynthesis, hydride delivery occurs at the lower Si face of the molecule to give the 3R product, whereas the semi-synthetic sodium borohydride attack occurs at the opposite Re face to give the 3S epimer 154.

3.3.4.2 1-Substituted Discorhabdin G*/I Analogues - Discorhadinfs K (21) and 1-Thiomethyl G*/I (108)

Three new C-1 substituted discorhabdin G*/I analogues were isolated from New Zealand-sourced Latrunculia spp. sponges, (+)-(6S,8S,7'S*)-discorhabdin K (21), (-)-(6R,8R,7'S*)-discorhabdin K (21) and (+)-(6S,8S)-1-thiomethyl discorhabdin G*/I (108). Proposed biosynthesis of the 1-substituted discorhabdin G*/I-type compounds (Scheme 3.28) starts from a nucleophilic addition of an appropriate thiol onto the C-1 position in a
discorhabdin B-type molecule, followed by the resulting enamine proton abstraction and $N$-18-C-2 ring closure to give a 1-substituted discorhabdin D-type compound 155. From 155, H-1 proton abstraction and consequent $N$-18-C-2 ring opening completes the structure of a 1-substituted discorhabdin G*/I analogue.

According to the pathway outlined in Scheme 3.28, discorhabdin H (16) is the biosynthetic precursor of discorhabdin K (21). Both the (+) and (-)-16 and (+) and (-)-21 diastereomers were isolated from New Zealand-sourced sponges in this work. In order to experimentally confirm the proposed biosynthetic origin of discorhabdin K, an attempt was made to react Wellington-sourced (-)-16 in dry acetone and an excess of K$_2$CO$_3$. However, compound 16, as well as all other 1-substituted discorhabdin D-type analogues, was unstable in an excess of base and only a mixture of insoluble brown decomposition products were recovered.

Scheme 3.28. Biosynthetic proposal for the formation of 1-substituted discorhabdin G*/I analogues.
3.3.4.3 16a,17a-Dehydro Discorhabdin W (109)

(-)$_{578}$-(6S,6aS)-109 and the antipode (+)$_{578}$-(6R,6aR)-109 were isolated from New Zealand-sourced *Latrunculia* spp. sponges. Synthesis of discorhabdin W (37) was experimentally shown to go through thioether bridge opening on discorhabdin B (8) and subsequent disulfide bond formation to give the dimer structure of 37 (Scheme 2.4). The biosynthesis of the new natural product 109 is hypothesized to follow the same mechanism with discorhabdin B (8) and Q (25) thioether opened monomeric subunits joining through a disulfide bond (Scheme 3.29).%

**Scheme 3.29.** Biosynthetic proposal for the formation of 16a,17a-dehydro discorhabdin W (109).

Alternatively, compound 109 could be generated from a C-16,17 oxidation on a single monomeric unit in the structure of discorhabdin W (37).
3.3.3 Biosynthesis of Enantiomeric Discorhabdins

This study identified antipodal pairs of discorhabdins B (8), G*/I (15) L (17), W (37) and 16a,17a-dehydro W (109) as well as diastereomeric pairs of discorhabdins H (16) and K (21). The proposed biosynthetic precursor of the discorhabdin alkaloids, makaluvamine F (69) was also isolated from several of the collections. In the original report on the isolation and structure elucidation of 69, the metabolite was found to be chiral ([α]D of -475.8 deg cm⁻³ g⁻¹ dm⁻¹) but no attempt was made to determine the stereochemistry at the C-10 stereogenic centre.⁵⁷ New Zealand-sourced makaluvamine F, isolated from sponges collected at Three Kings Islands (MNP 6117), Tutukaka (97 TUT 2-10) and Kaikoura (97 KK 1-17), was found in all three instances to be racemic ([α]D = 0, [α]L = 0, [α]S = 0, ECD = 0). In the Three Kings Island-sourced specimen compound 69 was the only metabolite isolated, however in the Tutukaka and Kaikoura-sourced collections racemic 69 was found together with chiral discorhabdins G*/I (15) and L (17).

It is unclear as to why New Zealand-sourced sponges produce racemic makaluvamine F (69), while the South Pacific/Australian collection yielded the chiral compound. An attempt at oxidation of rac-69 towards discorhabdin B with copper-(II)-chloride¹¹² was made but only starting material was recovered in the reaction. In the publication on total synthesis of discorhabdin/prianosin A (7), Kita and co-workers have reported failed oxidation attempts on a makaluvamine F-type molecule with both CuCl₂ and the hypervalent iodine reagent PIFA.¹¹³ The authors proposed the reactivity of the molecule and strain of the sulphur-cross linked system is hindering the cyclization reaction and developed an alternative approach where the thioether moiety in discorhabdin A was introduced in the last step of the synthesis (Scheme 3.8).

In an effort to explain the occurrence of racemic 69 as a natural product in New Zealand-sourced Latrunculia spp. sponges, the reverse reaction was attempted. Semi-synthetic
makaluvamine F (69) has been prepared via TCEP mediated reduction of (+)-discorhabdin B (8) (Scheme 3.30). Chiral Wellington-sourced (+)-(6S,8S)-discorhabdin B stirring at room temperature overnight in an aqueous solution of three molar equivalents of TCEP yielded a racemic mixture of makaluvamine F ([α]D = 0, [α]578 = 0, [α]546 = 0, ECD = 0).

Since all of the sponge material used in this work was kept in the freezer for a number of years prior to extraction, it is possible that the isolated makaluvamine F is an artefact of discorhabdin B rather than a natural product. However, the recovery of racemic biosynthetic precursor from a reduction of a chiral discorhabdin B has provided evidence for a new biosynthetic proposal for the generation of chiral thioether-bridged discorhabdin-type molecules.

Assuming that discorhabdin B and other thioether-bridged analogues can be generated either via sulphur insertion on a discorhabdin E-type substrate or by intramolecular phenolic coupling of a makaluvamine F-type precursor, four possible scenarios, outlined in Table 3.16 are presented. Stereoselective sulphur insertion on chiral discorhabdin E would yield a chiral discorhabdin B molecule. Although this proposal is viable, naturally-occurring New Zealand-sourced discorhabdin E (12) was found to be racemic. A stereoselective sulphur insertion on rac-discorhabdin E outlined as pathway B in Table 3.16, would yield chiral discorhabdin B as well as the unreacted other enantiomer of discorhabdin E, which yet again is not supported by an observation of the naturally-occurring rac-12. As an alternative, makaluvamine F (69) can also be a possible precursor to chiral discorhabdin B. Starting from chiral makaluvamine F, non-selective intramolecular ring closure can occur but will give only one stable chiral discorhabdin B product, outlined as pathway C. This route is viable and has evidence in the literature as
chiral makaluvamine F (69) was isolated together with chiral discorhabdin A (7) from a Fijian collection of the sponge *Z. fuliginosa*. However makaluvamine F samples isolated from three different collections of New Zealand-sourced sponges in this study were found to be racemic. A stereoselective closure on *rac-69* substrate would yield chiral discorhabdin B and the unreacted other makaluvamine F enantiomer shown as pathway D in Table 3.16.
Table 3.16. Possible reaction pathways for the biosynthesis of chiral discorhabdin B (8).

In section 3.2 it was shown that the C-7 methylene pair on the discorhabdin B (8) molecule were acidic and upon exposure to base, mediated the thioether bridge opening. Since a TCEP reduction of chiral discorhabdin B yielded racemic makaluvamine F it is
likely that the electron withdrawing effects of the bromophenol ring in 69 have an effect in making the C-11 methylene pair acidic and opening the thioether moiety in a similar manner to that observed for discorhabdin B. Therefore given that the C-10-S-C13 thioether functionality in makaluvamine F is susceptible to ring opening, in the biosynthesis of discorhabdin B the chirality of the discorhabdin molecule would be solely dependant on the phenolic coupling step towards the spiro-ring formation rather than the chirality, if any, contained in the makaluvamine F thioether bridge moiety. Since discorhabdins B (8), H (16), K (21) and W (37) were shown to be enantiopure this crucial oxidative coupling reaction is likely to be an enzyme-mediated process rather than a non-specific cyclization reaction. Chapter two of this thesis presented the occurrence of enantiomeric discorhabdins in New Zealand-sourced Latrunculia spp. sponges. The compounds were found to be enantiopure and to have consistent stereochemistry within a collection. Thus, the biosynthesis of enantiomeric discorhabdin alkaloids must go through two antipodal pathways.

The isolation of enantiopure antipodal pairs of marine natural products is uncommon however several examples exist in the literature. Recently Tsukamoto and co-workers reported the isolation of (-)-stephacidin A (156) and (+)-notoamide B (157) from a marine-derived fungus Aspergillus sp.,93 while the antipodes of the two compounds (+)-156 and (-)-157 were isolated by Williams and co-workers from a terrestrial fungus Aspergillus versicolor.114 Enantiopurity of the metabolites was confirmed by chiral HPLC and the absolute stereochemistry assigned upon comparison of ECD spectra with that of other known analogues in the series with known absolute stereochemistry.114
Williams and co-workers proposed that biosynthesis of the enantiomeric stephacidin A and notoamide B proceeds via two enantioselective enzymes to give two stereochemically parallel pathways. The authors are currently examining a biomimetic total synthesis of the series.

New Zealand-sourced *Latrunculia* spp. sponges appear to have the biosynthetic machinery to generate both antipodal pairs of the thioether-containing discorhabdin alkaloids. Specific stereochemical orientation of the *spiro*-ring is consistent within a location as all three sponge specimens worked on from Doubtful Sound area contained (-)-discorhabdin B (8) and the >10 Wellington-sourced replicate samples consistently yielded (+)-8. The enantiomeric pairs of discorhambdins B (8), G*/I (15), L (17) and W (37) exhibited nearly identical potency against the P388 cell line indicating that the observed activity was independent of the stereochemistry of the alkaloids. Assuming that cytotoxicity against a single mammalian tumour cell line can be equated to biological activity in general, for equipotent (+) and (-) end-products the selective pressure on the evolution of two different stereospecific enzymes is expected to be low, yet all of the replicates within the same collection produced discorhabdins with identical stereochemistry. Miller *et al.* have reported the New Zealand-sourced *Latrunculia* spp. sponges to be genetically distinct between populations, but found a large number of clones within a population. The source-specific distribution of the chiral discorhabdin alkaloids shown in Figure 2.39, may therefore represent a limited number of replicates and a large number of clones that this work potentially dealt with rather then differential selective pressures between geographically isolated populations evolving for two different stereospecific pathways. Ecological roles of enantiomeric discorhabdins have not been examined, and may in future provide insight into stereochemical differentiation of the discorhabdin alkaloids.
3.4 Biological Activity of the Discorhabdin Alkaloids

Discorhabdin alkaloids have been reported to exhibit a wide range of anti-proliferative \textit{in vitro} activities against multiple mammalian cancer cell lines and Gram-positive and -negative bacteria. In addition, the compounds are known to induce Ca$^{2+}$ release from sarcoplasmic reticulum$^{23}$ and inhibit the activity of calcineurin and caspase CPP32 enzymes involved in the immune system regulation.$^{41}$ However little is known about the mechanism of action of the specific group. Related pyrroloiminoquinone and pyrroloamino\textit{ortho}quinone analogues makaluvamines A (64) and C-F (66-69) were shown to inhibit the enzyme topoisomerase II, intercalate into DNA and, in the reduced form, cause single-stranded DNA cleavage.$^{57}$ In the same assay discorhabdin A (7) was not a topoisomerase II inhibitor, did not significantly intercalate into the double helix of DNA but did show a small measure of single-stranded DNA breakage, causing a 50% cleavage of plasmid DNA at a concentration of 33 $\mu$M. Reduction potentials of makaluvamines A (64), C-F (66-69) and discorhabdin A (7) were all found to be in biologically relevant ranges. Ireland and co-workers proposed that cytotoxicity of the makaluvamines was a factor of several mechanisms of action including DNA intercalation and generation of a semiquinone radical.$^{57}$ Recently Dijoux \textit{et al.} established that makaluvamine alkaloids have a high affinity for DNA binding but found that intercalation efficiency did not correlate with DNA damage potency, confirming that multiple mechanisms are responsible for the observed cytotoxicity of the group.$^{53}$ No mechanism of action has ever been proposed for the discorhabdin alkaloids.

A comparison of IC$_{50}$ values for the murine leukaemia P388 cell line assay for different discorhabdin B-type, U-type, G*/I-type, D-type and C-type compounds used in this study (Table 3.17) has revealed that compounds with an C-2 bromo-enone moiety such as 6, 7, 8, 29, 110 and 142 display the most potent anti-proliferative activity, while the discorhabdin Q (25) and discorhabdin D-type analogues 11, 17 and 111 are significantly less active. Reduction at C-3 as seen in 34 and 107 also reduces cytotoxic potency.
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-type</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.10</td>
<td>0.10</td>
<td>0.11a</td>
<td>1.83</td>
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<table>
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<tr>
<th></th>
<th>(+)-U (29)</th>
<th>(+)-N-13-deMe U (141)</th>
<th>(-)-DiBz U (142)</th>
<th>(-)-W (37)</th>
<th>(-)578-16a-17a dehydro W (109)</th>
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<tbody>
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<td><strong>U-type</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.15</td>
<td>1.30</td>
<td>0.13</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>(+)-G*/I (15)</th>
<th>(+)-1S-Me G* (101)</th>
<th>(+)-K (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>G</em>/I-type</em>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.65</td>
<td>0.28</td>
<td>&gt;8.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(-)-546-D (11)</th>
<th>(-)578-L (17)</th>
<th>(+)-2-Br D (110)</th>
<th>(-)-1-discorhabdyl D (111)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D-type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.9b</td>
<td>1.08</td>
<td>0.14</td>
<td>2.85</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C (6)</th>
<th>P (24)</th>
<th>3-OH C (34)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C-type</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.11</td>
<td>0.31</td>
<td>0.91c</td>
</tr>
</tbody>
</table>

Table 3.17. P388 *In vitro* biological activities of a range of naturally-occurring and semi-synthetic discorhabdin analogues. Values are presented as μM.

* value taken from Perry *et al.*

* value taken from Perry *et al.*

* value taken from Copp *et al.*

Two different hypotheses for the possible mechanism of action of the discorhabdins were considered in this work. Firstly, the importance of substitution of the *spiro*-ring was examined and used a sand dollar embryo development model to identify the structural characteristics that correlate to the highest observed cytotoxic effect. The second hypothesis attempted to relate P388 cytotoxicity to one-electron reduction potentials of the iminoquinone moiety in discorhabdin B-type, U-type, D-type, C-type and Q-type molecules.
3.4.1 Importance of the Spiro-Ring Moiety Exemplified by the Sand Dollar *Fellaster zelandiae* Embryo Development Assay

The echinoderm embryo development assay is a useful tool for obtaining anti-proliferative information in a short time frame and at a relatively low cost. The model, widely used for effluent toxicity testing\textsuperscript{115,116} has also found use in forward chemical genomics of small molecules. Numerous marine natural products have been reported to induce developmental effects in echinoderm embryos. A member of the pyrroloorthoquinone series, zyzzyanone A (88) was shown to inhibit cellular division of fertilised sea urchin *Strongylocentrotus nudus* eggs at a concentration of 25 μg/mL.\textsuperscript{66}

There are no reports on the effect of natural products on the embryonic development of any of the local echinoderm species. In this work, an attempt was made to develop an in-house procedure for echinoderm embryo development assay using the common intertidal sand dollar *Fellaster zelandiae* (Gray, 1855). Assay conditions and methodology used are outlined in Appendix I.

Discorhabdin alkaloids were shown to inhibit *F. zelandiae* embryonic development in a concentration dependent manner. Preliminary studies noted the most dramatic effects were observed within the first seven hours of division, before the onset of tissue differentiation, and consequently the assay was terminated at this time. Figure 3.16 shows the control embryo at a blastomere stage (A) and three of the developmental abnormalities commonly recorded: inhibition of first cleavage (B), embryo at an irregular fifth cleavage (C) and an embryo at an abnormal blastula stage with no signs of regular differentiation (D). The assay response of a range of discorhabdin molecules are shown in Table 3.18.

![Figure 3.16. *F. zelandiae* developmental abnormalities montage A) control (unhatched) blastula , B) zygote, C) 16-celled blastomere and D) abnormal (unhatched) blastula.](image-url)
### Table 3.18. *F. zelandiae* assay response to various concentrations of discorhabdins C (6), A (7), B (8), D (11), G*/I (15), L (17) and U (29).

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>10</th>
<th>5</th>
<th>2.5</th>
<th>1</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discorhabdin C (6)</td>
<td>zygote</td>
<td>&lt;4 cell blastomere</td>
<td>&lt;256 cell blastomere</td>
<td>abnormal blastulae</td>
<td>abnormal blastulae</td>
</tr>
<tr>
<td>(+)-Discorhabdin A (7)</td>
<td>&lt;256 cell blastomere</td>
<td>&gt;256 cell blastomere</td>
<td>abnormal blastulae</td>
<td>abnormal blastulae</td>
<td>normal blastulae</td>
</tr>
<tr>
<td>(+)-Discorhabdin B (8)</td>
<td>zygote</td>
<td>&lt;4 cell blastomere</td>
<td>&lt;4 cell blastomere</td>
<td>&lt;256 cell blastomere</td>
<td>abnormal blastulae</td>
</tr>
<tr>
<td>(-)5α-Discorhabdin D (11)</td>
<td>abnormal blastulae</td>
<td>abnormal blastulae</td>
<td>normal blastulae</td>
<td>normal blastulae</td>
<td>normal blastulae</td>
</tr>
<tr>
<td>(+)-Discorhabdin G*/I (15)</td>
<td>&gt;256 cell blastomere</td>
<td>abnormal blastulae</td>
<td>abnormal blastulae</td>
<td>abnormal blastulae</td>
<td>normal blastulae</td>
</tr>
<tr>
<td>(-)5β-Discorhabdin L (17)</td>
<td>abnormal blastulae</td>
<td>abnormal blastulae</td>
<td>normal blastulae</td>
<td>normal blastulae</td>
<td>normal blastulae</td>
</tr>
<tr>
<td>(+)-Discorhabdin U (29)</td>
<td>&lt;4 cell blastomere</td>
<td>&lt;256 cell blastomere</td>
<td>&gt;256 cell blastomere</td>
<td>abnormal blastulae</td>
<td>abnormal blastulae</td>
</tr>
</tbody>
</table>

Structural characteristics that correlated to the greatest degree of *F. zelandiae* developmental inhibition were the presence of a spiro-dienone moiety and a C-2 bromine substituent. At a concentration of 10 μg/mL discorhabdins C (6), B (8) and U (29) showed the greatest inhibitory effect with compounds 6 and 8 completely inhibiting first cleavage in all embryos and 29 inhibiting second and third cleavage. Discorhabdins A (7) and G*/I (15) at the highest run concentration of 10 μg/mL inhibited development at 256 cell stage or the eight cleavage. Discorhabdins D (11) and L (15) were least active as at 10 μg/mL the embryos developed up to an abnormal blastula stage. At 2.5 μg/mL compounds 11 and 15 did not cause any developmental abnormalities, whereas discorhabdin B (8) at the same concentration completely inhibited the third cleavage. These findings were consistent with the results of the P388 anti-proliferative assay presented in Table 3.17 where the N-18-C-2 ring closure was shown to drastically reduce cytotoxicity of the discorhabdin alkaloids. In contrast to the P388 assay, sand dollar development inhibition model also showed that there were differences in the substitution pattern of the spiro-ring that correlated with an anti-proliferative effect. Compounds 6, 8 and 29 with a C-2 bromo substituent and a di-enone moiety were most active, while debromo 15 and the enone 7 were shown to be less active (Figure 3.17).
Figure 3.17. Comparison of the structural features on the spiro-ring moiety that correlated with the greatest anti-proliferative effect in the sand dollar embryo development assay.

The proposed mechanism of action responsible for the observed variation in the cytotoxic effect involves a Michael-type nucleophilic attack on the spiro-ring C-1 position in a discorhabdin molecule. Compounds with an α, β unsaturated carbonyl moiety necessary for the Michael-type addition to take place showed the greatest cytotoxic potency. In addition, C-2 bromine substituent was also recognized as an important element for the observed cytotoxicity.

This chapter has already shown semi-synthetic routes from discorhabdin B (8) towards two C-1 substituted discorhabdin D-type analogues, 1-discorhabdyl discorhabdin D (111) and 1-epi discorhabdin L (150) utilising a Michael-type nucleophilic attack on C-1 followed by a N-18-C-2 ring closure and loss of bromine. The proposed mechanism for the nucleophilic addition of the cellular target in the biological mechanism of action shown in Scheme 3.31 is similar to the one stated for the formation of C-1 substituted discorhabdin D analogues 110 and 111 from 8. A nucleophilic site on the target structure undergoes a Michael-type addition onto C-1 of the discorhabdin B-type substrate to give the enolate 158. The enolate then abstracts a proton, possibly from the iminoquinone moiety on the discorhabdin molecule to give a 1-substituted 1,2-dihydro discorhabdin B-like structure 159. Since there are currently no examples of this type of molecule in the literature, and the relative stability of the proposed structure is unknown, a further...
N-18-C-2 ring closure with the expulsion of bromine is proposed, to give the final structure of a 1-substituted discorhabdin D-type derivative 160.

**Scheme 3.31.** Proposal for the biological mechanism of action of the discorhabdin alkaloids exemplified by discorhabdin B (8).

The identity of a possible nucleophilic biological target that can substitute at C-1 is unknown. Romagnoli et al. proposed that compounds with an α-bromoacryloyl moiety, present in discorhabdins A (7) and G (14), can act as thiol trapping agents.\(^{117}\) Expanding this argument on to the entire series, all of the discorhabdin B-type, C-type, G*/I-type, U-type and W-type compounds can react with intracellular reactive nucleophilic species in this manner.

As part of a structure-activity relationship study on the DNA minor groove binding agent distamycin A (161) Cozzi and co-workers have identified an α-bromoacrylamido derivative brostallicin (162) that was two orders of magnitude more potent against the L-1210 murine leukaemia cell line then 161.\(^ {118} \)
Surprisingly, in standard _in vitro_ assays, **162** did not alkylate DNA and the compounds anti-tumour potency was markedly improved in the presence of reduced glutathione species (GSH).\textsuperscript{119} Beria and co-workers proposed a mechanism of action for **162** where in the first step a reactive thiol species such as GSH adds on to the β-olefinic carbon in a Michael-type manner to give the intermediate **162a** (Scheme 3.32).\textsuperscript{120} With an activated second electrophilic centre, **162a** would then be able to further covalently bind to DNA. In _in vivo_ models brostallicin was shown to be a DNA minor groove binding agent and is currently in Phase II clinical trials for soft-tissue sarcoma in Europe and the United States.\textsuperscript{120}

**Scheme 3.32.** Proposed mechanism of action for brostallicin (**162**), adapted from Cozzi _et al._\textsuperscript{118} and Beria _et al._\textsuperscript{120}

For the discorhabdin alkaloids, the C-1 nucleophilic attack is likely to be only a part of a scaffold of mechanisms of action responsible for the activity of the group, as compounds such as 1-substituted discorhabdin G*/I and 1-substituted discorhabdin D-type derivatives still retain a measure of cytotoxic potency. The enhanced P388 activity and anti-developmental effect observed for the Michael acceptor discorhabdin-type molecules may be due to the ability of the compounds to react with intracellular nucleophilic species such as GSH or thiol groups of proteins, but certainly does not appear to be a primary mechanism responsible for the observed activity of the discorhabdin alkaloids.
3.4.2 One-Electron Reduction Potentials

Three electrochemical studies on the pyrroloiminoquinone alkaloids have been reported. Makaluvamines A (64), C-F (66-69), makaluvone (80), damirone B (45) and discorhabdin A (7) were shown to have reduction potentials in the biologically relevant ranges of -285 to -130 mV by Radisky et al. In the same study, the fully aromatised analogue makaluvamine B (65) could not be reduced under the experimental conditions. Recently Dijoux et al. reported the half wave reduction potentials and a reversibility measure parameter (δE_reduction) for makaluvamines A (64) and C-F (66-69), H (71), I (72), L (75), N (77) and V (81). All of the reduction potentials reported were in the biologically relevant ranges and the one-electron reductions were found to be reversible. Current proposal for the mechanism of action of the makaluvamine-type alkaloids involves semiquinone radical generation as well as DNA intercalation ability.

