Synthesis of triprenylated toluquinone and toluhydroquinone metabolites from a marine-derived *Penicillium* fungus.

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By

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Abstract

This project forms part of a collaborative effort between the marine natural products chemists at Rhodes University and the medical biochemists at the University of Cape Town's School of Medicine. Our UCT collaborators tested the cytotoxicity of a group of toluhydroquinones and toluquinones (**9**-**15**) against the oesophageal cancer cell line WHCO1 and revealed that the triprenylated toluhydroquinone **11** and it's oxidised analogue **12** were the most active. This thesis presents an investigation into the role of the polyprenyl side-chain in the cytotoxicity of compound **11** and it's oxidised analogue **12** by synthesizing and testing the cytotoxicity of simplified analogues of this compound.

The synthesis of the two *ortho*-prenylated toluhydroquinone analogues 5-methyl-2-[(2'*E*,6'*E*)-3',7' -dimethyl-2',6'-octadienyl]-1,4-benzenediol (**19**) and 5-methyl-2-[(2'*E*,6'*E*)-3',7',11'-trimethyl-2',6',10'-dodecatrienyl]-1,4-benzenediol (**21**) and their two *ortho*-prenylated toluquinone analogues, 5-methyl-2-[(2'*E*,6'*E*)-3',7'-dimethyl-2',6'-octadienyl]-2,5-cyclohexadiene-1,4-dione (**20**) and 5-methyl-2-[(2'*E*,6'*E*)-3',7',11'-trimethyl-2',6',10'-dodecatrienyl]-2,5-cyclohexadiene-1,4-dione (**22**) is described. Our initial attempts to couple geranyl bromide, farnesyl bromide and farnesal to the aromatic precursors *m*-cresol and 1,4-dimethoxy-2-methylbenzene using directed *ortho*-prenylation and phenoxide carbon-alkylation were unsuccessful. The four target analogues were eventually synthesized *via* the initial metal halogen exchange reaction between 1-bromo-2,5-dimethoxy-4-methylbenzene and geranyl bromide/farnesyl bromide using *n*-BuLi and TMEDA in ditheyl ether at 0 ^{o}C to yield **92** and **104** respectively in moderate yield. The demethylation of both compounds preceded smoothly using AgO giving the target analogues **20** and **22** in good yield (approx. 90 %). The reduction of quinones **20** and **22** with sodium dithionite gave **19** and **21** in quantitative yield. The synthesis reported here is the first regioselective synthesis of these compounds. The anti-oesophageal cancer activity of **19**-**22** and two commercially available non-prenylated analogues **17** and **18** were tested against WHCO1. The conclusion drawn from the anti-oesophageal cancer study was that the polyprenyl side-chain plays a negligable role in the cytotoxicity of compounds such as **11** and **9** against the oesophageal cancer cell line WHCO1.

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Table of contents

Abstract……II
Acknowledgements……..III
Table of Contents……IV
List of Figures……VII
List of Schemes……X
List of Tables…… XIII
Glossary……XIV

Chapter 1

1 General introduction to marine natural products chemistry…… 2
1.1 Marine natural products research within a South African context…… 6
1.2 Oesophageal cancer in South Africa…….. 7
1.2.1 Oesophageal cancer - possible causes……....8
1.2.2 Oesophageal cancer - stages and treatment……8
1.3 Southern African marine invertebrates as a potential source of new anti-oesophageal cancer drugs……..…….9
1.4 Thesis aims and objectives……...13

Chapter 2

2 *Ortho*-prenylated quinones and hydroquinones from marine organisms……...16
2.1 Fungi…… 18
2.2 Brown Algae…… 19
2.3 Green Algae……..23
2.4 Marine Invertebrates…….23
2.4.1 Sponges…… 23
2.4.2 Ascidians……..26
2.4.3 Gorgonians……...27
2.4.4 Soft Corals……28
2.4.5 Nudibranchs……30
Synthesis of *ortho*-prenylated compounds……...31
2.4.6 Directed *ortho*-Metalation (DoM)……32

2.4.7 Metal-halogen exchange (MHE)……..35
2.4.8 Phenoxide carbon-Alkylation……...36
2.4.9 Claisen rearrangement……..37
2.4.10 Friedal-Crafts-type *ortho*-prenylations…….37

Chapter 3

3 The synthesis of target compounds……..…..39
3.1 An overview of the synthetic approach to **19**–**22**…….40
3.2 Directed *ortho*-Metalation……41
3.3 Phenoxide carbon-alkylation……49
3.4 Metal Halogen exchange……..53
3.5 Future work…….. 73

Chapter 4

4 Anti-oesophageal cancer properties of synthetic target compounds…….. 75
4.1 Quinones as anti-cancer drugs……..76
4.2 The crystal violet and MTT anti-cancer bioaasays…….. 79

Chapter 5

5 Experimental……..86
5.1 Synthetic procedures……87
5.1.1 The methylation of compound **79**……..87
5.1.2 Directed *ortho* Metalation……..89
5.1.2.1 Attempted synthesis of compound **89**……..89
5.1.2.2 Attempted synthesis of compound **89** using a similar protocol to that used by Harder *et al*[127] to prepare compound **85**……..89
5.1.2.3 Synthesis of compounds **89** and **90** using Shirley *et al's*. synthetic protocol.[125]……90
5.1.2.4 Oxidation of farnesol to farnesal……..91
5.1.2.5 Attempted coupling of compound **80** with farnesal using Shirley *et al's*. synthetic protocol……91
5.1.2.6 Preparation of compound **17**……92
5.1.2.7 Preparation of compound **91**……92

5.1.2.8 Attempted preparation of compound **92** using the reaction protocol reported by Bouzbouz and Kirschleger.[128]…….. 93
5.1.3 Phenoxide carbon-alkylation……..94
5.1.3.1 Preparation of compound **95** using the synthetic strategy reported by *Kornblum et al*[120]……..94
5.1.4 Metal halogen exchange…….95
5.1.4.1 Preparation of **98** using the protocol outlined by Miller and Stewart..95
5.1.4.2 Preparation of **101**……95
5.1.4.3 Attempted coupling of compound **101** with geranyl bromide using the reaction conditions reported by Odejinmi and Wiemer[133]……...96
5.1.4.4 Demethylation of compound **91**……...97
5.1.4.4.1 Attempted demethylation of **91** using *L*-Selectride following a protocol reported by Majetich *et al*.[135]……...97
5.1.4.4.2 Attempted demethylation of **91** using Me_3SiI following a protocol reported by Jung and Lyster.[137]……..97
5.1.4.4.3 Preparation of AgO[140]……98
5.1.4.4.4 Preparation of **18** using Snyder and Rapoport's[139] AgO oxidative demethylation procedure……98
5.1.4.5 Preparation of compound **102**……..99
5.1.4.6 Attempted preparation of compound **92**…….100
5.1.4.7 Preparation of compound **103**……101
5.1.4.8 Preparation of compound **104**……101
5.1.4.9 Preparation of compound **92**……..102
5.1.4.10 Preparation of compound **22**……..103
5.1.4.11 Preparation of compound **20**……..104
5.1.4.12 Preparation of compound **21**……..105
5.1.4.13 Preparation of compound **19**……..105
5.2 The anti-oesophageal cancer bioaasays (performed in at UCT)……106
5.2.1 Cell culture…….. 106
5.2.2 The crystal violet bioassay……...106
5.2.3 The MTT bioassay used to determine IC_{50} values……... 107
Chapter 6
6 References……... 109

List of Figures

Figure 1: L. millecra photographed in Algoa Bay feeding on sea fans (gorgonians)……... 11

Figure 2: The distribution of hydroquinones and quinones across marine phyla.[6,29]……. 16

Figure 3: Hydroquinone substitution patterns in **A)** marine sponges, ascidians and one species of brown algae (*Dictyopteris undulata*)[30,31] **B)** marine fungi, gorgonians, soft corals and nudibranchs **C)** brown algae **D)** green algae **E)** ascidians. [R_1 = polyprenyl side chain and R_2 = alkyl group].[29]…….. 17

Figure 4: Expansions of the observed H-3, H-4 and H-6 peaks in the ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for **89**……. 45

Figure 5: Expansions of the observed H-4, H-5 and H-6 peaks in the ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for **90**……. 46

Figure 6: correlations Selected HMBC used to position the prenyl side-chain at C-4 in **95**……... 52

Figure 7: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **98**……... 55

Figure 8: GLC chromatographs of a. Standards, **18** (R_T = 2.59 min), **91** (R_T = 5.28 min), **17** (R_T = 6.17 min) ; b. oxidative demethylation of **91** using AgO. (GLC conditions: Duraband DB-1 column, initial temperature 100 ^{o}C, temperature ramp 15.0 ^{o}C/min, final temperature 200 ^{o}C, FID detector)……. 60

Figure 9: ^{1}H ($CDCl_3$, 400 MHz) NMR spectrum acquired for **103**……... 62

Figure 10: Selected HMBC correlations used to position the prenyl side-chain at C-2 in **92**……... 63

Figure 11: ^{1}H ($CDCl_3$, 400 Hz) and ^{13}C ($CDCl_3$, 100 Hz) NMR spectra acquired for **92**……... 64

Figure 12: Selected HMBC correlations to position the prenyl side-chain at C-2 in **104**……... 65

Figure 13: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **104**……. 66

Figure 14: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **19**……... 70

Figure 15: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **21**……... 72

Figure 16: Shows microscopy images of WHCO1 oesophageal cancer cells obtained using a Zeiss Axiovert 200 with an Axiocam camera and Axiovision 4.1 image software; a. untreated WHCO1 oesophageal cancer cells (cells are flat with well defined cell membranes) ; b. WHCO1 oesophageal cancer cells undergoing apoptosis (20x magnification) in the presence of **22** ; c. WHCO1 oesophageal cancer cells undergoing apoptosis (20x magnification) in the presence of **21** (in both images b and c. blebbing and loss of membrane integrity can be observed) ; d. WHCO1 oesophageal cancer cells undergoing apoptosis (40x magnification) in the presence of **21** (fragmentation of the cell into membrane bound vesicles)…….. 77

Figure 17: The crystal violet assay…….. 80

Figure 18: Crystal violet screening results for compounds **17**-**22**, **91**, **92** and **104**…….. 81

Figure 19: The determination of the IC_{50} value of **18** by plotting log concentration [μM] against the corrected optical density (OD) reading at 595 nm obtained from the MTT assay…….. 83

List of Schemes

Scheme 1: A proposed mechanism for the formation of the bicyclic polyprenylated marine secondary metabolites. [R = polyprenyl chain]…….. 19

Scheme 2: Reduction of the quinone moiety to the corresponding hydroquinone…….…….…. 31

Scheme 3: A summary of the DoM reaction. [105-107]…….. 33

Scheme 4: A rough hierarchy of DMG's using OMe as a reference group. Reagents a. Alkyllithium base, electrophile (E). [R = SO_2NR_2 > SO2N$^-$R > CON$^-$R > CH_2NR_2 > OMe > $CH_2CH_2NR_2$, NR_2 > CF_3, F, NR_2]…….…… 34

Scheme 5: An example of ortho-prenylation of a symmetrical phenol. Reagents a. s-BuLi, prenyl bromide (75 %)…….…. 34

Scheme 6: Synthesis of a simple ortho-prenylated phenyl using a MHE reaction. [P = protection group, X = halogen]……. 35

Scheme 7: Resonance stabilized phenoxide ion.[120]…….... 36

Scheme 8: The general strategy for a Claisen rearrangment……. 37

Scheme 9: General mechanism for a Friedal-Crafts type prenylation……... 37

Scheme 10: A proposed synthetic approach for the synthesis of target analogue **21** using DoM chemistry to couple the polyprenylated side-chain to **80**. Reagents: a. K_2CO_3, MeI ; b. n-BuLi ; c. farnesal ; d. Me_3SiCl, NaI ; e. PhMeNNa, $(Me_2N)PO$; f. $(KSO_3)_2NO$; g. Zn-ZnCl-EtOH……. 42

Scheme 11: Methylation of **79** with dimethyl sulfate……..…. 42

Scheme 12: The effect of the bidentate ligand TMEDA on the mechanism for the DoM reaction.[102]…… 43

Scheme 13: The coupling of benzaldehyde to m-methoxytoluene using DoM chemistry. Reagents: a. n-BuLi, ether, reflux for 15 min ; b. benzaldehyde, reflux for 4 h…… 44

Scheme 14: Proposed DoM synthetic strategy to couple **91** with geranyl bromide. Reagents: a. s-Buli, geranyl bromide, THF. (71 %)…..… 47

Scheme 15: Ortho-prenylation of para-dimethoxybenzene using DoM chemistry. Reagents: a. s-BuLi, THF, 0 ^{o}C, b. prenyl bromide, CuI (5 mol %), THF, -70 ^{o}C, (71 %).[128]…….…….. 48

Scheme 16: Coupling via phenoxide carbon-alkylation. Reagents: a. Prenyl bromide, Na, ether, reflux (85 %).[128]…….…….. 48

Scheme 17: The proposed strategy for the synthesis of analogues **19**-**22** using phenoxide carbon-prenylation. Reagents: a. geranyl bromide or farnesyl bromide, H_2O, RT ; b. $NO(SO_3Na)_2$; c. Zn-ZnCl, EtOH. 49

Scheme 18: Phenoxide carbon-prenylation of m-cresol. Reagents: a. NaOH, geranyl bromide, H_2O, 25 ^{o}C…….…….. 51

Scheme 19: Yamada et al.'s synthesis of **97** using phenoxide carbon-prenylation strategy. Reagents: a. Na metal, 3-methyl-2-butenyl chloride, ether (76%).[129]…….. 52

Scheme 20: Conversion of 1,4-benzoquinones to halogenated hydroquinones (X = halogen)…….…….. 53

Scheme 21: The proposed strategy for the synthesis of analogues **19-22** using MHE. Reagents: a. Me_3SiBr, NEt_4BF_4, MeCN, 25 ^oC (61 %) ; b. NaOH, Me_2SO_4, reflux (70 %) ; c. geranyl bromide [n = 2] or farnesyl bromide [n = 3] ; d. AgO, dioxane, HNO_3, 25 ^oC (90 %) ; e. $Na_2S_2O_4$ 1:3 DCM/Et_2O, 25 ^oC (90 %) ; f. $PhCH_2Br$, K_2CO_3, NaI, Ac_2O, reflux (80 %)…… 54

Scheme 22: Benzyl deprotection. Reagents: a. Na/s-BuOH, H^+, H_2O…….…... 56

Scheme 23: Demethylation of hydroquinone using *L*-Selectride. Reagents: a. *L*-Selectride (3 eq), 67 ^oC, 48 h. (71 %)…….…….. 57

Scheme 24: Four possible mechanisms (A-D) carbon-oxygen bond cleavage during the oxidative demethylation of 1,4-dimethoxy nuclei with AgO……..……. 59

Scheme 25: Coupling of phenyllithium (model organolithium intermediate) to geranyl bromide…….….. 61

Scheme 26: The conversion of the soluble yellow MTT salt to the insoluble purple formazan…….…….. 82

List of tables

Table 1: The comparative IC_{50} values of the linear triprenylquinones and hydroquinones (**9-13**, **15**) isolated from L. millecra and cisplatin (**16**) against the oesophageal cancer cell line WHCO1.[28]…… 12

Table 2: The IC_{50} values calculated for the cytotoxicity of **11** against a range of different cell lines.[28]…… 13

Table 3: Alkylation of sodium phenoxide resulting in both oxygen and carbon (*ortho* and *para*) alkylation products.[120]…….. 50

Table 4: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR aquired for **95**……... 51

Table 5: Reaction conditions used for the coupling of phenyllithium to geranyl bromide……... 62

Table 6: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **92**…… 65

Table 7: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **104**…….. 67

Table 8: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **19**…… 69

Table 9: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **21**…… 71

Table 10: The comparative IC_{50} values of analogues **17-22** against the oesophageal cancer cell line WHCO1……. 84

Glossary

^{13}C NMR	Carbon Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
Ac_2O	Acetone
AgO	Argentic oxide
Ar.	Argon
ATP	Adenosine triphosphate
br	Broad
BuLi	Butyllithium
Calcd	Calculated
C-alkylation	Carbon-alkylation
CD_3OD	Deuterated methanol
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm	Centimetre
CO_2	Carbon dioxide
Conc.	Concentrated
COSY	^{1}H-^{1}H Correlation Spectroscopy
CuBr·DMS	Copper bromide dimethyl sulfide complex
CuI	Copper (I) iodide
d	Doublet
DB	Duraband
DCM	Dichloromethane
dd	Doublet of doublets
DEPT	Distortionless Enhancement by Polarisation Transfer

DMG	Directing metalating group
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DoM	Directed ortho-metalation
DPPH	α,α-diphenyl-β-picrylhydrazyl radical
dquin.	Doublet of quintets
EIMS	Electon Impact Mass Spectrometry
eq	Equivalencies
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
eV	Electron volt
FT	Fourier transform
g	Grams
GLC	Gas Liquid Chromatography
h.	Hours
H_2O	Water
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation
HNO_3	Nitric acid
HPLC	High-Performance Liquid Chromatography
HRFABMS	High-Resolution Fast Atom Bombardment Mass Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	50 % Inhibitory Concentrations
Int.	Integration
IR	InfraRed

J	Scalar coupling constant
K_2CO_3	Potassium carbonate
km	Kilometre
M	Molarity
m	Multiplet
m/z	Mass to charge ratio
Me_2SO_4	Dimethyl sulfate
Me_3SiBr	Trimethylsilyl bromide
MeCN	Acetonitrile
MeI	Methyl iodide
mg	Milligram
$MgSO_4$	Anhydrous magnesium sulphate
MHE	Metal-halogen exchange
MHz	Megahertz
min	Minutes
mL	Millilitre
mmol	Millimole
MnO	Manganese dioxide
mol	Mole
MS	Mass spectrometry
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mult.	Multiplicity
N	Normal
N_2	Nitrogen
Na	Sodium

$Na_2S_2O_4$	Sodium dithionite
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide
NaH	Sodium hydride
NaOH	Sodium hydroxide
NCI	National Cancer Institute
NEt_4BF_4	Tetraethylammonium fluoroborate
NH_4Cl	Ammonium chloride
nm	Nanometer
NMR	Nuclear magnetic resonance
NPY	Neuropeptide Y
O_2	Oxygen molecule
OBn	Benzyl ether
Obsd	Observed
^oC	Degrees Celsius
OD	Optical density
OMe	Methyl ether
O-prenylation	Oxygen-prenylation
$PhCH_2Br$	Benzyl bromide
ppm	Parts per million
ROS	Reactive oxygen species
RT	Room temperature
R_T	Retention times
s	Singlet
SCC	Squamous cell carinoma

SOD	Superoxide dismutase
t	Triplet
TBARS	Thiobarbituric acid reactive substances
THF	Tetrahydrofuran
TLC	Thin-Layer Chromatography
TMEDA	Tetramethylethylenediamine
TPK	Tyrosine protein kinase
USA	United States of America
UV	UltraViolet
δ	Chemical shift (ppm)
μM	Micromolar
NH_4Cl	Ammonium chloride

Chapter 1.

1 General introduction to marine natural products chemistry

Secondary metabolites (natural products) are the metabolites that are not essential for the growth and day-to-day routine maintenance of cellular functions (primary metabolic activities) in an organism and unlike primary metabolites such as amino acids, carbohydrates and nucleotides, are not produced by all organisms. Secondary metabolite biosynthesis appears to be limited mainly to higher plants, marine invertebrates and microbes. The presence of a particular secondary metabolite is normally limited to one or at most a few species.[1] This peculiar phenomenon is evident in the review of marine *ortho*-prenylated quinones and hydroquinones presented in Chapter 2. Secondary metabolites are small to medium molecular weight compounds (MW < 1000 a.m.u.) that are often produced in response to external stimuli and have sparked the interest of many chemists and biochemists over the years.[1] This thesis focuses on the synthesis of two secondary metabolites and related compounds from a marine derived *Penicillium* fungus and it is thus important here to initially elaborate upon the role of secondary metabolism within marine organisms.

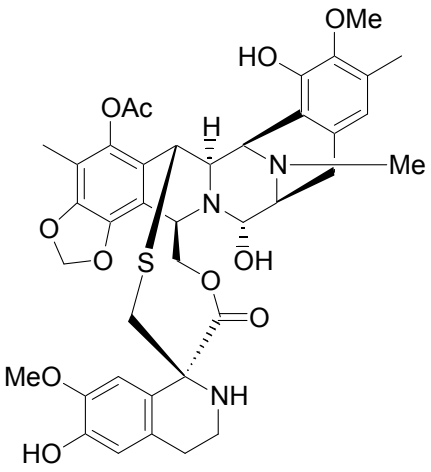
More than 70% of the earth's surface is covered with water and it is therefore not surprising that the oceans are home to thousands of marine species, many of which have yet to be discovered.[2,3] The ocean provides a stable physical and chemical environment in terms of both temperature and salinity, a variety of different habitats from the deep dark ocean floor to the shallow inter-tidal zone as well as a wealth of available and continually recycled nutrients. All of these factors have contributed to the large biodiversity of marine organisms found in the oceans today. Marine organisms (algae, invertebrates and microorganisms) are also a good source of novel secondary metabolites, not only because of the vast biodiversity in the marine environment, but also because marine organisms are well adapted to living in an environment with very different physiological and ecological challenges to those faced by terrestrial species. For example marine organisms such as algae and marine invertebrates have had to develop unique and interesting physiological adaptations for acquiring nutrients, respiring in an aqueous environment and reproduction.[3] Andersen and Williams have hypothesised that marine organisms which produce natural products will make use of unique biosynthetic pathways or adaptations of well

established pathways used by terrestrial organisms to biosynthesize novel natural products.[3]

Many marine secondary metabolites are toxic and a number of marine natural product researchers including Haefner[2] and Andersen and Williams,[3] have argued that a large proportion of marine organisms produce these compounds as a means of chemical-defence resulting from their sedentary or slow moving life-styles coupled in many instances with soft bodies that have no means of physical defence such as shells or spines.[2,3] Haefner has also hypothesized that in the marine environment many marine secondary metabolites serve as chemical mediators in the inter-species competition for space and nutrients on sub-tidal reefs and in reproduction.[2] It is important to note, however, that the ecological roles of many marine secondary metabolites are still unknown and much research still needs to be done. [3]

Marine natural products chemistry is a relatively new research field and incorporates the isolation and structural elucidation of secondary metabolites and the study of their biological activities, both ecological and pharmaceutical.[4,5] In the past the isolation and structural elucidation of compounds from marine invertebrates proved to be extremely difficult due to limitations in the techniques and technology available at that time *ca*. 1940-1970. The marine natural products research field has grown significantly since the 1970's due to improvements in techniques such as chromatography [especially high performance liquid chromatography (HPLC)] for dealing with more polar compounds and advances in nuclear magnetic resonance (NMR) for structural elucidation.[4] Initially, research focused mainly on the secondary metabolites isolated from marine algae, invertebrates and to a lesser extent marine microbes. However, over the past decade the focus has shifted away from algae and moved largely towards investigating the natural products of marine invertebrates and microorganisms.[5] This shift in focus may be attributed to the search for novel marine metabolites with anti-cancer bioactivity. The majority of marine metabolites exhibiting anti-cancer activity has thus far come from marine invertebrates, especially Porifera (sponges), Bryozoa (bryozoans) and Chordata (ascidians).[3,4] There are, for example, four potential anti-cancer compounds isolated from marine invertebrates currently in clinical trials [ecteinascidin 743 (**1**), aplidine (**2**), didemnin B (**3**) and

dolastatin 10 (**4**)] and two in preclinical trails [halichondrin B (**5**) and kahalalide F (**6**)]. [2,4]



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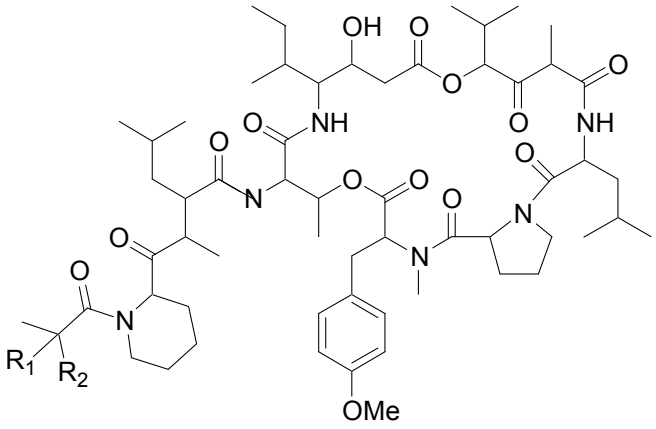

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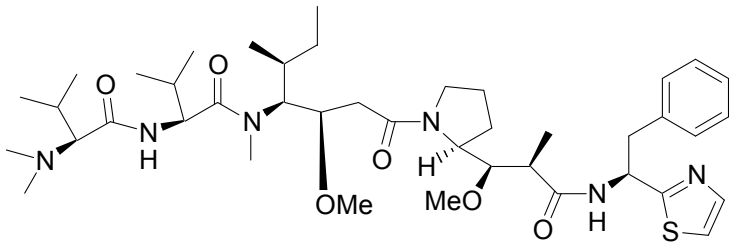

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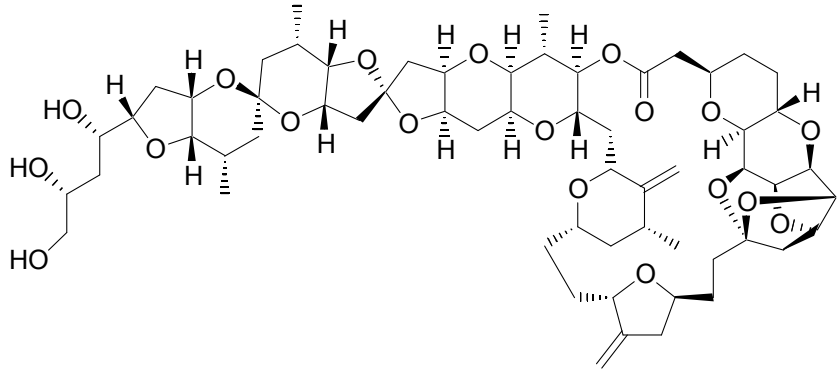

