New Halogenated Secondary Metabolites from Red Algae of the South Pacific

by

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Abstract

An NMR- and MS-directed study led to the isolation and structure elucidation of several halogenated secondary metabolites from a New Zealand and a Tongan red alga. An extensive investigation was carried out on the New Zealand red alga *Rhodophyllis membranacea* following mass spectrometric evidence for an unusual tetrahalogenated indole with the exceptionally rare inclusion of bromine, chlorine and iodine within a fraction of a semi-purified extract. Due to the difficulty associated with the structure elucidation of proton deficient molecules, a strategic isolation and structure elucidation of several polyhalogenated indoles was employed in order to unequivocally assign the halogen positions on the indolic core. This resulted in the isolation and characterisation of 11 new tetrahalogenated indoles (123–133), four of which contain bromine, chlorine and iodine (124 and 129–131) and represent the first isolation of such compounds. Additionally, four new pentahalogenated indoles (134–137) and an uncharacterised tribromo-trichloroindole were isolated. The synthetically known compound 4-chloroisatin (138) was isolated as a new marine natural product, while 4-chloro-3-hydroxyl-3-(2-oxopropyl)-2-oxindole (139) was established to be an artefact of isolation. Several compounds were found to exhibit antifungal properties against *Saccharomyces cerevisiae*.

A detailed examination of the Tongan alga *Callophycus serratus* led to the isolation of six new meroditerpenoids: callophycol C (227), iodocallophycols E (228) and F (229), iodocallophycoic acid B (230), deiodocallophycoic B (231) and callophycoic acid I (232). The relative configurations in compounds 228–231 are proposed to differ from closely related compounds in the literature. Iodocallophycol E (228) exhibited moderate cytotoxicity against the HL-60 cell line with an IC\textsubscript{50} value of 6.0 µM.
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<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>$\delta$</td>
<td>Chemical shift (ppm)</td>
</tr>
<tr>
<td>$^{13}$C NMR</td>
<td>Carbon-13 nuclear magnetic resonance</td>
</tr>
<tr>
<td>$^{15}$N NMR</td>
<td>Nitrogen-15 nuclear magnetic resonance</td>
</tr>
<tr>
<td>$^{1}$H NMR</td>
<td>Proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>$J$</td>
<td>Scalar coupling constant (Hz)</td>
</tr>
<tr>
<td>$T_1$</td>
<td>Spin-lattice relaxation</td>
</tr>
<tr>
<td>$m/z$</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>B-16</td>
<td>Murine melanoma cell line</td>
</tr>
<tr>
<td>BACE-1</td>
<td>Beta-site amyloid precursor protein cleaving enzyme</td>
</tr>
<tr>
<td>$br$</td>
<td>Broad</td>
</tr>
<tr>
<td>$C_{18}$</td>
<td>Octadecyl derivatised silica</td>
</tr>
<tr>
<td>$C_8$</td>
<td>Octyl derivatised silica</td>
</tr>
<tr>
<td>CIGAR</td>
<td>Constant time inverse-detected gradient accordion rescaled</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation spectroscopy ($^1$H to $^1$H shown as $\text{red}$)</td>
</tr>
<tr>
<td>$d$</td>
<td>Doublet</td>
</tr>
<tr>
<td>DESI</td>
<td>Desorption electrospray ionisation</td>
</tr>
<tr>
<td>DFT</td>
<td>Density functional theory</td>
</tr>
<tr>
<td>DIOL</td>
<td>2,3-dihydroxypropoxy-propyl-derivatised silica</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Effective concentration that gives 50% of the maximal response</td>
</tr>
<tr>
<td>GI&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Concentration that inhibits growth by 50%</td>
</tr>
<tr>
<td>HepG2</td>
<td>Human liver carcinoma cell line</td>
</tr>
<tr>
<td>HILIC</td>
<td>Hydrophilic interaction liquid chromatography</td>
</tr>
<tr>
<td>HL-60</td>
<td>Human promyelocytic cell line</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple-bond correlation ($^1$H to $^{13}$C shown as $\text{red}$)</td>
</tr>
<tr>
<td>HP20</td>
<td>PSDVB stationary support</td>
</tr>
<tr>
<td>HP20ss</td>
<td>PSDVB stationary support</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>HRESIMS</td>
<td>High-resolution electrospray ionisation mass spectrometry</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single-bond correlation</td>
</tr>
<tr>
<td>HT-29</td>
<td>Human colorectal adenocarcinoma cell line</td>
</tr>
<tr>
<td>IC&lt;sub&gt;100&lt;/sub&gt;</td>
<td>Inhibitory concentration in 100% test subjects</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Inhibitory concentration in 50% test subjects</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>LH20</td>
<td>Cross-linked dextran-based size-exclusion chromatographic medium</td>
</tr>
<tr>
<td>$m$</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser enhancement ((^1)H to (^1)H shown as →)</td>
</tr>
<tr>
<td>P388</td>
<td>Murine leukaemia cell line</td>
</tr>
<tr>
<td>Pos.</td>
<td>Position</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PSDVB</td>
<td>Poly(styrene-divinylbenzene)</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>quin</td>
<td>Quintet</td>
</tr>
<tr>
<td>R_f</td>
<td>Retention factor</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosyl-(l)-methionine</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure-activity relationship</td>
</tr>
<tr>
<td>SCUBA</td>
<td>Self contained underwater breathing apparatus</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TOCSY</td>
<td>Total correlated spectroscopy ((^1)H to (^1)H shown as →)</td>
</tr>
<tr>
<td>V-BrPO</td>
<td>Vanadium-dependent bromoperoxidase</td>
</tr>
<tr>
<td>V-ClPO</td>
<td>Vanadium-dependent chloroperoxidase</td>
</tr>
<tr>
<td>V-HPO</td>
<td>Vanadium-dependent haloperoxidase</td>
</tr>
<tr>
<td>V-IPO</td>
<td>Vanadium-dependent iodoperoxidase</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

The field of marine natural products chemistry has developed considerably since the discovery of sponge-derived nucleosides in the early 1950s.\textsuperscript{1,2} The literature contains a significant number of chemically intriguing, biologically active compounds sourced from a variety of marine organisms, including red algae.\textsuperscript{3-7}

1.1 Marine Red Algae

Fossil evidence shows algae to be an ancient group of organisms that have existed since the Cambrian period.\textsuperscript{8} Throughout this time, they have flourished, becoming an abundant and considerably diverse group of organisms. The presence of photosynthetic pigments give the characteristic green, golden, red and brown hues observed in many algae, and it is the colours exhibited by them that have influenced the names by which they are commonly known—the green, golden, red and brown algae.\textsuperscript{9} The largest of the three groups is the red algae.\textsuperscript{10}

Red algae (phylum Rhodophyta) consist of over 7000 described species.\textsuperscript{11} Their global distribution is widespread with speciation abundant in tropical and subtropical regions.\textsuperscript{10,12} While they can be found growing on coastal rocks alongside green and brown algae, their populations tend to be denser at greater depths than those of the other two algal types owing to the presence of accessory pigments (\textit{e.g.} phycoerythrin) that can capture the shorter wavelengths of light penetrating deeper waters.\textsuperscript{12,13} Of the macroalgae, red algae are the most prolific producers of secondary metabolites—molecules that are not involved in the normal metabolic processes required for life, but whose biochemical function contributes to the overall survival of the producing organism.\textsuperscript{12} Thus, secondary metabolites may inhibit the growth of nearby algae thereby reducing competition for space, or prevent the
overgrowth of fouling organisms, or provide chemical defence against grazing animals and pathogens.\textsuperscript{12} The levels and compositions of algal secondary metabolites are influenced by temporal and spatial variations and environmental factors such as light levels, temperature, nutrients, salinity and biotic interactions.\textsuperscript{14} Red algae were among the first organisms to be studied by marine natural product chemists due to their ease of accessibility before the arrival of SCUBA.\textsuperscript{12,13} Since then, the natural products literature has become rife with publications of biologically active compounds derived from red algae, many of which are halogenated. Examples include maharone (1) and 5-bromomaharone (2),\textsuperscript{15} the monoterpane plocamene (3),\textsuperscript{16} the diterpenes 4–7\textsuperscript{17} and the omaezallenes (8–10).\textsuperscript{18} Maharone (1) and 5-bromomaharone (2) were isolated from \textit{Asparagopsis taxiformis};\textsuperscript{15} an alga that has also been found by various research groups to produce more than 100 simple halogenated compounds, many of which are volatile.\textsuperscript{19–22} It has been suggested that volatile halocarbons such as those produced by \textit{A. taxiformis} could be the reason for the smell of the ocean.\textsuperscript{23} Both 1 and 2 exhibited activity in an assay against the bioluminescent bacteria \textit{Vibrio fischeri} (EC\textsubscript{50} 0.16 µM observed for both compounds).\textsuperscript{15} Plocamenone (3), originally isolated in 1979, was the subject of several structural revisions before the structure depicted below was reported in 2012. Potent cytotoxicity was observed for 3 against the P388 (murine leukemia) cell line (IC\textsubscript{50} 0.16 µg/mL).\textsuperscript{16} The diterpenes 4–7 were isolated from \textit{Sphaerococcus coronopifolius} and displayed varying levels of antiproliferative activity in HepG2 (human liver carcinoma) cells (IC\textsubscript{50} 42.9–279.9 µM). The highest activity was comparable to that shown by the positive control.\textsuperscript{17} Compounds 8–10 displayed antifouling activity against the cypris larvae of the barnacle \textit{Amphibalanus amphitrite} with EC\textsubscript{50} values of 0.22, 0.30 and 1.50 µg/mL, respectively.\textsuperscript{18} Chemically intriguing, biologically active halogenated metabolites such as 1–10 are the reason that red algae continue to captivate marine natural product researchers.
1.2 Halogenated Natural Products

The halogens are in group 17 of the period table† and comprise fluorine, chlorine, bromine, iodine and astatine. The first four are reactive non-metals that form a variety of compounds including diatomic, organic and inorganic molecules. Some of their physicochemical properties are presented in Table 1.1. Astatine is a highly unstable, radioactive element; its most stable isotope has a half-life of only eight hours. Compared with the other halogens, little is known about astatine.

Halogenated natural products were once considered to be artefacts of isolation or chemical accidents of nature but they are now recognised as authentic natural products. In 1968 there were fewer than 30 such known natural products from both the terrestrial and marine environments. Today, that number has significantly increased to more than 5000 with the largest proportion being represented by brominated molecules followed by chlorinated, iodinated and fluorinated compounds.

---

† Also in group 17, is the recently discovered tennessine, which is a highly radioactive element that is predicted to be metallic.24
Table 1.1. Some Physicochemical Properties of the Common Halogens.

<table>
<thead>
<tr>
<th>Bond dissociation energy</th>
<th>Bond length</th>
<th>Dipole moment</th>
<th>Hydration energy</th>
<th>Standard electrode potential, $E^\ominus$</th>
<th>Electronegativity ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$X</td>
<td>C—X</td>
<td>CH$_3$X</td>
<td>X$^-$</td>
<td>X$_2$+2e$^-\rightleftharpoons$2X$^-$</td>
<td>Pauling scale</td>
</tr>
</tbody>
</table>

The reverse half-cell reaction depicts oxidation and the corresponding magnitude of $E^\ominus$ is equal but opposite in sign.

The chemical and structural diversity of halogenated natural products varies considerably from simple compounds containing one carbon (e.g. haloform) to complex molecules comprised of more than 50 carbons. Equally diverse are the terrestrial and marine organisms that biosynthesise these extraordinary molecules, such as fungi, plants, sponges, algae, corals, tunicates, bacteria, cyanobacteria and even humans. In humans, iodine is an essential element required in the diet for the production of the metabolism-regulating hormone thyroxine (11). An imbalance in iodine can disrupt thyroxine (11) biosynthesis, which can lead to hypothyroidism. The vast majority of halogenated natural products have been covered in several reviews published by Gribble while Wang et al. have provided the most recent review of the naturally occurring organoiodides.

As with non-halogenated secondary metabolites, those that are halogenated are believed to facilitate advantages in the survival of an organism. In some compounds, the presence of halogen substituents show increased biological activity when compared to their non-halogenated counterparts. For example, rebeccamycin (12) exhibits strong cytotoxicity against the murine B16 (melanoma) and P388 cell lines, but the semi-synthetic dechlororebeccamycin (13) shows a significant decrease in activity. The antibiotic vancomycin (14) is another example. It
disrupts peptidoglycan cross-linking in susceptible bacteria by preferentially binding to intermediates terminating in specific amino acids. Upon the removal of both chlorine substituents the molecule has increased flexibility, which leads to a decreased specificity of the binding site and consequently reduced activity.\textsuperscript{41} Compounds 12 and 14 were originally isolated from terrestrial-derived bacteria,\textsuperscript{42,43} however, the largest proportion of the total number of halogenated natural products have originated from marine organisms.\textsuperscript{34} This is unsurprising considering the halogen-rich environment that is sea water (Table 1.2).\textsuperscript{37}

**Table 1.2.** Concentration of Common Halides in the Oceans and Sedimentary Rock.\textsuperscript{37}
1.2.1 Brominated and Chlorinated Marine Natural Products

The concentration of chloride ions in the oceans seemingly dwarfs that of bromide, fluoride and iodide by being ca. 600, 7000 and 1 million times more abundant, respectively (Table 1.2). Despite its great abundance, the relative proportion of reported chlorinated to either brominated or iodinated marine natural products is small; bromine and iodine-containing natural products occur in significantly higher numbers than their relative abundances would suggest. This is due to the mechanisms by which halogens are incorporated. Biohalogenation is typically an enzymatic oxidation process (see Section 1.2.4) and the oxidation potential increases in the order fluoride<chloride<bromide<iodide (see Table 1.1). Thus, the last two halogens are more readily susceptible to oxidation and this leads to a higher occurrence of brominated and iodinated natural products than is to be expected on the basis of their relative concentrations in seawater.

Perhaps the earliest known bromine-containing metabolite is 6,6’-dibromoindigo (15), more famously known as Tyrian purple. The intensely purple coloured compound has been used as a dye for centuries but it was only in 1909 that it was isolated from the mollusc Murex brandaris and characterised. Since then, the number of brominated metabolites derived from the marine environment has increased significantly.

A considerable number of marine-derived halogenated natural products carry both bromine and chlorine. Approximately 40% are substituted by at least one chlorine atom and 71% by one bromine atom. Red algae have been a major source of such compounds with numerous examples found in terpenes, such as 16–18 and C15 acetogenins, such as 19–21. Between these compounds moderate antifungal,
cytotoxic, and antibacterial activities was observed.

Halogenated marine natural products substituted by either bromine or chlorine have frequently been isolated from sponges. Unlike algal metabolites, the presence of both bromine and chlorine atoms in sponge-derived metabolites is relatively rare. Mollenynes A (22)\textsuperscript{51} and B (23)\textsuperscript{52} from *Spirastrella mollis*, are examples of sponge-derived bromochloro-substituted natural products. Compounds 22 and 23 differ by an interchange of the halogens and the configurations at positions C-8 and C-9. It is suggested that this could occur via a type I dyotropic rearrangement in 22, whereby a 1,2-shift of the halogens in a concerted manner leads to the formation of 23.\textsuperscript{52} Investigations of the sponge *Dysidea herbacea* have resulted in the isolation of polychlorinated compounds such as dysidamide (24)\textsuperscript{53} and dysideathiazole (25).\textsuperscript{54} Dysideathiazole (25) and closely related derivatives isolated in the same study were deduced as strongly deterrent in fish feeding experiments.\textsuperscript{54} A cyanobacterium symbiont is believed to be the true source of chlorinated compounds found in *Dysidea* sponges.\textsuperscript{55}

Other examples of marine-derived halogenated natural products have been isolated from ascidians (*e.g.* 26),\textsuperscript{56} seahares (*e.g.* 27 and 28),\textsuperscript{57,58} gorgonians (*e.g.* 29),\textsuperscript{59}
fungi \((e.g. \text{30})\), \(^{60}\) soft corals \((e.g. \text{31})\), \(^{61}\) bacteria \((e.g. \text{32})\) \(^{62}\) and molluscs \((e.g. \text{33})\). \(^{63}\) Compound \text{26} exhibited potent cytotoxicity against the P388 cell line \((\text{IC}_{50}\ 1 \text{ ng/mL}, \text{respectively})\). \(^{56}\) Compound \text{28} was ichthyotoxic\(^{†}\) to \text{Gambusia affinis} \((\text{IC}_{100}\ 3 \text{ mg/L over 24 h})\), \(^{58}\) while polycyclic \text{29} was noted to exhibit antifouling activity against \text{A. amphitrite} \((\text{EC}_{50}\ 4.1 \mu\text{g/mL})\). \(^{59}\) The polychlorinated sulfolipid \text{33}, isolated from the digestive glands of the bivalve mollusc \text{Mytilus galloprovincialis}, has a highly functionalised side-chain containing 11 chlorine atoms. While \text{33} was inactive in an antiproliferative bioassay, chlorolipids isolated from \text{Chrysophyte} species have been found to show antibiotic activity. \(^{63}\)

\(^{†}\)Toxic to fish.
1.2.2 Iodinated Marine Natural Products

The 1811 discovery of iodine was by the French chemist Bernard Courtois. Upon adding excess sulfuric acid to seaweed ash, molecular iodine and its concomitant violet vapour subsequently formed, which condensed to a lustrous black crystalline solid.\textsuperscript{64} Almost a century later, 3,5-diiodotyrosine (34) was discovered from the coral Gorgonia cavolinii as the first iodinated natural product.\textsuperscript{39}

Between 1896 and 2014 there were 182 naturally occurring organoiodides reported in the literature. More than two-thirds of iodine-containing natural products originated from the marine environment and close to half of those were sourced from marine algae.\textsuperscript{39} Within the halogenated marine natural products literature, 3\% contain
iodinated compounds. This is a significant percentage considering the concentration of iodide in sea water relative to chloride and bromide ions.\textsuperscript{44}

Of the isolated iodinated metabolites, several also contain either bromine or chlorine. Most of these are found in small molecules isolated from red algal species of the genera \textit{Asparagopsis}, \textit{Delisea} and \textit{Falkenbergia} and include halogenated derivatives of compounds such as propanones, acetic and propanoic acids, acetimides and butenols.\textsuperscript{39} Other examples of iodinated marine natural products include oxylipins (\textit{e.g.} \textsuperscript{35} and \textsuperscript{36} from a brown alga),\textsuperscript{65} acetylenes (\textit{e.g.} \textsuperscript{37}–\textsuperscript{39} from a sponge),\textsuperscript{66,67} furanones (\textit{e.g.} \textsuperscript{40}–\textsuperscript{42} from a red alga),\textsuperscript{68} prostaglandins (\textit{e.g.} \textsuperscript{43} from a soft coral),\textsuperscript{69} terpenes (\textit{e.g.} \textsuperscript{44} and \textsuperscript{45} from a red alga and soft coral, respectively),\textsuperscript{70,71} depsipeptides (\textit{e.g.} \textsuperscript{46} and \textsuperscript{47} from a sponge)\textsuperscript{72} and a trisulfated sterol (\textit{e.g.} \textsuperscript{48} from a sponge).\textsuperscript{73} Naturally occurring compounds substituted with chlorine, bromine and iodine are extremely rare with only three examples reported in the literature (see Section 3.5.5).\textsuperscript{19–21,74} A range of biological activities have been reported for iodinated natural products including cytotoxic, antiinflammatory, antibacterial, antifungal, anticonvulsant, antihistaminic, antifouling and enzyme-inhibiting.\textsuperscript{39}
1.2.3 Fluorinated Marine Natural Products

Fluorine-containing metabolites are rarely encountered. A number of factors contribute to the low occurrence of naturally occurring organofluorides. Despite fluorine being the thirteenth most common element present in the Earth’s crust, most is contained within insoluble minerals severely limiting bioavailability. Fluoride has the lowest oxidation potential of the halides, which prevents biohalogenation by the same mechanisms used for chlorine, bromine and iodine. Additionally, the high
hydration energy of the fluoride ion renders it a poor nucleophile in water (Table 1.1).26,75

From the marine environment only fluoroacetate (49) and five 5-fluorouracil analogues (50–54) have been isolated from a Streptomyces sp. bacterium and the sponge Phakellia fusca, respectively.76,77 Fluoroacetate (49), originally isolated in 1943 as the toxic component of the terrestrial plant Dichapetalum cymosum, is the most common organofluoride found from natural sources.26 Interestingly, compound 50 is an anticancer drug used in the treatment of head and neck, colorectal, bladder, and breast cancers,78 and surprisingly, it has been suggested that de novo biosynthesis of 50–54 is not carried out by P. fusca but instead 50 is accumulated by the sponge from industrial effluent.76

\[ \text{Fluoroacetate (49)} \]

\[ \text{50} \quad \text{R = OMe, H, NHNH}_2 \]

\[ \text{51} \quad \text{R = OMe} \]

\[ \text{52} \quad \text{R = H} \]

\[ \text{53} \quad \text{R = OMe} \]

\[ \text{54} \quad \text{R = NHNH}_2 \]

### 1.2.4 Vanadium-dependent Haloperoxidases

The insertion of halogens into biological substrates typically occurs via enzymatic oxidation. The incorporation of fluorine and in some instances chlorine, is an exception as the enzyme involved facilitates a nucleophilic substitution reaction. Several families of enzymes that catalyse biohalogenation have been identified, such as the vanadium-dependent and haem iron-dependent haloperoxidases and the non-haem iron-dependent, flavin-dependent and S-adenosyl-l-methionine (SAM)-dependent halogenases.79 Vanadium-dependent haloperoxidases (V-HPOs) are the predominant halogenating enzymes found in algae and have been studied extensively by Butler and co-workers.79–84
The first V-HPO was discovered in 1983 from the brown alga *Ascophyllum nodosum* and more have since been found in other macroalgae, fungi and bacteria.\(^{79,87}\) V-HPOs catalyse halide oxidation by hydrogen peroxide to form a two-electron oxidised species (*e.g.* hypohalous acid, hypohalite or an enzyme-bound halonium-type species), which can then proceed to react via electrophilic halogenation with the organic substrate or with a second equivalent of hydrogen peroxide when a suitable organic substrate is lacking (Scheme 1.1).\(^{79}\) The oxidation state of the vanadium centre remains constant throughout the process and thus, the V-HPO functions as a Lewis acid catalyst rather than a redox catalyst.\(^{79}\)

**Scheme 1.1.** Scheme of the halide and peroxide reaction with a vanadium haloperoxidase.\(^{80}\)

V-HPOs are categorised according to the enzyme’s ability to catalyse the oxidation of the halide with the lowest oxidation potential (for oxidation potentials see Table 1.1). Thus, a chloroperoxidase (V-ClPO) is capable of catalysing the oxidation of chloride, bromide and iodide ions; bromoperoxidase (V-BrPO) can catalyse the oxidation of bromide and iodide, and iodoperoxidase (V-IPO) can catalyse the oxidation of the iodide ion only.\(^{80}\) Thermodynamic studies indicate that it is impossible for the oxidation of fluoride ions to occur by the reduction of hydrogen peroxide (Scheme 1.2) implying that no such fluoroperoxidase exists.\(^{26}\) The V-BrPOs appear to be the most prevalent, particularly in algae, and research has been carried out on their catalytic oxidative properties. Two such studies have shown that the enzyme, in the presence of aqueous bromide and hydrogen peroxide, catalyses the regiospecific oxidation of an indole derivative\(^{82}\) and the stereoselective bromination and subsequent cyclisation of a terpene.\(^{81}\) Each reaction is thought to proceed through a bromonium ion intermediate. The observed regiospecificity and stereoselectivity suggests that the organic substrate is oriented in a specific way.
within the active site of V-BrPO. Organic substrate selectivity is also known for the V-BrPOs, although the factors governing this selectivity are yet to be determined. It is also intriguing that similar V-HPOs can exist in two related algae, yet halogenated natural products can be predominant in one but not the other.

Scheme 1.2. Standard electrode potential and redox equilibria of fluoride and hydrogen peroxide.

1.3 Proposed Research

The research described in this thesis was aimed at identifying and characterising new secondary metabolites from marine sponges and red algae, particularly those that are halogenated. An NMR and MS-guided isolation methodology was employed to facilitate these efforts. In particular, it was anticipated that using MS to guide the isolation process would be extremely powerful due to the isotopic nature of bromine and chlorine. Where possible, compounds isolated in this study were submitted to the School of Biological Sciences for biological activity assessment.
Once an organism has been selected for investigation, it is extracted with organic solvent. A typical crude extract contains a vast array of compounds ranging in polarities from hydro- to lipophilic. In the scope of polarities present, compounds at the outer extremes are largely primary metabolites (compounds that are essential to normal metabolic processes within an organism) that constitute a large proportion of the total number of compounds present within a crude extract. By comparison, secondary metabolites represent a much smaller amount of the crude extract mass and generally possess amphiphilic properties with a combination of polar and non-polar functional groups. Within a biological system, this amphiphilic nature enables partial solubility in water and the ability to traverse across a cell-membrane to interact with the substrate. It is partly for this reason that secondary metabolites entice the interest of natural product chemists.

### 2.1 Targeting Secondary Metabolites

The large chemical diversity and range of polarities within a crude extract often results in solubility issues, making analyses at this early stage very problematic. A preliminary purification step performed on the crude extract either substantially minimises or completely circumvents these issues. The hypothetical diagram illustrated in Figure 2.1 can be used as a chromatographic guideline for the preliminary purification of a crude extract so that further analyses can be carried out with ease. The majority of the mass is anticipated to elute from reversed-phase chromatographic media in the early (polar compounds such as salts and sugars) and late stages (non-polar compounds such as lipids), leaving the central region or ‘mass-window’ to contain compounds of intermediate polarity. Therefore, the aim
would be to target this region during the purification of a crude extract, as it should be comprised of mostly secondary metabolites.

**Figure 2.1.** Mass distribution of a biological extract and the elution profile from a reversed-phase chromatographic medium. Adapted from Ryan.91

### 2.1.1 PSDVB and Cyclic Loading

Our research group utilises the ‘mass-window’ concept (Figure 2.1) during the early purification of a crude extract. Before it can be employed, the extract must first be loaded onto a chromatographic medium for purification—cyclic loading serves this purpose. The technique ‘cyclic loading’ uses reversed-phase poly(styrene-divinylbenzene) (PSDVB) beads and was developed by Northcote and West at VUW for the preliminary purification of a crude sponge extract.90 While cyclic loading can be employed using any chromatographic adsorbent medium, PSDVB is particularly attractive as it is inexpensive, chemically inert and stable throughout the pH range. Respectively, the latter two properties mean that PSDVB can be recycled repeatedly without concern and the adsorption and retention of compounds can be facilitated with the use of ionic buffers. In addition to sponges,89,91–93 the technique has since been used successfully by VUW natural products research groups for the initial fractionation of algal,13 sea cucumber,94,95 nudibranch92 and extremophilic microbial96 extracts, thus becoming an integral method in our laboratories.

Conceptually, cyclic loading is the reverse of chromatography—instead of sequentially eluting compounds of interest from the chromatographic medium, the
technique sequentially adsorbs them.\textsuperscript{89} The general cyclic-loading procedure is as
follows: an organism of interest is extracted with organic solvent and the crude
extract is passed through a column containing PSDVB where most of the non-
polar compounds adsorb to the stationary phase. The eluent is subsequently
diluted with an equal volume of H\textsubscript{2}O (100\% v/v) and recycled through the column.
The dilution and recycling steps are repeated until all compounds of interest are
adsorbed to the PSDVB; usually a four-fold dilution of the extract is sufficient.
Adding H\textsubscript{2}O incrementally leads to a gradual increase in eluent polarity. This
effectively minimises any risk of precipitation and also increases the affinity of
compounds, still in solution, to the stationary phase, thus ensuring their adsorption.
Once cyclic loading is complete, the column is washed with \( \text{H}_2\text{O} \) to remove salts,
then successively eluted with \( \text{H}_2\text{O}/\text{organic modifier mixtures of decreasing polarity.} \)
Cyclic loading is pictorially presented in Figure 2.2.

The resulting fractions obtained from the elution of a PSDVB column are sub-
sequently back-loaded (a modified cyclic loading technique) onto another PSDVB
column. This is to effect a solvent change due to the tendency of some \( \text{H}_2\text{O}/\text{organic} \)
modifier mixtures frothing vigorously under reduced pressure. The fraction is
diluted with \( \text{H}_2\text{O} \) (100\% v/v) and recycled (back-loaded) onto PSDVB. The eluent is
collected and the process repeated once more—at this stage most of the compounds
should have re-adsorbed onto the PSDVB column. The column is then blown dry to
minimise the amount of \( \text{H}_2\text{O} \) present after which point elution is carried out using a
pure organic solvent that can be concentrated without difficulty to provide samples
that are ready for screening.

2.2 Natural Products Screening

Screening a partially purified organic extract of a given organism serves to determine
whether the organism is worthy of a more detailed scientific investigation before
considerable time and resources have been invested. Two main screening techniques
exist: bioassay-guided screening and spectroscopy-guided screening. The former technique tends to be more commonly used among natural product researchers as a primary method.

2.2.1 Bioassay-guided Screening

Bioassay-guided fractionation has been used extensively to screen and guide the isolation of natural products. A source organism is selected following a positive result of the extract against a specific biological assay. The extract is then purified and the fractions reassessed for biological activity in the same assay. Those producing a positive result undergo repeated purification/testing processes until the biologically active compound is isolated. As a result, the method ultimately yields a compound of potential pharmaceutical interest and forms the reason (validly) for the preference of this method among researchers in the field. However, the

Figure 2.2. Schematic of the cyclic loading procedure. Adapted from West. 

18
complex mixtures of compounds (even after one purification step) is problematic because the biological activity of major components could mask minor, potentially novel active compounds that may pass through the assay undetected. Additionally, assay results can be confounded by interference compounds (those causing false positives) or synergistic effects between two or more components both of which can be misleading.\textsuperscript{98}

### 2.2.2 Spectroscopy-guided Screening

Spectroscopy-based screening focuses on the chemical make-up of compounds within a crude extract. No information on biological activity is offered but there is perception is that structural novelty frequently leads to interesting biological activity.\textsuperscript{89}

Mass-spectrometry coupled with liquid chromatography (LC-MS) is an example of a widely-used spectroscopic screening tool. High sensitivity and mass accuracy in addition to the wealth of characteristic information provided, such as chromatographic retention time, molecular mass and molecular formula, make it a particularly powerful screening tool.\textsuperscript{99} Further advantages are offered by the use of tandem MS which can provide structural information in the form of fragment ions generated from the parent molecular ion. The main downfall in the technique is in the difficulty of optimising ionisation conditions to suit all compounds present within the matrix.\textsuperscript{100}

Nuclear magnetic resonance (NMR) spectroscopy is an alternative spectroscopic screening tool. NMR has the capability of providing elaborate structural information with regards to chemical motifs and substructures of molecules within the often complex mixture of a partially purified extract. Gaining this information early makes it a useful dereplication tool when used in conjunction with structural library databases, offering a greater chance for the isolation of novel compounds. Knowledge of chemical functionalities, such as acidic or basic moieties, is particularly valuable for guiding decisions in successive chromatographic techniques, the purification of
which may benefit from the use of ion-exchange media or pH buffers. However, when compared with MS- and bioassay-guided screening, the technique is comparatively insensitive. Although, sensitivity has significantly improved with technological advancements in NMR field strength, micro-coils and cryogenic systems, coupled with the development of capillary and micro-probes. These developments have facilitated the structure elucidation of sub-milligram quantities of natural product compounds, including that of hemi-phorboxazole A (55), the structure of which was elucidated from a mere total mass of 16.5 $\mu$g.$^{101-104}$

\[
\begin{align*}
\text{55} \\
\end{align*}
\]

### 2.2.3 NMR-guided Screening, VUW

NMR screening of partially purified extracts is the method employed in our research group. The extracts of a selected organism are cyclic loaded onto PSDVB (Section 2.1.1) and the column is sequentially eluted using solutions of 1) 30% Me$_2$CO/H$_2$O 2) 75% Me$_2$CO/H$_2$O and then 3) Me$_2$CO. The Me$_2$CO fraction can be immediately concentrated under reduced pressure but the 30% and 75% Me$_2$CO/H$_2$O fractions require back-loading onto PSDVB for the reason outlined in Section 2.1.1. These three fractions consecutively represent the polar, intermediate, and non-polar regions of the diagram provided in Figure 2.1 with the 75% Me$_2$CO/H$_2$O fraction thereby constituting the ‘mass-window’ and therefore, theoretically, contains most of the compounds of interest. Once concentrated, the fractions can be analysed using NMR spectroscopy.

The NMR screening methods of our research laboratory have developed over
time. Naturally, the first screening method began with the simple 1D $^1$H NMR experiment. The success of this screening tool was observed through the isolation of several novel compounds. However, signals attributed to primary metabolites obscured those belonging to potentially interesting secondary metabolites, especially in the region $\delta_\text{H} 0.5–5.5$, and structural information was limited to olefins and aromatics.