Antunes et al. have reported one-electron reduction potentials (E_{1/2}) for a range of fourteen pyrroloquinoline, pyrroloaminoorthoquinone, discorhabdin and tsitsikammamine-type alkaloids. In the discorhabdin series, structural features that correlated with a low E_{1/2} included the presence of a C-14 bromine substituent, C-5-C-8 thioether bridge, Δ4,5 olefin moiety and an additional ring between N-18 and C-2. Reduction of the C-3 ketone functionality was related to a slight increase of the reduction potential. Bis-pyrroloiminoquinones tsitsikammamines A - D (83-86) were more difficult to reduce, with the E_{1/2} values ranging from -395 to -669 mV. The N-oxime compounds 85 and 86 had the lowest recorded E_{1/2} values.

In this work one-electron reduction potentials of five discorhabdin analogues were determined at pH 7.0 by establishing redox equilibria between three mixtures of the one-electron reduced compounds and the reference compounds 2,3,5-trimethylbenzoquinone (E(TMBQ/TMBQ^-) = -165 mV), duroquinone (E(DQ/DQ^-) = -260 ± 7 mV) and benzyl viologen (E(BV2+/BV+) = -380 ± 10 mV). Reduction potentials shown are the work of Associate Professor Robert F. Anderson and Dr. Sujata S. Shinde. Results are presented with the permission of the two collaborators.
Five different compounds, discorhabdins C (6), B (8), L (17), Q (25) and U (29), representing main structural types of the discorhabdin analogues were chosen. The aim of the study was to attempt to correlate reduction potentials with the observed P388 cytotoxicity values, mainly the apparent significant drop in the bioactivity of discorhabdin Q (25) compared to the other analogues (Table 3.17). It was hypothesized that an additional element of conjugation of the discorhabdin Q-type structure would donate electrons into the system and effectively make compound 25 harder to reduce. One-electron reduction potentials and equilibrium constants for discorhabdins C (6), B (8), L (17), Q (25) and U (29) at pH 7.0 are presented in Table 3.19.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Redox indicator</th>
<th>K</th>
<th>ΔE/mV</th>
<th>$E^{\text{A/A}^-}/$mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discorhabdin C (6)</td>
<td>DQ</td>
<td>2.2 ± 0.7</td>
<td>20 ± 7</td>
<td>-243 ± 10</td>
</tr>
<tr>
<td></td>
<td>BV$^{2+}$</td>
<td>246 ± 13</td>
<td>141 ± 2</td>
<td>-232 ± 10</td>
</tr>
<tr>
<td>Discorhabdin B (8)</td>
<td>TMBQ</td>
<td>3.6 ± 0.4</td>
<td>33 ± 2</td>
<td>-136 ± 8</td>
</tr>
<tr>
<td>Discorhabdin L (17)</td>
<td>TMBQ</td>
<td>15.2 ± 1.7</td>
<td>70 ± 3</td>
<td>-98.8 ± 8</td>
</tr>
<tr>
<td>Discorhabdin Q (25)</td>
<td>DQ</td>
<td>2.5 ± 0.4</td>
<td>24 ± 3</td>
<td>-236 ± 8</td>
</tr>
<tr>
<td>Discorhabdin U (29)</td>
<td>TMBQ</td>
<td>0.7 ± 0.04</td>
<td>10 ± 1</td>
<td>-171 ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.97 ± 0.18*</td>
<td>17 ± 3*</td>
<td>-148 ± 8*</td>
</tr>
</tbody>
</table>

Table 3.19. One-electron reduction potentials and equilibrium constants for discorhabdin analogues. * denotes the absence of a phosphate buffer.

One-electron reduction potentials of discorhabdins C (6), B (8), L (17), Q (25) and U (29) were all shown to be in the biologically relevant ranges. Once inside a biological system, the iminoquinone moiety can therefore undergo one-electron reduction via oxidative enzymes such as NADPH cytochrome P-450 reductase and generate a semiquinone reactive intermediate. The proposed one-electron reduction of discorhabdin B (8) and the resulting semiquinone intermediate 163 are shown in Scheme 3.33. The semiquinone 163 can further abstract a proton to form the radical 164 which can disproportionate to generate aminophenol 165 and the di-radical 166. The resulting radical complex can then take part in redox cycling with oxygen to generate superoxide or other reactive oxygen species and consequently disrupt cellular electron transport or cause DNA damage.
Scheme 3.33. Formation of radical species 163, 164, 166 and the aminophenol 165 from discorhabdin B (8).

A plot of $E_7$ against P388 IC$_{50}$ values (Figure 3.18) shows a bimodal distribution of data. The reduction potential range from -170 to -120 mV that correlated to the greatest cytotoxic potency is centred on discorhabdins B (8) and U (29). Unexpectedly, discorhabdin C (6) with a P388 IC$_{50}$ value of 0.11 μM had a nearly identical $E_7$ value (-238 ± 20 mV) to that of discorhabdin Q (25) (-236 ± 8 mV) with a P388 IC$_{50}$ value of 3.0 μM. Clearly compound 25 could be reduced under the experimental conditions and the observed loss of cytotoxic potency against a P388 cell line of the Δ16,17 discorhabdin series was not due to the inability of the compounds to accept an electron.
Figure 3.18. Plot of reduction potentials of discorhabdins C (6), B (8), L (17), Q (25) and U (29) versus P388 IC_{50} values.
3.4.3 Anti-Parasitic Activity of the Discorhabdin Alkaloids

Discorhabdins C (6), (+)-B (8), (+)-Q (25), (+)-U (29) and (-)-1-discorhabdyl D (111) were evaluated for growth inhibition of *Plasmodium falciparum*, *Leishmania donovani*, *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi* and for cytotoxicity towards the mammalian L-6 cell line at the Swiss Tropical Institute. Compounds 6, 8 and 29 showed strong activity against all four parasitic strains while 25 and 111 were selective for *T. b. rhodesiense*; and *T. b. rhodesiense* and *P. falciparum* respectively. Table 3.20 presents IC₅₀ values for (+)-discorhabdin Q (25) and (-)-1-discorhabdyl discorhabdin D (111) against *T. b. rhodesiense* and *P. falciparum*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>L-6 IC₅₀ (μg/mL)</th>
<th>T. b. rhodesiense IC₅₀ (μg/mL)</th>
<th>P. falciparum IC₅₀ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-25</td>
<td>4.6</td>
<td>0.52</td>
<td>nd</td>
</tr>
<tr>
<td>(-)-111</td>
<td>40.3</td>
<td>0.32</td>
<td>0.078</td>
</tr>
</tbody>
</table>

**Drug standards**

- Melarsoprol 0.004
- Chloroquine 0.074

*Table 3.20. Anti-parasitic activity of compounds (+)-25 and (-)-111.*

(-)-1-Discorhabdyl discorhabdin D (111) exhibited high selectivity for *P. falciparum* and greatly reduced toxicity towards a mammalian skeletal muscle cell line L-6. At an IC₅₀ of 0.078 μg/mL, (-)-111 was shown to be equally potent to one of the current antimalarial drugs chloroquine (167), at 0.074 μg/mL. Due to it’s high therapeutic index, compound (-)-111 has been selected for further study and is currently awaiting delivery of 30 mg for in vivo testing.
3.4.4 Summary

Two different structural features implicated with the mechanisms of action of the discorhabdin alkaloids were identified in this work, namely the 2-bromo spiro-enone ring and the iminoquinone moiety (Figure 3.19).

There was no clear pattern of structure-activity relationship that could be assigned to a single proposed mechanism. While Michael-type acceptors discorhabdin B-type, C-type, and U-type compounds displayed the most dramatic sand dollar embryo anti-developmental effect and the greatest cytotoxic potency against the murine leukaemia P388 cell line, there was a significant amount of activity associated with discorhabdin D-type and 1-substituted G*/I-type molecules that do not have the electrophilic C-1 centre and would not react by this mechanism. An electrochemical investigation has shown that one-electron reduction potentials of discorhabdins C (6), B (8), L (17), Q (25) and U (29) show a bimodal distribution when compared against P388 cytotoxicity data, with discorhabdins B (8) and U (29) showing the optimal reduction potential range. Clearly both of the possible mechanisms of action identified in this study, a Michael-type addition on C-1 and the generation of a semiquinone radical are likely to be only a subset of the many pathways responsible for the bioactivity of the discorhabdin alkaloids.

Figure 3.19. Structural features on a discorhabdin B (8) molecule implicated in the proposed mechanisms of action of the series.
Finding of potent anti-malarial activity associated with (-)-1-discorhabdyl discorhabdin D (111) shows a promising lead in the future of the discorhabdin alkaloids. In a series of fifteen naturally-occurring and nine semi-synthetic discorhabdins from New Zealand Latrunculia spp. sponges presented in this thesis, at an IC₅₀ of 2.85 μM, compound (-)-111 was reported to have one of the least active cytotoxic values against the murine leukaemia P388 cell line. The anti-parasitic assay clearly demonstrated the limitations of equating drug potential of a compound with cytotoxic potency against a single cell line.

Since their first discovery over thirty years ago, many discorhabdins have shown biological activity in the nano-molar ranges but their high toxicity towards somatic cells and lack of selectivity have hampered the progress towards drug development of the group. In this work, a lead towards the discovery of new of anti-malarial agents was identified. Identification of less cytotoxic but more selective analogues opens new possibilities for future work on the discorhabdin project.
4. EXPERIMENTAL
4.1 General Experimental Procedures

Optical rotations were recorded on a Perkin Elmer 341 Polarimeter using a 0.1 dm cell in the solvent indicated. Absolute values were calculated according to: $[\alpha]_D = (100 \times a)/(c \times 1)$, where $a =$ observed absorbance, $c =$ concentration in g/100 mL and $l =$ path length in dm. Infrared spectra were recorded using a Perkin-Elmer spectrum One Fourier-Transform IR spectrometer as a dry film. Absorption maxima are expressed as wavenumbers (cm$^{-1}$).

Ultraviolet-visible spectra were run as methanol solutions on a UV-2102 PC Shimadzu UV-Vis scanning spectrophotometer. ECD spectra were recorded on an Applied Photophysics Pi star spectrophotometer. NMR spectra were recorded on either a Bruker Avance DRX-600 spectrometer operating at 600 MHz for $^1$H nuclei and 150 MHz for $^{13}$C nuclei, a Bruker Avance DRX-400 spectrometer operating at 400 MHz for $^1$H nuclei and 100 MHz for $^{13}$C nuclei or a Bruker Avance DRX-300 spectrometer operating at 300 MHz for $^1$H nuclei and 75 MHz for $^{13}$C nuclei. Residual solvent signals were used as reference (DMSO-$d_6$: $\delta_H$ 2.50, $\delta_C$ 39.43; CD$_3$OD: $\delta_H$ 3.30, $\delta_C$ 49.05; acetone-$d_6$, $\delta_H$ 2.05, $\delta_C$ 206.18; CDCl$_3$: $\delta_H$ 7.25, $\delta_C$ 77.0). $^1$H NMR data is reported as position ($\delta$), relative intergral, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), coupling constant ($J$, Hz), and the assignment of the atom. $^{13}$C NMR data is reported as position ($\delta$) and assignment of the atom. High resolution mass spectra were recorded on either a VG-7070 or a Thermo LQT-FT mass spectrometer.

Analytical reversed-phase HPLC was run on a Waters 600 HPLC photodiode array system using either an Alltech C$_{18}$ or C$_8$ column (3 mm Econosphere Rocket, 7 x 33 mm) and eluting with a linear gradient of H$_2$O (0.05% TFA) to MeCN over 13.5 min at 2 mL/min. Reversed-phase flash column chromatography was carried out on C$_{18}$, C$_8$, and CN LiChroprep stationary support with a pore size 40-63 $\mu$m. Gel filtration flash chromatography was carried out using Sephadex LH-20 (Pharmacia).

Samples were freeze-dried in an Edwards Micro Modulo freeze drier. Reactions were heated by immersion in oil while the temperature was taken from a thermometer touching the bottom of the bath. All solvents used were distilled analytical grade of better. Chemical reagents used were purchased from standard chemical suppliers.
P388 cytotoxicity results were reported as concentration of a compound required to reduce in vitro P388 D1 murine leukemia cell population by 50%. Antimicrobial activity was reported as zone of microbial inhibition against the Gram-negative bacterium *Escherichia coli* and Gram-positive bacterium *Bacillus subtilis*. The sample was applied to a 6 mm paper disc and incubated for 18 hrs at 35 ºC. Zones measured are excess radii in mm. Cytotoxicity against the NCI 60-cell line human tumor panel was reported as a mean GI₅₀ (50% growth inhibition) value in μM. *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum* and L-6 in vitro assay results were reported as concentration of a compound required to reduce the cell population by 50%.

### 4.2 Work on Chapter Two

#### 4.2.1 Wellington-Sourced *L. (Biannulata) wellingtonesis* Metabolite Extraction and Isolation Procedures

#### 4.2.1.1 Barret’s Reef (BR) Collection

Metabolite extraction and isolation has previously been outlined in section 2.1.1.

(+)-(5R,6S,8S)-Discorhabdin A (7)

![Discorhabdin A](image)

TFA salt purple-brown oil; [α]D = +310 (c 0.05, MeOH); UV (MeOH) λ (ε) 202 (14100), 248 (21810), 349 (8170), 399 (6800), 566 (1070) nm; ECD (MeOH) λmax (Δε) 235 (0), 244 (+2.4), 253 (0), 265 (-5.6), 306 (0), 340 (+5.8) nm; ¹H NMR (DMSO-d₆, 300 MHz) δ 13.18 (1H, br s, NH-13), 10.36 (1H, d, J = 3.7 Hz, NH-9), 9.42 (1H, br s, NH-18), 7.47 (1H, s, H-1), 7.38 (1H, d, J = 3.7 Hz, H-14), 5.33 (1H, br s, H-8), 4.58 (1H, dd, J = 12.0,
6.5 Hz, H-5), 3.70 (under solvent peak, H-17), 2.94 (5H, m, H-4/7A/16), 2.50 (under solvent peak, H-7B); HRFABMS m/z [M+H]+ 416.00761 (calcd for C_{18}H_{15}^{79}BrN_{3}SO_{2}, 416.00683), 418.00465 (calcd for C_{18}H_{15}^{81}BrN_{3}SO_{2}, 418.00479).

(+)-Discorhabdin A (7) TFA salt (3.1 mg, 5.9 μmol) was dissolved in dry acetone (2 mL) to which K₂CO₃ (2 mg) was added. Solution was stirred under N₂ for 10 min, during which the color changed from green to bright orange. The solvent was removed in vacuo and the product recovered by an ethyl acetate/water partition, yielding discorhabdin A (7) as a free base (0.7 mg, 1.7 μmol).

Free base orange solid; [α]D = +200, [α]_{578} = 0, [α]_{546} = -320 (c 0.05, MeOH); UV (MeOH) λ (ε) 203 (18660), 227 (16210), 248 (17340), 331 (10330); ECD (MeOH) λ_{max} (Δε) 234 (0), 246 (+3.8), 253 (0), 262 (-5.1), 288 (0), 319 (+5.6), 366 (0), 379 (-1.4), 412 (0); ¹H NMR (CDCl₃, 400 MHz) δ 8.82 (1H, br s, NH-13), 7.64 (1H, s, H-11), 6.85 (1H, br s, H-14), 5.90 (1H, br s, NH-9), 5.30 (1H, br s, H-8), 4.69 (1H, dd, J = 12.0, 7.6 Hz, H-5), 4.24 (1H, m, H-17A), 3.96 (1H, m, H-17B), 2.92 – 2.68 (6H, m).

(--)-(6S,8S)-Discorhabdin B (8)

TFA salt purple-brown oil; [α]D = +320, [α]_{578} = +200, [α]_{546} = -90 (c 0.05, MeOH); UV (MeOH) λₜₜ (ε) 246 (20650), 306 (8110), 358 (7290), 400 (6640), 562 (840) nm; ECD (MeOH) λ (Δε) 209 (-16.7), 225 (0), 234 (+3.5), 240 (0), 256 (-18.1), 281 (-3.8), 302 (-8.7), 320 (0), 350 (+13.6), 429 (0) nm; ¹H NMR (CD₃OD-d₄, 400 MHz) δ 7.88 (1H, s, H-1), 7.23 (1H, s, H-14), 6.27 (1H, s, H-4), 5.73 (1H, d, J = 4.0 Hz, H-8), 3.96 (1H, m, H-17A), 3.84 (1H, m, H-17B), 2.93 (2H, m, H-16), 2.82 (1H, d, J = 11.6 Hz, H-7A), 2.57 (1H, dd, J = 11.6, 4.0 Hz, H-7B); ¹³C NMR (CD₃OD-d₄, 100 MHz) δ 175.8 (C-3), 172.9 (C-5), 166.2 (C-11), 156.9 (C-19), 153.1 (C-10), 147.8 (C-1), 129.7 (C-2), 127.8 (C-14),
125.5 (C-12), 124.3 (C-21), 121.9 (C-15), 119.6 (C-4), 98.5 (C-20), 62.6 (C-8), 53.0 (C-6), 46.3 (C-17), 43.7 (C-7), 19.2 (C-16); $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 13.28 (1H, br s, NH-13), 10.65 (1H, br s, NH-9), 8.62 (1H, br s, H-18), 7.74 (1H, s, H-1), 7.35 (1H, br s, H-14), 6.26 (1H, br s, H-4), 5.73 (1H, d, $J$ = 3.1 Hz, H-8), 3.85 (1H, m, H-17A), 3.68 (1H, m, H-17B), 2.79 (2H, m, H-16), 2.70 (1H, d, $J$ = 11.6 Hz, H-7A), 2.42 (1H, d, $J$ = 11.7, 3.7 Hz, H-7B); HRFABMS m/z [M+H]$^+$ 413.99074 (calcd for C$_{18}$H$_{13}$BrN$_3$SO$_2$, 413.99118), 415.98971 (calcd for C$_{18}$H$_{13}$BrN$_3$SO$_2$, 415.98914).

$(-)_{546}$-(2$S$,6$R$,8$S$)-Discorhabdin D (11)

TFA salt green oil, $[\alpha]_D = 0$, $[\alpha]_{578} = 0$, $[\alpha]_{546} = -58$ (c 0.05, MeOH); UV (MeOH) $\lambda_{max}$ 203 (16800), 249 (17340), 283 (11220), 321 (6840), 397 (6540), 584 (780) nm; ECD (MeOH) $\lambda_{max} (\Delta \varepsilon)$ 256 (-14.8), 281 (-0.3), 303 (-6.5), 325 (0), 360 (+11.5), 420 (0) nm; $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 13.16 (1H, br s, NH-13), 10.71 (1H, br s, NH-9), 7.28 (1H, br s, H-14), 6.13 (1H, s, H-4), 5.67 (1H, d, $J$ = 2.4 Hz H-8), 4.37 (1H, br s, H-2), 4.04 (1H, m, H-17A), 3.83 (1H, m, H-17B), 3.03 (2H, m, H-16), 2.91 (1H, dd, $J$ = 13.4, 2.3 Hz, H-1A), 2.70 (1H, dd, $J$ = 12.1, 3.4 Hz, H-7A), 2.56 (1H, d, $J$ = 10.0 Hz, H-7B) 2.44 (1H, dd, $J$ = 13.5, 3 Hz, H-1B); HRFABMS m/z [M+H]$^+$ 336.08051 (calcd for C$_{18}$H$_{14}$N$_3$SO$_2$, 336.08067).
(+)-(6S,8S)-Discorhabdin G*/I (15)

TFA salt brown oil; $[\alpha]_D = +540$, $[\alpha]_{578} = +450$, $[\alpha]_{546} = -520$ (c 0.05, MeOH); UV (MeOH) $\lambda_{max} (\epsilon)$ 229 (19620), 295 (9150), 355 (6330), 405 (5360), 577 (1030); ECD (MeOH) $\lambda (\Delta \varepsilon)$ 216 (0), 224 (+12.0), 231 (0), 251 (-35.9), 282 (-3.3), 301 (-8.7), 316 (0), 346 (+14.1), 410 (0) nm; $^1$H NMR (CD$_3$OD-$d_4$, 600 MHz) $\delta$ 7.43 (1H, d, $J = 9.9$ Hz, H-1), 7.19 (1H, s, H-14), 6.66 (1H, dd, $J = 9.9$, 1.5 Hz, H-2), 6.15 (1H, d, $J = 1.3$ Hz, H-4), 5.67 (1H, dd, $J = 3.7$, 0.9 Hz, H-8), 3.94 (1H, m, H-17A), 3.82 (1H, m, H-17B), 2.88 (2H, m, H-16), 2.81 (1H, d, $J = 11.8$ Hz, H-7A), 2.46 (dd, $J = 11.9$, 3.9 Hz, H-7B); $^{13}$C NMR (CD$_3$OD-$d_4$, 150 MHz) $\delta$ 183.6 (C-3), 173.3 (C-5), 166.3 (C-11), 157.0 (C-19), 153.2 (C-10), 147.7 (C-1), 134.0 (C-2), 127.8 (C-14), 125.5 (C-12), 124.3 (C-21), 121.9 (C-15), 120.6 (C-4), 98.4 (C-20), 62.0 (C-8), 50.5 (C-6), 45.9 (C-17), 43.6 (C-7), 19.2 (C-16); $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 13.30 (1H, br s, NH-13), 10.70 (1H, br s, NH-9), 8.45 (1H, br s, NH-18), 7.37 (1H, s, H-14), 7.31 (1H, d, $J = 9.9$ Hz, H-1), 6.53 (1H, dd, $J = 9.9$, 1.4 Hz, H-2), 6.17 (1H, d, $J = 1.3$ Hz, H-4), 5.69 (1H, d, $J = 3.1$ Hz, H-8), 3.89 (1H, m, H-17A), 3.74 (1H, m, H-17B), 2.78 (2H, m, H-16), 2.73 (1H, d, $J = 11.2$ Hz, H-7A), 2.32 (1H, dd, $J = 11.9$, 3.9 Hz, H-7B); HRFABMS $m/z$ [M+H]$^+$ 336.08012 (calcd for C$_{18}$H$_{14}$N$_3$SO$_2$, 336.08067).
(-)-(1R,2R,6R,8S,7'S*)-Discorhabdin H (16)