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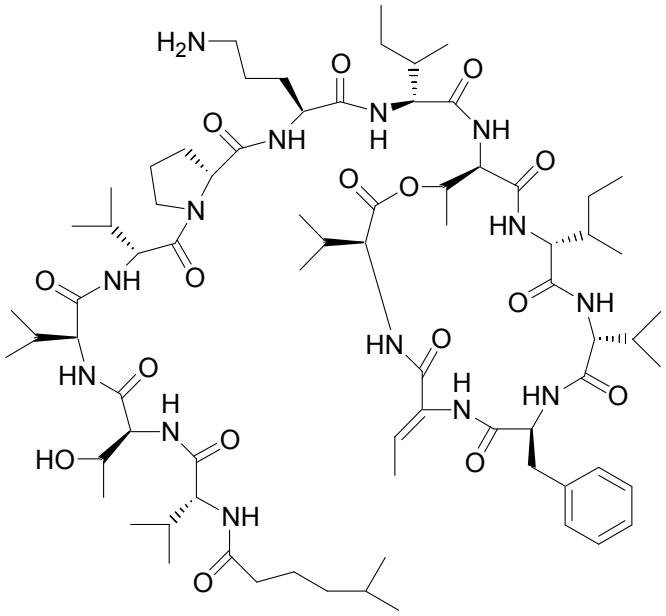


6

The most important factor hindering new drug discovery from marine natural products today is the potentially limited supply of these compounds from their natural sources. In general, marine organisms only produce very small quantities of secondary metabolites and as a result large quantities of an organism theoretically needs to be harvested to obtain a suitable amount of a particular metabolite for preclinical trials.[6] The negative environmental impact of harvesting large amounts of marine invertebrate often makes harvesting an unacceptable solution to the supply problem. Two other alternatives to harvesting marine organisms to obtain marine natural products on a large scale are:

1) Aquaculture of marine sponges and bryozoans and fermentation of marine microorganisms. This approach requires much prior research into the conditions necessary for growth and reproduction.[4]
2) Synthesize: Many marine natural products have proved to be difficult to synthesis industrially because of their complex structures incorporating many stereogenic centres.[4,7]

Natural products isolated from marine microbes are an important resource for new drug discovery.[6,8] Although technical problems associated with the culture of marine microorganisms still persist, significant progress in the production of natural products

from marine actinomycete bacteria has been made.[9] Nearly two thirds of the known naturally derived antibiotics have been discovered in the fermentation broth of terrestrial actinomycetes and exploration of natural products produced by actinomycetes found in deep water marine sediments provides an excellent opportunity for new drug discovery.[8] An interesting recent discovery is that many of the metabolites isolated from marine microbes have previously been associated with marine invertebrates suggesting a symbiotic relationship between a number of members of these two diverse groups of organisms.[3] Therefore, one of the current debates in the marine natural products community centres around the possible symbiotic microbe origin of many marine invertebrate natural products and how this symbiotic relationship can be exploited in new drug discovery.[10]

1.1 Marine natural products research within a South African context

South Africa's coastline extends across more than 2500 km from the Namibian border on the west to Mozambique on the east[11,12] and can be divided into three bio-geographical zones: the cold waters of the Benguela current on the west coast, the warm waters of the south east coast and the subtropical waters of the Agulhas current on the east coast. These three bio-geographical zones support their own unique population of diverse marine organisms and as a result South Africa has a huge diversity of marine organisms with great potential for the discovery of new pharmaceuticals.[11,12] The natural product chemistry of marine life in South African waters has recently been substantially reviewed.[11,12] The link between biodiversity and structural diversity means enormous biological diversity in the oceans off South Africa and makes South Africa's marine biodiversity an attractive unexplored resource for the discovery of new biologically active pharmaceuticals.[2,3,11,12]

Historically, marine natural products chemistry research in South Africa began at the University of Cape Town (UCT) in 1977 through the pioneering investigations of Elsworth and Cragg.[11,12] However, the limited access of the UCT researchers to modern HPLC and NMR techniques (available to emerging research groups especially in the USA and Europe) meant that marine natural products chemistry research progressed far slower in South Africa than elsewhere in the world. The situation changed in the early 1990's when Kashman from the University of Tel Aviv

in Israel, in collaboration with marine biologist Schleyer from the Oceanographic Research Institute in Durban, embarked on a four year investigation of the natural product chemistry of tropical marine invertebrates collected from Sodwana Bay in north eastern South Africa.[11,12] Simultaneously a collaborative programme between Faulkner (Scripps Institution of Oceanography, USA) and Davies-Coleman (Rhodes University) also began investigating the natural product chemistry and pharmaceutical potential of the marine invertebrates of southern Mozambique and the temperate south east coast of South Africa. International pharmaceutical companies and the National Cancer Institute (NCI) provided ready access to high throughput, anti-cancer drug discovery screening facilities (unavailable in South Africa at that time) for the screening of southern African marine invertebrate extracts. Although, no new marketable pharmaceutical has as yet been discovered from southern African marine organisms a large number of new bioactive marine natural products have been discovered and their structures and bioactivities have recently been comprehensively reviewed by Davies-Coleman and Beukes[11] and Davies-Coleman.[12]

Laws to protect South African intellectual property rights in bioprospecting programmes have been incorporated into the new South African Biodiversity Bill and this new legislation has had negative implications for international collaborative bioprospecting programmes in this country. In response to these changes the Rhodes University marine natural products chemistry group has focussed on building national bioprospecting collaborations and has worked with medical biochemists at the University of Cape Town's School of Medicine to investigate the potential of southern African marine natural products to provide a source of new drugs for the treatment of oesophageal cancer.[13,14] The research presented in this thesis forms part of this collaborative effort.

1.2 Oesophageal cancer in South Africa

Oesophageal cancer, as the name suggests, is cancer of the oesophagus and can be classified into two main categories: epithelial and non-epithelial tumours. The two epithelial tumours most commonly diagnosed are squamous cell carinoma (SCC) and adenocarcinoma.[15] SCC is a malignant tumour that affects the middle of the epithelial cell layer (the squamous cells) and is found predominantly in developing counties

such as South Africa.[15] This form of cancer commonly occurs in the upper portion of the oesophagus and accounts for 50 – 60 % of all oesophageal cancers worldwide.[16] Conversely, adenocarcinoma is a malignant tumour that originates in the epithelial cells of glandular tissue. This form of cancer predominantly develops in patients suffering from Barrett's Oesophagus,[15] a condition in which the flat squamous cells of the mucosa are replaced with cube like cells in a process known as metaplasia. Adenocarcinoma is found mainly in developed countries and accounts for 30 – 40 % of all oesophageal cancers worldwide.[16]

Oesophageal cancer affects primarily black males in South Africa in areas where poverty and malnutrition is a serious problem. Oesophageal cancer is the second most prominent form of cancer affecting black males and treatment is often difficult as symptoms are only evident after the cancer is well developed and has metastasized. Early detection is thus essential if the cancer is to be treated using radiation, chemotherapy or surgery.[17]

1.2.1 Oesophageal cancer - possible causes

While adenocarcinoma is commonly associated with patients suffering from Barrett's Oesophagus and gastric reflux from the stomach,[18] the causes underlying SCC are far less defined. At present the main causes are thought to be diet, cultural practises around the storage of food cereals and genetics.[15] In developed countries such as the USA, France and Italy for example there is a strong correlation between smoking, drinking and the contraction of SCC. In developing counties such as South Africa, maize meal is the staple food amongst the poor black community and research has shown that ingesting maize contaminated with a *Fusarium* fungus (that produces the carcinogen fumonisin) may indirectly result in the development of oesophageal cancer. A diet poor in fresh fruit and vegetables may also contribute to the onset of oesophageal cancer.[15]

1.2.2 Oesophageal cancer - stages and treatment

Oesophageal cancer can be classified into five stages:[19]

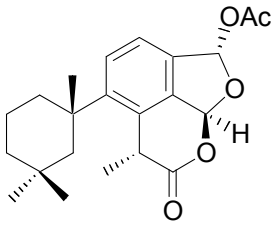
- Stage 0: The cancer is only present in the inner lining of the oesophagus.
- Stage 1: The cancer has spread to the epithelial layer and the submucosa.
- Stage 2: The cancer has reached the lymph nodes
- Stage 3: The cancer has infected all the layers of the oesophagus and local lymph nodes
- Stage 4: The cancer has spread to distant tissues.

The 5-year survival rate for patients diagnosed with oesophageal cancer is extremely poor (14 %)[17] due to the lack of effective screening tests to detect this cancer in its early stages. The late detection of the disease means that there is a high risk of the cancer returning even after surgery or chemotherapy.[20] There is thus a great deal of interest in developing an early screening test for the identification of patients with early malignant tumours. If the cancer is detected early enough (Stage 1 or 2) surgery and chemotherapy are the treatments of choice. Unfortunately, as mentioned earlier, symptoms of the disease often present themselves only once the cancer has spread to other areas of the body (metastasis) which in turn results in late diagnoses *i.e.* stage 3 and 4. Once the cancer has metastasized, treatment becomes far more complicated and chemotherapy and radiotherapy are often utilised to reduce the size of the tumour before attempting surgery.[21] Currently, the most common treatment for advanced oesophageal cancer is cisplatin/5-fluorouracil based chemotherapy and surgery.[22] Other chemotherapeutic agents include paclitaxel, ironetecan and docetaxal.[21]

1.3 Southern African marine invertebrates as a potential source of new anti-oesophageal cancer drugs

The collaborative drug discovery programme involving marine natural product chemists at Rhodes University and medical biochemists at the University of Cape Town has used a dual approach to identify marine natural products with anti-oesophageal cancer activity.[14] The first approach is bioassay guided drug discovery in which extracts of marine invertebrates are screened for their general cytotoxicity. Any extracts exhibiting reasonable cytotoxicity to oesophageal cancer cells are then fractionated and chromatographed further (with constant monitoring of bioactivity in the different fractions) to isolate the active compound(s) in the extract. This approach has been reasonably successful and several marine natural products *e.g.* **7** and **8**, with

50 % cell division inhibitory concentrations (IC_{50}) of 1-10 μM have been isolated and identified.[14]



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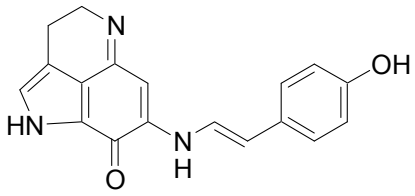

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The second approach is a random screening of a library of pure natural products isolated from Southern African marine invertebrates. Of particular interest amongst the cytotoxic compounds identified, was a group of *ortho*-prenylated toluhydroquinones and toluquinones (**9-15**) isolated by McPhail *et al*.[23] from an endemic nudibranch (sea slug) *Leminda millecra* (Figure 1) collected in Algoa Bay near Port Elizabeth. The word nudibranch comes from the Latin word "*nudibranchia*" where "*nudus*" means naked and "*branchia*" means gills.[24] These animals are found all over the oceans of the world, at any depth and as their name suggests they do not have shells and breathe through a branchial plume (gills) situated on their backs (mantles). Nudibranchs lack the physical protection provided by shells which means they have had to develop other means of protecting themselves for example by utilizing a chemical defence system sequestered from their diet of octocorals *e.g.* soft corals and seafans (also known as gorgonians).[23,25] Linear polyprenyl hydroquinones and quinones have been extracted from octocorals of the genera *Nephthea*[26,27] and *Sinularia*[27] supporting the suggestion that *L. millecra* might sequester these secondary metabolites from their diet of soft corals and related sea fans.[23]

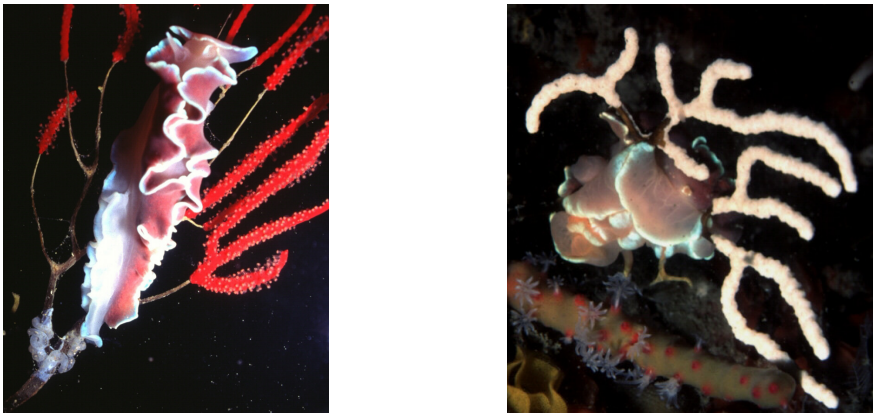
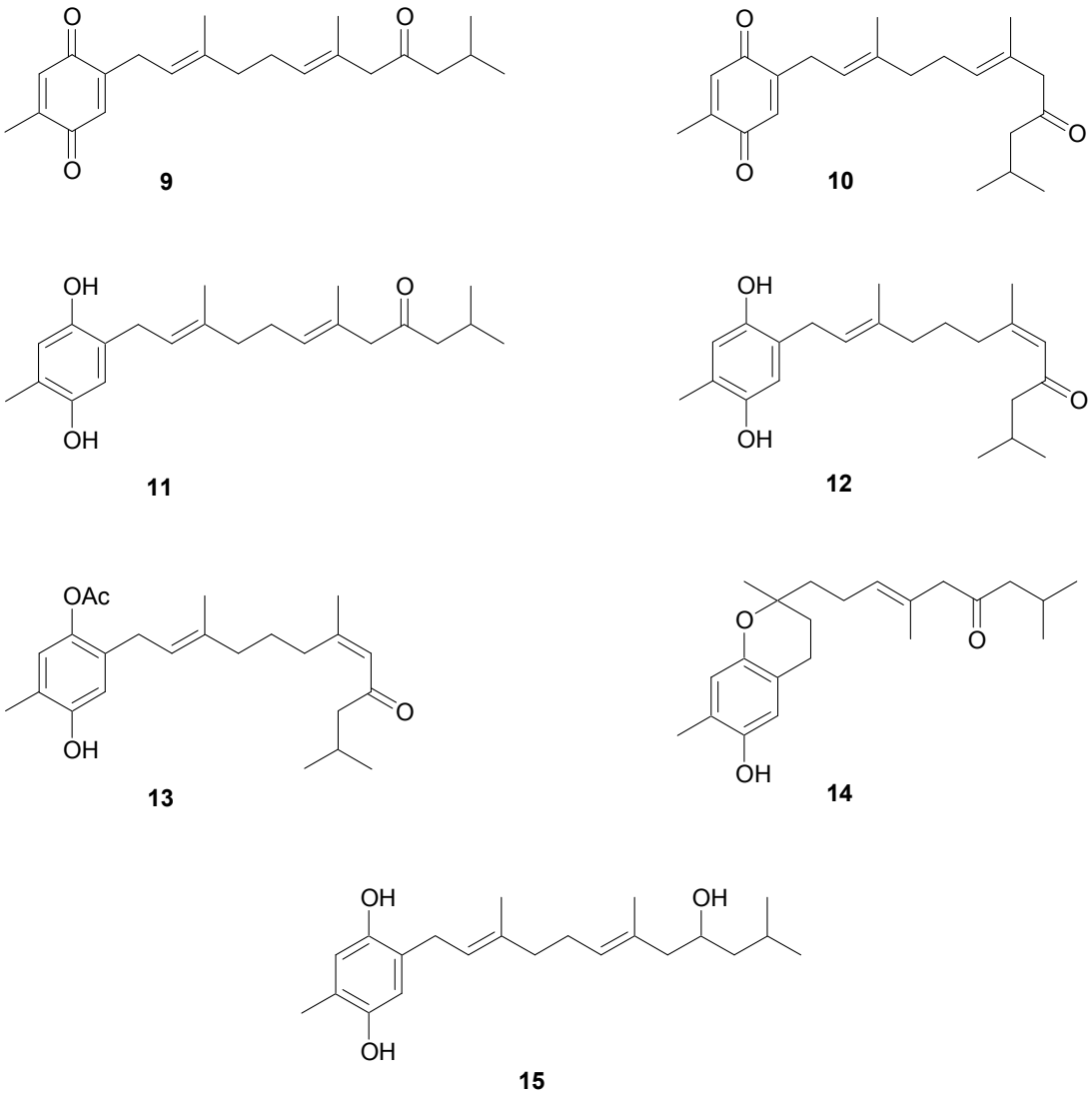
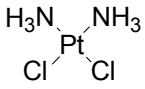


Figure 1: *L. millecra* photographed in Algoa Bay feeding on sea fans (gorgonians).





16

The cytotoxicity of the linear (non-cyclized) triprenylquinones and hydroquinones (**9-13**, **15**) were tested against the oesophageal cancer cell line WHCO1 using the (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (see Chapter 4). The results of the assay (Table 1) revealed that **11** and **12** had IC_{50} values similar to that of cisplatin (**16**), one of the main compounds used in the treatment of advanced oesophageal cancer today (Section 1.2.2). As observed from the IC_{50} values in Table 1, small changes in structure and double bond geometry within the side chain appear to result in possibly significant changes in the cytotoxicity of the compounds towards the oesophageal cancer cell line WHCO1.[28]

Table 1: The comparative IC_{50} values of the linear triprenylquinones and hydroquinones (**9-13, 15**) isolated from *L. millecra* and cisplatin (**16**) against the oesophageal cancer cell line WHCO1.[28]

Compound	IC_{50} [μM]
9	37.9
10	83.3
11	9.5
12	12.9
13	42.7
15	32.7
16	13.0

The most active compound isolated from *L. millecra*, (**11**) was tested against a range of other cells lines including the oesophageal cancer cell lines WHCO1 and WHCO6, cervical cancer cell lines ME180 and SiHa and a normal breast cell line MCF12A used as a control (Table 2). The IC_{50} values from Table 2 indicate that **11** is cytotoxic

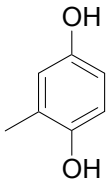
to four of the five cell lines (including the control) with only SiHa proving to be the exception.[28]

Table 2: The IC_{50} values calculated for the cytotoxicity of **11** against a range of different cell lines.[28]

Cell line	IC_{50} [μM]
WHCO1	9.5
WHCO6	5.8
ME180	33.9
SiHa	> 150
MCF12	32.0

1.4 Thesis aims and objectives

The mechanism by which **11** induces apoptosis in oesophageal cancer cells was established by our University of Cape Town collaborators and is outlined in Chapter 4. In support of the mechanistic studies, the aim of the research presented in this thesis was to synthesize simplified analogues of **11** and it's oxidized analogue **9** to explore the role of the prenylated side-chain in the cytotoxicity exhibited by this compound. Therefore to meet the aim of the thesis six compounds (**17**-**22**) were identified as suitable synthetic targets in which the prenylated side-chain was non-oxygenated and fully unsaturated compared to **11** (**21** and **22**), non-oxygenated, fully unsaturated and shortened (**19** and **20**) and absent altogether (**17** and **18**). The successful synthesis of four of the six compounds is discussed in Chapter 3 (**17** and **18** are commercially available) while Chapter 4 will present the anti-oesophageal cancer activity of these compounds. Chapter 2 provides an overview of the structural diversity of *ortho*-prenylated hydroquinones and quinones isolated from marine organisms and a discussion of the various synthetic strategies that can be used to synthesize these prenylated quinones and hydroquinones.



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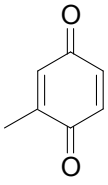

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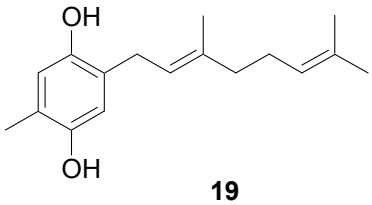

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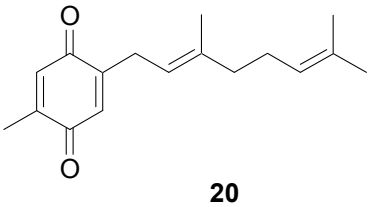

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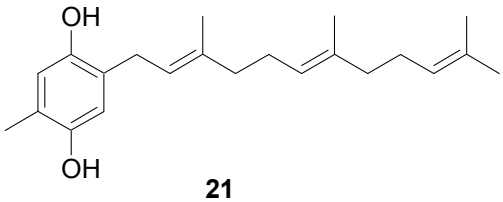

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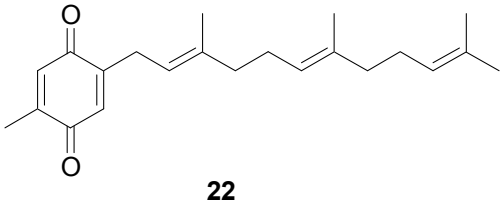

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Chapter 2.

2 *Ortho*-prenylated quinones and hydroquinones from marine organisms

Polyprenylquinones and hydroquinones are an important subclass of marine metabolites of mixed biosynthesis. The prenylated portion of these quinones and hydroquinones can vary in length from one to nine isoprene units and are products of the mevalonate biosynthetic pathway while the hydroquinone or quinone moiety is derived from shikimic acid. Although marine brown algae are the most common sources of these polyprenylated metabolites, they have also been extracted from marine fungi and various marine invertebrates including sponges, ascidians, gorgonians, soft corals and nudibranchs (Figure 2).[29] The results from various assays such as the α,α-diphenyl-β-picrylhydrazyl radical (DPPH) and thiobarbituric acid reactive substances (TBARS) assays have revealed that many of these prenylated metabolites exhibit powerful antioxidant properties with the hydroquinones not surprisingly being more powerful antioxidants than their corresponding quinones.[30]

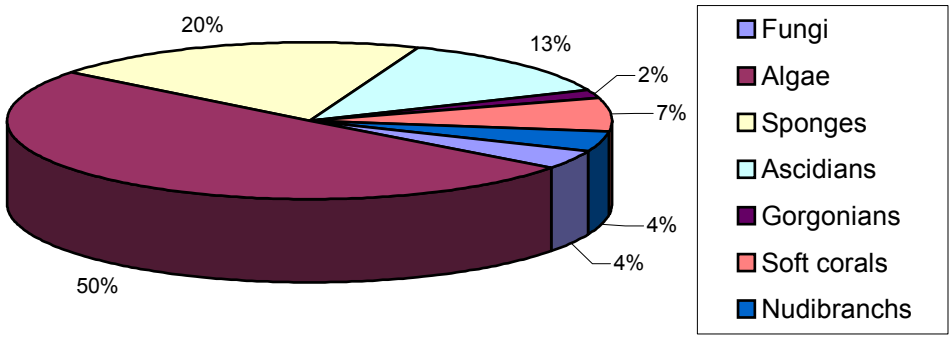


Figure 2: The distribution of hydroquinones and quinones across marine phyla.[6,29]

A survey of the marine natural product database MarinLit[29] and annual reviews in this field[6] has revealed that marine prenylated hydroquinones and quinones can be grouped into five different structural classes (Figure 3). It is clear from this survey that brown algae characteristically have produced hydroquinone and quinone metabolites where the alkyl and prenylated side-chains are placed *meta* relative to each other on the hydroquinone nucleus. The one exception to this trend is the monosubstituted hydroquinone metabolites found in *Dictyopteris undulata*[30,31] and may suggest a symbiotic fungal origin for these metabolites. The ubiquitous occurrence of structural class A and B (Figure 3) in fungi, sponges, soft corals and sea fans may also indicate a symbiotic fungal origin for these metabolites where they occur in marine invertebrates. Brominated and macrocylic hydroquinones (D and E in Figure 3) appear to be restricted to green algae and ascidians respectively. Selected examples of these structural classes, within the context of the phyla from which they originate, will be presented in Sections 2.1 – 2.4.

