CHEMICAL STUDIES OF ANTI-INFLAMMATORY SECONDARY METABOLITES FROM MARINE SPONGES

by

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SHIP is a 145 kDa SH2-domain-containing inositol 5-phosphatase. It has been proposed that upregulation of SHIP activity with small molecule agonists of SHIP could control inflammation. At the outset of the research there were no known modulators of SHIP activity. A library of marine invertebrate extracts was screened for SHIP activators. A previously described meroterpenoid, pelorol, that represents the first known SHIP activator was isolated from a tropical sponge *Dactylospongia elegans* (Thiele, 1899). *In vitro* studies of pelorol have shown that it is able to activate SHIP's enzyme activity in intact cells and inhibits activation of macrophages, an essential component of the innate and acquired immune response in inflammatory diseases. A total synthesis of pelorol and its analogs was undertaken in order to flesh out the structure activity of this family of anti-inflammatory compounds. One of these analogs, AQX-16A, showed a 3-fold higher activation of SHIP than pelorol at the same molar concentration. It represents a novel class of drugs which activates a physiologically important negative regulator of the PI3K pathway in hemopoietic cells. Stereoselectivity is an important impact factor in drug action. Several stereo- and regio-isomers of pelorol and its analogs have been synthesized to answer the questions of how

![Molecular structures of Pelorol and AQX-16A](image-url)
the regiochemistry of the aromatic ring of pelorol affects its activity and whether
the stereochemistry of the C-ring is important for the SHIP activating properties
of pelorol.

Contignasterol was isolated by our group in 1992 from the sponge Petrosia
contignata collected in Papua New Guinea. It was the first example of an
emerging family of sponge steroids that have a number of unprecedented
structural features. Contignasterol was found to inhibit the release of histamine
from sensitized rat mast cells stimulated with anti-Ige. IZP576-092, an
antiasthma drug developed from the contignasterol lead structure, is now in
Phase II human clinical trials. The structure of contignasterol was initially solved
by interpretation of spectroscopic data. At the time, the absolute configuration of
the side chain chiral centers was not determined. A reinvestigation of the
structure of contignasterol using a combination of spectroscopic analysis and
chemical degradation has now resulted in the determination of the complete
absolute configuration of the molecule.

4.1 Contignasterol
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<tr>
<td>°</td>
<td>-degree(s)</td>
</tr>
<tr>
<td>1D</td>
<td>-one-dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>-two-dimensional</td>
</tr>
<tr>
<td>([\alpha]^{25}_{D})</td>
<td>-specific rotation at wavelength of sodium D line at 25°C</td>
</tr>
<tr>
<td>Ac</td>
<td>-acetate</td>
</tr>
<tr>
<td>AcOH</td>
<td>-acetic acid</td>
</tr>
<tr>
<td>Ac(_2)O</td>
<td>-acetic anhydride</td>
</tr>
<tr>
<td>AIDS</td>
<td>-acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>AlCl(_3)</td>
<td>-aluminum chloride</td>
</tr>
<tr>
<td>AML</td>
<td>-acute myelogenous leukemia</td>
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<tr>
<td>Ar</td>
<td>-argon</td>
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<tr>
<td>BBr(_3)</td>
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<td>Bl(_3)</td>
<td>-boron iodide</td>
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<tr>
<td>BMMCs</td>
<td>-bone-marrow-derived mast cells</td>
</tr>
<tr>
<td>BMmsΦs</td>
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<tr>
<td>br</td>
<td>-broad</td>
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<td>Br(_2)</td>
<td>-bromine</td>
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<tr>
<td>cm(^{-1})</td>
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<tr>
<td>CML</td>
<td>-chronic myelogenous leukemia</td>
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<tr>
<td>COSY-gr</td>
<td>-gradient selected two-dimensional correlation spectroscopy</td>
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<tr>
<td>δ</td>
<td>-chemical shift in parts per million</td>
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<td>-doublet</td>
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<td>DAHP</td>
<td>-3-deoxy-D-arabino-heptulosonic acid 7-phosphate</td>
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<td>DIBAL-H</td>
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<td>DMAP</td>
<td>-4-(dimethylamino)pyridine</td>
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<td>DNFB</td>
<td>-dinitrofluorobenzene</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<td>D₂O</td>
<td>deuterium oxide</td>
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<td>enzyme linked immuno sorbent assay</td>
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<tr>
<td>Et₃N</td>
<td>triethyl amine</td>
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<tr>
<td>Et₂O</td>
<td>diethyl ether</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FPP</td>
<td>farnesyl diphosphate</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
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<tr>
<td>H₂</td>
<td>hydrogen</td>
</tr>
<tr>
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</tr>
<tr>
<td>H₂O</td>
<td>water</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
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<td>human immunodeficiency virus</td>
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<tr>
<td>HMBC</td>
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</tr>
<tr>
<td>HMQC</td>
<td>two-dimensional heteronuclear multiple quantum coherence spectroscopy</td>
</tr>
<tr>
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<td>high-performance liquid chromatography</td>
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<tr>
<td>HREIMS</td>
<td>high-resolution electron impact mass spectrometry</td>
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<tr>
<td>HRESIMS</td>
<td>high-resolution electrospray ionisation mass spectrometry</td>
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<tr>
<td>HRFABMS</td>
<td>high-resolution fast atom bombardment mass spectrometry</td>
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<td>HSA</td>
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<tr>
<td>HSQC</td>
<td>two-dimensional heteronuclear single quantum coherence spectroscopy</td>
</tr>
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<td>high-throughput screening</td>
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<tr>
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<td>hertz</td>
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<tr>
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<td>o-iodoxybenzoic acid</td>
</tr>
<tr>
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<td>immunoglobulin E</td>
</tr>
<tr>
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<td>interleukin 3</td>
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<tr>
<td>IP₄</td>
<td>inositol-1,3,4,5-tetrakisphosphate</td>
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<tr>
<td>IP₃</td>
<td>inositol-1,3,4-trisphosphate</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant in hertz</td>
</tr>
<tr>
<td>K</td>
<td>degrees Kelvin</td>
</tr>
<tr>
<td>K₂CO₃</td>
<td>potassium carbonate</td>
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<tr>
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<td>lithium diisopropylamine</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>λₘₐₓ</td>
<td>wavelength at maximum intensity</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>M⁺</td>
<td>molecular ion</td>
</tr>
<tr>
<td>mCPBA</td>
<td>m-chloroperbenzoic acid</td>
</tr>
<tr>
<td>M-CSF</td>
<td>macrophage colony stimulating factor</td>
</tr>
<tr>
<td>MeAlCl₂</td>
<td>methyl aluminum dichloride</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>MeCN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>MeI</td>
<td>methyl iodide</td>
</tr>
<tr>
<td>MeOD-d4</td>
<td>deuterated methanol</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
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<tr>
<td>mg</td>
<td>milligram(s)</td>
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<tr>
<td>MgSO4</td>
<td>magnesium sulphate</td>
</tr>
<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre(s)</td>
</tr>
<tr>
<td>mmol</td>
<td>millimol(s)</td>
</tr>
<tr>
<td>MoOPH</td>
<td>oxodiperoxomolybdenum (pyridine)-(hexamethylphosphoric triamide)</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>MPA</td>
<td>methoxy phenyl acetic acid</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MTG</td>
<td>monothioglycolate</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>N</td>
<td>normal</td>
</tr>
<tr>
<td>N₂</td>
<td>nitrogen</td>
</tr>
<tr>
<td>Na</td>
<td>sodium</td>
</tr>
<tr>
<td>NaBH₄</td>
<td>sodium borohydride</td>
</tr>
<tr>
<td>NaCNBH₃</td>
<td>sodium cyanoborohydride</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>sodium bicarbonate</td>
</tr>
<tr>
<td>NaOH</td>
<td>sodium hydroxide</td>
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<tr>
<td>NHPI</td>
<td>N-hydroxyphthalimide</td>
</tr>
<tr>
<td>nBuLi</td>
<td>n-butyl lithium</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre(s)</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser enhancement spectroscopy</td>
</tr>
<tr>
<td>ORD</td>
<td>optical rotatory dispersion</td>
</tr>
<tr>
<td>PCC</td>
<td>pyridinium chlorochromate</td>
</tr>
<tr>
<td>Pd on C</td>
<td>palladium on charcoal</td>
</tr>
<tr>
<td>PEP</td>
<td>phosphoenolpyruvate</td>
</tr>
<tr>
<td>PGE₂</td>
<td>prostaglandin E₂</td>
</tr>
<tr>
<td>PH</td>
<td>pleckstrin homology</td>
</tr>
<tr>
<td>PI-3,4,5-P₃</td>
<td>phosphatidylinositol-3,4,5-triphosphate</td>
</tr>
<tr>
<td>PI-4,5-P₂</td>
<td>phosphatidylinositol-4,5-bisphosphate</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphatidylinositol-3 kinase</td>
</tr>
<tr>
<td>PLA₂</td>
<td>phospholipase A₂</td>
</tr>
<tr>
<td>PMA</td>
<td>phorbol myristate acetate</td>
</tr>
<tr>
<td>PPA</td>
<td>polyphosphoric acid</td>
</tr>
<tr>
<td>&quot; PrOH</td>
<td>1-propanol</td>
</tr>
<tr>
<td>p.s.i.</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>PTB</td>
<td>phophotyrosine binding</td>
</tr>
<tr>
<td>PTEN</td>
<td>phosphatase and tensin homologue deleted on chromosome ten</td>
</tr>
<tr>
<td>PtO₂</td>
<td>platinum oxide</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
</tbody>
</table>
Rh/C - rhodium on charcoal
(R)-MPA - (R)-\(\alpha\)-Methoxy-\(\alpha\)-phenylacetic acid
(R)-MTPACI - (R)-(\(-\))\(\alpha\)-Methoxy-\(\alpha\)-(trifluoromethyl)phenylacetyl chloride
ROESY - rotating frame NOESY
RT - room temperature
RTX - resiniferatoxin
s - singlet
SAM - S-Adenosylmethionine
SAR - structure-activity relationship
Sc(OTf)\(_3\) - scandium triflate
SCUBA - self-contained underwater breathing apparatus
SEM - standard error in the mean
SeO\(_2\) - selenium dioxide
SHIP - Src Homology 2-containing Inositol 5'-Phosphatase
(S)-MPA - (S)-\(\alpha\)-Methoxy-\(\alpha\)-phenylacetic acid
(S)-MTPACI - (S)-(\(-\))\(\alpha\)-Methoxy-\(\alpha\)-(trifluoromethyl)phenylacetyl chloride
SnCl\(_4\) - tin tetrachloride
sp\(^2\) - sp\(^2\) hybrid orbital
sp\(^3\) - sp\(^3\) hybrid orbital
t - triplet
tBuLi - tert-butyl lithium
TFA - trifluoroacetic acid
THF - tetrahydrofuran
TLC - thin-layer chromatography
TMEDA - N,N,N',N'-tetramethylethylenediamine
TMS - tetramethylsilane
TNF\(_{\alpha}\) - tumor necrosis factor \(\alpha\)
TsOH - \(p\)-toluenesulfonic acid
\(\mu\)M - micromolar
\(\mu\)g - microgram(s)
\(\mu\)L - microlitre(s)
UV - ultraviolet
VT - varied temperature
W - watt(s)
ZnI\(_2\) - zinc iodide
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For his selfless devotion of time and energy to my education I offer my most sincere thanks to my supervisor, Professor Raymond J. Andersen. My respect and admiration are immeasurable.

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To Dr. Christopher Gray I give my appreciation for his helpful proof-reading of this dissertation.

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To my wife and parents I owe whatever measure of success I've managed to achieve. My accomplishments are theirs.
Chapter 1: General Introduction.

1.1 Introduction

The prevalence of fatal diseases such as cancer and AIDS, for which no effective cures are available, has stimulated tremendous interest among researchers in natural products chemistry, pharmacology, and biochemistry in discovering new chemotherapies. Nature has been the prime source of such discoveries for centuries. Terrestrial plants and other materials are still used for the preparation of galenicals in many countries where traditional medicine is important, such as China and India. As chemical techniques have improved, the active constituents of traditional medicines have been isolated from the original source, structurally characterized, and synthesized in the laboratory. Drugs of natural origin have been classified into three groups: the original active natural products, semi-synthetic analogues produced by chemical modification of natural products to give more potent and better tolerated drugs, and total synthetic analogues based on natural product pharmacophores.¹ Natural products make a core contribution on a global basis to the maintenance and enhancement of human health by providing drugs for treating diseases and nutritional supplements to increase the efficacy of foods. This is particularly evident in the areas of cancer and infectious diseases, where over 60% and 75% of current drugs used to treat these diseases, respectively, are of natural origin.²
With new natural products consistently being discovered in substantial numbers, this field has been broadened and diversified to include more scientific directions. These include research in the areas of chemical ecology, biosynthesis, total synthesis, enzymology, spectroscopy, and genetics. Natural products are often referred to as 'secondary metabolites', a term invented by plant physiologists and brought into general use for microbial products by J. D. Bu’Lock in 1961. This terminology is often used in reference to small chemical substances found in nature that have distinct pharmacological effects, such as the antibiotic penicillin.

Secondary metabolites are not necessarily produced under all conditions. Unlike the intermediates and cofactors that take part in cell-structure syntheses and energy transduction, these substances are not essential for growth and reproductive metabolism. From this perspective, the majority of secondary metabolites are considered to show no benefit to the producer. However, the opposite view, now widely held, is that every secondary metabolite is present because it displays a biological activity at some stage in evolution that endows the producer with increased fitness. Even though in the vast majority of cases, the function of these compounds and their benefit to the organism is still not yet known, some are undoubtedly produced for easily appreciated reasons, e.g. as toxic materials providing defense against predators, as volatile attractants towards the same or other species, or as colouring agents.
1.2 Plant and marine secondary metabolites

Natural products researchers first focused their attention on plants due to the abundance of the source materials and relatively easy methods of collection. Plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides. It has been reported that up to 80% of the population in developing countries are totally dependent on plants for their primary health care. Despite the remarkable progress in the synthetic organic chemistry of the twentieth century, it is still the case that over 25% of prescribed medicines in US are derived from plants, directly or indirectly.

A high profile example of a plant secondary metabolite is paclitaxel (1.1), which is a complex diterpene isolated from the bark of the *Taxus brevifolia*. It is a widely used anticancer agent due to its unique mode of action as a stabilizer of microtubules.

The discovery that marine organisms constitute a rich source of previously undescribed secondary metabolites is not entirely surprising, considering that many algal and invertebrate phyla reside exclusively in the sea. Today, both academic and industrial interest in marine organisms is on the rise, given the fact that the findings of unique, biologically active marine secondary metabolites are increasing. Many of these compounds have shown significant pharmacological
activities and some have reached advanced stages of clinical trials. A prominent example is didemnin B (1.2), a member of the family of cytotoxic cyclic depsipeptides isolated from the Caribbean tunicate *Trididemnum solidum*, which progressed to phase II clinical trials as an anticancer drug.

![Taxol](image1.png)

1.1 Taxol

![Didemnin B](image2.png)

1.2 Didemnin B

### 1.2 Microbial secondary metabolites

Even though natural drugs derived from microorganisms have a much shorter history than from plants, the investigation into secondary metabolites isolated from microorganisms has dramatically increased the number of novel bioactive compounds. Microbially produced antibiotics account for a very high portion of the drugs commonly prescribed nowadays. Microbial secondary metabolites have been found to have a variety of other biological activities as well.¹¹

The discovery of penicillin (1.3), a β-lactam compound first found to be produced by a mold called *Penicillium notatum* by a British scientist Alexander Fleming in 1928,¹² has become recognized as one of the greatest advances in medical
history. However, the major impact goes back to the mass production and clinical utilization of penicillin produced by *Penicillium chrysogenum*, which is presently used in producing penicillin commercially, during the second half of the last century.\textsuperscript{13} Since then, more and more compounds with microbial origins are used due to their effect on the health, nutrition and economics of our society.

![Penicillin Structure](attachment://penicillin.png)

1.3 Drugs from the sea

With approximately 70\% of our earth's surface covered with marine ecosystems, which also represent 95\% of the biosphere, we are living on a planet of oceans.\textsuperscript{14} Until the 1960s, our understanding of marine natural products chemistry was quite limited, largely due to technical barriers to collecting the source organisms. The situation changed with the advent of SCUBA which first found its scientific utilization in marine biology 60 years ago. In the mid-1960s, natural products chemists also started moving their eyes from terrestrial organisms to their marine counterparts.\textsuperscript{15} Inspired by the pioneering work of the late Dr. John Faulkner, Dr. Paul J. Scheuer, etc. on bioactive marine natural products, over the past three decades, we have witnessed well over 14000 new natural products being isolated from marine organisms.\textsuperscript{16} Most of these compounds have unique structures without direct precedent from terrestrial organisms. Moreover, at least
300 patents have been issued between 1969 and 1999 covering mainly potential anticancer agents from the sea.\textsuperscript{15} Although the successful commercialization of the sponge-derived cytosine arabinoside (Ara-C),\textsuperscript{17} as a potent antileukemic agent, and its analog adenine arabinoside (Ara-A), as an antiviral drug, has validate the potential in drug discovery from the sea, it could not be regarded as the milestone in this field, since the real break-through in marine drug discovery has not yet happened.

Since the early 1980s, a significant number of marine-derived compounds entered into preclinical and clinical antitumor trials. Prialt, generically called ziconotide,\textsuperscript{18} is the synthetic form of the cone snail peptide w-cenotoxin M-VII-A and the first in a new class of painkillers approved by the FDA in December 2004. There are 20 (Table 1.1, see page 8) marine natural products currently in different stages of clinical trials, mostly targeted towards cancer, pain, and inflammatory diseases, and there is an encouraging and still increasing list of marine natural products that are in preclinical trials. It seems possible that more marine natural products will be licensed for clinical use in the near future. The most promising candidates are ecteinascidin 743 (ET-743) and Neovastat. Ecteinascidin 743 (1.4), a tetrahydroisoquinoline alkaloid isolated from the colonial tunicate \textit{Ecteinascidia turbinata},\textsuperscript{19,20} is the only known chemotherapy agent that binds to the minor groove of DNA, bending the DNA towards the major groove. It exerts its therapeutic effect through several pathways, including interference with cellular transcription-coupled nucleotide excision repair (TC-
NER) to induce cell death, causing slowing and arrest of tumour cell division and subsequent p53-independent apoptosis (programmed cell death), and inhibiting transcriptional activation of inducible genes. Early efforts at drug development of ET-743 were very difficult because 1 tonne (wet weight) of \textit{E. turbinata} yielded only 1 g of the promising anti-tumor agent ET-743. This example illustrates a serious problem associated with development of drugs from marine natural products, namely the 'supply problem'. Close inspection of Table 1.1 reveals that since the majority of promising compounds have complex structures, it would be a formidable task in most cases to provide enough material for drug development by total synthesis.

The first total synthesis of ET-743 was accomplished by Corey and co-workers using a multistep enantiocontrolled process. However, the 'supply problem' was solved by Pharma Mar chemists through a semisynthesis from cyanosafracin B (1.5), a metabolite of the marine microbe \textit{Pseudomonas fluorescens}. Their 21-step synthetic route provided the large scale preparation of material required for
clinical trials. Although this approach can not be generalized, in certain instances it could be expected to be a satisfactory solution to the supply problem. Another approach to solving the supply problem is mariculture. A notable example of this approach is the aquaculture of the bryozoan *Bugula neritina* (the source of the bryostatins), which can provide 100-200g of bryostatin 1 per year for each aquaculture unit at a cost of $700,000. This approach can be extremely useful when mated with chemical synthesis to find new analogs with enhanced potencies or lower toxicities. The last but not the least important approach to solve the supply problem is to synthesize a basic core structure by aiming to mimic some properties of natural compounds, then using this as a template for combinatorial chemistry to generate a pool of chemical entities that is substantially more diverse and has greater biological relevance than a pure combinatorial chemistry library.

We have come a long way in our understanding of marine natural products chemistry since the identification of the Caribbean sponge *Cryptotheca crypta* derived antiviral compounds in the 1950s. Given the enormous bio-diversity of the oceans, more novel marine natural products with various bioactivities will be identified and this vast repository of esoteric structures will always complement terrestrial natural products chemistry.

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Compound Type</th>
<th>Source</th>
<th>Discovering laboratory</th>
<th>Clinical Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ara-A</td>
<td>Nucleoside</td>
<td>Gorgonian</td>
<td>Glaxo Smith-Kline</td>
<td>In Clinical Use</td>
</tr>
<tr>
<td>Ara-C</td>
<td>Nucleoside</td>
<td>Sponge</td>
<td>Pharmacia</td>
<td>In Clinical Use</td>
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<tr>
<td>Ziconotide</td>
<td>NRP</td>
<td>Mollusc</td>
<td>Olivera-Pfizer</td>
<td>In Clinical Use</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----</td>
<td>---------</td>
<td>---------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Didemnin B</td>
<td>PK-NRPS</td>
<td>Tunicate</td>
<td>Rinehart</td>
<td>Phase II (dropped 90s)</td>
</tr>
<tr>
<td>Dolastatin 10</td>
<td>NRP</td>
<td>Sea Hare</td>
<td>Petit</td>
<td>Phase II (dropped 90s)</td>
</tr>
<tr>
<td>Giroline</td>
<td>Imidazole deriv.</td>
<td>Sponge</td>
<td>Potier</td>
<td>Phase I (dropped 90s)</td>
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<tr>
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<td>Sponge</td>
<td>Scheuer-Allergan</td>
<td>Phase II (dropped 90s)</td>
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<tr>
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<td>Sponge</td>
<td>Crews</td>
<td>Phase I (dropped 2002)</td>
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<tr>
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<td>Merck</td>
<td>Phase I (dropped 2002)</td>
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<tr>
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<td>Mollusc</td>
<td>Olivera</td>
<td>Phase I (discontinued)</td>
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<td>Alkaloid</td>
<td>Tunicate</td>
<td>Rinehart-PharmaMar; Ortho Biotech (J&amp;J)</td>
<td>Phase III</td>
</tr>
<tr>
<td>Neovastat</td>
<td>Mixture</td>
<td>Shark</td>
<td>Sorbera</td>
<td>Phase III</td>
</tr>
<tr>
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<td>PK-NRPS</td>
<td>Bryozoan</td>
<td>Petit</td>
<td>Phase II</td>
</tr>
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<td>Cematoxin</td>
<td>NRP</td>
<td>Synthetic deriv. of dolastatin15</td>
<td>BASF Pharma</td>
<td>Phase II</td>
</tr>
<tr>
<td>Dehydrodidemnin B</td>
<td>NRP</td>
<td>Sponge</td>
<td>Andersen-Wyeth</td>
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<tr>
<td>(Aplidin)</td>
<td>Terpene</td>
<td>Deriv. of 576092</td>
<td>Andersen-Aventis</td>
<td>Phase II</td>
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<td>Zasloff</td>
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<td>Kahalalide F</td>
<td>NRP</td>
<td>Synthetic deriv. of dolastatin15</td>
<td>BASF Pharma</td>
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<td>Aminosteroid</td>
<td>Synthetic deriv. of dolastatin15</td>
<td>Teikoku Hormine</td>
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<td>NRP</td>
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<td>Kem</td>
<td>Phase I</td>
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<td>Sponge</td>
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1.5 Antiinflammatory marine natural products

Inflammation is caused by trauma of some kind which causes tissue injury. Our understanding of inflammation can be traced back to the first century when Celsus described the inflammatory response in terms of cardinal signs of redness, oedema, heat, pain, and loss of function. Since then, innumerable individuals in inflammation research have described many of the mechanisms associated with tissue injury. These include Lewis (1881-1945), who described the Triple Response; Cohnheim and Samuel (late 1880s), who reported on leukocyte emigration and vascular permeability; and Metchnikoff (1845-1916), who defined phagocytosis. As colorful as the history of inflammation research is, the treatment of inflammatory diseases with natural products has also come a long way since the great physician Hippocrates used salicylates in the form of preparations of willow bark in the treatment of pain, inflammation, and fevers. Aspirin, derived from salicylic acid, is probably the most well-known drug in the world today. It is still in use for treatment of inflammatory diseases. It is noteworthy that natural products and their derivatives from terrestrial origins have been the major source of antiinflammatory drugs currently in clinical use.

To date, there is no marine natural product that can be found on the shelves of pharmacies for treating inflammatory diseases. The reason for this situation can be partly attributed to the coincident situation at present in the field of marine drug development. However, compared with the number of antitumor marine
natural products in clinical trials and preclinical research, the number of antiinflammatory marine natural products under evaluation is insignificant. The deeper reason may be that pharmaceutical companies have given top priority to focusing on developing therapies for the fatal diseases, such as cancer.

The first antiinflammatory marine natural product emerged in the investigation of the marine sponge *Halichondria moorei* by a group of Roche scientists. They found fluorine was the major constituent of this sponge and it occurred as $K_2SiF_6$, which was a potent antiinflammatory agent. This was followed by another report from the same group on the isolation of three antiinflammatory compounds 6-n-tridecylsalicylic acid, flexibilide, and dendalone 3-hydroxybutyrate from a brown alga, a soft coral, and a dictyoceratid sponge, respectively. Since then, various classes of novel marine natural products with different antiinflammatory activities have been reported.

An updated review by Keyzers and Davies-Coleman provided the first holistic overview of the sponge derived antiinflammatory metabolites. It is not surprising that antiinflammatory marine natural products are dominated by sponge-derived metabolites, since marine sponges are the cornucopia of novel marine natural products. The forerunners of antiinflammatory sponge metabolites in terms of drug development are manoalide and contignasterol. Manoalide (1.6), a sesterterpenoid isolated from the Palauan sponge *luffariella variabilis*, is the first inhibitor of phospholipase $A_2$ (PLA$_2$). It was found to irreversibly inhibit the
release of arachidonic acid (the precursor of the acute inflammatory response mediator eicosanoids) from membrane phospholipids by the enzyme PLA$_2$, thus terminating the inflammatory response. The chemical basis of manoalide’s inhibitory activity is the formation of a Schiff base between the manoalide hemiacetals and a lysine residue of the PLA$_2$ protein, which prevents the enzyme from binding to membranes. Manoalide was licensed to Allergan Pharmaceuticals, and placed into clinical trials for treatment of psoriasis. It advanced to phase II, but was discontinued due to formulation problems.

Contignasterol (1.7) is the first naturally occurring sponge steroid with the ‘unnatural’ H-14 β configuration.$^{33,34}$ Shortly after its discovery, a pharmacological evaluation revealed that contignasterol could inhibit the anti-immunoglobulin E stimulated release of histamine from sensitized rat mast cells. Several synthetic
derivatives (IPL576,092 (1.8) and two others) showing the same activity have been advanced to various stages of clinical trials as antiasthma agents by Inflazyme in conjunction with Aventis Pharma. Izzo et al. recently reported the synthesis of hybrid structures of manoalide and IPL576,092 (eq. 1.9). This approach was inspired by the perspective of ‘natural product hybrids’, that combination of parts of structurally different naturally occurring bioactive products to yield hybrid structures can in principle, exceed the activities of their parent compounds. These analogs inhibited human synovial sPLA$_2$-IIA by 63% at 100µM and also reduced nitric oxide and PGE$_2$ (two important mediators of the inflammatory process) by 64 and 72%, respectively, at 10µM on lipopolysaccharide stimulated human monocytes. However, in consideration of the different mechanisms that manoalide and IPL576,092 have utilized to inhibit the inflammatory response, Izzo’s approach is questionable. 

![Image of chemical structure](image)

Antiinflammatory marine natural products from many other resources have also been discovered. Some of them have been shown to possess interesting chemistry and remarkable activity. The pseudopterosins (1.10a-d), a family of diterpene glycosides from the gorgonian *Pseudopterogorgia elisabethea,*
exhibited potent antiinflammatory and analgesic activities by inhibiting pancreatic PLA$_2$ and affecting both the cyclooxygenase and lipoxygenase pathways.

Cyclomarin A (1.11), a cyclic peptide produced by a marine bacterium *Streptomyces* sp., displayed significant antiinflammatory activity in a PMA-induced (PMA-phorbol myristate acetate) mouse ear edema assay and in an *in vitro* assay.

Oxepinamide A (1.12), the first of a family of alkaloids isolated from a marine fungal extract, exhibited good antiinflammatory activity in a topical RTX-induced mouse ear edema assay, to name but a few.
An important requirement for a compound to be approved as a drug is that it is safe to use. However, the antiinflammatory drugs currently on the market all have side-effects. This includes the best selling antiinflammatory drug VIOXX, which increases the relative risk for confirmed cardiovascular events, such as heart attack and stroke. This imposes serious limitations on the use of these drugs. Developing new antiinflammatory drugs with higher potency and lower or no side-effects is the desired outcome for the workers in this field.
Chapter 1: General Introduction.

1.6 References

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Chapter 1: General Introduction.

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Chapter 2: Synthesis of Pelorol and Analogs

2.1 A brief review of the SHIP assay

The phosphatidylinositol-3 kinase (PI3K) signaling pathway plays an important role in regulating various cellular activities. These include, depending on the cell type, survival, adhesion, movement, proliferation, differentiation and end cell activation. A key second messenger in this pathway is the membrane-associated phosphatidylinositol-3,4,5-triphosphate (PI-3,4,5-P₃) (Figure 2.1), which is present in low concentration in resting cells, but is transiently synthesized from PI-4,5-P₂ by PI3K in response to a diverse array of extracellular stimuli, and attracts pleckstrin homology (PH) domain containing proteins to the plasma membrane to mediate its effects.¹ To ensure the activation of this signaling pathway is properly restrained, the ubiquitously expressed tumor suppressor PTEN (phosphatase and tensin homologue deleted on chromosome ten) reverses PI3K activity by catalyzing the hydrolysis of PI-3,4,5-P₃ back to PI-4,5-P₂. Around 50% of human cancers contain biallelic inactivating mutations of PTEN and familial cancer predisposition disorders can be mimicked in PTEN knock-out mice, which illustrates the importance of these phospholipid phosphatases in preventing uncontrolled cell growth.⁵

¹ A version of this chapter has been published. Yang, Lu; Williams, David E.; Mui, Alice; Ong, Christopher; Krystal, Gerald; van Soest, Rob; Andersen, Raymond J. (2005) Synthesis of Pelorol and Analogs: Activators of the Inositol 5-Phosphatase SHIP. Organic Letters, 7(6), 1073-1076.
Similar to PTEN, the hemopoietic-restricted Src homology 2-containing inositol 5'-phosphatases SHIP, sSHIP, and SHIP2 hydrolyze PI-3,4,5-P$_3$ to PI-3,4-P$_2$.

SHIP, also known as SHIP1, is a 145kDa protein that is widely expressed in hemopoietic cells. It contains an amino-terminal src homology 2 (SH2) domain, a centrally located 5'-phosphatase domain that selectively hydrolyses PI-3,4,5-P$_3$ and inositol-1,3,4,5-tetrakiphosphate (IP$_4$) in vitro, two phosphotyrosine binding (PTB) consensus sequences, and a proline-rich region at the carboxyl tail.
sSHIP is a 104kDa protein that is only expressed in murine embryonic stem cells and hemopoietic stem cells. The function of sSHIP is to regulate PI-3,4,5-P$_3$ levels in stem cells in response to extracellular stimuli.