TFA salt green oil, [α]_D = -77, [α]_578 = -213, [α]_850 = -330 (c 0.05, MeOH); UV (MeOH) λ<sub>max</sub> 246 (20590), 288 (12140), 322 (8150), 397 (8330), 585 (790) nm; ECD (MeOH) λ (Δε) 212 (0), 220 (+11.0), 233 (0), 255 (-23.6), 283 (-2.9), 305 (-8.1), 333 (0), 366 (+14.9) 427 (0) nm; <sup>1</sup>H NMR (CD<sub>3</sub>OD-<sup>d4</sup>, 600 MHz) δ 8.14 (1H, s, H-2'), 7.09 (1H, s, H-14), 6.19 (1H, s, H-4), 5.67 (1H, dd, J = 3.5, 1.0 Hz, H-8), 4.42 (1H, d, J = 3.1 Hz, H-2), 4.18 (1H, dd, J = 9.4, 6.6 Hz, H-7'), 4.16 (1H, d, J = 3.2 Hz, H-1), 3.98 (1H, ddd, J = 14.2, 7.3, 2.8 Hz, H-17A), 3.87 (1H, m, H-17B), 3.79 (3H, s, H-9'), 3.39 (1H, m, H-6'A), 3.32 (1H, m, H-6'B), 3.24 (1H, dd, J = 12.2, 3.7 Hz, H-7A), 3.13 (1H, m, H-16A), 3.02 (1H, ddd, J = 16.3, 6.8, 2.7 Hz, H-16B), 2.82 (1H, d, J = 12.2 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD-<sup>d4</sup>, 150 MHz) δ 183.7 (C-3), 171.2 (C-5), 170.7 (C-8'), 167.2 (C-11), 150.4 (C-19), 148.4 (C-10), 141.0 (C-2'), 133.5 (C-4'), 127.4 (C-14), 127.2 (C-5'), 125.5 (C-12), 122.8 (C-21), 119.4 (C-15), 114.5 (C-4), 101.7 (C-20), 66.8 (C-2), 64.0 (C-8), 52.9 (C-17), 52.7 (C-7), 50.7 (C-1), 47.0 (C-6), 39.3 (C-7), 33.6 (C-9'), 26.0 (C-6'), 20.7 (C-16); <sup>1</sup>H NMR (DMSO-<sup>d6</sup>, 400 MHz) δ 13.22 (1H, br s, NH-13), 10.99 (1H, br s, NH-9), 8.58 (3H, br s, NH-10'), 8.11 (1H, s, H-2'), 7.29 (1H, s, H-14), 6.31 (1H, s, H-4), 5.75 (1H, d, J = 2.4 Hz, H-8), 4.43 (1H, d, J = 3.2 Hz, H-2), 4.02 (1H, d, J = 3.2 Hz, H-1), 3.98 (1H, m, H-7'), 3.76 (2H, m, H-17), 3.68 (3H, s, H-9'), 3.16 (2H, m, H-6'), 3.02 (3H, m, H-7A/16), 2.71 (1H, d, J = 11.6 Hz, H-7B); HRFABMS m/z [M+H]<sup>+</sup> 535.12218 (calcd for C<sub>25</sub>H<sub>23</sub>N<sub>6</sub>S<sub>2</sub>O<sub>4</sub>, 535.12222).
(-)\textsubscript{578}(1R,2S,6R,8S)-Discorhabdin L (17)

\[\text{[M+H]}^+ \text{ m/z } 352.07573 \text{ (calcd for C}_{18}\text{H}_{14}\text{N}_3\text{SO}_3, 352.07559).}\]
(-)-(1R,2S,6R,8S)-Discorhabdin N (18)

TFA salt dark green oil; [α]_D = -160, [α]_578 = -260, [α]_546 = -520 (c 0.05, MeOH); UV (MeOH) λ_{max} 203 (34030), 249 (20710), 285 (12730), 323 (9170), 399 (8480), 580 (1060) nm; ECD (MeOH) λ_{max} (Δε) 258 (-21.9), 284 (-5.4), 299 (-8.7), 321 (0), 360 (+20.0), 407 (0), 430 (-2.7), 467 (0) nm; \(^1\)H NMR (DMSO-d_6, 600 MHz) δ 13.18 (1H, br s, NH-13), 10.76 (1H, br s, NH-9), 7.28 (1H, s, H-14), 6.18 (1H, s, H-4), 5.68 (1H, d, J = 2.5 Hz, H-8), 4.34 (1H, d, J = 3.1 Hz, H-2), 4.01 (1H, ddd, J = 14.3, 7.1, 3.2 Hz, H-17A), 3.78 (1H, m, H-17B), 3.62 (1H, d, J = 3.2 Hz, H-1), 3.47 (1H, d, J = 18.0 Hz, H-23A), 3.40 (1H, d, J = 18.1 Hz, H-23B), 3.07 (1H, dd, J = 12.4, 3.7 Hz, H-7A), 3.03 (2H, m, H-16), 2.47 (1H, under solvent peak, H-7B); \(^13\)C NMR (DMSO-d_6, 100 MHz) δ 183.1 (C-3), 173.6 (C-24), 168.7 (C-5), 166.4 (C-11), 147.2 (C-19), 146.5 (C-10), 126.8 (C-14), 123.6 (C-12), 121.2 (C-21), 117.6 (C-15), 113.2 (C-4), 100.5 (C-20), 63.7 (C-2), 62.3 (C-8), 56.5 (C-1), 51.1 (C-17), 47.8 (C-23), 45.9 (C-6), 36.3 (C-7), 19.2 (C-16); HRFABMS m/z [M+H]^+ 409.09880 (calcd for C_20H_17N_4SO_4, 409.09705).

Discorhabdin Q (25)

TFA salt dark brown oil; \(^1\)H NMR (DMSO-d_6, 300 MHz) δ 8.27 (1H, s, H-14), 8.18 (1H, d, J = 5.8 Hz, H-17), 7.82 (1H, s, H-1), 7.51 (1H, d, J = 5.8 Hz, H-17), 6.01 (1H, s, H-4), 5.76 (1H, d, J = 3.2 Hz, H-8), 2.89 (1H, d, J = 11.4 Hz, H-7A), 2.60 (dd, J = 12.4, 4.2 Hz, H-7B).
(+)-(6R,8S)-1-Thiomethyl Discorhabdin G*/* (108)

TFA salt purple-brown oil; [α]D = +640, [α]578 = +480, [α]546 = -160 (c 0.05, MeOH); IR (smear) νmax 3122, 2911, 1671, 1614, 1523, 1414, 1330, 1173, 1120, 1018 cm⁻¹; UV (MeOH) λmax (ε) 245 (16190), 281 (10130), 334 (8520), 407 (5070), 568 (760) nm; ECD (MeOH) λ (Δε) 230 (-7.9), 237 (0), 239 (+0.5), 240 (0), 260 (-12.0), 299 (-5.7), 317 (-8.7), 334 (0), 360 (+16.5), 438 (0) nm; ¹H NMR (CD3OD-d4, 600 MHz) δ 7.20 (1H, s, H-14), 6.40 (1H, d, J = 1.1 Hz, H-2), 6.12 (1H, d, J = 0.6 Hz, H-4), 5.58 (1H, dd, J = 3.8, 1.2 Hz, H-8), 3.96 (1H, td, J = 14.1, 6.4 Hz, H-17A), 3.85 (1H, m, H-17B), 2.96 (1H, d, J = 12.0 Hz, H-7A), 2.89 (2H, m, H-16), 2.66 (1H, dd, J = 12.0, 3.9 Hz, H-7B), 2.56 (3H, s, H-22); ¹³C NMR (CD3OD-d4, 150 MHz) δ 181.6 (C-3), 170.7 (C-5), 166.0 (C-11), 164.8 (C-1), 156.7 (C-19), 153.4 (C-10), 128.0 (C-14), 125.6 (C-12), 124.2 (C-21), 123.3 (C-2), 122.0 (C-15), 119.2 (C-4), 100.1 (C-20), 60.6 (C-8), 52.9 (C-6), 45.9 (C-17), 45.2 (C-7), 19.2 (C-16), 14.6 (C-22); ¹H NMR (DMSO-d6, 600 MHz) δ 13.28 (1H, br s, NH-13), 10.83 (1H, br s, NH-9), 8.02 (1H, br s, NH-18), 7.39 (1H, d, J = 2.4 Hz, H-14), 6.31 (1H, s, H-2), 6.17 (1H, s, H-4), 5.60 (1H, d, J = 3.0 Hz, H-8), 3.93 (1H, m, H-17A), 3.80 (1H, m, H-17B), 2.82 (1H, d, J = 11.8 Hz, H-7A), 2.80 (2H, m, H-16), 2.54 (1H, dd, J = 11.8, 4.1 Hz, H-7B), 2.54 (3H, s, H-22); ¹³C NMR (DMSO-d6, 150 MHz) δ 179.0 (C-3), 167.1 (C-5), 164.7 (C-11), 160.0 (C-1), 153.8 (C-19), 151.4 (C-10), 127.5 (C-14), 123.6 (C-21), 122.9 (C-2), 122.7 (C-12), 121.6 (C-21), 120.4 (C-15), 118.4 (C-4), 98.2 (C-2-0), 50.7 (C-6), 44.6 (C-17), 17.7 (C-16), 143.8 (C-22); HRFABMS m/z [M+H]^+ 382.06874 (calcd for C19H16N3O2S2, 382.06840).
(-)-(2R,6R,8S,27S)-1-Discorhabdyl Discorhabdin D (111)  

TFA salt dark green oil; [α]_D = -120, [α]_578 = -140, [α]_546 = -240 (c 0.05, MeOH); IR (smear) ν_{max} 2925, 1673, 1620, 1202, 1135 cm\(^{-1}\); UV (MeOH) λ_{max} (ε) 203 (36130), 245 (30180), 293 (17820), 391 (10080), 593 (1140) nm; ECD (MeOH) λ (Δε) 256 (-14.3), 269 (0), 280 (+4.4), 291 (0), 315 (-4.5), 334 (0), 364 (+14.0), 406 (0), 443 (-4.1) nm; \(^1\)H NMR (CD\(_3\)OD-\(d_4\), 600 MHz) δ 7.87 (1H, s, H-22), 7.24 (1H, s, H-35), 7.14 (1H, s, H-14), 6.66 (1H, s, H-25), 6.56 (1H, d, J = 7.5 Hz, H-29), 6.15 (1H, s, H-4), 5.60 (1H, dd, J = 3.6, 1.1 Hz, H-8), 4.77 (1H, d, J = 7.5 Hz, H-28), 4.73 (1H, d, J = 3.0 Hz, H-1), 4.45 (1H, d, J = 3.0 Hz, H-2), 4.12 (1H, d, J = 13.9, 7.3, 2.6 Hz, H-17A), 3.90 (3H, m, H-17B/38), 3.22 (1H, m, H-16A), 3.08 (1H, d, J = 16.7, 6.7, 2.6 Hz, H-16B), 2.97 (2H, m, H-37), 2.89 (under an impurity, H-7A), 2.72 (1H, d, J = 11.9 Hz, H-7B); \(^{13}\)C NMR (CD\(_3\)OD-\(d_4\), 150 MHz) δ 182.4 (C-3), 176.2 (C-24), 171.0 (C-5), 167.0 (C-11), 166.5 (C-32), 163.5 (C-26), 160.3 (C-40), 150.5 (C-19/22), 148.6 (C-10), 147.2 (C-31), 127.7 (C-14), 127.6 (C-35), 126.8 (C-29), 125.8 (C-33), 125.6 (C-12), 124.7 (C-23), 123.2 (C-36), 122.8 (C-15), 122.3 (C-25), 121.2 (C-42), 119.5 (C-21), 115.1 (C-28), 114.5 (C-4), 101.2 (C-20), 95.8 (C-41), 65.6 (C-2), 63.8 (C-8), 52.9 (C-17), 50.8 (C-27), 46.6 (C-6), 46.2 (C-38), 46.1 (C-1), 38.9 (C-7), 20.8 (C-16), 19.3 (C-37); HRESIMS m/z [M+H]\(^+\) 747.0467 (calcd for C\(_{36}\)H\(_{24}\)BrN\(_6\)O\(_4\)S\(_2\) 747.0478), 749.0453 (calcd for C\(_{36}\)H\(_{26}\)BrN\(_6\)O\(_4\)S\(_2\) 749.0463).
4.2.1.2 Barret’s Reef (SEAS-LAT-2-BR-15-1) Collection

Metabolite extraction and isolation has previously been outlined in section 2.1.1.

(+)-(6S,8S)-Discorhabdin B (8)

Free base orange solid; \([\alpha]_D = +600 \ (c \ 0.05, \text{MeOH})\); UV (MeOH) \(\lambda_{max} (\varepsilon)\) 225 (7280), 260 (6050), 292 (4670) 350 (shoulder 2950) nm; ECD (MeOH) \(\lambda (\Delta\varepsilon)\) 219 (0), 227 (+1.4), 241 (0), 264 (-8.7), 285 (0), 288 (+0.3), 290 (0), 316 (-4.5), 339 (0), 362 (+4.7), 542 (+0.9) nm; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 12.17 (1H, br s, NH-13), 7.74 (1H, br s, NH-9), 7.62 (1H, s, H-1), 7.06 (1H, d, \(J = 2.1\) Hz, H-14), 5.97 (1H, s, H-4), 5.63 (1H, br s, H-8), 4.06 (1H, td, \(J = 17.6,\ 6.7\) Hz, H-17A), 3.67 (1H, m, H-17B), 2.74 (1H, d, \(J = 11.4\) Hz, H-7A), 2.52 (2H, m, H-16), 2.31 (1H, dd, \(J = 11.2,\ 4.2\) Hz, H-7B); \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz) \(\delta\) 176.2 (C-3), 172.8 (C-5), 170.0 (C-11), 153.9 (C-19), 153.6 (C-10), 148.7 (C-1), 125.0 (C-2), 124.2 (C-14), 122.1 (C-11), 121.1 (C-21), 117.3 (C-15), 114.9 (C-4), 108.3 (C-20 from HMBC), 63.5 (C-8), 52.3 (C-6), 50.3 (C-17), 41.0 (C-7), 17.5 (C-16).

(+)-(6S,8S)-Discorhabdin Q (25)

Free base orange solid; \([\alpha]_D = +720 \ (c \ 0.025, \text{MeOH})\); UV (MeOH) \(\lambda (\varepsilon)\) 224 (6010), 288 (3480), 408 (2180), 418 (2110), 428 (2150), 666 (150) nm; ECD (MeOH) \(\lambda (\Delta\varepsilon)\) 255 (-1.4), 268 (-6.5), 283 (0), 296 (+2.6), 315 (0), 334 (-4.4), 350 (0), 389 (+5.5) nm; \(^1\)H NMR (acetone-\(d_6\), 400 MHz) \(\delta\) 8.27 (1H, d, \(J = 5.8\) Hz, H-17), 8.23 (1H, s, H-14), 7.75 (1H, s, H-1), 7.51 (1H, d, \(J = 5.8\) Hz, H-17), 7.34 (1H, br s, NH-9), 5.94 (1H, s, H-4), 5.92 (1H, d, \(J = 4.3\) Hz, H-8), 3.03 (1H, d, \(J = 11.4\) Hz, H-7A), 2.60 (1H, dd, \(J = 12.4,\ 3.1\) Hz, H-7B); HRESIMS \(m/z\) [M-H]\(^–\) 409.9607 (caled for C\(_{18}\)H\(_9\)\(^{79}\)BrN\(_3\)SO\(_2\), 409.9604), 411.9594 (caled for C\(_{18}\)H\(_9\)\(^{81}\)BrN\(_3\)SO\(_2\), 411.9585).
(-)-(6S,6'S)-Discorhabdin W (37)

TFA salt dark green oil; $[\alpha]_D = -80$ (c 0.05, MeOH); ECD (MeOH) $\lambda_{\text{max}} (\Delta\varepsilon)$ 213 (0), 229 (-39.1), 250 (0), 253 (+0.8), 256 (0), 263 (-4.2), 272 (0), 291 (+6.6), 310 (0), 326 (-2.6), 342 (0), 379 (+4.3), 428 (0), 467 (-2.8), 515 (0) nm; $^1$H NMR (CD$_3$OD-$d_4$, 400 MHz) $\delta$ 7.84 (1H, s, H-1), 7.25 (1H, s, H-14), 6.67 (1H, d, $J = 7.5$ Hz, H-8), 6.31 (1H, s, H-4), 4.73 (1H, d, $J = 7.5$ Hz, H-7), 4.18 (1H, m, H-17A), 3.90 (1H, m, H-17B), 3.16 (1H, m, H-16A), 3.01 (1H, m, H-16B); $^{13}$C NMR (CD$_3$OD-$d_4$, 100 MHz) $\delta$ 176.4 (C-3), 166.4 (C-11), 162.6 (C-5), 160.2 (C-19), 150.5 (C-1), 147.1 (C-10), 127.7 (C-14), 126.9 (C-8), 125.8 (C-12), 125.1 (C-2), 123.3 (C-21), 121.5 (C-15), 120.9 (C-4), 114.2 (C-7), 95.7 (C-20 from HMBC), 49.9 (C-6), 46.7 (C-17), 19.3 (C-16); HRESIMS $m/z$ 824.9549 [M+H]$^+$ (calcd for C$_{36}$H$_{23}$Br$_2$N$_6$S$_2$O$_4$, 824.9589), 826.9535 (calcd for C$_{36}$H$_{23}$$_{79}$Br$^{81}$N$_6$S$_2$O$_4$, 826.9568), 828.9521 (calcd for C$_{36}$H$_{23}$$_{79}$Br$_2$N$_6$S$_2$O$_4$, 828.9548).

Free base orange solid; $[\alpha]_D = -260$ (c 0.05, MeOH); ECD (MeOH) $\lambda$ (D$\varepsilon$) 217 (0), 233 (-38.8), 253 (-4.1), 271 (-17.4), 288 (0), 307 (+21.7), 332 (0), 345 (-4.1), 405 (0) nm; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.31 (1H, br s, NH-13), 8.94 (1H, d, $J = 4.8$ Hz, NH-9), 7.90 (1H, s, H-1), 7.15 (1H, s, H-14), 6.46 (1H, dd, $J = 7.6$, 5.0 Hz, H-8), 5.85 (1H, s, H-4), 4.33 (1H, m, H-17A), 3.99 (1H, d, $J = 7.4$ Hz, H-7), 3.84 (1H, m, H-17B), 2.70 (2H, m, H-16); HRESIMS $m/z$ [M+H]$^+$ 824.9558 (calcd for C$_{36}$H$_{23}$$_{79}$Br$_2$N$_6$S$_2$O$_4$, 824.9589), 826.9555 (calcd for C$_{36}$H$_{23}$$_{79}$Br$^{81}$N$_6$S$_2$O$_4$, 826.9568), 828.9558 (calcd for C$_{36}$H$_{23}$$_{79}$Br$_2$N$_6$S$_2$O$_4$, 828.9548).
(-)578-(3R,5R,6S,8S)-3-Dihydro Discorhabdin A (107)

(-)578-(3R,5R,6S,8S)-107

TFA salt blue-green powder; [α]_D = +40, [α]_578 = -160, [α]_546 = -480 (c 0.05, MeOH); IR (smear) ν_max 3232, 3102, 1667, 1532, 1179, 1123, 1024 cm⁻¹; UV (MeOH) λ_max 204 (20700), 249 (18910), 347 (8130), 402 (6990), 586 (1130) nm; ECD (MeOH) λ (Δε) 219 (-14.5), 240 (-1.9), 258 (-7.5), 281 (0), 352 (+6.4), 419 (0) nm; ¹H NMR (DMSO-d₆, 600 MHz) δ 13.18 (1H, br s, NH-13), 10.51 (1H, br s, NH-9), 7.94 (1H, br s, NH-18), 7.37 (1H, s, H-14), 6.42 (1H, s, H-1), 5.21 (1H, d, J = 2.1 Hz, H-8), 4.28 (1H, dd, J = 13.2, 4.9 Hz, H-5), 4.16 (1H, br s, H-3), 3.89 (1H, m, H-17A), 3.83 (1H, m, H-17B), 2.88 (2H, m, H-16), 2.50 (under solvent peak, H-7A) 2.40 (1H, d, J = 12.0 Hz, H-7B), 2.11 (1H, td, J = 13.6, 3.0 Hz, H-4A), 1.90 (1H, ddd, J = 13.6, 4.8, 3.1 Hz, H-4B); ¹³C NMR (DMSO-d₆, 150 MHz) δ 165.9 (C-11), 153.7 (C-19), 150.9 (C-10), 132.6 (C-1), 127.3 (C-14), 126.5 (C-2), 123.2 (C-12), 123.0 (C-21), 119.6 (C-15), 103.3 (C-20), 69.4 (C-3), 58.8 (C-8), 50.0 (C-6), 48.9 (C-5), 43.7 (C-17), 40.7 (C-7), 38.3 (C-4), 18.1 (C-16); ¹H NMR (CD₃OD-d₄, 400 MHz) δ 7.16 (1H, s, H-14), 6.46 (1H, s, H-1), 5.20 (1H, dd, J = 3.5, 1.1 Hz, H-8), 4.33 (2H, m, H-3/H-5), 3.91 (2H, m, H-17), 2.95 (2H, m, H-16), 2.59 (1H, dd, J = 12.2, 3.7 Hz, H-7A), 2.46 (1H, d, J = 12.0 Hz, H-7B), 2.17 (1H, td, J = 13.7, 3.0 Hz, H-4A), 2.00 (1H, ddd, J = 13.9, 5.0, 3.1, H-4B); HRFABMS m/z [M+H]^+ 418.02314 (calcd for C_{18}H_{17}^{79}BrN_{3}SO_{2}, 418.02248), 420.02051 (calcd for C_{18}H_{17}^{81}BrN_{3}SO_{2}, 420.02044).
(-)-578-(6S,6aS)-16a,17a-Dehydro Discorhabdin W (109)

TFA salt green-brown oil; [α]D = 0, [α]578 = -200, [α]546 = -160 (c 0.025, MeOH); IR (smear) νmax 3434, 1678, 1656, 1497, 1480, 1203, 1136 cm⁻¹; UV (MeOH) λ (ε) 204 (33440), 226 (31440), 283 (16980), 296 (16530), 357 (3840), 421 (10320), 439 (11810), 544 (2860) nm; ¹H NMR (CD3OD-d4, 600 MHz) δ 8.44 (1H, d, J = 6.0 Hz, H-17a), 8.15 (1H, s, H-14a), 7.95 (1H, s, H-1a), 7.75 (1H, d, J = 7.8 Hz, H-16a), 7.25 (1H, s, H-14), 6.65 (1H, d, J = 7.8 Hz, H-8a), 6.57 (1H, d, J = 7.8 Hz, H-8), 6.15 (1H, s, H-4), 6.09 (1H, s, H-4a), 4.61 (1H, d, J = 7.8 Hz, H-7), 4.31 (1H, m, H-17A), 4.11 (1H, d, J = 7.8 Hz, H-7a), 3.91 (1H, m, H-17B), 3.18 (1H, m, H-16a), 3.06 (1H, m, H-16B); ¹³C NMR (CD3OD-d4, 150 MHz) δ 178.3 (C-3a), 176.0 (C-3), 170.6 (C-5a), 166.4 (C-11), 165.5 (C-11a), 163.5 (C-5), 160.6 (C-19), 157.1 (C-1a), 149.6 (C-1), 148.4 (C-19a), 147.2 (C-10), 144.2 (C-17a), 142.5 (C-10a), 130.1 (C-14a), 129.1 (C-8a), 127.7 (C-14), 126.7 (C-15a), 126.6 (C-8), 125.9 (C-12), 125.6 (C-2), 123.3 (C-21), 121.5 (C-15), 121.2 (C-21a), 120.9 (C-12a), 120.7 (C-4), 119.9 (C-2a), 116.4 (C-4a), 116.3 (C-16a), 114.6 (C-7), 107.1 (C-20a), 104.2 (C-7a), 95.6 (C-20), 52.0 (C-6a), 49.6 (C-6), 48.4 (C-17), 19.4 (C-16); HRESIMS m/z 822.9413 [M+H]+ (calcd for C36H2179Br2N6S2O4, 822.9427), 824.9420 (calcd for C36H2179Br81BrN6S2O4, 824.9409), 826.9433 (calcd for C36H2181Br2N6S2O4, 826.9395).

