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Total Synthesis of Furospongolide and Related Furanolipid Analogues as Potential Anti-tumour Agents

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A Thesis presented for the Degree of Doctor of Philosophy to
THE NATIONAL UNIVERSITY OF IRELAND, CORK.

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December 2013
# Table of Chapters

*Acknowledgments*  
*Abstract*  

**Chapter 1**  
Introduction  

**Chapter 2**  
Results and Discussion  

**Chapter 3**  
Biological Results and Discussion  

**Chapter 4**  
Experimental  

*Abbreviations*  

*Appendices can be found on a CD attached to this thesis*
DECLARATION BY CANDIDATE

I declare that this thesis contains my own work and has not been submitted for another degree, either at University College Cork, or elsewhere

___________________________

Donal P. Harrold
Acknowledgements

I would like to take this opportunity to sincerely thank all the people who helped me throughout my academic years in UCC. First and foremost, I would like to express my sincere gratitude to my supervisor Dr. Stuart Collins for all his help, encouragement and support throughout my research project and especially during the writing of my thesis. He is a true friend who I’m personally indebted to. Special thanks to Dr. Dan McCarthy and Dr. Lorraine Bateman for NMR work conducted; Dr. Florence McCarthy and Mick O’Shea for the mass spectrometry service; Dr. Nuala Maguire for HPLC assistance; Helen Kelly and Barry O’Mahony for microanalysis and the technical staff in the chemistry department. Without funding this thesis would not have been possible, therefore I would like to acknowledge IRCSET for financial support throughout my PhD studies.

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_________________

Donal P. Harrold
Abstract

This thesis details the design, development and execution of innovative methodology in the total synthesis of the terpene-derived marine natural product, furospongolide. It also outlines the synthetic routes used to prepare a novel range of furanolipids derivatives and subsequent evaluation of their potential as antitumour agents.

The first chapter is a review of the literature describing efforts undertaken towards the synthesis of biologically active furanosesterterpenoid marine natural products. A brief discussion on the sources and biological activity exhibited by furan natural products is also provided. In addition, a concise account of the role of hypoxia in cancer, and the increasing interest in HIF-1 inhibition as a target for chemotherapeutics is examined.

The second chapter discusses the concise synthesis of the marine HIF-1 inhibitor furospongolide, which was achieved in five linear steps from (E,E)-farnesyl acetate. The synthetic strategy features a selective oxidation reaction, a Schlosser sp³-sp³ cross-coupling, a Wittig cross-coupling and an elaborate one-pot selective reduction, lactonisation and isomerization reaction to install the butenolide ring. The structure-activity relationship of furospongolide was also investigated. This involved the design and synthesis of a library of structurally modified analogues sharing the same C1-C13 subunit. This was achieved by exploiting the brevity and high level of convergence of our synthetic route together with the readily amenable structure of our target molecule. Exploiting the Schlosser cross-coupling allowed for replacement of furan with other heterocycles in the preparation of various furanolipid and thiophenolipid derivatives. The employment of reductive amination and Wittig chemistry further added to our novel library of structural derivatives.

The third chapter discusses the results obtained from the NCI from biological evaluation. From a collection of 28 novel compounds evaluated against the NCI-60 cancer cell array, six drug candidates were successfully selected for further biological evaluation on the basis of antitumour activity. COMPARE analysis revealed a strong correlation between some of our design analogues and the blockbuster anticancer agent tamoxifen, further supporting the potential of furanolipids in the treatment of breast cancer.

The fourth chapter, details the full experimental procedures, including spectroscopic and analytical data for all the compounds prepared during this research.
To Mom and Dad
“Live as if you were to die tomorrow. Learn as if you were to live forever.”

Mahatma Gandhi
Chapter 1

Introduction
Table of Contents

1.1 Background .................................................................................................................... 3
  1.1.1 Overview ................................................................................................................. 3
  1.1.2 History of Furan ..................................................................................................... 4
  1.1.3 Physical properties of furan .................................................................................... 5
  1.1.4 Chemical properties of furan .................................................................................. 6
  1.1.5 Synthesis of furans and thiophenes ....................................................................... 8
  1.1.6 Natural products containing the furan ring ............................................................ 13
  1.1.7 Isolation of furospongolide .................................................................................... 15
    1.1.7.1 Introduction to furan derivatives isolated .......................................................... 15
    1.1.7.2 Biological evaluation of furospongolide ............................................................... 18
  1.1.8 Biological Background .......................................................................................... 21
    1.1.8.1 Cancer ................................................................................................................ 21
    1.1.8.2 Hypoxia .............................................................................................................. 23
    1.1.8.3 Hypoxia Inducible Factor (HIF) ....................................................................... 23
    1.1.8.4 Hypoxia-induced mitochondrial reactive oxygen species ............................... 27
    1.1.8.5 Small molecule HIF-1 inhibitors and chemoprevention .................................... 29
    1.1.8.6 HIF-1 inhibitors from marine life ....................................................................... 31
  1.1.9 Sesterterpenoids .................................................................................................... 35
    1.1.9.1 Introduction to sesterterpenoids ......

References ............................................................................................................................. 59
1.1 Background

1.1.1 Overview

The purpose of this introduction is to give a comprehensive overview on both the chemical and biological significance of furanolipids to modern day science. With respect to the latter, a furanolipid based natural product known as furospongolide was isolated from an Indonesian marine sponge and was found to possess promising antitumour activity towards the treatment of breast cancer. Today, furospongolide is recognised as a small molecule inhibitor of hypoxia-induced HIF-1 activation, which has recently become an attractive target for advanced stage cancer therapy. In this chapter, we will discuss the role of hypoxia in cancer, and the increasing interest in HIF-1 inhibition as a target for chemotherapeutics. In addition, we will examine recent advances in the discovery and development of small molecule inhibitors that target the HIF-1 pathways as potential antitumour agents.

As furospongolide is a member of a large family of terpene-derived furanosesterterpenoids, we will be reviewing the literature describing efforts undertaken towards the synthesis of structurally related sesterterpenoids, focusing on completed total syntheses of biologically active marine natural products. This review will provide us with innovative methodology to achieve the concise total synthesis of furospongolide as well as promising concepts to alter its structure and increase its potency as an antitumour agent.

We will begin this chapter by discussing the history of furan, its physical and chemical properties; traditional and modern methods towards the synthesis of this heterocycle as well as describing some diverse biologically active natural products containing furan(s).
1.1.2 History of Furan

The earliest furan compound discovered is pyromucic acid 1, more commonly known as furoic or furan-2-carboxylic acid (Figure 1.1). Pyromucic acid 1 was first isolated by Carl Wilhelm Scheele in 1780 by the dry distillation of mucic acid. In 1832 furfural 2, or furan-2-carbaldehyde, was obtained by the action of sulfuric acid and manganese dioxide on sugar, but these furans were not related until 1860, when furfural 2 was oxidized to furoic acid 1 with silver oxide (Scheme 1.1). 1, 2 Furan 3 itself was first prepared by Heinrich Limpricht in 1870 by treating barium furoate with soda lime. 3 In 1922 furfural 2 became commercially available, economically and in large-scale quantities from Quaker Oats Company from the acid hydrolysis of cereal waste. At this time the outlets for furfural 2 was limited except for its occasional use in perfume, but its low cost and availability greatly stimulated the search for uses. Today furfural 2 is still produced from agricultural by-products like sugarcane bagasse and corncobs and is the usual starting material for commercial preparation of other simple furans. This industrially used method of preparation of furans is rare as it is based on a renewable starting material rather than on oil, gas, or coal. 4-6

![Figure 1.1](image)

The positions in the furan ring are usually numbered, but in older literature a less convenient lettering system was sometimes used as illustrated in Figure 1.1. The compounds commonly known as furoic acid 1 and furfural 2 could therefore be named furan-2-carboxylic acid and furan-2-carbaldehyde respectively. 1
1.1.3 Physical properties of furan

Furan 3 itself is a colourless, volatile and highly flammable oil (b.p. 31-36 °C), which is slightly soluble in water and miscible with most organic solvents. The arrangement of the atoms in the ring was proved by Baeyer in 1877 following the conversion of furfural 2 into furan 3 as exemplified in Scheme 1.1.¹

\[ \text{Furan} \]

Scheme 1.1

Furan cannot be accurately represented by classical structure 3, as it is best considered as a resonance hybrid of the formulae 3a-3d. The furan molecule is far from a regular pentagon in shape and the bond lengths suggest that structure 3 is the major contributor to the resonance hybrid (Figure 1.2).¹
1.1.4 Chemical properties of furan

Furan 3 and thiophene 4 can usefully be examined in parallel, comparing one with the other and with pyrrole.7 Furan 3 is an electron-rich heterocycle and is generally less reactive than pyrrole towards electrophiles (by a factor of about $10^5$) although it is still much more reactive than benzene. Although furan 3 clearly exhibits aromatic stabilisation, its resonance energy (~16 kcal/mol) is significantly less than that of benzene (~36 kcal/mol), and thus it can be converted to non-aromatic derivatives under fairly mild conditions (most like a 1,3-diene). In the trio of heteroaromatic systems, furan 3 is much less aromatic than pyrrole (~22 kcal/mol) and thiophene 4 (~30 kcal/mol) is the most synthetically flexible of the aromatic heterocycles. For example, thiophene 4 undergoes normal nitration, with selectivity for α-substitution (5) rather than β-substitution (6) similar to pyrrole. In contrast, furan 3 tends to produce 2,5-dihydrofuran adducts (stable isolatable intermediate) in which the elements of the attacking agent have added to the heterocycle with subsequent loss of aromaticity. The aromatic substitution product 7 can be easily obtained from the adduct by base-induced loss of acetic acid as shown in Scheme 1.2.1,6,7 Nitration of furan illustrates how both addition-elimination and normal substitution mechanisms can operate together.

\[
\begin{align*}
\text{Furan} & \quad \text{HNO}_3, \text{Ac}_2\text{O} \\
& \quad \text{Nitration of thiophene and furan using acetyl nitrate.}
\end{align*}
\]