Further developments of the NMR screen led to the generation of a paper mask complied from the 75% Me$_2$CO/H$_2$O COSY and HSQC screens and later a digital HSQC mask.

The paper mask was generated from the collation of several COSY or HSQC screen spectra with correlations occurring regularly at any given position being traced onto paper. A screen spectrum was subsequently overlaid on the appropriate mask to identify unique correlations within the screen, and was assigned a rating depending on the position, number and signal intensity of unique correlations present, and the availability of a given organism. Though the COSY is significantly more sensitive, preference was given to the HSQC mask due to the additional information obtained from the carbon dimension enabling the ability to determine distinct chemical functionalities. The paper mask was further developed into a computer-based adaptation—the digital HSQC mask. A selected HSQC screen applied to the digital mask (compilation and superimposition of the 75% Me$_2$CO/H$_2$O HSQC screen spectra) was evaluated by comparing common (correlations appearing frequently at a given position) and uncommon correlations (those appearing less frequently at a given position) between the mask and the screen. As a result, common correlations appear strong and easily discernible to those that are uncommon, which are weak by comparison.

While the HSQC screen successfully served its purpose, leading to the isolation of a multitude of novel compounds, the capacity to only detect protonated carbon centres presented a limiting factor and indicated room for improvement. A greater level of information is provided by the HMBC experiment and accordingly, developments towards an HMBC screen were instigated. Information obtained about non-
protonated functional groups and chemical motifs and the ability to elucidate substructures are huge advantages offered by the use of this experiment as a screening tool. Families of compounds can be identified at the earliest stage of the purification process providing a powerful tool to aid in the decision of whether of an organism is worthy of further investigation. While the HMBC is theoretically less sensitive than the HSQC experiment, its simple pulse sequence and the absence of carbon decoupling make it more robust and almost as sensitive in practise. A generic HMBC spectrum generated from a 75% Me₂CO/H₂O screen fraction is provided in Figure 2.3.

![Figure 2.3](image_url)

**Figure 2.3.** A generic HMBC spectrum (600 MHz, CD₃OD) of a 75% Me₂CO/H₂O screen fraction showing a multitude of correlations throughout the spectrum.

Research conducted within our laboratory has mainly focused on sponges and algae, owing to the interesting, often novel secondary metabolites that originate from these organisms. In this study, the screening approach (see Appendix A for the screening and NMR protocol) initially involved both of these taxonomic groups but quickly progressed towards and remained focused on red algae of either Tongan or
New Zealand origin. Using an NMR-based spectroscopic screening methodology, six marine organisms were screened, of which two red algae were investigated extensively.

2.3 Marine Sponges

Sponges produce a diverse array of interesting, biologically active compounds. Owing to their soft-bodies and sessile nature, these organisms tend to produce toxic secondary metabolites as chemical defences to facilitate their survival by deterring predators, preventing competitor encroachment, parasitic infection or overgrowth of fouling organisms. For example, the strong odorous exudate of *Latrunculia magnifica* (now re-classified as *Negombata magnifica*) causes the immediate fleeing of fish when released into the sea and has been found to induce rapid death in aquarium fish. The major metabolite of the sponge is the cytotoxin latrunculin A (56).

Between 20–30% of all marine natural products reported in recent years have been from sponges alone. Many of these exhibit cytotoxic activity in tumour-based cell line assays (*e.g.* monanchomycalin A and B, 57 and 58), but sponge-derived metabolites have also been found to display other biological activities including antifungal (*e.g.* ceratinadin A and B, 59 and 60), antimalarial (*e.g.* tsitsikammamine C, 61), anti-inflammatory (*e.g.* tedanol, 62) and antiviral (*e.g.* polybrominated diphenyl ethers 63 and 64) activity. With such a broad range of biological activities and a large diversity of chemically intriguing molecules, it is not difficult to see why sponges are popular organisms to study in marine natural products laboratories.

In this research, two Tongan sponge specimens were selected for screening. A brief investigation of each ensued, the results of which are detailed below.
2.3.1 PTN4_08B

The unidentified sponge, PTN4_08B, was collected from Vava’u, Tonga, in 2009 (Figure 2.4). The sponge was soft, springy and irregularly shaped with a smooth pale-brown surface. The $^1$H NMR spectrum of the 75% Me$_2$CO/H$_2$O screen fraction (Figure 2.5) consisted of an almost pure compound. Further analysis of the 2D data revealed the presence of an atypical steroid as indicated by the three deshielded oxymethines: a triplet of doublets resonating at $\delta_H$ 4.16, $\delta_C$ 78.7 and two broad singlets at $\delta_H$ 4.74, $\delta_C$ 75.5 and $\delta_H$ 4.80, $\delta_C$ 75.5. In contrast, the $^1$H NMR spectrum of the 100% Me$_2$CO/H$_2$O screen fraction (Figure 2.6) displayed at least two steroidal compounds, each containing the typical A-ring CH-3 oxymethine proton resonance...
(dddd, δ_H 3.52, δ_C 71.8 and dddd, δ_H 3.59, δ_C 71.3). While the regular steroid failed to attract further attention, the unusual steroid confined to the 75% Me₂CO/H₂O fraction was intriguing and warranted additional purification and analyses.

![Figure 2.4. Photo of PTN4_08B. Image courtesy of Rob Keyzers, 2009.](image)

The 75% Me₂CO/H₂O fraction was partitioned on a second PSDVB column, then a DIOL column and then reversed-phase HPLC (C₁₈). This final purification step resulted in the isolation of a 1:1 mixture of the trisulfated steroids, halistanol sulfate (65) and topsentinol K trisulfate (66). Compound 65 was originally isolated from a Japanese collection of Halicondria cf. moorei as the active component in an undescribed antimicrobial assay. An Indonesian Topsentia sp. sponge was reported as the source of 66, which was subsequently discovered to inhibit the aspartic protease BACE-1. A multitude of compounds have been reported within this class of steroids and as such further analyses were discontinued.

![Chemical structures](image)
2.3.2 PTN3_19C

PTN3_19C is a sponge that is easily recognised because of its striking blood-red appearance (Figure 2.7). Collected from ‘Eua, Tonga, during a 2008 expedition, the sponge was originally screened by Singh\(^9\) and was tentatively identified as *Plakortis quasiamphiaster* based on the morphological and chemotaxonomic similarity. The 75% Me\(_2\)CO/H\(_2\)O screen fraction displayed a simple \(^1\)H NMR spectrum with one or
two major compounds, and fractionation of the sample by Singh led to the isolation and identification of two known pyrroloacridines, plakinidine A (67) and plakinidine B (68). The isolation of these compounds concluded Singh’s investigation of the sponge and the 30% Me$_2$CO/H$_2$O and 100% Me$_2$CO fractions remained unexplored, providing an opportunity for a re-examination of the sponge.

![Figure 2.7. Underwater photo of PTN3_19C. Image courtesy of Dan Crossett, 2016.](image)

During this study, PTN3_19C was extracted with MeOH and the extract subjected to the preliminary purification procedures outlined in Section 2.1.1. This resulted in three strongly pigmented screen fractions: 1) 30% Me$_2$CO/H$_2$O (intense red) 2) 75% Me$_2$CO/H$_2$O (intense red) and 3) 100% Me$_2$CO (vibrant purple). The $^1$H NMR spectra of both the 30% (Figure 2.8) and 75% Me$_2$CO/H$_2$O screen fractions (Figure 2.9) showed each to be almost pure with clear resonances attributable to plakinidines. Conversely, the 100% Me$_2$CO fraction (Figure 2.10) lacked any plakinidine-related signals but consisted mainly of two compounds. Further purification of the 100% Me$_2$CO fraction on silica gel afforded the major compounds, which were determined to be the new $\alpha$-exomethylene-$\gamma$-methyl-$\gamma$-tetradecyl-$\gamma$-
butyrolactone (69) (2.7 mg) and $\alpha$-exomethylene-$\beta$-hydroxyl-$\gamma$-methyl-$\gamma$-tetradecyl-$\gamma$-butyrolactone (70) (13.4 mg) (Scheme 2.1).

Figure 2.8. $^1$H NMR spectrum of the 30% Me$_2$CO/H$_2$O screen fraction from PTN3.19C (600 MHz, CDCl$_3$).

Figure 2.9. $^1$H NMR spectrum of the 75% Me$_2$CO/H$_2$O screen fraction from PTN3.19C (600 MHz, CD$_3$OD).
**Figure 2.10.** $^1$H NMR spectrum of the 100% Me$_2$CO screen fraction from PTN3_19C (600 MHz, CDCl$_3$).

**Scheme 2.1.** Isolation scheme of γ-butyrolactone compounds $69$ and $70$ from PTN3_19C.

α-Exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69)

A protonated molecule was detected using HRESIMS at $m/z$ 309.2778, consistent with the molecular formula C$_{20}$H$_{36}$O$_2$, which required three double bond equivalents. All protons and carbons were observed in their respective $^1$H and $^{13}$C NMR spectra.
The protonated centres were attributed to an exomethylene (methylidene), five distinct methylenes, a saturated alkyl chain and two methyls on the basis of the 1D and multiplicity-edited HSQC NMR spectra. Three non-protonated carbons were identified as an ester carbonyl ($\delta_C$ 170.2), olefinic carbon ($\delta_C$ 136.1) and a quaternary oxygenated carbon ($\delta_C$ 84.1).

Beginning with the methylidene protons of CH$_2$-19 ($\delta_H$ 6.22, 5.60; $\delta_C$ 122.0), HMBC correlations were observed to C-1 ($\delta_C$ 170.2), C-2 ($\delta_C$ 136.1) and C-3 ($\delta_H$ 2.79, 2.67; $\delta_C$ 39.6) establishing the attachment point as C-2. Allylic (long-range) COSY correlations between the protons of H$_2$-19 and H$_2$-3, and HMBC correlations from H$_2$-3 to C-1, C-2 and C-19 confirmed this connectivity. This segment was further extended to include the oxygenated quaternary carbon C-4 ($\delta_C$ 84.1), the methylenes CH$_2$-5 ($\delta_H$ 1.64; $\delta_C$ 41.5) and CH$_2$-6 ($\delta_H$ 1.34; $\delta_C$ 23.9), and the methyl CH$_3$-20 ($\delta_H$ 1.38; $\delta_C$ 26.7) through the following correlations: in the HMBC experiment, H$_2$-3 correlated to C-4, C-5 and C-20, H$_3$-20 correlated to C-3, C-4 and C-5 and in the COSY experiment, a correlation was observed between H$_2$-5 and H$_2$-6. The C-1 carbonyl and $\Delta^{2,19}$ accounted for two double bond equivalents and with no other candidates remaining to account for the third, a ring was implied leading to the establishment of a $\gamma$-lactone (Figure 2.11). This was supported by three observations: 1) the C-1 $\delta_C$ chemical shift was indicative of an ester carbonyl 2) the C-4 $\delta_C$ chemical shift suggested oxygenation at this centre and 3) the molecular formula required two oxygens. Compounds bearing this $\alpha$-exomethylene-$\gamma$-lactone moiety are not without precedence in the literature.$^{120,121}$

The $\alpha$-exomethylene-$\gamma$-lactone motif of 69 (Figure 2.11) satisfied all double bond equivalents as required by the molecular formula, indicating the remainder of the structure to be acyclic and fully saturated, which was supported by the chemical shifts of the unassigned carbons. The presence of a lone methyl triplet (CH$_3$-18: $\delta_H$ 0.88; $\delta_C$ 14.3), a pair of methylenes (CH$_2$-17: $\delta_H$ 1.25–1.35; $\delta_C$ 22.9 and CH$_2$-16: $\delta_H$ 1.25–1.35; $\delta_C$ 32.1) and a cluster of methylenes ($\delta_H$ 1.25–1.35; $\delta_C$ 29.5–29.9) accounting for 18 protons and comprising the remaining nine carbon resonances,
Figure 2.11. COSY and HMBC correlations establishing the cyclic substructure of the α-exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69).

implied the rest of the structure to be a saturated C_{12} linear alkyl chain extending from the methylene CH_{2}-6. A selective 1D TOCSY experiment (200 ms spin-lock mixing time) irradiating the H_{3}-18 proton resonance displayed coherence transfer through the alkyl chain to H_{2}-5 (Figure 2.12), validating the methyl connection to the structure and providing the planar structure of 69. NMR data is presented in Table 2.1.

Figure 2.12. 1D TOCSY coherence transfer from H_{3}-18 to H_{2}-5 in α-exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69).

α-Exomethylene-β-hydroxyl-γ-methyl-γ-tetradecyl-γ-butyrolactone (70)

For compound 70, a protonated molecule was detected at m/z 325.2729 using HRESIMS. This was found to be consistent with the molecular formula C_{20}H_{36}O_{3}, which required three double bond equivalents and differed only by one additional oxygen with respect to 69. All 20 carbons were observed in the $^{13}$C NMR spectrum and the 1D and 2D NMR spectra revealed the compound to be closely related to 69. The multiplicity-edited HSQC experiment revealed an exomethylene, four distinct
Table 2.1. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for α-Exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69) (CDCl$_3$).

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<th>$^J_{\text{CH}}$ (Hz)</th>
<th>$\delta$ (ppm)</th>
<th>mult</th>
<th>$J$ (Hz)</th>
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†Measured from the unsuppressed $^1J_{\text{CH}}$ in the HMBC experiment.

methylenes, a saturated alkyl chain, an oxymethine and two methyls. Similarly to 69, three non-protonated carbons were identified as an ester carbonyl ($\delta_C$ 168.2), olefinic carbon ($\delta_C$ 139.6) and a quaternary oxygenated carbon ($\delta_C$ 87.7).

As with 69, analysis of the 1D and 2D NMR spectra of 70 began at the exomethylene. The methyldiene protons of CH$_2$-19 ($\delta_H$ 6.31, 5.91; $\delta_C$ 125.2) correlated to C-1 ($\delta_C$ 168.2), C-2 ($\delta_C$ 139.6), and to the oxymethine CH-3 ($\delta_H$ 4.56; $\delta_C$ 74.5) in the HMBC spectrum and to H-3 in the COSY spectrum. HMBC correlations from H-3 to C-1, C-2 and C-19 validated the connectivity, with additional correlations to the quaternary oxygenated carbon C-4 ($\delta_C$ 87.7), the methylene CH$_2$-5 ($\delta_H$ 1.67; $\delta_C$ 39.7) and to the methyl CH$_3$-20 ($\delta_H$ 1.32; $\delta_C$ 19.2) extending the substructure. The methyl protons H$_3$-20 displayed HMBC correlations to C-3, C-4 and C-5, supporting the established C-3–C-5 connectivity (Figure 2.13).

TOCSY experiments (200 ms spin-lock mixing time) showed the remainder of the structure to be analogous to 69 with irradiation of the methyl H$_3$-18 ($\delta_H$ 0.87; $\delta_C$ 14.1) exhibiting transfer to H$_2$-5 and vice versa for the irradiation of H$_2$-6, thus establishing
the planar structure of 70. NMR data is provided in Table 2.2.

Figure 2.13. COSY and HMBC correlations establishing the cyclic substructure in α-exomethylene-β-hydroxyl-γ-methyl-γ-tetradecyl-γ-butyrolactone (70).

Table 2.2. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for α-Exomethylene-β-hydroxyl-γ-methyl-γ-tetradecyl-γ-butyrolactone (70) (CDCl$_3$).

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<th>$^1$J$_{CH}$ (Hz)</th>
<th>$\delta$ (ppm)</th>
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$^\dagger$Measured from the unsuppressed $^1$J$_{CH}$ in the HMBC experiment

Compounds 69 and 70 were submitted to the School of Biological Sciences for assessment of cytotoxicity in the HL-60 (human promyelocytic leukaemia) cell line bioassay. Each exhibited moderate activity with IC$_{50}$ values of 1.13 and 2.70 µM for 69 and 70, respectively.
Natural products containing the β-hydroxyl-γ-lactone moiety are not without precedence in our laboratory. A compound labelled AJS12_36A (71) featuring a triunsaturated alkyl chain was isolated by Singh from an undescribed sponge also collected from ‘Eua, Tonga.\textsuperscript{93} Other structurally related compounds to 69 and 70 in the chemical literature were isolated from sponges of the genus \textit{Plakortis}. An investigation of a Canary Islands \textit{Plakortis} sp. sponge resulted in the isolation of plakolide A (72), which demonstrated moderate to weak cytotoxicity, ranging from 1.1–5.0 µg/mL in several mammalian cell line bioassays.\textsuperscript{120} \textit{Plakortis quasiamphiaster}, collected from Vanuatu, was the source of 17 closely related compounds named the amphiasterins (73–89). Compounds 73–89 are γ-lactones that vary in the functionality at the α-position and in the length and degree of unsaturation of the alkyl side chain.\textsuperscript{121}

At this time, the configurational assignments of 69 and 70 remain undetermined due to the degradation of both compounds. Although, procurement of either compound was completed with ease, the focus of this research had switched towards red algal specimens and as such, further analyses on PTN3_19C were discontinued.
2.4 Marine Red Algae

A variety of compounds have been isolated from red algae, with terpenoids such as the diterpenes 90–92 and bromophenols such as 93–95 amongst the most prevalent. Additionally, red algal metabolites typically contain a high level of halogen substitution and a large number of novel terpenoid carbon skeletons. These structural characteristics make red algae a particularly attractive source of interesting, potentially bioactive secondary metabolites.

A total of four red algal specimens were selected for screening using our spectroscopic-screening protocol. Where possible, algae were identified by Dr Joe Zuccarello at the School of Biological Sciences, VUW. Only two were chosen for further study.
2.4.1 PTN4.34B

PTN4.34B (Figure 2.14) is an unidentified red alga collected from ‘Eua, Tonga in June 2016. The 75% Me₂CO/H₂O screen fraction displayed resonances in the 1D and 2D NMR spectra attributed to a sugar moiety with the anomeric proton doublet clearly visible at δ_H 4.76 in the ^1H NMR spectrum (Figure 2.15). Aromatic singlet resonances above δ_H 8.50 (Figure 2.16) attached to highly shielded carbons resonating between δ_C 92–108 were observed in the 1D and 2D NMR spectra of the 100% Me₂CO fraction, which revealed the presence of a class of molecules containing a porphyrin core. Other interesting olefinic and aromatic resonances were observed between δ_H 5.50–8.50, however, further investigation of both screens was discontinued in favour of other red algal specimens.

Figure 2.14. Underwater photo of PTN4.34B. Image courtesy of Dan Crossett, 2016.
2.4.2 PTN4_35B

The unidentified red alga, PTN4_35B (Figure 2.17) was collected from ‘Eua, Tonga, in June 2016 and was selected for screening. Despite obvious morphological differences between PTN4_34B and PTN4_35B, the NMR profile observed for the 75% Me₂CO/H₂O screen fraction of PTN4_35B (Figure 2.18) was very similar to...
that of PTN435B (Figure 2.15). Notably, resonances pertaining to a similar sugar moiety to that found in PTN434B were observed. As such, this alga was discounted from further investigation.

Figure 2.17. Photo of PTN435B. Image courtesy of Dan Crossett, 2016.

Figure 2.18. \(^1\)H NMR spectrum of the 75\% Me\(_2\)CO/H\(_2\)O screen fraction from PTN435B (600 MHz, CD\(_3\)OD).

2.4.3 PTN420A

The red alga PTN420A (Figure 3.1) was collected in January 2012 and 2014 from Wellington, New Zealand, and was identified as *Rhodophyllis membranacea*. 
An extensive natural product investigation ensued following mass spectrometric evidence for the presence of a multitude of polyhalogenated indoles. Further details of this investigation are described in Chapter 3.

2.4.4 PTN4_34F

PTN4_34F (Figure 4.1) was collected in June 2016 from ‘Eua, Tonga, and was identified as *Callophycus serratus*. Signals in the aromatic region, in addition to a deshielded gem-dimethyl, were identified in the screen 1D and 2D NMR spectra. This result prompted a more detailed study of this alga, the results of which are outlined in Chapter 4.
Chapter 3

Polyhalogenated Indoles from the New Zealand Red Alga \textit{Rhodophyllis membranacea}

The research described in this chapter includes and expands on work that was published in the Journal of Natural Products.\textsuperscript{125}


3.1 Secondary Metabolites of New Zealand Red Algae

New Zealand red algal metabolites have predominantly been reported by the Blunt and Munro group at the University of Canterbury. Over the period between 1978 and 1988, Blunt and Munro contributed a total of 24 new red algae compounds to the marine natural products literature.\textsuperscript{126–133} Algae of the genera \textit{Laurencia}, \textit{Plocamium} and \textit{Delisea} were among those studied and examples of halogenated compounds include the brominated polyether thyrsiferol (96),\textsuperscript{128} the isomeric acetylenic cyclic ethers 97 and 98,\textsuperscript{132} the sesquiterpene isolaurenisol (99)\textsuperscript{130} and a series of 2(5\textit{H})-furanones (100-104)\textsuperscript{133} were isolated. Thyrsiferol (96) was found to be inactive against several strains of bacteria,\textsuperscript{128} but was subsequently shown to possess potent cytotoxicity against the P388 cell line (IC\textsubscript{50} 16 nM).\textsuperscript{134} Compounds 97–104 were not assessed in any biological assay.\textsuperscript{128,130,132}

In addition to the research of the Blunt and Munro group, three other independent
groups have worked on New Zealand red algal specimens and reported halogenated compounds. König and Wright isolated several compounds from a *Laurencia* sp., including four new C_{15} acetogenins (105–108) and a structural isomer of isolaurenisol (109). Compounds 105–109 were not assessed for biological activity, but the subsequent re-isolation of 109 by a separate research group showed that it lacked cytotoxicity (IC_{50} > 88.6 µM) against several mammalian cell lines. Popplewell and Northcote reported the isolation of the nitrogenous bromophenol, colensolide A (110), from Northland specimens of *Osmundaria colensoi*. While colensolide A (110) lacked activity against the HL-60 cell line and the MC²_{55} strain of *Mycobacterium smegmatis*, several other known compounds isolated alongside 110 demonstrated moderate cytotoxicity and antibacterial activity, respectively. The third research group reported polyhalogenated indoles isolated from *Rhodophyllis membranacea*. Their work is discussed in Section 3.2.
3.2 *Rhodophyllis membranacea*

The red alga *R. membranacea* (Figure 3.1) is one of 49 accepted species within the genus *Rhodophyllis* and it is distributed throughout Australia and New Zealand. A specimen collected off the Kaikoura Coast of New Zealand was investigated by Brennan and Erickson in 1978 following a positive hit of the crude extract in an antifungal bioassay. Their report is amongst the earliest record of natural products derived from New Zealand marine organisms with Blunt and Munro acknowledged for facilitating algal collection and performing the biological assays. Several polyhalogenated indoles were reported, including 2,3,4- and 2,3,7-trihalogenated indoles (111–115) and a number of 2,3,4,7-tetrahalogenated indoles (116–122). While the structures of 112–115 were verified via synthesis, the others were not and the $^1$H NMR data of many of the tetrahalogenated indoles could not be unambiguously assigned. As such, there was obvious scope for a re-examination of *R. membranacea*.

*R. membranacea* has been found by our research group to grow abundantly in the subtidal region along the shoreline of Moa point, Wellington, New Zealand—while searching for a morphologically similar red alga for the undergraduate
teaching laboratory, *R. membranacea* was inadvertently and repeatedly collected over several years. Initially, the alga was disregarded as a prospective organism for research purposes and instead was screened by Cori Jones (a visiting student from the University of Kansas) for its potential applicability in the teaching laboratory. During that enquiry, however, the alga became interesting from a research perspective following mass spectrometric evidence for the presence of more than 20 tri-, tetra-, penta- and hexahalogenated indoles. Consequently, *R. membranacea* became the immediate focus of this study in the search to isolate and
characterise any unusual polyhalogenated species.

A spectroscopic-guided isolation methodology led to the isolation of 21 compounds. Three are known tetrahalogenated indoles (116, 119 and 121) previously reported by Brennan and Erickson\textsuperscript{138} but herein presented with full assignment of \textsuperscript{1}H and \textsuperscript{13}C NMR data, and 11 are new tetrahalogenated indoles (123–133). In addition, four new pentahalogenated (134–137), one hexahalogenated indole identified only by HRESIMS data as a tribromo-trichloroindole, and the synthetically known, albeit new marine natural products, 4-chloroisatin (138) and the aldol product of 138, 4-chloro-3-hydroxyl-3-(2-oxopropyl)-2-oxindole (139) (see Section 3.8), were isolated.
3.3 Purification and Isolation of Polyhalogenated Indoles

Specimens of *R. membranacea* were collected from Moa Point, Wellington, New Zealand, in January 2012 and 2014. The alga was extracted with MeOH and the extracts were cyclic loaded onto PSDVB (as described in Chapter 2.1.1) then eluted with 30% Me₂CO/H₂O, 75% Me₂CO/H₂O and Me₂CO. The 30% Me₂CO/H₂O fraction appeared to contain one major component and purification of the sample by flash column chromatography on Diol yielded 4-chloro-3-hydroxyl-3-(2-oxopropyl)-2-oxindole (139) (0.70 mg). The Me₂CO fraction was further purified on silica, followed by size-exclusion chromatography using Sephadex LH20, then C₁₈ HPLC to yield 4,7-dibromo-2,3-dichloroindole (119) (1.3 mg) and 7-bromo-2,3-dichloro-6-iodoindole (129) (0.70 mg) (Scheme 3.1).

Additional extractions of the alga were treated in a similar fashion. Fractions eluting from the Sephadex LH20 column, that contained the compounds of interest (determined using LC-MS), were dissolved in 75% MeOH/MeCN, centrifuged,† and the supernatant was separated and combined as one sample. A portion (280 mg) was partitioned on C₁₈ HPLC to provide six fractions (Scheme 3.1)—under these HPLC conditions (85% MeOH/H₂O), the elution profile displayed distinct separation of the halogenated indoles according to the number of halogens present on the indolic core (Figure 3.2). These were identified using HRESIMS as tri-, tetra-, penta-, and hexahalogenated indoles.

3.3.1 Purification of Tetrahalogenated Indoles

Further purification of the HPLC fraction containing the tetrahalogenated indoles was carried out on C₁₈ HPLC. This provided more of 119 (11.7 mg) and 129 (5.6 mg), in addition to 5-bromo-2,3,4-trichloroindole (133) (1.3 mg) and 14 other fractions.

†Attempts to re-dissolve these fractions using a variety of solvent mixtures persistently left some undissolved material.
Scheme 3.1. Isolation scheme of compounds 119, 129, 138, 139 and fractions containing tri-, tetra-, penta- and hexahalogenated indoles from *Rhodophyllis membranacea*. 
fractions. C$_8$ HPLC was used to purify one of these fractions, which yielded 7-bromo-2,3,4-trichloroindole (121) as the major compound (1.5 mg). Other fractions were purified either directly from Diol, or Diol then C$_{18}$ HPLC to provide 2,3,4,7-tetrabromoindole (116) (1.0 mg), 2,3,4-trichloro-7-iodoindole (123) (1.3 mg), 2-bromo-3,4-dichloro-7-iodoindole (124) (0.13 mg), 2,3,6,7-tetrabromoindole (125) (1.2 mg), 3,6,7-tribromo-2-chloroindole (126) (1.3 mg), 2,6,7-tribromo-3-chloroindole (127) (3.7 mg), 6,7-dibromo-2,3-dichloroindole (128) (7.6 mg), 2,7-dibromo-3-chloro-6-iodoindole (130) (0.70 mg), 3,7-dibromo-2-chloro-6-iodoindole (131) (0.08 mg) and 2,3,7-trichloro-6-iodoindole (132) (0.06 mg) (Scheme 3.2).

3.3.2 Purification of Pentahalogenated Indoles

Excellent separation of the fraction containing the pentahalogenated indoles was achieved using C$_{18}$ HPLC eluting with 55% MeCN/H$_2$O (Figure 3.3). The first fraction was collected at $t_R = 52$ min and the final fraction of interest was collected at $t_R = 84$ min. A series of later eluting peaks (present in low levels) were collected together. However, each chromatographic runtime was 138 min with the fractions of interest concentrated within a relatively short 32 min window in the middle of the analysis. To shorten the overall HPLC runtime, the pentahalogenated indoles were separated from the late-eluting compounds using C$_{18}$ HPLC eluting from 85-100% MeOH/H$_2$O, then the fraction containing the pentahalogenated indoles
Scheme 3.2. Isolation scheme of tetrahalogenated indoles from *Rhodophyllis membranacea.*
was further purified using C\textsubscript{18} HPLC (55% MeCN/H\textsubscript{2}O) as a single continuous chromatographic run with sample injected at 45 min intervals (Figure 3.4). Further purification using the same column and conditions led to the purification of 5,7-dibromo-2,3,4-trichloroindole (137) (1.2 mg). Other fractions were purified using DIOL and C\textsubscript{18} HPLC to yield pure 2,3,4,5,7-pentabromoindole (134) (1.6 mg), 2,3,4,6,7-pentabromoindole (135) (0.30 mg) and 4,5,7-tribromo-2,3-dichloroindole (136) (1.7 mg). The isolation scheme of the pentahalogenated indoles is presented in Scheme 3.3.

**Figure 3.3.** Reversed-phase HPLC elution profile of pentahalogenated indoles from 55% MeCN/H\textsubscript{2}O.

**Figure 3.4.** Reversed-phase HPLC elution profile of pentahalogenated indoles from 55% MeCN/H\textsubscript{2}O as a single continuous run with sample injected at 45 min intervals.
3.3.3 Purification of Hexahalogenated Indole

Purification of the fraction containing the hexahalogenated indoles was carried out in a similar manner to that used for the pentahalogenated indoles. The sample was first fractionated using C$_{18}$ HPLC to remove late-eluting non-hexahalogenated indoles, before a single continuous chromatographic run was performed using C$_{18}$ HPLC with sample being injected at ca. 35 min intervals. Five fractions were collected (Scheme 3.4) and based on the $^1$H NMR spectrum (using the NH proton resonance as a guide) and the HRESIMS data, the third fraction appeared most pure.

3.3.4 Isolation of 4-Chloroisatin

A separate methanolic extract of R. membranacea was fractionated on a PSDVB column, eluting with mixtures of MeOH and H$_2$O. Further purification using Diol and C$_{18}$ HPLC led to the isolation of 4-chloroisatin (138) (1.3 mg) in the presence of its inseparable tautomer. The isolation of 138 is detailed in Scheme 3.1.
3.4 Structure Elucidation

Evidence for the presence of the indolic core (140) was found using UV spectrophotometry. The number of bromines and chlorines for each molecule was confirmed from the characteristic molecular ion cluster in the mass spectrum. The presence of iodine was evidenced by the significantly increased mass and match to the high mass accuracy data.

![Structure 140]

Given the low H:C,N,X ratio, the difficulty in elucidation lay in where the protons and the halogens were located around the aromatic core. The structure elucidation began with the tetrahalogenated indoles since these presented the least challenging structures of the polyhalogenated indoles isolated in this study. Once the tetrahalogenated indoles were unequivocally assigned, the structures of the pentahalogenated indoles were elucidated.

3.5 Tetrahalogenated Indoles

The combination of NMR spectroscopy and HRESIMS was instrumental in guiding the isolation and differentiation between the different tetrahalogenated indoles. In
many cases, the $^1$H NMR spectrum of two closely related indoles differing only by the substitution of one halogen for another appeared almost indistinguishable (Figure 3.5). The aromatic protons of all isolated tetrahalogenated indoles appeared as doublets with ca. 8 Hz coupling constants, diagnostic of ortho- substitution on the benzenoid ring; vicinally-coupled protons on pyrrole rings exhibit a much smaller coupling constant (2–3 Hz).\textsuperscript{140} In contrast, but equally diagnostic, the one-bond proton-carbon coupling ($^1J_{CH}$) constants observed for an indole standard were found to be significantly larger for those located around the pyrrole ring than the benzenoid ring (Tables 3.1 and 3.2), consistent with those reported for benzene\textsuperscript{141} and pyrrole.\textsuperscript{142} Since the magnitude of the $^1J_{CH}$ value increases with increasing s-character of the C-H bond, angular distortion and electronegative substituents,\textsuperscript{140} this result is expected. The $^1J_{CH}$ values were measured for tetrahalogenated indoles 116, 119, 121 123–133 and were not consistent with proton substitution on the pyrrole ring of the indole. Consequently, the ortho- protons were limited to three possible regioisomers providing either a 2,3,4,5-, 2,3,4,7-, or 2,3,6,7-tetrahalogenated indole.