Free base orange solid; [α]D = -120, [α]578 = -320 (c 0.025, MeOH); UV (MeOH) λmax (ε) 204 (49630), 222 (shoulder 40770), 283 (shoulder 25540), 416 (9530), 438 (9910) nm; ECD (MeOH) λ (Δε) 217 (0), 232 (-32.7), 249 (0), 251 (+2.9), 252 (0), 270 (-16.2), 285 (0), 307 (+23.3), 336 (0), 345 (-1.11), 355 (0) 402 (+4.0) nm.
4.2.1.3 Mahanga Bay (LAT-SCA-L2) Collection

A single specimen of freeze dried sponge material (7.355 g) was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (1.321 g). A portion of the crude extract (610 mg) was subjected to Sephadex LH-20 and C_{18} flash (MeOH, H_{2}O-TFA (0.05%)) chromatography, yielding discorhabdin B (8) (3.2 mg, 0.09% dry weight, R_T 5.99 min), discorhabdin W (37) (2.8 mg, 0.08% dry weight, R_T not visible on the HPLC trace), 16a,17a-dehydro discorhabdin W (109) (0.7 mg, 0.02% dry weight, R_T not visible on the HPLC trace) and 2-bromo discorhabdin D (110) (2.2 mg, 0.06% dry weight, R_T 5.99 min). Metabolite isolation procedure is outlined in Scheme 4.1

Figure 4.1. Analytical HPLC trace (C_{18} rocket column) of the MeOH extract of the Wellington-sourced L. (Biannulata) wellingtonesis (LAT-SCA-L2).

(+)-(6S,8S)-Discorhabdin B (8), (-)-(6S,6'S)-Discorhabdin W (37) and (-)\textsubscript{578}-(6S,6aS)-16a,17a-Dehydro Discorhabdin W (109) chiroptical and spectroscopic data were identical with, or consistent with, previously presented Wellington-sourced material.

(-)-(2R,6S,8S)-2-Bromo Discorhabdin D (110)

\[
\begin{align*}
\text{TFA salt dark green oil; } [\alpha]_D &= -140, [\alpha]_{578} = -180, [\alpha]_{546} = -360 (c 0.05, \text{MeOH}); \\
\text{IR (smear) } &\nu_{\text{max}} 3078, 2918, 2833, 1660, 1555, 1503, 1432, 1175, 1120, 994 \text{ cm}^{-1}; \\
\text{UV (MeOH)} &\lambda_{\text{max}} (\varepsilon) 250 (12650), 285 (9300), 319 (shoulder 4840), 403 (5430) \text{ nm}; \\
\text{ECD (MeOH) } &\lambda_{\text{max}} (\Delta\varepsilon) 214 (0), 233 (+6.4), 240 (0), 255 (-21.0), 288 (0), 284 (+1.7), 290 (0), 305 (-4.7), 324 (0), 355 (+11.5) 406 (+3.9), 438 (0) \text{ nm}; \\
\text{\textsuperscript{1}H NMR (CD}_3\text{OD-}d_4, 600 \text{ MHz}) &\delta 7.13 (1H, s, H-14), 6.12 (1H, s, H-4), 5.68 (1H, dd, \text{J} = 3.4, 1.3 \text{ Hz}, H-8), 4.60 (1H, ddd, \text{J} = 13.2, 5.4, 2.4 \text{ Hz}, H-17A), 3.64 (1H, br m, H-17B), 3.56 (1H, d, \text{J} = 13.3 \text{ Hz}, H-1A),
\end{align*}
\]
3.22 (1H, d, J = 13.3 Hz, H-1B), 3.06 (2H, m, H-16), 2.83 (1H, dd, J = 12.1, 3.5 Hz, H-7A), 2.65 (1H, d, J = 12.1 Hz, H-7B); \(^{13}\)C NMR (CD\textsubscript{3}OD-\textit{d}_4, 150 MHz) \(\delta\) 178.1 (C-3), 174.4 (C-5), 166.8 (C-11), 151.6 (C-19), 149.8 (C-10), 127.5 (C-14), 125.8 (C-12), 123.7 (C-21), 120.8 (C-15), 112.3 (C-4), 101.9 (C-20), 79.7 (C-2), 64.6 (C-8), 51.7 (C-17), 45.9 (C-6), 43.9 (C-1), 40.2 (C-7), 21.6 (C-16); HRFABMS \textit{m/z} [M+H]\(^+\) 413.99168 (calcd for C\textsubscript{18}H\textsubscript{13}\textsuperscript{79}BrN\textsubscript{3}SO\textsubscript{2}, 413.99118), 415.98761 (calcd for C\textsubscript{18}H\textsubscript{13}\textsuperscript{81}BrN\textsubscript{3}SO\textsubscript{2}, 415.98914).

4.2.2 Kaikoura-Sourced \textit{Latrunculia} spp. Metabolite Extraction and Isolation Procedures

4.2.2.1 \textit{Latrunculia} (Biannulata) kaikoura (97 KK 1-17) Collection

Figure 4.2. Analytical HPLC trace (C\textsubscript{8} rocket column) of the MeOH extract of the Kaikoura-sourced \textit{L. (Biannulata) kaikoura} (97 KK 1-17).

A single specimen of freeze dried sponge material (11.30 g) was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (2.48 g). A portion of the crude extract (460 mg) was subjected to Sephadex LH-20, C\textsubscript{18} and C\textsubscript{8} flash (MeOH, H\textsubscript{2}O-TFA (0.05%)) chromatography, yielding
discorhabdin G*/I (15) (1.33 mg, 0.06% dry weight, R_T 5.59 min), discorhabdin L (17) (2.80 mg, 0.13% dry weight, R_T 5.11 min), makaluvamine D (67) (1.50 mg, 0.07% dry weight, R_T 5.73 min) and makaluvamine F (69) (4.25 mg, 0.2% dry weight, R_T 5.92 min). Metabolite isolation procedure is outlined in Scheme 4.2.

(+)-(6S,8S)-Discorhabdin G*/I (15)

TFA salt brown oil; [α]_D = +330 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 225 (+2.6), 230 (0), 254 (-7.9), 277 (-1.8), 303 (-3.9), 320 (0), 351 (+3.9), 408 (0) nm; ^1H NMR data were identical with, or consistent with, previously presented data; HRFABMS m/z [M+H]^+ 336.08003 (calcd for C_{18}H_{14}N_3SO_2, 336.08067).

(-)-578-(1R,2S,6R,8S)-Discorhabdin L (17)

TFA salt dark green oil; [α]_D = -80, [α]_{578} = -200, [α]_{546} = -360 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 256 (-15.9), 282 (-3.2), 304 (-6.8), 326 (0), 359 (+12.6), 417 (0) nm; ^1H NMR data were identical with, or consistent with, previously presented data; HRFABMS m/z [M+H]^+ 352.07545 (calcd for C_{18}H_{14}N_3SO_3, 352.07559).
Makaluvamine D (67)

TFA salt green oil; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 13.09 (1H, br s, NH-1), 10.42 (1H, br s, NH-5), 9.27 (1H, br s, OH), 9.02 (1H, t, \(J = 6.0\) Hz, NH-9), 7.34 (1H, d, \(J = 2.7\) Hz, H-2), 7.04 (2H, d, \(J = 8.4\) Hz, H-13/17), 6.70 (2H, d, \(J = 8.4\) Hz, H-14/16), 5.46 (1H, s, H-6), 3.81 (2H, dt, \(J = 10.3, 2.8\) Hz, H-4), 3.46 (under solvent signal, H-10); HRFABMS \(m/z\) [M+H]+ 308.13939 (calculated for C\(_{18}\)H\(_{18}\)N\(_3\)O\(_2\), 308.13990).

Makaluvamine F (69)

TFA salt red powder; \([\alpha]_D = 0\), \([\alpha]_{378}^\circ = 0\), \([\alpha]_{546} = 0\) (c 0.05, MeOH); UV (MeOH) \(\lambda_{\text{max}}\) 214 (23930), 248 (24510), 308 (6170), 350 (9920), 389 shoulder (6870), 540 (990) nm; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\) 13.16 (1H, br s, NH-1), 11.11 (1H, br s, NH-5), 10.28 (1H, s, OH), 9.21 (1H, d, \(J = 7.4\) Hz, NH-9), 7.38 (1H, s, H-13), 7.33 (1H, s, H-2), 6.80 (1H, s, H-16), 5.80 (1H, dd, \(J = 7.4, 4.5\) Hz, H-10) 5.53 (1H, s, H-6), 3.89 (2H, t, \(J = 7.8\) Hz, H-4), 3.71 (1H, dd, \(J = 16.4, 3.0\) Hz, H-11A), 3.54 (under solvent signal, H-11B), 2.92 (2H, t, \(J = 7.7\) Hz, H-3); \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz) \(\delta\) 167.5 (C-8), 157.6 (C-7), 153.4 (C-15), 151.1 (C-5a), 138.6 (C-17), 130.1 (C-12), 129.0 (C-13), 126.6 (C-2), 124.1 (C-8a), 121.6 (C-8b), 118.7 (C-2a), 109.4 (C-16), 105.2 (C-14), 88.0 (C-6), 63.7 (C-10), 42.9 (C-4), 39.2 (C-11 under solvent peak), 17.9 (C-3); \(^1\)H NMR (CD\(_3\)OD-\(d_4\), 400 MHz) \(\delta\) 7.40 (1H, s, H-13), 7.16 (1H, s, H-2), 6.73 (1H, s, H-16), 5.71 (1H, dd, \(J = 7.1, 3.2\) Hz, H-10), 5.54 (1H, s, H-6), 3.93 (2H, t, \(J = 7.6\) Hz, H-4), 3.60 (2H, m, H-11), 3.00 (2H, t, J
4.2.2.2 Latrunculia (Biannulata) kaikoura (UOC 91 K-1-1) Collection

A single specimen of freeze dried sponge material (25.835 g) was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (5.940 g). A portion of the crude extract (310 mg) was subjected to Sephadex LH-20 and C\textsubscript{18} flash (MeOH, H\textsubscript{2}O-TFA (0.05%)) chromatography, yielding discorhabdin K (21) (1.10 mg, 0.08% dry weight, R\textsubscript{T} 5.62 min). Other discorhabdin alkaloids were present in the sponge crude but no attempt was made to purify the compounds. Metabolite isolation procedure is outlined in Scheme 4.3.
Scheme 4.3. Kaikoura-sourced *L. (Biaannulata) kaikoura* (UOC 91 K-1-1) metabolite isolation scheme.

(+)-(6S,8S,7'S*)-Discorhabdin K (21)

TFA salt brown oil; $[\alpha]_D = +340$, $[\alpha]_{578} = +200$, $[\alpha]_{546} = -200$ (c 0.05, MeOH); UV (MeOH) $\lambda_{\text{max}} (\varepsilon)$ 240 (18980), 280 (shoulder 11150), 328 (9350), 399 (5870) 565 (740) nm; ECD (MeOH) $\lambda (\Delta \varepsilon)$ 236 (-4.9), 256 (-14.7), 298 (-5.9), 312 (-7.6), 332 (0), 358 (+12.5), 433 (0) nm; $^1$H NMR (CD$_3$OD-$d_4$, 600 MHz) $\delta$ 7.94 (1H, s, H-2'), 7.23 (1H, s, H-14), 6.09 (1H, br s, H-4), 5.90 (1H, d, $J = 1.1$ Hz, H-2), 5.64 (1H, d, $J = 2.9$ Hz, H-8), 4.10 (1H, m, H-17A), 4.05 (1H, t, $J = 7.8$ Hz, H-7'), 3.89 (1H, m, H-17B), 3.79 (3H, s, H-9'), 3.37 (1H, dd, $J = 15.3$, 8.3 Hz, H-6'A), 3.24 (1H, dd, $J = 15.3$, 7.3 Hz, H-6'B), 3.10 (1H, d, $J = 11.9$ Hz, H-7A), 2.96 (2H, m, H-16), 2.74 (1H, dd, $J = 11.9$, 3.8 Hz, H-7B); $^{13}$C NMR (CD$_3$OD-$d_4$, 150 MHz) $\delta$ 181.9 (C-3), 171.2 (C-5), 171.1 (C-8'), 166.1 (C-11), 163.5 (C-1), 156.5 (C-19), 153.5 (C-10), 142.9 (C-2'), 134.7 (C-5'), 128.1 (C-14), 126.0 (C-2), 125.6 (C-12), 124.9 (C-4'), 124.3 (C-21), 122.2 (C-15), 119.3 (C-4), 99.3 (C-20), 60.8 (C-8), 53.3 (C-7'), 52.8 (C-6), 46.2 (C-17), 44.4 (C-7), 33.1 (C-9'), 26.1 (C-6'), 19.1 (C-16); HRFABMS m/z [M+H]$^+$ 535.12221 (caled for C$_{25}$H$_{23}$N$_6$S$_2$O$_4$, 535.12222).
4.2.2.3 *Latrunculia (Latrunculia) trivetricillata* (NMP-6116) Collection

Freeze dried sponge material (12.363 g) was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a purple-brown crude extract (2.740 g). A portion of the crude extract (506 mg) was subjected to Sephadex LH-20 (MeOH (0.05% TFA)) and C$_8$ flash (MeOH, H$_2$O-TFA (0.05%)) chromatography, yielding discorhabdin C (6) (48.9 mg, 2.13% dry weight, R$_T$ 5.75 min), 2-hydroxy discorhabdin D (10) (0.6 mg, 0.026% dry weight, R$_T$ 5.33 min, not visible on HPLC trace), discorhabdin D (11) (3.7 mg, 0.16% dry weight, R$_T$ 5.75 min) and 3-dihydro discorhabdin C (34) (11.0 mg, 0.48% dry weight, R$_T$ 5.75 min). Metabolite isolation procedure is outlined in Scheme 4.4.

**Figure 4.4.** Analytical HPLC trace (C$_8$ rocket column) of the MeOH extract of Kaikoura-sourced *L. (Latrunculia) trivetricillata* (MNP 6116).
Scheme 4.4. Kaikoura-sourced *L. (Latrunculia) trivetricillata* (MNP 6116) metabolite isolation scheme

Discorhabdin C (6)

TFA salt dark purple solid; $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 13.29 (1H, br s, NH-13), 10.16 (1H, br s, NH-18), 8.22 (1H, br s, NH-9), 7.73 (2H, s, H-1/5), 7.38 (1H, d, $J = 2.3$ Hz, H-14), 3.68 (4H, m, H-8/17), 2.81 (2H, t, $J = 7.4$ Hz, H-16), 2.02 (2H, br m, H-7); HRFABMS $m/z$ [M+H]$^+$ 461.94353 (calcd for C$_{18}$H$_{14}$Br$_2$N$_3$O$_2$, 461.94527), 463.94375 (calcd for C$_{18}$H$_{14}$Br$_7$N$_3$O$_2$, 463.94323), 465.94288 (calcd for C$_{18}$H$_{14}$Br$_{10}$N$_3$O$_2$ 465.94118).
(+)-(2R,6R,8S)-2-Hydroxy Discorhabdin D (10)

TFA salt green oil; [α]D = +80 (c 0.05, MeOH); UV (MeOH) λmax 249 (11540), 283 (7290), 321 (4540), 396 (4520), 578 (600) nm; ECD (MeOH) λmax (Δε) 235 (0), 255 (-11.3), 277 (0), 281 (+1.1), 286 (0), 303 (-4.2), 323 (0), 354 (+8.6), 452 (0) nm; 1H NMR (CD3OD-d4, 400 MHz) δ 7.11 (1H, s, H-14), 6.08 (1H, s, H-4), 5.63 (1H, dd, J = 3.6, 1.6 Hz, H-8), 4.04 (2H, m, H-17), 3.50 (1H, m, H-16A), 3.08 (1H, m, H-16B), 2.85 (1H, d, J = 13.0 Hz, H-1A), 2.80 (1H, dd, J = 12.1, 3.6 Hz, H-7A), 2.62 (2H, d, J = 13.1 Hz, H-1B/H-7B); HRFABMS m/z [M+H]+ 352.07589 (calcd for C18H14N3SO3, 352.07559).

(-)546-(2S,6R,8S)-Discorhabdin D (11)

TFA salt green oil; [α]D = 0, [α]578 = -40, [α]546 = -200 (c 0.05, MeOH); ECD (MeOH) λmax (Δε) 253 (-12.0), 278 (0), 282 (+0.7), 286 (0), 305 (-4.5), 329 (0), 362 (+8.9), 419 (0) nm; 1H NMR data were identical, or consistent with, previously presented data; HRFABMS m/z [M+H]+ 336.08084 (calcd for C18H14N3SO2, 336.08067).

3-Dehydro Discorhabdin C (34)

TFA salt dark purple oil; 1H NMR (D2O-d2, 400 MHz) δ 7.15 (1H, s, H-14), 6.43 (2H, s, H-1/5), 4.82 (1H, s, H-3), 3.73 (2H, t, J = 7.6 Hz, H-17), 3.60 (2H, t, J = 5.4 Hz, H-8), 2.80 (2H, t, J = 7.6 Hz, H-16), 1.89 (2H, t, J = 5.6 Hz, H-7); HRFABMS m/z [M+H]+
463.96134 (calcd for $\text{C}_{18}\text{H}_{16}^7\text{Br}_2\text{N}_3\text{O}_2$, 463.96092), 465.95789 (calcd for $\text{C}_{18}\text{H}_{16}^7\text{Br}_8\text{N}_3\text{O}_2$, 456.95888), 467.95792 (calcd for $\text{C}_{18}\text{H}_{16}^8\text{Br}_2\text{N}_3\text{O}_2$, 467.95683).

4.2.3 Tutukaka-Sourced *Latrunculia (Biannulata) procumbens* (97 TUT 2-10)

Metabolite Extraction and Isolation Procedures

Freeze dried sponge material (9.680 g) was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (1.260 g). A portion of the crude extract (708 mg) was subjected to Sephadex LH-20, C$_{18}$, and CN flash (MeOH, H$_2$O-TFA (0.05%)) chromatography, yielding discorhabdin L (17) (0.86 mg, 0.015% dry weight, $R_T$ 4.61 min) and makaluvamine F (69) (1.18 mg, 0.02% dry weight, $R_T$ 5.75 min). Metabolite isolation procedure is outlined in Scheme 4.5.
Scheme 4.5. Tutukaka-sourced *L. (Biannulata) procumbens* metabolite isolation scheme

(−)578-(1R,2S,6R,8S)-Discorhabdin L (17)

TFA salt dark green oil; [α]D = 0, [α]546 = −200 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 255 (-14.1), 284 (-1.9), 303 (-5.3), 324 (0), 360 (+12.2), 413 (0), 438 (-2.4) nm; 1H NMR data were identical or consistent with that previously presented; HRFABMS m/z [M+H]+ 352.07544 (calcd for C18H14N3SO3, 352.07559).

Makaluvamine F (69)

TFA salt red powder; [α]D = 0, [α]546 = 0 (c 0.05, MeOH); 1H NMR data were identical or consistent with that previously presented; HRFABMS m/z [M+H]+ 416.00635 (calcd for C18H1579BrN3SO2, 416.00683), 418.00477 (calcd for C18H1581BrN3SO2, 418.00479).
4.2.4 Three Kings Islands-Sourced *Latrunculia* spp. metabolite Extraction and Isolation Procedures

Freeze dried sponge material (1.387 g) was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (283 mg). The crude extract was subjected to Sephadex LH-20 and CN flash (MeOH, H₂O-TFA (0.05%)) chromatography, yielding makaluvamine F (69) (0.80 mg, 0.06% dry weight, Rₜ 5.68 min. Metabolite isolation procedure is outlined in Scheme 4.6.

**Figure 4.6.** Analytical HPLC trace (C₈ rocket column) of the MeOH extract of the Three Kings Islands-sourced collection of *Latrunculia* spp. (MNP 6117).

**Scheme 4.6.** Three Kings islands-sourced *Latrunculia* spp. (MNP6117) metabolite isolation scheme.
Makaluvamine F (69)

TFA salt: red powder; \([\alpha]_D = 0 \ (c \ 0.05, \text{MeOH})\); \(^1\text{H} \) NMR data were identical or consistent with that previously presented; HRFABMS m/z [M+H]\(^+\) 416.00502 (calculated for C\(_{18}\)H\(_{15}\)\(^{79}\)BrN\(_3\)SO\(_2\), 416.00683), 418.00524 (calcd for C\(_{18}\)H\(_{15}\)\(^{81}\)BrN\(_3\)SO\(_2\), 418.00479).

4.2.5 Antarctic-Sourced *Latrunculia* spp. Metabolite Extraction and Isolation Procedures

![Analytical HPLC trace](image)

**Figure 4.7.** Analytical HPLC trace (C\(_{18}\) rocket column) of the MeOH extract of the Antarctic-sourced collection of *Latrunculia* spp. (MNP 7829).

Freeze dried sponge material was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (2.120 g). A portion of the crude extract (540 mg) was subjected to Sephadex LH-20 and C\(_{18}\) flash (MeOH, H\(_2\)O-TFA (0.05%)) chromatography, yielding discorhabdin B (8) (1.02 mg, 0.03% dry weight, R\(_T\) 6.50 min), discorhabdin Q (25) (1.71 mg, 0.06% dry weight, R\(_T\) 6.50 min) and discorhabdin W (37) (1.40 mg, 0.05% dry weight, R\(_T\) 8.34 min). Metabolite isolation procedure is outlined in Scheme 4.7.
(+)-(6S,8S)-Discorhabdin B (8)

TFA salt purple-brown oil; [α]D = +460, (c 0.05, MeOH); ECD (MeOH) λ (Δε) 208 (-25.0), 222 (0), 231 (+7.6), 241 (0), 257 (-25.2), 282 (-5.1), 302 (-12.6), 321 (0), 351 (+19.1) 418 (0) nm; 1H NMR data were identical or consistent with that previously presented; HRFABMS m/z [M+H]+ 413.99007 (calcd for C18H1379BrN3SO2, 413.99118), 415.98993 (calcd for C18H1381BrN3SO2, 415.98914).

(+)-(6S,8S)-Discorhabdin Q (25)

Free base orange solid; ECD (MeOH) λ (Δε) 244 (-1.0), 263 (-3.0), 284 (0), 290 (+0.7), 309 (0), 331 (-1.6), 350 (0), 381 (+1.7) nm; 1H NMR data were identical or consistent with that previously presented.

(-)-(6S,6'S)-Discorhabdin W (37)

TFA salt dark green oil; [α]D = -80 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 214 (0), 228 (-23.1), 247 (0), 253 (+7.3), 265 (+0.9), 292 (+10.4), 310 (0), 324 (-1.9), 337 (0), 376 (+4.1), 431 (0), 475 (-3.2) nm; 1H NMR data were identical or consistent with that previously presented.
4.2.6 Doubtful Sound-Sourced *Latrunculia* spp. Metabolite Extraction and Isolation Procedures

A single specimen of freeze dried sponge material (4.782 g) was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (1.290 g). A portion of the crude extract (420 mg) was subjected to Sephadex LH-20 and C\textsubscript{18} flash (MeOH, H\textsubscript{2}O-TFA (0.05%)) chromatography, yielding discorhabdin C (6) (0.1 mg, 0.006% dry weight, R\textsubscript{T} 5.68/5.82 min), discorhabdin B (8) (2.49 mg, 0.16% dry weight, R\textsubscript{T} under 5.68/5.82 min peak), discorhabdin G* (15) (1.50 mg, 0.096% dry weight, R\textsubscript{T} 5.68/5.82 min), discorhabdin L (17) (1.80 mg, 0.12% dry weight, R\textsubscript{T} 4.48 min), discorhabdin K (21) (2.14 mg, 0.14% dry weight, R\textsubscript{T} 5.68/5.82 min), discorhabdin W (37) (2.13 mg as free base, 0.13% dry weight; and 5.53 mg converted to a TFA salt, 0.36% dry weight, R\textsubscript{T} not visible on the crude HPLC trace) 16a,17a dehydro discorhabdin W (109) (converted to a TFA salt 0.6 mg, 0.04% dry weight, R\textsubscript{T} not visible on the crude HPLC trace) and 1-discorhabdyl discorhabdin D (111) (0.60 mg, 0.039% dry weight, R\textsubscript{T} 7.56 min). Metabolite isolation procedure is outlined in Scheme 4.8.
Scheme 4.8. Doubtful Sound-sourced *Latrunculia* spp. (95 DS (1-10)) metabolite isolation scheme.

