Studies in Marine Quinone Chemistry

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Abstract

This thesis is divided into two parts and the rationale of the research conducted is based on the cytotoxicity of the prenylated quinones **1.24**-**1.29**, isolated from the South African nudibranch *Leminda millecra*, against oesophageal cancer cells.

The first part (Chapters 2 and 3) of the thesis initially documents the distribution of cytotoxic and antioxidant prenylated quinones and hydroquinones in the marine environment. We have been able to show, for the first time, that these compounds can be divided into eight structural classes closely related to their phyletic distribution. Secondly, we attempted to synthesize the two marine natural products **1.24** and **1.26** in an effort to contribute to an ongoing collaborative search with the Division of Medical Biochemistry at the University of Cape Town for new compounds with anti-oesophageal cancer activity. Accordingly, we followed the published synthetic procedure for **1.26** and, although we were unable to reproduce the reported results, we have generated five new prenylated quinone analogues **3.53**-**3.55**, **3.63** and **3.71**, which are a potentially viable addition to our ongoing structure-activity relationship (SAR) studies. Moreover, we embarked on a ^{7}Li NMR mechanistic study for the synthesis of **3.2** from **3.1** which rewarded us with an improved and reproducible methodology for this crucial reaction that is detailed in Chapter 3.

The second part of this thesis (Chapters 4 and 5) is concerned with a synthetic, structural, electrochemical and biological exploration of the 1,4-naphthoquinone nucleus as a primary pharmacophore in our search for new chemical entities which can induce apoptosis in oesophageal cancer cells, thus contributing to our overall ongoing SAR study in this class of compounds. Seven new naphthoquinone derivatves (**4.19**, **4.30**, **4.31**, **4.33** and **4.46-4.48**) of the natural products 2-deoxylapachol (**2.44**), lapachol (**4.1**) and β-lapachone (**5.2**) were synthesized and 2-(1`-hydroxy-1`-phenylmethyl)-1,4-naphthoquinone (**4.29**) was found to be the most cytotoxic (IC_{50} 1.5 μM) against the oesophageal cancer cell line WHCO1, while **5.2**,

which is currently in phase II clinical trials as an anticancer drug, was found to be similarly active (IC_{50} 1.6 μM). Electrochemical investigations of the redox properties of the benzylic alcohol derivatives **4.29**-**4.31** indicated a higher reduction potential compared to their oxidized counterparts **4.45**-**4.48**, and this finding has been correlated to the increased activity of **4.29**-**4.31** against the WHCO1 cell line. Additionally, **4.29** is synthetically more accessible than either **1.26** or **5.2** and potentially a lead compound in our search for new and more effective chemotherapeutic agents against oesophageal cancer.

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List of Abbreviations

1D	one dimensional
2D	two dimensional
$[\alpha]_D$	specific rotation
17-AAG	17-allylamino-17-dimethoxy-geldanamycin
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
aq	aqueous
br	broad
BuLi	butyllithium
c	concentration (quoted in g/100 mL)
calcd	calculated
CAN	cerium ammonium nitrate
conc.	concentrated
CV	cyclic voltammetry
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
ddd	doublet of double doublets
DEPT	distortionless enhancement of polarisation transfer
DIP-Cl	*β*-chlorodiisopinocamphenylborane
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMG	directing metaling group
DMS	dimethyl sulfide
DMSO	dimethylsulfoxide
D*o*M	directed *ortho*-metalation
dt	double triplet
EC_{50}	median effective concentration
ED_{50}	median effective dose
EIMS	electron impact mass spectrometry
eq	molar equivalent
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
eV	electron-volt
FT	Fourier transform
GC	gas chromatography
gCOSY	gradient ^1H-^1H homonuclear correlation spectroscopy
gHMBC	gradient heteronuclear multiple bond correlation
gHSQC	gradient heteronuclear single quantum coherence
h	hour(s)
HPLC	high performance liquid chromatography
HREIMS	high resolution electron impact mass spectrometry
HRFABMS	high resolution fast atom bombardment mass spectrometry
Hsp90	heat-shock protein 90
HTS	high throughput screening

IC_{50}	median inhibitory concentration
int.	integration
IR	infrared
LAH	lithium aluminium hydride
lit.	literature
m	multiplet
MeOH	methanol
MHE	metal-halogen exchange
min	minute
mmHg	millimetres of mercury
mmol	millimoles
mol	moles
mp	melting point
MS	mass spectrometry
MTPA	α-methoxy-α-trifluoromethylphenylacetic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mult.	multiplicity
NBS	*N*-bromosuccinimide
NMO	*N*-methyl morpholine-*N*-oxide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser enhancement
NOESY	nuclear Overhauser enhancement spectroscopy
PPh_3	triphenyl[hosphine
ppm	parts per million
q	quartet
R	alkyl group
rel. int	relative intensity
ROS	reactive oxygen species
RT	room temperature
s	singlet
sat.	saturated
SAR	structure-activity relationship
SCC	squamous cell carcinoma
SCOC	squamous cell oesophageal cancer
SCUBA	self-contained underwater breathing apparatus
sp.	species
t	triplet
TBAP	tetrabutylammonium perchlorate
TBAF	tetrabutylammonium fluoride
TBHP/*t*-BuOOH	*tert*-butylhydroperoxide
TEATFB	tetraethylammonium tetrafluoroborate
tert	tertiary
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	*N,N,N`,N`*-tetramethylethylene diamine
TsOCl	toluene-*p*-sulphonyl chloride
TPAP	tetrapropylammonium perruthenate
UV	ultraviolet
V	volt
mV	milivolt
W	watt

Declaration

The following thesis has not been submitted to a university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author unless otherwise stated.

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Chapter One

General Introduction

1.1 Pharmaceuticals from marine organisms

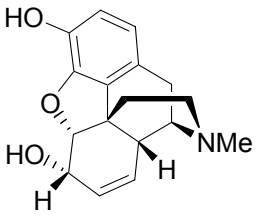
Oceans cover more than 70% of the Earth's surface and comprise approximately 95% of the biosphere making them the most biodiverse environments on the planet.[1;2] A function of marine biodiversity is a unique source of novel natural products, also known as secondary metabolites.[2;3] The ocean is a very stable chemical and physical environment where nutrients are readily accessible and constantly being recycled, thus contributing to the development of the high biodiversity inherent in this environment.[2-5] Marine organisms (invertebrates, algae and micro-organisms) utilize their secondary metabolites as a form of chemical defence against predators or in a chemically mediated response to inter-species competition for limited resources, *e.g.* for nutrients or space on a coral reef.[1-3] Not surprisingly, many chemists in the 1960's turned to marine organisms, then a previously unexplored source of chemical diversity, for possible new drug leads.[1;2] Over the last five decades, the collaborative efforts between marine natural product chemists and pharmacologists have yielded numerous lead compounds for the pharmaceutical industry, particularly novel anticancer drugs.[1;6] According to Andersen and Williams,[2] the complexity and uniqueness of many marine natural products isolated thus far reflects possible novel biosynthetic pathways or evolutionary adaptations of ubiquitous pathways in marine organisms, resulting from the enormous evolutionary time frame (over 500 million years in sponges) in which these pathways have been able to evolve in the unique environment provided by the sea.

With the problems associated with the increasing incidence of cancer in an ageing society, the steady spread of HIV-AIDS, malaria and other diseases and a growing resistance to current antibiotics, there is a continuous need for new drug development.[2] Through the historical use of plant-based traditional medicines in all cultures and societies, a very close relationship has been established between natural products and medicine.[7;8] Almost 80% of the current anticancer drugs and about 68% of the drugs presently used against infectious diseases are either naturally derived or are natural product derivatives, whilst truly synthetic

drugs, *i.e.* devoid of natural inspiration, account for only about 37% of the listed 1024 small drug molecules that have appeared in the market between 1981 and 2008.[8] Nearly all of these natural product derived drugs are of terrestrial origin, for example; the analgesic morphine (**1.1**) that was isolated from the poppy *Papaver somniferum*, the antimalarial drug artemisinin (**1.2**) isolated from the Chinese plant *Artemisica annua* and the anticancer drug paclitaxel or Taxol® (**1.3**) originally isolated from the bark of the Pacific Yew tree *Taxus brevifolia*.[2] Paclitaxel is currently used in the treatment of breast, ovarian and non-small cell lung cancer and is also potentially effective against multiple sclerosis and rheumatoid arthritis.[9] Marine natural products derived drugs currently on the market include the antiviral drug Acyclovir® (**1.4**), the anti-inflammatory pseudopterosin A (**1.5**) and ecteinascidin 743 (ET-743, **1.6**).[2;5;6;9;10] Acyclovir® is a synthetic analogue of the metabolites spongothymidine (**1.7**) and spongouridine (**1.8**) originally isolated from the sponge *Cryptoethya crypta* and also later found in the gorgonian *Eunicella cavolini*.[2;6] There are several examples of marine natural products derived drugs currently in anticancer clinical trials,[1;2;9] *e.g.* bryostatin-1 (**1.9**) isolated from the bryozoan *Bugula neritina* and halichondrin B (**1.10**) isolated from the Japanese sponge *Halichondria okadai*, as anticancer drugs.[2;9] Compound **1.6** was originally isolated from the ascidian *Ecteinascidia turbinate* and is currently in several phase II/III clinical trials for a number of different cancers, *e.g.* ovarian, breast, prostate and non-small cell lung.[8;9] About 25% of the clinical trials for **1.9** have completed both phases I and II, while the compound E7389 (**1.11**) which is a simplified synthetic analogue of the marine natural product halichondrin B, **1.10**, is currently in phase III clinical trials against refractory breast carcinoma.[9] Encouragingly, there has been a dramatic increase, in the last fifteen years, in the number of preclinical anticancer lead compounds, isolated from a diverse array of marine organisms, that have entered human clinical trials.[3;11;12]

It is interesting to note that natural product chemists initially noticed an unsuspected close similarity between the metabolites isolated from marine invertebrates and compounds isolated from terrestrial organisms of completely different taxa, *e.g.* the current drug ecteinascidin 743,

1.6, previously isolated from the tunicate *Ecteinascidia turbinate* bears a striking similarity to the saframycin type compounds isolated from various terrestrial actinomycetes.[13]



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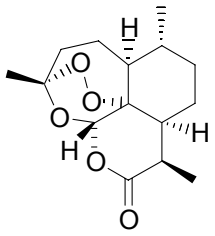

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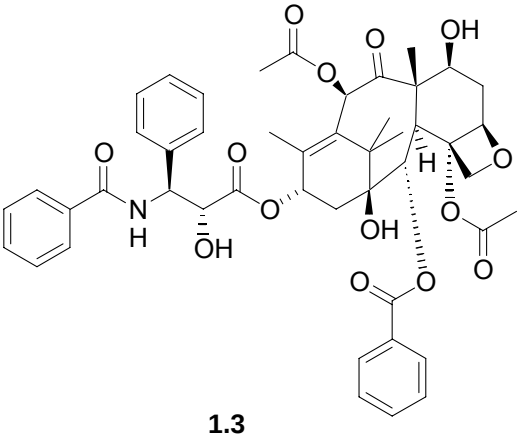

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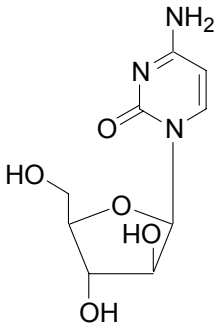

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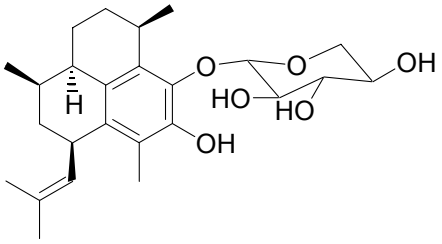

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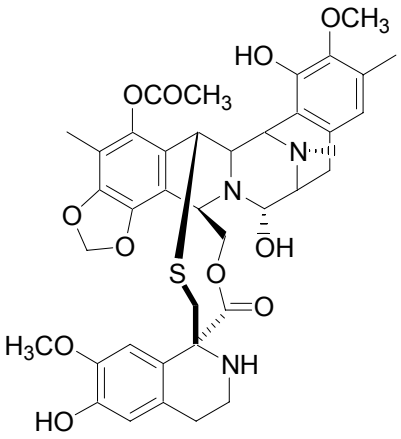
Li and Vederas have painted a bright future for natural product-based drug discovery where, in their recent Science paper, they highlighted new tools that are emerging in the discovery of novel drugs from natural products.[14] Amongst these tools was the return to the screening of microorganisms for novel natural products and the potential for microbial metagenomics and bioinformatics, aided by the falling costs of genome sequencing, providing access to new libraries of natural product analogues through modification of biosynthetic genes transposed

into heterologous hosts, *e.g. Escherichia coli*.[13;14] In the marine environment, there may be as many as 3.7 x 10^{30} microorganisms, of which an estimated 99% are unknown, therefore providing an enormous untapped reservoir of chemical diversity in the oceans.[15] Significant advances in high throughput screening (HTS) are opening new avenues in drug discovery and, in some cases, new uses for old and well-known natural products. Additionally, structure elucidation techniques continue to improve and it is now possible to establish chemical structures on diminishing amounts of material.[14]



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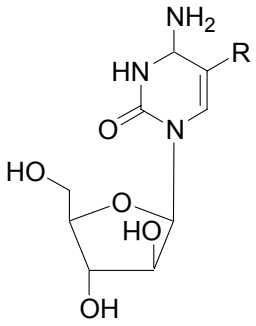

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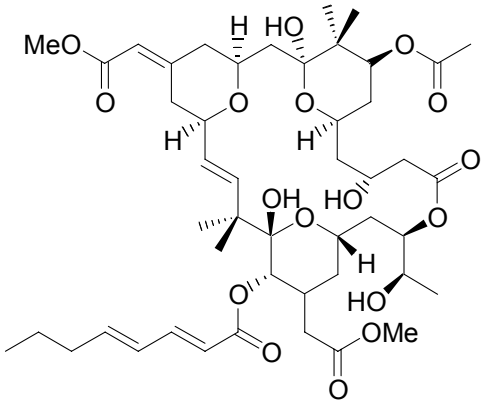

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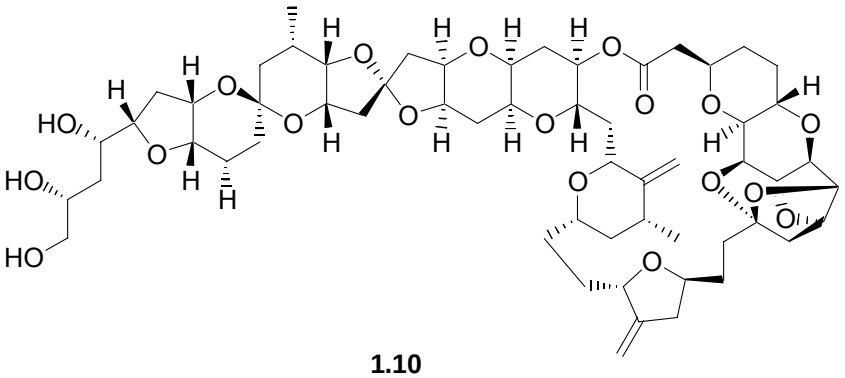

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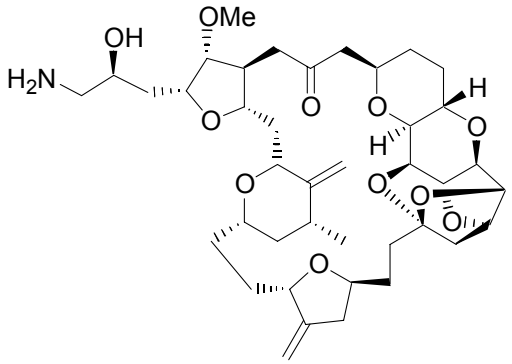

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1.2 Potential anticancer compounds from Southern African marine invertebrates

The natural product diversity or biomedical potential of the extensive and uniquely diverse inter-tidal and benthic marine invertebrate populations living off the South African coast is relatively unknown,[16;17] whilst the bioprospecting of southern Africa's terrestrial phytochemical resources for new pharmaceuticals is well-established.[18] The South African coastline stretches across approximately 3000 km from southern Namibia in the west to southern Mozambique in the east and can be categorized into three main biogeographical zones: the cool temperate west coast cooled by the cold waters of the Benguela current, the warm temperate southeast coast and the subtropical east coast warmed by the warm Agulhas current (Figure 1.1).[16;17] Each of these three biogeographical zones is host to its own unique

population of diverse marine organisms with an immense potential for the discovery of new pharmaceuticals.[16;17]

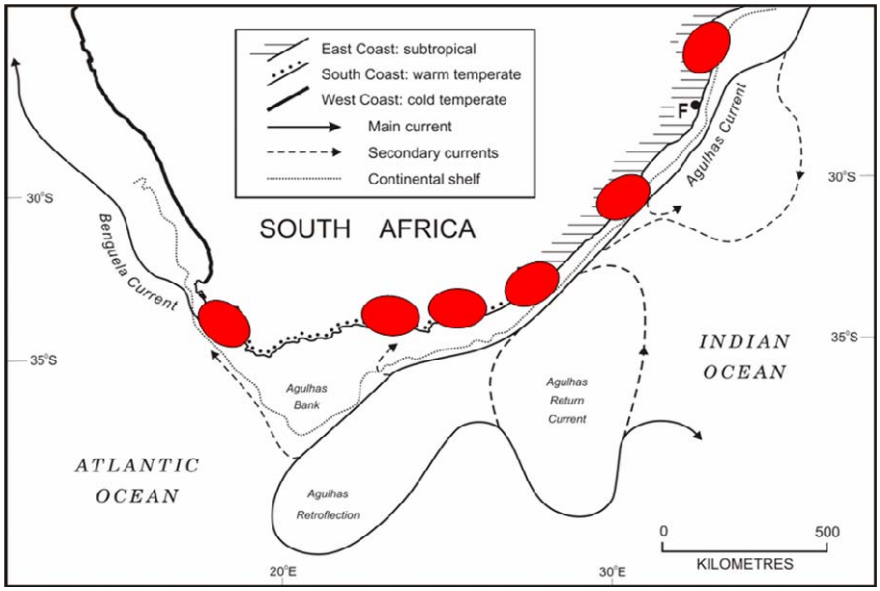
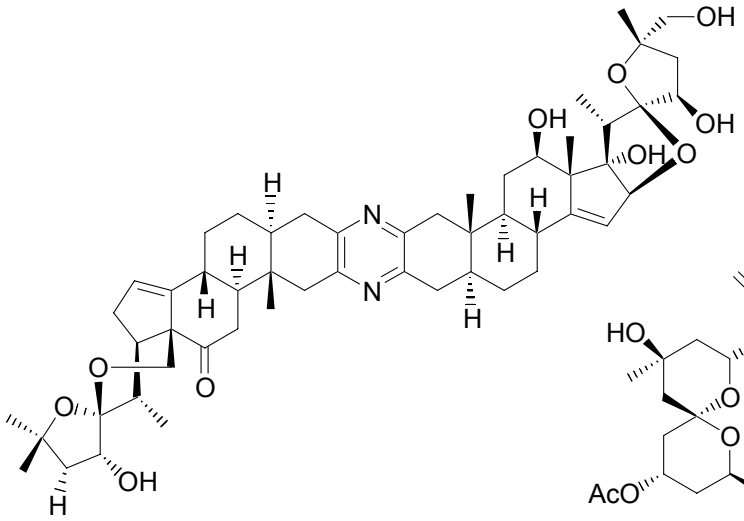


Figure 1.1 Map of the South African coastline differentiating the three biogeographical zones, continental shelf and main currents. The red ellipses indicate the collection areas explored by the Rhodes research group.[17]

1.2.1 Contribution of Pettit and Kashman to marine drug discovery in South Africa.

Three research groups have dominated marine drug discovery in South Africa. The pioneering work of Pettit and co-workers off Cape Town in the 1970's and early 1980's was followed by that of Kashman and Schleyer in the Sodwana Bay region of Kwazulu-Natal in the mid 1990's.[17] Davies-Coleman and co-workers continue to explore all regions of the South African coast for new potential pharmaceuticals.[16;17]

Pettit *et al*.[19] reported the isolation of the bioactive metabolite cephalostatin 1 (**1.12**) from two collections (1972 and 1981) of the marine tube worm *Cephalodiscus gilchristi* off Cape Town. Compound **1.12** was found to be strongly active against the P-388 lymphocytic leukemia (ED_{50} of 1.1 x 10^{-3} – 1.1 x 10^{-5} nM),[19] and its preclinical development has been hampered by the problems of supply. Successful syntheses[20;21] of this compound have unfortunately not resolved this problem.[22] Pettit *et al*.[23] also isolated a group of compounds known as the spongiostatins from the bright orange "wall-sponge" *Spirastrella spinispirulifer*. Amongst the metabolites isolated from this particular sponge was spongiostatin 4 (**1.13**) which exhibited the highest activity (GI_{50} 1.02 nM) in the National Cancer Institute's (NCI) *in vitro* screening panel of various cancer cell lines.[23] Again, problems of supply have hampered further development of this compound.



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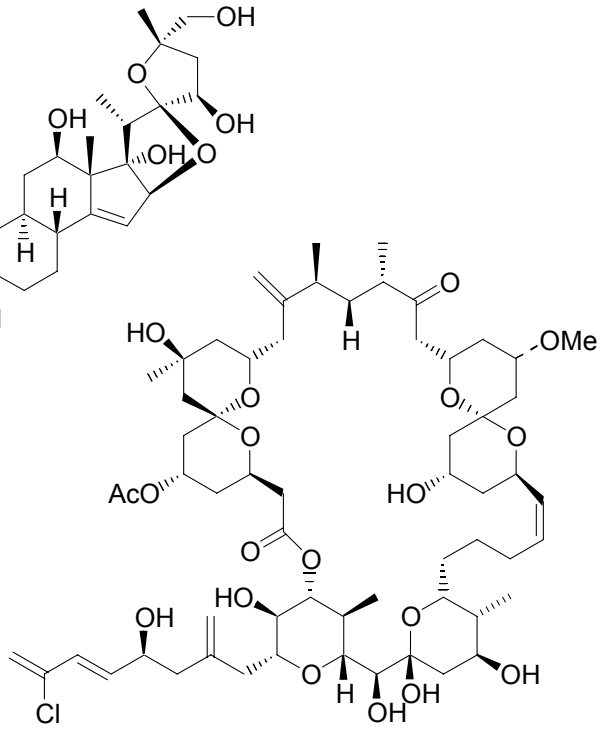

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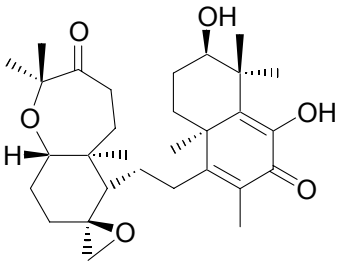

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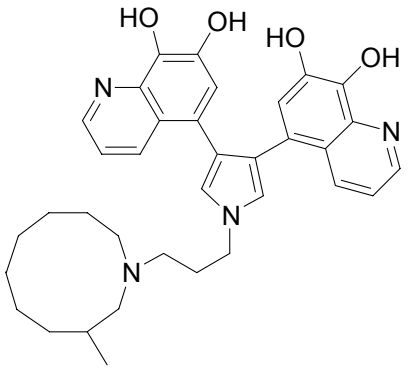

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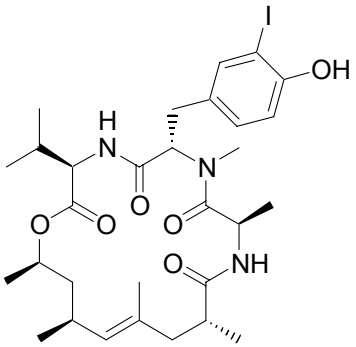

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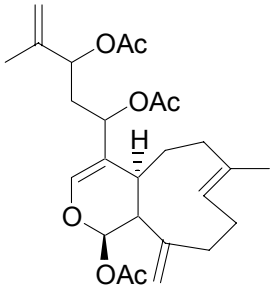


1.17

In a review of the bioactive natural products from South African marine invertebrates, Davies-Coleman[17] highlights the contribution of Yoel Kashman who has isolated and patented several bioactive metabolites (**1.14**-**1.17**) from marine invertebrates collected in the Sodwana Bay region of South Africa. Sodwanone H (**1.14**, patent no. WO 9701334) was isolated from the marine sponge *Axinella weltneri* and was shown to be highly cytotoxic (IC_{50} 0.02 μM) against the human lung carcinoma cell line A-549.[24] The alkaloid halitulin (**1.15**, patent no. WO 0020411) was extracted from the marine sponge *Haliclona tulearensis* from Sodwana Bay and exhibited very strong cytotoxicity (IC_{50} 0.04, 0.02, 0.02 and 0.04 μM) against the P-388 murine leukemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 melanoma cell lines respectively.[25] Heinrich *et al.*[26] have recently reported the total synthesis of **1.15**. The peptide geodiamolide TA (**1.16**, patent no. US 5661175) was originally isolated from the marine sponge *Hemiasterella minor* (Kirkpatrick) and exhibited cytotoxicity against the P-388 leukemia cell line at relatively low (*ca.* 0.015 μM) concentrations.[27] Rudi *et*

al.[28] obtained the xenicane diterpene zahavin A (**1.17**, patent no. WO 9632388) from the soft coral *Eleutherobia aurea* and have reported that **1.17** is cytotoxic towards P-388 (IC_{50} 1.7 μM) and A-549 (IC_{50} 1.7 μM), MEL-28 (IC_{50} 0.20 μM) and HT-29 (IC_{50} 2.0 μM) cancer cell lines.

Although the isolation of the two anticancer compounds **1.12** and **1.13** is possibly the most significant discovery of such bioactive compounds from South African organisms,[16] recent work conducted by our research group has confirmed the potential of South African marine invertebrates to still provide anticancer compounds, the details of which are discussed in the following sections.

1.3 Oesophageal cancer in South Africa

Globally, oesophageal cancer is one of the most prevalent forms of cancer and occurs in two main, but aetiologically unrelated, subtypes; adenocarcinoma and squamous cell carcinoma.[29;30] Adenocarcinoma occurs mainly in developed countries and is principally associated with gastric reflux and Barrett's oesophagus.[29-31] Barrett's oesophagus is a metaplastic change of the flat squamous cells of the oesophageal mucus layer to columnar epithelium and has been found, in patients suffering from this condition, to increase the risk of developing adenocarcinoma.[31] Squamous cell carcinoma (SCC) is the most common form of oesophageal cancer and is found predominantly in developing countries such as South Africa.[29;31] Squamous cell oesophageal cancer (SCOC) is the second most common cancer reported in poor rural and peri-urban populations in South Africa, with an age-standardized incidence rate of 16.22 per hundred thousand people.[32] The relatively high incidence of SCOC in South Africa has been reported to be linked to extraneous factors such as smoking, alcohol consumption, diets poor in fresh fruit and vegetables, as well as the consumption of foods contaminated with a *Fusarium* fungus (which produces a carcinogen called fumonisin).[29;30] SCOC is a significant health problem due to the late diagnosis of the disease,

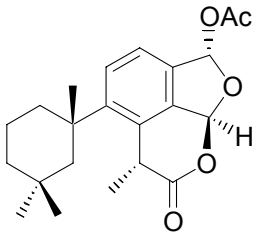
which is a direct consequence of the asymptomatic nature of the tumour which only becomes evident once the cancer has metastasized to the extent where it hinders the swallowing of food.[29] Unfortunately, late diagnosis results in only a 10% survival rate in affected patients,[29;32] and the likelihood of low levels of remission in patients after either surgery or chemotherapy.[33] The prevention of SCOC can be achieved through changes in lifestyle, including diversification of diet.[30] Presently, the most common and favourable form of chemotherapeutic intervention for SCOC are the anticancer drugs cisplatin and 5-fluorouracil,[33-35] resulting in complete remission in only 20-30% of early diagnosed cases.[34] Combination chemotherapy using a cisplatin-containing treatment, with other active agents such as either irinotecan, epirubicin, docetaxel or paclitaxel **1.3**, is also attempted in some cases.[33] It has become increasingly apparent in the last few years that the modest success rate of the current conventional chemotherapeutic agents against SCOC have reached a plateau.[35;36] Evidence also suggests that radiotherapy and surgery as treatment options will only yield improved remission rates if more advanced techniques are developed in order to detect and diagnose the disease at an early stage.[36]

The need for the discovery of new drugs to treat oesophageal cancer is clear. A recent emphasis in oesophageal cancer drug discovery has been placed on the identification of molecular markers, *e.g*. growth factor receptor pathways and protein kinase pathways such as the c-Jun (AP1), as potential targets in SCOC chemotherapy.[30;35] The discovery of marine natural product derived agonists of the c-Jun signalling pathway in WHCO1 (an oesophageal cancer cell line derived from a South African patient) underpins much of the work described in this thesis.

1.4 Southern African marine invertebrates as possible sources of new lead compounds for anti-oesophageal cancer drugs

Since there is an obvious need for the development of new and more effective chemotherapeutic agents against SCOC, an ongoing collaboration with the Division of Medical Biochemistry at the Medical School of the University of Cape Town has identified several natural products, isolated from South African marine invertebrates, with significant cytotoxicity to oesophageal cancer cells.[36] This collaboration involves two different approaches to the search for new compounds with anti-oesophageal cancer activity.

Firstly, a bioassay-guided approach where crude extracts of marine invertebrates are screened for their cytotoxicity to WHCO1 cells, and the relevant active fractions are fractionated and purified to yield the active component(s). This approach has afforded several marine natural products (**1.18**-**1.23**) with significant cytotoxicity against SCOC (Table 1.1).[36-38]



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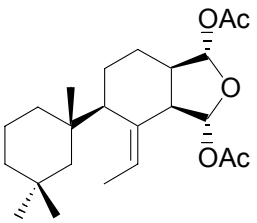

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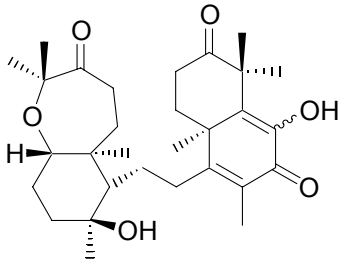

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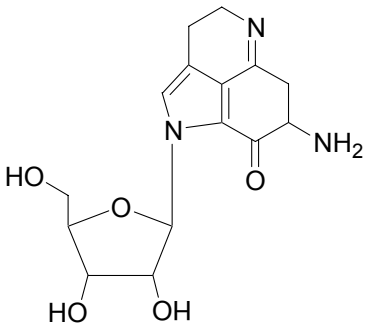

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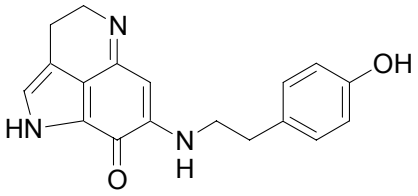

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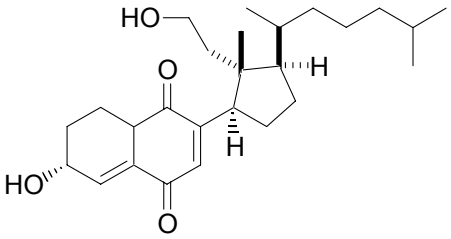

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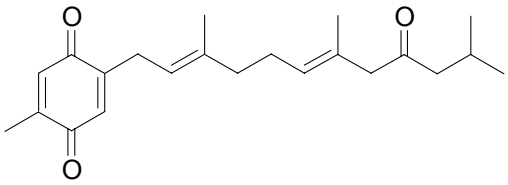
Table 1.1 IC_{50} values for selected compounds isolated from South African marine organisms against the WHCO1 cell line.[36]

	Compounds tested					
	1.18	**1.19**	**1.20**	**1.21**	**1.22**	**1.23**
(IC_{50} μM)	3.5	1.1	1.2	1.6	0.7	3.0

The second approach involves the random screening of a library of pure compounds isolated from South African marine invertebrates. This approach has yielded a group of *ortho*-prenylated toluquinones and quinones (**1.24**-**1.29**),[39] previously isolated by our research group from an endemic nudibranch *Leminda millecra* (Figure 3.1) collected from Algoa Bay in Port Elizabeth.[40] Compounds **1.24**-**1.29** induce apoptosis in WHCO1 cells by triggering the JNK/c-Jun signalling pathway, through the generation of reactive oxygen species (ROS), and this mechanism of action has been extensively studied,[39] and is reviewed in more detail in Chapter 5.

Prenylated quinones and hydroquinones are regularly isolated in bioassay-guided natural product investigation of South African marine invertebrates,[16] *e.g*. the cytotoxic nephthoside (**1.30**) was extracted from the soft coral *Nephthea* sp. collected in Sodwana Bay,[41] while the moderately cytotoxic sesquiterpene hydroquinone rietone (**1.31**) was isolated from the South African soft coral *Alcyonium fauri* by Hooper *el al*.[42] Rietone has also been shown to have

moderate anti-HIV properties[42] and, more recently, moderate cytotoxicity against the WHCO1 cell line.[43]



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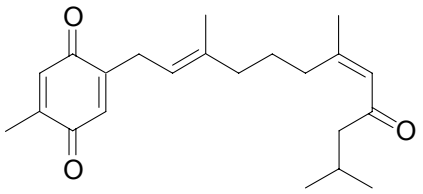

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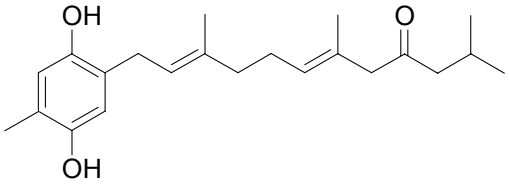

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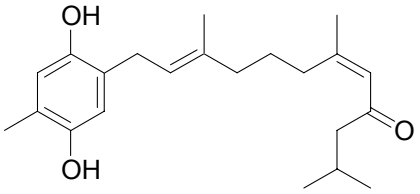

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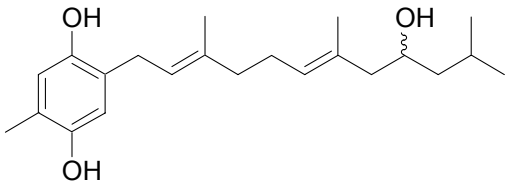

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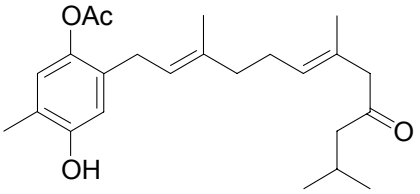

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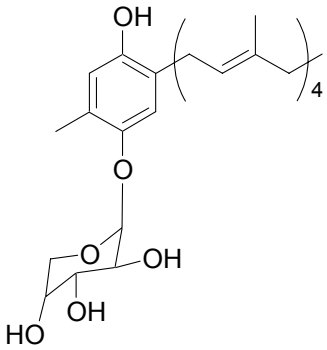

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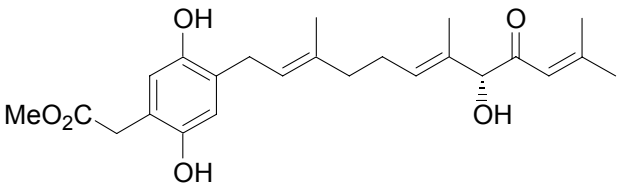


1.31

1.5 Thesis Structure

The research described in this thesis builds on the mechanistic studies of apoptosis induced in oesophageal cancer cells by the prenylated quinones **1.24**-**1.29** isolated from the South African nudibranch *L. millecra*.[39;40]

To provide the necessary background to the thesis research, the extent of cytotoxicity inherent in prenylated quinones, hydroquinones and naphthoquinones isolated from various marine phyla is comprehensively reviewed in Chapter 2. Marine secondary metabolites with reported antioxidant activity are also included in this review because of the well-established link between the redox properties and cytotoxicity in quinone compounds.

The problem of supply haunts any marine drug discovery programme, especially when the active compounds are isolated from an invertebrate predator, *e.g.* a nudibranch. In an attempt to resolve the problem of supplying sufficient amounts of **1.26**, found to be the most active against the WHCO1 cell line, to our collaborators for further biochemical studies (including combination chemotherapy with 17-AAG), we investigated the laboratory synthesis of this compound from simple precursors (Chapter 3).

In parallel with the attempted synthesis of **1.26**, we expanded our structure-activity (SAR) studies to initially synthesize a marine prenylated naphthoquinone analogous to **1.26** (Chapter 4). This was followed by exploration of cytotoxic synthetic mono-substituted naphthoquinones, with the aim of finding simple compounds with improved cytotoxicity to oesophageal cancer cells relative to **1.26**.

The redox cycling properties of quinones are well established and, in Chapter 5, we investigate the relationship between the electrochemical properties of the most active

synthetic naphthoquinones and their cytotoxicities. Additionally, the apoptosis mechanism of action of the most active synthetic naphthoquinone is discussed.

Experimental details for all procedures discussed in Chapters 3 – 5 are provided in Chapter 6.

Chapter Two

A Review of Cytotoxic and Antioxidant Marine Prenylated Quinones and Hydroquinones

2.1 Introduction

Quinones and hydroquinones are ubiquitous in nature and have been found to have a wide range of biological activities, including anticancer properties.[44-47] A survey of the chemistry literature has revealed that over 400 prenylated quinones, toluquinones, toluhydroquinones and naphthoquinones were isolated from marine organisms between 1974 and 2009, and about a quarter of these have been reported to be cytotoxic and/or have antioxidant properties (Figure 2.1).[48] There is a close relationship between the redox and cytotoxic properties of quinone compounds,[49;50] and this correlation will be explored in Chapter 5.

The review presented here focuses on cytotoxic and/or antioxidant prenylated marine quinones and their reduced hydroquinone analogues. The review also highlights an interesting structural trend amongst this class of compounds where the positioning of the prenyl chain and other substituents on the aromatic ring system can be classified into eight different structural classes which, in turn, are related to the phylum of the source organism (Figure 2.2). Brown algae are the most prolific producers of prenylated toluquinones and hydroquinones, of which only *ca.* 20% are reportedly cytotoxic. Conversely, 45% of the total number of prenylated quinones and hydroquinones isolated from marine sponges are cytotoxic (Figure 2.1). The *ortho* substitution pattern of the prenyl side chain in the metabolites isolated from sponges and ascidians is restricted to the structural class A (Figure 2.2), with the single exception of the compounds isolated from Okinawan sponge *Hippospongia* cf. *metachromia*[51] which fall into the structural class B, otherwise exclusively associated with ascidians. Ascidians have also been reported to produce a very complex group of cyclized prenylated compounds, longithorones and longithorols, assigned as Class F (Figure 2.2). Prenylated toluquinone and hydroquinones isolated from brown algae are confined without exception to structural Class C (Figure 2.2), which contains the largest number of prenylated toluquinones and hydroquinones (Figure 2.1). Structural Class D is interesting given the diversity of marine organisms that reportedly produce this class of

prenylated toluquinone and hydroquinones. The South African nudibranch *Leminda millecra* sequesters prenylated toluquinones and toluhydroquinones from its diet of octocorals (gorgonians and soft corals). The co-occurrence of this class of compounds in marine fungi begs the question as to the true source of these compounds and the possibility exists that compounds of this class, attributed to various marine invertebrates, may actually be of fungal origin. The only halogenated examples of prenylated quinones are found in green algae (Class E, Figure 2.2). Prenylated naphthoquinones are rare in the marine environment with only seven examples of these cytotoxic compounds in two structural classes G and H reported from a brown alga and a soft coral

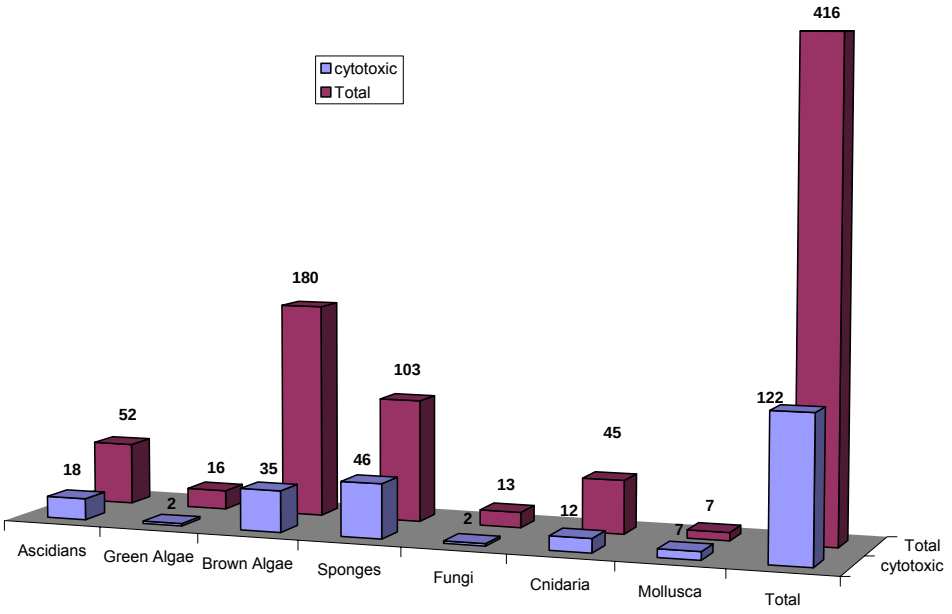


Figure 2.1: Graphical representation of the distribution of prenylated quinones across the marine phyla between 1974 and 2009.[48]

The phyletic distribution of marine cytotoxic prenylated quinones and hydroquinones is described in the following sections. The review is not confined to compounds containing a linear prenylated side-chain, but prenylated quinones and hydroquinones in which cyclization

appears to have occurred in the side-chain are also included. Syntheses confirming the structures of this ubiquitous group of marine secondary metabolites are included where relevant, while the structures themselves are presented, where possible, in order of increasing structural complexity.

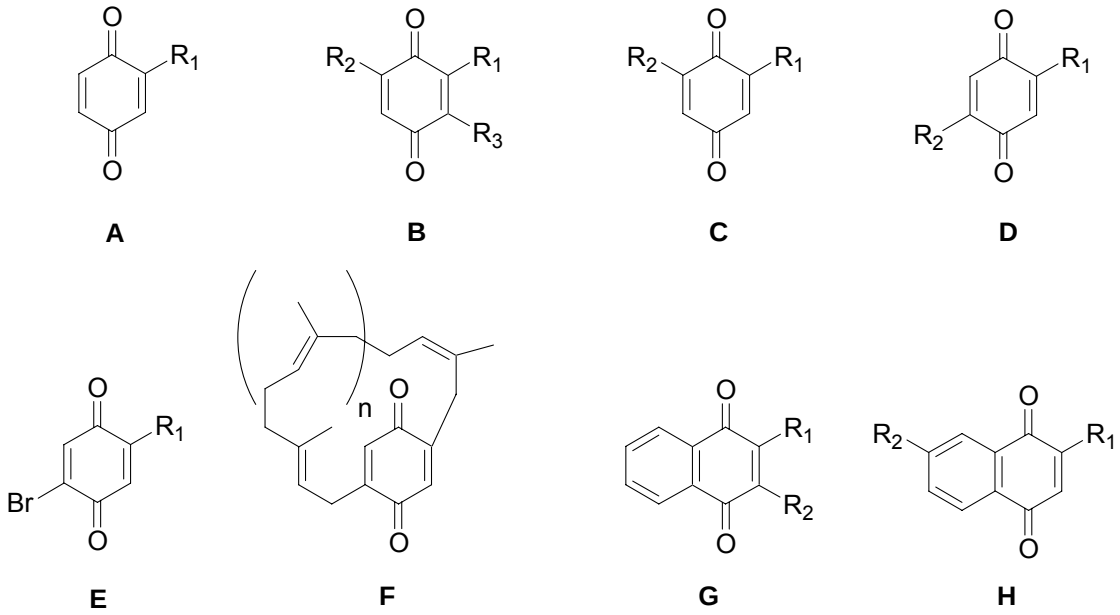


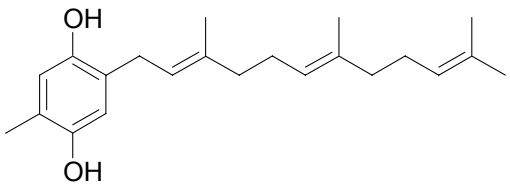
Figure 2.2 Substitution patterns of prenylated marine quinones in **A**) sponges, ascidians **B**) two sponges[51-56] **C**) brown algae **D**) fungi, gorgonians, soft corals, one brown alga[57] and a nudibranch[40] **E**) green algae **F**) ascidians **G**) one brown alga[58] **H**) one soft coral[59;60]

2.2 Fungi

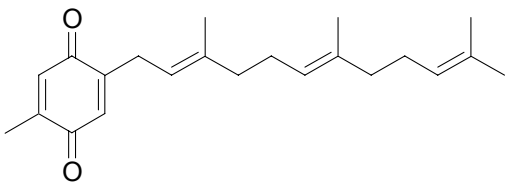
Terrestrial fungi have been extensively studied and a wide range of bioactive metabolites, with pharmaceutical properties, have been isolated from this group of microorganisms, *e.g.* the antibiotic penicillin.[61] Even though marine fungi have not been studied as extensively as their terrestrial counterparts, they have nevertheless been shown to produce a extensive array of structurally interesting and biologically active secondary metabolites, which have

been recognized to exhibit significant antimicrobial activity.[48;61;62] Interestingly, many of the metabolites isolated from marine microbes have previously been associated with marine invertebrates, therefore suggesting probable symbiotic relationships between these two distinct groups of organisms.[2;13]

The marine-derived fungus *Penicillium sp.* has yielded five farnesylated toluquinones and hydroquinones (**2.1**-**2.5**), of which the farnesyltoluhydroquinone **2.1** and deacetoxyyanuthone A **2.3** were found to be moderately cytotoxic (IC_{50} values ranging from 3.86 to 10.07 μM) *in vitro* against a panel of five tumour cell lines.[62;63] More recently, a naphthoquinone (**2.6**) was isolated from another marine-derived *Penicillium sp.* fungus that was collected from mangrove plants in South China.[64] The structure of **2.6** may be conceived to be derived from enzyme-mediated cyclization of a highly oxygenated diprenylated naphthoquinone precursor (**2.7**).

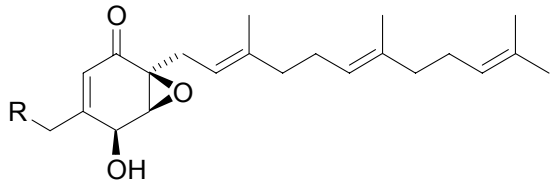


2.1

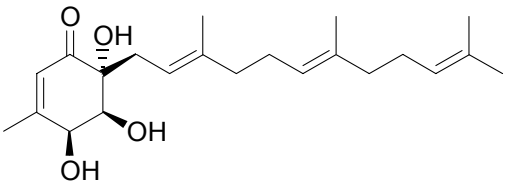


2.2

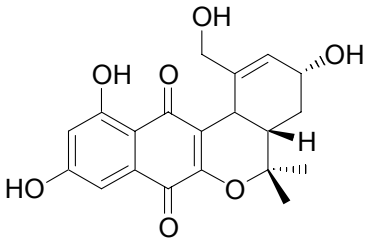
Farnesyltoluhydroquinone **2.1** is not restricted to the marine environment and has been isolated from the Canadian terrestrial fungus *Phellinus pini*[65] and three species of terrestrial plant (Genus *Seseli*).[66] The possibility exists that the source of **2.1** in these plants may be of fungal origin and not originating from the plants themselves. Eight antimicrobial yanuthones or farnesyl epoxy compounds, similar to **2.3** and **2.4**, were isolated from the marine fungus *Aspergillus niger* obtained from the orange ascidian *Aplidium* sp. collected in Benga, Fiji.[61] There is, however, no reported cytotoxicity data for these compounds.



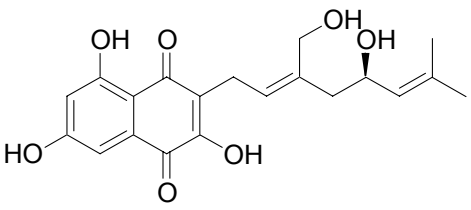
2.3 R = H
2.4 R = OAc



2.5



2.6



2.7

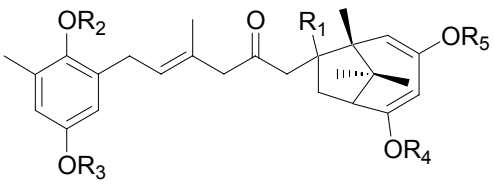
2.3 Brown algae

The highest number of marine prenylated toluquinones and hydroquinones (180 compounds) reported in the chemistry literature has been isolated from brown algae and they can all be categorized under structural class C (Figure 2.2), with only two exceptions. About one sixth of these secondary metabolites have been shown to exhibit cytotoxic and/or antioxidant properties (Figure 2.1) and these bioactive compounds are discussed here. Most of the prenylated secondary metabolites from brown algae have been isolated from the genus *Cystoseira* (Order Fucales, Family Cystoseiraceae) and their initial discovery in this particular genus can be largely attributed to the pioneering work of Amico.[67] Brown algae of the genus *Bifurcaria* have also afforded cytotoxic prenylated toluquinones and hydroquinones, while *Landsburgia* is the only genus to have produced prenylated naphthoquinone metabolites.[67]

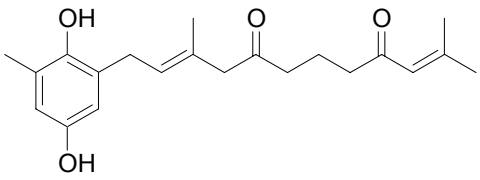
About 80% of the global distribution of *Cystoseira* species are found in the Mediterranean Sea and along the adjacent Atlantic coast, while *Cystophora* species are mostly found along the

coasts of Australia and New Zealand.[67] Although *Cystoseira* species appear to biosynthesize tetraprenyltoluquinols exclusively, this class of compounds has also been isolated from species of the genera *Halidrys* (northen coasts of England),[67] *Bifurcaria* (coasts of the Galapagos Islands),[68] *Cystophora*[69] and *Sargassum*.[70] As the structural complexity of the generally highly oxygenated tetraprenylated toluquinones and hydroquinone metabolites found in brown algae is similar across the phylum, the structures of the cytotoxic compounds are presented according to their occurrence in the various genera and not according to their increasing structural complexity.

The bioactive metabolites mediterraneol A (**2.8**),[71;72] mediterraneol B (**2.9**)[72] and mediterraneone (**2.10**)[73] were isolated from the marine brown alga *Cystoseira mediterranea*. The mediterraneols A and B (**2.8** and **2.9**) were found to inhibit mitotic cell division (ED_{50} = 2 μM) in fertilized urchin eggs and also exhibited *in vivo* cytotoxicity (T/C = 128% at 32 mg/Kg) against P-388 leukemia cells,[72] while the antineoplastic potential of **2.10** was tenuously extrapolated from the positive results obtained in the crown-gall potato disc bioassay.[73] Kakiuchi *et al.*[74] have reported the total synthesis of the tetramethyl derivative of mediterraneol B (**2.11**) where they indicated some inconsistencies in the NMR data of the synthetic **2.11**, pertaining specifically to the complex prenylated side chain, when compared with the NMR data of the natural product **2.9** and suggested a revision of the structure of the natural product.

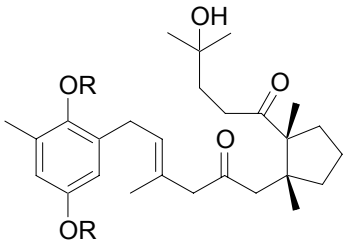


2.8 R_1 = β-Me, R_2 = R_3= R_4 = R_5 = H
2.9 R_1 = α-Me, R_2 = R_3= R_4 = R_5 = H
2.11 R_1 = α-Me, R_2 = R_3= R_4 = R_5 = Me

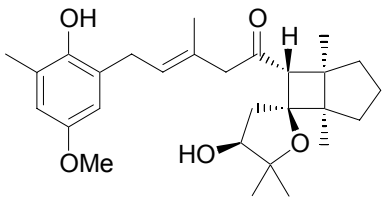


2.10

Foti *et al.*[75] have reported the antioxidant properties of two prenylated compounds (**2.12** and **2.13**) that have a cyclopentane ring included in the side-chain. The tetraprenyl compound **2.12** was first isolated from the brown alga *Cystoseira algeriensis*, along with four other related tetraprenylhydroquinols,[76] while balearone **2.13** was originally isolated from *Cystoseira balearica* collected at Portopalo in Sicily, Italy.[77] The monoquinol (**2.14**) isolated from *Cystoseira amentacea* var. *stricta* was found to have antimitotic activity (ED_{50} = 12 μM) against fertilized sea urchin eggs.[78] The oxygenation and relative cyclic complexity observed in **2.8**, **2.9** and **2.12**-**2.14** isolated from the genus *Cystoseira* is also observed in the genus *Cystophora*.[69] However, no cytotoxic or antioxidant activity was reported for similar *Cystophora* metabolites.



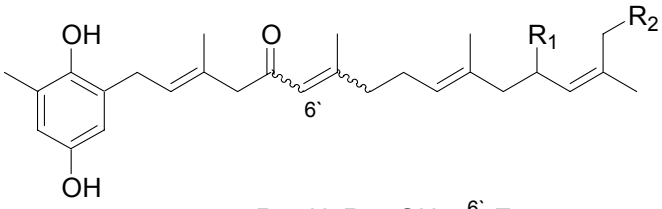
2.12 $R_1 = R_2$ = Me
2.14 R_1 = H, R_2 = Me



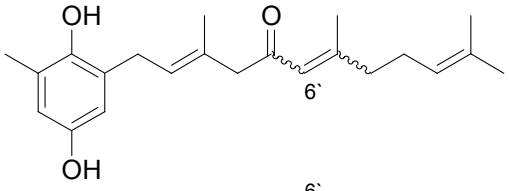
2.13

Recently, Fisch *et al.*[79] have reported the isolation of six new tetraprenylquinols (**2.15**-**2.20**), two new triprenyltoluquinols (**2.21** and **2.22**) and two new tetraprenyltoluquinones (**2.23** and **2.24**) from *Cystoseira crinita*, collected from the south coast of Sardinia, along with four known antimicrobial tetraprenyltoluquinols (**2.25**-**2.28**) that were previously isolated by Amico *et al.* [80] from *Cystoseira spinosa* var. *squarrosa* collected from Portopalo in Sicily. Only the hydroquinones **2.15**, **2.16**, **2.25**, **2.26** and **2.28** showed potent antioxidant activity and these compounds were also found to be moderately cytotoxic to HMO2, HepG2 and MCF7 cancer cell lines.[79] The cytotoxicities for the geometric isomer pairs **2.15** and **2.16**, **2.25** and **2.26** were very similar, thus suggesting that the effect on cytotoxicity of the *E/Z* geometry around the $\Delta^{6'}$ double bond is insignificant.[79] Fisch *et al.*[79] further postulated that the

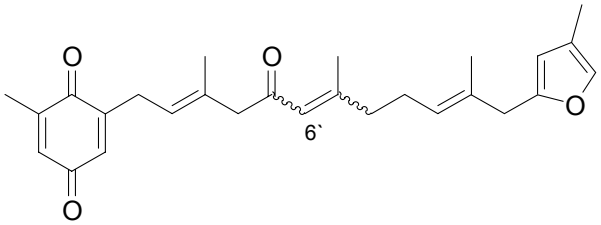
toluhydroquinone moiety in these compounds is the major pharmacophore and thus responsible for the observed cytotoxicity in these compounds.



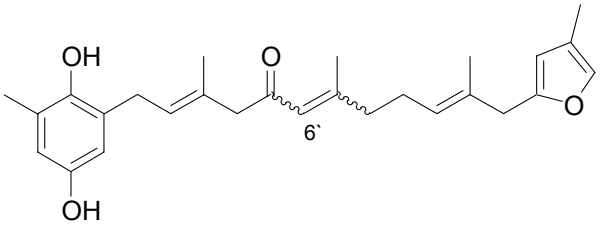
2.15 R_1= H, R_2= OH, $\Delta^{6`}$ *E*
2.16 R_1= H, R_2= OH, $\Delta^{6`}$ *Z*
2.17 R_1= OH, R_2= H, $\Delta^{6`}$ *E*
2.18 R_1= OH, R_2= H, $\Delta^{6`}$ *Z*
2.19 R_1= R_2= H, $\Delta^{6`}$ *E*
2.20 R_1= R_2= H, $\Delta^{6`}$ *Z*



2.21 $\Delta^{6`}$ *E*
2.22 $\Delta^{6`}$ *Z*



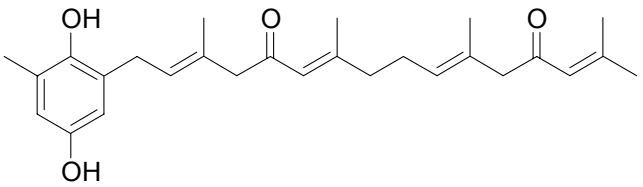
2.23 $\Delta^{6`}$ *E*
2.24 $\Delta^{6`}$ *Z*



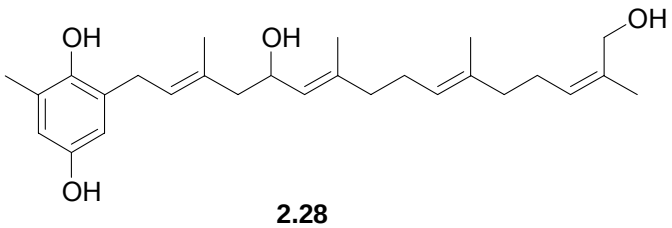
2.25 $\Delta^{6`}$ *E*
2.26 $\Delta^{6`}$ *Z*

As part of their ongoing investigations of the metabolites of *Cystoseira mediterranea*, Francisco *et al.*[72] isolated the bioactive molecule bifurcarenone (**2.29**) which was originally obtained from the marine brown alga *Bifurcaria galapagensis* collected from the Galapagos Islands.[68] Bifurcarenone was found to inhibit mitotic cell division in fertilized sea urchin eggs (ED_{50} = 4 $\mu g/mL^2$).[68] Interested in both the structural uniqueness and the possible biological importance of **2.29**, Mori *et al.*[81] synthesized both *E* and *Z* isomers of bifurcarenone and unequivocally established that the natural product has a $\Delta^{2`}$ *E*-configuration (**2.30**), and not a $\Delta^{2`}$ *Z*-configuration as proposed by Francisco *et al*.[72]

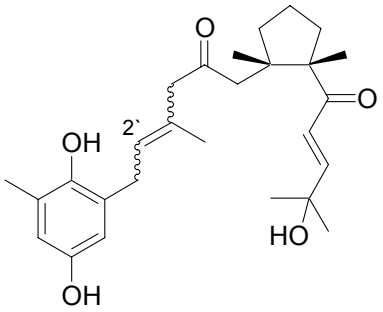
The brown algae of genus *Sargassum* is a prolific source of structurally unique bioactive secondary metabolites,[82] and a recent investigation of the brown alga *Sargassum micracanthum* by Mori *et al.*[70;82;83] yielded six bioactive compounds (**2.31**-**2.36**). The plastoquinones **2.31-2.35** and the chromene **2.36** all exhibited antioxidant properties.[70;83] Compounds **2.33** and **2.35** were strongly cytotoxic (IC_{50} 1.51 and 1.69 μM respectively) against the colon 26-L5 cancer cell line, while **2.34** exhibited only moderate cytotoxicity (IC_{50} 17.5 μM).[70] Plastoquinones **2.31** and **2.32**, along with the chromene **2.36**, have also been shown to exhibit potential antiulcer properties.[83]



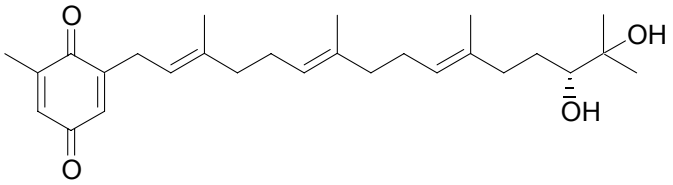
2.27



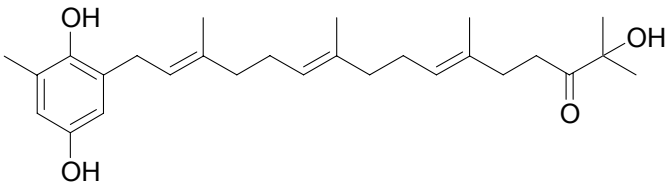
2.28



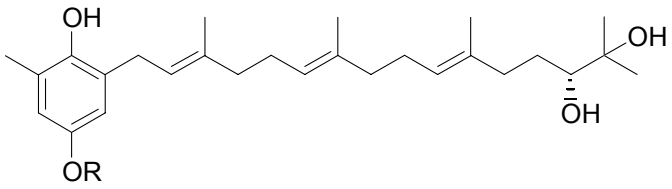
2.29 $\Delta^{2`}$ *Z*
2.30 $\Delta^{2`}$ *E*



2.31

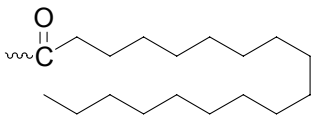


2.32

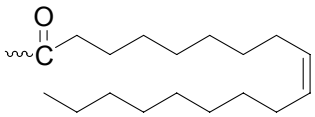


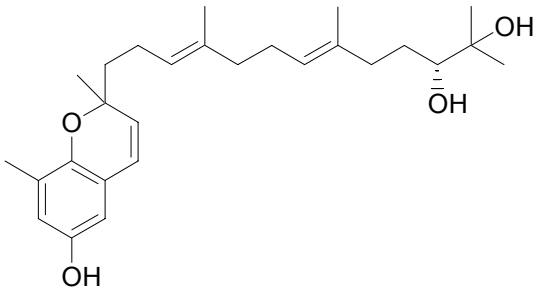
2.33 R = H

2.34 R =



2.35 R =



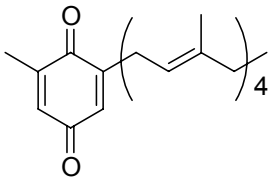


2.36

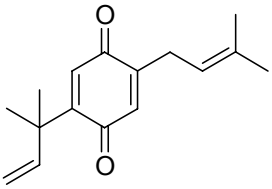
Fenical *et al.*[84] isolated the simple cytotoxic farnesylated toluquinone and toluhydroquinone (**2.37** and **2.38** respectively) from the brown alga *Stypopodium zonale* (L.) Lamouroux collected in Belize. Compounds **2.37** and **2.38** appear to be the only examples of cytotoxic prenylated toluquinones and toluhydroquinones to be isolated from this particular genus.

As mentioned in the introduction to this chapter, there are only two exceptions to the typical *meta* disubstitution pattern of the methyl and prenyl groups exhibited by the toluquinones and toluhydroquinone metabolites isolated from brown algae (Figure 2.2). The one exception is the occurrence of the *para*-disubstituted prenylated quinones (**2.39** and **2.40**) in the New Zealand brown alga *Perithalia capillaris* reported by Sansom *et al.*[57] The quinone **2.39** was found to inhibit superoxide production by human neutrophils (IC_{50} = 2.1 μM) and was also

strongly cytotoxic towards human leukemia HL-60 cells (IC_{50} = 0.34 μM) while the cyclic ether **2.40** was comparatively less cytotoxic (IC_{50} = 5.6 μM) against the same cancer cell line.[57]



2.37



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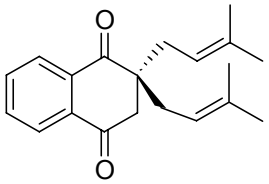

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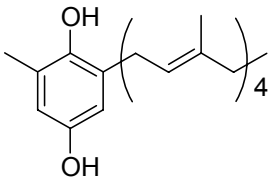

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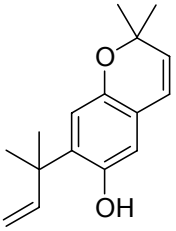

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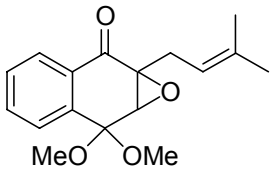
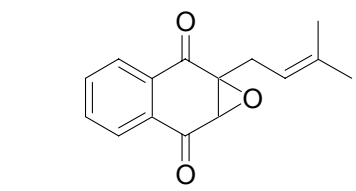


2.42

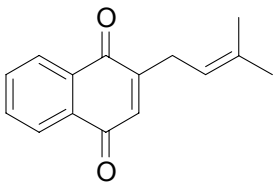
The second exception to the ubiquitous distribution of prenylated meta-disubstituted toluquinones and toluhydroquinones in brown algae is the isolation of prenylated naphthoquinones, naphthoquinone acetal and naphthohydroquinones (**2.41**-**2.45**) from the New Zealand brown alga *Landsburgia quercifolia* by Perry *et al*.[58] Amongst this cohort of compounds, 2-deoxylapachol **2.44** exhibited the strongest cytotoxicity (IC_{50} = 2.7 μM) against P-388 leukemia cells in addition to showing antifungal activity. According to Perry *et al*.,[58] most algae produce structurally similar quinones to those found in higher plants, including phylloquinone or vitamin K_1 (**2.46**). To the best of our knowledge, compounds **2.41**-**2.45** are the only secondary metabolite examples of prenylated naphthoquinones isolated from marine organisms. The cytotoxic **2.44** was originally isolated from the teak wood tree *Tectona grandis*.[85]

2.4 Green algae

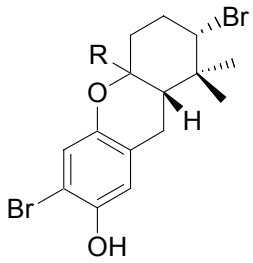
There a very few examples (only 16) in the literature of prenylated quinones and hydroquinones isolated from green algae and all of these metabolites are brominated and confined to the structural class E (Figure 2.2).[86-90] Two cyclic epimeric bromohydroquinones cymobarbatol (**2.47**) and 4-isocymobarbatol (**2.48**) were isolated from the green alga *Cymopolia barbata* (L.) Lamouroux collected off the north coast of Puerto Rico, and exhibited antimutagenic activity against *Salmonella typhimurium* (T-98).[90]



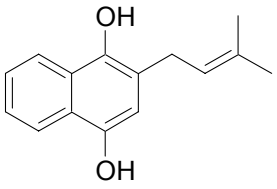
2.43



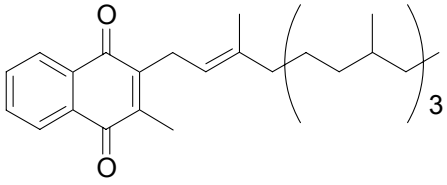
2.44



2.47 R = β-Me
2.48 R = α-Me



2.45



2.46

2.5 Marine invertebrates

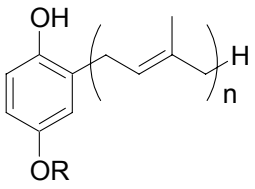
2.5.1 Sponges

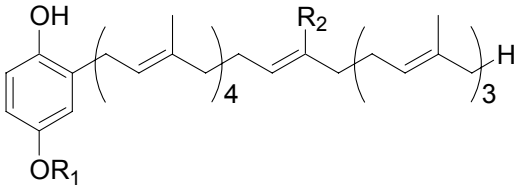
The highest number of cytotoxic prenylated quinones and hydroquinones (*ca.* 46) have been isolated from marine sponges (Figure 2.1), even though sponges are only the second most prolific source, after brown algae, of this particular class of compounds in the marine environment. Cytotoxic prenylated quinones and hydroquinones have mostly been isolated

from the sponge genera *Hippospongia*, *Ircinia*, *Dysidea*, *Spongia*, *Coscinoderma*, *Halichondria* and *Reniera*. The structures of the metabolites isolated from sponges generally adopt the structural substitution pattern assigned as class A (Figure 2.2), with the exception of the metabolites isolated from the sponge *Hippospongia metachromia* and *Spongia sp.*[51-56] which fall under the structural class B.

Amongst the marine sponges, the genus *Ircinia* is the major source of polyprenylated benzoquinones and the more abundant polyprenylated benzohydroquinones.[91;92] Polyprenylated benzohydroquinones have also been isolated from the related *Hippospongia communis*[93] and from an Australian *Spongia sp.*[94] Erdogan-Orhan *et al.*[95] have recently identified two polyprenylated benzohydroquinones (**2.49** and **2.50**) and three sulfated polyprenyl benzohydroquinones (**2.51**, **2.52** and **2.53**) as possessing activity against the CDC25A phosphatase enzyme, which is involved in the G1/S transition of the cell cycle and is utilized as a biological target and indicator for oncogenic inhibitors. Compound **2.49** was originally isolated from the sponge *Ircinia muscarum*, while the hexaprenyl hydroquinone **2.50** was first isolated from the *Ircinia spinolusa* collected in the bay of Naples.[92;96] Compound **2.50** has also recently been isolated from the marine sponge *Sarcotragus muscarum* collected in Mersin, Turkey.[97] The sulfated polyprenyl benzohydroquinones **2.51**-**2.53** were isolated from *I. spinosula* collected at Sutomiscica (Croatia),[98] along with the benzohydroquinone derivative (**2.54**) which was originally found in the same *I. spinosula* sample that yielded **2.50**.[92;96] The biological investigation conducted by Erdogan-Orhan *et al.*[95] revealed that **2.49** and **2.50** were moderately active (IC_{50} 40 and 30 μM respectively) against the CDC25A phosphatase enzyme, whilst the three sulfated polyprenyl hydroquinones **2.51**-**2.53** exhibited stronger activities (IC_{50} 4-5 μM) in the same assay. The benzohydroquinone **2.54**, in an earlier study, was also found to be cytotoxic (IC_{50} 27.5 μM) against the C98 clone of the human non-small cell lung cancer cell line L16.[99] Compounds **2.51**-**2.56** were subjected to the brine shrimp and fish lethality assays and the sulfated hydroquinones **2.51**-**2.53** were found to be more active than their corresponding

benzohydroquinones **2.54**-**2.56**.[98] The increased toxicity of the sulfated benzohydroquinones may be attributed to their detergent-like properties in which a long hydrophobic polyprenyl chain is attached to a relatively hydrophilic benzohydroquinone moiety. de Rosa *et al*.,[98] have suggested that these sponges may be utilizing these sulfated metabolites as a means of chemical defence against macro-symbionts, *e.g.* worms or crabs, living in the sponges.





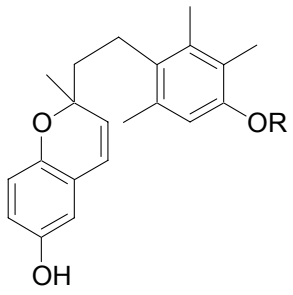
2.49 R = OH, n = 4
2.50 R = OH, n = 6
2.51 R = SO_3Na, n = 7
2.52 R = SO_3Na, n = 8
2.55 R = OH, n = 7
2.56 R = OH, n = 8
2.57 R = OH, n = 9
2.58 R = SO_3Na, n = 9

2.53 R_1 = SO_3Na, R_2 = CH_2OH
2.54 R_1 = H, R_2 = CH_2OH

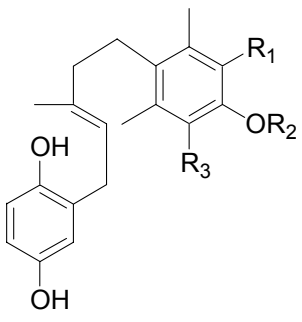
The *I. spinosula* specimen investigated by Cimino *et al.*[92;96] (*vide supra*) was also the source of the hepta- and octaprenyl benzohydroquinones (**2.55** and **2.56**), while the nonaprenyl benzohydroquinone (**2.57**) has only recently been isolated from the same Turkish *Sarcotragus muscarum* sample mentioned above,[97] unlike its sulfated analogue (**2.58**) which was isolated in the mid 1990's from the Croatian *I. spinosula* (*vide supra*).[98] Wätjen *et al*.[97] have recently shown that the polyprenyl compounds **2.50**, **2.55** and **2.57** are cytotoxic against H4IIE rat hepatoma cells, with **2.50** being the most cytotoxic (EC_{50} 2.5 μM). Additionally, these three compounds all exhibited strong antioxidant potential.[97] In some older studies, the sulfated hydroquinone **2.51** was found to be cytotoxic against KB cells,[91] while the polyprenylated hydroquinones **2.50**, **2.55** and **2.56** were shown to be highly toxic (LD_{50} 0.63-0.98 ppm) in the brine shrimp assay. The lethality results of the brine shrimp assay are often correlated with the results obtained from cytotoxicity studies using the cancer cell lines such as KB, P-388, L5178Y and L1210.[100] However, in the study conducted by Erdogan-Orhan *et*

al.,[95] **2.55** and **2.56** were found to be only mildy active (IC_{50} 30-190 and 400 μM respectively) against the CDC25A phosphatase enzyme.

A group of cyclized benzohydroquinones, the paniceins (**2.59**-**2.64**), in which cyclization and aromatization of the polyprenylated side-chain has occurred, were obtained from the marine sponge of genus *Reniera*.[101;102] The paniceins **2.59**-**2.61** were isolated from the sponge *Reniera mucosa* collected near Tarifa Island (Spain) and exhibited significant *in vitro* cytotoxicity against P-388 lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma cell lines.[102] Panicein A_2 , **2.59**, was significantly cytotoxic (ED_{50} 14.8 μM) against all four cancer cells lines, while panicein F_1, 2.61, exhibited the same cytotoxicity against all but the HT29 cancer cell line.[102] Although Zubia *et al*.[102] have alluded to the cytotoxicity of panicein F_2, **2.60**, no details of its activity was reported. The paniceins **2.62**-**2.64** have originally been isolated by Cimino *et al*.[103] from the sponge *Halichondria panacea* that was collected in the bay of Naples but have also, more recently, been isolated from a Mediterranean sponge *Reniera fulva*.[101] Casapullo *et al*.[101] have reported panicein B_3 (**2.62**) and panicein C (**2.63**) to be cytotoxic against both NCI-H522 non-small lung cancer cells and CCRF-CEM leukemia cells, while panicein A hydroquinone (**2.64**) was found to be selectively cytotoxic against the CCRF-CEM leukemia cells.[101] The quinone analogue of **2.64** (panicein A) has been synthesized by Davis *et al*.[104]



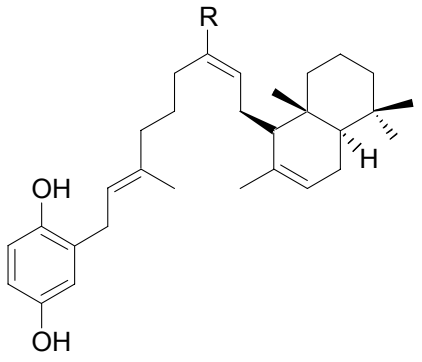
2.59 R = Me
2.60 R = CH_2OH



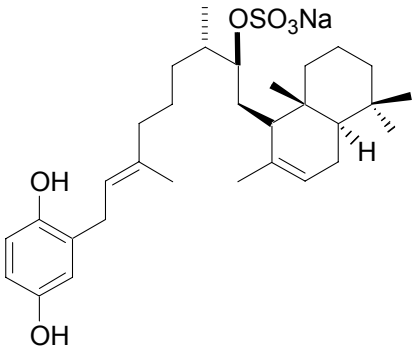
2.61 $R_1 = CH_2OH$, R_2 = Me, R_3 = H
2.62 R_1 = CHO, $R_2 = R_3$ = H
2.63 R_1 = CHO, R_2 = H, R_3 = OH
2.64 $R_1 = R_2$ = Me, R_3 = H

The cytotoxic benzohydroquinone coscinoquinol (**2.65**) was isolated from the Australian marine sponge *Coscinoderma sp.* and was shown to be very active against various cancer cell lines, *e.g.* P-388 (IC_{50} 0.55 μM), A-549 (IC_{50} 1.1 μM) and HT-29 (IC_{50} 0.55 μM).[105] Kernan *et al*.[106] have reported the cytotoxic properties (70% inhibition against fertilized sea urchin eggs) of the halisulfate (**2.66**) isolated from a Californian sponge of the family *Halichondriidae*. More recently, Musman *et al*.[107] have isolated a similar metabolite (**2.67**) isolated from the Okinawan sponge *Hippospongia* cf. *metachromia*. Hipposulfate A, **2.67**, was found to be strongly cytotoxic (IC_{50} 3.6 μM) against the P-388, A-549, HT-29 and MEL-28.[107]

As mentioned before, there are two exceptions to the typical substitution pattern (Class A, Figure 2.2) exhibited by the metabolites isolated from sponges. All the prenylated quinones or hydroquinones isolated from the Okinawan sponge *Hippospongia* cf. *metachromia*[51-53;108] (with the exception of the Hipposulfates *vide supra*) and the marine sponge *Spongia sp.*[54-56] can be generally categorized under structural class B.