Figure 3.5. Stacked $^1$H NMR spectra (600 MHz, CDCl$_3$) of (a) 123 (blue) and 124 (black), (b) 125 (blue) and 126 (black); (c) 127 (blue) and 128 (black); and (d) 129 (blue) and 130 (black).
Table 3.1. \(^{15}\text{N} \text{ (60 MHz)}, \, ^{13}\text{C} \text{ (150 MHz)} \text{ and } ^{1}\text{H} \text{ (600 MHz)} \text{ NMR Data for Indole (140) (CDCl}_3\text{).}

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†Determined from a \(^{15}\text{N} \text{ CIGAR experiment}
(w)Weak

Table 3.2. \(^{15}\text{N} \text{ (60 MHz)}, \, ^{13}\text{C} \text{ (150 MHz)} \text{ and } ^{1}\text{H} \text{ (600 MHz)} \text{ NMR Data for Indole (140) (DMSO–d}	ext{)}_6\text{).}

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†Determined from a \(^{15}\text{N} \text{ CIGAR experiment}
(w)Weak

In order to differentiate between the regioisomers and determine the proton positions, the conspicuous carbon chemical shifts of C-3, C-7 and C-7a (indole, 140: \(\delta_C\) 102.7, 111.1, 135.8 (CDCl\textsubscript{3}), respectively, see Tables 3.1 and 3.2 for full NMR data) coupled with the incidence of three-bond correlations in the HMBC spectrum appearing stronger than two-bond correlations, were used as diagnostic tools. A close examination of how many strong three-bond correlations from the protons to these carbons was particularly valuable. To illustrate this point, strong three-bond correlations to C-3, C-7 and C-7a would be observed from protons situated on C-
4 and C-5 (Figure 3.6a), protons positioned on C-5 and C-6 would show strong correlations to C-7 and C-7a (Figure 3.6b), and protons on C-6 and C-7 would display a strong correlation to C-7a only (Figure 3.6c). Additionally, of the three possibilities, the 2,3,4,5 tetrahalogenated indole was expected to be the only one that would contain a proton connected to a carbon resonating near $\delta_C$ 111 (CH-7) similarly to that found for indole (Tables 3.1 and 3.2).

Figure 3.6. Three-bond HMBC correlations to C-3, C-7 and C-7a in (a) 2,3,6,7-, (b) 2,3,4,7- and (c) 2,3,4,5-tetrasubstituted indole.

The next challenge was to determine where the halogens were situated. While iodinated carbon resonances are very distinct from that of brominated and chlorinated carbons, similarities often observed in the carbon chemical shifts of the latter two can complicate the assignment of these centres. NMR methods can be employed to facilitate with the differentiation between brominated and chlorinated carbons. Measurements of the $^{13}$C spin-lattice relaxation ($T_1$) times and integration of the halogenated carbon centres in question have been used to distinguish between the two, which works on the basis of brominated carbons generally having shorter $T_1$ times than chlorinated carbons.\textsuperscript{145} Other methods involve the acquisition of high-resolution NMR data to observe the $^{35}$Cl/$^{37}$Cl isotope shift in a 1D $^{13}$C NMR spectrum,\textsuperscript{146} and in 2D band-selective HSQC\textsuperscript{52,147} and HSQMBC experiments.\textsuperscript{148}

In this study, a strategy was employed that involved the isolation and full NMR and mass spectrometric characterisation of a tetrahalogenated indole containing one halogen type. From there, the successive isolation of tetrahalogenated indoles constituting the same substitution pattern, but where one halogen was replaced with another, were targeted and systematically characterised accordingly. Two isomeric

\textsuperscript{†}This is well known phenomenon described by a variety of explanations and is referred to as either the heavy halogen, heavy atom or spin-orbit coupling effect.\textsuperscript{143,144}
tetrabromoindoles were isolated, one exhibiting a 2,3,4,7- substitution pattern (116) and the other a 2,3,6,7-tetrabromoindole (125). With the exception of 133, all of the tetrahalogenated indoles isolated displayed either of these two substitution patterns. Where possible, high-resolution $^{13}$C NMR data was acquired by using a 6.0 s acquisition time so that the $^{35}$Cl/$^{37}$Cl isotope shift could be observed to give further support towards the proposed assignment of the chlorinated carbons. While some of the tetrahalogenated indoles isolated in this study were successfully crystallised, their quality was insufficient for single-crystal X-ray diffraction.

3.5.1 2,3,6,7-Tetrabromoindole

Negative-ion mode HRESIMS analysis of 125 provided evidence for the molecular formula C$_8$H$_3$Br$_4$N ($m/z$ 427.6928, [M – H]$^-$) (Figure 3.7), which required six degrees of unsaturation, fully accounted for by the indolic core. All protons and carbons were identified in the $^1$H and $^{13}$C NMR spectra. Two were assigned as aromatic methine doublets (CH 4: $\delta_H$ 7.31; $\delta_C$ 119.1 and CH 5: $\delta_H$ 7.42; $\delta_C$ 126.2) in a multiplicity-edited HSQC experiment, thus indicating one exchangeable proton ($\delta_H$ 8.45) and six non-protonated sp$^2$ hybridised carbons ($\delta_C$ 135.4, 127.2, 119.2, 111.5, 106.8, 95.6) were present.

![Figure 3.7. Experimental and calculated mass spectra for 2,3,6,7-tetrabromoindole (125).](image)

The methine protons correlated to each other in a COSY spectrum, as was the case for all the tetrahalogenated indoles isolated in this study. Analysis of the HMBC spectrum indicated a 2,3,6,7-tetrahalogenated indole through three-bond
correlations to the three distinctive carbons C-3, C-7 and C-7a, as illustrated in Figure 3.6a. HMBC correlations were observed from H-4 to C-3 (δ_C 95.6), C-6 (δ_C 119.2) and C-7a (δ_C 135.4), and from H-5 to C-3a (δ_C 127.2), C-7 (δ_C 106.8) and weakly to C-6. With one carbon left to assign, this could therefore be attributed to C-2 (δ_C 111.5). The chemical shifts of C-3a and C-7a were comparatively similar to indole (140; Tables 3.1 and 3.2). In contrast, the chemical shifts of C-2, C-3, C-6, and C-7 were more shielded than the corresponding carbons of indole (140), and as required by the molecular formula must therefore contain bromine at these sites to provide the structure of 125 as 2,3,6,7-tetrabromoindole. NMR data is provided in Tables 3.3 and 3.4.

Table 3.3. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 2,3,6,7-Tetrabromoindole (125) (CDCl$_3$).

<table>
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<tr>
<th>Pos.</th>
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<th>$^1$J_{CH} (Hz)</th>
<th>$^1$H (ppm)</th>
<th>mult</th>
<th>J (Hz)</th>
<th>COSY ($^1$H to $^{13}$C)</th>
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<td>119.2 C</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>106.8 C</td>
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<td></td>
</tr>
<tr>
<td>7a</td>
<td>135.4 C</td>
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</tr>
</tbody>
</table>

†Determined from a $^{15}$N CIGAR experiment
Table 3.4. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 2,3,6,7-Tetrabromoindole (125) (DMSO−d$_6$).

<table>
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<th>δ (ppm)</th>
<th>mult</th>
<th>$^J$ (Hz)</th>
<th>COSY ($^1$H to $^{13}$C)</th>
<th>HMBC</th>
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<tr>
<td>7a</td>
<td>135.5$^\dagger$</td>
<td>C</td>
<td></td>
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</tr>
</tbody>
</table>

$^\dagger$Not observed

$^\dagger$Determined from an HMBC experiment

3.5.2 3,6,7-Tribromo-2-chloroindole

The molecular formula of 126 was determined to be C$_8$H$_3$Br$_3$ClN from the HRESIMS data ($m/z$ 383.7438, [M − H]$^-$) (Figure 3.8). Inspection of the 1D and 2D NMR data revealed close similarities to 125 with the exception of the appearance of a carbon resonance at δ$_C$ 124.0 in place of δ$_C$ 111.5. HMBC correlations were observed from H-4 (δ$_H$ 7.31) to C-3 (δ$_C$ 91.5), C-6 (δ$_C$ 119.2) and C-7a (δ$_C$ 133.8), and from H-5 (δ$_H$ 7.43) to C-3a (δ$_C$ 126.8) C-6 and C-7 (δ$_C$ 106.9). The similarity in chemical shifts of C-3, C-6 and C-7 to those of 125 clearly pointed to bromination at those positions. Consequently, C-2 (δ$_C$ 124.0) was assigned as the chlorinated centre based on its higher chemical shift compared to that of 125. Thus, the structure 126 was deduced as 3,6,7-tribromo-2-chloroindole. NMR data is provided in Tables 3.5 and 3.6.

Figure 3.8. Experimental and calculated mass spectra for 3,6,7-tribromo-2-chloroindole (126).
Table 3.5. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 3,6,7-Tribromo-2-chloroindole (126) (CDCl$_3$).

![Structure of 126]

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<th>$^1$J$_{CH}$ (Hz)</th>
<th>$\delta$ (ppm)</th>
<th>mult</th>
<th>$^1$J (Hz)</th>
<th>COSY ($^1$H to $^{13}$C)</th>
<th>HMBC</th>
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<tr>
<td>7a</td>
<td>133.8</td>
<td>C</td>
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</tbody>
</table>

†Determined from a $^{15}$N CIGAR experiment
‡Interchangeable

Table 3.6. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 3,6,7-Tribromo-2-chloroindole (126) (DMSO–d$_6$).

<table>
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<tr>
<th>Pos.</th>
<th>$\delta$ (ppm)</th>
<th>mult</th>
<th>$^1$J$_{CH}$ (Hz)</th>
<th>$\delta$ (ppm)</th>
<th>mult</th>
<th>$^1$J (Hz)</th>
<th>COSY ($^1$H to $^{13}$C)</th>
<th>HMBC</th>
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<td>C</td>
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</table>

–Not observed
†Determined from an HMBC experiment

3.5.3 2,6,7-Tribromo-3-chloroindole

Compound 127 provided an identical molecular formula to 126 (C$_8$H$_3$Br$_3$ClN; m/z 383.7435, [M – H]$^-$), but differences in the $^1$H NMR spectrum suggested that it was a regioisomer. Through comparison of HMBC data, it was established that the structural alteration was an exchange of bromine and chlorine at C-2 ($\delta$C 108.8) and C-3 ($\delta$C 109.0) yielding the structure 2,6,7-tribromo-3-chloroindole (127). NMR data is presented in Tables 3.7 and 3.8.
Table 3.7. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 2,6,7-Tribromo-3-chloroindole (127) (CDCl$_3$).

<table>
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<th>$^1$H (ppm)</th>
<th>mult</th>
<th>$^1$J$_{CH}$ (Hz)</th>
<th>$^1$C or $^{15}$N†</th>
<th>$^1$H (ppm)</th>
<th>mult</th>
<th>$^1$J (Hz)</th>
<th>COSY ($^1$H to $^{13}$C)</th>
<th>HMBC</th>
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†Determined from a $^{15}$N CIGAR experiment
‡Interchangeable
(w)Weak

Table 3.8. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 2,6,7-Tribromo-3-chloroindole (127) (DMSO–d$_6$).

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<th>$^1$J (Hz)</th>
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</table>

–Not observed

3.5.4 6,7-Dibromo-2,3-dichloroindole

A molecular formula of C$_8$H$_3$Br$_2$Cl$_2$N was indicated for 128 through the HRESIMS data ($m/z$ 339.7936, [M – H]$^-$) (Figure 3.9). The analogous $^1$H and HMBC NMR data to 127 inferred identical halogen substitution at positions C-3, C-6 and C-7. This was supported through the close comparison of the carbon chemical shifts of C-3 ($\delta_{C}$ 105.2) with that of compound 127 and of C-6 ($\delta_{C}$ 119.2) and C-7 ($\delta_{C}$ 107.1) with those of compounds 125–127, establishing the substitution of C-3.
with chlorine and both C-6 and C-7 with bromine. This indicated that the remaining chlorine was positioned at C-2 (δC 121.8). In the high-resolution 13C NMR spectrum, the 35Cl/37Cl isotope shift was visualised—two peaks were observed resonating at δC 105.202 and δC 105.198 with 0.6 Hz difference between them, supporting the assignment of chlorine at C-3 (Figure 3.10). The effect was less pronounced in the C-2 resonance, which instead was visualised as a broadened peak. Although the reason for this is uncertain, it is perhaps attributed to a combination of factors. Despite the clear observation of the 35Cl/37Cl isotope shift for C-3, non-protonated sp² hybridised carbons show the isotope shift to a significantly smaller extent than protonated sp² hybridised carbons. For the C-2 resonance, this is probably further exacerbated by unresolved scalar coupling of the quadrupolar 14N (I=1) of the pyrrole which causes broader linewidths. Accordingly, the structure of 128 was assigned as 6,7 dibromo-2,3-dichloroindole with full NMR data presented in Tables 3.9 and 3.10.

Figure 3.9. Experimental and calculated mass spectra for 6,7-dibromo-2,3-dichloroindole (128).

Figure 3.10. High-resolution (top; at = 6.0 s) and default (bottom; at = 1.5 s) 13C NMR spectra of 6,7-dibromo-2,3-dichloroindole (128) showing the 35Cl/37Cl isotope shift (150 MHz, CDCl₃).
Table 3.9. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 6,7-Dibromo-2,3-dichloroindole (128) (CDCl$_3$).

\[
\begin{array}{cccccc}
\text{Pos.} & \delta \text{ (ppm)} & \text{mult} & J_{\text{CH}} \text{ (Hz)} & \delta \text{ (ppm)} & J \text{ (Hz)} \text{ COSY (}^1\text{H to} ^{13}\text{C)} \\
1 & -251 & \text{NH} & & 8.47 & \text{br s} & \\
2 & 121.8 & C & & & & \\
3 & 105.2 & C & & & & \\
3a & 125.2 & C & & & & \\
4 & 118.2 & \text{CH} & 168 & 7.35 & \text{d} & 8.4 & 5 & 3,6,7a \\
5 & 126.1 & \text{CH} & 168 & 7.42 & \text{d} & 8.4 & 4 & 3a,6,7 \\
6 & 119.2 & C & & & & \\
7 & 107.1 & C & & & & \\
7a & 133.0 & C & & & & \\
\end{array}
\]

†Determined from a $^{15}$N CIGAR experiment

Table 3.10. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 6,7-Dibromo-2,3-dichloroindole (128) (DMSO−d$_6$).

\[
\begin{array}{cccccc}
\text{Pos.} & \delta \text{ (ppm)} & \text{mult} & J_{\text{CH}} \text{ (Hz)} & \delta \text{ (ppm)} & J \text{ (Hz)} \text{ COSY (}^1\text{H to} ^{13}\text{C)} \\
1 & -239 & \text{NH} & & 12.76 & \text{br s} & 3,3a \\
2 & 122.9 & C & & & & \\
3 & 102.1 & C & & & & \\
3a & 124.5 & C & & & & \\
4 & 117.7 & \text{CH} & 168 & 7.40 & \text{d} & 8.4 & 5 & 3,6,7a \\
5 & 125.3 & \text{CH} & 169 & 7.47 & \text{d} & 8.4 & 4 & 3a,7 \\
6 & 118.2 & C & & & & \\
7 & 107.0 & C & & & & \\
7a & 133.1 & C & & & & \\
\end{array}
\]

†Determined from $^{15}$N CIGAR experiment

3.5.5 7-Bromo-2,3-dichloro-6-iodoindole

The molecular formula of 129 was identified as C$_8$H$_3$BrCl$_2$IN from the HRESIMS spectrum ($m/z$ 387.7799, [M − H]$^-$) (Figure 3.11). All proton and carbon resonances were observed in their corresponding 1D NMR spectrum. Strong HMBC correlations were displayed from H-4 ($\delta_H$ 7.25) to C-3 ($\delta_C$ 105.2), C-6 ($\delta_C$ 94.3) and C-7a ($\delta_C$ 132.4) and from H-5 ($\delta_H$ 7.63) to C-3a ($\delta_C$ 125.9) and C-7 ($\delta_C$ 111.9) with an additional weak correlation observed to C-6. Without the weak two bond correlation from H-5 to C-6, the chemical shift at $\delta_C$ 94.3 would suggest
bromination at C-3. From the previously identified tetrahalogenated indoles 125–128, it was evident that the chemical shift of C-6 was not in accordance with bromine substitution. On the other hand, the carbon chemical shifts of C-5 and C-6 were consistent with iodine substitution, given that iodine causes significant shielding of the ipso- ($\delta_C$ 94.5, CDCl$_3$) and deshielding of the ortho- carbons ($\delta_C$ 137.5, CDCl$_3$) in iodobenzene.$^\dagger$ With this in mind, C-7 was assigned to be substituted with bromine and was relatively more deshielded when compared with compounds 125–128 due to the neighbouring iodine. The remaining two chlorines were therefore positioned on carbons C-2 ($\delta_C$ 121.7) and C-3 ($\delta_C$ 105.2) with their respective chemical shifts corresponding to those observed in 128 supporting this assignment. Additionally, the high-resolution $^{13}$C NMR spectrum showed a similar result to that observed for 128 (Figure 3.12). As such, the structure of 129 was provided as 7-bromo-2,3-dichloro-6-iodoindole. This completed the full structural characterisation of a naturally occurring bromochloroiodo- compound for the first time. Full NMR data is provided in Tables 3.11 and 3.12.

Natural products containing three different halogens are extremely rare, with bromochloroiodomethane (141),$^{19,20}$ 1-bromo-1-chloro-3-iodoisopropanol (142)$^{21}$ and a bromochloroiodoketone (143)$^{74}$ comprising the only literature examples. However, 141 and 142 were never physically isolated but were rather detected from a mixture of compounds using GC-MS analysis,$^{19–21}$ and although 143 was claimed as being isolated, the report lacks evidence pertaining to this compound; it does not describe the isolation procedure utilised to obtain the compound, no details were provided as to how its structure was determined and no experimental data was reported.$^{74}$

\[\begin{align*}
141 & \quad \begin{array}{c}
\text{Br} \\
\text{Cl} \\
\text{I}
\end{array} \\
142 & \quad \begin{array}{c}
\text{Br} \\
\text{Cl} \\
\text{I}
\end{array} \\
143 & \quad \begin{array}{c}
\text{Br} \\
\text{Cl} \\
\text{I}
\end{array}
\end{align*}\]

$^\dagger$ Determined from NMR data acquired on iodobenzene
Figure 3.11. Experimental and calculated mass spectra for 7-bromo-2,3-dichloro-6-iodoindole (129).

Figure 3.12. High-resolution (top; at = 6.0 s) and default (bottom; at = 1.5 s) $^{13}$C NMR spectra of 7-bromo-2,3-dichloro-6-iodoindole (129) showing the $^{35}$Cl/$^{37}$Cl isotope shift (150 MHz, CDCl$_3$).

Table 3.11. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 7-Bromo-2,3-dichloro-6-iodoindole (129) (CDCl$_3$).

<table>
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<th>$^1$J$_{CH}$ (Hz)</th>
<th>$^1$H</th>
<th>$^{1}$H to $^{13}$C</th>
<th>HMBC</th>
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<td>mult</td>
<td>δ (ppm)</td>
<td>mult</td>
<td>J (Hz)</td>
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<td>CH</td>
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<td>d</td>
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<td></td>
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<td>7a</td>
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<td>C</td>
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</tbody>
</table>

$^\dagger$Determined from a $^{15}$N CIGAR experiment

(w)Weak
Table 3.12. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for 7-Bromo-2,3-dichloro-6-iodoindole (129) (DMSO–d$_6$).

<table>
<thead>
<tr>
<th>Pos.</th>
<th>δ (ppm)</th>
<th>mult</th>
<th>$^1$J$_{CH}$ (Hz)</th>
<th>$^1$H</th>
<th>δ (ppm)</th>
<th>mult</th>
<th>$^1$J (Hz)</th>
<th>COSY ($^1$H to $^{13}$C)</th>
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</tr>
</tbody>
</table>

†Determined from $^{15}$N CIGAR experiment
(w)Weak

3.5.6 2,7-Dibromo-3-chloro-6-iodoindole

HRESIMS analysis determined the molecular formula of 130 as C$_8$H$_3$Br$_2$ClIN ($m/z$ 431.7283, [M − H]$^-$). The proton and carbon chemical shifts of the aromatic methines closely resembled those of compound 129 suggesting that the halogen substitutions at C-3 and C-6 were identical. Analogous $^{13}$C and HMBC NMR data to 129 verified the substitution of C-3 ($\delta_C$ 109.0) and C-6 ($\delta_C$ 94.4) with chlorine and iodine, respectively, and further indicated substitution of C-7 ($\delta_C$ 112.0) with bromine. The remaining bromine was therefore positioned on C-2 ($\delta_C$ 108.9) and the structure of 130 was assigned as 2,7 dibromo-3-chloro-6-iodoindole. The $^1$H and $^{13}$C NMR data for 130 and tetrahalogenated indoles henceforth (116, 119, 121, 123, 124, 131–133) are tabulated together in Tables 3.13–3.16.

3.5.7 3,7-Dibromo-2-chloro-6-iodoindole

The molecular formula of 131 was determined as C$_8$H$_3$Br$_2$ClIN ($m/z$ 431.7280, [M − H]$^-$), regioisomeric with 130 as indicated by dissimilarities in the proton chemical shifts. This was confirmed following an analysis of the HMBC data, and the structure was established as 3,7-dibromo-2-chloro-6-iodoindole (131).
### 3.5.8 2,3,7-Trichloro-6-iodoindole

Compound 132 revealed a molecular formula of C₈H₃Cl₃IN from the HRESIMS spectrum (m/z 343.8302, [M – H]⁻) (Figure 3.13). The placement of iodine was established at C-6 (δ_C 91.1) through its similar carbon chemical shift to those observed in 129–131, and the strong three-bond HMBC correlation from H-4 (δ_H 7.20). As such, the chlorines were situated at C-2, C-3 (δ_C 105.2) and C-7 (δ_C 120.9) and the structure of 132 was deduced as 2,3,7-trichloro-6-iodoindole.

![Figure 3.13. Experimental and calculated mass spectra for 2,3,7-trichloro-6-iodoindole (132).](image)

### 3.5.9 2,3,4,7-Tetrabromoindole

A deprotonated molecule observed using HRESIMS indicated a molecular formula of C₈H₃Br₄N for 116 (m/z 427.6920, [M – H]⁻). The ¹H NMR spectrum was evidently dissimilar to tetrabromoindole 125, indicating this to be a regioisomer. All protons and carbons were accounted for in the ¹H and ¹³C NMR spectra, two of which were identified as protonated aromatic methines (CH-5: δ_H 7.24; δ_C 127.1 and CH-6: δ_H 7.19; δ_C 126.3) in a multiplicity-edited HSQC experiment. The presence of a 2,3,4,7-tetrahalogenated indole was indicated through HMBC correlations to C-7 and C-7a, as illustrated in Figure 3.6b. Strong three-bond correlations were observed in the HMBC spectrum from H-5 to C-3a (δ_C 125.0) and C-7 (δ_C 103.6), and from H-6 to C-4 (δ_C 112.6) and C-7a (δ_C 135.0). Additionally, weak two-bond correlations from H-5 to C-4 and H-6 to C-7 were observed. The final two brominated

---

¹Due to paucity of mass, this resonance was not observed in the ¹³C NMR spectrum.
carbons could therefore be assigned to C-2 ($\delta_C 113.8$) and C-3 ($\delta_C 95.1$) and their congruent chemical shifts with those of 125 supported this assignment. Thus, the structure of 116 was established as 2,3,4,7-tetrabromoindole.

### 3.5.10 2-Bromo-3,4-dichloro-7-iodoindole

The molecular formula of 124 was determined as $C_8H_3BrCl_IN$ using negative-ion mode HRESIMS ($m/z$ 387.7804, [M − H]$^-$). The near indistinguishable chemical shifts of CH-5 ($\delta_H 6.93$; $\delta_C 124.0$) and CH-6 ($\delta_H 7.44$; $\delta_C 132.2$) with those of 123 (See Tables 3.13–3.16), coupled with similarities in the HMBC data, indicated an identical halogen substitution pattern at C-4 (chlorinated; $\delta_C 126.0$) and C-7 (iodinated; $\delta_C 74.0$). The remaining two carbons, C-2 ($\delta_C 110.3$) and C-3 ($\delta_C 108.6$), exhibited chemical shifts analogous to those found in compounds 127 and 130, indicating these positions to be substituted by bromine and chlorine, respectively. Thus, the structure was determined to be 2-bromo-3,4 dichloro-7-iodoindole (124).

The structures of 119, 121 and 123 were deduced in a similar fashion to compounds 116 and 124.

### 3.5.11 5-Bromo-2,3,4-trichloroindole

HRESIMS analysis identified the molecular formula $C_8H_3BrCl_3N$ for compound 133 ($m/z$ 295.8444, [M − H]$^-$) (Figure 3.14). All protons and carbons were observed in the $^1$H and $^{13}$C NMR spectra. A 2,3,4,5-tetrasubstituted indole was suggested from the shielded carbon chemical shift of the protonated carbon C-7 ($\delta_C 110.8$) and by the observation of an HMBC correlation to C-7a in accordance with Figure 3.6c. The HMBC experiment displayed strong correlations from H-6 ($\delta_H 7.43$) to C-4 ($\delta_C 125.2$) and C-7a ($\delta_C 133.0$) and a weak correlation to C-5 ($\delta_C 116.1$), whereas H-7 ($\delta_H 7.08$) strongly correlated to C-5 and C-3a ($\delta_C 123.3$) and weakly to C-4. The chemical shift of C-4 was similar to that observed in compounds 121, 123.
and 124, indicating chlorine substitution at that position. The shielded carbon chemical shift of C-5, relative to indole (140), was in agreement with bromine substitution. The final two carbons, C-2 and C-3 (δC 121.0 and 104.0, respectively), must therefore be substituted with chlorine, yielding the structure of 133 as 5-bromo-2,3,4-trichloroindole.

Figure 3.14. Experimental and calculated mass spectra for 5-bromo-2,3,4-trichloroindole (133).

Table 3.13. ¹H (600 MHz) NMR Data for Tetrahalogenated Indoles 116, 119, 121, 123, 124 and 130–133 (CDCl₃).
Table 3.14. $^{15}\text{N}$ (60 MHz)† and $^{13}\text{C}$ (150 MHz) NMR Data for Tetrahalogenated Indoles 116, 119, 121, 123, 124 and 130–133 (CDCl$_3$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pos.</th>
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<th>121</th>
<th>123</th>
<th>124</th>
<th>130</th>
<th>131</th>
<th>132</th>
<th>133</th>
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–Not observed
†Determined from a $^{15}\text{N}$ CIGAR experiment

Table 3.15. $^1\text{H}$ (600 MHz) NMR Data for Tetrahalogenated Indoles 116, 119, 121, 123, 124 and 130–132 (DMSO–d$_6$).

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<tr>
<th>Compound</th>
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<td>7.32</td>
<td>7.35</td>
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<td>7.57</td>
<td>7.55</td>
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</tr>
<tr>
<td>7a</td>
<td>7.32</td>
<td>7.35</td>
<td>7.41</td>
<td>7.57</td>
<td>7.55</td>
<td></td>
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</tbody>
</table>

Table 3.16. $^{15}\text{N}$ (60 MHz)† and $^{13}\text{C}$ (150 MHz) NMR Data for Tetrahalogenated Indoles 116, 119, 121, 123, 124 and 130–132 (DMSO–d$_6$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pos.</th>
<th>116</th>
<th>119</th>
<th>121</th>
<th>123</th>
<th>124</th>
<th>130</th>
<th>131</th>
<th>132</th>
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<td>-239</td>
<td>-239</td>
<td>-235</td>
<td>-</td>
<td>-237</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>116.4</td>
<td>124.6</td>
<td>124.4</td>
<td>124.1</td>
<td>-</td>
<td>111.1</td>
<td>124.9</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>92.2</td>
<td>101.9</td>
<td>101.3</td>
<td>101.2</td>
<td>-</td>
<td>105.7</td>
<td>89.1</td>
<td>102.4†</td>
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</tr>
<tr>
<td>3a</td>
<td>124.2</td>
<td>122.6</td>
<td>121.6</td>
<td>120.6</td>
<td>121.3†</td>
<td>125.5</td>
<td>126.8†</td>
<td>126.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>110.9</td>
<td>110.2</td>
<td>122.6</td>
<td>123.4</td>
<td>126.0†</td>
<td>117.8</td>
<td>118.9</td>
<td>117.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
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<td>126.2</td>
<td>122.9</td>
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<td>132.1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>126.3</td>
<td>126.5</td>
<td>126.2</td>
<td>132.5</td>
<td>132.4</td>
<td>95.5</td>
<td>95.4</td>
<td>92.3†</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>103.9</td>
<td>104.1</td>
<td>103.4</td>
<td>76.0</td>
<td>76.3†</td>
<td>111.8</td>
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<td>120.8†</td>
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<tr>
<td>7a</td>
<td>135.2</td>
<td>133.0</td>
<td>133.1</td>
<td>136.0</td>
<td>138.7†</td>
<td>134.2</td>
<td>133.5</td>
<td>131.2</td>
<td></td>
</tr>
</tbody>
</table>

–Not observed
†Determined from a $^{15}\text{N}$ CIGAR experiment
‡Determined from an HMBC experiment
Further verification towards the proposed structure of tetrahalogenated indoles 116, 119, 121 and 123–132 was found using the \(^{15}\)N CIGAR NMR experiment. The CIGAR (Constant Time Inverse-detection Gradient Accordian Rescaled) is an HMBC experiment that is optimised for multiple \(^nJ_{XH}\) \((X = C, N)\) values\(^{150}\) making it particularly suited for detecting long-range \(^1\)H–\(^{15}\)N correlations due to the large variation in \(^nJ_{NH}\) coupling constants.\(^{151,152}\) Using indole (140) as a guide, correlations were expected to be observed from H-2, H-3 and H-7 (weakly) to N-1 (Tables 3.1 and 3.2). No correlations were observed in the \(^{15}\)N CIGAR experiments for 116, 119, 121 and 123–132. This observation provided further evidence that neither C-2, C-3 or C-7 were protonated. With regard to 5-bromo-2,3,4-trichloroindole (133), the expected correlation from H-7 to N-1 is lacking, but this is likely due to paucity of material.

When the tetrahalogenated indoles were grouped and the nitrogen chemical shift\(^\dagger\) analysed, it became apparent that the nitrogen chemical shift, in conjunction with other experimental data, could also be used as a diagnostic tool. Relative to indole, all exhibited shielded nitrogen chemical shifts, and this appeared to be dependent on the halogen substituted at C-2, C-3 and C-7 (or lack of halogen at C-7 in the case of 133). For example, 9 or 10 ppm differences were observed between tetrahalogenated indoles with bromine substituted at these three sites and that with bromine at C-7 and chlorine at both C-2 and C-3 (Figure 3.15). Interestingly, the DMSO–\(\text{d}_6\) \(^{15}\)N data appeared more shielded than the CDCl\(_3\) \(^{15}\)N data by ca. 10 ppm.

### 3.6 Pentahalogenated Indoles

With one less proton to work from, when compared with the tetrahalogenated indoles, the structure elucidation of the pentahalogenated indoles using NMR

\(^\dagger\)CH\(_3\)NO\(_2\) as the reference standard
spectroscopy was far more challenging because all six positions needed to be considered; the lack of $^{3}J_{HH}$ coupling constants meant that the aromatic proton could be positioned on either the benzenoid or pyrrole ring. However, the magnitude of the $^{1}J_{CH}$ could be used as a diagnostic tool to determine which ring the proton was located on. Of the six possibilities, the most readily assigned compounds would be presented by 2,4,5,6,7- or 2,3,4,5,6-pentahalogenated indole due to the shielded nature of the protonated carbon (i.e. CH-3 and CH-7, respectively) of each indole substitution type. The others, however, were less obvious and a similar logic to that used for the tetrahalogenated indoles was used to reason the substitution pattern of the remaining four possible pentahalogenated indoles. Thus, a proton situated on C-4 would display strong correlations to C-3 and C-7a (Figure 3.16a); a proton positioned on C-5 would correlate strongly to C-7 (Figure 3.16b); and a proton on either C-2 or C-6 would exhibit a strong correlation to C-7a (Figure 3.16c and d, respectively). To distinguish between a protonated C-2 and C-6, the $^{15}$N CIGAR experiment would be expected to show a correlation from H-2 to N-1 if the proton was positioned at C-2.

Once the position of the proton and corresponding substitution pattern was ascertained, the halogen positions needed to be determined. The unequivocal assignment of the tetrahalogenated indoles isolated in this study provided a gauge by which the $^{13}$C and $^{15}$N NMR data of the pentahalogenated indoles could be compared to in order to ascertain their structures. Although far fewer compounds were isolated, a similar strategy to that used for the elucidation of
the tetrahalogenated indoles was employed, which began with pentabromoindole, of which two regioisomers (134 and 135) were isolated. The NMR data for the pentahalogenated indoles (134–137) is presented in Tables 3.17 and 3.18.

**Figure 3.16.** Three-bond HMBC correlations to C-3, C-7 and C-7a in 2,3,5,6,7-, 2,3,4,6,7-, 2,3,4,5,7-, and 3,4,5,6,7-pentasubstituted indole.