One attribute that makes furan 3 such a useful building block is its ability to undergo a wide range of reactions and serve as a precursor to many important substructures (Scheme 1.3). Combined with its easy accessibility and its unique reactivity, furan 3 has achieved a prominent role in synthetic chemistry.
The ring of furan 3 is opened easily by acids and can be hydrolysed under acidic conditions to give the saturated dicarbonyl derivative 8. Furan 3 can be converted to alcohol 9 through a Vilsmeier-Haack reaction followed by subsequent reduction. Furan 3 is susceptible to reduction and oxidation reactions and can provide the unsaturated dialdehyde 12 by oxidative cleavage with \textit{meta}-chloroperbenzoic acid. The cycloaddition of furan 3 with singlet oxygen gives cyclic peroxides, which break down with loss of carbon dioxide revealing five-membered lactones (10), which are a very common occurrence in nature and are usually referred to as butenolides. Catalytic hydrogenation of furan leads to the formation of tetrahydrofuran derivatives (11), which are also common features in a vast array of natural products. Furan 3 reacts rapidly with bromine in dioxane at 0 °C to form 2-bromofuran 13. However, if a nucleophilic solvent like methanol was employed, furan can be oxidised to a stable adduct, 2,5-dihydro-2,5-dimethoxyfuran 14 resulting from nucleophilic displacement of the bromide. Furan 3 is the least aromatic 5-membered ring and therefore reacts readily
with electron-deficient dienophiles to afford oxabicyclo[2.2.1]heptane derivatives (19). Furan also participates in [4+3] cycloadditions with oxyallyl cations to give oxabicyclo[3.2.1]octane derivatives (18). In furan 3, the 2 and 5 positions are the sites of maximum π-electron density as a result of electron donation from the heteroatom and consequently they are the favoured positions for sulfonation (17), acetylation (15), metallation (20) and condensation reactions (9) (Scheme 1.3).

1.1.5 Synthesis of furans and thiophenes
Numerous routes have been developed for the ring synthesis of thiophenes and furans. With respect to the latter, two classical methods of furan ring synthesis are the Paal-Knorr synthesis and the Feist-Benary synthesis (Figure 1.3).

One of the most important methods in organic chemistry for the synthesis of furans, thiophenes and pyrroles from 1,4-dicarbonyl compounds through a dehydration reaction is known as a Paal-Knorr synthesis. Although the Paal-Knorr synthesis has seen widespread use, the mechanism wasn’t fully understood until it was elucidated by van Amarnath et al. in the 1995. The most likely sequence has intramolecular addition of enolic oxygen to the other carbonyl group requiring, loss of water to provide furan 24. 1,4-Diketones 23 can be obtained in several ways; the alkylation of a 1,3-keto-ester 22 with α-haloketone 21 is one such method as demonstrated below in Scheme 1.4.
Scheme 1.4: Paal Knorr synthesis.

The synthetic strategy for the preparation of the thiophene ring through Paal Knorr methodology first involves exposure of an appropriate 1,4-dicarbonyl precursor to conditions that convert a carbonyl group into a thiocarbonyl group (25-26). The reagent of choice for this transformation is Lawesson’s reagent as it is more soluble in organic solvents than traditional thionation reagents like phosphorous pentasulfide. To date there is still confusion whether both carbonyl groups are converted, but exactly comparable sequences lead to the aromatic thiophene 27, with loss of H₂S (if both have been thionated) or H₂O (if one has been thionated) (Scheme 1.5).
Conversely, the *Feist-Benary synthesis* occurs when an $\alpha$-halocarbonyl reacts with a $\beta$-dicarbonyl in the presence of base. The resulting product is a 3-furoate that incorporates substituents present in both starting materials (*Scheme 1.6*).\textsuperscript{5,6,9,10}

\begin{center}
\includegraphics[width=0.5\textwidth]{scheme16}
\end{center}

*Scheme 1.6: Feist-Benary synthesis.*

A common use of the *Feist-Benary furan synthesis* is for the preparation of 2-substituted-3-furoates. The most popular synthetic target is the furan originally prepared by Benary et al., namely ethyl 2-methyl-3-furoate ($R^1$ and $R^3 = H; R^2 = Me; R^4 = Et$, *Scheme 1.6*).\textsuperscript{11} Although the *Paal-Knorr* synthesis and the *Feist-Benary* have proven very useful for the synthesis of furan derivatives, there are some limitations, including the difficulty in accessing furans that contain sensitive functional groups (unstable to basic conditions) and the inability to provide furans with high flexibility regarding their substitution pattern. For this reason, the development of innovative and more efficient methods for the synthesis of highly functionalized furans under milder conditions remains an area of on-going interest.

Functionalised furans are frequent subunits in a variety of biological active molecules and are useful intermediates in synthetic chemistry.\textsuperscript{12-14} A well-known approach for the synthesis of functionalised furans is transition metal-catalysed cyclisation of alkynyl,\textsuperscript{15,16} alkenyl,\textsuperscript{17} or allenyl ketones,\textsuperscript{18} alcohols or epoxides.\textsuperscript{19-21}

With respect to alkynyl ketones (28), Gevorgyan *et al.* investigated their utility as readily available starting materials for the synthesis of 2,5-disubstituted furans (29).\textsuperscript{22} The Cu(I)-catalysed and base-assisted cycloisomerisation is believed to proceed via an intermediary allenyl isomer. This method yields substituted furans possessing different functional groups, such as alkenes, ethers, acetals, esters and alcohols (*Scheme 1.7*).
Scheme 1.7: Cu(I)-catalysed synthesis of 2,5-disubstituted furans.

An extension of this methodology was further achieved by Gevorgyan et al. for the preparation of trisubstituted furans 32 and 33 by employing 4-thio and 4-acyloxybut-2-yrones 30 and 31 in an innovative, Cu(I)-catalysed, synthetic route as illustrated in Scheme 1.8. 23, 24

Scheme 1.8: Synthesis of 3-thio- and 3-acyloxy-substituted furans. 1,2 migration has been proposed as the key step in the copper-catalysed propargyl-propenyl isomerisation in the synthesis of trisubstituted furans. 25

Since olefins are more readily available than are alkynes and allenes, Widenhoefer et al. exploited alkenyl substrates as suitable starting materials for the transition metal catalysed synthesis of furan via heterocyclization. 26 In this scenario, α-alkenyl-β-diketone 34 underwent furan formation using a Pd(II) source and CuCl2 as an oxidant to afford the trisubstituted furan 35 (Scheme 1.9).
Scheme 1.9: Palladium-catalysed oxidative alkoxylation of an α-alkenyl-β-diketone to form a functionalised trisubstituted furan.\textsuperscript{25}

Synthesis of simple 3-substituted furans can be achieved via reductive annulation of 1,1,1-trichloroethyl propargyl ethers employing catalytic Cr(II)Cl\textsubscript{2} regenerated by Mn/TMSCl (Scheme 1.10).\textsuperscript{27} This reaction is synthetically useful in the synthesis of 3-substituted natural products.

Scheme 1.10: Reductive annulation using catalytic Cr(II) regenerated from Mn/TMSCl

The field of furan synthesis has attracted significant interest in the last century with the emergence of many natural and pharmaceutically important furan-containing substances.\textsuperscript{28} The literature is saturated with concise methodology for accessing mono-, di-,tri-, or tetra-substituted furans and will continue to rapidly advance with the discovery of more complex and challenging furan containing natural products. An extensive review by Kirsch \textit{et al.} on recent developments in the synthesis of polysubstituted furans in available in the literature.\textsuperscript{25}
1.1.6 Natural products containing the furan ring

The furan ring system is found in many naturally occurring compounds, either as a fully unsaturated structure or in a reduced or partly reduced form. The majority of the naturally occurring compounds containing a fully unsaturated furan ring are terpenoid in character; like the di-substituted rosefuran 37 (rose oil) and mono-substituted perillene 36 (secondary plant metabolite). However, simple furan-containing natural products like furfuryl thiol 41 (aroma in roasted coffee) do exist. Furans that occur in nature in a reduced or otherwise modified form include pentose sugars such as ribose 38 and deoxyribose 39, which are components of nucleic acids and several types of unsaturated γ-lactone like ascorbic acid 40 (vitamin C) as seen in Figure 1.4.6,29

![Figure 1.4](image)

The furan ring is also present in a huge variety of natural products, such as polyketides, phenylpropanoids, alkaloids and terpenes. In general, the natural compounds containing the furan rings and its derivatives (di, tetrahydro, γ-lactone) have been found in all classes of terrestrial (fungi, bacteria, insects, plants) and marine (bacteria, fungi, algae, mollusc, sponge, seaweed) organisms.30

The different structural types of natural products with furan rings cover the acetogenins (asimicin),31 morphinananes (morphine), cembranolides (deoxypukalide),32 manzamine-related alkaloid (nakadomarin),33 macrolide antibiotics, polycyclic ethers, lignans (podophyllotoxin), ginkgolides (ginkgolide B),34,35 quassinoids, limonoids, furanflavonides, furanoquinones, steroidal glycosides (cephalostatins,36 ritterazines37), macrodiolides (pamamycin),38 among others.30 Table 1.1 shows representative examples of the families of natural products containing furans.