Besides SHIP and sSHIP, there is a more widely expressed 150kDa SHIP2 protein, which is encoded by a separate gene and has a structure similar to SHIP. Like SHIP and sSHIP, SHIP2 specifically hydrolyzes the 5'-phosphate from PI-3,4,5-P$_3$ and IP$_4$ in vitro.

In 1998, Krystal and co-workers generated mice containing a homozygous deletion of SHIP (SHIP$^{-/-}$ mice) by replacing SHIP's first exon with the neomycin resistance gene in an antisense orientation.$^6$ Although these animals are viable and fertile, they have a shortened lifespan and normally do not survive beyond 14 weeks. They overproduce granulocytes and macrophages, suffer from progressive splenomegaly, extramedullary hemopoiesis, massive myeloid infiltration of the lung, and osteoporosis. Experiments with SHIP$^{-/-}$ mice and with SHIP$^{-/-}$ bone-marrow-derived mast cells (BMMC$s$) and macrophages (BMM$\Phi$s) obtained from these mice, have demonstrated that SHIP is a negative regulator of immunoglobulin E (IgE) or Steel Factor induced mast cell activation,$^7$ a negative regulator of lipopolysaccharide (LPS) induced macrophage activation, and a negative regulator of osteoclast formation and resorptive function. There is also evidence that SHIP acts as a tumor suppressor in both acute myelogenous leukemia (AML)$^8$ and in chronic myelogenous leukemia (CML).$^9$ Macrophages
from older SHIP−/− mice display an impaired LPS-induced inflammatory cytokine production, which suggests that regulation of the PI3K signalling pathway may play an important role in programming the macrophage innate immune response.\textsuperscript{10} It has been suggested that therapeutic manipulation of the PI3K pathway might be beneficial in treating osteoporosis, inflammatory disorders, and cancers.

Current attempts to develop drugs based on intervention in signaling pathways are overwhelmingly biased toward finding selective kinase inhibitors. There has been some recent interest in examining the therapeutic potential of phosphatase inhibitors,\textsuperscript{11} but there has been virtually no effort to explore the usefulness of small molecule phosphatase activators. The important role of SHIP as a negative regulator of mast cell and macrophage activation, osteoclast formation and resorptive function, as well as in AML and CML, combined with its occurrence only in hemopoietic cells, makes it an attractive drug target. Krystal hypothesized that selective activators of SHIP would be useful experimental tools and potential drug candidates to provide proof of principle validation for a new approach to the treatment of inflammation, osteoporosis, and leukemia.
2.2 Introduction

As part of the ongoing efforts in the Andersen lab to search for bioactive natural products from marine sources through bioassay-guided separation, crude extracts of marine invertebrates were screened for in vitro activation of the SHIP-catalyzed conversion of inositol-1,3,4,5-tetrakisphosphate (IP$_4$) to inositol-1,3,4-trisphosphate (IP$_3$). Out of approximately 2000 extracts tested, 10 exhibiting SHIP activation were identified. A MeOH extract of the sponge *Dactylospongia elegans* (Thiele, 1899), collected in Papua New Guinea, exhibited promising activity in the assay. Bioassay-guided fractionation of the extract led to the identification of pelorol (2.1) as the sole SHIP-activating component (Figure 2.2).
Three related sesquiterpenes, illimaquinone (2.2), mamanuthaquinone (2.3), and dactyloquinone A (2.4) were also isolated from the *D. elegans* extract, but were not active in the assay.

Pelorol (2.1) and dactyloquinone A (2.4) were undescribed when first isolated in our laboratory, but while further biological studies were in progress, pelorol (2.1) was isolated by Konig’s group, also from *D. elegans*, and by Schmitz’s group from *Petrosaspongia metachromia*. Dactyloquinone A (2.4) was subsequently isolated from the Okinawan sponge *D.elegans* by Yamada’s group in 2001.

A review of the literature reveals that 36 sesquiterpene hydroquinones and quinones have been isolated from the species *D. elegans* to date and half of them were new compounds when they were initially identified. All of these compounds have a 4,9-friedodrimane (A,B) or drimane (C) skeleton, which couples with a hydroquinone, quinone, or lactone moiety. An early paper from Crews classified quinone (hydroquinone) sesquiterpenoids from sponges and algae in accordance with their structure type and the corresponding marine resource. As he indicated, quinone (hydroquinone) sesquiterpenoids from *D. elegans* had the largest variety of structures among the sponge families Dysideidae, Thorectidae, Spongiidae, and Haploscleridae. Subsequent work on this species enriched the sesquiterpene quinone and hydroquinone family which comprises four terpene skeletons (A,B,C,D) and four different benzenoid-derived substructures (W, X,Y,Z) (Figure 2.3). The benzenoid-derived substructures W-Z...
could be further categorized into different subunits (a-i) as a function of the heteroatom substitution, presence or absence of an additional carbon and oxidative transformations. Of interest, ilimaquinone (2.2) that we have isolated in our current work, is the only component present in all investigations on this species by six research groups (Andersen, Crews, Kobayashi, Konig, Riguela,
Figure 2.4 Proposed biogenesis of pelorol related compounds from *D. elegans*

Yamada) sampled from seven different geographical sites (Papua New Guinea, Fiji & Thailand, Indonesia, Australia, Malaysia, Japan), which exemplifies its chemotaxonomic importance to the species *D. elegans*. More interesting is that only three groups have reported the isolation of pelorol (2.1)\textsuperscript{16,20} from *D. elegans*. Recently, the other groups isolated either (-)smenodiol (2.6),\textsuperscript{17,23} which was believed to be the formal biogenetic precursor of pelorol, or smenospondiol (2.7),\textsuperscript{17,24} the rearrangement product. Riguera’s examination of this species led to the isolation of some ring-contracted derivatives of ilimaquinone,
dactylospongenones A-D (2.8-2.11)\textsuperscript{17}, which were originally discovered by Faulkner's group from the Palauan sponge *Dactylospongia* sp. together with smenospondiol (2.7).\textsuperscript{25} Faulkner refuted the hypothesis that dactylospongenones might be artifacts formed from ilimaquinone during the isolation and separation. On the basis of this evidence, we proposed that pelorol (2.1), smenodiol (2.6) and smenospondiol (2.7) may have a common precursor (Figure 2.4), which is the bicyclic cationic cyclization intermediate (2.5). This intermediate could undergo elimination to form smenodiol (2.6), cyclization to give pelorol (2.1), and the Wagner Meerwein rearrangement of the drimane terpene skeleton to give smenospondiol (2.7), which could be further oxidized, decarboxylated and cyclized to yield the four diastereomers dactylospongenone A-D (2.8-2.11).

*Delegans* is a rich source of sesquiterpene quinones / hydroquinones that possess interesting biological and pharmacological properties. Similar structural motifs in the sesquiterpene quinone and hydroquinone family members often translates to a variety of exciting biological properties unique to individual compounds. For example, ilimaquinone (2.2) was found to exhibit anti-HIV,\textsuperscript{26} antimicrobial, antimitotic, and antiinflammatory activities,\textsuperscript{25} in addition to inhibiting the cytotoxicity of ricin and diphtheria toxin,\textsuperscript{27} selectively fragmenting the Golgi apparatus,\textsuperscript{12} and interacting with methylation enzymes,\textsuperscript{28} as well as inhibiting the lyase activity of DNA polymerase β.\textsuperscript{29} Smenospongine (2.12) (Figure 2.5), an aminoquinone sesquiterpene, was reported to have antimicrobial, and
antileukemic activities\textsuperscript{17,24} and was found to induce the differentiation of human chronic myelogenous Leukemia (CML) K562 cells into erythroblasts along with cell-cycle arrest at the G1 phase. It also exhibits strong inhibition of DNA synthesis in L1210 leukemia cells and inhibition of the proliferative response of mitogens in murine splenocytes and human peripheral lymphocytes\textsuperscript{22,30}. The akylated aminoquinone sesquiterpene smenospongiarine (2.13), which has a similar structure to smenospongine (2.12), is less toxic to L1210 leukemia cells and shows moderate toxicity to tumor cells\textsuperscript{17,24,30}. Another aminoquinone sesquiterpene, smenospongidine (2.14), was found to have antimicrobial activity and differentiation-inducing activity for the conversion of CML K562 cells into erythroblasts, but was not cytotoxic to L1210 leukemia cells\textsuperscript{17,22,24}. This is consistent with the activity of (2.13), that a free amino group is essential to the cytotoxicity. 5-Epi-smenospongiarine (2.15) (Figure 2.6) was reported to show strong \textit{in vitro} antileukemic activity and potent \textit{in vitro} toxicity to solid tumor models. 5-Epi-smenospongidine (2.16) was found to have moderate toxicity to leukemia cells (P388) and solid tumor cells, in addition to exhibiting differentiation inducing activity in K562 leukemia cells\textsuperscript{17,22}. Dactylospongenone B (2.9) is the only non-quinone containing derivative that exhibits \textit{in vitro} activity against
leukemia cells (P388).\textsuperscript{17} Neodactyloquinone (2.17)\textsuperscript{21} expresses moderate cytotoxic activity toward Hela cells and mamanuthaquinone (2.3)\textsuperscript{13} is toxic to human colon tumor cells with an IC\textsubscript{50} of 2\textmu g/mL. As we noted above, while the interesting structure of pelorol (2.1) was reported by the Konig and Schmitz group,\textsuperscript{15,16} and Konig \textit{et al} described anti/protozoan activity of this compound, neither group appreciated the ability of pelorol to activate SHIP or modify the biology of mammalian cells.
2.3 Isolation and Characterization of Pelorol (1)

2.3.1 Isolation

Specimens of the brownish sheet sponge *Dactylospongia elegans* (Thiele, 1899) (order Dictyoceratida, family Spongiidae) (Figure 2.7) were collected by hand using SCUBA at a depth of 5-10 m from a protected overhang in Rasch Passage on the outer reef of Madang Lagoon, Papua New Guinea, in January 1995. Freshly collected sponge was frozen on site and transported to Vancouver over dry ice. The sponge was identified by Professor Rob van Soest, University of Amsterdam, and a voucher sample has been deposited at the Zoological Museum of Amsterdam (ZMA POR. 15986).

![Image of the sponge *Dactylospongia elegans* (Thiele, 1899)](image)

Figure 2.7 Image of the sponge *Dactylospongia elegans* (Thiele, 1899)

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Isolation and characterization work for pelorol, ilimaquinone, mamanuthaquinone, and actyloquinone A was performed by Dr. D.E. Williams, Andersen research group, University of British Columbia.
The frozen sponge (120 g) was cut into small pieces, immersed in and subsequently extracted with MeOH (3 x 250 mL). The combined methanolic extracts were concentrated in vacuo, and partitioned between EtOAc (4 x 100 mL) and H₂O (300 mL). The combined EtOAc extract was evaporated to dryness in vacuo to yield 490 mg of a brown purply oil that exhibited significant activity at 2 μM in a SHIP assay. The EtOAc soluble material was chromatographed on Sephadex LH-20 with 4:1 MeOH/CH₂Cl₂ as eluent to give two SHIP active fractions. One fraction (14.9 mg) consisted primarily of the novel metabolite 2.1 and lipid material. The second active strongly coloured fraction (30.5 mg) (appearing orange under basic and blue/purple under acidic conditions) contained a mixture of ilimaquinone (2.2), mamanuthaquinone (2.3), dactyloquinone A (2.4) and fats. A pure sample of 2.1 (8.9 mg) was obtained by semi-preparative reversed-phase HPLC, using a Whatman Magnum-9 Partisil 10 ODS-3 column, with 13:7 MeCN/0.05% aqueous TFA) as eluent. The mixture of ilimaquinone (2.2) and mamanuthaquinone (2.3), dactyloquinone A (2.4) was further fractionated by semi-preparative reversed-phase HPLC, using the same conditions as for the purification of 2.1, to give pure dactyloquinone A (2.4) (1.9 mg), pure ilimaquinone (2.2) (17.0 mg) and mamanuthaquinone (2.3) contaminated with a small quantity of 2.2. Pure 2.3 (0.8 mg) was obtained after an additional step of HPLC purification using the same conditions. The structures of 2.2 and 2.3 were solved by spectroscopic analysis and the data obtained were then compared to those found in the literature.¹²,¹³
It is important to note that the same products were isolated in a subsequent workup in which MeOH was substituted for EtOH throughout. Hence, compounds 2.1, 2.2, 2.3 and 2.4 can all be considered to be natural products and not artifacts of isolation.

**Pelorol (2.1):** Isolated as a pale oil; \([\alpha]_{D}^{25} -37.1^\circ\) (c 0.275, CH\(_2\)Cl\(_2\)); UV (CH\(_2\)Cl\(_2\)) \(\lambda_{max}\) 227 (e 7436), 253 (e 6557), 289 (e 2519) nm; \(^1\)H NMR, see Table 2.1; \(^{13}\)C NMR, see Table 2.1; positive ion HRFABMS [M+H]\(^+\) m/z 373.23808 (calcd for C\(_{23}\)H\(_{33}\)O\(_4\), 373.23799).

**Dactyloquinone A (2.4):** Isolated as a pale oil; \([\alpha]_{D}^{25} -46.3^\circ\) (c 0.095, CH\(_2\)Cl\(_2\)); UV (CH\(_2\)Cl\(_2\)) \(\lambda_{max}\) 290 (e 15469), 406 (e 394) nm; \(^1\)H NMR, see Table 2.1; \(^{13}\)C NMR, see Table 2.1; negative ion HRFABMS [M]\(^-\) m/z 356.19950 (calcd for C\(_{22}\)H\(_{28}\)O\(_4\), 356.19884).
Figure 2.8 $^1$HNMR of pelorol (2.1) isolated from sponge *D. elegans* recorded in CDCl$_3$ at 400MHz
Figure 2.9 $^{13}$CNMR of pelorol (2.1) isolated from sponge *D. elegans* recorded in CDCl$_3$ at 100MHz
Table 2.1 NMR Data for 2.1 and 2.4 recorded in CDCl<sub>3</sub>\(^1\)

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\(^a\)500 MHz. \(^b\)100 MHz.

\(^1\)Assignments within a column are interchangeable. Assignments based on HMQC, HMBC, COSY, ROESY and 1D NOESY data.
2.3.2 Characterization

Pelorol (2.1) was obtained as an optically active amorphous solid that gave a \([M+H]^+\) ion in HRFABMS at \(m/z\) 373.2381 appropriate for a molecular formula of \(C_{23}H_{32}O_4\) (calculated for \(C_{23}H_{32}O_4\) 373.2380), requiring eight sites of unsaturation. The \(^{13}\text{C}\) NMR spectrum (CDCl\(_3\)) obtained for 2.1 contained resonances accounting for 23 carbon atoms in agreement with the HRFABMS data. HMQC data showed that only 30 hydrogen atoms were attached to carbons (5xCH\(_3\), 6xCH\(_2\), 3xCH, 9xC), requiring the presence of 2 OHs. Broad singlets at \(\delta\) 5.17 and \(\delta\) 5.37 in the \(^1\text{H}\) NMR spectrum, that were not correlated to carbon resonances in the HMQC spectrum, were assigned to the OH protons.

Preliminary analysis of the \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra indicated that pelorol (2.1) had both benzenoid and sesquiterpenoid fragments. Four aliphatic methyl singlets (\(\delta\) 0.83, Me-12; 0.84, Me-11; 1.04, Me-14; 1.21, Me-13), one methoxyl singlet (\(\delta\) 3.81, Me-23), a benzylic methylene [\(\delta\) 2.48 (m, H-15) and \(\delta\) 2.60 (dd, J= 14.3, 6.2 Hz, H-15')] coupled to a methine (\(\delta\) 1.64-1.67, H-9), and an aromatic hydrogen (\(\delta\) 7.08, H-19) were present in the \(^1\text{H}\)NMR spectrum of 2.1. Resonances that could be assigned to a pentasubstituted benzene ring [\(\delta\) 114.9 (C-19), 118.1 (C-20), 130.0 (C-16), 140.5 (C-18), 143.5 (C-17), 149.5 (C-21)] and an ester carbonyl (\(\delta\) 168.2, C-22) were observed in the \(^{13}\text{C}\)NMR spectrum. The aromatic ring and ester functionalities accounted for 5 of the 8
required sites of unsaturation and the remaining 3 sites of unsaturation had to be rings. Structural features of 2.1 identified from the NMR data were similar to the structural components of (-)-smenodiol (2.6), previously isolated from the same species *D. elegans*, suggesting that the compounds were related and the $^{13}$C NMR data of 2.1 fit well for the decalin ring system. COSY correlations identified the spin system extending from the methylene protons at C-1 (δ 0.98 and 1.54) through to the methylene protons at C-3 (δ 1.14 and 1.40), and starting from H-5 (δ 0.99) through to H-7 (δ 1.39 and 2.51), from H-9 (δ 1.64-1.67) to H-15 (δ 2.48 and 2.60). This information suggested that 2.1 had the A/B rings of a drimane skeleton (Type C terpene skeleton in Figure 2.3). HMBC correlations observed between Me-11 (δ 0.84), Me-12 (δ 0.83) and C-5 (δ 57.1), C-3 (δ 42.5), C-4 (δ 33.1); between Me-14 (δ 1.04) and C-1 (δ 40.2), C-5 (δ 57.1), C-9 (δ 65.2), C-10 (δ 37.2); between Me-13 (δ 1.21) and C-7 (δ 36.5), C-8 (δ 48.5), C-9 (δ 65.2); between H-9 (δ 1.64-1.67) and C-8 (δ 48.5), C-10 (δ 37.2), C-15 (δ 24.3), C-13 (δ 19.9), C-14 (δ 16.3) confirmed the presence of the A/B ring system in 2.1.

The proton resonance at δ 7.08 (H-19) showed strong HMBC correlations to C-17 (δ 143.5), C-18 (δ 140.5), C-21 (δ 149.5) and C-22 (δ 168.2). This set of HMBC correlations confirmed the presence of a pentasubstituted benzene ring in 2.1. The Me-13 (δ 1.21) showed a strong HMBC correlation to C-21 (δ 149.5) indicating that the benzene ring C-21 was bonded to C-8. The benzylic methylene proton resonances at δ 2.48 (H-15ax) and 2.60 (H-15eq) showed
correlations to C-16 (δ 130.0) and C-21 (δ 149.5) establishing the connection of the drimane skeleton and the aromatic ring through the C-15 and C-16 bond to give the final ring required by the molecular formula of pelorol (2.1) and generating a constitution that was consistent with all of the spectroscopic data.

1D NOESY data provided evidence for the regiochemistry of the aromatic ring and the relative stereochemistry of pelorol (2.1). Correlations observed between Me-23 (δ 3.81) and H-7_{ax} (δ 1.38-1.40, m) and H-19 (δ 7.08) placed the methyl ester at C-20 and the single aromatic proton at C-19, leaving the two hydroxyls to be situated at C-17 and C-18. NOE correlations between Me-14 (δ 1.04), Me-13 (δ 1.21), Me-12 (δ 0.83), H-6_{ax} (δ 1.64-1.67, m), and H-15_{ax} (δ 2.48) are only possible if the decalin system of pelorol (2.1) adopts the fairly typical trans fused double-chair conformation and the drimane skeleton and the aromatic ring are connected by a trans fused five membered ring.
2.4. Total synthesis of pelorol

2.4.1 Introduction

Pelorol (2.1) is the first known SHIP activator. *In vitro* biological studies treating macrophages with lipopolysaccharide (LPS) in the presence or absence of pelorol showed that pelorol inhibited the release of the pro-inflammatory mediator TFNα production as efficiently as interleukin-10, a potent, physiological inhibitor of macrophage activation.

These exciting bioassay results prompted us to carry out further biological studies on this compound, but the limited quantity (8.9 mg) of pelorol (2.1) available from the source sponge *D. elegans* was inadequate to support a detailed *in vitro* and *in vivo* evaluation of its ability to activate SHIP. In order to satisfy the need for additional material, confirm the absolute configuration of the natural product, and generate analogs to flesh out the structure activity relationship for this family of anti-inflammatory compounds, the total synthesis of pelorol (2.1) and analogs, where the methyl ester at C-20 was replaced by methyl and ethyl residues, was undertaken.
2.4.2 Proposed biogenesis of pelorol

As mentioned above, pelorol (2.1) and its biogenetically related compounds smenodiol (2.6), smenospondiol (2.7), etc. might be derived from a common precursor, which is the bicyclic cationic intermediate 2.5. It is fairly common for nature to synthesize 2.5 from the linear polyene derivative, which could be the coupling product of the farnesyl diphosphate (FPP) and the shikimate unit.\textsuperscript{31,32} On the basis of this evidence, a biosynthetic pathway for pelorol was proposed as shown in Figure 2.10. In this proposal, the shikimate building block of the molecule, protocatechuic acid, was synthesized beginning with the coupling of phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate to give 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP), followed by elimination of phosphoric acid and an intramolecular aldol reaction to form the six-membered ring intermediate, 3-dehydroquinic acid. Dehydration of 3-dehydroquinic acid leads to 3-dehydroshikimic acid, which could lose another molecule of H\textsubscript{2}O and undergo enolization to give the protocatechuic acid.\textsuperscript{32} This simple phenolic acid could be alkylated at the position ortho to the hydroxyl group by a suitable alkylating agent, in this case farnesyl diphosphate. The newly introduced farnesyl group can then cyclize upon protonation of the terminal double bond to give the tertiary carbocation intermediate 2.5, which might undergo enzyme-catalysed Friedel-Crafts alkylation to give the meroterpenoid phenolic acid. O-methylation using SAM would lead to the natural product pelorol (2.1), though suspending the
phenolic acid in MeOH may give the same product. However, there was no evidence that pelorol (2.1) was an artifact formed during isolation and separation.

Figure 2.10 Proposed biogenesis of pelorol (2.1)
2.4.3 Proposed synthesis of pelorol

From the proposal for the biosynthesis of pelorol, it was natural to consider a biomimetic construction of this compound, although the question as to whether nature employed the polyene cyclization cascade in the biosynthesis of pelorol had not yet been experimentally answered. In spite of its productive and aesthetic appeal, the polyene cyclization approach would be difficult to execute in the laboratory. One of the anticipated difficulties was the nonenzymatic capture of the cationic intermediate 2.5 with a nucleophilic arene shown in Figure 2.10.

A recent publication by the Overman group (Figure 2.11) describing the enantioselective total synthesis of adociasulfate I was the only precedent that used an arene to terminate an epoxide-initiated polyene cyclization to afford a tetracyclic fragment similar to pelorol.\textsuperscript{33} In their early scouting studies, they found that treatment of A with a variety of Lewis acids under different conditions delivered only bi-, tri-, and tetracyclic products, not the desired pentacycle B. Although the transformation was eventually accomplished by addition of a

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{overman_study.png}
\caption{Overman's study on polyene tetracyclization}
\end{figure}
removable activating substituent at the 3'-position of the arene, the highest yield they could achieve was 15% after screening a range of Lewis acids and cyclization conditions. It seemed the polyene cyclization approach to pelorol was not suitable for large scale synthesis of the product and generating a variety of analogs with different functionalities on the arene ring, which would be highly important for a SAR study. A more succinct and conservative pathway for a synthesis of pelorol was, therefore, sought.

As shown in Figure 2.12, pelorol could be assembled in a convergent manner from two building blocks, the sesquiterpene and the arene. If sesquiterpene building block III (2.18 or 2.19) had a tertiary alcohol at C-8, which would act as the initiator of cyclization in the intermediate II upon treatment with Lewis acid, then the chirality of this alcohol was not important to the resulting carbocation I. On the basis of sound biogenetic arguments, Schmitz predicted that the absolute

![Figure 2.12 Retrosynthetic analysis of pelorol (2.1)](image)
configuration of pelorol was $5S,8R,9R,10S$. Therefore, a logical starting material for intermediate III would be (+)-sclareolide (2.20), which has the same absolute configurations at C-5, C-9, and C-10 as those predicted for pelorol.

Sclareolide is commercially available in gram quantities at an acceptable price. Vanillin was selected as the starting material for the arene unit. The key step in the convergent synthesis is the biomimetic carbocation-initiated cyclization of the intermediate I to generate the C-8/C-21 bond. Steric bulk associated with the C-14 axial methyl was expected to cause preferential approach of the phenyl ring from the bottom face of C-8, resulting in the formation of the required trans B/C ring fusion. Synthetic routes to both C-8 epimers of III starting from sclareolide have been reported by Quideau et al. in the synthesis of (+)-puupehenone and Chackalamannil et al in the preparation of wiedendiol. The plan was to examine both as intermediates in the preparation of direct precursors to the carbocation I and ultimately cyclized products.
2.4.4 Synthetic results

As presented in the retrosynthetic analysis of pelorol (Figure 2.12), the key intermediates were the drimane aldehydes 2.18 or 2.19. As already mentioned, aldehyde 2.18 has been prepared by Quideau et al. as a precursor in the synthesis of the antituberculosis marine sponge metabolite (+)-puupehenone.35

Following Quideau's procedure, commercially available (+)-sclareolide (2.20) was dissolved in a mixture of sulfuric acid and formic acid at room temperature for seven hours. These conditions brought about near quantitative inversion of the configuration at C-8. The ease of this reaction was due to the release of the 1,3-diaxial interaction between the 13- and 16-methyls in 2.20. The subsequent transformation required introduction of a hydroxyl functionality α to C-12 of 2.21.
This was accomplished by using Vedejs' MoO$_5$•pyridine•HMPA (MoOPH)-based protocol\textsuperscript{37} and the use of magnesium bis (dissopropylamide) [(DA)$_2$Mg] as a chelating agent.\textsuperscript{38} As described by Quideau \textit{et al.},\textsuperscript{35} along with the required α-hydroxylated product, a crystalline side-product \textbf{2.23} was also recovered after the standard workup conditions. The intermediate \textbf{2.22} was subjected to reduction by DIBAL-H at room temperature for 48 hours. After quenching the reaction slowly with dilute HCl at 0°C and workup, a mixture of lactol \textbf{2.25} and the desired triol \textbf{2.24} was obtained. The final step to generate the β-hydroxy drimane aldehyde \textbf{2.18} was realized by adding NaO$_4$ to the triol in THF/H$_2$O at 0°C for half an hour, which gives the oxidative cleavage product \textbf{2.18} in excellent yield. Of particular note is the oxidation of the side-product lactol \textbf{2.25} by NaO$_4$ in the last step, which led to the formate \textbf{2.26}. The formate was subsequently hydrolyzed by KOH to give the desired product \textbf{2.18}.

With the sesquiterpenoid unit of pelorol in hand, the next step was to synthesize the shikimate unit, which was elaborated from commercially available vanillin (\textbf{2.27}) (Figure \textbf{2.14}). As we have noted above, Overman had shown in his synthesis of adociasulfate that a highly nucleophilic arene was required to trap a carbocation such as \textbf{1} (Figure \textbf{2.12}) in preference to proton elimination to give an uncyclized olefin. Therefore, 1-bromo-2,3,5-trimethoxybenzene (\textbf{2.28}) was chosen as the appropriate shikimate unit to couple with the aldehyde \textbf{2.18}. Synthesis of the trimethoxyphenyl bromide \textbf{2.28} followed a known procedure by Furtser et al.,\textsuperscript{39} which started from the commercial product vanillin (\textbf{2.27}).
Vanillin was brominated regioselectively at the 5-position in methanol at 0°C by adding bromine slowly to the stirred solution to give compound 2.29. O-methylation of 2.29 by Mel in DMF gave quantitatively 6-bromoveratraldehyde (2.30), which was available commercially but is very expensive. Bayer-Villiger oxidation of 2.30 was carried out with mCPBA by refluxing in CH₂Cl₂ for 16 hours. Subsequent acid hydrolysis in aqueous MeOH for 30 minutes gave phenol 2.31. The phenol was O-methylated to give the trimethoxyphenyl bromide 2.28.

With both the sesquiterpenoid and aromatic units in hand, the way was cleared for the coupling reaction. nBuLi was slowly added to the trimethoxyphenyl bromide 2.28 in THF at -78°C. The mixture became a slurry after half an hour, which indicated completion of the halogen-metal exchange and formation of aggregates of trimethoxyphenyllithium 2.32. The drimane aldehyde 2.18 (0.5 equiv) was added to the slurry which was further stirred for 2 hours. After quenching with ice water, standard workup conditions followed by flash silica gel column chromatography afforded the diol 2.33. There are many methods
available for reducing benzylic alcohols to methylene groups. These fall into three general categories: i) dissolving metal reductions,\textsuperscript{40} ii) a mixture of Lewis acid (or protic acid) and a strong reducing agent,\textsuperscript{41} or iii) catalytic hydrogenation.\textsuperscript{42,43} Dissolving metal reduction was not suitable for selectively removing the benzylic alcohol of the diol 2.33, because the tertiary alcohol did not survive the reduction. The diol 2.33 was dissolved in triethylsilane, a hydride donor, and treated with TFA at $-78^\circ C$ for five minutes. TLC indicated that a less polar product formed, but subsequent NMR analysis revealed that instead of generating the desired reduction product 2.34, a retroaldol reaction product 2.35 was the only product. Changing the hydride donor to BH$_3$ or using a Lewis acid gave the same result. Sodium cyanoborohydride together with zinc iodide was reported to be an effective reducing combination for benzylic alcohols.\textsuperscript{44} However, when 2.33 was refluxed in dichloroethane containing sodium cyanoborohydride for 24 hours no trace of 2.34 was detected.

When all the other choices were exhausted, catalytic hydrogenation was explored as a method to obtain 2.34. Quideau \textit{et al.} \textsuperscript{35} successfully utilized
hydrogenolysis in removal of both benzyl protective groups and the benzylic hydroxyl group in their synthesis of puupehenone, providing a precedent that catalytic hydrogenation should be a feasible approach to 2.34. Diol 2.33 was dissolved in ethyl acetate containing 10% Pd on activated charcoal and hydrogenated at 45 psi in a Parr hydrogenator with agitation for 24 hours. TLC monitoring showed that all the starting material had disappeared. Subsequent NMR analysis confirmed that 2.34 was the only product. When the reaction was scaled up to 200mg, no reaction was observed. Elevating the pressure to 600 psi and extending the reaction time to 72 hours only led to the generation of a small amount (less than 5%) of the required product 2.34. Simply changing the catalyst from 10% Pd on activated carbon to 10% Pd on carbon successfully solved the problem. This result suggests that a trace amount of acidic impurity in the Pd on
carbon catalyst complexes with the benzylic hydroxyl group facilitating the breaking of the C-O bond and uptake of hydrogen. This was confirmed by carefully adding of microliter scale of conc. HCl to the hydrogenation reaction with 10% Pd on activated carbon as the catalyst, which gave the same hydrogenolysis product 2.34. It was found that 45 psi was the optimal pressure for the reaction. Higher pressures may lead to C-8 tertiary alcohol removal.