### 3.6.1 2,3,4,5,7-Pentabromoindole

The HRESIMS data for 134 provided the deprotonated molecular ion $m/z$ 505.6029, which was consistent with the molecular formula C$_8$H$_2$Br$_5$N (Figure 3.17). All carbon resonances ($\delta$C 133.6, 129.3, 126.2, 119.1, 115.0, 114.9, 103.7, 95.8) were observed in the $^{13}$C NMR spectrum. One protonated carbon (CH-6: $\delta$H 7.63; $\delta$C 129.3) was observed in the multiplicity-edited HSQC spectrum, indicating the other proton was exchangeable ($\delta$H 8.64). A $^{15}$N CIGAR experiment revealed the chemical shift of N-1 at $\delta$N -245.

**Figure 3.17.** Experimental and calculated mass spectra for 2,3,4,5,7-pentabromoindole (134).

On the basis of the deshielded carbon chemical shift of the protonated carbon CH-6, 2,4,5,6,7- and 2,3,4,5,6-pentabromoindole were discounted as possible structures.
The $^1J_{CH}$ value was measured at 174 Hz, which was suggestive of a proton situated on the pyrrole, however, a comparison made with indole (Table 3.1) showed this value to be inconsistent with that of CH-2 and therefore, both of C-2 and C-3 had to be substituted by bromine. In addition, no correlation to N-1 was observed in the $^{15}$N CIGAR experiment and an analogous chemical shift of N-1 ($\delta_N$ -245) to 116 and 125 supported bromination at C-2, C-3 and C-7. This eliminated 3,4,5,6,7-pentabromoindole and left 2,3,5,6,7-, 2,3,4,6,7- and 2,3,4,5,7-pentabromoindole as remaining possibilities.

The HMBC data was used to establish which of the three regioisomers was in hand. Strong three-bond correlations were observed from H-6 to C-4 ($\delta_C$ 115.0) and C-7a ($\delta_C$ 133.6). As depicted by Figure 3.16c, the correlation to C-7a is consistent with 2,3,4,5,7-substitution of the indole. Weaker two-bond correlations were also observed from H-6 to C-5 ($\delta_C$ 119.1) and C-7 ($\delta_C$ 103.7). The final carbons were assigned to C-2 ($\delta_C$ 114.9), C-3 ($\delta_C$ 95.8) and C-3a ($\delta_C$ 126.2) through a comparison of carbon chemical shifts to 116 and 125. The assignment of C-2 and C-3 were validated by HMBC correlations from the exchangeable proton NH-1 in DMSO–d$_6$ acquired data. Thus, the structure of 134 was deduced as 2,3,4,5,7-pentabromoindole. Compound 134 was crystallised and submitted to Associate Professor Martyn Coles for analysis using single X-ray crystallography, which confirmed the proposed structure of 134 (Figure 3.18). Although crystals were obtained for the additional pentahalogenated indoles isolated in this study, their quality was not sufficient for analysis by single X-ray crystallography.

### 3.6.2 2,3,4,6,7-Pentabromoindole

HRESIMS analysis of 135 gave rise to the deprotonated molecular ion $m/z$ 505.6032, which was consistent with the molecular formula $C_8H_2Br_5N$, but differences in the 1D and 2D NMR data indicated a regioisomer of 134. Both protons and all carbons ($\delta_C$ 135.7, 129.8, 123.9, 118.9, 114.2, 112.7, 106.6, 95.4) were observed in the 1D NMR spectrum. The multiplicity-edited HSQC identified the protonated carbon
(CH-5: $\delta_H$ 7.60; $\delta_C$ 129.8) and the $^{15}$N CIGAR spectrum revealed the N-1 chemical shift as $\delta_N$ -243.

**Figure 3.18.** X-ray crystal structure of 2,3,4,5,7-pentabromoindole.

Once again, both 2,4,5,6,7- and 2,3,4,5,6-pentabromoindole were eliminated as potential structures due to the deshielded carbon chemical shift of the protonated carbon (CH-6). The carbon and nitrogen chemical shifts supported bromination at both C-2 ($\delta_C$ 114.2), C-3 ($\delta_C$ 95.4) and C-7 ($\delta_C$ 106.6). These data suggested the compound to be either a 2,3,5,6,7- or 2,3,4,6,7-pentabromoindole. The former possibility was discounted following an analysis of the HMBC data, which did not show the strongest correlations to both C-3 and C-7a. Strong three-bond HMBC correlations from H-5 to C-3a ($\delta_C$ 123.9) and C-7, and additional weaker HMBC correlations to C-4 ($\delta_C$ 112.7) and C-6 ($\delta_C$ 118.9) were observed. The remaining carbon resonance was assigned to C-7a ($\delta_C$ 135.7) to give rise to 2,3,4,6,7-pentabromoindole (135).
3.6.3 4,5,7-Tribromo-2,3-dichloroindole

The molecular formula C$_8$H$_2$Br$_3$Cl$_2$N ($m/z$ 417.7031, [M – H]$^-$) for 136 was determined from the HRESIMS data (Figure 3.19). Almost identical chemical shifts to 134 for the protonated carbon (CH-6: $\delta_H$ 7.64; $\delta_C$ 129.4) superficially suggested that 136 possessed the same substitution pattern. The HMBC spectrum was also found to resemble that observed for 134. The strongest correlations were displayed from H-6 to C-4 ($\delta_C$ 114.5) and C-7a ($\delta_C$ 131.4) with weaker correlations to C-5 ($\delta_C$ 119.0) and C-7 ($\delta_C$ 104.0) also visible. Together, these data inferred substitution of C-4, C-5, and C-7 with bromine, and C-2 ($\delta_C$ 124.3) and C-3 ($\delta_C$ 105.9) with chlorine—the chemical shift of N-1 ($\delta_N$ -251) supported this assignment (Figure 3.15). Furthermore, the $^{35}$Cl/$^{37}$Cl isotope shift was observed for C-2 and C-3 in a high-resolution $^{13}$C NMR spectrum, providing additional evidence for substitution with chlorine these two carbons. Therefore, the structure of 136 is proposed.

![Graphical representation of Experimental and Calculated mass spectra for 4,5,7-tribromo-2,3-dichloroindole (136).](image)

3.6.4 5,7-Dibromo-2,3,4-trichloroindole

HRESIMS analysis for 137 revealed a deprotonated molecule at $m/z$ 373.7557 consistent with the molecular formula C$_8$H$_2$Br$_2$Cl$_3$N (Figure 3.20). The 1D and 2D NMR data were more similar to that of 136, suggesting the same substitution pattern and the N-1 chemical shift ($\delta_N$ -251) indicated chlorine substitution at C-2 ($\delta_C$ 123.5) and C-3 ($\delta_C$ 105.2) and bromine substitution at C-7. Furthermore, the
similar proton and carbon chemical shifts of CH-6 ($\delta_H 7.62; \delta_C 129.4$) suggested that the ortho-carbons were brominated. This was supported through the observation of strong HMBC correlations to C-4 ($\delta_C 124.9$) and C-7a ($\delta_C 131.7$) and weak correlations to C-5 ($\delta_C 116.2$) and C-7 ($\delta_C 103.1$), which also inferred chlorine substitution at C-4. The assignment of chlorine to C-2, C-3 and C-4, and bromine to C-5 agreed favourably with the carbon chemical shifts of 133. Thus, the structure of 137 is proposed as 5,7-dibromo-2,3,4-trichloroindole.

![Experimental and calculated mass spectra for 5,7-dibromo-2,3,4-trichloroindole (137).](image)

**Figure 3.20.** Experimental and calculated mass spectra for 5,7-dibromo-2,3,4-trichloroindole (137).

**Table 3.17.** $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Pentahalogenated Indoles 134–137 (CDCl$_3$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>134</th>
<th>135</th>
<th>136</th>
<th>137</th>
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</thead>
<tbody>
<tr>
<td>$\delta_C$ or $\delta_N$†</td>
<td>$\delta_H$ (ppm)</td>
<td>$\delta_C$ or $\delta_N$†</td>
<td>$\delta_H$ (ppm)</td>
<td>$\delta_C$ or $\delta_N$†</td>
</tr>
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<td>Pos.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>8.64</td>
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<td>114.9</td>
<td>114.2</td>
<td>124.3</td>
<td>123.5</td>
</tr>
<tr>
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<td>95.8</td>
<td>95.4</td>
<td>105.9</td>
<td>105.2</td>
</tr>
<tr>
<td>3a</td>
<td>126.2</td>
<td>123.9</td>
<td>125.0</td>
<td>123.9</td>
</tr>
<tr>
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</tr>
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<td>7a</td>
<td>133.6</td>
<td>135.7</td>
<td>131.4</td>
<td>131.7</td>
</tr>
</tbody>
</table>

† Determined from $^{15}$N CIGAR experiment
Table 3.18. $^{15}$N (60 MHz), $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Pentahalogenated Indoles 134, 136 and 137 (DMSO–d$_6$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>134</th>
<th>136</th>
<th>137</th>
</tr>
</thead>
<tbody>
<tr>
<td>δC or δN$^\dagger$ (ppm)</td>
<td>δH (ppm)</td>
<td>δC or δN$^\dagger$ (ppm)</td>
<td>δH (ppm)</td>
</tr>
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</tr>
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<td>2</td>
<td>117.5</td>
<td>124.0$^\dagger$</td>
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</tr>
<tr>
<td>3</td>
<td>93.9</td>
<td>106.7</td>
<td>101.6</td>
</tr>
<tr>
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<td>125.5</td>
<td>124.0$^\dagger$</td>
<td>122.38$^\dagger$</td>
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</tr>
<tr>
<td>7a</td>
<td>133.8</td>
<td>131.9</td>
<td>132.1</td>
</tr>
</tbody>
</table>

$^\dagger$Not observed
$^\dagger$Determined from $^{15}$N CIGAR experiment
$^\dagger$Interchangeable

3.7 Hexahalogenated Indole

The five fractions collected from the C$_{18}$ HPLC column described in Scheme 3.4, together contained a variety of hexahalogenated indoles ranging from dibromotetrachloro- to hexabromoindole. The third fraction appeared by NMR (by visualisation of the NH-1 resonance) and HRESIMS data to consist of mainly one compound identified as C$_8$HBr$_3$Cl$_3$N ($m/z$ 451.6667, [M − H]$^-$) (Figure 3.21). A permutation-combination calculation indicates that there are 20 constitutional isomers of a tribromo-trichloro indole where each halogen is connected to carbon. With only an exchangeable proton present on the molecule, it was clear that NMR spectroscopy would be ineffective for the complete structure elucidation—HMBC correlations from NH-1 in DMSO–d$_6$ combined with the carbon and nitrogen chemical shifts would have suggested the halogen types present at C-2 and C-3. However, paucity of mass meant that these data were unobtainable. Furthermore, efforts to crystallise the sample for X-ray diffraction were unsuccessful. As such, the structure of this compound remains unsolved.

The hexahalogenated indoles of R. membranacea are intriguing since all carbon centres in the molecule, that would otherwise be protonated, are substituted by a halogen. Synthetic hexachlorinated indole has been described in the chemical literature, but naturally-derived compounds with halogen substitution of every
available carbon is rare. Some examples have been found from the red alga *A. taxiformis* including carbon tetrabromide,\textsuperscript{19} perchloroacetone,\textsuperscript{19} and a number of trihaloacrylic acids.\textsuperscript{22}

![Figure 3.21. HRESIMS (left), and $^1$H (600 MHz, centre) and $^{13}$C (150 MHz, right) NMR spectra (CDCl$_3$) of a tribromo-trichloroindole.](image)

### 3.8 Isatin Derivatives

A deprotonated molecule detected using HRESIMS analysis provided the molecular formula of 4-chloro-3-hydroxyl-3-(2-oxopropyl)-2-oxindole (139) as C$_{11}$H$_{10}$ClNO$_3$ ($m/z$ 238.0276, [M − H]$^-$). The structure was elucidated through the examination of the 1D and 2D NMR spectra and confirmed by comparison with the literature.\textsuperscript{154} The methyl ketone moiety suggested an artefact of isolation, arising through an aldol condensation of the C-3 ketone of 4-chloroisatin (138) with acetone.\textsuperscript{154} This was verified upon the re-extraction of and subsequent purification (in the absence of acetone) that led to the isolation of a mixture of 4-chloroisatin and its tautomeric form (138, $m/z$ 179.9858, [M − H]$^-$) with no evidence for the presence of 4-chloro-3-hydroxyl-3-(2-oxopropyl)-2-oxindole (139). Interestingly, isatin (the dechlorinated version of 138) is believed to be the first recognised tautomeric molecule.\textsuperscript{155}
3.9 Biological Activity

Several compounds were submitted to the School of Biological Sciences where they were tested for biological activity. Antifungal activities of compounds 119, 125, 127–129, 138 and 139 were assessed against wild-type *Saccharomyces cerevisiae* (baker’s yeast) by monitoring inhibition of yeast growth in the presence of the compounds. These compounds were also tested in a standard 48 h MTT (3-(4,5-dimethyl-2-thiazolyl)2,5-diphenyl-2H-tetrazolium bromide) cell proliferation assay,\textsuperscript{156} against the HL-60 promyelocytic leukaemia cell line and showed similar activity to that observed against *S. cerevisiae* (Table 3.19). The similarities in IC\textsubscript{50} values between the two assays is unusual as yeast possess robust xenobiotic\textsuperscript{†} defence mechanisms and consequently, significantly higher doses of a compound (five to ten times) are typically required to inhibit yeast growth when compared with mammalian cell-based assays.\textsuperscript{157,158} The fractions containing tri-, penta- and hexahalogenated indoles (fractions D2, D4 and D5, respectively, from Scheme 3.1) were also tested and exhibited antifungal activity with the pentahalogenated indoles showing the greatest level of activity. These results are consistent with Brennan and Erickson’s observation of the extract of *R. membranacea* exhibiting antifungal activity\textsuperscript{138} and suggest that the polyhalogenated indoles may be responsible.

Table 3.19. IC\textsubscript{50} values of 119, 125, 127–129, 138 and 139 Against the HL-60 Cell Line and *Saccharomyces cerevisiae*,

<table>
<thead>
<tr>
<th>Compound</th>
<th>HL-60 (µM)</th>
<th><em>S. cerevisiae</em> (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>125</td>
<td>38</td>
<td>69</td>
</tr>
<tr>
<td>127</td>
<td>78</td>
<td>&gt;83\textsuperscript{†}</td>
</tr>
<tr>
<td>128</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>129</td>
<td>49</td>
<td>39</td>
</tr>
<tr>
<td>138</td>
<td>70</td>
<td>&gt;61\textsuperscript{†}</td>
</tr>
<tr>
<td>139</td>
<td>61</td>
<td>&gt;41\textsuperscript{†}</td>
</tr>
</tbody>
</table>

\textsuperscript{†}Highest concentration tested

\textsuperscript{†}A chemical substance found within an organism that is not naturally produced by that organism.
3.10 Related Halogenated Compounds in the Literature

The marine natural products literature contains a multitude of indoles and indole alkaloids with the Marinlit database returning 1130 results from an indole substructure search. These include compounds derived from hydrozoans, bryozoans, sponges, algae, tunicates and microbes. An exhaustive review is beyond the scope of this report but a few selected halogenated examples are detailed.

Indole alkaloids range from monomeric (e.g. 144–149) and dimeric indoles (e.g. 150–156) to more complex structures such as the enantiomeric compounds, similisine A (157) and B (158). Isolated from the red alga Laurencia similis, 157 and 158 are trisindole alkaloids with a [5,5]-spirooxindole moiety. Compounds 150 and 151 displayed cytotoxicity against HT-29 (human colorectal adenocarcinoma; 150 only) and P388 cell lines, although, no measured value was reported. Compounds 144–149 and 152–158 either lacked activity in the bioassay used or no biological activity was noted.

The chlorinated bisindole pyrroles lynamicins A–E (159–163) were isolated from a strain of actinomycetes and several exhibited broad-spectrum antimicrobial activity against a panel of both Gram-positive and Gram-negative bacteria. A deep-sea derived Streptomyces sp. yielded spiroindimicins A–D (164–167). Compounds 165–167 demonstrated moderate to weak cytotoxicity against a range of mammalian cell
Examples of iodinated indole alkaloids include hicksoanes A–C (168–170) from the gorgonian *Subergorgia hicksoni*,165 breitfussin A (171) and B (172) from the hydrozoan *Thuiaria breitfussi*166 and plakohypaphorines A–F (173–178) from the sponge *Plakortis simplex*.167,168 Hicksoanes A–C (168–170) were found to deter feeding by goldfish at concentrations between 5–100 μg/mL and are thought to provide chemical antifouling defences to the organism.165 Comprised of a
combination of pyrrole, oxazole and indole moieties, the structures of beitfussin A (171) and B (172) were determined using a combination of NMR spectroscopy, atomic-force microscopy, and computational methods.\textsuperscript{166} Neither were evaluated for biological activity. The isolation of plakohypaphorines A–D (173–176) constitute the first iodinated indoles and 174–176 were found to demonstrate antihistaminic activity.\textsuperscript{168}

3.11 Concluding Remarks

In summary, a spectroscopic-guided investigation of the methanolic extract of \textit{R. membranacea} was instigated due to mass spectrometric evidence for the presence of several polyhalogenated indoles. Following each stage of purification, NMR spectroscopy and MS were used to track and differentiate the polyhalogenated indoles.
present within each fraction. This led to the isolation of four naturally occurring
tetrahalogenated indoles with the rare inclusion of bromine, chlorine and iodine,
in addition to several other closely related tetra- and pentahalogenated indoles.
The unequivocal full NMR characterisation of the isolated bromochloroiodoindoles
would have been far more complicated without having isolated and characterised
the additional tetrahalogenated indoles described in this study.

The presence of a diverse array of tri- to hexahalogenated indoles within \textit{R. membranacea} is unusual, especially because there appeared to be little else in
the form of secondary metabolites—besides fatty acids and sugar derivatives,
other compounds also appeared to be indole derivatives. The impetus for the
biosynthesis of so many halogenated indoles, including regioisomers, is intriguing
and although these biological aspects were not examined in this study they are
possibly worth investigating in the future. Due to the metabolic expense incurred by
the biosynthesis of secondary metabolites and assuming that biohalogenation occurs
via a vanadium haloperoxidase, it seems unlikely that these are accidental products.
A vanadium bromoperoxidase has been found to show regiospecific oxidation of
an indole derivative at the C-2 position—an unsurprising result since the electron-
rich nature of the C-2–C-3 double bond in indole makes these centres particularly
susceptible to electrophilic attack. The reaction is suggested to proceed \textit{via} a
bromonium ion intermediate.\textsuperscript{82} Perhaps, the presence of many polyhalogenated
indoles act synergistically to provide improved chemical defences to the alga.
Chapter 4

New Halogenated Meroditerpenoids from

*Callophycus serratus*

4.1 The Genus *Callophycus*

The genus *Callophycus* (formerly *Thysanocladia*)\(^{11,160}\) encompasses ten species of red algae distributed across Eastern and Southern Africa, the Philippines, the South Pacific and Australia.\(^{11}\) Six of the ten species are found in Australia\(^{11}\) and as such, it is not surprising that the earliest literature record of natural products derived from the genus *Callophycus*, disseminated from this country.\(^{170}\)

There are three literature reports on Australian *Callophycus* natural products. Research conducted on all six species involved an investigation of the cell-wall polysaccharide contents for potential phycocolloids and also to assess their chemotaxonomic value.\(^{170}\) The main components were pyruvate- and sulfate-rich galactans comprised mostly of galactose and 3,6-anhydrogalactose. Further analyses determined the structure of the predominant repeating disaccharide found in all six species, to be a novel 4',6'-O-\((1\text{-carboxyethylidene})\)carrabiose 2-sulfate (179).\(^{170}\) The second publication, by a separate research group, sought the isolation of biologically active metabolites from *C. oppositifolius*. A bioassay-directed isolation procedure yielded callophycin A (180), a moderately cytotoxic tetrahydro-\(\beta\)-carboline that displayed activity against a range of human tumour cell-lines (GI\(_{50}\) values between 1.3 and 4.2 \(\mu\)M).\(^{171}\) The discovery of 180 and its associated biological activity initiated synthetic endeavours towards callophycin A analogues so that additional biological assessments could be made.\(^{108}\) The final publication, by a third research group, included *C. tridentifer* in a survey comprising 49 Australian red algal species, which aimed to search for and identify specific bromophenols recognised as key components responsible for seafood flavour.\(^{172}\) All other literature records of
4.2 Meroditerpenoids from *Callophycus* spp.

The prefix ‘mero’ in meroditerpene, is derived from the Greek word ‘meros’ meaning ‘part’ and relates to the concept that these class of compounds are derived from mixed biosynthetic pathways that include partial terpenoid origin;\textsuperscript{173} the suffix ‘diterpene’ indicates a C\textsubscript{20} terpene composed from the combination of four isoprene (C\textsubscript{5}H\textsubscript{8}) units.\textsuperscript{174}

Red algae of the genus *Callophycus* have been found by the Kubanek group to be prolific producers of a myriad of structurally diverse meroditerpenoids.\textsuperscript{44,175–181} Several Fijian specimens of this genus (mainly *C. serratus*) were the subject of an extensive study that resulted in discovery of eleven novel carbon skeletons and the isolation of 45 compounds that were new to the marine natural products literature.\textsuperscript{44,175–181} Each compound was found to posses either tri- or tetrasubstitution of the benzene ring and bromination was prevalent. Far fewer examples of iodinated and chlorinated compounds have been reported.\textsuperscript{44,181} A report featuring the first *Callophycus* derived di-, tri- and tetraiodinated meroditerpenes\textsuperscript{44} was published by Kubanek and co-workers simultaneously to the preparation of this dissertation. Even though a high level of structural diversity exists within this set of metabolites, they can be categorised into four simple classes: the \textit{p}-hydroxybenzoate macrolides,\textsuperscript{176–180} callophycoic acids,\textsuperscript{44,181} bromophycoic acids\textsuperscript{44,175} and callophycols.\textsuperscript{44,181}
4.2.1 \textit{p}-Hydroxybenzoate Macrolides

The largest class, which is comprised of 23 compounds isolated from \textit{C. serratus}, are the \textit{p}-hydroxybenzoate (diterpene-benzoate) macrolides—15- or 16-membered hybrid lactones containing a portion of each of the \textit{p}-hydroxybenzoate and diterpenoid moieties. With the exception of five macrolides, a brominated 6-membered ring substituted with either a methyl or exomethylene (methylidene), is contained within the macrocycle. Bromophycolides A and B and debromophycolide A (181–183) were the first of those isolated in the series.\textsuperscript{180} The relative configuration was determined from NMR data and X-ray crystallographic analysis provided the absolute configuration of compounds 181 and 182. In debromophycolide A (183), an analysis of the NOE data established the relative configuration at the epoxide while a comparison of the NMR data to 181 led to an identical configurational assignment at C-15. Ambiguous NOE data, coupled with the broad multiplicity of H-7 resulting in a lack of adequate information otherwise provided from $^1$H–$^1$H scalar couplings, meant that the configuration at C-7 could not be defined in 183.\textsuperscript{180}

An additional seven macrolides were reported in the year following that of 181–183. Bromophycolides C–I (184–190) share a common carbon backbone with either 181 or 182 and relative configurations were deduced based on $^1$H–$^1$H scalar couplings, NOE data and comparisons to 181 and 182.\textsuperscript{179} Synthetic approaches towards 181 and 185 from geranylgeranyl benzoate have since commenced although, no publications have appeared in the literature in recent years.\textsuperscript{182} Geranylgeranyl benzoate was proposed as the precursor molecule to \textit{Callophycus} meroditerpenes in the first publication that appeared in the literature,\textsuperscript{180} and recent work completed by the Kubanek group resulted in the isolation of 3-geranylgeranyl-4-hydroxybenzoic acid (191).\textsuperscript{44}

The isolation of bromophycolides J–Q (192–199) was accompanied by the discovery of a further two novel carbon skeletons, as exemplified by 192 and 193.\textsuperscript{178} Furthermore, this group of newly found bromophycolides revealed an additional subclass containing a tetrahydropyran ring within the macrocycle such as is found

85
in 198. Compounds 193 and 194 are examples of rearranged diterpenes and are thought to arise from a biosynthetic 1,2-methyl shift. Compound 192 is unusual in two respects: 1) to date, it is one of only two C$_{28}$ Callophycus meroditerpenes with the extra carbon present as an oxymethyl and 2) it contains an unusual bicyclo[3.1.0]hexane—although a cyclopentenyl unit was encountered in 183, its formation is envisioned through a naturally occurring cyclisation pathway. 180 Perhaps resulting from an artefact of isolation, this unique bicyclic system was suggested as potentially arising by methanolysis and homoallylic substitution of bromine in bromophycolide O (197). The relative configurations of 192–199 were established in a similar manner used for previously reported bromophycolides. 178 Completing the family of macrolides are bromophycolides R–U (200–203) and callophycolide A (204). 176, 177 While compounds 200–203 are structurally similar to previous bromophycolides, the non-brominated callophycolide A (204) barely holds a resemblance; the 15-membered macrocycle lacks a 6-membered ring and contains a longer alkyl chain substituted at the C-14 position. The absolute configuration of 14$S$,15$R$ was determined using a combination of Mosher’s ester data and CD analysis of a dimolybdenum tetraacetate complex (Snatzke’s method). 176
189  $R = \text{Br}  \quad 14R^\ast$
190  $R = \text{OH}  \quad 14S^\ast$
4.2.2 Callophycoic acids and Callophycols

The callophycoic acids and callophycols each comprise a relatively smaller group of meroditerpenes when compared with the $p$-hydroxybenzoate macrolides. Callophycoic acids A–H (205–212)$^{181}$ and iodocallophycoic acid A (213)$^{44}$ are either di-, tri- or tetracyclic $p$-hydroxybenzoic acid diterpenoids. Callophycols A (214) and B (215) and iodocallophycols A–D (216–219) lack the carboxyl group altogether in favour of halogen substitution at the corresponding aromatic carbon. Compounds 214 and 215 are regioisomeric through an exchange of halogen substitution and represent the only chlorinated Callophycus natural products reported in the literature.$^{181}$ Similarly, 213 and 216–219 constitute the only iodinated Callophycus meroditerpenes.$^{44}$ The relative and absolute configurations of 205 were established by NOE and X-ray crystallographic data, respectively.$^{181}$ NOE data and computational modelling using Spartan established the relative configuration for iodocallophycoic acid A (213). The absolute configuration for 213 was established using electronic circular dichroism spectroscopy and time-dependent density functional theory (DFT) calculations.$^{44}$ The known compound 3-geranylgeranyl-4-hydroxybenzoic acid (191) was also isolated alongside 213 and 216–219.$^{44}$

The isolation of 205–215 exclusive of bromophycolides, was originally thought to result from two genetically distinct C. serratus chemotypes, even co-existing within a few meters of each other. Environmental factors were also acknowledged as an alternative possibility.$^{183}$ However, it was later reasoned that non-macrocyclic compounds such as 205–215 originate from C. densus, while C. serratus specimens give rise to the macrocyclic bromophycolides.$^{175}$
$205 \ R = A$
$207 \ R = B$

$206 \ R = A$
$208 \ R = B$
$209 \ R = C$

$211 \ R = H$
$212 \ R = Br$

$214 \ R_1 = Br \ R_2 = Cl$
$215 \ R_1 = Cl \ R_2 = Br$

$213 \ R_1 = COOH \ R_2 = H$
$216 \ R_1 = Br \ R_2 = Br$
$217 \ R_1 = Br \ R_2 = I$
$218 \ R_1 = I \ R_2 = Br$
$219 \ R_1 = I \ R_2 = I$
4.2.3 Bromophycoic acids

The bromophycoic acids A–F (220–225) and bromophycoic acid A methyl ester (226) represent the final class of meroditerpenes isolated from a Callophycus specimen morphologically similar to *C. serratus*. Whilst having a similar carbon backbone to 211 and 212, bromophycoic acids A–D, F and bromophycoic acid A methyl ester (220–223, 225 and 226) follow a different cyclisation pathway with the isoprene methyl (generates the exomethylene observed in 211 and 212) being used to form the decalin system of 220–223, 225 and 226. Bromophycoic acid E (224) is unique among the bromophycoic acids as it possesses four fused ring systems and the carbon-carbon connectivity between the terpene and benzoic acid moieties, appears to be at a different attachment point on the diterpenoid portion. Unlike 192, the additional methyl (methyl ester) of 226 is considered to be of natural origin since an ethanolic extraction of *Callophycus* did not yield the ethyl ester equivalent. Compounds 225 and 226 are recent additions to this set of meroditerpenes whose absolute configurational assignments resulted in the revised configurational assignments for 220–224 as depicted below; the configurations of 220–224 in the original report were relative and inverted to 225 and 226.
4.2.4 Biological Activity

The *Callophycus* meroditerpenes were assessed for biological activity in a variety of assays, which allowed for consideration of a structure-activity relationship (SAR). The large majority were tested for antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), but only 198, 199, 203, 211, 213 and 220 displayed levels of inhibition (MIC = 0.8, 1.1, 0.5, 1.6, 1.4 and 1.6 µg/mL, respectively) comparable to the current vancomycin treatment (MIC = 2 µg/mL).\textsuperscript{175,178} Compared with other bromophycolides, the increased hydrophobicity, conformational restriction or both in 198, 199 and 203 were considered to be contributing factors.\textsuperscript{178} These structural features could also explain the significant decrease in antibacterial activity between 220 and 221 (MIC = 25.0 µg/mL).\textsuperscript{175} The presence of the carboxylic acid appeared to infer activity in iodocallophycoic acid A (213) as iodocallophycols A–D (216–219) were inactive in the same assay.\textsuperscript{44}

Most of the bromophycolides displayed modest activity when tested for cytotoxicity against a range of human tumour cell lines (mean IC\textsubscript{50} values varied between 2.0 and 42.6 µM). A superficial consideration of the SAR suggested that hydroxyl replacement of the isopropyl bromine (*e.g.* C-15 in 181) decreases cytotoxicity; a reduction in potency was observed between compounds 181 and 184 (*ca.* six times less active) and between 187 (*ca.* three times less active) and 201. The replacement of bromine by a hydroxyl group at other sites was less obvious\textsuperscript{177–180} and no inferences could be made into the SAR between the 15- and 16-membered macrolides.\textsuperscript{179}
When assessed for antimalarial activity against a drug-resistant *Plasmodium falciparum* parasite, the callophycoic acids, bromophycoic acids and callophycols were inactive,\(^{175,181}\) while the vast majority of bromophycolides exhibited low micromolar activity. The most potent activity was exhibited by bromophycolides A \(\text{(181)}\), D \(\text{(185)}\), E \(\text{(186)}\), H \(\text{(189)}\), M \(\text{(195)}\) and S \(\text{(201)}\) with IC\(_{50}\) values ranging between 0.3–0.9 \(\mu\text{M}\).\(^{177,178}\) These results suggest that the macrolide is required for activity, and similarly to the SAR observed in the MRSA bioassay, that bromine substitution at C-15 infers activity.\(^{178}\) Mechanism of action studies revealed that bromophycolide A \(\text{(181)}\) targets haem crystallisation. The release of free haem molecules, caused by the catabolism of haemoglobin by *P. falciparum*, is lethal to the parasite. To overcome this, the organism crystallises haem into the nontoxic haemozin molecule.\(^{184}\) The discovery of bromophycolide antimalarial activity and this mechanism of action has seen \(181–203\), and any other molecules with structurally similar scaffolds to the bromophycolides, the subject of a patent.\(^{185}\)

As a final note pertaining to biological activity, the bromophycolides are believed to provide fungal resistance to *C. serratus*. Using desorption electrospray ionisation mass-spectrometry (DESI-MS), the Kubanek group studied the distribution of the bromophycolides on the surfaces of *C. serratus*. They revealed that the sites of these compounds were localised over algal surfaces where tissue was compromised by mechanical abrasion.\(^{183,186}\) In the same study, ten samples containing natural concentrations of either bromophycolides or callophycoic acids and callophycols, displayed >95% growth inhibition against the pathogenic marine fungus *Lindra thalassiae*. The combination of these results suggest that the localisation of such compounds is the result of possible injury and serve to deter fungal infection.\(^{183,186}\)
4.3 Investigation of a Tongan Specimen of

*Callophycus serratus*

*Callophycus serratus* (Figure 4.1) is one of ten species included in the genus *Callophycus*. Certain morphological features shared with other species of this genus can cause confusion when taxonomic identification is concerned, although the manifestation of a mid-rib line traversing the main axes of dried *C. serratus* specimens is a distinctive characteristic. The distribution of the red alga is widespread with this species found in the Philippines, Australia, New Zealand, Papua New Guinea, the Solomon Islands, Fiji and Tonga.

![Figure 4.1. Underwater photo of *Callophycus serratus*. Image courtesy of Dan Crossett, 2016.](image)

A specimen of the fern-like red alga *C. serratus* (Figure 4.1) was collected from 'Eua, Tonga, and examined in this study. The alga was identified by Dr Joe Zuccarello in the School of Biological Sciences. In a similar approach to that used in the isolation of the polyhalogenated indoles (Chapter 3), purification was tracked using HRESIMS in addition to $^1$H NMR spectroscopy—fractions with data that displayed notable differences, when compared against the literature, were selected for further investigation. Using this strategy, a total of eight meroditerpenoids were purified—the two known macrolides, bromophycolides A ($181$) and T ($202$), and six new compounds, callophycol C ($227$), iodocallophycols E ($228$) and F ($229$),
iodocallophycoic acid B (230), deiodocallophycoic acid B (231) and callophycoic acid I (232). Iodocallophycols E (228) and F (229) and iodocallophycoic acid B (230) incorporate iodine within their structure bringing the total number of isolated iodinated Callophycus metabolites to eight.