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<tr>
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Table 1.1: Illustrates examples of the families of natural products containing furans*

<table>
<thead>
<tr>
<th>Product</th>
<th>Natural source</th>
<th>Biological activity</th>
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<tr>
<td>Asiminocin 42</td>
<td>Asimina triloba</td>
<td>Antitumour, pesticidal, antimalarial³⁹</td>
</tr>
<tr>
<td>Cantharidin 43</td>
<td>Mylabris phalerata</td>
<td>Hepatocarcinoma⁴⁰</td>
</tr>
<tr>
<td>Pamamycin 44</td>
<td>Streptomyces alboniger</td>
<td>Antibiotic⁴¹</td>
</tr>
<tr>
<td>Ginkgolide B 45</td>
<td>Ginkgo biloba</td>
<td>Dementia and age related cognitive decline³⁵,⁴²</td>
</tr>
<tr>
<td>Morphine 46</td>
<td>Daphniphyllum calycinum</td>
<td>Analgesic⁴³</td>
</tr>
<tr>
<td>Pallescensin A 47</td>
<td>Disidea pallescens</td>
<td>Defence⁴⁴</td>
</tr>
<tr>
<td>Podophyllotoxin 48</td>
<td>Podophyllum</td>
<td>Cytotoxic, antiviral⁴⁵</td>
</tr>
<tr>
<td>Salvinorin A 49</td>
<td>Salvia divinorum</td>
<td>Psychoactive⁴⁶</td>
</tr>
</tbody>
</table>

* Data sourced from ‘Heterocycles in Natural Product Synthesis’ by Boto et al.³⁰
1.1.7 Isolation of furospongolide

1.1.7.1 Introduction to furan derivatives isolated

The transcription factor hypoxia-inducible factor-1 (HIF-1) has stimulated significant interest as a novel molecular target for anticancer drug discovery. Following its discovery by Goldberg and Semenza et al. in the early 90’s, numerous clinical trials strongly support HIF-1 as a valid molecular target for drug discovery in the treatment of tumour hypoxia. Hypoxia influences many aspects of the biology of tumours and is directly associated with resistance to therapy, tumour progression, and patient mortality. Inhibition of HIF-1 activation has been shown to suppress the growth and spread of hypoxic tumours. To the best of our knowledge, despite extensive drug discovery research, there is no approved drug that directly targets tumour hypoxia. The majority of small molecule HIF-1 inhibitors discovered and developed in recent times are indirect inhibitors of HIF-1 activation.

Marine natural products have recently become an extraordinary resource for the discovery of new anticancer agents that effectively suppress numerous antitumour molecular targets. Several research programs have begun to examine their potential as HIF-1 activation inhibitors. Thus far, cell-based and in-vitro high-throughput screening has been effective in identifying new compounds that inhibit HIF-1. At the forefront of these investigations has been Dale. G. Nagle. His research group extensively explored the biomedical potential of marine natural products as new sources of drug leads for the treatment of cancer, whereby the group developed new molecular-based bioassays to investigate natural products for their potential to supplement existing chemotherapeutic agents.

Using a human breast carcinoma T47D cell-based reporter assay, Nagle et al. evaluated the HIF-1 inhibitory activity of over 15,000 natural product-rich extracts from various marine organisms and plants obtained from the NCI Open Repository. This screening effort has yielded an array of structurally diverse natural product-derived HIF-1 inhibitors such as manassantin B, 7-hydroxyneolamellarin A, laurenditerpenol, and tetrahydroisoquinoline alkaloids klugine and emetine (Figure 1.5).
As part of this molecular-targeted antitumour drug discovery program, an active extract of the tropical marine sponge *Lendenfeldia* sp. (5 µg mL\(^{-1}\)) collected from shallow water in Saipan was found to inhibit hypoxia (1% O\(_2\))-induced HIF-1 activation by 91%. Bioassay-guided chromatographic separation of this active extract from the NCI Open Repository of marine invertebrate extracts yielded the terpene-derived furanolipid furospongolide 55, one novel scalarane sesterterpene 56 and two previously reported scalaranes 57 and 58 as illustrated in Figure 1.6.\(^{63-65}\)
A photo of Lendenfeldia sp. which has been taken from the Wild Singapore website.\textsuperscript{66}

\begin{center}
\includegraphics[width=\textwidth]{figure1.jpg}
\end{center}

\textit{Figure 1.6: Illustration of the four components 55-58 isolated from an active extract of the marine sponge Lendenfeldia sp.}

The first reported isolation of furospongolide 55 was by Kashman \textit{et al.} in 1980 from the marine sponge \textit{Dysidea herbacea} collected in the Gulf of Suez (Red Sea).\textsuperscript{64} Following spectroscopic analysis of the C\textsubscript{21} metabolite 55, data was consistent with the presence of a $\alpha,\beta$-unsaturated-$\gamma$-lactone and a furan ring joined at the terminal ends of a linear carbon skeleton. The close relationship between 55 and the furospongins family led to the name, furospongolide 55.\textsuperscript{64,67}
1.1.7.2 Biological evaluation of furospongolide

The compounds 55-58 isolated from the active marine extract were tested independently in a concentration-response study to determine their effect on HIF-1 activation in T47D and PC-3 (human prostate tumour) cell-based reporter assay (Figure 1.6).\textsuperscript{52} Among the four Lendenfeldia sp. metabolites isolated, furospongolide 55 was the only relatively non-cytotoxic inhibitor of hypoxia (1% O\textsubscript{2})-induced HIF-1 activation with an IC\textsubscript{50} value of 2.9 µM in the T47D breast tumour cell line (Figure 1.7). Disappointingly, the three scalaranes 56-58 were found to be cytotoxic, showing only a narrow therapeutic window between the HIF-1 inhibitory activity and the suppression of cell proliferation/viability in T47D cells.

**Figure 1.7:** Concentration response effect of furospongolide 55 on HIF-1 activity in T47D cell reporter assays as reported by Liu et al.\textsuperscript{52} “pHRE_Hyp”: cells were transfected with the pHRE-TK-Luc reporter; “pGL3Control_Hyp”: cells transfected with the control plasmid pGL3-Control; “pHRE_1,10-phen”: Cells treated with a hypoxia mimetic, 1,10-phenanthroline (10 µM).

Utilising cutting edge molecular mechanism-targeted bioassay techniques, furospongolide 55 was found to suppress HIF-1 activation by inhibiting the hypoxia induction of HIF-1α protein. Furospongolide 55 was shown to block the hypoxia-induced production of the downstream HIF-1 target secreted Vascular Endothelial Growth Factor (VEGF) in a concentration dependant manner as illustrated in Figure 1.8. Surprisingly, furospongolide 55 only weakly inhibited the induction of HIF-1α protein by 1,10-phenanthroline (chemical hypoxia). Thus, 55 selectively inhibits hypoxic activation of HIF-1 by blocking the induction of HIF-1α protein.
Figure 1.8: Furospongolide 55 inhibited hypoxia induction of HIF-1 target VEGF protein (A) and blocks the induction of nuclear HIF-1α protein accumulation (B) as reported by Liu et al.52

The generation of mitochondrial reactive oxygen species (ROS) at complex III in hypoxic cells is generally believed to play a vital role in HIF-1 regulation. Mitochondria electron transport chain (ETC) inhibitors constitute one group of recently recognised small-molecule HIF-1 inhibitors.68,69 Inhibitors of the mitochondrial ETC could block the production of ROS-mediated signalling processes that stabilize HIF-1α protein under hypoxic conditions. Liu et al. used a Clark-type electrode system to measure mitochondrial respiration in the same T47D cells that were used to examine HIF-1 activation. Furospongolide 55 inhibited cellular oxygen consumption at concentrations as low as 10µM (38% inhibition) in a concentration dependant manner as illustrated in Figure 1.9.52 Additional mechanistic studies indicated that furospongolide 55 inhibits hypoxia-induced HIF-1 activity by blocking NADH-ubiquinone oxidoreductase (complex 1)-mediated mitochondrial electron transfer, thereby suppressing tumour cell respiration and hypoxic ROS generation.52
Figure 1.9: Furospongolide 55 inhibited oxygen consumption in T47D cells by disrupting the mitochondrial electron chain at complex I as reported by Liu et al.\textsuperscript{52}

Interestingly in 2013, Sagar \textit{et al.} made a hypothesis linking furospongolide 55 to angiogenesis via the VEGF. Inhibition of VEGF has been shown in studies to block angiogenesis in tumour cells.\textsuperscript{70} Thus it was proposed by Sagar \textit{et al.} that furospongolide 55 could potentially be used as a drug to block angiogenesis in solid tumours (\textit{Figure 1.10}).

\begin{center}
\includegraphics[width=\textwidth]{figure1_10}
\end{center}

\textit{Figure 1.10: A basic summary of the possible mechanism of action of furospongolide 55 in a tumour cell as depicted by Sagar \textit{et al.}}\textsuperscript{70}
While furospongolide 55 represents only a moderate potency inhibitor (IC$_{50}$ 2.9 $\mu$M), it is the first marine-derived furanolipid found to inhibit hypoxia-induced HIF-1 activation. The molecular target of 55 is linked with possible mitochondrial-associated toxicity and together with its arresting bioactivity and unusual natural structure, makes this furanolipid an attractive target for total synthesis, structural modification and possible pharmaceutical optimisation in the development of a potent anticancer agent.$^{52,56}$

### 1.1.8 Biological Background

#### 1.1.8.1 Cancer

Cancer is a term used to describe diseases in which abnormal cells divide without control and are able to invade other tissues. There are more than 100 different types of cancer and they are classified by the organ or type of cell in which they start. All cancers begin in cells, the body's basic unit of life. When the genetic material (DNA) of a cell becomes damaged or changed, this results in mutations that affect normal cell growth and division. When this happens, cells do not die when they should, and new cells form when the body does not need them, effectively forming a mass of tissue called a tumour. Cells that invade nearby tissues and spread to other parts of the body are cancerous and the spread of cancer from one part of the body to another is called metastasis.