![Figure 2.17 Lewis acid catalyzed cyclization of 2.34](image)

Treatment of 2.34 with SnCl₄ in CH₂Cl₂ at −20°C for 30 minutes afforded the cyclization product 2.36 in good yield (78%). We anticipated that the steric repulsion of the C-14 angular methyl would lead to the phenyl ring attacking from the bottom of the C-8 tertiary cation to give a trans fused five-membered ring. The 14-CH₃ peak at δ1.06 (s, 3H) in ¹H NMR spectrum of the product suggested the trans-annulation nature of the B/C rings in 2.36 and the corresponding methyl in the cis-annulation product was supposed to shift noticeably upfield to around δ 0.40 due to the shielding effect of the axial phenyl ring. This result meant that construction of a trans fused five-membered ring containing two aromatic carbons via the route we adopted was possible and high yielding. Comparing compound 2.36 with our target molecule pelorol (2.1), it was not difficult to see
that if the C-20 methoxy group could be replaced by a carbonyl group, the synthesis of pelorol would not be far away. The complicating factor in this approach was that there were 3 methoxyls on the phenyl ring and selectively deprotecting the C-20 methoxyl followed by triflation and Stille coupling\textsuperscript{45} to introduce the carbonyl group would be a real challenge. Unfortunately, all our attempts to selectively demethylate the C-20 methoxyl failed.

In order to overcome this problem, the dimethoxyethylphenyl bromide 2.38 was chosen as the aromatic unit to couple with the drimane aldehyde 2.18. There were two reasons to choose 2.38 as the candidate: 1) the ethyl group could act as an electron donating group along with the methoxyls on the ring, though its electron donating potential was weaker compared with the methoxyl group; 2) the
ethyl could be oxidized to a carboxylic acid which differentiates it from the methoxyls on the phenyl ring.

Synthesis of 2.38\textsuperscript{46} started from the 6-bromoveratraldehyde (2.30), which was refluxed in THF with MePPh\textsubscript{3}Br under basic conditions for two hours until TLC analysis indicated that all of the starting material had been converted to a less polar product with a strong UV absorbance. The Wittig reaction gave high yield of styrene 2.37, that was light sensitive. In our experience, avoiding exposure to light during the synthesis and following workup steps was essential. Numerous heterogeneous catalysts, such as PtO\textsubscript{2}, Raney Ni, Rh/C and Pd/C, can catalyze the hydrogenation of olefins. However these catalysts generally do not effect hydrogenation of olefins with a high degree of chemical selectivity (e.g., other sensitive groups such as nitro, ketone, arylhalide, benzyloxy, etc. also are reduced). In the initial trial, we found that hydrogenation of the styrene derivative 2.37 using a Pd/C catalyst provided mainly the debrominated product with only a small amount of the desired product 2.38. Facile and highly selective reduction of the olefinic functionality occurred with no overreduction of the aromatic bromide when the hydrogenation catalyst was changed to 5\% Rh/C. Thus, styrene 2.37

![Figure 2.19 Synthetic route to 2.38](image)

was smoothly and completely hydrogenated with Rh/C in CH\textsubscript{2}Cl\textsubscript{2} to afford the
ethyl benzene 2.38, which could be used for the next step without further purification.

Under the same protocol used to generate 2.34, dimethoxyethylphenyllithium 2.39 was coupled with the drimane aldehyde 2.18 to give the diol 2.40, which was further reduced to the tertiary alcohol 2.41 with Pd/C.

![Figure 2.20 Synthetic route to 2.41](image)

Initial reactions of 2.41 with SnCl₄ gave variable yields of the desired product 2.42 and the undesired elimination product 2.43 (Figure 2.21). In order to prompt the cyclization of the side product 2.43 to give 2.42, the alkene was dissolved in benzene with TsOH and refluxed overnight. We anticipated that electrophilic addition of the hydrogen to the double bond of 2.43 could regenerate the tertiary carbocation 2.44, which would readily cyclize to afford the expected product 2.42 under the forcing conditions. However, contrary to our expectation the reaction gave only the unanticipated cyclization product 2.45. Treatment of the tertiary alcohol 2.41 under the same conditions or the protic acid PPA afforded the identical product 2.45 (Figure 2.22).
Figure 2.21 Synthetic route to 2.42 and 2.43

Figure 2.22 Synthetic route to 2.45
We envisaged that the formation of 2.43 and 2.45 resulted from reduction of the nucleophilicity of the arene (R= Et vs R= OMe) making the elimination reaction leading to 2.43 and the Wagner Meerwein rearrangements leading to 2.45 competitive with direct trapping of the C-8 carbocation by the arene to give the desired product 2.42. After optimization of the reaction conditions by testing different Lewis acids (BF$_3$-EtO$_2$, Sc(OTf)$_3$, SnCl$_4$, MeAlCl$_2$) at various temperatures (-78°C, -20°C, 0°C, r.t.), it was found that the SnCl$_4$-catalyzed cyclization (-20°C) gave consistently high yields (~76%) for the conversion of 2.41 to 2.42.

Although it was possible to obtain 2.42 via the drimane aldehyde 2.18, the preparation of 2.18 from sclareolide was cumbersome. It not only required multiple chromatographic separations, but also preparation of the oxidizing agent

![Figure 2.23 Proposed synthetic route to 2.42 via 2.19.](image-url)
MoOPH (MoO$_5$·Py·HMPA) that was not commercially available, because it was not stable at room temperature to light or moisture. Therefore, we turned our attention to the aldehyde 2.19 (Figure 2.23), having an equatorial OH group at C-8, since it could couple with the shikimate unit 2.39, and after hydrogenolysis give a tertiary alcohol 2.47, which was the diastereomer of 2.41. Brief exposure of 2.47 to SnCl$_4$ in CH$_2$Cl$_2$ would lead to the same tertiary carbocation intermediate 2.44 as that generated from 2.41, which we expected would undergo cyclization to give the desired product 2.42.

There were several synthetic routes in the literature for the synthesis of 2.19 starting from (+)-sclareolide. Chackalamannil et al. reported a three-step procedure to synthesize 2.19 with an overall yield around 64%. Their procedure, which included using (+)-(10-camphorsulfonyl)oxaziridine as the $\alpha$-hydroxylation agent, was not reproducible in our hands.

![Figure 2.24](image)

**Figure 2.24** Chackalamannil's approach to 2.19 from (+)-sclareolide.$^{36}$

Recently, Hua et al. reported a route similar to Chackalamannil's.$^{47}$ They simply changed the oxidizing agent from (+)-(10-camphorsulfonyl)oxaziridine to MoOPH, which led to very good yield of 2.19, but they did not circumvent the arduous task
of making MoOPH. A search of the literature revealed that Kuchkova et al. had described a short efficient synthesis of drimane diol 2.52 from (+)-sclareolide.\textsuperscript{48} We felt this could be exploited to generate the aldehyde 2.19. Following Kuchkova's procedure, the reaction of sclareolide (2.20) with methyllithium in a molar ratio 1:2 afforded the desired hydroxy ketone 2.48 in 40% yield. The diol 2.49 was produced as the major product in 51% yield, which was much higher than Kuchkova's report of 18% yield for this compound. Decreasing the amount of methyllithium to 1 equivalent effectively suppressed the dialkylation product 2.49, but also increased the amount of unreacted sclareolide (2.20). In order to
find the optimal ratio between sclareolide (2.20) and methyllithium, various amounts of methyllithium were added to the substrate on different time scales. Eventually it was found that 1:1.2 was the best ratio for sclareolide and methyllithium, giving a 91% of 2.48 and only a trace amount of 2.49. The reaction time was optimized to 30 minutes to assure the adequate transformation of 2.20.

Ketone 2.48 existed in equilibrium with its hemiacetal form 2.50. With a rather good yield of 2.48 in hand, purification was not necessary and the methylation product could be subjected to the next step after a simple workup which removed the base. The oxidation of 2.48 to afford 2.51 in quantitative yield was accomplished with an excess of trifluoroperacetic acid, prepared in situ from trifluoroacetic anhydride and 50% hydrogen peroxide, in dichloromethane in the presence of sodium hydrogen carbonate. The successful Baeyer-Villiger transformation of 2.48 to 2.51 was largely dependent on the addition of NaHCO₃ to the reaction mixture. As Kuchkova et al. have mentioned, the ratio of sodium hydrogen carbonate and trifluoroacetic anhydride [molar ratio of NaHCO₃ and (CF₃CO)₂O, ca. 1:1] was critical to assure a high yield of hydroxyl ester 2.51. If this ratio is more than one, the yield of 2.51 is lowered because of the simultaneous formation of hydroperoxide 2.53. In practice, adding NaHCO₃ to the reaction mixture at a lower rate and vigorous stirring was found to result in higher yields; ineffective stirring led to a sharp increase in the side product 2.53. Finally, the hydroxyl ester 2.51 was hydrolyzed in basic MeOH at 0°C for 30 minutes. Standard workup conditions followed by flash silica gel column chromatography
(hexane: EtOAc = 70:30) gave the drimane diol 2.52. Thus, sclareolide (2.20) was converted to the diol 2.52 in excellent yield (90%) using a three-step sequence modification of the literature procedure.

The subsequent transformation required oxidation of the resulting diol 2.52 to the aldehyde 2.19. This was accomplished by three different reactions: i) Corey's PCC (pyridinium chlorochromate) oxidation,\textsuperscript{49} ii) Dess-Martin periodinane oxidation,\textsuperscript{50} and iii) Swern oxidation.\textsuperscript{51} Of particular note was that all these oxidation reactions gave the elimination products 2.54 and 2.55 in various yields. PCC oxidation of 2.52 was quite clean in the reaction vessel, but after the reaction was quenched with ether and passed through a pad of Silica gel during workup, nearly 50% of the required product 2.19 lost H\textsubscript{2}O to give the elimination side products. Changing the oxidant to Dess-Martin periodinane didn't improve

\begin{figure}  
\centering  
\includegraphics[width=\textwidth]{synthetic_route.png}  
\caption{Synthetic route to 2.19.}  
\end{figure}
2.19

Figure 2.27 Synthetic route to pelorol analog 2.57

the yield of 2.19. Initial trials of the Swern oxidation gave a mixture of 2.19 and the other three elimination products in varied ratios. Monitoring the reaction by TLC and $^1$HNMR showed that the ratio of 2.19 and the side products was directly related to the volume of triethylamine added in quenching the reaction and the time required to warm the mixture from $-78^\circ$C to room temperature. It was found that a high yield of the reaction product 2.19 could be produced by addition of 5 equivalents of triethylamine to quench the reaction and reducing the time for warming to 20 minutes. Either increasing the volume of triethylamine or extending the time resulted in more elimination products.

With the aldehyde 2.19 in hand, it could be coupled with the dimethoxyethylphenyl lithium 2.39 as shown in Figure 2.27 to give the epimeric
benzyl alcohols 2.56 in good yield. Hydrogenolysis of 2.56 cleanly removed the benzyl alcohols to afford 2.47, which underwent SnCl4 catalyzed cyclization to give the desired tetracyclic intermediate 2.42 in high yield, without any trace of the elimination product 2.43. Dimethyl ether 2.42 was converted to the catechol 2.57, which differed from pelorol (2.1) in the C-20 substituent.

![Figure 2.28 Synthetic route to 2.58](image_url)

Figure 2.28 Synthetic route to 2.58

To provide more pelorol (2.1) analogs for preliminary SAR study, 2.19 was also coupled with dimethoxymethylphenylbromide 2.58 produced from reduction of 6-bromovertraldehyde (2.30) with NaCNBH3 and ZnI2, a powerful deoxygenation combination described above. The entire sequence was repeated with

![Figure 2.29 Synthetic route to 2.63](image_url)

Figure 2.29 Synthetic route to 2.63
phenyllithium 2.59. After hydrogenolysis, cyclization, and demethylation, the methyl analog 2.63 was obtained. Both compounds 2.57 and 2.63 could be regarded as simplified versions of pelorol (2.1) and the short and efficient syntheses of these compounds made them attractive tools for further biological studies and ideal candidates for future drug development.

The next critical transformation in the pelorol synthesis was the regioselective introduction of oxygen at the C-22 methylene of 2.42. Conventional regioselective oxidation of C-H bonds requires neighboring activating groups and the oxidation of benzylic methylenes is common in synthesis. The most successful reagents for this transformation are derivatives of selenium (IV)\textsuperscript{52} and chromium (VI).\textsuperscript{53} The recent development of IBX\textsuperscript{54} by K.C.Nicolaou as an oxidant for benzylic carbons and Ishii's aerobic oxidation method\textsuperscript{55} which uses NHPI analogs as key catalysts, have provided new tools for our arsenal. Both 2.42 and 2.62 were subjected to the standard IBX-induced oxidation condition \([2.5 \text{ equiv IBX, fluorobenzene/DMSO (2:1) at 65}^\circ\text{C}]\) and more forcing conditions, but unfortunately no reaction occurred. Changing to Ishii's mild aerobic oxidation condition also failed to yield the desired product. Compound 2.42 was then refluxed in ethanol overnight in presence of SeO\textsubscript{2}, and again only starting material was recovered. Gentle refluxing of 2.42 with PCC\textsuperscript{56} in CH\textsubscript{2}Cl\textsubscript{2} for 20 hours and standard workup and purification of the reaction mixture with column chromatography gave methyl ketone 2.67. The disappearance of the C-22 methylene signal and the appearance of a methyl ketone signal at \(\delta2.49\) ppm in
the $^1$H-NMR of 2.67 indicated the reaction had proceeded as desired. The mechanism for this reaction might be as shown in Figure 2.30. Chromium trioxide might first add to the arene in an “ene” reaction to yield the allylchromic acid 2.65, which can rearrange to the benzyl chromoxylate 2.66. Compound 2.66 can then cleave to give the oxidation product 2.67. Repeating the same reaction on the methyl analog 2.62 didn’t afford the expected C-22 aldehyde. To complete the synthesis of pelorol, it was now necessary to convert the methyl ketone into a carboxylic acid.
Treatment of methyl ketone \(2.67\) with \(I_2\) in aqueous \(\text{NaOH}\),\(^{57}\) in an attempt to effect a haloform reaction to give benzoic acid \(2.72\), unexpectedly resulted in the near quantitative formation of the \(\alpha\)-ketoacid \(2.70\). This anomalous result might be caused by the steric bulk of the C-8 carbon ortho to the C-22 ketone preventing the formation of a triiodinated methyl. If a diiodinated methyl ketone \(2.68\) was attacked by hydroxide to give a tetrahedral intermediate, the diiodomethyl may not be a good enough leaving group to depart in the normal fashion to give a carboxylic acid. Instead, an intramolecular \(S_N\)\(^2\) displacement of iodide can form an epoxide, which after fragmentation as shown in Figure 2.31 would lead to the \(\alpha\)-ketoaldehyde \(2.69\). Oxidation of \(2.69\) via iodination of the aldehyde hydrate can generate the final \(\alpha\)-ketoacid \(2.70\). Simply changing the

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**Figure 2.31 Synthetic route to 2.70 with proposed mechanism**
halogen to Br₂ led to a clean transformation of methyl ketone 2.66 to the desired benzoic acid 2.72 (Figure 2.32).

The synthesis of pelorol (2.1) was completed by esterification of 2.72 with Mel followed by selective cleavage of the phenyl methyl ethers with BBr₃ at -78°C. Efforts in selective demethylation of 2.73 with BBr₃ led to the free carboxylic acid 2.74. Synthetic pelorol (2.1) was identical by NMR and MS comparison with the natural product. The [α]₀ of the synthetic material was -64⁰ compared with values of -69⁰ reported by König and -71⁰ reported by Schmitz, confirming that the absolute configuration is 5S,8R,9R,10S as predicted by Schmitz.¹⁶a
Figure 2.33 Comparison of $^1$HNMR of natural and synthetic pelorol (2.1) recorded in CDCl$_3$ at 400MHz.
Figure 2.34 Comparison of $^{13}$CNMR of natural and synthetic pelorol (2.1) recorded in CDCl$_3$ at 100MHz.
2.5 Biological activities of pelorol and its analog AQX-16A (2.63)

Because of the promising biological properties of pelorol, we undertook its total chemical synthesis and successfully developed an efficient, high yielding, 12-step protocol for the synthesis of pelorol from the commercially available terpenoid sclareolide. This allowed the production of sufficient quantities for testing in biological assays. Additionally, certain intermediate compounds derived during the synthesis, as well as structural analogs of pelorol, were tested for their ability to activate SHIP's enzymatic activity. One of these, designated AQX-16A (compound 2.63 generated in 9 chemical steps from sclareolide in section 2.4 of this chapter) showed a 3-fold higher activation of SHIP than pelorol at the same molar concentration (Figure 2.35). Due to the relative ease of synthesis of AQX-16A and its enhanced potency, most of the subsequent studies were performed with AQX-16A.

(AQX-16A) was the designation give to compound 2.63 by our biological collaborators, G. Krystal, A.Mui and C.Ong. The biological data presented here was generated by Krystal, Mui and Ong.
Figure 2.36 AQX-016A stimulates SHIP’s enzyme activity in intact cells. J16 cells were treated for 30 min with 5 μg/mL AQX-016A, 25 μM LY294002 or carrier prior to stimulation with 50 ng/mL of LPS with or without 100 ng/mL IL-10, as indicated, for 15 min. Cellular lipids were extracted and analyzed for PIP₃ (panel a) and PI-3,4-P₂ (panel b) levels as described in Methods. The error bars indicate the SEM of three independent replicates for each stimulation condition and the experiment was repeated twice.

To be an effective drug candidate, AQX-16A needs to be cell permeable and able to activate SHIP in intact cells. To directly test whether AQX-16A was indeed able to activate SHIP’s enzyme activity in intact cells the inositol phospholipid content of macrophages stimulated with lipopolysaccharide (LPS) in the presence and absence of AQX-16A was measured. As shown in Figure 2.36a, LPS stimulated a 3-5 fold increase in PIP₃ levels, in keeping with the ability of LPS to activate the PI3K pathway. The addition of AQX-16A abolished this increase (Figure 2.36a) and resulted in a corresponding increase in the SHIP hydrolysis product PI-3,4-P₂ (Figure 2.36b). For comparison, LPS-stimulated cells were also treated with the PI3K inhibitor LY294002 and, as expected, PIP₃ levels were diminished without a corresponding increase in PI-
3,4-P$_2$ levels. Thus, both AQX-16A and LY294002 inhibit the PI3K-mediated increase in intracellular PIP$_3$ levels, but through different mechanisms.

An important property of any potential drug is its specificity: it must act via its designated target and alter biological responses in a predictable manner. A rigorous way to test the specificity of AQX-16A for its target SHIP is to compare its effects on SHIP-regulated processes in wild-type SHIP$^{+/+}$ vs SHIP$^{-/-}$ cells. In this regard, it has been previously shown that activation of macrophages by LPS$^{58,60}$, and mast cells by IgE receptor (FceRI) cross-linking$^{61-63}$ is negatively regulated by SHIP. Macrophages are an essential component of the innate and acquired immune response, and stimulation with LPS is associated with a PIP$_3$-

**Figure 2.37a,2.37b** AQX-016A inhibits activation of SHIP$^{+/+}$ but not SHIP$^{-/-}$ macrophages and mast cells.

In panel a, SHIP$^{+/+}$ (■) and SHIP$^{-/-}$ (□) BMMφs were pretreated with the indicated concentration of AQX-016A or carrier 30 min prior to stimulation with 10 ng/mL of LPS at 37°C. Supernatants were collected 1 hr later for TNFα determination by ELISA. Values were plotted as a percentage of maximum TNFα production for SHIP$^{+/+}$ and SHIP$^{-/-}$ cells (623 and 812 pg/ml, respectively). This experiment was repeated 3 times, the error bars indicate the SEM of triplicates. In panel b, SHIP$^{+/+}$ (■) and SHIP$^{+/+}$ (□) BMMφs were pretreated with the indicated concentration of LY294002 or carrier for 30 min prior to stimulation with 10 ng/mL of LPS at 37°C. Supernatants were collected 1 hour later for determination by ELISA and values were plotted as a percentage of maximum TNFα production for SHIP$^{+/+}$ and SHIP$^{+/+}$ cells (693 and 921 pg/mL, respectively). Error bars indicate SEM of triplicate determinations, this experiment was repeated twice.
dependent release of pro-inflammatory mediators such as TNFα. The action of AQX-16A on wild-type vs SHIP−/− bone marrow derived macrophages (BMMφs) was examined, and as shown in Figure 2.37a, AQX-16A was far more effective at inhibiting LPS-stimulated TNFα production in wild-type than in SHIP−/− cells, while as expected, LY294002 inhibited TNFα production equally well in both wild-type and SHIP−/− BMMφs (Figure 2.37b). Mast cells also play a key role in triggering inflammation and in initiating allergic responses. Activation of mast cells results in the release of intracellular granule contents (i.e., degranulation) and this is preceded by an influx of extracellular calcium that plays an essential
role in triggering the degranulation process. As shown in Figure 2.37c, AQX-16A inhibited the IgE + antigen-induced calcium influx to a substantially greater degree in wild-type than in SHIP⁺⁻ bone marrow derived mast cells (BMMCs). To rule out that this difference was not simply due to SHIP⁺⁻ BMMCs being less sensitive to PI3K inhibition, the effect of LY294002 on IgE + antigen-induced calcium influxes was tested in these two cell types and it was found that SHIP⁺/+ and SHIP⁺⁻ BMMCs were inhibited to a similar extent by this PI3K inhibitor (Figure 2.37d). These data indicate AQX-16A inhibits both macrophage and mast cell activation in a SHIP-dependent manner.

The downstream signaling proteins that are activated by PIP₃-dependent pathways in macrophages⁵⁸,⁶⁰ and mast cells⁶³-⁶⁵ have been well characterized.
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Figure 2.38 AQX-016A inhibits PI3K-dependent signaling in SHIP^{+/+} but not SHIP^{-/-} macrophages and mast cells. In panel a, SHIP^{+/+} and ^{-/-} BMmφs were pretreated for 30 min with AQX-016A or carrier prior to stimulation with 10 ng/mL of LPS for 15 min. In panel b, SHIP^{+/+} and ^{-/-} BMMCs were pre-loaded overnight with 0.2 μg/ml anti-DNP-IgE and then treated for 30 min with 10 μg/ml AQX-016A or carrier prior to stimulation with 20 ng/ml DNP-HSA for 5 min. Total cell lysates were analyzed for the indicated phospho-proteins or proteins by immunoblot analysis. These experiments were repeated three times.

Therefore, the ability of AQX-16A to inhibit the activation (as assessed by site specific phosphorylation) of these proteins in wild-type vs SHIP^{-/-} cells was compared. LPS stimulation of macrophages results in the activation of the PIP₃-dependent protein kinase PKB (also known as Akt) and the downstream kinase p38^{MAPK}. As shown in Figure 2.38a, AQX-16A inhibited the LPS-stimulated phosphorylation of both PKB and p38^{MAPK} in wild-type but not in SHIP^{-/-} BMmφs. FcεRI cross-linking in mast cells results in the PIP₃-dependent activation of the kinases PKB, p38^{MAPK}, and MAPK (Erk1/2)⁶³. As shown in Figure 2.38b, AQX-16A inhibited the activation of all of these proteins in wild-type but not in SHIP^{-/-}
BMMCs. Thus, in both cell types, AQX-16A inhibits PIP$_3$-regulated intracellular signal transduction events in a SHIP-dependent manner.

The litmus test for the clinical utility of any potential therapeutic is the ability to exert effects in animal models. Therefore, the in vivo efficacy of AQX-16A was evaluated in mouse models of septicemia and acute cutaneous anaphylaxis. In humans, septic shock$^{66}$ is a condition in which uncontrolled bacterial infection leads to the over-enthusiastic production of TNF$_\alpha$ and nitric oxide (NO). Death often ensues due to hypotension. The mouse model of this condition involves intraperitoneal injection of bacterial LPS (also known as endotoxin) and measurement of serum TNF$_\alpha$ levels 2 hrs later.$^{67}$ AQX-16A or the steroidal drug dexamethasone were orally administered to mice 30 min prior to the LPS challenge. As predicted for an activator of SHIP and an inhibitor of macrophage

![Graphs showing TNF$\alpha$ levels and right ear/left ear response]

Figure 2.39 AQX-016A is protective in mouse models of septicemia and acute cutaneous anaphylaxis. In panel a, mice were administered 20 mg/kg AQX-016A or 0.4 mg/kg dexamethasone orally 30 min prior to an intraperitoneal injection of 20 mg/kg LPS. Blood was collected 2 hrs later for TNF$_\alpha$ determination by ELISA. In panel b, 10 $\mu$g of AQX-016A or vehicle was applied to the right ears of DNP-sensitized mice 30 min prior to application of DNFB to both ears. The no DNFB group had DNFB applied only to the left ear. Each symbol indicates one mouse and data are representative of three independent experiments.
activation, AQX-16A reduced the level of serum TNFα, and did so to the same extent as dexamethasone (Figure 2.39a).

Anaphylactic or allergic responses on the other hand are mediated by allergen-induced degranulation of pre-sensitized mast cells. The mouse ear edema/cutaneous anaphylaxis model reflects this process and involves pre-sensitization of mice with the allergen dinitrofluorobenzene (DNFB) one week prior to elicitation of the allergic reaction by painting DNFB onto the ears of the mice. The efficacy of potential anti-inflammatory compounds is tested by topical application of the test substance to one ear and comparing the resulting ear edema or inflammation of the two ears. As shown in Figure 2.39b, AQX-16A dramatically inhibited allergen-induced anaphylaxis in this model. Thus AQX-16A is protective in both septicemia and allergy models, and is orally bioavailable.

The data presented above suggest that AQX-16A might be a useful therapeutic agent for the treatment of various inflammatory disorders. However, AQX-16A may also be useful for the treatment of hematologic malignancies. Loss of SHIP protein has been implicated in human chronic myelogenous leukemia (CML) and acute myelogenous leukemia (AML). The transforming oncogene in CML is the tyrosine kinase BCR-abl and expression of BCR-abl has been shown to mediate its oncogenic effects via activation of the PI3K pathway and intriguingly has been shown to directly downregulate SHIP levels. The BCR-abl inhibitor Gleevec
very efficiently inhibits BCR-abl kinase activity and achieves astonishing remission rates when administered to CML patients. However, there are side effects associated with this drug and Gleevec resistant cells eventually appear in the treated patients. Thus, a great deal of effort is currently focused on discovering new BCR-abl inhibitors which inhibit using different mechanisms or developing combination therapies with other drugs to circumvent the problem of Gleevec side effects and resistance. Drugs inhibiting the PI3K pathway have attracted particular attention for use in combination with BCR-abl inhibitors so
AQX-16A was tested for the ability to synergize with Gleevec in inhibiting BCR-Abl transformed cells. As predicted, it was found that addition of AQX-16A substantially enhanced the sensitivity of BCR-abl expressing Ba/F3 (Figure 2.40b) cells to Gleevec while having no activity on control Ba/F3 cells (Figure 2.40a).

In summary, pelorol (2.1) and analogs such as AQX-16A represent a new class of anti-inflammatory and anti-leukemic drugs which target and activate the negative regulator, SHIP. This work marks the first time that a small molecule activator of a phosphatase has been described. The restricted expression of SHIP to immune/hematopoietic cells limits the action of these compounds to these cells. The novel mechanism of action of these compounds creates new treatment options as well as provides potential synergies with existing therapeutics.
2.6 Experimental

**General:** All starting materials and reagents were obtained from commercial sources and were used without further purification. Diethylether (Et₂O) and tetrahydrofuran (THF) were purified by distillation from sodium/benzophenone under argon immediately before use. Dichloromethane was distilled from CaH₂ under argon. Moisture and oxygen sensitive reactions were carried out in flame-dried glassware under N₂ or argon. Evaporations were conducted under reduced pressure at room temperature. Analytical thin layer chromatography was performed on silica gel 60 F-254 pre-coated aluminum plates (Merck). Column chromatography was carried out on 70-230 mesh silica gel and 10g normal phase Sepaks with the indicated solvent. UV spectra were recorded on a Waters 2487 spectrophotometer. Optical rotations were determined with a JASCO J-1010 polarimeter equipped with a halogen lamp (589 nm) and a 10 mm micro cell. NOESY, HMQC, and HMBC spectra were recorded on a Bruker Avance 400 NMR spectrometer. ¹³C spectra and ¹H spectra were recorded on Bruker AM400, Bruker AMX500, and Bruker Avance 400 NMR spectrometers. Chemical shifts were referenced to solvent peaks (δₓ 7.24, δₓ 77.0 for CDCl₃). Low resolution ESI mass spectra were recorded on a Bruker Esquire LC mass spectrometer. High resolution ESI mass spectra were recorded on a Macromass LCT mass spectrometer. Both low and high resolution FABMS were recorded on a Kratos Concept II HQ mass spectrometer. HPLC separations were achieved using a Waters 600 pump and a Waters 486 tuneable absorbance detector.
Solvents were all HPLC grade (Fisher) and those used for HPLC were filtered prior to use.

**Preparation of 1-bromo-2,3,5-trimethoxybezene (2.28)**

![Chemical structure of 2.28](image)

**2.28** was synthesized according to the reported procedure.\(^{39}\)

**Preparation of 1-bromo-2,3-dimethoxy-5-ethyl benzene (2.38)**

![Chemical structure of 2.38](image)

The precursor of **2.38**, 1-bromo-2,3-dimethoxy-5-ethenyl benzene, was synthesized according to the literature procedure.\(^{46}\) The hydrogenation of 1-bromo-2,3-dimethoxy-5-ethenyl benzene (5.0 g, 20.4 mmol) was carried out overnight in CH\(_2\)Cl\(_2\) (15 mL) under H\(_2\) (3 bars) in the presence of 5% Rh/C (800mg). The mixture was filtered and then washed with acetone, and the filtrate and washings were evaporated and lyophilized to afford 4.84 g of **2.38** as a colorless oil (96%) which was not purified and could be used in the next step.
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$^1$H NMR (CDCl$_3$) $\delta$ 1.20 (t, J= 7.6 Hz, 3H), 2.55 (q, J= 7.6 Hz, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 6.66 (d, J= 1.83 Hz, 1H), 6.95 (d, J= 1.83 Hz, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.4, 28.5, 56.0, 60.5, 111.5, 117.3, 123.7, 141.4, 144.4, 153.4;

HRESIMS calcd for C$_{10}$H$_{14}$O$_2$Br 247.0157, found 247.0157.

Preparation of 1-bromo-2,3-dimethoxy-5-methyl benzene (2.58)$^{44}$

To a stirred solution of 5-bromoveratraldehyde (2.0 g, 8.7 mmol) in 1,2-dichloroethane (45.0 mL) at ambient temperature were added zinc iodide (3.9 g, 12.2 mmol) and sodium cyanoborohydride (3.8 g, 61.2 mmol). The reaction mixture was heated under gentle reflux for 20 hrs and then filtered through celite. The filtrate was evaporated to dryness to afford 1.5 g of 2.58 as a light yellow oil (80%).