Salient features in the 1D and 2D NMR spectra of the 75% Me$_2$CO/H$_2$O (Figures 4.2 and 4.3) and 100% Me$_2$CO fractions (Figures 4.4 and 4.5) included several aromatic resonances and a deshielded gem-dimethyl at $\delta_H$ 1.81 and 1.79. The aromatic resonances of the major components were attributed to the 1,3,4-trisubstituted benzene ring present in the vast majority of Callophycus meroditerpenes, while the functionalised diterpene terminus in compounds such as bromophycolide A (181) accounted for the deshielded gem-dimethyl. At this stage, definitive structural progress of either moiety was impeded due to overlapping resonances of the many closely related compounds present within each sample, making identification of the major compound and detection of potentially new compounds difficult. Consequently, additional purification was required.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.2.png}
\caption{$^1$H NMR spectrum of the 75% Me$_2$CO/H$_2$O screen fraction from Callophycus serratus (600 MHz, CD$_3$OD).}
\end{figure}
Figure 4.3. HMBC spectrum of the 75% Me₂CO/H₂O screen fraction from *Callophycus serratus* (600 MHz, CD₃OD).

Figure 4.4. ¹H NMR spectrum of the 100% Me₂CO screen fraction from *Callophycus serratus* (600 MHz, CDCl₃).
4.3.1 The Isolation of New Callophycoic Acids and Callophycols

Analysis of the NMR spectra of the 75% Me₂CO/H₂O and 100% Me₂CO fractions showed that the latter contained several more resonances (Figures 4.2–4.5) and for this reason, it was selected first for further examination. Following purification on silica gel, fractions were spotted onto a TLC plate, eluted with CH₂Cl₂, and visualised after dipping the plate into an H₂SO₄, then a vanillin solution, followed by heating. A series of early eluting fractions were visualised as a purple char, which turned to a vibrant blue upon cooling to room temperature (Figure 4.6). These fractions were combined to form three samples based on slight differences in R_f (retention factor) values. Analysis of the NMR spectra of these samples established the presence of a tetrasubstituted benzene ring, vastly reducing the number of potentially known Callophycus compounds to just seven. Fraction two appeared to contain a pure compound by ¹H NMR and HRESIMS but was later determined to be a mixture of callophycols A (214) and B (215) (2.4 mg), while fraction three was found to be a new chlorinated congener, callophycol C (227)
(5.2 mg). Fractions eluting from silica gel between 50% DCM/\(n\)-hexane and DCM showed aromatic resonances between \(\delta_H\) 6.50–7.26 and were indicated by HRESIMS data to contain mixtures of halogenated C\(_{26}\) compounds. Subsequent purification using HILIC HPLC led to the isolation of iodocallophycols E (228) and F (229). Later eluting DCM fractions contained the major compounds of the original sample, which were tentatively identified as mixtures of bromophycolides on the basis of NMR and HRESIMS data.

The isolation of 228 and 229 prompted the extraction of a second batch of the alga and a more cautious purification approach was taken by substituting silica with DIOL. Silica gel, possibly due to its acidic nature, has been reported to cause the decomposition of iodinated compounds.\(^{19}\) Purification of iodocallophycols E (228) and F (229) from the second extraction was achieved using C\(_{18}\) HPLC, which also resulted in pure bromophycolide T (202) (0.10 mg). Samples containing iodocallophycol E (228) were pooled together (combined mass: 1.3 mg), as were samples of iodocallophycol F (229) (combined mass: 1.8 mg). Iodocallophycoic acid B (230) was purified from the relevant DIOL fraction on C\(_{18}\) HPLC (Scheme 4.1).

The 75% Me\(_2\)CO/H\(_2\)O PSDVB fractions obtained from the first and second batch extractions were partitioned separately on DIOL. Selected fractions that eluted between 70% DCM/\(n\)-hexane and DCM were subjected to repeated chromatography on C\(_{18}\) HPLC, affording bromophycolide A (181) (1.2 mg) as the major compound.
The 20% EtOAc/DCM DIOL fraction (from first extraction) was further purified using C\textsubscript{18} HPLC to yield deiodocallophycoic acid B (231). The 10% EtOAc/DCM DIOL fraction of the first extract, was combined with fractions eluting between 10–20% EtOAc/DCM of the second extract and the resulting sample was finally purified using C\textsubscript{18} HPLC to afford iodo- and deiodocallophycoic acid B (230 and 231, respectively) and callophycoic acid I (232) (0.60 mg) (Scheme 4.1). The duplicate samples of iodocallophycoic acid B (230) were combined (combined mass: 0.80 mg), as were those of deiodocallophycoic acid B (231) (combined mass: 1.0 mg).
Scheme 4.1. Isolation scheme of compounds isolated from *Callophycus serratus*. 
4.4 Callophycol C

Callophycol C (227) was isolated as a white amorphous solid. The most distinguishable feature observed in the $^1$H NMR spectrum was a pair of aromatic proton *meta*-coupled doublet resonances that pointed to the presence of a 1,2,4,6-tetrasubstituted aromatic ring. Notable differences in the region $\delta_H$ 3.80–4.40 were observed in the $^1$H NMR spectra of callophycols A–C (214, 215 and 227, respectively), suggesting that 227 was a potentially new analogue.

Negative-ion mode HRESIMS data provided a deprotonated molecule at $m/z$ 632.9787, consistent with the parent molecular formula $C_{26}H_{34}Br_3ClO$, which required eight double bond equivalents. The isotopic distribution pattern confirmed the presence of bromine and chlorine (Figure 4.7). All 26 carbons were observed in the $^{13}$C NMR spectrum and a multiplicity-edited HSQC experiment accounted for 33 protons connected to 17 carbons. The remaining nine non-protonated carbon centres were comprised of seven olefinic/aromatic carbons ($\delta_C$ 149.5; 146.6; 131.5; 127.6; 127.4; 112.3; 110.9) and two quaternary carbons ($\delta_C$ 42.0; 40.0). The absence of one proton resonance in the HSQC experiment indicated this to be exchangeable.

![Figure 4.7. Experimental and calculated mass spectra for callophycol C (227).](image)

Structure elucidation of callophycol C (227) was executed in a successive manner beginning with the aromatic proton resonances. Two aromatic proton doublets, CH-3 ($\delta_H$ 7.39; $\delta_C$ 131.2) and CH-5 ($\delta_H$ 7.12; $\delta_C$ 132.2) exhibited small $^1$H–$^1$H scalar
coupling constants (2.3 Hz) suggesting a 1,2,4,6-tetrasubstituted benzene moiety with a *meta*-relationship between the two protons. Reciprocal COSY correlations were observed between each proton and additional correlations were present between H-5 and H$_2$-7 ($\delta_H$ 2.75) (long-range), and H$_2$-7 and H-8 ($\delta_H$ 2.16). The non-protonated carbon C-1 ($\delta_C$ 149.5) was designated to the *meta*-position relative to each aromatic proton on the basis of strong three-bond HMBC correlations from both H-3 and H-5 to C-1 and to each other’s carbon. The deshielded chemical shift of C-1, combined with HMBC correlations from the exchangeable proton 1-OH ($\delta_H$ 5.75) to C-1, C-2 ($\delta_C$ 110.9) and C-6 ($\delta_C$ 131.5), indicated substitution of this carbon by a hydroxyl group. The placement of C-2 and C-6 to the appropriate *ortho*-positions relative to C-1 was established by the observation of a weak two-bond HMBC correlation from H-3 to C-2 and also HMBC correlations from H$_2$-7 to C-1, C-5 and C-6. The latter set of HMBC correlations placed the attachment point of CH$_2$-7 at C-6, establishing an aromatic ring. Additionally, an HMBC correlation was displayed from H-8 to C-6 (Figure 4.8).

![Figure 4.8. Key COSY and HMBC correlations establishing the tetrasubstituted aromatic ring substructure of callophycol C (227).](image)

Beginning from the highly deshielded proton resonances, CH$_2$-20 ($\delta_H$ 4.81, 4.61; $\delta_C$ 108.8), the partial structure outlined in Figure 4.8 was extended. From each proton resonance, HMBC correlations were observed to C-8 ($\delta_C$ 55.7), C-19
The proton and carbon chemical shifts of CH$_2$-20 indicated an exomethylene (methylidene) motif that was confirmed by the HMBC correlation to the non-protonated C-19 carbon, establishing the internal segment of the olefinic bond. COSY correlations were observed between H$_2$-20 and the resonances of both H-8 and H$_2$-21 ($\delta_H$ 2.39, 2.00) and could thus be identified as long-range allylic coupling. Additional COSY correlations between H$_2$-21 and H$_2$-22 ($\delta_H$ 1.81, 1.48), and H$_2$-22 and H-23 ($\delta_H$ 1.45) led to further extensions of this segment, supported by HMBC correlations from H$_2$-21 to C-8, C-20, and C-23 ($\delta_C$ 50.2), H$_2$-22 to C-19 and C-23 and H-23 to C-21 and C-22 (Figure 4.9).

**Figure 4.9.** Key COSY and HMBC correlations establishing the extended substructure of callophycol C (227).

The substructure in Figure 4.9 was further extended to establish a cyclohexane moiety. Four HMBC correlations were observed from the proton resonance of the quaternary methyl singlet H$_3$-24 ($\delta_H$ 0.87) to C-8, C-9 ($\delta_C$ 40.0), C-10 ($\delta_C$ 40.3) and C-23 with the non-protonated carbon C-9 being its attachment point. These observations established the link between C-8 and C-23 through C-9, giving rise to a cyclohexane ring. HMBC correlations observed from H-8, H$_2$-10 ($\delta_H$ 1.88, 1.36) and H-23 to C-24 ($\delta_C$ 15.3) confirmed this connectivity (Figure 4.10).
Methylene H$_2$-10 was further connected to a methylene and a methine through observed COSY correlations between H$_2$-10 and H$_2$-11 ($\delta_H$ 2.26, 2.19) and H$_2$-11 and H-12 ($\delta_H$ 4.24). HMBC correlations observed from H$_2$-10 to C-11 ($\delta_C$ 31.3) and C-12 ($\delta_C$ 63.8) and from H-12 to C-10 and C-11 validated this connectivity (Figure 4.11).

With six double bond equivalents established, two more were left to be determined as required by the molecular formula. Of those yet to be assigned, two olefinic carbons could account for only one of the two remaining double bond equivalents, a consequence of which implied the presence of a third ring within the structure. Evidence to support this was presented by HMBC correlations observed from H-23 to C-12 and C-13 ($\delta_C$ 42.0) giving rise to a decalin ring system. The methyl singlet H$_3$-25 ($\delta_H$ 0.97) displayed HMBC correlations to C-12, C-13, C-14 ($\delta_C$ 37.8) and
C-23, indicating its attachment to C-13 and supporting the elucidated decalin ring system (Figure 4.12).

![Diagram of the decalin ring system in callophycol C (227).](image)

**Figure 4.12.** Key HMBC correlations establishing the decalin ring system in callophycol C (227).

With the majority of carbons required by the molecular formula having been assigned within the structure, the assignment of two olefinic methyl singlets, two olefinic carbons and one methylene remained yet to be determined. A *gem*-dimethyl moiety was established based on the HMBC correlations from H₃-18 (δ_H 1.81) and H₃-26 (δ_H 1.78) to each other’s carbon and the shared correlations to C-16 (δ_C 127.6) and C-17 (δ_C 127.4) (Figure 4.13). The near identical chemical shifts of the olefinic carbons caused the appearance of a single correlation in the HMBC experiment. Visualisation of this region at higher resolution generated by a band-selective HMBC acquired over the bandwidth δ_C 105–155 confirmed that the methyls were indeed correlating to both olefinic carbons (Figure 4.14). COSY correlations observed between H₂-14 (δ_H 1.69) and H₂-15 (δ_H 2.45, 2.12), in addition to HMBC correlations from H₂-15 to C-16 and C-17, provided the connection between the *gem*-dimethyl and decalin ring motifs. Finally, the halogens were left to be assigned: the bromines were assigned to C-2, C-4, and C-12 on the basis of almost identical carbon chemical shifts with 214 and 215,¹⁸¹ which subsequently left chlorine to be positioned on the non-protonated olefinic C-16 carbon. The non-protonated chlorinated carbon chemical shift was in agreement with literature examples.¹⁸⁷ Thus, these assignments gave rise to the planar structure of 227 (Figure 4.13).
4.4.1 Relative Configuration

The congruent proton and carbon chemical shifts of the decalin ring systems in callophycols A–C (214, 215 and 227, respectively, see Table 4.1) suggest that 227 has the same relative configuration. NOE correlations in a 1D NOE experiment were observed from H$_3$-24 to H$_2$-7, H$_2$-10a, H$_2$-11a, H$_2$-22b and H$_3$-25 and from H$_3$-25 to H$_2$-11a, H$_2$-22b and H$_3$-24 implying these to be on the same face of the bicyclic ring system. These assignments were indirectly supported through correlations observed on the opposite face, from H-12 to H$_2$-10b, H$_2$-11b, H$_2$-15a, and H-23. These observations implied a trans-fused decalin system (Figure 4.15).
consistent with those found in callophycols A (214) and B (215), whereby CH$_3$-24 and CH$_2$-25 assume axial configurations and the bulky bromine substituent an equatorial configuration—the proton multiplicity (doublet of doublets) and $^3J_{HH}$ coupling constants of H-12 (12.5, 4.2 Hz), which are indicative of an axial–axial and an axial-equatorial coupling, support this assignment. Consequently, CH$_2$-7 and CH$_2$-14 were assigned to equatorial positions and the relative configuration of 227 is therefore provided as $8R^*,9S^*,12R^*,13R^*,23S^*$. Full NMR data for callophycol C (227) is presented in Table 4.2.

![Figure 4.15. 3D representation of callophycol C (227) with COSY correlations displaying W-coupling and selected NOE correlations. Double-headed arrows indicate reciprocal NOE correlations.](image)

### 4.4.2 Tandem Mass Spectrometry

Fragmentation of 227 using tandem mass spectrometry (MSMS) afforded two major and one weak fragment from the parent ion. One of the major fragments was
observed at m/z 553.0520 and was attributed to an [M – H – HBr]− ion. The second major fragment ion observed was at m/z 78.9193 and was attributed to a bromide ion. The weakest fragment was observed at m/z 261.8617 and was attributed to the dibrominated phenol through an [M – H – C_{19}H_{29}BrCl]− ion. Although the MSMS fragments were not greatly informative towards verifying the proposed structure of 227, the observation of fragments due to the loss of H-X is not unusual due to the weaker C–X bond relative to the C–C bond.

Table 4.1. Chemical Shift Differences Between the Decalin Ring Systems of Callophycols A (214),^{181} B (215)^{181} and C (227) (CDCl₃).

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Table 4.2. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Callophycol C (227) (CDCl$_3$).

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†Assignments interchangeable
‡Selected resonances
*Undetermined
(w)Weak
4.5 Iodocallophycol E

Iodocallophycol E (228) was isolated as a white amorphous solid. A prominent feature of the $^1$H NMR spectrum was the chemical shifts of the aromatic resonances that were all upfield of the residual CHCl$_3$ peak—all but three of the known *Callophycus* meroditerpenes possess an aromatic ring with proton chemical shifts spanning above and below $\delta_H$ 7.26. Most unusual however, was the presence of what initially appeared to be a phenolic exchangeable proton resonance; a broad singlet at $\delta_H$ 5.75. When the HSQC data was examined, this proton was surprisingly discovered to be connected to a carbon at $\delta_C$ 75.7 with a $^1J_{CH}$ of 192 Hz, suggesting this centre to be sp$^2$-hybridised; together, these data were very odd and enticing. HRESIMS data alluded to the most likely cause of the peculiar NMR data; iodine.

A molecular formula of C$_{26}$H$_{34}$Br$_3$IO was indicated by the observation of a deprotonated molecule at $m/z$ 724.9131 in negative-ion mode HRESIMS, which required eight double bond equivalents. A characteristic ‘quartet’ composed of an approximate 1:3:3:1 ratio of the [M − H]$^-$ to [M − H + 6]$^-$ ions supported the presence of three bromine atoms (Figure 4.16). Due to its monoisotopic nature, iodine, unlike bromine and chlorine, lacks an isotopic distribution pattern in a mass spectrum, making its detection less obvious. However, the increased mass of the parent ion and although extremely weak, the observation of an in-source fragment ion at $m/z$ 126.9052 both signified iodine, providing strong support for its presence (Figure 4.16). All 26 carbons were observed in the $^{13}$C NMR spectrum, 19 of which were protonated by a total of 33 out of the 34 protons. The seven non-protonated carbons were attributed to five aromatic/olefinic centres ($\delta_C$ 152.3, 146.7, 145.3, 129.5 and 113.1) and two quaternary carbons ($\delta_C$ 44.1 and 41.9). The sole exchangeable proton was observed as a broad singlet at $\delta_H$ 4.87 in the $^1$H NMR spectrum.

Beginning with the aromatic proton resonances, CH-2’ ($\delta_H$ 7.10; $\delta_C$ 132.8), CH-5’ ($\delta_H$ 6.59; $\delta_C$ 117.0) and CH-6’ ($\delta_H$ 7.14; $\delta_C$ 130.0), a trisubstituted benzene ring
was elucidated. The multiplicities and magnitude of the $^{1}J_{HH}$ coupling constants established an ortho- and meta-relationship between H-5'/H-6' and H-2'/H-6', respectively, supported by COSY correlations between H-6' and both H-2' and H-5'. Additional COSY correlations were displayed between H-2' and H$_2$-1 ($\delta_H$ 3.03, 2.75) (long-range) and H$_2$-1 and H-2 ($\delta_H$ 2.64). Strong three-bond HMBC correlations were observed from both H-2' and H-6' to each other’s carbon and to C-4' ($\delta_C$ 152.3), and from H-5' to the non-protonated carbons C-1' ($\delta_C$ 113.1) and C-3' ($\delta_C$ 129.5). The comparable carbon chemical shift of C-4’ to the corresponding carbon in 227 and observed HMBC correlations from the exchangeable proton 4’-OH ($\delta_H$ 4.87) to C-3’, C-4’ and C-5’ indicated the phenolic functionality was present in 228 with the hydroxyl positioned at this carbon. HMBC correlations from H$_2$-1 to C-2’, C-3’ and C-4’ established its attachment point at C-3’, which was confirmed by the HMBC correlation from H-2 to C-3’. Thus, C-1’ was placed at the ortho-position relative to both H-2’ and H-6’ (Figure 4.17).

Elucidation of the second fragment was initiated from the methyldiene, CH$_2$-18 ($\delta_H$ 4.94, 4.78; $\delta_C$ 109.8). Each proton resonance displayed HMBC correlations to the sp$^3$-hybridised carbons C-10 ($\delta_C$ 53.6) and C-12 ($\delta_C$ 37.0) and to the non-protonated olefinic carbon C-11 ($\delta_C$ 145.3) establishing a double bond between C-11–C-18 and indicating the connectivity of C-11 to C-10 and C-12. This was supported by COSY correlations (allylic coupling) between H$_2$-18 and H-10 ($\delta_H$ 1.64) and H$_2$-12 ($\delta_H$ 2.34, 2.02). Additional COSY correlations between H-10 and H$_2$-9
Figure 4.17. Key COSY and HMBC correlations establishing the aromatic substructure of iodocallophycol E (228).

(δH 1.61, 1.56), H2-9 and H2-8 (δH 1.83, 1.20), H2-12 and H2-13 (δH 2.24, 2.02) and between H2-13 and H-14 (δH 4.09) extended the substructure. This connectivity was supported by HMBC correlations observed from H2-8 to C-10, H2-9 to C-10 and C-11, H2-12 to C-10, C-13 (δC 36.0) and C-14 (δC 67.0), H2-13 to C-11, C-12 and C-14 and H-14 to C-12 and C-13 (Figure 4.18).

Figure 4.18. Key COSY and HMBC correlations establishing substructure two of iodocallophycol E (228).

The substructure outlined in Figure 4.18 was further extended through HMBC correlations from the methyl singlets, CH3-19 (δH 1.13; δC 28.7) and CH3-20 (δH 0.89; δC 17.5). Together the methyls formed a gem-dimethyl as evidenced by
HMBC correlations from the proton resonances of each methyl to the other’s carbon, C-10, C-14 and C-15 ($\delta_C 41.9$), establishing a link between C-10 and C-14 through C-15, to give rise to a cyclohexane moiety (Figure 4.19).

**Figure 4.19.** Key COSY and HMBC correlations establishing the cyclohexane substructure of iodocallophycol E (228).

Evidence connecting the aromatic ring (Figure 4.17) and cyclohexane (Figure 4.19) substructures was provided by the methyl singlet CH$_3$-17 ($\delta_H 1.06$; $\delta_C 19.7$). Four HMBC correlations were observed from H$_3$-17 to C-2, C-6 ($\delta_C 61.6$), C-7 ($\delta_C 44.3$), C-8, indicating its attachment to the non-protonated carbon C-7 and establishing the link between C-2 and C-8 through C-7 (Figure 4.20).

**Figure 4.20.** Key COSY and HMBC correlations establishing the link between the aromatic and cyclohexane substructures of iodocallophycol E (228).

Two double bond equivalents, four carbons, five protons, and the halogens were yet to be accounted for. The presence of the unassigned olefinic carbon C-3 ($\delta_C 146.7$) accounted for one of the two double bond equivalents indicating the other had to be
satisfied by another ring. Sequential COSY correlations from H-6 (δ_H 4.41) through H_2-5 (δ_H 2.24, 2.12) to H_2-4 (δ_H 2.81, 2.18), and HMBC correlations from H-6 to C-4 (δ_C 35.0) and C-5 (δ_C 33.4) and H-5 to C-7, C-4 and C-6 established the connectivity between these centres. Three-bond HMBC correlations from H_2-1 and H_2-5 to C-3 placed its carbon between C-2 and C-4 forming a second cyclohexane motif. While the carbon chemical shift of C-3 was clearly indicative of a double bond, there was no obvious olefinic partner that remained (i.e. > δ_H 4.0 or > δ_C 100). Initially, the proton and carbon chemical shifts of the terminal end of the double bond were assumed to be identical to, and overlapping with, those of CH_2-18. However, this scenario would have presented too many carbons. The only candidate yet to be assigned was that of the unusual CH-16 (δ_H 5.75; δ_C 75.7) whose proton chemical shift was within the typical range for an alkenyl proton but the carbon chemical shift was not. However, the magnitude of the 1^J_{CH} was measured at 192 Hz and was only consistent with an sp²-hybridised carbon, giving rise to a terminal vinyl halide. Specifically, this combination of proton and carbon chemical shifts was consistent with those observed for the vinyl iodide methine in the structures of 213 and 216-219 only very recently reported. Additional support towards the C-3–C-16 connectivity was obtained through HMBC correlations from H-16 to C-2, C-3 and C-4 and both H-2 and H_2-4 to C-16. Long-range allylic coupling between H-16 and H-2 was also observed. With all protons and carbons assigned within the structure, the placement of the halogens was determined based on chemical shift arguments. Iodine was placed at C-16 on the basis of similar proton and carbon chemical shifts to that of the iodinated Callophycus meroditerpenes. C-1’, CH-6 and CH-14 were substituted by bromine due to their comparable proton and carbon chemical shifts to 227. With all the elements indicated by the molecular formula assigned, the planar structure of iodocallophycol E (228) is proposed (Figure 4.21).
Figure 4.21. Key COSY and HMBC correlations establishing the planar structure of iodocallophycol E (228).

4.5.1 Relative Configuration within each Cyclohexane System

NOE data was obtained from a 1D NOE experiment and was used to establish the $E$-double bond geometry of $\Delta^3\text{,}16$ through an observed correlation between H-16 and H$_2$-1a. Reciprocal NOE correlations displayed between H-2 and H-6 in addition to NOE correlations from both of these protons to H$_2$-9 indicated these protons to be on the same face of the cyclohexane ring. H-5b and H$_2$-1 showed NOE correlations to H$_3$-17, suggesting these to be on the opposite face of the ring relative to H-2, H-6 and H$_2$-9. On the second cyclohexane, NOE correlations from H-14 to H-10, H-12b, H-13a and H$_3$-19, pointed towards these protons being on the same face, which was indirectly supported by an NOE correlation from H$_3$-20 to H-13b on the opposite face (Figure 4.22). These observations are consistent with those reported for 213 and 216–219, suggesting that the same relative configuration exists within each cyclohexane system of 228, with the large bulky groups such as the aromatic ring, bromines and the ethylene bridge (containing the other cyclohexane ring) assuming equatorial positions (Figure 4.22). H-6 and H-14 both appear as a doublet of doublets with large (axial–axial) and small (axial-equatorial) coupling constants providing evidence of both bromines occupying equatorial positions.
Figure 4.22. 3D representation showing key NOE correlations establishing the relative configurations within each cyclohexane ring of iodocallophycol E (228). Double-headed arrows indicate a reciprocal NOE correlation.

4.5.2 Relative Configuration between the Cyclohexane Systems

With the relative configuration within each cyclohexane system established as identical to those reported in the literature for 213, 216–219, it seemed likely that the relative configurations between the ring systems would also be the same. However, analysis of $^3J_{HH}$ coupling constants and NOE correlation data suggested that this was not the case. The multiplicities of H-8a (td, 12.9, 4.8 Hz), H-8b (ddd, 12.9, 12.1, 4.5 Hz) and H-10 (d, 10.7 Hz) suggested the molecule to be conformationally restricted and predominantly one conformation. The combination of large and small $^3J_{HH}$ coupling constants observed for each proton resonance of H$_2$-8 suggested an antiperiplanar conformation across C-8/C-9 with approximate dihedral angles of 180° and 60° between the vicinally-coupled protons. The apparent ‘doublet’ multiplicity of H-10 indicated 0 Hz (or close to) coupling to one proton of H$_2$-9 and a corresponding dihedral angle near 90°. This further signified a dihedral angle close to 150° with the other proton of H$_2$-9, which was supported by the large coupling constant of 10.7 Hz. Visualised through C-10 across the C-9/C-10 bond, four conformations of the possible two configurations at C-10 could account for these observations (Figure 4.23). Further analysis of the NOE data was required to narrow...

\[\text{†Estimated using the vicinal Karplus correlation.}\]
the possibilities to one.

**Figure 4.23.** Possible configurations and conformations (A, D, E, and H) deduced from the H-10 scalar coupling constant in iodocallophycol E (228). Options B, C, F and G could be discounted as possibilities based on the expected coupling constants.

NOE correlations were observed from H-2, H$_3$-19 and H$_3$-20 to H-9b and from H$_3$-19 to H-2' indicating that the *gem*-dimethyls were oriented towards the aromatic ring. An NOE correlation from H-8a to H-18b placed these two protons in close proximity to each other and indicated the methylidene moiety to be on the opposite side of the ethylene bridge, relative to the aromatic ring (and *gem*-dimethyls); an NOE correlation between H-1b and H-8b supported this assignment. These observations eliminated options ‘A’, ‘D’ and ‘E’ illustrated in Figure 4.23, indicating that option ‘H’ depicts the configuration at C-10 and preferred conformation across the C-9/C-10 bond. Additional evidence for this was provided by an NOE correlation observed from H-8b to H-10. These results suggest that the relative configurations between the two cyclohexane rings of 228 are different to those reported for 213 and 216–219 and therefore, the relative configuration of 228 is tentatively assigned as drawn in Figure 4.24. Key NOE correlations that established the relative configuration between the cyclohexane ring systems is also provided in Figure 4.24.

Finally, before a configurational assignment for 228 is proposed, International Union of Pure and Applied Chemistry (IUPAC) recommendations must be acknowledged. The IUPAC recommendation for relative configuration is that $R^*$ is designated to
the first configuration and all others are relative to that. The $R^*$ configuration would thus be designated to C-2 in 228, however, structural representation of this assignment is opposite to those reported in the literature. Furthermore, although the absolute configurations have only been solved for six compounds (i.e. 181, 180, 182, 205, 213, 225 and 226), the configurations of the stereogenic centres around the cyclohexane ring in these compounds (equivalent to C-2, C-6 and C-7 in 228) appears to be conserved. As a result, the relative configuration is proposed to be $2S^*, 6S^*, 7S^*, 10S^*, 14R^*$ with C-2, C-6 and C-7 reflecting those reported in the literature and C-10 and C-14 being the opposite configuration. Full NMR data for iodocallophycol E (228) is presented in Table 4.3.

![Figure 4.24. 3D representation of iodocallophycol E (228) showing key NOE correlations establishing the relative configurations between the cyclohexane ring systems.](image)

### 4.5.3 Tandem Mass Spectrometry

While tandem mass spectrometry was performed on iodocallophycol E (228) with the anticipation of further structure validation, the only fragment ions detected were iodide and bromide. This result was also observed for the other iodinated meroditerpenoids isolated in this study.
Table 4.3. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Iodocallophycol E (228) (CDCl$_3$).

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†Assignment interchangeable
‡Selected resonances
*Undetermined
(w)Weak
4.6 Iodocallophycol F

Iodocallophycol F (229) was isolated as a white amorphous solid. Negative-ion mode HRESIMS data provided a molecular formula of C_{26}H_{33}Br_{2}IO (m/z 644.9880, [M − H]−) indicating a difference of one additional double bond equivalent and the loss of one hydrogen and bromine from iodocallophycol E (228). A detailed analysis of the NMR data revealed close similarities to 228 and established that the p-bromophenol and bromo-(iodomethylene)cyclohexane moieties were conserved in 229. Further examination of the NMR data signified that 229 contained a disubstituted olefin—from the gem-dimethyls H_{3}-19 (δ_{H} 0.96) and H_{3}-20 (δ_{H} 0.95), a reciprocal HMBC correlation was observed to each other’s carbon and additionally, each shared HMBC correlations to C-10 (δ_{C} 53.3), quaternary C-15 (δ_{C} 37.2) and the olefinic carbon C-14 (δ_{C} 136.5) establishing that C-15 served as the attachment point. Extensions to this segment were initiated through COSY correlations between H-14 (δ_{H} 5.34) and H-13 (δ_{H} 5.50), and H-13 and H_{2}-12 (δ_{H} 2.80, 2.56). HMBC correlations from the methyldiene protons H_{2}-18 (δ_{H} 4.86, 4.69) to C-10, the non-protonated olefinic carbon C-11 (δ_{C} 145.6) and C-12 (δ_{C} 31.8) provided evidence for a link between C-10 and C-12 through C-11 establishing a methylene-cyclohexene motif. As with 228, an ethylene bridge was found to connect the two 6-membered ring systems.

NOE data was comparable to that found in 228 leading to an identical E-geometry of the vinyl iodide and the tentative assignment of relative configuration of 2S*,6S*,7S*,10S* for 229. Full NMR data is outlined in Table 4.4.
Table 4.4. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Iodocallophycol F (229) (CDCl$_3$).

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<th>(\delta) (ppm)</th>
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<th>(J) (Hz)</th>
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</table>

\(^\dagger\) Assignment interchangeable
\(^\dagger\) Selected resonances
\(*\) Undetermined
(w) Weak
4.7 Iodocallophycoic Acid B

Iodocallophycoic acid B (230), which was isolated as a white amorphous solid, dissolved readily in CDCl₃, however, broad signals were observed in the NMR spectra. When the sample was dissolved in CD₃OD all the peaks resolved to provide a spectrum like that of previously isolated callophycols. A singlet peak at δH 6.00, δC 75.3 indicated another vinyl iodide-containing metabolite.

Negative-ion mode HRESIMS produced a deprotonated molecule at m/z 611.0668 consistent with the parent molecular formula C₂₇H₃₄BrIO₃ for which ten double bond equivalents were required—one more than 229 and with the addition of a carboxyl group. The multiplicity-edited HSQC established that 32 protons were connected to 19 carbons indicating the presence of two exchangeable protons. When acquired using default parameters, only 25 out 27 carbon resonances were observed and some appeared extremely weak in the ¹³C NMR spectrum. Also, the HMBC experiment did not provide the chemical shift of one out of the two missing carbons. To overcome this issue, the ¹³C acquisition parameters were altered and this is discussed in more detail in Section 4.10.