A report from the National Cancer Registry of Ireland (NCRI) and the Central Statistics Office (CSO) shows the incidence, survival and mortality rates in Ireland from all cancers for men and women in 2013, the most recent reporting period available.$^{71}$ According to the statistics, it is estimated that one in three people will develop cancer during their lifetime. Worryingly, an average of 30,000 new cases of cancer are diagnosed each year in Ireland and this number is expected to rise dramatically to over 40,000 per year by 2020. The five most common cancers in Ireland are non-melanoma skin cancer, prostate cancer, breast cancer, bowel cancer and lung cancer respectively. The average incidence of each of these cancers between 2009 and 2010 inclusive is illustrated below in Figure 1.11.
The most common type of cancer in Ireland is non-melanoma skin cancer across both genders. Excluding skin cancer however, breast cancer was the most common among women (2,767) while prostate cancer was the most common cancer among men (3,014) (Figure 1.11). With respect to cancer mortality, cancer accounts for 25% of the annual death toll making it the second most common cause of death in Ireland. According to the most recent figures, 8,316 people died from cancer in 2010. Worldwide this figure was as high as 7.6 million. Thankfully, with recent advances in medical research and the implementation of new treatment options for patients, cancer is viewed as a condition from which people survive. Statistically speaking, 42% of men and 50% of women diagnosed with cancer currently survive for five years and longer. Amazingly, it is estimated that 280,000 people, diagnosed between 1995-2009, have survived their cancer in Ireland. Cancer is still a major problem and it goes without saying that numerous laboratories around the world are investing huge time and energy into addressing this problem by continually developing new and more innovative treatment options for cancer patients.
1.1.8.2 Hypoxia

Hypoxia is a common feature of most tumours that profoundly affects the biological behaviour, response to therapy and prognosis of human cancers.\(^{72,73}\) The presence of hypoxia in solid tumours has been recognised for more than 50 years.\(^{72}\) Hypoxia occurs when cells are located too far from a functional blood vessel for adequate supply of oxygen due to rapid cancer cell proliferation and the formation of blood vessels that are structurally and functionally abnormal. In the most extreme cases, oxygen supply is below that required for survival, resulting in cell death and the establishment of a selection of cancer cells in which the apoptotic pathways are inactivated and anti-apoptotic pathways are activated.\(^{51,74,75}\) These hypoxic cells are generally more resistant to killing by radiation and chemotherapy, are more invasive and metastatic, resistant to apoptosis, and genetically unstable.\(^{72,73}\) One of the major advances in cancer research over the last two decades has been the discovery of Hypoxia Inducible Factors (HIF), a family of transcription factors crucially involved in the response of mammalian cells to oxygen deprivation.\(^{48}\) The discovery of HIF-1 as a crucial player of the response to hypoxia has changed the perspective on how to target hypoxia for the development of cancer therapeutics by turning a strength of cancer cells into their Achilles’ heel.\(^{72}\) Clinical studies in cancer patients indicate that the expression of HIF-1 is directly correlated with poor patient prognosis and activation of HIF-1 contributes to advanced disease stages (malignant behaviour) and therapeutic resistance. Conversely, inhibition of HIF-1 activation has been shown to suppress growth, survival and metastatic spread of hypoxic tumours.\(^{52,56}\)

1.1.8.3 Hypoxia Inducible Factor (HIF)

Since its discovery in 1992 by Semenza et al., HIF-1 has been the subject of thousands of published studies.\(^{50}\) It is widely accepted among experts that the Hypoxia Inducible Factor is the primary transcription factor activated by hypoxia and is responsible for orchestrating a number of cellular responses such as angiogenesis and glycolysis that are important to tumour cell survival under hypoxic conditions. Hypoxia-inducible factor-1 is a heterodimeric transcriptional factor consisting of a HIF-1α (120-kDa) and a HIF-1β subunit (80-kDa).\(^{53,76}\) HIF-1 is a member of the rapidly growing Per-ARNT-Sim (PAS) family of basic helix-loop-helix (bHLH) transcription factors which is activated during dimerisation of HIF-1α and HIF-
HIF-1α is a protein that in humans is encoded by the HIF-1α gene. HIF-1α plays a major role in activating gene transcription, which is important for maintaining homeostasis under hypoxic conditions and is an obvious target for development of novel cancer therapeutics.

The level of HIF-1α protein is directly regulated by intracellular oxygen concentration. In the presence of oxygen (normoxic conditions) HIF-1α protein is rapidly degraded, while it is stabilized in the absence of oxygen (hypoxic conditions). HIF-1β protein on the other hand is always present (constitutively expressed). Upon hypoxic induction and activation, HIF-1α protein levels are dramatically increased and undergo heterodimerisation with HIF-1β protein. This activates HIF-1, which subsequently binds to the hypoxia response element (HRE) present in the promoters of target genes, which ultimately control angiogenesis, anaerobic metabolism, cell survival and metastasis as well as other cellular functions.

More than seventy target genes that are activated by HIF-1 have been identified. These genes are involved in many aspects of cancer progression, angiogenesis, cell survival, glucose metabolism and invasion. These include genes encoding for vascular endothelial growth factor (VEGF), erythropoietin (EPO) and many numerous enzymes involved in glucose, iron and nucleotide metabolism. Hypoxia-inducible factor 1 (HIF-1) is therefore a master regulator of this adaptive response to hypoxia. Given the central role that HIF-1-driven transcription factor activity has in compensating for loss of oxygen, it is clear that
modulation of that activity could be a potent mechanism for treating a wide range of hypoxia-related pathologies. A simplified representation of the processes influenced by HIF-1 was provided by Quintero et al. and is exemplified in Figure 1.13.

**Figure 1.13**

With respect to HIF-α, three homologs have been identified: HIF-1α, HIF-2α and HIF-3α. HIF-2α is closely related to HIF-1α and both are able to interact with hypoxia response elements, to up-regulate transcriptional activity. By contrast, HIF-3α is involved in down-regulation of the hypoxic response via an alternatively spliced transcription factor, which may function as an inhibitor of HIF-1α adding to the complexity in the regulation of hypoxia-inducible genes by the HIF family of transcription factors. HIF-1α has two transactivation domains located in its COOH-terminal half, which are termed the NH2-terminal transactivation domain or N-TAD (amino acids 531-575) and the COOH-terminal transactivation domain or C-TAD (amino acids 786-826) as illustrated in Figure 1.14.
The C-TAD of HIF-1α plays a key role in modulating the transcriptional activation of HIF-1α under hypoxic conditions. On the other hand, N-TAD is involved in the stabilisation of HIF-1α under anoxic conditions. Under hypoxia, the C-TAD is able to interact with transcriptional co-activators like p300/CBP at N803. However under normoxic conditions, hydroxylation of N803 is mediated by an asparaginyl hydroxylase, known as factor inhibiting HIF-1 (FIH-1), which prevents HIF-1α from interacting with the transcriptional co-activator p300/CBP (Figure 1.14).[^84]

The von Hippel-Lindau protein (pVHL) is involved in the regulation of HIF-1α. Under normoxic conditions, oxygen-dependant hydroxylation of proline residues Pro^{402} and Pro^{564} in HIF-1α by three prolyl-4-hydroxylase domain containing enzymes (PHD1-3) is required for binding of the von Hippel-Lindau tumour-suppressor protein, which is the recognition component of an E3 ubiquitin-protein ligase. VHL binding is also promoted by acetylation of K532 residue by the ARD1 acetyltransferase. This prolyl-hydroxylation ‘tags’ HIF-1α protein for polyubquitination mediated by the pVHL E3 ubiquitin ligase complex, followed by rapid degradation through a 26S proteasome-dependent mechanism (Figure 1.14).[^85]
Under hypoxic conditions, the rate of N803 and K532 hydroxylation decreases dramatically and VHL protein can no longer bind to HIF-1α that is not prolyl-hydroxylated, resulting in a decreased rate of HIF-1 degradation. As previously stated, p300/CBP can now bind to HIF-1α that is not asparaginyl-hydroxylated, resulting in transcriptional activation of HIF-1 target genes (Figure 1.14).^53,83^

**1.1.8.4 Hypoxia-induced mitochondrial reactive oxygen species**

Natural product-based small molecule inhibitors of the mitochondrial electron transport chain (ETC) have been found to inhibit hypoxia-induced HIF-1 activation.\(^{68,69,86-88}\) The mechanism is not fully understood, but several opposing theories have emerged to explain the role of mitochondria in the regulation of HIF-1 activation.\(^{89,90}\) Under hypoxic conditions, reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide are produced by the Qp site of mitochondrial complex III (Figure 1.15). These hypoxia-induced ROS are believed to function as signalling molecules that oxidise the catalytic Fe(II) in Fe(II)-dependent HIF-prolyl hydroxylase that is essential in the initial steps of ubiquitin-mediated proteasomal degradation of HIF-1α protein.\(^{68,86,87,91,92}\) Furthermore, mitochondrial ROS have also been linked to the inhibition of the Fe(II)-dependent asparaginyl hydroxylase [factor inhibiting HIF, (FIH)] which interferes with HIF-1 transcriptional activation by hydroxylating the N803 asparagine in the C-TAD of HIF-1α (Figure 1.14).
Figure 1.15: The mitochondria of hypoxic cells release superoxide from the Qp site of mitochondria complex III. This prevents the transfer of electrons required to drive the hypoxia-induced production of reactive oxygen species at complex III. The marine natural product furospongolide 55 inhibits the mitochondrial electron transport chain at the NADH-ubiquinone oxidoreductase (complex I) site.\textsuperscript{56}

As previously stated in Section 1.1.7.2, it has been postulated that the mechanism through which furospongolide 55 inhibits hypoxia-induced HIF-1 activity is linked to inhibition of the mitochondrial ETC by suppressing the production of ROS signalling pathways in hypoxic tumours. This stabilises HIF-1α protein by preventing proteasomal degradation under hypoxic conditions and activates HIF-1 by interfering with the ability of asparaginyl hydroxylase to suppress HIF-1 activation.
1.1.8.5 Small molecule HIF-1 inhibitors and chemoprevention

In brief, the progression of cancer is correlated with the development of hypoxic regions within solid tumours. HIF-1 is a transcriptional factor activated by hypoxia, which orchestrates the expressions of specific genes associated with conferring radio- and chemo resistance while simultaneously inducing angiogenesis, enhancing tumour development and promoting metastasis. As a result, HIF-1 has emerged as a key molecular target for anticancer drug discovery.\(^5\) Over the last decade intense efforts has been made to investigate natural products that can be potentially used as HIF-1 inhibitors. In general, natural products have been a major source of new drugs for centuries and statistics show that nearly 50% of approved anticancer agents are derived directly or indirectly from natural products.\(^9\) The importance of natural products in drug discovery has been discussed in several reviews and reports.\(^94-97\)