**Discorhabdin C (6)**

TFA salt dark purple oil; HRFABMS \(m/z\) [M+H]\(^+\) 461.94613 (calcd for C\(_{18}H_{14}^{79}\)Br\(_2\)N\(_3\)O\(_2\), 461.94527), 463.94324 (calcd for C\(_{18}H_{14}^{79}\)Br\(^{80}\)N\(_3\)O\(_2\), 463.94323), 465.94161 (calcd for C\(_{18}H_{14}^{80}\)Br\(_2\)N\(_3\)O\(_2\) 465.94118).
(-)-(6R,8R)-Discorhabdin B (8)

![Chemical structure of (-)-(6R,8R)-Discorhabdin B](image)

TFA salt purple-brown oil; \([\alpha]_D = -400 (c 0.05, \text{MeOH})\); ECD (MeOH) \(\lambda (\Delta\varepsilon)\) 225 (0), 234 (-2.7), 240 (0), 256 (+14.5), 280 (+3.2), 303 (+7.6), 320 (0), 350 (-11.8), 428 (0) nm; \(^1\)H NMR (CD\(_3\)OD-\(d_4\), 400 MHz) \(\delta\) 7.83 (1H, s, H-1), 7.20 (1H, s, H-14), 6.24 (1H, s, H-4), 5.69 (1H, dd, \(J = 3.9, 1.4\) Hz, H-8), 3.94 (1H, m, H-17A), 3.81 (1H, m, H-17B), 2.92 (2H, m, H-16), 2.78 (1H, d, \(J = 11.6\) Hz, H-7A), 2.54 (1H, dd, \(J = 11.9, 4.0\) Hz, H-7B); HRFABMS \(m/z\) [M+H]\(^+\) 413.99082 (calcd for C\(_{18}\)H\(_{13}\)\(^{79}\)BrN\(_3\)SO\(_2\), 413.99118), 415.99016 (calcd for C\(_{18}\)H\(_{13}\)\(^{81}\)BrN\(_3\)SO\(_2\), 415.98914).

(-)-Discorhabdin B (8) TFA salt (1.1 mg, 2.1 \(\mu\)mol) was dissolved in dry acetone (1.5 mL) to which K\(_2\)CO\(_3\) (2 mg) was added. Solution was stirred under N\(_2\) for 10 min, during which the color changed from green to bright orange. The solvent was removed in vacuo and the product recovered by an ethyl acetate/water partition, yielding discorhabdin B (8) as a free base (0.7 mg, 1.6 \(\mu\)mol).

Free Base orange solid; \([\alpha]_D = -460 (c 0.05, \text{MeOH})\); ECD (MeOH) \(\lambda (\Delta\varepsilon)\) 219 (0), 224 (-1.4), 241 (0), 264 (+8.8), 286 (0), 290 (-0.4), 295 (0), 315 (+4.9), 337 (0), 362 (-4.9) 540 (-1.0) nm.
(-)-(6R,8R)-Discorhabdin G*/I (15)

TFA salt brown oil; [α]D = -720 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 217 (0), 225 (-14.1), 230 (0), 252 (+41.5), 281 (+4.3), 300 (+10.9), 316 (0), 349 (-15.3), 405 (0) nm; 1H NMR (CD3OD-d4, 400 MHz) δ 7.42 (1H, d, J = 10.1 Hz, H-1), 7.19 (1H, s, H-14), 6.66 (1H, dd, J = 9.8, 1.5 Hz, H-2), 6.16 (1H, d, J = 1.3 Hz, H-4), 5.66 (1H, dd, J = 3.8, 1.3 Hz, H-8), 3.93 (1H, m, H-17A), 3.81 (1H, m, H-17B), 2.88 (2H, m, H-16), 2.47 (1H, d, J = 11.6 Hz, H-7A), 2.47 (1H, dd, J = 11.8, 3.9 Hz, H-7B); 13C NMR (CD3OD-d4, 100 MHz) δ 183.6 (C-3), 173.3 (C-5), 166.4 (C-11), 157.0 (C-19), 153.2 (C-10), 147.6 (C-1), 134.0 (C-2), 127.8 (C-14), 125.6 (C-12), 124.4 (C-21), 121.9 (C-15), 120.7 (C-4), 98.5 (C-20), 62.0 (C-8), 50.5 (C-6), 45.9 (C-17), 43.6 (C-7), 19.2 (C-16); HRFABMS m/z [M+H]+ 336.08049 (calcd for C18H14N3SO2, 336.08067).

(+)-578-(1S,2R,6S,8R)-Discorhabdin L (17)

TFA salt dark green oil; [α]D = 0, [α]578 = +140, [α]346 = +200 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 255 (+18.9), 282 (+2.8), 302 (+7.7), 324 (0), 358 (-16.8), 416 (0), 446 (+2.8), 488 (0) nm; 1H NMR (CD3OD-d4, 400 MHz) δ 7.10 (1H, s, H-14), 6.13 (1H, s, H-4), 5.58 (1H, dd, J = 3.5, 1.2 Hz, H-8), 4.62 (1H, d, J = 3.6 Hz, H-1), 4.14 (1H, d, J = 3.6 Hz, H-2), 4.01 (1H, m, H-17A), 3.90 (1H, m, H-17B), 3.16 (1H, m, H-16A), 3.06 (1H, ddd, J = 16.7, 6.8, 2.9 Hz, H-16B), 2.96 (1H, dd, J = 11.9, 3.6 Hz, H-7A), 2.56 (1H, d, J = 11.8 Hz, H-7B); 1H NMR (DMSO-d6, 400 MHz) δ 13.15 (1H, br s, NH-13), 10.79 (1H, br
m/z $[M+H]^+$ 352.07558 (calcd for C$_{18}$H$_{14}$N$_3$SO$_3$, 352.07559).

(-)-(6$R$,8$R$,7'S*)-Discorhabdin K (21)

TFA salt brown oil; $[\alpha]_D = -260$, $[\alpha]_{578} = -180$, $[\alpha]_{546} = +80$ (c 0.05, MeOH); IR (smear) $\nu_{\text{max}}$ 2932, 1664, 1616, 1523, 1411, 1327, 1178, 1120 cm$^{-1}$; UV (MeOH) $\lambda_{\text{max}}$ (s) 230 (20270), 241 (20909), 280 (12570), 327 (9960), 392 (shoulder 6990) nm; ECD (MeOH) $\lambda_{\text{max}}$ ($\Delta\varepsilon$) 255 (+17.2), 305 (+7.9), 313 (+8.7), 333 (0), 359 (-12.3), 415 (0) nm; $^1$H NMR (CD$_3$OD-$_d$$_4$, 600 MHz) $\delta$ 7.94 (1H, s, H-2'), 7.22 (1H, s, H-14), 6.09 (1H, br s, H-4), 5.94 (1H, d, $J = 1.3$ Hz, H-2), 5.64 (1H, d, $J = 2.7$ Hz, H-8), 4.09 (1H, m, H-17A), 4.06 (1H, t, $J = 8.9$ Hz, H-7'), 3.90 (1H, m, H-17B), 3.44 (1H, dd, $J = 15.4$, 7.8 Hz, H-6'A), 3.79 (3H, s, H-9'), 3.19 (1H, dd, $J = 15.4$, 7.8 Hz, H-6'B), 3.11 (1H, d, $J = 11.7$ Hz, H-7A), 2.96 (2H, m, H-16), 2.74 (1H, dd, $J = 12.0$, 3.9 Hz, H-7B); $^{13}$C NMR (CD$_3$OD-$_d$$_4$, 150 MHz) $\delta$ 171.3 (C-5), 171.0 (C-8' from HMBC), 163.3 (C-1), 156.5 (C-19), 153.6 (C-10 from HMBC), 142.8 (C-2'), 134.7 (C-5'), 128.1 (C-14), 126.3 (C-2), 125.5 (C-12 from HMBC), 124.7 (C-4'), 124.0 (C-21 from HMBC), 122.2 (C-15), 119.2 (C-4), 99.1 (C-20 from HMBC), 60.8 (C-8), 53.5 (C-7' from HMBC), 52.8 (C-6), 46.2 (C-17), 44.3 (C-7), 33.1 (C-9'), 26.2 (C-6'), 19.0 (C-16); HRFABMS m/z [M+H]$^+$ 535.12262 (calcd for C$_{25}$H$_{23}$N$_6$S$_2$O$_4$, 535.12222).
(+)-(6R,6'R)-Discorhabdin W (37)

TFA salt dark green oil; [α]D = +80 (c 0.05, MeOH); UV (MeOH) λmax (ε) 240 (24890), 267 (shoulder 17330), 295 (14650), 435 (8030) 600 (2030) nm; ECD (MeOH) λ (Δε) 213 (0), 227 (+19.9), 245 (0), 255 (-7.7), 266 (-2.6), 291 (-8.9), 310 (0), 325 (+1.8), 340 (0), 382 (-2.9), 427 (0), 465 (+3.3), 502 (0) nm; 1H NMR (CD3OD-d4, 400 MHz) δ 7.85 (1H, s, H-1), 7.24 (1H, s, H-14), 6.67 (1H, d, J = 7.3 Hz, H-8), 6.33 (1H, s, H-4), 4.74 (1H, d, J = 7.3 Hz, H-7), 4.14 (1H, td, J = 15.2, 6.9, H-17A), 3.89 (1H, m, H-16A), 3.01 (1H, m, H-16B); 13C NMR (CD3OD-d4, 100 MHz) δ 176.3 (C-3), 166.5 (C-11), 162.5 (C-5), 160.3 (C-19), 150.5 (C-1), 147.2 (C-10), 127.7 (C-14), 126.7 (C-8), 125.8 (C-12), 125.1 (C-2), 123.3 (C-21), 121.5 (C-15), 121.0 (C-4), 114.2 (C-7), 95.6 (C-20), 49.9 (C-6), 46.6 (C-17), 19.3 (C-16); HRESIMS m/z [M+H]+ 824.9565 (calcd for C36H2379Br2N6S2O4, 824.9589), 826.9541 (calcd for C36H2381BrN6S2O4, 826.9568), 828.9541 (calcd for C36H2381Br2N6S2O4, 828.9548).

Free base orange solid; [α]D = +240 (c 0.05, MeOH); UV (MeOH) λmax (ε) 204 (46870), 229 (shoulder 36370), 282 (25240), 366 (11790) 435 (4070) 500 (2300) nm; ECD (MeOH) λ (Δε) 217 (0), 233 (+36.2), 251 (+5.9), 271 (+16.3), 288 (0), 305 (-19.5), 331 (0), 344 (+4.2) 405 (0) nm; 1H NMR (DMSO-d6, 400 MHz) δ 12.31 (1H, br s, NH-13), 8.94 (1H, d, J = 4.8 Hz, NH-9), 7.90 (1H, s, H-1), 7.15 (1H, br s, H-14), 6.46 (1H, dd, J = 7.6, 5.2 Hz, H-8), 5.85 (1H, s, H-4), 4.33 (1H, td, J = 17.6, 7.2 H-17A), 3.98 (1H, dd, J = 7.4, 1.0 Hz, H-7), 3.83 (1H, m, H-17B), 2.71 (2H, m, H-16); 13C NMR (DMSO-d6, 400 MHz) δ 175.7 (C-3), 168.7 (C-5), 168.2 (C-11), 155.8 (C-1), 155.0 (C-19), 138.5 (C-10), 127.8 (C-8), 124.8 (C-14), 122.2 (C-12), 121.5 (C-21), 117.8 (C-2), 117.1 (C-15), 114.4 (C-4), 105.2 (C-20), 102.7 (C-7), 50.0 (C-17), 49.5 (C-6), 17.7 (C-16); HRESIMS m/z [M+H]+ 824.9555 (calcd for C36H2379BrN6S2O4, 824.9589), 826.9558 (calcd for C36H2379Br81BrN6S2O4, 826.9568), 828.9562 (calcd for C36H2381Br2N6S2O4, 828.9548).
(+)-578-(6R,6aR)-16a,17a-Dehydro Discorhabdin W (109)

TFA salt green-brown oil; $[\alpha]_D = 0$, $[\alpha]_{578} = +80$, $[\alpha]_{546} = +160$ (c 0.025, MeOH); UV (MeOH) $\lambda$ (c) 203 (41620), 221 (35090), 284 (18360), 295 (18390), 356 (3850), 421 (11840), 440 (13720), 550 (3340) nm; $^1$H NMR (CD$_3$OD-$d_4$, 400 MHz) $\delta$ 8.44 (1H, d, $J = 5.9$ Hz, H-17a), 8.17 (1H, s, H-14a), 7.92 (1H, s, H-1a), 7.76 (1H, s, H-1), 7.72 (1H, d, $J = 6.0$ Hz, H-16a), 7.25 (1H, s, H-14), 6.65 (1H, d, $J = 7.7$ Hz, H-8a), 6.57 (1H, d, $J = 7.5$ Hz, H-8), 6.15 (1H, s, H-4), 6.09 (1H, s, H-4a), 4.61 (1H, d, $J = 7.6$ Hz, H-7), 4.31 (1H, m, H-17a), 4.10 (1H, d, $J = 7.5$ Hz, H-7a), 3.92 (1H, m, H-17b), 3.18 (1H, m, H-16a), 3.05 (1H, m, H-16b); HRESIMS $m/z$ 822.9416 [M+H]$^+$ (calcd for C$_{36}$H$_{21}$Br$_2$N$_6$S$_2$O$_4$, 822.9427), 824.9428 (calcd for C$_{36}$H$_{21}$Br$_2$N$_6$S$_2$O$_4$, 824.9409), 826.9450 (calcd for C$_{36}$H$_{21}$Br$_2$N$_6$S$_2$O$_4$, 826.9395).

Free base orange solid; $[\alpha]_D = +80$, $[\alpha]_{578} = +80$, $[\alpha]_{546} = +120$ (c 0.025, MeOH); ECD (MeOH) $\lambda$ (Deltae) 231 (+26.4), 246 (0), 248 (-3.9), 252 (0), 270 (+17.0), 283 (0), 307 (-22.2), 331 (0), 343 (+2.5), 356 (0) 400 (-2.5), 453 (0) nm.
(+)-1-(2S,6S,8R,27R)-Discorhabdyl Discorhabdin D (111)

TFA salt dark green oil; [α]D = +60, [α]578 = 0, [α]546 = +60, (c 0.05, MeOH); UV (MeOH) λmax (ε) 204 (32670), 243 (29540), 294 (18070), 395 (10110), 591 (1780) nm; ECD (MeOH) λ (Δε) 255 (+11.2), 269 (0), 285 (-6.7), 299 (0), 323 (+4.6), 347 (0), 379 (-5.7), 426 (0), 458 (+2.0) 487 (0) nm; 1H NMR (CD3OD-d4, 400 MHz) δ 7.88 (1H, s, H-22), 7.23 (1H, s, H-35), 7.14 (1H, s, H-14), 6.64 (1H, s, H-25), 6.56 (1H, d, J = 7.6 Hz, H-29), 6.16 (1H, s, H-4), 5.60 (1H, d, J = 2.4 Hz, H-8), 4.77 (under solvent peak H-7), 4.70 (1H, d, J = 3.0 Hz, H-1), 4.44 (1H, d, J = 3.1 Hz, H-2), 4.12 (1H, m, H-17A), 3.90 (3H, m, H-17B/38), 3.07–2.89 (7H, m, H-7A/16/37/38), 2.70 (1H, d, J = 11.6 Hz, H-7B); LRESI m/z [M+H]+ 747/749.
4.2.7 Milford Sound-Sourced *Latrunculia* spp. Metabolite Extraction and Isolation Procedures

Freeze dried sponge material was extracted with MeOH (4 x 200 mL). The solvent was filtered and then removed in vacuo to give a dark brown crude extract (2.790 g). A portion of the crude extract (370 mg) was subjected to Sephadex LH-20 and C18 flash (MeOH, H2O-TFA (0.05%)) chromatography, yielding discorhabdin B (8) (1.12 mg, 0.06% dry weight, R_T 6.56 min), discorhabdin G*/I (15) (0.71 mg, 0.039% dry weight, R_T 6.56 min), discorhabdin H (16) (3.50 mg, 0.19% dry weight, R_T 6.14 min), discorhabdin L (17) (1.35 mg, 0.073% dry weight, R_T 6.56 min) and discorhabdin W (37) (6.77 mg, 0.37% dry weight, not present on the crude HPLC trace). Metabolite isolation procedure is outlined in Scheme 4.9.
Scheme 4.9. Milford Sound-sourced *Latrunculia* spp. (95 MS-1-(1-10)) metabolite isolation scheme.

**(-)-(6R,8R)-Discorhabdin B (8)**

TFA salt brown oil; $[\alpha]_D = -420$ (c 0.05, MeOH); $^1$H NMR data were identical or consistent with that previously presented; HRFABMS $m/z$ [M+H]$^+$ 413.98985 (calcd for $C_{18}H_{13}^{79}$Br$N_3$SO$_2$, 413.99118), 415.99015 (calcd for $C_{18}H_{13}^{81}$Br$N_3$SO$_2$, 415.98914).

**(-)-(6R,8R)-Discorhabdin G*/I (15)**

TFA salt brown oil; $[\alpha]_D = -640$ (c 0.05, MeOH); $^1$H NMR data were identical or consistent with that previously presented; HRFABMS $m/z$ [M+H]$^+$ 336.08106 (calcd for $C_{18}H_{14}N_3$SO$_2$, 336.08067).
(+)-(1S,2S,6S,8R,7'S*)-Discorhabdin H (16)

TFA salt green oil; \([\alpha]_D = +40, [\alpha]_{378} = +80, [\alpha]_{546} = +180 \ (c \ 0.05, \text{MeOH}); \) UV (MeOH) \(\lambda_{\text{max}} \) 247 (21340), 288 (13150), 322 (8840), 395 (8500), 585 (870) nm; ECD (MeOH) \(\lambda (\Delta\varepsilon) \) 256 (+22.2), 282 (+4.1), 305 (+9.2), 334 (0), 365 (-13.1) 417 (0) nm; \(^1H\) NMR (CD\(_3\)OD-\(d_4\), 600 MHz) \(\delta \) 7.90 (1H, s, H-2'), 7.09 (1H, s, H-14), 6.19 (1H, s, H-4), 5.67 (1H, dd, \(J = 3.5, 1.1 \text{ Hz}, H-8\)), 4.46 (1H, d, \(J = 3.1 \text{ Hz}, H-2\)), 4.10 (1H, t, \(J = 7.9 \text{ Hz}, H-7\)), 4.07 (1H, ddd, \(J = 14.0, 7.3, 2.7 \text{ Hz}, H-17A\)), 4.01 (1H, d, \(J = 3.0 \text{ Hz}, H-1\)), 3.88 (1H, td, \(J = 13.6, 7.0 \text{ Hz}, H-17B\)), 3.75 (3H, s, H-9'), 3.43 (1H, dd, \(J = 15.3, 8.3 \text{ Hz}, H-6'A\)), 3.34 (1H, dd, \(J = 12.0, 3.6 \text{ Hz}, H-7A\)), 3.23 (1H, ddd, \(J = 15.3, 7.5 \text{ Hz}, H-6'B\)), 3.16 (1H, m, H-16A), 3.03 (1H, ddd, \(J = 16.8, 6.9, 2.7 \text{ Hz}, H-16B\)), 2.76 (1H, d, \(J = 12.1 \text{ Hz}\)); \(^13C\) NMR (CD\(_3\)OD-\(d_4\), 150 MHz) \(\delta \) 183.7 (C-3), 171.8 (C-5), 171.2 (C-8'), 167.3 (C-11), 150.4 (C-19), 148.4 (C-10), 141.3 (C-2'), 132.9 (C-4'), 128.7 (C-5'), 127.4 (C-14), 125.6 (C-12), 122.8 (C-21), 119.4 (C-15), 114.5 (C-4), 101.7 (C-20), 67.0 (C-2), 64.1 (C-8), 53.4 (C-7'), 52.8 (C-17), 51.2 (C-1), 47.3 (C-6), 39.4 (C-7), 33.2 (C-9'), 26.3 (C-6'), 20.7 (C-16); \(^1H\) NMR (DMSO-\(d_6\), 300 MHz) \(\delta \) 13.18 (1H, br s, NH-13), 10.95 (1H, br s, NH-9), 8.49 (3H, br s, NH-10'), 7.87 (1H, s, H-2'), 7.30 (1H, d, \(J = 2.1 \text{ Hz}, H-14\)), 6.31 (1H, s, H-4), 5.75 (1H, br s, H-8), 4.50 (1H, d, \(J = 3.1 \text{ Hz}, H-2\)), 4.01 (1H, m, H-7'), 3.93 (1H, d, \(J = 3.5 \text{ Hz}, H-1\)), 3.75 (2H, m, H-17), 3.64 (3H, s, H-9'), 3.25 (2H, m, H-6'), 3.06 (3H, m, H-7A/16), 2.64 (1H, d, \(J = 11.7 \text{ Hz}, H-7B\)); HRFABMS \(m/z \ [M+H]^+ \) 535.12372 (caled for C\(_{25}\)H\(_{23}\)N\(_6\)S\(_2\)O\(_4\), 535.12222).
(+)$^{578}$(1$S$,2$R$,6$S$,8$R$)-Discorhabdin L (17)

TFA salt dark green oil; [α]$^D_0$ = 0, [α]$^{578} = +200$ [α]$^{546} = +500$ (c 0.05, MeOH); ECD (MeOH) $λ$ (Δε) 256 (+22.1), 282 (+4.8), 302 (+10.1), 324 (0), 357 (-20.6), 409 (0), 439 (+4.9), 496 (0) nm; $^1$H NMR data were identical or consistent with that previously presented; $^{13}$C NMR (CD$_3$OD-$d_4$, 150 MHz) δ 184.9 (C-3), 171.6 (C-5), 167.5 (C-11), 150.4 (C-19), 148.6 (C-10), 127.3 (C-14), 125.6 (C-12), 122.8 (C-21), 119.3 (C-15), 114.1 (C-4), 101.9 (C-20), 68.6 (C-1), 67.9 (C-2), 63.7 (C-8), 52.9 (C-17), 48.5 (C-6), 37.5 (C-7), 20.7 (C-16); HRFABMS $m/z$ [M+H]$^+$ 352.07558 (calcd for C$_{18}$H$_{14}$N$_3$SO$_3$, 352.07559).

(+)-(6$R$,6'$R$)-Discorhabdin W (37)

TFA salt dark green oil; [α]$^D_0$ = +80 (c 0.05, MeOH); ECD (MeOH) $λ$ (Δε) 215 (0), 231 (+63.5), 251 (0), 255 (-4.0), 261 (0), 264 (+1.8), 271 (0), 295 (-9.3), 323 (0), 333 (+1.7), 342 (0), 383 (-6.7) nm; $^1$H and $^{13}$C NMR data were identical or consistent with that previously presented.
4.2.8 South African-Sourced *Cyclacanthia bellae* Metabolite Extraction and Isolation Procedures

Figure 4.10. Analytical HPLC trace (C₈ rocket column) of the n-butanol extract of South African-sourced *C. bellae*.

A portion of the n-butanol crude extract (155 mg) was subjected to Sephadex LH-20, C₁₈, C₈ and CN flash (MeOH, H₂O-TFA (0.05%)) chromatography, yielding discorhabdin G*/I (15) (0.15 mg, 0.097% crude weight, Rₜ 5.56 min), discorhabdin L (17) (2.25 mg, 1.5% crude weight, Rₜ 4.52 min), damirone B (47) (3.15 mg, 2% crude weight, Rₜ 4.78 min) and makaluvamine C (66) (12.40 mg, 8.0% crude weight, Rₜ 5.19 min). Metabolite isolation procedure is outlined in Scheme 4.10.
Scheme 4.10. South African-sourced *C. bellae* metabolite isolation scheme.