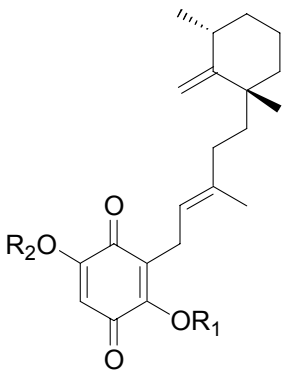
2.65 R = Me
2.67 R = OSO_3Na

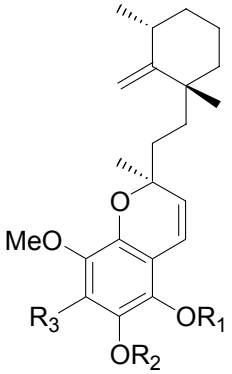


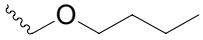
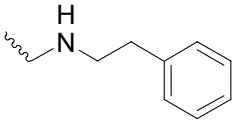
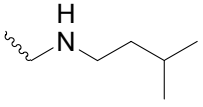
2.66

A class of antineoplastic compounds (**2.68**-**2.75**) known as metachromins have been isolated from the Okinawan sponge *Hippospongia* cf. *metachromia*,[51-53] along with the hydroquinone hippochromin A (**2.76**).[108] Metachromin A (**2.68**) and metachromin B (**2.69**) exhibited potent *in vitro* antitumour activity (IC_{50} 6.7 and 4.5 μM respectively) against the L-1210 leukemia cell

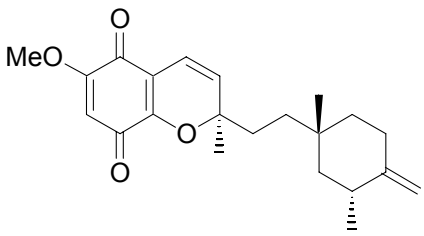
line.[51] Almeida *et al*.[109] have reported the stereoselective total synthesis of the antineoplastic **2.68** in 16 steps. Since the isolation of **2.68** and **2.69**, Kobayashi *et al*.[52;53] have extracted the metachromins C-H (**2.70**-**2.75**) from the same sponge. Metachromin C (**2.70**) was found to have strong *in vitro* cytotoxicity against the L-1210 lymphoma and L-5178Y leukemia cells (IC_{50} 5.6 and 2.6 μM respectively).[52] The metachromins D-H (**2.71**-**2.75**) also exhibited cytotoxicity against murine L-1210 cancer cells (IC_{50} 7.5, 0.56, 1.4, 2.8 and 4.7 μM respectively) and against human epidermoid carcinoma KB cells (IC_{50} 24.8, 1.1, 4.6, > 21.6 and 14.9 μM respectively).[53]





2.68 R_1 = H, R_2 = Me
2.70 R_1 = H, R_2 = Me
2.73 R_1 = H, R_2 = 
2.74 R_1 = H, R_2 = 
2.75 R_1 = H, R_2 = 

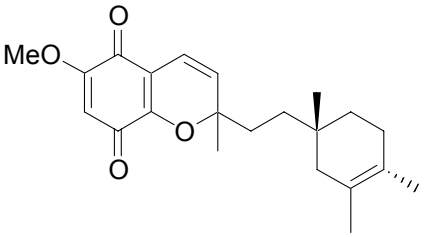
2.69 R_1 = R_2 = H, R_3 = H
2.71 R_1 = H, R_2 = Me, R_3 = OMe
2.76 R_1 = R_2 = H, R_3 = OMe
2.77 R_1 = R_2 = Ac, R_3 = OMe



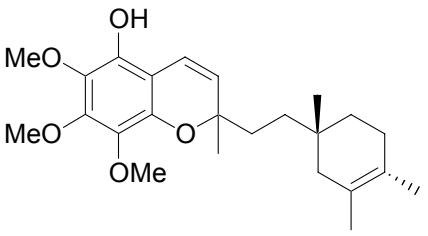
2.72

The sponge *Hippospongia metachromia* has also yielded hippochromin A (**2.76**) which was reported as being unstable and was, hence, isolated as its diacetate derivative (**2.77**) and found to be strongly cytotoxic (IC_{50} 0.47 μM) against the COLO-205 human colon cancer cell

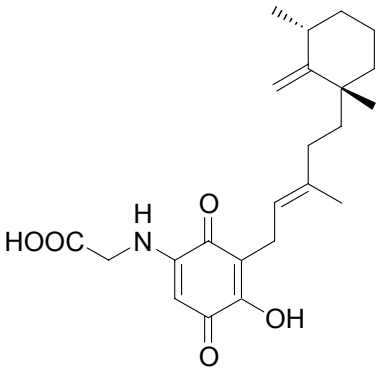
line.[108] The structure of these metachromins is unprecedented and, according to Ishibashi *et al*.,[51] possess a biogenetically unusual carbon skeleton that is similar to that of the paniceins (**2.59** and **2.60**) isolated from the marine sponge *Halichondria panacea*.[103]



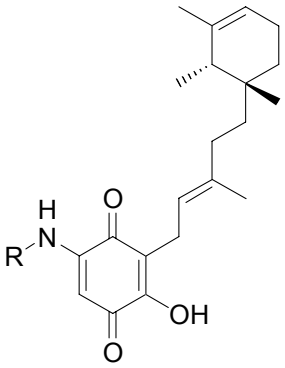
2.78

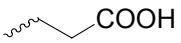


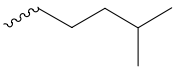
2.79



2.80

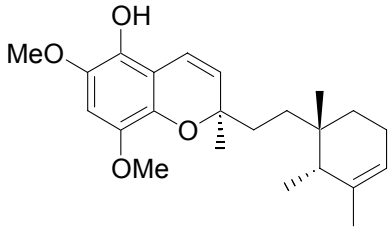


2.81 R = 

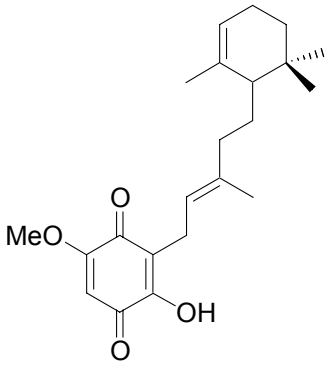
2.82 R = 

The marine sponge *Spongia sp.* yielded the cytotoxic metachromins J and K (**2.78** and **2.79**),[54] L and M (**2.80** and **2.81**),[55] S and T (**2.82** and **2.83**),[56] and isometachromin (**2.84**).[110] Metachromins J and K (**2.78** and **2.79**) exhibited *in vitro* cytotoxicity (IC_{50} 2.8 and 31.3 μM respectively) against murine lymphoma L-1210 cells and human KB carcinoma cells (IC_{50} 27.8 and > 49.7 μM respectively).[54] The metachromins L, M, S and T (**2.80**-**2.83**) all exhibited *in vitro* cytotoxicity against the L-1210 murine leukemia (IC_{50} 10.0, 8.4, 12.2 and 8.1 μM respectively) and KB human epidermoid carcinoma cells (IC_{50} 10.0, 13.0, > 23.4 and 15.0 μM respectively).[55;56] Similarly, isometachromatin **2.84** was found to be cytotoxic against the

human lung cancer A-549 (IC_{50} 7.3 μM) and the P-388 leukemia (IC_{50} > 27.9 μM) cancer cell lines.[110]



2.83

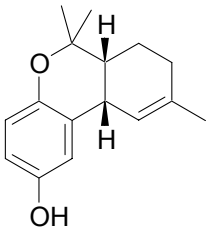


2.84

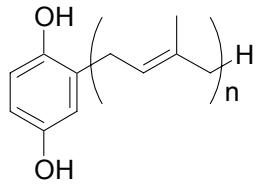
2.5.2 Ascidians

Ascidians or tunicates belong to the phylum Chordata and are regarded as one of the most productive sources of bioactive and cytotoxic natural products in the marine environment,[10;111] Out of the first six marine-derived antitumour compounds to reach clinical trials, three are derived from ascidians, *e.g.* the recently approved anticancer drug (ET-743, **1.6**) from the ascidian *Ecteinascidia turbinata*.[5;10] The metabolites isolated from ascidians can be divided in two groups; nitrogenous and non-nitrogenous.[111] Prenylated quinones and hydroquinones (cyclic or linear) are the most common non-nitrogenous metabolites isolated from ascidians,[111] and the majority of the cytotoxic members of this class of compounds are apparently confined to the genus *Aplidium* (Family Polyclinidae), with the exception of the cyclized hydroquinone (**2.85**).[112] These compounds generally adopt the structural class A (Figure 2.2), with the exception of the non-cytotoxic verapliquinones A-D,[113] and the cytotoxic longithorone/longithorol complex metabolites isolated from *Aplidium longithorax*.

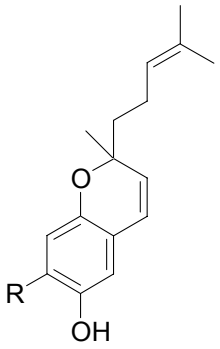
The cyclized diprenylated benzohydroquinone **2.85** was isolated from the Australian ascidian *Synoicum castellatum* and exhibited mild cytotoxicity (IC_{50} 40.9 μM) against P388 murine leukemia, A-549 human lung carcinoma and HT-29 human colon cancer cells.[112]



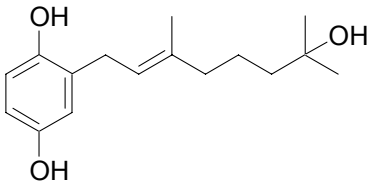
2.85



2.86 n = 2
2.94 n = 1



2.87 R = H
2.93 R = OMe

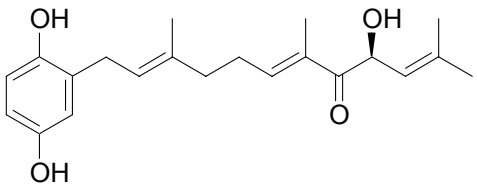


2.88

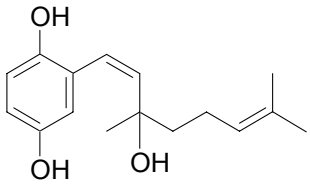
The first linear prenylated benzohydroquinone (**2.86**) from an ascidian was isolated by Fenical *et al*.[114] in 1974 from an *Aplidium sp.* and exhibited cytotoxicity against leukemia cell lines, Rous sarcoma and mammary cincinoma in test animals. The discovery of this cytotoxicity along with the fact that, at the time, there was no record of neoplasms in ascidians, contributed to a renewed interest in the research on ascidians.[115] Subsequently, ascidians have been the source of numerous metabolites with attractive biological activities.[115] In a recent study, Benslimane *et al*.[116] have reported the isolation of **2.86** from *Aplidium antillense* collected in Guadeloupe, together with the chromenol cordiachromene (**2.87**). Both compounds **2.86** and **2.87** were found to be cytotoxic against P-388 leukemia (IC_{50} 17.5 and 0.14 μM respectively) and against KB human epidermoid carcinoma cells (IC_{50} 146 and 2.0

*μ*M respectively).[116] Cordiachromene A (**2.87**) was originally isolated from the marine ascidian *Aplidium constellatum* collected from the Canadian east coast[117] and from the heartwood of a tropical American tree, *Cordia alliodora*.[118]

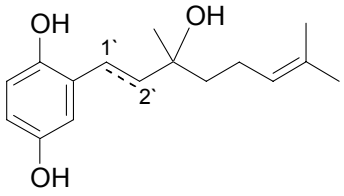
The Mediterranean tunicate *Aplidium sp.* also yielded geranylhydroquinone **2.86**, along with the benzohydroquinone (**2.88**).[119] The hydroquinones **2.86** and **2.88** were both found to have very good cytotoxicity against P-388 (IC_{50} 0.81 and 4.5 *μ*M) and against various other cancer cell lines, thus, according to Rueda *et al.*,[119] indicating that the hydroxylation of the prenyl chain may result in a marginal decrease in the cytotoxicity.



2.89



2.90

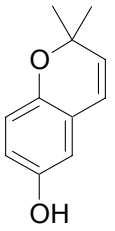


2.91 C-1` - C2` = single bond
2.92 C-1` - C2` = *E* double bond

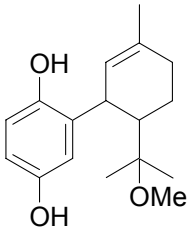
The hydroxylated prenylated hydroquinones (**2.89**-**2.92**) have been reported as being either cytotoxic or having antioxidant properties.[120-122] Rossinone A (**2.92**) was isolated from an Antarctic *Aplidium* species and exhibited significant antiproliferative activity (IC_{50} 1.5 *μ*M) against the P-388 murine leukemia cell line.[121] The tunicate *Aplidium savignyi* collected in the Indian Ocean near the Comoro Islands yielded the hydroquinone (**2.90**) and the two known hydroquinones (**2.86** and **2.91**), all of which were found to have antioxidant properties.[120] The benzohydroquinone **2.91** was first isolated from the tunicate *Amaroucium multiplicatum*, along

with the novel metabolites (**2.92** and **2.93**), all of which were shown to have potent antioxidant properties.[122]

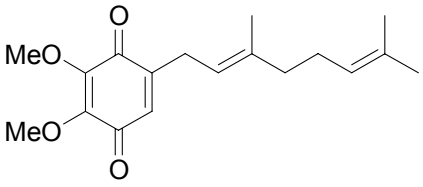
The Californian tunicate *Aplidium californicum* afforded two novel cytotoxic metabolites (**2.94** and **2.95**).[123] The prenylhydroquinone **2.95** exhibited *in vivo* activity (T/C = 138) against P-388 lymphocytic leukemia,[123] and has, more recently, been shown to be slightly cytotoxic (IC_{50} 41 μM) against the P-388 murine leukemia cell line.[121] Both prenylhydroquinone **2.94** and the chromenol **2.95** were found to have significant antimutagenic and antioxidant properties.[47;123] However, Pettit *et al.*[124] have suggested that the cytotoxic properties of the extracts of the marine ascidian *Aplidium californicum* can be attributed to the metabolites isolated from the symbiotic bryozoan *Bugula neritina*.



2.95



2.96

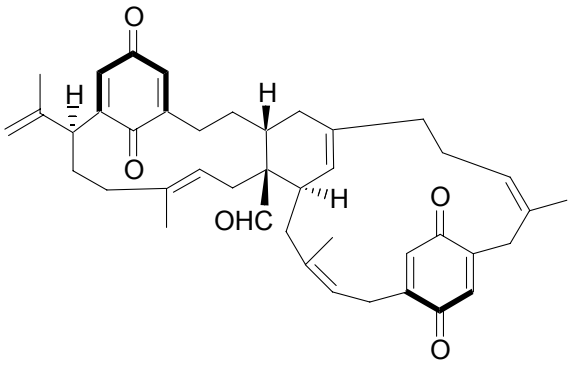


2.97

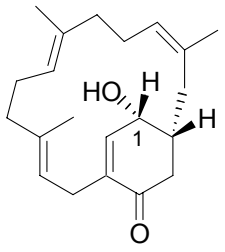
The meroterpene methoxyconidiol (**2.96**) was isolated from the *Aplidium aff. densum* collected in Oman by Simon-Levert *et al*.,[125] and was subsequently found to have antimitotic activity against sea urchin eggs, making **2.96** a potential antiproliferative agent for cancer cells.[126] The far-eastern ascidian *Aplidium glabrum* yielded the bioactive metabolite glabruquinone (**2.97**), which exhibited moderate cytotoxicity against various cancer cell lines.[127] A five step synthesis of glabruquinone was used to confirm its structure.[128;129]

The unprecedented and unusual macrocyclic metabolite longithorone A (**2.98**) was first isolated from the tunicate *Aplidium longithorax* collected in Palau, and was found to be weakly

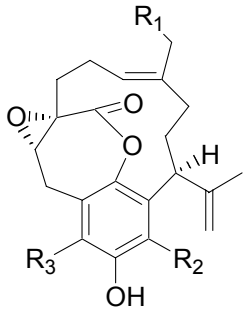
cytotoxic (ED_{50} ~ 15.8 μM) against P-388 murine leukemia cells.[130] The complex and elaborate structure of **2.98** was determined by X-ray analysis,[130] and the absolute configuration has been confirmed by its enantioselective synthesis using a series of Diels-Alder reactions.[131;132] The structure of this class of compounds is characterized by a farnesyl unit that bridges the 2,5-positions of the benzoquinone moiety to form a macrocycle with two such units fusing to yield a new carbocyclic structure.[111;130] The Australian *Aplidium longithorax* has yielded the moderately cytotoxic (IC_{50} 63.6 μM) longithorone J (**2.99**) and its absolute stereochemistry at C-1 was determined using the advanced Mosher's method.[133;134] The structural similarity of the longithorones to the yanuthones (Section 2.2) isolated from the symbiotic *Aspergillus niger* fungus in the *Aplidium sp.* sample, has prompted Bugni *et al*.[61] to propose that the quinone moiety of the longithorones may, in fact, originate from the shikimate biosynthetic pathway in marine fungi.



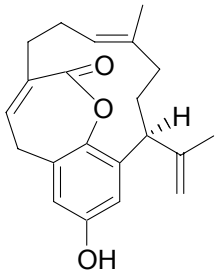
2.98



2.99



2.100 $R_1 = R_2 = R_3 = H$
2.102 $R_1 = OH$, $R_2 = R_3 = Br$



2.101

The Indonesian *Aplidium sp.* specimen collected from Flores Island yielded three novel bioactive metabolites (**2.100**-**2.102**) known as floresolides.[135] These compounds are similar to the longithorone/longithorol family and were found to be moderately cytotoxic (IC_{50} 2.9 – 30.8 μM) against KB cells.[135]

2.5.3 Cnidarians

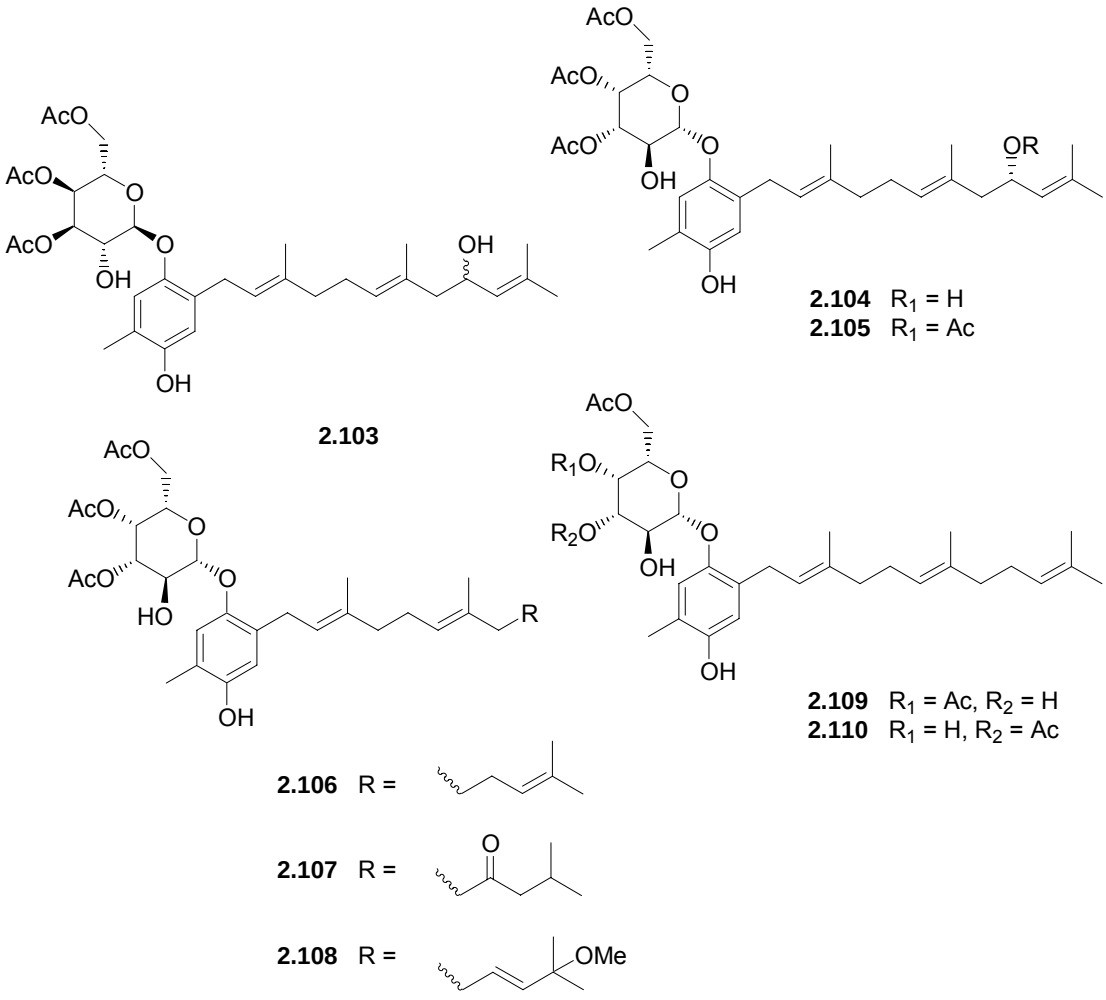
2.5.3.1 Gorgonians

The first reported cytotoxic prenylated toluhydroquinone, moritoside (**2.103**), was isolated from the gorgonian *Euplexaura sp.* collected from the Gulf of Sagami (Japan) in 1985.[136] Compound **2.103** is a farnesylated hydroquinone bearing a glycoside moiety and is, possibly, the first example of the occurrence of a β-D-altrose sugar in natural products.[136] Moritoside was found to inhibit cell division at 0.16 μM in the fertilized starfish egg assay.[136]

Subsequently, a series of similar farnesylhydroquinone glycosides (**2.104**-**2.110**), with cytotoxic properties, were isolated from the Korean gorgonian *Euplexaura anastomosans*.[137;138] Compounds **2.104**-**2.108**, also known as euplexides A-E, are structurally similar to **2.103** except that a β-D-galactose sugar moiety replaces the β-D-altrose moiety of the latter compound.[137] Additionally, Shin *et al.*[137] have assigned the absolute stereochemistry of the oxygenated chiral centre on the farnesyl side chain of **2.104** and **2.105**, using the modified Mosher's method. The euplexides A-E, **2.104**-**2.108**, exhibited moderate cytotoxicity (IC_{50} 4.1, 4.6, 8.4, 12.8 and 14.54 μM respectively) against the K-462 human leukemia cell line and, additionally, all the compounds, except for **2.107**, showed antioxidant properties.[137]

The isolation of the cytotoxic euplexides F and G (**2.109** and **2.110**) from *Euplexaura anastomosans* was reported in 2001 by Seo *et al.*,[138] and these two novel euplexides were

found to be modestly cytotoxic (LC_{50} 15.17 and 19.7 μM respectively) against the K-562 human leukemia cell line.[138]



2.103

2.104 R_1 = H
2.105 R_1 = Ac

2.109 R_1 = Ac, R_2 = H
2.110 R_1 = H, R_2 = Ac

2.106 R =

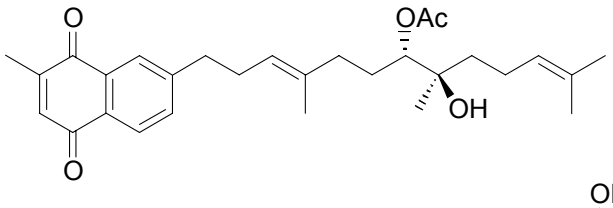
2.107 R =

2.108 R =

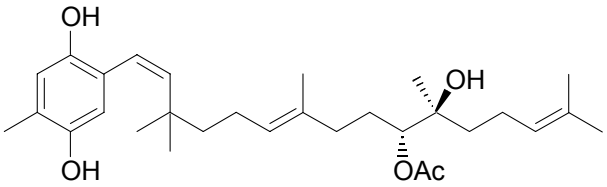
2.5.3.2 Soft corals

Cytotoxic prenylated toluquinones and hydroquinones have been isolated from the soft coral genera *Nephthea*,[41;59;60] *Sinularia*[41] and *Alcyonium*.[42] Su *et al*.[59] have isolated a novel naphthoquinone derivative (**2.111**) and toluhydroquinone (**2.112**) from the Formosan soft coral *Nephthea chabrolii*. Both compounds were shown to be weakly cytotoxic against the Hep-G2 (IC_{50} 26.6 and 37.8 μM respectively) and the MDA-MB-231 breast (IC_{50} 10.1 and 37.0 μM respectively) cancer cell lines. The naphthoquinone (**2.113**), subsequently isolated from

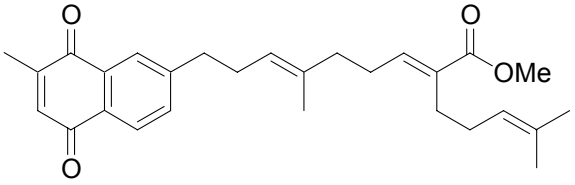
the same soft coral, was also found to be moderately cytotoxic against various cancer cell lines.[60]



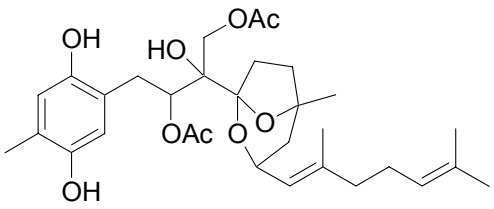
2.111



2.112



2.113



2.114

The South African soft coral *Nephthea sp.* collected in Sodwana Bay yielded the bioactive nephthoside **1.30**, while the soft coral *Sinularia dura* from the same collection afforded the bioactive metabolite sindurol (**2.114**).[41] Both nephthoside and sindurol were found to be cytotoxic (IC_{50} 3.8 and 2.1 μM respectively) to P-388 mouse leukemia cells.[41]

The soft coral *Alcyonium fauri* collected at Riet Point, near Port Alfred (South Africa), yielded the cytotoxic sesquiterpene hydroquinone rietone **1.31**.[42] Compound **1.31** exhibited activity (IC_{50} 9.3 μM) in the NCI's CEM-SS cell line screen, used to identify metabolites with anti-HIV properties,[42] and has recently been shown to be mildly cytotoxic against several oesophageal cancer cell lines.[43]

2.5.4 Molluscs

In our search of cytotoxic prenylated toluquinones and hydroquinones across the various marine phyla, the only example of such compounds within the phylum Mollusca is the series of *ortho*-prenylated toluquinones and quinones (**1.24**-**1.29**, Section 1.4) that were previously isolated by our research group from an endemic nudibranch *Leminda millecra* (Figure 3.1) collected from Algoa Bay in Port Elizabeth.[40] An approach to the synthesis of the most cytotoxic member of this series (**1.26**) is described in the next Chapter and the cytotoxicities of these compounds against the oesophageal cancer cell line WHCO1 is presented and discussed in Chapter 5 (Section 5.3, Table 5.3).

Chapter Three

An Approach to the Synthesis of a Marine Triprenylated Toluhydroquinone

3.1 Introduction

This chapter describes our attempt to synthesize the two marine natural products **1.24** and **1.26** that were both isolated from the endemic South African nudibranch *Leminda millecra* by McPhail *et al*.[40] as part of an ongoing collaboration with the Division of Medical Biochemistry at the Medical School of the University of Cape Town to identify and characterize possible novel marine-derived metabolites with significant anti-oesophageal cancer activity.[36] Amongst the secondary metabolites isolated by McPhail *et al*.,[40] were a series of six cytotoxic triprenylated toluquinones and toluhydroquinones **1.24**-**1.29** (Section 1.4). These metabolites initiate apoptotic cell death in oesophageal cancer cells after a cell cycle block, with **1.26** exhibiting the strongest cytotoxic activity[39] (Chapter 5, Table 5.3). Compound **1.26** has also been shown to increase the cytotoxicity of 17-allylamino-17-demethoxygeldanamycin (17-AAG) to oesophageal cancer cells when added in combination with this drug candidate.[139] 17-AAG is currently in Phases I and II of clinical trials as an anticancer drug (Section 5.5.1).[140;141]

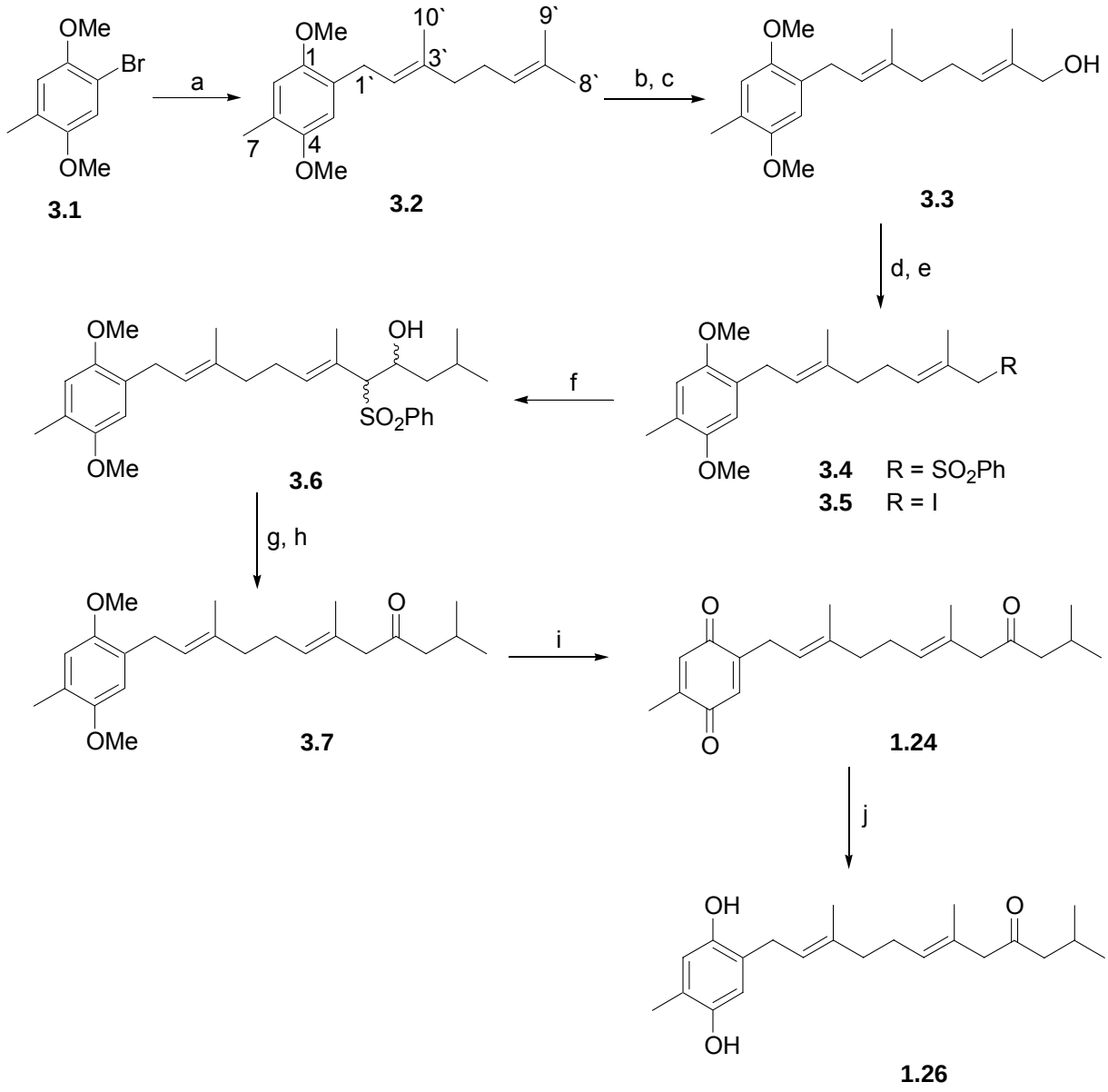
Figure 3.1 The nudibranch *Leminda millecra* photographed in Algoa Bay, South Africa

The availability of marine natural products for biological studies is often hampered by the inherent difficulties in isolating sufficient material from the original marine resource *viz* the

expense of SCUBA recollections, the possibility of not relocating the source organism and the improbability of obtaining prerequisite collecting permits for large scale recollections.[6] Nudibranchs are predators of other marine invertebrates which places them, as a predatory species, at the top of the marine invertebrate food chain.[142] Accordingly, large scale removal of nudibranchs for chemical ecology and/or bioactivity studies, would have obvious negative ramifications for the marine environment. The potential susceptibility of *L. millecra* to over-exploitation for harvesting compounds with oesophageal cancer cytotoxicity and other bioactivity studies of the secondary metabolites, which this species sequesters from its diet of octocorals, prompted us to attempt the syntheses of **1.24** and **1.26** described in this chapter.

3.2 First total synthesis of the marine natural product 1.26

Li *et al*.[143] reported the first total synthesis of **1.26** from the simple aromatic bromide precursor (**3.1**) in ten steps with an overall yield of 7% (Scheme 3.1). Their synthesis involved a modified Grignard addition of **3.1** to geranyl bromide in the presence of catalytic amounts of dilithium tetrachlorocuprate in tetrahydrofuran to afford the prenylated intermediate (**3.2**, 73%).[144] The second step of this synthesis involved a regioselective oxidation of the terminal allylic methyl group using a modified Sharpless oxidation[145;146] followed by a reduction of the resultant aldehyde with sodium borohydride ($NaBH_4$) to yield the alcohol (**3.3**, 40%). The sulfone (**3.4**) was reportedly synthesized in almost quantitative yield (92%) by the iodination of **3.3** followed by sulfonation of the iodide (**3.5**) using sodium benezenesulfinate.[146] A classical Julia coupling[147] of **3.4** with isovaleraldehyde afforded the hydroxy sulfone **3.6** (85%) which was then oxidized to the ketone **3.7** *via* a Swern oxidation followed by a reductive desulfonylation using sodium-mercury amalgam in methanol.[148] An oxidative demethylation of **3.7** using cerium ammonium nitrate (CAN) afforded the quinone **1.24** in 52% yield before being reduced to the desired hydroquinone **1.26** (71%).[149]

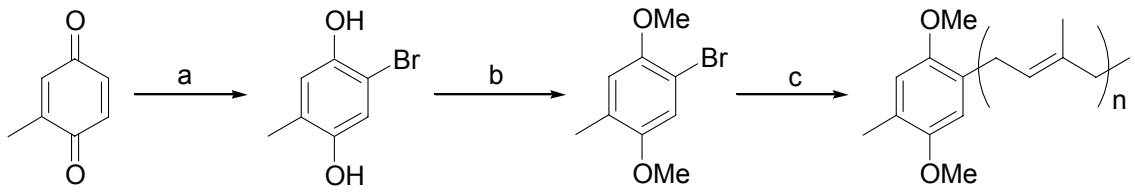


Scheme 3.1 Total synthesis of **1.24** and **1.26**.[143] *Reagents and conditions*: a) Mg, Li_2CuCl_4, geranyl bromide, THF, -78 °C – 0 °C, 1.5 h; b) SeO_2, *t*-BuOOH; c) $NaBH_4$, EtOH; d) Ph_3P, imidazole, I_2; e) $PhSO_2Na$, DMF, RT, 40 h; f) *n*-BuLi, THF, -78 °C, isovaleraldehyde; g) DMSO, $(COCl)_2$, Et_3N, -78°C; h) Na(Hg) (6%), Na_2HPO_4, MeOH; i) CAN, MeCN; j) $NaHSO_3$, THF.

3.3 Attempted synthesis of 1.24 and 1.26

In our endeavour to circumvent the supply problem of the marine natural product **1.26** and generate sufficient material to extend our bioactivity studies of these compounds, we decided to follow the synthetic procedure of Li *et al.*[143] starting with the same aryl bromide **3.1**. The aryl bromide **3.1** has previously been prepared from 2,5-dimethoxytoluene (**3.8**) using bromine in dichloromethane[150] or a buffered solution of sodium acetate and acetic acid[151;152] and *N*-bromosuccinimide (NBS) in acetonitrile,[153] while Bloomer and Zheng[154] have reported the bromination of similar aromatics, *e.g.* dimethoxybenzene, with NBS in dichloromethane.[154] From previous work accomplished in our laboratory, Scheepers *et al*.[155] have also reported the synthesis of **3.1** from commercially available 2-methyl-1,4-benzoquinone (**3.9**) in two steps in their synthesis of triprenylated toluquinone and toluhydroquinone metabolites from a marine-derived *Penicillium* fungus (Scheme 3.2). Scheepers *et al*.[155] firstly converted **3.9** to the bromohydroquinone (**3.10**, 61%) by employing the one-pot reductive bromination procedure by Miller and Steward,[156] using trimethylsilyl bromide in acetonitrile and tetraethylammonium tetrafluoroborate (TEATFB) as a phase-transfer catalyst. Subsequent methylation of **3.10** by refluxing in dimethyl sulfate and aqueous sodium hydroxide afforded **3.1** in 70% (Scheme 3.2).[155] Surprisingly, our attempts to reproduce the two-step synthesis by Scheepers *et al*.[155] of **3.1** from **3.9** gave us low and variable yields (20-40%). Accordingly, we opted for the direct bromination of commercially available **3.8** using NBS (Scheme 3.3). Bromination of **3.8** with NBS in dichloromethane[154] afforded **3.1** (40-50%) whilst the same reaction carried out in acetonitrile as the solvent generated **3.1** in 82% yield (Scheme 3.3).[153] Both 1D and 2D NMR data were used to confirm the structure of **3.1** and the molecular formula ($C_9H_{11}O_2Br$) was established by HRFABMS data. Both ^1H and ^{13}C NMR data obtained for **3.1** were consistent with reported values.[151-153] The low yields for Bloomer and Zheng's[154] method may be attributed to the relative insolubility of NBS in dichloromethane, while NBS was observed to be completely soluble in acetonitrile and quantitative conversion of **3.8** to **3.1** was obtained, as monitored by analytical thin layer chromatography (TLC).

Carreño *et al*.[153] have also highlighted the advantage of using acetonitrile to achieve regiospecific bromination of alkyl substituted methoxybenzenes, compared to using carbon tetrachloride (in which NBS is also only partially soluble) which often results in the bromination of the alkyl substituent.

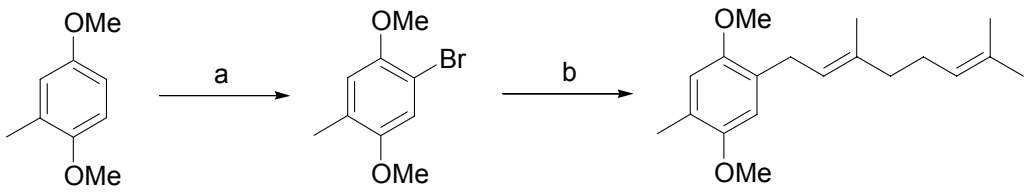


3.9 **3.10** **3.1** **3.2** n = 2 **3.33** n = 3

Scheme 3.2 Scheepers *et al*.[155;157] approach to the synthesis of **3.2**. *Reagents and conditions*: a) Me_3SiBr, MeCN, TEATFB, RT, overnight; b) NaOH, Me_2SO_4, reflux; c) *n*-BuLi, TMEDA, dry Et_2O, 0 °C.

Having successfully synthesized **3.1**, we were now able to perform a standard Grignard procedure to access the diprenylated quinone **3.2** (Scheme 3.3). Grignard reactions involve the initial displacement of a bromine atom from an alkyl or aromatic halide by magnesium metal (activated by the addition of a small amount of iodine) to generate the Grignard reagent.[158] The activation of the magnesium for the preparation of Grignard reagents can be a challenging process as outlined by Baker *et al*.[159] Grignard reactions are known to undergo side- or so-called 'abnormal' reactions, such as reduction, condensation and conjugate addition, which inevitably leads to a loss in chemoselectivity and therefore results in poor yields of the desired products.[160;161] To circumvent this problem, additives or catalysts, such as cerium (III) halides[160-162] and copper reagents[144;163] have been shown to enhance both chemoselectivity and reactivity of Grignard reactions.[160;163] The use of cerium (III) halides, *e.g.* cerium chloride ($CeCl_3$), as catalysts is more pertinent in reactions involving the addition of carbonyl compounds to Grignard reagents.[160] The ability of $CeCl_3$ to improve yields in Grignard reactions has recently been investigated in our laboratory and has been found to

enhance product formation from 0-5% to 20-57% in some instances.[164] However, for our synthetic requirements, we opted for the copper based lithium tetrachlorocuprate (II) catalyst (Li_2CuCl_4) since Li *et al*.[143] had reported the synthesis of **3.2** in 73% yield by making use of this particular reagent (Scheme 3.1).



3.8 **3.1** **3.2**

Scheme 3.3 Synthesis of **3.2** from **3.8.** *Reagents and conditions*: a) NBS, MeCN, RT, overnight;[153] b) Mg, Li_2CuCl_4, geranyl bromide, Et_2O, -10 °C.[143;165]

Consequently, we attempted the synthesis of **3.2** under the same conditions described by Li *et al*.,[143] by adding a solution of the arylbromide **3.1** in anhydrous diethyl ether to a stirred solution of iodine-activated magnesium turnings in anhydrous diethyl ether under argon to generate the aryl Grignard reagent *in situ*.[165] The resulting Grignard solution was cooled on ice before dropwise addition to a solution of geranyl bromide and the Li_2CuCl_4 catalyst in tetrahydrofuran at -10 °C to afford the desired product in low (15-30%) yields. Both 1D and 2D NMR spectroscopy (Figure 3.2 and 3.3) were used to unequivocally assign the structure of **3.2** as the NMR data for **3.2** was not reported by Li *et al*.[143] A comparison of the ^{1}H NMR spectrum indicated obvious similarities, *e.g.* H-1` ($\delta_H$ 3.30, d, *J* = 7.3 Hz), H-2`(δ_H 5.30, t, *J* = 7.3 Hz) and H-6` ($\delta_H$ 5.10, t, *J* = 6.8 Hz), between **3.2** (Figure 3.2) and similar compounds, previously synthesized in our laboratory,[155;157] while gHMBC correlations between H-1` and C-1, C-2, C-3, C-2` and C-3` (Figure 3.3) were used to confirm the successful *ortho*-prenylation of **3.1**.

Even though the yields obtained for this Grignard reaction were low (15-30%), we noticed a marginal improvement on our previous yields (10-15%) for this approach, prior to using the

Li_2CuCl_4 catalyst. Frustratingly, we were unable to reproduce the results reported by Li *et al*.,[143] and this resulted in the need to develop a new synthetic strategy for this crucial prenylation step.

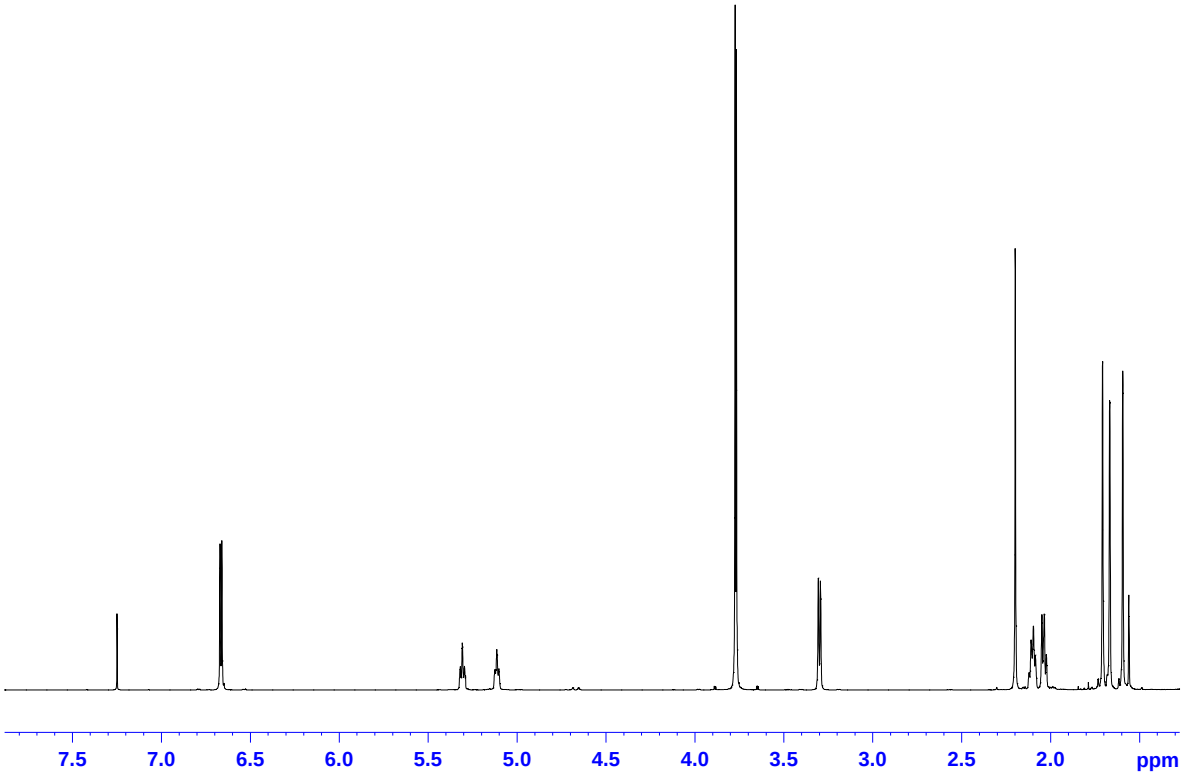


Figure 3.2 ^1H spectrum (600 MHz, $CDCl_3$) for **3.2**.

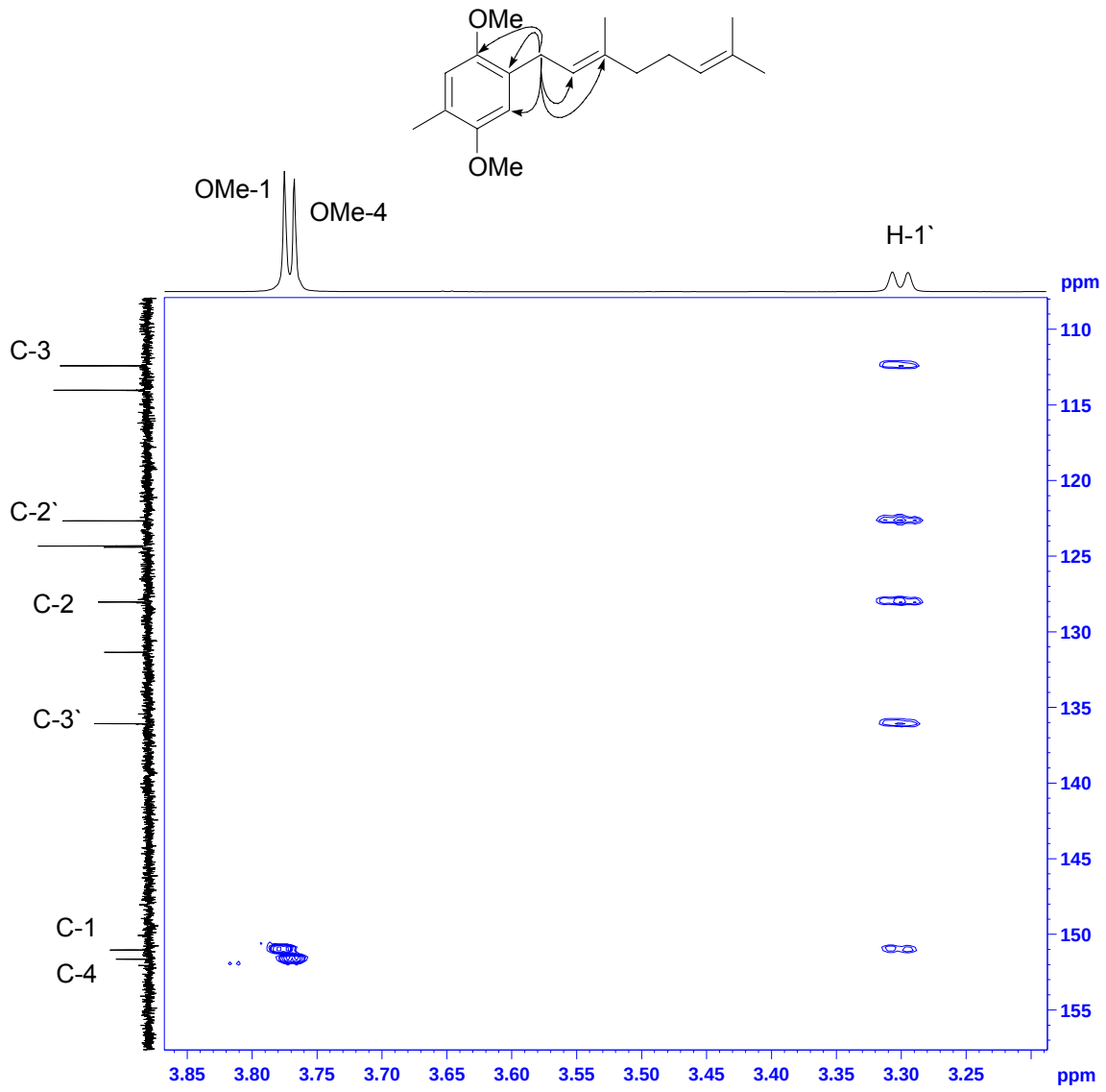


Figure 3.3 A region (F1 = δ_C 107.9 – 157.6; F2 = δ_H 3.19 – 3.87) of the gHMBC spectrum (600 MHz, $CDCl_3$) obtained for **3.2**. The accompanying figure indicates the key gHMBC correclations.

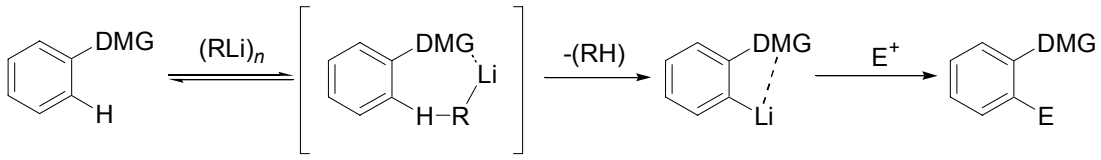
3.3.1 Different synthetic strategies towards the synthesis of *ortho*-prenylated phenols

Over three decades ago, Snyder and Rapoport[166] proposed that one of the paramount concerns for a synthetic organic chemist attempting the synthesis of prenylated quinones was the retention of the configurational integrity of the unsaturated prenyl side chain. Stereocontrolled organometallic cross-coupling reactions using allylsilanes, allyltin or allylnickel reagents allows the *ortho*-coupling of simple (C_5-C_{20}) prenyl groups to phenolic compounds and, while this methodology retains the configurational integrity of the prenyl side-chain, this approach can be problematic.[167] For example the synthesis of the marine natural product **2.44** (Scheme 4.1, Section 4.1.1) by Claessens *et al*.[168] uses cross-coupling synthetic methodology requiring costly chemicals that are highly toxic and extremely sensitive to moisture and air. Claisen rearrangements, directed *ortho*-metallation (D*o*M), phenoxide carbon-alkylation, metal-halogen exchange (MHE) and Friedel-Crafts-type prenylations are some of the other strategies also available for the regioselective synthesis of *ortho*-prenylated phenols and are reviewed here.[169;170]

3.3.1.1 Directed *ortho*-metalation (D*o*M)

Directed *ortho*-metalation (D*o*M) originated from the independent discoveries of Gilman[171] and Wittig[172] and has been comprehensively reviewed. [169;173-176] The popularity of D*o*M chemistry has led to the development of several synthetic protocols since it is regarded as a very attractive strategy for the regiospecific functionalization of aromatic compounds.[169;175;176] The complementary technique of metal-halogen exchange (MHE) (Section 3.3.1.5) contributed to further advancement in D*o*M chemistry,[175;177;178] when the key observation was made that, at very low temperatures, MHE occurs rapidly and preferentially to deprotonation by BuLi.[175] The mechanism for D*o*M reaction is believed to occur through a three-step

process that involves an equilibrium between the alkyllithium aggregates $(RLi)_n$ and the prelithiated complex (**3.11**) of the alkyllithium coordinated to the directing metalating group (DMG) (**3.12**) *vide infra* (Scheme 3.4). The complex **3.11** subsequently undergoes a slow and irreversible deprotonation at the *ortho*-carbon relative to the DMG, thus resulting in an *ortho*-lithiated intermediate (**3.13**) that can consequently react with a suitable electrophile to yield a 1,2-substituted species (**3.14**) (Scheme 3.4).[171;175;179]



3.12 **3.11** **3.13** **3.14**

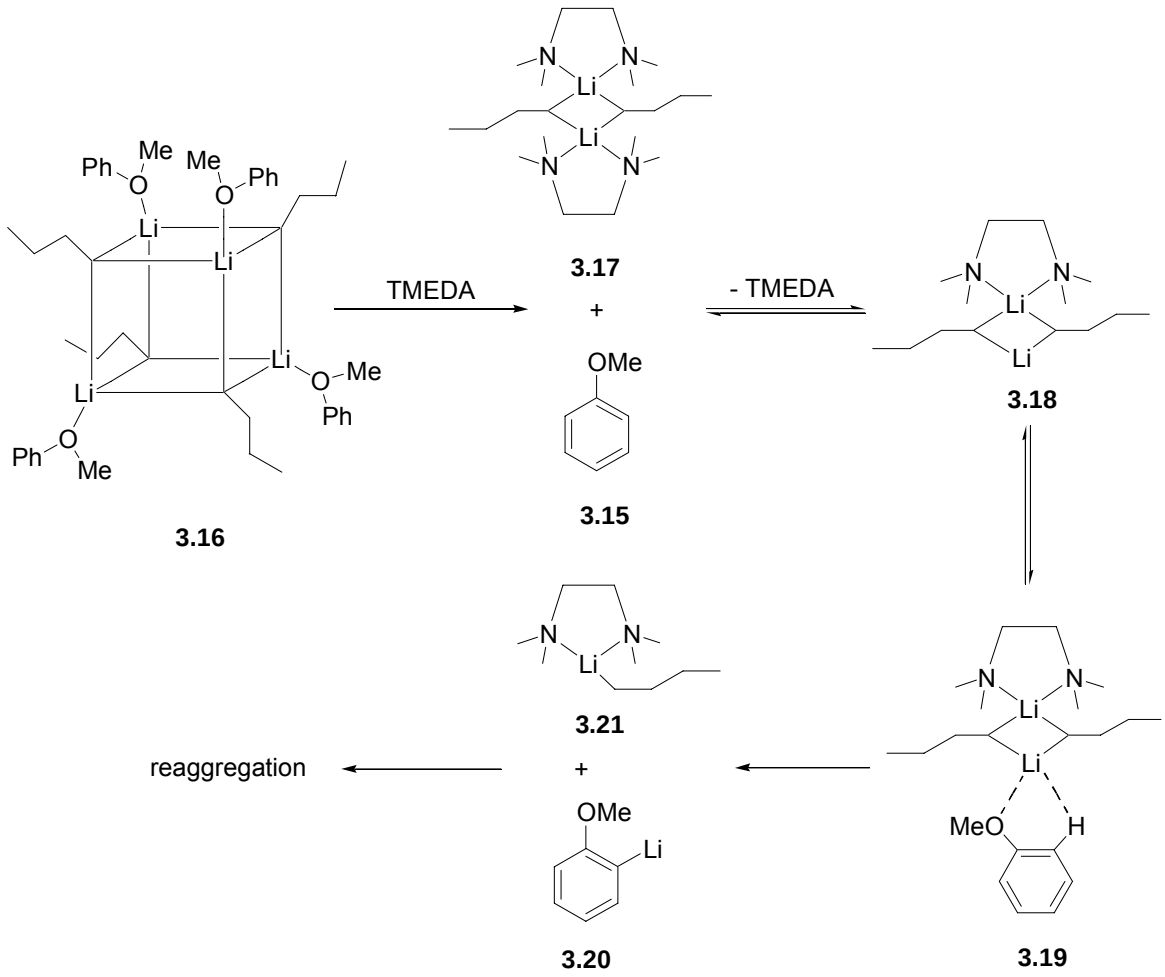
Scheme 3.4 A putative mechanism of the D*o*M reaction.[175;180]

Generally, the deprotonation step of the D*o*M reaction requires strong alkyllithium bases that are highly soluble in organic solvents.[175;181] In his excellent review of D*o*M chemistry, Snieckus[175] alludes to the structure of alkylithiums in solution as being essentially viewed as bridged electron-deficient arrangements of polycovalent C-Li bonds, which can undergo rapid equilibrium between the C-Li and Li-ligand bonds. The high solubility of these alkylithium bases, *e.g. n*-BuLi, is due to the formation of defined aggregates in solution where they have the propensity to mainly form hexamers in hydrocarbon solvents like hexane, while in solvents such as tetrahydrofuran and diethyl ether, these alkyllithium bases tend to rather adopt a tetrameric and/or dimeric structure.[175] Alkyllithiums are believed to react as aggregates and mixtures of aggregate or dissociated species in hydrocarbon solvents.[175] The addition of aprotic solvents, *e.g.* diethyl ether, tetrahydrofuran or amine solvents, induces the dissociation of these aggregates through an acid-base type reaction.[175;181] Additionally, the addition of the bidentate ligand tetramethylethylene diamine (TMEDA) effectively dissociates alkyllithium aggregates into monomers and dimers in solution.[175;181]

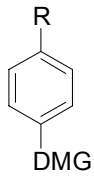
Bauer and Schleyer[182] investigated the *ortho*-directed lithiation of anisole (**3.15**) with *n*-BuLi in toluene at -64 °C in a detailed NMR study. *n*-BuLi exists as a tetrameric complex (**3.16**) in solution,[175;181] and, upon the addition of one equivalent of TMEDA, the formation of the dimeric form of *n*-BuLi (**3.17**) was observed as the TMEDA initiates the dissociation and disaggregation of the tetrameric form of *n*-BuLi (**3.16**, Scheme 3.5). [175;181-183] The dimeric *n*-BuLi-TMEDA complex **3.17** is much more reactive than its tetrameric counterpart **3.16** and accounts for the facile *ortho*-lithiation under these conditions, since the lithium atom is more accessible for effective lithiation.[182;183] TMEDA also enhances reactivity by moderating the probable internal coordination between the oxygen of the methoxy group and lithium and this coordination has been shown to render anisole **3.15** almost completely unreactive to electrophiles.[184;185] The free anisole **3.15** does not undergo *ortho*-lithiation,[175] but is rather thought to coordinate *via* the two free coordination sites on the Li atom of the *n*-BuLi-TMEDA aggregate (**3.18**) and the anisole oxygen, thus forming the intermediate (**3.19**).[175] The *ortho*-lithiated species (**3.20**) is then formed by the irreversible deprotonation of **3.19**, which also yields 1 : 1 *n*-BuLi-TMEDA complex (**3.21**, Scheme 3.5).[175] Both **3.20** and **3.21** subsequently undergo aggregation to form **3.15** and **3.17** in solution.[175]

The efficiency of *ortho*-lithiation also depends on the nature of the DMG, which can also serve as a protecting group in some instances.[175] DMG's can be classified as either heteroatom based ($OCONR_2$, OMe) or carbon based (CON^-R, $CONR_2$).[169] The characteristics of a good DMG are that it must contain a heteroatom so that the strong alkyllithium base can coordinate to it and also be a poor electrophile to prevent nucleophilic attack by the strong alkyllithium at the DMG, instead of deprotonation. The effectiveness of a good DMG is also determined by steric hindrance and/or charge deactivation.[175] The efficiency of DMGs has been found to decrease in the order where R = SO_2NR_2, > SO_2N^-R > CON^-R > CH_2NR_2 > OMe > $CH_2CH_2NR_2$ > F when using (**3.22**) as a model system.[186] Slocum and Jennings[186] used the 4-OMe substituent in **3.22** as a reference group to propose the hierarchy of DMG efficiency presented above, based on the assumption that lithiation prior to prenylation tends to occur

ortho to the strongest DMG in an aromatic system that contains more than one competing DMG.[180;187;188] Contrastingly, Beak *et al*.[180;187] used a tertiary amide reference group (**3.23**) and proposed the following relative reactivities of various DMG's where R = CON^-R > $CONR_2$ > SO_2NR_2 > SO_2N^-R > CH_2NR_2, OMe, Cl. Interestingly, however, the relative DoM reactivity of DMGs is subject to other factors, for instance solvent and/or the presence of additives such as TMEDA, that may influence the outcome of the reaction.[181]



Scheme 3.5 The effect of TMEDA on alkyllithium and anisole **3.15** aggregation in hydrocarbon solvents.[175]



3.22 DMG = OMe
3.23 DMG = $OCONEt_2$

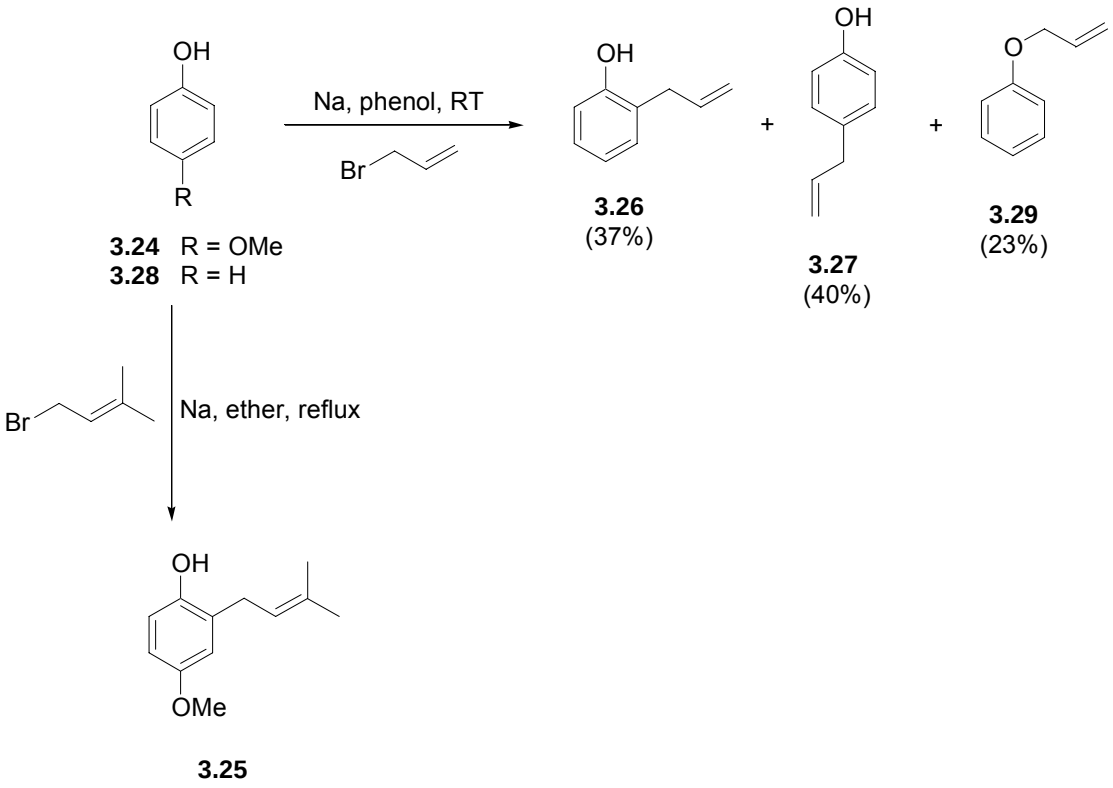
The major attraction to D*o*M chemistry is its regiospecificity in the functionalizing of aromatic compounds,[169;175;176] *e.g.* the *ortho*-prenylation of symmetrical phenol derivatives with one or two oxygen substituents (**3.22** and **3.23**) has proven to be highly successful.[175] However, compounds containing more than two oxygen substituents are usually difficult to deprotonate and are thus unlikely to undergo prenylation *via* D*o*M chemistry.[175]

3.3.1.2 Phenoxide carbon-alkylation

Phenoxide carbon-alkylation is a fairly popular strategy for the synthesis of *ortho*-prenylated phenols using mild basic conditions despite the fact that competing side reactions such as *para*-prenylation, bis-prenylation and oxygen-prenylation limit the yields of this reaction to a modest 30-50%.[169;189] The phenoxide carbon-alkylation reaction requires the initial deprotonation of the methoxyphenol (**3.24**) to form a phenoxide-enolate anion species that can subsequently undergo *ortho*-prenylation with the prenyl bromide, by refluxing with sodium metal in ether, *via* a nucleophilic substitution reaction to give the prenylated compound (**3.25**, 85%) (Scheme 3.6).[169] Both *ortho* and *para* carbon-prenylations (**3.26** and **3.27**) of phenol (**3.28**) are achieved using sodium phenoxide whilst potassium or lithium phenoxides favour oxygen prenylations (**3.29**).[169;190]

Carbon-alkylation induced prenylation reactions are affected by solvation and the dielectric constant of solvents used in this reaction, thus making the choice of solvent crucial to the selective formation of the different prenylated products (Scheme 3.6). Solvents that promote

selective solvation of the phenoxide ion, which is achieved through hydrogen bonding between the oxygen atom of the phenoxide ion and the solvent, limit the access of the oxygen to electrophilic attack and thus favour carbon-prenylation.[189;191] Oxygen-prenylation is promoted by the use of solvents with high dielectric constants causing a shielding effect on the leaving group relative to the attractive force of the sodium ion, [189;191] *e.g.* a mixture of the oxygen allyl compound **3.29** and the *ortho* and *para*-allyl compounds **3.26** and **3.27** respectively are obtained when using phenol as the solvent (Scheme 3.6).[189]

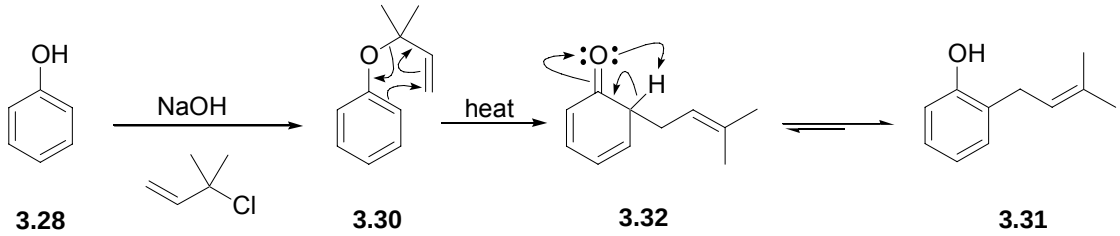


Scheme 3.6 A summary of the phenoxide carbon-alkylation reaction.[169;189]

3.3.1.3 Claisen rearrangement

The Claisen rearrangement is a relatively mild synthetic procedure that allows the formation of several substitution permutations but, more importantly, it uses reverse prenylation of **3.28**

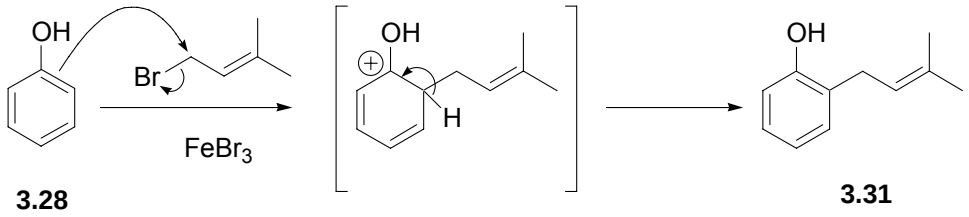
to form a prenyl aryl ether (**3.30**) that can then be manipulated to obtain the required *ortho*-prenylated compound (**3.31**).[169;175] This reverse prenylation process involves the deprotonation of **3.28** with a strong base, *e.g.* NaOH, to form the phenoxide ion which undergoes a nucleophilic substitution reaction with a tertiary allyl halide.[158;169;175] The resulting allyl ether **3.30** undergoes a [3,3]-sigmatropic rearrangement to form the intermediate (**3.32**) which subsequently tautomerizes to the enol form to afford the *ortho*-prenylated compound **3.31** (Scheme 3.7).[158;169;175] Despite the regioselectivity of the Claisen rearrangement, this synthetic strategy is limited for the synthesis of prenylated compounds by the accessibility of suitable tertiary alkyl halide reagents.[169;175]



Scheme 3.7 Prenylation of **3.28** *via* a Claisen rearrangement strategy.[158;169]

3.3.1.4 Friedel-Crafts-type *ortho*-prenylation

The Friedel-Crafts type reaction is an electrophilic aromatic substitution reaction that usually occurs between electrophilic carbon of the halogenated prenyl precursor and the most nucleophilic site of the aromatic compound (Scheme 3.8).[169] The Friedel-Crafts-type *ortho*-prenylation approach therefore requires an aromatic system suitably substituted with multiple electron donating substitutents in order to guarantee success and modest regiocontrol. Unfortunately, this prenylation method also promotes polyalkylation since the addition of a prenyl moiety increases the nucleophilicity of the aromatic ring which therefore becomes more susceptible to a second addition.[169]



3.28 **3.31**

Scheme 3.8 The general mechanism for a Friedel-Crafts prenylation reaction.[158]

3.3.1.5 Metal-halogen exchange (MHE)

Metal-halogen exchange (MHE) generally occurs under mild conditions and involves the exchange of an element, *e.g.* I, Sn, Br, Se, Cl, with a metal ion such as Li.[192-194] The nature of each of these different elements offers both advantages as well as drawbacks. For instance, the fastest MHE reaction occurs between iodine and Li with the added advantage of minimal chances of side reactions, but this reaction is only feasible for primary alkyl iodides and unsuccessful for both secondary and tertiary alkyl iodides.[195;196] The MHE methodology is complementary to D*o*M chemistry (Section 3.3.1.1) and they both share similar characteristics. The most convenient MHE applicable for allyl, vinyl and aryl bromides is the Li-Br exchange and it is our methodology of choice for our first step in our effort to synthesize the marine natural product **1.26**. MHE between bromine and lithium, however, has the disadvantage of being slower than the Li-I exchange thus allowing for competing side reactions such as α and *β*-metalation.[197] Interestingly, in the case of MHE reactions, the concern of regiocontrol is only limited to the regioselective synthesis of the *ortho*-halogenated precursor which Hoarau and Pettus have reported as challenging.[169] The methyl and dimethoxy substitution pattern inherent in **3.8** ensured that our synthetic strategy was not jeopardized by difficulties associated with regioselective bromination of this compound at the beginning of our synthesis (Section 3.3.1).

3.4 Prenylation of the activated aryl bromide 3.1 using MHE methodology

Our previous[155;198] successful use of the butyllithium-mediated MHE methodology in the synthesis of prenylated marine natural products provided us with the confidence to adopt this synthetic route instead of the Grignard approach. However, Scheepers *et al.*[155;157] only obtained **3.2** and the farnesyl analogue (**3.33**) in a moderate 27% yield (Scheme 3.2) and, subsequently, all our attempts to synthesize **3.2**, using the same procedure, gave similarly low and frustratingly variable isolated yields (5-40%). Given the apparent vagaries of this crucial MHE-mediated prenylation step, we initiated a detailed mechanistic study in an effort to understand the factors affecting the outcome of this reaction.[199] Analysis of the crude reaction mixture by HPLC was of paramount importance in this study and the results obtained (Figure 3.4) suggested the presence of variable amounts of the same five components (Figure 3.4) each time the experiment was repeated (on 100 mg of **3.1**), both with and without the addition of TMEDA (Table 3.1). In order to shed light on this reaction, we initially investigated the composition of the different HPLC fractions obtained from the reaction mixture. Analysis of the ^{1}H NMR spectrum (Figure 3.5, top) for fraction A revealed the presence of only aliphatic and olefinic signals and suggested a mixture of two products, *i.e.* unreacted geranyl bromide (**3.34**) and compound (**3.35**) which is formed by the coupling of geranyl bromide **3.34** and *n*-BuLi.

HRFABMS data for fraction B established the molecular formula as ($C_{19}H_{28}O_2$) (obsd. *m/z* 288.2090, calcd. for $C_{19}H_{28}O_2$ [M^+] 288.2089) and both ^{1}H and ^{13}C NMR data were consistent with previous results obtained (Figures 3.3 and 3.4), confirming the structure of the prenylated product **3.2**.

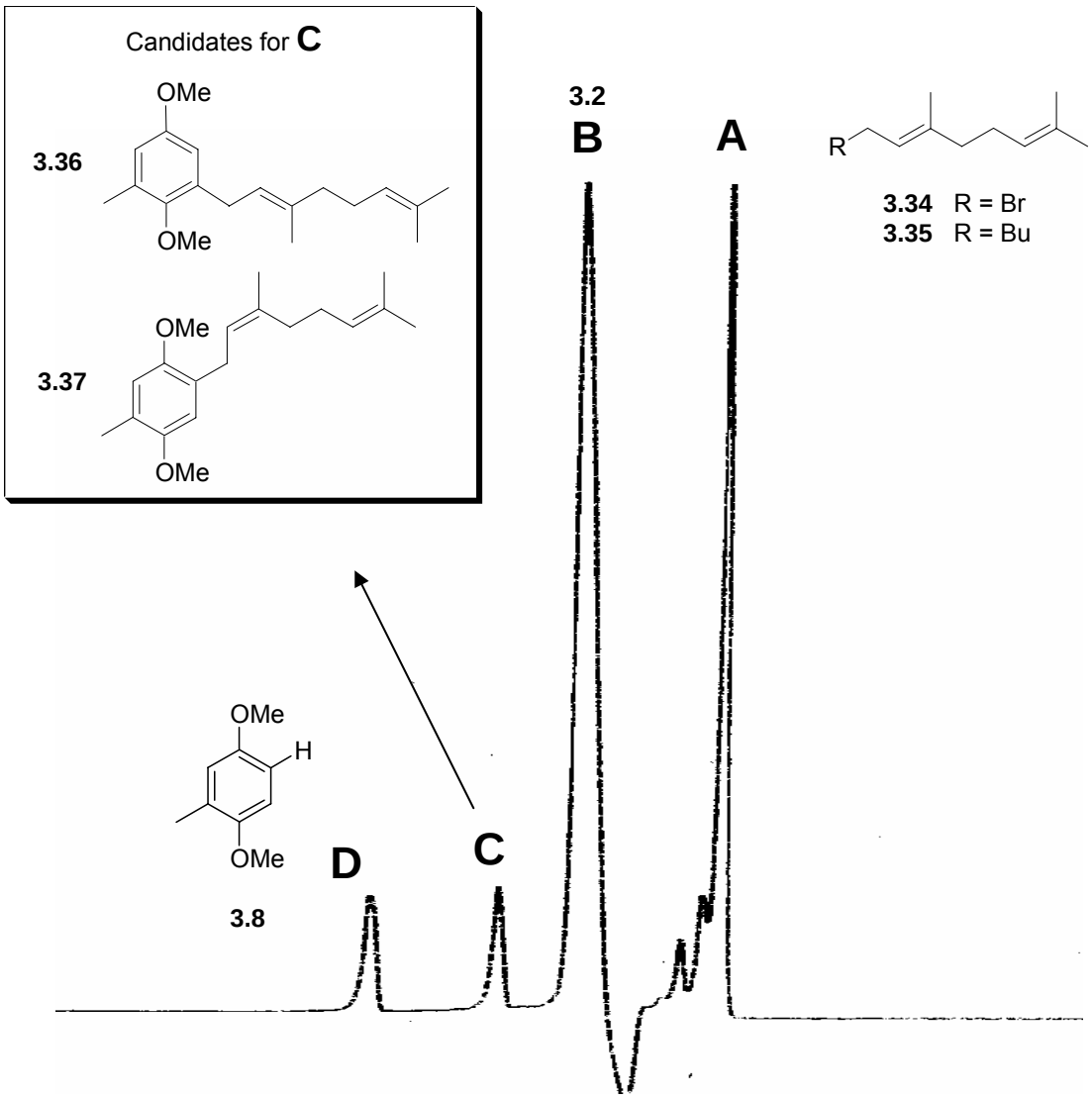


Figure 3.4 HPLC chromatogram of the different products obtained form the prenylation of **3.1**.[199]

Examination of the ^{1}H spectrum (Figure 3.5, bottom) for fraction C revealed the duplication of the proton resonances H_2-1` ($\delta_H$ 3.30), $H_3$-7 ($\delta_H$ 2.18) and the methyl signals for $H_3$-8`, H_3-9` and $H_3$-10` (δ_H 1.67, 1.59 and 1.70 respectively) for **3.2**, thus suggesting the presence of either an alternative coupling product or a geometric isomer of the target compound **3.2**. Unfortunately, all our efforts to separate the different components of fraction C by normal and reversed phase semi-preparative HPLC, and normal phase analytical HPLC, using various mixtures of solvents, proved unsuccessful. EIMS data obtained for this intractable mixture of

products showed a molecular ion peak *m/z* of 288 which, along with the duplication of the key proton signals (Figure 3.5) mentioned above, suggested that the unknown compound was possibly an isomer (**3.36** or **3.37**) of the major product **3.2**.