As our knowledge of the tumour cell continues to expand, stronger evidence suggests that inhibition of the HIF-1 pathway represents an attractive approach to cancer therapy. Recently, numerous laboratories have joined in the race to discover small molecule HIF-1 inhibitors,\(^72,98,99\) most of which use a synthetic or commercial compound library-based screening approach. Small molecules aim to suppress tumour hypoxia and to increase the susceptibility of tumour cells to radiotherapy and chemotherapy thereby improving patient outcome. They also serve as important molecular probes to investigate the pathways that regulate HIF-1 activity. Xia et al. disclosed a review on recent advances in discovery and development of small molecule HIF-1 inhibitors.\(^5\) Representative HIF-1 inhibitors discovered from compound library screening efforts include topotecan,\(^10\) echinomycin,\(^11\) chetomin,\(^10\) a benzopyran derivative 103D5R, analogues of emetine and actinomycin D,\(^10\) a pyrroloquinoline derivative DJ12,\(^10\) and a group of structurally diverse compounds including alkyliminophenylacetates that affect mitochondrial function.\(^10\) For many of these, the mechanism of action has been established and involves a reduction of HIF-1α mRNA or protein levels, HIF-1 DNA-binding activity, or HIF-1 mediated transactivation of target genes (Table 1.2).\(^74\)
Table 1.2: Anticancer agents that inhibit HIF-1 activity*

<table>
<thead>
<tr>
<th>Mechanism of action</th>
<th>Drug molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease HIF-1α mRNA levels</td>
<td>GL331\textsuperscript{106}</td>
</tr>
<tr>
<td>Decrease HIF-1α protein levels</td>
<td>Ibuprofen, Celecoxib\textsuperscript{107}</td>
</tr>
<tr>
<td>Topoisomerases</td>
<td>Topotecan\textsuperscript{100}</td>
</tr>
<tr>
<td>Cyclin-dependent kinases</td>
<td>Flavopiridol\textsuperscript{108}</td>
</tr>
<tr>
<td>Microtubule targeting agents</td>
<td>2-Methoxyestradiol\textsuperscript{109}</td>
</tr>
<tr>
<td>Decreased binding of HIF-1 to DNA</td>
<td>Echinomycin,\textsuperscript{101} DJ12\textsuperscript{104}</td>
</tr>
<tr>
<td>Decreased HIF-1 mediated transactivation</td>
<td>Bortezomib,\textsuperscript{110} Chetomin\textsuperscript{102}</td>
</tr>
<tr>
<td>Inhibits mitochondrial ETC</td>
<td>Laurenditerpenol,\textsuperscript{60} Furospongolide\textsuperscript{52}</td>
</tr>
<tr>
<td>Unknown</td>
<td>Manassantins,\textsuperscript{57} 103D5R\textsuperscript{111}</td>
</tr>
</tbody>
</table>

* Information was sourced from a review by Semenza et al.\textsuperscript{74}*

Unfortunately, many of the identified compounds have poor water solubility and are very toxic and therefore cannot be used in human therapy. Using a natural product chemistry-based approach, several groups have discovered chemically and mechanistically diverse HIF-1 inhibitors. Some of these HIF-1 inhibitors function at low nanomolar concentrations (e.g. manassantins) with a wide therapeutic window between their HIF-1 inhibitory activity and cytotoxicity.\textsuperscript{56,57} A number of fantastic reviews have been published discussing natural compounds with HIF-1 inhibitory activity that have been discovered to date.\textsuperscript{112-114}
1.1.8.6 HIF-1 inhibitors from marine life

The potential of marine life as a source of novel molecules for the treatment of human diseases is extraordinary. Recent technological and methodological advances in structure elucidation, organic synthesis and biological assay has resulted in the isolation and clinical evaluation of various novel anticancer agents from marine organisms.\textsuperscript{115,116} Natural products, especially those from terrestrial plants and microbes, have long been a traditional source of drug molecules (morphine and penicillin). Modern pharmaceutical discovery programmes are indebted to natural products as active compounds from plants and microbes represent an invaluable pipeline for new investigational drugs.\textsuperscript{117,118} On the other hand, marine organisms possess a greater molecular diversity than their terrestrial counterparts due to their longer evolutionary history. Recent research has discovered that marine life produce more antibiotic, anticancer and anti-inflammatory substances than any group of organisms on land.\textsuperscript{119} The major problem with developing drugs from a marine source is that procurement or manufacture of quantities of rare compounds to ensure a sustainable supply to industry was essentially a bottleneck. Sponges and their microbial fauna are largely unculturable, and the valuable compounds they produce must be extracted and purified from specimens collected by hand from shallow to deep waters. Nevertheless, the unrivalled potential of marine natural products as antitumour agents has inspired innovative solutions to the supply problem ranging from aquaculture to total synthesis.\textsuperscript{120}

The past two decades has seen a dramatic increase in the number of preclinical anticancer lead compounds from diverse marine life enter human clinical trials (Figure 1.16). Ziconotide \textsuperscript{59} (Prialt\textsuperscript{®}, Elan Pharmaceuticals), a peptide originally discovered in a tropical cone snail (\textit{Conus magus}) was the first marine-derived compound to be approved in the US in December 2004 for the treatment of pain (non opioid and non NSAID analgesic).\textsuperscript{121,122} In October 2007, trabectedin \textsuperscript{60} (Yondelis\textsuperscript{®}, PharmaMar) became the first marine anticancer drug to be approved in the EU.\textsuperscript{123} Currently, the antitumour agent plitidepsin \textsuperscript{61} (Aplidin\textsuperscript{®}, PharmaMar) is in phase III clinical trials for the treatment of multiple myeloma.\textsuperscript{124,125}
Numerous other marine natural products, primarily invertebrates (e.g., sponges, tunicates, bryozoans, and mollusks) have shown potent antimitotic and/or antitumour properties and have advanced to late-stage clinical trials. The literature has many comprehensive reviews discussing the importance of marine natural products in anticancer drug discovery. With respect to Aplidin®, links have been made which suggest it functions as an inhibitor of HIF-1 activation by suppressing the expression of HIF and angiogenesis-related HIF-1 target genes in vivo.

Several research programs have recently begun to examine marine natural products as a potential source of HIF-1 activation inhibitors. The first marine natural product found to inhibit hypoxia-induced HIF-1 activation was laurenditerpenol, a diterpene first isolated in 2004 from the marine red algae Laurencia intricate (Table 1.3, Entry 1). Interestingly,
Despite having little to no structural homology, both laurenditerpenol 51 and furospongolide 55 inhibit hypoxia-induced HIF-1 activation in breast cancer cells by inhibiting NADH-ubiquinone oxidoreductase-mediated mitochondrial signalling pathways.\textsuperscript{52,56,60} Bioassay-guided isolation has since yielded an array of HIF-1 inhibitors from sponges and other marine organisms. Two good examples include the macrolide macrolide latrunculin A 62, isolated from a Red Sea sponge \textit{Negombata magnifica},\textsuperscript{130} which is now commercially available from Sigma Aldrich,\textsuperscript{131} and the phenolic pyrrole alkaloid 7-hydroxyneolamellarin A 52 isolated from the sponge \textit{Dendrilla nigra} (\textit{Table 1.3, Entry 2 and 3}).\textsuperscript{58} Within our research group, studies are currently in progress towards the synthesis and biological evaluation of novel neolamellarin analogues as potential antitumour agents.\textsuperscript{132} To date, the most potent marine natural product inhibitor of hypoxia induced HIF-1 activation is mycothiazole 63, a polyketide isolated in 2002 from the marine sponge \textit{Cacospongia mycofifiensis} (\textit{Table 1.3, Entry 4}).\textsuperscript{133,134} As illustrated in \textit{Table 1.3}, these marine natural products vary widely in potency and selectivity to target HIF-1 and ultimately tumour hypoxia.
Table 1.3: Marine natural products found to inhibit hypoxia-induced HIF-1 activation in T47D human breast cancer cells*.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>Natural source</th>
<th>IC₅₀ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Laurenditerpenol <strong>51</strong></td>
<td><em>Laurencia intricata</em></td>
<td>0.4 µM</td>
</tr>
<tr>
<td>2</td>
<td>7-Hydroxyneolamellarin <strong>52</strong></td>
<td><em>Dendrilla nigra</em></td>
<td>1.9 µM</td>
</tr>
<tr>
<td>3</td>
<td>Latrunculin A <strong>62</strong></td>
<td><em>Negombata magnifica</em></td>
<td>6.7 µM</td>
</tr>
<tr>
<td>4</td>
<td>Mycothiazole <strong>63</strong></td>
<td><em>Cacospongia mycofijiensis</em></td>
<td>1 nM</td>
</tr>
</tbody>
</table>

* Data sourced from a review on marine natural products as HIF-1 inhibitors by Nagle et al.⁵⁶

One of the intended applications of natural product-based HIF-1 inhibitors is as adjunct agents to be used in combination with other cancer treatment options.⁵⁶ Preclinical studies strongly support this treatment regime of combining HIF-1 inhibition with radiation and chemotherapy to improve patient outcome.⁵⁶,¹³⁵,¹³⁶ As the exploration for HIF-1 targeted antitumour natural products continues to expose innovative lead compounds, a greater prospect of identifying a potent clinically useful inhibitor of HIF-1 activation is at hand.
1.1.9 Sesterterpenoids

1.1.9.1 Introduction to sesterterpenoids

Terpenes are an enormous class of natural products spanning well over 30,000 members. They have been used throughout history for a broad variety of purposes including perfumes, medicine and flavouring. Terpenoids are derived from C₅ isoprene units joined in a head to-tail fashion. Typical structures contain carbon skeletons represented by (C₅)ₙ, and are classified as hemiterpenes (C₅), monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes (C₂₀), sesterterpenes (C₂₅), triterpenes (C₃₀) and tetraterpenes (C₄₀) as illustrated in Scheme 1.11.
The two biosynthetic pathways leading to terpenoids are the mevalonate pathway and the more recently discovered mevalonate-independent deoxyxylulose pathway for the production of isopentyl diphosphate (IPP) and dimethyl allyl diphosphate (DMAPP), which are the biochemically active isoprene units (Scheme 1.12).  