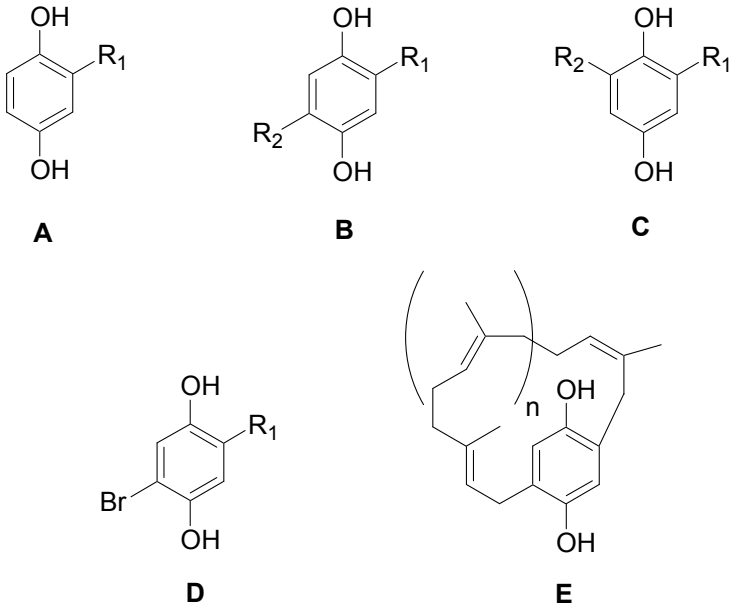
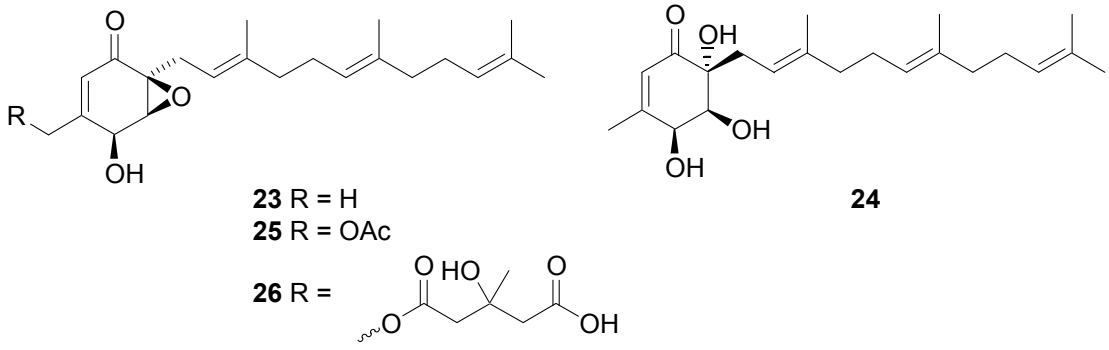


Figure 3: Hydroquinone substitution patterns in **A)** marine sponges, ascidians and one species of brown algae (*Dictyopteris undulata*)[30,31] **B)** marine fungi, gorgonians, soft corals and nudibranchs **C)** brown algae **D)** green algae **E)** ascidians. [R_1 = polyprenyl side chain and R_2 = alkyl group].[29]

2.1 Fungi

Terrestrial fungi have been a major source of a wide variety of pharmaceuticals especially antibiotics *e.g.* penicillin.[1] Marine fungi have not been studied as extensively as their terrestrial counterparts, but nonetheless have produced a variety of bioactive metabolites. A number of studies have shown that metabolites isolated from marine-derived fungi also exhibit significant antibiotic activity.[32] As alluded to in Chapter 1 and above, symbiotic marine microorganisms may be responsible for producing many of the metabolites previously attributed to marine invertebrates.[3]

Triprenylated toluquinone and toluhydroquinone secondary metabolites have been isolated from both marine and terrestrial fungi. A marine derived *Penicillium* species afforded farnesyltoluhydroquinone (**21**) along with its quinone analogue (**22**).[33,34] Farnesyltoluhydroquinone **21** is not confined to the marine environment and has also been isolated from the terrestrial fungus *Phellinus pini*[35] and three species of terrestrial plant genus *Seseli*.[36] The anti-oxidant activity of both compounds were tested using the DPPH radical scavenging assay and the results of this assay revealed that hydroquinone **21** showed potent radical scavenging activity with an IC_{50} = 12.5 μM.[34] Two polyoxygenated farnesylcyclohexenones (**23** and **24**) were also later isolated from this fungus.[33] The cytotoxicity of metabolites **21, 22** and **23** were tested against five human solid tumor cells as well as methicillin-resistant and multidrug resistant *Staphylococcus aureus*. Compounds **21** and **23** showed moderate *in vitro* cytotoxicity against the tumor cell lines as well as antimicrobial activity towards the methicillin-resistant and multidrug resistant *S. aureus*.[33]



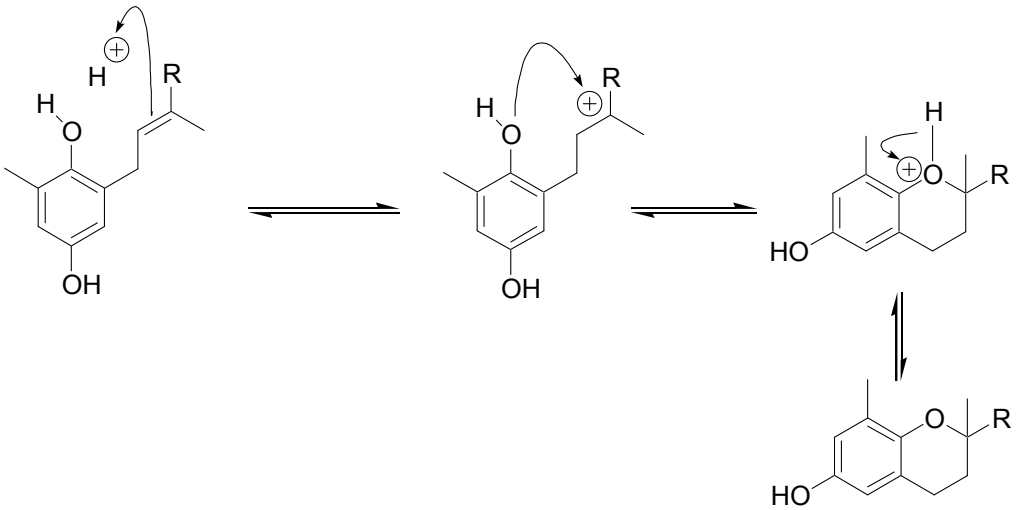
23 R = H
25 R = OAc
26 R =

24

Other metabolites exhibiting epoxidation similar to that observed in **23** have also been isolated from the marine fungus *Aspergillus niger e.g.* compound **25**.[32] The ester substituent in this group of compounds can also be further oxygenated as observed in compound **26**.[32] Eight cytotoxic farnesylated epoxy cyclohexenones similar in structure to **25** and **26** were isolated from the fungus *A. niger* which exists in a symbiotic relationship with an orange ascidian (*Aplidium* sp) collected in Benga, Fiji.[32] Longithorols (cyclo-farnesylated quinones. See Section 2.4.2) have also been isolated from the ascidian *Aplidium longithorax*[37-41] which could imply that the quinone moiety is produced by symbiotic fungi that co-occur with ascidians of the genus *Aplidium*.

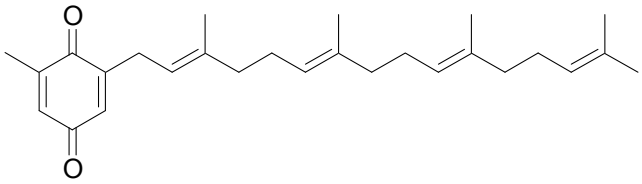
2.2 Brown Algae

A large number of prenylated hydroquinone and quinone derivatives have been isolated from brown algae of the Order Fucales especially from the Families Cystoseiraceae and Sargassaceae. In the group of metabolites isolated from brown algae, cyclization between the hydroxyl of the hydroquinone and the C-3′ of the polyprenyl chain to form various chromenol derivatives, is a common feature. A putative mechanism for this cyclization is presented in Scheme 1. Interestingly, this cyclization appears to be confined to hydroquinone metabolites isolated from Mediterranean brown algal species suggesting that the cyclization is not an artifact of the isolation procedure.[42]



Scheme 1: A proposed mechanism for the formation of the bicyclic polyprenylated marine secondary metabolites. [R = polyprenyl chain].

The majority of marine prenylated secondary metabolites isolated from the Family Cystoseiraceae originate from the genus *Cystoseira* collected from the Mediterranean Sea and along the Atlantic coast.[43] The genus *Cystoseira* is characterized by the production of tetraprenylquinone derivatives such as **27** isolated from the chloroform extract of *C. jabukae*.[44]



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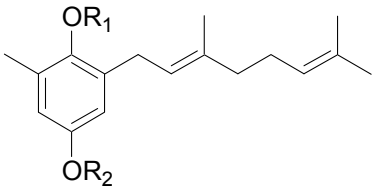
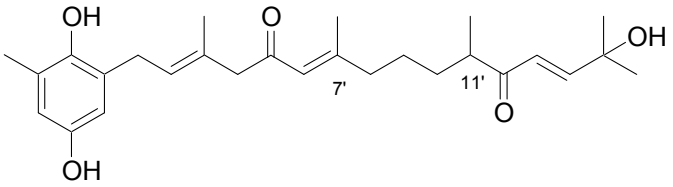
Three geranyl toluquinol derivatives (**28**-**30**) were isolated from a C*ystophora* sp. collected near Hamelin Bay in Australia and serve as typical examples of methylated and non-methylated prenylated toluhydroquinones found in brown algae.[45]



28 $R_1 = R_2 = Me$
29 $R_1 = Me, R_2 = H$
30 $R_1 = R_2 = H$

A number of compounds exhibiting oxygenation within the polyprenyl side-chain *e.g.* compound **31** have also been isolated from *Cystoseira*. *stricta var*. *amentacea Bory* found in the central Mediterranean Sea along the coast of Sicily.[46] Oxygenation however, is not confined to the polyprenyl side-chain and there is one instance where the methyl substituent of the aromatic ring has been oxidized to an aldehyde *e.g.* desmarestial (**32**) from *Desmarestia menziesii* collected in Antarctica.[47,31]



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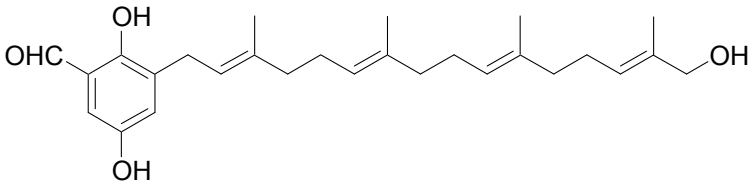
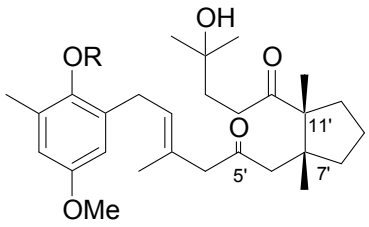
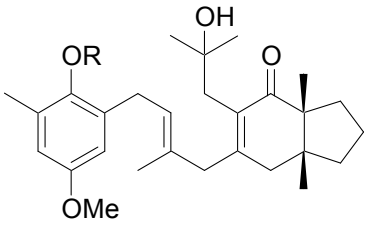


32

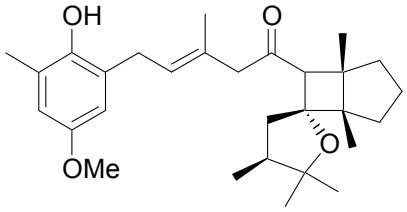
Amico *et al*.[48] hypothesized that polyprenylated diketones, similar in structure to **31,** could be precursors to very similar metabolites possessing cyclopentane, bicyclo[4.3.0]nonane or bicyclo[3.2.0]heptane ring systems within their prenylated side-chains. A Michael type reaction, resulting in bond formation between C-11' and C-7', would yield a cyclopentane ring system within the polyprenyl side-chain *e.g.* **33** and **34** isolated from *Cystoseira elegans* and the hybrid plant *Cystoseira elegans x Cystoseira algeriensis*.[49-51] If this is followed by an aldol condensation reaction and subsequent dehydration it would lead to ring closure between C-5' and C-13' and the formation of the bicyclo[4.3.0]nonane system as seen, for example, in compounds **35** and **36** from *Cystoseira algeriensis* and the hybrid *Cystoseira elegans x Cystoseira algeriensis.*[49-51] Finally, if ring closure were to occur from C-6' to C-12', the highly strained bicyclo[3.2.0]heptane ring system would result[46,48] as observed in compound **37** isolated from *Cystoseira balearica* collected at Portopalo, Sicily.[42] A further group of tetraprenyltoluhydroquinones, exhibiting even more complex elaboration within the polyprenyl side-chain, have also been isolated from brown algae *e.g.* **38** and **39**.[52,53]



33 R = Me
34 R = H



35 R = Me
36 R = H



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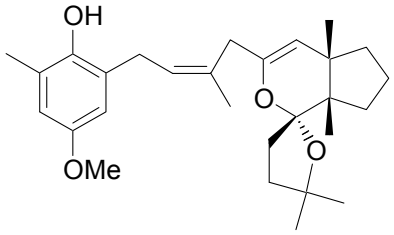

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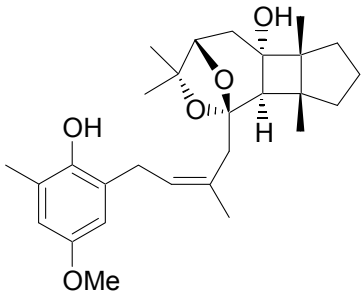
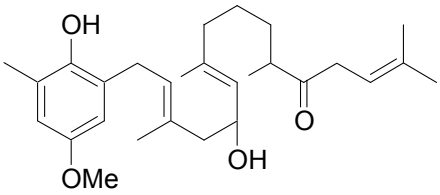


39

An additional feature observed throughout the plethora of prenylated hydroquinone and quinone metabolites isolated from brown algae, is double bond isomerism *e.g.* compound **40** isolated from an unidentified Sicilian species of *Cystoseira*, C. *elegans*[54] in addition to another brown alga *Halidrys siliquosa*.[54-58] The prenylated quinone (**41**) isolated from the methanol extract of *Sargassum serratifolium* is another good example of double bond isomerism in the prenylated side-chain of a brown algal metabolite.[59]



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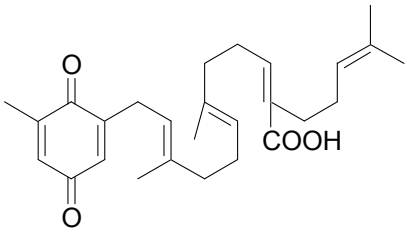
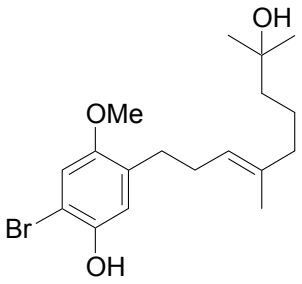


41

2.3 Green Algae

There is only one instance where polyprenylated hydroquinones have been isolated from green algae.[60] Interestingly, it would appear that these metabolites are characterised by a bromine substituent on the hydroquinone nucleus *e.g.* **42** and **43**.



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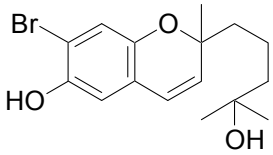
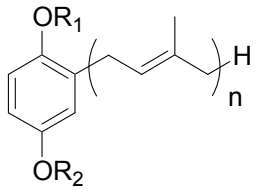


43

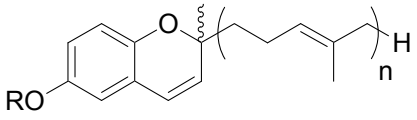
2.4 Marine Invertebrates

2.4.1 Sponges

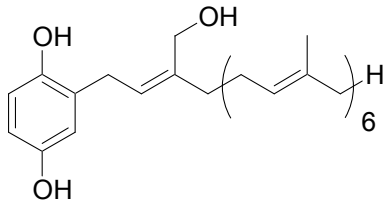
Amongst marine sponges, the genus *Ircinia* is the predominant source of polyprenylated quinones regularly isolated with their corresponding and often more abundant hydroquinones.[61] The secondary metabolites (**44-57**) exemplify the major types of polyprenylhydroquinones, their sulfated derivatives, chromenols and polyprenylquinones found in a variety of sponge species from the genera, *Ircinia*,[61-63] *Smenospongia*,[64] *Hippospongia*,[65-72] *Sarcotragus*,[73,74] *Spongia*,[75] *Dysidea*,[76,77] *Siphonodictyon,*[78] *Reniera*,[79] *Cacospongia,*[80] and *Psammocinia*.[81]



44 R_1, R_2 = H, n = 4-8
45 R_1 = SO_3Na, R_2 = H, n = 6-7, 9
46 R_1 = SO_3Na, R_2 = Me, n = 7
47 R_1 = H, R_2 = SO_3Na, n = 5-8



48 R = H, n = 6
49 R = Me n = 6
50 R = SO_3Na, n = 5-7



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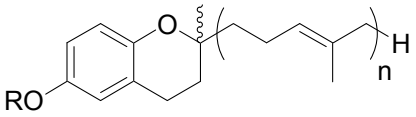
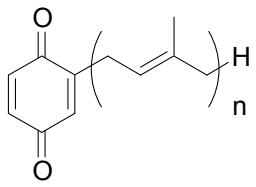
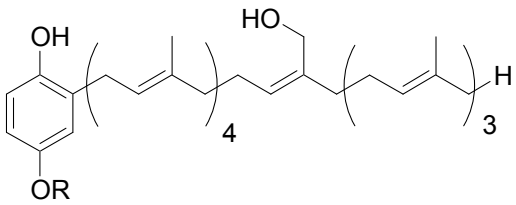


52 R = SO_3Na, n = 5
53 R = H n = 4-6



54 n = 4-8



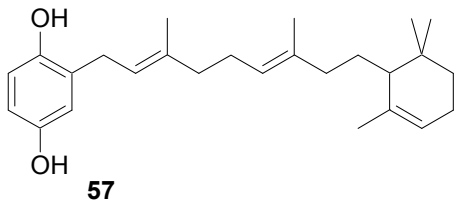
55 R = H
56 R = SO_3Na

Studies of the biological activity of prenylated hydroquinone and quinone metabolites isolated from sponges have focused on their activity against isolated enzymes of biomedical significance and also their role in chemical ecology. For example the compounds **44** (n = 4, 6-8), **45** (n = 6), **47** (n = 6-8) and chromenols **50** (n = 5-7) have shown activity against H^+, K^+ and Na -ATPase.[61,62] Conversely, the sulfated derivatives **47** (n = 5-7) inhibit the neuropeptide Y (NPY) receptor, tyrosine protein kinase (TPK) and even HIV-integrase enzymes. Compounds **44** (n = 4-6), **54** (n = 4-5), **51** and the chromenols **52** and **53** (n = 4-6) also showed activity in TPK and HIV integrase assays.[62] Compounds **45** (n = 6) and **47** (n = 8) isolated from an Australian

sponge *Sarcotragus* sp. inhibits the α-1,3-fucosyltransferase VII, enzymes responsible for the synthesis of selectin ligands.[74]

Three polyprenylated quinones **54** (n = 6-8) along with their hydroquinones **44** (n = 6-8) and hydroxylated 2-octaprenylquinol **55** were isolated from the methanol extract of the sponge species *I. spinosula* collected in the Bay of Naples.[82,83] Metabolites **44** (n = 6-8), **55**, **56** and **47** (n = 7-8) were also isolated from *I. spinosula* collected near Zadar, Croatia, using the brine shrimp assay to guide the fractionation.[84] *Artemia salina* shrimp lethality and fish lethality assays were performed using metabolites **44** (n = 7-8), **55**, **56** and **47** (n = 7-8) . The results of the assays showed that the sulfated metabolites **47** (n = 7-8) and **56** were more toxic than the corresponding hydroquinones **44** (n = 7-8) and **55.** This is not surprising given the "detergent-like" properties of the sulfated prenylated hydroquinones in which a long "hydrophobic tail" is attached to a "hydrophilic head", a common characteristic of soaps and detergents. De Rosa *et al*.[84] have since hypothesized that the sponges use the sulfated derivatives as a means of chemical defense against macro-symbionts such as marine worms.

Unlike the prenylated secondary metabolites isolated from brown algae there seems to be very few examples of cyclization occurring within the prenylated side-chains of secondary metabolites isolated from sponges. Cacospongins B (**57**) and C (**58**) isolated from the sponge genus *Cacospongia*[80] serve as rare examples.



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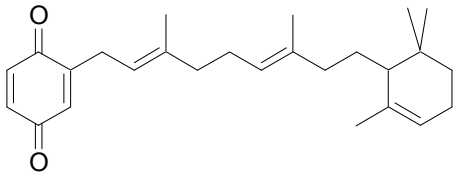
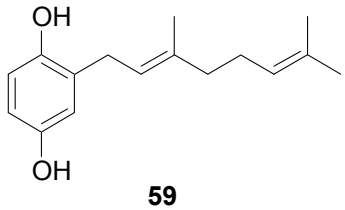


58

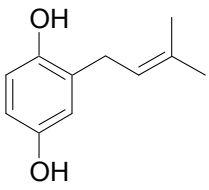
2.4.2 Ascidians

Ascidians belong to the Phylum Chordata, and are the most advanced filter feeding marine invertebrates commonly studied by organic chemists. The first ascidian prenylated hydroquinone metabolite was isolated by Fenical in 1974 from an *Aplidium* sp. (**59**) This compound exhibited cytotoxicity against leukaemia, Rous sarcoma and mammary cincinoma in test animals, which in turn lead to further research on this ascidian species. The results of the research proved to be fruitful yielding a variety of structurally diverse compounds with interesting biological activities.[85] The metabolites isolated from ascidians can be grouped into two categories: nitrogenous and non-nitrogenous metabolites. Prenylated quinone and hydroquinone derivatives are the most common non-nitrogenous metabolites that have been isolated to date.[85]



59

Simple prenylated hydroquinone and quinone derivatives such as those isolated from the marine urochordate *Aplidium californicum* (**60**-**62**) are fairly common.[86] Bicyclic hydroquinone derivatives *e.g.* **61** have also been isolated.[87-90]



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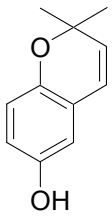

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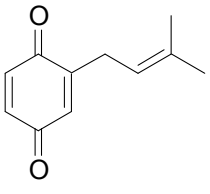
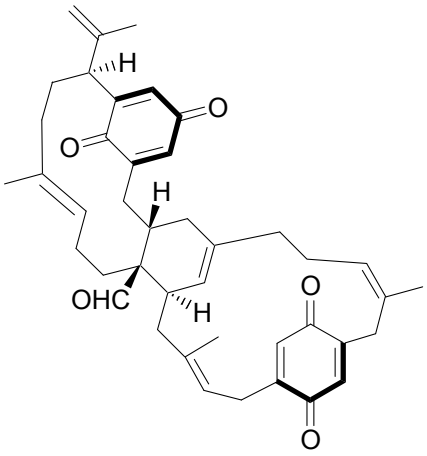


62

Ascidians also contain some metabolites in which the prenylated side-chain has cyclized to form a large macrocyclic ring. The longithorones *e.g.* **63** (cyclo-farnesylated quinones) and the longithorols *e.g.* **64** isolated from the tunicate *Aplidium longithorax* serve as good examples.[37-41]



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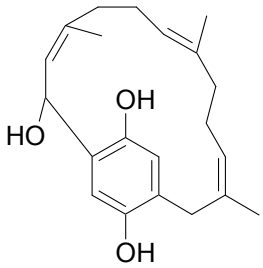
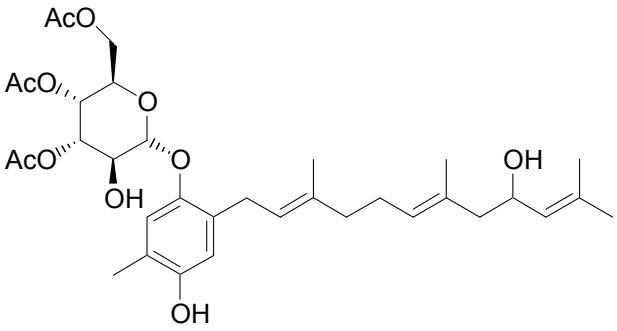


64

2.4.3 Gorgonians

Marine natural products research on various gorgonian (sea fan) species over the years has lead to the isolation of many important bioactive compounds such as sesquiterpenes, diterpenes, steroids and a number of postaglandins. Moritoside (**65**) isolated from the lipophilic extract of a Japanese gorganian: *Euplexaura sp*. is an unusual farnesyl hydroquinone glycoside, which was found to be the active compound for inhibiting cell division in the fertilized starfish egg assay. The role of this compound in the gorgonian is unknown.[91] Two very similar metabolites called euplexides F (**66**) and G (**67**) were isolated from *E. anastomosans.* [92] The only other prenylated compounds isolated from sea fans include two diterpene cyclohexanes namely eunicones A (**68**) and B (**69**) isolated from the genus *Eunicea.*[93]



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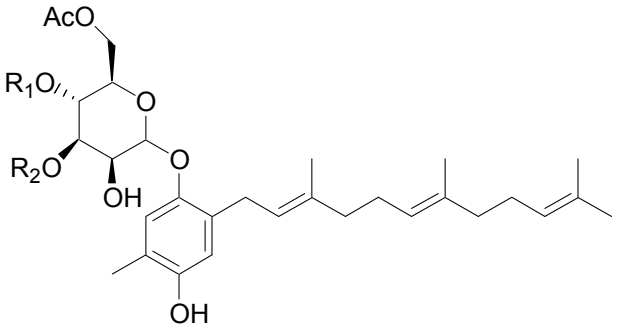
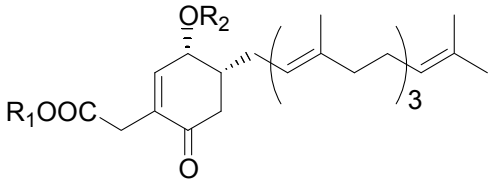


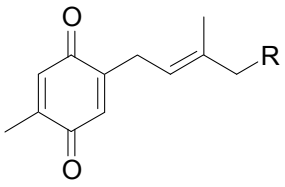
66 R_1 = Ac, R_2 = H
67 R_1 = H, R_2 = Ac



68 R_1 = Me; R_2 = H
69 R_1 = H; R_2 = Ac

2.4.4 Soft Corals

The simplest tetraprenylbenzoquinone derivatives isolated from soft corals were those from the genus *Nephthea* (**70**-**72**).[26] These compounds serve as good representative examples of the types of prenylated metabolites (including chromenols) isolated from soft corals.