The ^{1}H and ^{13}C NMR data obtained for fraction D were in accordance with that of the commercially available **3.8** used in our synthesis.

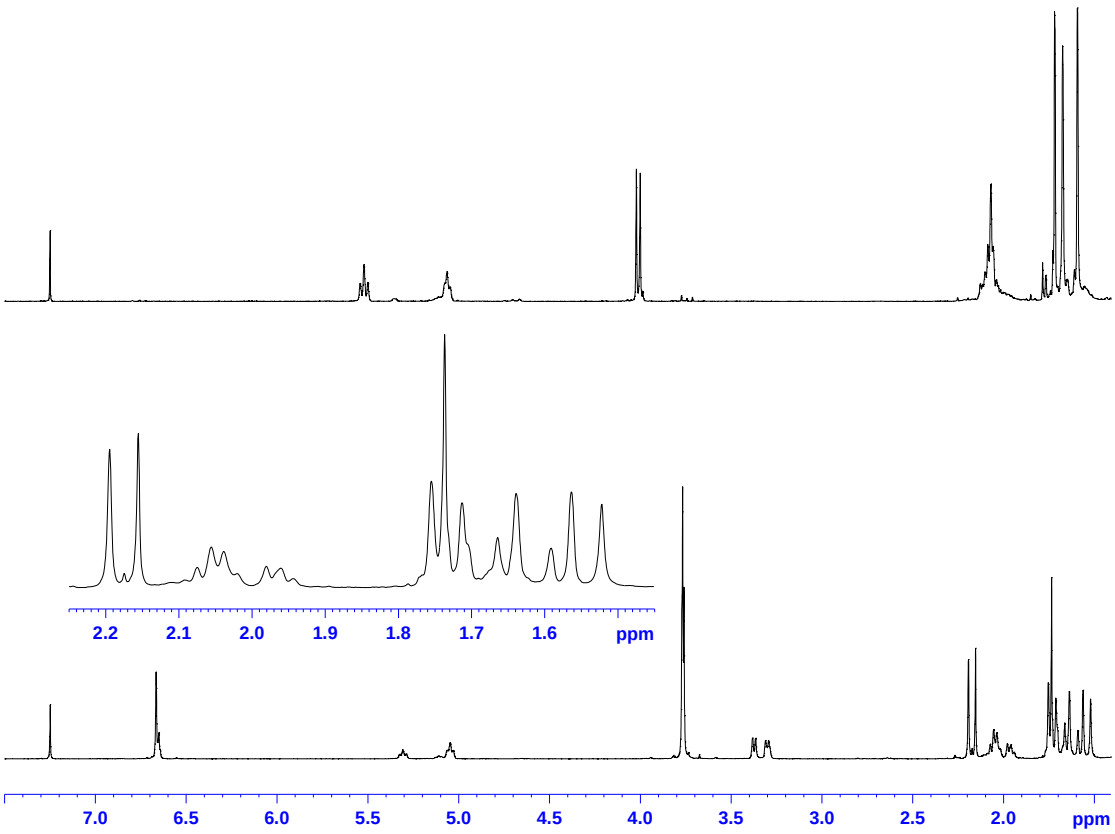


Figure 3.5 ^{1}H spectra (400 MHz, $CDCl_3$) for HPLC fraction A (top), fraction C (bottom) and expansion of upfield section (δ_H 1.45 – 2.25) shown inset.

Analysis of the results obtained from several experiments (Table 3.1), indicated low ratios of the desired product **3.2** to the undesirable products **3.36** and **3.37** (4 : 5) when TMEDA was omitted from the reaction, thus reinforcing the importance of TMEDA as a crucial additive in these MHE reactions, in order to break up the aggregates formed and enhance reactivity of the *n*-BuLi as discussed above (Section 3.3.1.1).[182;183] Additionally, the addition of one

equivalent of TMEDA to the ether solution of **3.1** and *n*-BuLi resulted in an obvious increase in the proportion of the prenylated product **3.2** to **3.36** and **3.37** (6 : 1), whilst the addition of two equivalents of TMEDA to the reaction mixture apparently provided no added advantage (Table 3.1). This observation implies that no more than one equivalent of TMEDA is involved in the rate-determining step as suggested by Galiano-Roth and Collum.[200] Frustratingly, we were unable to reproducibly extrapolate our results from this small scale study to give a comparable isolated yield of **3.2** when the reaction was scaled up. Therefore, the need to investigate the nuances of this particular MHE reaction was warranted.

Table 3.1 Product ratios as determined from HPLC analysis of reaction mixtures (using a differential refractometer)[a].[199]

Fraction	Compound	Without TMEDA	1 eq. TMEDA	2 eq. TMEDA
A	**3.34, 3.35**	5	1	1
B	**3.2**	4	6	6
C	**3.2, 3.36/3.37**	0.6	1	1
D	**3.8**	0.6	0.8	1

[a]There were also traces of unidentified products.

3.4.1 ^{7}Li NMR study of the MHE reaction of 3.1

According to Zuend *et al*.,[201] ^{7}Li and ^{13}C NMR can both be utilized to investigate the mechanism of lithium-mediated reactions. With this in mind, we initiated a ^{7}Li NMR study of the MHE reaction of **3.1** and monitored the course of the reaction between **3.1** and *n*-BuLi using ^{7}Li and ^{13}C NMR, both in the presence and absence of TMEDA (Figures 3.6 and 3.7 respectively).

The organolithium samples were prepared by reaction of **3.1** with *n*-BuLi. Samples of **3.1** were weighed into oven dried 5 mm NMR tubes fitted with a screw cap and a deuterated

chloroform ($CDCl_3$) insert (*vide infra*) and flushed with argon. After dilution with diethyl ether and cooling in a dry ice/acetone bath, each sample was treated with a stoichiometric amount of *n*-BuLi (~1.5 eq of 1.5 M solution) and 0-2 equivalents of TMEDA and mixed by shaking and applying a vortex. The resulting organolithium samples were then inserted into the NMR probe precooled to -80 °C. After a short time for temperature equilibration, the field was locked and shimmed using the $CDCl_3$ insert. A ^{13}C (proton decoupled) spectrum (referenced to $CDCl_3$) and a ^{7}Li-NMR spectrum (referenced to an external standard of 1M LiBr in D_2O) was acquired. Additional lithium spectra were subsequently obtained at intervals as the temperature was increased. The temperature of the NMR probe was calibrated with a 4% methanol in d_4-methanol sample using a standard protocol, *i.e.* by monitoring the chemical shift difference between the hydroxyl proton and the methyl protons of the 4% methanol in d_4-methanol.[202]

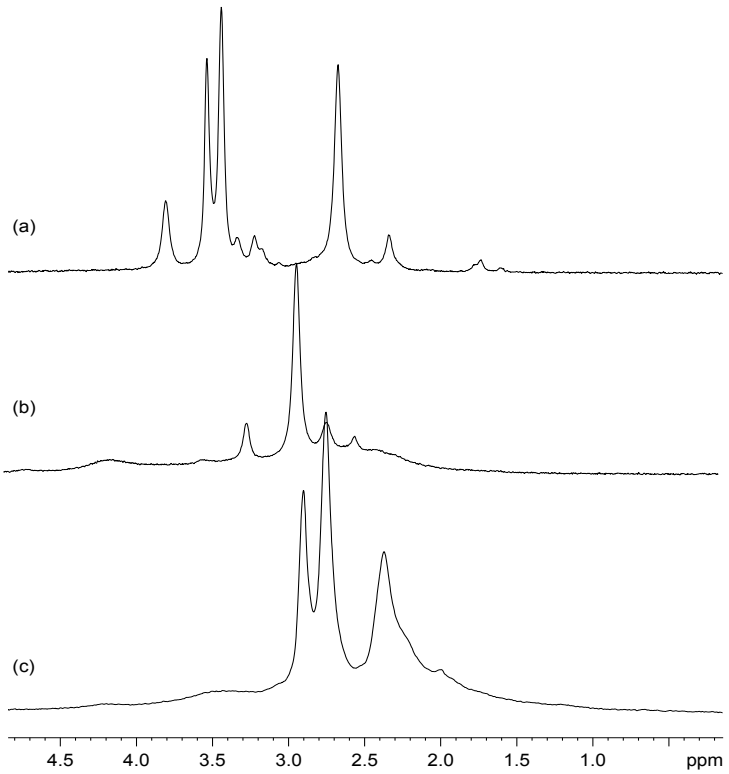


Figure 3.6 ^{7}Li spectra (155.5 MHz, δ_{Li} 0 – 5.5, LiBr) of (a) *n*-BuLi added to **3.1** (b) *n*-BuLi and TMEDA added to **3.1** and (c) **3.1** added to *n*-BuLi and TMEDA.

Analysis of the ^{7}Li and ^{13}C NMR data, along with our proposed structures for the various products obtained in this reaction suggested that the MHE was successful. The disappearance of the sharp brominated carbon signal (δ_C 108) of **3.1** and the emergence of the two broad carbon resonances (δ_C 108 and δ_C 122) were consistent with those of a lithiated carbon (Figure 3.7), according to Bauer and Schleyer[182] who have previously reported that the chemical shift of the lithiated aromatic carbon signals usually appear in the region of 110-220 ppm, *e.g.* phenyllithium (dimer, δ_C 188)[203] and *ortho*-lithio-anisole (δ_C 155).[182] The broad signals in the ^{13}C NMR spectrum (Figure 3.7) can be attributed to the typical quadrupolar broadening of the lithiated carbon resonances in the mélange of mixed aggregates resulting from the unsymmetrical system around ^{7}Li.[204;205] The quadrupolar broadening effect, in our case, is also caused by the competitive coordination between the Li and the ether, as well as between the TMEDA and the aromatic methoxy groups of **3.1**.[206] Other factors such as solvent exchange processes around the lithium contribute to additional signal broadening in the ^{13}C NMR spectrum,[182] and are responsible for the analogous broadening of the signals in the ^{7}Li NMR spectra (Figure 3.6b).[182]

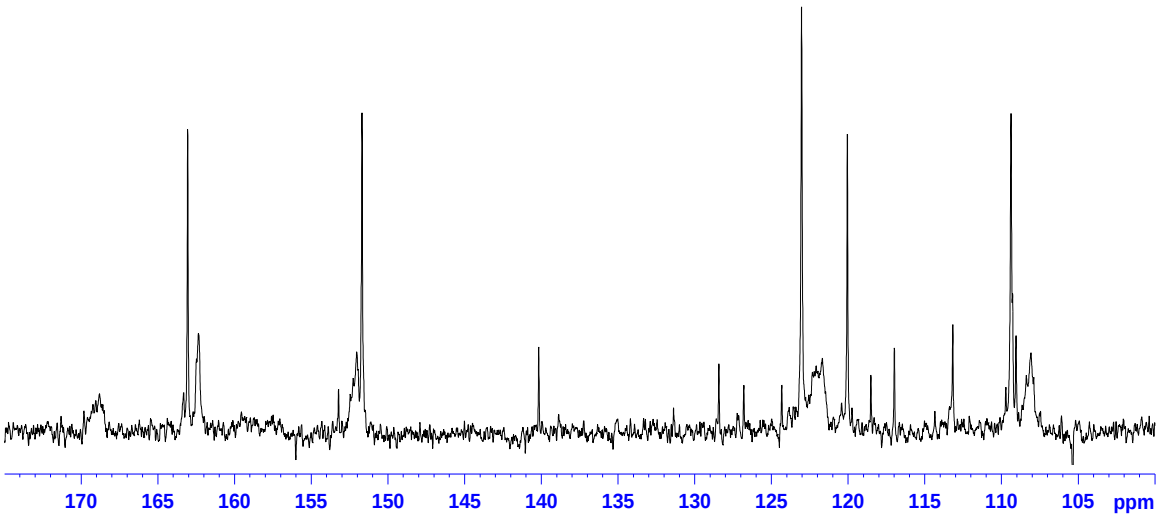
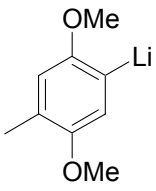


Figure 3.7 Downfield section (δ_C 100-175) of the ^{13}C NMR spectrum (100MHz, $CDCl_3$) of **3.1** in ether, *n*-BuLi and TMEDA at -60 °C, showing the emergence of broad signals attributed to lithiated carbon resonances (δ_C 108 and δ_C 122).

As discussed previously in Section 3.3.1.1, D*o*M in anisole, **3.15**, involves the coordination of the *n*-BuLi dimer to the methoxy/electron-rich aromatic ring before the metal proton exchange occurs,[175;179;182;207] resulting in the formation of a complex comprising of butyllithium and aryllithium.[182;183] We expected a similar occurrence of the same process in our MHE reaction of **3.1** with *n*-BuLi and hoped to observe the formation of the mixed dimer **3.17** (Scheme 3.5) in the ^{7}Li and ^{13}C NMR spectra of the reaction mixture. Frustratingly, after numerous attempts, it was evident that we could not conclusively identify the presence of a mixed dimer or ascertain the structure of any of the aryllithium aggregates. However, we were reassured by the presence of several signals in both the ^{7}Li and ^{13}C NMR spectra which confirmed the occurrence of more than one aggregate (Figures 3.6 and 3.7 respectively).

The ^{7}Li NMR spectrum of the resulting aryllithium (**3.38**), in the absence of TMEDA (Figure 3.6a), revealed the presence of three major (δ_{Li} 3.6, 3.5, 2.7) and several minor aggregates. Expectedly, as discussed before in Section 3.3.1.1, upon the addition of TMEDA to the organolithium **3.38**, the emergence of one major signal (δ_{Li} 2.9) was observed suggesting the presence of a single new dominant aggregate, in conjunction with a significant reduction in the number of minor aggregates present (Figure 3.6b).



3.38

In an effort to investigate the effect of changes in temperature on lithium aggregation, we initiated a dynamic NMR investigation of the TMEDA/organolithium **3.38** complex, and the results of this study are depicted in Figure 3.8. The ^{7}Li NMR spectrum at around -80 °C clearly shows the presence of one dominant and a few minor aggregates. As the temperature of both the probe and sample was increased, the ^{7}Li signals (Figure 3.8) began to merge until

they finally coalesced at about -15 °C, therefore suggesting that they exist in various dynamic equilibria with one another.[208] Anisole, **3.15**, has been shown to form a plethora of lithium aggregates in the presence of butyllithium, ether and various equivalents of TMEDA.[209] The NMR evidence described above suggests that a solution of **3.1**, butyllithium and TMEDA aggregates in a similar fashion.

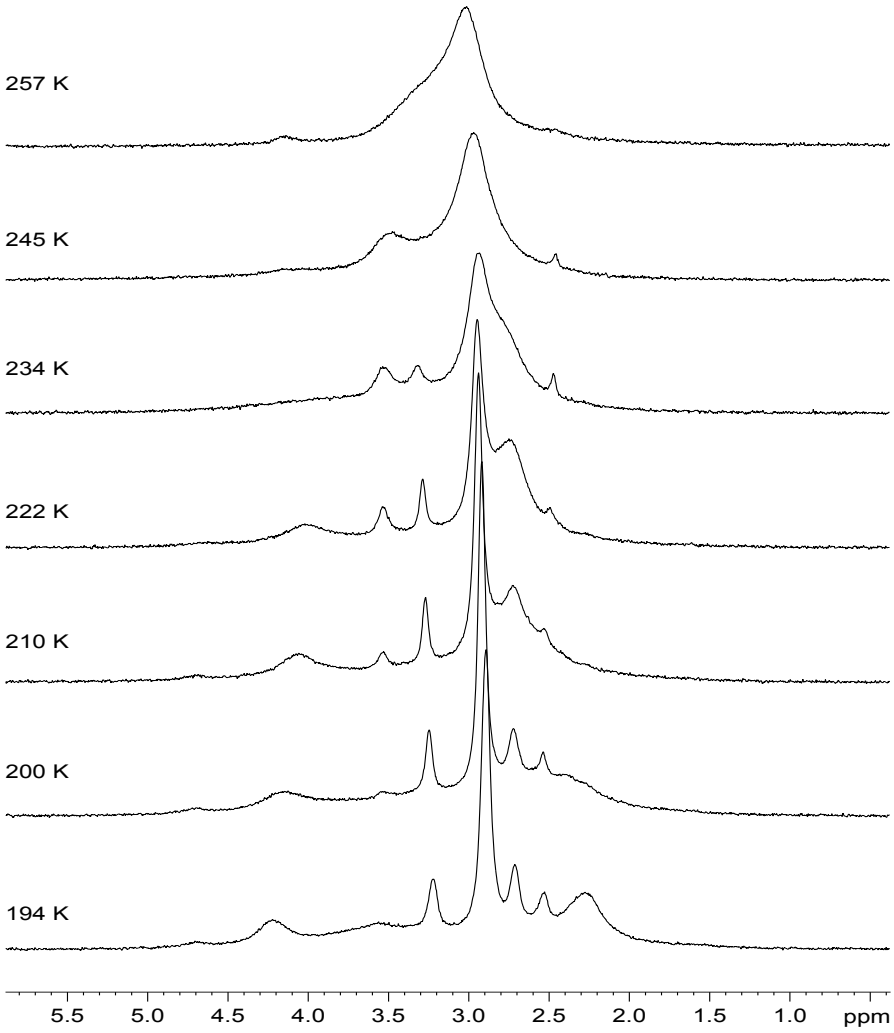


Figure 3.8 ^{7}Li NMR study of **3.38** in TMEDA (155.5 MHz, δ_{Li} 0 – 5.5, LiBr) showing the dynamic relationship between several less populated aggregates and the sustained dominance of a single species.

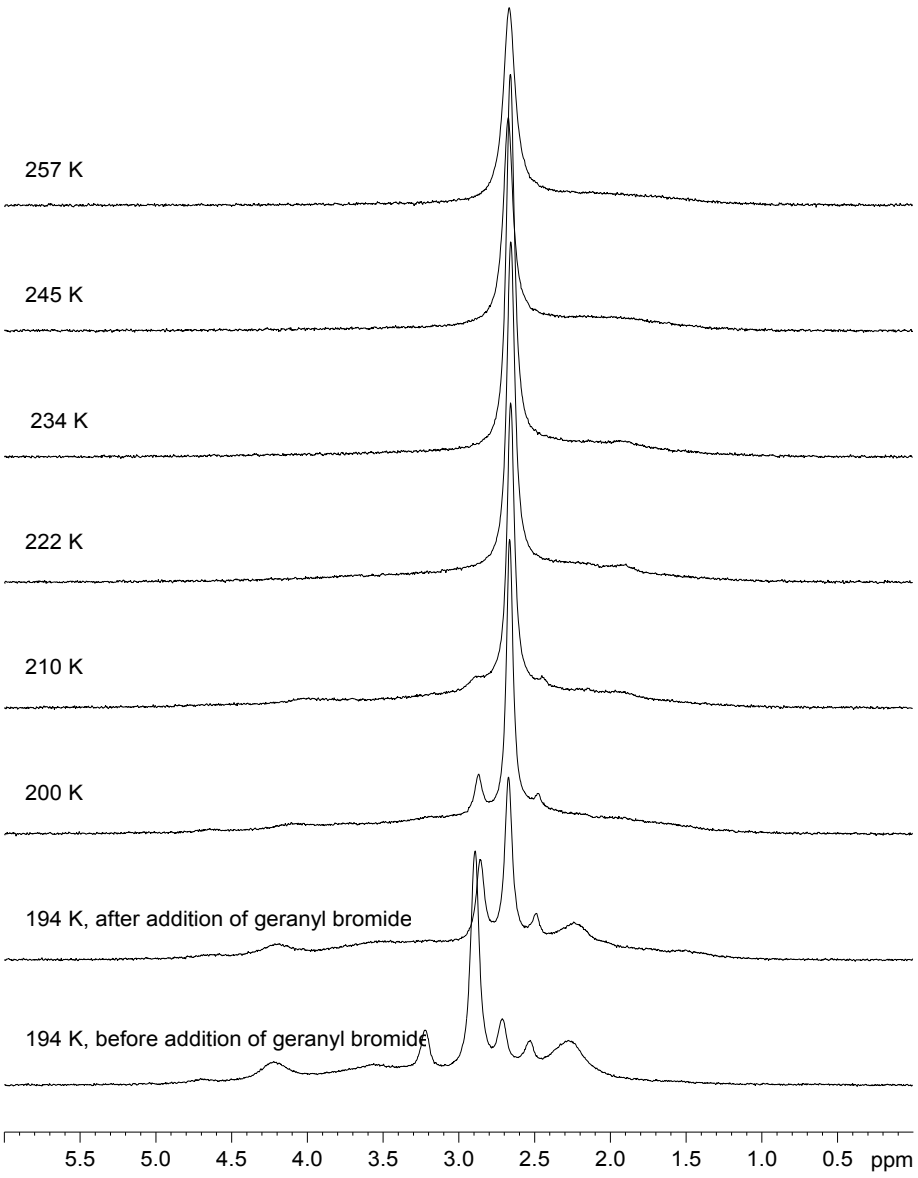


Figure 3.9 Variable temperature NMR study of **3.38** in TMEDA following the addition of geranyl bromide (155.5 MHz, δ_{Li} 0 – 5.5, LiBr).

Interestingly, upon the addition of geranyl bromide to the solution of **3.38**, TMEDA and the mixed aggregates in ether at about -80 °C, the immediate emergence of a new signal (δ_{Li} 2.7) in the ^{7}Li NMR spectrum (Figure 3.9), followed by the mild decrease in intensity of the major aggregate and the loss of resonances attributed to minor aggregates. A steady increase in the temperature from -80 °C to -15 °C induced an upfield shift of the broad signal at δ_{Li} 4.3

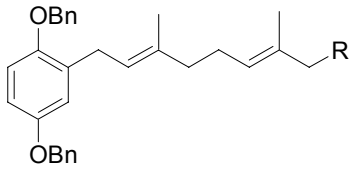
until its eventual disappearance. At about -50 °C the major aggregate (δ_{Li} 2.9), formed prior to the addition of geranyl bromide, was totally consumed, along with all the minor aggregates present, leaving only one predominant signal in the ^{7}Li NMR spectrum (Figure 3.9). Regrettably, we were unable to establish if these changes in the ^{7}Li NMR spectrum were as a result of the temperature increase or rather a direct consequence of the coupling of geranyl bromide with the aryllithium **3.38**.

During the course of our detailed ^{7}Li NMR investigation, we were still unsuccessful at synthesizing the desired product **3.2** in reproducible and satisfactory yields. We therefore examined the possibility that adventitious water might be affecting the outcome of the MHE reaction, despite all our efforts to work under strictly anhydrous conditions. Additionally, we titrated the solution of *n*-BuLi against a solution of diphenylacetic acid in tetrahydrofuran, using the method outlined by Kofron and Baclawski,[210] to assay the actual lithium concentration to ensure that the required stoichiometric amounts of *n*-BuLi were being added to the reaction. The first approach to promoting a rigorously anhydrous environment for the prenylation reaction was to ensure the purity and dryness of the aromatic bromide **3.1**. Therefore, **3.1** was purified by sublimation at 65 °C *in vacuo* (*ca.* 7.5 x 10^{-4} mmHg) and subsequently stored under inert and anhydrous conditions until required. Frustratingly, sublimation of **3.1** neither improved the yields nor the reliability of the MHE reaction and therefore led us to assume that the possible presence of water in the MHE reaction, especially when working in such small quantities (mmol), was hydrolyzing the *n*-BuLi prior to the reaction. In order to circumvent the hydrolytic effect of water in the MHE reaction, we decided to add an excess of *n*-BuLi to the anhydrous diethyl ether solvent, prior to the addition of the bromide **3.1**, in order to 'mop up' any traces of water present. This decision therefore entailed a reversal in the order of addition of the reagents where *n*-BuLi, followed by TMEDA, was added to diethyl ether at -10 °C followed by the dropwise addition of a solution of **3.1** in ether. We rationalized that the absence of water in the reaction would lessen the likelihood of the aryllithium **3.38** being protonated. When this new method was monitored by

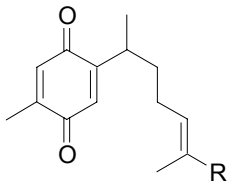
^{7}Li NMR (Figure 3.6c), it was evident from the spectrum that most of the minor aggregates had been eliminated, providing us with a clear indication that this revised method of reagent addition limits the number and nature of organolithium species in solution, thus diminishing the chances of side reactions. Gratifyingly, this revised addition method not only increased the isolated yield (65%) of this problematic MHE reaction but has also led to the elimination of the alternative coupled product (**3.36/3.37**). To our satisfaction, this ^{7}Li NMR study provided valuable insight into this previously problematic MHE prenylation, thus enabling our successful improvement of the procedure to achieve higher yields and guaranteed reproducibility, previously unattainable in our laboratory.[155;157]

3.5 Modified Sharpless oxidation of 3.2

With a reproducible synthesis of **3.2** now in hand, we turned our attention to the selective selenium diodxide allylic oxidation of **3.2** as the second step (Scheme 3.1) in our attempted synthesis of the marine natural product **1.26**. The modified Sharpless oxidation[146] is a well-established procedure in the literature[145;211-218] for the stereospecific allylic oxidation of *gem*-dimethyl olefins, *e.g.* the oxidation of (**3.39**) to (**3.40**, 48%),[218] and (**3.41**) to (**3.42**, 41%).[217] However, the major drawback of this reaction is the unavoidable production of reduced selenium waste, in a colloidal form, and organoselenium byproducts with problematic waste disposal requirements,[146] thus posing environmental and safety concerns.[219] Umbreit and Sharpless[146] have circumvented the waste issues associated with this reaction with their carboxylic acid-catalyzed catalytic selenium dioxide oxidation protocol, using an alkyl hydroperoxide as an oxidant (Scheme 3.9).

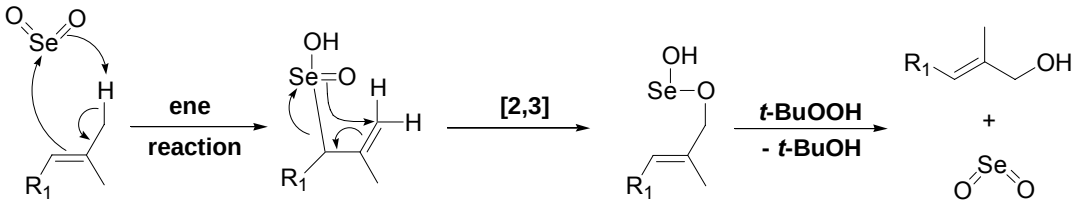


3.39 R = H
3.40 R = OH (48%)



3.41 R = CH_3
3.42 R = CHO (41%)

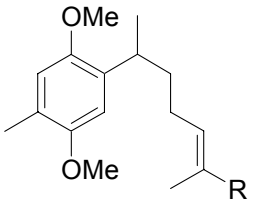
The proposed mechanism of this selective selenium dioxide allylic oxidation is summarized in Scheme 3.9.[219-221] Generally, it is believed that this reaction catalyzed with *tert*-butyl hydroperoxide (*t*-BuOOH or TBHP), as the oxidant, involves an initial cycloaddition or "ene reaction" of SeO_2 and the olefin, followed by a [2,3] sigmatropic rearrangement with the resulting Se (II) being reoxidized by the TBHP (Scheme 3.9).[219-222] The stereoselectivity of this reaction seems to be determined in the intial step of this reaction mechanism and is assumed to arise due to steric factors around the olefinic bond which favours the attack of the *E*-methyl group.[158] The stereochemistry is, however, lost after the initial ene reaction but is then restored during the [2,3]-sigmatropic rearrangement step that follows (Scheme 3.9).[158]



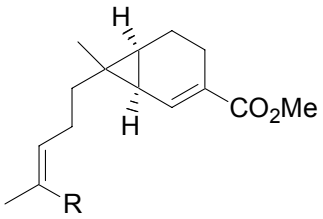
Scheme 3.9 The general mechanism of the acid-catalyzed Modified Sharpless oxidation procedure.[219-221]

We applied the modified Sharpless oxidation to our prenylated compound **3.2**. A solution of **3.2** in anhydrous dichloromethane at -20 °C was accordingly added dropwise to a stirred suspension of catalytic amounts of SeO_2, salicylic acid and TBHP in anhydrous dichloromethane at -20 °C. The solution was stirred at this temperature for 4 h and then allowed to gradually warm to room temperature and stirred overnight. Analysis of both the ^{1}H and ^{13}C NMR data, after purification by normal phase semi-preparative HPLC, indicated no

concurrent loss in methyl signals and appearance of an oxymethylene signal, thus suggesting that the reaction was unsuccessful, as were our subsequent attempts at accessing the allylic alcohol **3.3** using the reagents we had at hand. We had anticipated that this crucial step could be problematic, given the variability in yields (15-90%) reported in the literature.[145;211-217] Fairlamb *et al*.[213] investigated the effect of different protecting groups, *e.g.* $OCOCH_3$, SO_2Ph, SO_2Me, on the allylic oxidation of various geranyl analogues, and concluded that the outcome of the modified Sharpless reaction does in fact depend on the nature of the protecting group, which would therefore account for some of the low yields reported in the literature. Cognizant of Fairlamb's findings,[213] we were, however, certain that the methyl ether protecting groups on **3.3** were not responsible for the ineffective allylic oxidation, given that Vyvyan *et al*.[217] have recently reported the preparation of the aldehyde (**3.43**, 55%) from the similar 1,4-dimethoxybenzoquinone (**3.44**).

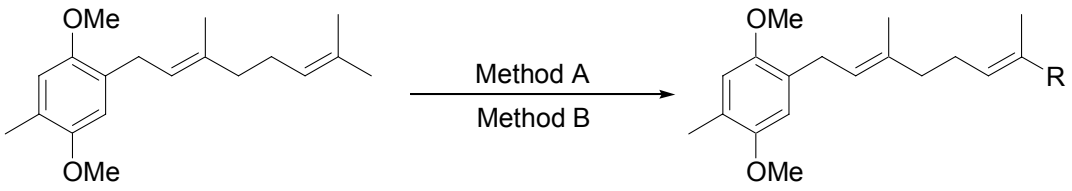


3.43 R = CHO (55%)
3.44 R = Me



3.45 R = CH_2OH
3.46 R = CHO
3.47 R = Me

Rapoport and Bhalerao[215] have previously reported the synthesis of allylic alcohols and aldehydes by SeO_2 oxidation in ethanol, *e.g.* (**3.45**) and (**3.46**) from (**3.47**), while, as mentioned above, the preparation of the aldehyde **3.43** from **3.44** was achieved using the same procedure. Frustratingly, our attempts to synthesize **3.3** and/or the aldehyde (**3.48**), using Rapoport and Bhalerao's method,[215] by refluxing a solution of **3.2** and SeO_2 (0.5-1.0 eq) in 95% ethanol gave us a complex mixture of products without any evidence, *i.e.* the disappearance of the methyl carbon resonance (δ_C 25.7) in the ^{13}C NMR spectrum, of the formation of the expected alcohol **3.3** and/or the aldehyde **3.48** (Scheme 3.10).

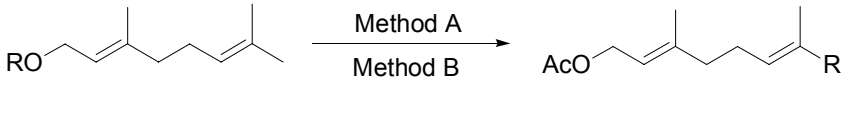


3.2

3.3 R = CH_2OH
3.48 R = CHO

Scheme 3.10 Selenium dioxide oxidation of **3.2**. *Reagents and conditions*: Method A.[146] a) SeO_2, *t*-BuOOH, CH_2Cl_2, 0 °C; b) $NaBH_4$, EtOH, 0 °C. Method B.[215] SeO_2, EtOH, reflux.

With these results in hand, we realised the need to perform these reactions on a model system. A search of the literature, revealed numerous articles pertaining to the selective allylic oxidation of geranyl acetate (**3.49**) using both the method introduced by Sharpless[146;212;213;216;223] and Bhalerao's protocol[215;224-226] (Scheme 3.11).



3.50 R = H

3.49 R = Ac

3.51 R = CHO
3.52 R = CH_2OH

Scheme 3.11 Selenium dioxide oxidation of **3.49**. *Reagents and conditions*: Method A.[146] a) SeO_2, *t*-BuOOH, CH_2Cl_2, 0 °C; b) $NaBH_4$, EtOH, 0°C; Method B.[215] SeO_2, EtOH, reflux.

Our model study therefore began with the standard acetylation of the commercially available geraniol (**3.50**), with acetic anhydride, DMAP and pyridine at room temperature to yield **3.49** (80%). The relative yields of allylic oxidation from both the Bhalerao and Sharpless procedures were investigated using purified geranyl acetate **3.49** as the model system (Scheme 3.11). We were successful in obtaining the aldehyde (**3.51**) using the former procedure,[215] whilst, surprisingly, the latter oxidation[146] failed to provide the anticipated alcohol (**3.52**).

Following method B for the formation of the aldehyde **3.51**, a solution of **3.49** and SeO_2 (1.5 eq) in 95% ethanol was refluxed for 1 h and the formation of the product **3.51** was monitored by analytical TLC until the complete consumption of the starting material **3.49** was observed. After standard workup, a preliminary examination of the ^{1}H (Figure 3.10) and ^{13}C NMR spectra of the crude reaction mixture revealed the disappearance of a methyl signal (δ_H 1.64) and the appearance of an aldehydic proton signal (δ_H 9.37) and its corresponding carbon resonance (δ_C 195.1) suggesting the sucessful transformation of **3.49** to **3.51**. Normal phase semi-preparative HPLC (67% ethyl acetate, 33% hexane) of the reaction mixture afforded the aldehyde **3.51** (45% yield). The ^{1}H NMR data (Figure 3.10) obtained for **3.51** was consistent with literature values,[226;227] and no further characterization for this compound was deemed necessary.

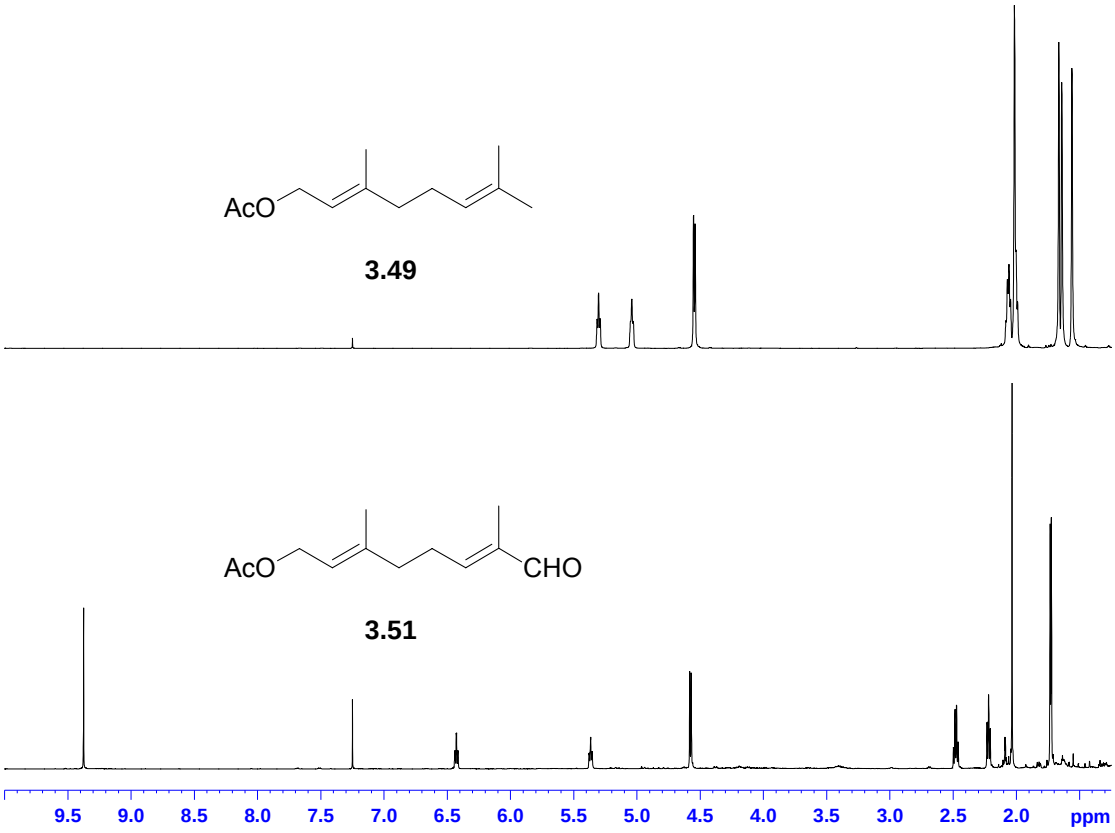


Figure 3.10 ^{1}H spectra (600 MHz, $CDCl_3$) showing the conversion of **3.49** (top) to **3.51** (bottom).

The capricious nature of this crucial allylic oxidation step in our synthesis required a closer examination of the reagents utilized, *e.g.* TBHP, in this Sharpless oxidation reaction. The instability of peroxides over time when left to stand at room temperature is well-established.[228] Iodometric titration of the 70% TBHP reagent bottle (Batch no. 5979MJ) supplied by Sigma-Aldrich revealed that the actual peroxide concentration was a mere 5% (see Section 6.2.8), thus probably accounting for a lack of oxidative reactivity that was reflected by the absence of the expected product. A new bottle of TBHP (from a different batch) was acquired from the same supplier (three weeks for delivery from Sigma-Aldrich, Germany) and the peroxide concentration was similarly assayed *vide supra*, and found to be a modest 20%. Cognizant of this, we altered the number of equivalents of TBHP accordingly when we attempted the modified Sharpless oxidation procedure, as described before, on the model system **3.49** and obtained *ca*. 60% conversion (determined by NMR, Figures 3.11 and 3.12) to the alcohol **3.52**. Analysis of the ^{1}H and ^{13}C spectra (Figures 3.11 and 3.12) of the reaction mixture, after workup, revealed the appearance of the characteristic oxymethylene signal (δ_H 3.97 and δ_C 68.8) and the appearance of a methyl proton signal (δ_H 1.64) and its corresponding carbon resonance (δ_C 13.6), which are consistent with reported values.[223;227]

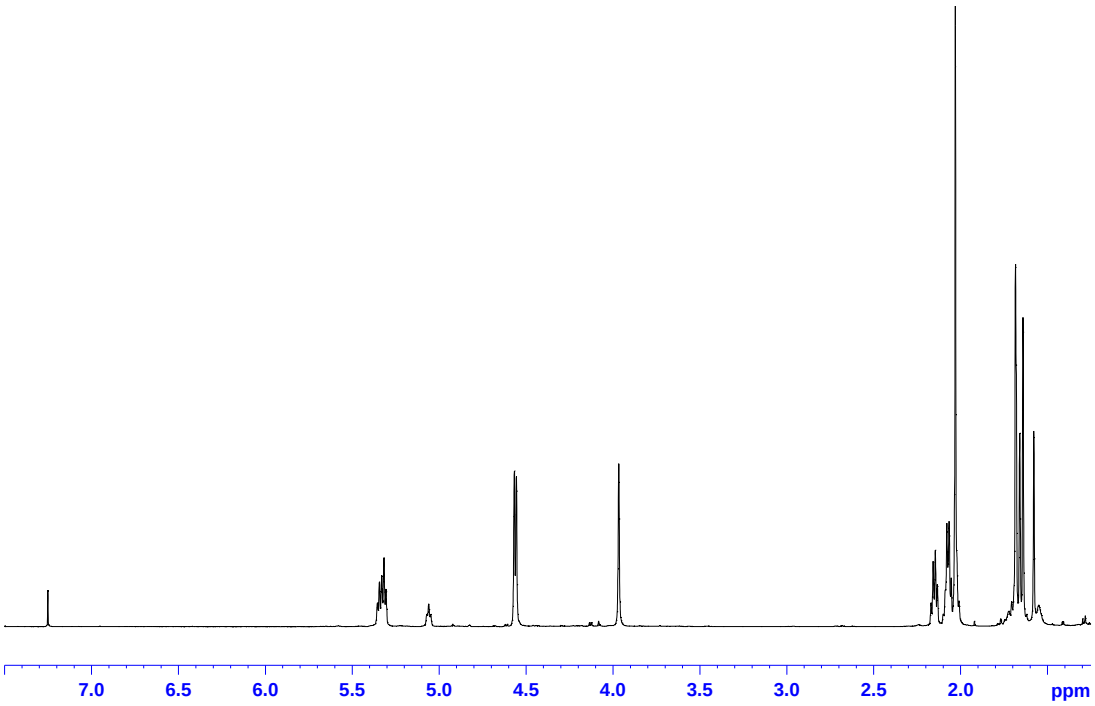


Figure 3.11 ^{1}H spectrum (600 MHz, $CDCl_3$) of a mixture **3.49** and **3.52**.

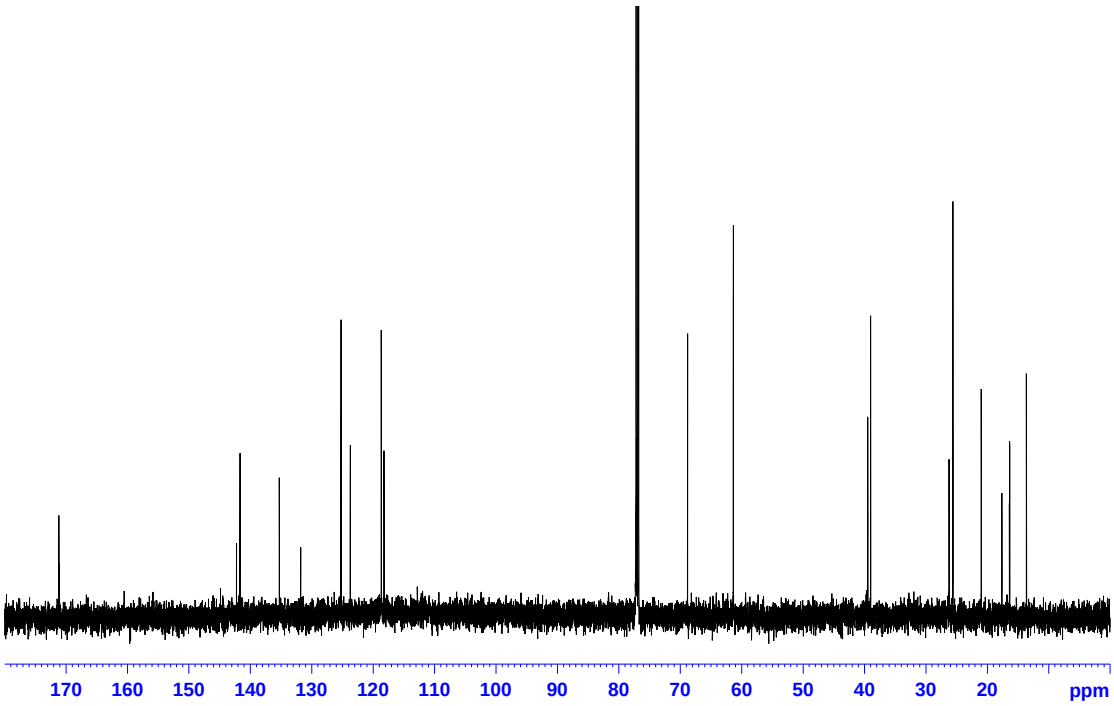
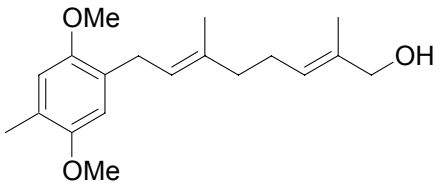
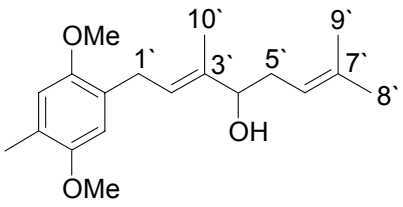


Figure 3.12 ^{13}C spectrum (150 MHz, $CDCl_3$) of a mixture **3.49** and **3.52**.

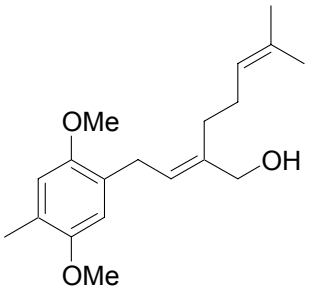
Encouraged by the success of the model reactions performed on geranyl acetate **3.49**, we returned to the preparation of **3.3**. Accordingly, we successfully synthesized the alcochol **3.3** using the same modified Sharpless oxidation procedure applied to the model compound **3.49** (Scheme 3.10). Chromatographic separation of the crude reaction mixture using normal phase semi-preparative HPLC (33% ethyl acetate, 67% hexane) afforded four fractions (A – D, Figure 3.13). A cursory examination of the ^{1}H and ^{13}C NMR spectra of all four fractions, suggested that each fraction contained a pure compound. HREIMS analysis of the more polar fractions B – D (Figure 3.13) established the molecular formula of each compound ($C_{19}H_{28}O_3$) thus suggesting three different monohydroxylated isomers, whilst the ^{1}H and ^{13}C NMR spectra of fraction A indicated the presence of unreacted starting material **3.2** (~60%). From a careful analysis of both 1D and 2D NMR data of the three isomers, we identified the desired product **3.3** (16%) and the two regioisomers as (**3.53**, 20%) and (**3.54**, 4%).



3.3



3.53



3.54

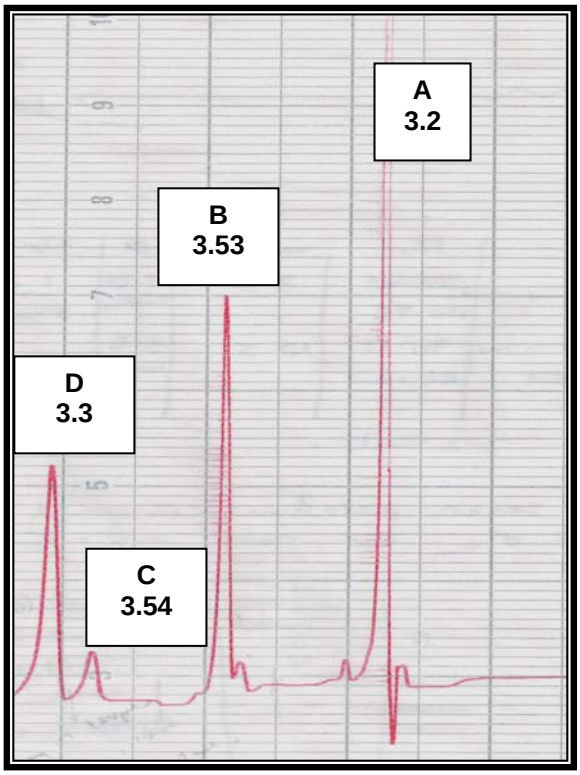


Figure 3.13 HPLC trace showing the different fractions obtained from the SeO_2 oxidation of **3.2** and the structures of the various products obtained.

Preliminary analysis of the ^1H NMR spectrum of the major product **3.53** indicated no loss of the methyl signals H_3-8`, $H_3$-9` and H_3-10` (δ_H 1.70, 1.63, 1.73) and the emergence of a downfield proton signal (δ_H 4.02) characteristic of an oxygenated methine proton. The ^{13}C NMR and DEPT 135 spectra of **3.53** revealed the presence of only two of three expected methylene carbons (δ_C 28.0, 34.2) and the appearance of an oxymethine resonance (δ_C 77.2) suggesting oxidation within the prenylated side chain as opposed to oxidation of an allylic methyl group. gCOSY correlation between H_2-5` (δ_H 2.22, 2.31) and the oxymethine signal

(δ_H 4.02) suggested their vicinal proximity to each other, while the gHMBC correlations from H_3-10` ($\delta_H$ 1.73), $H_2$-5` (δ_H 2.22, 2.31), H_2-1` ($\delta_H$ 3.33), H-2` (δ_H 5.56) to C-4` ($\delta_C$ 77.2) secured the position of a hydroxyl functionality at C-4`and hence confirmed the structure of **3.53**. The formation of **3.53** is consistent with the relative order of reactivity (CH_2 > CH_3 > CH) proposed for allylic alkyl groups,[215;222] as well as previous evidence of similar oxdiation of an allylic methylene in the Sharpless oxidation reaction.[223;227;229-231]

The disappearance of the methyl carbon resonance in the ^{13}C NMR spectrum of **3.3** (δ_C 25.7), coupled with the appearance of a deshielded oxymethylene singlet (δ_H 3.96) in the ^{1}H NMR spectrum suggested that **3.3** was our target compound. Further evidence in the form of NOESY data (Figure 3.14) indicated NOE correlations between the olefinic methine H-6` ($\delta_H$ 5.38) and the oxymethylene signal ($\delta_H$ 3.96) and between the methylene H-5` (δ_H 2.15) and the methyl H-9` (δ_H 1.65) thus confirming the *E* stereochemistry of the $\Delta^{6,7}$ olefin of **3.3**.

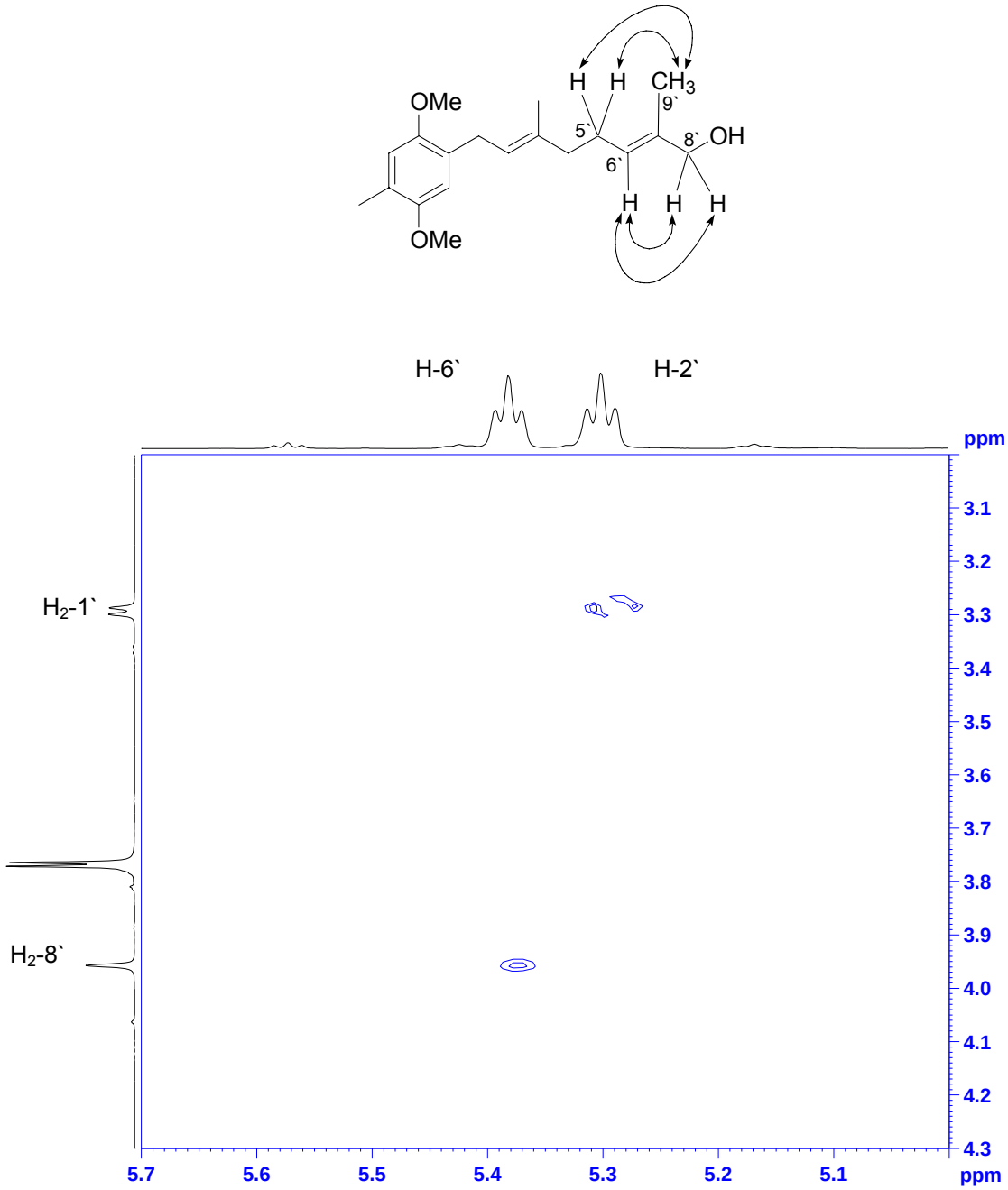


Figure 3.14 An upfield section (F1 = δ_H 3.0 – 4.3, F2 = δ_H 5.0 – 5.7) of the NOESY spectrum (600 MHz, $CDCl_3$) of **3.3**.

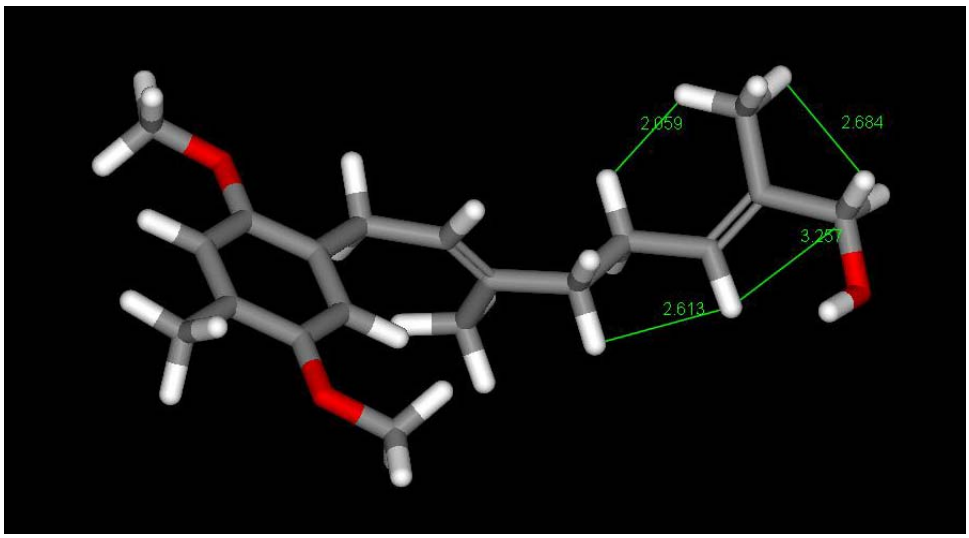


Figure 3.15 Stick molecular model representation of **3.3** showing the distances between hydrogen atoms around the C-6` and C-7` double bond. All DFT calculations (energy optimizations and frequency analyses) were performed using the Gaussian 03[232] suite of algorithms with the 6-31G(d) vector basis set and the B3LYP[233;234] energy gradient correcting functional. Models were constructed and visualized using DS Visualizer.[234]

The structure of the minor isomer **3.54** was not immediately apparent after a cursory examination of the NMR data, but upon closer inspection of both 1D and 2D NMR data, we were able to assign the structure as **3.54**. Our first clue as to positioning the hydroxyl at C-10` was the disappearance of the methyl signal ($H_3$-10`, δ_H 1.71) and the continued presence of terminal methyl carbon resonances (δ_C 25.7, 17.7) for C-8` and C-9` respectively. This was further corroborated by long-range gCOSY correlations between the oxymethylene H_2-10` ($\delta_H$ 4.06), the benzylic methylene $H_2$-1` (δ_H 3.36) and olefinic methine H-2` ($\delta_H$ 5.57) protons, whilst the long range gCOSY correlations between the allylic methyls $H_3$-8` and H_3-9` ($\delta_H$ 1.68, 1.61) confirmed the integrity of the terminal prenyl group (Figure 3.16). A four bond gHMBC correlation from $H_2$-10` to benzylic C-1` ($\delta_C$ 28.2) indicated the proximity of the oxymethylene $H_2$-10` to the aromatic ring, while the three bond gHMBC correlation from H_2-10` to C-2` (δ_C 125.6) and two bond correlation to C-3` ($\delta_C$ 139.4) confirmed its position on the prenylated side chain. NOESY correlations between $H_2$-10` and H-2` ($\delta_H$ 5.57) and $H_2$-1`

and H_2-4` confirmed the *E* configuration (Figure 3.17) around $\Delta^{2,3}$ and hence the structure for **3.54**. The presence of **3.54** in the reaction mixture is unexpected and is not readily explained. It is possible that **3.54** may form from a small amount of **3.37** (Section 3.4, Figure 3.4) suspected to be present in the MHE reaction product.

Our investigation of the selenium dioxide oxidation of **3.2** and the isolation of the target compound **3.3** in low yield (16%), in addition to the two isomers **3.53** (20%) and **3.54** (4%), calls into question the regioselectivity and yield of this reaction (40%) claimed by Li *et al*.[143]

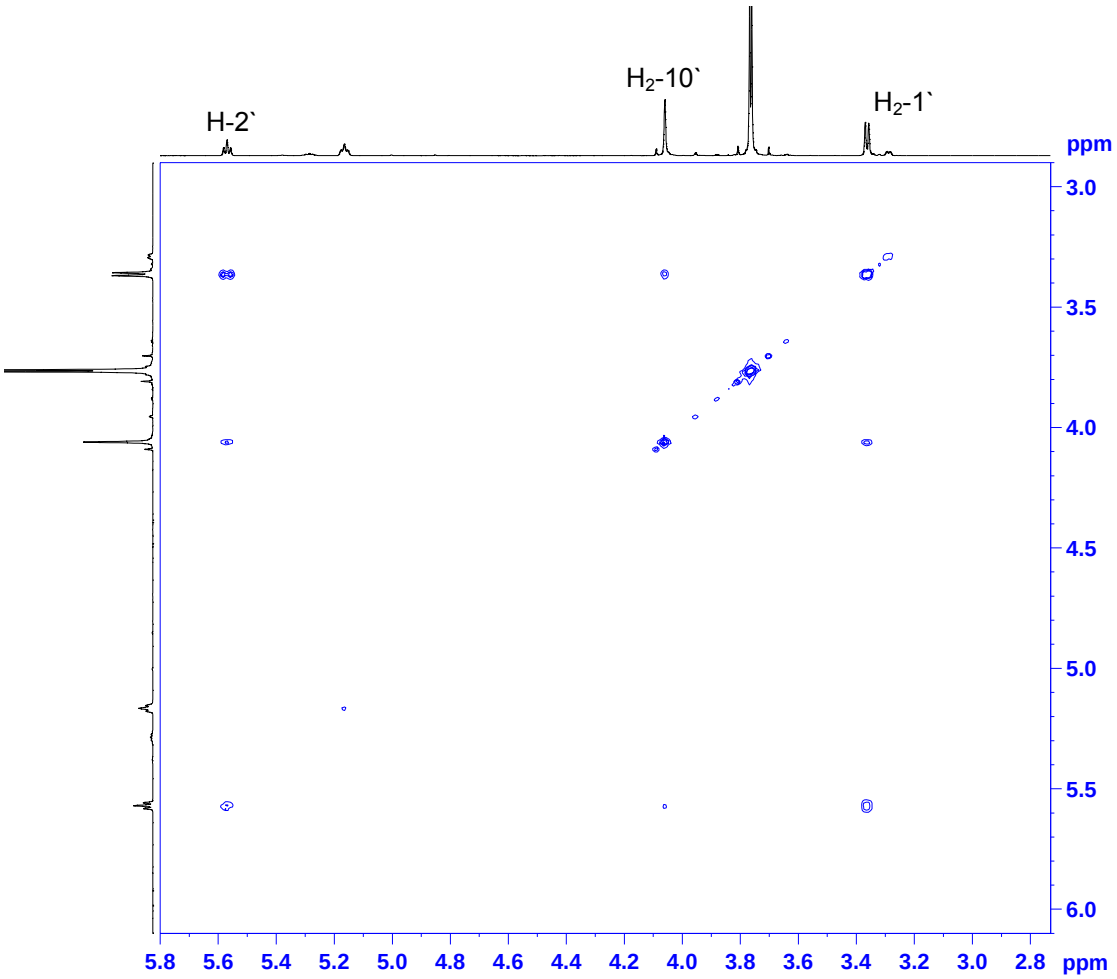


Figure 3.16 A section (F1 δ_H 2.73 – 5.8; F2 δ_H 2.90 – 6.10) of the gCOSY (600 MHz, $CDCl_3$) spectrum of **3.54**.

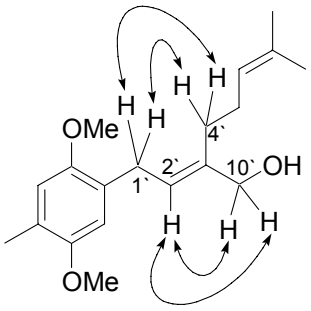
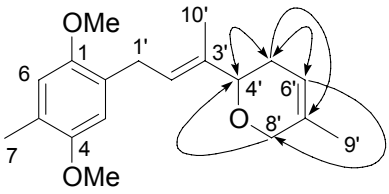


Figure 3.17 Key NOESY correlations for compound **3.54**.

Additionally, when we used a solution of 5-6M TBHP in decane for the modified Sharpless oxidation we obtained very different results. Purification of the crude reaction mixture by semi-preparative HPLC (75% ethyl acetate, 25% hexane) afforded two major fractions. Initial analysis of the ^{1}H and ^{13}C NMR data for these two fractions indicated the presence of a single compound (**3.55**, 3.5% yield) in the less polar fraction and a mixture of two closely related compounds (15.6% yield) in the more polar fraction. Several attempts to separate this latter mixture *via* semi-preparative HPLC were unsuccessful.

HRFABMS established a molecular structure of $C_{19}H_{26}O_3$ for **3.55**, implying seven degrees of unsaturation for this compound. Four of these degrees of unsaturation could be accounted for by the benzene ring and a further two by the two trisubstituted olefins (δ_C 124.3, 124.8, 137.5, 138.4). The remaining degree of unsaturation implied the presence of an additional ring system. The loss of a methyl signal (H_3-8`, δ_H 1.67) in the ^{1}H spectrum and the emergence of the oxymethine (δ_C 76.3) and oxymethylene (δ_C 61.3) carbon resonances in the ^{13}C NMR spectrum of this compound tentatively placed an oxygenated ring at the end of the prenylated side chain. Three bond gHMBC correlations between H-4` ($\delta_H$ 4.00), H-6` (δ_H 5.32), H_3-9` ($\delta_H$ 1.81) and C-8` (δ_C 61.3) confirmed both the pyran structure of the ring and its terminal position (Table 3.2, Figures 3.18 and 3.19).



3.55

Figure 3.18 Key gCOSY (double headed arrows) and gHMBC correlations (single arrows) that established the cyclic portion in **3.55**.

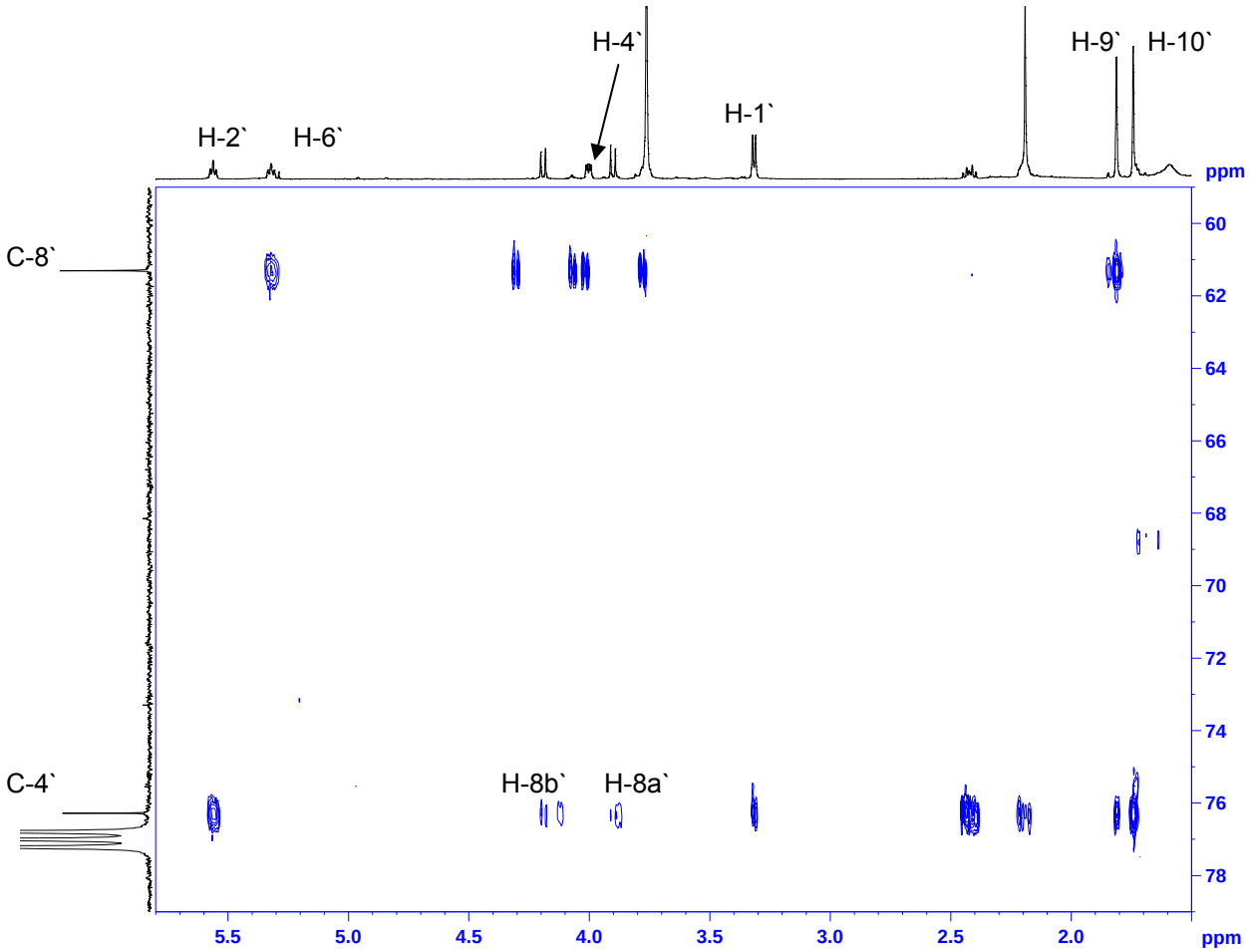
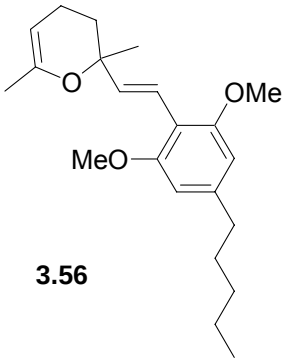


Figure 3.19 A section (F1 = δ_C 59.0 – 79.0; F2 = δ_H 1.50 – 5.80) of the HMBC spectrum (600 MHz, $CDCl_3$) of **3.55**.

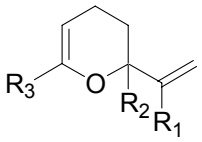
Table 3.2 ^{1}H (600 MHz), ^{13}C (150 MHz), gCOSY and gHMBC NMR data for **3.55** in $CDCl_3$.

Carbon	δ_c (mult.)	δ_H (int., mult., *J*/Hz)	gHMBC	gCOSY
1	151.0	q_c	-	-
2	127.0	q_c	-	-
3	112.6	6.62 (1H, s)	1, 2, 4, 5, 6, 7, 1'	7, 1', OMe-4
4	151.6	q_c	-	-
5	124.9	q_c	-	-
6	114.0	6.67 (1H, s)	1, 2, 3, 4, 5, 7, 1'	7, OMe-1
7	16.1	2.19 (3H, s)	1, 2, 3, 5, 6	
OMe-1	56.2	3.76 (3H, s)	1, 6	6
OMe-4	56.2	3.76 (3H, s)	3, 4	3
1'	28.2	3.32 (2H, d, 7.1)	1, 2, 3, 3'	3, 2', 5', 10'
2'	124.8	5.56 (1H, t, 7.3)	5, 1', 4', 10'	1', 10'
3'	137.5	q_c	-	-
4'	76.3	4.00 (1H, dd, 8.6, 3.7)	3', 5', 8', 10'	5'
5a'	33.7	2.42 (1H, dt, 14.3, 8.8)	4', 6', 7'	4', 6'
5b'	33.7	2.20 (1H, m)	-	4', 6'
6'	124.3	5.32 (1H, t, 7.9)	5', 8', 9'	5', 8', 9'
7'	138.4	q_c	-	-
8a'	61.3	3.90 (1H, d, 11.6)	4', 6', 7', 9'	8b
8b'	61.3	4.20 (1H, d, 11.6)	6', 7', 9'	8a
9'	22.4	1.81 (3H, s)	6', 7', 8'	5', 6'
10'	12.0	1.74 (3H, s)	2', 3', 4'	1', 2'

The structure of **3.55** is unprecedented in prenylated compounds and the mechanism leading to its formation is unclear. However, the synthetic pyran compounds (**3.56**)[235] and (**3.57**-**3.59**)[236] have been reported in the literature, unfortunately without adequate NMR spectroscopic data for comparison.



3.56



3.57 $R_1 = R_2 = R_3 = H$
3.58 $R_1 = R_3 = CH_3$; $R_2 = H$
3.59 $R_1 = R_3 = H$; $R_2 = CH_3$

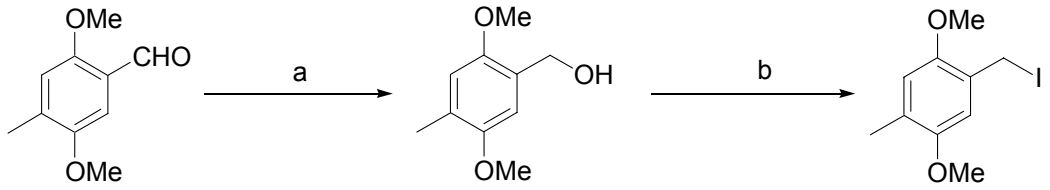
3.6 Attempted iodination of 3.3 following Li's procedure[143]

In our endeavour to synthesize the marine natural product **1.26** following Li *et al*.'s procedure, [143] we realised that we had a very limited supply of **3.3**, given the lack of regioselectivity and modest isolated yield obtained from the selenium dioxide oxidation step (Section 3.5).

Consequently, in order to avoid wasting valuable synthetic material, we used the benzylic alcohol (**3.60**) as a model compound to practice and test the iodination reaction. Firstly, the aldehyde (**3.61**, 80%) was prepared from 2,5-dimethoxytoluene **3.8** *via* a standard Vilsmeier-Haack formylation,[237] and subsequently reduced using sodium borohydride ($NaBH_4$) in ethanol to provide access to the benzylic alcohol **3.60** (80% yield), as white needles after recrystallization from hexane. The aldehyde **3.61** has previously been synthesized[238;239] but, to the best of our knowledge, no associated NMR data have been reported.

The transformation from **3.8** to **3.61** was, however, simple thus rendering the NMR spectroscopic assignment unambiguous. The appearance of the downfield signal (δ_H 10.37) in the ^1H NMR spectrum (Figure 3.20, top) corresponding to the aldehyde proton suggested the successful formation of **3.61**, while both 1D and 2D NMR spectroscopy were used to unequivocally assign the structure as **3.61**. No further characterization of **3.61** was deemed necessary.

The reduction of **3.61** to the alcohol **3.60** (Scheme 3.12) was monitored by analytical TLC until total consumption of the aldehyde was evident. Analysis of the NMR data revealed the disappearance of the aldehyde proton signal (10.37 ppm) and the emergence of an oxymethylene signal (δ_H 4.64) in the ^{1}H NMR spectrum (Figure 3.20, middle) and its corresponding carbon resonance (δ_C 62.0) suggesting the successful reduction of **3.61**. HREIMS data was used to establish the molecular formula of **3.60** ($C_{10}H_{14}O_3$) while both 1D and 2D NMR spectroscopy were used to confirm the structure of **3.60**. The ^{1}H NMR data obtained, as was the melting point (78-80 °C), were both consistent with literature values.[239]



3.61 **3.60** **3.62**

Scheme 3.12 Model reaction system for the iodination reaction. *Reagents and condition*: a) $NaBH_4$, EtOH, 0 °C, 1h; b) PPh_3, imidazole, I_2, CH_2Cl_2.

Standard iodination methodology[240-242] was applied to **3.60** (Scheme 3.12) and provided the benzylic iodide as a brown oil (**3.62**, 100% conversion by NMR). Examination of the ^{1}H NMR spectrum of the crude product (Figure 3.20, bottom) revealed the upfield shift of the methylene signal (δ_H 3.89). Accordingly, a marked upfield shift of the C-1` methylene (δ_C 29.7) was observed in the ^{13}C NMR spectrum suggesting that successful iodination had occurred. Both 1D and 2D NMR data were used to assign the structure of **3.62**. Crystallization of the crude product (from dichloromethane and hexane mixture) afforded **3.62** as colourless plates (20% yield) and HREIMS data confirmed the molecular formula $C_{10}H_{13}IO_2$. Subsequent attempts to grow more crystals from the mother liquor were unsuccessful and analysis of the ^{1}H NMR spectrum, following the attempted crystallization, indicated the appearance of numerous originally unobserved signals, suggesting that **3.62** was unstable after prolonged standing in solution and/or exposure to light.