**Scheme 1.12:** The chemistry of the mevalonate and deoxyxylulose pathway as by Dewick et al.  

---

**Mevalonate Pathway**

1. **Claisen condensation**
   - HMG-CoA
   - SCoA
   - acetoacetyl-CoA

2. **Stereospecific aldol reaction**
   - mevaldic acid
   - homithioacetals
   - NADPH

3. **NADPH**
   - mevaldic acid
   - hemithioacetals
   - 2 x ATP

4. **Isomerase**
   - isopentenyl PP (IPP)

5. **Deoxynucleotide Pathway**
   - Pinacol-type rearrangement
   - 1-deoxy-D-xylulose 5-P
   - (from pyruvic acid)

6. **HMG-CoA reductase**
   - HMG-CoA
   - NADPH

7. **2-phosphate-4-(CDP)-2-C-methyl-D-erythritol**

8. **Steps not fully determined**

9. **Isomerase**
   - IPP

10. **DMAPP**

---

**Deoxynucleotide Pathway**

1. **Claisen condensation**
   - HMG-CoA
   - SCoA
   - acetoacetyl-CoA

2. **Stereospecific aldol reaction**
   - mevaldic acid
   - homithioacetals
   - NADPH

3. **NADPH**
   - mevaldic acid
   - hemithioacetals
   - 2 x ATP

4. **Isomerase**
   - dimethyl allyl PP (DMAPP)
Within the terpenoid family, sesterterpenoids probably form the smallest class, comprising less than a thousand known compounds. They comprise of two and a half (sester in latin) terpene units, which for historic reasons were defined as pieces made of ten carbons. As such, sesterterpenoids contain 25 (or slightly fewer) carbon atoms. Their sources are widespread, having been isolated from terrestrial fungi, lichens, higher plants, insects and various marine organisms especially sponges.

Sesterterpenoids are a group of pentaprenyl substances formed from combination of geranylgeranyl diphosphate (GGPP) and IPP via the enzyme prenyl transferase, which yields geranylfarnesyl diphosphate (GFPP). This is believed to involve ionisation of GGPP to the allylic cation, addition to the double bond of IPP, followed by loss of a proton. This produces a sesterterpene diphosphate, geranylfarnesyl PP, in which the new double bond is trans (E) (Scheme 1.13).
1.1.9.2 Synthetic approaches towards sesterterpenoids

Terpenoids have played an important role in developing organic synthesis as far back as Komppa’s pioneering work on camphor in the early 1900’s. Many important concepts in organic chemistry such as Wagner-Meerwein rearrangements, Diels-Alder reactions, or polyolefin cyclization, were first explored with members of this large natural product class. With huge advancements in organic synthesis in recent years, complex terpenoids have been targeted and their total syntheses must now number in the hundreds.

Sesterterpenoids represent an attractive target for both biomedical and synthetic purposes, as this structurally complex terpenoid family exhibit diverse biological activity such as anti-inflammatory, cytotoxic, anticancer, antimicrobial, antitubercular, and anti-biofilm activities. They exhibit inhibitory activity against parasitic protozoa, suppression of the expression of cyclooxygenase-2 and inducible nitric oxide synthase, and act as inhibitors of hypoxia-inducible factor-1 (HIF-1). The natural products of the sesterterpenoid subclass possess a linear carbon chain, which in most cases has been partially oxidized leading to cyclization at the terminal ends of the terpenoid chain resulting in furans and/or lactones. Although numerous linear sesterterpenoids have been reported, very few have been targeted by synthetic chemists to date.

In this short section, I will be reviewing efforts undertaken towards the synthesis of linear and bicarbocyclic sesterterpenoids, focusing on completed total syntheses of marine natural products, which have shown promising biological activity towards the treatment of human related diseases.

A number of reviews have been published by Hanson et al. and Liu et al., which extensively cover the isolation, structure and biological evaluation of sesterterpenoid marine natural products from 1970 to 2013.
1.1.9.2.1 Synthesis of furospinosulin-1

Marine organisms, particularly sponges, have continued to provide a source of linear sesterterpenoids. The terminal units often comprise either of a furan, a \(\gamma\)-lactone or a tetronic acid moiety. Furospinosulin-1 69 was first isolated in 1972 from the marine sponge *Ircinia spinosula* by Cimino et al. and has very recently shown promising activity as a hypoxia-selective growth inhibitor following its isolation from an Indonesian marine sponge *Dactylospongia elegans*.\(^{158,159}\) Furospinosulin-1 69 showed selective antiproliferative activity against DU145 human prostate cancer cells under hypoxic conditions and exhibited antitumour activity at a level of 10-50 mg kg\(^{-1}\) after oral administration to a mouse model inoculated with sarcoma S180 cells. Mechanistic analysis revealed that 69 suppressed the transcription of the insulin-like growth factor-2 gene (IGF-2), which is selectively induced under hypoxic conditions through presentation of the binding of nuclear protein to the Sp1 consensus sequence in the IGF-2 promoter region.\(^{158}\) These intriguing bioactivities prompted Kotoku et al. to develop a concise total synthesis of furospinosulin-1 69 and further investigate its structural-activity relationship (*Scheme 1.14*).\(^{160}\)

![Scheme 1.14](image)

The first geometry-selective total synthesis of furospinosulin-1 69 is illustrated in *Scheme 1.14*. The route-defining step involved a sulfone-mediated cross coupling reaction between allylic bromide 67 and the known farnesyl phenylsulfone 68. The allylic bromide 67 was conveniently prepared from commercially available 3-bromofuran 64. The first step involved
reacting trimethyleneoxide with the derived organolithium species of 3-bromofuran 64 to afforded 3-substituted furan alcohol 65. Oxidation and subsequent Wittig olefination provided the enoate 66. Diisobutylaluminium hydride reduction of the ester moiety in 66 and subsequent bromination with CBr₄/PPh₃ under standard Appel conditions furnished the allylic bromide 67. The coupling reaction between the allylic bromide 67 and the sulfone 68 proceeded smoothly using potassium tert-butoxide. Subsequent reductive desulfonylation using Super-Hydride® lithium triethylborohydride in the presence of Pd(dppp)Cl₂ successfully furnished furospinosulin-1 69 in seven linear steps from 64. Since furospinosulin-1 69 is structurally similar to furospongolide 55, quite a number of synthetic transformations discussed here can be utilised in the synthesis of our target molecule 55 especially methodology for attaching the furan moiety to the lipophilic terpenoid sidechain.

Living organisms produce sesterterpenoids for certain physiological functions. Given the different selection pressures under which organisms have evolved over time, an enormous number of structural diversity is expected. With regard to furospinosulin-1 69, both the butenolide derivative 71 and the thiophene derivative 70 have been isolated from marine origin and have shown interesting bioactivity (Figure 1.17).¹⁶¹,¹⁶²

![Figure 1.17](image)

The butenolide 71 was first isolated from the Caribbean sponge, *Thorecta horridus* and has been found to possess marked inflammatory activity inducing the release of histamine and cause oedema in the paw of test animals.¹⁵⁷,¹⁶¹ On the other hand, the thiophene sesterterpene 70 has recently been isolated from an active extract from the Sikao Bay sponge *Xestospongia sp.*¹⁶² Surprisingly, this was the first reported discovery and isolation of a thiophene containing sesterterpene produced by a marine sponge. Worth mentioning, sulfur is often found in marine natural products in the form of the isothiocyanate, thiocyanate, thioacetate,
thiol, sulfone and sulphate functional groups, whereas the thiazole is a common natural heterocyclic moiety. Furthermore, sesterterpenoid 70, which is almost identical in structure to furospinosulin-1 69 was found to be cytotoxic against Vero cells. 

With regard to the length of the acyclic terpenoid chain, two structural derivatives of furospinosulin-1 69 have been isolated from the marine sponge *Ircinia spinosula*, difurospinosulin 75 and furospinosulin-3 73 (*Figure 1.18*). Interestingly, it has been suggested by Cimino et al. that difurospinosulin 75 could be derived from the C35 linear furanoterpene, furospinosulin-3 73 by the loss of four carbon atoms following biological inter-conversions involving enzyme catalysed oxidation, reduction and proton initiated cyclisation. Similarly, it has been proposed that anhydrofurospongin-1 74 can be derived from the C25 linear furanoterpene, furospinosulin-1 72 through an identical metabolic pathway. Further enzyme catalysed oxidation would potentially afford furospongolide 55.

![Figure 1.18](image)

When furospongolide 55 was isolated back in 1980 by Kashman et al. from the marine sponge *Dysidea herbacea*, furanoterpenes that terminated in a lactone ring were completely unknown in the C21-furanoterpene series but not unfamiliar in other marine metabolites. In the last 3 decades, very few novel sesterterpenoids encompassing both a furan and lactone ring have been isolated from marine life. One obvious example is the C22 lactone 77, which is associated with furospongolide 55 both in structure and origin as it was isolated from the same species of Madagascan sponges of the genus *Lendenfeldia* (*Figure 1.19*).
Dehydrofurodendin 77 contains a \( \beta,\gamma \)-unsaturated-\( \delta \)-lactone ring which is extremely rare among marine metabolites and in fact only exists in dehydrofurodendin 77, furodendin 76 and in another C_{21} terpenoid. The C_{22} lactone, furodendin 76, has been reported in the sponge *phyllosponngia dendyi*.\textsuperscript{165} It should be noted that dehydrofurodendin 77 differs from furodendin 76 only in the additional double bond at C_{14}-C_{15}, which probably results from oxidation occurring at a late stage in the biosynthesis. Interestingly, dehydrofurodendin 77 has been found to be a potent inhibitor of HIV-1 RT-associated DNA polymerase activity. It is important to highlight here how making simple changes to the structure of the molecule can affect its bioactivity and alter its therapeutic potential.
1.1.2.2 Synthesis of variabilin