$^1$H NMR (CDCl$_3$) $\delta$ 2.26 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 6.63 (d, J= 1.4 Hz, 1H), 6.92 (d, J= 1.4 Hz, 1H);
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\[ ^{13} \text{C NMR (CDCl}_3 \right) \delta 20.9, 56.0, 60.4, 112.6, 117.2, 124.9, 135.0, 144.2, 153.3; \]

HRESIMS calcd for C\(_9\)H\(_{11}\)O\(_2\)Na\(^{81}\)Br 254.9820, found 254.9830.

**Preparation of tertiary alcohol 2.41**

A 1.7 M solution of t-BuLi in pentane (1.74 mL, 2.79 mmol) was added slowly to a stirred solution of 2.38 (627.4 mg, 2.54 mmol) in dry THF (20.0 mL) at -78\(^0\)C. After stirring for 30 minutes, a solution of 2.18 (300mg, 1.26mmol) in dry THF (5.0 mL) was added. The mixture was further stirred at -78\(^0\)C for 2hrs. Then \( \text{H}_2\text{O} \) (10.0 mL) was added and the mixture was extracted with Et\(_2\)O (120mL twice). The combined Et\(_2\)O extracts were washed with saturated brine, dried (MgSO\(_4\)) and concentrated to give a residue, which was chromatographed on a normal phase Seppak (hexane:ethyl acetate 9:1) to give 306 mg (60%) of 2.40 as a white solid.

\[ ^1\text{H NMR (CDCl}_3 \right) \delta 0.87 (s, 3H), 0.88 (s, 3H), 1.03 (s, 3H), 1.21 (t, J= 7.6 Hz, 3H), 1.36 (s, 3H), 1.92 (brd, J= 12.5Hz, 1H), 2.59 (q, J= 7.7 Hz, 2H), 3.77 (s, 3H), 3.84 (s, 3H), 5.49 (s, 1H), 6.63 (d, J= 1.7Hz, 1H), 7.03 (d, J= 1.7 Hz, 1H); \]
Chapter 2: Synthesis of Pelorol and Analogs.

\[ { } \]

\[ ^{13} \text{C NMR (CDCl}_3 \text{)} \delta 15.7, 16.3, 18.4, 18.8, 21.8, 29.0, 32.8, 33.5, 33.8, 39.3, 39.9, 41.9, 44.1, 55.6, 55.7, 59.1, 60.0, 69.5, 75.4, 76.7, 77.0, 77.3, 110.0, 118.9, 139.2, 139.8, 143.3, 151.9; \]

HRESIMS calcd for C\(_{25}\)H\(_{40}\)O\(_4\)Na 427.2824, found 427.2828.

Hydrogenolysis of 2.40 (202 mg, 0.5 mmol) was carried out in EtOAc (8.0 mL) under H\(_2\) (3 bars) in the presence of 10 % Pd/C (150 mg) overnight. The reaction mixture was filtered and concentrated to afford a brown oil, which was further purified by column chromatography to give 2.41 (165 mg, 85%) as a colorless oil.

\[ ^{1} \text{H NMR (CDCl}_3 \text{)} \delta 0.85 (s, 3H), 0.87 (s, 3H), 0.90 (s, 3H), 1.06 (s, 3H), 1.21 (t, J= 7.6 Hz, 3H), 1.73 (dt, J= 13.1, 2.7 Hz, 1H), 1.79 (brd, J= 12.3 Hz, 1H), 2.57 (q, J= 7.5 Hz, 2H), 2.60 (dd, J= 15.7, 2.9 Hz, 1H), 2.91 (dd, J= 15.7, 7.3 Hz, 1H), 3.78 (s, 3H), 3.82 (s, 3H), 6.54 (d, J= 1.8 Hz, 1H), 6.63 (d, J= 1.8 Hz, 1H); \]

\[ ^{13} \text{C NMR (CDCl}_3 \text{)} \delta 15.2, 15.8, 18.5, 18.5, 21.9, 23.4, 28.9, 31.6, 33.4, 33.6, 39.0, 40.0, 42.0, 42.9, 55.6, 56.0, 58.9, 60.6, 73.3, 109.0, 120.5, 137.9, 139.7, 144.5, 152.4; \]

HRESIMS calcd for C\(_{25}\)H\(_{40}\)O\(_3\)Na 411.2875, found 411.2880.
Preparation of tetracycle 2.36

The procedure for the synthesis of the tertiary alcohol 2.34 from the diol 2.33 was the same as the synthesis of 2.41 from 2.40 and afforded a colorless oil (20mg, 60%). It was dissolved in 5mL of CH$_2$Cl$_2$ and SnCl$_4$ (0.05 mL) was added slowly during stirring at -20°C under argon for 2 min. The resulting mixture was further stirred for 20 min and then diluted with CH$_2$Cl$_2$ (10mL) and poured into ice. The aqueous phase was extracted with CH$_2$Cl$_2$ twice (10mL) and the combined extracts were washed with sat. NaHCO$_3$, sat. brine and dried over MgSO$_4$. Evaporation afforded 2.36 (14 mg, 70%) as a colorless oil.

$^1$H NMR (CDCl$_3$) δ 0.84 (s, 3H), 1.01 (s, 3H), 1.06 (s, 3H), 1.23 (s, 3H), 1.71 (dd, J= 12.8, 6.1 Hz, 1H), 2.40 (m, 1H), 2.50 (dd, J= 14.7, 12.9 Hz, 1H), 2.69 (dd, J= 14.7, 6.2 Hz, 1H), 3.74 (s, 3H), 3.76 (s, 3H), 3.82 (s, 3H), 6.28 (s, 1H);

$^{13}$C NMR (CDCl$_3$) δ 16.2, 18.4, 19.5, 20.7, 21.1, 25.9, 33.1, 33.4, 37.0, 38.4, 40.2, 42.6, 46.8, 55.9, 56.3, 57.5, 60.6, 64.5, 96.3, 134.3, 136.8, 139.8, 150.9, 150.9;

HRESIMS calcd for C$_{24}$H$_{36}$O$_3$Na 395.2562, found 395.2564.
Preparation of tetracycle 2.45

Tertiary alcohol 2.41 (100 mg, 0.26 mmol) was dissolved in 3.0 mL of polyphosphoric acid (PPA) and the mixture was heated under argon at 60°C for 30 minutes. The resulting orange mixture was diluted with ice water and extracted with ether. The combined extracts were washed with brine repeatedly and concentrated to give a brown oil, which was further purified by column chromatography to give 76.3 mg (80%) of 2.45.

$^1$H NMR (CDCl$_3$) $\delta$ 0.73 (s, 3H), 0.75 (s, 3H), 0.82 (s, 3H), 0.99 (d, J= 7.2 Hz, 3H), 1.21 (t, J= 7.5 Hz, 3H), 2.13 (d, J= 18.4 Hz, 1H), 2.61 (m, 2H), 3.08 (m, 1H), 3.20 (d, J= 18.4 Hz, 1H), 3.76 (s, 3H), 3.82 (s, 3H), 6.59 (s, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 14.8, 15.9, 20.6, 21.6, 24.4, 26.3, 27.3, 28.8, 30.5, 32.1, 32.9, 33.7, 34.7, 37.6, 38.2, 38.9, 42.1, 55.5, 59.8, 111.4, 130.2, 132.0, 138.1, 144.4, 149.4;

HRESIMS calcd for C$_{25}$H$_{38}$O$_2$Na 393.2770, found 393.2768.
Preparation of diol 2.56

The procedure for the synthesis of diol 2.56 from the aldehyde 2.19 and the dimethoxyethylithium 2.39 was the same as the synthesis of 2.40 from the aldehyde 2.18 and the dimethoxyethylithium 2.39 and afforded a white solid (63%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.28 (td, J= 13.1, 3.4 Hz, 1H), 0.76 (s, 3H), 0.81 (s, 3H), 1.03 (s, 3H), 1.21 (t, J= 7.6 Hz, 3H), 1.54 (s, 3H), 1.85 (dt, J= 12.2, 3.2 Hz, 1H), 2.11 (d, J= 8.4 Hz, 1H), 2.60 (q, J= 7.6 Hz, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 5.25 (d, J= 8.4 Hz, 1H), 6.65 (d, J= 1.7 Hz, 1H), 6.90 (d, J=1.7 Hz, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.7, 15.9, 18.4, 20.0, 21.7, 25.8, 28.9, 33.3, 33.6, 38.9, 40.0, 41.5, 44.7, 55.7, 56.0, 61.3, 62.7, 69.5, 74.7, 111.0, 119.6, 140.5, 140.6, 143.5, 152.3;

HRESIMS calcd for C$_{25}$H$_{40}$O$_4$Na 427.2824, found 427.2828.
Preparation of tertiary alcohol 2.47

The procedure for the synthesis of tertiary alcohol 2.47 from the diol 2.56 was the same as the synthesis of tertiary alcohol 2.41 from the diol 2.40 and afforded colorless oil (87%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.78 (s, 3H), 0.83 (s, 3H), 0.89 (s, 3H), 1.08 (td, $J$ = 13.3, 3.8Hz, 1H), 1.19 (t, $J$ = 7.6Hz, 3H), 1.26 (s, 3H), 1.82 (dt, $J$ = 12.5, 3.2Hz, 1H), 2.54 (q, $J$ = 7.6Hz, 2H), 2.81 (dd, $J$ = 14.5, 5.5Hz, 1H), 3.81 (s, 6H), 6.54 (d, $J$ = 1.8Hz, 1H), 6.64 (d, $J$ = 1.8Hz, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.4, 15.7, 18.6, 20.3, 21.6, 24.3, 25.3, 28.8, 33.3, 33.5, 39.4, 40.3, 41.8, 43.7, 55.6, 56.1, 60.6, 62.8, 73.7, 109.6, 122.0, 137.6, 140.0, 144.3, 152.3;

HRESIMS calcd for C$_{25}$H$_{46}$O$_3$Na 411.2875, found 411.2871.
Preparation of diol 2.60

The procedure for the synthesis of diol 2.60 from the aldehyde 2.19 and the dimethoxymethylithium 2.59 was the same as the synthesis of 2.40 from the aldehyde 2.18 and the dimethoxyethylithium 2.39 and afforded a white solid (61%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.27 (td, J= 12.8, 3.1 Hz, 1H), 0.75 (s, 3H), 0.80 (s, 3H), 1.02 (s, 3H), 1.53 (s, 3H), 1.84 (dt, J= 12.2, 2.9 Hz, 1H), 2.10 (d, J= 8.5 Hz, 1H), 2.31 (s, 3H), 3.82 (s, 3H), 3.84 (s, 3H), 5.26 (d, J= 8.5 Hz, 1H), 6.61 (d, J= 1.5 Hz, 1H), 6.89 (d, J= 1.5 Hz, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.8, 18.4, 20.0, 21.5, 21.6, 25.9, 33.2, 33.6, 38.8, 41.5, 44.6, 55.6, 55.9, 61.4, 62.6, 69.0, 74.7, 112.1, 120.5, 134.1, 140.7, 143.3, 152.2;

HRESIMS calcd for C$_{24}$H$_{30}$O$_4$Na 413.2668, found 413.2664
Preparation of tertiary alcohol 2.61

The procedure for the synthesis of tertiary alcohol 2.61 from the diol 2.60 was the same as the synthesis of tertiary alcohol 2.41 from the diol 2.40 and afforded a colorless oil (89%).

$^1$H NMR (CDCl$_3$) δ 0.78 (s, 3H), 0.83 (s, 3H), 0.88 (s, 3H), 1.27 (s, 3H), 1.76 (brd, J = 12.6 Hz, 1H), 1.82 (dt, J = 12.5, 3.2 Hz, 1H), 2.25 (s, 3H), 2.54 (dd, J = 14.5, 4.6 Hz, 1H), 2.78 (dd, J = 14.5, 5.6 Hz, 1H), 3.79 (s, 3H), 3.80 (s, 3H), 6.52 (d, J = 1.7 Hz, 1H), 6.62 (d, J = 1.7 Hz, 1H);

$^{13}$C NMR (CDCl$_3$) δ 15.4, 18.6, 20.3, 21.4, 21.6, 24.3, 25.0, 33.3, 33.5, 39.4, 40.3, 41.8, 43.7, 55.6, 56.1, 60.6, 62.8, 73.7, 110.8, 123.1, 129.6, 133.6, 137.7, 152.3;

HRESIMS calcd for C$_{24}$H$_{36}$O$_3$Na 397.2719, found 397.2713.
Preparation of tetracycle 2.42

To a stirred solution of 2.41 or 2.47 (38.8 mg, 0.1 mmol) in CH₂Cl₂ (10 mL), SnCl₄ (0.1 mL) was added slowly at -20°C under argon for 2 min. The resulting mixture was further stirred for 20 min and then diluted with CH₂Cl₂ (20 mL) and poured into ice. The aqueous phase was extracted with CH₂Cl₂ twice (20 mL) and the combined extracts were washed with sat. NaHCO₃, sat. brine and dried over MgSO₄. Evaporation afforded 29.5 mg (76%) of 2.42 as a colorless oil.

¹H NMR (CDCl₃) δ 0.85 (s, 6H), 1.02 (s, 3H), 1.08 (s, 3H), 1.22 (t, J= 7.6 Hz, 3H), 2.36 (dt, J= 12.0, 3.4 Hz, 1H), 2.49 (dd, J= 14.5, 13.0 Hz, 1H), 2.56 (dd, J= 14.5, 7.5 Hz, 1H), 2.69 (m, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 6.49 (s, 1H);

¹³C NMR (CDCl₃) δ 16.0, 16.1, 18.3, 19.7, 21.1, 21.3, 24.7, 25.2, 33.1, 33.4, 37.1, 39.3, 40.2, 42.5, 47.9, 55.9, 57.1, 60.4, 64.3, 111.1, 133.7, 135.8, 143.6, 144.8, 150.3;

HRESIMS calcd for C₂₅H₃₈O₂Na 393.2770, found 393.2768.
Preparation of tetracycle 2.62

The procedure for the synthesis of tetracycle 2.62 from the tertiary alcohol 2.61 was the same as the synthesis of tetracycle 2.42 from the tertiary alcohol 2.41 or 2.47 and afforded a colorless oil (85%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.85 (s, 6H), 1.02 (s, 3H), 1.06 (s, 3H), 2.24 (s, 3H), 2.32 (dt, J= 11.7, 3.1 Hz, 1H), 2.49 (dd, J= 14.8, 13.0 Hz, 1H), 2.69 (dd, J= 14.8, 6.1 Hz, 1H), 3.47 (s, 6H), 6.41 (s, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 16.1, 18.3, 18.6, 19.6, 20.5, 21.1, 25.3, 33.1, 33.4, 37.1, 38.8, 40.1, 42.5, 47.6, 55.9, 57.1, 60.4, 64.4, 113.0, 127.1, 135.8, 143.7, 145.2, 150.0;

HRESIMS calcd for C$_{24}$H$_{38}$O$_2$Na 379.2613, found 379.2614
Chapter 2: Synthesis of Pelorol and Analogs.

Preparation of catechol 2.57

To a stirred solution of 2.42 (38.6mg, 0.1 mmol) in CH$_2$Cl$_2$ (1 mL) under argon at 0°C, BBr$_3$ in CH$_2$Cl$_2$ (2.0 mL 1M) was added, and stirring was continued for 1.5 h at room temperature. The mixture was then poured into H$_2$O and extracted with CH$_2$Cl$_2$ (50 mL). The combined extracts were then dried over MgSO$_4$, filtered and concentrated. The residue was purified by flash chromatography (hexane:EtOAc=7:3) to afford 32mg (90%) of 2.57 as a white solid.

$^1$H NMR (CDCl$_3$) $\delta$ 0.85 (s, 6H), 1.02 (s, 3H), 1.20 (s, 3H), 2.35 (dt, J= 12.2, 3.4 Hz, 1H), 2.43 (dd, J= 13.9, 13.6 Hz, 1H), 2.57 (m, 2H), 2.61 (dd, J= 14.5, 7.3 Hz, 1H), 6.47 (s, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.9, 16.1, 18.3, 19.6, 21.0, 21.3, 24.1, 24.4, 33.0, 33.3, 37.0, 40.1, 42.4, 48.0, 57.0, 64.5, 113.5, 128.4, 130.8, 137.8, 141.3, 144.7;

HRESIMS calcd for C$_{23}$H$_{34}$O$_2$Na 365.2457, found 365.2456
Preparation of catechol 2.63

The procedure for the synthesis of catechol 2.63 from the tetracycle 2.62 was the same as the synthesis of catechol 2.57 from the tetracycle 2.42 and afforded a white solid (85%).

$^1\text{H NMR} \ (\text{CDCl}_3) \ \delta \ 0.85 \ (s, \ 6\text{H}), \ 1.02 \ (s, \ 3\text{H}), \ 1.04 \ (s, \ 3\text{H}), \ 1.74 \ (dd, \ J= 12.6, \ 6.4 \text{ Hz}, \ 1\text{H}), \ 2.17 \ (s, \ 3\text{H}), \ 2.31 \ (dt, \ J= 11.6, \ 3.0 \text{ Hz}, \ 1\text{H}), \ 2.43 \ (dd, \ J= 14.0, \ 12.5 \text{ Hz}, \ 1\text{H}), \ 2.57 \ (dd, \ J= 14.3, \ 6.4 \text{ Hz}, \ 1\text{H}), \ 6.40 \ (s, \ 1\text{H});$

$^{13}\text{C NMR} \ (\text{CDCl}_3) \ \delta \ 16.1, \ 18.2, \ 18.3, \ 19.5, \ 20.5, \ 21.1, \ 24.5, \ 33.0, \ 33.4, \ 37.0, \ 38.7, \ 40.0, \ 42.5, \ 47.8, \ 57.0, \ 64.6, \ 115.7, \ 124.1, \ 128.7, \ 138.0, \ 141.0, \ 145.2;$

HRESIMS calcd for $\text{C}_{22}\text{H}_{32}\text{O}_2\text{Na} \ 351.2300$, found 351.2303.
Preparation of methyl ketone 2.67

Pyridium chlorochromate (416mg, 1.92mmol) was added to 2.42 (74mg, 0.2mmol) dissolved in 20mL of CH₂Cl₂. The mixture was stirred at gentle reflux for 20 hours under argon. The reaction was diluted with Et₂O (80mL) and the resulting dark solution was filtered through a pad of silica gel. Concentration of the filtrates and further purification afforded 40.7 mg (53 %) of 2.67 as colorless oil.

¹H NMR (CDCl₃) δ 0.82 (s, 3H), 0.84 (s, 3H), 1.03 (s, 3H), 1.22 (s, 3H), 2.31 (dt, J= 12.5, 3.7Hz, 1H), 2.49 (s, 3H), 2.54 (dd, J= 14.9, 12.5Hz, 1H), 2.69 (dd, J= 14.9, 6.7Hz, 1H), 3.84 (s, 3H), 3.86 (s, 3H), 6.79 (s, 1H);

¹³C NMR (CDCl₃) δ 16.2, 18.3, 19.4, 20.5, 21.1, 25.2, 30.2, 33.1, 33.4, 36.4, 37.1, 40.2, 42.5, 48.0, 56.2, 57.2, 60.4, 64.9, 110.1, 130.5, 137.4, 147.6, 147.9, 149.6, 201.8;

HRESIMS calcd for C₂₅H₃₆O₃Na 407.2562, found 407.2563.
Preparation of α-Keto ester 2.71

\[
2.67 \text{ (15.0 mg, 0.04 mmol) was dissolved and stirred in 20.0 mL of NaOH (10\%) solution (containing 5.0 mL THF). 50 mg of iodine was added subsequently and the mixture was further stirred for 20 min and acidified by adding 30.0 mL of 10\% H}_2\text{SO}_4. \text{ The solution was extracted with 150.0 mL of Et}_2\text{O, washed with sat. brine and concentrated to afford a residue, which was dissolved in 10.0 mL of DMF. Potassium carbonate (0.5 g) and Mel (1.0 mL) were added to the mixture and stirring was continued overnight. The reaction was quenched by adding 10.0 mL of brine and the resulting mixture was extracted with Et}_2\text{O, washed with brine, dried with MgSO}_4 \text{ and evaporated to dryness. The residue was further purified by column chromatography to give 15.0 mg of 2.71 (95\%).}
\]

\[^1\text{H NMR (CDCl}_3\text{) } \delta \text{ 0.84 (s, 3H), 0.87 (s, 3H), 1.19 (s, 3H), 1.41 (s, 3H), 1.79 (dd, J= 11.9, 7.8 Hz, 1H), 2.41 (brd, J= 11.9 Hz, 1H), 2.74 (dd, J= 15.7, 12.5 Hz, 1H), 2.93 (dd, J= 15.7, 8.1 Hz, 1H), 3.81 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.93 (s, 1H);}\]
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\[ ^{13}\text{C NMR (CDCl}_3\) \delta 18.4, 18.5, 21.9, 24.2, 24.5, 30.1, 32.9, 33.5, 36.6, 36.7, 38.4, 42.4, 47.9, 48.1, 52.8, 53.4, 56.2, 60.4, 63.2, 114.1, 118.2, 125.3, 136.4, 149.4, 150.6, 164.6, 187.4; \]

HRESIMS calcd for C_{26}H_{35}O_{3}Na 451.2460, found 451.2457.

**Preparation of methyl ester 2.73**

![Molecular Structure of 2.73](attachment:image.png)

A solution of 0.21 g of sodium hydroxide in 2.0 mL of water was added 0.15 mL of bromine at 0°C over 10 minutes and 38.0 mg (0.1 mmol) of 2.67 in 1.0 mL of dioxane was added subsequently. After being stirred at 40°C for 30 minutes, the yellow mixture was quenched by sodium bisulfite. After acidification with 10% H$_2$SO$_4$, the mixture was extracted with Et$_2$O, washed with brine and dried to furnish a residue, which was dissolved in 10.0 mL of DMF. Potassium carbonate (1.0 g) and Mel (2.0 mL) were added to the mixture and stirring was continued overnight. The reaction was quenched by adding 20.0 mL of brine and the resulting mixture was extracted with Et$_2$O, washed with brine, dried with MgSO$_4$. 

and evaporated to dryness. The residue was further purified by column chromatography to give 32.0 mg of 2.73 (80%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.83 (s, 3H), 0.84 (s, 3H), 1.04 (s, 3H), 1.22 (s, 3H), 2.44 (dt, J = 12.3, 3.4Hz, 1H), 2.54 (dd, J = 14.3, 12.9Hz, 1H), 2.69 (dd, J = 14.3, 6.3Hz, 1H), 3.82 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 7.00 (s, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 16.3, 18.3, 19.5, 19.9, 21.1, 25.2, 33.1, 33.3, 36.5, 37.1, 40.2, 42.5, 48.1, 51.7, 56.1, 57.1, 60.4, 64.8, 111.6, 121.2, 137.0, 148.3, 148.7, 149.8, 168.3;

HRESIMS calcd for C$_{25}$H$_{36}$O$_4$Na 423.2511, found 423.2513.

Preparation of pelorol (2.1)

To a solution of 2.73 (5.0 mg, 0.013 mmol) in 2.0 mL of CH$_2$Cl$_2$ at -78°C under argon was added Bi$_3$ (60.0 mg) in 2.0 mL of CH$_2$Cl$_2$ slowly and the stirring was continued for 0.5 h. The mixture was then poured into H$_2$O and extracted
with CH$_2$Cl$_2$ (50.0 mL). The combined extracts were then dried over MgSO$_4$, filtered and concentrated. The residue was purified by flash chromatography to furnish 1 (2.3 mg, 50%).

$[\alpha]_D^{22.8} = -63.5$ (0.06, CHCl$_3$);

$^1$H NMR (CDCl$_3$) $\delta$ 0.83 (s, 3H), 0.84 (s, 3H), 0.97 (td, J= 12.8, 3.7 Hz, 1H), 1.04 (s, 3H), 1.21 (s, 3H), 1.66 (dd, J= 12.5, 6.4 Hz, 1H), 2.48 (dd, J= 14.6, 12.6 Hz, 1H), 2.51 (m, 1H), 2.60 (dd, J= 14.3, 6.4 Hz, 1H), 3.81 (s, 3H), 7.07 (s, 1H);

$^{13}$C NMR (CDCl$_3$) $\delta$ 16.3, 18.4, 19.5, 19.8, 21.1, 24.3, 33.0, 33.3, 36.5, 37.1, 40.2, 42.5, 48.5, 51.7, 57.1, 65.2, 114.8, 118.2, 130.0, 140.5, 143.4, 149.5, 168.0;

HRESIMS calcd for C$_{23}$H$_{32}$O$_4$Na 395.2198, found 395.2199.
In vitro SHIP enzyme assay. The SHIP enzyme assay was performed in 96-well microtitre plates with 10 ng of enzyme/well in a total volume of 25 μL of 20 mM Tris HCl, pH 7.5 and 10 mM MgCl₂. SHIP enzyme was incubated with extract or vehicle for 15 min at 23°C before the addition of 200 mM inositol-1,3,4,5-tetrakisphosphate (Echelon Biosciences Inc, Salt Lake City, Utah). The reaction was allowed to proceed for 20 min at 37°C and the amount of inorganic phosphate released assessed by the addition of Malachite Green reagent followed by an absorbance measurement at 650 nm.

Inositol phospholipid analysis. J16 cells, a macrophage cell line immortalized from C57Bl6 mice, were grown in 10% FCS in IMDM supplemented with 10 μM 2-mercaptoethanol, 150 μM monothioglycolate (MTG) and 1 mM glutamine. 5x10⁶ cells were plated the night before in 10 cm tissue culture dishes. The next day, cells were washed three times with phosphate-free medium before being starved in phosphate free RPMI (MP Biomedicals, Irvine, CA) supplemented with 10% dialyzed FCS (Invitrogen, Burlington, Ont) and 1% RPMI for 2 hrs. Cells were then labeled with 1.0 mCi of orthophosphate (MP Biomedicals, Irvine, CA)/ml for 2 hrs at 37°C. Cells were pretreated for 30 min with 2.63, LY294004 or vehicle prior to stimulation with LPS (50 ng/ml) for 15 min, or directly treated with LPS and IL-10 (100 ng/ml) for 15 min. Extraction of inositol phospholipids and HPLC analysis of deacylated lipids were performed as previously described. The amount of radioactivity contained in the elution peak for each lipid (two to five

The biological experiments presented here were carried out by our collaborators, G. Krystal, A.Mui and C.Ong.
fractions) was summed to give the total counts for each lipid, and data were normalized to the first 60 fractions to adjust for fluctuations in total lipid labeling and recovery between samples.

Production of SHIP^{+/+} and SHIP^{-/-} bone marrow derived mast cells (BMMCs) and macrophages (BMMφs). To obtain BMMCs, bone marrow cells were aspirated from 4 to 8 week old C57Bl6 x 129Sv mixed background mice and SHIP^{+/+} and SHIP^{-/-} BMMCs prepared as described previously. After 8 weeks in IMDM + 15% FCS (StemCell Technologies, Vancouver, Canada) + 150 μM MTG + 30 ng/ml IL-3 (BMMC medium) more than 99% of the cells were c-kit and FcεR1 positive as determined by flow cytometry with FITC-labeled anti-c-kit (BD Pharmingen, Mississauga, Canada) and FITC-labeled IgE (anti-Epo 26), respectively. BMMφs from SHIP^{+/+} and SHIP^{-/-} mice were obtained as described previously and maintained in IMDM supplemented with 10% FCS, 150 μM MTG, 2% C127 cell conditioned medium as a source of macrophage colony stimulating factor (M-CSF) (BMMφ medium)

LPS stimulation of BMMφs. For the analysis of LPS-stimulated TNFα production, 2 x10^5 cells were plated the night before in 24 well plates in BMMφ medium. The next day, the medium was changed and 2.63 or carrier was added to cells at the indicated concentrations for 30 min prior to the addition of 10 ng/mL LPS. Supernatants were collected for TNFα determination by ELISA (BD Biosciences, Mississauga, ON, Canada). For analysis of intracellular signaling, 2
x$10^6$ cells were plated the night before in 6 cm tissue culture plates. The next day, the cells were cultured in BMmφ medium without M-CSF for 1 hr at 37°C and then pretreated with 2.63 or carrier for 30 min prior to the addition of 10 ng/mL of LPS for 15 min. Cell lysates were rinsed with PBS and collected into lysis buffer (50 mM Hepes, 2 mM EDTA, 1mM NaVO$_4$ , 100 mM NaF, 50 mM NaPP$_i$ and 1%NP40) supplemented with Complete Protease Inhibitor Cocktail (Roche, Montreal, Canada). Lysates were rocked at 4°C for 30 min and clarified by centrifuging 20 min at 12000 x g. Lysates were then made 2 x in Laemmli’s buffer, boiled 2 min and loaded onto 7.5% SDS polyacrylamide cells. Immunoblot analysis for phospho PKB, phospho p38$^{\text{MAPK}}$ (Cell Signalling, Mississauga, Ont), SHIP$^{60}$ and actin (Santa Cruz, Santa Cruz, CA) were carried out as described previously.$^{60}$

**Stimulation of BMMCs by FceRI crosslinking.** SHIP$^{+/+}$ and SHIP$^{-/-}$ BMMCs were pre-loaded overnight in BMMC medium lacking IL-3 in the presence of 0.1 μg/ml anti-DNP IgE (Sigma, Oakville, Ont). For calcium flux measurements, the cells were incubated with 2 μM fura 2-acetoxymethyl ester (Molecular Probes, Eugene, OR) in Tyrode’s buffer$^{59}$ at 23°C for 45 min. The cells were then washed and incubated in the presence of vehicle control, LY294002 or 2.63 30 min prior to stimulation with the indicated concentration of DNP-human serum albumin (DNP-HSA). Calcium influx was then monitored by spectrofluorometry as described previously$^{59}$. For analysis of intracellular signaling, cells were pre-loaded with anti-DNP IgE as above, pre-treated with 2.63 or buffer control for 30 min at 37°C and stimulated with 20 ng/ml DNP-HSA for 5 min. Total cell lysates
were then prepared and analyzed for phospho-PKB, phospho-p38MAPK, phospho-MAPK, Grb-2 (Cell Signalling, Mississauga, Ont) and SHIP\textsuperscript{60} by immunoblot analysis as described.\textsuperscript{63}

**Mouse septicemia model.** 6-8 week old C57Bl6 mice (VCHRI Mammalian Model of Human Disease Core Facility, Vancouver, BC) were orally administered 20 mg/kg 2.63, 0.4 mg/kg dexamethasone or carrier 30 min prior to an intraperitoneal injection of 20 mg/kg of LPS (*E. Coli* serotype 0111:B4, Sigma, Oakville, Ont). Blood was drawn 2 hr later for determination of plasma TNF\textsubscript{\alpha} by ELISA.