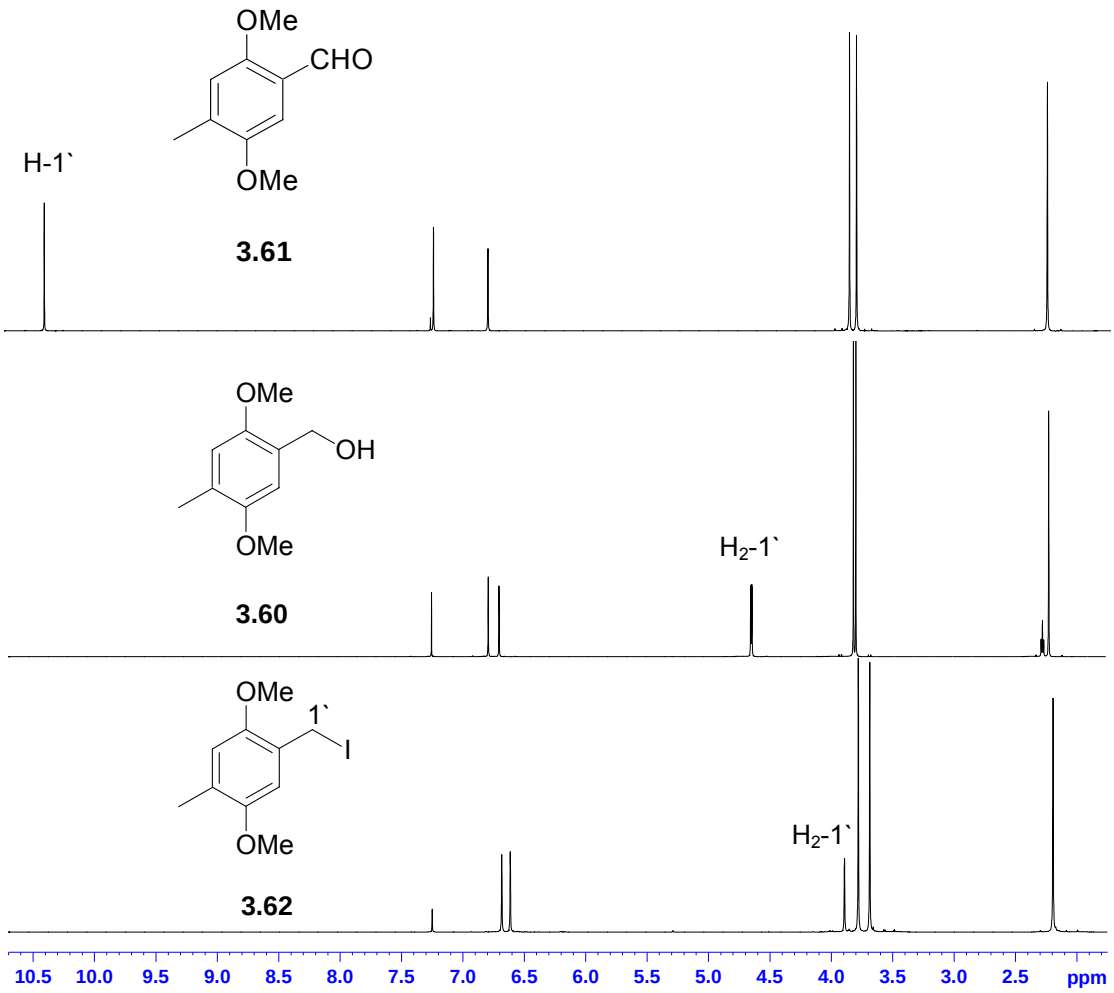
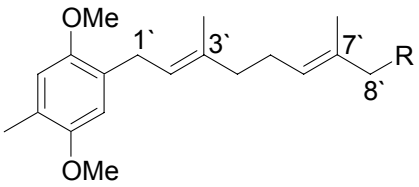


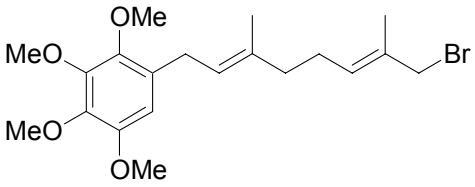
Figure 3.20 ^{1}H NMR spectra (600 MHz, $CDCl_3$) for **3.61** (top), **3.60** (middle) and **3.62** (bottom).

Encouraged by success of the model reaction performed on **3.60**, we attempted to reproduce the results obtained by Li *et al.*[143], using the same procedure outlined above.[240-242] Compound **3.3** was added to a stirred solution of triphenylphosphine, imidazole and iodine at 0 °C in anhydrous dichloromethane. The reaction was stirred at room temperature for 2 h in the dark, to avoid the possibility of photochemical decomposition, and monitored by analytical TLC, which indicated the conversion of the alcohol to a more non-polar product. This initial result seemed promising but examination of the ^{1}H and ^{13}C NMR data was inconclusive. Purification of the crude material using semi-preparative HPLC (25 % EtOAc, 75% hexane) afforded four different fractions. A cursory examination of the ^{1}H and ^{13}C NMR spectra for the

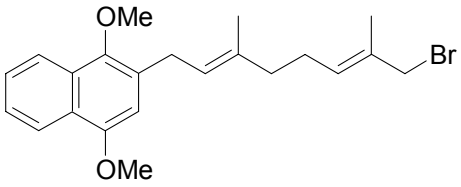
different fractions gave no clear evidence, *i.e.* no apparent upfield shift of the C-8` methylene signals that would support the formation of the compound **3.5**.



3.5 R = I
3.63 R = Br
3.64 R = Cl



3.65



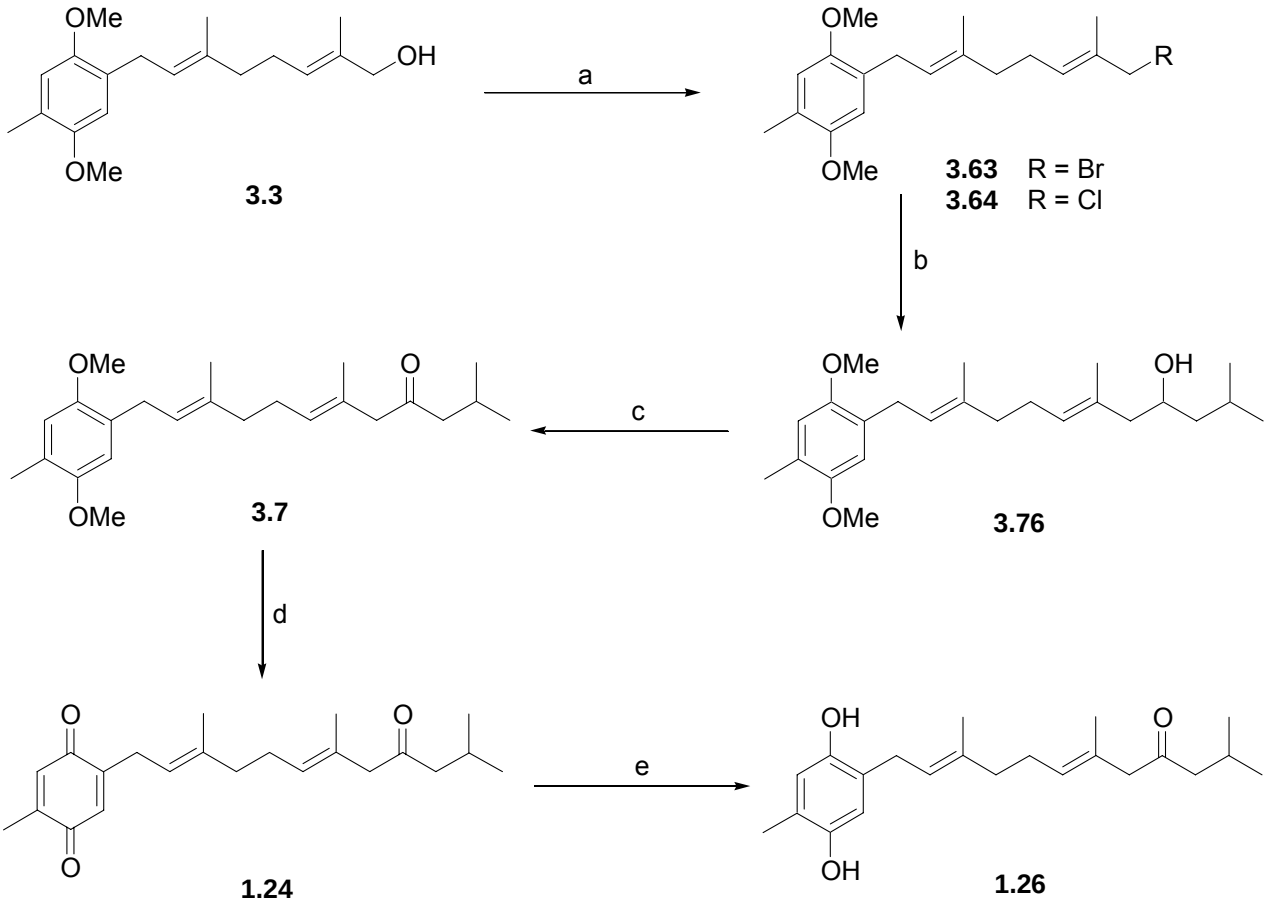
3.66

To the best of our knowledge, apart from Li *et al.*'s[143] claim to have made this compound en route to **1.26** (Scheme 3.1), compound **3.5** has not previously been reported in the literature and neither have the halogenated analogues (**3.63** and **3.64**). However, Masaki *et al.*[243] have reported the synthesis of the similar quinones (**3.65** and **3.66**) and refer to the characteristic sharp singlet signal (δ_H 8.50-8.90) in the ^{1}H NMR spectrum (60 MHz, CCl_4) for the allylic bromomethylene H_2-8`.

The failure of the iodination reaction implied that we were unable to proceed with Li *et al.*'s[143] synthesis (Scheme 3.1) and were faced with the necessity of finding an alternative synthetic route for the total synthesis of **1.26**. The synthetic protocol reported by Li *et al*.[143] claimed an overall yield of 7% from the simple precursor **3.1** in ten steps (Scheme 3.1). However, in our hands, the first three steps were all problematic while the fourth step failed to work completely.

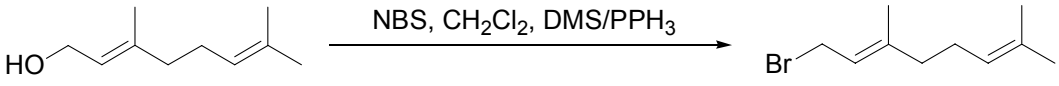
3.7 Allylic bromination of 3.3

Following our unsuccessful attempt at the iodination of the alcohol **3.3**, we envisaged an alternative approach to **1.26** *via* the bromide **3.63**, followed by its coupling to isovaleraldehyde through either a standard metal-halogen exchange (MHE) with *n*-BuLi or a Grignard procedure (Scheme 3.13). We were initially attracted by Corey's procedure[244] of selectively converting an allylic alcohol to its corresponding bromide, under mild conditions, a procedure with apparent broad applicability.[212;218;244-246]



Scheme 3.13 Proposed alternative synthesis of **1.24** and **1.26**. *Reagents and conditions*: a) NBS or NCS, DMS or PPh_3, CH_2Cl_2 b) *n*-BuLi, THF, -78 °C, isovaleraldehyde; c) NMO, TPAP, CH_2Cl_2, 3h ; d) AgO, dioxane, HCl, 2 mins; e) $Na_2S_2O_4$, $CHCl_3/Et_2O$.

We first attempted Corey's bromination procedure on the commercially available geraniol (**3.50**, Scheme 3.14), in order to conserve our limited stock of **3.3**. A solution of **3.50** in anhydrous dichloromethane was slowly added to a stirred suspension of NBS and dimethyl sulfide (DMS) in anhydrous dichloromethane at -10 °C under an atmosphere of argon. The reaction was monitored over time using gas chromatography (GC) (Table 3.3) with **3.50** and geranyl bromide (**3.67**) as standards.

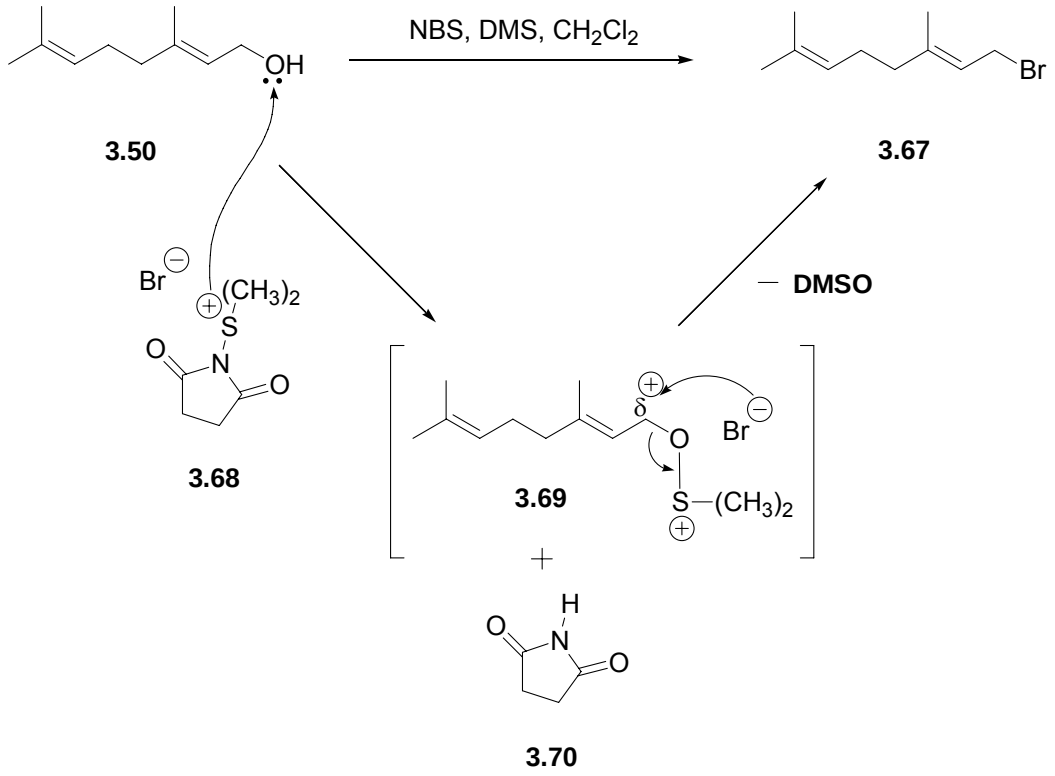


3.50 **3.67**

Scheme 3.14 Conversion of geraniol to geranyl bromide.

The DMS is believed to form a 1 : 1 complex (**3.68**) with NBS, which subsequently attacks the oxygen atom of the hydroxyl group to form the sulfoxonium intermediate (**3.69**) and succinimide (**3.70**) as a by-product (Scheme 3.15).[244] Nucleophilic attack of the carbocation of **3.69** by the negatively charged bromine atom affords the desired **3.67** and dimethylsulfoxide (DMSO) as the other by-product.[244]

According to Fairlamb,[247] allylic brominations can be capricious and, on the basis of his suggestions, we substituted DMS with triphenylphosphine (PPh_3). The results of a comparative GC study of the allylic bromination of **3.50** with both DMS and PPh_3 is presented in Table 3.3. For both methods, reactions were carried out in the dark (reaction vessels were wrapped in aluminium foil), to eliminate the possibility of photochemical side reactions, on 0.1 g (0.65 mmol) and 1.0 g (6.5 mmol) scales and aliquots of the reaction mixture were subjected to GC analysis at one hour intervals over a 4 h period (Table 3.3).



Scheme 3.15 Mechanism for allylic bromination of **3.50** adapted from Corey's method.[244]

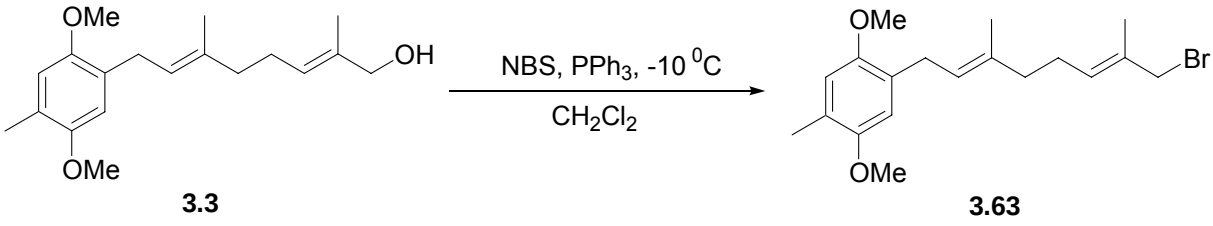
Gratifyingly, both sets of reactions appeared to achieve some level of conversion to the product and it was interesting to observe the apparent concentration effect on the course of these reactions (Table 3.3). The large scale (6.5 mmol) reaction with DMS went to completion while the smaller scale (0.65 mmol) reaction gave only a 10% conversion to the product. Conversely, the use of PPh_3 in this allylic bromination gave a 55% conversion to the product when working on small scale, and a 75% conversion with the large scale reaction (Table 3.3). This latter method therefore provided the best compromise between the two concentrations, bearing in mind our limited reserves of **3.3**.

Accordingly, we attempted the allylic bromination of the alcohol **3.3** using the method by Fairlamb (Scheme 3.16).[247] PPh_3 was added to a suspension of NBS in anhydrous dichloromethane at -10 °C and stirred for about 45 mins, followed by the dropwise addition of

a solution of **3.3** in anhydrous dichloromethane. The resulting mixture was stirred at -10 °C for 1hr and then room temperature (in the dark) for 4 h.

Table 3.3 Percentage conversion of **3.50** to **3.67** as monitored by GC.

	DMS (mmol)		PPh_3 (mmol)	
Time	0.65	6.5	0.65	6.5
After 1 h	5%	6%	33%	43%
After 2 h	8%	7%	48%	56%
After 3 h	10%	29%	55%	68%
After 4 h	10%	100%	55%	75%



3.3 **3.63**

Scheme 3.16 Allylic bromination of alcohol **3.3** using Fairlamb's method.[247]

Purification of the crude reaction mixture by flash chromatography (50% hexane : 50% dichloromethane) afforded **3.63** in a modest 33% yield. The disappearance of the oxymethylene carbon resonance (δ_C 69.0) and the emergence of a shielded halogenated methylene signal at δ_C 41.8 suggested that the bromination was successful. Subsequent acquisition of HREIMS data confirmed the molecular formula of **3.63** as $C_{19}H_{27}O_2Br$, and the isotopic bromine peaks, consistent with monobromination, were clearly evident in the low resolution mass spectrum (Figure 3.21).

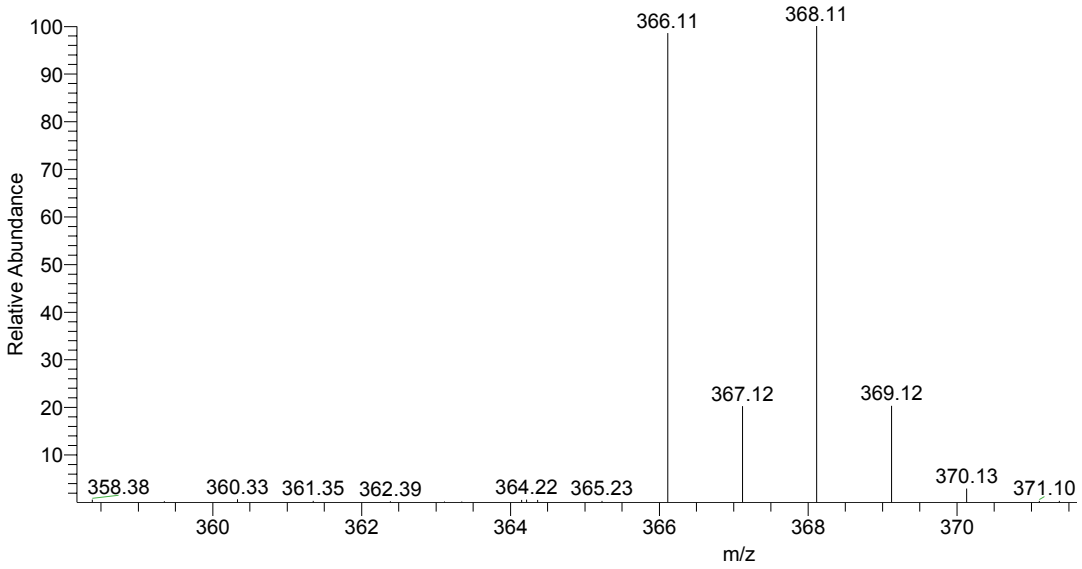
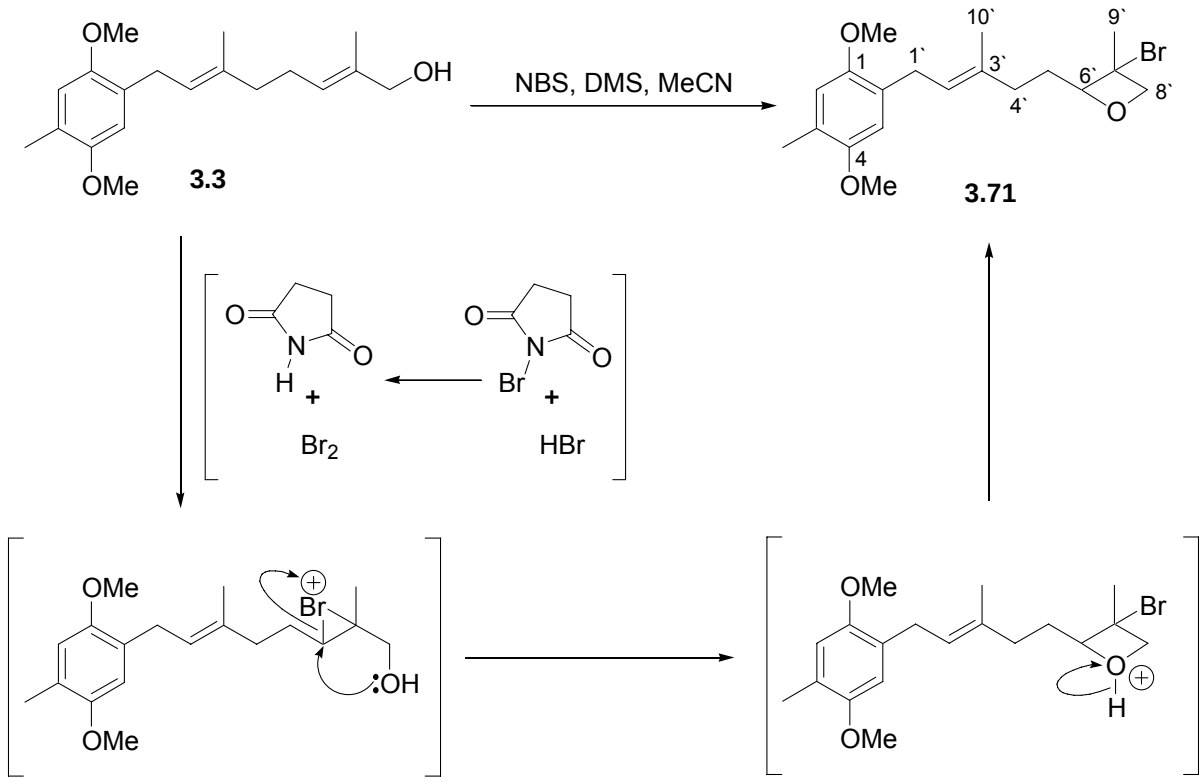


Figure 3.21 LREI mass spectrum for **3.63** indicating the presence of the isotopic bromine peaks.

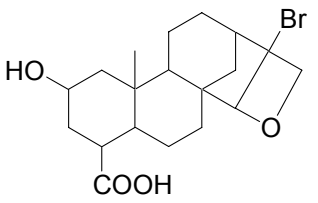
Given our previous success in improving the yields of the bromination of **3.8** by using a different solvent (Section 3.3), we attempted Corey's bromination[244] of **3.3** using acetonitrile as the solvent. Interestingly, instead of the expected product **3.63**, we isolated the totally unexpected bromo oxetane (**3.71**, 46% yield) after purification by semi-preparative HPLC (25% EtOAc, 75% hexane). The molecular formula of $C_{19}H_{27}O_3Br$ was established from HREIMS data and implied six degrees of unsaturation, of which four could be attributed to the aromatic ring, one to the single olefin from the ^{13}C NMR data (δ_C 124.8, 133.7) and the remaining double bond equivalent was accordingly assigned to a ring. Initial analysis of the ^1H NMR spectrum of **3.71** revealed the retention of only one of the olefinic protons (δ_H 5.43, H-2`) and the upfield shift of the other methine resonance ($\delta_H$ 4.40, H-6`), inidicative of an oxymethine proton (Table 3.4). The chemical shift of an oxymethylene signal (δ_C 71.9, C-8`) and both the quaternary resonance ($\delta_C$ 76.3, C-7`) and the oxymethine (δ_C 59.5, C-6`) (Table 3.4) suggested the loss of the $\Delta^6$ olefin and positioned the ring at the end of the side chain. The oxetane ring structure was established by a three bond gHMBC correlation from $H_2$-8` (δ_H 3.81, 3.96) and H_3-9` ($\delta_H$ 1.74) to C-6` (δ_C 59.5) (Figure 3.22), and possibly arises *via* an

intramolecular bromohydrin mechanism (Scheme 3.17), which is initiated by the electrophilic attack of bromine liberated from NBS in the presence of trace amounts of HBr.[158]

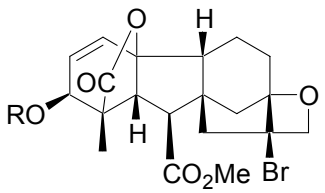


Scheme 3.17 A proposed mechanism for the formation of bromo oxetane **3.71**.

Bromo oxetanes are not unprecedented in the literature, *e.g.* compounds (**3.72**)[248] and (**3.73** and **3.74**)[249] have previously been synthesized. Interestingly, this unexpected product **3.71** was not observed when the bromination was performed in dichloromethane and may thus provide a previously unexplored route to bromo oxetanes from allylic alcohols.



3.72



3.73 R = H
3.74 R = Ac

Table 3.4 ^{1}H (600 MHz), ^{13}C (150 MHz), gCOSY and gHMBC NMR data for **3.71** in $CDCl_3$.

Carbon	δ_c (mult.)	δ_H (int., mult., *J*/Hz)	gHMBC
1	151.1	q_c	
2	127.7	q_c	
3	112.7	6.67 (1H, s)	1, 4, 2, 1`, 2`, 7`, 10`
4	151.7	q_c	
5	124.7	q_c	
6	114.0	6.67 (1H, s)	1, 4, 2, 1`, 2`, 7`, 10`
7	16.1	2.20 (3H, s)	3, 4, 5, 6
OMe-1	56.4	3.78 (1H, s)	1, 3, 4, 6
OMe-4	56.3	3.78 (1H, s)	1, 3, 4, 6
1'	28.3	3.32 (2H, d, 7.3)	1, 2, 3, 2`, 3`, 10`
2'	124.8	5.43 (1H, t, 7.3)	2, 1`, 4`, 10`
3'	133.7	q_c	
4a'	37.5	2.22 (1H, dt, 13.9, 8.0)	2`, 3`, 5`, 6`, 10`
4b'		2.39 (1H, m)	2`, 3`, 5`, 6`, 10`
5a'	32.9	1.89 (1H, dddd, 14.9, 11.6, 7.8, 4.4)	3`, 4`, 6`
5b'		2.52 (1H, dtd, 14.6, 8.3, 1.3)	3`, 4`, 7`
6'	59.5	4.4 (1H, dd, 11.3, 1.1)	4`, 5`, 7`, 8`, 9`
7'	76.3	q_c	
8a'	71.9	3.96 (1H, d, 12.5)	
8b'		3.81 (1H, d, 12.5)	6`, 7`, 9`
9'	22.1	1.74 (3H, s)	6`, 7`, 8`
10'	15.9	1.74 (3H, s)	2`, 3`, 4`

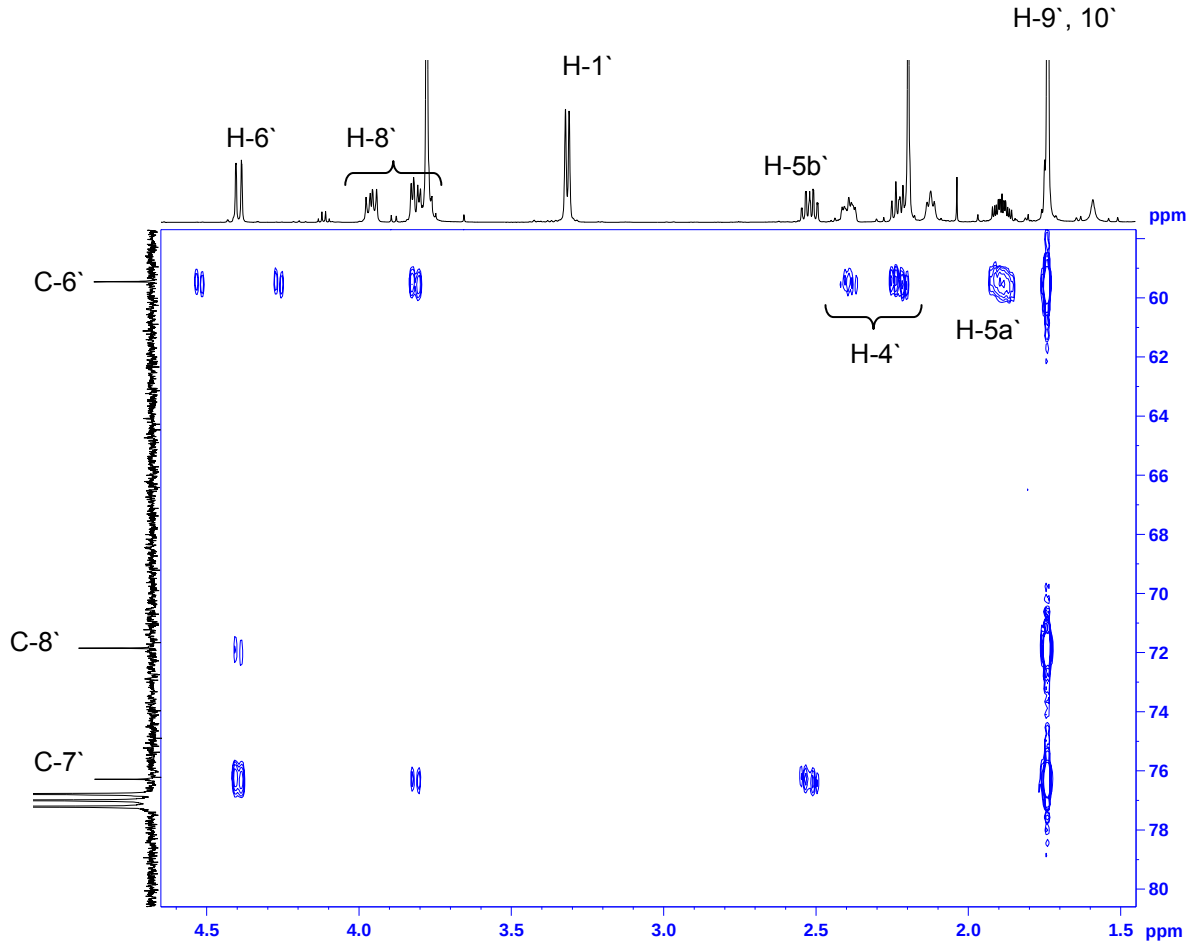


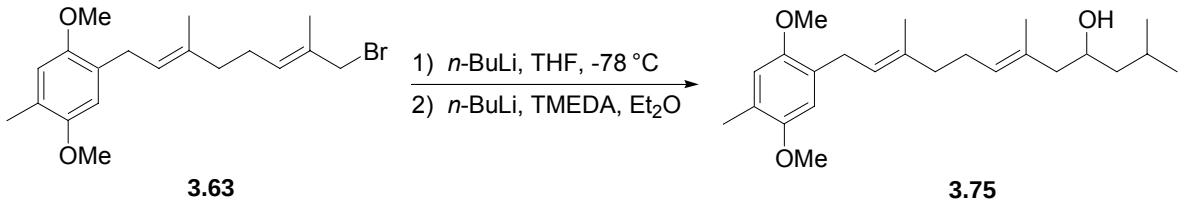
Figure 3.22 A section (F1 = δ_C 57.7 – 80.6; F2 = δ_H 1.50 – 4.60) of the HMBC spectrum (600 MHz, $CDCl_3$) of **3.71**.

3.8 Attempted addition of isovaleraldehyde to 3.63

Cognizant of the challenges faced thus far in following Li's procedure[143], we opted to divert from our initial synthetic route (Scheme 3.1), which would have entailed a typical Julia coupling[147] reaction of isovaleraldehyde to afford the hydroxy sulfone **3.6**. Instead, given our previous success using MHE methodology (Section 3.4), we rationalized a MHE reaction between the bromide **3.63** and isovaleraldehyde. This option was more appealing to our synthetic needs, as it would ideally reduce the number of steps from ten to eight in our

attempt to synthesize **1.24** and **1.26**. Additionally, this procedure would circumvent the use of highly toxic and environmentally unfriendly reagents such as the sodium-amalgam used in the desulfonization of the hydroxy sulfone **3.6** (Scheme 3.1).

A solution of the bromide **3.63** in tetrahydrofuran at -78 °C was added dropwise to a stirred solution of *n*-BuLi in tetrahydrofuran at the same temperature. The MHE process between the bromine and lithium was monitored by analytical TLC and was deemed to be complete after 5 mins and the aldehyde was added. The resulting reaction mixture was stirred at -78 °C for 3 h and then room temperature for 1 h. ^{1}H and ^{13}C NMR analysis of the crude reaction mixture revealed a complex mixture of products with, unfortunately, no clear evidence of formation of (**3.75**). Additionally, our attempt to access **3.75** using our improved MHE method (Section 3.4.1) using *n*-BuLi and TMEDA in anhydrous ether also gave us a complex mixture of products that showed no evidence of product formation (Scheme 3.18).

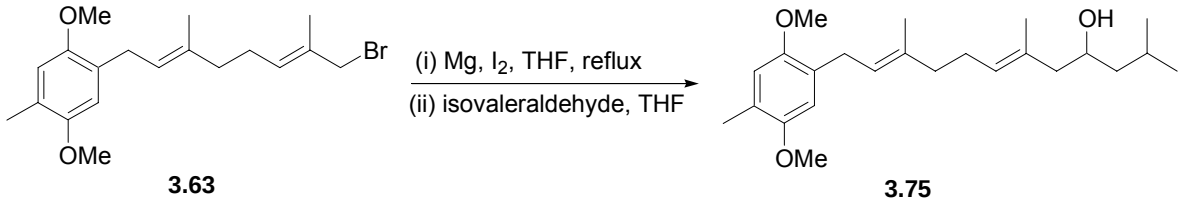


3.63 **3.75**

Scheme 3.18 Attempted MHE reaction of **3.63** with isovaleraldehyde.

Encouraged by our previous success at Grignard addition reactions (Sections 4.1.1 and 4.1.2), we attempted to synthesize **3.75** by treating a solution of the bromide **3.63** in anhydrous tetrahydrofuran with magnesium turnings and iodine in order to obtain the Grignard derivative (Scheme 3.19). After this suspension was refluxed for 1 h, the reaction was cooled on ice and added dropwise *via* cannula to a solution of the aldehyde in anhydrous tetrahydrofuran at -10 °C. The resulting reaction mixture was stirred at this temperature for 2 h and then at room temperature overnight. A complex mixture of products was observed from

the ^{1}H and ^{13}C NMR data of the crude reaction mixture with no clear evidence of the formation of **3.75**.



3.63 **3.75**

Scheme 3.19 Attempted Grignard addition of **3.63** to isovaleraldehyde.

3.9 Summary and concluding remarks

In this chapter we attempted to reproduce the total synthesis of the marine natural products **1.24** and **1.26** (Scheme 3.1) reported by Li *et al*.[143] However, in our endeavour, we were faced with significant problems at each step of the synthetic protocol, calling into question the published synthesis of Li *et al*.[143]

The Grignard synthesis of **3.2** was the first step in Li *et al*.'s[143] synthetic protocol but failed to give reproducible results in our hands. Therefore, we adopted the MHE methodology which also proved, initially, to be problematic. Problems with the MHE approach necessitated a detailed study[199] of the MHE reaction mechanism using ^{7}Li NMR spectroscopy which, consequently, significantly improved the reproducibility and the yield (65%) of this reaction. The second step of Li *et al*.'s[143] synthesis involved a modified Sharpless oxidation of **3.2**, for which we were only able to obtain a low yield (16%) for the target alcohol **3.3**. Subsequent synthesis of the iodide **3.5**, prior to the sulfonation to afford **3.4** in preparation for the Julia coupling step, proved unsuccessful and evoked the need to change our approach towards the synthesis of the marine natural product **1.26**. Accordingly, we synthesized the bromide **3.63** in modest yield (33%) *via* the allylic bromination of the alcohol **3.3**, but the subsequent

addition of **3.63** to isovaleraldehyde using both Grignard and MHE methodologies was unsuccessful. Regrettably, due to inevitable time constraints placed upon the laboratory aspect of this research, we were unable to further investigate this reaction in order to synthesize **3.75** and complete our required synthesis to obtain **1.24** and **1.26** (Scheme 3.13).

We have learnt valuable lessons in our endeavour to synthesize the marine natural product **1.26** and can therefore propose different avenues for future research. The fact that we were unable to successfully achieve the MHE of **3.63** can possibly be rectified as follows. At the time that this research was conducted, we were working with relatively small amounts of material that could explain our inability to get the MHE reaction to work. Therefore, performing the MHE reaction on a one-gram scale could be a possible solution to this problem, since it would reduce the negative impact of adventitious water on this reaction. The synthesis of **3.64** through the allylic chlorination of the alcohol **3.3** using Corey's method[244] may be a possible alternative precursor in the MHE reaction with *n*-BuLi. The successful coupling of isovaleradehyde with either halogenated compounds **3.63** or **3.64** would entail a reduction in the total number of steps towards the total synthesis of **1.26** from ten to eight (Scheme 3.13). Additionally, for the subsequent final three steps, we would then apply synthetic procedures that have been carried out in our laboratory and proved to be very effective and high yielding. These would involve the oxidation of the secondary alcohol of **3.75** using *N*-morpholine oxide (NMO) and TPAP (Scheme 3.13, step c). Oxidative demethylation of methyl benzoquinones using argentic oxide (AgO) in dioxane has granted us excellent results (90-95% yields) in the past.[155;157] Finally, reduction of prenylated quinones using sodium dithionite to afford the corresponding hydroquinones has previously given us consistent quantitative yields (90-98%).[155;157] However, failing the MHE reaction (Scheme 3.13, step b), it is also possible to revert to Li's synthetic route[143] (Scheme 3.1) by sulfonating the bromide **3.63** to yield **3.5**.[145]

Although the synthesis the marine natural product **1.26** was ultimately unsuccessful, we have identified flaws in the single published synthesis of this compound and have, concurrently,

generated five new prenylated quinone analogues **3.53**-**3.55**, **3.63** and **3.71**, which are a potentially viable addition to our ongoing structure-activity relationship (SAR) studies. In addition, we have serendipitously discovered a new possible method of converting allylic alcohols to bromo oxetanes, *e.g.* conversion of **3.3** to **3.71**, using NBS in acetonitrile.

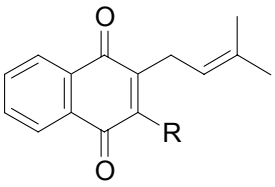
Chapter Four

Synthesis of 2-substituted-1,4-naphthoquinones

4.1 Introduction

This chapter describes a parallel component to the research described in Chapter 3 where we attempted to synthesize the marine natural products **1.24** and **1.26** as part of our ongoing collaborative research with the Division of Medical Biochemistry at the Medical School of the University of Cape Town. We describe here an exploration of the 1,4-naphthoquinone nucleus as opposed to the toluhydroquinone moiety as a primary pharmacophore in our search for new chemical entities which can induce apoptosis in oesophageal cancer cells. While our initial aim was the synthesis of the prenylated marine naphthoquinone 2-deoxylapachol (and a subsequent comparison of the oesophageal cancer cytotoxicity of this compound with the anticancer natural product lapachol), we expanded our synthetic endeavours to include the synthesis of a wide variety of simple naphthoquinone derivatives. The direction of this latter aspect of our research was dictated by the continual cytotoxic evaluation of our synthetic naphthoquinones against the oesophageal cancer cell line WHCO1.

1,4-Naphthoquinones have well-established cytotoxic properties[250-253] and have also been recently evaluated as being potential inhibitors of the heat-shock protein Hsp90,[254] which has become a novel molecular target in cancer research (Section 5.5.1).[141;255-259] The prenylated marine 1,4-naphthoquinone **2.44**, which is structurally related to the plant natural product lapachol (**4.1**), has been shown to be cytotoxic (IC_{50} of 2.7 μM) against P338 leukemia cells (Section 2.3).[58] Lapachol and a cohort of related structural analogues have been exhaustively studied over the last decade,[253;260-267] whilst to the best of our knowledge, **2.44** has not been extensively investigated as a potential antitumour agent. Cognizant of this, and the recent synthetic and biological interests directed towards **4.1** and its analogues,[58;261;268-273] we embarked on a structure-activity relationship (SAR) study using 1,4-naphthoquinones as our molecular scaffold and pharmacophore.



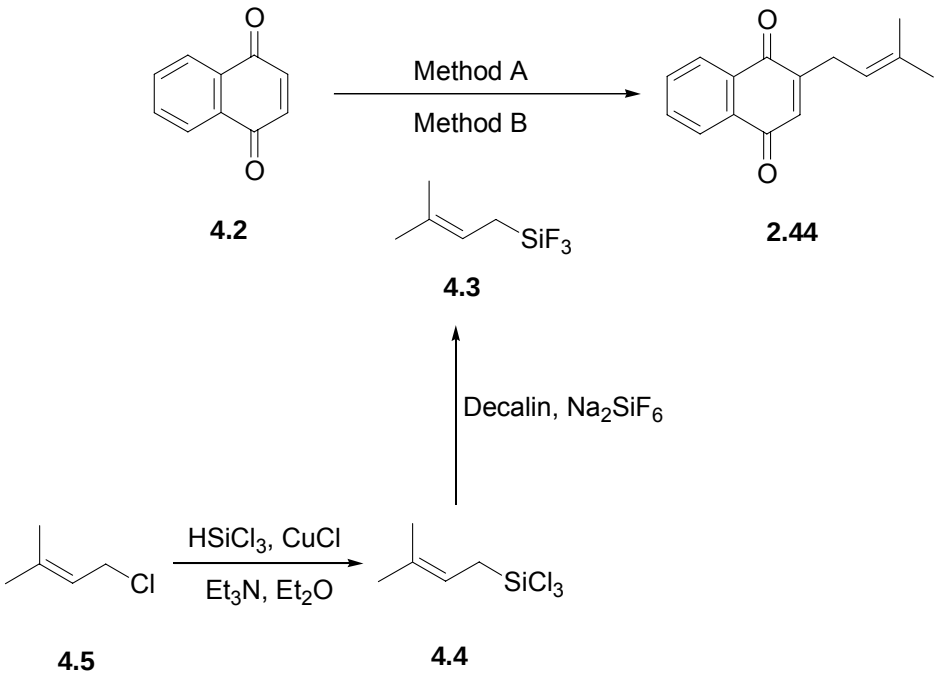
2.44 R = H
4.1 R = OH

Our synthesis of **2.44** and a plethora of synthetic analogues has, not only provided compounds more active than both **1.26** and **2.44** in the WHCO1 cell line assay, but has also provided useful data that has contributed to our ongoing SAR studies. Selected synthetic naphthoquinones arising from the syntheses described in this chapter were evaluated for their activity against the oesophageal cancer cell line WHCO1, the details of which are discussed in Section 5.4.

4.1.1 Synthesis of the marine natural product 2.44

The marine natural product **2.44** has previously been synthesized by Claessens *et a*l.[168] as a key step in their total synthesis of mollugin. The two approaches employed towards the synthesis of **2.44** from 1,4-naphthoquinone (**4.2**) are summarized in Scheme 4.1. Firstly, Claessens *et al*.[168] tried to access **2.44** *via* the radical prenylation of quinone **4.2** with 4-methyl-3-pentenoic acid (Scheme 4.1, method A),[274;275] which only gave them a low yield of 36% for **2.44**.[168] Finding this yield unacceptable, Claessens *et al*.[168] consequently obtained 2-deoxylapachol in almost quantitative yield (96%) *via* the allylation[276] of **4.2** using prenyltrifluorosilane (**4.3**) in the presence of the Lewis acid $FeCl_3.6H_2O$ (Scheme 4.1, method B).[276] We had previously envisaged the use of method B to access **2.44**, only to find out that not only the chemicals are extremely costly but the reagents used are also highly toxic, reactive and extremely sensitive to moisture and air. Nevertheless, we attempted to utilize this methodology for the synthesis of **2.44**. The reagent **4.3** was unfortunately not available

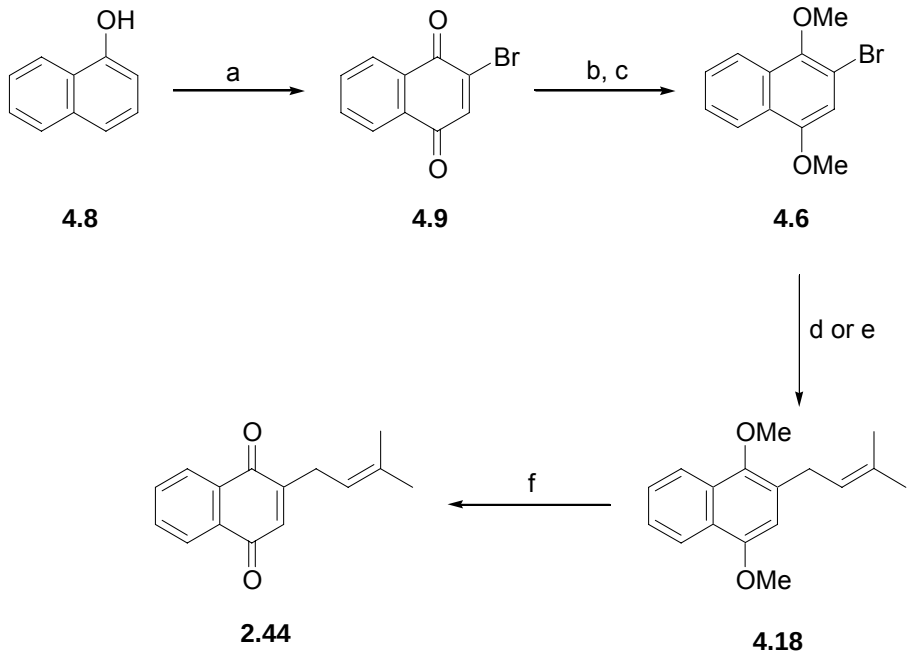
commercially, and thus its *in situ* preparation was required. Accordingly, we attempted to access **4.3** by firstly synthesizing the prenyltrichlorosilane (**4.4**) from 1-chloro-3-methyl-but-2-ene (**4.5**) and trichlorosilane in triethylamine and anhydrous diethyl ether (Scheme 4.1).[277;278] Several attempts to prepare **4.4** from **4.5** failed to give the desired product. Analysis of both ^{1}H and ^{13}C NMR spectra indicated a complex mixture of products that was not investigated further. Upon closer inspection of the synthetic protocol for method B employed by Claessens *et al.*[168], we noticed that the high yield of **2.44** reported by Claessens *et al.*[168] was only obtained when the reaction was performed on a small scale (100 mg of **4.2**) using method B to afford 140 mg of **2.44**, compared to 7.12 g using method A.



Scheme 4.1 Synthesis of 2-deoxylapachol, **2.44**.[168;277;278] *Reagents and conditions* : Method A. 4-methyl-3-pentenoic acid, $AgNO_3$, $(NH_4)_2S_2O_8$, MeCN, H_2O, 65-70 °C, 6 h; Method B. prenyltrifluorosilane (**4.3**), $FeCl_3.6H_2O$, DMF, MeCN, RT, 63 h.

In our approaches to the synthesis of 2-deoxylapachol, **2.44**, we envisaged either a MHE reaction or a Grignard reaction, given our previous familiarity with both methodologies (Chapter 3). With these preferences in mind, we had to find an appropriate method of

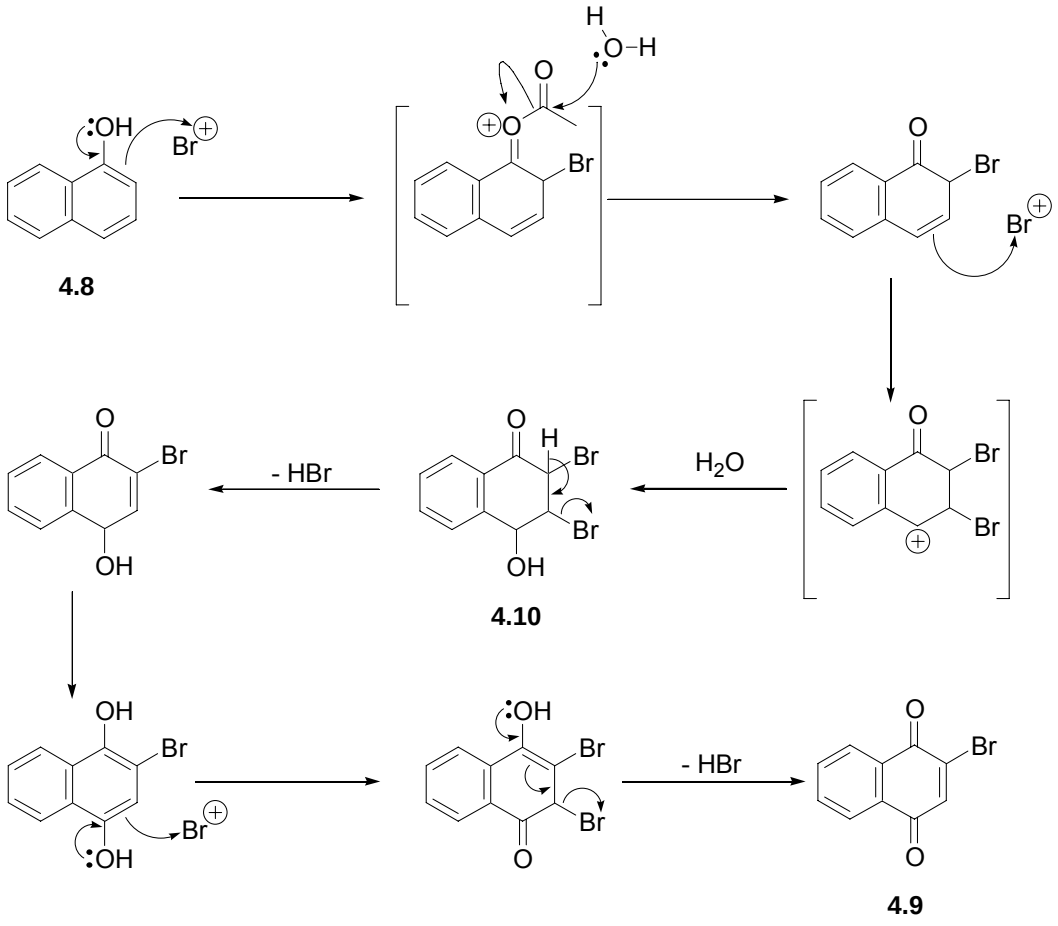
preparing the brominated precursor (**4.6**), suitable for both MHE and Grignard reactions. 2-Bromo-1,4-dimethoxynaphthalene, **4.6**, has previously been accessed either through the initial direct bromination of the quinone **4.2** or 1,4-dimethoxynaphthalene (**4.7**, Scheme 4.5), using bromine and acetic acid in chloroform[279-282] or through an oxidative bromination of 1-naphthol (**4.8**) using *N*-bromosuccinimide (NBS) in acetic acid and water to yield 2-bromo-1,4-naphthoquinone (**4.9**).[283-286]



Scheme 4.2 Proposed synthesis of **2.44**. *Reagents and conditions* : (a) NBS, AcOH, H_2O, 65 °C; (b) TEAB, $Na_2S_2O_4$, THF; (c) KOH, Me_2SO_4, RT; (d) *n*-BuLi, TMEDA, Et_2O, -10 °C - RT; (e) Mg, I_2, THF, reflux, 1h, then prenyl bromide, Li_2CuCl_4, THF, -10 °C – RT; (f) CAN, H_2O, MeCN.

In order to avoid the obvious safety risks associated with the use of bromine, we chose to begin our synthesis (Scheme 4.2) with the oxidative bromination protocol.[283] This regiospecific *ortho*-directed bromination was first developed by Heinzman and Grunwell[286] who proposed the mechanism outlined in Scheme 4.3 for this reaction. The reaction

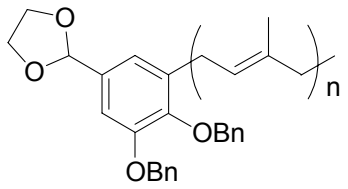
proceeds through the formation of the dibromo intermediate (**4.10**) from **4.8** which is then hydrolyzed to the desired bromoquinone **4.9**. Treatment of **4.8** with NBS in acetic acid and water at 65 °C, according to Heinzman and Grunwell's[286] procedure, afforded **4.9** as a yellow crystalline solid (90%), after recrystallization of the crude product from ethanol (Scheme 4.2). Interestingly, we noticed that the addition of the acetic acid solution to the 1-naphthol has to be very slow in order for this reaction to work. Both ^{1}H and ^{13}C NMR data for **4.9** were consistent with previously reported values.[283]



Scheme 4.3 Mechanism for the regiospsecific *ortho*-bromination of **4.8** (adapted from Heinzman and Grunwell).[285]

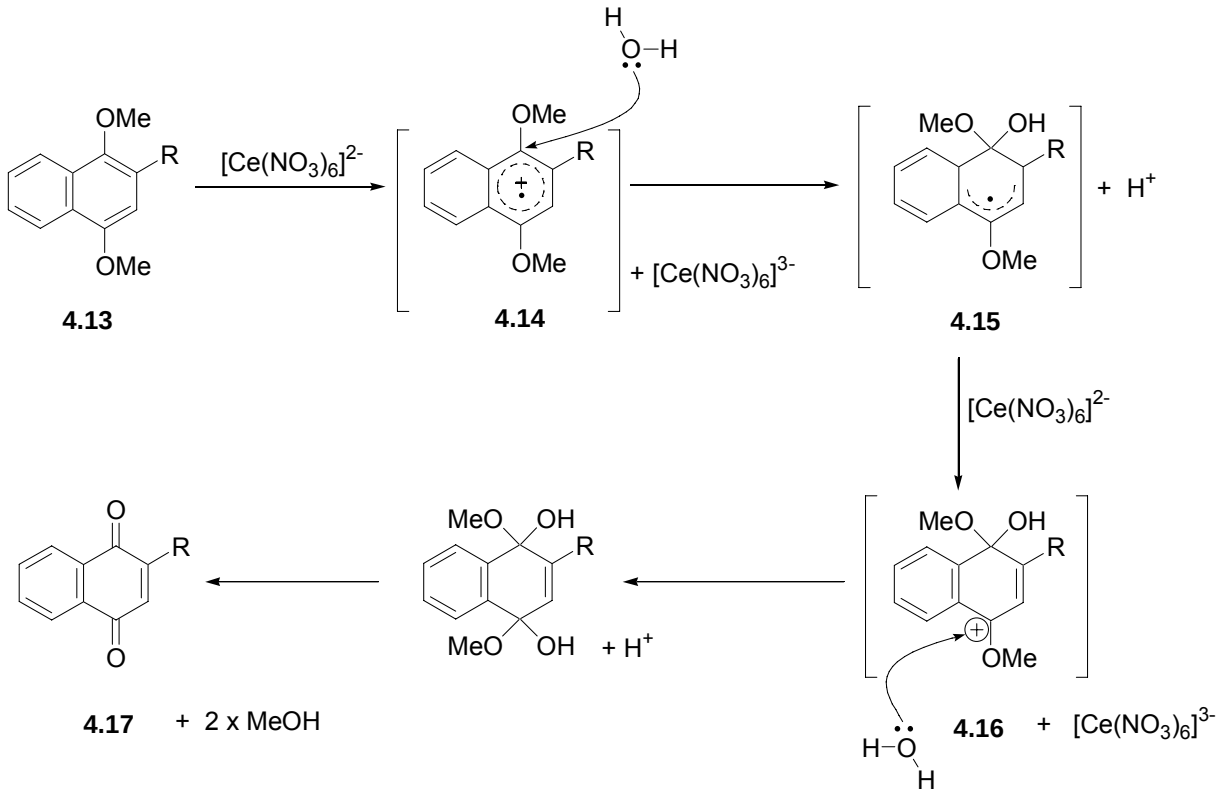
The selection of a suitable protecting group is crucial to a synthetic organic chemist.[287] Therefore, in order to protect the carbonyl groups in **4.9** so we could proceed with our synthesis, we needed to select a protecting group that would not only endure the initial strong

basic conditions imposed by the MHE reaction (Scheme 4.2, step d), but also one that could be removed without comprising the integrity of the olefinic prenyl side chain. Ester protecting groups are often deprotonated or halogenated when subjected to MHE or D*o*M whilst the use of silyl ethers can potentially undergo rearrangement reactions under the same conditions.[170] Strongly acidic reagents are not desirable as they are prone to form undesired cyclized products with the olefin side chain.[288] Odejinmi and Wiemer[170] have recently reported the use of benzyl ethers as suitable protecting groups for two prenylated aromatic compounds (**4.11** and **4.12**) and their subsequent removal by heating in the presence of metallic sodium, without affecting the olefinic side chain. Scheepers *et al*.,[155;157] on the other hand, opted for the use of methyl ethers as their protecting group (Section 3.3, Scheme 3.2), followed by the quantitative (90-95% yield) deprotection using Snyder and Rapoport's oxidative demethylation protocol employing argentic oxide (AgO).[289] However, Syper *et al*.[290] commented that this latter method is not applicable to the synthesis of a wide group of alkenylquinones and have pointed out the efficient use of cerium ammonium nitrate (CAN) by Jacob *et al*.[291] as a milder and more convenient alternative to the oxidative demethylation of substituted 1,4-dimethoxynaphthalenes. The proposed general mechanism for this oxidative demethylation reaction, adapted from Tanoue and Terada,[292] is outlined in Scheme 4.4. Firstly, the 2-substituted-1,4-dimethoxynaphthalene (**4.13**) forms a radical cationic intermediate (**4.14**) through a one-electron oxidation process as a result of the dissociation of CAN to $[Ce(NO_3)_6]^2$ in an aqueous solution. Nucleophilic addition of water to the intermediate **4.14** affords a further radical (**4.15**) which is subsequently oxidized by another $[Ce(NO_3)_6]^{2-}$ at the *para*-methoxy position (**4.16**) to finally yield the desired quinone (**4.17**) and methanol as a byproduct (Scheme 4.4).[290;292]



4.11 n = 1
4.12 n = 3

Accordingly, we proceeded to protect the carbonyl groups of **4.9** by reductive methylation[293] using sodium dithionite as the reducing agent and dimethyl sulfate as the methylating reagent (Scheme 4.2). Flash chromatography of the crude product in pure hexane yielded **4.6** (75% after two steps). Analysis of the ^{1}H and ^{13}C NMR spectra indicated the loss of two carbonyl resonances (δ_C 177.6 and 182.2) and the appearance of two methoxy signals (δ_H 3.95 and 3.97), and both ^{1}H and ^{13}C NMR data were consistent with literature values.[154;281]



Scheme 4.4 Proposed general mechanism for the general oxidative demethylation of 2-substituted dimethoxynaphthalenes.[292] [R = halogen, alkyl, alkenyl, benzyl]

With the brominated precursor **4.6** in hand, we were now able to investigate the feasibility of either an MHE reaction or a Grignard approach to the synthesis of the non-cytotoxic marine natural product 2-(3-methyl-2-butene)-1,4-dimethoxynaphthalene (**4.18**), that was isolated by Perry *et al*.[58] from the same New Zealand marine brown alga as **2.44**. Firstly, we attempted to synthesize **4.18** using standard MHE methodology by treating a solution of **4.6** in

anhydrous ether with *n*-BuLi in the presence of TMEDA, followed by the addition of prenyl bromide (Scheme 4.2, step d). Standard workup and purification of the crude material using semi-preparative HPLC (4% ethyl acetate, 96% hexane) afforded 2-(3-methyl-2-butene)-1,4-dimethoxynaphthalene **4.18** in only *ca*. 3% yield. Regrettably, at this point in our research, we had not yet accomplished the improvement (Section 3.4.1) on the MHE procedure employed and, therefore, we turned our attention to the Grignard methodology. The Grignard derivative of **4.6**, 1,4-dimethoxynaphthalenylmagnesium bromide, was generated *in situ* (*ca.* 8 eq.) and transferred *via* cannula to a solution of prenyl bromide and the catalyst dilithiumtetrachloro cuprate (Li_2CuCl_4) in anhydrous tetrahydrofuran at -10 °C, according to the same procedure discussed earlier (Section 3.3).[143;144] After purification by semi-preparative HPLC (4% ethyl acetate, 96% hexane), the expected product, **4.18**, was obtained in 67% yield. Standard oxidative demethylation of **4.18** (Scheme 4.2, step f), using a solution of CAN in acetonitrile, quantitatively afforded the marine natural product **2.44** (92%). HRFABMS data was used to establish the molecular formulae of **2.44** and **4.18**, while both 1D and 2D NMR spectroscopy were used to confirm the respective chemical structures. Furthermore, the ^{1}H and ^{13}C NMR data for **4.18** and **2.44** are summarized in Tables 4.1 and 4.2 respectively, and are consistent with the data reported by Perry and Blunt (also presented in Tables 4.1 and 4.2).[58] Unassigned signals for C-1, C-2, C-4, C-4a and C-8a (Table 4.1) in the marine natural product **4.18**[58] were readily obtainable from gHMBC data (Figure 4.1). gHMBC correlations from H_2-1` (δ_H 3.52) to C-1 (δ_C 146.56), C-2 (δ_C 129.52), from OMe-1 (δ_H 3.86) to C-1 (δ_C 146.56) and from OMe-4 (δ_H 3.96) to C-4 (δ_C 151.83) unequivocally confirmed the assignment of the ^{13}C chemical shifts for the C-1 and C-4 positions on the aromatic ring, while the three-bond gHMBC correlation from H-3 (δ_H 6.60) to C-4a (δ_C 125.35) established the chemical shift for C-4a (Figure 4.1).

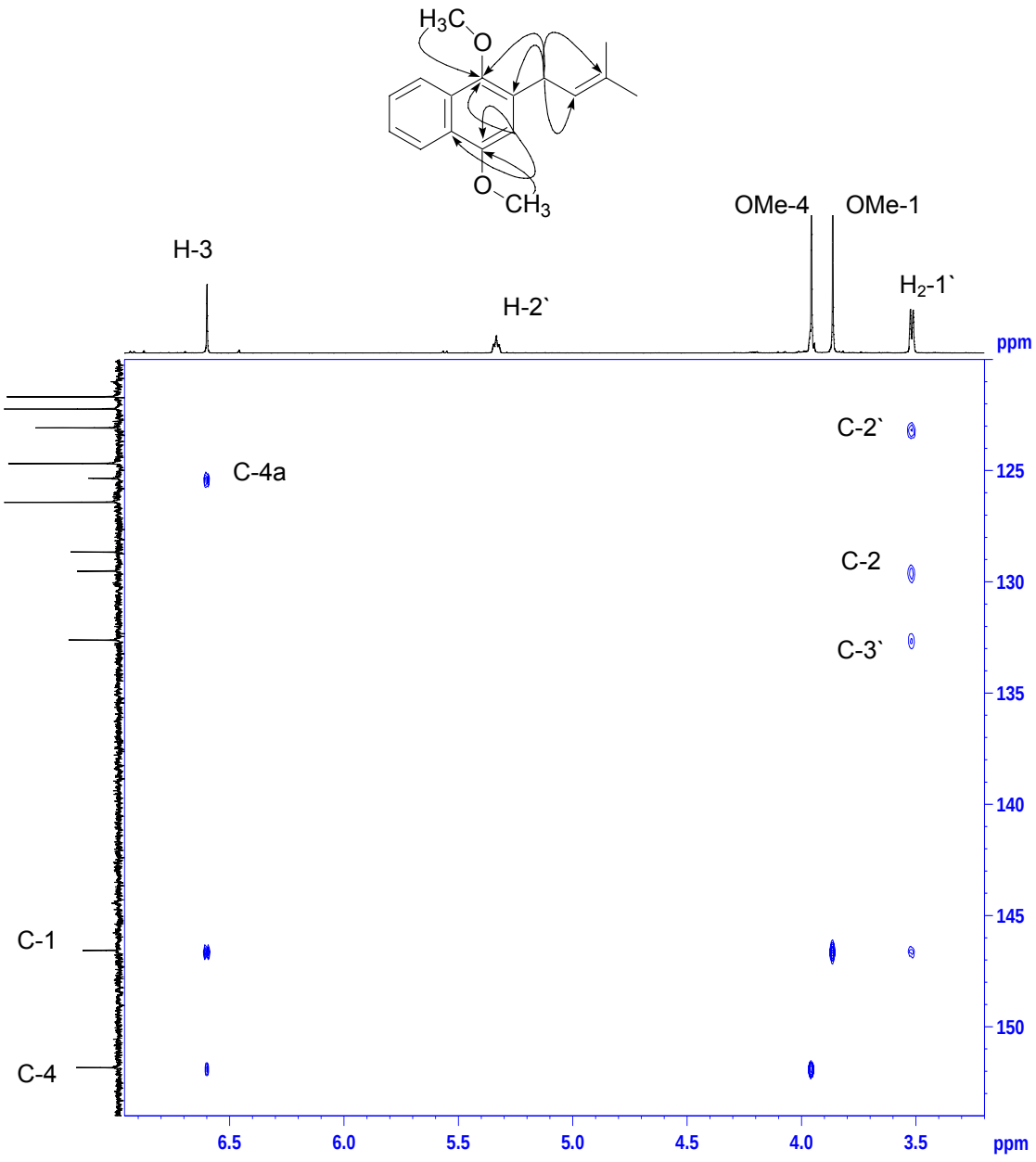


Figure 4.1 A section (F1 = δ_C 120.0 – 154.0, F2 = δ_H 3.20 – 6.96) of the gHMBC spectrum (600 MHz, $CDCl_3$) of **4.18**. The accompanying figure indicates the key gHMBC correlations of **4.18**.

Table 4.1 ^{1}H and ^{13}C NMR data for **4.18**.

	4.18†		Literature data‡	
Position	δ_c (mult.)	δ_H (int., mult., *J*/Hz)	δ_c (mult.)	δ_H (int., mult., *J*/Hz)
1	146.56		151.80[a]	
2	129.52		129.48[b]	
3	105.52	6.60 (1H, s)	105.47	6.60
4	151.83		146.53[a]	
4a	125.35		125.33[b]	
5	122.23	8.18 (1H, dt, 8.1, 1.0)	122.21	8.18 (8.3, 1.5, 0.8)
6	124.68	7.42 (1H, td, 7.6, 1.1)	124.64	7.41 (8.2, 6.8, 1.3)
7	126.42	7.50 (1H, td, 7.6, 1.1)	126.39	7.50 (8.2, 6.9, 1.4)
8	121.68	8.01 (1H, dt, 8.0, 0.9)	121.64	8.02 (8.3, 1.4, 0.8)
8a	128.66		128.64[b]	
OMe-1	61.97	3.86 (3H, s)	61.91	3.86
OMe-4	55.60	3.96 (3H, s)	55.54	3.96
1'	28.67	3.52 (2H, d, 7.1)	28.65	3.51 (dm, 7.1, 1.1)
2'	123.08	5.34 (1H, tquint, 7.1, 1.1)	123.07	5.34 (tm, 7.1, 1.4)
3'	132.61		132.54	
4'	25.77	1.75 (3H, q, 1.3)	25.63	1.75 (q, 1.3)
5'	18.00	1.80 (3H, br s)	17.95	1.80 (br d, 1.0)

† ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) data recorded for synthetic **4.18** (Scheme 4.2); ‡ ^{1}H (300 MHz, $CDCl_3$) and ^{13}C (75 MHz, $CDCl_3$) data recorded for **4.18** isolated by Perry and Blunt.[58] [a, b] Values in the reported data for **4.18** are interchangeable within column.

Table 4.2 ^{1}H and ^{13}C NMR data for **2.44**.

	2.44†		Literature data‡	
Position	δc (mult.)	δH (int., mult., J/Hz)	δc (mult.)	δH (int., mult., J/Hz)
1	184.34		185.38[a]	
2	150.82		150.84	
3	134.64	6.76 (1H, s)	134.66	6.76 (t, 1.7)
4	185.36		185.35[a]	
4a	132.14		132.17[b]	
5	126.54	8.03 - 8.11 (1H, m)	126.55[c]	8.02 - 8.12 (m)[a]
6	133.59	7.69 - 7.78 (1H, m)	133.59[d]	7.68 - 7.75 (m)[a]
7	133.64	7.69 - 7.78 (1H, m)	133.64[d]	7.68 - 7.75 (m)[a]
8	126.05	8.03 - 8.11 (1H, m)	126.07[c]	8.02 - 8.12 (m)[a]
8a	136.42		136.42[b]	
1'	28.00	3.26 (2H, d, 7.4)	28.03	3.26 (br d, 7.3)
2'	118.18	5.21 (1H, t, 7.3)	118.20	5.21 (tm, 7.3, 1.4)
3'	132.34		132.36[b]	
4'	25.77	1.78 (3H, s)	25.78	1.77 (q, 1.2)
5'	17.80	1.66 (3H, s)	17.81	1.66 (br d, 1.2)

† ^{1}H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) data recorded for synthetic **2.44** (Scheme 4.2); ‡ ^{1}H (300 MHz, $CDCl_3$) and ^{13}C (75 MHz, $CDCl_3$) data recorded for **2.44** isolated by Perry and Blunt.[58] *a, b, c, d* Values in the reported data for **2.44** are interchangeable within column.

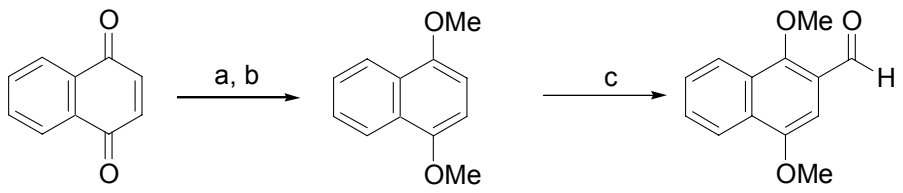
4.1.2 Grignard synthesis of benzylic alcohol analogues

In our endeavour to explore the naphthoquinone pharmacophore, we wanted to investigate the cytotoxic effect on WHCO1 cancer cells of transposing the hydroxyl substituent (C-3) of lapachol onto the benzylic position (C-1`) of the prenyl chain. We therefore rationalized the synthesis of the structural analogue (**4.19**) of **2.44** by the Grignard addition of

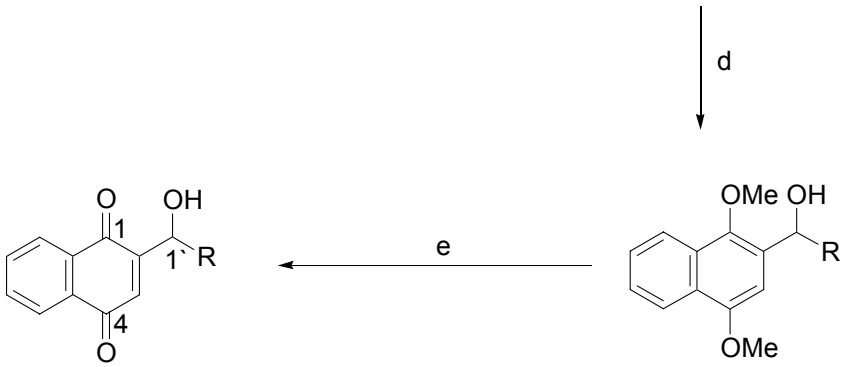
isobutenylmagnesium bromide to the aromatic aldehyde (**4.20**) (Scheme 4.5.). A one-pot reductive methylation of **4.2** (Scheme 4.2, steps b and c) yielded 1,4-dimethoxynaphthalene (**4.7**, 81%). The appearance of a methoxy proton signal (δ_H 3.97, 6H) and the absence of carbonyl resonances in the ^{13}C NMR spectrum suggested that the reaction was successful. The NMR data acquired for **4.7** was also consistent with literature values.[281] 1,4-dimethoxynaphthalene, **4.7**, was then subjected to a standard Vilsmeier-Haack formylation (Scheme 4.5) to afford 2-carbaldehyde-1,4-dimethoxynaphthalene (**4.20**, 91%), after recrystallization from hexane. The appearance of the carbonyl resonance (δ_C 189.6) and the aldehyde proton (δ_H 10.57) in **4.20**, together with the HRFABMS data (*m*/*z* 216.0786, $C_{13}H_{12}O_3$), suggested that the formylation was successful. Further validation of the structure followed from comparison of the spectroscopic data with those previously reported.[280;294]

With aldehyde **4.20** in hand, we now turned our attention to the addition of various Grignard reagents to **4.20** to afford the respective benzylic alcohol products (Scheme 4.5, step d). While methylmagnesium bromide, vinylmagnesium bromide, phenylmagnesium bromide and phenylethylmagnesium bromide are commercially available reagents, isobutenylmagnesium bromide[295] and naphthalenylmagnesium bromide[296] were prepared *in situ*. The oxidative demethylation of the Grignard addition products (**4.21**-**4.26**) proceeded smoothly to afford the 2-substituted-1,4-naphthoquinone target molecules **4.19** and (**4.27-4.31**) in 77-94% yields (Scheme 4.5).

Although the target compound **4.27** has previously been synthesized from **4.2** by Nguyen Van *et al*.[297] in six steps through a circuitous Fries rearrangement (Scheme 4.6), we have successfully accessed **4.27** in only four steps (Scheme 4.5) with the use of more cost-effective reagents. Kobayashi *et al*.[298] have also synthesized **4.27** by employing a similarly more direct synthetic route through the addition of acetaldehyde to the Grignard derivative of **4.6** followed by a standard CAN deprotection.



4.2 **4.7** **4.20**

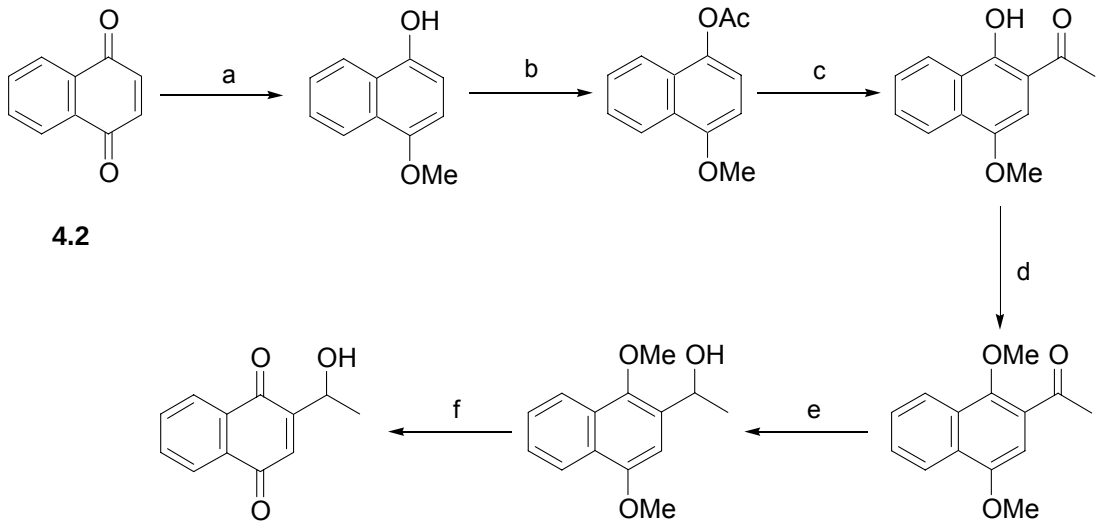


4.19	R = CH=C(CH_3)$_2$	(23%)	**4.21**	R = CH=C(CH_3)$_2$	(62%)
4.27	R = CH_3	(77%)	**4.22**	R = CH_3	(100%)
4.28	R = CH=CH_2	(70%)	**4.23**	R = CH=CH_2	(83%)
4.29	R = Ph	(94%)	**4.24**	R = Ph	(93%)
4.30	R = CH_2CH_2Ph	(80%)	**4.25**	R = CH_2CH_2Ph	(60%)
4.31	R = Naph	(50%)	**4.26**	R = Naph	(85%)

Scheme 4.5 General procedure for the preparation of **4.19** and **4.27**-**4.31**. *Reagents and conditions* : (a) $Na_2S_2O_4$, TEAB, THF, RT; (b) KOH, Me_2SO_4, RT, overnight; (c) $POCl_3$, DMF, $CHCl_3$, reflux, 96 h; (d) R-MgBr, THF, -10 °C - RT, overnight; (e) CAN (2-3 eq), H_2O, MeCN. Yields for the respective steps are presented in parentheses.