As previously stated, the terminal units of linear sesterterpenoids often comprise of a furan, lactone or a tetronic acid moiety. These natural products presumably occur following partial oxidation of the linear chain followed by cyclisation. Quite a number of the sesterterpenoid family contain a tetronic acid moiety and show strong antibiotic activity against *staphylococcus aureus*.\(^{155}\) Common examples are variabilin \(78\) from *Ircinia variabilis*,\(^{166}\) its double bond isomer strobilinin \(79\) from *Ircinia strobilina*,\(^{167}\) and fasciculatin \(80\) from *Ircinia fasciculate* (Figure 1.20)\(^{168}\)

![Chemical structures of variabilin, strobilinin, and fasciculatin](image)

**Figure 1.20**

Variabilin \(78\) was first isolated by Flaukner *et al.* in 1973 from the marine sponge *Ircinia variabilis* (*I. variabilis*).\(^{169}\) A variety of geometric, stereo- and regioisomers of this natural product and other related types have since been isolated and have shown to possess remarkable antiviral and cytotoxic activity.\(^{170-173}\) Variabilin \(78\) is recognised as a novel RGD-containing antagonist of glycoprotein IIb-IIIa and a platelet aggregation inhibitor.\(^{174}\) It is also a dual inhibitor of human secretory and cytosolic phospholipase A\(_2\) (PLA\(_2\)) with anti-inflammatory activity.\(^{172}\)
More than 30 years after its isolation, the naturally occurring linear furanesterterpene tetronic acid, (18S)-variabilin 78 was first synthesised by Yoda et al.\textsuperscript{171}

Scheme 1.15

The synthetic route began with the lipase PS-catalysed asymmetric desymmetrization of the 1,3-propanediol 81 to furnish the mono acetate 82 in both high chemical and enantiomeric excesses (98% ee) respectively, to install the sole stereocenter (Scheme 1.15). The monoacetate 82 was converted into the silyl ether 83 through a three-step sequence and was subsequently coupled with the furanyl side chain 84 leading to the silyl sulfone 85 (Scheme 1.16).

Scheme 1.16: Total synthesis of variabilin 78 by Yoda et al.\textsuperscript{171}
Oddly, the synthesis of the furanyl side chain 84 was not fully disclosed in the report by Takabe et al. It is presumed that 84 was prepared by furanylation of allylic sulfone 89 with 3-furylmethyl bromide 90 through an sp³-sp³ cross coupling reaction to afford dendrolasin 91 followed sequentially by allylic oxidation and chlorination chemistry (Scheme 1.17).

Scheme 1.17: Preparation of the furanyl side chain 84.

Compound 85 was then subjected to desulfonylation and deprotection chemistry to furnish 86, the chiral segment of variabilin 78 (Scheme 1.16). In order to introduce the conjugated tetronic acid moiety, a coupling reaction of 86 after TPAP-induced oxidation to an aldehyde intermediate was effected with methyl tetronate 87 in the presence of LDA. Finally, dehydroxylation under basic conditions and demethylation of 88 completed the first asymmetric synthesis of variabilin 78 (Scheme 1.16).
1.1.9.2.3 Synthesis of palinurin

Similar to variabilin 78, the first reported isolation of (+)-palinurin 103 was from the Mediterranean marine sponge Ircinia variabilis.\textsuperscript{175} Palinurin 103 has emerged as a non-ATP competitive glycogen synthase kinase 3β (GSK-3β) inhibitor, a kinase implicated in Alzheimer’s disease.\textsuperscript{176-178} Recently, Gomez et al. accomplished the first enantioselective synthesis of (+)-palinurin 103 starting from commercially available furaldehyde 92 and (R)-Roche ester 99.\textsuperscript{179} The key step in the synthesis was a Horner-Wadsworth-Emmons reaction to construct the alkene unit by coupling the phosphine oxide 95 and the tetronic moiety 102 (Scheme 1.18).

Scheme 1.18: Total synthesis of palinurin 103 by Gomez et al.\textsuperscript{179}
Preparation of the phosphine oxide 95 involved 9 linear steps from furaldehyde 92 (Scheme 1.18). The 3-substituted furan alcohol 93 was prepared by a Wittig reaction of 92 followed sequentially by reduction and catalytic hydrogenation. The alcohol 92 was converted to its corresponding iodide and then nitrile before reacting it with methyllithium to afford a ketone substrate, which was subjected to a Horner-Wadsworth-Emmons reaction to obtain the α,β-unsaturated ester 94. Reduction of 94 with DIBAL-H gave the corresponding allylic alcohol, which was finally converted into phosphine oxide 95.

Preparation of the requisite tetronic moiety 102 began with reacting methyl tetronic acid 96 with pyrrolidine 97 under Dean-Stark conditions to afford the chiral enamino-furanone 98. Cross coupling of 98 with the allylic bromide 100 under the influence of the chiral tether provided the chiral furanone 101. Removal of the silyl protecting group using TBAF and the chiral auxiliary with aqueous hydrochloric acid gave the corresponding alcohol, which was subsequently reacted with methanol under Mitsunobu conditions followed by Swern oxidation to afford the target aldehyde 102.

With respect to the Wittig-Horner reaction, treatment of phosphine oxide 95 with n-butyllithium generated its corresponding anion, which was subsequently coupled with the aldehyde 102. Finally, demethylation concluded the first enantioselective total synthesis of palinurin 103 (Scheme 1.18).

### 1.1.9.2.4 Synthesis of (-)-ircinianin and (+)-wistarin

Two bicarboyclic sesterterpenoids marine natural products, (-)-ircinianin 104 and its cyclic isomer (+)-wistarin 105 have been the subject of numerous synthetic studies.180-182 (-)-Ircinianin 104 was isolated in 1977 by Hofheinz et al. from the marine sponge, genus *Ircinia*,183 while (+)-wistarin 105 was isolated from the marine sponge, *Ircinia wistarii*,184 by Gregson et al. in 1982. Despite the additional complexity in structure, both 104 and 105 are members of the same family of furanosesterterpenetonic acids as variabilin 78 and palinurin 103. As expected, much of the same methodology previously described for 78 and 103 was utilised in the synthesis of 104 and 105. The first total synthesis of ircinianin 104 was achieved in 1986 by Takeda et al. inspired by a biogenetic hypothesis proposed by Hofheinz et al. on the construction of the spirotetronic ring by intramolecular Diels-Alder
(IMDA) cyclisation. Uenishi et al. revised this route almost eleven years later, completing an enantioselective synthesis of (-)-ircinianin 104 in 1997 as illustrated in Scheme 1.19.

Scheme 1.19: Total synthesis of (-)-ircinianin 104 and (+)-wistarin 105 by Uenishi et al. 182

Biogenetically, it was assumed that (-)-ircinianin 104 was formed enzymatically or thermally by intramolecular Diels-Alder reaction from an acyclic tetronic acid precursor similar in structure to 108 in nature. The key step therefore in the synthesis of 104 involved a NiCl2-CrCl2 mediated coupling reaction of aldehyde 106 and conjugated triene 107. The synthesis of the chiral aldehyde 106 began from (R)-Roche ester 99 (Scheme 1.19) with preparation of the γ-methylene-butenoide substructure in a similar fashion to that shown previously for variabilin 78 (Scheme 1.16). Likewise, synthesis of the conjugate triene 107 was achieved using a similar pathway previously described for palinurin 103 (Scheme 1.18).
The Nozaki-Hiyama-Kishi (NHK) reaction of 106 and 107, followed by an intramolecular Diels-Alder reaction furnished the tricyclic adduct 110. Interestingly, Ueniski et al observed that isomer 108 obtained from the NHK reaction was spontaneously cyclised to 110, whereas the isomer 109 remained unreacted under the same conditions and could easily be isolated from the reaction mixture. Finally, Barton deoxygenation and demethylation led to (-)-ircinianin 104 (Scheme 1.19). Moreover, intramolecular iodo-etheration for the formation of a tetrahydropyran ring followed by radical-induced reductive deiodination accomplished the total synthesis of (+)-wistarin 105 (Scheme 1.19). It is worth noting that wistarin 105 was the first example of a sesterterpenoid that occurs naturally in both enantiomeric forms.185

1.1.9.2.5 Synthesis of ircinin-4

The 2,4-disubstituted furan motif is present in various physiologically active natural products such as 111-114 isolated from marine sources. Ircinin-4 111 is a member of a large family of furanoterpenes, some of which are illustrated in Figure 1.21 and deserve mentioning due to issues related to their biosynthesis,186 and more importantly, because of the interesting biological effects exerted by these compounds.187-189

Figure 1.21: 2,4-Disubstituted furanosesterterpenoid marine natural products.
The first total synthesis of ircinin-4 \textit{111} was achieved by Frustner \textit{et al.} in 1999,\textsuperscript{188} almost two decades after its isolation from the Mediterranean marine sponge \textit{Ircinia oros} by Cimino \textit{et al.}\textsuperscript{190} Synthesis of ircinin-4 \textit{111} began with the preparation of segment \textit{119}. Reaction of 3-furylacetaldehyde \textit{115} with the sulfur ylide of \textit{116} formed by deprotonation with \textit{tert}-butyllithium gave the epoxide \textit{117} (\textit{Scheme 1.20}). The functionalised sulfonium salt \textit{116} was a valuable building block frequently used within Furstner’s research group especially in the concise synthesis of the antitumour alkaloid roseophilin.\textsuperscript{191,192}

\textit{Scheme 1.20: The first total synthesis of the marine natural product ircinin-4 \textit{111}.}

Synthesis of the allylic alcohol \textit{118} was innovatively accomplished \textit{via} a palladium catalysed ring opening of the vinyloxirane \textit{117} employing Pd(PPh\textsubscript{3})\textsubscript{4}. The mechanism involved deprotonation of bis(phenylsulfonyl)methane by the alkoxide unit of the \textit{π}-allylpalladium complex resulting in regioselective attack at the electrophilic organopalladium species to furnish \textit{118}. Transformation of \textit{118} into the desired furanylmethylfuran \textit{119} was achieved by temporary protection of \textit{118} as a THP acetal followed by desilylation and selective oxidation. Subsequent treatment of the resulting aldehyde with aqueous hydrochloric acid cleanly furnished the furanylmethylfuran \textit{119} (\textit{Scheme 1.20}).
Segment 122 was prepared in 4 linear steps from commercially available citronellol 120 (Scheme 1.20). The two initial steps involved O-silylation and oxidation using ozone to furnish the aldehyde 121. A modified Wittig reaction delivered the \((Z)\)-configured allyl alcohol as a single isomer.\(^{193-195}\) The final step in the synthesis of the allylic bromide 122 involved a bromination reaction using NBS.