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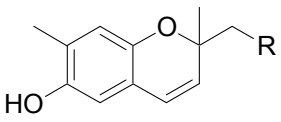

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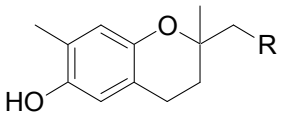
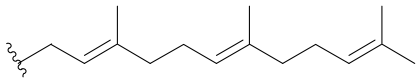
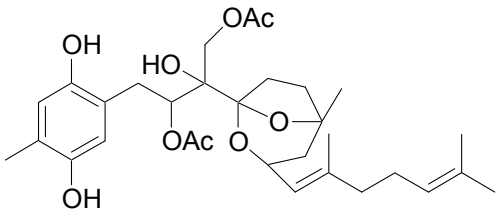


72

R =

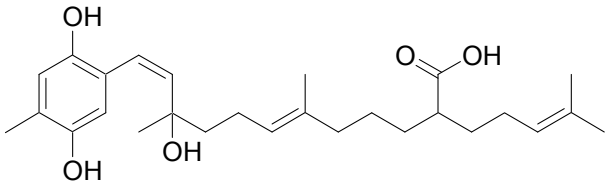


These secondary metabolites are similar to those isolated from sponges, in that cyclization is rarely observed within their polyprenyl side-chains. A rare example is that of the tetraprenylhydroquinone derivative, sindurol (**73**) isolated from *Sinularia dura* collected in Sodwana Bay, South Africa.[27]



73

Oxidation, as well as double bond isomerism observed within the polyprenyl side-chain of the secondary metabolites isolated from brown algae, is also often observed in secondary metabolites produced by soft corals *e.g.* **74** and **75** isolated from the Taiwanese soft coral *Nephthea chabrolii.*[94] Another similarity between a number of secondary metabolites from soft corals and those from brown algae is that the oxidation is not limited to the polyprenyl side-chain *e.g.* rietone (**76**) isolated of the South African soft coral *Alcyonium fauri* collected at Riet Point near Port Alfred.[95]



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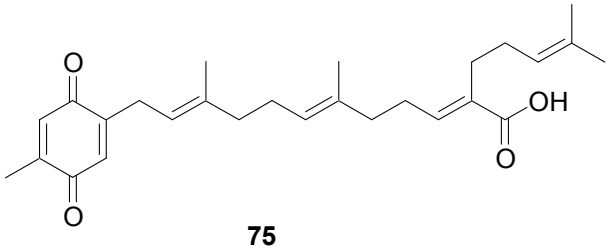

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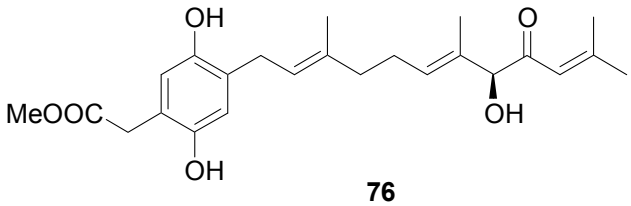

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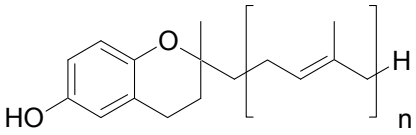
2.4.5 Nudibranchs

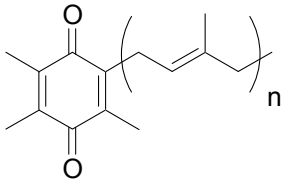
Nudibranchs as mentioned in Section 1.3, are brightly coloured animals that sequester toxic secondary metabolites from the marine organisms (octocorals and sponges) on which they feed as a means of fending off predators.[23,25] Several polyprenylated secondary metabolites have been isolated from nudibranchs including the seven triprenylquinones and hydroquinones (**9**-**15**) isolated from *Leminda millecra.*[23] Not surprisingly similar compounds have been isolated from the sponges, soft corals and sea fans on which nudibranchs are known to feed. A mixture of prenylated chromenols *e.g.* **77** have also been isolated from the digestive glands of the nudibranch *Dendrodoris grandiflora*.[96]



77 n = 1 - 6

2.5 Synthesis of *ortho*-prenylated compounds

Ortho-prenylated phenolic compounds *e.g.* hydroquinones play an important role in many biological processes including cellular respiration, photosynthesis and electron transport. Prenylated quinones are highly hydrophobic because of their long aliphatic side-chains and thus form part of a group of mitochondrial membrane associated electron carriers whose role is to conserve energy released during electron transport for ATP production *via* oxidative phosphorylation.[97] A prenylated quinone *e.g.* ubiquinone (**78**), whose side-chain length varies depending on the source of the mitochondria,[98] accepts electrons from an electron donor *e.g.* NADH and is reduced to the prenylated hydroquinone. The electrons are subsequently passed onto another donor *e.g.* cytochrome bc_1, resulting in the oxidation of the hydroquinone back to the quinone (Scheme 2).[1,98-100]



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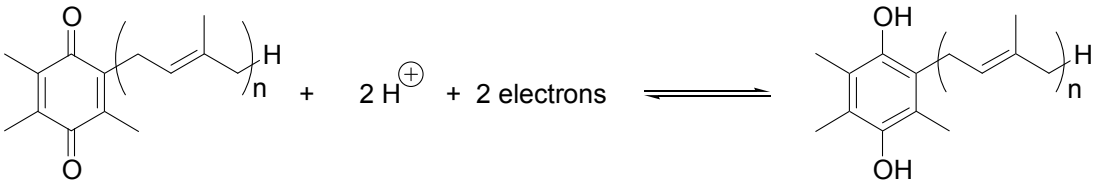
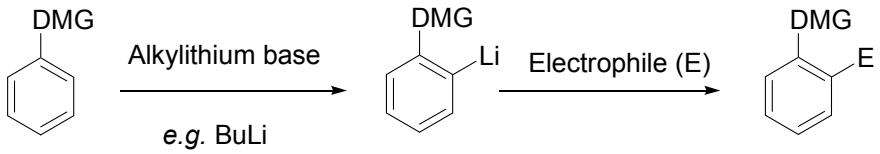


Scheme 2: Reduction of the quinone moiety to the corresponding hydroquinone.

The biological activities of numerous marine polyprenylated quinones and hydroquinones have been investigated using a variety of assays and these compounds have been shown to exhibit powerful antioxidant properties, signifying the potential of these compounds for the treatment of diseases commonly associated with free radical reactions such as arteriosclerosis, dementia and cancer.[30] As described previously there is evidence which suggests that the various sulfated derivatives of prenylated hydroquinones, quinones and chromenols extracted mainly from sponges inhibit the NPY receptor, the tyrosine protein kinase (TPK) and HIV-integrase enzymes as well as H^+, K^+ and Na -ATPase enzymes.[61,62] There is also recent evidence which suggests that these *ortho*-prenylated quinones and hydroquinones may be useful as anti-oesophageal cancer agents[28] (Section 1.3 and Chapter 4). Clearly, *ortho*-prenylated hydroquinones and quinones are important biologically active compounds and it is because of their importance that many chemists have tried to develop an effective means of synthesizing them. Over the past few years a number of synthetic strategies have been developed including directed *ortho*-metalation (DoM), metal-halogen exchange (MHE), phenoxide carbon-alkylation, Claisen rearrangement and Friedel-Crafts-type prenylations.[101] The various approaches to the synthesis of *ortho*-prenylated phenolic compounds have been recently reviewed.[101,102] In order to provide a suitable background to our synthesis of *ortho*-prenylated hydroquinone and quinone metabolites **19-22** described in the next chapter, a brief overview is provided in the following sections of the five general synthetic strategies mentioned above.

2.5.1 Directed *ortho*-Metalation (DoM)

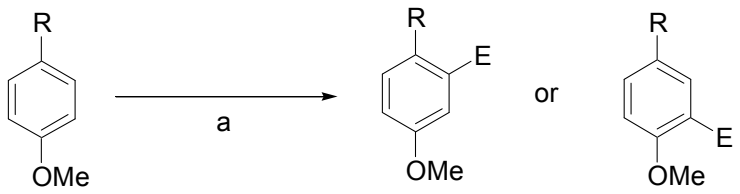
Directed *ortho*-metalation chemistry has been comprehensively reviewed by Gilman and Morton[103] and Mallan and Bebb.[104] DoM is a two-step process that involves firstly the deprotonation of the carbon *ortho* to the heteroatom containing "directing metalating group" (DMG). The resulting *ortho*-lithiated intermediate can then react with a suitable electrophile to give the 1,2-disubstituted product (Scheme 3).[102,105-107]



Scheme 3: A summary of the DoM reaction. [105-107]

The strong alkyllithium bases used during the deprotonation step are highly soluble in organic solvents where they tend to form defined aggregates with electron deficient bonding in solution. Alkylithium bases form mainly hexamers in hydrocarbon solvents (*e.g.* cyclohexane) and tetramers-dimers in electron donor, Lewis base-type solvents *e.g.* THF or diethyl ether. As a general rule: the stronger the electron donor (Lewis base) the less electron deficient are the aggregates that form in solution.[102,108] Tetramethylethylenediamine (TMEDA) is a bidentate ligand that effectively breaks down electron deficient alkyllithium aggregates into monomers and dimers and in so doing, increases the basicity of the alkyllithium reagent[108] (see Chapter 3).

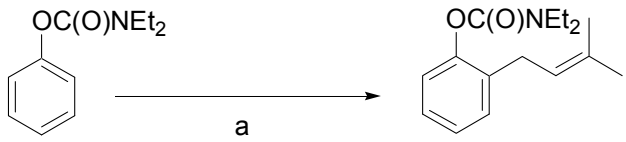
There are currently a large number of DMG's used in DoM chemistry some, which can also serve as protecting groups. These DMG/protecting groups can be divided into carbon based ($CON^{-}R$ and $CONR_2$) and heteroatom based ($OCONR_2$, OMe).[101,108,109] A suitable DMG must contain a heteroatom onto which the powerful alkyllithium base can coordinate but must also be a poor electrophilic site to prevent nucleophilic attack by strong alkyllithium bases at the DMG instead of deprotonation. Steric hinderance and charge deactivation are two other factors that also play a role in determining the effectiveness of a DMG.[102] Slocum and Jennings using a 4-OMe substituent as a reference group (Scheme 4) proposed a rough hierarchy of DMG's *i.e.* SO_2NR_2 > $SO2N^{-}R$ > $CON^{-}R$ > CH_2NR_2 > OMe > $CH_2CH_2NR_2$, NR_2, CF_3, F[102,110] based on the hypothesis by Gschwend and Rodriguez[109] that lithiation followed by prenylation tends to occur *ortho* to the strongest DMG in aromatic systems that contain two or more competing DMG's.[102,107,111,112]



Scheme 4: A rough hierarchy of DMG's using OMe as a reference group. Reagents a. Alkyllithium base, electrophile (E). [R = SO_2NR_2 > $SO2N^-R$ > CON^-R > CH_2NR_2 > OMe > $CH_2CH_2NR_2$, NR_2,CF_3, F].

Beak *et al*. utilized a tertiary amide as their reference group and suggested the relative reactive rates of various DMG's as follows: $CON^-R \geq CONR_2 > SO_2NR_2$, SO_2N^-R, CH_2NR_2, OMe, Cl.[107,111] It is important to note, however, that the relative DoM reactivity of these DMG's may change depending on solvent and/or the presence of additives such as TMEDA.[108]

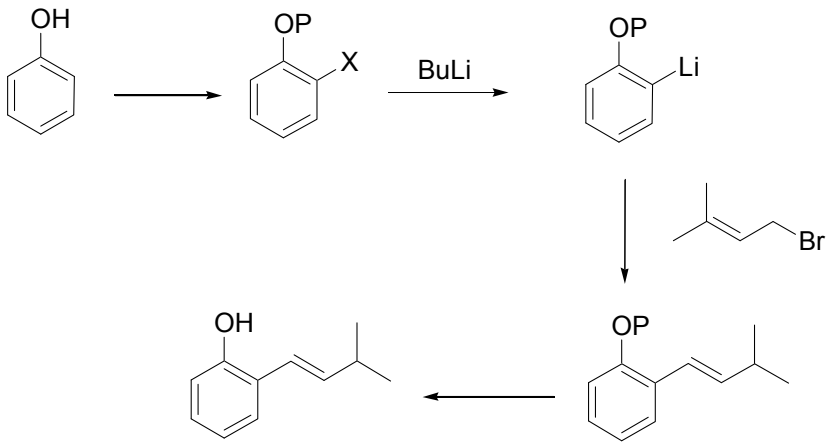
One of the greatest advantages of DoM chemistry is its regioselectivity. *Ortho*-prenylation of symmetrical phenol derivatives with one or two oxygen substituents has proven to be highly successful (Scheme 5). The disadvantages associated with DoM chemistry is that derivatives containing more than two oxygen substituents are usually difficult to deprotonate because proton transfer can and often does, take place from the neighbouring protecting group (OBn or OMOM), which in turn quenches the aryl anion.[102]



Scheme 5: An example of *ortho*-prenylation of a symmetrical phenol. Reagents a. *s*-BuLi, prenyl bromide (75 %).

2.5.2 Metal-halogen exchange (MHE)

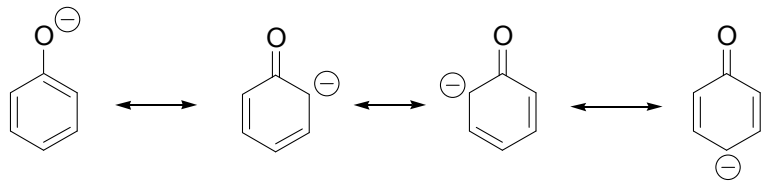
In a metal halogen exchange reaction an element *e.g.* I, Te, Sn, Br, Se, Cl, S, P, Si, Ge is exchanged with a metal *e.g.* Li using generally mild reaction conditions.[113-115] Each element has it's own advantages and disadvantages when used in the preparation of organolithium intermediates, for example iodine undergoes the fastest MHE reaction with Li and thus reduces the potential for side reactions. A significant disadvantage of the Li/I strategy is that the reaction is only successful for primary alkyl iodides and often fails for secondary and tertiary alkyl iodides.[116,117] Li/Br exchange is the best MHE reaction for vinyl and aryl bromides and thus proved suitable for our synthesis of the prenylated hydroquinones **19**-**22** (described in Chapter 3). The disadvantage is that the reaction of this particular MHE is considerably slower than the Li/I MHE reaction which accordingly sometimes allows for competing side reactions such as α and β-metalation.[108,118] Once formed the lithiated substrate is reacted with a suitable electrophile *e.g.* an alkyl halide in a nucleophilic substitution reaction as outlined in Scheme 6



Scheme 6: Synthesis of a simple *ortho*-prenylated phenyl using a MHE reaction. [P = protection group, X = halogen].

2.5.3 Phenoxide carbon-Alkylation

Phenoxide *ortho*-alkylation is one of the more useful methods for the preparation of *ortho*-prenylated compounds under mild basic conditions. This method initially requires the hydroxyl group of the phenol be deprotonated to form a resonance-stabilized phenoxide ion (Scheme 7).[102] The phenoxide anion can then undergo *ortho*-prenylation with a prenyl bromide in a nucleophilic substitution reaction. A major disadvantage of this method however is that the yields are often poor due to competing reactions that can result in prenylation at both the *para* position and the phenoxide oxygen.[119,120]

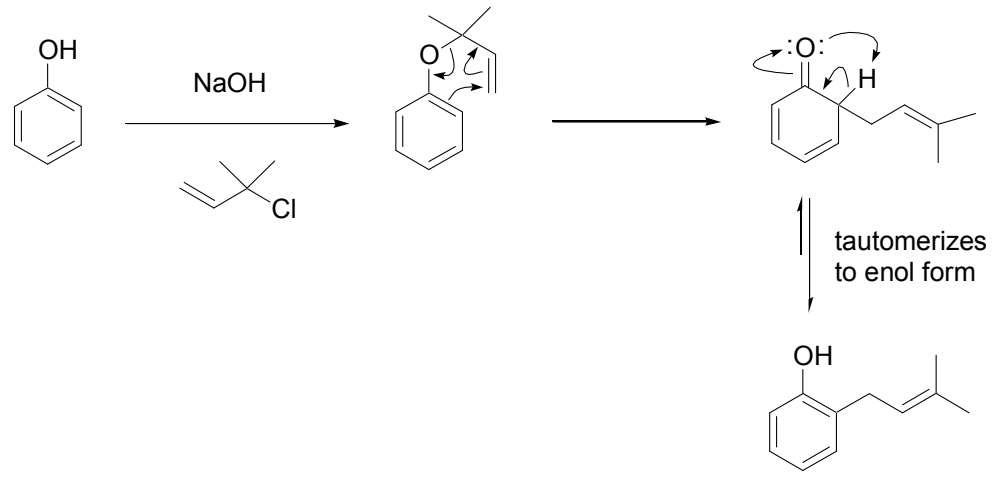


Scheme 7: Resonance stabilized phenoxide ion.[120]

Sodium phenoxide favours carbon-prenylation (*ortho* and *para*) while potassium and lithium phenoxides favour oxygen prenylation.[102,121] Solvation and the dielectric constant of solvents (the ability of a solvent to reduce electrostatic interactions), also play an important role in phenoxide carbon-alkylation. The choice of solvent is therefore essential in determining whether oxygen or carbon alkylation is dominant. Carbon-alkylation/prenylation (mixture of *ortho*/*para* products) requires solvents, which promote "selective solvation" of the phenoxide ion (the oxygen of the phenoxide is bound or at least partially bound by hydrogen-bonds from the solvent thus reducing it's accessibility to electrophiles and favouring carbon-alkylation).[109,119,120] Solvents with high dielectric constants shield the leaving group *e.g.* Br^- from the attractive force of the sodium ion (Na^+) during the transition state thus favouring oxygen prenylation, [109,119,120,122] Kornblum *et al*. proposed that oxygen prenylation is dominate for solvents with high dielectric constants, whereas, carbon prenylation is favoured for solvents with small dielectric constants *e.g.* H_2O, phenol or fluorinated alcohols.[121]

2.5.4 Claisen rearrangement

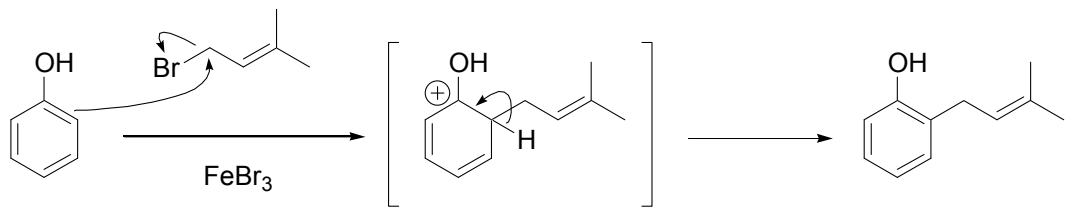
The Claisen rearrangement reaction utilizes reverse prenylation where a strong base *e.g.* NaOH, first deprotonates the hydroxyl group on the phenol to form a phenoxide ion. The phenoxide then reacts with an allyl halide in a nucleophilic substitution reaction. The resultant allyl ether subsequently undergoes rearrangement and tautomerizes to yield the *ortho*-prenylated compound (Scheme 8). Although this rearrangment is regioselective the synthetic applicability of this approach is limited by the availability of a suitable alkyl halide reagent.[101,102]



Scheme 8: The general strategy for a Claisen rearrangment.

2.5.5 Friedal-Crafts-type *ortho*-prenylations

Friedal-Crafts alkylation is an electrophilic aromatic substitution reaction (Scheme 9). The Friedal-Crafts-type reaction is not suitable for *ortho*-prenylations because of several limitations including polyalkylation and rearrangement reactions especially with activated aromatic compounds *e.g.* phenols.[122]

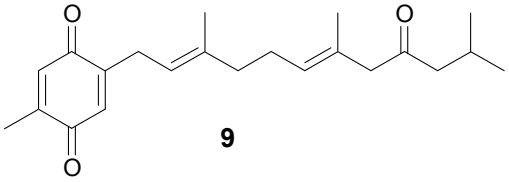


Scheme 9: General mechanism for a Friedal-Crafts type prenylation.

Chapter 3.

3 The synthesis of target compounds

As mentioned in Section 1.4, the aim of this thesis was to synthesize and test the anti-oesophageal cancer properties of simplified structural analogues **17**-**22** of a triprenylated marine hydroquinone **11** and its corresponding quinone **9.** In the process we hoped to investigate the importance of the prenylated side-chain in the cytotoxicity of these compounds against oesophageal cancer.



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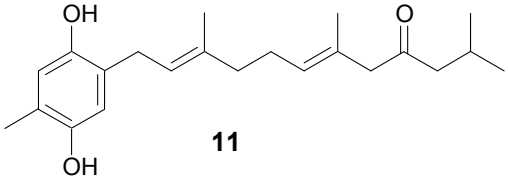

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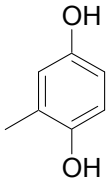

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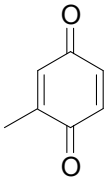

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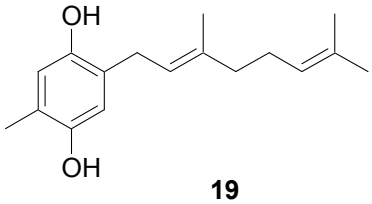

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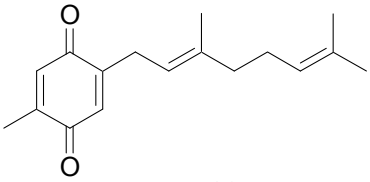

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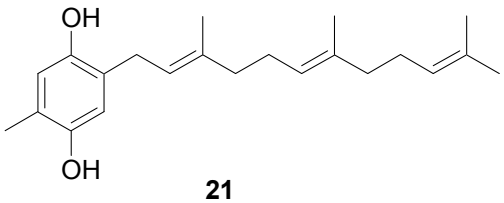

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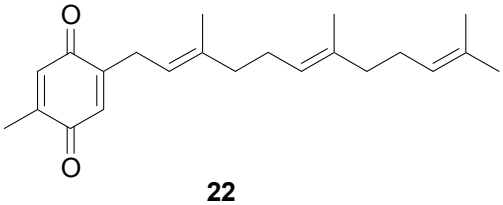


22

3.1 An overview of the synthetic approach to 19–22

Before attempting the synthesis of **19-22**, a literature review was carried out to discover whether these analogues, or compounds similar to them, had been previously synthesized or isolated from marine or terrestrial organisms. To the best of our knowledge compounds **19** and **20** have not been isolated from natural sources before, however, compound **21** and it's corresponding quinone **22** were originally isolated from a marine derived fungus *Penicillium* species,[33,34] a terrestrial fungus *Phellinus pini*[35] and three species of terrestrial plant from the genus *Seseli*[36] (Section 2.1). Bohlmann *et al*. reported a synthesize of compound **22** using a Friedal Crafts-type prenylation reaction in which two reagents, farnesol and 1,4-dihydroxytoluene were reacted together in the presence of the Lewis acid catalyst, BF_3-etherate, to yield the triprenylated hydroquinone **21**. Oxidation of **21** using activated manganese dioxide (MnO_2) followed by distillation of the oxidation products gave the target compound **22**.[36] There was, however, no mention by Bohlmann *et al*. of the regioselectivity or isolated yield of **22** from their reaction. Friedal Crafts-type syntheses are notorious for the formation of multiple products (polyalkylation), especially when this reaction is carried out with highly activated aromatic precursors such as 1,4-dihydroxytoluene.[122] In this Chapter we describe a regioselective method for synthesizing the prenylated hydroquinone derivatives **19-22** which is an improvement on Bohlmann *et al's*. non-regioselective approach. As mentioned in Section 2.5, there are currently a number of methods that have been developed for the regioselective synthesis of prenylated hydroquinones.[101] Methylhydroquinone **17** and 2-methyl-1,4-benzoquinone **18** are both commercially available. We attempted three synthetic strategies (DoM chemistry, phenoxide carbon-alkylation and MHE) to regioselectively synthesis analogues **19-22** in good yield. At the outset we anticipated that the most challenging component of our synthesis would be the coupling of a polyprenyl side-chain to an aromatic nucleus. We were not encouraged by reports of prenylated compounds only being synthesised in moderate yields (40 – 66 %).[123]

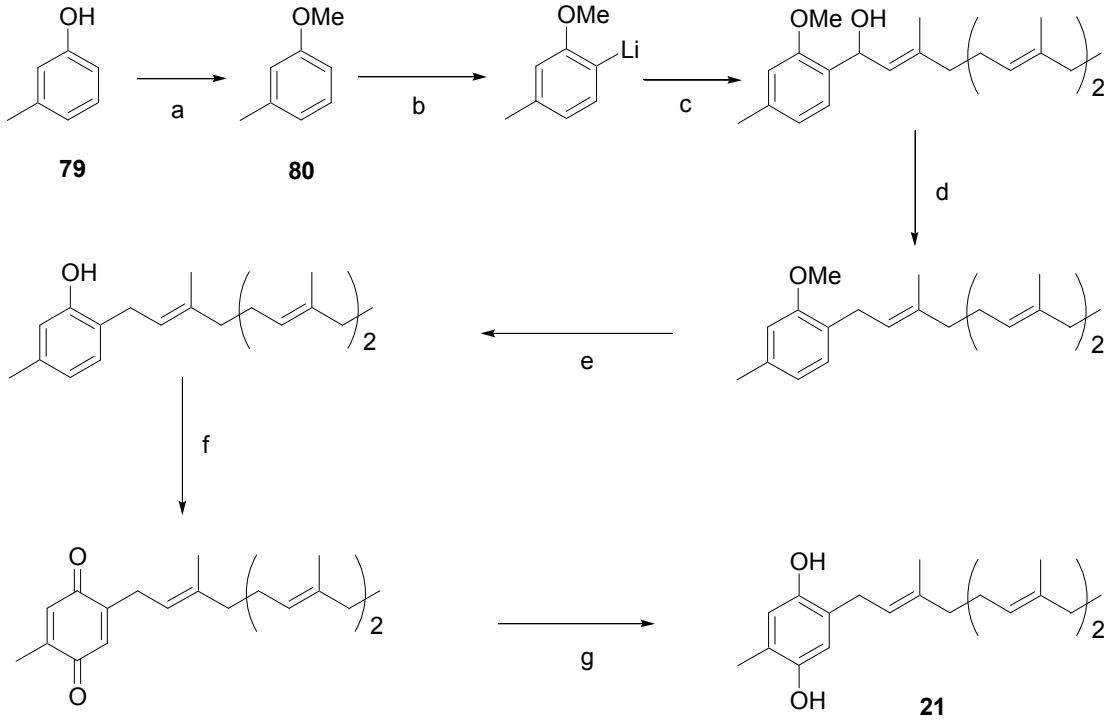
All the synthetic products described in this chapter were fully characterized where possible by a combination of NMR [^{1}H, ^{13}C, Distortionless Enhancement by Polarisation Transfer (DEPT), ^{1}H-^{1}H Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond

Correlation (HMBC)], infra red spectroscopy (IR), ultraviolet spectroscopy (UV) and mass spectrometry (MS) techniques [Electon Impact Mass Spectrometry (EIMS) and High Resolution Fast Atom Bombardment Mass Spectroscopy (HRFABMS)].