**Mouse acute cutaneous anaphylaxis model.** 6-8 week old CD1 mice (University of British Columbia Animal Facility, Vancouver, BC) were sensitized to the hapten DNP by cutaneous application of 25 \(\mu\)L of 0.5% dinitrofluorobenzene (DNFB) (Sigma, Oakville, Ont) in acetone to the shaved abdomen of mice for two consecutive days. One week after the first application, 20 \(\mu\)Ci of tritiated thymidine ([\(\text{H}\)]-Tdr (GE Healthcare, Piscataway, NJ) was injected intra-peritoneally. 24 hr later, test substances (dissolved in 10 \(\mu\)L of 1:2 DMSO:MeOH) were painted on the left ear while the right ear received vehicle control. 30 min after drug application, DNFB was applied to both ears to induce mast cell degranulation. The resulting neutrophil infiltration was quantitated by taking a 6mm diameter punch from the ear 1 hr later for dissolution in Solvable (Perkin Elmer-Packard, Woodbridge, Ont) and scintillation counting. The ability of test substances to inhibit mast cell degranulation was then determined by
calculating the ratio of $[^3\text{H}]-\text{Tdr}$ in the test (right) ear vs the control (left) ear. One group of mice had DNFB applied only to the left ear leaving the right ear non-inflamed, in order to control for basal $[^3\text{H}]-\text{Tdr}$ incorporation into ear parenchymal cells.

**Effect of 48 on the proliferation of BCR-abl-expressing Ba/F3 cells.** The hematopoietic cell line Ba/F3 (control cells) or Ba/F3 cells expressing the oncogene BCR-Abl (BCR-abl cells)$^{75}$ were maintained in RPMI supplemented with 10% FCS and 1 $\mu$g/mL IL-3. To test the effect of 2.63 on cell proliferation, 2 $\times$ 10$^4$ cells/well were plated into 96 well round bottom microtitre plates with 1 $\mu$g/mL of 2.63 or carrier, the indicated concentrations of Gleevec in RPMI + 1 $\mu$g/mL IL-3 + 10% FCS. Cells were cultured for 18 hrs and pulsed with 1 $\mu$Ci/well of $^3\text{H}$-Tdr for 2 hrs and harvested for liquid scintillation counting.
2.7 References


Chapter 2: Synthesis of Pelorol and Analogs.


3.1 Introduction

A typical natural product drug discovery process starts from the identification of the bioactive sample with HTS (high-throughput screening), then the natural product is extracted from the source material, concentrated, fractionated and purified to yield essentially a single biologically active component. In those cases in which the biological activity profile of this component meets criteria for potency and selectivity, preliminary SAR studies are conducted. We have described our discovery and synthesis of the sponge meroterpenoid pelorol and its two analogs, AQX-16A and AQX-18A, which represent a new class of anti-inflammatory and anti-leukemic drugs that specifically target and activate the negative regulator, SHIP. The promising biological study results of AQX-16A, which produced a 3-fold higher activation of SHIP than the natural product pelorol at the same molar concentration, provides sound reasons to carry out further SAR research on pelorol. The goal of the study is to determine which functionality is essential for the activation of the SHIP enzyme and which part of the molecule is not important for the biological activity, thus simplifying the pharmacophore. More importantly, the rational generation of chemical diversity by synthesis of a library of pelorol analogs may lead to the discovery of more promising drug development candidates having improved potency and drug-like properties, essentially optimizing the pelorol drug lead.
As Koehn et al.\textsuperscript{1} mentioned in their paper, one of the distinguishing features of natural products is their frequent occurrence as suites or complexes of structurally related analogues. It might be the consequence of an organism's need to generate its own chemical diversity to optimize the activity of its secondary metabolites, essentially performing its own SAR optimization. There are two recent reports concerning the structure-activity relationship studies of marine sesquiterpenoid quinones based on the compound pool generated by marine organisms.\textsuperscript{2,3} Aoki et al. evaluated the differentiation-inducing activity to ATRA-nonresponsive chronic myelogenous leukemia cells (K562 cells) into erythroblasts of ten sesquiterpene quinones isolated from \textit{D.elegans} (Figure 3.1). The structure-activity relationship study of these compounds clarified that the quinone skeleton (a to h) is indispensable and the configuration at C-5 in the sesquiterpene fragment is not important for their differentiation-inducing activity to K562 cells.\textsuperscript{2} A similar investigation by Prokof'eva et al. on the SAR of some sesquiterpene quinones for cytotoxic and hemolytic activities, using developing
sea urchin eggs, Ehrlich carcinoma cells, and mouse red blood cells has shown that the quinone ring is essential for both cytotoxic and hemolytic activities and the structure of the terpenoid portions of the compounds have no significant influence on biological activity. Although the SAR studies of these sesquiterpene quinone/hydroquinone families on different cell lines can not be directly compared, there are some common structural features that are required for the biological activity in both of these series. First, the quinone or hydroquinone moiety is essential for activity and modifying the substitution pattern on this moiety has a major input on its activity. Second, the sesquiterpene skeleton is not important to activity.

In our synthesis of pelorol (Chapter 2), pelorol (2.1), dimethylpelorol 2.73, the analogs 2.57 and 2.63, the corresponding methyl ethers 2.42 and 2.62, the

![Figure 3.2 Preliminary SAR study of pelorol by evaluation of their ability to supress TNFα production in murine mast cells stimulated with IgE.](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>% max TNFα production</th>
</tr>
</thead>
<tbody>
<tr>
<td>no drug</td>
<td>110</td>
</tr>
<tr>
<td>Pelorol</td>
<td>90</td>
</tr>
<tr>
<td>AQX-16A</td>
<td>80</td>
</tr>
<tr>
<td>AQX-18A</td>
<td>70</td>
</tr>
</tbody>
</table>

Figure 3.2 Preliminary SAR study of pelorol by evaluation of their ability to supress TNFα production in murine mast cells stimulated with IgE.
trimethoxy pelorol analog \(2.36\), and the uncyclized precursor \(2.61\) were tested for \textit{in vitro} activation of SHIP and the ability to suppress degranualation and TNF\(\alpha\) production in murine mast cells stimulated with IgE (Figure 3.2). Synthetic pelorol \(2.1\), the ethyl analog \(2.57\), and the methyl analog \(2.63\) showed significant activity in all three assays, but the methyl ethers \(2.36, 2.42, 2.61, 2.62\) and \(2.73\) were inactive. The relative effectiveness of the active compounds in the SHIP activation assay was \(2.63 \gt 2.57 \approx 2.1\), showing that replacement of the methyl ester at C-20 in pelorol with a methyl group gives enhanced activity. Lack of activity in the dimethyl ethers \(2.62, 2.42\), and \(2.73\) demonstrates that at least one phenol is required for activity. Based on these preliminary SAR study results in combination with the previous SAR results on sesquiterpene quinones, we proposed that: i) the aromatic ring of pelorol would be important for its activity and changing the substitutions on the ring may significantly alter its activity, ii) the five-membered C ring should also play a significant role in its activity, opening of this ring would result in loss of activity, and iii) the A, B ring of pelorol would not have remarkable impact on its activity.

This chapter will be focused on answering the questions of how the functionality on the aromatic ring of pelorol affects its activity and whether the stereochemistry of the C-ring is important for the SHIP activating properties of pelorol.
3.2 Synthesis of C-8 and C-9 stereoisomers of pelorol

Biological systems are largely constructed from chiral molecules such as L-amino acids or D-sugars. It is then understandable that some drugs, which have a chiral center or centers exhibit a high degree of stereoselectivity in their interactions with biological macromolecules. Stereoselectivity in drug action at specific receptors with enzymes or ion channels, is well known. For example, the antihypertensive agent labetol is both an α- and β-adrenoceptor antagonist. The drug possesses two chiral centers and is used as a mixture of four diastereomers. The R, R form provides most of the β-blocking activity while the S, R diastereomer is an α-receptor antagonist. The S, S and R, S forms do not contribute significantly to the pharmacology of the drug.  

Although the mechanism of how pelorol activates the SHIP enzyme is still not clear, it seems reasonable to predict that the stereochemistry of pelorol may be an important factor in the interaction of pelorol with the enzyme. Pelorol has four stereogenic centers at C-5, C-8, C-9, and C-10 on the sesquiterpene skeleton. As we proposed above, varying the A, B ring stereochemistry of pelorol may not lead to a significant change in its activity and it is the C-8, C-9 stereochemistry that is likely most critical for its activity. Our interest was in how the stereochemistry of the C ring fusion affected pelorol's activity. To examine this
SAR issue, four stereoisomers, pelorol, 8-epi-pelorol, 9-epi-pelorol, and 8-epi,9-epi-pelorol, had to be synthesized, and their activity in the SHIP assay compared.

In our synthesis of pelorol, we synthesized an intermediate 3.1, which underwent a Nazarov cyclization reaction under acidic conditions at \(-78^\circ\text{C}\) as shown in Figure 3.3, to give the tetracycle 3.2 and trace amount of the C-8 epimer 3.2a. This result led us to postulate that varying the cyclization conditions, especially the reaction temperature, might increase the yield of the 3.2a epimer. Furthermore, we anticipated that the \(\Delta^9\) double bond of 3.2 and its epimer could be reduced to generate two of the four possible stereoisomers at C-8 and C-9. More importantly, 8-epi-pelorol, 9-epi-pelorol, and 8-epi,9-epi-pelorol could be synthesized in this approach by simply replacing the C-20 methoxyl with an ethyl group.

**Figure 3.3** Synthetic route to 3.2
The synthesis of pelorol stereoisomers started with diol 2.52. As mentioned in Chapter 2, all the oxidation conditions tried gave the elimination side products 2.54 and 2.55. To increase the yield of the desired enal 2.54, the volume of Et₃N used to quench the reaction was doubled and the duration of the Swern oxidation was extended from 20 minutes to 3 hours. As expected, the major product under these conditions was the desired enal 2.54 and the minor elimination product 2.55 could be converted to the thermodynamically favored product 2.54 by dissolving the alkene mixture in MeOH and treating with KOH at room temperature. Compared to the reported procedures for preparation of this key intermediate 2.54, the new synthetic route is superior in yield and succinctness.
With the enal **2.54** in hand, it was added to one equivalent of phenyl lithium **24** in THF at $-78^\circ$C. The mixture was stirred for 2 hours at $-78^\circ$C and then allowed to slowly warm to room temperature. TLC analysis indicated the disappearance of **2.54**, which was characterised by its strong UV absorbance, and two polar spots with weak UV absorbance were generated. Standard workup followed by normal phase chromatography (hexane : EtOAc = 93:7) yielded two epimeric benzylic alcohols **3.3a** and **3.3b** in a ratio of 8.4:1 (Figure 3.5). Initial attempts to acquire NMR data on these epimers failed due to their instability. It was found that the benzylic alcohols cyclized readily at room temperature in protic or acidic NMR solvents such as MeOD, CDCl$_3$, etc. C$_6$D$_6$ was tried but some of the aliphatic signals from **3.3a/b** were obscured by the solvent peak in the carbon spectrum. Finally, CD$_3$CN was chosen as a suitable solvent, because it didn’t cause the decomposition of **3.3a** and **3.3b** and all the signals were well resolved.

![Synthetic route to 3.4 and 3.5](image)

**Figure 3.6** Synthetic route to **3.4** and **3.5**
Treatment of 3.3a and 3.3b with SnCl₄ in CH₂Cl₂ resulted in cyclization to give a mixture of the C-8 epimers 3.4 and 3.5 in high yield (Figure 3.6). Assuming that the driving force for this cyclization is the formation of the stable indene structure within the tetracycle product, then the nucleophilicity of the arene is not so important to a successful cyclization. This explains why this acid-catalyzed cyclization is much easier to achieve than the acid-catalyzed Friedel-Crafts alkylicyclization that we utilized in the synthesis of pelorol. It is worth noting that the ratio of the cyclization products varied with the temperature used in the cyclization step. At -78°C, compound 3.4 was the major product, while at -20°C, a 6:4 mixture of 3.4 and 3.5 was formed. We envisioned that the steric bulk of the C-14 methyl played a major role in determining the ratio of isomers formed in the cyclization. At the lower temperature (-78°C), the steric strain between the C-14 axial methyl and the nucleophilic aromatic ring made attack of the arene from the bottom of C-8 predominant. The higher temperature (-20°C) would provide sufficient energy for the arene to overcome the steric hinderance, facilitating attack from the top face of C-8 resulting in a higher yield of 3.5. After passing through a normal phase Seppak, the mixture formed at -20°C was subjected to HPLC separation with a chiral column, eluted with hexane: EtOAc (97:3) to give pure samples of 3.4 and 3.5. The stereochemistries of 3.4 and 3.5 were confirmed by 2D NOESY experiments (Figure 3.7 and 3.8).
Figure 3.7 Expansion of the NOESY spectrum for 3.4
Figure 3.8 Expansion of the NOESY spectrum of 3.5
Catalytic hydrogenation of 3.4 over Pd/C in ethanol gave exclusively 3.6 in excellent yield, and under the same conditions compound 3.5 cleanly gave 3.7, also in high yield (Figure 3.9). The stereochemistry of catalytic hydrogenation of the cycloalkenes is strongly affected by the catalyst and slightly affected by the hydrogen pressure. A palladium catalyst was found to promote the generation of cis products in both reactions. Either low pressure (30 psi) or high pressure (600 psi) did not change the reaction outcome. Efforts to synthesize the C-9 epimer of 3.7, which was the precusor of 8-epi,9-epi-pelorol, by homogeneous catalytic hydrogenation using chiral catalyst (Wilkinson’s catalyst) did not afford the desired product. Reduction of 3.5 with sodium borohydride (NaBH₄) in the presence of nickel dichloride was attempted, but no reaction was observed. The stereochemistry of compound 3.6 was confirmed by a 2D-NOESY experiment (Figure 3.10), which showed strong correlations between the C-14 methyl (δ1.22)
Chapter 3: Structure Activity Relationship Study of Pelorol.

Figure 3.10 Expansion of the NOESY spectrum for 3.6
Figure 3.11 Expansion of the NOESY spectrum of 3.7
Figure 3.12 Synthetic routes to 9-epi-pelorol (3.10) and 8-epi-pelorol (3.13)
and C-15 methyl (δ 1.61) resonances and was devoid of correlations between the C-11 methylene (δ 2.66 and 2.90) and C-15, C-14 methyl resonances. The stereochemistry of 3.7 was also determined by a 2D-NOESY experiment (Figure 3.11) and the characteristic C-14 methyl chemical shift at δ 0.44, which moved upfield due to the shielding effect of the aromatic ring.

Following the same procedure used in the synthesis of pelorol, 3.6 and 3.7 were oxidized by PCC to give the corresponding ketones 3.8 and 3.11. A haloform reaction using Br₂/NaOH followed by methylation of the resulting acid with Mel afforded the methyl ethers 3.9 and 3.12. As in the synthesis of pelorol, the phenyl methyl ethers were selective removed by treating with Bi₃ in CH₂Cl₂ at −78°C to give the 9-epi-pelorol (3.10) and 8-epi-pelorol (3.13).
3.3 Synthesis of stereoisomers of 2.63 (AQX-16A)

Our synthesis of pelorol 2.1, the ethyl analog 2.57, and the methyl analog 2.63 which had significantly improved potency, answered the question of whether a methyl ester on C-20 was essential for activity. Furthermore, the synthesis of 8epi-pelorol 3.13 and 9epi-pelorol 3.10 was expected to reveal how the stereochemistries of C-8 and C-9 in pelorol affect its activity. Another question arises at this point, would the stereochemical changes at C-8 or C-9 and the functionality replacement at C-20 have a synergistic effect in enhancing activity or cancel each other out? To answer this question, both C-8 and C-9 stereoisomers of 2.63 were synthesized by adopting a similar synthetic route to the one used in the synthesis of 8-epi and 9-epi-pelorols.

Coupling the dimethoxymethylphenyllithium 2.59 with enal 2.54 yielded two epimers 3.14a and 3.14b in high yield with an isolated ratio of 8:2 (Figure 3.13). As with their ethyl analogs, 3.14a and 3.14b were quite unstable in protic
solvents or under acidic conditions. Therefore, CD$_3$CN was used as NMR solvent. Cyclization of 3.14a and 3.14b catalysed by SnCl$_4$ at $-20^\circ$C in CH$_2$Cl$_2$ afforded two epimers 3.15 and 3.16, in high yield, in a ratio of 1:1, which were separated by chiral HPLC. The same reaction run at $-78^\circ$C gave a mixture of
3.15 and 3.16 in a ratio of 8:2 (Figure 3.14). The stereochemistries of 3.15 and 3.16 were confirmed by 2D NOESY experiments. It was interesting to note that the proportion of the epimer 3.16 in the mixture was higher compared to its ethyl analog 3.5 under both conditions (–20°C and –78°C), which was probably due to less steric strain between the C-10 angular methyl and the C-20 aromatic methyl than the bulky ethyl in 3.5. Catalytic hydrogenation of 3.15 and 3.16 over Pd/C gave the corresponding cis products 3.17 and 3.18. Their stereochemistries were characterised by ¹H NMR and 2D NOESY experiments. Demethylation of 3.17 and 3.18 with BBr₃ at room temperature furnished the desired catechols 3.19 and 3.20 in excellent yield.
3.4 Synthesis of desmethyl-2.63 and its stereoisomers

The preliminary SAR study of pelorol showed that at least one phenol was required for activity and replacement of the methyl ester with methyl gave enhanced activity. This led us to ask whether the C-20 functionality was required for activity. To answer this question, desmethyl-2.63 (3.25) and its stereoisomers were synthesized.

![Chemical structures and reaction schemes](image)

Figure 3.15 Synthetic route to 3.25

One equivalent of aldehyde 2.19 was reacted with 2 equivalents of 3-lithioveratrole generated in situ by reaction of veratrole with butyllithium in the
Chapter 3: Structure Activity Relationship Study of Pelorol.

Figure 3.16 Synthetic route to 3.26a and 3.26b

Scheme 3.17 Synthetic route to 3.31 and 3.32
presence of TMEDA, to give the epimeric diols 3.22, which on hydrogenolysis with Pd/C in ethanol afforded 3.23 in 81% yield. Reaction of 3.23 with SnCl₄ in CH₂Cl₂ at -20°C furnished the tetracycle 3.24 nearly quantitatively. It was a surprise to find that one para methoxyl provided sufficient nucleophilicity in the arene to bring about the Friedel Crafts cyclization. This was in contrast to our initial expectation that both para and ortho activation were required for a successful cyclization. BBr₃ deprotected the methyl ether to give the desired catechol 3.25 (Figure 3.16).

Following the procedure used in the synthesis of 3.19 and 3.20, one equivalent of enal 2.54 was coupled with one equivalent of the 3-lithioveratrole 3.21 to give the two epimers 3.26a and 3.26b (8:2), which underwent cyclization to afford the epimers 3.27 and 3.28 in a ratio of 6:4 at -20°C. The two epimers were subsequently separated by chiral HPLC. Stereospecific hydrogenation of 3.27 and 3.28 led to the tetracycles 3.29 and 3.30, respectively, which after treatment with BBr₃ gave the desired catechols 3.31 and 3.32 in high yield.
3.5 Synthesis of a regioisomer of pelorol

As part of our efforts to synthesize a small library of pelorol analogs for SAR evaluation, we tried to synthesize regioisomers of pelorol that differed in the position of the C-20 methyl ester. In our model study to synthesize the regioisomer of 9-epi-pelorol 3.38 as shown in Figure 3.18, enal 2.54 was coupled...
with dimethoxyethyl-lithium 3.33, the regioisomer of 2.42. The coupling products 3.34a and 3.34b (9:1) were reacted with SnCl$_4$ in CH$_2$Cl$_2$ at $-20^\circ$C to give exclusively one epimer 3.35 as well as small amount of elimination side product 3.35a. This reaction outcome is different from all the cyclization results we obtained before, which may due to the decrease of nucleophilicity on C-21. Catalytic hydrogenation of 3.35 afforded the cis product 3.36 quantitatively. Unfortunately, 3.36 failed to undergo the desired oxidation reaction upon treatment with PCC and other oxidants to furnish 3.37.

![Figure 3.19 Model study in the synthesis of a pelorol regioisomer](image)

To circumvent this problem, 3.29 was treated with BuLi in TMEDA at $-78^\circ$C. After warming to room temperature for half an hour, the mixture was cooled down to $0^\circ$C and quenched with Br$_2$. However, the envisioned directed ortho metalation

![Figure 3.20 Model study in synthesis of pelorol regioisomer](image)
reaction proceeded with partial decomposition of the starting material 3.29 and no desired product 3.39 was recovered (Figure 3.19).

Since direct bromination of 3.29 with \( \text{Br}_2 \) in AcOH gave the undesired C-20 brominated product, catechol 3.31 was subjected to bromination (Figure 3.20). Bromine was added dropwise to catechol 3.31 dissolved in MeOH at 0\(^\circ\)C. The mixture was gradually warmed to room temperature and the reaction was monitored by TLC. After quenching the reaction mixture with \( \text{H}_2\text{O} \) and followed by standard workup, the \( ^1\text{H} \) NMR of the product showed the signal of the unreacted 3.31, characterised by the two doublets at \( \delta 6.50 \) (d, J= 7.9 Hz, 1H), 6.67 (d, J= 7.9 Hz, 1H) and some singlets around \( \delta 6.90 \), which might be the signals of 3.41 and 3.42. This reaction could be optimized to selectively forming 3.41 by using a literature procedure that utilizes \( \text{Br}_2 \) and t-butylamine. The synthesis of 3.41 would provide a practical approach to the desired pelorol isomer.
Three years after the first identification of pelorol by Schmitz's group, another meroterpenoid, akaol (3.43), was isolated by the same group from a marine sponge of the genus *Aka* collected at Yap Island, Federated States of Micronesia. Akaol was tested for activity in a CDK4/cyclin D1 assay at a concentration of 10 μg/mL, but was found to be inactive. However, the $8\alpha, 9\alpha$ stereochemistry of akaol's B/C ring fusion drew our interest because it shared the same $cis$ B/C ring fusion as the $8$-epi-pelorol that we had synthesized. Akaol
differed from 8-epi-pelorol in the C-20 substituent, which was a benzyl methyl ether. Though it was possible to prepare akaol by transforming the C-20 methyl ester in 8epi-pelorol to the methyl ether, we thought there was a shorter route to akaol by manipulation of the protecting groups on the aromatic portion of the starting material. Figure 3.21 shows our proposed synthetic route to akaol, which was initiated from the same terpene portion as we used in the synthesis of stereoisomers of pelorol. The aromatic portion 3.44 was the benzyl methyl ether with the two phenols protected by benzyl groups and anion generation on the ring was conducted in situ by lithium-halogen exchange. As with the many successful precedents, the coupled products 3.45a and 3.45b could cyclize under acidic conditions to give 3.46, though we might face the problem of separating 3.46 from its epimer. The advantage of this route is the last step where low pressure catalytic hydrogenation could be used to reduce the double bond and remove the benzyl protecting groups in one step. Though this approach seemed simpler than our synthesis of 8-epi-pelorol, there were a number of potential problems underlying this synthetic plan. For example, it was not clear that all the protecting groups would survive the cyclization conditions and what conditions would be suitable for 3.45a and 3.45b to cyclize. To answer these questions, a model study was carried out on 3.48 and 3.26.

\[\text{Figure 3.22 Synthetic route to 3.48}\]
Compound 3.48 (Figure 3.22) was synthesized from 5-bromoveratraldehyde 2.30 by demethylation with pyridine and AlCl₃ in refluxing CHCl₃ to expose the phenols,⁸ which were protected by benzyl groups under basic conditions to give 3.47. Reduction of 3.47 with NaBH₄ and methylation of the benzyl alcohol give model compound 3.48.

Figure 3.23 Model study of 3.48 under various cyclization conditions

As shown in Figure 3.23, 3.48 was exposed to various cyclization conditions. It was stable when treated with excess TsOH at 0°C or TFA at −20°C. At higher temperatures, treatment with TFA or with the Lewis acid SnCl₄ was found to remove the protecting groups on the phenols. Once the cyclization conditions suitable for the protecting groups were chosen, it was necessary to optimize the specific conditions for cyclization. 3.26a and 3.26b were selected as the substrates for this purpose. When 3.26a and 3.26b were treated with TsOH at 0°C in CH₂Cl₂, some rearrangement products were formed but no cyclization product was observed in the reaction mixture. 3.26a and 3.26b reacted with TFA at −20°C cleanly to give the epimers 3.27a and 3.27b (6:4). It was obvious that
TFA at $-20^\circ{\mathrm{C}}$ would be the optimal condition for the cyclization of $3.45\text{a}$ and $3.45\text{b}$.

\[ \text{OMe} \]  
\[ \text{(TsOH, CH}_2\text{Cl}_2 \]  
\[ 0^\circ{\mathrm{C}} \]  
\[ \text{No cyclization product} \]  
\[ \text{3.26a} \]  
\[ \text{3.26b} \]  

\[ \text{TFA, CH}_2\text{Cl}_2 \]  
\[ -20^\circ{\mathrm{C}} \]  
\[ \text{3.27a} \]  
\[ \text{3.27b} \]  

**Figure 3.24** Model study of $3.26\text{a}$ and $3.26\text{b}$ under various cyclization conditions

The enal $2.54$ was reacted with phenyllithium $3.44$ (Figure 3.21) in THF at $-78^\circ{\mathrm{C}}$ for two hours and warmed to $-20^\circ{\mathrm{C}}$ over a period of two hours. The reaction was quenched with $\text{H}_2\text{O}$ followed by standard workup. However, $^1\text{H}$ NMR indicated that the envisioned coupling reaction proceeded with unacceptably low yield. The

\[ i) \text{NaBH}_4, \text{EtOH, r.t.} \]  
\[ ii) \text{NaH, THF, r.t.} \]  
\[ 95\% \]  

**Figure 3.25** Synthetic route to $3.50$
Figure 3.26 Synthetic route to 3.51

Scheme 3.27 Synthetic route to akaol (3.43)
discouraging result of the coupling reaction may be explained by the shielding effect of the bulky benzyl protecting group ortho to the anion, which made the coupling difficult. To overcome this problem, substrate 3.50 was prepared in two steps from 2.30 (Figure 3.25). The coupling reaction between 3.50 and 2.54 proceeded smoothly to give a 41% yield of 3.51 (Figure 3.26).

Cyclization was realized with TFA at $-20^\circ$C to give epimers 3.52 and 3.53, as well as around 20% of the elimination product 3.54, which was presumably the result of decreased nucleophilicity of the arene with a benzylic methoxyl at C-20. 3.52, 3.53, and 3.54 were separated on a chiral HPLC column. 3.52 and 3.53 were subjected to catalytic hydrogenation using 5% Rh/C as catalyst to afford stereospecific products 3.55 and 3.56. The stereochemistries of 3.55 and 3.56 were confirmed by 2D NOESY experiments. Removing the methyls of 3.55 with BBr$_3$ at 0°C, followed by methylation at C-20 with TsOH in MeOH did not give the desired product akaol. Simply changing BBr$_3$ to Bf$_3$ and keeping the temperature at $-78^\circ$C furnished the demethylation product, which after remethylation in MeOH with TsOH gave akaol.
3.7 Biological Activities of Pelorol analogs and Discussion of SAR

3.7.1 *In vitro* activation of SHIP.

Pelorol (2.1), the stereoisomers 8-epi-pelorol (3.13) and 9-epi-pelorol (3.10), AQX-16A (2.63), desmethylpelorol (2.74), AQX-18A (2.57) were evaluated side by side for *in vitro* activation of SHIP (15 min, 30μM) as shown in Figure 3.28.

![Figure 3.28 In vitro SHIP activation activities of pelorol and its analogs](image-url)
AQX-16A (2.63) stands out as the most active compound in this bioassay. Pelorol (2.1), 9-epi-pelorol (3.10), AQX-18A (2.57), and desmethylpelorol (2.74) exhibited similar activity, which is weaker than that of 8-epi-pelorol (3.13).
3.7.2 Inhibition of LPS-stimulated TNFα production from J774 macrophage.

Pelorol (2.1), and its analogs were also tested on LPS stimulated TNFα production from J774 macrophage cells. The J774 assay can be used to assess the ability of test compounds to cross the cell membrane and to activate SHIP in intact cells. Test compounds were dissolved in cyclodextrin (CD) and then tested in the J774 assay at 2 μg/mL. Cells were stimulated with 10 ng/mL LPS in the presence of the compounds or DMSO (the same volume of DMSO diluted into CD as in the compounds) for 4 hours. Supernatants were collected and assayed for TNFα by ELISA.

Figure 3.29 Inhibition of LPS-stimulated TNFα production from J774 macrophage by pelorol and its analogs.
As shown in Figure 3.29, AQX-16A (2.63) has greater activity in inhibition of TNFα production than other analogs. Both pelorol (2.1) and AQX-18A (2.57) exhibited comparable activity to AQX-16A (2.63). It is worth noting that 9-epi-pelorol (3.10) shows similar activity to AQX-16A (2.63), however, 8-epi-pelorol (3.13) is inactive. This result is opposite to what we observed in the SHIP activation assay. The reciprocal biological activity of 9-epi (3.10) and 8-epi-pelorol (3.13) in two assays probably reflects 9-epi-pelorol has better cell permeability. The same tendency could be observed when comparing the three stereoisomers desmethyl-2.63 (3.25), 9-epi-desmethyl-2.63 (3.31) and 8-epi-desmethyl-2.63 (3.32). The relative activities of these compounds in the bioassay are $3.25 \approx 3.31 > 3.32$. The dimethylAQX-18A (2.57) didn’t show inhibitory
activity, which is in good agreement with the previous conclusion that at least one phenol group is required for activity. One exception in this assay is that the trimethoxy analog (2.36) shows somewhat inhibitory activity when dissolved into CD. Further work is being carried out to confirm this result.

3.7.3 Discussion of SAR.

On the basis of the structure activity relationship studies, the following evidence was obtained (Figure 3.30).

1) At least one phenol is required for activity. This was demonstrated by the prominent activity of pelorol (2.1), AQX-16A (2.63), AQX-18A (2.57) and lack of activity in the dimethyl ethers 2.73, 2.62, 2.42 (Figure 3.2).

2) The substituent at C-20 is important for activity. A distinct decrease of activity is observed when there is no substituent at C-20 of pelorol and 9epi-pelorol (Figure 3.29). The C-20 methyl analog AQX-16A (2.63) is the most active compound in all assays.

3) The stereochemistry at C-9 has no drastic effect on in vitro SHIP activation (Figure 3.28) and inhibition of TNFα production in intact cells (Figure 3.29).

4) The stereochemistry at C-8 is critical for activity in intact cells. All C-8 epi stereoisomers are less active than the C-9 epi stereoisomers in the J774 assay (Figure 3.29).
5) The five-membered C-ring is required for activity. The open ring compound is inactive (Figure 3.2).