Reductive metalation of bis-sulfone 119 with lithium naphthalenide followed by addition of the allylic bromide 122 provided the desired coupling product 123. Desulfonylation and deprotection chemistry gave the corresponding primary alcohol. Due to the inherent labile nature of the furan to oxidation, transformation to ircinin-4 111 was successfully achieved employing a carefully controlled two-step protocol using PDC and silver nitrate (Scheme 1.20).

1.1.9.2.6 Synthesis of (+)-manoalide and related monocarbocyclic derivatives

Monocarbocyclic sesterterpenoids are an important series of marine sponge metabolites belonging to the sesterterpene class. (+)-Manoalide 124 is the parent compound in this series and was first isolated in 1980 by Scheuer et al. from a Pacific sponge *Luffariella variablis.*\(^{196}\) One year later, Scheuer et al. reported three additional related metabolites from the same Pacific sponge, namely secomanoalide 125, \((E)\)-neomanoalide 126 and \((Z)\)-neomanoalide 127. All three compounds, as well as the parent compound, displayed antibacterial activity against *Gram*-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) (Figure 1.22).
Since its discovery, (+)-manoalide 124 has attracted considerable attention from synthetic organic chemists with the first reported racemic synthesis in 1985 by Katsumara et al.,\textsuperscript{197} which was followed by six additional syntheses of the racemate by the groups of Garst,\textsuperscript{198} Katsamura,\textsuperscript{199} Kocienski,\textsuperscript{200} and Hoffmann.\textsuperscript{201} The structure of manoalide 124 is relatively simple as it only embodies one defined stereogenic center. The interesting fact about manoalide 124 is its biological profile, showing fantastic activity as a potent and irreversible inhibitor of phospholipase A\textsubscript{2}, which is the enzyme that catalyses arachidonic acid release in the formation of pro-inflammatory factors.\textsuperscript{202,203} Despite the number of syntheses available, only two enantioselective routes towards (+)-manoalide 124 have been reported in the literature.\textsuperscript{204,205}

The first enantioselective synthesis of manoalide 124 was accomplished by Soriente et al. nearly twenty years after its isolation (\textbf{Scheme 1.21}).\textsuperscript{204} The alkyl iodide 131 was prepared from β-ionone 130 utilizing the same synthetic procedure first reported by Hoffmann et al.\textsuperscript{201} The second subunit 129 bearing the stereogenic centre with the desired (\textit{R})-configuration was prepared in two steps as illustrated in \textbf{Scheme 1.21}. 

\textbf{Figure 1.22}
Scheme 1.21: First enantioselective synthesis of (+)-manoalide 124 reported by Soriente et al.\textsuperscript{204}

The stereogenic centre at C(4) was introduced by exploiting an aldol condensation reaction.\textsuperscript{206} Subjecting 3-furylaldehyde 92 and silyloxydien 128 to a mixture of Ti(0i-Pr)\textsubscript{4} and (R)-BINOL gave the corresponding aldol (65% yield, 88% ee), which was subsequently converted to its corresponding ester 129 by microwave irradiation (Scheme 1.21).\textsuperscript{206}

The alkylation reaction between homoallyl iodide 131 and the ester 129 required the presence of tetrabutylammonium salt 132 as a phase transfer catalyst to furnish, after diastereoselective ketone reduction, furan 133. An ensuing three-step protocol generated lactone 134 via ester hydrolysis, acetylation with simultaneous lactonization and finally elimination of acetate in the presence of DBU. Treatment with DIBAL-H to afford the lactol followed by photooxygenation of the furan moiety successfully furnished (+)-manoalide 124 (Scheme 1.21).
Four years later in 2003, Kocienski et al. reported the second enantioselective synthesis of (+)-manoalide \textbf{124} (\textit{Scheme 1.22}). In contrast to Sodano’s methodology, the stereogenic centre was introduced using a Sharpless kinetic resolution. Similar to Sodano’s synthesis, Kocienski’s route employed the same homoallyl iodide \textbf{131}, which was prepared from β-ionone \textbf{130} but using a different 8 step procedure.

\begin{center}
\textbf{Scheme 1.22: Second enantioselective synthesis of (+)-manoalide 124 reported by Kocienski et al.}
\end{center}

The second subunit was derived from furyl aldehyde \textbf{135}, which was reacted with propargyl magnesium bromide to afford a racemic propargylic alcohol. Sharpless asymmetric epoxidation of the alcohol subsequently afforded the desired (\textit{R})-configured alcohol \textbf{136} in 41\% yield. Successive Mo-catalysed cycloisomerisation in the presence of Bu$_3$SnOTf led to the vinyl stannane \textbf{137}, which is the key intermediate for the Cu-mediated 1,2-metalate rearrangement. To facilitate this cross coupling reaction, the vinyl lithium species of \textbf{137} was generated \textit{in-situ} using \textit{s}-butyllithium, which was subsequently added to the mixed cuprate \textbf{138} previously prepared from homoallyl iodide \textbf{131} employing \textit{t}-butyllithium and 1-pentynylcopper. The vinyl cuprate species was quenched with iodine to generate vinyl iodide \textbf{139}. The last three steps in the synthetic route involved a palladium-catalysed carbonylation reaction to afford a lactone, which was subsequently reduced to its corresponding lactol using
DIBAL-H. Similar to Sodano et al., the final step was a photooxidation of the furan moiety using rose bengal to furnish (+)-manoalide 124 (Scheme 1.22). Manoalide 124 was licensed to Allergan Pharmaceuticals and reached phase II clinical trials as a topical antipsoriatic. Its development was however discontinued due to formulation problems. The compound is now commercially available as a biochemical standard tool to block the action of PLA₂.²⁰⁷,²⁰⁸

With regard to the first synthesis of (E)-neomanoalide 126 and (Z)-neomanoalide 127, this was first achieved by Jefford and Boukouvalas et al. in 1994.²⁰⁹ Charles Jefford and John Boukouvalas have been involved in the synthesis of a number of synthetically novel furanolipid marine natural products in the last two decades. With respect to the early 90’s, they applied versatile furanolate technology which worked efficiently for the synthesis of γ-lactone and furan natural products like freelingnite,²¹⁰ (+/-)-eldanolide and siphonodictidine.²¹¹,²¹² Logically, this methodology, which is illustrated in Scheme 1.23, was again utilised in the concise synthesis of (E)-neomanoalide 126 and (Z)-neomanoalide 127.
Scheme 1.23: Concise synthesis of (E)-neomanoalide 126 and (Z)-neomanoalide 127 as developed by Jefford et al.\textsuperscript{209}

The key step in preparation of (E)-neomanoalide 126 and (Z)-neomanoalide 127 involved attachment of the two coupling partners 147 and 149. This was achieved by generating the 5-lithio derivative of 149 using tert-butyl lithium and subsequent cross coupling with 147. Furan 149 was prepared from the γ-lactone 148 by treatment with (tert-butyl)dimethylsilyl trifluoromethanesulphonate in the presence of triethylamine.

Preparation of the allyl bromide 147 was accomplished by modifying and extending the side chain of (E)-methyl monocyclofarnesate 140 (Scheme 1.23). Reduction and bromination of 140 gave the bromide 142. The hydroxy acetone element of the molecule was attached by employing hydrazone 143. The anion of 143 was generated in-situ by the action of lithium...
diisopropylamine and alkylated with 142 to give hydrazone 144. Hydrolysis with copper acetate gave the ketone 145 which was submitted to a Wittig reaction with ethyl (diethoxyphosphoryl)acetate to furnish both the 2E and 2Z-isomers of ethyl ester 146 in a 1:1 ratio, which were separated by column chromatography. Each isomer was converted separately to its corresponding (2E)- and (2Z)-bromide 147 (Scheme 1.23).

Following attachment of the two coupling partners 147 and 149, hydrolysis with aqueous hydrochloric acid successfully delivered (Z)-neomanoalide 127 or (E)-neomanoalide 126 depending on the isomer of 147 employed (Scheme 1.23).
1.1.9.3 Summary

Herein, I have reported on the total synthesis of a variety of biologically active sesterterpenoid marine natural products of varying complexity ranging from simple linear furanolipid molecules like furospinosulin-H 69 to more complex molecules containing stereogenic centers and cyclic systems like (-)-ircinianin 104 and (+)-wistarin 105. All syntheses reported in this section feature much of the repertoire of modern chemistry, including elaborate intramolecular Diels-Alder reactions and exciting transition-metal catalysed C-C-bond formations and rearrangements. As we reflect on the syntheses featured in the section, a number of plausible methods to achieve the concise total synthesis furospongolide 55 have been identified. Elegant concepts for introducing the furan and butenolide ring onto a central linchpin unit have been discussed as well as exciting and convenient functional group transformation reactions. These reactions will all be considered as practical approaches towards the synthesis of our target molecule 55.

Despite the uniqueness of each sesterterpenoid reported in this section, they all have a common structural homology containing either/both a furan or lactone ring, which must at some level be connected to their inherent biological activity. This comparative study between sesterterpenoids provided promising concepts on how to amend the structure of furospongolide 55 to increase its potency as an antitumour agent. Standard oxidation and reduction chemistry can also be performed on our target molecule 55 to alter its structure to imitate other known biological active marine natural products.

As new members of the sesterterpenoid family are continually being discovered, and largely forgotten ones unearthed, modern synthetic chemistry will continue to be developed and refined to meet the needs for synthetically targeting these important and attractive molecules of this fascinating class of natural products.
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6636.

Please note that Chapters 2-4 (pp.65-450) are unavailable due to a restriction requested by the author.

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## Table of Abbreviations

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<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
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<td>NMR</td>
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<td>Vascular Endothelial Growth Factor</td>
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