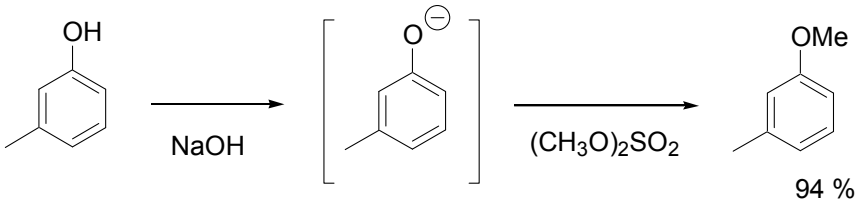
3.2 Directed *ortho*-Metalation

The metalation of *m*-methoxytoluene at the *ortho* position has been extensively studied[105,124,125] and we initially proposed that *m*-cresol (**79**) would be a suitable starting point for the synthesis of analogues **19**-**22** using DoM chemistry (Scheme 10). The first step in our DoM approach to the synthesis of **21** was the methylation of **79**. Our first attempt made use of dry potassium carbonate (K_2CO_3) to generate the phenoxide ion of **79** followed by the addition of methyl iodide (MeI). Surprisingly this standard protocol for preparing aromatic methyl ethers gave poor yields (< 20 %) of *m*-methoxytoluene (**80**) from NMR analysis. The poor yields obtained from the first procedure suggested that the use of a stronger base *i.e.* sodium hydride (NaH), might be more appropriate and the yield of **80** was accordingly improved (45 %). Not satisfied with this latter yield we explored a third approach and were able to synthesize **80** in 94 % yield using sodium hydroxide (NaOH) to form the phenoxide ion and dimethyl sulfate (Me_2SO_4) as the methylating electrophile (Scheme 11).

The next step in the proposed synthesis (Scheme 10) involved the coupling of the electrophile farnesal to **80**. Initially, benzaldehyde was used as a model electrophile to ensure that coupling could be achieved in good yield before attempting the reaction with farnesal. As mentioned in the previous chapter, organolithium reagents such as *n*-BuLi are inclined to form electron deficient aggregates in solution. In a Lewis base solvent such as diethyl ether, *n*-BuLi exists as tetrameric aggregates (Scheme 12) and in an even more basic solvent such as THF, exists as either monomeric or dimeric aggregates at low temperatures (-108 ^{o}C).[108] The smaller the aggregates formed between *n*-BuLi and the solvent the more available the electrons of the *n*-BuLi to accept a proton (*ortho* to the DMG) and thus theoretically, the more effective it would be at forming the organolithium intermediate essential for the coupling reaction. The coupling of benzaldehyde to **80** was therefore initially attempted in THF at –78 ^{o}C using *n*-BuLi (1 *eq*). Unfortunately, however, no coupling with benzaldehyde was observed in the ^{1}H NMR spectrum

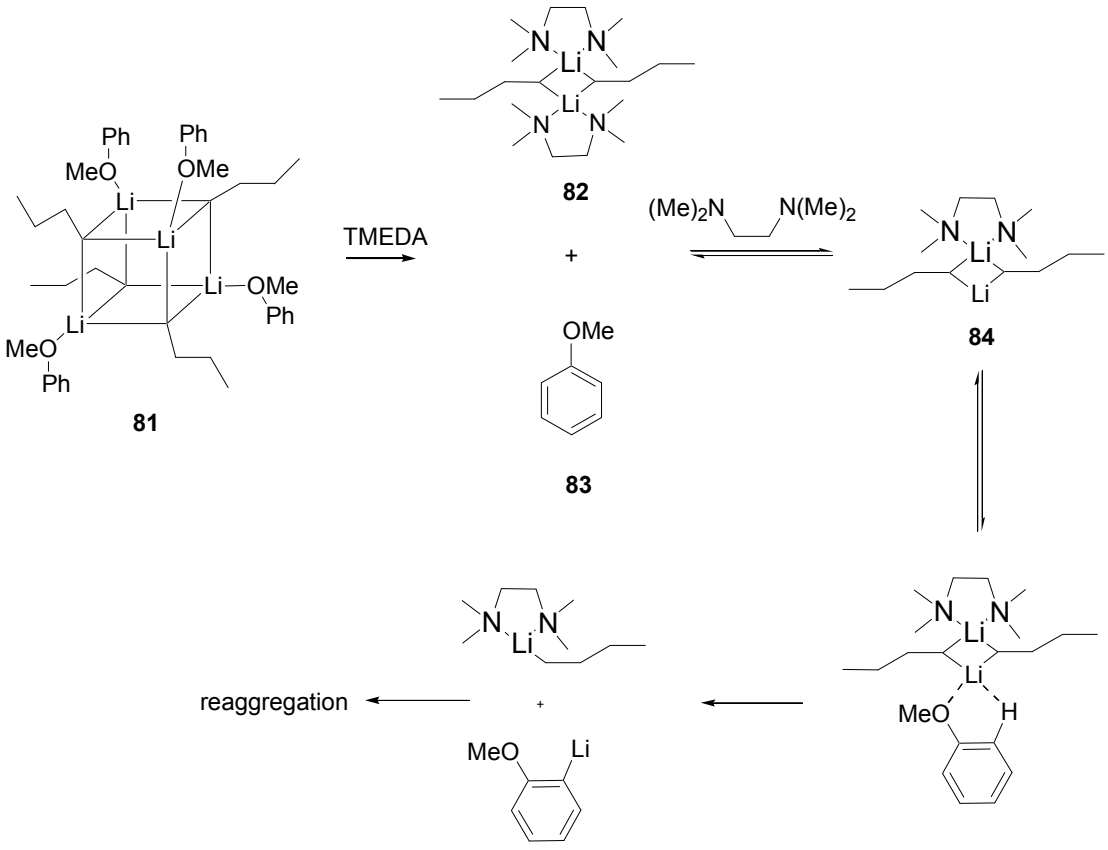


Scheme 10: A proposed synthetic approach for the synthesis of target analogue **21** using DoM chemistry to couple the polyprenylated side-chain to **80**. Reagents: a. K_2CO_3, MeI ; b. *n*-BuLi ; c. farnesal ; d. Me_3SiCl, NaI ; e. PhMeNNa, $(Me_2N)PO$; f. $(KSO_3)_2NO$; g. Zn-ZnCl-EtOH.



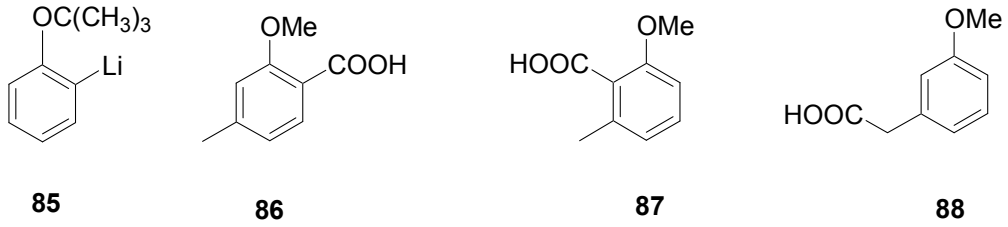
Scheme 11: Methylation of **79** with dimethyl sulfate.

Additives such as TMEDA are commonly used to break-up organolithium aggregates in solution and in so doing, increases the basicity of the alkyllithium reagent *i.e. n*-BuLi.[108] For example in toluene at –64 ^{o}C, anisole and *n*-BuLi (**81)** exist as tetrameric aggregates (Scheme 12). The addition of 1 *eq* of TMEDA results in a 1:1 ratio of *n*-BuLi-TMEDA dimer (**82**) and the free anisole (**83**). The free anisole **83** is thought to be deprotonated by a small residual concentration of **84** (Scheme 12) *via* the two free coordination sites on the Li atom. The deprotonation yields a 1:1 ratio of the *ortho*-lithiated intermediate and *n*-BuLi-TMEDA, which then re-aggregates to form **82** in solution.[102]

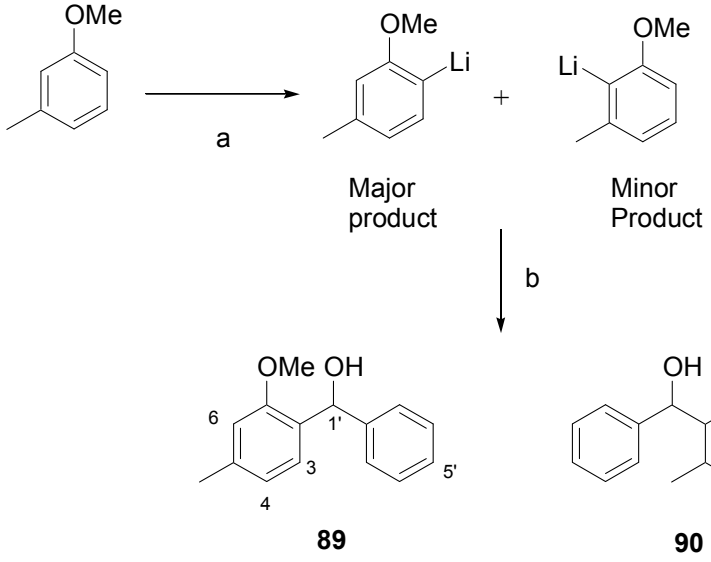


Scheme 12: The effect of the bidentate ligand TMEDA on the mechanism for the DoM reaction.[102]

Harder *et al*. prepared 1-lithio-2-tert-butoxybenzene (**85**) by reacting *n*-BuLi (1.6 M, 1 eq) with *t*-butoxybenzene in the presence of TMEDA (2 eq) using hexane as the solvent.[127] Our attempt to couple **80** and benzaldehyde using a similar protocol to that used by Harder *et al*. proved unsuccessful. Shirley *et al*. coupled carbon dioxide (CO_2) to **80** using *n*-BuLi in diethyl ether under reflux conditions and obtained three products **86**, **87** and **88** in 59-61 %, 39-41 % and 0.5 % yield respectively.[125] We were able to successfully use Shirley *et al's*. synthetic protocol to couple benzaldehyde to **80.** After work-up and purification of the reaction products using normal phase HPLC (10:1 ; hexane/EtOAc) compounds **89** and **90** were obtained in similar yields (26 and 23 % respectively. Scheme 13).



85 **86** **87** **88**



89 **90**

Scheme 13: The coupling of benzaldehyde to *m*-methoxytoluene using DoM chemistry. Reagents: a. *n*-BuLi, ether, reflux for 15 min ; b. benzaldehyde, reflux for 4 h.

HRFABMS data confirmed that **89** and **90** were isomeric with both compounds possessing a molecular formula of $C_{15}H_{16}O_2$ (obsd. for **89** *m/z* 228.1151 and **90** *m/z* 228.1150, calcd. for $C_{15}H_{16}O_2$ *m/z* 228.1150). Only thirteen of the expected fifteen ^{13}C NMR resonances were observed in the ^{13}C spectra of both **89** and **90** suggesting two pairs of overlapped resonances in each spectrum. The key to establishing the structures of these two compounds, however, lay in the examination of the aromatic region of their ^1H NMR spectra (Figure 4 and 5). The ^1H NMR spectrum of **89** clearly shows two coupled doublets (δ_H 7.09 J = 7.6 Hz and 6.75 J = 7.8 Hz) and an isolated singlet (δ_H 6.71). HMBC correlations from the C-7 methyl protons to C-4 and C-6 enabled us to unequivocally assign the shielded doublet and the singlet in the ^1H NMR spectrum of **89** as H-3 and H-6 respectively. COSY NMR data confirmed that both the aromatic proton doublets (δ_H 6.85 J = 7.60 Hz and 6.80 J = 8.27 Hz) in the ^1H NMR spectrum of **90** (Figure 5) were coupled to a double doublet at δ_H 7.20 which is the expected proton splitting pattern for a 1,2,3-trisubstituted benzene ring. HMBC data as described previously for **89** were used to unequivocally assign protons H-4 – H-6 and the other ^{13}C and ^1H NMR resonances in this compound.

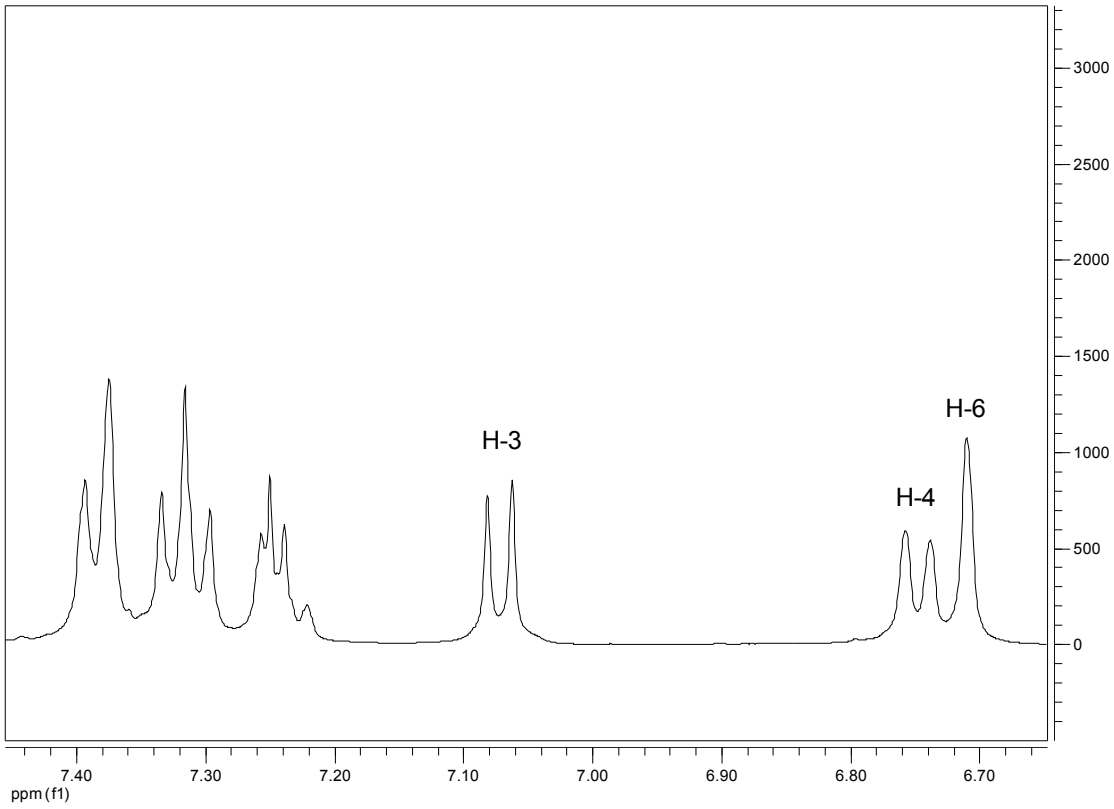


Figure 4: Expansions of the observed H-3, H-4 and H-6 peaks in the ^1H NMR ($CDCl_3$, 400 MHz) spectrum for **89**.

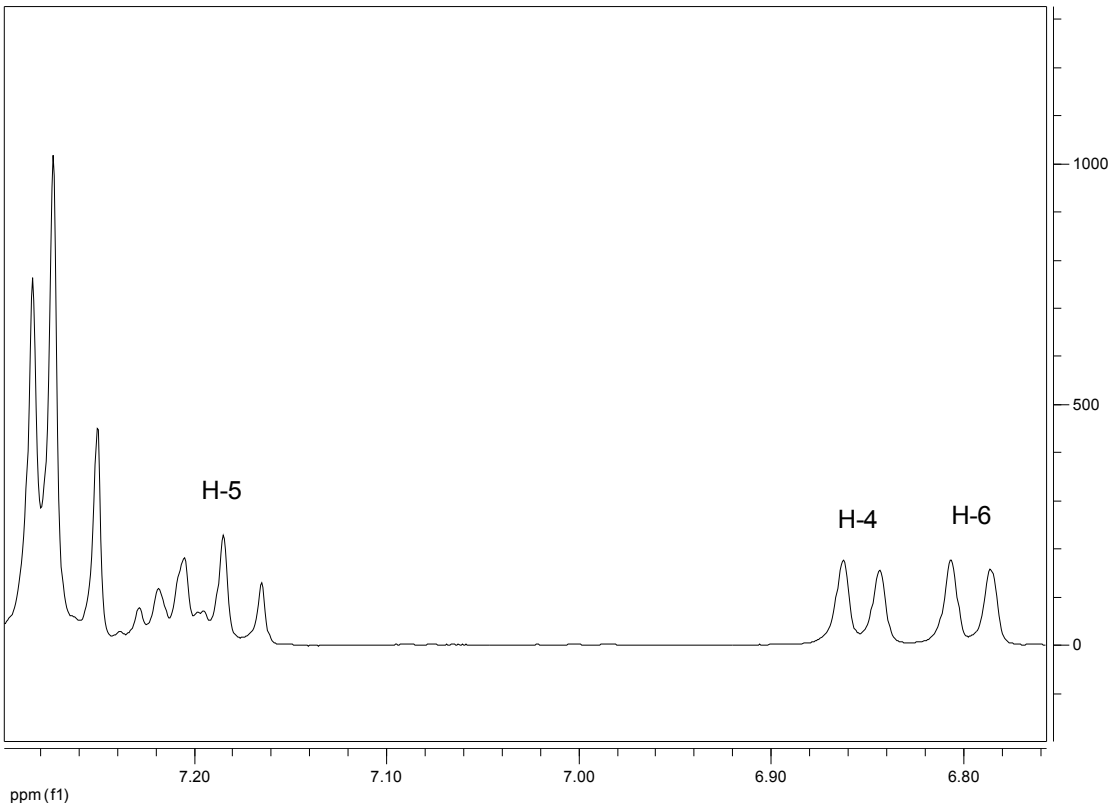
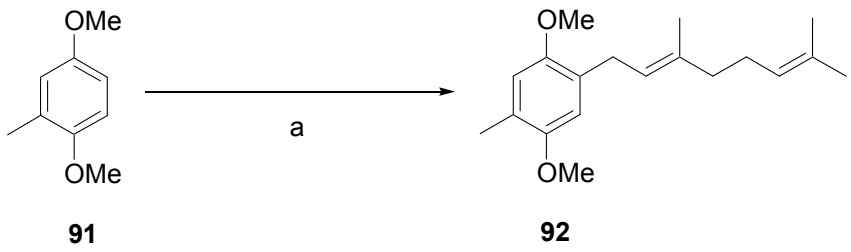


Figure 5: Expansions of the observed H-4, H-5 and H-6 peaks in the ^{1}H NMR ($CDCl_3$, 400 MHz) spectrum for **90**.

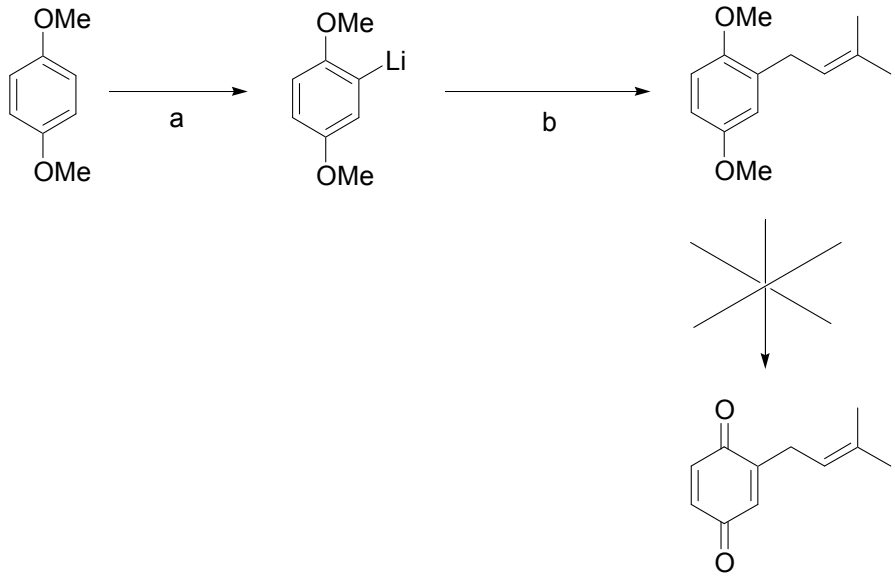
We used the same reaction conditions in an attempt to couple farnesal (prepared by the oxidation of farnesol with MnO_2) to **80** but unfortunately the reaction proved unsuccessful. Only resonance's corresponding to the two starting materials *i.e.* **80** and farnesal, could be observed in the ^{1}H NMR spectrum. The reaction was repeated with the addition of TMEDA (1 eq), without any improvement in yield and after numerous attempts, ensuring that all the reagents were effectively dry and the reactions carried out under an inert atmosphere [dry nitrogen (N_2) or argon gas (Ar)] we decided to attempt the DoM synthetic strategy with 1,4-dimethoxy-2-methylbenzene (**91**) and a different electrophile *i.e.* geranyl bromide (Scheme 14). Compound **91** is similar in structure to the aromatic precursor used by Bouzbouz and Kirschleger[128] who discovered that *ortho*-prenylation of *para*-dimethoxybenzene with geranyl bromide could be achieved in 71 % yield using *s*-BuLi (1 *eq*) in the presence of the catalyst copper (I) iodide (Scheme 15). Our attempts to couple geranyl bromide to **91,** using

the same reaction conditions regrettably were unsuccessful and once again ^{1}H NMR analysis revealed only resonances corresponding to the two starting materials geranyl bromide and **91**. Although Bouzbouz and Kirschleger[128] achieved the coupling of *para*-dimethoxybenzene with prenyl bromide, their attempts at demethylating the coupled product using numerous known techniques (no details were provided by the authors) were unsuccessful and their focus shifted from a DoM synthetic strategy to a phenoxide carbon-alkylation strategy (Scheme 16). The success of Bouzbouz and Kirschleger[128] using the phenoxide carbon-alkylation strategy to achieve effective coupling and foreseeing similar problems with the demethylation of **92,** we were encouraged to abandon the DoM synthetic strategy for a phenoxide carbon-alkylation strategy.

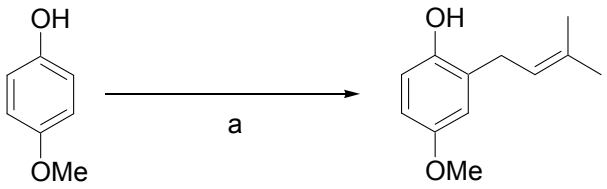


91 **92**

Scheme 14: Proposed DoM synthetic strategy to couple **91** with geranyl bromide. Reagents: a. *s*-BuLi, geranyl bromide, THF. (71 %).



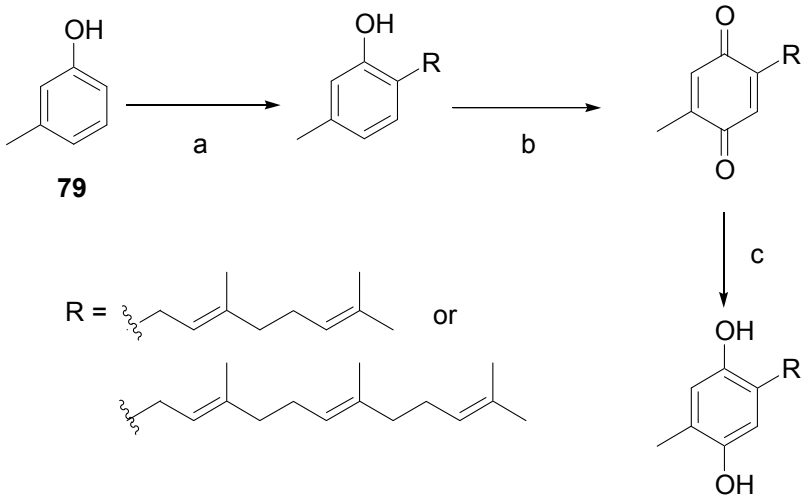
Scheme 15: *Ortho*-prenylation of *para*-dimethoxybenzene using DoM chemistry. Reagents: a. *s*-BuLi, THF, 0 ^{o}C, b. prenyl bromide, CuI (5 mol %), THF, -70 ^{o}C, (71 %).[128]



Scheme 16: Coupling *via* phenoxide carbon-alkylation. Reagents: a. Prenyl bromide, Na, ether, reflux (85 %).[128]

3.3 Phenoxide carbon-alkylation

Compound **79** was determined to be a suitable starting point having decided on phenoxide carbon-alkylation as the next synthetic strategy in an attempt to synthesize analogues **19-22.** (Scheme 17). The advantage of this synthetic strategy (Scheme 17) over the one proposed earlier (Scheme 10) is that the number of steps involved is reduced from seven to three. No DMG/protecting groups *e.g.* -OMe are required and the use of geranyl/farnesyl bromide over farnesal as an electrophile, prevents the formation of an alcohol at C-1' which would require removal later in the synthetic sequence. However, a disadvantage associated with this strategy is the number of possible products which could be produced arising from the resonance stabilized phenoxide ion formed during the coupling reaction, which allows for *ortho*, *para* and O-prenylation (Scheme 17). As discussed in Section 2.5.3 sodium phenoxide tends to favour carbon-prenylation and the choice of solvent is also essential in determining whether oxygen or carbon prenylation will prevail. Generally solvents with low dielectric constants and which promote "selective solvation" of the phenoxide ion favour carbon alkylation *e.g.* H_2O, phenol or fluorinated alcohols (see Section 2.5.3).

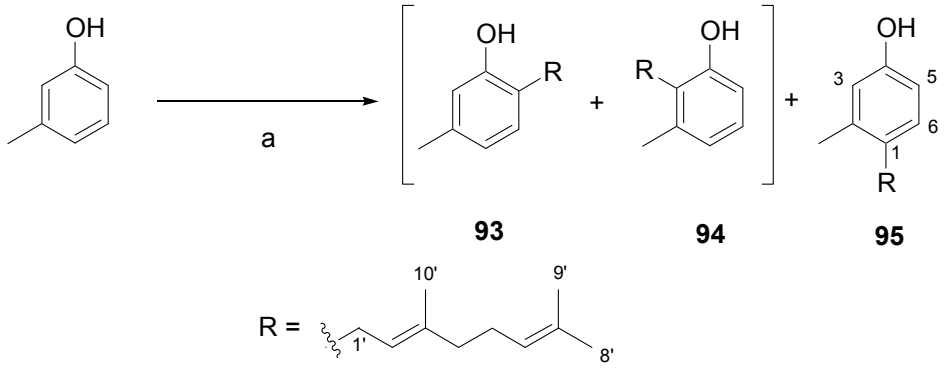


Scheme 17: The proposed strategy for the synthesis of analogues **19-22** using phenoxide carbon-prenylation. Reagents: a. geranyl bromide or farnesyl bromide, H_2O, RT ; b. $NO(SO_3Na)_2$; c. Zn-ZnCl, EtOH.