(-)-(6*R*,8*R*)-Discorhabdin G*/I (15)

TFA salt brown oil; \( [\alpha]_D = -400 (c 0.05, \text{MeOH}) \); ECD (MeOH) \( \lambda (\Delta \varepsilon) \) 222 (-3.8), 231 (0), 251 (+24.0), 281 (+2.5), 299 (+6.9), 320 (0), 357 (-10.7) 415 (0) nm; \(^1\)H NMR data were identical or consistent with that previously presented; HRFABMS m/z \([M]^+ 336.08014\) (calcd for C\(_{18}\)H\(_{14}\)N\(_3\)SO\(_2\), 336.08067).

(+)-578-(1*S*,2*R*,6*S*,8*R*)-Discorhabdin L (17)

TFA salt dark green oil; \( [\alpha]_D = 0, [\alpha]_{578} = +160, [\alpha]_{546} = +360 (c 0.05, \text{MeOH}) \); ECD (MeOH) \( \lambda (\Delta \varepsilon) \) 257 (+14.7), 281 (+2.7), 301 (+5.8), 325 (0), 359 (-12.9), 414 (0), 440 (+2.4), 483 (0) nm; \(^1\)H NMR data were identical or consistent with that previously presented; HRFABMS m/z \([M+H]^+ 352.07594\) (calcd for C\(_{18}\)H\(_{14}\)N\(_3\)SO\(_3\), 352.07559).
Damirone B (47)

![Image of Damirone B](image)

TFA salt red oil; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 12.42 (1H, br s, NH-1), 7.09 (1H, d, $J$ = 2.8 Hz, H-2), 5.14 (1H, s, H-6), 3.61 (2H, t, $J$ = 6.8 Hz, H-4), 3.04 (3H, s, H-9), 2.81 (2H, t, $J$ = 7.2 Hz, H-3); HRFABMS $m/z$ [M+H]$^+$ 203.08210 (calculated for C$_{11}$H$_{11}$N$_2$O$_2$, 203.08205).

Makaluvamine C (66)

![Image of Makaluvamine C](image)

TFA salt red oil; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$ 13.05 (1H, br s, NH-1), 9.33 (1H, br s, NH-9A), 8.67 (1H, br s, NH-9B), 7.30 (1H, d, $J$ = 2.8 Hz, H-2), 5.67 (1H, s, H-6), 3.91 (2H, t, $J$ = 7.6 Hz, H-4), 3.32 (3H, s, H-10), 2.93 (2H, t, $J$ = 7.2 Hz, H-3); $^{13}$C NMR (DMSO-$d_6$, 400 MHz) $\delta$ 167.4 (C-8), 156.5 (C-7), 155.7 (C-5a), 126.6 (C-2), 123.2 (C-8b), 123.4 (C-2a), 117.9 (C-8a), 85.4 (C-6), 52.6 (C-4), 39.0 (C-10), 18.8 (C-3); $^1$H NMR (CD$_3$OD-$d_4$, 400 MHz) $\delta$ 7.12 (1H, s, H-2), 5.74 (1H, s, H-6), 3.95 (2H, t, $J$ = 7.3 Hz, H-4), 3.39 (3H, s, H-10), 3.01 (2H, t, $J$ = 7.7 Hz, H-3); HRFABMS $m/z$ [M+H]$^+$ 202.09810 (calcld for C$_{11}$H$_{12}$N$_3$O, 202.09804).
4.2.9 Enantiomeric Purity of Discorhabdin B (8)

4.2.9.1 Discorhabdin B (8) (+) and (-) Enantiomers Racemic Mixture

Rac-8 was prepared by titrating the (-)-8 enantiomer at a concentration of 0.5 mg/mL into a 1.0 mL solution of the (+)-8 enantiomer at a concentration of 0.5 mg/mL. Optical rotation measurements were taken at 0.2 mL increments until $[\alpha]_D = 0$.

Rac-8: TFA salt purple-brown oil; $[\alpha]_D = 0$ (c 0.05, MeOH); $^1$H NMR (D$_2$O, 400 MHz) $\delta$ 7.96 (1H, s, H-1), 7.19 (1H, s, H-14), 6.32 (1H, s, H-4), 5.75 (1H, dd, $J = 3.8, 0.9$ Hz, H-8), 3.91 (1H, m, H-17A), 3.67 (1H, m, H-17B), 2.84 (2H, m, H-16), 2.80 (1H, d, $J = 11.7$ Hz, H-7A), 2.59 (1H, dd, $J = 12.1, 4.0$ Hz, H-7B).

$\beta$-CD (1.1 mg, 1.0 µmol) was added to a solution of Rac-8 (0.5 mg, 0.95 µmol) in D$_2$O (0.5 mL) and the $^1$H NMR spectrum of the mixture recorded. At 1:1, 1:2 and 1:3 molar ratio of rac-8:$\beta$-CD no doubling up of discorhabdin B-specific resonances was observed.

4.2.9.2 Semi-Synthetic (+)-(6S)-Discorhabdin U (29)

Wellington-sourced (+)-(6S,8S)-Discorhabdin B (8) TFA salt (2.1 mg, 4.0 µmol) was dissolved in trimethylphosphate (1 mL) to which an excess of K$_2$CO$_3$ (6 mg) was added. The reaction mixture was kept at reflux at 90 ºC for 3 hrs under N$_2$. The crude reaction mixture was loaded on a C$_8$ flash column and the product eluted with 50% MeOH, 50% H$_2$O-TFA (0.05%) yielding (+)-(6S)-discorhabdin U (29) (1.7 mg, 3.0 µmol, 75% yield).

TFA salt dark green oil; $[\alpha]_D = +222$, $[\alpha]_{578} = +180$, $[\alpha]_{546} = +220$ (c 0.05, MeOH); IR (smear) $\nu_{max}$ 3296, 2923, 1680, 1651, 1489, 1357, 1198, 1129 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ ($\epsilon$) 203 (15770), 247 (7340), 309 (4640), 431 (2290), 603 (700) nm; ECD (MeOH) $\lambda$ ($\Delta\epsilon$) 215
4.2.9.3 Semi-Synthetic (--)(6R)-Discorhabdin U (29)

Doubtful Sound-sourced (--)(6R,8R)-Discorhabdin B (8) TFA salt (1.1 mg, 2.1 µmol) was dissolved in trimethylphosphate (1 mL) to which an excess of K_2CO_3 (5 mg) was added. The reaction mixture was kept at reflux at 90 °C for 3 hrs under N_2. The crude reaction mixture was loaded on a C_8 flash column and the product eluted with 50% MeOH, 50% H_2O-TFA (0.05%) yielding (--)(6R)-discorhabdin U (29) (0.8 mg, 1.4 µmol, 70% yield).

TFA salt dark green oil; [α]_D = -220 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 215 (0), 228 (+18.1), 242 (0), 251 (-7.5), 260 (0), 267 (+3.8), 275 (0), 288 (-4.8), 307 (-0.3), 327 (-6.8), 353 (-1.4), 377 (-3.6), 436 (0) nm; ^1^H NMR (DMSO-d_6, 600 MHz) δ 10.70 (1H, br s, NH-9), 8.84 (1H, br s, NH-18), 7.77 (1H, s, H-1), 7.41 (1H, br s, H-14), 6.54 (1H, dd, J = 7.5, 4.8 Hz, H-8), 6.07 (1H, s, H-4), 4.71 (1H, d, J = 6.6 Hz, H-7), 3.94 (3H, s, H-22), 3.77 (2H, t, J = 7.9 Hz, H-17), 2.84 (2H, m, H-16), 2.42 (3H, s, H-23); ^13^C NMR (DMSO-d_6, 100 MHz) δ 174.1 (C-3), 168.3 (C-5), 165.8 (C-11), 156.7 (C-19), 147.8 (C-1), 144.4 (C-10), 131.6 (C-14), 124.8 (C-8), 123.3 (C-2), 122.9 (C-12), 121.6 (C-21), 118.9 (C-15), 117.5 (C-4), 114.4 (C-7), 95.7 (C-20), 48.3 (C-6), 44.4 (C-17), 36.2 (C-22), 17.7 (C-16), 14.7 (C-23); HRFABMS m/z [M+H]^+ 442.02189 (calcd for C_{20}H_{17}^{79}BrN_3O_2S 442.02248) 444.01979 (calcd for C_{20}H_{17}^{81}BrN_3O_2S, 444.02044).
(C-10), 131.5 (C-14), 124.7 (C-8), 123.3 (C-2), 122.9 (C-12), 121.6 (C-21), 118.9 (C-15),
117.5 (C-4), 114.4 (C-7), 95.7 (C-20), 48.3 (C-6), 44.4 (C-17), 36.1 (C-22), 17.6 (C-16),
14.6 (C-23); HRFABMS m/z [M+H]^+ 442.02209 (calcd for C_{20}H_{17}^{79}\text{Br}N_3O_2S 442.02248)
444.02064 (calcd for C_{20}H_{17}^{81}\text{Br}N_3O_2S, 444.02044).

4.2.9.4 Discorhabdin U (29) (+) and (-) Enantiomers Racemic Mixture

Rac-29 was prepared by titrating the (-)-29 enantiomer at a concentration of 0.5 mg/mL
into a 1.0 mL solution of the (+)-29 enantiomer at a concentration of 0.5 mg/mL. Optical
rotation measurements were taken at 0.2 mL increments until [\alpha]_D = 0.

Rac-29: TFA salt dark green oil; [\alpha]_D = 0 (c 0.05, MeOH); \textsuperscript{1}H NMR (D_2O, 400 MHz) \delta
7.84 (1H, s, H-1), 7.10 (1H, s, H-14), 6.58 (1H, d, J = 7.6 Hz, H-8), 6.21 (1H, s, H-4), 3.91
(3H, s, H-22), 3.78 (2H, t, J = 8.4 Hz, H-17), 2.86 (2H, t, J = 7.6 Hz H-16), 2.46 (3H, s,
H-23).

\beta-CD (1.1 mg, 1.0 \mu mol) was added to a solution of Rac-29 (0.5 mg, 0.90 \mu mol) in D_2O
(0.5 mL) and the \textsuperscript{1}H NMR spectrum of the mixture recorded. At 1:1, 1:2 and 1:3 molar
ratio of rac-29;\beta-CD doubling up of discorhabdin U-specific resonances for H-1, H-4, H-
16 and H-23 was observed.

4.2.10 Enantiomeric Purity of Discorhabdin W (37)

4.2.10.1 Discorhabdin W (37) (+) and (-) Enantiomers Racemic Mixture

Rac-37 was prepared by titrating the (-)-37 enantiomer at a concentration of 0.5 mg/mL
into a 1.0 mL solution of the (+)-37 enantiomer at a concentration of 0.5 mg/mL. Optical
rotation measurements were taken at 0.2 mL increments until [\alpha]_D = 0.

Rac-37: TFA salt dark green oil; [\alpha]_D = 0 (c 0.05, MeOH); \textsuperscript{1}H NMR (D_2O, 400 MHz) \delta
7.92 (1H, s, H-1), 7.20 (1H, s, H-14), 6.69 (1H, d, J = 7.6 Hz, H-8), 6.15 (1H, s, H-4), 4.64
(1H, d, J = 7.5 Hz, H-7), 3.98 (1H, m, H-17A), 3.84 (1H, m, H-17B), 3.03 (2H, m, H-16).
β-CD (0.5 mg, 0.44 µmol) was added to a solution of Rac-37 (0.5 mg, 0.45 µmol) in D₂O (0.5 mL) and the ¹H NMR spectrum of the mixture recorded. At a 1:1 molar ratio of rac-37:β-CD no doubling up of discorhabdin W-specific resonances was observed.

4.2.10.2 (-)-Discorhabdin W (37)-Sourced Semi-Synthetic (+)-(6S,8S)-Discorhabdin B (8)

Naturally-occurring (-)-(6S,6'S)-discorhabdin W (37) TFA salt (2.5 mg, 2.4 µmol) was dissolved in MilliQ water (2 mL) to which an aqueous solution of TCEP (2.5 µmol) was added. The reaction mixture was stirred at room temperature in air for five minutes at which time the analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the solid purified by C₁₈ flash chromatography (MeOH, H₂O-TFA (0.05%)), yielding (+)-(6S,8S)-discorhabdin B (8) (1.7 mg, 3.1 µmol, 66% yield).

TFA salt purple-brown oil; [α]D = +340 (c 0.05 MeOH); UV (MeOH) \( \lambda_{\text{max}} (\varepsilon) \) 208 (14760), 248 (17440), 308 (6680), 359 (6300), 558 (700) nm; ECD (MeOH) \( \lambda (\Delta\varepsilon) \) 209 (-19.5), 223 (0), 232 (+4.8), 241 (0), 257 (-19.4), 281 (-4.9), 303 (-10.7), 320 (0), 348 (+15.4) nm; ¹H NMR (CD₃OD-\( d_4 \), 300 MHz) \( \delta \) 7.83 (1H, s, H-1), 7.21 (1H, s, H-14), 6.25 (1H, s, H-4), 5.70 (1H, dd, \( J = 3.9, 1.2 \) Hz, H-8), 3.94 (1H, s, H-17A), 3.83 (1H, m, H-17B), 2.92 (2H, m, H-16), 2.78 (1H, d, \( J = 11.7 \) Hz, H-7A), 2.54 (1H, dd, \( J = 11.8, 3.8 \) Hz, H-7B); HRFABMS m/z [M+H]⁺ 413.99089 (calcd for C₁₈H₁₃⁷⁹BrN₃SO₂, 413.99118), 415.98958 (calcd for C₁₈H₁₃⁸¹BrN₃SO₂, 415.98914).

4.2.10.3 (+)-Discorhabdin W (37)-sourced Semi-Synthetic (-)-(6R,8R)-Discorhabdin B (8)

Naturally-occurring (+)-(6R,6'R)-discorhabdin W (37) TFA salt (1.50 mg, 1.4 µmol) was dissolved in MilliQ water (1 mL) to which an aqueous solution of TCEP (1.4 µmol) was added. The reaction mixture was stirred at room temperature in air for five minutes at which time analytical HPLC indicated complete consumption of starting material. The
product was purified by C₈ flash chromatography (MeOH, H₂O-TFA (0.05%)), yielding (-)-(6R,8R)-discorhabdin B (8) (1.40 mg, 2.7 μmol, 95% yield).

TFA salt purple-brown oil; [α]D = -720 (c 0.05, MeOH); ¹H NMR (CD₃OD-d₄, 400 MHz) δ 7.84 (1H, s, H-1), 7.21 (1H, s, H-14), 6.25 (1H, s, H-4), 5.70 (1H, dd, J = 4.0, 1.2 Hz, H-8), 3.94 (1H, m, H-17A), 3.82 (1H, m, H-17B), 2.92 (2H, m, H-16), 2.78 (1H, d, J = 11.2 Hz, H-7A), 2.54 (1H, dd, J = 11.6, 3.9 Hz, H-7B); HRFABMS m/z [M+H]+ 413.99036 (calcd for C₁₈H₁₃⁷⁹BrN₃SO₂, 413.99118), 415.98948 (calcd for C₁₈H₁₃⁸¹BrN₃SO₂, 415.98914).

4.2.11 Absolute Stereochemistry Calculations

DFT calculations, using Gaussian 03, were employed to optimize the ground state geometries at 298K in the gas phase at B3LYP/6-31G** level by using default convergence. Harmonic frequencies were calculated to confirm the minima. The geometries of the ground states were used to calculate the ECD at the same level by using time dependent density functional theory (TDDFT). The calculated excitation energies ΔEᵢ (in nm) and rotatory strength (Rᵢ) were then simulated into ECD curves by using the Gaussian function:

\[ \Delta \epsilon(E) = \frac{1}{2.297 \times 10^{-10}} \frac{1}{\sqrt{2\pi}\sigma} \sum_i \Delta E_i R_i e^{-\left(\frac{E - \Delta E_i}{\sigma}\right)^2} \]

where σ is the width of the band at 1/e height and ΔEᵢ and Rᵢ are the excitation energies and rotatory strengths for transition i, respectively. In the current work a value of σ = 0.15 eV and rotatory strength in the dipole length form (Rlen) were used. Solvent effects on the ECD were considered by using Self-Consistent Reaction Field method (SCRF) with COnductor-like continuum Solvent MOdel (COSMO) in MeOH (dielectric constant ε = 32.63) at B3LYP-SCRF/6-31G**/B3LYP/6-31G** level.
4.3 Work on Chapter Three

4.3.1 Semi-Synthetic Discorhabdin P (24)

Discorhabdin C (6) TFA salt (70.7 mg, 0.12 mmol) was dissolved in dry acetone (20 mL) to which CH₃I (100µL, 1.61 mmol) and K₂CO₃ (40 mg) were added. The reaction mixture was kept at reflux at 70 °C for 6 hrs under N₂. The solvent was removed in vacuo and the solid purified by C₁₈ flash (MeOH, H₂O-TFA (0.05%)) and Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding discorhabdin P (24) (30.7 mg, 0.052 mmol, 42% yield).

Discorhabdin C (6) TFA salt (5.5 mg, 9.5 µmol) was dissolved in trimethylphosphate (1 mL) to which an excess of K₂CO₃ (5 mg) was added. The reaction mixture was kept at reflux at 90 °C for 2 hrs under N₂. The crude reaction mixture was loaded on a C₁₈ flash column and the product eluted with 50% MeOH, 50% H₂O-TFA (0.05%) yielding discorhabdin P (24) (4.92 mg, 8.3 µmol, 87 % yield).

TFA salt purple oil; ¹H NMR (DMSO-ｄ₆, 400 MHz) δ 10.13 (1H, br s, NH-9), 8.18 (1H, br s, NH-18), 7.71 (2H, s, H-1/5), 7.41 (1H, s, H-14), 3.92 (3H, s, H-22), 3.66 (4H, m, H-8/17), 2.79 (2H, t, J = 7.6 Hz, H-16), 2.01 (2H, t, J = 5.2 Hz, H-7); ¹³C NMR (DMSO-ｄ₆, 100 MHz) δ 171.3 (C-3), 165.7 (C-11), 152.6 (C-19), 151.6 (C-10), 151.1 (C-1/5), 132.0 (C-14), 123.3 (C-21), 122.6 (C-2/4), 122.4 (C-12), 119.2 (C-15), 91.8 (C-20), 44.6 (C-6), 43.4 (C-17), 38.2 (C-8), 36.0 (C-22), 33.6 (C-7), 17.9 (C-16); ¹H NMR (CD₃OD-ｄ₄, 400 MHz) δ 7.70 (2H, s, H-1/5), 7.16 (1H, s, H-14), 3.97 (3H, s, H-23), 3.74 (4H, m, H-8/17), 2.85 (2H, t, J = 7.6 Hz, H-16), 2.08 (2H, t, J = 5.7 Hz, H-7); HRFABMS m/z [M+H]+ 475.96128 (calcd for C₁₉H₁₆⁷⁹Br₂N₃O₂, 475.96092), 477.95950 (calcd for C₁₉H₁₆⁷⁹Br₂N₃O₂, 477.95888), 479.95799 (calcd for C₁₉H₁₆⁸₁Br₂N₃O₂, 479.95683).
4.3.2 N-Alkyl Analogues of Discorhabdin B

4.3.2.1 N-13 Demethyl Discorhabdin U (141)

(+)-(6S,8S)-Discorhabdin B (8) TFA salt (5.5 mg, 10.4 µmol) was dissolved in dry acetone (5.5 mL) to which CH₃I (1.3 µL, 20.7 µmol) and K₂CO₃ (15 mg) were added. The reaction mixture was kept at reflux at 80 ºC for 2 h under N₂ at which time the analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the product purified by C₁₈ and C₈ flash (MeOH, H₂O-TFA (0.05%)) and Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding 141 (4.6 mg, 8.5 µmol, 82% yield).

TFA salt dark green oil; [α]D = +120 (c 0.05, MeOH); IR (smear) νmax 3129, 2925, 1672, 1664, 1616, 1552, 1481, 1415, 1355, 1176, 1120, 1009 cm⁻¹; UV (MeOH) λmax (ε) 204 (4790), 247 (4030), 312 (2590), 433 (1280), 609 (360) nm; ECD (MeOH) λ (Δε) 215 (0), 225 (-14.1), 240 (0), 250 (+9.8), 262 (0), 268 (-1.5), 271 (0), 285 (+5.0), 307 (+1.8), 327 (+6.4), 350 (+0.2), 386 (+2.7), 442 (0) nm; ¹H NMR (CD₃OD–d₄, 400 MHz) 7.78 (1H, s, H-1), 7.22 (1H, s, H-14), 6.54 (1H, d, J = 7.5 Hz, H-8), 6.15 (1H, s, H-4), 4.73 (1H, d, J = 7.5 Hz, H-7), 3.86 (2H, td, J = 8.2, 3.0 Hz, H-17), 2.94 (2H, td, J = 7.5, 3.0 Hz, H-16), 2.47 (3H, s, H-22); ¹³C NMR (CD₃OD–d₄, 100 MHz) δ 176.2 (C-3), 171.8 (C-5), 166.8 (C-11), 160.4 (C-19), 150.0 (C-1), 146.5 (C-10), 127.5 (C-14), 125.7 (C-8/12), 124.8 (C-2), 123.3 (C-15), 121.1 (C-21), 118.4 (C-4), 115.9 (C-7), 97.3 (C-20), 50.2 (C-6), 46.0 (C-17), 19.3 (C-16), 15.2 (C-22); HRFABMS m/z [M+H]⁺ 428.00735 (calcd for C₁₉H₁₅⁷⁹BrN₃O₂S 428.00683), 430.0067 (calcd for C₁₉H₁₅⁸¹BrN₃O₂S 430.00479).
**4.3.2.2 Discorhabdin U (29)**

(+)-(6S,8S)-Discorhabdin B (8) TFA salt (15.4 mg, 29.2 µmol) was dissolved in dry acetone (2 mL) to which CH3I (20 µL, 318 µmol) and K2CO3 (20 mg) were added. The reaction mixture was kept at reflux at 80 °C for 3 h under N2 at which time analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the solid purified by C18, C8 (MeOH, H2O-TFA (0.05%)) and Sephadex LH-20 (MeOH (0.05% TFA)) flash chromatography, yielding (+)-(6S)-discorhabdin U (29) (6.3 mg, 11.3 µmol, 39% yield) and (+)-(6S)-N-13 demethyl discorhabdin U (141) (3.6 mg, 6.6 µmol, 22% yield).

Alternative route via trimethylphosphate and full chiroptical, NMR and mass spectrometry data for the TFA salt form of (+)-(6S)-29 are outlined in section 4.2.6.1.

Free base dark brown oil; 1H NMR (CDCl3, 300 MHz) δ 7.59 (1H, s, H-1), 6.64 (1H, s, H-14), 6.39 (1H, d, J = 7.6 Hz, H-8), 5.90 (1H, s, H-4), 4.11 (1H, d, J = 7.5 Hz, H-7), 3.96 (2H, m, H-17), 3.95 (3H, s, H-22), 2.60 (2H, t, J = 8.1 Hz, H-16), 2.31 (3H, s, H-23).

**4.3.2.3 Dibenzyl Discorhabdin U (142)**

(+)-(6S,8S)-Discorhabdin B (8) TFA salt (8.5 mg, 16.1 µmol) was dissolved in dry acetone (2 mL) to which benzyl bromide (20 µL, 168.2 µmol) and K2CO3 (20 mg) were added. The reaction mixture was kept at reflux at 80 °C for 3 hrs under N2 at which time analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the solid purified by C18 flash (MeOH, H2O-TFA (0.05%))
chromatography, yielding (-)-(6S)-dibenzyl discorhabdin U (142) (5.0 mg, 7.1 µmol, 44% yield).