Figure 3.30 Structure activity relationships of Pelorol (2.1)

3.8 Experimental

General: See chapter 2.
Preparation of enal (2.54)

To a solution of oxalyl chloride (1.4 mL, 15.8 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (50 mL) was added dropwise DMSO (1.3 mL, 18.9 mmol) at −78°C. After the mixture was stirred for 20 minutes at the same temperature, a solution of alcohol 2.52 (1.51 g, 6.3 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (2 mL) was added. The resulting reaction mixture was stirred for 40 minutes at −78°C and triethylamine (8.8 mL, 62.8 mmol) was added. The cold dewar was removed and the mixture was allowed to warm to room temperature and further stirred for 3 hours after which brine (50 mL) was added and the mixture was extracted with CH\textsubscript{2}Cl\textsubscript{2} (200 mL, twice). The combined CH\textsubscript{2}Cl\textsubscript{2} extracts were washed with H\textsubscript{2}O, dried (MgSO\textsubscript{4}), and concentrated to give a residue, which was dissolved in 20% KOH in MeOH at room temperature and stirred for another 20 minutes. After addition of H\textsubscript{2}O (80 mL), the mixture was extracted with Et\textsubscript{2}O (200 mL, twice). The combined Et\textsubscript{2}O extracts were washed with saturated brine, dried (MgSO\textsubscript{4}), and concentrated to give a brown residue, which was chromatographed on a normal phase silica gel column to give 1.18 g (85%) of 2.54 (light yellow oil). The spectral data of 2.54 were identical to reported values.\textsuperscript{5}
Preparation of benzyl alcohol (3.3a) and (3.3b)

A solution of 1.7 M tBuLi in pentane (3.48 mL, 5.58 mmol) was added slowly to a stirred solution of 2.38 (1.25 g, 5.08 mmol) in dry THF (40.0 mL) at -78°C. After stirring for 30 minutes, a solution of 2.54 (1.1g, 5 mmol) in dry THF (10.0 mL) was added. The mixture was stirred at -78°C for another 30 minutes and the cold dewar was removed to allow the mixture warm to room temperature. Stirring was continued for 2 hours after which H₂O (20 mL) was added and the mixture was extracted with Et₂O (120 mL, twice). The combined extracts were washed with brine, dried (MgSO₄), and concentrated to yield a residue. Silica gel column chromatography give 1.63 g (84%) of 3.3a and 0.2 g (10%) of 3.3b as colorless oils.

3.3a ¹H NMR (CD₃CN) δ 0.87 (s, 3H), 0.93 (s, 3H), 1.00 (s, 3H), 1.16 (t, J= 7.6 Hz, 3H), 1.33 (td, J= 13.0, 3.5 Hz, 1H), 1.43 (s, 3H), 1.64 (dt, J= 13.7, 3.4 Hz, 1H), 2.55 (q, J= 7.6 Hz, 2H), 3.65 (d, J= 3.7 Hz, 1H), 3.78 (s, 3H), 3.82 (s, 3H), 5.64 (d, J= 3.7 Hz, 1H), 6.73 (d, J= 1.8 Hz, 1H), 6.77 (d, J= 1.8 Hz, 1H)
Chapter 3: Structure Activity Relationship Study of Pelorl.

$^{13}$C NMR (CD$_3$CN) $\delta$ 16.3, 19.9 (C×2), 21.0, 22.2, 22.3, 29.6, 33.9, 34.2, 35.6, 37.9, 40.2, 42.5, 53.4, 56.4, 60.7, 68.7, 112.2, 120.6, 132.9, 138.8, 140.2, 141.0, 146.0, 153.4

HRESIMS calcd for C$_{25}$H$_{38}$O$_3$Na 409.2719, found 409.2715

3.3b $^1$H NMR (CD$_3$CN) $\delta$ 0.76 (td, J= 13.6, 4.0 Hz, 1H), 0.86 (s, 3H), 0.91 (s, 3H), 1.07 (td, J= 13.3, 3.7 Hz, 1H), 1.13 (s, 3H), 1.17 (t, J= 7.6 Hz, 3H), 1.34 (brd, J= 12.9 Hz, 1H), 1.65 (s, 3H), 1.73 (dd, J= 13.1, 7.2 Hz, 1H), 2.55 (q, J= 7.6 Hz, 2H), 3.28 (d, J= 3.5 Hz, 1H), 3.83 (s, 3H), 3.84 (s, 3H), 5.56 (d, J= 3.5 Hz, 1H), 6.70 (d, J= 1.7 Hz, 1H), 6.80 (d, J= 1.7 Hz, 1H)

$^{13}$C NMR (CD$_3$CN) $\delta$ 16.3, 19.6, 19.8, 20.3, 22.1, 22.9, 29.6, 33.7, 34.0, 35.4, 37.0, 39.8, 42.4, 52.8, 56.5, 61.1, 67.9, 112.9, 121.2, 132.8, 137.7, 140.3, 141.0, 146.5, 153.6

HRESIMS calcd for C$_{25}$H$_{38}$O$_3$Na 409.2719, found 409.2726
Preparation of indenes 3.4 and 3.5

To a stirred solution of 3.3a or 3.3b (98 mg, 0.25 mmol) in CH$_2$Cl$_2$ (25 mL), SnCl$_4$ (0.25 mL) was added slowly at -20°C under argon for 2 minutes. The mixture was further stirred for 5 minutes, and diluted with CH$_2$Cl$_2$ (40 mL) and then poured into ice water. The aqueous phase was extracted with CH$_2$Cl$_2$ twice (40 mL) and the combined extracts were washed with saturated NaHCO$_3$, H$_2$O, and dried (MgSO$_4$). Concentration of the CH$_2$Cl$_2$ gave a brown oil, which was passed through a normal phase Seppak eluted with Hexane : EtOAc (93:7) to give an yellow oil. The mixture was subjected to HPLC separation with a chiral column to afford 46 mg of 3.4 and 30 mg of 3.5 as colorless oils.

![Chemical structure of 3.4](image)

3.4 $^1$H NMR (CDCl$_3$) δ 0.83 (s, 3H), 0.91 (s, 3H), 1.21 (t, J= 7.6 Hz, 3H), 1.24 (s, 3H), 1.41 (s, 3H), 1.86 (brd, J= 11.7 Hz, 1H), 2.43 (dt, J= 12.8, 3.0 Hz, 1H), 2.70 (m, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 6.33 (s, 1H), 6.45 (s, 1H)

$^{13}$C NMR (CDCl$_3$) δ 16.3, 18.6, 19.2, 19.5, 21.6, 23.3, 24.5, 33.5, 33.7, 37.8, 38.5, 39.3, 42.2, 52.6, 56.0, 56.1, 61.2, 108.9, 114.1, 133.9, 135.9, 140.0, 144.3, 151.1, 168.3
HRESIMS calcd for C_{25}H_{37}O_{2} [M+H]^+ 369.2794, found 369.2794

3.5 $^1$H NMR (CDCl$_3$) $\delta$ 0.90 (s, 3H), 0.92 (s, 3H), 1.15 (s, 3H), 1.22 (t, J=7.5 Hz, 3H), 1.49 (s, 3H), 1.71 (dt, J= 13.5, 3.3 Hz, 1H), 2.01 (dd, J= 12.6, 5.8 Hz, 1H), 2.36 (m, 1H), 2.70 (m, 2H), 3.83 (s, 3H), 3.84 (s, 3H), 6.43 (s, 1H), 6.47 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 16.1, 17.6, 19.5, 21.5, 24.7, 25.5, 26.1, 28.0, 33.2, 33.7, 38.9, 40.3, 42.4, 45.2, 52.3, 56.0, 61.2, 108.5, 117.8, 133.6, 136.7, 139.7, 144.8, 151.2, 170.4

HRESIMS calcd for C_{25}H_{36}O_{2}Na 391.2613, found 391.2613
Preparation of tetracycle 3.6

Catalytic hydrogenation of 3.4 (25 mg, mmol) was carried out in EtOH (3.0 mL) under H₂ (45 psi) in the presence of 10% Pd/C (50 mg) overnight. The reaction mixture was filtered and concentrated to afford an oil, which was purified with normal phase Seppak to give 23 mg of 3.6 (90%) as a colorless oil.

¹H NMR (CDCl₃) δ 0.83 (s, 3H), 0.85 (s, 3H), 1.22 (t, J= 7.5 Hz, 3H), 1.22 (s, 3H), 1.61 (s, 3H), 1.68 (brd, J= 13.7 Hz, 1H), 1.81 (dd, J= 12.3, 8.2 Hz, 1H), 1.97 (brd, J= 11.1 Hz, 1H), 2.66 (dd, J= 15.7, 12.3 Hz, 1H), 2.54-2.77 (m, 2H), 2.90 (dd, J= 15.7, 8.1 Hz), 3.80 (s, 3H), 3.81 (s, 3H), 6.52 (s, 1H)

¹³C NMR (CDCl₃) δ 16.3, 18.4, 18.5, 21.9, 24.5, 25.2, 26.9, 30.1, 32.9, 33.5, 36.6, 37.6, 38.3, 42.4, 47.6, 47.9, 55.9, 60.3, 62.4, 112.3, 134.3, 135.0, 142.8, 144.2, 150.4

HRESIMS calcd for C₂₅H₃₈O₂Na 393.2770, found 393.2772
Preparation of tetracycle 3.7

The procedure for the preparation of tetracycle 3.7 from the indene 3.5 was the same as that used in the preparation of tetracycle 3.6 from the indene 3.4, and afforded 21 mg of 3.7 (87%) as a colorless oil.

$^1$H NMR (CDCl$_3$) δ 0.44 (s, 3H), 0.77 (s, 3H), 0.86 (s, 3H), 0.96 (dd, J= 11.3, 4.1 Hz, 1H), 1.19 (s, 3H), 1.22 (t, J= 7.6 Hz, 3H), 2.49 (dt, J= 14.2, 5.3 Hz, 1H), 2.56-2.74 (m, 2H), 2.75 (brd, J= 16.9 Hz, 1H), 2.86 (dd, J= 16.9, 7.6 Hz, 1H), 3.78 (s, 3H), 3.81 (s, 3H), 6.47 (s, 1H)

$^{13}$C NMR (CDCl$_3$) δ 15.5, 16.0, 18.5, 19.8, 21.9, 25.4, 28.3, 32.1, 33.2, 33.4, 35.3, 37.3, 41.0, 42.1, 48.8, 52.7, 55.8, 59.9, 62.7, 111.8, 134.2, 137.3, 141.4, 142.4, 150.5

HRESIMS calcd for C$_{25}$H$_{38}$O$_2$Na 393.2770, found 393.2773
Preparation of methylketone 3.8

The procedure for the preparation of methylketone 3.8 from the tetracycle 3.6 was the same as that used in the preparation of methylketone 2.67 from the tetracycle 2.42, and it afforded 11 mg of 3.8 (53%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.83 (s, 3H), 0.87 (s, 3H), 1.18 (s, 3H), 1.35 (s, 3H), 1.75 (dd, J= 11.9, 8.2 Hz, 1H), 2.28 (m, 1H), 2.53 (s, 3H), 2.74 (dd, J= 15.5, 12.3 Hz, 1H), 2.90 (dd, J= 15.5, 7.9 Hz, 1H), 3.83 (s, 3H), 3.86 (s, 3H), 6.75 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 18.4, 18.6, 21.9, 24.2, 24.6, 29.9, 30.9, 32.9, 33.5, 36.6, 37.7, 38.5, 42.4, 47.6, 47.9, 56.3, 60.3, 63.3, 110.7, 132.9, 136.0, 146.3, 147.0, 149.6, 203.6

HRESIMS calcd for C$_{25}$H$_{36}$O$_3$Na 407.2562, found 407.2569
Preparation of methylketone 3.11

The procedure for the preparation of methylketone 3.11 from the tetracycle 3.7 was the same as that used in the preparation of methylketone 2.67 from the tetracycle 2.42, and it afforded 7 mg of 3.11 (47%) as a colorless oil.

\[^1\text{H} \text{NMR (CDCl}_3\text{)} \delta 0.28 (s, 3H), 0.71 (s, 3H), 0.84 (s, 3H), 1.29 (s, 3H), 1.65 (d, J= 7.0 Hz, 1H), 1.71 (brd, J= 12.9 Hz, 1H), 2.27 (m, 1H), 2.54 (s, 3H), 2.76 (d, J= 16.7 Hz, 1H), 2.90 (dd, J= 16.7, 7.0 Hz, 1H), 3.82 (s, 3H), 3.84 (s, 3H), 6.66 (s, 1H)\]

\[^{13}\text{C} \text{NMR (CDCl}_3\text{)} \delta 15.3, 18.4, 19.6, 22.1, 28.3, 31.1, 32.6, 33.0, 33.6, 34.2, 37.2, 40.7, 42.0, 48.4, 53.4, 56.1, 60.0, 63.3, 109.3, 132.3, 139.1, 142.3, 146.3, 150.0, 204.2\]

HRESIMS calcd for C\text{_{25}H\text{_{36}O\text{_{3}Na}}} 407.2562, found 407.2570
Preparation of methylester 3.9

The procedure for the preparation of methylester 3.9 from the methylketone 3.8 was the same as that used in the preparation of methylester 2.73 from the methylketone 2.67, and it afforded 5 mg of 3.9 (64%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.84 (s, 3H), 0.87 (s, 3H), 1.19 (s, 3H), 1.42 (s, 3H), 1.77 (dd, J = 11.9, 7.6 Hz, 1H), 2.36 (m, 1H), 2.73 (dd, J = 15.5, 12.2 Hz, 1H), 2.90 (dd, J = 15.5, 8.1 Hz, 1H), 3.82 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 6.98 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 18.4, 18.6, 21.9, 24.2, 24.7, 29.8, 32.9, 33.5, 36.6, 36.6, 38.4, 42.4, 47.5, 47.9, 51.9, 56.1, 60.3, 63.4, 112.5, 123.0, 135.6, 147.4, 147.4, 149.9, 169.3

HRESIMS calcd for C$_{25}$H$_{36}$O$_4$Na 423.2511, found 423.2513
Preparation of methylester 3.12

The procedure for the preparation of methylester 3.12 from the methylketone 3.11 was the same as that used in the preparation of methylester 2.73 from the methylketone 2.67, and it afforded 3 mg of 3.12 (71%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.27 (s, 3H), 0.70 (s, 3H), 0.84 (s, 3H), 1.31 (s, 3H), 1.67 (d, $J=7.2$ Hz, 1H), 1.71 (brd, $J=12.8$ Hz, 1H), 2.37 (dt, $J=14.5$, 4.0 Hz, 1H), 2.77 (d, $J=16.6$ Hz, 1H), 2.89 (dd, $J=16.6$, 7.2 Hz, 1H), 3.82 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 6.89 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.3, 18.4, 19.7, 22.0, 28.3, 31.7, 33.0, 33.6, 34.2, 37.2, 40.8, 42.0, 48.4, 51.9, 53.3, 56.0, 60.0, 63.4, 111.4, 122.5, 138.8, 143.8, 146.8, 150.2, 169.6

HRESIMS calcd for C$_{25}$H$_{36}$O$_{4}$Na 423.2511, found 423.2505
Preparation of 9-epi-pelorol (3.10)

The procedure for the preparation of 9-epi-pelorol (3.10) from the methylester 3.9 was the same as that used in the preparation of pelorol (2.1) from the methylester 2.73, and it afforded 1 mg of 3.10 (38%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.84 (s, 3H), 0.87 (s, 3H), 1.19 (s, 3H), 1.43 (s, 3H), 1.81 (dd, J = 12.0, 8.5 Hz, 1H), 2.42 (brd, J = 11.7 Hz, 1H), 2.70 (dd, J = 15.4, 12.2 Hz, 1H), 2.82 (dd, J = 15.4, 8.4 Hz, 1H), 3.82 (s, 3H), 7.03 (s, 3H)

HRESIMS calcd for C$_{23}$H$_{32}$O$_4$Na 395.2198, found 395.2202
Preparation of 8-epi-pelorol (3.13)

The procedure for the preparation of 8-epi-pelorol 3.13 from the methylester 3.12 was the same as that used in the preparation of pelorol (2.1) from the methylester 2.73, and it afforded 0.8 mg of 3.13 (42%) as a colorless oil.

\[ ^1H \text{NMR (CDCl}_3) \delta 0.31 (s, 3H), 0.72 (s, 3H), 0.84 (s, 3H), 1.32 (s, 3H), 2.39 (dt, J= 14.3, 4.3 Hz, 1H), 2.67 (d, J= 16.0 Hz, 1H), 2.84 (dd, J= 16.0, 7.4 Hz, 1H), 3.83 (s, 3H), 6.97 (s, 3H) \]

HRESIMS calcd for C\textsubscript{23}H\textsubscript{32}O\textsubscript{4}Na 395.2198, found 395.2205
Preparation of benzyl alcohol 3.14a and 3.14b

A solution of 1.7 M tBuLi in pentane (1.16 mL, 1.86 mmol) was added slowly to a stirred solution of 2.38 (0.42 g, 1.70 mmol) in dry THF (17.0 mL) at -78°C. After stirring for 30 minutes, a solution of 2.54 (367 mg, 1.67 mmol) in dry THF (4.0 mL) was added. The mixture was stirred at -78°C for another 30 minutes and the cold dewar was removed to allow the mixture warm to room temperature. Stirring was continued for 2 hours after which H₂O (10.0 mL) was added and the mixture was extracted with Et₂O (40 mL, twice). The combined extracts were washed with brine, dried (MgSO₄), and concentrated to yield a residue. Silica gel column chromatography give 417 mg of 3.14a (67%) and 104 mg of 3.14b (17%) as colorless oils.

3.14a ¹H NMR (CD₃CN) δ 0.87 (s, 3H), 0.93 (s, 3H), 1.01 (s, 3H), 1.42 (s, 3H), 2.25 (s, 3H), 3.61 (d, J= 3.7 Hz, 1H), 3.76 (s, 3H), 3.80 (s, 3H), 5.61 (d, J= 3.7 Hz, 1H), 6.71 (d, J= 1.2 Hz, 1H), 6.74 (d, J= 1.2 Hz, 1H)
$^{13}$C NMR (CD$_3$CN) $\delta$ 19.9, 20.0, 20.9, 21.6, 22.2, 22.3, 33.9, 34.2, 35.7, 37.9, 40.2, 42.4, 53.4, 56.4, 60.7, 68.5, 113.2, 121.7, 132.9, 133.7, 138.9, 141.0, 145.8, 153.3

HRESIMS calcd for C$_{24}$H$_{36}$O$_3$Na 395.2562, found 395.2560

3.14b $^1$H NMR (CD$_3$CN) $\delta$ 0.77 (td, J= 12.9, 3.4 Hz, 1H), 0.86 (s, 3H), 0.91 (s, 3H), 1.13 (s, 3H), 1.22 (dd, J= 12.3, 1.5 Hz, 1H), 1.65 (s, 3H), 1.73 (dd, J= 12.9, 6.9 Hz, 1H), 2.25 (s, 3H), 3.27 (d, J= 3.5 Hz, 1H), 3.81 (s, 3H), 3.83 (s, 3H), 5.55 (d, J= 3.5 Hz, 1H), 6.66 (d, J= 1.7 Hz, 1H), 6.78 (d, J= 1.7 Hz, 1H)

$^{13}$C NMR (CD$_3$CN) $\delta$ 19.6, 19.8, 20.3, 21.6, 22.1, 22.9, 33.7, 33.9, 35.3, 36.9, 39.7, 42.3, 52.6, 56.4, 61.1, 67.8, 113.9, 122.3, 132.7, 133.8, 137.7, 140.9, 146.4, 153.5

HRESIMS calcd for C$_{24}$H$_{36}$O$_3$Na 395.2562, found 395.2565
Preparation of indenes 3.15 and 3.16

The procedure for the preparation of indenes 3.15 and 3.16 from the benzyl alcohols 3.14a and 3.14b was the same as that used in the preparation of indenes 3.4 and 3.5 from the benzyl alcohols 3.3a and 3.3b, and it afforded 21 mg of 3.15 (43%) and 20 mg of 3.16 (42%) as colorless oils.

\[
\begin{align*}
\text{3.15} & \quad \text{H NMR (CDCl}_3\text{)} \quad \delta 0.83 (s, 3H), 0.91 (s, 3H), 1.23 (s, 3H), 1.41 (s, 3H), 1.86 \text{ (brd, } J= 11.7 \text{ Hz, } 1H), 2.34 (s, 3H), 2.48 \text{ (dt, } J= 12.6, 3.1 \text{ Hz, } 1H), 3.82 (s, 3H), 3.85 (s, 3H), 6.32 (s, 1H), 6.38 (s, 1H) \\
\text{13C NMR (CDCl}_3\text{)} & \quad \delta 18.5, 18.6, 19.1, 19.4, 21.6, 22.0, 33.5, 33.7, 36.7, 38.5, 39.3, 42.2, 52.5, 56.0, 56.1, 61.3, 110.8, 114.0, 127.2, 136.0, 140.1, 144.9, 150.7, 168.4
\end{align*}
\]

HRESIMS calcd for C_{24}H_{35}O_{2} [M+H]^+ 355.2637, found 355.2634
3.16 $^1$H NMR (CDCl$_3$) $\delta$ 0.90 (s, 3H), 0.92 (s, 3H), 1.15 (s, 3H), 1.49 (s, 3H), 2.34 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 6.36 (s, 1H), 6.46 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 17.5, 18.6, 19.5, 21.5, 24.4, 26.0, 26.8, 33.2, 33.7, 39.0, 40.3, 42.4, 45.2, 52.2, 56.0, 61.3, 110.4, 117.8, 127.0, 136.8, 139.8, 145.3, 150.9, 170.5

HRESIMS calcd for C$_{24}$H$_{35}$O$_2$ [M+H]$^+$ 355.2637, found 355.2638
Preparation of tetracycle 3.17

The procedure for the preparation of tetracycle 3.17 from the indene 3.15 was the same as that used in the preparation of tetracycle 3.6 from the indene 3.4, and it afforded 15 mg of 3.17 (88%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.85 (s, 3H), 0.86 (s, 3H), 1.23 (s, 3H), 1.60 (s, 3H), 1.81 (dd, J= 12.0, 8.4 Hz, 1H), 1.98 (brd, J= 12.2 Hz, 1H), 2.32 (s, 3H), 2.68 (dd, J= 15.7, 12.2 Hz, 1H), 2.90 (dd, J= 15.8, 8.2 Hz, 1H), 3.80 (s, 6H), 6.46 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 18.4, 18.4, 19.9, 21.9, 24.5, 26.0, 30.1, 32.9, 33.5, 36.6, 36.7, 38.3, 42.4, 47.4, 47.9, 56.0, 60.3, 62.3, 114.3, 128.2, 134.4, 143.0, 144.7, 150.0

HRESIMS calcd for C$_{24}$H$_{37}$O$_2$ [M+H]$^+$ 357.2794, found 357.2790
Preparation of tetracycle 3.18

The procedure for the preparation of tetracycle 3.18 from the indene 3.16 was the same as that used in the preparation of tetracycle 3.6 from the indene 3.4, and it afforded 14 mg of 3.18 (83%) as a colorless oil.

$^{1}$H NMR (CDCl$_3$) $\delta$ 0.43 (s, 3H), 0.77 (s, 3H), 0.86 (s, 3H), 0.96 (dd, J= 11.4, 4.3 Hz, 1H), 1.17 (s, 3H), 1.48 (tt, J= 13.6, 3.2 Hz, 1H), 1.72 (brd, J= 12.6 Hz, 1H), 2.31 (s, 3H), 2.53 (dt, J= 13.9, 5.8 Hz, 1H), 2.75 (d, J= 16.8 Hz, 1H), 2.88 (dd, J= 16.9, 7.8 Hz, 1H), 3.78 (s, 3H), 3.79 (s, 3H), 6.41 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.5, 18.5, 19.5, 19.7, 21.9, 28.4, 31.0, 33.2, 33.4, 34.7, 37.3, 41.1, 42.1, 48.6, 52.6, 55.9, 59.9, 62.6, 113.7, 127.4, 137.2, 142.0, 142.7, 150.2

HRESIMS calcd for C$_{24}$H$_{37}$O$_2$ [M+H]$^+$ 357.2794, found 357.2789
Preparation of catechol 3.19

The procedure for the synthesis of catechol 3.19 from the tetracycle 3.17 is the same as the synthesis of catechol 2.57 from the tetracycle 2.42 and it afforded a white solid 9.1 mg (85%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.83 (s, 3H), 0.85 (s, 3H), 1.22 (s, 3H), 1.59 (s, 3H), 1.67 (tt, J= 14.5, 3.1 Hz, 1H), 1.85 (dd, J= 11.3, 8.4 Hz, 1H), 1.97 (brd, J= 12.3 Hz, 1H), 2.24 (s, 3H), 2.64 (dd, J= 17.1, 11.9 Hz, 1H), 2.79 (dd, J= 15.2, 8.2 Hz, 1H), 4.70 (brs, 1H), 4.78 (brs, 1H), 6.42 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 18.4 (2xC), 19.5, 21.9, 24.5, 26.0, 29.4, 32.9, 33.5, 36.6, 36.8, 38.3, 42.4, 47.7, 48.0, 62.5, 116.8, 125.0, 127.2, 137.5, 140.9, 144.7

HRESIMS calcd for C$_{22}$H$_{33}$O$_2$ [M+H]$^+$ 329.2481, found 329.2467
Preparation of catechol 3.20

The procedure for the synthesis of catechol 3.20 from the tetracycle 3.18 is the same as the synthesis of catechol 2.57 from the tetracycle 2.42 and it afforded a white solid 7 mg (83%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.44 (s, 3H), 0.78 (s, 3H), 0.86 (s, 3H), 0.97 (dd, J= 11.3, 4.6 Hz, 1H), 1.15 (s, 3H), 1.48 (tt, J= 13.6, 3.4 Hz, 1H), 2.23 (s, 3H), 2.48 (dt, J= 13.9, 5.8 Hz, 1H), 2.62 (d, J= 16.3 Hz, 1H), 2.81 (dd, J= 16.5, 7.9 Hz, 1H), 4.78 (brs, 1H), 4.91 (brs, 1H), 6.40 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.4, 18.5, 19.2, 19.4, 21.8, 27.7, 31.0, 33.2, 33.3, 34.4, 37.3, 41.1, 42.1, 48.8, 52.4, 62.8, 116.5, 124.4, 129.9, 137.2, 141.0, 142.2

HRESIMS calcd for C$_{22}$H$_{32}$O$_2$Na 351.2300, found 351.2304
Preparation of diol 3.22

To a stirred solution of veratrol (552 mg, 4 mmol), 6 mmol of TMEDA (0.91 mL) and 6 mL THF at 0°C was added 5 mmol BuLi (3.125 mL, 1.6M). The reaction mixture was stirred at room temperature for 1 hour, then cooled to -78°C, and 380 mg of 2.19 in 5 mL of THF was added dropwise. After stirring at -78°C for another hour, the reaction was quenched with water and partitioned with Et₂O. The ether layer was washed with sat. brine successively and evaporated to dryness. The residue was subjected to silica gel column chromatography and eluted with Hexane: EtOAc (100:0-100:7) to give 438 mg solid of 3.22 (73%).

\[
\begin{align*}
\text{H NMR (CDCl}_3\text{)} & \delta 0.27 (td, J= 13.4, 3.8 Hz, 1H), 0.75 (s, 3H), 0.80 (s, 3H), 0.86 (dd, J= 12.2, 2.1 Hz, 1H), 0.94 (dd, J= 13.6, 4.0 Hz, 1H), 1.04 (s, 3H), 1.55 (s, 3H), 1.63 (brd, J= 14.0 Hz, 1H), 1.85 (dt, J= 12.8, 3.5 Hz, 1H), 2.12 (d, J= 8.4 Hz, 1H), 3.85 (s, 3H), 3.89 (s, 3H), 5.28 (d, J= 8.4 Hz, 1H), 6.81 (dd, J= 8.1, 1.5 Hz, 1H), 7.05 (t, J= 7.9 Hz, 1H), 7.11 (dd, J= 7.9, 1.5 Hz, 1H) \\
\text{C NMR (CDCl}_3\text{)} & \delta 15.9, 18.4, 20.0, 21.6, 25.9, 33.3, 33.6, 38.9, 39.9, 41.5, 44.8, 55.7, 56.0, 61.3, 62.8, 69.2, 74.8, 111.2, 120.6, 124.4, 141.3, 145.6, 152.6
\end{align*}
\]
Preparation of catechol 3.25

\[ \text{HRESIMS calcd for } \text{C}_{23}\text{H}_{36}\text{O}_4\text{Na } 399.2511, \text{ found } 399.2514 \]

The procedure for the synthesis of catechol 3.25 from the tetracycle 3.24 is the same as the synthesis of catechol 2.57 from the tetracycle 2.42 and it afforded a white solid 15 mg (81%).

\[ ^1\text{H NMR (CDCl}_3\text{)} \delta 0.85 \text{ (s, 6H), 0.95 (dd, } J= 10.5, 3.7 \text{ Hz, 1H), 1.00 (s, 3H), 1.02 (s, 3H), 1.17 (m, 1H), 2.07 (m, 1H), 2.49 (dd, } J= 14.0, 12.5 \text{ Hz, 1H), 2.59(dd, } J= 14.3, 6.4 \text{ Hz, 1H), 4.82 (s, 1H), 4.93 (s, 1H), 6.46 (d, } J= 7.9 \text{ Hz, 1H), 6.64 (d, } J= 7.8 \text{ Hz, 1H)} \]

\[ ^13\text{C NMR (CDCl}_3\text{)} \delta 16.2, 18.3, 19.5, 21.1, 23.3, 24.6, 33.2, 33.5, 37.0, 37.4, 40.2, 42.6, 46.0, 57.5, 64.9, 112.0, 113.3, 128.1, 140.2, 141.3, 149.6 \]

\[ \text{HRESIMS calcd for } \text{C}_{21}\text{H}_{31}\text{O}_2 [\text{M+H}]^+ 315.2324, \text{ found } 315.2326 \]
Preparation of benzyl alcohols 3.26a and 3.26b

To a stirred solution of veratrol (414 mg, 3 mmol), 4.5 mmol of TMEDA (0.68 mL) and 4.5 mL THF at 0°C was added 3.75 mmol BuLi (2.34 mL, 1.6M). The reaction mixture was stirred at room temperature for 1 hour, then cooled to −78°C, and 260 mg of 2.54 in 4 mL of THF was added dropwise. After stirring at −78°C for another hour, the mixture was allowed to warm to room temperature over one hour. The reaction was quenched with water and partitioned with ether. The ether layer was washed with saturated brine successively and evaporated to dryness. The residue was subjected to silica gel column chromatography and eluted with Hexane: EtOAc (100:0-100:7) to give 260 mg of 3.26a (65%) and 63 mg 3.26b (16%).