Having considered the information mentioned above, in Section 2.5.3, as well as the results of the alkylation reactions carried out on sodium phenoxide by Kornblum *et al*.[120] (Table 3) water was initially chosen as the solvent, for the phenoxide carbon-prenylation of **79**. *m*-Cresol (**79**) was accordingly dissolved in a NaOH solution and allowed to stir at room temperature (25 ^{o}C) in the presence of geranyl bromide for 48 h as described by Kornblum *et al*.[120] As expected the prenylation of **79** yield a mixture of *ortho*/*para* C-alkylation products as well as un-reacted **79**. Surprisingly, there was no evidence of any O-alkylation having occurred (Scheme 18). ^{1}H NMR analysis of the normal phase HPLC (7:1 ; Hex:EtOAc) fractions revealed a mixture of the two *ortho* isomers (**93** and **94**) which could not be separated, the *para* product (**95**) and un-reacted starting material. HRFABMS was also used to confirm the structure of **95**. (obsd. *m/z* 244.1827 calcd. for $C_{17}H_{24}O$ [M^{+}], *m/z* 244.1827). A detailed analysis of two and three bond proton carbon correlations in the HMBC spectrum (Figure 6) in combination with other 2D spectra such as HSQC and COSY confirmed the structure of the *para* product **95**. The fully assigned NMR data for **95** is presented in Table 4

Table 3: Alkylation of sodium phenoxide resulting in both oxygen and carbon (*ortho* and *para*) alkylation products.[120]

Solvent	Salt	Alkylating agent	Yield (%)	
			O-alkylation	C-alkylation
Water	Sodium phenoxide	Allyl chloride	49	41
		Allyl Bromide	51	38
		Benzyl Chloride	65	24
Phenol	Sodium phenoxide	Allyl chloride	22	78
		Allyl Bromide	23	77
		Benzyl Chloride	22	69



Scheme 18: Phenoxide carbon-prenylation of *m*-cresol. Reagents: a. NaOH, geranyl bromide, H_2O, 25 ^oC.

Table 4: ^1H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR aquired for **95**.

Carbon	δ_c ppm	δ_H ppm (int., mult., *J*/Hz)	HMBC correlation to	COSY coupling to
1	132.0			
2	137.7			
3	116.8	6.63 (1H, d, 2.1)	C-1, C-2, C-4	H-5
4	153.6			
5	112.4	6.59 (1H, dd, 8.0, 2.5)	C-1, C-3, C-4	H-3, H-6
6	129.6	6.98 (1H, d, 8.1)	C-2, C-4, C-5, C-1'	H-5
7	19.5	2.22 (3H, s)	C-1, C-2, C-3	
1'	31.2	3.22 (1H, d, 7.0)	C-1, C-6, C-2', C-3'	H-2'
2'	122.9	5.21 (1H, t, 6.9)		H-1'
3'	135.7			
4'	39.7	2.08 (2H,m)		
5'	26.6	2.08 (2H, m)		
6'	124.3	5.09 (1H, t, 6.6)	C-8', C-9'	H-5'
7'	131.4			
8'	25.7	1.67 (3H, s)	C-6', C-7', C-9'	
9'	17.7	1.59 (3H, s)	C-6', C-7', C-8'	
10'	16.0	1.69 (3H, s)	C-2', C-3', C-4'	

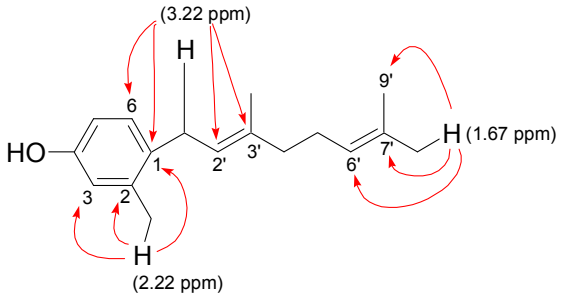
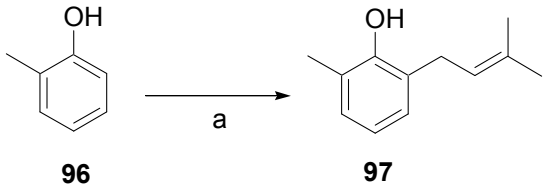
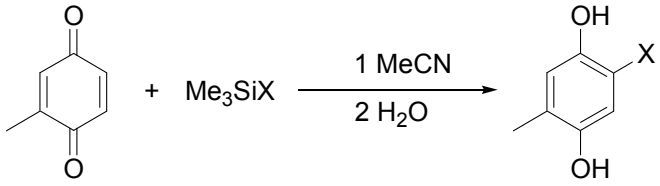


Figure 6: Selected HMBC correlations used to position the prenyl side-chain at C-4 in **95**.

According to the results obtained by Kornblum *et al.*[120] in Table 3 the use of the starting material (phenol) as a solvent during the phenoxide carbon-alkylation reaction increases the yield of C-alkylation products (*ortho*/*para* mixture) by approximately 39 % compared to when water was used as a solvent. We were unaware at the time of a procedure reported by Yamada *et al.*[129] in which *o*-cresol (**96**) was reacted with metallic sodium in ether followed by the addition of 3-methyl-2-butenyl chloride to afford 2-methyl-6-(3-methyl-2-butenyl)phenol (**97**) in a 76 % yield (Scheme 19). However, given the mixture of isomers **93** and **94** obtained in our reaction with *m*-cresol (Scheme 18) we anticipated a similar scenario if we had repeated this reaction with *m*-cresol. Before repeating the prenylation reaction using the starting material **79** as the solvent, a paper by Miller and Stewart[130] involving the conversion of 1,4-benzoquinones to halogenated hydroquinones came to our attention (Scheme 20). With a viable route to a suitable halogenated precursor available, metal halogen exchange immediately became a plausible alternative synthetic approach for the regioselective synthesis of our target analogues (**19-22**) and we discontinued our phenoxide carbon-alkylation strategy.



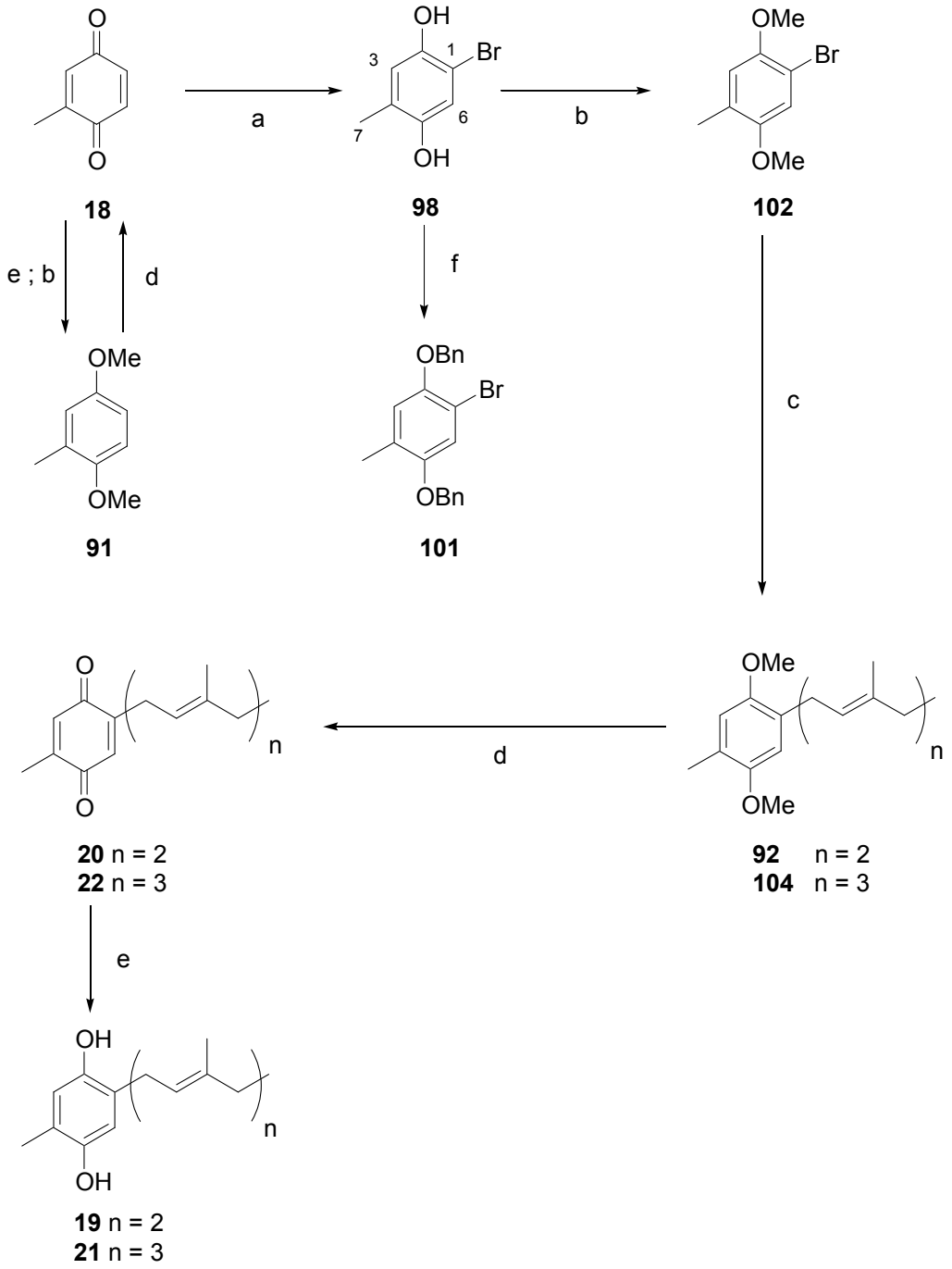
Scheme 19: Yamada *et al.'s* synthesis of **97** using phenoxide carbon-prenylation strategy. Reagents: a. Na metal, 3-methyl-2-butenyl chloride, ether (76%).[129]



Scheme 20: Conversion of 1,4-benzoquinones to halogenated hydroquinones (X = halogen)

3.4 Metal Halogen exchange

Encouraged by the work of Miller and Stewart[130] mentioned above and our laboratory's previous success in synthesizing the marine sponge metabolite tsitsikammafuran using MHE,[131] a new synthetic approach was formulated with 2-methyl-1,4-benzoquinone (**18**) as a suitable starting material (Scheme 21). The conversion of **18** to the corresponding halogenated hydroquinone **98** was achieved using trimethylsilyl bromide (Me_3SiBr) in the presence of the catalyst tetraethylammonium fluoroborate (NEt_4BF_4) followed by a hydrolytic work up. The reaction proceeded smoothly and the reaction products were purified using normal phase column chromatography (5:1 ; Hexane:EtOAc) to give **98** as a white amorphous solid in 61 % yield. The molecular mass of **98** was confirmed from the EIMS spectrum where two molecular ion peaks (*m/z* 202 and 204) were observed. The 1:1 ratio of the molecular ion peaks *m/z* 202 and 204 clearly indicated the presence of one bromine atom in this compound. The ^1H NMR spectrum of **98** revealed three sharp singlets (δ_H 6.87, 6.69 and 2.12) and one broad exchangeable proton signal (δ_H 4.85). These resonances were assigned to the two aromatic methines H-3 and H-6, the methyl moiety (H_3-7) and the two phenolic protons respectively. All seven expected resonances were evident in the ^{13}C NMR spectrum of **98** (Figure 7) while a broad absorbance at 3212 cm^{-1} in the IR spectrum of this compound provided further evidence for the two phenolic functional groups in this compound. It is important to note that the reaction does not proceed without the presence of the NEt_4BF_4. However, the mechanism for this reaction has not yet been established and the role played by the fluoroborate is not completely clear.[130]



Scheme 21: The proposed strategy for the synthesis of analogues **19**-**22** using MHE. Reagents: a. Me_3SiBr, NEt_4BF_4, MeCN, 25 ^oC (61 %) ; b. NaOH, Me_2SO_4, reflux (70 %) ; c. BuLi, geranyl bromide [n = 2] or farnesyl Bromide [n = 3] ; d. AgO, dioxane, HNO_3, 25 ^oC (90 %) ; e. $Na_2S_2O_4$ 1:3 DCM/Et_2O, 25 ^oC (90 %) ; f. $PhCH_2Br$, K_2CO_3, NaI, Ac_2O, reflux (80 %).

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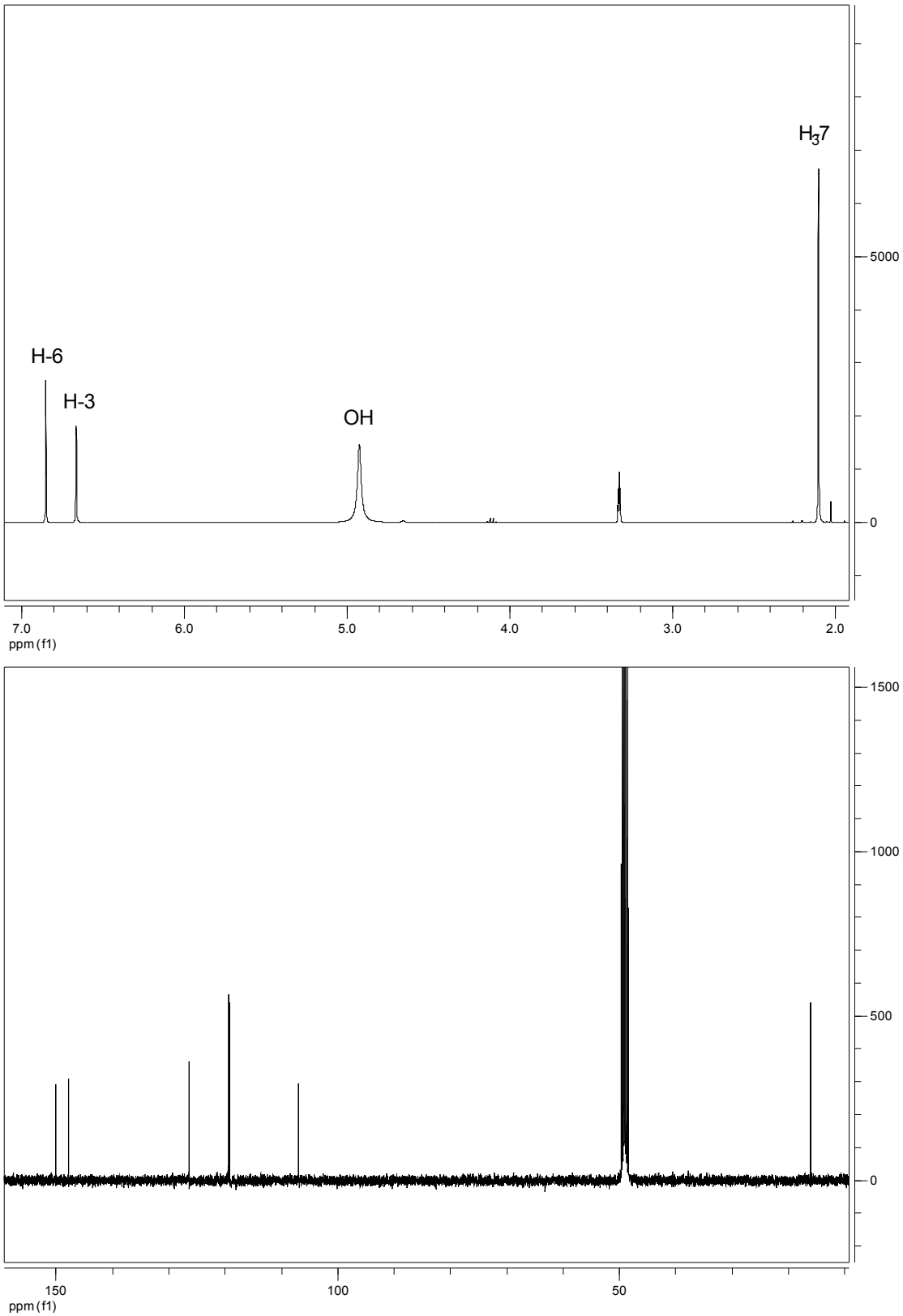
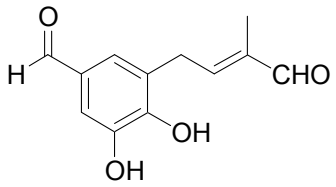


Figure 7: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **98**.

The next step in our synthesis of analogues **19-22** involved the protection of the hydroxyl groups in **98,** in preparation for the MHE coupling of the organolithium intermediate, derived from **98**, with a polyprenyl electrophile *e.g.* geranyl bromide and farnesyl bromide. Care was taken to select a protection group that would survive the initial strong basic conditions required for MHE and which could be removed (deprotection of the hydroxyl groups) without compromising the unsaturated polyprenyl side-chain. Ester protecting groups are often deprotonated by BuLi and silyl ether protecting groups are capable of undergoing retro-Brook rearrangement,[132,133] therefore, these protection groups were rejected in favour of aryloxy ether protection *e.g.* OBn or OMe. Odejinmi and Wiemer[133] recently reported the potential of benzyl ethers as suitable phenol protection groups in the synthesis of two prenylated aromatic compounds, montadial (**99**) and the *E,E*-isomer of piperoic acid (**100**). An attractive feature of the benzyl protection was that the olefins in the farnesyl side-chain of **100** remained unaffected by the mild conditions used for deprotection *viz* heating in the presence of sodium metal (Scheme 22). Unfortunately, our attempts to couple the benzyl protected precursor **101** with geranyl bromide using the MHE reaction conditions reported by Odejinmi and Wiemer[133] were unsuccessful.



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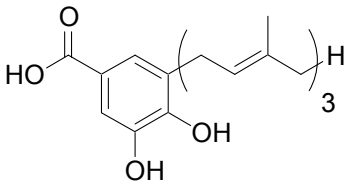

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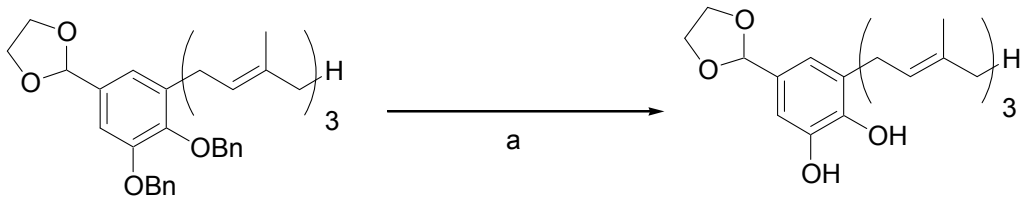
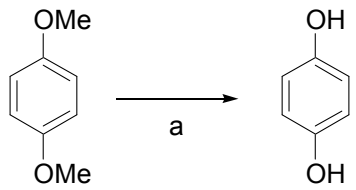


Scheme 22: Benzyl deprotection. Reagents: a. Na/*s*-BuOH, H^+, H_2O.

After this initial drawback we decided to protect the hydroxyl functionalities of **98** as methyl ethers bearing in mind that this protection group is notoriously difficult to remove and that the polyprenylated side-chain would more than likely not survive the harsh Lewis acid conditions commonly required for effective deprotection. However, our proposed use of the -OMe protecting group was encouraged by the discovery of a number of deprotection protocols making use of milder conditions *e.g.* *L*-Selectride,[134,135] trimethylsilyliodide,[136,137] as well as an intriguing "phase vanishing" reaction using perfluorohexane as a phase screen and boron tribromide.[138] An additional option was oxidative demethylation using argentic oxide (AgO).[139]

Before methylating **98** a number of demethylation studies were carried out on **91**, and *L*-Selectride was the first dealkylating agent tested following the protocol reported by Majetich *et al.*[135] who successfully used *L*-Selectride to demethylate a methylated hydroquinone in 71 % yield (Scheme 23). Unfortunately, our attempts to demethylate **91** were unsuccessful, the two ^{1}H NMR resonance's at δ_H 3.82 and 3.77 corresponding to the two OMe protecting groups could be seen clearly and each integrated perfectly for three protons with respect to the resonances at δ_H 6.74, 6.73 and 2.17 corresponding to H-3, H-6 and H-7 of **91**. The second dealkylating agent investigated was Me_3SiI following the protocol reported by Jung and Lyster[137] and only resulted in partial demethylation of **91** according to our ^{1}H NMR integration analysis of the pertinent peaks. Regrettably the time delays associated with importing boron tribromide by sea freight prevented us from attempting Ryn *et al.'s*[138] "phase vanishing" deprotection strategy. Our final option was therefore oxidative demethylation with AgO

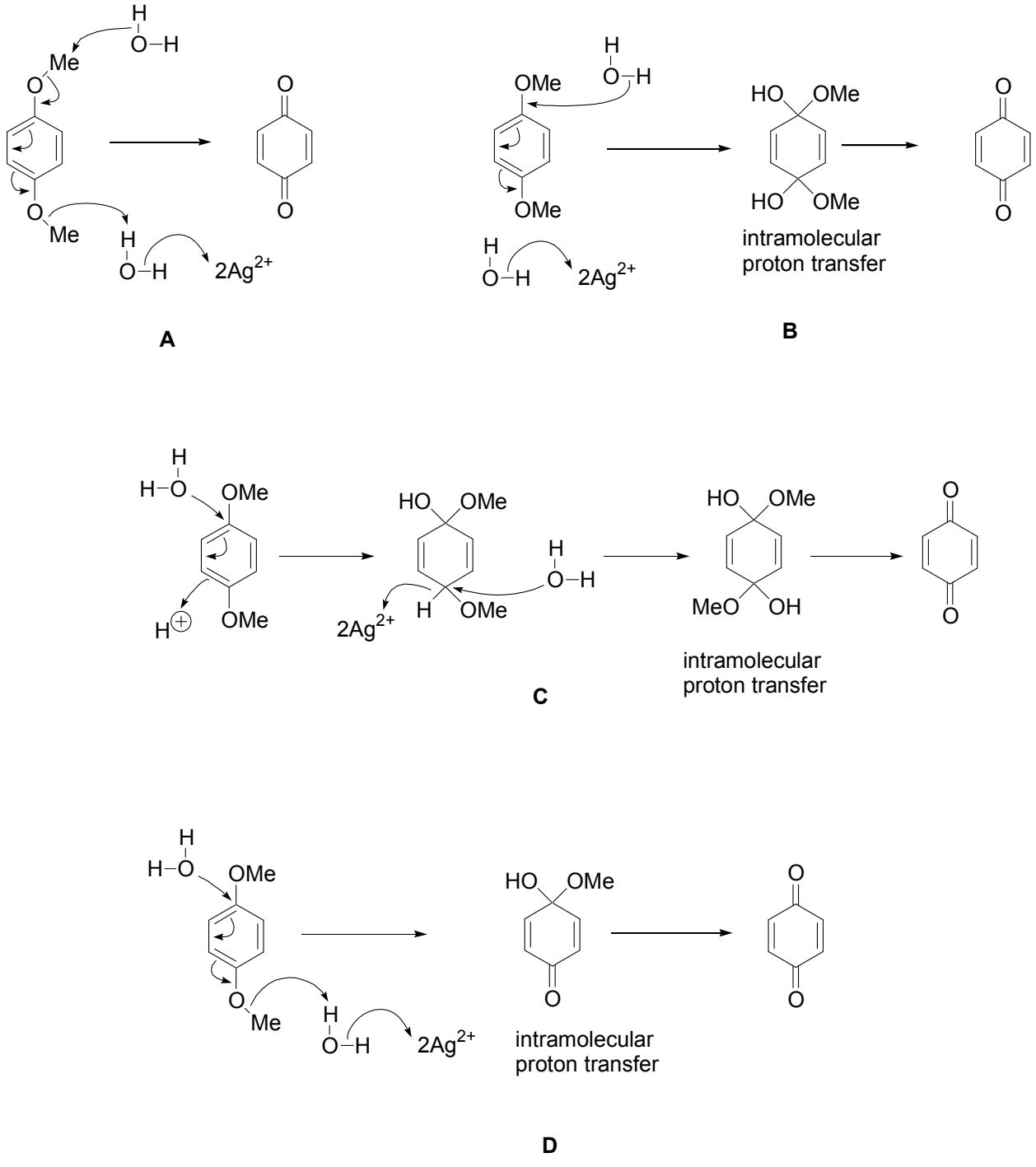


Scheme 23: Demethylation of hydroquinone using *L*-Selectride. Reagents: a. *L*-Selectride (3 eq), 67 ^{o}C, 48 h. (71 %).

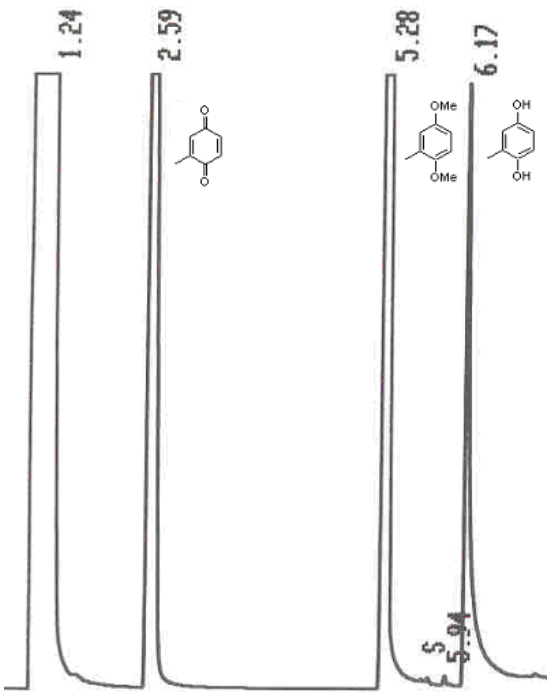
The oxidative demethylation of **91**, a suitable standard compound, using AgO was monitored using gas liquid chromatography (GLC). The AgO was prepared, prior to use, by adding an aqueous slurry of potassium peroxydisulfate followed by silver(I) nitrate to a NaOH solution maintained at approximately 85 ^{o}C. The temperature was then raised to 90 ^{o}C and stirred for 15 min during which time AgO precipited out of solution. The AgO was filtered, washed with a slightly alkaline solution (NaOH) and air dried overnight.[140] Some important features to note about the AgO mediated demethylation reaction, is that the reaction does not proceed unless a mineral acid such as HNO_3 is present. The oxidizing agent (AgO) must commonly be present in four fold molar excess if quantitative demethylation is required. The mineral acid is also normally used in molar excess and should contain a sufficient amount of water to prevent silver salt precipitation. The silver salt precipitates may impede the oxidative potential of AgO because of their tendency to form layers around un-reacted AgO. Acids such as HNO_3, which form water-soluble silver salts, are thus commonly used for the oxidative demethylation of 1,4-dimethoxy aromatic compounds using AgO. Another concern regarding oxidative demethylation using AgO was the highly acidic medium [HNO_3 (6M)] in which these reactions are carried out. However, because of the speed at which the oxidation reaction takes place (usually < 2 min) we hypothesized that the polyprenyl side-chain of the target analogues **19**-**22** would not be adversely affected.[139] Snyder and Rapoport.[139] proposed four possible mechanisms for carbon-oxygen bond cleavage (Scheme 24) during the oxidative demethylation of 1,4-dimethoxy aromatic compounds with AgO, each one resulting in the same reaction products, namely, quinone, methanol and silver ions.