An analysis of the NMR data of 230 established the presence of the methylene-cyclohexene moiety connected to the bromo-(iodomethylene)cyclohexane via the ethylene bridge, analogous to the structure of 229. The structural change from 229 was found in the aromatic ring where a carboxylic acid replaced the bromine. COSY correlations were observed between H-2’ (δH 7.76) and H-6’ (δH 7.68) and H-5’ (δH 6.78) and H-6’. The multiplicity and ⁵JHH coupling constants indicated an ortho-relationship between H-5’/H-6’ and a meta-relationship between H-2’/H-6. HMBC correlations from both H-2’ and H-6’ to C-4’ (δC 160.1) established the meta-position of the hydroxyl group, consistent with the previously isolated compounds. From H-5’ two HMBC correlations were observed, one to C-4’ and the other, which was much stronger in magnitude, to C-3’ (δC 127.8). Within the aromatic/olefinic region of the ¹³C NMR spectrum, one carbon was clearly absent. The assignment
of the majority of aromatic/olefinic carbons within the structure left only that of C-1’. The apparent lack of an HMBC correlation to C-1’ from H-5’ led to the conclusion that the chemical shifts of C-1’ and C-3’ were overlapping making the correlation appear more intensely in the HMBC spectrum; no additional carbon resonance appeared in the specified spectral region when the $^{13}$C NMR experimental parameters were altered to increase signal-to-noise (Section 4.10). As with the previous elucidations (i.e. 227–229), HMBC correlations from the methylene H$_2$-1 ($\delta_H$ 3.02, 2.77) to C-2’ ($\delta_C$ 132.9), C-3’ and C-4’ established the connection of the aromatic ring to the remainder of the structure. This left a carboxylic acid functional group (C-7’: $\delta_C$ 171.8) to be assigned to the structure, which could only be placed on the non-protonated C-1’ carbon.

NOE correlations were analogous to those observed for 228 and 229 leading to the assignment of an E-double bond geometry of the vinyl iodide and a 2S*,6S*,7S*,10S* relative configuration for iodocallophycoic acid B (230). NMR data is presented in Table 4.5.
Table 4.5. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Iodocallophycoic acid B (230) (CD$_3$OD).

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<th>mult</th>
<th>$^1$J$_{CH}$ (Hz)</th>
<th>δ (ppm)</th>
<th>mult</th>
<th>J (Hz)</th>
<th>COSY ($^1$H to $^{13}$C)</th>
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$^a$Assignments interchangeable
$^b$Selected resonances
$^b$Measured from the unsuppressed $^1$J$_{CH}$ in the HMBC experiment
(w)Weak
4.8 Deiodocallophycoic Acid B

Deiodocallophycoic acid B (231) was isolated as a white amorphous solid. Of interest in the $^1$H NMR spectrum of 231 was two pairs of overlapping methyldiene protons, deceptively masked as doublets. The occurrence of two methyldienes on the same molecule is less common among closely related compounds reported in the literature,\textsuperscript{44,175–181} and further examination of the NMR data pointed towards that of a new meroditerpene. With the exception of the additional methyldiene, and the lack of a singlet at $\delta_H$ 5.75, the proton NMR spectrum resembled that of 230 (Figure 4.25).

Deiodocallophycoic acid B (231) formed a deprotonated molecule at $m/z$ 485.1684 in negative-ion mode HRESIMS. A significant $[M - H + 2]^{-}$ indicated the presence of bromine, giving rise to the molecular formula C_{27}H_{35}BrO_{3} for which ten double bond equivalents were required and differing from 230 by the replacement of iodine with an additional hydrogen. All COSY and HMBC correlations determined the carbon framework to be analogous to 230 and a complete methyldene motif (CH$_2$-16: $\delta_H$ 4.87, 4.73; $\delta_C$ 110.6) was found in 231 at the position where the vinyl iodide was observed in 230.

The relative configuration of 231 is proposed to be 2$R^*$,6$S^*$,7$S^*$,10$S^*$ on the basis of analogous NOE correlations to 228–230 and also the close agreement of proton and carbon chemical shifts in the cyclohexane and cyclohexene systems of 229 and 231 (Table 4.6). NMR data for 231 is presented in Table 4.7.
Figure 4.25. Comparison of the $^1$H NMR spectra (600 MHz, CDCl$_3$) of iodocallophyic acid B (230) (blue) and deiodocallophyic acid B (231) (black).

Table 4.6. Chemical Shift Differences Between the Cyclohexane Ring Systems of Iodocallophycol F (229) and Deiodocallophycoic acid B (231) (CDCl$_3$).

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<th>229 δH (ppm)</th>
<th>231 δC (ppm)</th>
<th>231 δH (ppm)</th>
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Table 4.7. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Deiodocallophyic acid B (231) (CDCl$_3$).

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†Assignments interchangeable
‡Selected resonances
§Tentatively assigned from the COSY experiment
*Undetermined
4.9 Callophycoic Acid I

Callophycoic acid I (232) was isolated as a white amorphous solid. A prominent feature observed in the $^1$H NMR spectrum of 232 was that of a highly deshielded pair of proton resonances at $\delta_H$ 6.08 and 5.83, neither of which matched with signals reported in any other Callophycus meroditerpene.$^{44,175–181}$

A deprotonated molecule at $m/z$ 501.1650 was detected using HRESIMS which was suitable for the molecular formula C$_{27}$H$_{35}$BrO$_4$. An approximate 1:1 ratio of the [M − H]$^-$ and the [M − H + 2]$^-$ ions verified the presence of bromine. Ten double bond equivalents were required by the molecular formula.

Sequential COSY and HMBC correlations led to the elucidation of the trisubstituted p-hydroxybenzoic acid and a decalin ring system analogous to that in callophycol C (227). Compared with 227, the isoprene head group presented a point of difference. In compound 232, the deshielded methyl H$_3$-16 ($\delta_H$ 1.88) protons correlated to the ketone carbonyl C-14 ($\delta_C$ 201.9), the non-protonated olefin C-15 ($\delta_C$ 144.2) and the methylidene C-27 ($\delta_C$ 125.2). From H$_2$-27 ($\delta_H$ 6.08, 5.83), HMBC correlations were observed to C-14, C-15 and C-16 ($\delta_C$ 17.7). These observations established the attachment of CH$_3$-16 and CH$_2$-27 to C-15 with the ketone carbonyl carbon adjacent to C-15 forming an $\alpha,\beta$-unsaturated ketone.

Similarities in chemical shift data about the decalin ring systems of 211, 212 and 232 (see Table 4.8) suggest that 232 contains an identical relative configuration to the former two compounds and this is supported by analogous NOE correlations. As such, the relative configuration of 6$^R\ast$,7$S\ast$,10$R\ast$,13$R\ast$,23$S\ast$ is proposed. NMR data is provided in Table 4.9.
Table 4.8. Chemical Shift Difference Between the Decalin Ring Systems of Callophycoic acids G (211), H (212) and I (232) (CDCl₃).

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Table 4.9. $^{13}$C (150 MHz) and $^1$H (600 MHz) NMR Data for Callophycoic acid I (232) (CDCl$_3$).

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†Assigned from the HSQC and HMBC experiments
‡Assignments interchangeable
§Tentatively assigned by comparison to the CD$_3$OD data
§Determined from CD$_3$OD acquired data
(w)Weak

[Diagram of structure 232]
4.10 Weak resonances in the $^{13}$C NMR Spectra of Callophycoic Acids

Some of the carbon signals in 230–232 were either extremely weak or absent entirely from the $^{13}$C NMR spectrum (Figure 4.26). Variation in spin-lattice (longitudinal) relaxation ($T_1$) and enhanced signals of protonated carbons through NOE are two major factors that influence $^{13}$C NMR peak intensities.$^{140}$ Spin-lattice relaxation occurs as the nuclei net magnetisation, following irradiation by a radio-frequency pulse to excite and flip the nuclei net magnetisation onto the $xy$-plane, relaxes back along the $z$-axis in alignment with the externally-applied magnetic field. While $T_1$ relaxation times are short for proton nuclei (on the order of seconds to fractions of a second), they are much longer and vary greatly for carbon nuclei, meaning that those with long $T_1$ times often result in only partial recovery of the signal.$^{140}$

As a consequence, the nuclei of non-protonated carbons, such as carbonyls, which generally have longer $T_1$ relaxation times and are not affected by NOE because of the lack of proton attachment, typically exhibit weaker signals in a $^{13}$C NMR spectrum than those of protonated carbons.$^{140}$ This explanation, however, did not account for the very weak signals in the $^{13}$C NMR spectrum of 230–232 that were attributed to methines, methylenes and even methyls (Figure 4.27). As may be expected, the carbonyl carbon resonances of the carboxylic acids were not observed.

This was particularly problematic in the structure elucidation of iodocallophycoic acid B (230) because evidence of a carboxylic acid carbonyl was also lacking in the HMBC experiment. Although the functionality of a carboxylic acid was certain in 230, as all atoms required by the molecular formula were established within the molecule except for ‘COOH’, a carbon signal needed to be observed in the $^{13}$C spectrum to unequivocally prove its existence.

Signals in a $^{13}$C spectrum resulting from nuclei with long $T_1$ times, can be amplified by increasing the relaxation delay in the pulse sequence. The longer delay allows more time for the nuclei net magnetisation to re-establish along the $z$-axis, which
Figure 4.26. Stacked $^{13}$C NMR spectra of iodocallophycoic acid B (230) (blue, CD$_3$OD), deiodocallophycoic acid B (231) (black, CDCl$_3$) and callophycoic acid I (232) (red, CD$_3$OD) (150 MHz). Each should contain 27 resonances but many are either absent or extremely weak.

Figure 4.27. $^{13}$C NMR spectrum of iodocallophycoic acid B (230) (CDCl$_3$) magnified to show the weak resonances (150 MHz).

consequently leads to more coherent magnetisation being flipped onto the $xy$-plane when the next 90° pulse-width is applied. As a result, when compared to a $^{13}$C NMR spectrum acquired from default parameters, the same overall experimental time (at the expense of the number of iterative scans) leads to signal amplification of nuclei with long $T_1$ times. In an attempt to increase the weakened signals in 230 and also to observe the carbonyl carbon of the carboxylic acid, the relaxation delay was increased to 2.00 s (default: 0.01 s) and the experiment was acquired
over the same period of time as the default $^{13}$C NMR experiment. Unexpectedly, instead of increasing the signals this adjustment proved to be detrimental with some of the weaker signals worsening (relative to other carbons) and thus indicated that the problem was not related to long $T_1$ relaxation times. This result suggested that the $T_1$ relaxation was occurring at a faster rate than the average carbon. To test this theory, the acquisition time was decreased from 1.60 s to 0.20 s and the overall experimental time kept the same. The shorter acquisition time meant that more scans could be acquired in the same period of time. The resulting $^{13}$C NMR spectrum showed that the weaker resonances were stronger (relative to the other carbon resonances) and although very weak and broad, evidence of the carbonyl carbon appeared at $\delta_C$ 171.6 (Figure 4.28). To validate the carbonyl carbon as the carboxylic acid, both sets of $^{13}$C NMR spectra (one with a long relaxation delay and the other with a decreased acquisition time) were acquired for deiodocallophycoic acid B (231). The $^{13}$C NMR spectrum acquired with a shorter acquisition time provided the resonance corresponding to the carbonyl carbon (Figure 4.29) and was consistent with the carbon chemical shift (pertaining to a carboxylic acid carbonyl) observed in the HMBC experiment. Similarities in carbon chemical shifts and the weak broad signal that arose in the spectrum for 230 and 231, supported that the carbon observed at $\delta_C$ 171.6 in the $^{13}$C NMR spectrum of 230 indeed belonged to the carboxylic acid.

The short $T_1$ relaxation times can be explained by the tumbling rate of the molecule. Hydrogen bonding leads to the dimerisation of carboxylic acids in solution, thus increasing the molecule’s effective size. Larger species resulting from this intermolecular association substantially decreases the tumbling rate in solution and enables more efficient $T_1$ relaxation, thus shortening the $T_1$ time of the nuclei, particularly that of the carbonyl carbon.\textsuperscript{189} Reducing the acquisition time in the pulse sequence enabled more scans to be sampled in the same overall experimental time, which led to stronger $^{13}$C signals of those with short $T_1$ times. It should be noted that for 230–232, a combination of the short $T_1$ relaxation (possibly caused by dimerisation) and paucity of material were likely contributing factors to
the weaker signals observed in the $^{13}$C NMR spectrum for each compound.

**Figure 4.28.** $^{13}$C NMR spectra of iodocallophycoic acid B (230) acquired from default parameters (black), increased relaxation delay (2.0 s; blue) and decreased acquisition time (0.2 s; red) (150 MHz).

**Figure 4.29.** $^{13}$C NMR spectra of deiodocallophycoic acid B (231) acquired from default parameters (black), increased relaxation delay (2.0 s; blue) and decreased acquisition time (0.2 s; red) (150 MHz).

### 4.11 Biological Activity

The samples of bromophycolide A (181) and T (202) isolated during this study, in addition to iodocallophycols E (228) and F (229) were submitted to the School of
Biological Sciences to test for cytotoxicity against the HL-60 cell line. The results showed moderate cytotoxicity for (181), (202) and (228) with IC$_{50}$ values of 5.1, 6.2 and 6.0 µM ($n=3$ experimental repeats), respectively. At this stage, the bioassay result for 229 is inconclusive as it showed a similar level of activity to 228 in the first assay, but lacked activity in the last two assays.

4.12 Proposed Biogenesis

There are two pathways by which a meroterpene can be biosynthesised. One involves a combination of polyketide and terpene biosynthesis and the other is non-polyketide (Shikimate) combined with terpene biosynthesis. The meroditerpenes of *Callophycus* fall into the latter category.

A putative biogenesis has been proposed by Kubanek and co-workers for 181–226. Coupling between geranylgeranyl pyrophosphate (GGPP) and an aromatic moiety *via* electrophilic aromatic substitution generates the meroditerpene carbon backbone present in the *Callophycus* meroditerpenes. Biogenesis of the aromatic moiety is suggested to arise through the Shikimate pathway. The proposed biogenesis depicts terpene cyclisation after the C–C coupling reaction with their recent isolation of 3-geranylgeranyl-4-hydroxybenzoic acid (191) offering support towards this notion. The large structural diversity exhibited by compounds 181–226 indicates the presence of promiscuous terpenoid cyclisation processes and as is typical in the cyclisation of brominated terpenes, those containing bromine in their structure are thought to follow a bromonium ion induced terpene cyclisation. The site of bromination is consistent with sites of unsaturation in the acyclic diterpene precursor.

A common structural framework is observed for the known compounds callophycols A (214), B (215), callophycoic acids G (211) H (212) and the new compounds, callophycol C (227) and callophycoic acid I (232), suggesting a mutual precursor exists between them. The 2,4-dibromophenol functionality of 214, 215 and 227
is thought to arise from a decarboxylation of the benzoic acid functionality, such as that observed in 232, during the electrophilic aromatic substitution step in the biogenesis.44,181 Terpene cyclisation is proposed181 to be initiated by the selective addition of an electrophilic bromonium ion across the Δ10,11 double bond† similarly to that previously reported in the literature by Butler and co-workers.80,81,83 This addition induces a cascade cyclisation of the terpene to form the brominated decalin ring system observed in 211, 212, 214, 215,181 227 and 232. Callophycol B (215) could be the precursor to callophycol C (227) through a dehydrobromination (Scheme 4.2). Callophycoic acid I (232) is possibly derived from callophycoic acid G (211) through a C-14/C-15 epoxide, followed by β-elimination of a methyl proton, then oxidation of the allylic alcohol to form the ketone (Scheme 4.2). Epoxidation is envisioned to happen either via a bromohydrin intermediate formed by nucleophilic attack of a hydroxide on a bromonium ion in a similar fashion to that proposed for the epoxide-containing bromophycolides such as 183,180 or through a biologically equivalent Prilezhaev-type (also referred to as Prileschajew) reaction‡ with epoxidation occurring directly from the Δ14,15 olefin. In this proposed biogenesis, β-elimination of a methylene CH2-13 proton is conceivable, which would generate a tertiary allylic alcohol. Such a structural motif is exhibited in the acyclic terpene portion of bromophycoic acid B (221), while bromophycoic acids C (222) and D (223) contain a hydroperoxide and α,β-unsaturated ketone,175 respectively, lending support to the proposed biogenesis.

The newly found iodocallophycols E (228) and F (229) and iodo- and deiodocallophycoic acid B (230) and (231, respectively) isolated in this study closely resemble callophycoic acids C–E (207–209, respectively), albeit without the ether linkage to the aromatic ring. The proposed biogenesis of 207–209 provides a convincing possible route to their formation,181 although it is tempting to suggest the cyclic ether derives from a p-hydroxybenzoic acid-diterpene equivalent that contains a vinyl iodide motif such as that found in 230 (Scheme 4.3).

†The numbering system here is for the acyclic geranylgeranyl pyrophosphate and should not be confused with that used for compounds 227–232.
‡The Prilezhaev reaction involves olefin oxidation via reaction with a peroxy acid, typically m-chloroperoxybenzoic acid, to form an epoxide.190
Scheme 4.2. Possible biogenesis of callophycol C (227) and callophycoic acid I (232).
Scheme 4.3. Hypothetical biogenesis of the formation of the cyclic ether functionality in callophycoic acids C–E (207–209, respectively) from a vinyl iodide precursor.

A putative biogenesis can be readily deduced for deiodocallophycoic acid B (231) via typical bromonium induced cyclisation (two such events would be required to form the separate cyclohexane motifs) and then dehydrobromination. From a biosynthetic perspective, the question thus appears to be, how is the iodine in 228, 229 and 230 incorporated? Vanadium haloperoxidase (V-HPOs) enzymes are believed to be responsible for biohalogenation in marine algae. These enzymes oxidise halides in the presence of H$_2$O$_2$, rendering a source of an electrophilic halonium ion that proceeds to react with either an organic substrate or a second equivalent of H$_2$O$_2$ (see Scheme 1.1).$^{80,82}$ As was mentioned in Section 1.2.4, three such V-HPOs exist (i.e. V-ClPO, V-BrPO and V-IPO) and are named on the basis of the enzymes ability to oxidise the most electronegative halide.$^{80,82}$

Unlike V-BrPOs, research carried out on V-IPOs is scarce and little is known about iodination of natural products.$^{85,87,191}$ Two pathways leading to the vinyl iodide formation in 228, 229 and 230 can be envisioned, one incorporating iodine through an electrophilic iodonium ion (Scheme 4.4) and the other through a nucleophilic iodide (Scheme 4.5). Compound 230 could be derived from 231 by the electrophilic addition of an iodonium ion across the $\Delta^{3,16}$ olefin in a similar fashion to that observed for bromonium ion formation.$^{80,81,83}$ This could be ring-opened to form either a halohydrin or dihalide, which would then proceed through a dehydration
or dehydrohalogenation reaction, respectively, to generate the vinyl iodide (Scheme 4.4). A hypothetical precursor containing the cyclised terpene of 231 but with a $p$-bromophenol would lead to 228 and 229. This pathway is in agreement with that proposed in the literature. The alternative pathway could lead to the iodinated compounds from a cyclised precursor, such as callophycoic acid E (209), through a selective anti-Markovnikov hydroiodination across the enol ether, then subsequent proton abstraction and re-establishment of the phenol moiety to form the vinyl iodide (Scheme 4.5). The implication of nucleophilic iodide in this scheme is somewhat questionable because biosynthetically, halogenation is generally believed to occur via an electrophilic source. However, this potential pathway should not be excluded as it indicates that little knowledge exists on the iodination of organic substrates in Nature.

Interestingly, this study resulted in the isolation of both macrolide and non-macrolide meroditerpenes from a single specimen of Callophycus serratus. The presence of both types of meroditerpenes from a single organism rules out two out of three suggestions made in the literature to explain experimental findings of only one type of compound, pointing towards environmental differences between their collections as the probable underlying cause.

4.13 Concluding Remarks

The investigation of a Tongan specimen of Callophycus serratus has yielded six new meroditerpenes, callophycol C (227), iodocallophycols E (228) and F (229), iodocallophycoic acid B (230), deiodocallophycoic acid B (231) and callophycoic acid I (232), bringing the total number of Callophycus meroditerpene isolates to 52. The isolation was guided using a combination of MS and NMR spectroscopy and was exceptionally successful from a dereplication perspective when considering the large number of metabolites reported from C. serratus. Taking into consideration the putative biogenesis proposed by Kubanek and co-workers, an alternative pathway is
Scheme 4.4. Possible biogenesis of iodocallophycols E (228) and F (229) and iodo- and deiodocallophycoic acids B (230 and 231, respectively).
Scheme 4.5. Alternative possible biogenesis of iodocallophycols E (228) and F (229) and iodocallophycoic acids B (230).

proposed that leads to the cyclisation of callophycoic acids C–E through a vinylic iodide motif precursor like that observed in 228, 229 and 230. Interestingly, this study is the first to result in the isolation of macrolide meroditerpenes alongside those of non-macrolide meroditerpenes suggesting that in previous literature reports, environmental, rather than genotypical differences were the probable reason behind their observations of finding one type of compound exclusive of the other. To the best of the author’s knowledge, the organism under study was that of a single species identified as *Callophycus serratus*. 
Chapter 5

Concluding Remarks

This study involved an extensive investigation into the halogenated secondary metabolites of the New Zealand red alga *Rhodophyllis membranacea*. From this organism, a total of 21 compounds were isolated, of which 16 were found to be new. These consisted of 11 tetra-, four penta- and one hexahalogenated indole. The most exceptional compounds isolated from this alga, were four tetrahalogenated indoles substituted with bromine, chlorine and iodine—a rare feature observed among halogenated natural products. The biohalogenation processes leading to the production of polyhalogenated indoles in this alga are assumed to be catalysed by a vanadium-dependent haloperoxidase. It is particularly intriguing that the alga would produce so many congeners, including regioisomers and hexahalogenated indoles. This could be of interest to researchers working in the field of biohalogenation.

While an extensive investigation was carried out on the polyhalogenated indoles of *R. membranacea* further work is envisioned. Although the experimental data supports the proposed structures of the tetra- and pentahalogenated indoles isolated in this study, X-ray crystallographic data would solidify the arguments put forward and should be considered in future work. At this stage, the hexahalogenated indole is simply identified as a tribromo-trichloroindole and the constitution remains unsolved. Future directions for the elucidation of this compound would primarily involve isolating more mass to crystallise the compound so that an X-ray crystallographic analysis can be carried out. More mass would also mean that $^{13}$C, $^{15}$N and HMBC (acquired in DMSO–$d_6$ to potentially observe correlations from the NH proton) NMR data could be obtained. Also, the antifungal activity exhibited by the polyhalogenated indoles deserves further consideration. Those that were isolated as pure compounds, but not included in antifungal testing, should be submitted for biological testing. This would give insight into the SAR, if any exists. Furthermore, if these tests were to be performed, it would be interesting to compare the activity of
the pure compounds with that of the mixtures of tri-, penta- and hexahalogenated indoles.

An investigation of *Callophycus serratus* resulted in the isolation of six new halogenated meroditerpenes, of which three were found to be iodinated. NMR spectroscopic data suggests that the relative configuration of four of the new compounds (228–231), differs from that of literature reports of closely related compounds isolated from *Callophycus*. As a result, the relative configuration of compounds 228–231 is tentatively assigned based on $^1$H—$^1$H scalar couplings and NOE spectroscopic data. Additional work to investigate this further could involve molecular modelling so that comparisons can be made with experimental findings. Also, any future work should attempt to determine the absolute configurations.

Overall, this work resulted in nine new iodine-containing secondary metabolites from two different species of algae. At first, this seemed to be an extraordinary result because of the ostensible rarity of iodinated marine natural products reported in the literature. However, this is not unusual at all. Considering the relative halide concentrations in seawater, the proportion of iodinated to brominated and chlorinated metabolites, should be significantly lower than that which is actually present in the literature. Biohalogenation processes that incorporate halides into organic substrates and the higher reactivity of the iodide ion due to its enhanced oxidation potential are responsible for the greater proportion of iodinated marine natural products. The success of this research is accredited to the use of both NMR and MS as complementary tools during the isolation process. Specifically, the deceptively simple NMR spectra of the polyhalogenated indoles would have undoubtedly led to the algae being discounted from this study if an exclusively NMR-guided isolation strategy was employed. It is often challenging for a natural product chemist to find new secondary metabolites from an organism that has well established chemistry, as was the case with *Callophycus*. With over forty meroditerpenes sourced from this algae and reported in the literature, the challenge of dereplication was achieved.
through the use of both NMR and HRESIMS data and by careful examination and comparison of these data to those in the literature.
Chapter 6

Experimental

6.1 General Experimental Procedures

Optical rotations were recorded on a Rudolph Research analytical Autopol IV polarimeter. UV spectra were obtained on an Agilent 8453 UV-visible spectrophotometer. IR spectra were recorded on a Bruker Alpha Platinum ATR FT-IR spectrometer. A 600 MHz Varian Direct Drive spectrometer equipped with a triple resonance HCN cryogenic probe operating at 25 K was used to record all NMR spectra (600 MHz for $^1$H nuclei, 150 MHz for $^{13}$C nuclei, and 60 MHz for $^{15}$N nuclei). The residual solvent peak was used as an internal reference for $^1$H ($\delta^H$ 7.26, CDCl$_3$; 3.31, CD$_3$OD; 2.50, DMSO–$d_6$) and $^{13}$C ($\delta^C$ 77.16, CDCl$_3$; 49.00, CD$_3$OD; 39.52, DMSO–$d_6$) chemical shifts. $^{15}$N chemical shifts were internally calibrated by the spectrometer to CH$_3$NO$_2$ ($\delta$ scale, $^{15}$NO$_2$ $\delta^N_0$). Samples were quantified by $^1$H NMR spectroscopy using an internal CH$_3$NO$_2$ standard and the acquisition parameters described by West.$^{90}$ $^1$H, $^{13}$C, 1D NOE, TOCSY, COSY, HSQC, HMBC, band-selective HMBC and CIGAR NMR spectroscopic experiments were used throughout this research. High-resolution (ESI) mass spectrometric data were obtained with an Agilent 6530 Accurate-Mass Q-TOF LC-MS equipped with a 1260 Infinity binary pump. Low-resolution mass spectrometric data were acquired from a Shimadzu GC-2010 operating with a GCMS-QP2010 MS detector. HPLC purifications were carried out using a Rainin Dynamax SD-200 solvent delivery system with 25 mL pump heads (analytical and semi-preparative chromatography) and a Varian Prostar 335 diode array detector. Samples were submitted to Associate Professor Martyn Coles for analysis by single-crystal X-ray crystallography and the data obtained on an Agilent SuperNova diffractometer.

TLC analyses were carried out using Machery-Nagel Polygram Sil G/UV$_{254}$ plates.
Developed plates were visualised under UV light ($\lambda = 254 \text{ nm}$) before analysing by dipping in 5% H$_2$SO$_4$/MeOH followed by 0.1% vanillin/EtOH and heating. The mobile phase used for TLC was 5% MeOH/DCM unless otherwise stated. Normal-phase bench-top column chromatography was performed using Pure Science silica gel 60, 40-63 $\mu$m or YMC DIOL (2,3-dihydroxypropoxy-propyl-derivatised silica) 12 nm, S-50 $\mu$m. Reversed-phase bench-top column chromatography was carried out using Mitsubishi Dianon HP20 or Supelco Dianon HP20SS poly(styrene-divinylbenzene) (PSDVB). Size-exclusion column chromatography was performed using Sigma Sephadex LH20. Normal-phase HPLC was performed using Phenomenex Lichrospher DIOL (analytical: $4.0 \times 250 \text{ mm, 5 } \mu\text{m}$; semi-preparative: $10 \times 250 \text{ mm, 5 } \mu\text{m}$) or Phenomenex Luna hydrophilic interaction liquid chromatography (HILIC) (analytical: $4.6 \times 250 \text{ mm, 5 } \mu\text{m}$). Reversed-phase HPLC was carried out with Phenomenex Luna octyl-derivatised silica gel ($C_8$) (analytical: $4.6 \times 250 \text{ mm, 5 } \mu\text{m}$) or Phenomenex Prodigy octadecyl-derivatised silica gel ($C_{18}$) (analytical: $4.6 \times 250 \text{ mm, 5 } \mu\text{m}$; semi-preparative: $10 \times 250 \text{ mm, 5 } \mu\text{m}$). Analytical HPLC was performed at a flowrate of 1 mL/min and semi-preparative HPLC at 5 mL/min. All solvents used for column chromatography were of HPLC grade, and H$_2$O was glass distilled. Solvent mixtures are reported as % v/v unless otherwise stated.

**Bioassays:** Samples were submitted to the School of Biological Sciences, where they were tested for biological activity. A standard 48 h MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) cell proliferation assay was used to evaluate cytotoxic activity against the HL-60 promyelocytic leukaemia cell line ($n=1$–3 independent experiments with duplicate wells per experiment). Cells were treated with compound at various concentrations, and a dose-response generated relative to a control of untreated HL-60 cells. A bakers yeast (*Saccharomyces cerevisiae*) wild-type strain BY4741 was used to assess antifungal activity. Concentrations that inhibited growth of wild-type yeast by 50% relative to untreated controls, were determined in liquid synthetic complete media *via* measurements of cell density at $A_{600}$. Positive controls were peloruside A (HL-60: IC$_{50}$ 10 ± 4 nM)
and cycloheximide \((S.\ cerevisiae: IC_{50}\ 40\ nM)\).

### 6.2 Isolation of Compounds from PTN4_08B

PTN4_08B was collected from Vava’u, Tonga in 2009. The sample was stored at \(-20\ ^\circ C\) until extraction. A voucher specimen is held at the School of Chemical and Physical Sciences.

A section (19 g, wet weight) of PTN4_08B was extracted overnight in MeOH (100 mL). The extract was filtered and set aside and the sponge re-extracted in MeOH (100 mL) overnight. The second extract was passed through PSDVB (80 mL) and then the first extract was passed through the same column. The eluents were combined, diluted with 200 mL \(H_2O\) (100% v/v) and re-cycled through the PSDVB column. The eluent was collected and diluted with 400 mL \(H_2O\) (100% v/v) and re-cycled through the column. The column was washed with \(H_2O\) (240 mL) and then eluted with 1) 30% Me\(_2\)CO/\(H_2O\), 2) 75% Me\(_2\)CO/\(H_2O\) and 3) Me\(_2\)CO \((A1-\ A3)\). Fraction \(A2\) was cyclic loaded onto HP20ss (7 mL) until a 4-fold dilution was achieved and then the column was washed with NaCl solution (150 mL, 2 M), then \(H_2O\) (600 mL) before eluting with MeOH (100 mL). Once dry, the sample seemed to flake and was consequently cyclic loaded back onto HP20ss (7 mL) and washed with more \(H_2O\) (900 mL). The column was eluted with MeOH (200 mL), which dried to form a glass-like film \((B1)\). Fraction \(B1\) was loaded onto DIOL (10 mL) that was pre-equilibrated in DCM, and eluted with 30 mL portions of DCM, 50% EtOAc/DCM, EtOAc, 5%, 10%, 20%, 50% MeOH/EtOAc and 20% MeOH/DCM. This provided 48 fractions that were combined based on TLC to give five fractions \((C1-C5)\). Further purification was carried out on fraction \(C4\) using C\(_{18}\) HPLC (50% MeCN/0.1 M NH\(_4\)COOH), which yielded five fractions \((D1-D5)\). Fraction \(D4\) (5.3 mg) was found to contain a 1:1 mixture of the sulfated steroids halistanol sulfate \((65)\) and topsentinol K trisulfate \((66)\).
6.3 Isolation of Compounds from PTN3_19C

PTN3_19C was collected by hand from ‘Eua, Tonga in 2009. The sample was stored at −20 °C until extraction. A voucher specimen is held at the School of Chemical and Physical Sciences.

A sample of the sponge PTN3_19C (68 g, wet weight) was sectioned and extracted twice in MeOH (300 mL) overnight. The second, followed by the first extracts were passed through PSDVB (100 mL) and the eluents were combined, then diluted with H₂O (100% v/v) and re-cycled through the column. The eluent was collected, diluted with H₂O and passed through the column again. The column was washed with H₂O and eluted with 1) 30% Me₂CO/H₂O, 2) 75% Me₂CO/H₂O and 3) Me₂CO (A1–A3). A3 was fractionated by flash column chromatography on silica gel (10 mL) eluting with n-hexane, 50% DCM/n-hexane, DCM, 1%, 2%, 5%, 10%, and 20% MeOH/DCM, which yielded the α-exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69) (2.7 mg) and α-exomethylene-β-hydroxyl-γ-methyl-γ-tetradecyl-γ-butyrolactone (70) (13.4 mg) compounds from 50% DCM/n-hexane and 1% MeOH/DCM, respectively.

α-Exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69): White amorphous solid; ¹H and ¹³C NMR data see Table 2.1; HRESIMS (15 V) m/z (% relative intensity) 309.2793 [M + H]⁺ (20.6), 291.2681 (23.1), 273.2569 (16.6), 263.2732 (57.0), 137.1315 (20.1), 123.1164 (40.9), 109.1012 (72.3), 97.1014 (33.0), 95.0858 (100), 83.0853 (27.3), 81.0703 (68.2), 57.0703 (28.5); HRESIMS m/z 309.2788 [M + H]⁺ (calcd for C₂₀H₃₆O₂, 309.2788).