The 52% overall yield in Kobayashi's synthesis[298] of **4.27** was, however, significantly less than the overall yield of 80% that we obtained for the preparation of the same compound from the aldehyde **4.20** (Scheme 4.5). Although the phenyl derivative **4.29** has previously been reported as a synthetic precursor, no details of its preparation were provided,[299] while compounds **4.23**-**4.26**, **4.28**, **4.30** and **4.31** are, to the best of our knowledge all new. HRFABMS data for all the synthetic compounds prepared established their molecular formulae, whilst both 1D and 2D NMR spectroscopy were used to confirm their chemical

structures. The key gHMBC correlations from H-1` to C-1 and C-3 for all the protected benzylic alcohols (**4.21**-**4.26**) was used as evidence for the successful addition of the Grignard reagents to the aldehyde **4.20**. An example of this is shown for compound **4.24** in Figure 4.2. Details of the cytotoxicities of **4.19**-**4.31** against the WHCO1 cell line is discussed in Section 5.4, and the redox properties of selected synthetic naphthoquinones is investigated in Section 5.2.2.



4.2 **4.22** **4.27**

Scheme 4.6 Nguyen Van *et al*.[297] synthesis of **4.27** from **4.2**. *Reagents and conditions*: (a) $SnCl_2$, 12 M HCl, MeOH, reflux, 3 h (90%); (b) Ac_2O, pyridine, RT, 12 h (97%); (c) $BF_3.Et_2O$, 120 °C, 5 min (96%); (d) $(CH_3O)_2SO_2$, K_2CO_3, acetone, reflux, 12 h (98%); (e) $NaBH_4$, MeOH, RT, 1 h (86%); (f) CAN, MeCN, H_2O, RT, 0.5 h (87%).

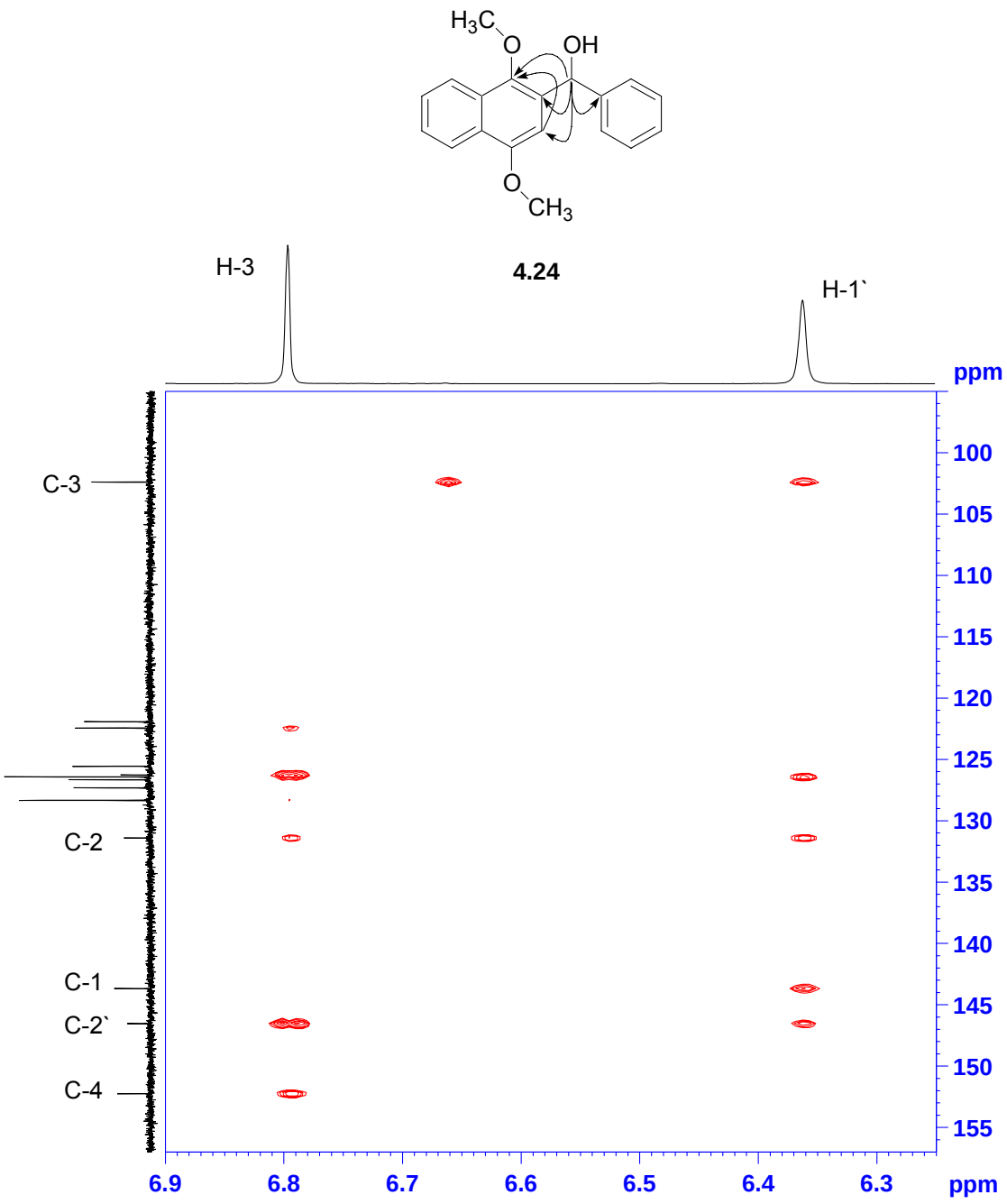
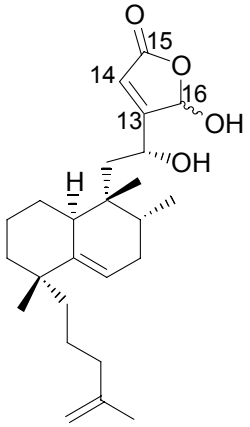


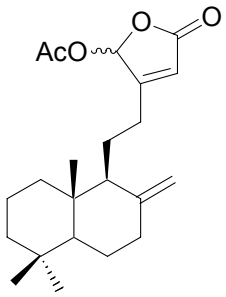
Figure 4.2 Downfield section (F1 = δ_C 95 – 157, F2 = δ_H 6.25 – 6.90) of the gHMBC spectrum (600 MHz, CDCl3) of **4.24.** The accompanying figure indicates the key gHMBC correlations of **4.24**.

4.1.3 Synthesis of the furanyl analogues (4.32 and 4.33) *via* MHE and attempted synthesis of γ-hydroxybutenolide derivates (4.34 and 4.35)

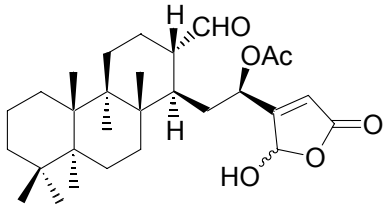
The marine natural product dysidiolide (**4.36**), isolated from a Carribean sponge, has been shown to have cytotoxic properties that maybe be useful in cancer treatment.[300;301] The pendant γ-hydroxybutenolide functionality in **4.36** is also present in various other marine secondary metabolites, *e.g.* (+)-langerstronolide (**4.37**) and luffolide (**4.38**), with important biological activites[302] and this functionality has consequently generated a lot of interest over the last few years.[300-304] Cognizant of this, we envisaged the addition of this functionality to our naphthoquinone structural motif in order to assess the biological activity of this class of new compounds against the WHCO1 cell line.



4.36



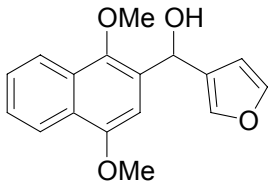
4.37



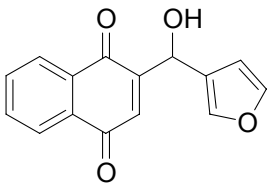
4.38

Our strategy to access the furanyl and γ-hydroxybutenolide derivatives **4.32**-**4.35** is summarized in Scheme 4.7. However, before attempting this synthesis and, in an effort to conserve valuable synthetic material, we wanted to investigate the feasibility of the photochemical oxidation employed by Kernan and Faulkner,[305] Corey and Roberts[301] and, more recently, by Marcos *et al*.[304] on a readily available standard compound. Therefore, we

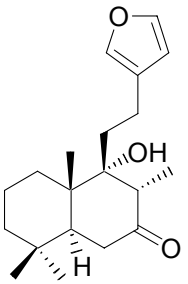
initiated a model study for this reaction using the furanoid diterpene hispanolone (**4.39**), previously isolated by members of our research group.[306]



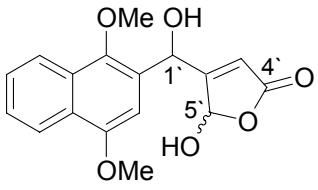
4.32



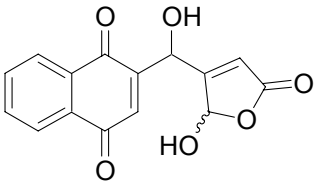
4.33



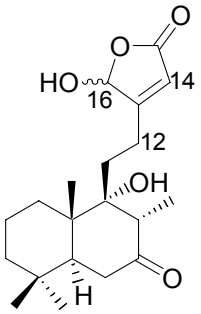
4.39



4.34



4.35



4.40

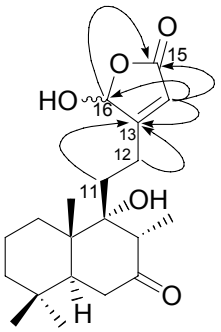
Pre-dried oxygen was bubbled for ten minutes through a solution of **4.39**, Rose Bengal and diisopropylethylamine (DIPEA) in dry dichloromethane at room temperature. The reaction mixture was then irradiated with a 200 W lamp at -78 °C for 4 h under an atmosphere of oxygen and then passed through a short silica plug, without further work up or purification. 1D and 2D NMR data for the crude reaction mixture suggested that the oxidation of **4.39** to (**4.40**) was successful. Analysis of the ^{13}C NMR spectrum revealed the disappearance of the furan methine signal for C-15 (δ_c 143.7) and the downfield shift of the other furan methine signal for C-14 (δ_c 110.7), coupled with the appearance of both the quaternary resonance at δ_c 171.0 and oxymethine resonance (δ_c 99.0) (Table 4.3), suggesting the successful oxidation at C-15 and C-16 respectively. Duplication of the γ-hydroxybutenolide signals in the ^{13}C NMR spectrum, due to the epimeric proton at position C-16, along with the similar chemical shifts for the γ-hydroxybutenolide carbons and the respective proton signals in the marine natural

product **4.36** (Table 4.3), provided further evidence to support the successful oxidation of the furanoid ring. The chemical shift assignments of **4.40** presented in Table 4.3 were supported by gHMBC correlations from H-14 (δ_H 5.84) to C-13, C-15 and C-16 and between the methylene protons H_2-11 (δ_H 2.06) and H_2-12 (δ_H 2.52) and C-13 (Figure 4.3). Having proved the success of this oxidation reaction *via* NMR spectroscopy, further purification of the crude material and additional characterization were deemed unnecessary and we proceeded to attempt the same procedure on the furan compound **4.32** (Scheme 4.7).

Table 4.3 Selected ^{1}H and ^{13}C NMR data (C-11 to C-16) for **4.36**, **4.39** and **4.40**.

	4.39†		**4.40**		**4.36**‡	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
11	34.8	1.89	34.8	2.06		
12	21.6	2.47	21.2	2.52		
13	124.8		169.4		172.0 (br)	
14	110.7	6.35	117.4	5.84	116.2 (br)	5.91
15	143.0	7.33	171.0		170.4	
16	138.6	7.21	96.9/99.0	6.00	97.6/98.1	6.09

† ^{1}H and ^{13}C NMR data (75.4 MHz, $CDCl_3$) for **4.39** obtained from Davies-Coleman and Rivett.[306] ‡ ^{1}H (500 MHz, $CDCl_3$) and ^{13}C NMR data (101 MHz, $CDCl_3$) for **4.36** obtained from Corey and Roberts.[301]



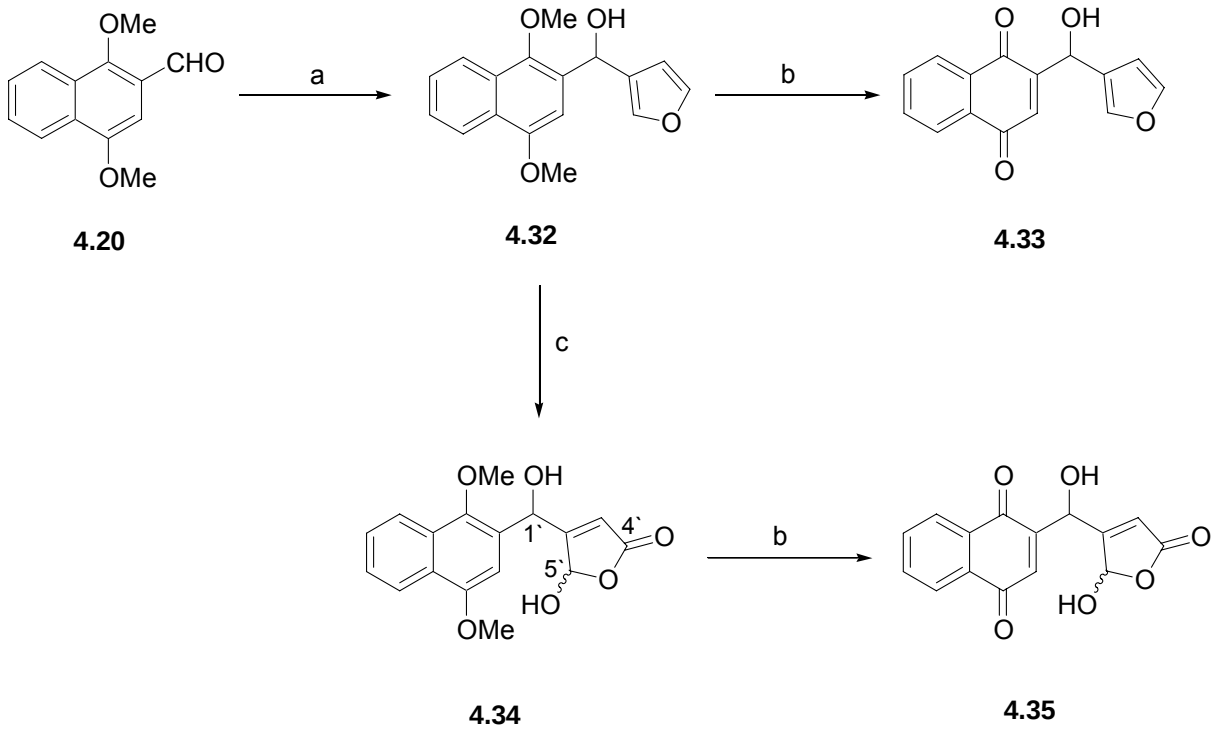
4.40

Figure 4.3 KEY gHMBC correlations for **4.40**.

Standard MHE methodology was used to synthesize the furanyl analogue **4.32** by reaction of *n*-BuLi with 3-bromofuran in anhydrous tetrahydrofuran at -78 °C, followed by the addition of the aldehyde **4.20** (Scheme 4.7). The reaction was monitored by analytical TLC and Ehrlich's reagent (5% dimethylaminobenzaldehyde in ethanol) was used to develop the TLC plate. The Ehrlich test is based on the reaction of pyrroles, furans or indoles with dimethylaminobenzaldehyde to form a coloured cation, *e.g.* deep red or violet.[307] Therefore, the visualization of a purple spot on the TLC plate, after treatment with Ehrlich's reagent, indicated the formation of the desired furanyl product, **4.32**, and the reaction was subsequently quenched with sat. ammonium chloride after stirring at -78 °C for 0.5 h and at room temperature for 15 mins. After subsequent workup, the reaction mixture was purified by semi-preparative HPLC (25% ethyl acetate, 75% hexane) to yield **4.32** in 73% isolated yield. The molecular formula ($C_{17}H_{16}O_4$) of **4.32** was established from HRFABMS data and the structure was confirmed using both 1D and 2D NMR spectroscopy. Standard oxidative demethylation of **4.32** afforded the quinone **4.33** in 55% yield (Scheme 4.7). The appearance of two carbonyl resonances (δ_C 185.2 and 185.3) in the ^{13}C NMR spectrum and the loss of the two methoxy proton signals at δ_H 3.84 and 3.94 confirmed the successful deprotection and quinone formation.

Having successfully performed the Rose Bengal oxidation on **4.39**, we attempted this transformation on **4.32**. However, all our efforts to convert the furan to a γ-hydroxybutenolide functionality in the naphthoquinone system **4.32** were unsuccessful. Preliminary analysis of the ^1H and ^{13}C NMR spectra for the crude reaction mixtures were acquired after each attempted oxidation and revealed a mixture of complex products with no indication of oxygenation at C-4` and C-5` of the furanyl moiety, as evidenced by deshielded carbonyl or hemiacetal resonances respectively in the ^{13}C NMR spectrum.

Despite the fact that we were unable to successfully synthesize the target compounds **4.34** and **4.35**, we still tested the cytotoxicity of **4.33** against the oesophageal cancer cell line WHCO1 (Section 5.4).



Scheme 4.7 General procedure for the preparation of **4.32**-**4.35**. *Reagents and conditions* : (a) *n*-BuLi, 3-bromofuran, THF, -78 °C; (b) CAN (2-3 eq), H_2O, MeCN; (c) Rose Bengal, DIPEA, CH_2Cl_2, O_2, -78 °C, 200 W.

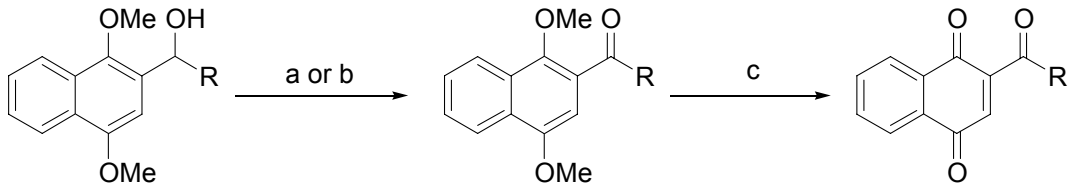
4.1.4 Oxidation of selected benzylic alcohols

We hypothesized that a simple oxidation of the benzylic alcohol would produce more analogues for screening against the oesophageal cancer cell line WHCO1 and hence contribute to furthering our SAR study. We were particularly interested in exploring the contribution of the benzylic alcohol to the cytotoxicity of the most active compounds, **4.24**-

4.26 and **4.32**, in the cohort of synthetic compounds we prepared. Both oxidation of the benzylic alcohol to a ketone and complete removal of the alcohol functionality were envisaged as the next logical step in our SAR study.

Two methods were used for the oxidation transformation of the benzylic alcohol to a ketone. Firstly, we treated the alcohols **4.24**-**4.26** and **4.32** with manganese dioxide (~30 eq.) in anhydrous dichloromethane at room temperature over a period of two days (Scheme 4.8). The oxidized products (**4.41**-**4.43**) were obtained in 80%, 91% and 72% yields respectively while the oxidation of **4.25** was surprisingly unsuccessful. All the oxidized products, with the exception of **4.42**, required separation of the product from the starting material by normal phase semi-preparative HPLC. Consequently, we decided to explore an alternative oxidation protocol for **4.25**. In their synthesis of novel exocyclic ketone analogue of the natural product **4.36**, Marcos *et al*.[304] have reported the combined use of the Ley-Griffith reagent as a catalytic oxidant and *N*-methyl morpholine-*N*-oxide (NMO) as the co-oxidant. The Ley-Griffith reagent, also known as tetrapropylammonium perruthenate (TPAP) is a ruthenium-based catalyst that has been extensively used as a catalytic oxidant in organic synthesis.[279;302;304;308;309] This oxidation protocol appealed to us as it has been reported to afford high yields in short reaction times.[304;308;309] Although we only needed to oxidize the outstanding benzylic alcohol **4.25** to obtain (**4.44**) using this new methodology, we decided to attempt this oxidation on **4.24** and **4.32** for comparative purposes. Therefore, *N*-methyl morpholine-*N*-oxide (3.0 eq.), powdered 4Å molecular sieves and catalytic amounts of tetrapropylammonium perruthenate were added to the respective benzylic alcohol solutions in anhydrous dichloromethane and stirred at room temperature (2-3 h) under an atmosphere of dry argon (Scheme 4.8). The reactions were monitored by analytical TLC until the complete consumption of starting material was evident. Gratifyingly, this new approach gave us the expected product **4.41** and the previously unaccessible **4.44** in quantitative yields (98% and 100% respectively). While we only obtained **4.43** in a modest 60% yield, this latter compound was obtained pure and did not require further chromatographic purification. HRFABMS data

confirmed the molecular formulae of **4.41**-**4.44**. The loss of the oxymethine signals in the ^{1}H and ^{13}C spectra, coupled with the appearance of a carbonyl resonance (δ_C 189.9-202.1) in the ^{13}C spectrum, *e.g.* Figure 4.4, indicated the formation of the oxidized products.



4.24	R = Ph	**4.41**	R = Ph	**4.45**	R = Ph
4.25	R = CH_2CH_2Ph	**4.42**	R = Naph	**4.46**	R = CH_2CH_2Ph
4.26	R = Naph	**4.43**	R = Fur	**4.47**	R = Naph
4.32	R = Fur	**4.44**	R = CH_2CH_2Ph	**4.48**	R = Fur

Scheme 4.8 Oxidation of benzylic alcohols **4.24**-**4.26** and **4.32**. *Reagents and conditions* : (a) MnO_2 (~30 eq.), CH_2Cl_2, 48 h; (b) NMO (3.0 eq.), TPAP, CH_2Cl_2, 2 h; (c) CAN (2-3 eq), H_2O, MeCN.

The use of the Ley-Griffith reagent was clearly a better oxidation procedure than the manganese dioxide oxidation as it required only catalytic amounts of the oxidizing agent tetrapropylammonium perruthenate, shorter reaction times (2-3 h) and no purification after workup. Conversely, a large excess (~30 eq.) of manganese dioxide was required for the other oxidation, concomitant with a long reaction time (48 h) and the need for the final purification of the ketones *via* HPLC.

Having successfully oxidized the benzylic alcohols **4.24**-**4.26** and **4.32**, we were now able to perform the oxidative demethylation (Scheme 4.8) of **4.41**-**4.44** in order to yield the desired quinones (**4.45**-**4.48**, 56-97% yield). HRFABMS for the products confirmed their respective molecular formulae, while 1D and 2D NMR spectroscopy were used to confirm their chemical structures. Again, the loss of the methoxy proton signals (δ_H 3.74-3.99) and the emergence of two carbonyl resonances at *ca.* 184 ppm in the ^{13}C NMR spectrum were key indicators to

confirm that the deprotections were successful (Figure 4.4). The cytotoxicities of **4.45**-**4.48** against the WHCO1 cancer cell line were obtained and the IC_{50} values for these compounds are summarized and discussed in Section 5.4.

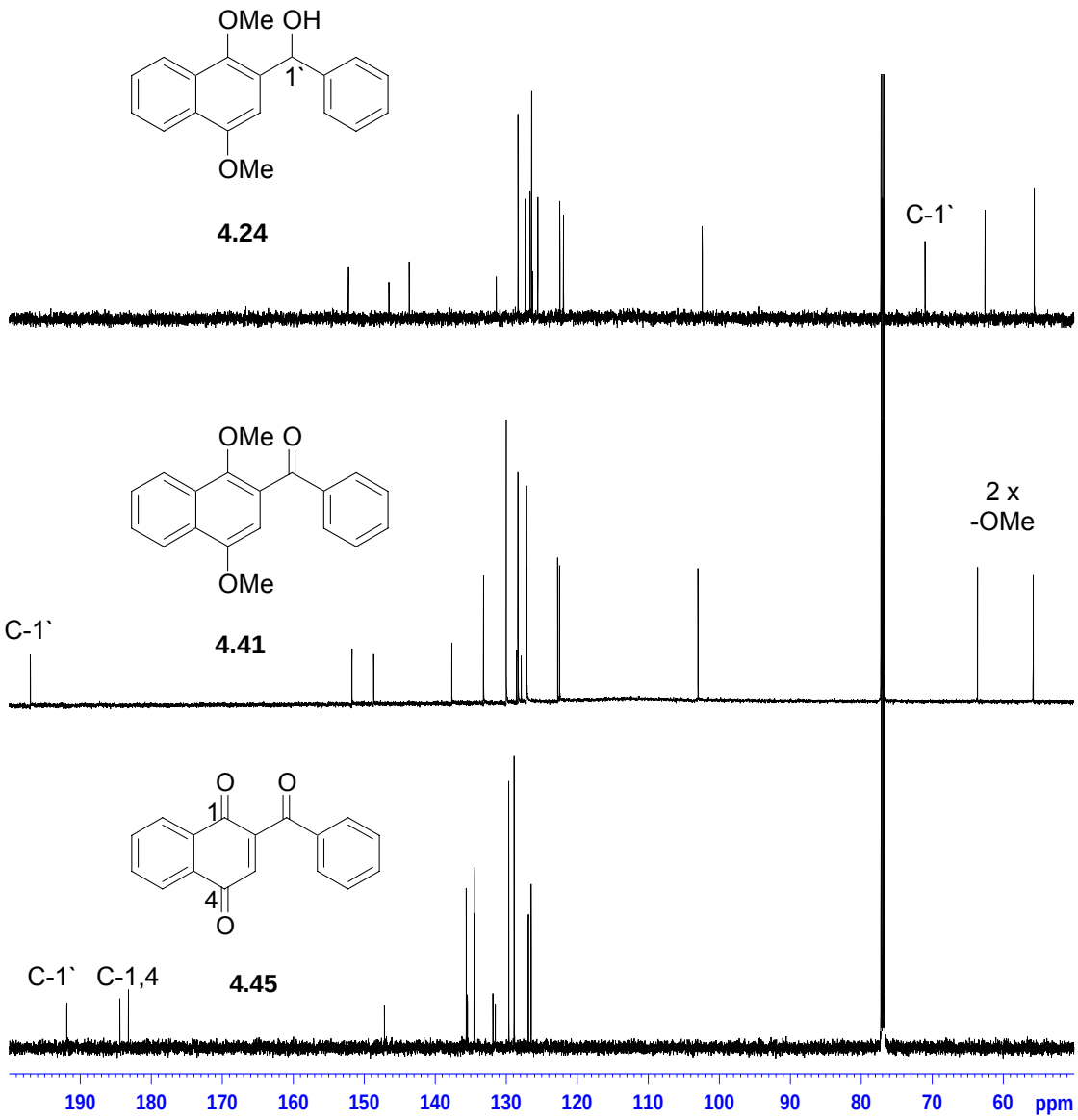
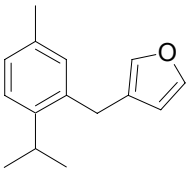


Figure 4.4 The ^{13}C NMR spectra (150 MHz, $CDCl_3$) of **4.24** (top), **4.41** (middle) and **4.45** (bottom) (structures shown inset).

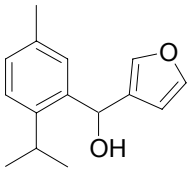
4.1.5 Reductive dehydroxylation of 4.24 and 4.41

During our SAR study (Chapter 5), it was clearly evident that **4.29** was the most active against the oesophageal cancer cell line WHCO1. Of interest to us was the effect on cytotoxicity of the removing the benzylic alcohol and our initial approach was, therefore, the direct dehydroxylation of the protected benzylic alcohol **4.24.**

Chauhan and Frost[310] have previously highlighted the efficient use of chlorodimethylsilane in combination with indinium catalysts to achieve reductive deoxygenation in secondary benzylic alcohols. Although the reported yields (*ca*. 99%)[310] for this procedure were extremely appealing, we were unfortunately limited in our access to indinium catalysts thus rendering this approach non-viable. From previous work done in our laboratory, McPhail *et al*.[198] have reported the synthesis of the marine natural product tsitsikammafuran (**4.49**) from its benzylic alcohol analogue (**4.50**), using a reductive dehydroxylation method adapted from Perry *et al*.[311] as the final step in their synthesis.



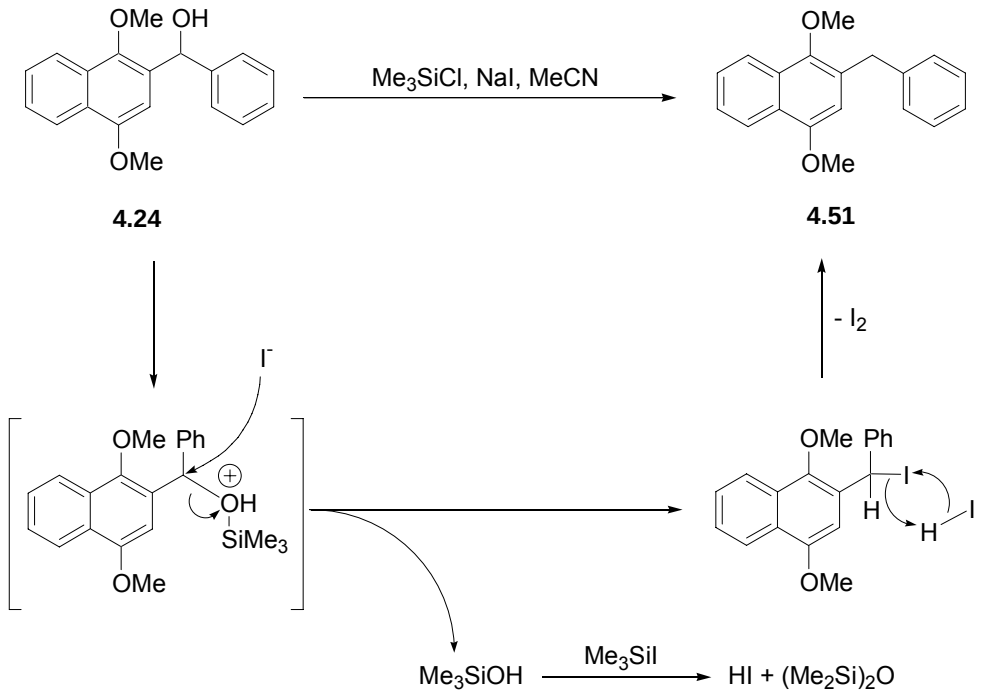
4.49



4.50

Though we had a supply of **4.41** in our hands and could apply the silane reduction methodology by West *et al*.[312] and Joshi *et al*.[284] to synthesize the compound (**4.51**), we rather opted for Perry's[311] and McPhail's[198] approach to reductively dehydroxylate **4.24** (Scheme 4.9), since it omits one additional step, *i.e.* the benzylic oxidation, in our synthetic route towards **4.51** and the quinone (**4.52**).

The mechanism of this reaction, adapted from Perry *et al*.[311] (Scheme 4.9), involves the initial formation of a silyl ether intermediate which is then cleaved by the attack of an iodine anion to form a silyl alcohol. This resulting alcohol reacts with additional iodotrimethylsilane to release hydrogen iodide (HI) which subsequently iodates and deiodates the biaryl species to yield the reduced product (Scheme 4.9).



Scheme 4.9 Mechanism for the attempted reductive dehydroxylation of **4.24** (adapted from Perry *et al*.)[311]

Consequently, using the procedure employed by McPhail *et al*.,[198;311] we treated the alcohol **4.24** with iodotrimethylsilane, which was generated *in situ* from chlorotrimethylsilane and sodium iodide, in anhydrous acetonitrile under an atmosphere of nitrogen. A cursory examination of the ^{1}H and ^{13}C NMR spectra of the crude reaction mixture suggested the possible formation of the expected product but, upon closer investigation after purification *via* HPLC, it was apparent that the reaction was unsuccessful, as evidenced by the presence of the oxymethine resonances (δ_H 3.96 and δ_C 71.0). This approach to the dehydroxylation of

4.24 was therefore deemed unsuitable. Similarly, the attempted dehydroxylation of **4.24** *via* standard tosylation/LAH methodology[306] also proved to be unsuccessful. The alcohol **4.24**, toluene-*p*-sulfonyl chloride (ToSCl) and 4-dimethylaminopyridine (DMAP) were taken up in anhydrous pyridine and left to stand at 0 °C for 1 day. After standard workup, examination of the ^{1}H NMR spectrum indicated the presence of mostly starting material since there was no disappearance of the oxymethine proton signal (δ_H 3.96) and/or the emergence of a new methylene signal.

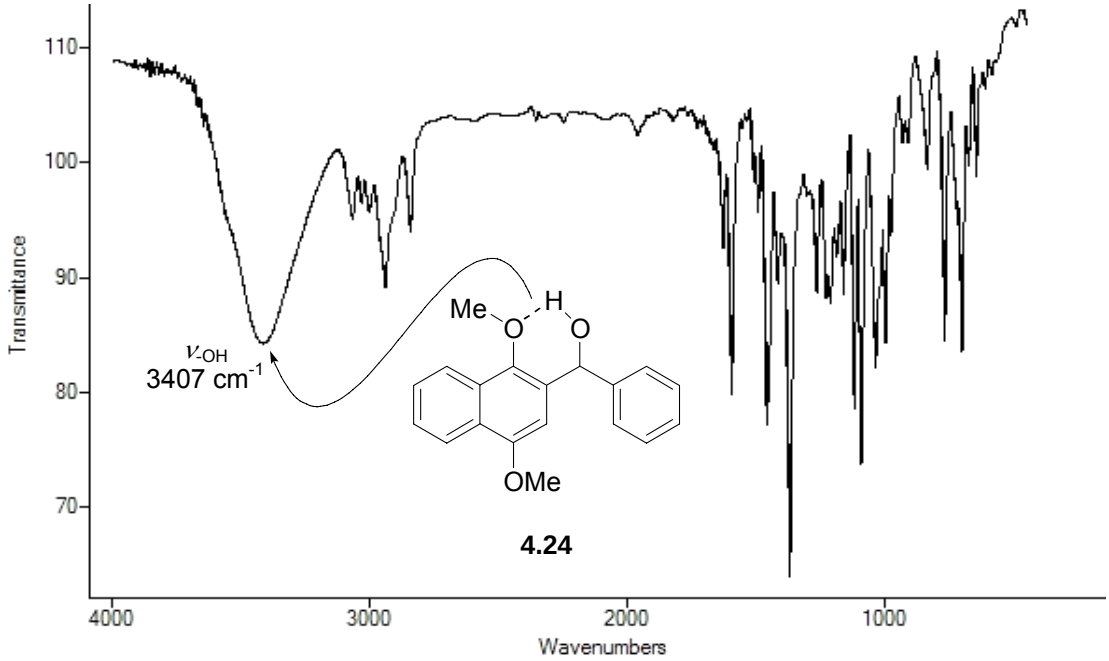
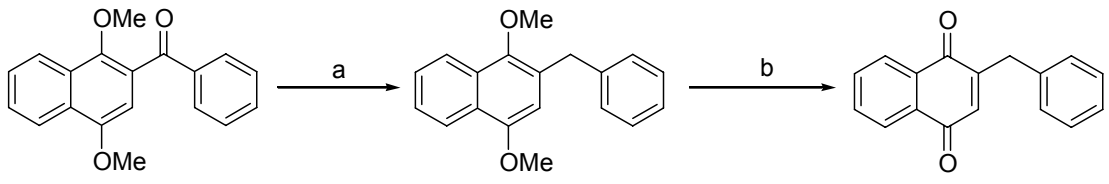


Figure 4.5 Infrared spectrum of **4.24** (structure shown inset) indicating the hydrogen-bonded hydroxyl frequency.

Puzzled by our inability to successfully dehydroxylate **4.24**, we turned our attention to the IR data for **4.24** and noticed the relatively low frequency of the broad hydroxyl band (ν_{-OH} 3407 cm^{-1}) (Figure 4.5), which is indicative of a hydrogen-bonded hydroxyl (3600-3200 cm^{-1}).[313] Conversely, a free O-H stretching frequency is normally observed as a sharp 'monomeric' band (3650-3590 cm^{-1}).[313] The interpretation of the IR data led us to predict the possible

intramolecular hydrogen bonding in **4.24** between the proton of the hydroxyl moiety and the oxygen atom of the methyl ether on the naphthoquinone (Figure 4.5), which may impede the formation of both the trimethylsilyl and tosyl ethers required for the two dehydroxylation methods outlined above.

Evidently, we required a new synthetic approach to obtain the target compounds **4.51** and **4.52**. Joshi *et al*.[284] have previously reported the synthesis of **4.51** from **4.7** (Scheme 4.5) in a two-step process. Firstly, **4.7** was refluxed at 60 °C (12 h) in a mixture of benzoic acid, trifluoroacetic acid (TFA) and trifluoroacetic anhydride (TFAA) to yield **4.41** which is then reduced with triethylsilane in an acidic medium to afford the desired **4.51** in an overall yield of 82%.[284;312;314] Further examination of the literature revealed the possibility of converting a ketone functionality directly to a methylene group *via* two different routes.[315] In his new approach to the synthesis of Strigol, Dailey[315] firstly considered a two-step conversion using 1,2-ethanedithiol and boron trifluoride etherate ($BF_3.Et_2O$) to form a dithioketal which is then subjected to a standard Raney-Nickel desulfurization to afford an olefin. We had previously considered this synthetic route to synthesize **4.51** but a closer look at the second alternative put forward by Dailey[315] changed our original choice since the latter strategy only involved a one step process. Additionally, this alternative procedure using triethylsilane and boron trifluoride etherate evokes the silane reduction methodology by Joshi *et al*.,[284] as discussed above, to synthesize **4.51**. Our attraction to this silane reduction protocol was three-fold; firstly it is a solvent-free reaction and secondly, it circumvents both the tedious and time-consuming task of preparing fresh Raney-Nickel and finally, excludes the use of the odorous 1,2-ethanedithiol.



4.41 **4.51** **4.52**

Scheme 4.10 Dehydroxylation of **4.41** using the procedure by Joshi *et al.*[284] *Reagents and conditions* : (a) $BF_3.Et_2O$, Et_3Si, 80-95 °C ; (b) CAN (2-3 eq), H_2O, MeCN.

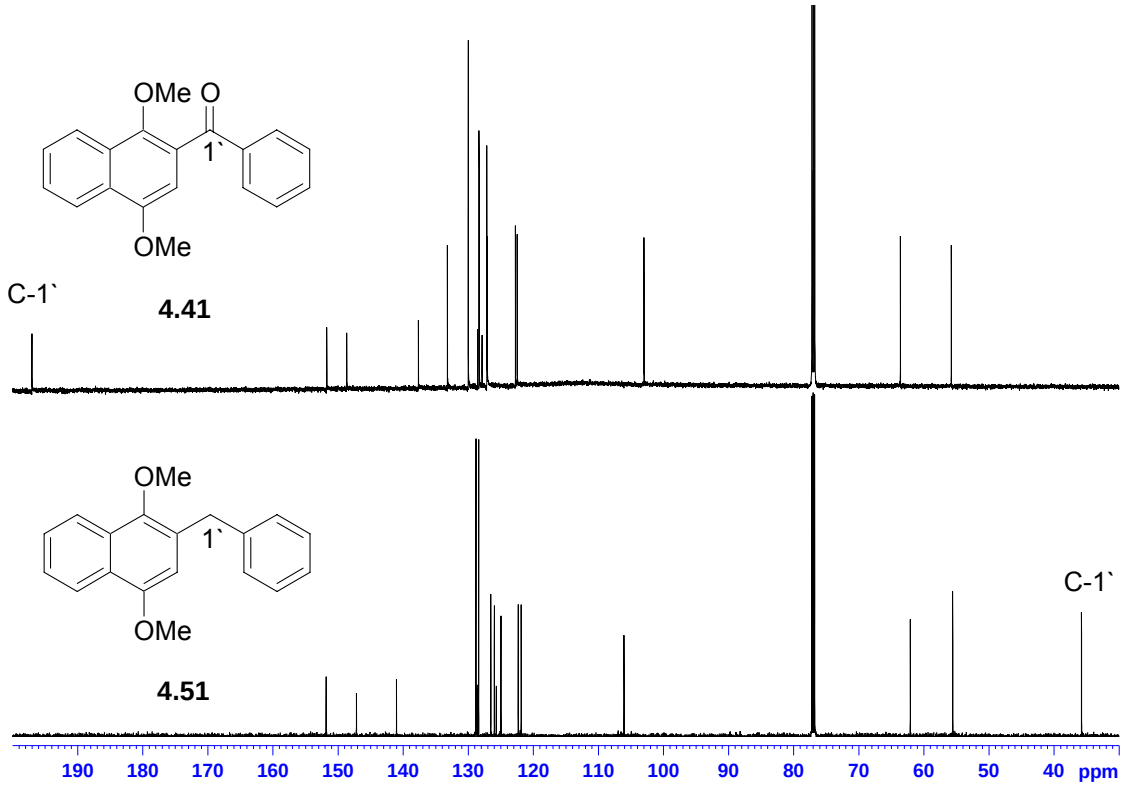


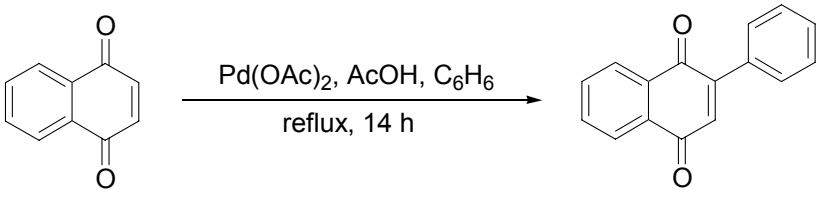
Figure 4.6 The ^{13}C NMR spectra (150 MHz, $CDCl_3$) of **4.41** (top) and **4.51** (bottom) (structures shown inset).

Consequently, a solution of **4.41**, triethylsilane and boron trifluoride etherate was refluxed for 2 h at 80-95 °C (Scheme 4.10). Standard workup of the reaction and subsequent purification by semi-preparative HPLC (25% ethyl acetate, 75% hexane) afforded the target compound

4.51 (58% yield). The loss of the carbonyl resonance (δ_C 197.0) for C-1` coupled with the appearance of a methylene proton signal (δ_H 4.18) suggested the successful dehydroxylation and the ^{1}H and ^{13}C NMR data obtained were consistent with literature values for **4.51** (Figure 4.6).[284] Oxidative demethylation of **4.51** with CAN in acetonitrile afforded the target compound **4.52** in 84% yield. The ^{1}H and ^{13}C NMR data obtained for **4.52** were also in accordance with literature values.[316]

4.1.6 Oxidative coupling of 1,4-naphthoquinone

Having successfully synthesized **4.29**, **4.45** and **4.52**, we realised that attaching an aromatic ring directly onto our 1,4-naphthoquinone scaffold would enhance our overall SAR study. A search through the literature revealed the previous synthesis of the target compound 2-phenyl-1,4-naphthoquinone (**4.53**, Scheme 4.11).[317] Itahara[317] accessed **4.53** *via* the oxidative coupling of 1,4-naphthoquine **4.2** with benzene, using palladium acetate in acetic acid. We attempted this simple arylation reaction to obtain **4.53** in 80% yield, after purification by flash chromatography using benzene as the eluent. The structure of **4.53** was confirmed by both 1D and 2D NMR spectroscopy and both ^{1}H and ^{13}C NMR data were congruent with literature values.[318]



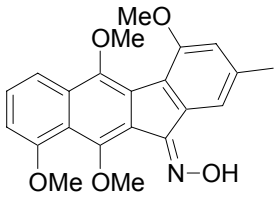
4.2 **4.53**

Scheme 4.11 Oxidative coupling of **4.2** to benzene.[317]

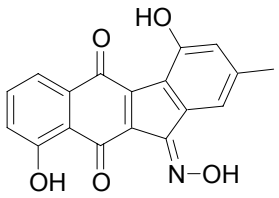
The cytotoxicity of **4.53** against the WHCO1 oesophageal cancer cell line was assayed and the significance of this biological activity in the overall SAR study is presented in Section 5.4.

4.1.7 Attempted synthesis of hydroxylamine

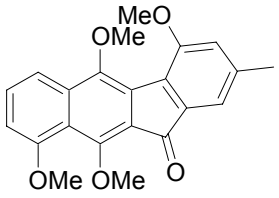
For the purpose of our SAR study, we also wanted to investigate the effect which a nitrogen atom, within our phenyl substituted-1,4-naphthoquinone scaffold, would have on the cytotoxicity against the WHCO1 cancer cell lines. Gould *et al*.[319] have previously reported the synthesis of the quinone-based oximes (**4.54** and **4.55**) from their ketone precursor (**4.56**), using hydroxylamine and pyridine in ethanol, and, more recently, Álvarez *et al*.[320] have described the synthesis of the oximes (**4.57** and **4.58**) from their respective carbonyl precursors (**4.59** and **4.60**) using the same methodology. Compounds **4.59** and **4.60** were assayed for cytotoxic activity against cancer cell lines and indicated promising IC_{50} values ranging from 0.4-10 μM.[320] Consequently, we hoped to ultimately synthesize the amine derivative (**4.61**) of **4.41**, through the Raney-Nickel reduction of the oxime (**4.62**, Scheme 4.13). In order to familiarize ourselves with the first step of our proposed synthesis and to also avoid the unnecessary wastage of valuable synthetic material, we decided to perform the transformation on a model system. Our molecule of choice for the model study was again the readily available furan diterpene **4.39** (Scheme 4.12).



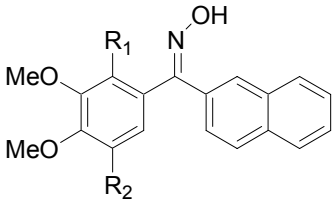
4.54



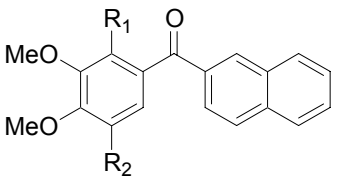
4.55



4.56

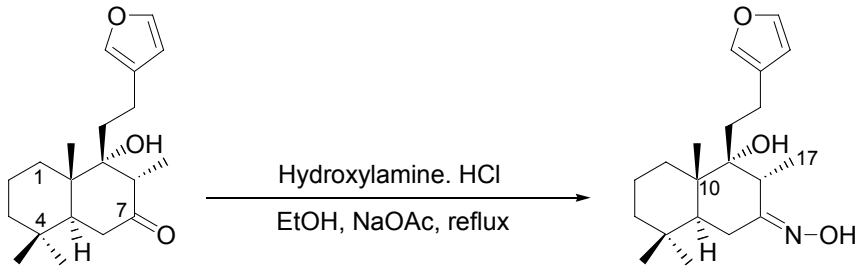


4.57 R_1 = H, R_2 = OMe
4.58 R_1 = OMe, R_2 = H



4.59 R_1 = H, R_2 = OMe
4.60 R_1 = OMe, R_2 = H

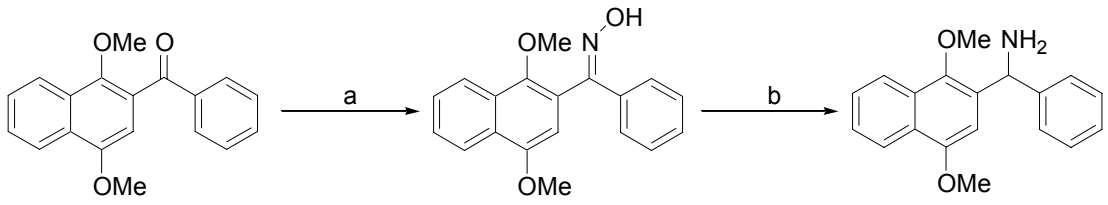
A mixture of **4.39**, hydroxylamine.HCl and sodium acetate in ethanol was heated at 80 °C for 4.5 h and then allowed to stir at room temperature for a further 16 h. The reaction was monitored by analytical TLC and, after standard workup, preliminary analysis of the ^{13}C NMR data revealed the disappearance of the carbonyl resonance (δ_C 211.8) for C-7,[306] and the appearance of a quaternary carbon resonance at 162.2 ppm, consistent with the formation of an oxime at C-7.[319;320] The formation of the oxime was quantitative and hence no further purification for **4.63** was required.



4.39 **4.63**

Scheme 4.12 Hydroxylamine model reaction on hispanolone, **4.39**.

The same procedure as described before was applied to **4.41** and, after standard workup, the crude reaction mixture was purified *via* semi-preparative HPLC (25% ethyl acetate, 75% hexane) to afford the oxime **4.62** in only 10% yield. The disappearance of the carbonyl resonance (δ_C 197.0) and the emergence of the quaternary resonance at 157.0 ppm in the ^{13}C NMR spectrum suggested the successful formation of oxime **4.62** (Figure 4.7).



4.41 **4.62** **4.61**

Scheme 4.13 Reductive amination of **4.41** *via* oxime **4.62**. *Reagents and conditions*: (a) Hydroxylamine.HCl, EtOH, NaOAc, reflux; (b) Raney-Nickel, H_2, 800 KPa.

Previous reports in the literature of similar oximes (**4.54**, **4.55**, **4.57** and **4.58**) indicate that the ^{13}C chemical shift of the oxime carbon resonance (C = N) is generally in the region of *ca*. 150-160 ppm, [319;320] thus further corroborating our evidence for the formation of **4.62**. Both 1D (Figure 4.7) and 2D NMR spectroscopy were utilized to confirm the structure of **4.62**. Several subsequent attempts to improve the yield of this transformation proved unsuccessful and we therefore decided to abandon the inclusion of either the oxime or the amine in our SAR study.

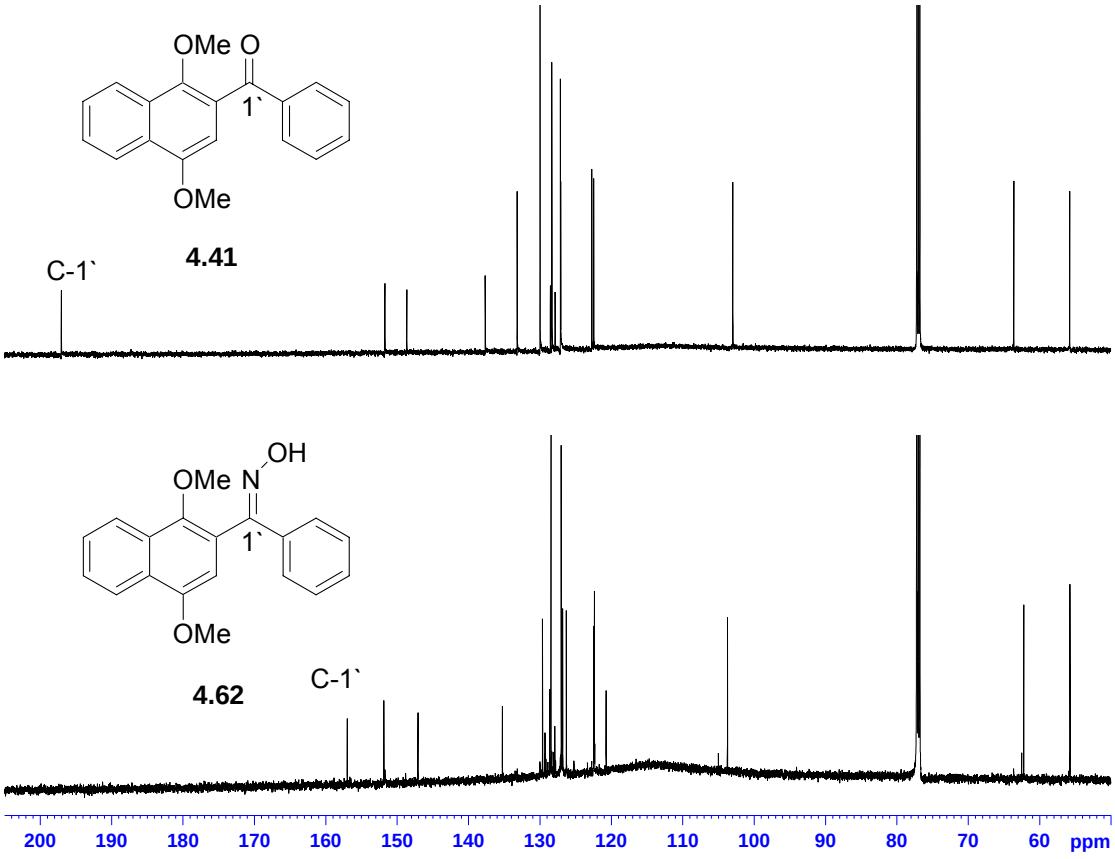


Figure 4.7 The ^{13}C NMR spectra (150 MHz, $CDCl_3$) of **4.41** (top) and **4.62** (bottom) (structures shown inset).

4.2 Importance of optical resolution in medicinal chemistry

The issue of chirality is a crucial aspect of the design, discovery and development of new drugs.[321;322] Consequently, in their constant search for drugs with improved therapeutic advantages, low toxicity and minimal side effects, the pharmaceutical industry has emphasized the importance of producing enantiomerically pure compounds for biological evaluation.[321-323] If one enantiomer is responsible for the activity of interest, then its paired enantiomer maybe be inactive, possess some of activity of interest, be an antagonist of the active enantiomer or have separate activity that could be either desirable or undesirable.[321;322;324] The thalidomide tragedy of 1961 is a classic example of the detrimental effect that one enantiomer, in a racemic drug, can cause along with the desirable effect of the other enantiomer.[321]

In drug discovery, the use of enantioselective chromatographic techniques, *e.g.* HPLC and supercritical fluid chromatography (SFC) on chiral stationary phases, have become popular in order to obtain small quantities (from mg to multigrams) of pure enantiomers rapidly and efficiently for preliminary biological testing.[323;325] However, when larger quantities of enantiomerically pure chiral molecules are needed, these techniques for chiral resolution are neither cost-effective nor time-effective.[323;325] The cost of chiral HPLC columns and the reality that no general chiral stationary phase exists that will afford resolution of every analyte mixture is often a limitation in the separation of racemates by HPLC.[326] The asymmetric synthetic approach to accessing enantiomerically pure chiral compounds only accounts for *ca.* 20% of the total number of chiral centres generated in drug syntheses.[327] Chiral resolution of racemates, especially if performed early in the synthetic sequence, is the simpler and most commonly used method in the pharmaceutical industry for producing enantiomerically pure chiral compounds.[325;327] Carey *et al*.[327] have also highlighted the synthetic advantage of the efficient construction, followed by chiral resolution, rather than the application of asymmetric methodology.

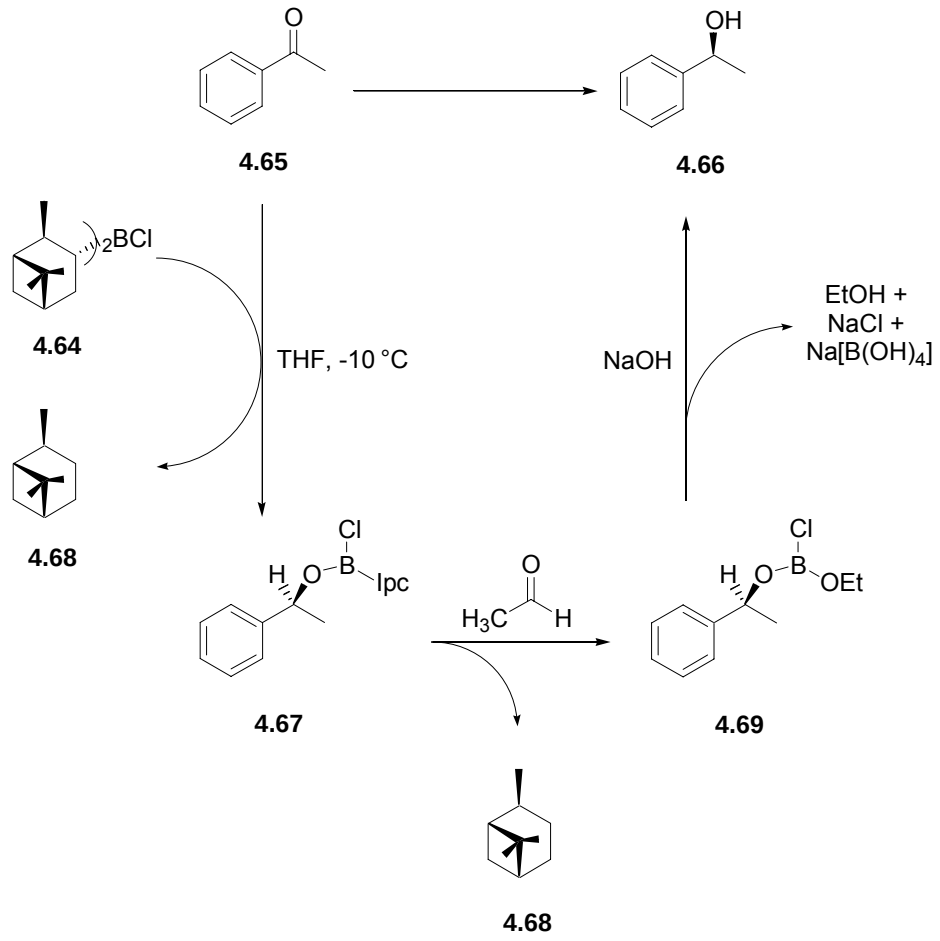
Cognizant of the importance of the biological testing of enantiomerically pure chiral compounds, we therefore endeavoured to generate both enantiomers of our most active compound **4.29**. We opted to use chiral HPLC, asymmetric synthesis and the chiral resolution of the racemic mixture to obtain pure enantiomers of the dimethoxy derivative **4.24**.

The analytical chiral HPLC column Lux 5u Cellulose-1 was utilized to attempt the enantiomeric separation of the racemic mixture of **4.24**, using gradient elution with acetonitrile/water, and hexane/isopropyl alcohol mixtures. Regrettably, this approach did not afford respectable resolution of the enantiomers.

4.2.1 Attempted asymmetric reduction of the benzylic ketone 4.41.

The asymmetric reduction of prochiral ketones is one of the best methods used to prepare enantiomerically enriched secondary alcohols.[328-330] Brown *et al*.[330] have investigated the relative efficiency of several reducing agents for the asymmetric reduction of various ketones and have consequently reported the excellent efficiency of β-chlorodiisopinocamphenylborane (DIP-Cl or Ipc_2BCl, **4.64**).[328] The asymmetric reduction of acetophenone (**4.65**) using the dimer (-)-DIP-Cl has been shown to yield the desired (*S*)-phenylethanol (**4.66**) in 72% yield and 98% enantiomeric excess (*ee*) (Scheme 4.14).[331;332] The reaction is carried out in dry tetrahydrofuran at low temperatures (-10 °C) under strict anhydrous conditions. During the reduction process, the one monomer of **4.64** bonds to the benzylic oxygen of **4.65** to form the borane complex (**4.67**) while the other monomer is converted to α-pinene (**4.68,** Scheme 4.14).[328;331] Brown *et al*.[330] had originally employed a non-oxidative removal of the boron byproducts using diethanolamine during their workup, before simplifying the procedure with the treatment of the reaction mixture with acetaldehyde, which displaces **4.68** to form the complex (**4.69,** Scheme 4.14), at room temperature. Finally, the addition of sodium hydroxide to **4.69** yields the desired alcohol **4.66**.[328;331]

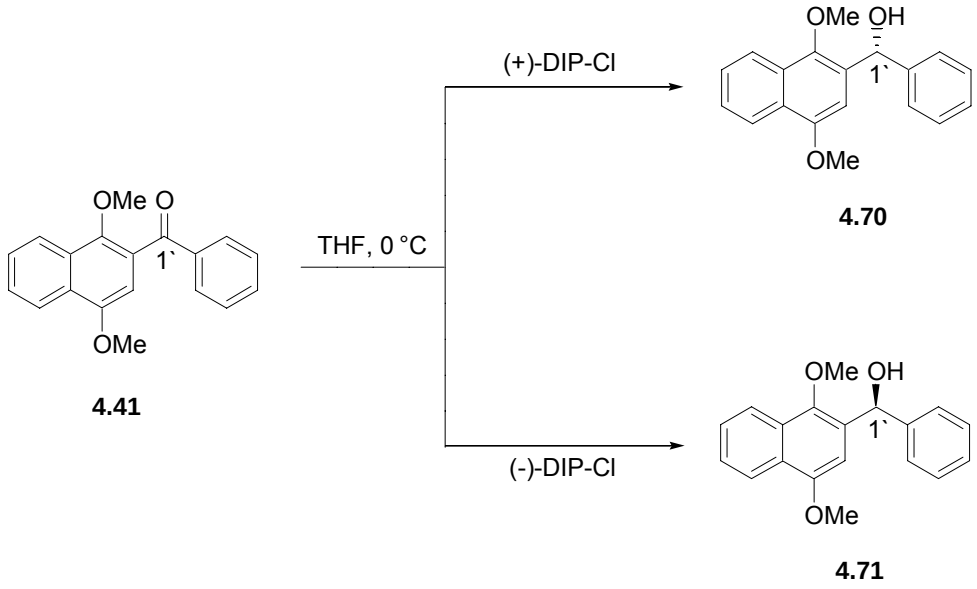
Ramachandran *et al*.[331] have observed that this simplified workup not only facilitated the isolation of the alcohol products but also achieved higher yields of the desired alcohols with the complete recycling of α-pinene, **4.68**, and the disposal of water soluble and environmentally safe byproducts, such as ethanol and sodium chloride (Scheme 4.14).



Scheme 4.14 Asymmetric reduction of acetophenone, **4.65**, using (-)-DIP-Cl.[331;332]

In order to conserve valuable synthetic material, we attempted this asymmetric transformation on **4.65** as a model system. A solution of (-)-DIP-Cl in anhydrous tetrahydrofuran was added dropwise to a solution of **4.65** in anhydrous tetrahydrofuran at -10 °C under argon. The reaction mixture was stirred at this temperature for 3 h and then at room temperature overnight. No purification of the crude product was required after standard workup with acetaldehyde and sodium hydroxide. A cursory examination of the ^{1}H spectrum revealed the

appearance of a oxymethine signal (δ_H 4.76, q, *J* = 6.4 Hz) and the splitting of the methyl signal into a doublet (δ_H 1.37, *J* = 6.4 Hz), due to the presence of the benzylic proton. The ^{13}C NMR spectrum indicated the complete loss of the carbonyl resonance (δ_C 196.7) in **4.65** and the emergence of an oxymethine signal (δ_C 70.0) for the benzylic alcohol, thus signifying the quantitative formation of the desired **4.66**. Final confirmation of the asymmetric reduction of **4.65** to **4.66** was provided by the optical rotation ($[\alpha]^{22}_D$ -48.2), which was in accordance with the reported value ($[\alpha]^{20}_D$ -42.6).[328]



Scheme 4.15 Asymmetric reduction of **4.41** using both (+) and (-)-DIP-Cl.[331;332]

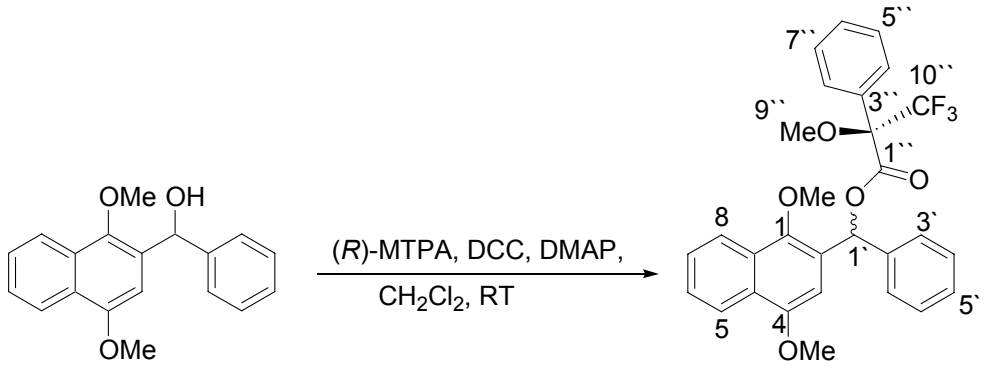
Following our success with the asymmetric reduction procedure of Brown and Ramachandran,[328;331] we attempted this asymmetric transformation on **4.41**, using both (+) and (-)-DIP-Cl to afford the two enantiomers (**4.70** and **4.71** respectively) of **4.24** (Scheme 4.15). Analysis of the ^1H and ^{13}C NMR spectrum of the crude reaction mixture, after standard workup, revealed the presence of predominantly unreacted starting material, with no clear evidence of the formation of the expected alcohol **4.70**/**4.71**, *i.e.* the continued presence of the carbonyl resonance for C-1` (δ_C 197.0) and no oxymethine signal for **4.70**/**4.71** (δ_H 6.36 and δ_C 71.0).

Repeated attempts at this asymmetric reduction of **4.41** proved unsuccessful, thus compelling us to abandon this synthetic approach as a means of accessing enantiomerically pure **4.24** and, ultimately, the quinone **4.29**.

4.2.2 Preparation of the MTPA esters of 4.24

With our lack of success at obtaining enantiomerically pure **4.24** through the asymmetric reduction of **4.41** (Scheme 4.15), we next attempted the chiral resolution of the racemate **4.24** using the modified Mosher's method,[333] using α-methoxy-α-trifluoromethylphenylacetic acid (MTPA)[334;335] as a chiral derivitazing agent. Although, MTPA is a popular reagent in both natural products and synthetic chemistry as a tool to determine the absolute configuration of compounds using NMR spectroscopy,[326;333] Jackson *et al*.[326] have highlighted the use of MTPA and camphanic acid in the derivatization of enantiomeric alcohols to diastereomers, thus allowing the use of achiral media for separation and/or NMR analysis. With this in mind, we prepared the (*R*)-MTPA ester of **4.24** by treatment of the alcohol with (*R*)-MTPA, 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in anhydrous dichloromethane at room temperature (Scheme 4.16). Semi-preparative normal phase HPLC (25% ethyl acetate, 75% hexane) of the crude reaction mixture afforded a mixture of the two (*R*)-MTPA diastereomers (**4.72**). Several attempts to separate this mixture of diastereomers on semi-preparative HPLC, using a variety of solvent mixtures, failed to give any separation of the two diastereomers. However, after a few trial injections (0.5 mg/50 μL) onto an analytical normal phase HPLC system, using gradient elution with hexane and dichloromethane, we achieved a respectable resolution (20% dichloromethane, 80% hexane) of the two diastereomers of **4.72**. Even though this technique of separation proved to be time-consuming, given that we could only load a maximum of 1 mg of the mixture onto the analytical HPLC column at a time, we reasoned that it was worth our while to persevere. At this stage, we hoped that we would be able to crystallize one of the diastereomers to enable

us to obtain a crystal structure using X-ray crystallography and accordingly assign the stereochemistry of the benzylic chiral centre. Unfortunately, all our attempts at growing crystals of either diastereomer of **4.72** using a variety of solvents (*e. g.* hexane, ethyl acetate, methanol, ethanol, diethyl ether, petroleum ether) and mixtures of these solvents failed to yield crystals suitable for X-ray analysis.



4.24 **4.72**

Scheme 4.16 (*R*)-MTPA esterification of **4.24**.

The molecular formula for each diastereomer of **4.72** was established as $C_{29}H_{25}O_5F_3$ from HRFABMS data. The ^1H (Figure 4.8) and ^{13}C NMR resonances of each diastereoisomer were fully assigned with the aid of gCOSY, gHSQC and gHMBC NMR data.

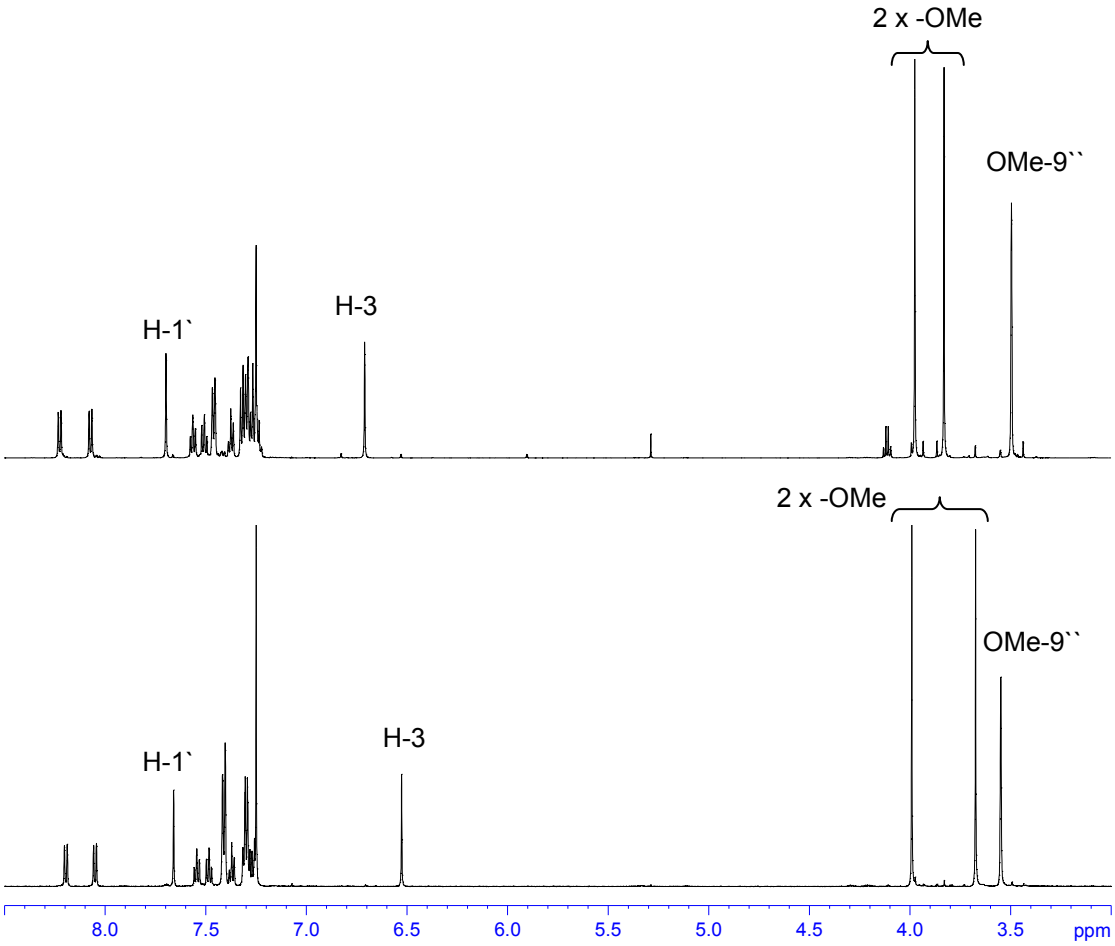
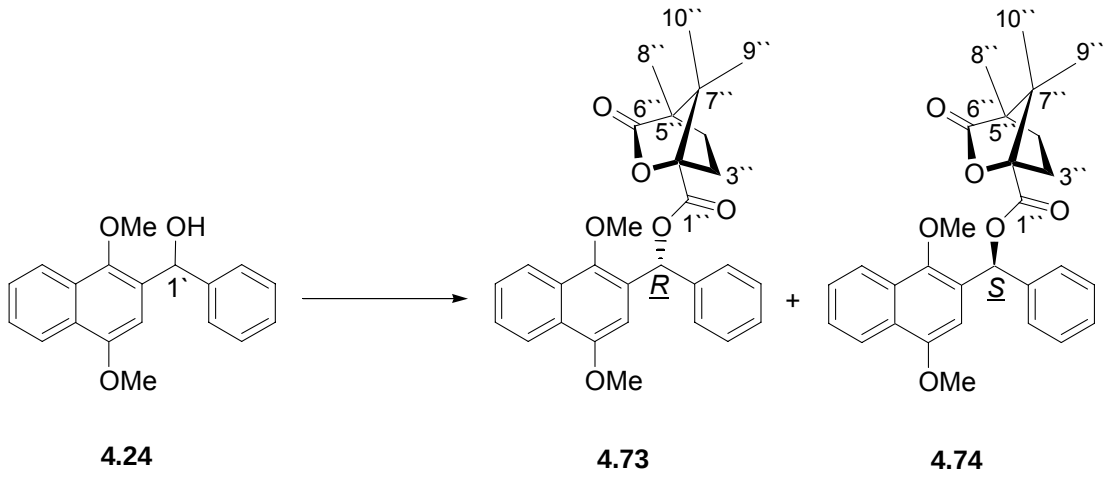


Figure 4.8 The ^{1}H NMR spectra (600 MHz, $CDCl_3$) of the two unassigned diastereomers of **4.72**.

4.2.3 Preparation of the camphanate esters of 4.24

As mentioned earlier in section 4.2.2, Jackson *et al*.[326] have reported the previous use of camphanic acid in the conversion of enantiomeric alcohols to diastereomeric esters. Camphanate esters produced from alcohols can either be separated by chromatography or fractional crystallization.[336] Again, we hoped to use X-ray analysis of one of the crystalline diastereomers to assign the absolute configuration of the chiral carbon (C-1`) eminating from the secondary alcohol **4.24**. This particular method has previously been successfully applied

in our laboratory,[38] thus giving us added confidence in adopting this approach. Compound **4.24** was accordingly derivatized with (-)-camphanic chloride, in the presence of triethylamine and 4-dimethylaminopyridine (DMAP) in anhydrous dichloromethane at room temperature to yield a mixture of the diastereomers (**4.73** and **4.74**, Scheme 4.17). Both normal and reverse phase semi-preparative HPLC, with a variety of mixed solvents (hexane and ethyl acetate, acetonitrile and water) failed to separate the diastereomeric mixture of esters **4.73** and **4.74**.



Scheme 4.17 Preparation of camphanate ester of **4.24**. *Reagents and conditions* : (-) camphanic chloride, Et_3N, DMAP, CH_2Cl_2, RT.

Since the use of a normal phase analytical column gave us good separation for the (*R*)-MTPA esters **4.72**, we proceeded to use the same chromatographic technique for the separation of the camphanate esters of **4.24**. Various solvent mixtures were investigated and finally a combination of 99% dichloromethane and 1% ethyl acetate afforded reasonable separation of the two diastereoisomers **4.73** and **4.74** (70% overall yield), and crystallization was attempted from a variety of solvents. Fine white needles (suitable for X-ray crystallography) of one of the diastereomers were obtained from the initial dissolution of the compound in hot methanol, followed by the slow diffusion of water into the solution over a few days. Single crystal analysis (Figure 4.9) established the *R*-configuration of C-1` and a molecular formula $C_{29}H_{30}O_6$ for **4.73** was established by HRFABMS. The structure of **4.73** was unequivocally

assigned using both 1D and 2D NMR spectroscopy. gHMBC correlations between the oxymethine proton H-1` (δ_H 7.60) and C-1, C-1``, C-2`, C-3` and C-7` were used to confirm the ester linkage between the camphanate moiety and the oxygen at C-1` (Figure 4.10).

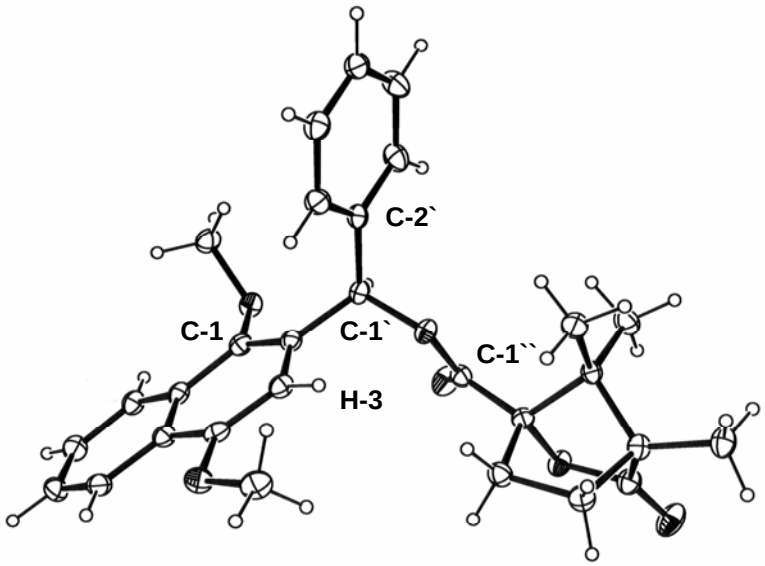


Figure 4.9 Perspective view of the diastereomer **4.73**.