An aliquot of the reaction mixture (oxidative demethylation of **91**) and three standards [2-methyl-1,4-benzoquinone (**18**), 1,4-dimethoxy-2-methylbenzene (**91**) and 2-methyl-benzene-1,4 diol (**17**)] were compared using a non-polar GLC column (Duraband DB-1) in order to determine the degree of demethylation from the AgO reaction. The retention times (R_T) of the three standards on a non-polar DB-1 column were found to be 2.59, 5.28 and 6.17 min respectively (Figure 8a). The aliquot of the reaction mixture revealed two peaks *i.e.* the major peak had a retention time of 2.49 min implying that **18** was the major product with a yield of 90 % and the minor peak

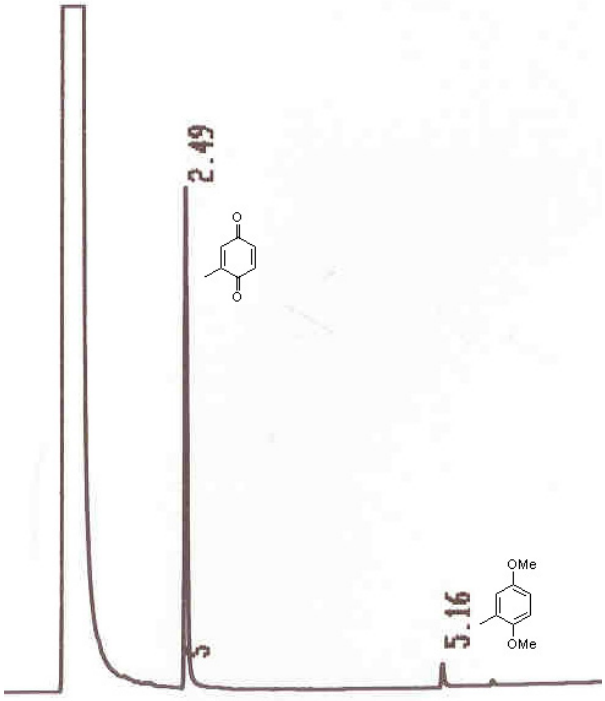
had a retention time of 5.16 min corresponding to un-reacted starting material **91** as shown in Figure 8.



Scheme 24: Four possible mechanisms (A-D) carbon-oxygen bond cleavage during the oxidative demethylation of 1,4-dimethoxy nuclei with AgO.



a

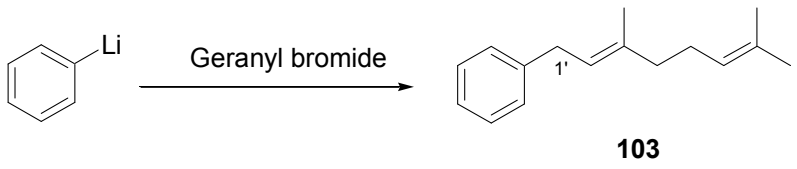


b

Figure 8: GLC chromatographs of a. Standards, **18** (R_T = 2.59 min), **91** (R_T = 5.28 min), **17** (R_T = 6.17 min) ; b. oxidative demethylation of **91** using AgO. (GLC conditions: Duraband DB-1 column, initial temperature 100 ^oC, temperature ramp 15.0 ^oC/min, final temperature 200 ^oC, FID detector)

The halogenated hydroquinone (**98**) was methylated with Me_2SO_4 as described earlier to give 1-bromo-2,5-dimethoxy-4-methylbenzene (**102)** in 68 % yield.[126] The MHE coupling of **102** with geranyl bromide was exhaustively attempted varying the type of alkylithium base (*n* and *t*-BuLi), solvent (THF, diethyl ether), solvent additives (TMEDA, CuI) and temperature (-78, -55 ^oC), frustratingly, without any success. Interestingly, Odejinmi and Wiemer[133] experienced similar problems with their MHE coupling reaction while trying to synthesis the natural products montadial A **99** and piperoic acid **100**. It was only after changing their solvent system from THF to a mixture of benzene and diethyl ether and using the additive copper bromide dimethyl sulfide complex (CuBr·DMS) that effective coupling was achieved.

In order to establish the appropriate reaction conditions for the coupling of an organolithium intermediate with an electrophile, we performed trial reactions in an attempt to couple phenyllithium (a model organolithium intermediate) to geranyl bromide (Scheme 25) using a variety of reaction conditions outlined in Table 5. ^1H NMR spectroscopic analysis confirmed the coupling of phenyllithium to geranyl bromide at 0 ^oC in diethyl ether in the presence of TMEDA. The up-field, shift of the methylene doublet (2H, d, H-1') from δ_H 4.00 for the starting material geranyl bromide to δ_H 3.35 is diagnostic of successful coupling (Figure 9). ^1H NMR spectroscopic integration as well as HRFABMS confirms the structure of **103** (obsd. *m/z* 214.1721 calcd. for $C_{16}H_{22}$ [M^+], *m/z* 214.1724).



103

Scheme 25: Coupling of phenyllithium (model organolithium intermediate) to geranyl bromide.

Table 5: Reaction conditions used for the coupling of phenyllithium to geranyl bromide.

Organolithium	Electrophile	Solvent and Additives	Temperature (^{o}C)	Evidence of coupling
phenyllithium	Geranyl bromide	THF	-78	No
		Diethyl Ether (TMEDA)	0	Yes
		Diethyl ether (TMEDA)	25	No

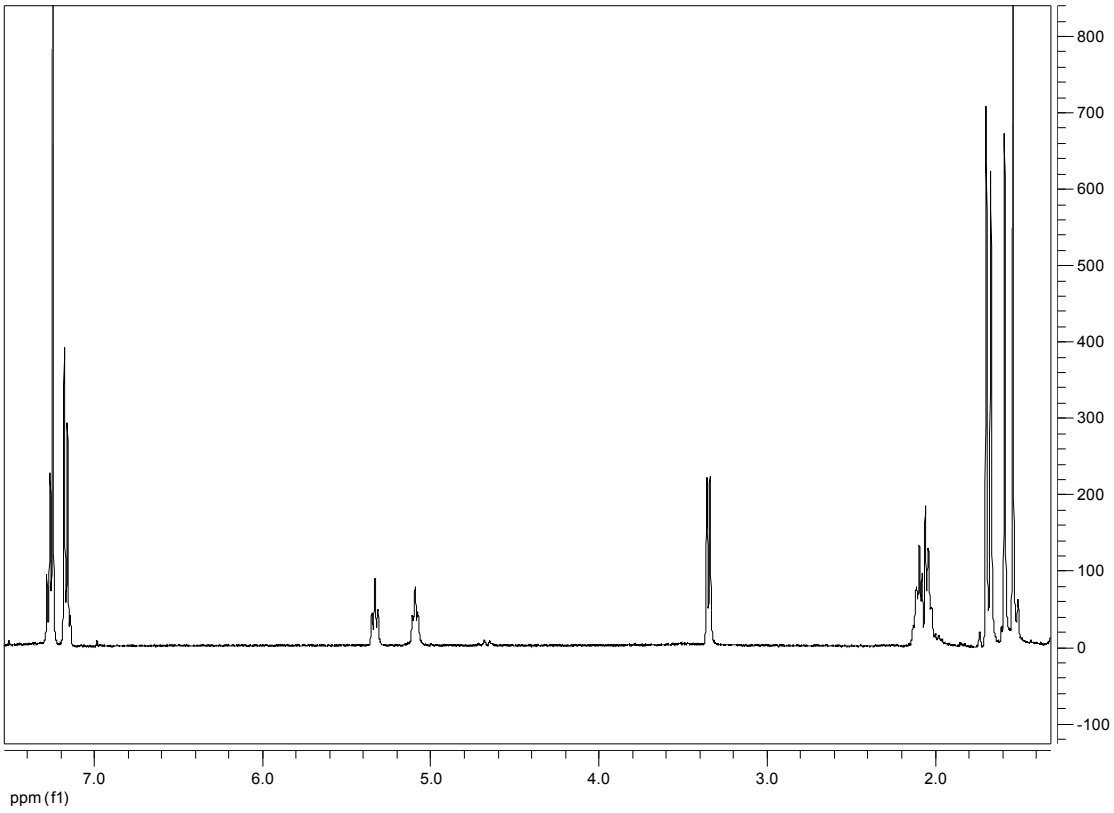


Figure 9: ^{1}H ($CDCl_3$, 400 MHz) NMR spectrum acquired for **103.**

Encouraged by the success of the coupling between phenyllithium and geranyl bromide, the MHE coupling between **102** and geranyl bromide was repeated using *n*-BuLi and TMEDA in diethyl ether at 0 ^{o}C. The reaction was quenched with saturated aqueous ammonium chloride (NH_4Cl) and the product extracted in the usual manner. Normal phase HPLC (10:1 ; hexane:EtOAc) of the reaction products gave pure **92** in 40 % yield. The structure of **92** was elucidated using NMR spectroscopic data (Table 6 and Figure 10 and 11) The doublet at approximately δ_H 4.00, representing the methylene protons on C-1' for the starting material, geranyl bromide, shifted up-field to δ_H 3.30 in the ^{1}H NMR spectrum which was the first indication that coupling had been successfully achieved. Integration on the ^{1}H NMR spectrum, the number of carbons observed in the ^{13}C NMR spectrum and an in depth analysis of 2D NMR (COSY, HSQC, HMBC) confirmed that coupling between **102** and geranyl bromide had been successful. HRFABMS confirmed the structure of **92** (obsd. *m/z* 288.2090 calcd. for $C_{19}H_{28}O_2$ [M^+], *m/z* 288.2089). Compound **104** was prepared using the same reaction procedure in comparable yield and its structure confirmed with NMR spectroscopy in the usual manner (Table 7 and Figures 12 and 13) and HRFABMS confirmed the structure of **104** (obsd. *m/z* 356.2714 calcd. for $C_{24}H_{36}O_2$ [M^+], *m/z* 356.2715).

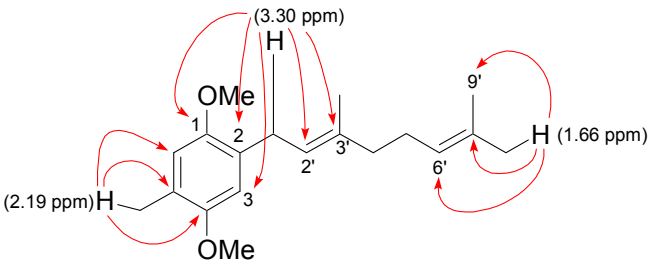


Figure 10: Selected HMBC correlations used to position the prenyl side-chain at C-2 in **92**.

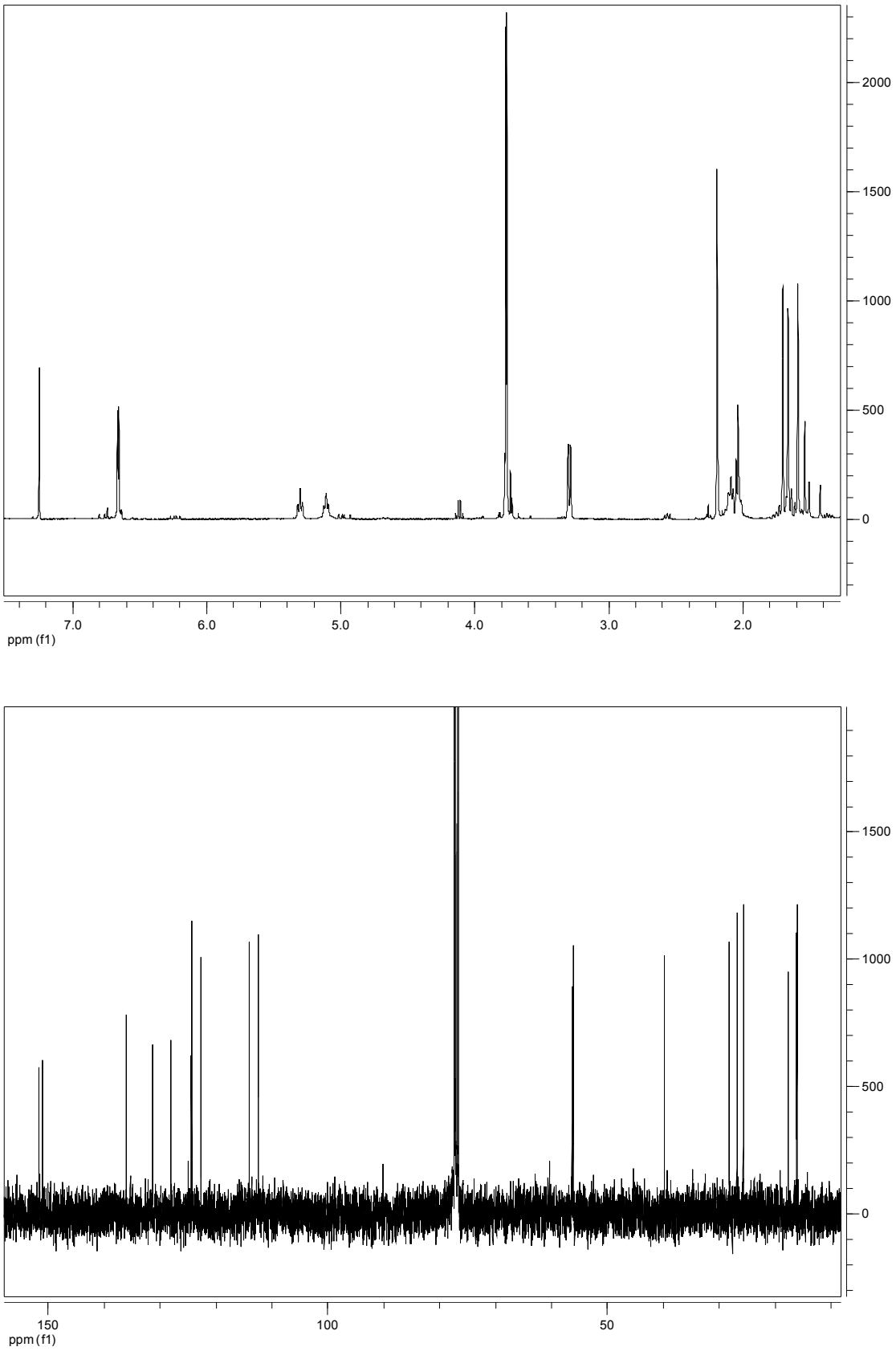


Figure 11: ^{1}H ($CDCl_3$, 400 Hz) and ^{13}C ($CDCl_3$, 100 Hz) NMR spectra acquired for **92**.

Table 6: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **92**.

Carbon	δ_c ppm	δ_H ppm (int., mult., *J*/Hz)	HMBC correlation to	COSY coupling to
1	151.0			
2	128.0			
3	112.4	6.66 (1H, s)	C-4, C-5, C-1'	
4	151.6			
5	124.3			
6	114.1	6.67 (1H, s)	C-1, C-2, C-7	
7	16.1	2.19 (3H, s)	C-4, C-5, C-6	
1'	28.2	3.30 (2H, d, 7.23)	C-1, C-2, C-3, C-2', C-3'	H-2'
2'	122.7	5.30 (1H, t, 7.25)	C-1', C-4', C-10'	H-1'
3'	136.1			
4'	39.8	2.10 (2H, m)		H-5'
5'	26.8	2.10 (2H, m)		H-4', H-6'
6'	124.4	5.11 (1H, t, 6.58)		H-5'
7'	131.3			
8'	25.7	1.66 (3H, s)	C-6', C-7', C-9'	
9'	17.7	1.59 (3H, s)	C-6', C-7', C-8'	
10'	16.1	1.70 (3H, s)	C-2', C-3', C-4'	
	56.1	3.76 (3H, s, OMe)		
	56.3	3.77 (3H, s, OMe)		

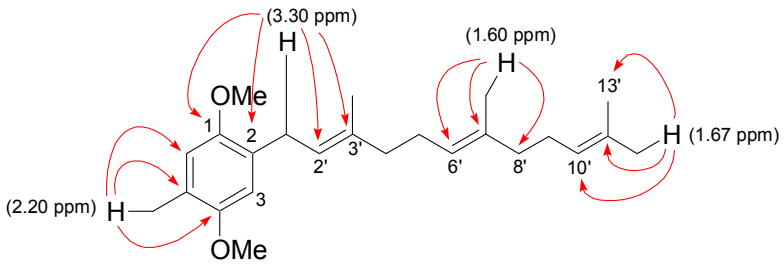


Figure 12: Selected HMBC correlations to position the prenyl side-chain at C-2 in **104**.

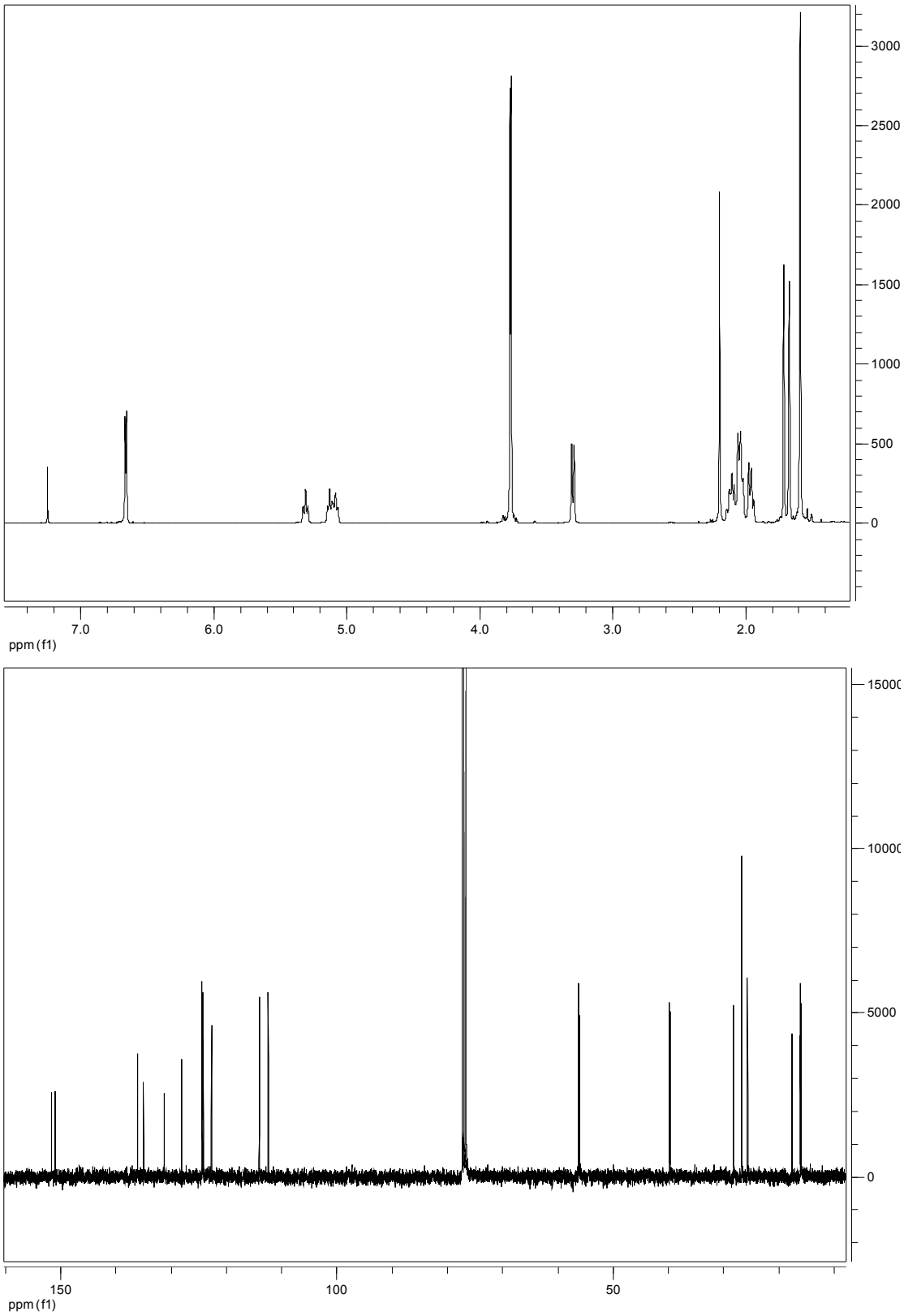


Figure 13: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **104**.

Table 7: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **104**.

Carbon	δ_c ppm	δ_H ppm (int., mult., *J*/Hz)	HMBC correlation to	COSY coupling to
1	151.0			
2	128.0			
3	112.4	6.66 (1H, s)	C-1', C-4, C-5	
4	151.6			
5	124.2			
6	114.1	6.67 (1H, s)	C-1, C-2, C-7	
7	16.0	2.20 (3H, s)	C-4, C-5, C-6	
1'	28.2	3.30 (2H, d, 7.21)	C-1, C-2, C-2', C-3'	H-2'
2'	122.7	5.31 (1H, t, 7.23)	C-1', C-4', C-15'	H-1'
3'	136.1			
4'	39.8	2.05 (2H, m)		
5' ; 9'	26.8	2.05 (2 x 2H, m)		
6'	124.4	5.13 (1H, t, 7.0)		H-5', H-9'
7'	135.0			
8'	39.7	2.05 (2H, m)		
10'	124.4	5.09 (1H, t, 7.4)		H-5', H-9'
11'	131.2			
12'	25.7	1.67 (3H, s)	C-10', C-11', C-13'	
13'	17.7	1.60 (3H, s)	C-9', C-10', C-11', C-12'	
14'	16.1	1.60 (3H, s)	C-6', C-7', C-8'	
15'	16.1	1.71 (3H, s)	C-2', C-3', C-4'	
	56.1	3.76 (3H, OMe)		
	56.3	3.77 (3H, OMe)		

Having successfully achieved coupling between **102** and both geranyl bromide and farnesyl bromide to prepare **92** and **104** respectively in moderate yield, the demethylation of both compounds proceeded smoothly using AgO giving the target analogues **20** and **22** in good yield (approximately 90 %). The structures for both compounds were once again confirmed using NMR. The characteristic resonances at δ_H 3.77 and 3.76 for the OMe protecting groups in the ^{1}H NMR spectrum observed in both **92** and **104** disappeared which was the first indication that the demethylation reactions were highly successful. The resonance's present at δ_C 188.3 and 187.8 in the ^{13}C NMR spectrum are characteristic of carbonyl carbons, the only functional group observed at the extreme low field of the ^{13}C NMR spectrum (δ_C 170 – 210). IR spectroscopy also confirmed the presence of the carbonyl carbon atoms in both **20** and **22**. Normally carbonyl functional groups exhibit intense absorption bands between 1670 - 1780 cm^{-1}. The IR spectrum for **20** and **22** showed strong absorption bands at 1657 and 1658 cm^{-1} respectively, thus confirming the presence of the carbonyl groups and the premise that the demethylation of **92** and **104** was successful. The AgO mediated oxidative demethylation used for the preparation of **20** and **22** showed no evidence of cyclization, oxidation, isomerization or degredation of the polyprenyl side-chains from NMR (1D and 2D NMR were used to elucidate the structures of both compounds) and HRFABMS analysis confirmed the molecular formula for **20** (obsd. *m/z* 259.1698 calcd. for $C_{17}H_{23}O_2$ [M$^+$], *m/z* 259.1697) and **22** (obsd. *m/z* 327.2323 calcd. for $C_{22}H_{31}O_2$ [M$^+$], *m/z* 327.2324). The reduction of quinones **20** and **22** with sodium dithionite gave **19** and **21** in quantitative yield and again the structures were confirmed with NMR. This time the characteristic carbonyl peaks observed at δ_C 188.3 and 187.8 in the ^{13}C NMR spectrum for **20** and **22** shifted far up-field to δ_C 147.8 and 147.4 which was diagnostic for the reduction of the quinones **20** and **22** to hydroquinones **19** and **21**. Further evidence suggesting the success of the reduction reactions, was provided by the strong broad band observed at approximately 3394 cm^{-1} in the IR spectrum for both compounds **19** and **21** which is characteristic of OH functional groups. 1D and 2D NMR were used in the usual manner to elucidate the structures of compounds **19** (Table 8 and Figure 14) and **21** (Table 9 and Figure 15). HRFABMS confirmed the molecular formala for **19** (obsd. *m/z* 260.1776 calcd. for $C_{17}H_{24}O_2$ [M$^+$], *m/z* 260.1775) and for **21** (obsd. *m/z* 328.2403 calcd. for $C_{22}H_{31}O_2$ [M$^+$], *m/z* 328.2402). The ^{1}H and ^{13}C NMR data for synthetic 21 and 22 were identical

with those reported for the natural products isolated from a marine derived Penicillium fungus.[33,34]

Table 8: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **19**.