3.26a

$^1$H NMR (CD$_3$CN) δ 0.86 (s, 3H), 0.91 (s, 3H), 0.99 (s, 3H), 1.30 (td, J= 12.9, 3.7 Hz, 1H), 1.38 (s, 3H), 1.59 (dt, J= 13.7, 3.5 Hz, 1H), 3.58 (d, J= 3.7 Hz, 1H), 3.76 (s, 3H), 3.79 (s, 3H), 5.61 (d, J= 3.7 Hz, 1H), 6.90 (m, 3H)
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$^1$H NMR (CD$_3$CN) $\delta$ 0.73 (td, J= 13.0, 3.5 Hz, 1H), 0.85 (s, 3H), 0.90 (s, 3H), 1.05 (td, J= 13.6, 4.0 Hz, 1H), 1.33 (brd, J= 13.1 Hz, 1H), 1.64 (s, 3H), 1.72 (dd, J= 13.1, 6.7 Hz, 1H), 3.29 (d, J= 3.7 Hz, 1H), 3.83 (s, 3H), 3.87 (s, 3H), 5.58 (d, J= 3.5 Hz, 1H), 6.85 (dd, J= 6.7, 2.6 Hz, 1H), 6.95 (m, 2H)

$^{13}$C NMR (CD$_3$CN) $\delta$ 19.6, 19.8, 20.3, 22.1, 22.9, 33.7, 33.9, 35.3, 36.9, 39.7, 42.3, 52.7, 56.5, 61.2, 67.8, 113.2, 122.0, 124.2, 132.8, 138.2, 141.0, 148.7, 153.8

HRESIMS calcd for C$_{23}$H$_{34}$O$_3$Na 381.2406, found 381.2407
Preparation of indenes 3.27 and 3.28

The procedure for the preparation of indenes 3.27 and 3.28 from the benzyl alcohols 3.26a and 3.26b was the same as that used in the preparation of indenes 3.4 and 3.5 from the benzyl alcohols 3.3a and 3.3b, and it afforded 39.5 mg of 3.27 (47%) and 25.5 mg of 3.28 (31%) as colorless oils.

\[
(3.27) ^1H NMR (CDCl_3) \delta 0.84 (s, 3H), 0.92 (s, 3H), 1.23 (s, 3H), 1.36 (s, 3H), 1.42 (brd, 1H), 1.55 (dt, J= 13.3, 3.5 Hz, 1H), 1.85 (brd, 1H), 2.16 (dt, J= 12.6, 3.1 Hz, 1H), 3.84 (s, 3H), 3.91 (s, 3H), 6.37 (s, 1H), 6.66 (d, J= 7.9 Hz, 1H), 6.86 (d, J= 7.9 Hz, 1H)
\]

\[
^{13}C NMR (CDCl_3) \delta 18.6, 19.2, 19.3, 21.6, 25.0, 33.6, 33.8, 38.2, 39.1, 39.4, 42.3, 51.0, 56.2, 56.7, 61.2, 108.3, 113.8, 115.8, 135.2, 142.0, 148.8, 151.0, 168.1
\]

HRESIMS calcd for C_{23}H_{33}O_{2} [M+H]^+ 341.2481, found 341.2478
1H NMR (CDCl$_3$) $\delta$ 0.90 (s, 3H), 0.91 (s, 3H), 1.14 (s, 3H), 1.23 (td, $J$= 13.4, 3.7 Hz, 1H), 1.42 (s, 3H), 1.69 (tt, $J$= 13.6, 3.4 Hz, 1H), 2.04 (dd, $J$= 12.6, 6.1 Hz, 1H), 2.11 (ddd, $J$= 12.3, 9.9, 1.8 Hz, 1H), 3.83 (s, 3H), 3.89 (s, 3H), 6.51 (s, 1H), 6.63 (d, $J$= 8.1 Hz, 1H), 6.83 (d, $J$= 8.1 Hz, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 17.1, 19.5, 21.5, 26.4, 27.0, 28.2, 33.3, 33.7, 38.9, 40.2, 42.5, 45.2, 51.2, 56.2, 61.2, 108.1, 116.0, 117.9, 136.2, 141.8, 148.7, 151.1, 170.5

HRESIMS calcd for C$_{23}$H$_{33}$O$_2$ [M+H]$^+$ 341.2481, found 341.2479
Preparation of tetracycle 3.29

The procedure for the preparation of tetracycle 3.29 from the indene 3.27 was the same as that used in the preparation of tetracycle 3.6 from the indene 3.4, and it afforded 29 mg of 3.29 (89%) as a colorless oil.

$^1$H NMR (CDCl$_3$) δ 0.85 (s, 3H), 0.87 (s, 3H), 1.18 (s, 3H), 1.81 (dd, J= 11.6, 8.2 Hz, 1H), 2.78 (dd, J= 15.8, 11.7 Hz, 1H), 2.90 (dd, J= 15.7, 8.1 Hz, 1H), 3.81 (s, 3H), 3.85 (s, 3H), 6.72 (t, J= 9.0 Hz, 2H)

$^{13}$C NMR (CDCl$_3$) δ 18.2, 18.8, 22.0, 23.8, 25.8, 29.8, 33.0, 33.5, 36.5, 38.5, 39.2, 42.5, 45.1, 47.8, 56.1, 60.3, 62.9, 110.8, 116.2, 133.5, 145.3, 148.5, 150.7

HRESIMS calcd for C$_{23}$H$_{35}$O$_2$ [M+H]$^+$ 343.2637, found 343.2640
Preparation of tetracycle 3.30

The procedure for the preparation of tetracycle 3.30 from the indene 3.28 was the same as that used in the preparation of tetracycle 3.6 from the indene 3.4, and it afforded 18 mg of 3.30 (88%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.32 (s, 3H), 0.72 (s, 3H), 0.87 (s, 3H), 1.05 (s, 3H), 1.14 (td, J= 12.3, 3.8 Hz, 1H), 1.70 (brd, J= 6.7 Hz, 1H), 2.28 (dt, J= 13.9, 3.5 Hz, 1H), 2.76 (d, J= 16.6 Hz, 1H), 2.91 (dd, J= 16.6, 6.9 Hz, 1H), 3.81 (s, 3H), 3.83 (s, 3H), 6.69 (s, 2H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.2, 18.4, 19.4, 22.1, 28.6, 33.0, 33.7, 34.0, 35.1, 37.0, 41.3, 42.1, 45.9, 53.0, 56.0, 59.9, 62.3, 110.3, 114.9, 136.3, 145.0, 145.8, 150.8

HRESIMS calcd for C$_{23}$H$_{35}$O$_2$ [M+H]$^+$ 343.2637, found 343.2643
Preparation of catechol 3.31

The procedure for the synthesis of catechol 3.31 from the tetracycle 3.29 was the same as the synthesis of catechol 2.57 from the tetracycle 2.42 and it afforded a white solid 3.0 mg (87%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.84 (s, 3H), 0.85 (s, 3H), 1.18 (s, 3H), 1.42 (s, 3H), 1.56 (brd, $J= 13.4$ Hz, 1H), 1.84 (dd, $J= 10.4, 8.4$ Hz, 1H), 2.71 (dd, $J= 15.1, 11.4$ Hz, 1H), 2.80 (dd, $J= 15.4, 8.2$ Hz, 1H), 4.89 (s, 1H), 4.99 (s, 1H), 6.50 (d, $J= 7.9$ Hz, 1H), 6.67 (d, $J= 7.9$ Hz, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 18.4, 18.8, 22.0, 23.7, 25.8, 29.0, 33.0, 33.5, 36.4, 38.5, 39.3, 42.5, 45.4, 47.9, 63.1, 113.2, 113.5, 126.5, 139.8, 141.4, 148.6

HRESIMS calcd for C$_{21}$H$_{31}$O$_2$ [M+H]$^+$ 315.2324, found 315.2326
Preparation of catechol 3.32

The procedure for the synthesis of catechol 3.32 from the tetracycle 3.30 was the same as the synthesis of catechol 2.57 from the tetracycle 2.42 and it afforded a white solid 2.6 mg (84%).

$^1$H NMR (CDCl$_3$) $\delta$ 0.32 (s, 3H), 0.72 (s, 3H), 0.86 (s, 3H), 1.74 (brd, $J = 6.9$ Hz, 1H), 2.25 (m, 1H), 2.63 (d, $J = 16.4$ Hz, 1H), 2.83 (dd, $J = 16.0$, 6.9 Hz, 1H), 4.86 (s, 1H), 4.95 (s, 1H), 6.47 (d, $J = 8.0$ Hz, 1H), 6.65 (d, $J = 8.0$ Hz, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.0, 18.4, 19.4, 22.1, 27.7, 33.1, 33.6, 33.9, 34.9, 37.0, 41.3, 42.1, 46.2, 52.8, 62.6, 112.1, 113.3, 129.2, 139.6, 141.6, 146.1

HRESIMS calcd for C$_{21}$H$_{30}$O$_2$Na 337.2144, found 337.2147
Preparation of benzyl alcohols 3.34a and 3.34b

The procedure for the preparation of benzyl alcohols 3.34a and 3.34b was the same as that used in the preparation of benzyl alcohols 3.14a and 3.14b, and it afforded 102 mg of 3.34a (74%) and 11 mg of 3.34b (8%) as colorless oils.

3.34a $^1$H NMR (CD$_3$CN) $\delta$ 0.87 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.17 (t, J= 7.6 Hz, 3H), 1.34 (td, J= 13.1, 3.8 Hz, 1H), 1.43 (s, 3H), 2.61 (q, J= 7.6 Hz, 2H), 3.56 (d, J= 3.8 Hz, 1H), 3.79 (s, 3H), 3.85 (s, 3H), 5.64 (d, J= 3.7 Hz, 1H), 6.85 (d, J= 8.1 Hz, 1H), 6.97 (d, J= 8.1 Hz, 1H)

$^{13}$C NMR (CD$_3$CN) $\delta$ 15.5, 19.9, 20.0, 21.0, 22.2, 22.4, 23.5, 33.8, 34.2, 35.7, 37.9, 40.2, 42.4, 53.4, 60.7, 60.9, 68.3, 124.1, 124.4, 133.0, 137.4, 138.2, 140.8, 151.9, 152.3

HRESIMS calcd for C$_{25}$H$_{38}$O$_3$Na 409.2719, found 409.2715
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3.34b $^{1}$H NMR (CD$_{3}$CN) $\delta$ 0.75 (td, $J= 13.0$, 3.5 Hz, 1H), 0.86 (s, 3H), 0.91 (s, 3H), 1.07 (td, $J= 13.4$, 3.8 Hz, 1H), 1.14 (s, 3H), 1.18 (t, $J= 7.6$ Hz, 3H), 1.34 (brd, $J= 13.1$ Hz, 1H), 1.64 (s, 3H), 1.73 (dd, $J= 13.0$, 6.6 Hz, 1H), 2.62 (q, $J= 7.6$ Hz, 2H), 3.26 (d, $J= 3.5$ Hz, 1H), 3.80 (s, 3H), 3.91 (s, 3H), 5.56 (d, $J= 3.5$ Hz, 1H), 6.85 (d, $J= 7.9$ Hz, 1H), 6.93 (d, $J= 7.9$ Hz, 1H)

$^{13}$C NMR (CD$_{3}$CN) $\delta$ 15.5, 19.6, 19.8, 20.3, 22.1, 22.9, 23.5, 33.7, 34.0, 35.3, 37.0, 39.7, 42.4, 52.7, 60.9, 61.1, 67.8, 124.1, 125.0, 132.8, 136.3, 138.9, 141.0, 152.1, 152.8

HRESIMS calcd for C$_{28}$H$_{38}$O$_{3}$Na 409.2719, found 409.2714
Preparation of indene 3.35 and the elimination product 3.35a

The procedure for the preparation of indene 3.35 from the benzyl alcohol 3.34a and 3.34b was the same as that used in the preparation of indene 3.4 from the benzyl alcohol 3.3a and 3.3b, and it afforded 17 mg of 3.35 (84%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.83 (s, 3H), 0.92 (s, 3H), 1.20 (t, J= 7.6 Hz, 3H), 1.20 (s, 3H), 1.22 (s, 3H), 2.14 (dd, J= 9.8, 3.0 Hz, 1H), 2.63 (q, J= 7.6 Hz, 2H), 3.82 (s, 3H), 3.92 (s, 3H), 6.31 (s, 1H), 6.75 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.5, 18.6, 19.3, 19.4, 21.6, 23.3, 25.0, 33.6, 33.8, 38.3, 39.0, 39.3, 42.3, 51.3, 56.8, 60.8, 60.9, 113.7, 116.4, 132.8, 133.9, 145.7, 148.8, 151.3, 166.9

HRESIMS calcd for C$_{25}$H$_{37}$O$_2$ [M+H]$^+$ 369.2794, found 369.2795
(3.35a)

$^1$H NMR (CDCl$_3$) $\delta$ 0.86 (s, 3H), 0.93 (s, 3H), 1.02 (s, 3H), 1.18 (t, $J$ = 7.6 Hz, 3H), 1.47 (s, 3H), 2.24 (brd, $J$ = 9.8 Hz, 1H), 2.61 (m, 2H), 3.78 (s, 3H), 3.83 (s, 3H), 5.56 (brs, 1H), 6.25 (s, 1H), 6.78 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.1, 19.3, 19.7, 21.9, 22.9, 23.1, 25.3, 32.6, 33.8, 38.1, 38.7, 42.4, 48.2, 60.1, 60.8, 115.7, 123.1, 126.3, 128.7, 131.5, 132.7, 136.6, 150.4, 150.5, 151.4

HRESIMS calcd for C$_{25}$H$_{37}$O$_2$ [M+H]$^+$ 369.2794, found 369.2791
Preparation of tetracycle 3.36

The procedure for the preparation of tetracycle 3.36 from the indene 3.35 was the same as that used in the preparation of tetracycle 3.6 from the indene 3.4, and it afforded 13 mg of 3.36 (88%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.85 (s, 3H), 0.86 (s, 3H), 1.17 (s, 3H), 1.18 (t, J= 7.6 Hz, 3H), 1.43 (s, 3H), 1.68 (brd, J= 14.3 Hz, 1H), 1.80 (dd, J= 11.3, 8.7 Hz, 1H), 2.60 (m, 2H), 2.74 (dd, J= 15.4, 11.6 Hz, 1H), 2.85 (dd, J= 15.5, 8.1 Hz, 1H), 3.80 (s, 3H), 3.86 (s, 3H), 6.61 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 15.4, 18.4, 18.8, 22.0, 23.3, 23.8, 25.7, 30.0, 33.0, 33.5, 36.4, 38.5, 39.2, 42.5, 45.4, 47.8, 60.0, 60.7, 62.8, 116.5, 130.7, 136.3, 148.5, 149.0, 150.8

HRESIMS calcd for C$_{25}$H$_{39}$O$_2$ [M+H]$^+$ 371.2950, found 369.2952
Preparation of benzyl alcohol 3.51

A solution of 1.6 M BuLi in pentane (1.95 mL, 3.12 mmol) was added slowly to a stirred solution of 3.50 (742 mg, 2.85 mmol) in dry THF (22.0 mL) at $-78^\circ$C. After stirring for 30 minutes, a solution of 2.54 (0.62 g, 2.8 mmol) in dry THF (6.0 mL) was added. The mixture was stirred at $-78^\circ$C for another 30 minutes and was allowed to warm to $-20^\circ$C in 2 hours, after which H$_2$O (10.0 mL) was added and the mixture was extracted with Et$_2$O (60mL, twice). The combined extracts were washed with brine, dried (MgSO$_4$), and concentrated to yield a residue. Silica gel column chromatography give 460 mg of 3.51 (41%) as colorless oil.

$^1$H NMR (CD$_3$CN) $\delta$ 0.87 (s, 3H), 0.93 (s, 3H), 1.02 (s, 3H), 1.40 (s, 3H), 3.28 (s, 3H), 3.55 (d, $J$= 3.9 Hz, 1H), 3.78 (s, 3H), 3.83 (s, 3H), 4.33 (s, 2H), 5.63 (d, $J$= 3.9 Hz, 1H), 6.88 (d, $J$= 1.7 Hz, 1H), 6.90 (d, $J$= 1.7 Hz, 1H)

$^{13}$C NMR (CD$_3$CN) $\delta$ 20.0 (C\times2), 21.0, 22.2, 22.2, 33.9, 34.2, 35.8, 38.0, 40.3, 42.5, 53.4, 56.5, 58.1, 60.7, 68.5, 75.3, 112.0, 120.9, 132.8, 134.5, 139.2, 141.3, 147.3, 153.6
Preparation of indenes 3.52 and 3.53 and uncyclized product 3.54

To a stirred solution of 3.51 (260 mg, 0.65 mmol) in CH$_2$Cl$_2$ (30 mL) at -20°C, TFA (2.72 g, 1.78 mL) was added slowly under argon for 5 minutes. The mixture was further stirred for 13 minutes and quenched with Sat. NaHCO$_3$ 10.0 mL, then diluted with CH$_2$Cl$_2$ (100 mL) poured into ice water. The aqueous phase was extracted with CH$_2$Cl$_2$ twice (100 mL) and the combined extracts were washed with saturated NaHCO$_3$, H$_2$O, and dried (MgSO$_4$). Concentration of the CH$_2$Cl$_2$ gave a brown oil, which was passed through a normal phase Seppak eluted with Hexane : EtOAc (90:10) to give an yellow oil. The mixture was subjected to HPLC separation with a chiral column to afford 77 mg of 3.52 (31%), 67 mg of 3.53 (27%) and 46 mg of 3.54 (19%) as colorless oils.

(3.52) $^1$H NMR (CDCl$_3$)  δ 0.90 (s, 3H), 0.92 (s, 3H), 1.14 (s, 3H), 1.49 (s, 3H), 1.71 (dt, J= 13.4, 3.4 Hz, 1H), 2.01 (dd, J= 12.5, 5.6 Hz, 1H), 2.31 (ddd, J= 12.5, 9.3, 2.4 Hz, 1H), 3.42 (s, 3H), 3.84 (s, 3H), 3.84 (s, 3H), 4.49 (d, J= 11.3 Hz, 1H), 4.54 (d, J= 11.3 Hz, 1H), 6.47 (s, 1H), 6.66 (s, 1H)
Chapter 3: Structure Activity Relationship Study of Pelorol.

$^{13}\text{C NMR (CDCl}_3\text{)} \ \delta \ 17.6, 19.5, 21.5, 26.0, 26.1, 28.0, 33.2, 33.7, 39.0, 40.3,$
$42.3, 45.2, 52.3, 56.0, 58.3, 61.2, 71.2, 108.6, 117.8, 127.5, 136.9, 141.2, 145.7,$
$151.3, 170.4$

HRESIMS calcd for $C_{25}H_{36}O_3Na$ 407.2562, found 407.2560

HRESIMS calcd for $C_{25}H_{37}O_3$ [M+H]$^+$ 385.2743, found 385.2744
(3.54) $^1$H NMR (CDCl$_3$) $\delta$ 0.88 (s, 3H), 0.97 (s, 3H), 1.04 (s, 3H), 1.21 (td, $J$ = 13.1, 3.9 Hz, 1H), 1.41 (dd, $J$ = 3.5, 1.9 Hz, 3H), 1.60 (dt, $J$ = 10.0, 3.5 Hz, 1H), 1.70 (tt, $J$ = 13.5, 3.1 Hz, 1H), 1.84 (brd, $J$ = 12.3 Hz, 1H), 3.29 (s, 3H), 3.69 (s, 3H), 3.81 (s, 3H), 4.31 (s, 2H), 5.61 (brs, 1H), 6.27 (brs, 1H), 6.61 (d, $J$ = 1.5 Hz, 1H), 6.83 (d, $J$ = 1.5 Hz, 1H)

$^{13}$C NMR (CD$_3$CN) $\delta$ 20.1, 20.2, 22.2, 23.6, 26.1, 33.0, 34.6, 39.1, 39.6, 43.1, 49.2, 56.4, 58.2, 60.4, 75.0, 111.6, 116.8, 123.5, 130.1, 132.2, 134.7, 135.5, 146.8, 152.9, 153.3

HRESIMS calcd for C$_{25}$H$_{36}$O$_3$Na 407.2562, found 407.2567
Chapter 3: Structure Activity Relationship Study of Pelorol.

Preparation of tetracycle 3.55

Catalytic hydrogenation of 3.52 (70 mg, 0.18 mmol) was carried out in CH$_2$Cl$_2$ (10 mL) under H$_2$ (45 psi) in presence of 5% Rh/C (300 mg) overnight. The reaction mixture was filtered and concentrated to afford an oil, which was purified by passing through a normal phase Seppak to give 58 mg of 3.55 (83%) as a colorless oil.

$^1$H NMR (CDCl$_3$) δ 0.36 (s, 3H), 0.74 (s, 3H), 0.86 (s, 3H), 0.93 (dd, J= 11.6, 3.2 Hz, 1H), 1.17 (s, 3H), 1.72 (brd, J= 14.2 Hz, 1H), 2.49 (m, 1H), 2.75 (d, J= 16.8 Hz, 1H), 2.88 (dd, J= 16.6, 7.0 Hz, 1H), 3.38 (s, 3H), 3.79 (s, 3H), 3.81 (s, 3H), 4.38 (d, J= 11.1 Hz, 1H), 4.50 (d, J= 11.1 Hz, 1H), 6.67 (s, 1H)

$^{13}$C NMR (CDCl$_3$) δ 15.5, 18.5, 19.8, 22.0, 28.3, 32.4, 33.1, 33.6, 35.3, 37.4, 40.8, 42.1, 48.9, 53.2, 55.9, 57.9, 59.9, 62.7, 72.4, 112.2, 127.6, 137.8, 142.6, 144.2, 150.3

HRESIMS calcd for C$_{25}$H$_{38}$O$_3$Na 409.2719, found 409.2715
Preparation of tetracycle 3.56

The procedure for the preparation of tetracycle 3.56 from the indene 3.53 was the same as that used in the preparation of tetracycle 3.55 from the indene 3.52, and it afforded 49 mg of 3.56 (81%) as a colorless oil.

$^1$H NMR (CDCl$_3$) $\delta$ 0.84 (s, 3H), 0.86 (s, 3H), 1.22 (s, 3H), 1.59 (s, 3H), 1.67 (brt, J = 13.6 Hz, 1H), 1.82 (dd, J = 12.2, 8.4 Hz, 1H), 1.98 (brd, J = 11.4 Hz, 1H), 2.70 (dd, J = 15.5, 12.2 Hz, 1H), 2.91 (dd, J = 15.8, 8.2 Hz, 1H), 3.37 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 4.47 (s, 2H), 6.75 (s, 1H)

$^{13}$C NMR (CDCl$_3$) $\delta$ 18.4, 18.5, 21.9, 24.5, 26.2, 30.2, 33.0, 33.5, 36.6, 37.6, 38.3, 42.4, 47.7, 47.9, 56.0, 58.0, 60.2, 62.4, 72.1, 112.4, 128.6, 134.7, 144.5, 145.2, 150.3

HRESIMS calcd for C$_{25}$H$_{38}$O$_3$Na 409.2719, found 409.2717
3.9 References


4.1 Introduction

Contignasterol (4.1),¹ isolated from Petrosia contignata collected in Papua New Guinea, was the first reported example of an emerging family of marine sponge

¹A version of this chapter has been published. Yang, Lu; Andersen, Raymond J. (2002) Absolute Configuration of the Antiinflammatory Sponge Natural Product Contignasterol. Journal of Natural Products. 65(12), 1924-1926.
steroids that are characterized by having in common the rare cis C/D ring junction and 15-keto, 6α-hydroxyl, and 7β-hydroxyl functionalities.

Other known members of this family include xestobergsterols A to C (4.2 to 4.4), 14-tamosterone sulfate (4.5), haliclostanone sulfate (4.6), and clathriol (4.7). Contignasterol and other members of this family of steroids inhibit the anti-Ige stimulated release of histamine from sensitized rat mast cells in a dose-dependent manner. On the basis of this promising biological activity, contignasterol was chosen as the lead structure for an analog synthesis program that identified IPL576-092 as a novel antiasthma/antiinflammatory agent. IPL576-092 has been advanced to phase II human clinical trials.

The initial structure elucidation of contignasterol (4.1) assigned the relative stereochemistry by analysis of the coupling constants and NOE data obtained for contignasterol tetraacetate. Diagnostic coupling constants indicated that H-3 and H-4 were equatorially oriented and H-5, H-6, H-7, H-8 and H-9 were all axially oriented. NOE experiments in conjunction with scalar coupling constants showed that the hemiacetal ring in the side chain occupied a chair conformation. A NOE between CH₃-18 and 14-H helped to establish the 14-H β configuration. To complete the structure elucidation of contignasterol (1), we set out to solve the absolute configuration of this compound ten years after it was originally identified. The relative stereochemistry at C-17 was assumed to be the "natural β configuration". Our efforts to establish the relative configuration between C-17, C-
Figure 4.1 Separate coupling systems A, B, and C of Contignasterol

20 and C-22 with NOE experiments were unsuccessful, thus the contignasterol structure was divided into three parts with the separate coupling systems shown in Figure 4.1. These were: A) the steroid core, B) C-20, and C) the hemiacetal ring in the side chain. Our goal was to determine the absolute configuration of the moieties A and C independently and then try to relate one or the other to moiety B.
4.2 Absolute configuration of Moiety A

The absolute stereochemistry of moiety A was solved by applying the exciton chirality circular dichroism method\textsuperscript{11} because the two α-diol systems of 3,4-OH and 6,7-OH provided easy access to this method. Contignasterol was treated with 4-methoxybenzoyl chloride and the 3,6,7-tri-p-methoxybenzoate derivative was obtained in high yield as shown in Figure 4.2.

**Figure 4.2** Synthesis of contignasterol 3,6,7-tri-p-methoxybenzoate

**Figure 4.3** CD spectra of contignasterol and contignasterol 3,6,7-tri-p-methoxybenzoate (4.9) in EtOH
The p-methoxybenzoylation of the 4β-hydroxyl group proved to be quite difficult due to the steric hindrance from the 19-CH₃, which simplified the interpretation of the CD spectrum. In this case, when the tri-p-methoxybenzoate derivative was employed for CD measurement, the observed Cotton effects were only affected by the benzoate groups at C-3, C-6, and C-7 on the steroid nucleus. The CD spectrum of contignasterol tri-p-methoxybenzoate in EtOH showed a pair of typical exciton-split Cotton effects with opposite signs centered upon the UV absorption (257nm) of the p-methoxybenzoate chromophore: \( \Delta\varepsilon_{264.0} \) -29.4 and \( \Delta\varepsilon_{246.8} \) +16.3. The negative longer wavelength Cotton effect clearly defines the negative chirality between the three p-methoxybenzoate chromophores, thus unequivocally assigning the absolute configuration at C-3 as R.

![Figure 4.4](image)

**Figure 4.4** Negative chirality between the three p-methoxybenzoate chromophores on moiety A

The CD spectrum of contignasterol (4.1) shows a negative Cotton effect at 300 nm attributed to the n to \( \pi^* \) transition of the C-15 ketone. Comparison of this CD
data with that for the model 15-keto-14 steroid 4.10, which is reported to have a negative Cotton effect at 300 nm in its ORD curve,\textsuperscript{12} confirmed that the nucleus of contignasterol has the standard steroidal configuration.
4.3 Absolute configuration of Moiety C

The secondary hemiacetal hydroxyl group suggested that the determination of side chain absolute configuration might be possible by using a modified Mosher's method. A potential complicating factor in the analysis of the side chain configuration was the polyhydroxyl nature of contignasterol, which we anticipated would result in the formation of multiple esters with the chiral reagent. To circumvent this complication, the hydroxyl groups on the nucleus were protected as acetate esters prior to selective exposure of the C-29 alcohol and forming the

Figure 4.5 Derivatization of contignasterol hemiacetal alcohol to (R)- and (S)-MTPA esters (4.13 and 4.14)
C-22 chiral auxillary esters. This sequence of transformations was accomplished as shown in Figure 4.5. Reaction of contignasterol with excess acetic anhydride in pyridine in the presence of a catalytic amount of (dimethylamino)pyridine gave pentaacetylcontignasterol (4.11), as a mixture of C-29 epimers, in good yield. Treatment of the pentaacetates with BF$_3$ etherate in aqueous acetonitrile selectively hydrolyzed the C-29 acetates to give the tetraacetate 4.12.\textsuperscript{14} (R) and (S)-MTPACl were coupled to the hemiacetal hydroxyl group in 4.12 and the two major diastereomers were isolated by normal phase HPLC (hexane:ethyl acetate=4:3).

\textbf{Figure 4.6} selected $\Delta \delta^{RS} \text{ } ^1$HNMR values in ppm for MTPA esters 4.13 and 4.14 ($\Delta \delta^{RS} = \delta_{(R)-MTPA} - \delta_{(S)-MTPA}$)

Comparison of the $^1$HNMR spectra of the (R) and (S)-MTPA esters was carried out between the two diastereomers that had the 29-\(\alpha\)Hs. The $^1$HNMR spectra indicated that H$_{28}$, H$_{24}$, H$_{25}$ and H$_{26,27}$ of (S)-MTPA ester were more shielded than in the (R)-MTPA ester, which suggested the C-22 configuration in contignasterol was (S). Comparing the H$_{22}$ and H$_{29}$ chemical shifts of the (S)-MTPA ester with the corresponding (R)-MTPA ester, we found they were also more shielded. This could be explained by conformational deviation of the MTPA moiety from the \textit{ideal} position which requires the carbinyl proton and ester carbonyl and
trifluoromethyl groups of MTPA to be synperplanar.\textsuperscript{13} Therefore, it was not safe to derive the absolute configuration from the MTPA esters of the hemiacetal alcohol. Also, there is almost no literature on hemiacetal Mosher esters. Further evidence was required to demonstrate the absolute configuration in the side chain.

Contignasterol has a cyclic hemiacetal in its side chain, which exists in equilibrium with the open chain 22-hydroxy-29-aldehyde form (Figure 4.7). The secondary alcohol at C- 22 appeared to be ideally suited for chiral auxiliary analysis of its absolute configuration using either MTPA or MPA esters. The C-22 alcohol could be liberated from the hemiacetal in 4.12 by trapping the C-29 aldehyde with 1,2- ethanedithiol to give the cyclic dithiane 4.15 (Figure 4.8).\textsuperscript{15} It's worth noting that the reaction condition applied to this transformation would not lead to epimerization of C-22 according to Miranda et al.'s recent report.\textsuperscript{16} Reaction of 4.15 with the (S)- and (R)-MPA and DCC gave the Mosher esters 4.16 and 4.17,\textsuperscript{17} while treatment with (R)- and (S)-MTPA acid chlorides gave the MTPA esters 4.18 and 4.19, respectively.

\[ \text{Figure 4.7} \]
Figure 4.8 chemical conversion of tetraacetate 4.12 into the MPA esters 4.16 and 4.17 and the MTPA esters 4.18 and 4.19
Table 4.1. Selected NMR data for (R), (S)-MPA esters (4.16) and (4.17), (R), (S)-MTPA esters (4.18) and (4.19) recorded at 400MHz in CD$_2$Cl$_2$ at 300K

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* $\Delta\delta_{RS} = \delta_{(R)}$-MPA - $\delta_{(S)}$-MPA
** $\Delta\delta_{SR} = \delta_{(S)}$-MTPA - $\delta_{(R)}$-MTPA
Figure 4.9 $^1$HNMR comparison of (R)- and (S)-MPA esters recorded at 300K in CD$_2$Cl$_2$
Figure 4.10 $^1$HNMR comparison of (R)- and (S)-MTPA esters recorded at 300K in CD$_2$Cl$_2$
### Table 4.2 Selected NMR data for dithioacetal (4.15) recorded at 400MHz in CD$_2$Cl$_2$ at 300K

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<td>38.39, 38.66</td>
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**Figure 4.11** Models proposed by Mosher with expected sign of $\Delta\delta_{SR}$ and $\Delta\delta_{RS}^{13,16}$
Table 4.1 lists the $^1$H chemical shifts for the esters 4.16, 4.17, 4.18, and 4.19 and the $\Delta \delta^{SR}$ and $\Delta \delta^{RS}$ value for each proton in both the MTPA and MPA (R/S) pairs. From a comparison of the chemical shifts of the (R) and (S)-MPA esters in the table, it was obvious that $\Delta \delta^{RS}$ values on the steroid core structure were all negative ranging from $-0.01$ ppm to $-0.42$ ppm and the positive $\Delta \delta^{RS}$ values are all on the side chain. The chemical shift difference becomes smaller when the proton is more remote from the chiral center C-22. It is worth noting that the $\Delta \delta^{RS}$ value of H-22 is only 0.05 ppm, which means the conformational composition and preference are quite similar in the two MPA esters and the assignment of the absolute configuration is reliable. The $\Delta \delta^{RS}$ values could be used to deduce the location of L₁ and L₂ as shown in Figure 4.11 and thus the absolute configuration at C-22. In this case, the steroid core was represented by L₂ in the model shown in Figure 4.11 and the side chain was represented by L₁. Thus C-22 of 4.12 has the R configuration and the C-22 of 4.1 should have the same configuration, which is opposite to our initial judgement of the chirality by comparing the (R) and (S)-MTPA ester of the hemiacetal alcohol. The absolute configuration of C-24 is R according to the relative stereochemistry of the side chain, which was previously deduced from NOE experiments. Applying the empirical rules¹³ to the $\Delta \delta^{RS}$ values of (R)- and (S)-MTPA esters in Table 1 gives the same conclusion.