α-Exomethylene-β-hydroxyl-γ-methyl-γ-tetradecyl-γ-butyrolactone (70): White amorphous solid; ¹H and ¹³C NMR data see Table 2.2; HRESIMS (15 V) m/z (% relative intensity) 307.2636 (100), 289.2529 (33.3), 261.2570 (67.4), 149.1321 (32.7), 135.1164 (46.7), 125.0604 (32.5), 121.1014 (29.5), 109.1007 (35.3), 107.0848 (24.8), 97.1008 (28.4), 95.0861 (54.9), 89.0594 (28.6), 85.1015 (27.0), 83.0859 (29.8), 81.0703 (50.7), 57.0703 (36.3); HRESIMS m/z 325.2729 [M + H]⁺ (calcd
6.4 Isolation of Indoles from *Rhodophyllis membranacea*

Specimens of *Rhodophyllis membranacea* were collected by hand using SCUBA at a depth of 3–10 m from Moa Point, Wellington, New Zealand in January 2012 and in 2014 and stored at $-20^\circ$C until extraction. The alga was identified by Dr Joe Zuccarello at the School of Biological Sciences, Victoria University of Wellington, Wellington, where a voucher specimen (PTN4.20A) is also stored.

The alga (230 g wet weight) was extracted with MeOH ($2 \times 800$ mL) twice overnight. The second extract, followed by the first was passed through a PSDVB column (200 mL) pre-equilibrated in MeOH. The combined eluents were loaded back onto the PSDVB column by successively diluting with H$_2$O until a two-fold dilution was achieved after which point the column was washed with H$_2$O. Elution of the column was performed with 1) 30% Me$_2$CO/H$_2$O, 2) 75% Me$_2$CO/H$_2$O and 3) Me$_2$CO (fractions A$_1$–A$_3$, respectively). Fraction A$_1$ was subjected to flash column chromatography on Diol (10 mL) eluting with DCM, 50% DCM/EtOAc, EtOAc, 50% DCM/MeOH then MeOH which yielded 4-chloro-3-hydroxy-3-(2-oxopropyl)-2-oxindole (139) (0.70 mg) in the 50% DCM/EtOAc fractions. Fraction A$_3$ was loaded onto silica (20 mL) and batch eluted with DCM (60 mL), then 20% MeOH/DCM (60 mL). The DCM fraction was then partitioned on Sephadex LH20 with 50% MeOH/DCM, and the resulting fractions were combined on the basis of TLC (fractions B$_1$–B$_{17}$). Fraction B$_9$ was further purified on a semi-preparative C$_{18}$ HPLC column (55% MeCN/H$_2$O) yielding the major compound 4,7-dibromo-2,3-dichloroindole (119) (1.3 mg, $t_R = 42.3$ min) and 7-bromo-2,3-dichloro-6-iodoindole (129) (0.70 mg, $t_R = 59.5$ min).

Second, third and fourth extractions of the alga (154, 781 and 951 g, respectively)
were treated in a similar fashion. Fractions eluting from the Sephadex LH20 column that contained the compounds of interest (determined using LC-MS) were dissolved in 75% MeOH/MeCN, centrifuged, and the supernatant was separated and combined as one sample (fraction C1). A portion (280 mg) was partitioned on C18 HPLC (85% MeOH/H2O) providing six fractions (D1–D6).

6.4.1 Fractionation of D3—Tetrahalogenated Indoles

Further semi-preparative HPLC (C18, 55% MeCN/H2O) on fraction D3 provided 17 fractions (E1–E17), including more of 4,7-dibromo-2,3-dichloroindole (119) (11.7 mg) and 7-bromo-2,3-dichloro-6-iodoindole (129) (5.6 mg) in addition to 5-bromo-2,3,4-trichloroindole (133) (1.3 mg, tR = 36.2 min). Fraction E4, was purified on C8 HPLC using 80% MeCN/H2O to give 7-bromo-2,3,4-trichloroindole (121) as the major compound (1.5 mg, tR = 6.8 min). The following fractions were subjected to further purification on Diol HPLC (1% IPA/n-hexane): Fraction E8, provided compounds 2,3,4-trichloro-7-iodoindole (123) (1.3 mg, tR = 20.1 min) and 2,3,4,7-tetrabromoindole (116) (1.0 mg, tR = 23.1 min); Fraction E9 afforded 6,7-dibromo-2,3-dichloroindole (128) (7.6 mg, tR = 19.0 min) and 2-bromo-3,4-dichloro-7-iodoindole (124) (0.13 mg, tR = 24.0 min); Fraction E11 gave rise to 2,3,6,7-tetrambromoindole (125) (1.2 mg, tR = 24.4 min). Fraction E10, provided 2,3,7-trichloro-6-iodoindole (132) (0.06 mg, tR = 24.8 min) in addition to two earlier eluting fractions, one of which was purified twice using C18 HPLC (70% MeOH/H2O) to afford compounds 3,6,7-tribromo-2-chloroindole (126) (1.3 mg, tR = 53.2 min) and 2,6,7-tribromo-3-chloroindole (127) (3.7 mg, tR = 67.5 min). Fractions E13–15 were fractionated on Diol HPLC (1% IPA/n-hexane) to afford four fractions each (F1–F12). Based on the 1H NMR spectrum and mass spectrometric analysis, fractions F3, F4, F8, and F12 were combined and subjected to further chromatography on C18 HPLC (70% MeOH/H2O) affording fractions G1–G5. Fractions G1 and G2 were purified further using C18 HPLC and eluting from 60% MeCN/H2O to provide compounds 2,7-dibromo-3-chloro-6-iodoindole (130)
(0.70 mg, \( t_R = 58.4 \) min) and 3,7-dibromo-2-chloro-6-iodoindole (131) (0.08 mg, \( t_R = 62.8 \) min) as the major compounds.

### 6.4.2 Fractionation of D4—Pentahalogenated Indoles

Semi-preparative HPLC of fraction D4 using C\(_{18}\) eluting with MeOH/H\(_2\)O (85% MeOH/H\(_2\)O 0–31 min, ramped to 100% from 31–31.5 min and eluted at 100% from 31.5–40 min) generated two fractions (H1 and H2). Fraction H1 was purified further using semi-preparative C\(_{18}\) HPLC (55% MeCN/H\(_2\)O) as a single continuous run and sample was injected at 45 min intervals, which provided 14 fractions (I1–I14). Using the same column and conditions, fraction I6 was purified to provide 5,7-dibromo-2,3,4-trichloroindole (137) (1.2 mg, \( t_R = 81.8 \) min). Further HPLC purification of fraction I7 was performed on DIOL with 1% IPA/n-hexane to yield fractions J1–J3. Fractionation of J2 on C\(_{18}\) HPLC (semi-preparative; 80% MeOH/H\(_2\)O) provided pure 4,5,7-tribromo-2,3-dichloroindole (136) (1.7 mg, \( t_R = 32.0 \) min). Fractions I9 and I12 were purified separately from 80% MeOH/H\(_2\)O using C\(_{18}\) HPLC to provide 2,3,4,5,7-pentabromoindole (134) (1.6 mg, \( t_R = 53.9 \) min) and 2,3,4,6,7-pentabromoindole (135) (0.30 mg, \( t_R = 56.7 \) min), respectively.

### 6.4.3 Fractionation of D5—Hexahalogenated Indoles

Fraction D5 was subjected to chromatography on C\(_{18}\) HPLC (semi-preparative) eluting with MeCN/H\(_2\)O (80% MeCN/H\(_2\)O 0–26 min, ramped to 100% between 26–27 min and eluted at 100% from 27–41 min) to yield two fractions (K1 and K2). Fraction K1 was further purified using C\(_{18}\) HPLC (55% MeCN/H\(_2\)O) in a continuous chromatographic run with sample being injected at ca. 35 min intervals. This provided five fractions (L1–L5) each containing a mixture of hexahalogenated indoles—based on the \(^1\)H NMR spectrum and the HRESIMS data, fraction L3 appeared to consist of mainly one hexahalogenated indole with the major compound
corresponding to a tribromo-trichloroindole.

6.4.4 Isolation of 4-Chloroisatin

A separate sample of the alga (96 g) was extracted in MeOH (2 × 400 mL) and loaded onto PSDVB (80 mL) using the same methodology described above. After washing with H₂O, the column was eluted with: 1) 20% MeOH/H₂O, 2) 40% MeOH/H₂O, 3) 60% MeOH/H₂O, 4) 80% MeOH/H₂O, 5) MeOH and 6) 20% MeOH/DCM (M1–M6). Further fractionation of M3 was performed on Diol (10 mL) eluting with DCM, 50% EtOAc/DCM, EtOAc, then MeOH. The DCM fraction was purified on C₁₈ HPLC using a gradient from acidified H₂O to 55% acidified MeOH/H₂O (each with 0.2% HCOOH) over 10 min, after which point the column was run isocratically at 55% acidified MeOH/H₂O for a further 10 min. This provided 4-chloroisatin (138) as the major compound (1.3 mg, tᵣ = 15.0 min; tautomer ratio: 1:2.5, CD₃OD).

2,3,4,7-Tetrabromoindole (116): White film; UV (MeOH): λₑₓᵣ (log ε) 290 (3.60), 237 (4.53); ¹H and ¹³C NMR data see Tables 3.13–3.16; GCMS m/z (% relative intensity) 436 (14.5), 434 (47.9), 432 (71.5), 430 (56.6), 428 [M − 1]+ (18.7), 350 (16.3), 271 (23.8), 192 (29.9), 165 (19.3), 113 (42.3), 112 (46.5), 86 (100), 85 (61.4); HRESIMS (30 V) m/z (% relative intensity) 427.6867 [M − H]⁻ (6.4), 347.7635 (9.9), 78.9187 (100.0); HRESIMS m/z 427.6920 [M − H]⁻ (calcd for C₈H₂Br₄N, 427.6926).

4,7-Dibromo-2,3-dichloroindole (119): White crystals; UV (MeOH): λₑₓᵣ (log ε) 290 (4.23), 229 (4.77); IR νₓᵢ₅ 3378 cm⁻¹; ¹H and ¹³C NMR data see Tables 3.13–3.16; GCMS m/z (% relative intensity) 349 (8.2), 347 (26.1), 345 (81.0), 343 (100.0), 341 [M]+ (30.6), 262 (32.6), 183 (49.0), 182 (26.6), 148 (53.0), 121 (34.3), 112 (25.8), 86 (44.9), 85 (38.4); HRESIMS (30 V) m/z (% relative intensity) 339.7857 [M − H]⁻ (3.7), 259.8681 (17.4), 78.9188 (91.9); HRESIMS m/z 339.7938 [M − H]⁻ (calcd for C₈H₂Br₂Cl₂N, 339.7937).
7-Bromo-2,3,4-trichloroindole (121): White film; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 288 (4.07), 229 (4.59); $^1$H and $^{13}$C NMR data see Tables 3.13–3.16; GCMS $m/z$ (% relative intensity) 303 (15.4), 301 (55.3), 299 (100.0), 297 [M]$^+$ (48.4), 262 (12.5), 218 (45.9), 191 (10.2), 183 (24.5), 182 (35.5), 148 (42.9), 121 (38.5), 112 (20.8), 109 (20.2), 91 (29.0), 86 (47.7) 85 (38.6); HRESIMS (30 V) $m/z$ (% relative intensity) 295.8403 [M − H]$^-$ (3.7), 215.9186 (11.9), 78.9190 (98.5); HRESIMS $m/z$ 295.8444 [M − H]$^-$ (calcd for C$_8$H$_2$BrCl$_3$N, 295.8442).

2,3,4-Trichloro-7-iodoindole (123): White film; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 290 (3.98), 235 (4.24) 226 (443); $^1$H and $^{13}$C NMR data see Tables 3.13–3.16; GCMS $m/z$ (% relative intensity) 351 (0.3), 349 (31.4), 347 (100.0), 345 [M]$^+$ (99.5), 218 (92.3), 191 (33.2), 183 (43.8), 148 (67.9), 121 (42.0), 113 (5.8), 112 (22.6), 86 (43.8) 85 (34.5); HRESIMS (30 V) $m/z$ (% relative intensity) 343.8230 [M − H]$^-$ (5.3), 216.9244 (13.9), 126.9043 (100), 34.9672 (9.2); HRESIMS $m/z$ 343.8313 [M − H]$^-$ (calcd for C$_8$H$_2$Cl$_3$IN, 343.8303).

2-Bromo-3,4-dichloro-7-iodoindole (124): White film; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 290 (4.35), 234 (4.81) 227 (4.83); $^1$H and $^{13}$C NMR data see Tables 3.13–3.16; GCMS $m/z$ (% relative intensity) 394 (5.1), 392 (40.4), 390 (100.0), 388 [M − 1]$^+$ (53.4), 262 (29.6), 183 (37.1), 182 (13.2), 148 (53.1), 121 (29.6), 113 (6.8), 112 (19.1), 86 (34.4) 85 (24.4); HRESIMS (30 V) $m/z$ (% relative intensity) 387.7816 [M − H]$^-$ (8.0), 260.8752 (22.3), 181.9576 (14.4), 126.9049 (100), 78.9194 (30.6), 34.9698 (2.4); HRESIMS $m/z$ 387.7804 [M − H]$^-$ (calcd for C$_8$H$_2$BrCl$_2$IN, 387.7798).

2,3,6,7-Tetrabromoindole (125): White film; UV (MeOH); $\lambda_{\text{max}}$ (log $\varepsilon$) 289 (4.06), 231 (4.66); IR $\nu_{\text{max}}$ 3400 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Tables 3.3 and 3.4; GCMS $m/z$ (% relative intensity) 436 (16.7), 434 (66.4), 432 (100.0), 430 (71.0), 428 [M − 1]$^+$ (18.0), 349 (11.5), 271 (18.0), 192 (23.7), 165 (16.5), 113 (25.7), 112 (39.1), 86 (54.0), 85 (35.0); HRESIMS (30 V) $m/z$ (% relative intensity) 427.6919 [M − H]$^-$ (17.6), 348.7729 (1.9), 347.7639 (4.1), 78.9190 (74.33); HRESIMS $m/z$ 427.6928 [M − H]$^-$ (calcd for C$_8$H$_2$Br$_4$N, 427.6926).
3,6,7-Tribromo-2-chloroindole (126): White film; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 288 (3.93), 230 (4.57); $^1$H and $^{13}$C NMR data see Tables 3.5 and 3.6; GCMS $m/z$ (% relative intensity) 392 (8.5), 390 (49.3), 388 (100.0), 386 (86.7), 384 [M – 1]$^+$ (26.7), 305 (14.7), 227 (30.1), 226 (14.7), 148 (30.9), 121 (18.6), 113 (19.5), 112 (28.6), 86 (36.1), 85 (24.9); HRESIMS (30 V) $m/z$ (% relative intensity) 383.7421 [M – H]$^-$ (12.7), 303.8141 (5.5), 225.9014 (1.6), 78.9188 (89.3); HRESIMS $m/z$ 383.7438 [M – H]$^-$ (calcld for C$_8$H$_2$Br$_3$ClN, 383.7431).

2,6,7-Tribromo-3-chloroindole (127): White film; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 289 (4.01), 231 (4.61); $^1$H and $^{13}$C NMR data see Tables 3.7 and 3.8; GCMS $m/z$ (% relative intensity) 392 (8.3), 390 (47.4), 388 (100.0), 386 (88.1), 384 [M – 1]$^+$ (26.1), 305 (21.0), 227 (28.3), 226 (15.1), 148 (23.5), 121 (18.6), 113 (17.9), 112 (22.1), 86 (26.2), 85 (17.5); HRESIMS (30 V) $m/z$ (% relative intensity) 383.7391 [M – H]$^-$ (13.9), 303.8133 (6.7), 225.8992 (2.4), 78.9185 (100); HRESIMS $m/z$ 383.7435 [M – H]$^-$ (calcld for C$_8$H$_2$Br$_3$ClN, 383.7431).

6,7-Dibromo-2,3-dichloroindole (128): White film; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 288 (4.00), 229 (4.63); IR $\nu_{\text{max}}$ 3415 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Tables 3.9 and 3.10; GCMS $m/z$ (% relative intensity) 348 (4.6), 346 (24.4), 344 (100.0), 342 (75.4), 340 [M – 1]$^+$ (26.9), 262 (21.7), 183 (54.4), 182 (20.2), 148 (39.8), 121 (38.6), 113 (12.8), 112 (33.3), 86 (49.1), 85 (36.4); HRESIMS (30 V) $m/z$ (% relative intensity) 339.7938 [M – H]$^-$ (9.9), 259.8639 (6.7), 78.9188 (98.1); HRESIMS $m/z$ 339.7936 [M – H]$^-$ (calcld for C$_8$H$_2$Br$_2$Cl$_2$N, 339.7937).

7-Bromo-2,3-dichloro-6-iodoindole (129): White crystals; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 289 (4.42), 235 (4.98); $\nu_{\text{max}}$ 3397 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Tables 3.11 and 3.12; GCMS $m/z$ (% relative intensity) 394 (6.2), 392 (41.2), 390 (89.2), 388 [M – 1]$^+$ (58.6), 262 (35.6), 226 (14.7), 183 (100.0), 148 (55.4), 121 (45.4), 113 (12.0), 112 (43.9), 86 (59.2) 85 (41.0); HRESIMS (30 V) $m/z$ (% relative intensity) 387.7782 [M – H]$^-$ (17.6), 260.8744 (33.2), 181.9546 (9.1), 78.9191 (97.9); HRESIMS $m/z$ 387.7799 [M – H]$^-$ (calcld for C$_8$H$_2$Br$_2$Cl$_2$N, 387.7798).
2,7-Dibromo-3-chloro-6-iodoindole (130): White film; UV (MeOH): \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 291 (4.13), 237 (4.71); IR \( \nu_{\text{max}} \) 3403 cm\(^{-1}\); \(^1\)H and \(^{13}\)C NMR data see Tables 3.13–3.16; GCMS \( m/z \) (% relative intensity) 438 (14.1), 436 (72.1), 434 (100.0), 432 \([M - 1]^+\) (45.4), 306 (14.06), 227 (38.5), 148 (25.4), 121 (20.1), 113 (11.0), 112 (25.8), 86 (28.7) 85 (18.4); HRESIMS (30 V) \( m/z \) (% relative intensity) 431.7319 \([M - H]^-\) (27.0), 304.8246 (26.8), 126.9045 (77.8), 78.9191 (98.2); HRESIMS \( m/z \) 431.7283 \([M - H]^-\) (calcd for \( C_8H_2Br_2ClIN \), 431.7293).

3,7-Dibromo-2-chloro-6-iodoindole (131): White film; UV (MeOH): \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 290 (4.23), 237 (4.84); \(^1\)H and \(^{13}\)C NMR data see Tables 3.13–3.16; GCMS \( m/z \) (% relative intensity) 439 (13.4), 437 (58.8), 435 (100.0), 433 \([M]^+\) (46.6), 306 (14.06), 227 (38.5), 148 (25.4), 121 (20.1), 113 (11.0), 112 (25.8), 86 (28.7) 85 (18.4); HRESIMS (30 V) \( m/z \) (% relative intensity) 431.7220 \([M - H]^-\) (29.0), 304.8211 (24.3), 126.9034 (72.4), 78.9192 (89.1); HRESIMS \( m/z \) 431.7280 \([M - H]^-\) (calcd for \( C_8H_2Br_2ClIN \), 431.7293).

2,3,7-Trichloro-6-iodoindole (132): White film; UV (MeOH): \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 288 (4.47), 234 (5.05); \(^1\)H and \(^{13}\)C NMR data see Tables 3.13–3.16; GCMS \( m/z \) (% relative intensity) 351 (0.3), 349 (32.1), 347 (97.4), 345 \([M]^+\) (100.0), 218 (73.2), 191 (15.6), 183 (24.2), 148 (31.0), 121 (29.3), 112 (18.3), 87 (42.1) 86 (25.0) 74 (65.2); HRESIMS (30 V) \( m/z \) (% relative intensity) 343.8292 \([M - H]^-\) (6.6), 216.9238 (20.3), 126.9042 (100.0), 34.9696 (6.6); HRESIMS \( m/z \) 343.8302 \([M - H]^-\) (calcd for \( C_8H_2Cl_3IN \), 343.8303).

5-Bromo-2,3,4-trichloroindole (133): White film; UV (MeOH): \( \lambda_{\text{max}} \) (log \( \varepsilon \)) 280 (4.59), 227 (5.17); \(^1\)H and \(^{13}\)C NMR data see Table 3.13 and 3.14; GCMS \( m/z \) (% relative intensity) 305 (2.2), 303 (17.8), 301 (60.7), 299 (100), 297 \([M]^+\) (54.1), 262 (15.2), 218 (56.8), 191 (11.4), 183 (25.2), 182 (23.5), 148 (42.6), 121 (39.3), 113 (5.1), 112 (15.3), 91 (39.9), 86 (32.1) 85 (26.4); HRESIMS (30 V) \( m/z \) (% relative intensity) 295.8431 \([M - H]^-\) (9.0), 215.9174 (18.9), 78.9193 (98.3); HRESIMS \( m/z \) 295.8444 \([M - H]^-\) (calcd for \( C_8H_2BrCl_3N \), 295.8442).
**2,3,4,5,7-Pentabromoindole (134):** White needles; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 294 (3.78), 236 (4.39); IR $\nu_{\text{max}}$ 3407 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Tables 3.17 and 3.18; HRESIMS (40 V) m/z (% relative intensity) 513.5984 (31.9), 511.6010 (98.2), 509.6028 (100), 507.6041 (46.7), 505.6083 [M − H]$^-$ (6.8), 432.6807 (44.7), 430.6836 (77.9), 429.6771 (42.8), 428.6853 (58.5), 427.6774 (28.6), 351.7643 (47.5), 349.7676 (59.2), 347.7684 (20.5), 80.9172 (59.4), 78.9190 (72.6); HRESIMS m/z 505.6029 [M − H]$^-$ (calcd for C$_8$HBr$_5$N, 505.6031).

**2,3,4,6,7-Pentabromoindole (135):** White needles; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 297 (4.96), 235 (5.54); IR $\nu_{\text{max}}$ 3394 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Tables 3.17 and 3.18; HRESIMS (40 V) m/z (% relative intensity) 513.5970 (30.1), 511.6016 (94.9), 509.6012 (100), 507.6024 (46.4), 505.5997 [M − H]$^-$ (7.2), 432.6818 (39.8), 430.6830 (67.5), 429.6750 (54.9), 428.6843 (50.9), 427.6799 (41.1), 351.7658 (30.1), 349.7640 (22.0), 80.9177 (70.1), 78.9191 (74.1); HRESIMS m/z 505.6032 [M − H]$^-$ (calcd for C$_8$HBr$_5$N, 505.6031).

**4,5,7-Tribromo-2,3-dichloroindole (136):** White needles; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 296 (4.34), 234 (4.92); IR $\nu_{\text{max}}$ 3423 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Tables 3.17 and 3.18; HRESIMS (40 V) m/z (% relative intensity) 423.7008 (23.9), 421.7035 (34.2), 419.7049 (20.8), 417.7089 [M − H]$^-$ (4.4), 343.7774 (12.7), 342.7843 (39.8), 341.7776 (38.8), 340.7858 (39.6), 339.7790 (38.5), 338.7881 (15.1), 337.7804 (12.3), 263.8644 (22.5), 261.8672 (58.0), 259.8699 (34.7), 80.9172 (97.2), 78.9193 (100); HRESIMS m/z 417.7031 [M − H]$^-$ (calcd for C$_8$HBr$_3$Cl$_2$N, 417.7042).

**5,7-Dibromo-2,3,4-trichloroindole (137):** White needles; UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) 295 (4.45), 234 (5.02); IR $\nu_{\text{max}}$ 3423 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Tables 3.17 and 3.18; HRESIMS (40 V) m/z (% relative intensity) 377.7509 (21.2), 375.7539 (18.0), 373.7564 [M − H]$^-$ (4.2), 298.9318 (25.9), 297.8259 (41.7), 296.8350 (40.1), 295.8281 (61.7), 294.8367 (18.0), 293.8301 (25.7), 80.9172 (100), 78.9192 (98.5); HRESIMS m/z 373.7557 [M − H]$^-$ (calcd for C$_8$HBr$_2$Cl$_3$N, 373.7547).
6.5 Isolation of Compounds from *Callophycus serratus*

A specimen of *Callophycus serratus* was collected by hand from ‘Eua, Tonga in June 2016 and stored at −20 °C until extraction. The alga was identified by Dr Joe Zuccarello at the School of Biological Sciences, Victoria University of Wellington, where a voucher specimen (PTN434F) is also stored.

The alga (102.5 g wet weight) was extracted in MeOH (400 mL) twice overnight. The second, then first extracts were cyclic loaded onto PSDVB (80 mL) and eluted with 1) 30% Me$_2$CO/H$_2$O 2) 75% Me$_2$CO/H$_2$O 3) 100% Me$_2$CO (A1–A3). Fraction A2 was loaded onto DIOL (15 mL) that was pre-equilibrated in n-hexane overnight, and batch-eluted with 45 mL portions of DCM, 10%, 20%, 50% EtOAc/DCM, EtOAc and MeOH (B1–B6). B3 was further purified using C$_{18}$ HPLC, 85% MeOH/H$_2$O isocratic, to afford deiodocallophycoic acid B (231) ($t_R = 12.2$ min). Fraction A3 was dry-loaded onto silica gel (20 mL) and flash column chromatography was performed eluting with 60 mL portions of n-hexane, 5%, 10%, 20%, 50% DCM/n-hexane, DCM, 25%, 50% EtOAc/DCM and 20% MeOH/EtOAc, which yielded 84 fractions that were combined on the basis of TLC to provide 22 fractions (C1–C22). Both C12 and C13 were separately subjected to further purification on a HILIC HPLC column, eluting with 10% EtOAc/n-hexane. This resulted in the purification of iodocallophycol F (229) from fraction C12 ($t_R = 24.4$ min) and both iodocallophycols E (228) and F (229) ($t_R = 16.6$ min) from C13.

A second batch of the alga (79 g) was extracted in MeOH (400 mL), cyclic loaded onto PSDVB and eluted with 1) 30% Me$_2$CO/H$_2$O 2) 75% Me$_2$CO/H$_2$O 3) 100% Me$_2$CO (D1–D3) in a similar fashion to that described above. The 100% Me$_2$CO fraction (D3) was further purified on a DIOL (15 mL) column from 45 mL volumes of n-hexane, 5%, 10%, 20%, 50% DCM/n-hexane, DCM, EtOAc, MeOH to generate 47 fractions that were combined, following a TLC analysis, into 14 samples (E1–E14). Samples E5–E7 were recombined due to similarities observed in their $^1$H
NMR spectra and the resulting sample was purified by HPLC (C18) using 80% MeCN/H2O to afford iodocallophycoic acid B (230) ($t_R = 16.6$ min). E9 was fractionated using C18 HPLC, eluting isocratically with 90% MeCN/H2O, which afforded pure bromophycolide T (202) (0.10 mg, $t_R = 4.8$ min) and additional mass of 228 ($t_R = 22.5$ min) and 229 ($t_R = 21.6$ min). All samples identified as 228 were combined (1.3 mg), as were those of 229 (1.8 mg). The 75% Me2CO/H2O fraction (D2) was further fractionated by flash column chromatography on DIOL, eluting with n-hexane, 30%, 50%, 70% DCM/n-hexane, DCM, 10%, 20%, 50% EtOAc/DCM, EtOAc and MeOH, which resulted in 50 fractions that were combined to form 11 samples (F1–F11). Additional purification of F5 was performed on C18 HPLC, eluting at 80% MeCN/H2O isocratically for 12.5 min, then ramping to 100% MeCN between 12.5–13.0 min and eluting for 12 min, providing fractions G1–G3. Bromophycolide A (181) was finally purified from G1 using C18 HPLC, 75% MeCN/H2O (1.2 mg, $t_R = 14.5$ min). Fractions B2 (first batch extraction) and G7 were subjected to HPLC C18 chromatography, eluting with 80% MeCN/H2O to afford iodocallophycoic acid B (230) ($t_R = 16.6$ min), deiodocallophycoic B (231) ($t_R = 11.6$ min) and callophycoic acid I (0.60 mg) (232) ($t_R = 7.0$ min). Fractions that contained 230 were combined (0.80 mg) as were those that contained 231 (1.0 mg).

**Bromophycolide A (181):** White amorphous solid; All NMR data as previously described.\(^{180}\)

**Bromophycolide T (202):** White amorphous solid; All NMR data as previously described.\(^{177}\)

**Callophycol C (227):** White amorphous solid; $^1$H and $^{13}$C NMR data see Table 4.2; HRESIMS (40 V) $m/z$ (% relative intensity) 638.9696 (0.4), 636.9715 (0.8), 634.9743 (0.7), 632.9812 [M − H]$^-$ (0.2), 559.0454 (9.5), 557.0483 (50.8), 555.0503 (76.2), 553.0520 (29.0), 265.8586 (4.6), 263.8604 (10.6), 261.8617 (5.7), 80.9171 (96.3), 78.9193 (100); HRESIMS $m/z$ 632.9787 [M − H]$^-$ (calcd for C26H32Br3ClO, 632.9776).
Iodocallophycol E (228): White amorphous solid; $\left[ \alpha \right]_{D}^{20} = -27.1$ (c 0.17, CHCl$_3$); IR $\nu_{\text{max}}$ 3378, 2953, 1267 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 4.3; HRESIMS (5 V) m/z (% relative intensity) 730.9107 (30.8), 728.9128 (100), 726.9143 (93.2), 724.9153 [M – H]$^-$ (20.4), 126.9054 (93.6), 80.91711 (7.3), 78.9192 (7.9); HRESIMS m/z 724.9131 [M – H]$^-$ (calcd for C$_{26}$H$_{33}$Br$_3$IO, 724.9132).

Iodocallophycol F (229): White amorphous solid; $\left[ \alpha \right]_{D}^{20} = -49.2$ (c 0.12, CHCl$_3$); IR $\nu_{\text{max}}$ 3378, 2956, 1265 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 4.4; HRESIMS (5 V) m/z (% relative intensity) 648.9877 (28.4), 646.9896 (53.3), 644.9900 ([M – H]$^-$ (17.5), 126.9058 (100), 80.9173 (3.6), 78.9195 (3.4); HRESIMS m/z 644.9880 [M – H]$^-$ (calcd for C$_{26}$H$_{32}$Br$_2$IO, 644.9870).

Iodocallophycoic Acid B (230): White amorphous solid; $\left[ \alpha \right]_{D}^{20} = -27.5$ (c 0.08, CHCl$_3$); IR $\nu_{\text{max}}$ 3320, 3068, 2925, 1683, 1273 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 4.5; HRESIMS (5 V) m/z (% relative intensity) 615.0654 (2.0), 613.0625 (30.3), 611.0630 [M – H]$^-$ (17.7), 126.9053 (100), 80.9173 (3.2), 78.9172 (14.3); HRESIMS m/z 611.0668 [M – H]$^-$ (calcd for C$_{27}$H$_{33}$BrIO$_3$, 611.0663).

Deiodocallophycoic Acid B (231): White amorphous solid; $\left[ \alpha \right]_{D}^{20} = +46.0$ (c 0.1, CHCl$_3$); IR $\nu_{\text{max}}$ 3291, 3068, 2952, 1683, 1274 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 4.7; HRESIMS (30 V) m/z (% relative intensity) 487.1723 (1.4), 485.1722 [M – H]$^-$ (1.0), 405.2445 (49.9), 150.0356 (24.8), 80.9171 (100), 78.9190 (78.9); HRESIMS m/z 485.1684 [M – H]$^-$ (calcd for C$_{27}$H$_{34}$BrO$_3$, 485.1697).

Callophycoic Acid I (232): White amorphous solid; $\left[ \alpha \right]_{D}^{20} = +19.4$ (c 0.06, MeOH); IR $\nu_{\text{max}}$ 3330, 3068, 2925, 1679, 1275 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 4.9; HRESIMS (30 V) m/z (% relative intensity) 505.1576 (0.8), 503.1650 (3.5), 501.1658 [M – H]$^-$ (2.2), 423.2413 (9.7), 422.2405 (37.0), 421.2378 (87.1), 377.247 (8.4), 80.9173 (100), 78.9194 (85.9); HRESIMS m/z 501.1650 [M – H]$^-$ (calcd for C$_{27}$H$_{34}$BrO$_4$, 501.1646).
Appendix A

Screening and NMR Protocol

The screening protocol utilised in this research was initially developed by West\textsuperscript{90} for New Zealand sponges.\textsuperscript{1} Where appropriate, masses and volumes were scaled accordingly.

Equipment

- 1 × screen column loaded with 80 mL of HP20 equilibrated in MeOH.
- 1 × backloading column loaded with 40 mL of HP20 equilibrated in MeOH.

Extraction

- Extract \textit{ca.} 100 g of sponge material in 400 mL MeOH overnight.
- Filter the first extract and set aside. Re-extract the sponge material (and filter paper/celite as necessary) in 400 mL of MeOH overnight.
- Filter the second extract.
- Keep all sponge material (and filter paper/celite as necessary) until the screen is complete at which time it may be discarded.

Cyclic Loading

- Pass the second extract through the screen column with a flow rate of \textit{ca.} 10 mL/min.
- Pass the first extract through the screen column with a flow rate of \textit{ca.} 10 mL/min. Combine the eluent with that of the second extract.