TFA salt dark green oil; [α]D = -80 (c 0.05, MeOH); IR (smear) νmax 2917, 2855, 1647, 1609, 1490, 1436, 1353, 1166, 1124, 1005 cm⁻¹; UV (MeOH) λmax (ε) 205 (36470), 249 (18680), 312 (12580), 432 (6210) 612 (2130) nm; ECD (MeOH) λ (Δε) 216 (0), 226 (-17.1), 241 (0), 253 (+8.3), 263 (0), 268 (-3.8), 274 (0), 292 (+5.8), 310 (+0.2), 365 (+5.5), 353 (+1.1), 384 (+2.9), 436 (0) nm; ¹H NMR (CD3OD-d4, 600 MHz) δ 7.72 (1H, s, H-1), 7.34 (1H, s, H-14), 7.26–7.40 (10H, m, aryl), 6.56 (1H, d, J = 7.5 Hz, H-8), 6.29 (1H, d, J = 14.7 Hz, H-22A), 5.48 (1H, d, J = 14.7 Hz, H-22B), 4.72 (1H, d, J = 7.5 Hz, H-7), 4.25 (2H, s, H-27), 3.75 (2H, m, H-17), 2.91 (2H, td, J = 7.7, 3.8 Hz H-16); ¹³C NMR (CD3OD-d4, 150 MHz) δ 176.2 (C-3), 169.6 (C-5), 167.2 (C-11), 159.6 (C-19), 149.7 (C-1), 146.5 (C-10), 137.5 (C-23), 136.4 (C-28), 131.5 (C-14), 129.0–130.1 (aryl CH), 125.8 (C-8), 124.8 (C-2), 124.5 (C-12), 123.7 (C-21), 121.2 (C-15), 119.4 (C-4), 115.6 (C-7), 97.4 (C-20), 53.6 (C-22), 50.1 (C-6), 45.7 (C-17), 37.1 (C-27), 19.3 (C-16); HRFABMS m/z [M+H]+ 594.08652 (calcd for C32H25⁷⁹BrN3O2S, 594.08509), 596.08357 (calcd for C32H25⁸¹BrN3O2S, 596.08304).

4.3.3 Discorhabdin W (37) as a Source of N-13 Alkyl Analogues of Discorhabdin B (8)

4.3.3.1 Semi-Synthetic (-)-(6S,6'S)-Discorhabdin W (37)

(+)-(6S,8S)-Discorhabdin B (8) TFA salt (12.5 mg, 23.7 µmol) was dissolved in methanol (50 mL) and irradiated with a sunlamp for six hours. In order for the reaction to remain at room temperature the sunlamp was turned on for 45 min, with 60 min off intervals. After six hours of total sunlamp time, analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the product purified by Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding (-)-(6S,6'S)-discorhabdin W (37) (5.0 mg, 4.7 µmol, 40% yield).
(+)-(6S,8S)-Discorhabdin B (8) TFA salt (9.3 mg, 17.6 µmol) was dissolved in methanol (50 mL) and left on a benchtop for two weeks, at which time the analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the product purified by Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding (-)-(6S,6'S)-discorhabdin W (37) (2.1 mg, 2.0 µmol, 23% yield).

(+)-(6S,8S)-Discorhabdin B (8) free base (2.2 mg, 5.3 µmol) was dissolved in dry DMF (2 mL) to which an excess of K₂CO₃ (5 mg) was added. The reaction was kept at reflux at 85 ºC for 3 hrs 30 min under N₂. Crude reaction mixture was loaded on a C₈ flash column, the product eluted with 50% MeOH, 50% H₂O-TFA (0.05%) and further purified by Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding (-)-(6S,6'S)-discorhabdin W (37) (0.9 mg, 0.9 µmol, 34% yield).

TFA salt dark green oil; [α]D = -80 (c 0.05, MeOH); ECD (MeOH) λ (Δε) 213 (0), 232 (-42.6), 248 (0), 253 (+3.8), 260 (0), 266 (-1.7), 271 (0), 293 (+7.4), 314 (0), 328 (-4.0), 350 (0), 380 (+3.4), 418 (0) nm; ¹H NMR (CD₃OD-d₄, 400 MHz) δ 7.83 (1H, s, H-1), 7.23 (1H, s, H-14), 6.67 (1H, d, J = 7.2 Hz, H-8), 6.33 (1H, s, H-4), 4.74 (1H, d, J = 7.6 Hz, H-7), 4.12 (1H, m, H-17A), 3.89 (1H, m, H-17B), 3.11 (1H, m, H-16A), 3.07 (1H, m, H-16B); ¹³C NMR (CD₃OD-d₄, 100 MHz) δ 176.4 (C-3), 166.5 (C-11), 162.8 (C-5), 160.3 (C-19), 150.7 (C-1), 147.1 (C-10), 127.6 (C-14), 127.0 (C-8), 125.8 (C-12), 125.0 (C-2), 123.3 (C-21), 121.4 (C-15), 120.9 (C-4), 114.1 (C-7), 95.9 (C-20 from HMBC), 49.9 (C-6), 46.7 (C-17), 19.3 (C-16); HRESIMS m/z [M+H]+ 824.9588 (calcd for C₃₆H₂₃⁷⁹Br₂N₆S₂O₄, 824.9589), 826.9574 (calcd for C₃₆H₂₃⁸¹BrN₆S₂O₄, 826.9568), 828.9556 (calcd for C₃₆H₂₃⁸¹Br₂N₆S₂O₄, 828.9548).
4.3.3.2 N-13 Methyl Discorhabdin B (144)

N-13-Dimethyl Discorhabdin W (143)

\[(6S,6'S)-143\]

\((-\)-(6\(S\),6\(S\))-Discorhabdin W (37) TFA salt (6.1 mg, 5.8 \(\mu\)mol) was dissolved in trimethylphosphate (0.75 mL) to which an excess of K\(_2\)CO\(_3\) (5 mg) was added. The reaction mixture was kept at reflux at 80 °C for 2 hrs under N\(_2\), after which the reaction was cooled to room temperature. Crude reaction mixture was loaded on a C\(_{18}\) flash column and two green bands eluted with 15% MeOH, 85% H\(_2\)O-TFA (0.05%) and 50% MeOH, 50% H\(_2\)O-TFA (0.05%), yielding \((+)-(6\(S\))-discorhabdin U (29) (2.9 mg, 5.1 \(\mu\)mol, 44% yield) and \((6\(S\),6\(S\))-N-13-dimethyl discorhabdin W (143) (3.6 mg, 3.3 \(\mu\)mol, 57% yield) respectively.

TFA salt green oil; \(^1\)H NMR (CD\(_3\)OD-\(d_4\), 400 MHz) \(\delta\) 7.84 (1H, s, H-1), 7.20 (1H, s, H-14), 6.67 (1H, d, \(J=7.6\) Hz, H-8), 6.31 (1H, s, H-4), 4.72 (1H, br s, H-7), 4.08 (1H, m, H-17A), 4.00 (3H, s, H-22), 3.87 (1H, m, H-17B), 3.02 (2H, m, H-16); LRESI m/z [M+H]\(^+\) 852.24/854.23/856.24.

N-13-Methyl Discorhabdin B (144)

\[(+)-(6\(S\),8\(S\))-144\]

\((6\(S\),6\(S\))-N-13-dimethyl discorhabdin W (143) TFA salt (3.5 mg, 3.2 \(\mu\)mol) was dissolved in MilliQ water (1 mL), to which a solution of TCEP (3.5 \(\mu\)mol) was added. The reaction mixture was stirred at room temperature in air for five minutes at which time the analytical
HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the solid purified by C18 flash chromatography (MeCN, H2O-TFA (0.05%)), yielding (+)-(6S,8S)-N-13-methyl discorhabdin B (144) (1.8 mg, 3.3 µmol, 52% yield, 30% overall yield).

TFA salt purple-brown oil; [α]D = +440 (c 0.05, MeOH); UV (MeOH) λmax (ε) 203 (14730), 250 (18440), 307 (6510), 357 (7190), 399 (shoulder 5300), 558 (720) nm; ECD (MeOH) λmax (Δε) 213 (-24.0), 225 (0), 235 (+5.7), 241 (0), 257 (-26.2), 281 (-5.9), 304 (-13.6), 321 (0), 349 (+21.7), 408 (0) nm; 1H NMR (CD3OD-d4, 600 MHz) δ 7.82 (1H, s, H-1), 7.17 (1H, s, H-14), 6.24 (1H, s, H-4), 5.70 (1H, dd, J = 3.6, 1.2 Hz, H-8), 3.96 (3H, s, H-22), 3.90 (1H, m, H-17A), 3.78 (1H, m, H-17B), 2.88 (2H, m, H-16), 2.77 (1H, d, J = 12.0 Hz, H-7A), 2.54 (1H, dd, J = 11.4, 3.6 Hz, H-7B); 13C NMR (CD3OD-d4, 150 MHz) δ 175.8 (C-3), 172.7 (C-5), 166.7 (C-11), 156.3 (C-19), 153.0 (C-10), 147.7 (C-1), 132.5 (C-14), 129.8 (C-2), 125.0 (C-12), 124.2 (C-21), 121.6 (C-15), 119.6 (C-4), 98.7 (C-20), 62.6 (C-8), 53.0 (C-6), 46.1 (C-17), 43.6 (C-7), 36.7 (C-22), 19.1 (C-16); HRFABMS m/z [M+H]+ 428.00676 (calcd for C19H1579BrN3SO2, 428.00683), 430.00411 (calcd for C19H1581BrN3SO2, 430.00479).

4.3.3.3 N-13 Benzyl Discorhabdin B (148)

N-13-Dibenzyl Discorhabdin W (147)

(-)-(6S,6'S)-Discorhabdin W (37) TFA salt (4.8 mg, 4.5 µmol) was dissolved in dry acetone (2 mL) to which BzBr (100 µL, 1.04 mmol) and K2CO3 (5 mg) were added. The reaction mixture was kept at reflux at 75 ºC for 3 hrs under N2 at which time analytical HPLC indicated complete consumption of starting material. The solvent was removed in
vacuo and the product purified by C$_{18}$ flash chromatography (MeOH, H$_2$O-TFA (0.05%)), yielding (6S,6'S) N-13-dibenzyl discorhabdin W (147) (2.8 mg, 2.3 µmol, 50% yield).

TFA salt dark green oil; $^1$H NMR (CD$_3$OD-$d_4$, 400 MHz) $\delta$ 7.82 (1H, s, H-1), 7.34 (6H, m, aryl), 6.64 (1H, d, $J$ = 7.2 Hz, H-8), 6.27 (1H, br s, H-4), 5.55 (1H, d, $J$ = 14.8 Hz, H-22A), 5.50 (1H, d, $J$ = 14.8 Hz, H-22B), 4.66 (1H, br s, H-7), 4.14 (1H, m, H-17A), 3.85 (1H, m, H-17B), 3.06 (1H, m, H-16A), 2.95 (1H, m, H-16B); HRESIMS $m/z$ [M+H]$^+$ 1005.0619 (calcd for C$_{50}$H$_{35}$Br$_2$N$_6$S$_2$O$_4$, 1005.0522), 1007.0606 (calcd for C$_{50}$H$_{35}$Br$_{81}$BrN$_6$S$_2$O$_4$, 1007.0507) 1009.0605 (calcd for C$_{50}$H$_{35}$Br$_2$N$_6$S$_2$O$_4$ 1009.0499).

**N-13-Benzyl Discorhabdin B (148)**

(6S,8'S)-N-13-dibenzyl discorhabdin W (147) TFA salt (2.5 mg, 2.0 µmol) was dissolved in 2 mL of 1:1 mixture of methanol/MilliQ water to which TCEP (4.9 µmol) was added. The reaction mixture was stirred at room temperature in air for five minutes. The solvent was removed in vacuo and the product purified by C$_{18}$ flash (MeOH, H$_2$O-TFA (0.05%)) and Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding (+)-(6S,8'S)-N-13-benzyl discorhabdin B (148) (0.9 mg, 1.4 µmol, 34% yield; 17% overall yield).

TFA salt purple-brown oil; [α]$_D$ = +400 (c 0.05, MeOH); UV (MeOH) $\lambda_{max}$ ($\varepsilon$) 204 (16910), 250 (12080), 305 (4890), 358 (4410), 414 (3330), 555 (750) nm; ECD (MeOH) $\lambda_{max}$ ($\Delta\varepsilon$) 232 (+1.3), 243 (0), 259 (-8.3), 285 (-0.8), 305 (-3.7), 321 (0), 348 (+6.9), 412 (+0.5) nm; $^1$H NMR (CD$_3$OD-$d_4$, 400 MHz) $\delta$ 7.80 (1H, s, H-1), 7.34 (1H, s, H-14), 7.32-7.34 (5H, m, aryl), 6.24 (1H, s, H-4), 5.69 (1H, dd, $J$ = 4.0, 1.2 Hz, H-8), 5.51 (1H, d, $J$ = 14.8 Hz, H-22A), 5.46 (1H, d, $J$ = 14.8 Hz, H-22B), 3.91 (1H, m, H-17A), 3.78 (1H, m, H-17B), 2.89 (2H, m, H-16), 2.77 (1H, dd, $J$ = 11.6 Hz, H-7A), 2.53 (dd, $J$ = 11.6, 4.0 Hz, H-7B); $^{13}$C NMR (CD$_3$OD-$d_4$, 150 MHz) $\delta$ 175.8 (C-3), 172.6 (C-5), 156.3 (C-19 from
HMBC), 153.2 (C-10 from HMBC), 147.6 (C-1), 137.7 (C-23 from HMBC), 131.8 (C-14), 129.0-130.0 (CH aryl), 125.0 (C-21 from HMBC), 124.5 (C-12 from HMBC), 122.0 (C-15), 119.7 (C-4), 99.0 (C-20), 62.6 (C-8), 53.6 (C-22), 53.0 (C-6 from HMBC), 46.0 (C-17), 43.6 (C-7), 19.1 (C-16); HRFABMS m/z [M+H]^+ 504.03693 (calcd for C_{25}H_{19}^{79}BrN_{3}SO_{2}, 504.03813), 506.03601 (calcd for C_{25}H_{19}^{81}BrN_{3}SO_{2}, 506.03609).

4.3.4 Semi-Synthetic Discorhabdin B (8) and U (29) Analogues

4.3.4.1 Semi-Synthetic (+)-(6S,8S)-Discorhabdin Q (25)

(+)-(6S,8S)-Discorhabdin B (8) TFA salt (6.0 mg, 11.4 µmol) was dissolved in aqueous acetone (5% H_{2}O) (6 mL) to which an excess of K_{2}CO_{3} (20 mg) was added. The reaction mixture was kept at reflux at 75 ºC for 1 hr under N_{2} at which time analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the solid purified by C_{8} flash (MeOH, H_{2}O-TFA (0.05%)) chromatography, and then washed with DCM to yield (+)-(6S,8S)-discorhabdin Q (25) free base (1.3 mg, 3.2 µmol, 28% yield).

Free base orange solid; [\alpha]_{D} = +400 (c 0.025, MeOH); ECD (MeOH) \lambda(\Delta\varepsilon) 237 (-5.0) 249 (-1.2), 268 (-5.5), 283 (0), 296 (+2.3), 314 (0), 336 (-3.7), 352 (0), 389 (+4.6) nm; \textsuperscript{1}H NMR (acetone-d_{6}, 400 MHz) \delta 8.27 (1H, d, J = 5.8 Hz, H-17), 8.23 (1H, s, H-1), 8.17 (1H, s, H-14), 7.51 (1H, d, J = 5.6 Hz, H-17), 5.94 (1H, s, H-4), 5.92 (1H, m, H-8), 3.03 (1H, d, J = 11.5 Hz, H-7A), 2.60 (1H, dd, J = 11.4, 4.3 Hz, H-7B).
4.3.4.2 Discorhabdin T (28)

(+)-(6S)-Discorhabdin T (28)

(+)-(6S)-Discorhabdin U (29) TFA salt (1.4 mg, 2.5 µmol) was dissolved in dry DMF (2 mL) to which an excess of K₂CO₃ (20 mg) was added. The reaction mixture was kept at reflux at 80 ºC for 4 hrs under N₂. Crude reaction mixture was loaded on a C₈ flash column and the solid purified eluting with 50% MeOH, 50% H₂O (0.05%), to yield (+)-(6S)-discorhabdin T (28) (0.60 mg, 1.4 µmol, 54% yield).

Free base orange powder; [α]₀ = +280 (c 0.025, DCM (10% MeOH)); ¹H NMR (CDCl₃ (10% CD₃OD), 400 MHz) [spectrum referenced on the CD₃OD resonance at 3.30 ppm] δ 8.23 (1H, d, J = 5.7 Hz, H-17), 7.68 (1H, s, H-1), 7.64 (1H, s, H-14), 7.34 (1H, d, J = 5.8 Hz, H-16), 6.48 (1H, d, J = 7.5 Hz, H-8), 5.98 (1H, s, H-4), 4.31 (3H, s, H-22), 4.16 (1H, d, J = 7.5 Hz, H-7), 2.21 (3H, s, H-23).

(-)-(6R)-discorhabdin T (28)

Semi-synthetic (-)-(6R,8R)-Discorhabdin B (8) TFA salt (4.5 mg, 8.5 µmol) was dissolved in trimethylphosphate (1 mL) to which K₂CO₃ (5 mg) was added. The reaction mixture was kept at reflux at 90 ºC for 4 hrs under N₂. The crude reaction mixture was loaded on a C₈ flash column and the products eluted with 15% MeOH, 85% H₂O-TFA (0.05%) and
50% MeOH, 50% H2O-TFA (0.05%) yielding (-)-(6R)-discorhabdin U (29) TFA salt (3.2 mg, 5.8 µmol, 68% yield) and (-)-(6R)-discorhabdin T (28) TFA salt (0.9 mg, 1.6 µmol, 19% yield) respectively.

TFA salt dark blue-green oil; ¹H NMR (CD₃OD-d₄, 400 MHz) δ 8.26 (1H, s, H-14), 7.94 (1H, d, J = 6.6 Hz, H-17), 7.88 (1H, s, H-1), 7.80 (1H, d, J = 6.7 Hz, H-16), 6.61 (1H, d, J= 7.6 Hz, H-8), 6.18 (1H, s, H-4), 4.55 (1H, d, J = 7.6 Hz, H-7), 4.39 (3H, s, H-22), 2.39 (3H, s, H-23); ¹³C NMR (CD₃OD-d₄, 100 MHz) δ 176.6 (C-3), 173.1 (C-5 from HMBC), (C-11 no value obtained), 150.3 (C-1), 145.6 (C-10 from HMBC), 144.3 (C-19 from HMBC), 134.3 (C-14), 132.9 (C-17), 126.6 (C-8), 125.8 (C15), 123.3 (C-2 from HMBC), 122.1 (C-12), 121.3 (C-21), 117.8 (C-4), 115.8 (C-16), 111.2 (C-7) 100.4 (C-20 from HMBC), 51.0 (C-6), 38.7 (C-22), 15.1 (C-23).

(-)-(6R)-Discorhabdin T (28) TFA salt was left to stand in a 1:1 solution of dichloromethane/methanol at room temperature for 4 hrs during which time the solution changed color from dark blue-green to bright orange, yielding (-)-(6R)-Discorhabdin T (28) free base.

Free base orange powder; [α]₀ = -320, [α]₅₇₈ = -280, [α]₅₄₆ = -440, (c 0.025, DCM (10% MeOH)).
4.3.5.1 2-Bromo Discorhabdin D (110)

Naturally-occurring (+)-(6S,8S)-discorhabdin B (8) free base (5.4 mg) was dissolved in methanol (20 mL) to which 5 mL of methanol (0.05% TFA) was added. The solution immediately changed color from bright orange to bright green and the analytical HPLC detected two compounds, discorhabdin B (8) and 2-bromo discorhabdin D (110). The mixture was loaded on a C18 column and compound 110 (1.9 mg) eluted with 20% MeOH, 80% H2O-TFA (0.05%).

TFA salt dark green oil; \(^{1}H\) NMR (CD\(_3\)OD-\(d_4\), 400 MHz) \(\delta\) 7.13 (1H, s, H-14), 6.13 (1H, s, H-4), 5.68 (1H, dd, \(J = 3.6, 1.2 \text{ Hz}, \text{H-8}\)), 4.60 (1H, ddd, \(J = 13.2, 4.8, 2.8 \text{ Hz}, \text{H-17A}\)), 3.64 (1H, m, H-17B), 3.56 (1H, d, \(J = 13.2 \text{ Hz}, \text{H-1A}\)), 3.22 (1H, d, \(J = 13.6 \text{ Hz}, \text{H-1B}\)), 3.06 (2H, m, H-16), 2.83 (1H, dd, \(J = 12.0, 3.6 \text{ Hz}, \text{H-7A}\)), 2.66 (1H, d, \(J = 12.0 \text{ Hz}, \text{H-7B}\)); HRFABMS \(m/z\) [M+H]\(^+\) 413.9942 (calcd for C\(_{18}\)H\(_{13}\)\(^{79}\)BrN\(_3\)SO\(_2\), 413.99118), 415.98754 (calcd for C\(_{18}\)H\(_{13}\)\(^{81}\)BrN\(_3\)SO\(_2\), 415.98914).

2-Br Discorhabdin D Methanol Adduct

2-Bromo discorhabdin D (110) (5 mg) was left to stand in an acidic solution of water/methanol (0.05%) TFA (40 mL) for seven days. The solvent was removed in vacuo and the product purified by C8 flash (MeOH, H\(_2\)O-TFA (0.05%)) chromatography yielding the unidentified methanol addition product the structure of which was identified as fragments 149a and 149b.

TFA salt dark green oil; \(^{1}H\) NMR (DMSO-\(d_6\), 600 MHz) \(\delta\) 13.36 (1H, br s, NH-13), 11.22 (1H, br s, NH-9), 7.35 (1H, s, H-14), 6.15 (1H, s, H-4), 5.11 (1H, d, \(J = 1.7 \text{ Hz}, \text{HF-2}\)), 4.75 (1H, br s, HF-1), 4.24 (1H, m, H-17A), 3.78 (1H, d, \(J = 12.5 \text{ Hz}, \text{H-1A}\)), 3.66 (1H, m, H-17B), 3.32 (3H, s, HF-3), 3.00 (2H, m, H-16), 2.90 (1H, d, \(J = 12.4 \text{ Hz}, \text{H-1B}\)); \(^{13}C\) NMR (CD\(_3\)OD -\(d_4\), 150 MHz) \(\delta\) 177.9 (C-5), 174.2 (C-3), 165.3 (C-11), 151.8 (C-19), 149.1 (C-10), 127.0 (C-14), 124.0 (C-12), 121.5 (C-21), 119.5 (C-15), 113.3 (C-4), 96.4
(C-20), 80.8 (C-2), 80.5 (CF-2), 56.4 (CF-3), 50.1 (C-17), 48.3 (C-6), 47.6 (C-1), 47.5 (CF-2), 19.7 (C-16); LRESMS m/z [M+H]+ 444/446.

4.3.5.2 1-Discorhabdyl Discorhabdin D (111)

Freshly extracted and purified (+)-(6S,8S)-discorhabdin B (8) free base (15.8 mg, 38.1 µmol) was dry left in the freezer for two weeks, after which time analytical HPLC showed one major and several minor non-discorhabdin B peaks. The crude mixture was dissolved in methanol (1 mL) and loaded on a C8 flash column. The major product was eluted with 40% MeOH:60% H2O-TFA (0.05%), and further purified by a combination of Sephadex LH-20 (MeOH (0.05% TFA)) and C8 flash chromatography (MeOH, H2O-TFA (0.05%)) chromatography, yielding 1-discorhabdyl discorhabdin D (111) (3.0 mg, 3.1 µmol, 16% yield).