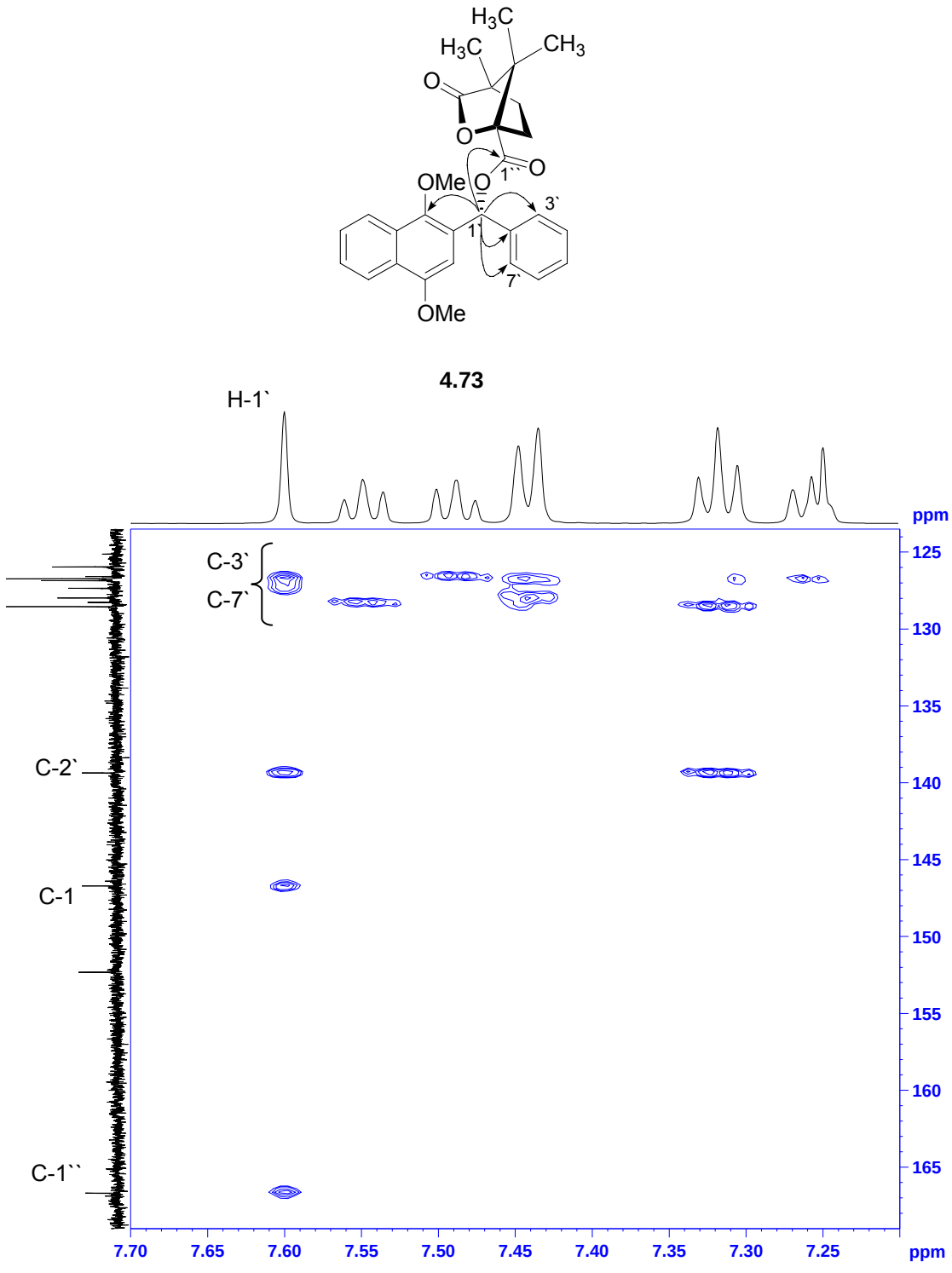
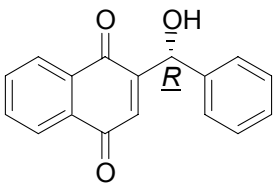


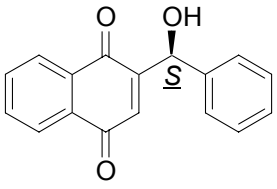
Figure 4.10 Downfield section (F1 = δ_C 123.5 – 169, F2 = δ_H 7.20 – 7.70) of the gHMBC spectrum (600 MHz, CDCl3) of **4.73**. The accompanying figure indicates the key gHMBC correlations of **4.73**.

4.2.4 Saponification of camphanate esters 4.73 and 4.74

Having successfully separated the diastereomeric camphanate esters **4.73** and **4.74** and consequently established the absolute configuration of the benzylic chiral centre for each diastereomer, we turned our attention to the hydrolysis of the ester functionality in both isomers **4.73** and **4.74** to afford the individual enantiomers **4.70** and **4.71** respectively. Accordingly, both **4.73** and **4.74** were hydrolyzed with potassium hydroxide in aqueous ethanol under reflux. The saponification was monitored by analytical TLC and quenched with acid as soon as there was no evidence of residual ester. Extraction with dichloromethane afforded the two enantiomers **4.70** in 93% yield and **4.71** in 100% yield, without the need for further purification. Both ^{1}H and ^{13}C NMR data for each enantiomer were congruent with previous spectral data obtained for the racemate **4.24** (Section 4.1.2). Standard oxidative demethylation with CAN in acetonitrile afforded the target enantiomeric quinones (**4.75** and **4.76**). A cursory examination of the ^{1}H and ^{13}C NMR spectra indicated the loss of the methoxy signals (δ_H 3.80 and 3.92) and the appearance of two carbonyl resonances (δ_c 184.9 and 185.3) as described previously in Section 4.1.1 for the corresponding racemate **4.29**. The optical rotation data obtained for **4.75** ($[\alpha]_D^{22}$ +39) and **4.76** ($[\alpha]_D^{22}$ -26) further corroborated our success in resolving the two enantiomers. To the best of our knowledge, the two enantiomers **4.75** and **4.76** have not been previously reported in the literature. The cytotoxicities and IC_{50} values for both **4.75** and **4.76** against the WHCO1 cell line were obtained and the significance of these results will be discussed in more detail in the next Chapter (Section 5.4).



4.75



4.76

4.3 Summary and conclusions

In this chapter we have reported the synthesis of the marine natural product 2-deoxylapachol, **2.44**, along with a suite of 13 synthetic naphthoquinone analogues using different synthetic strategies, *e.g*. MHE, Grignard and oxidative demethylation using cerium ammonium nitrate. Amongst this cohort of compounds, **4.19**, **4.30**, **4.31**, **4.33**, **4.46-4.48** are new while compounds **4.27**-**4.29**, **4.45**, **4.52** and **4.53** have previously been reported. All 14 compounds tested against the oesophageal cancer cell line WHCO1 and exhibited varying cytotoxicities, with 2-(1`-hydroxy-1`-phenylmethyl)-1,4-naphthoquinone (**4.29**) being the most cytotoxic (IC_{50} 1.5 μM) against WHCO1 cell line.

In our endeavor to study SAR amongst this class of naphthoquinone compounds, we successfully achieved the oxidation of **4.24** using the Ley-Griffith reagent (TPAP)[308;309] and NMO as the co-oxidant.[304] This oxidation procedure was found to be more suitable than the manganese dioxide (MnO_2) oxidation of benzylic hydroxyl groups in this class of compounds, since the latter method required a large excess (~30 eq.) of MnO_2, concomitant with a long reaction time (48 h) and the need for the final purification of the ketones *via* HPLC.

Additionally, we attempted the asymmetric reduction of the ketone **4.41** using (+/-)-DIP-Cl, in an effort to ultimately obtain the enantiomers **4.75** and **4.76**, in order to asses their cytotoxicity against the WHCO1 cell line. However, this asymmetric reduction approach was unsuccessful and we therefore turned our attention to the chiral resolution of the racemate **4.29** by preparing the (-)-camphanate esters of the dimethoxy derivative **4.24**. Separation of the resulting diastereomers followed by the crystallization of the (*R*)-camphanate ester **4.73** provided access (*via* X-ray crystallography) to the absolute configuration at the C-1` chiral centre. Subsequent saponification of the diastereomeric esters and oxidative demethylation afforded the anticipated enantiomers **4.75** and **4.76**.

Unfortunately, due to inevitable time constraints, we were unable to investigate the effect of substitution on the phenyl ring of **4.29**. Therefore for future work towards the SAR study in this class of compounds, we suggest the chlorination of the phenyl moiety of **4.29** which could potentially facilitate transport of the molecule across cell membranes and thus result in improved cytotoxicity against the oesophageal cancer cell line WHCO1.

Chapter Five

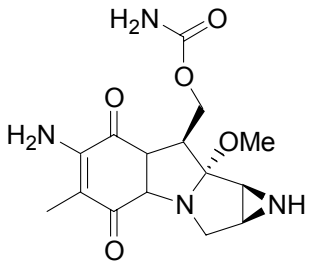
Electrochemical and Cytotoxic Investigations of Synthetic Naphthoquinones

5.1 The general mechanisms of quinone cytotoxicity

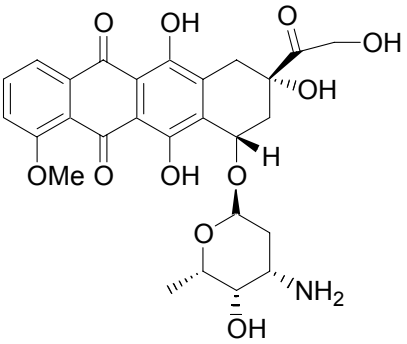
Quinones are ubiquitous in nature and comprise one of the largest classes of antitumour agents.[44-47] The anticancer activity of quinones has been extensively studied[44;47] and used as models to understand chemically induced toxicity in cellular mechanisms.[45] From a toxicological perspective, quinones possess two general mechanisms of action; redox cycling and conjugation reactions with bionucleophiles. However, the prediction of the mechanism responsible for cytotoxicity is difficult.[44;45] The conjugate addition of nucleophiles to quinones can be correlated with their electrophilic properties, which is determined by the nature of the substituents attached to the quinone moiety.[44;45] The presence of an electron-withdrawing group (*e.g.* Cl, Br, F, NO_2 or carbonyl) generally increases the conjugative addition properties of the quinone molecule.[44;45] Conversely, quinones bearing electron-donating groups (*e.g.* -NH_2, -OH and –OMe) are less likely to undergo conjugate addition reactions.[44;45] These properties are physiologically important since the degree of electrophilicity of quinones enables them to react, almost spontaneously, with thiol groups of proteins and glutathione (GSH).[44;45;337] GSH is a multifunctional peptide thiol which is a very good antioxidant within cells, and is highly abundant in the cystosol, nuclei and mitochndria.[337] GSH is involved in the protection of cells against oxidative stress, *i.e.* detoxification, and the regulation of gene expression or signal transduction through the catalyzed generation of glutathionyl conjugates by glutathione transferase enzymes (GST).[44;45;337] However, in some instances, the glutathionyl conjugates of some substituted 1,4-naphthoquinones have been reported to undergo redox cycling more readily than the original quinone.[44] GST is well-known for its crucial ability to regenerate the antioxidants, vitamins C & E, to their active forms.[337]

The second mechanism of action of quinone toxicity involves the reduction of the quinone molecule by enzymes.[45;337] This process can either involve a one or two electron reduction to yield a semiquinone (**B**) or hydroquinone (**C**) respectively (Figure 5.1).[44;45;47] The one electron reduction can be catalyzed by enzymes such as microsomal NADPH-cytochrome P-

450 reductase or mitochondrial NADH ubiquinone oxidoreductase to yield the appropriate semiquinone radical.[45;337] The resulting semiquinone radical can accept one more electron to yield the hydroquinone (**C**) or it can undergo redox cycling, *i.e.* can be oxidized to the original quinone (**A**) by molecular oxygen, to generate reactive oxygen species (ROS) as a by-product, *e.g.* superoxide anion radicals ($O_2^{\bullet-}$) and hydrogen peroxide, under aerobic conditions (Figure 5.1).[44;45;47;337] Not surprisingly, this process is reliant on oxygen and therefore persists until the system becomes anaerobic.[44] The two-electron reduction of quinones to the corresponding hydroquinone is generally catalyzed by the enzyme NADPH-quinone oxidoreductase or DT-diaphorase,[44;45] and the antitumour quinones mitomycin C (**5.1**) and adriamycin (**5.2**) are both activated by this two-electron reduction process.[45]



5.1



5.2

Under aerobic conditions, the semiquinone is basic in nature, compared to the parent quinone, and occurs as an anion radical at physiological pH 7.4, since it is still not sufficiently basic to be protonated under those conditions.[44] Superoxide radical anions are highly unstable in aqueous solution and tend to dissociate to molecular oxygen and, more importantly, hydrogen peroxide which then reacts with Fe^{2+} to generate hydroxyl radicals ($HO^{\bullet}$).[44;45] These hydroxyl radicals are extremely toxic and are believed to be responsible for the oxidative damage and, consequently, induction of programmed cell death (apoptosis) by ROS in the cell.[44;45;47]

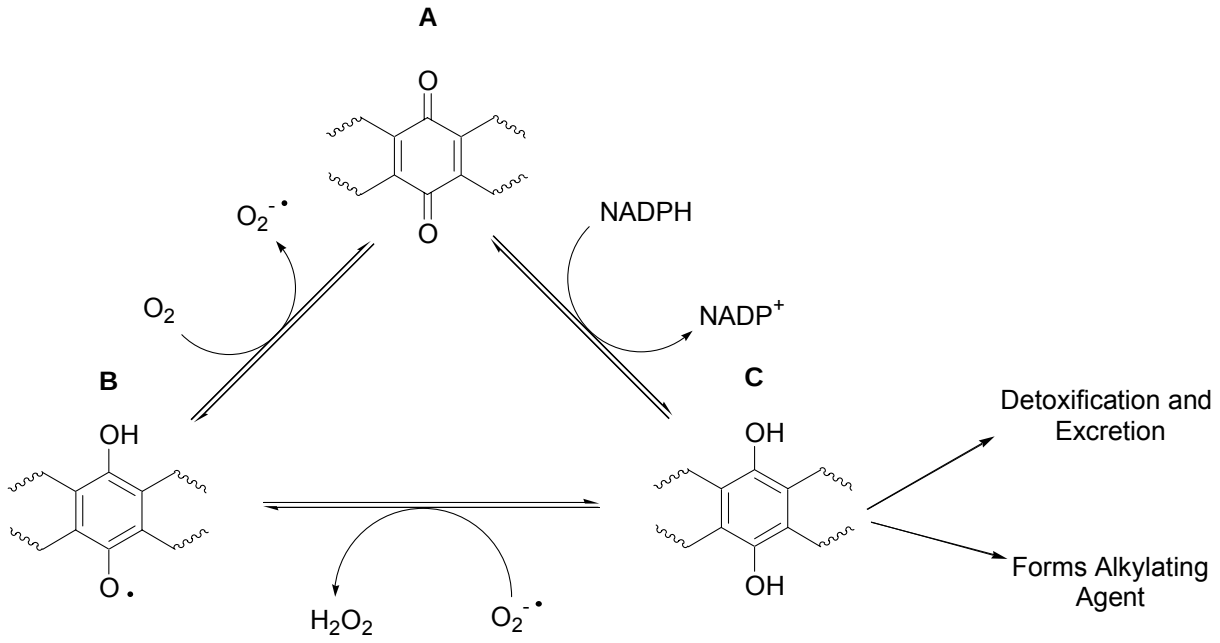


Figure 5.1 Quinone redox cycling; **A**) quinone **B**) semiquinone **C**) hydroquinone.[44]

The cytotoxicity of quinones can usually be related to their propensity to be reduced and therefore their ability to act as oxidizing agents.[44;45] Quinones are generally more cytotoxic than their hydroquinone counterparts, but their toxicity is highly dependant on the structure, stability and reduction potential of the quinone/hydroquinone pair.[44;45] The hydroquinone (**C**) can either be metabolized and excreted, but, in some cases, it undergoes rearrangement to form an alkylating agent which is more reactive than the parent quinone (**A**, Figure 5.1). This resulting alkylating agent can cause damage to the cell constituents, *e.g.* DNA and proteins, by generating ROS.[44;47]

5.2 Redox cycling and quinone cytotoxicity

In drug design, the mechanism of action of known drugs involving ROS and electron transfer is well known, while the drug interaction with the redox machinery of the cell is a relatively new field.[338] Quinones can be activated either by oxidation or reduction and can hence be

used to exploit the susceptibility of diseased cells to redox action.[338] The rationale for this strategy is to develop potential drugs that will selectively kill diseased cells, without affecting surrounding healthy cells.[338] Additionally, drugs that produce ROS are also very attractive since they can disrupt cellular homeostasis, especially in cancer cells that are subject to oxidative stress, where they tend to induce apotosis.[338] In order to determine physiological activities within structure-activity relationship studies for drug molecules, one of the most important factors to consider is the standard redox potential.[339;340]

5.2.1 Cyclic voltammetry

The study of redox reactions and measurement of standard redox potentials is generally achieved through electrochemistry.[338] Cyclic voltammetry (CV) is a popular electrochemical technique that allows the electrode potential to be scanned very rapidly, and the characterization of peak potentials and varying scan rates can be utilized to yield redox couples.[341;342] CV requires the application of a dynamic voltage to an analyte solution to yield a time-dependent characterization of a redox active system by plotting the current generated against the applied voltage.[338;341] The potential applied in CV is swept back and forth linearly with time, and the sweep direction is inverted at a chosen potential during the course of the experiment.[341] The current generated is continuously monitored and is used to examine the resulting oxidation or reduction of the surface species.[341]

From the CV obtained for ferrocene (Figure 5.2), the half-wave potential ($E_{1/2}$), which is characteristic of a particular species, can be calculated according to the following equation: $E^{0`} = E_{1/2} = (E_{pa} + E_{pc}) / 2$, where $E_{pa}$ is the anodic potential and $E_{pc}$ is the cathodic potential $E^{0`}$ is the formal potential which is corrected to the reference electrode used.

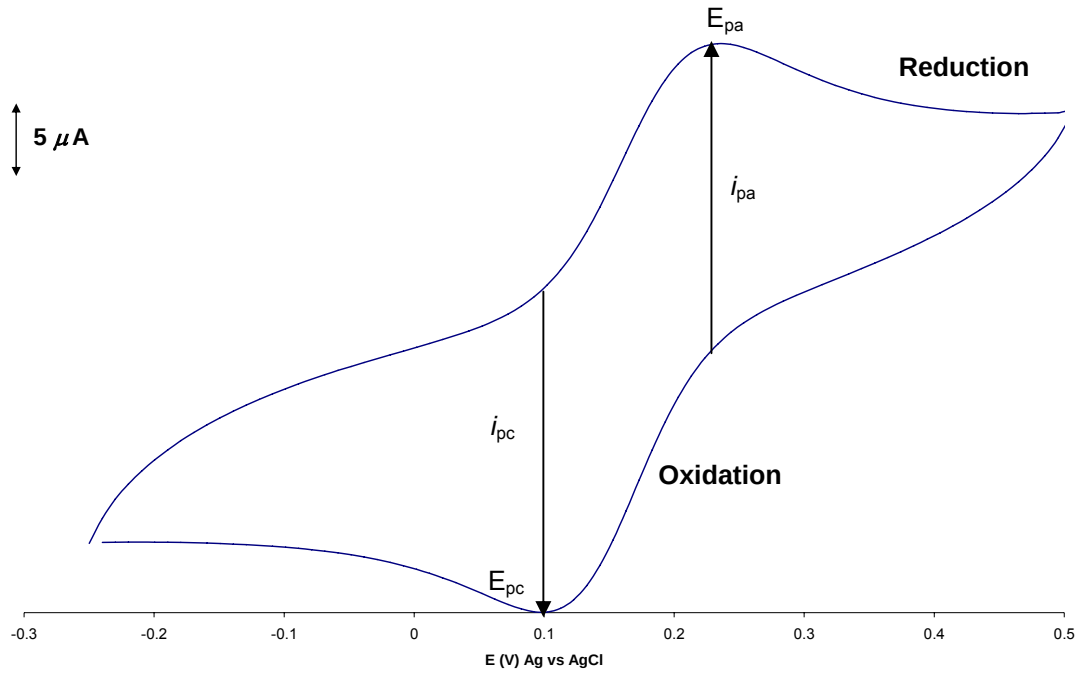


Figure 5.2 Cyclic voltammogram for ferrocene in anhydrous DMF containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte.

$\Delta E = E_{pa} - E_{pc}$, where the value of ΔE calculated indicates a one-electron or two-electron reversible process, when compared to the ΔE of a known compound, *e.g.* ferrocene which undergoes a one-electron reversible process with a ΔE of 131 mV (Figure 5.2). Therefore, if ΔE of a compound is ~ 131 mV or less, it can be assumed that it undergoes a one-electron reversible process. However, if ΔE is greater than or twice the value of ΔE for ferrocene, a two-electron quasi-reversible process is then assumed. The stability and true reversibility of the redox process for a compound can also be calculated from the current obtained from the CV (Figure 5.2), where $i_{pa}/i_{pc} = 1$.

5.2.2 Electrochemical evaluation of selected synthetic naphthoquinone analogues

The bioreductive activation of quinones is clearly very reliant on the electrochemical properties of the molecule.[338] There are several studies where definite correlations between standard redox potentials, derived from cyclic voltammetry experiments, and cytotoxic and/or inhibitory activities of quinone molecules have been reported.[49;50;338-340;343] Inbaraj *et al*.[49;50] have proposed that quinones with a high reduction potential are more cytotoxic since they favour the formation of semiquinone radicals that further generate the superoxide anion radicals $O_2^{\bullet-}$ and this has been recently further corroborated by Koyama *et al.*[339;340]

Cognizant of this, we have therefore investigated the redox properties of the cytotoxic benzylic alcohols **4.29**-**4.31** and **4.33** compared to their less cytotoxic ketone derivatives **4.45**-**4.48** respectively, and the dehydroxylated analogues **4.52** and **4.53**, in an effort to correlate the reduction potentials of these selected naphthoquinones with their cytotoxicity, thus further contributing to our SAR study of this class of compounds. Cyclic voltammetry was performed with the electrochemical analyser Autolab potentiostat PGSTAT 30 (Eco Chemie, Utretch, The Netherlands) using the General Purpose Electrochemical System data processing software (GPES, software version 4.9). A three-electrode assembly of a glassy-carbon working electrode, platinum wire auxiliary electrode and Ag/AgCl reference electrode was utilized. The glassy-carbon electrode was resurfaced with alumina and the solutions of the quinones (1 mg in 5 mL dimethylformamide) were prepared in freshly distilled dimethylformamide containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. Each solution was deoxygenated for 5 min with purified nitrogen prior to measurement and the cyclic voltammograms were recorded under nitrogen atmosphere. The redox potentials were recorded against the Ag/AgCl reference electrode and the cyclic voltammograms obtained are presented in figures 5.3-5.7.

A general trend can be observed from figures 5.3-5.6, where the oxidized quinones **4.45**-**4.48** (red CV) have a lower reduction potential than their corresponding benzylic alcohols **4.29**-**4.31** and **4.33** (blue CV). Additionally, cyclic voltammograms for **4.52** and **4.53** (Figure 5.7) were obtained and it is interesting to note that the CV for **4.53** has two distinct anodic potential peaks (E_a = -1.078 and -1.309 V).

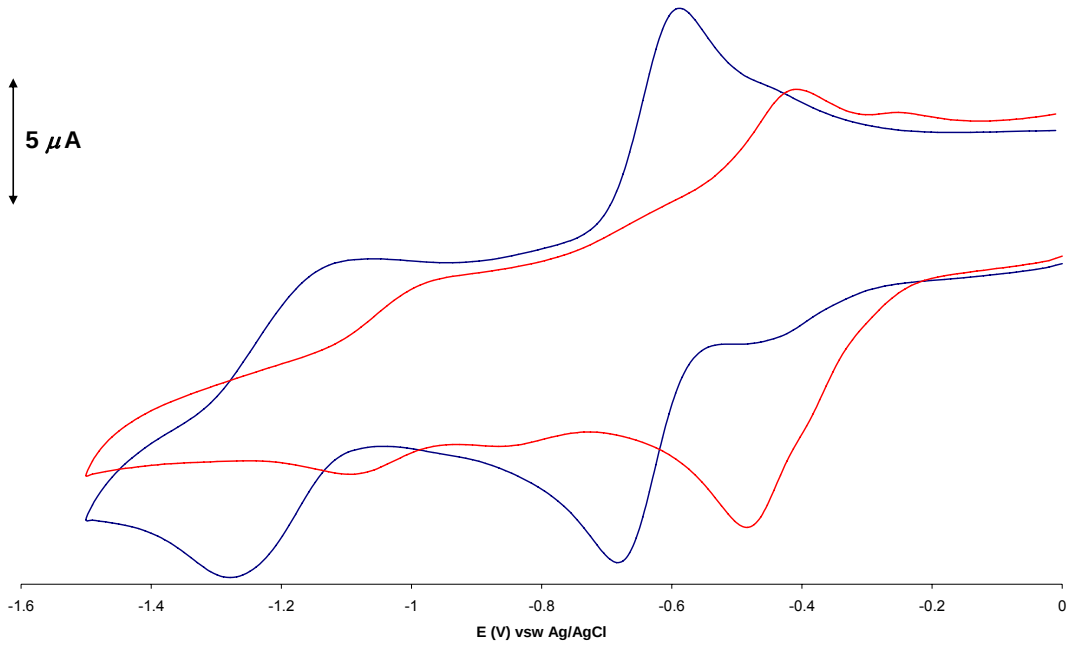


Figure 5.3 Cyclic Voltammograms of **4.29** (blue) and **4.45** (red) in anhydrous DMF containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte.

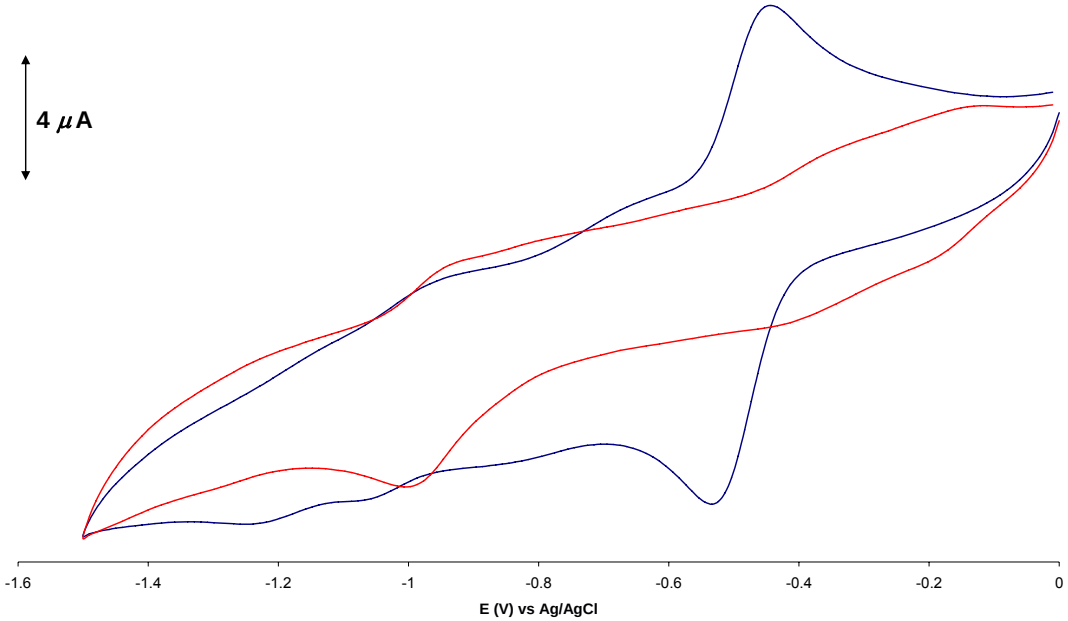


Figure 5.4 Cyclic Voltammograms of **4.30** (blue) and **4.46** (red) in anhydrous DMF containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte.

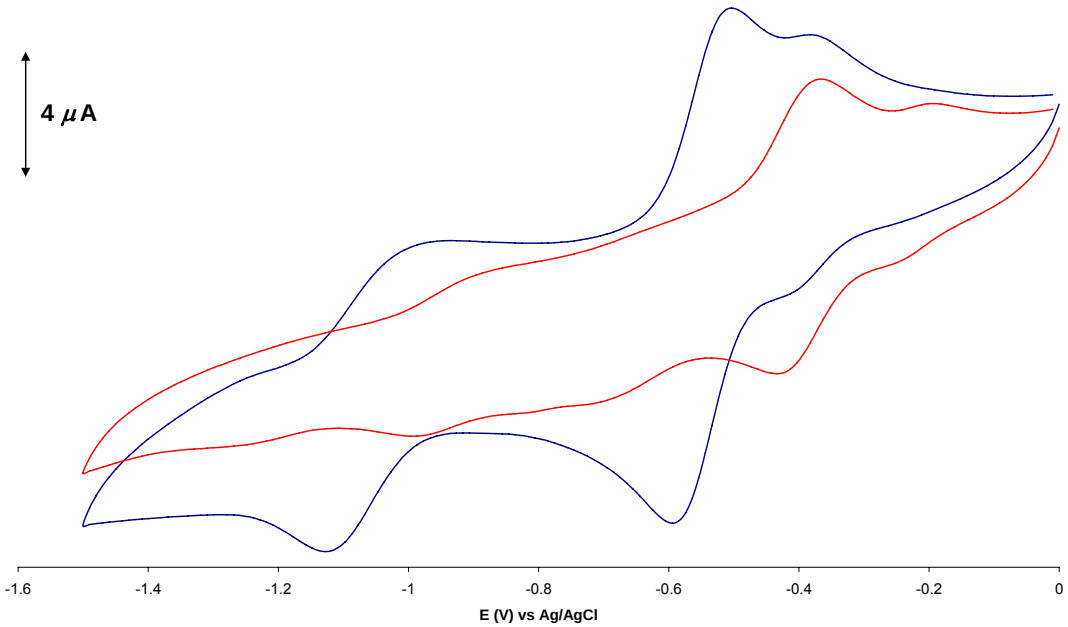


Figure 5.5 Cyclic Voltammograms of **4.31** (blue) and **4.47** (red) in anhydrous DMF containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte.

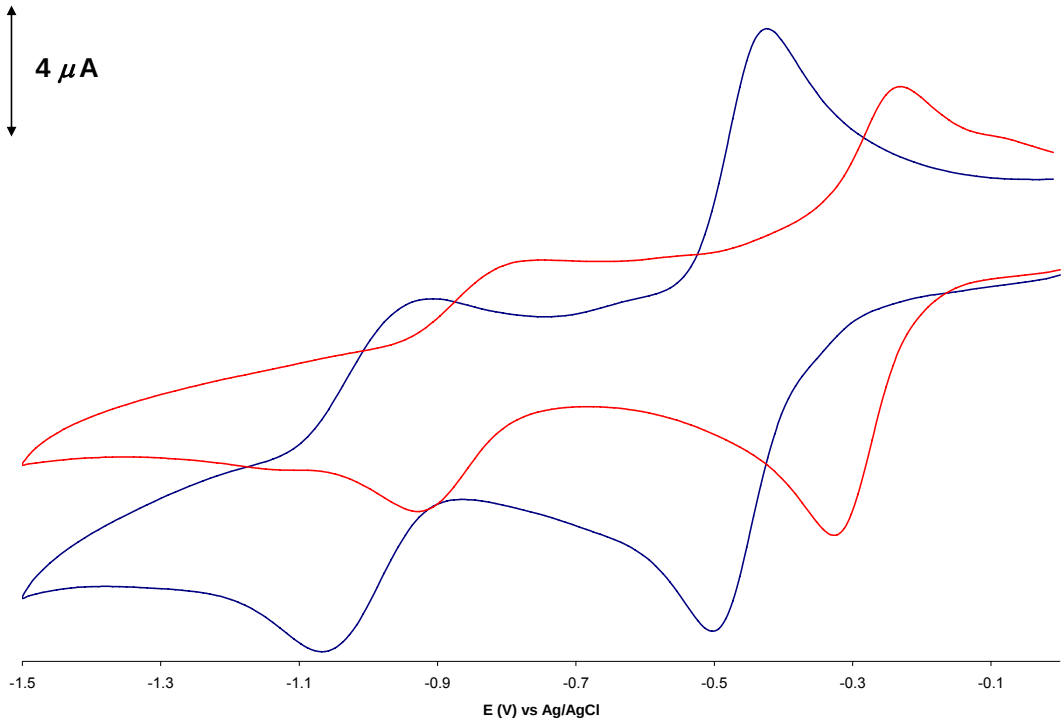


Figure 5.6 Cyclic Voltammograms of **4.33** (blue) and **4.48** (red) in anhydrous DMF containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte.

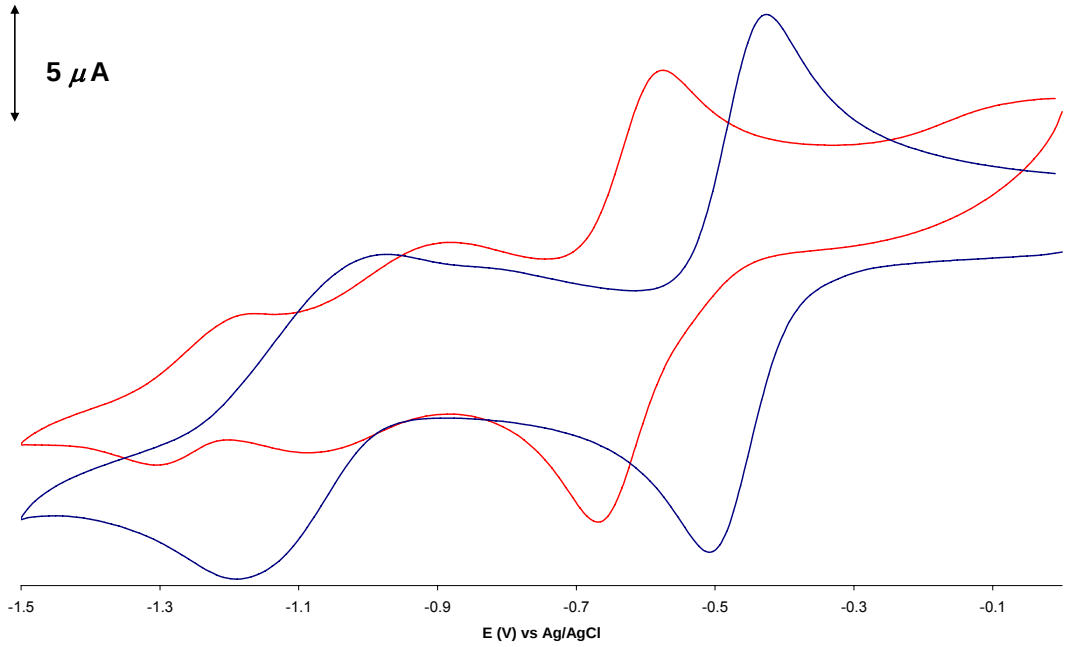


Figure 5.7 Cyclic voltammograms of **4.52** (blue) and **4.53** (red) in anhydrous DMF containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte.

The correlation between the higher reduction potential of the benzylic alcohols **4.29**-**4.31** and **4.33** (Table 5.1) and their potent cytotoxicities (Table 5.4) is in agreement with the findings of Inbaraj *et al*.[49;50] Conversely, the lower cytotoxicities of the oxidized naphthoquinones **4.45**-**4.48** (Table 5.5) coupled with their lower reduction potential (Table 5.2) further corroborates this general trend.

Table 5.1 Summary of the $E_{1/2}$ and ΔE values recorded for the benzylic alcohols **4.29**-**4.31**, **4.33** and the dehydroxylated analogues **4.52** and **4.53**.

	Compounds					
	4.29	**4.30**	**4.31**	**4.33**	**4.52**	**4.53**
$E_{1/2}$ (V)	-1.193	-1.098	-1.037	-0.988	-1.274	-1.309, -1.078
ΔE (mV)	252	212	212	151	211	151, 212

Additionally, the redox cycling of the benzylic alcohols **4.29**-**4.31** (ΔE 212-322 mV, Table 5.1) appears to occur through a quasi-reversible two-electron process, whilst the oxidized compounds **4.45**-**4.48** (ΔE 101-182 mV, Table 5.2) appear to under a reversible one-electron process.

Table 5.2 Summary of the $E_{1/2}$ and ΔE values recorded for the benzylic ketones **4.45**-**4.48**.

	Compounds			
	4.45	**4.46**	**4.47**	**4.48**
$E_{1/2}$ (V)	-1.098	-1.007	-0.987	-0.927
ΔE (mV)	182	101	152	111

5.3 Cytotoxicity of the metabolites from the South African nudibranch *L.millecra*.

As mentioned previously, through our collaborative effort with the Division of Medical Biochemistry at the Medical School of the University of Cape Town, our research group has identified a group of marine *ortho*-prenylated toluquinones and hydroquinones **1.24**-**1.29** that have shown significant cytotoxic activity against the oesophageal cancer cell line WHCO1, with **1.26** being the most active (IC_{50} 9.5 μM).[39;40] Whibley *et al*.[39] have also demonstrated that this group of compounds initiate apoptotic cell death of the WHCO1 cells through the initial production of ROS, followed by induction and activation of the c-Jun (AP1) signalling pathway, ultimately leading to a cell cycle block, activation of caspase-3 and apoptosis of the oesophageal cancer cells. Whibley *et al*.[39] also noticed a strong inverse correlation between the IC_{50} values of **1.24**-**1.29** and the production of ROS and, from their findings, have tentatively concluded that ROS generating ability of **1.26** is the main reason for its capacity to induce apoptosis in cancer cells.

The cytotoxicity of the linear triprenylquinones and hydroquinones **1.24**-**1.29** were tested against the oesophageal cancer cell line (WHCO1), using an MTT assay (Section 5.3.2), and indicated that compounds **1.26** and **1.27** had the lowest IC_{50} values (9.5 and 12.9 μM respectively, Table 5.3) and therefore were the most active against the WHCO1 oesophageal cancer cell line.[39] The most active compound from this screening, **1.26**, was also screened against another oesophageal cancer cell line WHCO6, a non-malignant breast epithelial cancer cell line MCF12 and the two cervical carcinoma cancer cell lines ME180 and SiHa. Compound **1.26** was found to be moderately active against most of these cell lines and more cytotoxic (IC_{50} = 5.8 μM) against the WHCO6 oesophageal cancer cell line.[39]

Table 5.3 Summary of the IC_{50} values of the linear triprenylquinones and hydroquinones **1.24**-**1.29** isolated from the *L. millecra* against the oesophageal cancer cell line WHCO1.[39]

	Compounds tested					
	1.24	**1.25**	**1.26**	**1.27**	**1.28**	**1.29**
IC_{50} (μM)	37.9	83.3	9.5	12.9	42.7	32.7
95% C.I.[a]	37.7-38.0	82.1-84.5	9.4-9.5	12.5-13.3	42.6-42.7	32.5-33.0

[a] Confidence Interval.

5.3.1 Significance and mechanism of ROS production

ROS are involved in regular cellular activities and the overall ROS levels are regulated by enzymes such as superoxide dismutase (SOD) in order to maintain physiological homeostasis.[338] However, when cells are exposed to increased levels of ROS, they experience a disruption in redox signalling and control, while causing damage to the cell constituents, *e.g.* DNA, lipids and proteins, and this condition is known as oxidative stress.[44;338] Cellular mechanisms exist within cells to deal with high levels of ROS, *e.g.* SOD is an antioxidant enzyme that catalyzes the scavenging of superoxide anion radicals,[338] and is often used as an indication of whether cancer cells are undergoing oxidative stress.[49] Cells also generally attempt to repair the oxidative damage caused to the various cellular components, and this is associated with a cell cycle block, which in turn can lead to cell death if the damage is irreparable.[39] After treatment with compound **1.26**, the WHCO1 cells experienced cell cycle arrest and apoptosis, which are both typical of ROS-induced cell death.[39]

Whibley *et al*.[39] have shown that **1.26** specifically activates the JNK/c-Jun pathway in the WHCO1 cells to subsequently induce apoptosis. c-Jun is both a pro- and anti-apoptotic protein, depending on the cellular environment and other signals, that enables cells to deal with damage caused by ROS production by regulating antioxidant genes.[39] The process of

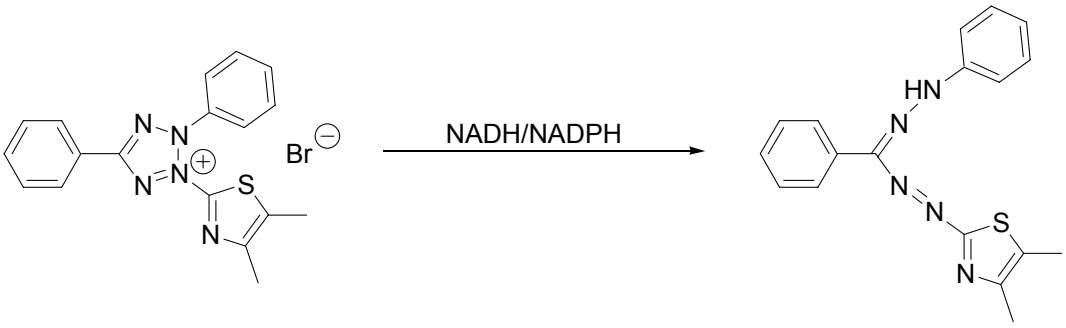
apoptosis causes the activation of a cascade of intracellular cysteine proteases known as caspases, of which caspase-3 has been shown to play an intrinsic role in the final stages of apoptosis.[270;344] The relationship between caspase and JNK is still elusive, although it has been suggested that they both are crucial mediators in the process of apoptosis.[270;344] As mentioned in Chapter 1, recent emphasis has been placed on the identification of molecular markers, *e.g*. growth factor receptor pathways and protein kinase pathways that include transcription factors such as the c-Jun (AP1), as potential targets in SCOC chemotherapy.[30;35]

5.3.2 The Crystal Violet and MTT anticancer assays

The crystal violet bioassay utilizes a crystal violet protein staining dye to stain cell proteins with a deep purple colour and the intensity of the colour increases proportionally as the cells progress through the G_1/S phase, divide and continue to proliferate. The assay makes use of a 96-well plate with an estimated 1500 oesophageal cancer cells (WHCO1) per well. Varying concentrations (0.1, 1.0, 10 and 100 μM) of the test compounds in DMSO were then added to the WHCO1 cells. The cells were incubated for 48 h before the media was discarded and the remaining living cells were stained with the crystal violet dye. The dye was solubilised with 50% acetic acid and the plates read at 595 nm on an Anthos microplate reader. The crystal violet assay is a cheap and relatively quick method of screening many compounds simultaneously, although it cannot be used as a quantitative method.

The MTT assay was therefore used to further investigate the cytotoxicity of the synthetic compounds and to calculate their IC_{50} values. This method is usually utilized as an indication of the cell viability, since metabolizing cells can reduce the yellow water-soluble salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the purple formazan (Scheme 5.1). The MTT assay, unlike the crystal violet assay, can be used to quantify cell death and

is, therefore, generally the assay of choice used to evaluate cytotoxicity of chemical compounds.



MTT (yellow salt) **Formazan (purple)**

Scheme 5.1 The reduction of the yellow MTT salt to the formazan by NADH/NADPH.

The same 96-well plate approach to the crystal violet assay was adopted for the MTT assay, except that the cells are incubated for 52 h. The amount of MTT metabolized in each well is directly proportional to the number of living cells still present. After the cells are read at 595 nm using the same microplate reader, the IC_{50} value for each compound can be calculated by plotting its log concentration [μM] against the corrected optical density reading at 595 nm.

5.4 Cytotoxicity of synthetic naphthoquinones

Eight of the synthetic naphthoquinones (**2.44**, **4.19**, **4.27**-**4.31** and **4.33**) discussed in Chapter 4 were sent to our collaborators at the Department of Medicinal Biochemistry at the University of Cape Town for screening against the oesophageal cancer cell line WHCO1. A dose response curve was obtained using the MTT assay and IC_{50} values were determined against the WHCO1 oesophageal cancer cell line and are summarized in Table 5.4. All the compounds tested showed high to moderate cytotoxicity against the oesophageal cancer cell line. The phenyl compound **4.29** was found to be the most active against the WHCO1 cancer cell line with an IC_{50} of 1.5 μM, while the marine natural product **2.44** was found to be the

least active (IC_{50} of 15.0 μM) in this panel of compounds. The naphthyl derivative **4.31** also exhibited good activity (IC_{50} of 2.4 μM) against the WHCO1 cell line.

Table 5.4 Summary of the IC_{50} values of compounds tested against the WHCO1 cell line.

	Compounds tested							
	2.44	**4.19**	**4.27**	**4.28**	**4.29**	**4.30**	**4.31**	**4.33**
IC_{50} (μM)	15.0	5.2	6.4	4.3	1.5	4.8	2.4	10.9
95% C.I.[a]	13.2-16.8	4.9-5.4	6.0-6.8	3.1-5.5	1.1-1.9	2.4-7.2	1.3-3.4	9.8-12.0

[a] Confidence Interval.

The biological activities of oxidized products **4.45**-**4.48** and the phenyl analogues **4.52** and **4.53** were evaluated against oesophageal cancer cell lines and the IC_{50} values obtained summarized in Table 5.5. The IC_{50} values for all four oxidized compounds **4.45**-**4.48** tested showed a marked increase, indicating a significant decrease in cytotoxicity against the WHCO1 cancer cell line, with **4.47** having no activity at all. The IC_{50} for **4.52** was found to be 21.6 μM while that of **4.53** was 11.7 μM. The hydroxyl group in the benzylic position therefore appears important for increased cytotoxicity against the WHCO1 cell line.

Table 5.5 Summary of the IC_{50} values of compounds tested against the WHCO1 cell line.

	Compounds tested					
	4.45	**4.46**	**4.47**	**4.48**	**4.52**	**4.53**
IC_{50} (μM)	63.4	83.7	No activity	96.9	21.6	11.7
95% C.I.[a]	54.6-73.6	78.2-89.4		92.3-101.7	19.6-23.6	10.6-12.8

[a] Confidence Interval.

As discussed previously in Section 4.2, in order to further our SAR study, we resolved the two enantiomers **4.75** and **4.76** of the corresponding racemate **4.29**. The cytotoxicities of the two

enantiomers were evaluated against the WHCO1 cell line and were found to exhibit similar activities (IC_{50} for **4.75** = 4.3 μM and **4.76** = 3.8 μM) as the racemate, suggesting that the absolute configuration at the benzylic position does not determine the cytotoxicity of the **4.29**. Additionally, our collaborators investigated the mode of action of **4.29** and its enantiomers against the WHCO1 cell line, by conducting a Western Blot analysis which involved the treatment of the oesophageal cancer cell line with all three chosen compounds. These blots were then probed for both poly adenosine-diphosphate ribose polymerase (PARP) and c-Jun, and the observed results after regular intervals, *i.e.* 6h, 24, and 48 h, are shown in Figure 5.8. Cleavage of the PARP into two distinct molecular fragments (116 kDa and 85 kDa) serves as a marker for apoptosis.

The results obtained indicated PARP cleavage after 24 h (Figure 5.8B) in, not only the WHCO1 cells treated with the positive control doxorubicin (dox), but also in experiments run with all three compounds, **4.29**, **4.76** and **4.75**, at their highest treatment concentration (20 μM). However, only slight PARP cleavage was observed in the lower treatment concentrations after 48 h (Figure 5.8C). The results obtained showed that c-Jun is induced in a concentration-dependent manner for all three compounds after the different incubation periods (Figure 5.8). The highest expression of c-Jun is after 6 h (Figure 5.8A), while a decrease in the c-Jun expression levels is noticed after 48 h (Figure 5.8C). These results demonstrate the activation of the JNK/c-Jun signalling pathway and induced cell death in WHCO1 cells *via* apoptosis, in an apparently identical manner to the marine natural product **1.26**.[39;139] This is the first example of JNK/c-Jun activation by naphthoquinone compounds.

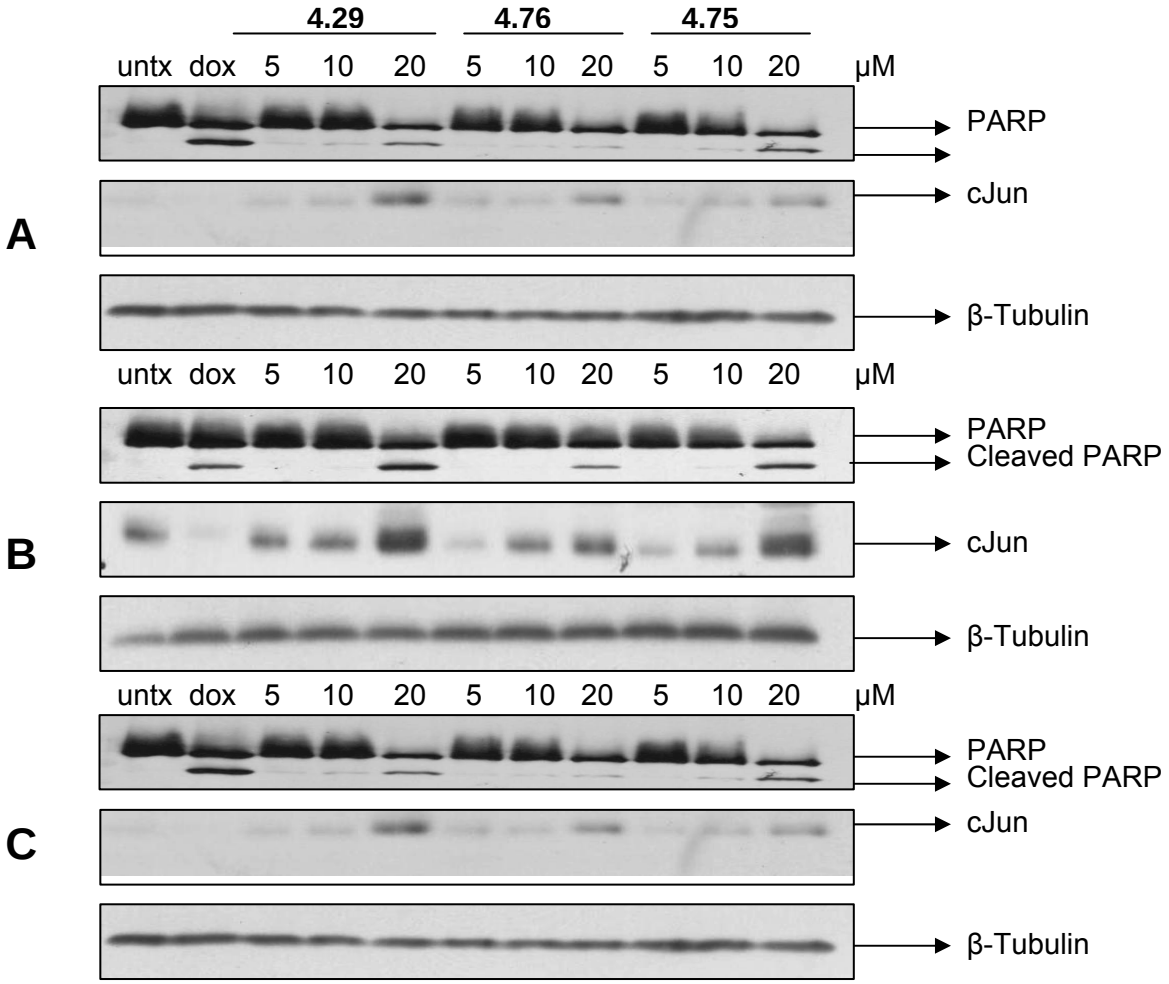
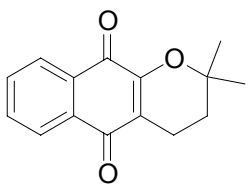


Figure 5.8 Western Blot analysis of WHCO1 cells treated with varying concentrations of racemic **4.29**, **4.76** and **4.75** at A) 6 hours B) 24 hours and C) 48 hours.

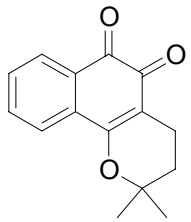
5.4.1 Cytotoxicity of some naturally occurring naphthoquinones against WHCO1

A sample of the natural product lapachol, **4.1**, and six of its structural analogues (**5.1**-**5.6**), were generously donated to us by Dr. A. V. Pinto from Núcleo de Pesquisas de Produtos Naturais at the Universidade Federal do Rio de Janeiro in Brazil. The compounds were all

tested against the oesophageal cancer cell line WHCO1 and the IC_{50} values obtained are tabulated in Table 5.6.



5.1



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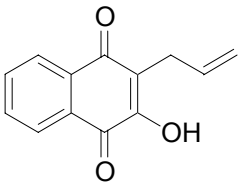

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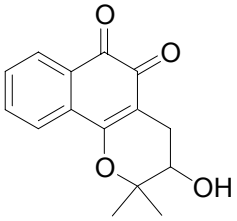

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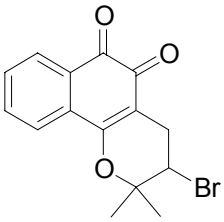
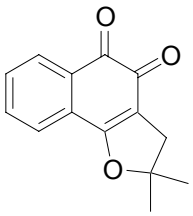


5.5



5.6

Table 5.6 Summary of the IC_{50} values of Brazilian compounds tested against WHCO1.

	Compounds tested						
	4.1	**5.1**	**5.2**	**5.3**	**5.4**	**5.5**	**5.6**
IC_{50} (μM)	24.1	28.7	1.6	Not Active	6.5	1.8	2.4
95% C.I.[a]	13.4-43.4	20.1-39.8	1.3-1.9		6.0-6.9	1.3-2.3	2.2-2.6

[a] Confidence Interval. Note the wide ranging C.I. values for **4.1** and **5.1** may be related to the intense colouration of these compounds interfering with the interpretation of the MTT results.

From the results obtained, both **4.1** and α-lapachol (**5.1**) were found to be only moderately cytotoxic (IC_{50} 24.1 and 28.7 μM respectively) against the WHCO1 cell line, while C-alil-lausona (**5.3**) exhibited no activity at all (Table 5.6). Interestingly, β-lapachone (**5.2**) exhibited the highest activity against the WHCO1 cell line with an IC_{50} value of 1.6 μM, followed very closely by Br-β-lapachone (**5.5**) and nor-β-lapachone (**5.6**) with IC_{50} values of 1.8 and 2.4 μM

respectively. OH-β-lapachone (**5.4**) also showed relatively strong cytotoxicity (IC_{50} 6.5 μM) against WHCO1.

The cytotoxicity results obtained for these Brazilian natural products are very interesting, especially the cytotoxicity of **5.2** against WHCO1 which is comparable to that established for **4.29**. Lapachol, **4.1**, is structurally similar to the marine natural product 2-deoxylapachol, **2.44**, and the relevance of these results to our research is discussed in the following section.

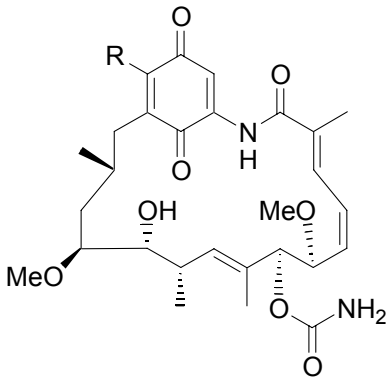
5.5 Lapachol and β-lapachone as potential anticancer drugs

Lapachol, **4.1**, is a natural naphthoquinone known as 'Pau D'Arco' in Brazil and was first isolated from the tree *Tabebuia avellanedae*.[267;271;345;346] Since it's discovery in the late 1950's, **4.1** has been used in traditional medicine for cases of stomatitis, inflammatory processes and, more recently, against cancer,[267;271;345] and a cohort of related structural analogues of **4.1** have been exhaustively studied over the last decade.[253;260-267] The first reported anticancer evaluation of lapachol was by Rao *et al.*[273] in 1968, followed by the advancement of this compound to phase I clinical trial in 1974.[347] However, this clinical trial was discontinued due to the lack of therapeutic effect with **4.1** without side effects.[347] Lapachol is currently licensed in Brazil for general clinical practice as a carcinostatic drug[346] and several studies pertaining to the anticancer properties of **4.1** have been reported,[252;267;268;272;345;348;349] along with various synthetic analogues[237;260-262;264-266;269;350;351] and two SAR studies that have also been conducted on lapachol and some of its analogues.[350;352] Recently, a review of **4.1** has been presented by Hussain *et al*.[271] Despite its reported significant antitumour properties, **4.1** is in fact not a very good anticancer drug in the clinic, but can, however, be utilized as a molecular template for the synthesis of analogues in order to establish a SAR understanding in this class of compounds in an effort to develop improved pharmacologically active compounds against cancer cells.[345]

β-Lapachone, **5.2**, is a naturally occurring naphthoquinone, similar in structure to **4.1**, that is derived from the same tree as lapachol.[270;271;345;353] β-Lapachone has demonstrated interesting molecular target activity in anticancer research[270;345;353;354] and is currently in phase II clinical trials in the USA for the treatment of advanced solid tumours.[8;9] Compound **5.2** has been shown to induce apoptosis in cancer cells,[270;345] and, more importantly, Lee *et al.*[270] have recently shown that β-lapachone-induced apoptosis in the human bladder carcinoma T24 cell line is partly mediated by an increases in caspase-3 activity. β-Lapachone has also been shown to induce apoptosis in cancer cells through the activation of c-Jun signalling pathway.[345] Hussain *et al*.[271] have reviewed some of the reported anticancer properties of **5.2**. Of interest to us is that we were able to obtain similar cytotoxicities to β-lapachone with the much simpler and more accessible synthetic analogue **4.29** (Tables 5.4 and 5.6).

5.5.1 The role of the heat-shock protein Hsp 90 and its inhibitors in anticancer research

As mentioned previously in section 3.1, 1,4-naphthoquinones have been recently evaluated as being potential inhibitors of the heat-shock protein 90 (Hsp90),[254] which has become a novel molecular target in cancer research.[141;255-259] Hsp90 is a key component of multichaperone complex that is required for stress-survival response, and is responsible for the refolding of denatured proteins and the conformational maturation of a variety of signalling proteins into a biologically active three-dimensional structures.[140;255;259] The Hsp90 protein folding machinery plays a crucial role in the development and progression of transformed and/or mutated cellular mechanisms.[141;255;259] The understanding of the role of Hsp90 in promoting cellular malignant transformation is mostly derived from studies of the Hsp90 inhibitors such as the natural product geldanamycin (**5.7**) and its derivative 17-allylamino-17-dimethoxy-geldanamycin (17-AAG, **5.8**).[141] Hsp90 inhibition is more prominent and also selectively toxic in cancer cells compared to normal cells.[141;255]



5.7 R = OMe
5.8 R = $NHCH_2CH{=}CH_2$

Geldanamycin was the lead compound in the search for Hsp90 inhibitors but proved to have several pharmacological limitations and was found to be too toxic for human clinical use.[140;141] However, compound **5.8**, the structural derivative of **5.7**, exhibited a more appealing and favourable toxicity profile against Hsp90 compared to its parent structure.[140;141] Compound **5.8** entered clinical testings in 1999 and is now currently in phases I and II clinical trials in patients with advanced cancer.[140;141] Several novel synthetic Hsp90 inhibitors have been developed, using diverse chemical scaffolds, and are currently undergoing phase I/II clinical trials as anticancer drugs.[141;256]

In our endeavour to identify a potential novel lead compound with significant anti-oesophageal activity, our collaborators have tested the activity of the South African marine natural product **1.26** in combination with the known Hsp90 inhibitor **5.8** against the WHCO1 cell line and, to our surprise, have recorded a 100-fold increase in the cytotoxicity of **1.26**.[139] Similar results, however, were not obtained with **4.29**,[139] suggesting that the triprenylated side chain in **1.26** (Section 1.4) plays a significant role in the activation of 17-AAG, **5.8**.

This finding therefore formed the rationale behind the attempted total synthesis of **1.26** described in Chapter 3 and the syntheses of the naphthoquinones described in Chapter 4

were in an effort to improve on the cytotoxicity of **1.26**, while attempting to shed some light on the SAR within this class of compounds.

5.6 Summary and conclusions

The cohort of synthetic naphthoquinones generated in our research have contributed to the overall SAR study in this class of compounds, especially when compared to the cytotoxicities of the marine natural products **1.26** and **2.44**, as well as the Brazilian plant-derived metabolites **4.1** and **5.2**, against the WHCO1 cell line. The study revealed that the 2-(1`-hydroxy-1`-phenylmethyl)-1,4-naphthoquinone (**4.29**) was the most cytotoxic (IC_{50} 1.5 μM) against WHCO1 cell line, while the naphthyl analogue **4.31** was the second most active compound with an IC_{50} value of 2.4 μM. The cytotoxicity of **4.29** represents a significant improvement over that observed for the marine natural products **1.26** (IC_{50} 9.5 μM) and **2.44** (IC_{50} 15.0 μM).

The oxidized analogues were found to be significantly less cytotoxic against the oesophageal cancer cells compared to their benzylic alcohol counterparts. Interested in exploring the contribution of the benzylic alcohol to the observed increased cytotoxicity, we investigated the redox properties of selected compounds. The study revealed that the benzylic alcohols **4.29**-**4.31** had a higher reduction potential compared to their oxidized naphthoquinone analogues **4.45**-**4.48**, which can be correlated to their respective cytotoxic values, as proposed by Inbaraj *et al*.[49;50]

Interestingly, the phenyl analogue **4.29** was found to have similar activity against the WHCO1 cell line as β-lapachone, which is currently in phase II clinical trials for the treatment of advanced tumours. Compound **4.29** has also been shown to activate the same JUNK/c-Jun signalling pathway and apoptotic-induced cell death in WHCO1 cells as the marine natural

product **1.26**. Additionally, **4.29** is synthetically more accessible than either **1.26** or **5.2** and potentially a lead compound in our search for new and more effective chemotherapeutic agents against oesophageal cancer.

Chapter Six

Experimental Details

6.1 General Experimental Procedures

6.1.1 Analytical

NMR spectra were acquired using standard pulse sequences on Bruker Avance 400 MHz and 600 MHz Avance II spectrometers. Chemical shifts are reported in ppm and referenced to residual protonated solvent resonances. Coupling constants are reported directly from the NMR spectra and corresponding coupling constants have not been matched. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at the sodium-D line (589 nm). Following standard protocol, the concentration of solutions used to determine optical rotations is expressed in g/100 mL. Infra-red spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer and Digilab FTS 3100 Excalibur HE Series with compounds as films (neat) on NaCl discs. Low resolution mass spectra were recorded on a Finnigan GCQ spectrometer at 70 eV. GC was performed using a Duraband DB-1 non-polar column. High resolution fast atom bombardment mass spectra (Micromass 70-70E spectrometer) were obtained by Prof. L. Fourie of the Mass Spectrometry Unit, Northwest University, Potchefstroom, South Africa. High-resolution mass spectrometry was performed on a Waters API Q-TOF Ultima instrument using electron-spray ionization in the positive ion mode (ESI+) by the University of Stellenbosch Central Analytical Facility.

6.1.2 Chromatography

General laboratory solvents were distilled from glass before use. Analytical normal phase thin layer chromatography was performed on DC-Plastikfolien Kieselgel 60 F_{254} plates and analytical reverse phase thin layer chromatography was performed on DC-Ferigplatten RP18 F_{254} plates. Plates were viewed under UV light (254 nm) and developed either with I_2, by spraying with 10% H_2SO_4 in MeOH followed by heating or using Ehrlich's reagent (5% dimethylaminobenzaldehyde in EtOH). Flash chromatography was performed using Kieselgel

60 (230-400 mesh) silical gel. Normal phase semi-preparative HPLC separations were performed on a Whatman Magnum 9 Partisil 10 column and normal phase analytical HPLC separations were performed on a Lux 5u Cellulose-1 0.25 *μ*m column using a Spectra-Physics Spectra-Series P100 isocratic pump and a Waters 410 Differential Refractometer. Reverse phase semi-preparative HPLC separations were performed on a Phenomenex Luna 10 *μ*m C18 column using a Spectra-Physics IsoChrom LC pump and a Waters R401 Differential Refractometer.

6.1.3 Synthesis

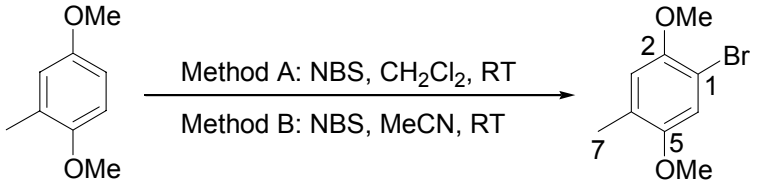
All reactions requiring anhydrous conditions were conducted in either flame-dried or oven-dried apparatus under an atmosphere of dry argon/nitrogen or using an anhydrous calcium chloride drying tube. Dry solvents were prepared by standard procedures as described by Perin and Armarego[355] and stored over appropriate drying agent under an atmosphere of dry nitrogen. Immediately prior to their use in dry reactions, Et_2O, THF and C_6H_6 were distilled from Na/benzophenone ketyl, while CH_2Cl_2, MeCN and Et_3N were distilled from calcium hydride. Organic extracts were dried over anhydrous $MgSO_4$ or $NaSO_4$. All reactions were magnetically stirred.

6.1.4 Computer modelling

Theoretical models were constructed and underwent structural optimization using DFT methods, followed by full frequency analyses to confirm that the final structures were true stationary points. All DFT calculations (energy optimizations and frequency analyses) were performed using the Gaussian 03[232] suite of algorithms with the 6-31G(d) vector basis set and the B3LYP[233;234] energy gradient correcting functional. Models were constructed and visualized using DS Visualizer.[234]

6.2 Chapter Three Experimental

*6.2.1 Bromination of **3.8***



3.8 **3.1**

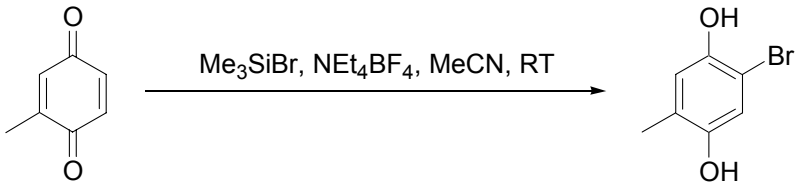
Method A :[154] *N*-bromosuccinimide (2.20 g, 12.4 mmol) was added to a solution of **3.8** (1.58 g, 10.4 mmol) in anhydrous CH_2Cl_2 (100 mL) and stirred overnight at RT. The solvent was then removed under reduced pressure, taken up in CH_2Cl_2 (25 mL) and washed with sodium sulfite solution (25 mL), followed by water (25 mL) before being dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a white solid (4.20 g). Flash chromatography of the crude product (10% ethyl acetate, 90% hexane), followed by recrystallization from hexane yielded pure 1-bromo-2,5-dimethoxytoluene (**3.1**, 1.20 g, 5.2 mmol, 50%) as white plates.

1-bromo-2,5-dimethoxytoluene (3.1) : white plates (from hexane); mp 82-86 °C, lit.[356] 81-82 °C; IR ν_{max} 2945, 2833, 1505, 1217, 1037, 866 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.98 (1H, s, H-3), 6.73 (1H, s, H-6), 3.83 (3H, s, OMe-5), 3.77 (3H, s, OMe-2), 2.17 (3H, s, H_3-7); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.2 (q_c, C-5), 149.7 (q_c, C-2), 126.8 (q_c, C-4), 115.4 (CH, C-6), 115.2 (CH, C-3), 108.0 (q_c, C-1), 57.0 (CH_3, 2-OMe), 56.1 (CH_3, 5-OMe), 16.3 (CH_3, C-7); EIMS *m/z* (rel. int.) 231 [M^+] (17), 230 (95), 217 (62), 216(7), 215 (43); HRFABMS *m/z* 229.9943 (calcd for $C_9H_{11}O_2Br$ [M^+], 229.9942).

Method B :[153] *N*-bromosuccinimide (4.40 g, 24.8 mmol) was added to a solution of **3.8** (3.15 g, 20.7 mmol) in 200 mL of MeCN and stirred overnight at RT. The solvent was then removed under reduced pressure, taken up in CH_2Cl_2 (50 mL) and washed with sodium sulfite solution (50 mL), followed by water (50 mL) before being dried over anhydrous $MgSO_4$ and

concentrated under vacuum to give a white solid (8.56 g). Flash chromatography of the crude product (10% ethyl acetate, 90% hexane) yielded pure **3.1** (3.94 g, 17.0 mmol, 82%).

*6.2.2 Reductive bromination **3.9***[156]

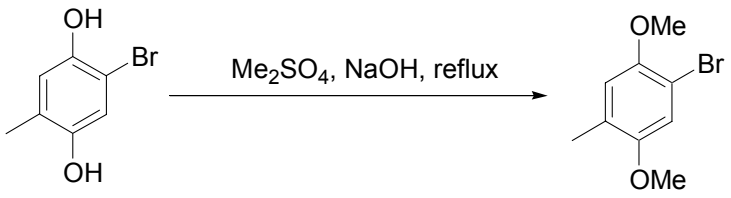


3.9 **3.10**

1-Methyl-1,4-benzoquinone (**3.9**, 490 mg, 4.0 mmol), Me_3SiBr (0.84 mL, 6.0 mmol) and NEt_4BF_4 (87 mg, 0.4 mmol) were dissolved in anhydrous MeCN and allowed to stir overnight at room temperature. The reaction was quenched with water (10 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with water (10 mL), sat. brine (10 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield the crude product as a pale brown solid (500 mg). Purification of the crude product using flash chromatography (17% ethyl acetate, 83% hexane) afforded 1-bromo-2,5-dihydroxytoluene (**3.10**, 320 mg, 1.6 mmol, 39%) as a white amorphous solid.

1-bromo-2,5-dihydroxytoluene (3.10) : white amorphous solid; IR ν_{max} 3212, 1191, 864, 799 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 6.87 (1H, s, H-3), 6.69 (1H, s, H-6), 4.85 (2H, s, 2 x –OH), 2.12 (3H, s, H_3-7); ^{13}C NMR (CD_3OD, 100 MHz) δ 150.4 (q_c, C-5), 148.1 (q_c, C-2), 126.7 (q_c, C-4), 119.6 (CH, C-3), 119.5 (CH, C-6), 107.3 (q_c, C-1), 16.3 (CH_3, C-7); EIMS *m/z* (rel. int.) 203 [M^+] (23), 202 (100), 124 (29), 123 (59).

*6.2.3 Preparation of **3.1** via methylation of **3.10***[157]

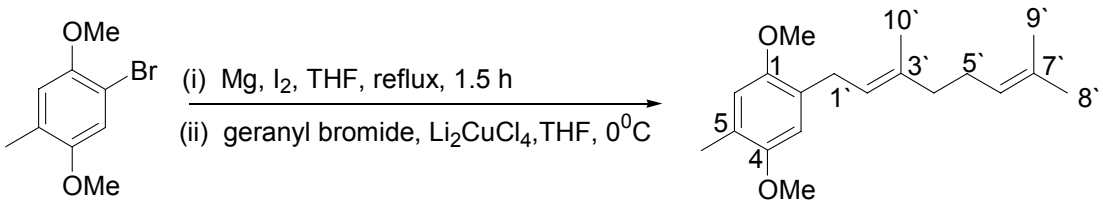


3.10 **3.1**

1-Bromo-2,5-dihydroxytoluene (**3.10**, 600 mg, 3.0 mmol) was dissolved in a NaOH solution (240 mg, 6.0 mmol) at RT and then cooled to 0 °C before Me_2SO_4 (0.57 mL, 6.0 mmol) was dropwise (with extreme caution) *via* a dropping funnel. The reaction mixture was refluxed overnight before being quenched with water (10 mL) and extracted with $CHCl_3$ (3 x 20 mL). The combined organic fractions were washed with water (2 x 20 mL) and sat. brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give a pale yellow solid (630 mg). Purification of the crude product using flash chromatography in pure hexane afforded pure 1-bromo-2,5-dimethoxytoluene (**3.1**, 420 mg, 1.8 mmol, 60%) as white plates (*vide infra*).

*6.2.4 Preparation of **3.2***

6.2.4.1 Using Grignard methodology[143;165]



3.1 **3.2**

This method is representative. A few drops of a solution of 1-bromo-2,5-dimethoxytoluene (**3.1**, 1.5 g, 6.5 mmol) in anhydrous THF (4 mL) were added to a suspension of Mg turnings (0.2 g, 8.1 mmol) and iodine in dry THF (3 mL). The mixture was gently warmed to initiate

reflux, upon which the rest of the solution of **3.1** was added dropwise over 5 mins. The mixture was gently refluxed for 1.5 h before being cooled to -10 °C. The supernatant containing excess 2,5-dimethoxy-4-methylbenzylmagnesium bromide (*ca.* 8.0 eq) was added dropwise *via* cannula to a solution of geranyl bromide (0.35 g, 1.6 mmol) and the Li_2CuCl_4 catalyst (1.5 mL, 0.15 mmol) in dry THF at -10 °C. The reaction mixture was gradually allowed to warm up and was stirred at RT overnight, quenched with water (10 mL) and extracted with Et_2O (3 x 5 mL). The combined organic phases were rinsed with 2.5M HCl (10 mL), dH_2O (10 mL) and sat. brine (10 mL), dried over anhydrous $MgSO_4$ and the solvent evaporated *in vacuo* to give a brown oil (1.45 g). Purification of the crude product using flash chromatography (40% CH_2Cl_2, 60% hexane) yielded 1-[(*2E*)-3,7-dimethylocta-2`,6`-dienyl]-2,5-dimethoxy-4-methyl-benzene (**3.2**, 138 mg, 0.48 mmol, 30%) as a pale yellow oil.

2-[(*2`E*)-3`,7`-dimethylocta-2`,6`-dienyl]-1,4-dimethoxy-5-methylbenzene (3.2) : IR ν_{max} 2915, 2849, 1506, 1400, 1211, 857; δ_H (600 MHz, $CDCl_3$) 6.67 (1H, s, H-6), 6.65 (1H, s, H-3), 5.30 (1H, t, *J* = 7.3 Hz, H-2'), 5.10 (1H, t, *J* = 6.8 Hz, H-6'), 3.77 (3H, s, OMe-1), 3.76 (3H, s, OMe-4), 3.30 (2H, d , *J* = 7.3 Hz, H_2-1'), 2.18 (3H, s, H_3-7), 2.10 (2H, q, *J* = 7.6 Hz, H_2-5'), 2.04 (2H, t, *J* = 7.5 Hz, H_2-4'), 1.70 (3H, s, H_3-10'), 1.67 (3H, s, H_3-8'), 1.59 (3H, s, H_3-9'); δ_C (150 MHz, $CDCl_3$) 151.6 (q_c, C-4), 151.0 (q_c, C-1), 136.1 (q_c, C-3`), 131.4 ($q_c$, C-7`), 128.0 (q_c, C-2), 124.4 (CH, C-6`), 124.3 ($q_c$, C-5), 122.7 (CH, C-2`), 114.0 (CH, C-6), 112.4 (CH, C-3), 56.3 (CH_3, 1-OMe), 56.1 (CH_3, 4-OMe), 39.8 (CH_2, C-4`), 28.2 ($CH_2$, C-1`), 26.8 (CH_2, C-5`), 25.7 ($CH_3$, C-8`), 17.7 (CH_3, C-9`), 16.1 ($CH_3$, C-10`); HRFABMS *m/z* 288.2090 (calcd for $C_{19}H_{28}O_2$ [M^+], 288.2089).