\textsuperscript{1}Last updated 13/11/2006
• Dilute the combined eluents with 800 mL of distilled H₂O. Pass the diluted eluents back through the screen column as a flow rate of ca. 10 mL/min.

• Dilute the eluent with 1.6 L of distilled H₂O. Pass the diluted eluent back through the screen column at a flow rate of ca. 10 mL/min.

• The eluent should be kept until the screen is complete at which time it may be discarded.

Elution

• Elute the screen column with 250 mL of distilled H₂O at a flow rate of ca. 10 mL/min. The H₂O eluent can be discarded immediately.

• Elute the screen column with 250 mL of 30% Me₂CO/H₂O (75 mL Me₂CO to 175 mL H₂O) at a flow rate of ca. 10 mL/min.

• Elute the screen column with 250 mL of 75% Me₂CO/H₂O (187.5 mL Me₂CO to 62.5 mL H₂O) at a flow rate of ca. 10 mL/min.

• Elute the screen column with 250 mL of Me₂CO at a flow rate of ca. 10 mL/min.

• The eluent should be kept until the screen is complete at which time it may be discarded.

Backloading the 75% Me₂CO/H₂O Fraction

• Dilute the 75% Me₂CO/H₂O fraction with 250 mL of distilled H₂O. Pass the diluted eluent through the backloading column at a flow rate of ca. 8 mL/min.

• Dilute the eluent with 500 mL of distilled H₂O. Pass the diluted eluent back through the backloading column at a flow rate of ca. 8 mL/min.

• The eluent should be kept aside until the screen is complete at which time it may be discarded.

• Elute the backloading column with 150 mL of Me₂CO.
Processing the 75% Me$_2$CO/H$_2$O Fraction

- Concentrate the Me$_2$CO eluent of the backloading column to dryness and transfer to a pre-weighed sample vial. Concentrate to dryness and record the mass.

- Subsample ca. 30 mg of material for NMR analysis if necessary.

- Prepare an NMR sample in ca. 700 µL of CD$_3$OD in a 5 mm NMR tube.

NMR Analysis of the 75% Me$_2$CO/H$_2$O Fraction

- Run a $^1$H NMR spectrum of the sample on the 600 MHz instrument using the standard Screen1H parameter set (experiment time: ca. 4 min).

- NMR parameters for $^1$H NMR screen
  - increments per scan (ni) = 64.
  - acquisition time (at) = 3.407 s.
  - relaxation delay (d1) = 0.01 s.
  - $^1$H spectral width: −1.0–9.0 ppm.
  - total experimental time: 3 min, 39 s.

- Run a COSY spectrum of the sample using the standard ScreenCOSY parameter set (experiment time: ca. 20 min).

- NMR parameters for COSY screen
  - increments per scan (ni) = 1.
  - number of scans (nt) = 512.
  - acquisition time (at) = 0.150 s.
  - relaxation delay (d1) = 2.0 s.
  - $^1$H spectral width: −1.0–9.0 ppm.
  - window functions: sinebell.
- total experimental time: 19 min, 56 s.

- Run an HSQC spectrum of the sample using the standard ScreenHSQC parameter set (experiment time: ca. 4 h).

- NMR parameters for HSQC screen
  - increments per scan (ni) = 8.
  - number of scans (nt) = 512.
  - acquisition time (at) = 0.150 s.
  - One-Bond coupling constant = 146 Hz.
  - relaxation delay (d1) = 1.5 s.
  - $^1$H spectral width: −1.0–9.0 ppm.
  - $^{13}$C spectral width: 0–160 ppm
  - $^1$H–$^{13}$C multiplicity editing: yes
  - window functions: cosine.
  - total experimental time: 3 h, 51 min.

- Run an HMBC spectrum of the sample using the default gHMBC parameter set (experiment time: ca. 8 h)

- NMR parameters for HMBC screen
  - increments per scan (ni) = 16.
  - number of scans (nt) = 512.
  - acquisition time (at) = 0.213 s.
  - coupling constant = 8 Hz.
  - relaxation delay (d1) = 1.5 s.
  - $^1$H spectral width: −1.0–9.0 ppm.
  - $^{13}$C spectral width: 0–220 ppm
  - window functions: sinebell.
Backloading the 30% Me$_2$CO/H$_2$O Fraction

- Dilute the 30% Me$_2$CO/H$_2$O fraction with 250 mL of distilled H$_2$O. Pass the diluted eluent through the backloading column at a flow rate of ca. 8 mL/min.
- Dilute the eluent with 500 mL of distilled H$_2$O. Pass the diluted eluent back through the backloading column at a flow rate of ca. 8 mL/min.
- The eluent should be kept aside until the screen is complete at which time it may be discarded.
- Elute the backloading column with 150 mL of Me$_2$CO.

Processing the 30% Me$_2$CO/H$_2$O Fraction

- Concentrate the Me$_2$CO eluent of the backloading column to dryness and transfer to a pre-weighed sample vial. Concentrate to dryness and record the mass.
- Subsample ca. 30 mg of material for NMR analysis if necessary.
- Prepare an NMR sample in ca. 700 µL of CD$_3$OD in a 5 mm NMR tube.

NMR Analysis of the 30% Me$_2$CO/H$_2$O Fraction

- Run a $^1$H NMR spectrum of the sample on the 600 MHz instrument using the standard Screen1H parameter set (experiment time: ca. 4 min).

Processing the 100% Me$_2$CO/H$_2$O Fraction

- Concentrate the Me$_2$CO eluent of the backloading column to dryness and transfer to a pre-weighed sample vial. Concentrate to dryness and record the mass.
- Subsample ca. 30 mg of material for NMR analysis if necessary.
• Prepare an NMR sample in ca. 700 µL of CD$_3$OD in a 5 mm NMR tube.

NMR Analysis of the 100% Me$_2$CO/H$_2$O Fraction

• Run a $^1$H NMR spectrum of the sample on the 600 MHz instrument using the standard Screen1H parameter set (experiment time: ca. 4 min).
Appendix B

NMR Spectra of Known Compounds

2,3,4,7-Tetrabromoindole

$^{1}$H NMR spectrum of 2,3,4,7-tetrabromoindole (116) (600 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 2,3,4,7-tetrabromoindole (116) (150 MHz, CDCl$_3$)
COSY spectrum of 2,3,4,7-tetrabromoindole (116) (600 MHz, CDCl$_3$)
HMBC spectrum of 2,3,4,7-tetramidoindole (116) (600 MHz, CDCl₃)

¹⁵N CIGARAD spectrum of 2,3,4,7-tetramidoindole (116) (600 MHz, CDCl₃)
\[
\text{\textsuperscript{1}H NMR spectrum of 2,3,4,7-tetrabromoindole (116) (600 MHz, DMSO-d\textsubscript{6})}
\]

\[
\text{\textsuperscript{13}C NMR spectrum of 2,3,4,7-tetrabromoindole (116) (150 MHz, DMSO-d\textsubscript{6})}
\]
COSY spectrum of 2,3,4,7-tetrabromoindole (116) (600 MHz, DMSO–d₆)

Coupled HSQC spectrum of 2,3,4,7-tetrabromoindole (116) (600 MHz, DMSO–d₆)
HMBC spectrum of 2,3,4,7-tetrabromoindole (116) (600 MHz, DMSO–d$_6$)

$^{15}$N CIGARAD spectrum of 2,3,4,7-tetrabromoindole (116) (600 MHz, DMSO–d$_6$)
4,7-Dibromo-2,3-dichloroindole

\[ \text{H NMR spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, CDCl}_3) \]

\[ \text{^1H NMR spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, CDCl}_3) \]

\[ \text{^13C NMR spectrum of 4,7-dibromo-2,3-dichloroindole (119) (150 MHz, CDCl}_3) \]
COSY spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, CDCl₃)
HMBC spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, CDCl₃)

¹⁵N CIGARAD spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, CDCl₃)
$^1$H NMR spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 4,7-dibromo-2,3-dichloroindole (119) (150 MHz, DMSO–d$_6$)
COSY spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, DMSO-\textit{d}_6)

Coupled HSQC spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, DMSO-\textit{d}_6)
HMBC spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, DMSO–d$_6$)

$^{15}$N CIGARAD spectrum of 4,7-dibromo-2,3-dichloroindole (119) (600 MHz, DMSO–d$_6$)
7-Bromo-2,3,4-trichloroindole

$\text{H NMR spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, CDCl}_3$)

$\text{C NMR spectrum of 7-bromo-2,3,4-trichloroindole (121) (150 MHz, CDCl}_3$)
COSY spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, CDCl₃)
HMBC spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, CDCl₃)

\[15^N\] CIGARAD spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, CDCl₃)
$^1$H NMR spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 7-bromo-2,3,4-trichloroindole (121) (150 MHz, DMSO–d$_6$)
COSY spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, DMSO–d₆)

Coupled HSQC spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, DMSO–d₆)
HMBC spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, DMSO–d₆)

\[ ^{15}N \text{ CIGARAD spectrum of 7-bromo-2,3,4-trichloroindole (121) (600 MHz, DMSO–d₆)} \]
Indole

\[
\begin{align*}
\text{\( ^1H \) NMR spectrum of indole (140) (600 MHz, CDCl}_3) \\
\text{\( ^{13}C \) NMR spectrum of indole (140) (150 MHz, CDCl}_3) 
\end{align*}
\]
COSY spectrum of indole (140) (600 MHz, CDCl$_3$)

Coupled HSQC spectrum of indole (140) (600 MHz, CDCl$_3$)
HMBC spectrum of indole (140) (600 MHz, CDCl₃)

¹⁵N CIGARAD spectrum of indole (140) (600 MHz, CDCl₃)
$^1$H NMR spectrum of indole (140) (600 MHz, DMSO-$d_6$)

$^{13}$C NMR spectrum of indole (140) (150 MHz, DMSO-$d_6$)
COSY spectrum of indole (140) (600 MHz, DMSO-d$_6$)

Coupled HSQC spectrum of indole (140) (600 MHz, DMSO-d$_6$)
HMBC spectrum of indole (140) (600 MHz, DMSO–d$_6$)

$^{15}$N CIGARAD spectrum of indole (140) (600 MHz, DMSO–d$_6$)
Bromophycolide A

$\text{HO} \quad \text{O} \quad \text{Br} \quad \text{OH} \quad \text{Br} \quad \text{Br}$

$\text{1H NMR spectrum of bromophycolide A (181) (600 MHz, CDCl}_3\text{)}$

$\text{13C NMR spectrum of bromophycolide A (181) (150 MHz, CDCl}_3\text{)}$
Bromophycolide T

\[
\begin{align*}
\text{HO} & \quad \text{O} \\
\text{Br} & \quad \text{Br} \\
\text{H} & \quad \text{O}
\end{align*}
\]

\[202\]

\[\begin{align*}
\text{1H NMR spectrum of bromophycolide T (202)} & \quad (600 \text{ MHz, CDCl}_3) \\
\text{13C NMR spectrum of bromophycolide T (202)} & \quad (150 \text{ MHz, CDCl}_3)
\end{align*}\]
Appendix C

NMR Spectra of α-Exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone

$^1$H NMR spectrum of α-exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69) (600 MHz, CDCl$_3$)
$^{13}$C NMR spectrum of α-exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69) (150 MHz, CDCl$_3$)
COSY spectrum of $\alpha$-exomethylene-$\gamma$-methyl-$\gamma$-tetradecyl-$\gamma$-butyrolactone (69) (600 MHz, CDCl$_3$)
HSQC spectrum of α-exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69) (600 MHz, CDCl₃)
HMBC spectrum of α-exomethylene-γ-methyl-γ-tetradecyl-γ-butyrolactone (69) (600 MHz, CDCl₃)
Appendix D

NMR Spectra of $\alpha$-Exomethylene-$\beta$-hydroxyl-$\gamma$-methyl-$\gamma$-tetradecyl-$\gamma$-butyrolactone

$\text{OH}$

$\text{O}$

$\text{O}$

$\text{OH}$

$\text{OH}$

$\text{f1 (ppm)}$

$\text{f1 (ppm)}$

$\text{1H NMR spectrum of } \alpha$-exomethylene-$\beta$-hydroxyl-$\gamma$-methyl-$\gamma$-tetradecyl-$\gamma$-butyrolactone (70) (600 MHz, CDCl$_3$)
$^{13}$C NMR spectrum of $\alpha$-exomethylene-$\beta$-hydroxyl-$\gamma$-methyl-$\gamma$-tetradecyl-$\gamma$-butyrolactone (70) (150 MHz, CDCl$_3$)
COSY spectrum of α-exomethylene-β-hydroxyl-γ-methyl-γ-tetradecyl-γ-butyrolactone (70) (600 MHz, CDCl₃)
HSQC spectrum of α-exomethylene-β-hydroxyl-γ-methyl-γ-tetradecyl-γ-butyrolactone (70) (600 MHz, CDCl₃)
HMBC spectrum of α-exomethylene-β-hydroxy-γ-methyl-γ-tetradecyl-γ-butyrolactone (70) (600 MHz, CDCl₃)
Appendix E

NMR Spectra of 2,3,4-Trichloro-7-iodoindole

\[
\begin{align*}
\text{H NMR spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, CDCl}_3) \\
\text{13C NMR spectrum of 2,3,4-trichloro-7-iodoindole (123) (150 MHz, CDCl}_3) 
\end{align*}
\]
COSY spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, CDCl₃)
HMBC spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, CDCl$_3$)

$^{15}$N CIGARAD spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, CDCl$_3$)
$^1$H NMR spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 2,3,4-trichloro-7-iodoindole (123) (150 MHz, DMSO–d$_6$)
COSY spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, DMSO–d$_6$)

Coupled HSQC spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, DMSO–d$_6$)
HMBC spectrum of 2,3,4-trichloro-7-iodoindole (123) (600 MHz, DMSO–d$_6$)
Appendix F

NMR Spectra of

2-Bromo-3,4-dichloro-7-iodoindole

$\begin{align*}
\text{H NMR spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, CDCl}_3) \\
\text{C NMR spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (150 MHz, CDCl}_3)
\end{align*}$
COSY spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, CDCl₃)
HMBC spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, CDCl₃)

¹⁵N CIGARAD spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, CDCl₃)
$^1$H NMR spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (150 MHz, DMSO–d$_6$)
COSY spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, DMSO–d<sub>6</sub>)

Coupled HSQC spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, DMSO–d<sub>6</sub>)
HMBC spectrum of 2-bromo-3,4-dichloro-7-iodoindole (124) (600 MHz, DMSO–d$_6$)
Appendix G

NMR Spectra of 2,3,6,7-Tetrabromoindole

\[
\begin{align*}
\text{H NMR spectrum of 2,3,6,7-tetrabromoindole (125) (600 MHz, CDCl}_3) \\
\text{13C NMR spectrum of 2,3,6,7-tetrabromoindole (125) (150 MHz, CDCl}_3)
\end{align*}
\]
COSY spectrum of 2,3,6,7-tetrabromoindole (125) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 2,3,6,7-tetrabromoindole (125) (600 MHz, CDCl₃)
HMBC spectrum of 2,3,6,7-tetabromoindole (125) (600 MHz, CDCl$_3$)

$^{15}$N CIGARAD spectrum of 2,3,6,7-tetabromoindole (125) (600 MHz, CDCl$_3$)
$^1$H NMR spectrum of 2,3,6,7-tetram bromoindole (125) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 2,3,6,7-tetram bromoindole (125) (150 MHz, DMSO–d$_6$)
COSY spectrum of 2,3,6,7-tetrabromoindole (125) (600 MHz, DMSO–d₆)

Coupled HSQC spectrum of 2,3,6,7-tetrabromoindole (125) (600 MHz, DMSO–d₆)
HMBC spectrum of 2,3,6,7-tetrabromoindole (125) (600 MHz, (DMSO–d$_6$))
Appendix H

NMR Spectra of 3,6,7-Tribromo-2-chloroindole

\[
\begin{align*}
\text{1H NMR spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, CDCl}_3) \\
\text{13C NMR spectrum of 3,6,7-tribromo-2-chloroindole (126) (150 MHz, CDCl}_3)
\end{align*}
\]
COSY spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, CDCl₃)
HMBC spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, CDCl$_3$)

$^{15}$N CIGARAD spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, CDCl$_3$)
$^1$H NMR spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 3,6,7-tribromo-2-chloroindole (126) (150 MHz, DMSO–d$_6$)
COSY spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, DMSO–d$_6$)

Coupled HSQC spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, DMSO–d$_6$)
HMBC spectrum of 3,6,7-tribromo-2-chloroindole (126) (600 MHz, (DMSO–d$_6$))
Appendix I

NMR Spectra of 2,6,7-Tribromo-3-chloroindole

$\text{NMR Spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, CDCl}_3$)

$\text{1^3C NMR Spectrum of 2,6,7-tribromo-3-chloroindole (127) (150 MHz, CDCl}_3$)
COSY spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, CDCl₃)
HMBC spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, CDCl₃)

^{15}N CIGARAD spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, CDCl₃)
$^1$H NMR spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 2,6,7-tribromo-3-chloroindole (127) (150 MHz, DMSO–d$_6$)
COSY spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, DMSO–d$_6$)

Coupled HSQC spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, DMSO–d$_6$)
HMBC spectrum of 2,6,7-tribromo-3-chloroindole (127) (600 MHz, DMSO–d6)
Appendix J

NMR Spectra of 6,7-Dibromo-2,3-dichloroindole

$\begin{align*}
\text{H} & \quad \text{NMR} \\
128 & \\
\text{Cl} & \\
\text{Br} & \\
\text{Cl} & \\
\text{Br} &
\end{align*}$

$^1$H NMR spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, CDCl$_3$)

$\begin{align*}
\text{C} & \quad \text{NMR} \\
231 & \\
\text{Cl} & \\
\text{Br} & \\
\text{Br} &
\end{align*}$

$^{13}$C NMR spectrum of 6,7-Dibromo-2,3-dichloroindole (128) (150 MHz, CDCl$_3$)
COSY spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, CDCl₃)
HMBC spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, CDCl₃)

$^{15}$N CIGARAD spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, CDCl₃)
$^1$H NMR spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, DMSO–$d_6$)

$^{13}$C NMR spectrum of 6,7-dibromo-2,3-dichloroindole (128) (150 MHz, DMSO–$d_6$)
COSY spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, DMSO–d₆)

Coupled HSQC spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, DMSO–d₆)
HMBC spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, DMSO–d$_6$)

$^{15}$N CIGARAD spectrum of 6,7-dibromo-2,3-dichloroindole (128) (600 MHz, DMSO–d$_6$)
Appendix K

NMR Spectra of

7-Bromo-2,3-dichloro-6-iodoindole

$\text{Br}$ $\text{Cl}$ $\text{Cl}$ $\text{I}$ $\text{Br}$

$^1$H NMR spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (150 MHz, CDCl$_3$)
COSY spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, CDCl$_3$)

Coupled HSQC spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, CDCl$_3$)
HMBC spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, CDCl$_3$)

$^{15}$N CIGARAD spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, CDCl$_3$)
$^1$H NMR spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (150 MHz, DMSO–d$_6$)
COSY spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, DMSO–d₆)

Coupled HSQC spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, DMSO–d₆)
HMBC spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, DMSO-d$_6$)

$^{15}$N CIGARAD spectrum of 7-bromo-2,3-dichloro-6-iodoindole (129) (600 MHz, DMSO-d$_6$)
Appendix L

NMR Spectra of

2,7-Dibromo-3-chloro-6-iodoindole

\[ \text{H NMR spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, CDCl}_3 \}\]

\[ \text{^13C NMR spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (150 MHz, CDCl}_3 \}\]
COSY spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, CDCl$_3$)

Coupled HSQC spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, CDCl$_3$)
HMBC spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, CDCl₃)

¹⁵N CIGARAD spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, CDCl₃)
$^1$H NMR spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, DMSO–d$_6$)

$^{13}$C NMR spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (150 MHz, DMSO–d$_6$)
COSY spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, DMSO–d$_6$)

Coupled HSQC spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, DMSO–d$_6$)
HMBC spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, DMSO–d$_6$)

$^{15}$N CIGARAD spectrum of 2,7-dibromo-3-chloro-6-iodoindole (130) (600 MHz, DMSO–d$_6$)
Appendix M

NMR Spectra of

3,7-Dibromo-2-chloro-6-iodoindole

$^1$H NMR spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (150 MHz, CDCl$_3$)
COSY spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, CDCl$_3$)

HSQC spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, CDCl$_3$)
HMBC spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, CDCl$_3$)

$^1$H NMR spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, DMSO–d$_6$)
$^{13}$C NMR spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (150 MHz, DMSO–d$_6$)

COSY spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, DMSO–d$_6$)
Coupled HSQC spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, DMSO-\(d_6\))

HMBC spectrum of 3,7-dibromo-2-chloro-6-iodoindole (131) (600 MHz, DMSO-\(d_6\))
Appendix N

NMR Spectra of 2,3,7-Trichloro-6-iodoindole

$\text{Cl} \quad \text{Cl} \quad \text{I} \quad \text{Cl}$

$\text{H NMR spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, CDCl}_3\text{)}$

$\text{13C NMR spectrum of 2,3,7-trichloro-6-iodoindole (132) (150 MHz, CDCl}_3\text{)}$
COSY spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, CDCl₃)
HMBC spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, CDCl₃)

¹H NMR spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, DMSO-d₆)
$^{13}$C NMR spectrum of 2,3,7-trichloro-6-iodoindole (132) (150 MHz, DMSO$-d_6$)

COSY spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, DMSO$-d_6$)
Coupled HSQC spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, DMSO–d$_6$)

HMBC spectrum of 2,3,7-trichloro-6-iodoindole (132) (600 MHz, DMSO–d$_6$)
Appendix O

NMR Spectra of 5-Bromo-2,3,4-trichloroindole

\[
\text{\begin{tabular}{c}
\text{\includegraphics[width=0.5\textwidth]{spectra.png}}
\end{tabular}}
\]

\( ^1\text{H NMR spectrum of 5-bromo-2,3,4-trichloroindole (133) (600 MHz, CDCl}_3) \)

\( ^{13}\text{C NMR spectrum of 5-bromo-2,3,4-trichloroindole (133) (150 MHz, CDCl}_3) \)
COSY spectrum of 5-bromo-2,3,4-trichloroindole (133) (600 MHz, CDCl₃)

Coupled HSQC spectrum of 5-bromo-2,3,4-trichloroindole (133) (600 MHz, CDCl₃)
HMBC spectrum of 5-bromo-2,3,4-trichloroindole (133) (600 MHz, CDCl₃)

¹⁵N CIGARAD spectrum of 5-bromo-2,3,4-trichloroindole (133) (600 MHz, CDCl₃)
Appendix P

NMR Spectra of 2,3,4,5,7-Pentabromoindole

$^1$H NMR spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 2,3,4,5,7-pentabromoindole (134) (150 MHz, CDCl$_3$)
Coupled HSQC spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, CDCl₃)

HMBC spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, CDCl₃)
$^{15}$N CIGARAD spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, CDCl$_3$)

$^1$H NMR spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, DMSO–d$_6$)
$^{13}$C NMR spectrum of 2,3,4,5,7-pentabromoindole (134) (150 MHz, DMSO–d$_6$)

Coupled HSQC spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, DMSO–d$_6$)
HMBC spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, DMSO–d<sub>6</sub>)

<figure>
<svg>
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<sup>15</sup>N CIGARAD spectrum of 2,3,4,5,7-pentabromoindole (134) (600 MHz, DMSO–d<sub>6</sub>)
Appendix Q

X-ray Diffraction Data for

2,3,4,5,7-Pentabromoindole

The following data was collected and solved by Associate Professor Martyn Coles

Data collection SuperNova, Dual, Cu at zero, Atlas, Program package WinGX, Abs. correction 'MULTISCAN' Refinement using SHELXL-97, Drawing using ORTEP-3 for Windows

Empirical formula $\text{C}_8\text{H}_2\text{Br}_5\text{N}$

Formula weight 511.66 g/mol

Temperature/K 120.01(10)

Crystal system monoclinic

Space group $P2_1/c$ (No.14)

a/Å 16.1303(8)

b/Å 3.9804(2)

c/Å 17.4061(9)

$\alpha/$° 90

$\beta/$° 105.163(5)

$\gamma/$° 90

Volume/Å³ 1078.66(10)

Z 4

$\rho_{\text{calc}}$/g/cm³ 3.151

$\mu$/mm⁻¹ 22.239
F(000) 928.0
Crystal size/mm$^3$ 0.11 × 0.03 × 0.03
Radiation Cu Kα ($\gamma = 1.54184$)
2Θ range for data collection/$^\circ$ 10.5316 to 143.421
Index ranges $-16 \leq h \leq 19, -4 \leq k \leq 4, -20 \leq l \leq 20$
Reflections collected 4636
Independent reflections 2079 ($R_{\text{int}} = 0.03, R_{\text{sigma}} = 0.048$)
Data/restraints/parameters 2079/0/127
Goodness-of-fit on $F^2$ 1.097
Final R indexes [I > = 2σ (I)] $R_1 = 0.036, wR_2 = 0.088$
Final R indexes [all data] $R_1 = 0.040, wR_2 = 0.091$
Largest diff. peak/hole / e Å$^{-3}$ 0.98/−0.74
Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($\text{Å}^2 \times 10^3$). $U_{eq}$ is defined as 1/3 of the trace of the orthogonalised $U_{ij}$ tensor.

<table>
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<th>Atom</th>
<th>$x$</th>
<th>$y$</th>
<th>$z$</th>
<th>$U_{eq}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br1</td>
<td>9054.0(4)</td>
<td>7059.2(17)</td>
<td>1894.0(3)</td>
<td>25.76(16)</td>
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<tr>
<td>Br2</td>
<td>9417.9(4)</td>
<td>6808.9(16)</td>
<td>3980.1(3)</td>
<td>26.47(16)</td>
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<tr>
<td>Br3</td>
<td>8082.0(4)</td>
<td>3828.3(17)</td>
<td>5103.4(3)</td>
<td>27.28(17)</td>
</tr>
<tr>
<td>Br4</td>
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Appendix R

NMR Spectra of 2,3,4,6,7-Pentabromoindole

$\text{H NMR spectrum of 2,3,4,6,7-pentabromoindole (135) (600 MHz, CDCl}_3)$

$\text{C NMR spectrum of 2,3,4,6,7-pentabromoindole (135) (150 MHz, CDCl}_3)$
Coupled HSQC spectrum of 2,3,4,6,7-pentabromoindole (135) (600 MHz, CDCl₃)

HMBC spectrum of 2,3,4,6,7-pentabromoindole (135) (600 MHz, CDCl₃)
$^{15}$N CIGARAD spectrum of 2,3,4,6,7-pentabromoindole (135) (600 MHz, CDCl$_3$)
Appendix S

NMR Spectra of

4,5,7-Tribromo-2,3-dichloroindole

\[ \begin{align*}
\text{Br} & \quad \text{Br} \\
\text{Br} & \quad \text{Cl} \\
\text{Br} & \\
\text{Br} & \quad \text{H} \\
\text{Cl} & \end{align*} \]

1H NMR spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (600 MHz, CDCl\(_3\))

13C NMR spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (150 MHz, CDCl\(_3\))
Coupled HSQC spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (600 MHz, CDCl₃)

HMBC spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (600 MHz, CDCl₃)
$^{15}$N CIGARAD spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (600 MHz, CDCl$_3$)

$^1$H NMR spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (600 MHz, DMSO–d$_6$)
\(^{13}\)C NMR spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (150 MHz, DMSO–d<sub>6</sub>)

HSQC spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (600 MHz, DMSO–d<sub>6</sub>)
HMBC spectrum of 4,5,7-tribromo-2,3-dichloroindole (136) (600 MHz, DMSO–d$_6$)
Appendix T

NMR Spectra of

5,7-Dibromo-2,3,4-trichloroindole

$^1$H NMR spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (600 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (150 MHz, CDCl$_3$)
HSQC spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (600 MHz, CDCl₃)

HMBC spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (600 MHz, CDCl₃)
$^{15}$N CIGARAD spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (600 MHz, CDCl$_3$)

$^1$H NMR spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (600 MHz, DMSO-d$_6$)
$^{13}$C NMR spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (150 MHz, DMSO-$d_6$)

HSQC spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (600 MHz, DMSO-$d_6$)
HMBC spectrum of 5,7-dibromo-2,3,4-trichloroindole (137) (600 MHz, DMSO–d$_6$)
Appendix U

NMR Spectra of Callophycol C

\[
\text{HO}
\begin{array}{c}
\text{Br} \\
\text{Br} \\
\text{Cl} \\
\text{Br} \\
\text{H}
\end{array}
\]

\[227\]

\[
\begin{array}{c}
\text{Br} \\
\text{Br} \\
\text{Cl}
\end{array}
\]

\[
\text{285}
\]

\[\text{H NMR spectrum of callophycol C (227) (600 MHz, CDCl}_3)\]

1
$^{13}$C NMR spectrum of callophycol C (227) (150 MHz, CDCl$_3$)
COSY spectrum of callophycol C (227) (600 MHz, CDCl₃)
HSQC spectrum of callophycol C (227) (600 MHz, CDCl$_3$).
HMBC spectrum of callophycol C (227) (600 MHz, CDCl₃)
Appendix V

NMR Spectra of Iodocallophycol E

$^1$H NMR spectrum of iodocallophycol E (228) (600 MHz, CDCl$_3$)
$^{13}$C NMR spectrum of iodocallophycol E (228) (150 MHz, CDCl$_3$)
COSY spectrum of iodocallophycol E (228) (600 MHz, CDCl₃)
HSQC spectrum of iodocallophycol E (228) (600 MHz, CDCl₃).
HMBC spectrum of iodocallophycol E (228) (600 MHz, CDCl₃)
Appendix W

NMR Spectra of Iodocallophycol F

\[
\begin{align*}
\text{HO} & \quad \text{Br} & \quad \text{Br} & \quad \text{I} \\
229
\end{align*}
\]

\[\text{f1 (ppm)}\]

\[\begin{align*}
0.5 & \quad 1.0 & \quad 1.5 & \quad 2.0 & \quad 2.5 & \quad 3.0 & \quad 3.5 & \quad 4.0 & \quad 4.5 & \quad 5.0 & \quad 5.5 & \quad 6.0 & \quad 6.5 & \quad 7.0 & \quad 7.5 & \quad 8.0
\end{align*}\]

\[\text{1H NMR spectrum of iodocallophycol F (229) (600 MHz, CDCl}_3)\]
$^{13}$C NMR spectrum of iodocallophycol F (229) (150 MHz, CDCl$_3$)
COSY spectrum of iodocallophycol F (229) (600 MHz, CDCl₃)
HSQC spectrum of iodocallophycol F (229) (600 MHz, CDCl₃).
HMBC spectrum of iodocallophycol F (229) (600 MHz, CDCl$_3$)
Appendix X

NMR Spectra of Iodocallophycoic acid B

$^1$H NMR spectrum of iodocallophycoic acid B (230) (600 MHz, CD$_3$OD)
$^1^3$C NMR spectrum of iodocallophycoic acid B (230) (150 MHz, CD$_3$OD)
COSY spectrum of iodocallophycoic acid B (230) (600 MHz, CD$_3$OD)
HSQC spectrum of iodocallophycoic acid B (230) (600 MHz, CD$_3$OD).
HMBC spectrum of iodocallophycoic acid B (230) (600 MHz, CD$_3$OD)
Appendix Y

NMR Spectra of Deiodocallophycoic acid B

\[ \text{\textsuperscript{1}H NMR spectrum of deiodocallophycoic acid B (231) (600 MHz, CDCl}_3) } \]
$^{13}$C NMR spectrum of deiodocallyphyoric acid B (231) (150 MHz, CDCl$_3$)
COSY spectrum of deiodocallophycoic acid B (231) (600 MHz, CDCl₃)
HSQC spectrum of deiodocallophycoic acid B (231) (600 MHz, CDCl₃).
HMBC spectrum of deiodocallophycoic acid B (231) (600 MHz, CDCl₃)
NMR Spectra of Callophycoic acid I

\[232\]

\[\text{H NMR spectrum of callophycoic acid I (232) (600 MHz, CDCl}_3\text{)}\]

1H NMR spectrum of callophycoic acid I (232) (600 MHz, CDCl\textsubscript{3})
$\text{C NMR spectrum of callophycoic acid I (232) (150 MHz, CD}_3\text{OD)}$
COSY spectrum of callophycoic acid I (232) (600 MHz, CDCl₃)
HSQC spectrum of callophycoic acid I (232) (600 MHz, CDCl$_3$).
HMBC spectrum of callophycoic acid I (232) (600 MHz, CDCl₃)
References


64. Dembitsky, V. M. Nat. Prod. Commun. 2006, 1, 139–175.