Recent work by Riguera's group has compared MTPA with MPA as reagents for determination absolute stereochemistry by NMR, which indicated that MTPA
Chapter 4: Absolute Configuration of Contignasterol.

Figure 4.12 Selected $\Delta \delta^1$HNMR values in ppm for the MPA esters 4.16 and 4.17 and the MTPA esters 4.18 and 4.19

esters were limited by the intrinsic greater complexity of their conformational composition, leading to diminished $\Delta \delta^{RS}$ values, and were consequently less reliable for configurational assignment of chiral alcohols than MPA.\textsuperscript{16} The $\Delta \delta^{RS}$ values of MPA esters and $\Delta \delta^{SR}$ values MTPA esters of contignasterol derivatives as shown in Figure 4.12 were in accordance with Riguera's conclusion. It was obvious that MPA was the preferred chiral auxiliary for determining the C-22 configuration of contignasterol. The decision to carry out the apparently redundant configurational analysis with MTPA was based on the report by Izzo et al.\textsuperscript{18} that they had synthesized a series of model compounds (i.e., 4.20)
containing the four possible C-22/C-24 stereoisomers for the contignasterol side chain and they had used (R) and (S) C-22 MTPA esters (4.21) to verify the side chain configurations in the diastereomers of the model compound 4.20. Using the same chiral auxiliary to make C-22 esters of 4.15 provided a more direct comparison of the C-22 configurations in the model compounds and contignasterol (4.1), and it provided independent confirmation of the C-22 configuration determined with the MPA esters (Figure 4.12). It was worth noting that on the basis of comparisons of the proton NMR data obtained for these four stereoisomers (4.20) with the NMR data for contignasterol derivative (4.22).

![Figure 4.13](image)

**Figure 4.13** Comparison of side chain chemical shifts of the contignasterol derivative 4.22 and Izzo’s synthetic product 4.23.

shown in Figure 4.13, it was proposed by Izzo et al.\textsuperscript{18} that the side chain configuration in the natural product was (22S, 24S). As shown in Figure 4.13, one of the stereoisomers 4.23 with (22S, 24S) configurations on the side chain had very similar \textsuperscript{1}HNMR values as the contignasterol derivative, which led to Izzo’s assignment of the side chain’s absolute configuration of contignasterol antipodal to our proposal that the side chain had (22R, 24R) configurations. This
discrepancy presumably reflects the sensitivity of the side chain proton chemical shifts to through-space shielding and deshielding interactions with functional groups in the steroid nucleus of contignasterol (4.1). It is likely that the unfunctionalized nucleus of the model compound 4.23 cannot accurately mimic these interactions, making a simple proton chemical shift comparison between the model compounds and contignasterol an unreliable predictor of the side chain stereochemistry in 4.1.

One of the recent advances in the assignment of absolute configuration by NMR with chiral auxiliary reagents was that the absolute configuration of a secondary alcohol could be deduced from the $^1$HNMR of a single methoxyphenylacetic ester derivative [MPA, either (R) or the (S)] recorded at two different temperatures.\textsuperscript{19}

![Figure 4.14](image)

**Figure 4.14** (R)-MPA ester of chiral secondary alcohol exists as two main conformers (sp and ap)

As shown in Figure 4.14, the (R)-MPA ester of chiral secondary alcohol exists as two main conformers (sp and ap) that are in equilibrium. The orientation of the phenyl ring produces different shieldings in the two forms, and consequently produces different chemical shifts for $L_1$ and $L_2$ in each of the conformers. The
direct consequence of this phenomenon is that at low temperature, the resonance of one of the substituents of the alcohol, L₁, is shifted upfield, and at the same time, another substituent L₂, is shifted downfield. In this way, the spatial arrangement of L₁ and L₂ around the chiral center can be interpreted by comparing the ¹H NMR spectra both at room and low temperatures. As a valuable complement to determination of the side chain absolute configuration by NMR, this method was applied in our current research. Variable temperature ¹H NMR spectra of the (R)-MPA ester of the contignasterol tetraacetate in CD₂Cl₂ were recorded at room (300K) and four low temperatures (273K, 253K, 223K, 203K). TMS was used as the internal standard in these experiments. Table 4.3 shows selected chemical shifts of the (R)-MPA ester of contignasterol tetraacetate taken at different temperatures. Two groups of data were distinguishable in the table. One group was formed by chemical shifts that shifted upfield in the low-temperature spectrum (Δδ¹H,T₁,T₂>0). They were H₃, H₆, H₁₄, H₁₆, H₁₈, H₂₀, H₂₁ that all reside on one side of the C-22 alcohol. This group was represented by L₁ in the model shown in Figure 4.14. The other group was formed by resonances that moved downfield in the low-temperature spectrum (Δδ¹H,T₁,T₂<0). They were H₂₆, H₂₇, H₂₉, H₃₀, H₃₁ that reside on the other side of the C-22 alcohol. This group was represented by L₂ in the model shown in Figure 4.14. Therefore, the C-22 configuration in contignasterol determined by this method was R, which confirmed the results we obtained from both the MTPA and MPA (R/S) pairs.
Table 4.3. Selected Chemical Shifts of (R)-MPA ester recorded in CD$_2$Cl$_2$ at different temperatures*

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<th>$\delta$ (R)-MPA 253K</th>
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* TMS was used as internal standard  ** overlapped with upfield signals
Figure 4.15 $^1$HNMR comparison of (R)-MPA ester recorded at 300K, 273K, 253K, 223K, and 203K in CD$_2$Cl$_2$. 
4.4 Absolute configuration of Moiety C

![Newman projections for the C-20/C-22 bond in the dithiane 5 in which C-22 has the R configuration and H-20 and H-22 are anti.](image)

The H-22 resonance (δ 3.33; benzene-d₆) of 4.1 showed a positive NOE when H-14 (δ 2.34) was irradiated in a difference NOE experiment,¹ confirming that the side chain was β at C-17. A coupling constant of 8.4 Hz was observed between H-20 (δ 1.84) and H-22 (δ 3.49) in the dithiane 4.15, and there was no appreciable NOE observed between these resonances in a 1D NOESY experiment (Figure 4.17), suggesting that the protons were anti to each other. Strong NOEs observed between H-22 (δ 3.49) and both the H-16 (δ 2.21) and CH₃-21 (δ 0.94) resonances in 1D NOESY experiments carried out on 4.15 were consistent with the proposed anti orientation of H-20 and H-22 and the 22-R configuration shown in the Newman projections in Figure 4.16a and 4.16b. Intense NOEs observed between the CH₃-21 (δ 0.94) and H-23 (δ 1.21)/H-23' (δ 1.60) resonances in 4.15 demonstrated that C-20 has the S configuration shown in Figure 3a, which is the normal C-20 configuration for a steroid.
Figure 4.17 The fragment structure of 4.15 with selected NOESY correlation

Figure 4.18 Expansion of the NOESY spectrum for 4.15
4.5 Experimental Section

General: Circular dichroism spectra were recorded on a JASCO J-76 spectropolarimeter. $^1$H and $^{13}$C NMR spectra were recorded at 400 and 100 MHz, respectively, on Bruker AV400, AV500, and AM400 spectrometers. $^1$H chemical shifts are referenced to the residual CH$_2$Cl$_2$-d$_2$ signal (5.32 ppm) and C$_6$H$_6$-d$_6$ signal (7.15 ppm), and $^{13}$C chemical shifts are referenced to the CH$_2$Cl$_2$-d$_2$ solvent peak (53.8 ppm). In VT $^1$HNMR, $^1$H chemical shifts are referenced to the residual TMS signal (0.0 ppm). FABMS were recorded on a Kratos Concept II HQ mass spectrometer. Commercially obtained reagents were used without further purification. All reactions were monitored by TLC (silica gel, 60F-54, Merck). HPLC was performed on a Alltech column (250 × 4.6 mm, n-hexane/ethyl acetate, 1 mL/min) monitored by a RI detector.

Isolation of Contignasterol (4.1)

Contignasterol (4.1) was isolated as a white solid from the extracts of the sponge *Petrosia contignata* Thiele using the same procedure as reported in the original paper.$^1$

Preparation of Contignasterol tri-p-methoxybenzoate (4.9)
Contignasterol (4.1) (15.0 mg) was dissolved in 2 mL of pyridine and 4 mL of 4-methoxybenzoyl chloride. The mixture was stirred at RT for 4 h. 3.0 mL of MeOH was added to the mixture and stirring was continued for 0.5 h. Pyridine and MeOH were removed in vacuo, and the residue was purified by normal-phase HPLC (eluent: hexane/EtOAc (4:6)).

$^1$H NMR (C$_6$D$_6$) δ 0.69 (d, J= 7.5 Hz, 3H), 0.71 (d, J= 7.0 Hz, 3H), 0.73 (d, J= 6.9 Hz, 3H), 1.16 (s, 3H), 1.42 (s, 3H), 2.64 (s, 1H), 2.96 (s, 3H), 3.00 (s, 3H), 3.12 (s, 3H), 3.44 (s, 3H), 3.94 (dd, J= 9.3, 1.8 Hz, 1H), 4.10 (d, J= 3.8 Hz, 1H), 4.18 (brs, 1H), 5.63 (brd, J= 2.4 Hz, 1H), 5.99 (dd, J= 12.0, 9.1 Hz, 1H), 6.40 (d, J= 8.7 Hz, 2H), 6.49 (d, J= 8.8 Hz, 2H), 6.52 (d, J= 8.5 Hz, 2H), 7.34 (dd, J= 10.5, 9.3 Hz, 1H), 8.08 (d, J= 7.8 Hz, 2H), 8.10 (d, J= 7.8 Hz, 2H), 8.15 (d, J= 9.0 Hz, 2H)

Preparation of Contignasterol Pentaacetates (4.11)
Contignasterol (4.1) (32.8 mg) was dissolved in 2 mL of pyridine and 2 mL of Ac₂O. A few crystals of DMAP were added, and the resulting mixture was stirred at RT for 18 h. The reaction solvents were removed by evaporation under high vacuum, and purification of the products was carried out by normal-phase HPLC, eluting with n-hexane/EtOAc (4:3) to yield the C-29α (6 mg) and C-29β (16 mg) contignasterol pentaacetates (4.11) (total isolated yield was 47.5%). The spectroscopic data for the pentaacetates (4.11) were identical to the literature values.¹

**Pentaacetate (C-29β)** \(^1\)H NMR (CDCl₃) δ 0.73 (d, J = 6.7 Hz, 3H), 0.75 (d, J = 7.0 Hz, 3H), 0.76 (d, J = 6.7 Hz, 3H), 0.93 (s, 3H), 1.24 (s, 3H), 1.52, 1.79, 1.85, 1.88, 1.95 (5XCH₃CO), 2.39 (brs, 1H), 3.31 (t, J = 9.9 Hz, 1H), 5.11 (brd, J = 2.7 Hz, 1H), 5.46 (dd, J = 12.2, 9.1 Hz, 1H), 5.49 (brs, 1H), 5.60 (dd, J = 9.6, 2.1 Hz, 1H), 6.56 (dd, J = 10.7, 9.1 Hz, 1H),

**Pentaacetate (C-29α)** \(^1\)H NMR (CDCl₃) δ 0.72 (d, J = 7.0 Hz, 3H), 0.74 (d, J = 7.8 Hz, 3H), 0.77 (d, J = 6.9 Hz, 3H), 0.95 (s, 3H), 1.13 (s, 3H), 1.51, 1.78, 1.81, 1.91, 1.95 (5XCH₃CO), 2.63 (brs, 1H), 3.77 (t, J = 8.7 Hz, 1H), 5.12 (brd, J = 2.7 Hz, 1H), 5.48 (m, 2H), 6.24 (brd, J = 2.3 Hz, 1H), 6.54 (dd, J = 10.4, 9.0 Hz, 1H)
Preparation of Contignasterol Tetraacetate (4.12)

The pentaacetates (4.11) of contignasterol (either C-29 or C-29) (21.2 mg, 0.0295 mmol) were dissolved in 0.8 mL of MeCN containing 7.3 μL of H₂O and treated with BF₃·OEt₂ (6 μL, 0.048 mmol) at 0 C for 1.5 h. The reaction was quenched with saturated NaHCO₃ (1 mL) and extracted with EtOAc (3 × 10 mL), and the EtOAc was dried with MgSO₄. After evaporation of the solvent, 18.4 mg (92%) of contignasterol tetraacetate (4.12) was obtained as a white solid, which was used without further purification. The ¹H NMR spectrum of 4.12 was not assigned. It is very complex because 4.12 exists as a slowly equilibrating mixture of C-29 epimers.

¹H NMR (C₆D₆) δ 0.66 (d, J= 7.9 Hz, 3H), 0.68 (d, J= 7.5 Hz, 3H), 0.72 (d, J= 6.2 Hz, 3H), 0.74 (d, J= 5.9 Hz, 3H), 0.80 (d, J= 6.7 Hz, 3H), 0.82 (d, J= 6.9 Hz, 3H), 0.93 (s, 3H), 0.95 (s, 3H), 1.11 (s, 3H), 1.14 (s, 3H), 1.51, 1.64, 1.78, 1.80, 1.81, 1.84, 1.91, 2.05 (8XCH₃CO), 2.63 (t, J= 8.8 Hz, 1H), 2.92 (brs, 1H), 3.16 (brs, 1H), 3.68 (t, J= 9.5 Hz, 1H), 5.13 (m, 3H), 5.47 (m, 4H), 6.56 (t, J= 9.8 Hz, 1H), 6.69 (t, J= 9.4 Hz, 1H)
FABHRMS [M + Na]^+ m/z 699.3715 (calcd for C_{37}H_{56}NaO_{11}, 699.3720).

Preparation of MTPA ester of Contignasterol Tetraacetate (4.13) and (4.14)

(R)- or (S)-MTPA chloride (0.5 mL) and 1 mL of pyridine were added to 4.12, and the mixture was stirred at RT overnight. The pyridine was removed in vacuo, and the (R)- and (S)-MTPA esters were purified by normal-phase HPLC (eluent: hexane/EtOAc (7:3)).

(R)-MTPA ester (4.13) $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 0.87 (d, J= 6.7 Hz, 3H), 0.89 (d, J= 6.4 Hz, 3H), 0.99 (d, J= 7.2 Hz, 3H), 1.09 (s, 3H), 1.29 (s, 3H), 1.92, 1.92, 1.97, 2.06 (4XCH$_3$CO), 2.24 (brs, 1H), 3.57 (s, 3H), 4.81 (brs, 1H), 4.97 (brs, 1H), 5.03 (dd, J= 11.9, 9.1 Hz, 1H), 5.82 (dd, J= 9.9, 2.1 Hz, 1H), 5.96 (dd, J= 10.8, 9.3 Hz, 1H), 7.49 (m, 5H)

(S)-MTPA ester (4.14) $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 0.87 (d, J= 6.7 Hz, 3H), 0.88 (d, J= 6.7 Hz, 3H), 0.97 (d, J= 7.2 Hz, 3H), 1.10 (s, 3H), 1.24 (s, 3H), 1.92, 1.93, 1.98, 2.06 (4XCH$_3$CO), 2.30 (brs, 1H), 2.53 (dd, J= 19.8, 10.1 Hz, 1H), 3.41 (s, 3H),
4.22 (dd, J = 9.6, 2.0 Hz, 1H), 4.82 (brd, J = 2.9 Hz, 1H), 4.98 (brs, 1H), 5.03 (dd, J = 12.0, 9.2 Hz, 1H), 5.98 (dd, J = 10.8, 9.2 Hz, 1H), 7.51 (m, 5H)

Preparation of Dithiane 4.15

Contignasterol tetraacetate (4.12) (10 mg in 28.7 µL of AcOH) was treated with a solution of 1,2-ethanediethiol (9.6 µL) and BF₃·OEt₂ (1.2 µL), and the resulting mixture was stirred at room temperature overnight. Aqueous NaHCO₃ (1 mL of a 1 M solution) and 7 mL of CH₂Cl₂ were added, and the organic layer was separated, evaporated in vacuo, and subjected to normal-phase HPLC (elucent: EtOAc/hexane (1:2)) to give pure dithiane 4.15 (yield 7.8 mg, 70%):

\(^1\)H NMR (CD₂Cl₂) δ 0.80 (d, J = 6.9 Hz, 3H), 0.90 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.9 Hz, 3H), 1.10 (s, 3H), 1.20 (s, 3H), 1.92 (s, 3H, CH₃CO), 1.92 (s, 3H, CH₃CO), 1.98 (s, 3H, CH₃CO), 2.06 (s, 3H, CH₃CO), 2.21 (dd, J = 19.6, 1.9 Hz, 1H), 2.41 (dd, J = 19.6, 10.2 Hz, 1H), 2.74 (brs, 1H), 3.22 (m, 4H), 3.49 (t, J = 8.7
Hz, 1H), 4.57 (dd, J= 8.9, 5.7 Hz, 1H), 4.82 (brd, J= 2.8 Hz, 1H), 4.97 (brs, 1H), 5.03 (dd, J= 12.0, 9.1 Hz, 1H), 5.96 (dd, J= 10.6, 9.1 Hz, 1H)

$^{13}$C NMR δ 14.3, 17.6, 18.0, 20.0, 20.0, 21.0, 21.0, 21.1, 21.1, 21.4, 21.9, 29.8, 32.0, 36.4, 37.0, 37.3, 37.8, 38.4, 38.7, 39.2, 40.3, 41.1, 41.4, 42.0, 44.4, 46.5, 46.6, 51.0, 53.0, 67.3, 68.6, 70.8, 72.6, 74.8, 169.9, 170.0, 170.5, 170.9, 218.8

FABHRMS [M + H]$^+$ m/z 753.3701 (calcd for C$_{39}$H$_{61}$O$_{10}$S$_2$, 753.3706).

**Preparation of (R)- and (S)-MPA Esters 4.16 and 4.17**

(R)- or (S)-MPA, DCC, and the dithiane 4.15 in the molar ratio of 5:7:1 were added into 1 mL of anhydrous CH$_2$Cl$_2$ along with a few crystals of DMAP. The mixture was stirred at RT for 10 h, then filtered to remove the dicyclohexylurea, and the (R)- and (S)-MPA esters were purified by normal-phase HPLC (eluent: hexane/EtOAc (2:1)).
(R)-MPA ester 4.16: $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 0.77 (d, J= 6.9 Hz, 3H), 0.82 (d, J= 6.9 Hz, 3H), 0.88 (d, J= 6.9 Hz, 3H), 1.05 (s, 3H), 1.06 (s, 3H), 1.91 (s, 3H, CH$_3$CO), 1.92 (s, 3H, CH$_3$CO), 1.97 (s, 3H, CH$_3$CO), 2.06 (s, 3H, CH$_3$CO), 2.14 (brs, 1H), 3.25 (m, 4H), 3.45 (s, 3H), 4.54 (dd, J= 10.0, 4.9 Hz, 1H), 4.80 (brd, J= 2.7 Hz, 1H), 4.96 (brs, 1H), 5.00 (dd, J= 12.0, 9.2 Hz, 1H), 5.12 (brt, J= 8.0 Hz, 1H), 5.88 (dd, J= 10.7, 9.1 Hz, 1H), 7.39 (m, 5H)

FABHRMS [M + H]$^+$ m/z 901.4229 (calcd for C$_{48}$H$_{69}$O$_{12}$S$_2$, 901.4230).

(S)-MPA ester 4.17: $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 0.63 (d, J= 6.9 Hz, 3H), 0.65 (d, J= 7.0 Hz, 3H), 1.01 (d, J= 7.0 Hz, 3H), 1.08 (s, 3H), 1.16 (s, 3H), 1.92 (s, 3H, CH$_3$CO), 1.94 (s, 3H, CH$_3$CO), 1.97 (s, 3H, CH$_3$CO), 2.06 (s, 3H, CH$_3$CO), 2.24 (brs, 1H), 2.42 (dd, J= 19.7, 10.0 Hz, 1H), 3.21 (m, 4H), 3.40 (s, 3H), 4.33 (d, J= 8.7, 5.7 Hz, 1H), 4.81 (brd, J= 2.6 Hz, 1H), 4.97 (brs, 1H), 5.02 (dd, J= 11.9, 9.1 Hz, 1H), 5.08 (m, 1H), 5.92 (dd, J= 10.9, 9.3 Hz, 1H), 7.41 (m, 5H)

FABHRMS [M + H]$^+$ m/z 901.4229 (calcd for C$_{48}$H$_{69}$O$_{12}$S$_2$, 901.4230).
Preparation of the (R)- and (S)-MTPA Esters 4.18 and 4.19

(R)- or (S)-MTPA chloride (0.5 mL) and 1 mL of pyridine were added to the dithiane 4.15, and the mixture was stirred at RT overnight. The pyridine was removed in vacuo, and the (R)- and (S)-MTPA esters were purified by normal-phase HPLC (eluent: hexane/EtOAc (7:3)).

(R)-MTPA ester 4.18: $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 0.74 (d, J = 6.8 Hz, 3H), 0.85 (d, J = 6.8 Hz, 3H), 1.09 (d, J = 6.8 Hz, 3H), 1.10 (s, 3H), 1.21 (s, 3H), 1.92 (s, 3H, CH$_3$CO), 1.92 (s, 3H, CH$_3$CO), 1.98 (s, 3H, CH$_3$CO), 2.06 (s, 3H, CH$_3$CO), 2.24 (dd, J = 19.3, 2.3 Hz, 1H), 2.27 (brs, 1H), 2.46 (dd, J = 19.3, 9.7 Hz, 1H), 3.17 (m, 4H), 3.45 (dd, J = 3.1, 1.6 Hz, 1H), 3.55 (s, 3H), 4.33 (dd, J = 9.2, 5.4 Hz, 1H), 4.82 (brd, J = 2.8 Hz, 1H), 4.98 (brs, 1H), 5.03 (dd, J = 12.0, 9.2 Hz, 1H), 5.26 (dd, J = 10.6, 2.7 Hz, 1H), 5.93 (dd, J = 10.9, 9.3 Hz, 1H), 7.50 (m, 5H)

FABHRMS [M + H]$^+$ m/z 969.4103 (calcd for C$_{49}$H$_{68}$F$_3$O$_{12}$S$_2$, 969.4104).
(S)-MTPA ester 4.19: $^1$H NMR (CD$_2$Cl$_2$) $\delta$ 0.76 (d, J= 6.9 Hz, 3H), 0.88 (d, J= 6.9 Hz, 3H), 0.90 (d, J= 6.9 Hz, 3H), 1.11 (s, 3H), 1.17 (s, 3H), 1.92 (s, 3H, CH$_3$CO), 1.95 (s, 3H, CH$_3$CO), 1.98 (s, 3H, CH$_3$CO), 2.06 (s, 3H, CH$_3$CO), 2.21 (dd, J= 19.4, 2.3 Hz, 1H), 2.25 (brs, 1H), 2.42 (dd, J= 19.5, 9.5 Hz, 1H), 3.22 (m, 4H), 3.45 (dd, J= 3.1, 1.4 Hz, 1H), 3.57 (s, 3H), 4.43 (dd, J= 9.1, 5.3 Hz, 1H), 4.82 (brd, J= 2.8 Hz, 1H), 4.98 (brs, 1H), 5.04 (dd, J= 12.1, 9.2 Hz, 1H), 5.20 (brd, J= 10.5 Hz, 1H), 5.94 (dd, J= 10.8, 9.2 Hz, 1H), 7.48 (m, 5H)

FABHRMS [M$^+$ + H]$^+$ m/z 969.4105 (calcd for C$_{49}$H$_{68}$F$_3$O$_{12}$S$_2$, 969.4104).
4.6 References

18. Izzo, I.; Pironti, V.; Della Monica, C.; Sodano, G.; De Riccardis, F.
Chapter 4: Absolute Configuration of Contignasterol.


Small molecules derived from natural sources or syntheses are invaluable tools for biochemists to probe the behavior of biological systems. Discovering new chemical tools can improve our knowledge of biology and also lead to the development of new drugs. The work presented in chapter two represents a successful example of this type of research. Our biology collaborators hypothesized that a SHIP activator could inhibit inflammatory cell activation and leukemic cell proliferation, based on the fact that SHIP deficient mice overproduce neutrophils, macrophages and osteoclasts and are hyper-responsive to inflammatory stimuli, while the loss of SHIP in humans has been implicated in allergies and various leukemias. Identification of the first known SHIP activator, pelorol (2.1), which inhibits macrophage activation in intact cells supported the initial hypothesis that an activator of SHIP might indeed be a potent anti-inflammatory compound. Our synthesis of pelorol starting from the plant natural product sclareolide confirmed its proposed structure, absolute configuration, and biological activity. In the process, several pelorol analogs were prepared. One of them, AQX-16A (2.63), showed significantly enhanced bioactivity. AQX-16A has been shown to have excellent in vivo activity in two mouse models of inflammation, which provides proof of principle validation of the original hypothesis. This work appears to be the first demonstration that small molecule activators of phosphatases can be found and they represent viable drug leads.
A rational drug design process always requires modification of the lead compound to identify the pharmacophore and to enhance potency and selectivity. Chapter three described an investigation of the structure activity relationship (SAR) for the pelorol pharmacophore. Since the molecular target of pelorol is chiral, it is not surprising that the individual stereoisomers of pelorol and pelalogs may have differential bioactivity. A series of C-ring stereoisomers of pelorol and its analogs have been synthesized in high yield with a Nazarov cyclization as the key step. The biological activity of these stereoisomers help us understand the relationship between the stereochemistry of pelorol and its bioactivity.

It is widely recognized that the stereochemistry of an organic compound often determines its chemical, physical, biological, and pharmacological properties. The final portion of the thesis presents the determination of the absolute stereochemistry of the anti-inflammatory sponge metabolite contignasterol (4.1) by a combination of chemical degradation and spectroscopic methods. Some recent developments in the assignment of absolute configuration by NMR have found their application in this work. Our assignment of the 22R, 24R contignasterol side chain configurations is antipodal to Izzo's result. The nucleus of contignasterol has the standard steroidal configuration. Solution of the absolute configuration of contignasterol cleared the way to the asymmetric total synthesis of this polyoxygenated compound.
Appendix for Chapter 2 and 3

Spectra of Compounds submitted for SHIP assay
Figure A.1: $^1$H NMR of 2.36 recorded in CDCl$_3$ at 400 MHz

- 7.2400
- 6.2836
- 3.8204
- 3.7579
- 3.7386
- 2.7141
- 2.6985
- 2.6774
- 2.6618
- 2.5290
- 2.4969
- 2.4602
- 1.2346
- 1.0592
- 1.0072
- 0.8415
Figure A.2 $^{13}$C NMR of 2.36 recorded in CDCl$_3$ at 100MHz
Figure A.3, H NMR of 2.42 recorded in CDCl₃ at 400 MHz.
Figure A.4 $^{13}$C NMR of 2.42 recorded in CDCl$_3$ at 100MHz
Figure A.6 $^{13}$C NMR of 2.57 recorded in CDCl$_3$ at 100MHz
Figure A.7: 1'H NMR of 2.61 recorded in CDCl₃ at 400 MHz.
Figure A.8 $^{13}$C NMR of 2.61 recorded in CDCl$_3$ at 100MHz
Figure A.9, H NMR of 2.62 recorded in CDCl₃ at 400 MHz.
Figure A.10 $^{13}$C NMR of 2.62 recorded in CDCl$_3$ at 100MHz
Figure A.11  $^1$H NMR of 2.63 recorded in CDCl$_3$ at 400 MHz

Chemical Shifts (ppm):
- 7.2400
- 6.3963
- 4.7966
- 4.7165
- 2.5905
- 2.5743
- 2.5543
- 2.5390
- 2.4685
- 2.4360
- 2.4017
- 2.3274
- 2.3197
- 2.3045
- 2.2978
- 2.1710
- 1.0403
- 1.0156
- 0.8459

Appendix
Figure A.12 $^{13}$C NMR of 2.63 recorded in CDCl$_3$ at 100MHz
Figure A.13: \( \text{H}_1 \text{NMR of 3.19 recorded in CDCl}_3 \text{ at } 400 \text{MHz} \)

- 7.2400
- 6.4248
- 4.7441
- 4.6794
- 2.8172
- 2.7963
- 2.7788
- 2.7582
- 2.6722
- 2.6418
- 2.6037
- 2.2403
- 1.5906
- 1.2237
- 0.8538
- 0.8386
Figure A.14 $^{13}$C NMR of 3.19 recorded in CDCl$_3$ at 100MHz
Figure A.15: H NMR of 3.20 recorded in CDCl₃ at 400MHz.
Figure A.16 $^{13}$C NMR of 3.20 recorded in CDCl$_3$ at 100MHz
Figure A.17: $^1$H NMR of 3.25 recorded in CDCl$_3$ at 400 MHz.
Figure A.18 $^{13}$C NMR of 3.25 recorded in CDCl$_3$ at 100MHz
Figure A.19, H NMR of 3.31 recorded in CDCl₃ at 400 MHz.
Figure A.20 $^{13}$C NMR of 3.31 recorded in CDCl$_3$ at 100MHz
Figure A.21, {subscript}H NMR of 3.33 recorded in CDCl₃ at 400 MHz.

- 7.2400
- 6.6546
- 6.6356
- 6.4630
- 6.4630
- 4.9548
- 4.8595
- 2.8574
- 2.8403
- 2.8174
- 2.8002
- 2.6544
- 2.6134
- 1.0403
- 0.8630
- 0.7210
- 0.3186
Figure A.22 $^{13}$C NMR of 3.32 recorded in CDCl$_3$ at 100MHz