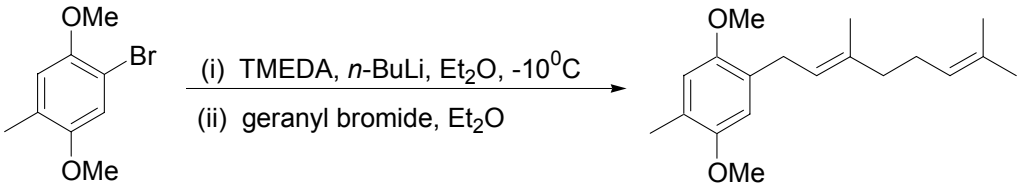
*6.2.4.2 General procedure for the preparation of NMR samples of **3.1** for the ^{7}Li NMR study*

Method A: The organolithium samples were prepared by reaction of **3.1** with *n*-BuLi. The samples of **3.1** (28 mg, 0.12 mmol) were weighed into oven dried 5 mm NMR tubes fitted with

a screw cap and a $CDCl_3$ insert and flushed with argon. After dilution with ether and cooling in a dry ice/acetone bath, each sample was treated with a stoichiometric amount of *n*-BuLi (~1.5 eq of 1.5 M solution) and 0, 1, or 2 equivalents of TMEDA and mixed by shaking and applying a vortex. The resulting organolithium samples were then inserted into the NMR probe precooled to -80 °C. After a short time for temperature equilibration, the field was locked and shimmed. This was followed by acquisition of a ^{13}C (proton decoupled) spectrum (referenced to $CDCl_3$) and a ^{7}Li-NMR spectrum (referenced to an external standard of 1M LiBr in D_2O at 30 °C). Additional lithium spectra were subsequently obtained at intervals as the temperature was increased. The temperature of the NMR probe was calibrated using a 4% MeOH in d_4-methanol sample.

Method B: The *n*-BuLi aliquots were transferred into oven dried 5 mm NMR tubes fitted with a screw cap and a $CDCl_3$ insert and flushed with argon. After dilution with ether, each sample was treated with a stoichiometric amount of TMEDA and mixed by shaking and applying a vortex before cooling in a dry ice/acetone bath. The solution of (**3.1**, 28 mg, 0.12 mmol) in ether (0.2 mL) was added dropwise and the resulting solution mixed carefully using a vortex mixer. The resulting organolithium samples were then inserted into the NMR probe precooled to -80 °C. After a short time for temperature equilibration, the field was locked and shimmed. This was followed by acquisition of a ^{13}C (proton decoupled) spectrum (referenced to $CDCl_3$) and a ^{7}Li-NMR spectrum (referenced to an external standard of 1M LiBr in D_2O at 30 °C). Further lithium spectra were then acquired at intervals as the temperature was increased.

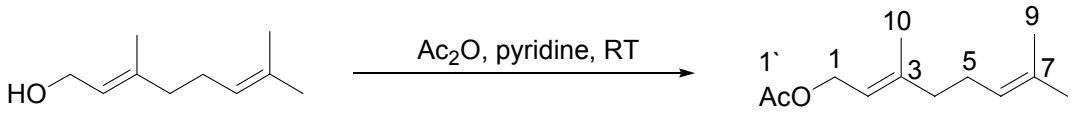
6.4.2.3 Using MHE methodology[199]



3.1 **3.2**

TMEDA (1.01 g, 8.65 mmol, 2.0 eq) followed by 1.6M *n*-BuLi (5.4 mL, 8.65 mmol) was added to anhydrous Et_2O (1 mL) at -10 °C and allowed to stir for 15 mins before a solution of (**3.1**, 1.0 g, 4.33 mmol, 1.0 eq) in 5 mL of dry Et_2O was added. The resulting mixture was stirred at -10 °C for 15 mins before geranyl bromide (**3.34**, 1.88 g, 8.65 mmol, 2.0 eq) was added dropwise and the mixture was allowed to stir overnight at RT. The reaction was thereafter quenched with sat. NH_4Cl (10 mL) and the aqueous solution extracted with Et_2O (3 x 5 mL). The combined ethereal extracts were washed with water (10 mL) and sat. brine (10 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo* to afford the crude mixture (1.97 g) as a brown oil. Purification using flash chromatography (40% CH_2Cl_2, 60% hexane) yielded pure **3.2** (810 mg, 2.8 mmol, 65%) as a pale yellow oil.

*6.2.5 Acetylation of **3.50***



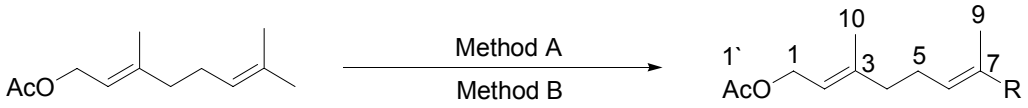
3.50 **3.49**

A mixture of **3.50** (5.0 g, 32.4 mmol) and acetic anhydride (20 mL) in anhydrous pyridine (20 mL) was stirred at RT for 48 h. The reaction was quenched with MeOH (20 mL) and the

pyridine and acetic anhydride were removed *in vacuo* (0.5 mm Hg) to yield 3,7-dimethyl-2(*E*)-6-octadien-1-yl acetate (**3.49**, 5.6 g, 88%) as a clear yellow oil.

3,7-dimethyl-2(*E*)-6-octadien-1-yl acetate (3.49) : [216;357] ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.30 (1H, t, *J* = 7.3 Hz, H-6), 5.04 (1H, t, *J* = 6.8 Hz, H-2), 4.55 (2H, d, *J* = 7.3 Hz, H_2-1), 2.06 (2H, m, H_2-5), 2.02 (3H, s, H_3-2`), 2.00 (2H, m, $H_2$-4), 1.66 (3H, s, $H_3$-10), 1.64 (3H, s, $H_3$-8), 1.56 (3H, s, $H_3$-9). $^{13}$C NMR ($CDCl_3$, 100 MHz) δ 170.9 ($q_c$, C-1`), 142.1 (q_c, C-3), 131.7 (q_c, C-7), 123.9 (CH, C-6), 118.6 (CH, C-2), 61.4 (CH_2, C-1), 39.7 (CH_2, C-4), 26.4 (CH_2, C-5), 25.7 (CH_3, C-8), 20.9 (CH_3, C-2`), 17.7 (CH_3, C-9), 16.5 (CH_3, C-10).

*6.2.6 Selenium dioxide oxidation of **3.49***



3.49

3.51 R = CH_2OH
3.52 R = CHO

Method A : see Section 6.2.7

3,7-dimethyl-8-hydroxy-2(E),6(E)-octadienyl acetate (3.51) :[223;357]

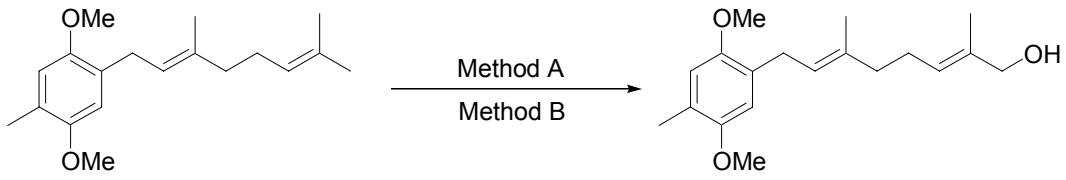
^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.34 (2H, m, H-2, H-6), 4.56 (2H, d, *J* = 7.1 Hz, H_2-1), 3.97 (2H, br s, H_2-8), 2.15 (2H, q, *J* = 7.3 Hz, H_2-5), 2.07 (2H, m, H_2-4), 2.03 (3H, s, H_3-2`), 1.68 (3H, s, H_3-10) , 1.64 (3H, s, H_3-9).

Method B : see Section 6.2.7

3,7-dimethyl-8-al-2(E),6(E)-octadien-1-yl acetate (3.52) :[226]

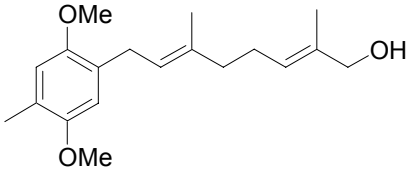
^{1}H NMR ($CDCl_3$, 600 MHz) δ 9.38 (1H, s, H-8), 6.43 (1H, td, *J* = 7.3, 1.2 Hz, H-6), 5.37 (1H, td, *J* = 7.1, 1.1 Hz, H-2), 4.58 (2H, d, *J* = 7.1 Hz, H_2-1), 2.48 (2H, q, *J* = 7.3 Hz, H_2-5), 2.22 (2H, t, *J* = 7.6 Hz, H_2-4), 2.04 (3H, s, H_3-2`), 1.73 (3H, s, H_3-10), 1.72 (3H, s, H_3-9).

*6.2.7 Selenium dioxide oxidation of **3.2***



3.2 **3.3**

Method A :[146] A solution of **3.2** (1.0 g, 3.5 mmol) in anhydrous CH_2Cl_2 (5 mL) was added to a stirred mixture of SeO_2 (19 mg, 0.17 mmol), 70% *t*-BuOOH (1.70 mL, 12.5 mmol) and salicylic acid (48 mg, 0.35 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C under an atmosphere of dry argon, and stirred at this temperature for 2 h and then at RT for a further 20 h. The resulting reaction mixture was washed with 20% Na_2SO_3 (2 x 20 mL), H_2O (20 mL) and sat. brine (10 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a yellow oil (1.05 g). This resultant oil was then dissolved in EtOH (20 mL) and $NaBH_4$ (0.19 g, 5.1 mmol) was added at 0 °C, in small portions over 20 mins. The reaction mixture was stirred at 0 °C for 1 h and then quenched with sat. NH_4Cl (15 mL) at the same temperature, gradually allowed to reach RT and then extracted with Et_2O (3 x 20 mL). The combined organic fractions were washed with H_2O (20 mL) and sat. brine (10 mL), dried over anhydrous Na_2SO_4 and the solvent evaporated *in vacuo* to yield a yellow oil (950 mg). Purification *via* semi-preparative HPLC (33% ethyl acetate, 67% hexane) afforded (**3.3**, 170 mg, 0.56 mmol, 16%), (**3.53**, 206 mg, 0.68 mmol, 20%) and (**3.54**, 45 mg, 0.15 mmol, 4%).

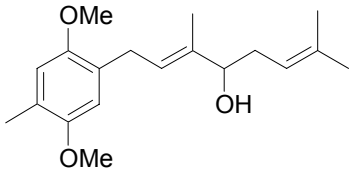


3.3

2-[(*2`E*)-3`,7`-dimethyl-8`-hydroxyocta-2`,6`-dienyl]-1,4-dimethoxy-5-methylbenzene

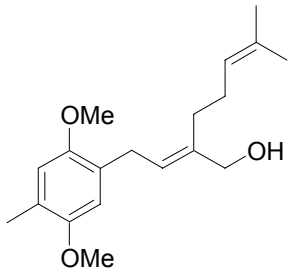
(3.3) : pale yellow oil; IR ν_{max} cm^{-1}; 3418, 2926, 2350, 1691, 1458, 1209, 1044; ^1H NMR

($CDCl_3$, 600 MHz) δ 6.67 (1H, s, H-6), 6.65 (1H, s, H-3), 5.38 (1H, t, *J* = 6.8 Hz, H-6'), 5.30 (1H, t, *J* = 7.1 Hz, H-2'), 3.96 (2H, s, H_2-8'), 3.77 (3H, s, OMe-4), 3.77 (3H, s, OMe-1), 3.29 (2H, d , *J* = 7.2 Hz, H_2-1'), 2.20 (3H, s, H_3-7), 2.15 (2H, q, *J* = 7.4 Hz, H_2-5'), 2.07 (2H, t, *J* = 7.6 Hz, H_2-4'), 1.71 (3H, s, H_3-10'), 1.65 (3H, s, H_3-9'); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.6 (q_c, C-4), 151.0 (q_c, C-1), 135.5 (q_c, C-3`), 131.8 ($q_c$, C-7`), 128.0, (q_c, C-2), 125.9 (CH, C-6`), 124.6 ($q_c$, C-5), 123.1 (CH, C-2`), 114.1 (CH, C-6), 112.6 (CH, C-3), 69.0 (CH_2, C-8`), 56.3 ($CH_3$, 1-OMe), 56.1 ($CH_3$, 4-OMe), 39.3 ($CH_2$, C-4`), 28.3 (CH_2, C-1`), 26.2 ($CH_2$, C-5`), 16.0 (CH_3, C-7), 16.0 (CH_3, C-10`), 13.6 ($CH_3$, C-9`); EIMS *m/z* (rel. int.) 304 [M^+] (18), 286 (24), 219 (14), 205 (27), 165 (100), 135 (35), 105 (15), 91 (22); HREIMS *m/z* 304.2045 (calcd for $C_{19}H_{28}O_3$ [M^+], 304.2038).



3.53

2-[(*2`E*)-3`,7`-dimethyl-4`-hydroxyocta-2`,6`-dienyl]-1,4-dimethoxy-5-methylbenzene (3.53) : pale yellow oil; IR ν_{max} cm^{-1} 3442, 2936, 1729, 1511, 1279, 1049; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.66 (1H, s, H-6), 6.63 (1H, s, H-3), 5.56 (1H, t, *J* = 7.1 Hz, H-2'), 5.10 (1H, t, *J* = 6.8 Hz, H-6'), 4.02 (1H, dd, *J* = 7.3, 5.8 Hz, H-4'), 3.76 (3H, s, OMe-1), 3.76 (3H, s, OMe-4), 3.33 (2H, d , *J* = 7.0 Hz, H_2-1'), 2.31 (1H, dt, *J* = 14.4, 7.5 Hz, H-5'b), 2.22 (1H, dt, *J* = 14.6, 6.9 Hz, H-5'a), 2.19 (3H, s, H_3-7), 1.73 (3H, s, H_3-10'), 1.70 (3H, s, H_3-8'), 1.63 (3H, s, H_3-9'); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.6 (q_c, C-4), 151.0 (q_c, C-1), 137.6 (q_c, C-3`), 134.6 ($q_c$, C-7`), 127.3 (q_c, C-2), 124.7 (q_c, C-5), 124.7 (CH, C-2`), 120.2 (CH, C-6`), 114.1 (CH, C-6), 112.5 (CH, C-3), 56.2 (CH_3, 1-OMe), 56.1 (CH_3, 4-OMe), 34.2 (CH_2, C-5`), 28.0 ($CH_2$, C-1`), 25.9 (CH_3, C-8`), 18.0 ($CH_3$, C-9`), 16.1 (CH_3, C-7), 11.8 (CH_3, C-10`); EIMS *m/z* (rel. int.) 304 [M^+] (8), 286 (35), 243 (20), 206 (10), 165 (100), 135 (26); HREIMS *m/z* 304.2031 (calcd for $C_{19}H_{28}O_3$ [M^+], 304.2038).

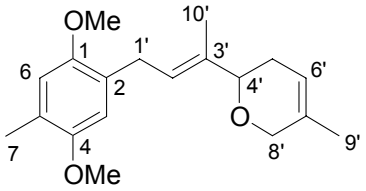


3.54

2-[(*2`E*)-3`-hydroxyethyl-7`-methyl-2`,6`-dienyl]-1,4-dimethoxy-5-methylbenzene (3.54) : pale yellow oil; IR ν_{max} cm^{-1} 3427, 2927, 2351, 1692, 1512, 1209, 1043; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.67 (1H, s, H-6), 6.63 (1H, s, H-3), 5.57 (1H, t, *J* = 7.3 Hz, H-2'), 5.17 (1H, t, *J* = 7.2 Hz, H-6'), 4.06 (2H, s, H_2-10'), 3.77 (3H, s, OMe-1), 3.76 (3H, s, OMe-4), 3.36 (2H, d , *J* = 7.3 Hz, H_2-1'), 2.25 (2H, dd, *J* = 8.4, 7.3 Hz, H_2-4'), 2.19 (3H, s, H_3-7), 2.15 (2H, q, *J* = 7.6 Hz, H_2-5'), 1.68 (3H, s, H_3-8'), 1.61 (3H, s, H_3-9'); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.6 (q_c, C-4), 151.0 (q_c, C-1), 139.4 (q_c, C-3`), 132.0 ($q_c$, C-7`), 127.1 (q_c, C-2), 125.6 (CH, C-2`), 124.9 ($q_c$, C-5), 124.1 (CH, C-6`), 114.0 (CH, C-6), 112.6 (CH, C-3), 67.3 (CH_2, C-10`), 56.2 ($CH_3$, 1-OMe), 56.1 ($CH_3$, 4-OMe), 28.2 ($CH_2$, C-4`), 28.1 (CH_2, C-1`), 27.2 ($CH_2$, C-5`), 25.7 (CH_3, C-8`), 17.8 ($CH_3$, C-9`), 16.1 (CH_3, C-7); EIMS *m/z* (rel. int.) 304 [M^+] (16), 286 (22), 243 (15), 165 (100), 149 (24), 97 (35), 57 (90); HREIMS *m/z* 304.2047 (calcd for $C_{19}H_{28}O_3$ [M^+], 304.2038).

Method B :[215] SeO_2 (0.35 g, 3.1 mmol) was added to a solution of **3.2** (0.6 g, 2.1 mmol) in EtOH (5 mL) and the mixture was refluxed for 1 h. The reaction mixture was allowed to cool and filtered through Celite (545 coarse), after which the solvent was removed *in vacuo*. The reaction mixture was taken up in EtOAc (15 mL), flushed through a small silica (25 mL) plug with EtOAc and concentrated under reduced pressure to give a dark green oil (580 mg). Examination of the crude product by ^1H and ^{13}C NMR spectroscopy indicated a complex mixture of products that was not investigated further.

When using 5-6 M TBHP solution instead of the 70% solution, and without salicylic acid, we followed the same procedure as described in Method A. Purification of the crude reaction mixture by semi-preparative HPLC (25% ethyl acetate, 75% hexane) afforded (**3.55**, 3.5%).



3.55

Cyclic ether (3.55) : pale yellow oil; IR ν_{max} cm^{-1} 3418, 3027, 2350, 1690, 1523, 1217, 1039, 757; For ^{1}H and ^{13}C NMR data, see Table 3.2; HRFABMS *m/z* 303.1960 (calcd for $C_{19}H_{27}O_3$ $[(M+H)^+]$, 302.1882).

6.2.8 Titration of 70% t-butylhydroperoxide (TBHP)

Standardization of Sodium Thiosulfate solution:

Made up an approximated 0.1 M $Na_2S_2O_3$ solution (25 mL)

M_w $Na_2S_2O_3$	= 248.18 $g.mol^{-1}$
25 mL solution => 2.5 mmol	= 0.62 g $Na_2S_2O_3$

Used KIO_3

6 moles thiosulfate	→ 1 mole KIO_3
	(1)
∴ 0.25 mmol thiosulfate	→ 0.042 mmol KIO_3
2.5 mL thiosulfate	→ 2 mL KIO_3
2 mL solution KIO_3	→ 0.042 mmol
25 mL solution KIO_3	→ 0.521 mmol = 0.112 g KIO_3

M_w KIO_3 = 214 $g.mol^{-1}$

Average volume of thiosulfate = 2.65 mL (for endpoint)

∴ from equation (1)

In 2.65 mL thiosulfate → 0.25 mmol thiosulfate

=> In 100 mL thiosulfate → 0,094 moles thiosulfate

∴ Concetration of $Na_2S_2O_3$ solution = 0.094 M

Titration of TBHP Solution

M_w TBHP = 90.12 $g.mol^{-1}$

Used 2.5 mL of TBHP to make up 25 mL solution

=> 2.5 mL TBHP → 19.4 mmol TBHP

25 mL solution → 19.4 mmol TBHP

Used 2 mL of solution

Endpoint of titration = 2.5 mL thiosulfate

Concentration of thiosulfate solution = 0.094 M

∴ In 2.5 mL solution of thiosulfate → 0.235 mmol

2 moles thiosulfate → 1 mole I_2

(2)

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$$

(3)

$$H_2O_2 \rightarrow O_2 + 2H^+ 2e^-$$

(4)

∴ 1 mole H_2O_2 reacts with 2 moles thiosulfate

=> 0.235 mmol thiosulfate → 0.118 mmol TBHP

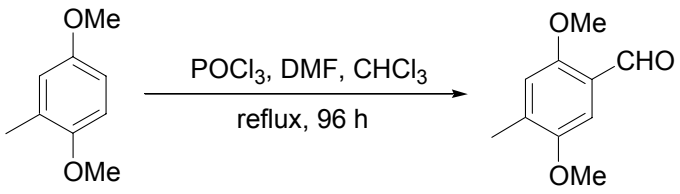
∴ In 2mL TBHP solution → 0.118 mmol TBHP

=> In 100 mL TBHP solution → 0.059 moles TBHP

∴ Concentration of TBHP = (0.059 moles) × 90.12 $g.mol^{-1}$

= 5.4%

6.2.9 Vilsmeier-Haack formylation of **3.8**[237]

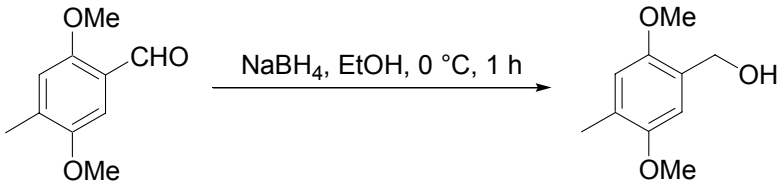


3.8 **3.61**

N,N-Dimethylformamide (DMF) (2.40 g, 32.8 mmol) and phosphoryl chloride (5.04 g, 32.8 mmol) were added to a solution of **3.8** (0.58 g, 3.8 mmol) in $CHCl_3$ (10 mL) at 0 °C. The reaction mixture was allowed to reach RT and then refluxed for 96 h. The reaction was carefully quenched with ice cold water and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were washed with water (10 mL) and sat. brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give a reddish solid (0.63 g). Flash chromatography of the crude product (17% ethyl acetate, 83% hexane) afforded 2-benzaldehyde-1,4-dimethoxytoluene (**3.61**, 0.54 g, 3.0 mmol, 80 %) as pale yellow needles (from Et_2O).

2-benzaldehyde-1,4-dimethoxytoluene (3.61) : pale yellow needles (from Et_2O); mp 80- 82 °C, lit.[238] 77-78 °C; IR (film) ν_{max} 3446, 3018, 1672, 1216, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 10.37 (1H, s, H-1`), 7.22 (1H, s, H-3), 6.78 (1H, s, H-6), 3.85 (3H, s, OMe-1), 3.80 (3H, s, OMe-4), 2.25 (3H, s, $H_3$-7); $^{13}C$ NMR ($CDCl_3$, 150 MHz) δ 189.2 (CH, C-1`), 156.5 (q_c, C-4), 151.9 (q_c, C-1), 136.5 (q_c, C-5), 122.8 (q_c, C-2), 114.6 (CH, C-6), 107.6 (CH, C-3), 56.1 (CH_3, 1-OMe), 55.7 (CH_3, 4-OMe), 17.2 (CH_3, C-7).

*6.2.10 Sodium borohydride reduction of **3.61***

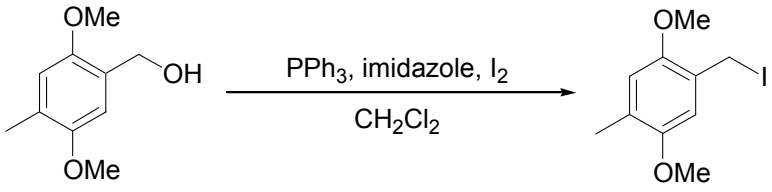


3.61 **3.60**

$NaBH_4$ (131 mg, 3.5 mmol) was added in small portions over 20 mins to a solution of **3.61** (0.5 g, 2.3 mmol) in EtOH (10 mL) at 0 °C, and the mixture was stirred at this temperature for 1.5 h. The reaction mixture was quenched by the slow addition of saturated NH_4Cl at 0 °C and extracted with Et_2O (3 x 10 mL). The combined organic fractions were washed with dH_2O (2 x 20 mL) and sat. brine (20 mL), dried over anhydrous Na_2SO_4 and the solvent removed *in vacuo* to give a white solid (380 mg). Recrystallization of the crude product from hexane yielded 2-hydroxymethyl-1,4-dimethoxytoluene (**3.60**, 337 mg, 1.85 mmol, 80%) as fine white needles.

2-hydroxymethyl-1,4-dimethoxytoluene (3.60) : Fine white needles (from hexane); mp 78-80 °C, lit.[239] 77-78 °C; IR (film) ν_{max} 3444, 3017, 2405, 1465, 1214, 1041, 753; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.79 (1H, s, H-6), 6.70 (1H, s, H-3), 4.64 (2H, d, *J* = 6.0 Hz, H_2-1`), 3.81 (3H, s, OMe-1), 3.79 (3H, s, OMe-4), 2.22 (3H, s, $H_3$-7); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 151.6 ($q_c$, C-4), 151.0 ($q_c$, C-1), 126.8 ($q_c$, C-2), 126.6 ($q_c$, C-5), 113.6 (CH, C-6), 111.6 (CH, C-3), 62.0 ($CH_2$, C-1`), 56.0 (CH_3, 1-OMe), 55.8 (CH_3, 4-Ome), 16.3 (CH_3, C-7); EIMS *m/z* (rel. int.) 182 [M^+] (100), 165 (23), 139 (30), 107 (22), 91 (29), 77 (27); HREIMS *m/z* 182.0949 (calcd for $C_{10}H_{14}O_3$ [M^+], 182.0943).

*6.2.11 Iodination of **3.60***[240-242]

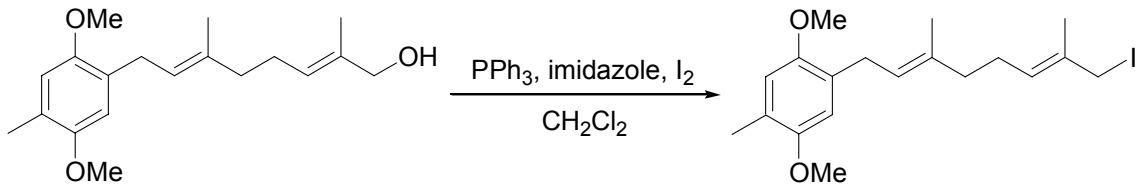


3.60 **3.62**

A solution of **3.60** (100 mg, 0.46 mmol) in CH_2Cl_2 (2 mL) was added to a stirred solution of PPh_3 (180 mg, 0.69 mmol), imidazole (47 mg, 0.69 mmol) and iodine (175 mg, 0.69 mmol) in CH_2Cl_2 (5 mL) at 0°C. The reaction was stirred at this temperature for 10 mins and then at RT for 1.5 h, before being filtered through Celite (545 coarse) with CH_2Cl_2 (5 mL). Removal of the solvent under reduced pressure gave a yellow oil (95 mg). Crystallization of the crude product from a mixture of hexane/CH_2Cl_2 afforded 2-iodomethyl-1,4-dimethoxytoluene (**3.62**, 27 mg, 0.092 mmol, 20%) as colourless plates.

2-iodomethyl-1,4-dimethoxytoluene (3.62) : Colourless plates (from hexane/CH_2Cl_2); mp 95-99 °C; IR (film) ν_{max} 3013, 2397, 1693, 1465, 1211, 1042, 757; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.68 (1H, s, H-6), 6.61 (1H, s, H-3), 3.89 (2H, s, H_2-1`), 3.78 (3H, s, OMe-1), 3.69 (3H, s, OMe-4), 2.19 (3H, s, $H_3$-7); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 151.6 ($q_c$, C-4), 151.3 ($q_c$, C-1), 127.2 ($q_c$, C-2), 124.7 ($q_c$, C-5), 113.9 (CH, C-6), 113.4 (CH, C-3), 56.2 ($CH_3$, 1-OMe), 56.1 ($CH_3$, 4-Ome), 29.7 ($CH_2$, C-1`), 16.1 (CH_3, C-7); EIMS *m/z* (rel. int.) 291 [M^+] 269 (35), 239 (10), 211 (8), 165 (50), 151 (84), 135 (83), 91 (6); HREIMS *m/z* 291.9889 (calcd for $C_{10}H_{13}O_2I$ [M^+], 291.9960).

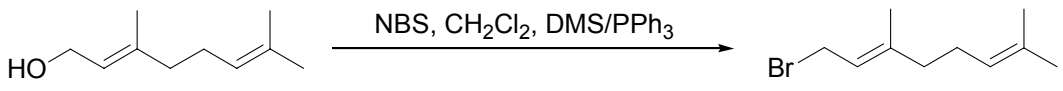
*6.2.12 Attempted iodination of **3.3***[240-242]



3.3 **3.5**

A solution of **3.3** (90 mg, 0.30 mmol) in CH_2Cl_2 (2 mL) was added to a stirred solution of PPh_3 (116 mg, 0.45 mmol), imidazole (30 mg, 0.45 mmol) and iodine (113 mg, 0.45 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction was wrapped in tin foil and stirred in the dark at this temperature for 10 mins and then at RT for 1.5 h, before being filtered through Celite (545 coarse) with CH_2Cl_2 (5 mL). Removal of the solvent under reduced pressure gave a yellow solid (210 mg) that was purified by semi-preparative HPLC (25% ethyl acetate, 75% hexane). Examination of the ^1H and ^{13}C NMR spectra for the different fractions obtained showed no clear evidence of the formation of the desired product.

*6.2.13 Allylic bromination of **3.50***[244]

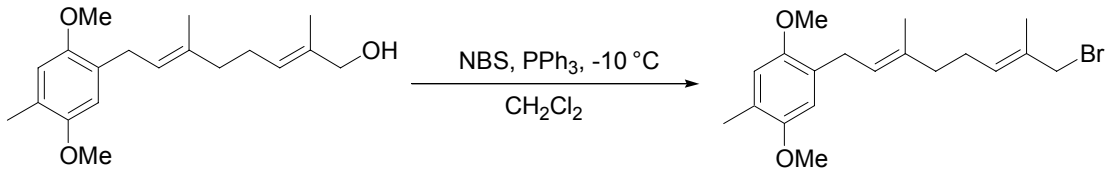


3.50 **3.67**

This method is representative. A solution of **3.50** (0.1 g, 0.65 mmol) in anhydrous CH_2Cl_2 (2 mL) was added dropwise to a stirred suspension of NBS (172 mg, 0.97 mmol) and DMS (72.5 mg, 1.2 mmol) in anhydrous CH_2Cl_2 (3 mL) at -10 °C. The reaction was carried out in the dark for 4 h, and aliquots of the reaction mixture were subjected to GC analysis at one hour intervals. Commercially available **3.50** and **3.67** were used as standards for the GC study,

and their retention times were used. Additionally, the same procedure was followed using 1.0 g of **3.50**. For our comparative study, the same conditions and scales, *i.e.* 0.1 g and 1.0 g, as described above, were utilized except for using PPh_3 (1.05 eq) instead of DMS and also the reactions were stirred at -10 °C for only 0.5 h and then at RT for 3.5 h. The percentage conversion of **3.50** to **3.67** for all four reactions were monitored by GC and the results obtained are summarized in Table 3.3. No further characterization was deemed necessary.

*6.2.14 Allylic bromination of **3.3***[244;247]



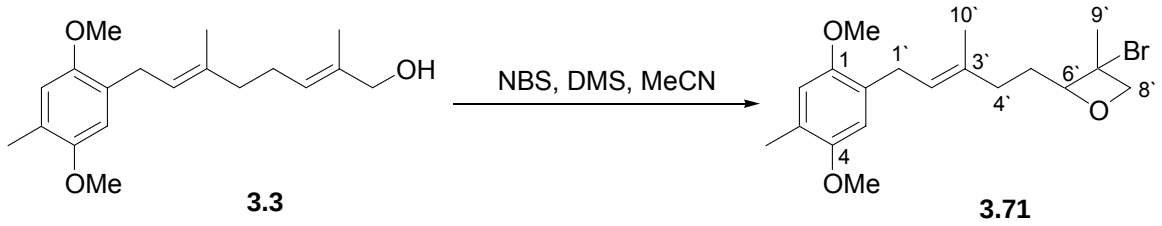
3.3 **3.63**

The same method as outlined above was applied to **3.3** (0.4 g, 1.31 mmol) using PPh_3 (362 mg, 1.38 mmol, 1.05 eq) instead of DMS. Strictly anhydrous conditions were maintained and the reaction was carried out at -10 °C in the dark for 0.5 h and then at RT for 3.5 h. The solvent was removed under reduced pressure to give a dark brown oil (1.33 g). The crude product was purified by flash chromatography (50% CH_2Cl_2, 50% hexane) to afford (**3.63**, 160 mg, 0.44 mmol, 33%) as a colourless oil.

2-[(*2`E*)-3`,7`-dimethyl-8`-bromoocta-2`,6`-dienyl]-1,4-dimethoxy-5-methylbenzene (3.63) : colourless oil; IR (film) ν_{max} 3852, 3438, 2353, 1649, 1537, 1042, 768; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.67 (1H, s, H-6), 6.64 (1H, s, H-3), 5.60 (1H, t, *J* = 6.9 Hz, H-6'), 5.30 (1H, t, *J* = 7.4 Hz, H-2'), 3.94 (2H, s, H_2-8'), 3.77 (3H, s, OMe-1), 3.77 (3H, s, OMe-4), 3.29 (2H, d , *J* = 7.0 Hz, H_2-1'), 2.20 (3H, s, H_3-7), 2.15 (2H, q, *J* = 7.6 Hz, H_2-5'), 2.06 (2H, t, *J* = 7.7 Hz, H_2-4'), 1.73 (3H, s, H_3-10'), 1.71 (3H, s, H_3-9'); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.6 (q_c, C-4), 151.0

(q_c, C-1), 135.1 (q_c, C-3`), 132.0 ($q_c$, C-7`), 131.1 (CH, C-6`), 127.8 ($q_c$, C-2), 124.5 ($q_c$, C-5), 123.4 (CH, C-2`), 114.0 (CH, C-6), 112.5 (CH, C-3), 56.3 (CH_3, 1-OMe), 56.1 (CH_3, 4-Ome), 41.8 (CH_2, C-8`), 38.8 ($CH_2$, C-4`), 28.3 (CH_2, C-1`), 26.9 ($CH_2$, C-5`), 16.1 (CH_3, C-10`), 14.6 ($CH_3$, C-9`); EIMS *m/z* (rel. int.) 367 [M^+] (8), 286 (15), 219 (13), 205 (24), 189 (18), 165 (39), 149 (20), 105 (16); HREIMS *m/z* 366.1196 (calcd for $C_{19}H_{27}O_2Br$ [M^+], 366.1194).

*6.2.15 Bromination of **3.3** in acetonitrile*[153;246]

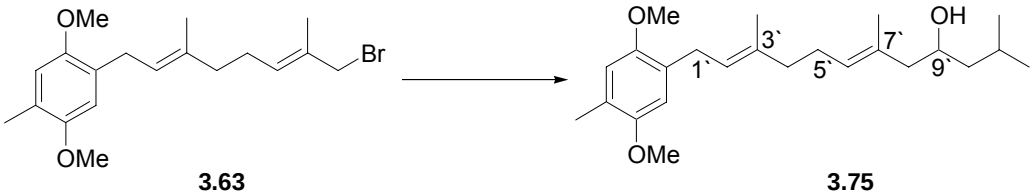


3.3 **3.71**

A solution of **3.3** (103 mg, 0.34 mmol) in anhydrous MeCN (2 mL) was added slowly to a solution of NBS (105 mg, 0.59 mmol) and DMS (37 mg, 0.59 mmol) in anhydrous MeCN (3 mL) at -10 °C in the presence of light. The reaction was stirred at this temperature for 4 h, before being quenched with sat. NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL) and the combined organic fractions washed with sat. brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to yield a pale brown oil (135 mg). Semi-preparative HPLC (25% ethyl acetate, 75% hexane) of the crude reaction mixture afforded (**3.71**, 60 mg, 0.16 mmol, 46%) as a pale yellow oil.

Bromo oxetane (3.71) : pale yellow oil; IR (film) ν_{max} 3458, 2935, 1702, 1461, 1210, 1045; For ^1H and ^{13}C NMR data, see Table 3.4; EIMS *m/z* (rel. int.) 383 [M^+] (5), 302 (52), 277 (10), 205 (26), 179 (23), 165 (100), 135 (30), 105 (10), 91 (25); HREIMS *m/z* 382.1155 (calcd for $C_{19}H_{27}O_3Br$ [M^+], 382.1144).

*6.2.16 Attempted coupling of isovaleraldehyde to **3.63***



3.63 **3.75**

Method A : Using MHE methodology with *n*-BuLi in THF

A solution of **3.63** (100 mg, 0.27 mmol) in anhydrous THF (2 mL) was added dropwise to a stirred solution of 1.6M *n*-BuLi (0.26 mL, 0.41 mmol) in anhydrous THF (3 mL) at -78 °C under an atmosphere of dry argon. After 15 mins, a solution of isovaleraldehyde (23 mg, 0.27 mmol) in dry THF was cannulated into the reaction mixture and the mixture stirred at -78 °C for 3 h and at RT for 1 h, before being quenched with sat. NH_4Cl (5 mL) and the aqueous solution extracted with Et_2O (3 x 5 mL). The combined ethereal fractions were washed with water (10 mL), sat. brine (10 mL), dried over anhydrous $MgSO_4$. Removal of the solvent *in vacuo* gave a pale yellow oil (135 mg). Purification of the crude reaction mixture *via* normal phase semi-preparative HPLC (20% ethyl acetate, 80% hexane) yielded five different fractions. Examination of both ^1H and ^{13}C NMR spectra of these fractions gave no indication of the presence of the expected product **3.75**, *i.e.* no indication of an oxymethine resonance corresponding to C-9` (δ_H 3.5-4.0 and δ_C 60-70).

Method B : Using MHE methodology with *n*-BuLi and TMEDA in Et_2O

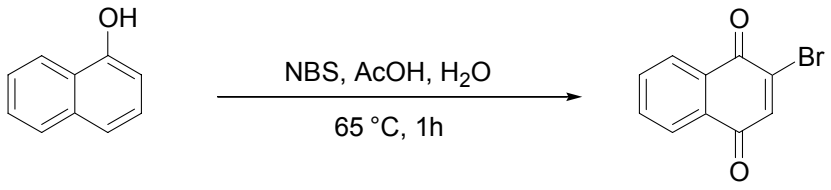
A solution of **3.63** (100 mg, 0.27 mmol) in anhydrous Et_2O (2 mL) was added dropwise to a stirred solution of 1.6M *n*-BuLi (0.26 mL, 0.41 mmol) and TMEDA (48 mg, 0.41 mmol) in anhydrous Et_2O (3 mL) at -10 °C under an atmosphere of dry argon. After 15 mins, a solution of isovaleraldehyde (23 mg, 0.27 mmol) in dry Et_2O was cannulated into the reaction mixture and the mixture stirred at -10 °C for 3 h and at RT overnight. The usual workup (*vide supra*) afforded a pale yellow oil (122 mg) and ^1H and ^{13}C NMR spectroscopy indicated the presence of a complex mixture of products that were not investigated further.

Method C : Using Grignard approach with Mg

The Grignard derivative of **3.63** (200 mg, 0.54 mmol) was generated *in situ* by gentle reflux for 1.5 h with Mg (20 mg, 0.82 mmol) and iodine in anhydrous THF (8 mL), according to the procedure described above in section 7.3.4. The resulting Grignard solution was cooled to -10 °C and transferred *via* cannula into a stirred solution of isovaleraldehyde (47 mg, 0.54 mmol) in anhydrous THF at -10 °C. The reaction mixture was stirred at this temperature for 3 h and then at RT overnight. The usual workup yielded a dark green oily residue (190 mg) that was purified by normal phase semi-preparative HPLC (20% ethyl acetate, 80% hexane) to give five different fractions. Analysis of these fractions using ^{1}H and ^{13}C NMR spectroscopy gave no indication of the presence of the expected product **3.75**.

6.3 Chapter Four Experimental

*6.3.1 Synthesis of **4.9** from **4.8***[283]

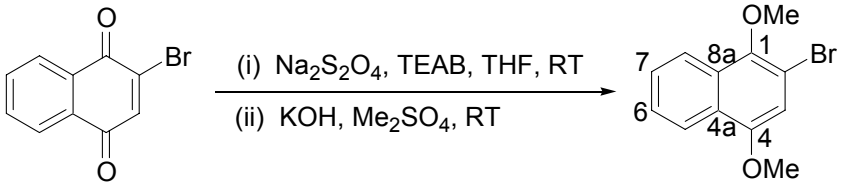


4.8 **4.9**

A solution of **4.8** (144 mg, 1.0 mmol) in 10 mL glacial AcOH was added dropwise to a mixture of NBS (712mg, 4 mmol) in 10 mL glacial AcOH and 20 mL H_2O at 65 °C. The resulting mixture was stirred at this temperature for 1 h, then cooled to RT before being diluted with 20 mL H_2O and extracted with $CHCl_3$ (3 x 10 mL). The combined organic fractions were washed with 5% $NaHCO_3$ (3 x 20 mL), H_2O (2 x 20 mL) and sat. brine (20 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent *in vacuo* yielded the crude product as a light brown solid (225.2 mg). Recrystallization from EtOH afforded the pure 2-bromo-1,4-naphthoquinone (**4.9**, 215mg, 90%) as a yellow crystalline solid.

2-bromo-1,4-naphthoquinone (4.9) : yellow crystalline solid (from EtOH); mp 132-134 °C, lit.[285] 131-132 °C; IR (film) ν_{max} cm^{-1} 3440, 1970, 1720, 1500, 1250, 1120, 996, 845, 769; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.11 – 8.06 (1H, m, H-5), 8.02 – 8.00 (1H, m, H-8), 7.75 – 7.69 (2H, m, H-7, H-6), 7.44 (1H, s, H-3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 182.2 (q_c, C-4), 177.6 (q_c, C-1), 140.2 (CH, C-3), 140.0 (q_c, C-2), 134.3 (CH, C-7), 134.0 (CH, C-6), 131.5 (q_c, C-8a), 130.8 (q_c, C-4a), 127.6 (CH, C-5), 126.7 (CH, C-8); EIMS *m/z* (rel. int.) 237 [M^+] (17), 236 (77), 210 (11), 157 (100), 129 (85), 101 (23), 75 (34), 50 (17).

*6.3.2 Reductive methylation of **4.9***[293]

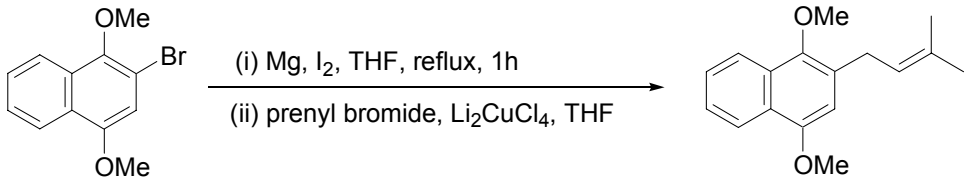


4.9 **4.6**

Tetraethylammonium bromide (TEAB) (170 mg) was added to a solution of **4.9** (1.21g, 5.11 mmol) in 10 mL THF and 5 mL H_2O. Aqueous sodium dithionite (6.68 g, 31.8 mmol, 6 eq) was added and the mixture was stirred at RT for 20 mins. An aqueous KOH (6.85 g, 122 mmol) solution was added to the reaction mixture and, after 5 mins, dimethyl sulfate (15.40 g, 122 mmol) was added dropwise with caution. The solution was allowed to stir overnight (16 h) before the reaction was quenched with water (15 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic fractions were washed with water (10 mL) and sat. brine (5 mL), dried over $MgSO_4$ and concentrated under reduced pressure to give a reddish brown solid (2.63 g). Purification of the crude product using flash chromatography in pure hexane afforded 2-bromo-1,4-dimethoxynaphthalene (**4.6**, 1.02g, 3.82 mmol, 75%) as a white crystalline solid.

2-bromo-1,4-dimethoxynaphthalene (4.6) : white crystalline solid (from hexane); mp 53-55 °C, lit.[280] 54-55 °C; IR (film) ν_{max} cm^{-1} 3441, 3073, 1959, 1729, 1583, 1456, 1261, 1161, 1026, 995, 833, 768; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.21 (1H, d, *J* = 8.4 Hz, H-5), 8.07 (1H, d, *J* = 8.4 Hz, H-8), 7.56 (1H, td, *J* = 7.6, 1.0 Hz, H-7), 7.50 (1H, td, *J* = 7.6, 1.1 Hz, H-6), 6.88 (1H, s, H-3), 3.97 (3H, s, OMe-4), 3.95 (3H, s, OMe-1); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.2 (q_c, C-4), 146.7 (q_c, C-1), 128.9 (q_c, C-8a), 127.3 (CH, C-7), 125.7 (q_c, C-4a), 125.7 (CH, C-6), 122.6 (CH, C-5), 121.8 (CH, C-8), 111.9 (q_c, C-2), 107.9 (CH, C-3), 61.4 (CH_3, 1-OMe), 55.8 (CH_3, 4-OMe); EIMS *m*/*z* (rel. int.) 267 [M^+] (2), 220 (100), 205 (55), 189 (37), 175 (25), 165 (23), 145 (11), 135 (15), 92 (6), 91 (10).

*6.3.3 Grignard synthesis of **4.18***[237]

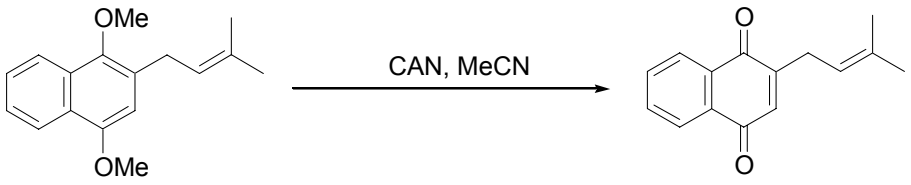


4.6 **4.18**

A few drops of a solution of **4.6** (0.250 g, 0.94 mmol) in dry THF (1 mL) was added to a suspension of Mg turnings (0.036 g, 1.4 mmol) and iodine in dry THF (2 mL). The mixture was gently warmed to initiate reflux, upon which the rest of the solution of **4.6** was added very slowly over 5 mins. The resulting mixture was refluxed for 1h before being cooled to -10 °C. The supernatant containing excess 1,4-dimethoxynaphthalenylmagnesium bromide (*ca.* 8 eq.) was transferred *via* cannula to a solution of prenyl bromide (0.017 g, 0.12 mmol), Li_2CuCl_4 (1 mL, 0.1 mmol) and dry THF (2 mL) at -10 °C. The reaction mixture was slowly warmed to RT and left to stir overnight, quenched with water (5 mL) and extracted with CH_2Cl_2 (3 x 3 mL). The combined organic phases were washed with 10% HCl (5 mL), H_2O (5 mL) and sat. brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield a dark brown oil (0.20 g). NP HPLC of the oil in (4% EtOAc, 96% hexane) yielded **4.18** as a dark yellow oil (0.020 g, 0.08 mmol, 67%).

2-(3-methyl-2-butene)-1,4-dimethoxynaphthalene (4.18) : Dark yellow oil; IR (film) ν_{max} cm^{-1} 3384, 2922, 2853, 1729, 1596, 1454, 1370, 1226, 1091, 1005, 768, 714; For ^{1}H and ^{13}C NMR data see Table 4.1; EIMS *m/z* (rel. int.) 256 [M^{+}] (100), 241 (23), 209 (30), 181 (38), 171 (74), 149 (29), 141 (20), 127 (9), 91 (11), 77 (15); HRFABMS *m/z* 257.1541(calcd for $C_{17}H_{21}O_2$ [$(M + H)^{+}$] 257.1542).

*6.3.4 Oxidative demethylation of **4.18***[237]

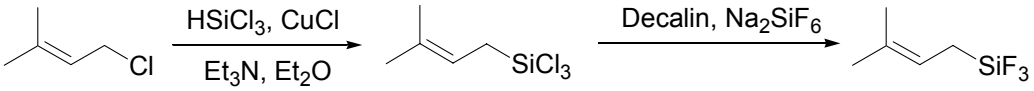


4.18 **2.44**

A solution of cerium ammonium nitrate (CAN) (0.065 g, 0.12 mmol) in water (0.1 mL) was added dropwise to a solution of **4.18** (0.015 g, 0.06 mmol) in MeCN (1.5 mL) at 0 °C. The mixture was stirred (15 mins) at 0 °C, diluted with water (2 mL) and extracted with ether (3 x 2 mL). The combined organic extracts were washed with water (5 mL), sat. brine (5 mL), dried over $MgSO_4$ and the solvent evaporated *in vacuo* to give **2.44** as a brown oil (0.0125 g, 0.06 mmol, 92%).

2-deoxylapachol (2.44) : brown oil; IR (film) ν_{max} cm^{-1} 3420, 3090, 1673, 1594, 1475, 1302, 1151, 1047, 950, 770; For ^{1}H and ^{13}C NMR data see Table 4.2; HRFABMS *m/z* 227.1072 (calcd for $C_{15}H_{15}O_2$ [$(M + H)^{+}$] 227.1072).

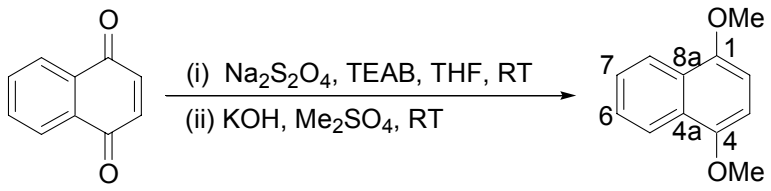
*6.3.4.1 Attempted synthesis of **4.3***[168;277;278]



4.5 **4.4** **4.3**

A mixture of trichlorosilane (2.98 g, 22.0 mmol) and 1-chloro-3-methyl-but-2-ene (**4.5**, 2.30 g, 22.0 mmol) in anhydrous Et_2O (5 mL) was added dropwise *via* a dropping funnel to a stirred mixture of CuCl (0.11 g, 1.1 mmol) and dry Et_3N (2.23 g, 22.0 mmol) in anhydrous Et_2O (5 mL) in a two-neck round-bottom flask fitted with a condensor under an atmosphere of dry nitrogen. This reaction mixture was stirred at RT for 4 h before the white solids were filtered and rinsed with Et_2O (5 mL). Removal of the solvent *in vacuo*, followed by distillation of the residue afforded a brown oil (1.0 g). Analysis of both ^1H and ^{13}C NMR spectra indicated a complex mixture of products that was not investigated further.

*6.3.5 Reductive methylation of **4.2***[293]



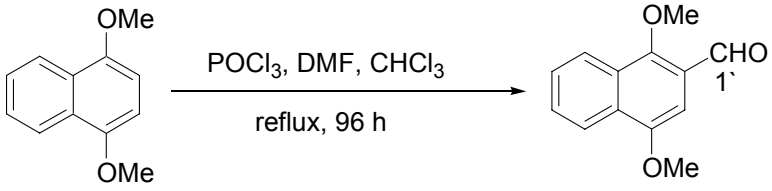
4.2 **4.7**

Tetraethylammonium bromide (TEAB) (500 mg) was added to a solution of the **4.2** (2.0 g, 12.6 mmol) in THF (30 mL) and water (12 mL). Aqueous sodium dithionite (13.16 g, 75.6 mmol, 6 eq) was added and the mixture was stirred at RT for 20 mins. An aqueous KOH (16.32 g, 291 mmol) solution was added to the reaction mixture and, after 5 mins, dimethyl sulfate (36.70 g, 291 mmol) was added dropwise with caution. The solution was allowed to stir overnight (16 h) before the reaction was quenched with water (30 mL) and extracted with

CH_2Cl_2 (3 x 20 mL). The combined organic fractions were washed with water (20 mL) and sat. brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give a reddish oil (2.63 g). Flash chromatography of the crude product in pure hexane afforded 1,4-dimethoxynaphthalene (**4.7**, 1.91g, 10.1 mmol, 81 %) as a white crystalline solid.

1,4-dimethoxynaphthalene (4.7) : white crystalline solid (from hexane); mp 87-88 °C, lit.[281] 84-86 °C; IR (film) ν_{max} cm^{-1} 3444, 1633, 1279, 1088, 765; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.24 (2H, dd, *J* = 6.4, 3.3 Hz, H-8, H-5), 7.52 (2H, dd, *J* = 6.4, 3.3 Hz, H-7, H-6), 6.70 (2H, s, H-2, H-3), 3.97 (6H, s, OMe-1, OMe-4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.5 (q_c, C-1, C-4), 126.3 (q_c, C-4a, C-8a), 125.8 (CH, C-6, C-7), 121.7 (CH, C-5, C-8), 103.2 (CH, C-2, C-3), 55.7 (2 x CH_3, 1-OMe, 4-OMe); EIMS *m*/*z* (rel. int.) 173 [M^+] 188 (94), 145 (35), 130 (6), 115 (11).

*6.3.6 Vilsmeier-Haack formylation of **4.7***[294]

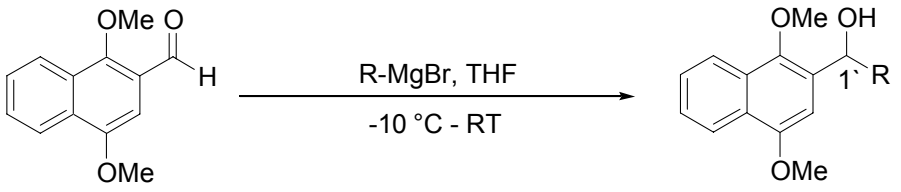


4.7 **4.20**

A solution of **4.7** (500 mg, 2.7 mmol) in chloroform (10 mL) was added to a mixture of phosphoryl chloride (4.72 g, 31 mmol) and *N*,*N*-dimethylformamide (DMF) (2.25 g, 31 mmol). The resulting solution was refluxed for 96 h before the reaction was carefully quenched with cold water. The mixture was extracted with CH_2Cl_2 (3 x 10 mL) and the combined organic fractions washed with water (1 x 10 mL) and sat. brine (1 x 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure (0.5-10 mmHg) to give a reddish brown solid (660 mg). Recrystallisation from hexane afforded 1,4-dimethoxy-2-naphthalenecarbaldehyde (**4.20**, 530 mg, 91 %) as pale yellow needles (from hexane).

1,4-dimethoxy-2-naphthalenecarbaldehyde (4.20) :[294] pale yellow needles (from hexane); mp 122-123 °C, lit.[280] 119.5-120 °C; IR (film) ν_{max} cm^{-1} 1677, 1373, 1205, 993, 771; ^1H NMR ($CDCl_3$, 400 MHz) δ 10.57 (1H, s, H-1`), 8.27 (1H, d, *J* = 7.5 Hz, H-5), 8.19 (1H, d, *J* = 7.5 Hz, H-8), 7.62 (2H, m, H-7, H-6), 7.11 (1H, s, H-3), 4.09 (3H, s, OMe-1), 4.01 (3H, s, OMe-4); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 189.6 ($q_c$, C-1`), 157.0 (q_c, C-4), 152.3 (q_c, C-1), 130.3 (q_c, C-2), 128.9 (CH, C-6), 128.5 (q_c, C-4a), 127.3 (CH, C-7), 124.7 (q_c, C-8a), 123.0 (CH, C-5), 122.9 (CH, C-8), 98.3 (CH, C-3), 65.8 (CH_3, 1-OMe), 55.8 (CH_3, 4-OMe); EIMS *m/z* (rel. int.) 216 [M^+] 201 (74), 187 (18), 173 (83), 159 (26), 145 (93), 115 (42); HRFABMS *m/z* 216.0786 (calcd for $C_{13}H_{12}O_3$ [M^+], 216.0786).

*6.3.7 Synthesis of **4.21** – **4.26** via Grignard addition to **4.20***



4.20

4.21 R = $CH{=}C(CH_3)_2$
4.22 R = CH_3
4.23 R = $CH{=}CH_2$
4.24 R = Ph
4.25 R = CH_2CH_2Ph
4.26 R = Naph

2-(1-hydroxy-3-methyl-2-butenyl)-1,4-dimethoxynaphthalene (4.21) :[237] A solution of isobutenylmagnesium bromide (1.5 g, 11.1 mmol) was prepared *in situ* as described previously (Section 6.3.3), and added dropwise *via cannula* to a stirred solution of the aldehyde **4.20** (0.255 g, 1.2 mmol) in dry THF (10 mL) at -10 °C. The resulting solution was stirred for 2 h at -10 °C and then gradually warmed to RT. The mixture was stirred for 1h before being quenched with sat. NH_4Cl (10 mL) and extracted with Et_2O (3 x 5 mL). The combined organic extracts were washed with water (2 x 10 mL) and sat. brine (1 x 10 mL),

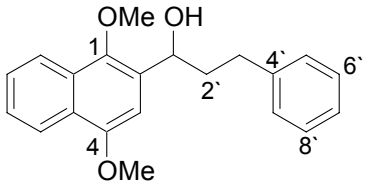
dried over $MgSO_4$ and concentrated under vacuum to yield a brown oil (0.407 g). NP HPLC (40% EtOAc, 60% hexane) of the crude product afforded **4.21** (0.202 g, 0.74 mmol, 62%) as a pale yellow oil. IR (film) ν_{max} cm^{-1} 3407, 2937, 1596, 1371, 1092, 846, 770; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.21 (1H, d, *J* = 8.5 Hz, H-5), 8.01 (1H, d, *J* = 8.5 Hz, H-8), 7.52 (1H, t, *J* = 7.5 Hz, H-7), 7.45 (1H, t, *J* = 7.5 Hz, H-6), 6.92 (1H, s, H-3), 6.02 (1H, d, *J* = 8.9 Hz, H-1`), 5.53 (1H, d, *J* = 8.8 Hz, H-2`), 4.0 (3H, s, OMe-4), 3.9 (3H, s, OMe-1), 1.88 (3H, s, H_3-5`), 1.76 (3H, s, $H_3$-4`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.3 (q_c, C-4), 145.8 (q_c, C-1), 135.9 (q_c, C-3`), 131.8 ($q_c$, C-2), 128.4 ($q_c$, C-8a), 127.0 (CH, C-2`), 126.6 (CH, C-7), 126.1 (q_c, C-4a), 125.3 (CH, C-6), 122.4 (CH, C-5), 121.9 (CH, C-8), 101.6 (CH, C-3), 65.6 (CH, C-1`), 62.5 ($CH_3$, 1-OMe), 55.7 ($CH_3$, 4-OMe), 25.9 ($CH_3$, C-4`), 18.4 (CH_3, C-5`); HRFABMS *m/z* 273.1490 (calcd for $C_{17}H_{21}O_3$ [$(M + H)^+$] 273.1412).

2-(1`-hydroxyethyl)-1,4-dimethoxynaphthalene (4.22) :[237] This method is representative. A solution of methylmagnesium bromide (3M, 1.4 mmol, 3.0 eq) was added to a cooled solution (-10 °C) of the aldehyde **4.20** (100 mg, 0.46 mmol) in anhydrous THF under an Ar atmosphere. The resulting solution was stirred for 1 h at -10 °C and gradually allowed to reach RT. The mixture was stirred for a further 16 h at RT before being quenched with sat. NH_4Cl (10 mL) and extracted with $CHCl_3$ (3 x 3 mL). The combined organic extracts were washed with water (2 x 5 mL) and sat. brine (1 x 5 mL), dried over Na_2SO_4 and concentrated under vacuum. NP HPLC (50% EtOAc, 50% hexane) of the crude products afforded 2-(1`-hydroxyethyl)-1,4-dimethoxynaphthalene (**4.22**, 107 mg, 100%) as a white crystalline solid (from hexane); mp 104-105 °C; lit.[297] 101-103 °C; IR (film) ν_{max} cm^{-1} 3401, 1598, 1373, 1000, 770; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.21 (1H, d, *J* = 8.5 Hz, H-5), 8.0 (1H, d, *J* = 8.5 Hz, H-8), 7.52 (1H, t, *J* = 7.5 Hz, H-7), 7.46 (1H, m, H-6), 6.90 (1H, s, H-3), 5.46 (1H, q, *J* = 6.4 Hz, H-1`), 3.98 (3H, s, OMe-4), 3.90 (3H, s, OMe-1), 1.56 (3H, d, *J* = 6.4 Hz, $H_3$-2`); ^{13}C (150 MHz, CDCl3) δ 152.4 (q_c, C-4), 145.5 (q_c, C-1), 133.1 (q_c, C-2), 128.3 (q_c, C-8a), 126.6 (CH, C-7), 126.1 (q_c, C-4a), 125.4 (CH, C-6), 122.4 (CH, C-5), 121.8 (CH, C-8), 101.0 (CH, C-3), 64.7 (CH, C-1`), 62.7 ($CH_3$, 1-OMe), 55.6 ($CH_3$, 4-OMe), 24.3 ($CH_3$, C-2`); EIMS *m/z* (rel. int.) 232

[M^+] (90), 215 (56), 202 (26), 189 (60), 175 (100), 159 (46), 115 (26), 77 (12), 43 (79); HRFABMS *m/z* 233.1177 (calcd for $C_{14}H_{17}O_3$ [(M + H)$^+$], 233.1099).

2-(1`-hydroxy-2`-propenyl)-1,4-dimethoxynaphthalene (4.23) :[237] (92 mg, 0.38 mmol, 83%); pale yellow oil; IR (film) ν_{max} cm^{-1} 3397, 2940, 1596, 1461, 1370, 1090, 770; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.21 (1H, d, *J* = 8.3 Hz, H-5), 8.02 (1H, d, *J* = 8.3 Hz, H-8), 7.53 (1H, t, *J* = 7.5 Hz, H-7), 7.47 (1H, t, *J* = 7.6 Hz, H-6), 6.78 (1H, s, H-3), 6.15 (1H, ddd, *J* = 17.6, 10.4, 5.2 Hz, H-2`), 5.77 (1H, br d, *J* = 4.9 Hz, H-1`), 5.41 (1H, d, *J* = 17.1 Hz, H-3b`), 5.23 (1H, d, *J* = 10.5 Hz, H-3a`), 3.96 (3H, s, 4-OMe), 3.90 (3H, s, 1-OMe); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.3 (q_c, C-4), 146.4 (q_c, C-1), 140.0 (CH, C-2`), 130.1 ($q_c$, C-2), 128.4 ($q_c$, C-8a), 126.7 (CH, C-7), 126.3 ($q_c$, C-4a), 125.6 (CH, C-6), 122.4 (CH, C-8), 122.0 (CH, C-5), 114.9 ($CH_2$, C-3`), 101.8 (CH, C-3), 69.7 (CH, C-1`), 62.9 (CH_3, 1-OMe), 55.6 (CH_3, 4-OMe); EIMS *m/z* (rel. int.) 244 [M^+] (94), 227 (58), 213 (10), 201 (55), 197 (18), 169 (58), 143 (16), 115 (43), 91 (6), 77 (13), 55 (100), 43 (5); HRFABMS *m/z* 245.1177 (calcd for $C_{15}H_{17}O_3$ [(M + H)$^+$], 245.1099).

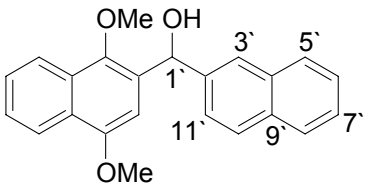
2-(1`-hydroxy-1`-phenylmethyl)-1,4-dimethoxynaphthalene (4.24) :[237] (125 mg, 0.43 mmol, 93%); white crystalline solid (from hexane); mp 123-125 °C; IR (film) ν_{max} cm^{-1} 3407, 2937, 1596, 1370, 998, 770, 702; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.22 (1H, d, *J* = 8.4 Hz, H-5), 8.02 (1H, d, *J* = 8.4 Hz, H-8), 7.52 (1H, t, *J* = 7.6 Hz, H-7), 7.47 (1H, m, H-6), 7.45 (2H, d, *J* = 7.5 Hz, H-3`, H-7`), 7.32 (2H, t, *J* = 7.6 Hz, H-4`, H-6`), 7.24 (1H, t, *J* = 7.4 Hz, H-5`), 6.80 (1H, s, H-3), 6.36 (1H, s, H-1`), 3.92 (3H, s, OMe-4), 3.80 (3H, s, OMe-1); 152.2 (q_c, C-4), 146.5 (q_c, C-1), 143.7 (q_c, C-2'), 131.4 (q_c, C-2), 128.4 (q_c, C-8a), 128.4 (2 x CH, C-4`, 6`), 127.3 (CH, C-5`), 126.7 (CH, C-7), 126.4 (2 x CH, C-3`, C7`), 126.3 ($q_c$, C-4a), 125.6 (CH, C-6), 122.5 (CH, C-8), 122.0 (CH, C-5), 102.4 (CH, C-3), 71.0 (CH, C-1`), 62.6 (CH_3, 1-OMe), 55.6 (CH_3, 4-OMe); EIMS *m/z* (rel. int.) 294 [M^+] (17), 277 (11), 251 (12), 202, (8), 189 (37), 174 (10), 149 (15), 105 (100), 77 (19); HRFABMS *m/z* 295.1335 (calcd for $C_{19}H_{19}O_3$ [(M + H)$^+$], 295.1256).



4.25

Phenylethylmagnesium bromide (1M, 3.5 mL, 3.5 mmol) was added to a stirred solution of **4.20** (250 mg, 1.16 mmol) in dry THF (3 mL) at -10 °C. The resulting solution was stirred for 1 h at -10 °C and then gradually allowed to reach RT. The mixture was allowed to stir overnight at RT before being quenched with sat. NH_4Cl (10 mL) and extracted with Et_2O (3 x 3 mL). The combined organic extracts were washed with water (2 x 5 mL) and sat. brine (1 x 5 mL), dried over Na_2SO_4 and concentrated *in vacuo* to yield a brown oil (462 mg). Trituration with 1:1 hexane/EtOAc afforded 2-(1`-hydroxy-1`-ethyl-3`-phenyl)-1,4-dimethoxynaphthalene (**4.25**) as fine white needles.

2-(1`-hydroxy-3`-phenylpropyl)-1,4-dimethoxynaphthalene (4.25) : (222 mg, 0.70 mmol, 60 %) as fine white needles (from 1:1 hexane/EtOAc); mp 109-110 °C; IR (film) ν_{max} cm^{-1} 3438, 3020, 1597, 1460, 1217, 1000, 770; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.25 (1H, d, *J* = 8.2 Hz, H-5), 8.03 (1H, d, *J* = 8.2 Hz, H-8), 7.53 (1H, td, *J* = 7.6, 1.1 Hz, H-7), 7.47 (1H, td, *J* = 7.6, 1.1 Hz, H-6), 7.28 (2H, t, *J* = 7.5 Hz, H-6`, H-8`), 7.23 (2H, d, *J* = 7.0 Hz, H-5`, H-9`), 7.18 (1H, t, *J* = 7.3 Hz, H-7`), 6.89 (1H, s, H-3), 5.29 (1H, dd, *J* = 8.3, 4.7 Hz, H-1`), 3.99 (3H, s, OMe-4), 3.82 (3H, s, OMe-1), 2.90 (1H, ddd, *J* = 14.3, 10.0, 5.2 Hz, H-2b`), 2.74 (1H, ddd, *J* = 13.9, 9.6, 6.8 Hz, H-2a`), 2.23 (1H, dddd, *J* = 13.8, 9.4, 8.8, 5.2 Hz, H-3b`), 2.07 (1H, m, H-3a`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.4 (q_c, C-4), 146.0 (q_c, C-1), 141.8 (q_c, C-4`), 132.0 ($q_c$, C-2), 128.5 (2 x CH, C-5`, C-7`), 128.4 (2 x CH, C-6`, C-8`), 128.4 ($q_c$, C-8a), 126.6 (CH, C-7), 126.2 ($q_c$, C-4a), 125.8 (CH, C-7`), 125.4 (CH, C-6), 122.4 (CH, C-5), 121.9 (CH, C-8), 101.6 (CH, C-3), 68.2 (CH, C-1`), 62.6 ($CH_3$, 1-OMe), 55.7 ($CH_3$, 4-OMe), 39.9 ($CH_2$, C-2`), 32.4 (CH_2, C-3`); EIMS *m/z* (rel. int.) 322 [M^+] (73), 304 (12), 217 (100), 202 (17), 189 (29), 174 (12), 159 (9), 105 (5), 93 (3); HRFABMS *m/z* 322.1569 (calcd for $C_{21}H_{22}O_3$ [M^+], 322.1569).

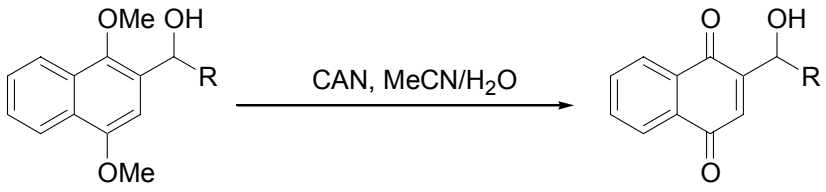


4.26

A solution of 2-naphthylmagnesium bromide (1.20 g, 5.8 mmol) was prepared *in situ* according to the procedure by Beck *et al*.,[296] and added dropwise *via* cannula to a stirred solution of the **4.20** (250 mg, 1.16 mmol) in anhydrous THF (5 mL) at -10 °C. The resulting solution was stirred for 1 h at -10 °C and then gradually allowed to reach RT. The resulting mixture was allowed to stir overnight at RT before being quenched with sat. NH_4Cl (10 mL) and extracted with Et_2O (3 x 3 mL). The combined organic extracts were washed with water (2 x 5 mL) and sat. brine (1 x 5 mL), dried over Na_2SO_4 and concentrated under vacuum to yield a brown solid (858 mg). NP semi-preparative HPLC of the crude product (25% EtOAc, 75% hexane) afforded 2-(1`-hydroxy-1`-naphthylmethyl)-1,4-dimethoxynaphthalene (**4.26**, 340 mg, 0.99 mmol, 85%) as a pale yellow oil.

2-(1`-hydroxy-1`-naphthylmethyl)-1,4-dimethoxynaphthalene (4.26) : yellow oil; IR (film) ν_{max} cm^{-1} 3407, 3009, 2938, 1508, 1459, 1369, 1092, 999, 756; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.23 (1H, d, *J* = 8.3 Hz, H-5), 8.04 (1H, d, *J* = 8.4 Hz, H-8), 7.95 (1H, s, H-3`), 7.83 (1H, m H-5`), 7.80 (1H, m, H-8`), 7.78 (1H, d, *J* = 8.6 Hz, H-10`), 7.55 (1H, t, *J* = 7.5 Hz, H-7), 7.52 (1H, dd, *J* = 8.6, 1.6 Hz, H-11`), 7.49 (1H, t, *J* = 7.5 Hz, H-6), 7.47 (H, m, H-6`), 7.46 (1H, m, H-7`), 6.81 (1H, s, H-3), 6.53 (1H, s, H-1`), 3.90 (3H, s, OMe-4), 3.83 (3H, s, OMe-1); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.3 (q_c, C-4), 146.7 (q_c, C-1), 141.1 (q_c, C-2`), 133.2 ($q_c$, C-4`), 132.7 (q_c, C-9`), 131.3 ($q_c$, C-2), 128.4 ($q_c$, C-8a), 128.1 (CH, C-10`), 128.1 (CH, C-5`), 127.6 (CH, C-8`), 126.7 (CH, C-7), 126.4 (q_c, C-4a), 126.1 (CH, C-6`), 125.8 (CH, C-7`), 125.6 (CH, C-6), 124.9 (CH, C-11`), 124.8 (CH, C-3`), 122.5 (CH, C-5), 122.0 (CH, C-8), 102.5 (CH, C-3), 71.0 (CH, C-1`), 62.7 (CH_3, 1-OMe), 55.6 (CH_3, 4-OMe); EIMS *m/z* (rel. int.) 344 [M^+] (100), 313 (15), 301 (27), 269 (9), 241 (5), 215 (21), 201 (14), 189 (28), 173 (40), 145 (14), 133 (4); HRFABMS *m/z* 344.1425 (calcd for $C_{23}H_{20}O_3$ [M^+], 344.1412).

*6.3.8 Oxidative demethylation of **4.21** – **4.26***



4.21	R = $CH=C(CH_3)_2$	**4.19**	R = $CH=C(CH_3)_2$
4.22	R = CH_3	**4.27**	R = CH_3
4.23	R = $CH=CH_2$	**4.28**	R = $CH=CH_2$
4.24	R = Ph	**4.29**	R = Ph
4.25	R = CH_2CH_2Ph	**4.30**	R = CH_2CH_2Ph
4.26	R = Naph	**4.31**	R = Naph

This method is representative. A solution of 2 eq of cerium ammonium nitrate (CAN) (0.262 g, 0.48 mmol) in water (0.3 mL) was added dropwise to a solution of **4.21** (0.065 g, 0.24 mmol) in MeCN (5 mL) until a deep yellow colour persisted. The mixture was diluted with water (5 mL) and extracted with Et_2O (3 x 2 mL). The combined organic extracts were washed with water (5 mL) and sat. brine (5 mL), dried over Na_2SO_4 and the solvent evaporated *in vacuo* to give 2-(1`-hydroxy-3`-methyl-2`-butenyl)-1,4-naphthoquinone as a dark yellow oil (0.013 g, 0.05 mmol, 23%).

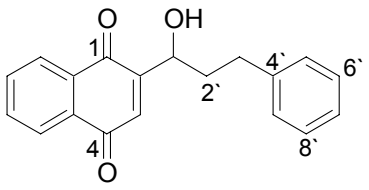
**2-(1`-hydroxy-3`-methyl-2`-butenyl)-1,4-naphthoquinone (4.19) :**[237] IR (film) $\nu_{max}$ $cm^{-1}$ 3416, 2923, 1662, 1300, 1073, 843, 775; $^1H$ NMR ($CDCl_3$, 600 MHz) δ 8.06 (2H, m, H-8, H-5), 7.73 (2H, m, H-7, H-6), 6.99 (1H, s, H-3), 5.57 (1H, d, *J* = 8.6 Hz, H-1`), 5.25 (1H, d, *J* = 8.6 Hz, H-2`), 1.83 (3H, s, $H_3$-5`), 1.76 (3H, s, H_3-4`); $^{13}C$ NMR ($CDCl_3$, 150 MHz) δ 185.6 ($q_c$, C-4), 185.5 ($q_c$, C-1), 150.9 ($q_c$, C-2), 138.7 ($q_c$, C-3`), 134.0 (CH, C-7), 133.8 (CH, C-6), 133.2 (CH, C-3), 132.3 (q_c, C-8a), 131.9 (q_c, C-4a), 126.5 (CH, C-5), 126.2 (CH, C-8), 123.8 (CH, C-2`), 66.5 (CH, C-1`), 25.9 (CH_3, C-4`), 18.6 ($CH_3$, C-5`); HRFABMS *m/z* 242.1196 (calcd for $C_{15}H_{14}O_3$ $[M]^+$ 242.0943).

2-(1`-hydroxyethyl)-1,4-naphthoquinone (4.27) :[237] (35 mg, 0.17 mmol, 77%) yellow powder; mp 86-87 °C; lit.[358] 87-88 °C; IR (film) ν_{max} cm^{-1} 3430, 1662, 1303, 1250, 1019, 781, 720; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.02 (2H, m, H-8, H-5), 7.70 (2H, m, H-7, H-6), 6.99 (1H, s, H-3), 5.00 (1H, q, *J* = 6.4 Hz, H-1`), 1.48 (3H, d, *J* = 6.4 Hz, $H_3$-2`); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.4 (q_c, C-4, C-1), 152.9 (q_c, C-2), 134.0 (CH, C-7), 133.8 (CH, C-6), 132.8 (CH, C-5), 132.1 (q_c, C-8a), 131.8 (q_c, C-4a), 126.4 (CH, C-8), 126.1 (CH, C-3), 65.0 (CH, C-1`), 22.6 ($CH_3$, C-2`); EIMS *m/z* (rel. int.) 202 [M^+] (82), 187 (80), 184 (56), 175 (3), 160 (100), 149 (11), 131 (49), 102 (33), 91 (8), 77 (29), 50, (10), 43 (4); HRFABMS *m/z* 203.0709 (calcd for $C_{12}H_{11}O_3$ [$(M + H)^+$], 203.0630).

2-(1`-hydroxyallyl)-1,4-naphthoquinone (4.28) :[237] (31 mg, 0.14 mmol, 70%) brown oil; IR (film) ν_{max} cm^{-1} 3419, 1662, 1594, 1302, 1151, 1047, 929, 754; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.05 (2H, m, H-8, H-5), 7.72 (2H, m, H-7, H-6), 7.00 (1H, s, H-3), 6.00 (1H, ddd, *J* = 16.7, 10.4, 5.8 Hz, H-2`), 5.46 (1H, d, *J* = 17.2 Hz, H-3a`), 5.36 (1H, br d, *J* = 4.2 Hz, H-1`), 5.27 (1H, d, *J* = 10.4 Hz, H-3b`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.2 (q_c, C-4), 185.1 (q_c, C-1), 150.0 (q_c, C-2), 136.9 (CH, C-2`), 134.0 (CH, H-7),133.8 (CH, C-6), 133.6 (CH, C-3), 132.1 ($q_c$, C-8a), 131.8 ($q_c$, C-4a), 126.5 (CH, C-5), 126.2 (CH, C-8), 117.3 ($CH_2$, C-3`), 69.8 (CH, C-1`); EIMS *m/z* (rel. int.) 214 [M^+] (5), 196 (34), 185 (100), 168 (31), 149 (96), 139 (17), 128 (20), 115 (10), 102 (17), 89 (4), 77 (17), 55 (10), 43 (8); HRFABMS *m/z* 215.0709 (calcd for $C_{13}H_{11}O_3$ [$(M + H)^+$], 215.0630).