TFA salt dark green oil; [α]D = -40, [α]578 = -120, [α]546 = -200, (c 0.05, MeOH); IR (smear) νmax 2920, 1654, 1616, 1180, 1127 cm⁻¹; 1H NMR (CD3OD-d4, 400 MHz) δ 7.88 (1H, s, H-22), 7.24 (1H, s, H-35), 7.14 (1H, s, H-14), 6.66 (1H, s, H-25), 6.56 (1H, d, J = 7.6 Hz, H-29), 6.16 (1H, s, H-4), 5.61 (1H, d, J = 2.4 Hz, H-8), 4.78 (1H, d, J = 7.6 Hz, H-28), 4.73 (1H, d, J = 3.2 Hz, H-1), 4.45 (1H, d, J = 2.8 Hz, H-2), 4.12 (1H, m, H-17A), 3.90 (3H, m, H-17B/38), 3.22 (1H, m, H-16A), 3.08 (1H, m, H-16B), 2.97 (2H, m, H-37), 2.89 (1H, m, H-7A), 2.71 (1H, d, J = 12.0 Hz, H-7B).
4.3.5.3 1-Epi-Discorhabdin L (150)

(+)-(6S,8S)-Discorhabdin B (8) trifluoroacetate salt (5.9 mg, 11.2 µmol) was dissolved in dry acetone (6 mL) to which an excess of K₂CO₃ (20 mg) was added. The reaction mixture was sealed and left to stand for three days at room temperature after which time analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the product purified by C₈ flash (MeCN, H₂O-TFA (0.05%)) chromatography, yielding (-)-(6S,6'S)-discorhabdin W (37) (1.2 mg, 1.1 µmol, 20% yield) and 1-epi-discorhabdin L (150) (0.8 mg, 1.72 µmol, 15% yield).

TFA salt dark green oil; ¹H NMR (DMSO-$_d$_6, 600 MHz) δ 13.11 (1H, br s, NH-13), 10.70 (1H, br s, NH-9), 7.29 (1H, d, J = 2.4 Hz, H-14), 6.48 (1H, br s, OH), 6.23 (1H, s, H-4), 5.63 (1H, br s, H-8), 4.72 (1H, d, J = 2.9 Hz, H-1), 4.24 (1H, d, J = 3.0 Hz, H-2), 4.00 (1H, m, H-17A), 3.85 (1H, m, H-17B), 3.05 (2H, m, H-16), 2.77 (1H, d, J = 3.5 Hz H-7A), 2.52 (1H, under solvent peak, H-7B); ¹³C NMR (DMSO-$_d$_6, 150 MHz) δ 184.5 (C-3), 172.3 (C-5), 166.3 (C-11), 147.7 (C-19), 147.2 (C-10), 126.8 (C-14), 123.6 (C-12), 121.2 (C-21), 117.5 (C-15), 113.4 (C-4), 98.0 (C-20), 67.7 (C-2), 63.9 (C-1), 62.3 (C-8), 51.0 (C-17), 46.8 (C-6), 36.3 (C-7), 19.2 (C-16); HRFABMS m/z [M+H]$^+$ 352.07691 (calcd for C$_{18}$H$_{14}$N$_3$SO$_3$, 352.07559).
4.3.6 Biosynthesis of the Discorhabdin Alkaloids

4.3.6.1 Semi-Synthetic 3-Dihydro Discorhabdin A (154)

(+)-(5R,6S,8S)-Discorhabdin A (7) TFA salt (1.9 mg, 3.6 μmol) was dissolved in dry methanol (2 mL) to which an excess of NaBH₄ was added. Solution was stirred for 10 min under N₂, during which the color changed from dark red to bright yellow. After stirring in air for 5 min, the solution had reverted to red. The solvent was removed in vacuo and the solid purified by C₈ flash (MeOH, H₂O-TFA (0.05%)) chromatography, yielding semi-synthetic 3-dihydro discorhabdin A (154) TFA salt (1.7 mg, 3.2 μmol, 89% yield).

TFA salt blue-green powder; [α]₀ = +200, [α]₅₇₈ = -40, [α]₅₄₆ = -400 (c 0.05, MeOH); IR (smear) νₘₐₓ 3102, 2926, 1676, 1525, 1184, 1123, 1024 cm⁻¹; UV (MeOH) λₘₐₓ 204 (20510), 250 (17180), 347 (8010), 402 (4740), 586 (1130) nm; ECD (MeOH) λ (Δε) 215 (-25.1), 258 (0), 343 (+8.0), 381 (0) nm; ¹H NMR (DMSO-d₆, 600 MHz) δ 13.14 (1H, br s, NH-13), 10.36 (1H, br s, NH-9), 8.50 (1H, br s, NH-18), 7.36 (1H, s, H-14), 6.21 (1H, s, H-1), 5.56 (1H, d, J = 7.8 Hz, OH), 5.20 (1H, d, J = 2.6 Hz, H-8), 4.49 (1H, br s, H-3), 4.10 (1H, dd, J = 12.8, 4.8 Hz, H-5), 3.89 (1H, br m, H-17A), 3.76 (1H, m, H-17B), 2.56 (1H, dd, J = 12.1, 2.9, H-7A), 2.50 (under solvent peak H-16), 2.36 (1H, d, J = 12.1 Hz, H-7B), 2.11 (1H, m, H-4A), 1.96 (1H, m, H-4B); ¹³C NMR (DMSO-d₆, 100 MHz) δ 165.8 (C-11), 153.4 (C-19), 150.5 (C-10), 133.5 (C-2), 130.0 (C-1), 127.2 (C-14), 123.2 (C-12/21), 119.6 (C-15), 103.3 (C-20), 66.8 (C-3), 58.5 (C-8), 53.5 (C-5), 49.2 (C-6), 44.4 (C-17), 41.4 (C-7), 40.5 (C-4), 18.1 (C-16); HRFABMS m/z [M+H]+ 418.02248 (calcd for C₁₈H₁₇⁷⁹BrN₅SO₂, 418.02248), 420.02115 (calcd for C₁₈H₁₇⁸¹BrN₅SO₂, 420.02044).
4.3.6.2 Semi-Synthetic Makaluvamine F (69)

(+)-(6S,8S)-Discorhabdin B (8) TFA salt (1.6 mg, 3.0 μmol) was dissolved in MilliQ water (1 mL) to which an aqueous solution of excess TCEP (9.8 μmol) was added. The reaction mixture was stirred at room temperature in air for 24 hours. The solvent was removed in vacuo and the solid purified by C18 flash chromatography (MeOH, H2O-TFA (0.05%)), yielding semi-synthetic rac-makaluvamine F (69) (0.6 mg, 1.0 μmol, 33% yield).

TFA salt red powder; [α]D = 0; 1H NMR (CD3OD-d4, 400 MHz) δ 7.40 (1H, s, H-13), 7.15 (1H, s, H-2), 6.73 (1H, s, H-16), 5.71 (1H, dd, J = 6.6, 2.7 Hz, H-10), 5.53 (1H, s, H-6), 3.93 (2H, t, J = 7.6 Hz, H-4), 3.60 (2H, m, H-11), 3.00 (2H, t, J = 7.6 Hz, H-3); HRFABMS m/z [M+H]+ 416.00612 (calculated for C18H1579BrN3SO2, 416.00683), 418.00554 (calcd for C18H1581BrN3SO2, 418.00498).

4.3.7 Bioactivity of the Discorhabdin Alkaloids

4.3.7.1 Sand Dollar Embryo Development Assay

General experimental procedures on animal rearing and gamete acquisition are given in Appendix I.

Test solutions of 10, 5, 2.5, 1 and 0.5 μg/mL of discorbhdins C (6) TFA salt, (+)-(5R,6S,8S)-A (7) TFA salt, (+)-(6S,8S)-B (8) TFA salt, (-)546-(2S,6R,8S)-D (11) TFA salt, (+)-(6S,8S)-G*/I (15) TFA salt, (-)578-(1R,2S,6R,8S)-L (17) TFA salt and (+)-(6S)-U (29) TFA salt were prepared by the addition of filtered seawater (9.8 mL), a solution of 200-300 fertilized eggs (0.2 mL) and the treatment compound in the appropriate solvent.
(5μL), all in a 30 mL test tube. The assay was kept at 20 ± 3 °C, at environmental light regime for 6 hours. The assay was terminated by the addition of borax buffered 50% formalin solution (0.5 mL) and the embryos were then examined under the light microscope with the relative proportion of abnormal development in each sample recorded. Each treatment was done in three replicates. Solvents used in the discorhabdin assay were MilliQ water for all of the TFA salt forms and methanol for discorhabdin C (6) TFA salt which is only sparingly soluble in water.

4.3.7.2 One-Electron Reduction Potentials

One-electron reduction potentials were determined at pH 7.0 by establishing redox equilibria between three mixtures of the one-electron reduced discorhabdin compounds and the reference compounds 2,3,5-trimethylbenzoquinone ($E_r(TMBQ/TMBQ^-) = -165$ mV), duroquinone ($E_r(DQ/DQ^-) = -260 ± 7$ mV) and benzyl viologen ($E_r(BV^{2+}/BV^+) = -380 ± 10$ mV). Details of the experimental procedure have been reported elsewhere by the collaborators.¹²²,¹²³
APPENDIX I

Sand Dollar Embryo Development Essay
General experimental procedure developed for the assay is as follows, adult *Fellaster zelandiae* were collected at low tide on Cheltenham beach, Auckland, and kept in a seawater aquarium in the laboratory. Spawning was induced by an intra-coelomic injection of 1.0 mL of 0.5 M KCl, and the gametes collected by inverting the sand dollars over 20 mL glass vials overfilled with filtered seawater. Sperm was kept on ice and the eggs from at least three females were collected, examined for viability under the microscope, and the good quality ones pooled together and washed with filtered seawater prior to fertilization. Fertilization was initiated by the addition of a single drop of dense sperm solution and the success was measured by the appearance of a fertilization membrane enveloping the zygote three minutes following the addition of sperm. All embryos were made at least half siblings by the addition of sperm from a single male. The embryos were added to the test solutions no later than ten minutes following fertilization. Test solutions were prepared by the addition of filtered seawater (9.8 mL), a solution of 200-300 fertilized eggs (0.2 mL) and the treatment compound in the appropriate solvent (5 \( \mu \)L), all in a 30 mL test tube. The assay was kept at 20 ± 3 ºC, at environmental light regime for either 6 or 28 hours. The assay was terminated by the addition of borax buffered 50 % formalin solution (0.5 mL) and the larvae were then examined under the microscope, with the first 100 counted and relative numbers of normal and abnormal larvae recorded for further statistical analysis.

The sand dollar *Fellaster zelandiae* (Gray, 1855) was chosen as the test animal for the assay due to several factors allowing for easy collection, relatively long spawning season and the ability to keep viable adults and embryos in the chemistry laboratory. Sand dollars are disk-shaped echinoderms, inhabiting shallow water benthic communities. A collection trip done at, or even ±2 hours of, low tide involves walking over the sand flats and feeling the animals under feet, with large beds of *F. zelandiae* on the west side of Cheltenham Beach in Auckland starting about 20 meters from the low tide mark. The collected animals were kept in the laboratory in a 20 L plastic aquarium, with daily fresh seawater change (2 L) keeping the animals in spawning condition for up to a week. Bunckenburg has shown that in Otago *F. zelandiae* produce mature gametes from September to May,\(^ {124} \) however successful spawnings with the local population was reliable in the summer months only. During the period of four years, from 2003 to 2008, the assays were run continuously with no complications in February, March and April, and with limited success and some
unsuccessful fertilizations during January and May. Another echinoderm species, the sea urchin *Evechinus chloroticus* (Valenciennes, 1846) was also considered as the test animal for the assay. However during the summer of 2003, *E. chloroticus* were producing viable gametes for only the first three weeks of March, and since this species was known to have a limited reproductive season, all further efforts were focused on developing the assay for *F. zelandiae* as the test organism.

The sand dollar *F. zelandiae* exhibits deuterostomus embryonic development. The zygote undergoes first cellular division 40 minutes post fertilization, at three hours the embryo is at the 8 cell blastomere stage and at seven hours the embryo hatches and is a swimming blastula. Thirty two hours post fertilization the embryo develops into a fully grown pluteus larva, with well defined four arms, primitive gut and skeletal elements (Figure A 1.1).

![Figure A 1.1](image)

*Figure A 1.1 F. zelandiae cleavage montage: A) fertilized and unfertilized eggs; B) 2 cell blastomeres; C) 8 cell blastomeres; D) blastulae; E) gastrula; F) pluteus larva*

Examples of abnormal development include embryos not undergoing or showing an irregular cellular division, developmental arrest at a certain embryonic stage and irregular plutei larvae (Figure A 1.2).
Preliminary studies on the response of *F. zelandiae* embryonic development to discorhabdin alkaloids have shown that the most dramatic effect was observed within the first seven hours of the assay. At seven hours post fertilization the embryo at a blastula stage hatches out of the fertilization membrane. Figure A 1.3. shows the control embryos at the blastula stage in A, and an example of abnormal development in B. Once hatched, the abnormal, undifferentiated embryos would lose the structure and rupture into individual cells. Since this made the identification of a single embryo impossible, the discorhabdin treatment assay was terminated one hour pre-hatching, at six hours post fertilization.

**Figure A 1.2.** *F. zelandiae* irregular cleavage montage: A) 16 cell blastomere; B) blastula; C) gastrula D) plutei larvae.

**Figure A 1.3.** *F. zelandiae* assay at seven hours post fertilization, A) control hatched blastulae B) discorhabdin-treated raptured blastomeres.
APPENDIX II
DISCORHABDIN INDEX

Discorhabdin A (7)  Discorhabdin B (8)  Discorhabdin C (6)  Discorhabdin D (11)

Discorhabdin E (12)  Discorhabdin F (13)  Discorhabdin G (14)  Discorhabdin G*/I (15)

Discorhabdin H (16)  Discorhabdin L (17)  Discorhabdin N (18)

Discorhabdin K (21)  Discorhabdin M (22)  Discorhabdin O (23)

Discorhabdin T (28)  Discorhabdin U (29)  Discorhabdin V (30)

Discorhabdin W (37)
APPENDIX III

Electronic copies of $^1$H and $^{13}$C NMR spectra of enantiomeric pairs of known discorhabdins, new natural products and semi-synthetic derivatives are given as jpeg. files on a CD ROM attached to the back cover page of the thesis.

**Figure A3.1** $^1$H NMR spectrum of (+)-(6$^S$,8$^S$)-discorhabdin B (8) TFA salt in CD$_3$OD.

**Figure A3.2** $^{13}$C NMR spectrum of (+)-(6$^S$,8$^S$)-discorhabdin B (8) TFA salt in CD$_3$OD.

**Figure A3.3** $^1$H NMR spectrum of (-)-(6$^R$,8$^R$)-discorhabdin B (8) TFA salt in CD$_3$OD.

**Figure A3.4** $^1$H NMR spectrum of (+)-(6$^S$,8$^S$)-discorhabdin G*/I (15) TFA salt in CD$_3$OD.

**Figure A3.5** $^{13}$C NMR spectrum of (+)-(6$^S$,8$^S$)-discorhabdin G*/I (15) TFA salt in CD$_3$OD.

**Figure A3.6** $^1$H NMR spectrum of (-)-(6$^R$,8$^R$)-discorhabdin G*/I (15) TFA salt in CD$_3$OD.

**Figure A3.7** $^{13}$C NMR spectrum of (-)-(6$^R$,8$^R$)-discorhabdin G*/I (15) TFA salt in CD$_3$OD.

**Figure A3.8** $^1$H NMR spectrum of (+)-(6$^R$,6'$^R$)-discorhabdin W (36) free base in DMSO-$d_6$.

**Figure A3.9** $^{13}$C NMR spectrum of (+)-(6$^R$,6'$^R$)-discorhabdin W (36) free base in DMSO-$d_6$.

**Figure A3.10** $^1$H NMR spectrum of (+)-(6$^R$,6'$^R$)-discorhabdin W (36) TFA salt in CD$_3$OD.

**Figure A3.11** $^{13}$C NMR spectrum of (+)-(6$^R$,6'$^R$)-discorhabdin W (36) TFA salt in CD$_3$OD.

**Figure A3.12** $^1$H NMR spectrum of (-)-(6$^S$,6'$^S$)-discorhabdin W (36) TFA salt in CD$_3$OD.

**Figure A3.13** $^{13}$C NMR spectrum of (-)-(6$^S$,6'$^S$)-discorhabdin W (36) TFA salt in CD$_3$OD.

**Figure A3.14** $^1$H NMR spectrum of (-)$_{378}$-(1$^R$,2$^S$,6$^R$,8$^S$)-discorhabdin L (17) TFA salt in CD$_3$OD.

**Figure A3.15** $^{13}$C NMR spectrum of (-)$_{378}$-(1$^R$,2$^S$,6$^R$,8$^S$)-discorhabdin L (17) TFA salt in CD$_3$OD.
Figure A3.16 $^1$H NMR spectrum of (+)$_{578}$-(1$S,2R,6S,8R$)-discorhabdin L (17) TFA salt in CD$_3$OD.

Figure A3.17 $^{13}$C NMR spectrum of (+)$_{578}$-(1$S,2R,6S,8R$)-discorhabdin L (17) TFA salt in CD$_3$OD.

Figure A3.18 $^1$H NMR spectrum of (-)-(1$R,2R,6R,8S,7'S^*$)-discorhabdin H (16) TFA salt in CD$_3$OD.

Figure A3.19 $^{13}$C NMR spectrum of (-)-(1$R,2R,6R,8S,7'S^*$)-discorhabdin H (16) TFA salt in CD$_3$OD.

Figure A3.20 $^1$H NMR spectrum of (+)-(1$S,2S,6S,8R,7'S^*$)-discorhabdin H (16) TFA salt in CD$_3$OD.

Figure A3.21 $^{13}$C NMR spectrum of (+)-(1$S,2S,6S,8R,7'S^*$)-discorhabdin H (16) TFA salt in CD$_3$OD.

Figure A3.22 $^1$H NMR spectrum of (-)$_{578}$-(3$R,5R,6S,8S$)-3-dihydro discorhabdin A (107) TFA salt in DMSO-$d_6$.

Figure A3.23 $^{13}$C NMR spectrum of (-)$_{578}$-(3$R,5R,6S,8S$)-3-dihydro discorhabdin A (107) TFA salt in DMSO-$d_6$.

Figure A3.24 $^1$H NMR spectrum of (+)-(6$R,8S$)-1-thiomethyl discorhabdin G*/I (108) TFA salt in CD$_3$OD.

Figure A3.25 $^{13}$C NMR spectrum of (+)-(6$R,8S$)-1-thiomethyl discorhabdin G*/I (108) TFA salt in CD$_3$OD.

Figure A3.26 $^1$H NMR spectrum of (-)-(6$S,8S,7'S^*$)-discorhabdin K (21) TFA salt in CD$_3$OD.

Figure A3.27 $^{13}$C NMR spectrum of (-)-(6$S,8S,7'S^*$)-discorhabdin K (21) TFA salt in CD$_3$OD.

Figure A3.28 $^1$H NMR spectrum of (+)-(6$R,8R,7'S^*$)-discorhabdin K (21) TFA salt in CD$_3$OD.

Figure A3.29 $^{13}$C NMR spectrum of (+)-(6$R,8R,7'S^*$)-discorhabdin K (21) TFA salt in CD$_3$OD.

Figure A3.30 $^1$H NMR spectrum of (-)$_{578}$-(6$S,6aS$)-16a,17a-dehydro discorhabdin W (109) TFA salt in CD$_3$OD.

Figure A3.31 $^{13}$C NMR spectrum of (-)$_{578}$-(6$S,6aS$)-16a,17a-dehydro discorhabdin W (109) TFA salt in CD$_3$OD.
Figure A3.32 $^1$H NMR spectrum of (+)$_{578}$-(6$R$,6a$R$)-16a,17a-dehydro discorhabdin W (109) TFA salt in CD$_3$OD.

Figure A3.33 $^{13}$C NMR spectrum of (+)$_{578}$-(6$R$,6a$R$)-16a,17a-dehydro discorhabdin W (109) TFA salt in CD$_3$OD.

Figure A3.34 $^1$H NMR spectrum of (+)-(6$S$)-N-13-demethyl discorhabdin U (141) TFA salt in CD$_3$OD.

Figure A3.35 $^{13}$C NMR spectrum of (+)-(6$S$)-N-13-demethyl discorhabdin U (141) TFA salt in CD$_3$OD.

Figure A3.36 $^1$H NMR spectrum of (+)-(6$S$)-discorhabdin U (29) TFA salt in DMSO-$d_6$.

Figure A3.37 $^{13}$C NMR spectrum of (+)-(6$S$)-discorhabdin U (29) TFA salt in DMSO-$d_6$.

Figure A3.38 $^1$H NMR spectrum of (-)-(6$R$)-discorhabdin U (29) TFA salt in DMSO-$d_6$.

Figure A3.39 $^{13}$C NMR spectrum of (-)-(6$R$)-discorhabdin U (29) TFA salt in DMSO-$d_6$.

Figure A3.40 $^1$H NMR spectrum of (-)-(6$S$)-dibenzyl discorhabdin U (142) TFA salt in CD$_3$OD.

Figure A3.41 $^{13}$C NMR spectrum of (-)-(6$S$)-dibenzyl discorhabdin U (142) TFA salt in CD$_3$OD.

Figure A3.42 $^1$H NMR spectrum of (+)-(6$S$,8$S$)-N-13-methyl discorhabdin B (144) TFA salt in CD$_3$OD.

Figure A3.43 $^{13}$C NMR spectrum of (+)-(6$S$,8$S$)-N-13-methyl discorhabdin B (144) TFA salt in CD$_3$OD.

Figure A3.44 $^1$H NMR spectrum of (+)-(6$S$,8$S$)-N-13-benzyl discorhabdin B (148) TFA salt in CD$_3$OD.

Figure A3.45 $^{13}$C NMR spectrum of (+)-(6$S$,8$S$)-N-13-benzyl discorhabdin B (148) TFA salt in CD$_3$OD.

Figure A3.46 $^1$H NMR spectrum of (-)-(2$R$,6$S$,8$S$)-2-bromo discorhabdin D (110) TFA salt in CD$_3$OD.

Figure A3.47 $^{13}$C NMR spectrum of (-)-(2$R$,6$S$,8$S$)-2-bromo discorhabdin D (110) TFA salt in CD$_3$OD.

Figure A3.48 $^1$H NMR spectrum of the (-)-(2$R$,6$S$,8$S$)-2-bromo discorhabdin D (110) methanol addition product TFA salt in DMSO-$d_6$.

Figure A3.49 $^{13}$C NMR spectrum of the (-)-(2$R$,6$S$,8$S$)-2-bromo discorhabdin D (110) methanol addition product TFA salt in DMSO-$d_6$. 
**Figure A3.50** $^1$H NMR spectrum of \((-\)-(2$R$,6$S$,8$S$,27$S$)-1-discorhabdyl discorhabdin D (111) TFA salt in CD$_3$OD.

**Figure A3.51** $^{13}$C NMR spectrum of \((-\)-(2$R$,6$S$,8$S$,27$S$)-1-discorhabdyl discorhabdin D (111) TFA salt in CD$_3$OD.

**Figure A3.52** $^1$H NMR spectrum of \((+\)-(2$S$,6$R$,8$R$,27$R$)-1-discorhabdyl discorhabdin D (111) TFA salt in CD$_3$OD.

**Figure A3.53** $^1$H NMR spectrum of \((1$S$,2$S$,6$R$,8$S$)-1-\textit{epi} discorhabdin L (150) TFA salt in DMSO-$d_6$.

**Figure A3.54** $^{13}$C NMR spectrum of \((1$S$,2$S$,6$R$,8$S$)-1-\textit{epi} discorhabdin L (150) TFA salt in DMSO-$d_6$.

**Figure A3.55** $^1$H NMR spectrum of \((+\)-(3$S$,5$R$,6$S$,8$S$)-3-dihydro discorhabdin A (154) TFA salt in DMSO-$d_6$.

**Figure A3.56** $^{13}$C NMR spectrum of \((+\)-(3$S$,5$R$,6$S$,8$S$)-3-dihydro discorhabdin A (154) TFA salt in DMSO-$d_6$. 

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