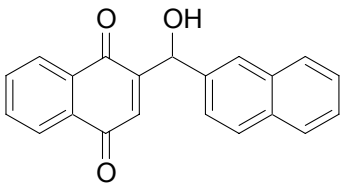
2-(1`-hydroxy-1`-phenylmethyl)-1,4-naphthoquinone (4.29) :[237] (41 mg, 0.16 mmol, 94%) brown oil; IR (film) ν_{max} cm^{-1} 3427, 1662, 1594, 1301, 1252, 1142, 1047, 941, 781, 726, 700; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.00 (1H, d, *J* = 7.4 Hz, H-5), 7.96 (1H, d, *J* = 7.4 Hz, H-8), 7.71 (1H, td, *J* = 7.5, 1.5 Hz, H-7), 7.69 (1H, td, *J* = 7.5, 1.5 Hz, H-6), 7.42 (2H, d, *J* = 7.5 Hz, H-7`, H-3`), 7.31 (2H, t, *J* = 7.5 Hz, H-6`, H-4`), 7.24 (1H, m, H-5`), 7.05 (1H, s, H-3), 5.90 (1H, s, H-1`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.3 (q_c, C-4), 184.9 (q_c, C-1), 151.0 (q_c, C-2), 140.3 (q_c, C-2`), 134.0 (CH, C-7), 133.8 (CH, C-6), 133.5 (CH, C-3), 132.1 (q_c, C-8a), 131.9 (q_c, C-

4a), 128.7 (2 x CH, C-6`, C4`), 128.4 (CH, C-5`), 126.9 (2 x CH, C-7`, C-3`), 126.5 (CH, C-5), 126.1 (CH, C-8), 70.8 (CH, C-1`); EIMS *m/z* (rel. int.) 264 [M^+] (3), 247 (36), 218 (22), 207 (5), 189 (48), 178 (12), 158 (10), 149 (20), 130 (15), 105 (53), 77 (100), 51 (18); HRFABMS *m/z* 264.0757 (calcd for $C_{17}H_{12}O_3$ [M^+], 264.0786).



4.30

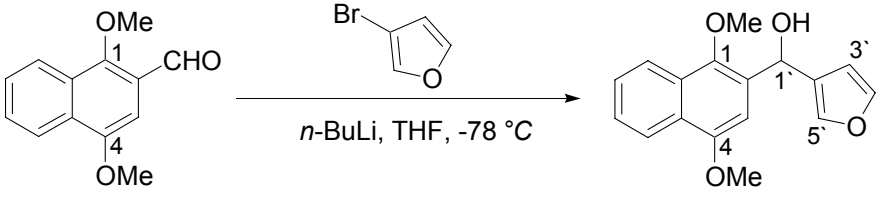
2-(1`-hydroxy-3`-phenylpropyl)-1,4-naphthoquinone (4.30) : (45 mg, 0.15 mmol, 80%) brown oil; IR (film) ν_{max} cm^{-1} 3442, 3020, 2401, 1662, 1303, 1216, 1064, 926, 756, 667; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.04 (2H, m, H-8, H-5), 7.72 (2H, m, H-7, H-6), 7.25 (2H, t, *J* = 7.5 Hz, H-8`, H-6`), 7.20 (2H, d, *J* = 7.0 Hz, H-9`, H-5`), 7.15 (1H, t, *J* = 7.3 Hz, H-7`), 6.97 (1H, br s, H-3), 4.83 (1H, dd, *J* = 8.3, 3.3 Hz, H-1`), 2.89 (1H, ddd, *J* = 14.3, 9.6, 5.5 Hz, H-3b`), 2.79 (1H, ddd, *J* = 14.2, 9.2, 7.0 Hz, H-3a`), 2.14 (1H, dddd, *J* = 13.7, 10.1, 6.9, 3.8 Hz, H-2b`), 2.00 (1H, dddd, *J* = 14.1, 9.1, 8.6, 5.5 Hz, H-2a`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.4 (q_c, C-4), 185.1 (q_c, C-1), 151.8 (q_c, C-2), 141.0 (q_c, C-4`), 134.0 (CH, C-7), 133.8 (CH, C-6), 133.5 (CH, C-3), 132.2 ($q_c$, C-8a), 131.8 ($q_c$, C-4a), 128.5 (2 x CH, C-8`-, C-6`), 128.5 (2 x CH, C-9`, C-5`), 126.4 (CH, C-5), 126.1 (CH, C-7`), 126.0 (CH, C-8), 68.7 (CH, C-1`), 37.8 ($CH_2$, C-2`), 31.8 (CH_2, C-3`); EIMS *m/z* (rel. int.) 292 [M^+] (12), 274 (43), 257 (9), 228 (3), 202 (5), 188 (100), 160 (41), 133 (6), 105 (9), 79 (4); HRFABMS *m/z* 292.1100 (calcd for $C_{19}H_{16}O_3$ [M^+], 292.1099).



4.31

2-(1`-hydroxy-1`-naphthylmethyl)-1,4--naphthoquinone (4.31) : (23.1 mg, 0.07 mmol, 50%) yellow oil; IR (film) ν_{max} cm^{-1} 3433, 3059, 2919, 1664, 1349, 1297, 1121, 818, 758; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.04 (1H, dd, *J* = 7.3, 1.2 Hz, H-5), 7.98 (1H, dd, *J* = 7.3, 1.1 Hz, H-8), 7.93 (1H, br s, H-3`), 7.82 (1H, d, *J* = 8.0 Hz, H-10`), 7.82 (1H, m, H-5`), 7.80 (1H, m, H-8`), 7.70 (1H, td, *J* = 7.1, 1.5 Hz, H-7), 7.67 (1H, td, *J* = 7.4, 1.5 Hz, H-6), 7.53 (1H, dd, *J* = 8.5, 1.6 Hz, H-11`), 7.47 (1H, td, *J* = 6.4, 1.6 Hz, H-6`), 7.46 (1H, td, *J* = 6.4, 1.7 Hz, H-7`), 7.12 (1H, br d, *J* = 1.2 Hz, H-3), 6.11 (1H, s, H-1`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.3 (q_c, C-4), 185.0 (q_c, C-1), 150.8 (q_c, C-2), 137.5 (q_c, C-2`), 134.0 (CH, C-7), 133.8 (CH, C-6), 133.6 (CH, C-3), 133.2 ($q_c$, C-4`, C9`), 132.1 ($q_c$, C-8a), 131.9 ($q_c$, C-4a), 128.7 (CH, C-10`), 128.1 (CH, C-5`), 127.7 (CH, C-8`), 126.5 (CH, C-8), 126.4 (CH, C-6`, C7`), 126.3 (CH, C-3`), 126.2 (CH, C-5), 124.4 (CH, C-11`), 71.0 (CH, C-1`); EIMS *m/z* (rel. int.) 314 [M^+] (33), 296 (100), 284 (13), 268 (21), 241 (9), 239 (21), 186 (2), 155 (13), 128 (8); HRFABMS *m/z* 314.0935 (calcd for $C_{21}H_{14}O_3$ [M^+], 314.0943).

6.3.9 Synthesis of **4.32** and **4.33**

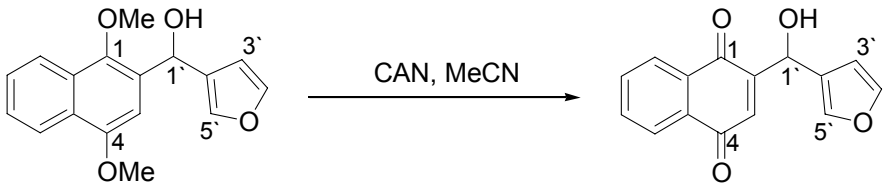


4.20 **4.32**

A solution of *n*-butyllithium (1.6 M, 2.9 mL, 4.6 mmol, 10 eq) was added to a solution of 3-bromofuran (680 mg, 4.6 mmol) in THF (5 mL) at -78 °C and the reaction mixture stirred for

30 mins before a solution of **4.20** (210 mg, 0.97 mmol) in dry THF (5 mL) was added dropwise *via* cannula. The reaction was stirred at -78 °C for 30 mins and gradually allowed to reach RT. The reaction mixture was quenched with sat. NH_4Cl (10 mL) and extracted with EtOAc (3 x 5 mL). The combined organic phases were washed with sat. brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo* to give a brown oil (339 mg). Analysis of the mixture by TLC using Ehrlich's reagent indicated the presence of the desired furanyl product. NP HPLC (25% EtOAc, 75% hexane) of the crude mixture afforded 2-(1`-hydroxy-1`-furanylmethyl)-1,4-dimethoxynaphthalene (**4.32**, 200 mg, 0.70 mmol, 73%) as a yellow oil.

2-(1`-hydroxy-1`-furanylmethyl)-1,4-dimethoxynaphthalene (4.32) : yellow oil; IR (film) ν_{max} cm^{-1} 3420, 3014, 2842, 1596, 1461, 1371, 1217, 1092,1000, 875, 758; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.23 (1H, d, *J* = 8.3 Hz, H-5), 8.02 (1H, d, *J* = 8.3 Hz, H-8), 7.54 (1H, td, *J* = 6.8, 1.1 Hz, H-7), 7.48 (1H, td, *J* = 6.8, 1.1 Hz, H-6), 7.37 (1H, t, *J* = 1.6 Hz, H-4`), 7.32 (1H, br s, H-5`), 6.83 (1H, s, H-3), 6.39 (1H, br d, *J* = 1.1 Hz, H-3`), 6.30 (1H, s, H-1`), 3.94 (3H, s, OMe-4), 3.84 (3H, s, OMe-1); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 152.3 (q_c, C-4), 146.3 (q_c, C-1), 143.3 (CH, C-4`), 139.8 (CH, C-5`), 130.6 (q_c, C-2), 128.9 (q_c, C-2`), 128.3 ($q_c$, C-8a), 126.7 (CH, C-7), 126.4 ($q_c$, C-4a), 125.7 (CH, C-6), 122.4 (CH, C-5), 122.0 (CH, C-8), 109.5 (CH, C-3), 102.0 (CH, C-3`), 64.6 (CH, C-1`), 62.8 (CH_3, 1-OMe), 55.7 (CH_3, 4-OMe); EIMS *m/z* (rel. int.) 284 [M^+] (54), 252 (100), 239 (24), 236 (10), 209 (29), 196 (15), 181 (25), 165 (15), 152 (42); HRFABMS *m/z* 284.1058 (calcd for $C_{17}H_{16}O_4$ [M^+], 284.1049).

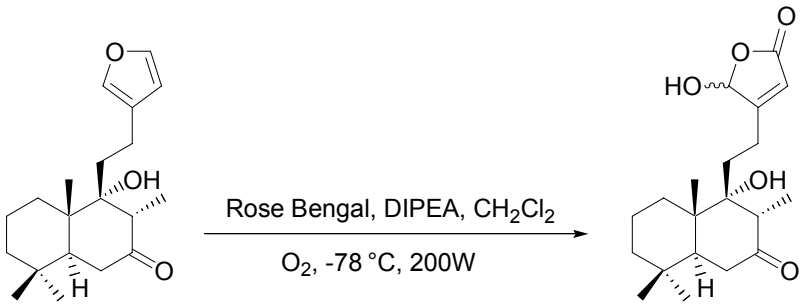


4.32 **4.33**

2-(1`-hydroxy-1`-furanylmethyl)-1,4- naphthoquinone (4.33) : (17.0 mg, 0.066 mmol, 55%) Brown solid; IR (film) ν_{max} cm^{-1} 3021, 2401, 1666, 1521, 1302, 1216, 929, 757, 669, 518; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.05 (2H, m, H-8, H-5), 7.74 (1H, td, *J* = 7.2, 1.9 Hz, H-7), 7.72 (1H,

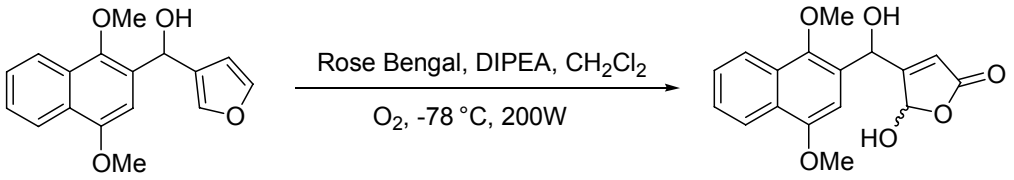
td, *J* = 7.5, 1.9 Hz, H-6), 7.49 (1H, m, H-5`), 7.37 (1H, t, *J* = 1.7 Hz, H-4`), 7.04 (1H, br d, *J* = 1.3 Hz, H-3), 6.41 (1H, br d, *J* = 1.7 Hz, H-3`), 5.94 (1H, s, H-1`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.3 (q_c, C-4), 185.2 (q_c, C-1), 150.2 (q_c, C-2), 143.6 (CH, H-4`), 140.4 (CH, C-5`), 134.1 (CH, C-7), 133.9 (CH, C-6), 133.5 (CH, C-3), 132.1 (q_c, C-8a), 131.9 (q_c, C-4a), 126.6 (CH, C-5), 126.3 (CH, C-8), 125.4 (q_c, C-2`), 108.8 (CH, C-3`), 64.2 (CH, C-1`); EIMS *m*/*z* (rel. int.) 254 [M^+] (16), 237 (11), 226 (71), 225 (100), 199 (30), 197 (39), 181 (14), 169 (13), 152 (20), 141 (14), 130 (8), 105 (11), 78 (8), 51 (3); HRFABMS *m/z* 254.0585 (calcd for $C_{15}H_{10}O_4$ [M^+], 254.0579).

*6.3.10 Attempted Rose Bengal oxidation of **4.32** and **4.39***[304]



4.39 **4.40**

This method is representative. Rose Bengal (6 mg) was added to a solution of **4.39** (20 mg, 0.063 mmol) and DIPEA (81 mg, 0.63 mmol) in anhydrous CH_2Cl_2 (10 mL) at RT. Anhydrous oxygen was bubbled through the reaction mixture for 10 mins and then stirred under an atmosphere of oxygen at -78 °C and irradiated with a 200 W lamp for 4 h. The reaction was allowed to gradually warm up to RT, quenched with sat. oxalic acid (5 mL) and this mixture was vigorously stirred for 30 mins. This mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3 x 5 mL). The combined organic fractions were washed with H_2O (10 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, taken up in EtOAc and flushed through a short (10 mL) silica plug to afford the crude product as a pink solid (**4.40**, 15.2 mg, 0.043 mmol, 69%). No further purification was deemed necessary. Selected ^{1}H and ^{13}C NMR shifts for **4.40** are summarized in Table 4.3.



4.32 **4.34**

The same procedure as outline above was carried out on **4.32**. Preliminary analysis of the ^{1}H and ^{13}C NMR spectra for the crude reaction mixtures obtained after every attempt revealed a mixture of complex products with no indication of oxygenation at C-4` and C-5` of the furanyl moiety.

*6.3.11 Oxidation of benzylic alcohols **4.24** – **4.26** and **4.32***

4.24 R = Ph
4.25 R = CH_2CH_2Ph
4.26 R = Naph
4.32 R = Fur

4.41 R = Ph
4.42 R = Naph
4.43 R = Fur
4.44 R = CH_2CH_2Ph

Method A: MnO_2 Oxidation of **4.26**[359]

This method is representative. The secondary alcohol **4.26** (100 mg, 0.29 mmol, 1 eq.) was dissolved in anhydrous CH_2Cl_2 and finely powdered MnO_2 (606 mg, 7.0 mmol, 30 eq.) was added. The solution was stirred at ambient temperature for 48 h and filtered through Celite (coarse 545). The crude product yielded 2-naphthoyl-1,4-dimethoxynaphthalene (**4.42**, 90.5 mg, 0.26 mmol, 91%) as a white amorphous powder, without any purification.

2-naphthoyl-1,4-dimethoxynaphthalene (4.42) : (91% using Method A without purification) Light yellow crystals (from hexane); mp 106-109 °C; IR (film) ν_{max} cm^{-1} 3019, 2938, 2400,

1656, 1461, 1372, 1216, 1111, 1032, 968, 770, 668; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.35 (1H, s, H-3`), 8.34 (1H, m, H-5), 8.18 (1H, m, H-5), 8.07 (1H, dd, *J* = 8.7, 1.5 Hz, H-11`), 7.93 (1H, d, *J* = 8.4 Hz, H-10`), 7.88 (2H, m, H-6`, H-5`), 7.62 (2H, m, H-7, H-6), 7.59 (1H, t, *J* = 7.3 Hz, H-7`), 7.51 (1H, d, *J* = 7.2 Hz, H-8`), 6.84 (1H, s, H-3), 3.99 (3H, s, OMe-4), 3.76 (3H, s, OMe-1); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 197.0 ($q_c$, C-1`), 151.7 (q_c, C-4), 148.7 (q_c, C-1), 135.7 (q_c, C-4`), 135.0 ($q_c$, C-2`), 132.5 (CH, C-3`), 132.5 ($q_c$, C-9`), 129.7 (CH, C-5`), 128.6 ($q_c$, C-8a), 128.5 (CH, C-8`), 128.2 (CH, C-10`), 127.9 ($q_c$, C-2), 127.8 (CH, C-6`), 127.3 (q_c, C-4a), 127.2 (CH, C-7), 127.1 (CH, C-6), 126.6 (CH, C-7`), 125.1 (CH, C-11`), 122.8 (CH, C-8), 122.5 (CH, C-5), 103.1 (CH, C-3), 63.6 (CH_3, 1-OMe), 55.8 (CH_3, 4-OMe); EIMS *m*/*z* (rel. int.) 342 [M^+] (100), 327 (40), 312 (33), 294 (6), 256 (11), 200 (30), 173 (2), 155 (9), 127 (6), 77 (1); HRFABMS *m/z* 342.1252 (calcd for $C_{23}H_{18}O_3$ [M^+], 342.1256).

Method B: TPAP Oxidation of **4.24**[304]

This method is representative. *N*-methylmorpholine-*N*-oxide (80 mg, 0.68 mmol, 3.0 eq), powdered 4Å molecular sieves (120 mg) and TPAP (30 mg, 0.09 mmol) were added to a solution of benzylic alcohol **4.24** (52 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (5 mL) under an Ar and stirred at ambient temperature for 2 h. The reaction mixture was filtered through Celite (545 coarse)/silica gel and washed with CH_2Cl_2 (10 mL), after which the solvent was removed *in vacuo* to yield 2-(1-formal-1-phenylmethyl)-1,4-dimethoxynaphthalene (**4.41**, 51.6 mg, 0.18 mmol, 98%) as yellow plates (from hexane/Ac_2O). No further purification was required.

2-benzoyl-1,4-dimethoxynaphthalene (4.41) : (80% yield after NP HPLC using Method A); yellow plates (from hexane/Me_2O); mp 109-110 °C; lit.[284] 100-102 °C; IR (film) ν_{max} cm^{-1} 3009, 2936, 2844, 1966, 1595, 1371, 1241, 1095, 1005, 956, 812, 769, 671; ^{1}H NMR $CDCl_3$, 600 MHz) δ 8.29 (1H, m, H-5), 8.14 (1H, m, H-8), 7.90 (2H, dd, *J* = 8.3, 1.1 Hz, H-7`, H-3`), 7.60 (2H, m, H-7, H-6), 7.58 (1H, m, H-5`), 7.45 (2H, t, *J* = 7.7 Hz, H-6`, H-4`), 6.77 (1H, s, H-3), 3.98 (3H, s, 4-OMe), 3.74 (3H, s, 1-OMe); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 197.0 ($q_c$, C-1`),

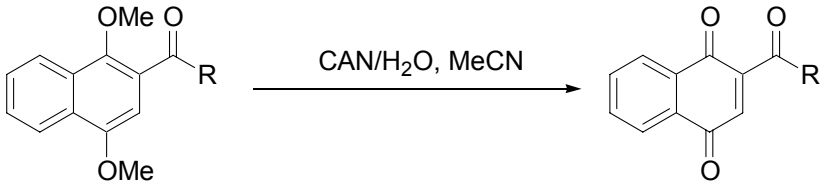
151.7 (q_c, C-4), 148.7 (q_c, C-1), 137.7 (q_c, C-2`), 133.2 (CH, C-5`), 130.0 (2 x CH, C-7`, C-3`), 130.0 (q_c, C-2), 128.5 (q_c, C-8a), 128.3 (2 x CH, C-6`, C4`), 127.9 (q_c, C-4a), 127.1 (CH, C-7), 127.1 (CH, C-6), 122.8 (CH, C-8), 122.5 (CH, C-5), 103.0 (CH, C-3), 63.6 (CH_3, OMe-1), 55.8 (CH_3, OMe-4); EIMS *m/z* (rel. int.) 292 [M^+] (100), 277 (56), 262 (33), 249 (15), 234 (28), 218 (18), 201 (16), 178 (10), 149 (11), 129 (11); HRFABMS *m/z* 293.1177 (calcd for $C_{19}H_{17}O_3$ [(M + H)]$^+$, 293.1099).

2-(1`-oxo-3`-phenylpropyl)-1,4-dimethoxynaphthalene (4.44) : (100% using Method B without purification); yellow crystals (from hexane/CH_2Cl_2); mp 80-82 °C; IR (film) ν_{max} cm^{-1} 3020, 2938, 2401, 1668, 1596, 1459, 1372, 1216, 1101, 966, 929, 758, 670; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.25 (1H, m, H-5), 8.14 (1H, m, H-8), 7.60 (1H, m, H-6), 7.56 (1H, m, H-7), 7.29 (2H, t, *J* = 6.7 Hz, H-8`, H-6`), 7.28 (2H, d, *J* = 6.5 Hz, H-9`, H-5`), 7.19 (1H, tt, *J* = 6.5, 2.1 Hz, H-7`), 6.99 (1H, s, H-3), 3.99 (3H, s, OMe-4), 3.87 (3H, s, OMe-1), 3.50 (2H, t, *J* = 7.5 Hz, $H_2$-2`), 3.10 (2H, t, *J* = 7.5 Hz, H_2-3`); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 202.1 ($q_c$, C-1`), 151.9 (q_c, C-4), 151.0 (q_c, C-1), 141.5 (q_c, C-4`), 128.8 ($q_c$, C-4a), 128.7 ($q_c$, C-8a), 128.5 (2 x CH, C-5`, C-9`), 128.4 (2 x CH, C-6`, C-8`), 127.6 (CH, C-7), 127.4 ($q_c$, C-2), 127.1 (CH, C-6), 126.0 (CH, C-7`), 123.1 (CH, C-8), 122.5 (CH, C-5), 102.2 (CH, C-3), 63.9 (CH_3, 1-OMe), 55.7 (CH_3, 4-OMe), 44.7 (CH_2, C-2`), 30.6 ($CH_2$, C-3`); LREIMS *m/z* (rel. int.) 320 [M^+] (100), 289 (15), 215 (80), 201 (13), 149 (6), 69 (11); HRFABMS *m/z* 321.1510 (calcd for $C_{21}H_{21}O_3$ [(M + H)]$^+$, 321.1412).

2-furanoyl-1,4-dimethoxynaphthalene (4.43) : (72% using Method A; 60% using Method B without purification); IR (film) ν_{max} cm^{-1} 3024, 2963, 2407, 1651, 1574, 1219, 1096, 926, 756; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.27 (1H, dd, *J* = 7.5, 1.6 Hz, H-5), 8.17 (1H, dd, *J* = 7.2, 1.8 Hz, H-8), 7.90 (1H, s, H-5`), 7.60 (1H, td, *J* = 6.9, 1.5 Hz, H-6), 7.57 (1H, td, *J* = 6.8, 1.5 Hz, H-7), 7.47 (1H, t, 1.6 Hz, H-4`), 6.92 (1H, br d, *J* = 1.5 Hz, H-3`), 6.79 (1H, s, H-3), 3.98 (3H, s, OMe-4), 3.83 (3H, s, OMe-1); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 189.9 ($q_c$, C-1`), 151.7 (q_c, C-4),

150.3 (CH, C-5`), 148.2 ($q_c$, C-1), 143.9 (CH, C-4`), 128.7 (q_c, C-8a), 128.0 (q_c, C-4a), 127.8 (2 x q_c, C-2, C-2`), 127.2 (CH, C-6), 127.1 (CH, C-7), 122.8 (CH, C-8), 122.4 (CH, C-5), 109.4 (CH, C-3`), 102.7 (CH, C-3), 63.8 (CH_3, 1-OMe), 55.8 (CH_3, 4-OMe); EIMS *m/z* (rel. int.) 282 [M^+] (100), 267 (13), 265 (21), 253 (13), 239 (68), 211 (31), 201 (26), 196 (24), 173 (11), 139 (11), 129 (16); HRFABMS *m/z* 282.0897 (calcd for $C_{17}H_{14}O_4$ [M]$^+$, 282.0892).

*6.3.12 Oxidative demethylation of **4.41** – **4.44***



4.41 R = Ph
4.42 R = Naph
4.43 R = Fur
4.44 R = CH_2CH_2Ph

4.45 R = Ph
4.46 R = CH_2CH_2Ph
4.47 R = Naph
4.48 R = Fur

2-benzoyl-1,4-naphthoquinone (4.45) : (97%); yellow needles (from hexane); mp 162-164 °C, lit.[360] 162-163 °C; IR (film) ν_{max} cm^{-1} 3020, 2400, 1671, 1599, 1450, 1348, 1216, 929, 756, 667; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.14 (1H, m, H-5), 8.11 (1H, m, H-8), 7.89 (2H, d, *J* = 7.3 Hz, H-7`, 3`), 7.82 (1H, td, *J* = 7.3, 1.8 Hz, H-7), 7.80 (1H, td, *J* = 7.3, 1.7 Hz, H-6), 7.64 (1H, t, *J* = 7.5 Hz, H-5`), 7.49 (2H, t, *J* = 7.6 Hz, H-6`, 4`), 6.99 (1H, s, H-3); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 191.9 ($q_c$, C-1`), 184.4 (q_c, C-4), 183.2 (q_c, C-1), 147.1 (q_c, C-2), 135.6 (CH, C-3), 135.5 (q_c, C-2`), 134.5 (CH, C-5`), 134.4 (CH, C-6), 134.4 (CH, C-7), 131.9 (q_c, C-4a), 131.5 (q_c, C-8a), 129.6 (2 x CH, C-7`, C-3`), 128.9 (2 x CH, C-6`, C-4`), 126.9 (CH, C-8), 126.5 (CH, C-5); EIMS *m/z* (rel. int.) 262 [M^+] (100), 236 (20), 234 (44), 206 (13), 178 (7), 157 (2), 129 (3), 106 (30), 78 (30); HRFABMS *m/z* 262.0628 (calcd for $C_{17}H_{10}O_3$ [M^+], 262.0630).

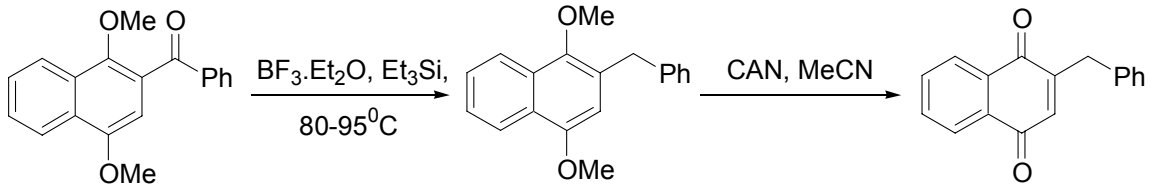
2-(1`-oxo-3`-phenylpropyl)-1,4-naphthoquinone (4.46) : Brown solid; IR (film) ν_{max} cm^{-1} 3020, 2410, 1670, 1579, 1463, 1218, 1104, 967, 933, 754, 669; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.10 (1H, dd, *J* = 7.1, 1.6 Hz, H-8), 8.07 (1H, dd, *J* = 7.0, 1.7 Hz, H-5), 7.80 (1H, td, *J* = 7.4, 1.6 Hz, H-7), 7.78 (1H, td, *J* = 7.3, 1.6 Hz, H-6), 7.27 (2H, t, *J* = 7.5 Hz, H-7`, H-6`), 7.22 (2H, d, *J* = 7.4 Hz, H-9`, H-5`), 7.18 (1H, t, *J* = 7.3 Hz, H-5`), 7.04 (1H, s, H-3); 3.30 (2H, t, *J* = 7.5 Hz, $H_2$-2`), 3.03 (2H, t, *J* = 7.5 Hz, H_2-3`); $^{13}C$ NMR ($CDCl_3$, 150 MHz) δ 199.7 ($q_c$, C-1`), 184.9 (q_c, C-4), 183.3 (q_c, C-1), 145.5 (q_c, C-2), 140.4 (q_c, C-4`), 137.0 (CH, C-3), 134.5 (CH, C-7), 134.4 (CH, C-6), 131.7 ($q_c$, C-8a), 131.7 ($q_c$, C-4a), 128.6 (2 x CH, C-8`, C-6`), 128.4 (2 x CH, C-9`, C-5`), 126.8 (CH, C-8`), 126.3 (CH, C-5`), 45.0 ($CH_2$, C-2`), 29.6 (CH_2, C-3`); EIMS *m*/*z* (rel. int.) 290 [M^+] (100), 274 (12), 237 (25), 202 (33), 187 (44), 105 (22), 91 (39)69 (24); HRFABMS *m/z* 290.0947 (calcd for $C_{19}H_{14}O_3$ [M^+], 290.0943).

2-naphthoyl-1,4-naphthoquinone (4.47) : (60%) Brown solid; IR (film) ν_{max} cm^{-1} 3020, 2401, 1662, 1589, 1469, 1216, 1076, 916, 757, 669; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.31 (1H, s, H-3`), 8.17 (1H, d, *J* = 7.1 Hz, H-5), 8.13(1H, d, *J* = 7.1 Hz, H-8), 8.02 (1H, d, *J* = 8.6 Hz, H-11`), 7.94 (1H, d, *J* = 8.6 Hz, H-10`), 7.91 (1H, d, *J* = 8.4 Hz, H-5`), 7.89 (1H, d, *J* = 8.4 Hz, H-8`), 7.84 (1H, td, *J* = 7.8, 1.5 Hz, H-6), 7.82 (1H, td, *J* = 7.8, 1.5 Hz, H-7), 7.63 (1H, t, *J* = 7.5 Hz, H-6`), 7.55 (1H, t, *J* = 7.5 Hz, H-7`), 7.06 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$, 150 MHz) δ 191.8 ($q_c$, C-1`), 184.5 (q_c, C-4), 183.3 (q_c, C-1), 147.2 (q_c, C-2), 136.2 (q_c, C-4`), 135.6 (CH, C-3), 134.5 (2 x CH, C-7, C-6), 132.9 ($q_c$, C-2`), 132.7 (CH, C-3`), 132.3 ($q_c$, C-9`), 131.9 (q_c, C-4a), 131.6 (q_c, C-8a), 129.8 (CH, C-5`), 129.4 (CH, C-6`), 129.0 (CH, C-10`), 127.9 (CH, C-8`), 127.2 (CH, C-7`), 126.9 (CH, C-8), 126.5 (CH, C-5), 123.9 (CH, C-11`); EIMS *m*/*z* (rel. int.) 312 [M^+] (100), 284 (41), 255 (12), 239 (3), 228 (5), 155 (37), 145 (13), 128 (10), 101 (2), 77 (5); HRFABMS *m/z* 312.0788 (calcd for $C_{21}H_{12}O_3$ [M^+], 312.0786).

2-furanoyl-1,4-naphthoquinone (4.48) : (56%) Fine yellow needles (from hexane/CH_2Cl_2); mp 138-141 °C; IR (film) ν_{max} cm^{-1} 3021, 2401, 1669, 1513, 1301, 1216, 1167, 928, 873, 769, 669; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.13 (1H, m, H-8), 8.12 (1H, m, H-5), 7.93 (1H, s, H-5`),

7.81 (2H, m, H-7, H-6), 7.51 (1H, t, *J* = 1.6Hz, H-4`), 7.02 (1H, s, H-3), 6.87 (1H, br d, *J* = 1.5 Hz, H-3`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 184.7 (q_c, C-4), 184.7 (q_c, C-1`), 182.7 ($q_c$, C-1), 150.0 (CH, C-5`), 146.3 (q_c, C-2), 144.9 (CH, C-4`), 135.2 (CH, C-3), 134.5 (CH, C-7), 134.5 (CH, C-6), 131.7 ($q_c$, C-4a), 131.5 ($q_c$, C-8a), 127.1 ($q_c$, C-2`), 126.9 (CH, C-8), 126.4 (CH, C-5), 108.8 (CH, C-3`); EIMS *m/z* (rel. int.) 252 [M^+] (54), 242 (4), 224 (100), 203 (3), 196 (25), 185 (3), 168 (17), 157 (6), 140 (8), 129 (5), 95 (47), 91 (2), 75 (10); HRFABMS *m/z* 252.0431 (calcd for $C_{15}H_8O_4$ [M^+], 252.0423).

*6.3.13 Reduction of ketone **4.41***[315]



4.41 **4.51** **4.52**

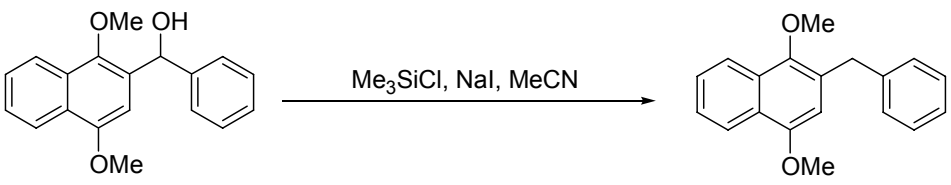
A red mixture of **4.41** (106 mg, 0.36 mmol), $BF_3.Et_2O$ (154 mg, 1.09 mmol, 137.5 μL) and Et_3Si (126.5 mg, 1.09, 174 μL) was heated at 80-95 °C for 2 h, upon which it turned colourless. The reaction mixture was allowed to cool and separated between water (3 mL) and Et_2O (3 x 3 mL). The combined organic fractions were washed with 10% saturated $NaHCO_3$ followed by sat. brine and dried over anhydrous $MgSO_4$. NP HPLC (25% EtOAc, 75% hexane) of the crude product afforded 2-benzyl-1,4-dimethoxynaphthalene **4.51** (58.4 mg, 0.21 mmol, 58%) which was crystallized by slow evaporation from hexane.

2-benzyl-1,4-dimethoxynaphthalene (4.51) : Colourless crystalline solid (from hexane); mp 62-64 °C, lit.[284] 61-62 °C; IR (film) ν_{max} cm^{-1} 3063, 2937, 1953, 1596, 1461, 1370, 1226, 1120, 1093, 1004, 979, 934, 848, 768; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.20 (1H, d, *J* = 8.5 Hz, H-5), 8.05 (1H, d, *J* = 8.5 Hz, H-8), 7.52 (1H, t, *J* = 7.7 Hz, H-7), 7.44 (1H, t, *J* = 7.6 Hz, H-6) , 7.24

(2H, d, *J* = 7.3 Hz, H-7`, H-3`), 7.22 (2H, m, H-6`, H-4`), 7.17 (1H, t, *J* = 7.1 Hz, H-5`), 6.52 (1H, s, H-3), 4.18 (2H, s, $H_2$-1`), 3.86 (3H, s, OMe-4), 3.84 (3H, s, OMe-1); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 151.8 (q_c, C-4), 147.2 (q_c, C-1), 141.0 (q_c, C-2`), 128.8 (2 x CH, C-6`, C-4`), 128.6 ($q_c$, C-2), 128.4 ($q_c$, C-8a), 128.4 (2 x CH, C-7`, C-3`), 126.5 (CH, C-7), 126.0 (CH, C-5`), 125.7 (q_c, C-4a), 125.0 (CH, C-6), 122.3 (CH, C-5), 121.9 (CH, C-8), 106.1 (CH. C-3), 62.1 (CH_3, 1-OMe), 55.6 (CH_3, 4-OMe), 35.8 (CH_2, C-1`); EIMS *m*/*z* (rel. int.) 278 [M^+] (100), 263 (75), 231 (45), 202 (32), 191 (7), 123 (10); HRFABMS *m/z* 278.1301 (calcd for $C_{19}H_{18}O_2$ [M^+], 278.1307).

2-benzyl-1,4-naphthoquinone (4.52) : (84%) Fine yellow needles (from hexane); mp 96-98 °C, lit.[316] 97-99 °C; IR (film) ν_{max} cm^{-1} 3021, 2401, 1666, 1596, 1496, 1364, 1302, 1216, 1030, 930, 756, 669, 533; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.10 (1H, m, H-8), 8.03 (1H, m, H-5), 7.72 (1H, m, H-7), 7.71 (1H, m, H-6), 7.33 (2H, t, *J* = 7.6 Hz, H-6`, H-4`), 7.26 (1H, d, *J* = 8.5 Hz, H-5`), 7.24 (2H, d, *J* = 7.5 Hz, H-7`, H-3`), 6.60 (1H, s, H-3), 3.90 (2H, s, $H_2$-1`); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 185.1 (q_c, C-4), 185.0 (q_c, C-1), 150.9 (q_c, C-2), 136.7 (q_c, C-2`), 133.7 (CH, C-7), 133.7 (CH, C-6), 132.1 ($q_c$, C-8a), 132.1 ($q_c$, C-4a), 129.4 (2 x CH, C-7`, C-3`), 128.8 (2 x CH, C-6`, C-4`), 126.9 (CH, C-5`), 126.6 (CH, C-8), 126.1 (CH, C-5), 35.7 (CH_2, C-1`); EIMS *m*/*z* (rel. int.) 248 [M^+] (100), 231 (57), 219 (7), 191 (10); HRFABMS *m/z* 248.0841 (calcd for $C_{17}H_{12}O_2$ [M^+], 248.0837).

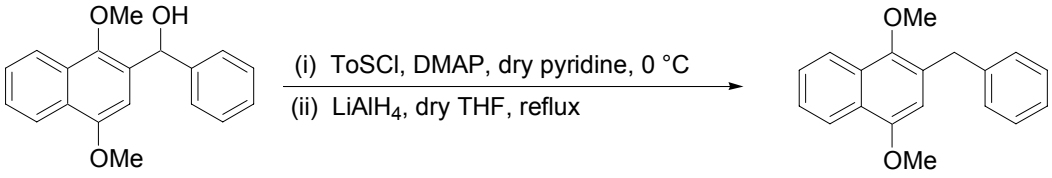
6.3.13.1 Attempted dehydroxylation of ***4.24***[198;311]



4.24 **4.51**

Iodotrimethylsilane was generated *in situ* through the slow addition of chlorotrimethylsilane (48 μL, 0.37 mmol) to a stirred suspension of NaI (55.8 mg, 0.37 mmol) in dry MeCN (1 mL). A solution of **4.24** (18.1 mg, 0.06 mmol) in dry MeCN (2 mL) was added dropwise to the pale yellow iodotrimethylsilane solution and the resulting reaction mixture was stirred for 0.5 h at ambient temperature, before being quenched with H_2O (5 mL). The aqueous solution was extracted with Et_2O (3 x 10 mL), the combined organic fractions washed with aq. $Na_2S_2O_3$ (2 x 10 mL) followed by H_2O (15 mL) before being dried over anhydrous $MgSO_4$. Removal of the solvent under reduced pressure gave a pale yellow oil (22.6 mg) that was purified using NP semi-preparative HPLC (33% EtOAc, 67% hexane) to yield five different fractions. Examination of the ^1H and ^{13}C NMR spectra of the different fractions showed no evidence of the presence of the expected product **4.51**.

*6.3.13.2 Attempted dehydroxylation of **4.24** via tosylation followed by LAH reduction* [306]

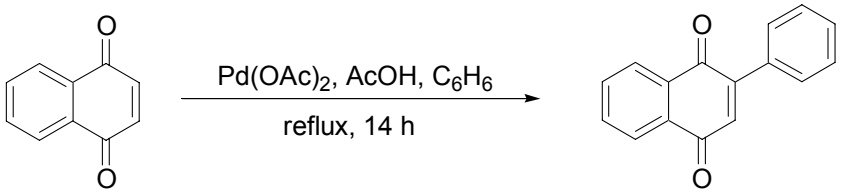


4.24 **4.51**

A solution of the alcohol **4.24** (25 mg, 0.085 mmol) in anhydrous pyridine (2 mL) was added to a stirred mixture of toluene-*p*-sulfonyl chloride (80 mg, 0.42 mmol) and DMAP (5 mg, 0.041 mmol) in dry pyridine (1 mL) at 0 °C. The reaction mixture was stirred at this temperature for 1 h and the left to stand at 0 °C overnight, before being diluted with dH_2O (2 mL) followed by addition of 2.5M HCl (3 mL) and ice. The aqueous solution was extracted with Et_2O (3 x 5 mL) and the combined organic layers washed with dH_2O (2 x 5 mL), sat. $NaHSO_3$ (5 mL) and dH_2O (2 x 5 mL) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to afford a clear yellow oil (15.8 mg). Analysis of the ^1H NMR spectrum of the oil indicated the

presence of mostly starting material and a complex mixture of products that was not investigated further.

6.3.14 Oxidative coupling of ***4.2*** [317]

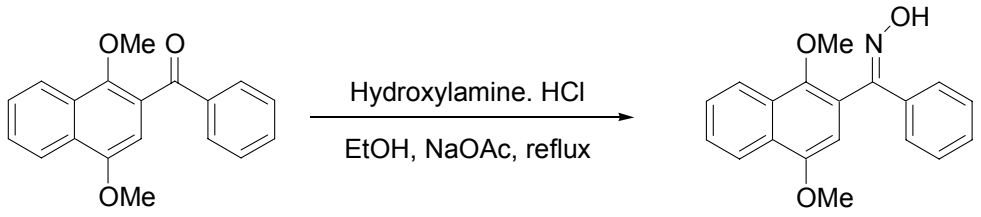


4.2 **4.53**

$Pd(OAc)_2$ (449 mg, 2.0 mmol) followed by anhydrous benzene (50 mL) were added to a solution of **4.2** (316mg, 2.0 mmol) in AcOH (50 mL). The reaction mixture was then refluxed for 14 h under nitrogen. This mixture was allowed to cool to RT and extracted with $CHCl_3$ (3 x 20 mL). The combined organic fractions were then filtered through Celite (coarse 545) and the solvent was removed under reduced pressure. Flash chromatography of the concentrated organic material in pure benzene yielded 2-phenyl-1,4-naphthoquinone (**4.53**, 375 mg, 1.6 mmol, 80%) as a fine yellow crystals (from hexane/CH_2Cl_2).

2-phenyl-1,4-naphthoquinone (4.53) : Fine yellow crystals (from hexane/CH_2Cl_2); mp 111-113 °C, lit.[361] 111-112 °C; IR (film) ν_{max} cm^{-1} 3020, 1665, 1596, 1446, 1306, 1215, 1020, 909, 852, 758, 669, 523; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.17 (1H, m, H-8), 8.11 (1H, m, H-5), 7.77 (1H, m, H-6), 7.76 (1H, m, H-7), 7.56 (2H, m, H-6`, H-2`), 7.47 (2H, m, H-5`, H-3`), 7.46 (1H, m, H-4`), 7.07 (1H, s, H-3); $^{13}C$ NMR ($CDCl_3$, 150 MHz) δ 185.1 ($q_c$, C-4), 184.3 ($q_c$, C-1), 148.1 ($q_c$, C-2), 135.2 ($q_c$, C-3), 133. 9 (CH, C-7), 133.8 (CH, C-6), 133.4 ($q_c$, C-1`), 132.4 (q_c, C-8a), 132.1 (q_c, C-4a), 130.0 (CH, C-4`), 129.4 (2 x CH, C-6`, 2`), 128.4 (2 x CH, C-5`, C-3`), 127.0 (CH, C-8), 125.9 (CH, C-5); EIMS *m/z* (rel. int.) 234 [M^+] (100) 206 (42), 178 (14), 104 (18), 76 (10); HRFABMS *m/z* 234.0673 (calcd for $C_{16}H_{10}O_2$ [M^+], 234.0681).

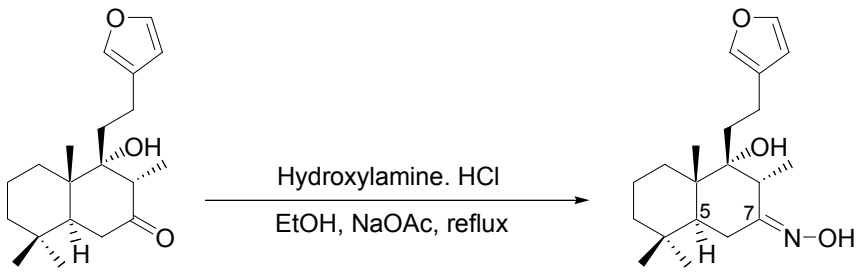
*6.3.15 Attempted synthesis of oximes **4.62** and **4.63***



4.41 **4.62**

This method is representative. Hydroxylamine.HCl (36 mg, 0.51 mmol) was added to EtOH (2 mL) and warmed up until all the solid dissolved. A solution of **4.62** (100 mg, 0.34 mmol) in warm EtOH was then added followed by NaOAc (70 mg, 0.51 mmol), and the reaction mixture was refluxed for 4.5 h and then stirred at RT overnight. The solvent was removed under reduced pressure, taken up in CH_2Cl_2 (5 mL) and 10% $NaHCO_3$ (5 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 5 mL) and the combined organic fractions were washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. NP HPLC (25% EtOAc, 75% hexane) of the crude product afforded 2-(1`-phenylmethanone oxime)-1,4-dimethoxynaphthalene (**4.62**, 10.4 mg, 0.034 mmol, 10%) as a pale yellow amorphous solid.

**2-(1`-phenylmethanone oxime)-1,4-dimethoxynaphthalene (4.62) :** pale yellow amorphous solid; $^1$H NMR ($CDCl_3$, 600 MHz) δ 8.29 (1H, m, H-5), 8.12 (1H, m, H-8), 7.58 (2H, m, H-7`, H-3`), 7.54 (2H, m, H-7, H-6), 7.36 (1H, m, H-5`), 7.32 (2H, m, H-6`, H-4`), 6.56 (1H, s, H-3), 3.96 (3H, s, OMe-4), 3.82 (3H, s, OMe-1); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 157.0 (q_c, C-1`), 151.8 ($q_c$, C-4), 147.1 ($q_c$, C-1), 135.3 ($q_c$, C-2`), 129.6 (CH, C-5`), 128.7 ($q_c$, C-8a), 128.5 (2 x CH, C-6`, C-4`), 127.0 (2 x CH, C-7`, C-3`), 127.0 (q_c, C-4a), 126.8 (CH, C-7), 126.3 (CH, C-6), 122.5 (CH, C-5), 122.4 (CH, C-8), 120.7 (q_c, C-2), 103.7 (CH, C-3), 62.2 (1-OMe), 55.7 (4-OMe).

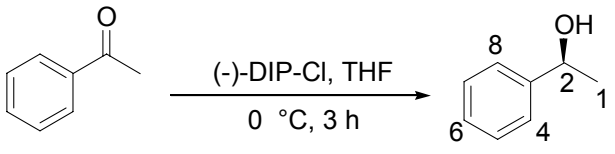


4.39 **4.63**

The same procedure as outlined above was used to afford **4.63** (90.0 mg, 0.25 mmol, 79%). No further purification was deemed necessary.

(4.63) : ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34 (1H, t, *J* = 1.7 Hz, H-15), 7.22 (1H, s, H-16), 6.27 (1H, s, H-14), 3.40 (1H, q, *J* = 10.5 Hz, H-12a), 2.66 (1H, q, *J* = 6.7 Hz, H-8), 2.48 (1H, pent, *J* = 5.7 Hz, H-12b), 2.00 (1H, m, H-1a), 1.76 (1H, m, H-1b), 1.62 (2H, m, H-1b, H-5), 1.59 (1H, m, H-2a), 1.50 (1H, m, H-2b), 1.49 (2H, m, H_2-11), 1.39 (1H, s, H-3a), 1.17 (1H, td, *J* = 13.4, 3.4 Hz, H-3b), 1.15 (3H, d, *J* = 6.6 Hz, H-17), 1.05 (3H, s, H-20), 0.96 (3H, s, H-18), 0.90 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 162.2 (q_c, C-7), 142.9 (CH, C-15), 138.5 (CH, C-16), 125.3 (q_c, C-13), 110.8 (CH, C-14), 79.4 (q_c, C-9), 45.9 (CH, C-5), 43.4 (q_c, C-10), 43.4 (CH , C-8), 41.5 (CH_2, C-3), 34.4 (CH_2, C-1), 31.6 (CH_2, C-11), 21.5 (CH_3, C-19), 21.4 (CH_2, C-12), 21.3 (CH_2, C-6), 18.5 (CH_2, C-2), 15.8 (CH_3, C-20), 9.9 (CH_3, C-17).

*6.3.16 Asymmetric reduction of **4.65** using (-)-DIP-Cl*[331;332]



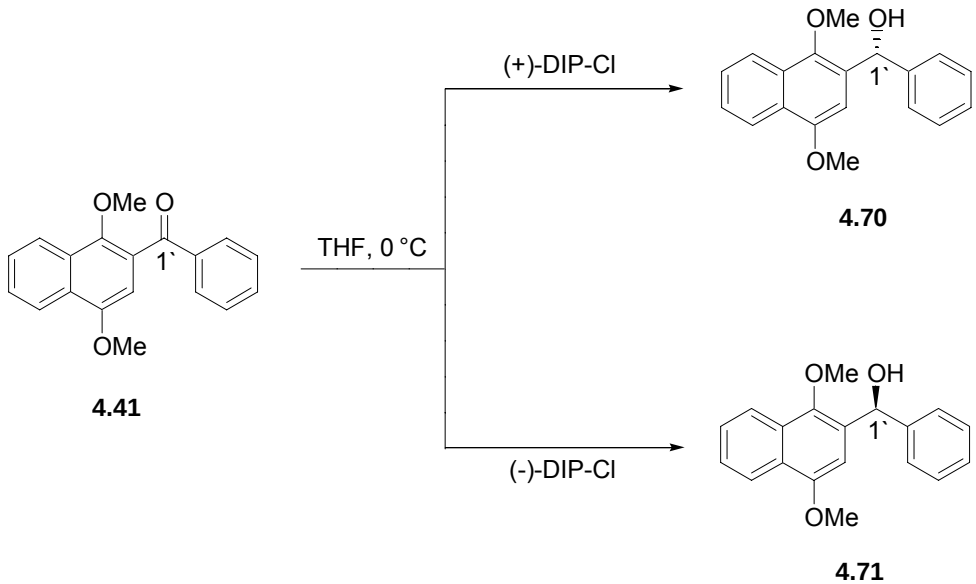
4.65 **4.66**

This method is representative. A solution of (-)-DIP-Cl (4.8 g, 15 mmol) in anhydrous THF (10 mL) at -10 °C was added dropwise to a solution of **4.65** (1.60 g, 13 mmol) in 5 mL anhydrous THF and the reaction mixture was stirred at this temperature for 3 h and then at RT overnight.

Subsequently, acetaldehyde (10 mL) was added to the reaction at 0 °C and the reaction was stirred at this temperature for another hr. NaOH (1.5M, 20 mL) was then added and the mixture was extracted with Et_2O (3 x 10 mL). The combined organic fractions were washed with water (2 x 10 mL) and sat. brine (10 mL) before being dried over anhydrous $MgSO_4$. Removal of the solvent under vacuum afforded the product in quantitative yield, as determined by 1D NMR and optical rotation.

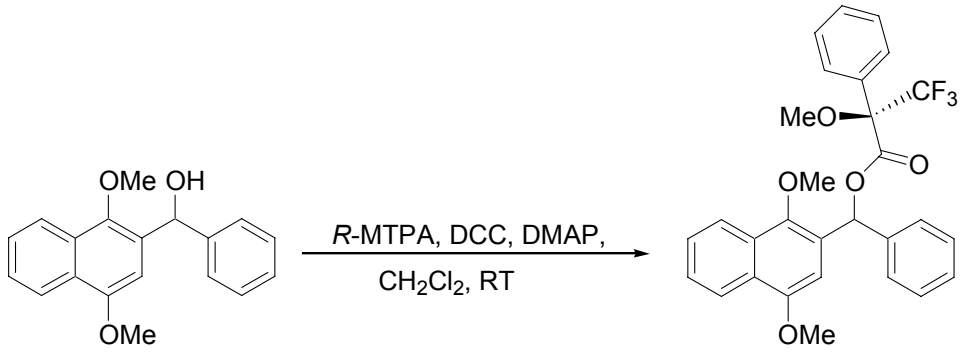
***S*-phenylethanol** (**4.66**) : ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.01-7.40 (Ar), 4.76 (1H, q, *J* = 6.4 Hz, H-2), 1.37 (3H, d, *J* = 6.5 Hz, H_3-1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.9 (q_c, C-3), 128.3 (2 x CH, C-5, C-7), 125.3 (2 x CH, C-4, C-8), 127.2 (CH, C-6), 70.2 (CH, C-2), 25.1 (CH_3, C-1). Final confirmation of the asymmetric reduction of **4.65** to **4.66** was provided by the optical rotation ($[\alpha]^{22}_D$ -48.2^0), which was in accordance with the reported value ($[\alpha]^{20}_D$ -42.6^0).[328]

*6.3.16.1 Attempted asymmetric reduction of **4.41** using both (+) and (-)-DIP-Cl*[331;332]



The same procedure as outlined above was followed. Repeated attempts at this transformation yielded a complex mixture of products that were not investigated further.

*6.3.17 Preparation of the MTPA esters of **4.24***



4.24 **4.72A** **4.72B**

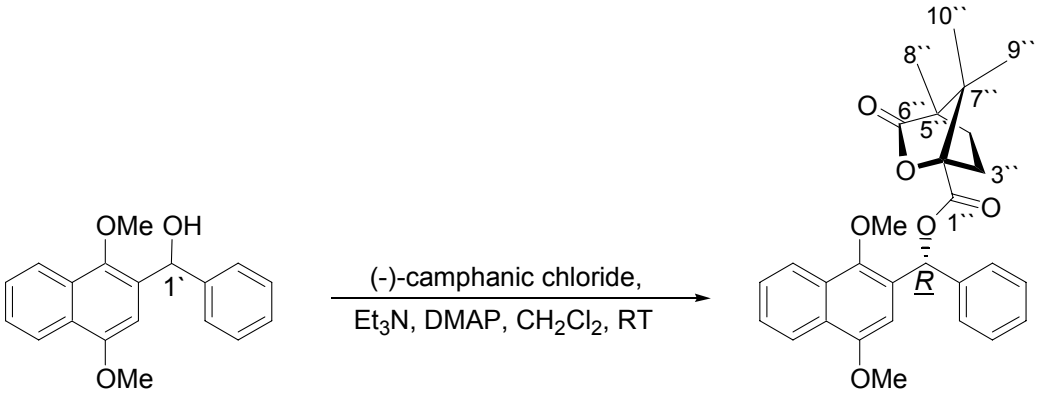
R-α-Methoxy-α-trifluoromethylphenylacetic acid (100 mg), DCC (70 mg) and DMAP (10 mg) were added to a solution of **4.24** (50 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (5 mL). The solution was stirred overnight at ambient temperature, diluted in EtOAc (5 mL) and H_2O (2 mL) and filtered. The resulting solution was washed with 1M HCl (2 mL), H_2O (2 mL), saturated aqueous $NaHCO_3$ (2 mL) and once more with H_2O (2 mL) in the order presented. The EtOAc solution was dried over anhydrous $MgSO_4$, the solvent removed under reduced pressure and the resultant yellow oil purified by semi-preparative HPLC (3: 1 hexane/EtOAc) to afford a mixture of the two *R*-MTPA diastereomers **4.72** as a yellow oil (61.2 mg, 0.12 mmol, 71%). The mixture was separated by analytical HPLC (20% CH_2Cl_2, 80% hexane) to afford the two diastereomers (**A** : **B** ; 14% : 56%), but all attempts at growing crystals of either stereoisomer using a variety of solvents (*e. g.* hexane, EtOAc, MeOH, EtOH, Et_2O, petroleum ether) and mixtures of these solvents failed to yield any crystals.

***R*-MTPA diastereomer (4.72A) :** off-white solid (2.2 mg, 4.31 *μ*mol, 14%); IR (film) ν_{max} cm^{-1} 3021, 2401, 1521, 1216, 1019, 929, 771, 670, 511; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.20 (1H, d, *J* = 8.4 Hz, H-5), 8.05 (1H, d, *J* = 8.4 Hz, H-8), 7.66 (1H, s, H-1`), 7.55 (1H, td, *J* = 7.3, 1.0 Hz, H-7), 7.48 (1H, td, *J* = 7.3, 1.0 Hz, H-6), 7.41 (2H, d, *J* = 8.0 Hz, H-7`, H-3`), 7.41 (2H, d, *J* = 8.0 Hz, H-8``, H-4``), 7.37 (1H, t, *J* = 7.4 Hz, H-6``), 7.30 (2H, m, H-6`, H-4`), 7.30 (2H, m, H-7``, H-5``), 7.27 (1H, m, H-5`), 6.53 (1H, s, H-3), 3.99 (3H, s, 1-OMe), 3.67 (3H, s, 4-OMe),

3.56 (3H, s, H_3-9``); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 165.5 ($q_c$, C-1``), 152.2 (q_c, C-4), 146.5 (q_c, C-1), 139.1 (q_c, C-2`), 132.3 (q_c, C-3``), 129.6 (CH, C-6``), 128.6 (2 x CH, C-7``, C-5``), 128.3 (2 x CH, C-6`, C-4`), 128.2 (q_c, C-8a), 128.2 (CH, C-5`), 127.3 (2 x CH, C-8``, C-4``), 126.9 (2 x CH, C-7`, C-3`), 126.9 (q_c, C-2), 126.8 (CH, C-7), 126.5 (q_c, C-4a), 125.9 (CH, C-6), 124.2 (q_c, C-10``) 122.5 ($q_c$, C-2``), 122.5 (CH, C-5), 122.3 (CH, C-8), 101.1 (CH, C-3), 74.1 (CH, C-1`), 62.3 (CH_3, OMe-1), 55.7 (CH_3, OMe-4), 55.3 (CH_3, C-9``); EIMS *m*/*z* (rel. int.) 510 [M^+] (100), 308 (7), 277 (783), 261 (32),247 (9), 202 (8), 189 (26), 167 (10), 149 (36), 105 (12), 91 (43); HRFABMS *m/z* 510.1682 (calcd for $C_{29}H_{25}O_5F_3$ [M]$^+$, 510.1654).

***R*-MTPA diastereomer (4.72B) :** pale brown solid (8.5 mg, 16.7 *μ*mol, 56%); IR (film) ν_{max} cm^{-1} 3020, 2401, 1749, 1598, 1372, 1216, 1019, 930, 757, 771, 669, 517; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.23 (1H, d, *J* = 8.3 Hz, H-5), 8.07 (1H, d, *J* = 8.3 Hz, H-8), 7.70 (1H, s, H-1`), 7.56 (1H, t, *J* = 7.4 Hz, H-7), 7.51(1H, t, *J* = 7.4 Hz, H-6), 7.46 (2H, d, *J* = 7.8 Hz, H-8``, H-4``), 7.38 (1H, t, *J* = 7.3 Hz, H-6``), 7.31 (2H, m, H-7``, H-5``), 7.29 (2H, m, H-7`, H-3`), 7.27 (2H, m, H-6`, H-4`), 7.23 (1H, m, H-5`), 6.71 (1H, s, H-3), 3.98 (3H, s, 1-OMe), 3.83 (3H, s, 4-OMe), 3.50 (3H, s, $H_3$-9``); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 165.6 (q_c, C-1``), 152.3 ($q_c$, C-4), 146.9 ($q_c$, C-1), 139.0 ($q_c$, C-2`), 132.1 ($q_c$, C-3``), 129.6 (CH, C-6``), 128.5 (2 x CH, C-6`, C-4`), 128.4 (2 x CH, C-7``, C-5``), 128.3 ($q_c$, C-8a), 128.0 (CH, C-5`), 127.6 (2 x CH, C-8``, C-4``), 126.9 (CH, C-7), 126.7 ($q_c$, C-2), 126.7 ($q_c$, C-4a), 126.6 (2 x CH, C-7`, C-3`), 126.1 (CH, C-6), 124.4 ($q_c$, C-10``), 122.5 (q_c, C-2``), 122.5 (CH, C-5), 122.4 (CH, C-8), 101.6 (CH, C-3), 73.9 (CH, C-1`), 62.6 ($CH_3$, OMe-1), 55.5 ($CH_3$, OMe-4), 55.5 ($CH_3$, H-9``); EIMS *m*/*z* (rel. int.) 510 [M^+] (100), 308 (13), 277 (75), 261 (39), 245 (10), 202 (11), 139 (34), 149 (12), 105 (14), 91 (51); HRFABMS *m/z* 510.1670 (calcd for $C_{29}H_{25}O_5F_3$ [M]$^+$, 510.1654).

*6.3.18 Preparation of the camphanate esters **4.73** and **4.74***



4.24 **4.73**

The benzylic alcohol **4.24** (300 mg, 1.02 mmol), camphanic chloride (508 mg, 2.34 mmol, 2.3 eq), Et_3N (853 µL, 6.12 mmol, 6.0 eq) and DMAP (63 mg, 0.51 mmol, 0.5 eq) were dissolved in anhydrous CH_2Cl_2 (30 mL) under Ar atmosphere and stirred at ambient temperature for 5 h. The reaction mixture was concentrated to dryness, taken up in Et_2O (20 mL) and washed with 1M HCl (10 mL) followed by H_2O (10 mL). The organic portion was dried over $MgSO_4$ and concentrated to give a pale yellow oil (659 mg). All our attempts at fractional crystallization of the mixture using a variety of solvents (*e. g.* hexane, EtOAc, MeOH, EtOH, H_2O, Et_2O, 2-methoxyethanol, petroleum ether) and mixtures of these solvents failed to afford any separation. Normal phase HPLC (99% CH_2Cl_2, 1% EtOAc) of the crude mixture (190 mg, 0.4 mmol) afforded *R*-camphanate ester (91 mg, 0.19 mmol, 50%) and *S*-camphanate ester (88 mg, 0.19 mmol, 50%) in 70% overall yield.

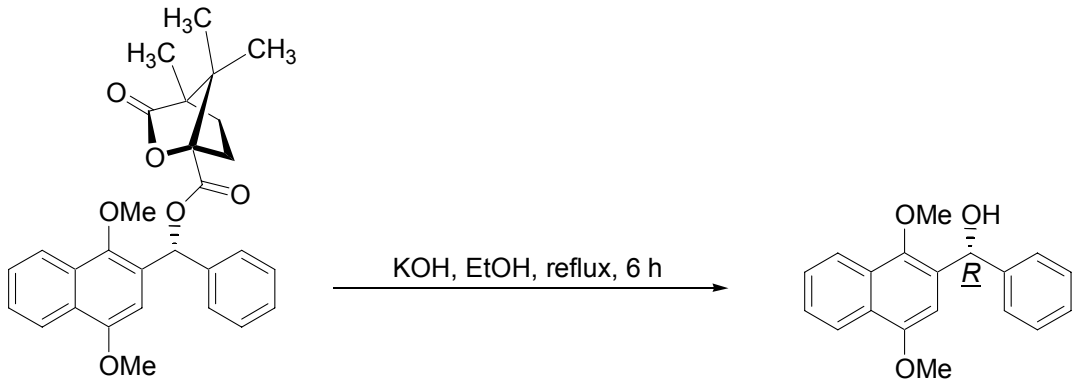
***R*-camphanate ester (4.73) :** Fine white needles (from MeOH/H_2O); mp 162-166 °C; $[\alpha]_D^{22}$ +39 (*c* 0.23, $CHCl_3$); IR (film) ν_{max} cm^{-1} 3686, 3021, 2401, 1785, 1667, 1522, 1423, 1215, 1019, 929, 757, 669, 511; ^1H NMR ($CDCl_3$, 600 MHz) δ 8.21 (1H, d, *J* = 8.4 Hz, H-5), 8.06 (1H, d, *J* = 8.4 Hz, H-8), 7.60 (1H, s, H-1`), 7.55 (1H, t, *J* = 7.6 Hz, H-7), 7.49 (1H, t, *J* = 7.6 Hz, H-6), 7.44 (2H, d, *J* = 7.8 Hz, H-7`, H-3`), 7.32 (2H, t, *J* = 7.6 Hz, H-6`, H-4`), 7.26 (1H, t, *J* = 7.4 Hz, H-5`), 6.78 (1H, s, H-3), 3.98 (3H, s, 1-OMe), 3.90 (3H, s, 4-OMe), 2.49 (1H, ddd, *J* = 13.8, 10.9, 4.2 Hz, H-3``b), 2.11 (1H, ddd, *J* = 13.8, 9.4, 4.6 Hz, H-3``a), 1.93 (1H, ddd, *J*

= 13.2, 10.9, 4.5 Hz, H-4``b), 1.71 (1H, ddd, *J* = 13.5, 9.5, 4.3 Hz, H-4``a), 1.10 (3H, s, H_3-8``), 1.02 (3H, s, $H_3$-10``), 0.90 (3H, s, H_3-9``); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 178.3 ($q_c$, C-6``), 166.7 (q_c, C-1``), 152.3 (q_c, C-4), 146.7 (q_c, C-1), 139.4 (q_c, C-2`), 128.5 (2 x CH, C-6`, C-4`), 128.3 ($q_c$, C-8a), 128.0 (CH, C-5`), 127.3 (q_c, C-2), 126.8 (CH, C-7), 126.7 (2 x CH, C-7`, C-3`), 126.6 (q_c, C-4a), 126.0 (CH, C-6), 122.5 (CH, C-5), 122.3 (CH, C-8), 101.4 (CH, C-3), 91.1 (q_c, C-2``), 72.9 (CH, C-1`), 62.4 ($CH_3$, OMe-1), 55.6 ($CH_3$, OMe-4), 54.9 ($q_c$, C-7``), 54.5 (q_c, C-5``), 30.8 ($CH_2$, C-3``), 28.9 (CH_2, C-4``), 16.9 ($CH_3$, C-9``), 16.7 (CH_3, C-10``), 9.7 ($CH_3$, C-8``); EIMS *m/z* (rel. int.) 474 [M^+] (100), 277 (35), 261 (19), 236 (6), 167 (13), 149 (27), 91 (22), 69 (15), 57 (8); HRFABMS *m/z* 474.2031 (calcd for $C_{29}H_{30}O_6$ [M^+], 474.2042). Crystal data for **4.73** : $C_{29}H_{30}O_6$, *M* = 474.53, 0.16 x 0.08 x 0.03 mm^3, orthorhombic, space group $P2_12_12_1$ (No. 19), *a* = 6.1443 (3) Å , *b* = 12.0135 (5) Å, *c* = 33.3284 (10) Å, *V* = 2460.08 (17) Å^3, *Z* = 4, D_c = 1.2812 g/cm^3, F_{000} = 1008, μ(MoKα) = 0.089 mm^{-1}, *T* = 100 K, $2\theta_{max}$ = 25984 reflections collected, 3077 unique (R_{int} = 1.023). R_1 = 1.023, wR_2 = 0.08, *R* indices based on 3077 reflections with I > 2σ(I) (refinement of F^2), 321 parameters, 0 restraint.

***S*-camphanate ester (4.74) :** White plates (from MeOH); mp 153-155 °C; $[\alpha]_D^{22}$ -13 (*c* 0.08, $CHCl_3$); IR (film) ν_{max} cm^{-1} 3684, 3020, 2401, 1523, 1423, 1372, 1216, 1101, 929, 759, 670, 512; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.21 (1H, d, *J* = 8.4 Hz, H-5), 8.06 (1H, d, *J* = 8.4 Hz, H-8), 7.60 (1H, s, H-1`), 7.55 (1H, t, *J* = 7.6 Hz, H-7), 7.49 (1H, t, *J* = 7.6 Hz, H-6), 7.41 (2H, d, *J* = 7.6 Hz, H-7`, H-3`), 7.31 (2H, t, *J* = 7.7 Hz, H-6`, H-4`), 7.26 (1H, t, *J* = 7.3 Hz, H-5`), 6.78 (1H, s, H-3), 3.98 (3H, s, 1-OMe), 3.93 (3H, s, 4-OMe), 2.46 (1H, ddd, *J* = 13.9, 10.8, 4.3 Hz, H-3``b), 2.05 (1H, ddd, *J* = 13.8, 9.4, 4.6 Hz, H-3``a), 1.92 (1H, ddd, *J* = 15.0, 10.7, 4.5 Hz, H-4``b), 1.70 (1H, ddd, *J* = 13.5, 9.4, 4.4 Hz, H-4``a), 1.11 (3H, s, H_3-8``), 1.07 (3H, s, $H_3$-10``), 0.90 (3H, s, H_3-9``); $^{13}$C NMR ($CDCl_3$, 150 MHz) δ 178.3 ($q_c$, C-6``), 166.7 (q_c, C-1``), 152.4 (q_c, C-4), 146.8 (q_c, C-1), 139.4 (q_c, C-2`), 128.5 (2 x CH, C-6`, C-4`), 128.3 ($q_c$, C-8a), 128.0 (CH, C-5`), 127.3 (q_c, C-2), 126.9 (CH, C-7), 126.6 (2 x CH, C-7`, C-3`), 126.6 (q_c, C-4a), 126.0 (CH, C-6), 122.5 (CH, C-5), 122.3 (CH, C-8), 101.5 (CH, C-3), 91.1 (q_c, C-2``), 72.9 (CH, C-1`), 62.4 ($CH_3$, OMe-1), 55.7 ($CH_3$, OMe-4), 54.9 ($q_c$, C-7``), 54.3 (q_c, C-5``), 30.8

(CH_2, C-3``), 28.9 ($CH_2$, C-4``), 16.8 (2 x CH_3, C-10``, 9``), 9.7 (CH_3, C-8``); EIMS *m/z* (rel. int.) 474 [M^+] (100), 294 (7), 277 (38), 261 (30), 202 (8), 167 (32), 149 (78), 91 (24), 57 (19); HRFABMS *m/z* 474.2040 (calcd for $C_{29}H_{30}O_6$ [M^+],474.2042).

*6.3.19 KOH Saponification of camphanate esters **4.73** and **4.74***



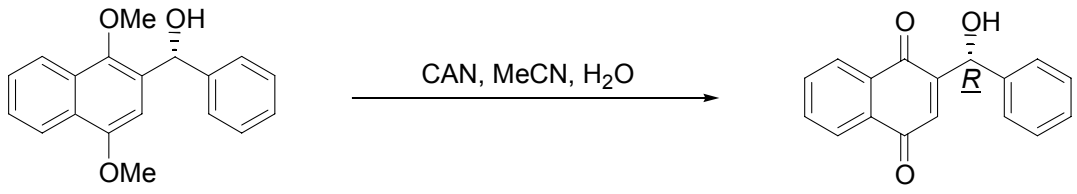
4.73 **4.70**

The *R*-camphanate ester **4.73** (46.0 mg, 0.097 mmol) and 1M KOH (10 mL) was refluxed in EtOH (15 mL) for 6 h. The reaction mixture was then concentrated *in vacuo*, acidified with 2.5M HCl and extracted with CH_2Cl_2 (3 x 5 mL). The organic phases were combined, washed with 10% $NaHCO_3$ (20 mL), dried over $MgSO_4$ and concentrated to afford **4.70** as an off-white oil (26.5 mg, 0.09 mmol, 93%). No further purification was required. The **4.71** enantiomer (25.3 mg, 0.086 mmol, 100%) was similarly prepared from the *S*-camphanate ester **4.74** (41.0 mg, 0.086 mmol).

***R*-2-(1`-hydroxy-2`-propenyl)-1,4-dimethoxynaphthalene (4.70) :** $[\alpha]_D^{22}$ +67 (*c* 1.05, $CHCl_3$); ^1H and ^{13}C NMR data identical as in Section 6.3.7.

***S*-2-(1`-hydroxy-2`-propenyl)-1,4-dimethoxynaphthalene (4.71) :** $[\alpha]_D^{22}$ -86 (*c* 1.32, $CHCl_3$); ^1H and ^{13}C NMR data identical as in section 6.3.7.

*6.3.20 Oxidative demethylation of **4.70** and **4.71***



4.70 **4.75**

Same procedure as described in section 6.3.8 was followed.

***R*-2-(1`-hydroxy-1`-phenylmethyl)-1,4-naphthoquinone (4.75) :** $[\alpha]_D^{22}$ +39 (*c* 1.29, $CHCl_3$); ^1H and ^{13}C NMR data identical as in Section 6.3.8.

***S*-2-(1`-hydroxy-1`-phenylmethyl)-1,4-naphthoquinone (4.76) :** $[\alpha]_D^{22}$ -26 (*c* 1.23, $CHCl_3$); ^1H and ^{13}C NMR data identical as in Section 6.3.8.

6.4 Chapter Five Experimental

6.4.1 Electrochemistry

Cyclic voltammetry was performed with the electrochemical analyser Autolab potentiostat PGSTAT 30 (Eco Chemie, Utretch, The Netherlands) using the General Purpose Electrochemical System data processing software (GPES, software version 4.9). A three-electrode assembly of a glassy-carbon working electrode, platinum wire auxiliary electrode and Ag/AgCl reference electrode was utilized. Glassy-carbon electrode was resurfaced with alumina, using a fine chamois leather, and the solutions of the quinones (1 mg in 5 mL dimethylformamide) were prepared in freshly distilled dimethylformamide containing 0.05 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. Each solution was deoxygenated for 5 min with purified nitrogen prior to measurement and the cyclic

voltammograms were recorded under nitrogen atmosphere. The redox potentials were recorded against the Ag/AgCl reference electrode.

6.4.2 Activity of synthetic and natural compounds against oesophageal cancer

6.4.2.1 Cell lines and screening protocol

Assays were performed at the department of Medicinal Biochemistry at the University of Cape Town by Dr. C. Whibley, Dr. O. Osoniyi and N. Shunmooganm-Gouden. The protocol described here has been taken from the thesis of Dr. C. Whibley and is presented in truncated form.[362]

The human oesophageal cancer cell line (WHCO1) screened against was obtained as a gift from Prof. Veale, University of Witswatersrand, South Africa and derived from South African patients with squamous cell carcinoma of the oesophagus. Samples for the MTT assay were plated in 96-well plates to a final volume of 100 μL. MTT reagent (10 μL, Roche cat # 1465007) was added and the cells incubated (4 h, 37 °C). Solubilization reagent (100 μL) was added to each well and incubation continued (16 h, 37 °C). Upon completion of the incubation time, plates were read (595 nm) on an Anthos microplate reader 2001. To determine IC_{50} values, 1500 cells per well were seeded in 90 μL Dulbecco/Vogt Modified Eagle's Minimal Essential Medium (DMEM) in Cellstar 96-well plates. After incubation (24 h), test samples were plated at a range of concentrations in 10 μL medium, with a final concentration of 0.2% DMSO and again incubated for 48 h. Observations were made and processed in the manner described for the MTT assay. A dose-response curve was analyzed by non-linear regression analysis [non-linear regression (sigmoidal dose response with variable slope)] using the GraphPad Prism 4.00 package of GraphPad software, San Diego, USA to determine the specific IC_{50} value for the compound tested against the WHCO1 cell line. The formula used was $Y = bottom + [(top-bottom)/(1 + 10^{(logIC50 - X) \times hillslope})]$, where Y is

the absorbance at 595 nm, X is the concentration of the test compound, bottom is the minimum absorbance (also the absorbance of the medium blank) and the hillslope is the slope of the curve.

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