Carbon	δ_c ppm	δ_H ppm (int., mult., *J*/Hz)	HMBC correlation to	COSY coupling to
1	147.4			
2	125.4			
3	116.2	6.53 (1H, s)	C-1', C-1, C-5	
4	147.8			
5	122.5			
6	118.1	6.58 (1H, s)	C-1, C-2, C-7	
7	15.4	2.16 (3H, s)	C-4, C-5, C-6	
1'	29.3	3.26 (2H, d, 7.02)	C-1, C-2, C-3, C-2', C-3'	H-2'
2'	121.7	5.28 (1H, t, 6.58)		H-1'
3'	138.2			
4'	39.7	2.09 (2H, m)		H-5'
5'	26.4	2.09 (2H, m)		H-6'
6'	123.9	5.07 (1H, t, 5.22)		H-5'
7'	131.9			
8'	25.7	1.68 (3H, s)	C-6', C-7', C-9'	
9'	17.7	1.60 (3H, s)	C-6', C-7', C-8'	
10'	16.1	1.73 (3H, s)	C-2', C-3', C-4'	

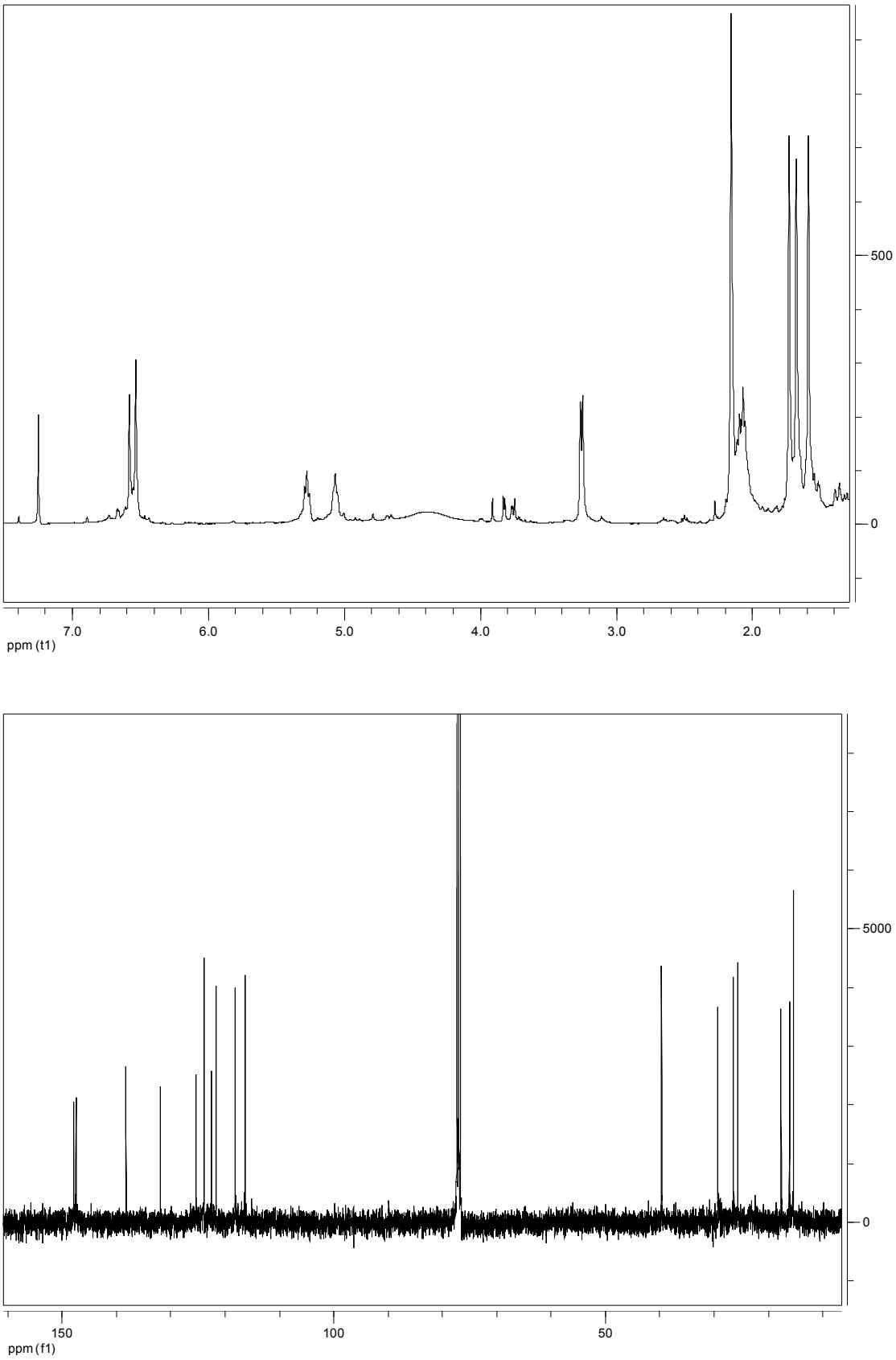


Figure 14: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **19**.

Table 9: ^{1}H ($CDCl_3$, 400 MHz), ^{13}C ($CDCl_3$, 100 MHz) and 2D NMR correlations for **21**.

Carbon	δ_c ppm	δ_H ppm (int., mult., *J*/Hz)	HMBC correlation to	COSY coupling to
1	147.8			
2	125.3			
3	116.2	6.53 (1H, s)	C-1, C-5, C-1'	
4	147.4			
5	122.5			
6	118.1	6.57 (1H, s)	C-1, C-2, C-7	
7	15.4	2.16 (3H, s)	C-4, C-5, C-6	
1'	29.3	3.26 (2H, d, 7.05)	C-1, C-2, C-3, C-2', C-3'	H-2'
2'	121.6	5.28 (1H, t, 7.00)		H-1'
3'	138.3			
4' ; 8'	39.7	2.00 (2 x 2H, m)		H-5', H-9'
5'	26.7	2.00 (2H, m)		H-4', H-6'
6'	123.7	5.08 (1H, t, 5.40)		H-5'
7'	135.5			
9'	26.4	2.00 (2H, m)		H-8', H-10'
10'	124.4	5.08 (1H, t, 5.40)		H-9'
11'	131.3			
12'	25.7	1.67 (3H, s)	C-10', C-11', C-13'	
13'	17.7	1.59 (3H, s)	C-10', C-11', C-12'	
14'	16.0	1.59 (3H, s)	C-6', C-7', C-8'	
15'	16.2	1.74 (3H, s)	C-2', C-3', C-4'	

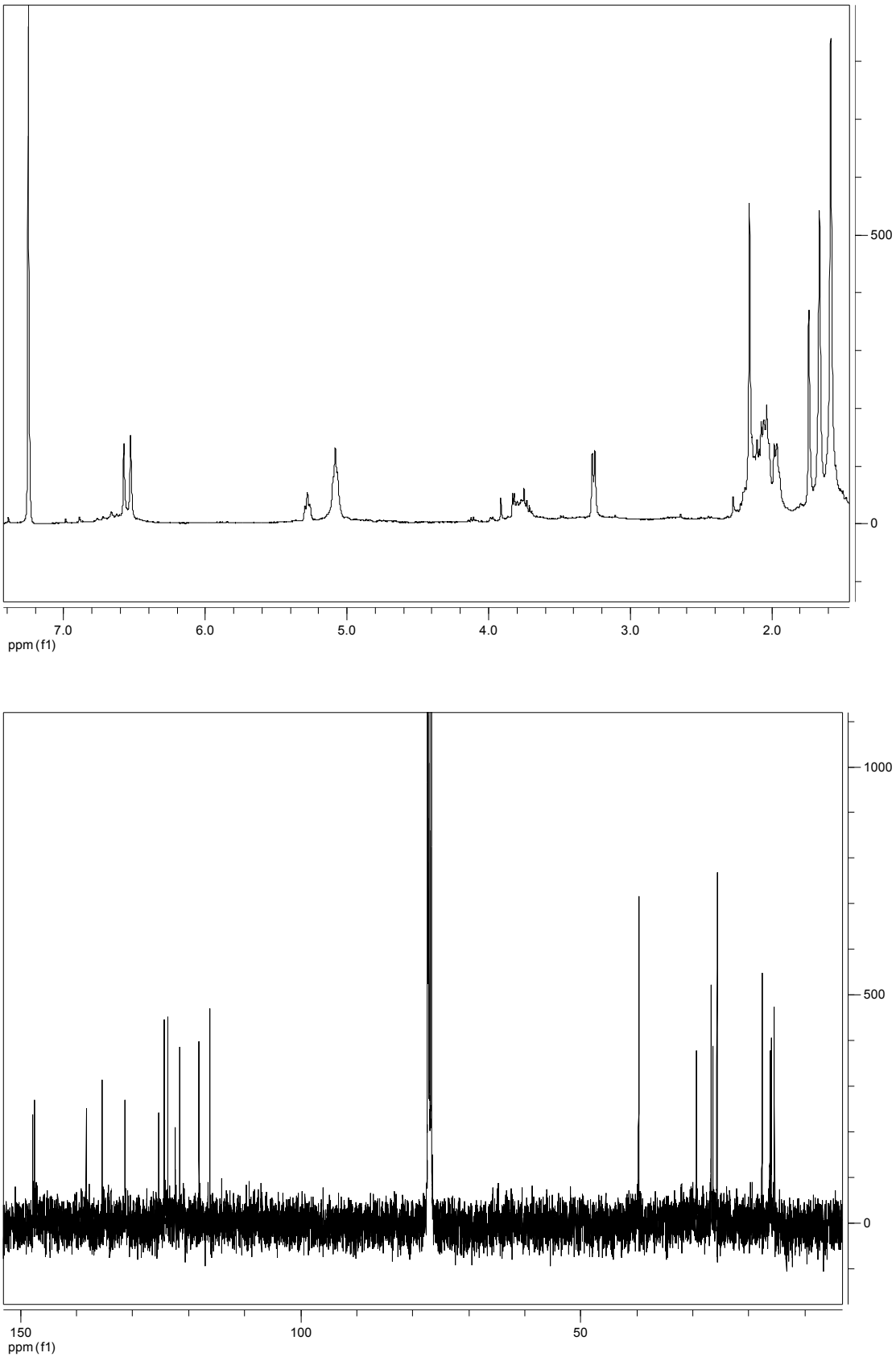


Figure 15: ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR spectra acquired for **21**.

Therefore, in conclusion, the two triprenylated toluquinone and toluhydroquinone marine fungal metabolites, 5-methyl-2-[(2'*E*,6'*E*)-3,'7',11'-trimethyl-2',6',10'-dodecatrienyl]-1,4-benzenediol, **21** and 5-methyl-2-[(2'*E*,6'*E*)-3',7',11'-trimethyl-2',6',10'-dodecatrienyl]-2,5-cyclohexadiene-1,4-dione, **22**, were synthesized in four and five steps respectively from 2-methyl-1,4-benzoquinone in overall yields of 15.0 % and 14.9 % respectively. The two analogues of **21** and **22** with a shorter polyprenylated side-chain *viz.* 5-methyl-2-[(2'*E*,6'*E*)-3',7' -dimethyl-2',6'-octadienyl]-1,4-benzenediol, **19**, and 5-methyl-2-[(2'*E*,6'*E*)-3',7'-dimethyl-2',6'-octadienyl]-2,5-cyclohexadiene-1,4-dione, **20** were similarly prepared in 14.3 % and 15.4 % overall yield respectively. The synthesis of these four compounds extends the applicability of the oxidative ether cleavage of hydroquinone dimethyl ethers with AgO under acidic conditions to include the oxidative demethylation of polyprenylated-1,4-dimethoxy-toluhydroquinones with quantitative survival of the supposedly oxidation- and acid-sensitive polyprenyl side chain.

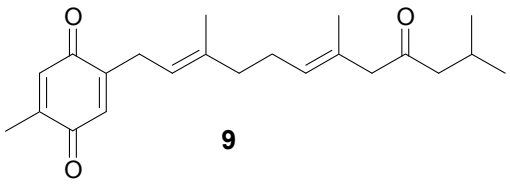
3.5 Future work

Towards the end of our synthesis of analogues **19-22** a paper by Li *et al*.[141] came to our attention in which the secondary metabolites **9** and **11** originally isolated from the South African nudibranch *L. millecra* were synthesized. Interestingly Li *et al*.[141] reacted a Grignard reagent [(2-(5-methyl-1,4-dimethoxy)phenylmagnesium bromide)] with geranyl bromide and achieved regioselective coupling in 73% yield. A Julia type reaction was then used to complete the synthesis of the secondary metabolites. The yield Li *et al*. obtained for the coupling of **93** with geranyl bromide using a Grignard reagent was double the yield we obtained using MHE. Therefore, it would be worthwhile to explore this Grignard strategy to improve the yield of the lowest yielding step in our synthetic sequence.

Chapter 4.

4. Anti-oesophageal cancer properties of synthetic target compounds

The main aim of this thesis, as mentioned in Section 1.4, was to examine the effect of the polyprenyl side-chain on the anti-oesophageal cancer properties originally exhibited by the triprenylated marine hydroquinone **11** and its corresponding quinone **9**. Simplified analogues **17**-**22** (as well as their methylated precursors **91**, **92** and **104**) of **11** and its oxidized analogue **9** were required in order to achieve our goal. Having successfully acquired the first two target analogues **17** and **18** commercially and synthesizing the remaining target analogues **19**-**22**, **91**, **92** and **104** as described in Chapter 3, an investigation into their anti-oesophageal cancer properties could now be executed.



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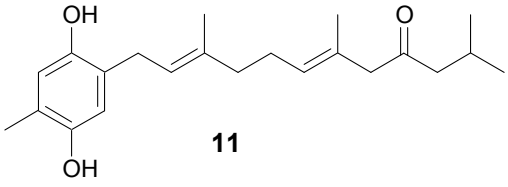

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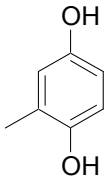

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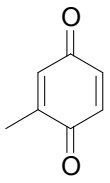

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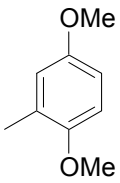
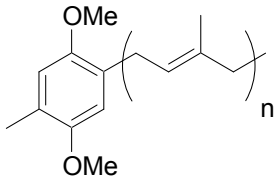
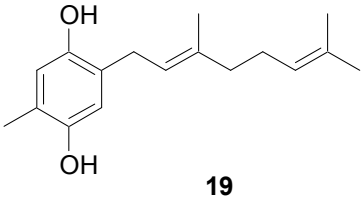


91



92 n = 2
104 n = 3



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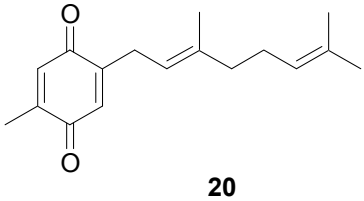

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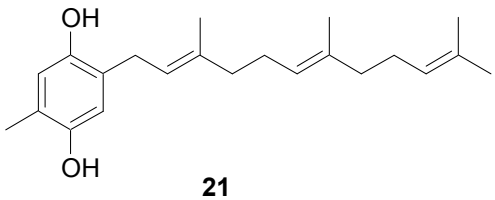
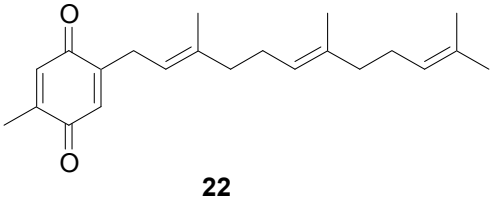


21



22

4.1 Quinones as anti-cancer drugs

Apoptosis is the term used to describe a common type of programmed cell death normally observed in multicellular organisms as a means of removing potentially dangerous cells as well as regulating cell numbers and tissue size.[142] The mechanism for apoptosis is not well known, however, it has been hypothesized that a set of cysteine proteases (protein family: caspases) are responsible for many of the morphological changes which occur in cells during apoptosis. Examples of the morphological features commonly observed in apoptotic cells include membrane blebbing, loss of cell shape, aggregation of the chromatin at the nuclear membrane, cell shrinkage and the eventual division of the cellular material into membrane bound vesicles. Conversely necrosis is uncontrolled cell death leading to the lysis of cells and inflammatory responses, which is an undesirable event when treating cancer. [143] Figure 16b-d shows examples of cells undergoing apoptosis in the presence of **21** and **22**.

Compounds containing a quinone moiety *e.g.* streptonigrin (**105**), mitomycine (**106**), diaziquone (**107**) and adriamycin (**108**) commonly exhibit anticancer properties [144,145,146] The mechanism of action of these quinone-containing compounds is not well known and a large amount of research still needs to be done. There are currently two theories describing the anti-cancer activity of quinone compounds, the first is redox cycling, which involves the reduction of the quinone moiety by a reductase enzyme to form an unstable semiquinone moiety. This semiquinone in turn donates an electron to an oxygen molecule (O_2) resulting in the production of oxidative radicals such as reactive oxygen species (ROS) superoxide radical anions, hydrogen peroxide and hydroxyl radicals that lead to apoptosis of the cell.[145,146] The second theory is alkylation, where the quinone-containing compound becomes activated by covalently binding to various target compounds in the cell such as proteins and DNA. The covalent binding of these compounds to DNA can activate various genes in the cancer cell leading to, amongst other things, apoptosis.[145,146]

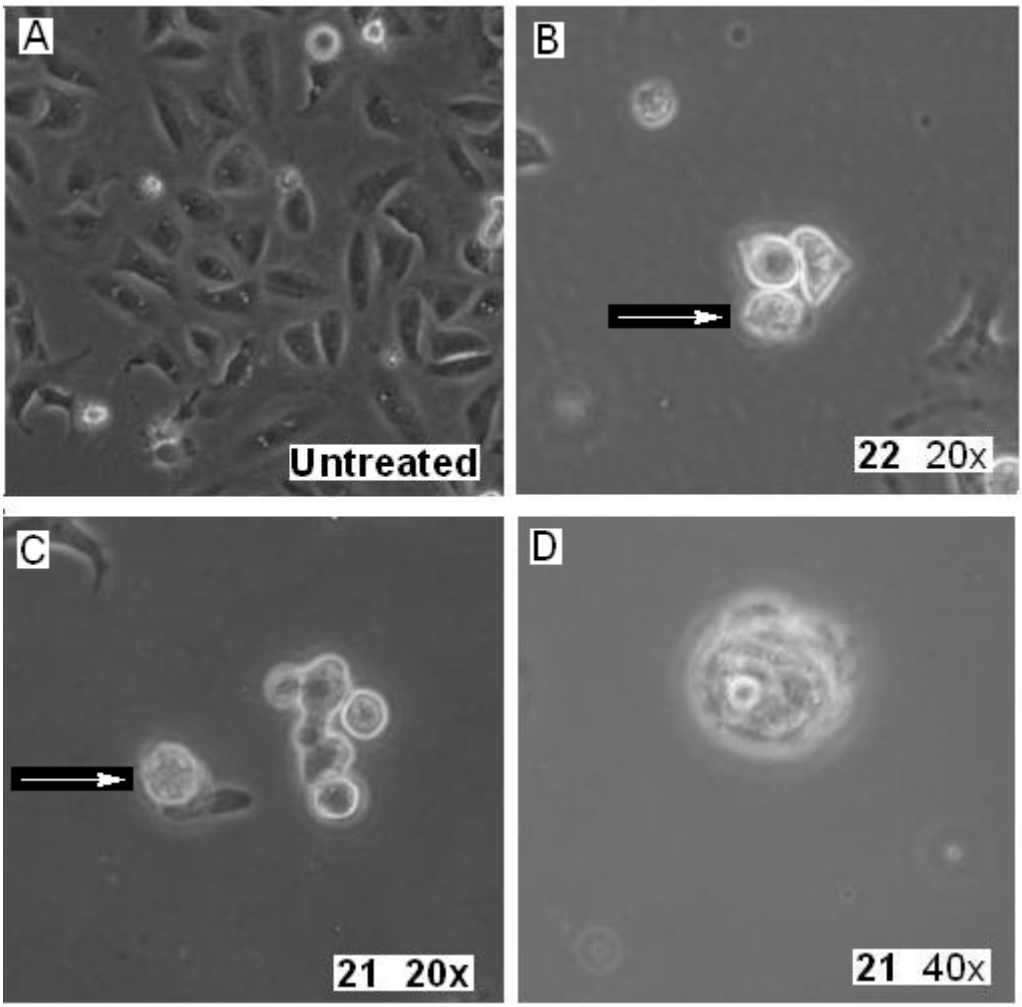
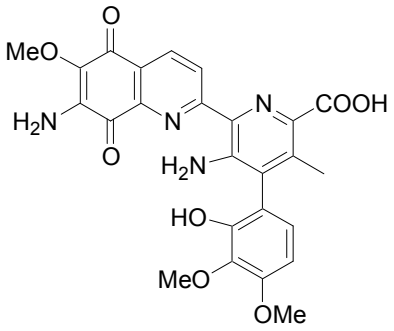
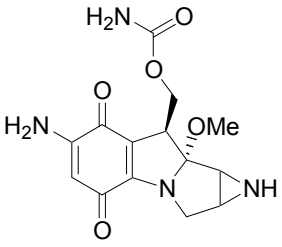


Figure 16: Shows microscopy images of WHCO1 oesophageal cancer cells obtained using a Zeiss Axiovert 200 with an Axiocam camera and Axiovision 4.1 image software; a. untreated WHCO1 oesophageal cancer cells (cells are flat with well defined cell membranes) ; b. WHCO1 oesophageal cancer cells undergoing apoptosis (20x magnification) in the presence of **22** ; c. WHCO1 oesophageal cancer cells undergoing apoptosis (20x magnification) in the presence of **21** (in both images b and c. blebbing and loss of membrane integrity can be observed) ; d. WHCO1 oesophageal cancer cells undergoing apoptosis (40x magnification) in the presence of **21** (fragmentation of the cell into membrane bound vesicles).



105



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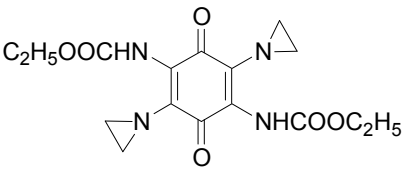

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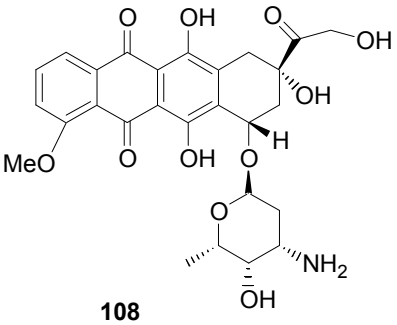


108

As described in Section 1.3, the cytotoxicity of the *ortho*-prenylated toluquinones and toluhydroquinones (**9**-**15**) isolated of the endemic nudibranch *L. millecra* were tested against the oesophageal cancer cell line WHCO1 using the MTT assay. The assay (Table 1) revealed that compounds **11** and **12** exhibited moderate cytotoxicity against WHCO1 cells.[28] Triprenylated toluquinone and toluhydroquinone (**9**-**15**) induces apoptosis following a cell cycle block. Molecular events leading to the cell cycle block include production of ROS, followed by activation of SPK/JNK/c-Jun(AP1) signal transduction.[28] It is reasonable to hypothesize that cancer cells are often more susceptible to ROS because of the central role played by ROS in other potential anti-cancer agents such as 4HPR in head and neck squamous carcinoma cell lines,[147] diallyl disulfide in neuroblastoma cells,[148] paclitaxel in T24 urothelial carcinoma cell lines[149] and heteroarotinoids in HNSCC cell lines.[150] Compounds **9**-**15**, therefore, present a reasonable lead for new anti-oesophageal cancer treatments, and is an indication of the potential of the South African marine environment to provide new chemical compounds with anti-cancer activity.[28]

Cancer cells generally are exposed to much higher levels of oxidative stress than normal cells.[151] This is based on direct measurements of ROS levels in tumour tissues,[152] accumulation of oxidative products in tumour tissue,[153] and the presence of oxidised DNA products in the urine of cancer patients.[154] Elevated levels of superoxide dismutase (SOD) have also been measured in squamous cell carcinomas of the oesophagus relative to normal tissues. Superoxide dismutase is an enzyme which catalyzes the dismutation of superoxide anion radicals to hydrogen peroxide. Hydrogen peroxide reacts with iron(II) ions via the Fenton reaction to generate highly oxidising cell-damaging species such as the hydroxyl radical. Therefore the presence of this enzyme is also an indication that cancer cells experience high levels of oxidative stress.[155] As a result of the constant assault of oxidative stress, cancer cells have an impaired ability to deal with oxidative stress rendering them sensitive to additional oxidative challenges. This diminished ability of cancer cells to mount an effective response to further oxidative challenges may represent a critical therapeutic window that could be exploited to develop more selective anticancer agents. The central role of ROS in mediating the cytotoxic activity of the family of triprenylquinones and triprenylhydroquinones described by the research of our UCT collaborators highlights the value of this family of compounds as potential lead chemotherapeutic agents for further structure/function analysis.

4.2 The crystal violet and MTT anti-cancer bioaasays

The crystal violet bioassay as the name suggests makes use of a protein straining dye called crystal violet. This dye stains cell proteins a deep purple and the intensity of the purple colour increases proportionally as cells divide. This assay initially involves the seeding of approximately 1500 oesophageal cancer cells (WHCO1) per well in 96 well plates. DMSO solutions of the test compounds (**17-22, 91**, **92** and **104**) each in varying concentrations (100, 10, 1 and 0.1 μM) were then added to the oesophageal cancer cells and incubated (48 h). Following this, the media was discarded and the remaining living cells stained with the crystal violet dye. The dye was solubilized with 50 % acetic acid (50 μL) and the plates read at 595 nm on an Anthos microplate reader (Figure 17). The advantage associated with this assay is that it is cheap, relatively rapid and a large number of compounds can be screened simultaneously.

The apparent disadvantage of the assay is that it is not quantitatively accurate *i.e.* all the proteins present in the well both from living and dying cells are stained and contribute to overall absorbance reading.

The initial results of crystal violet screening assay suggested that analogues **17-22** were all active at 100 μM, however, it can be noted that **21** only showed moderate activity in comparison to the other analogues. As expected all the methylated analogues **91**, **92** and **104** showed very little activity at the highest concentration (100 μM). These analogues (**91**, **92** and **104**) being methylated are unable to undergo redox-type reactions to produce ROS and thus served as controls during the crystal violet anti-cancer bioassay. The preliminary results from the assay supported the results of our UCT collaborators who established that triprenylated toluquinone and toluhydroquinone derivatives (**19-22**) induce apoptosis within oesophageal cancer cells by producing ROS superoxide radical anions, hydrogen peroxide and hydroxyl radicals as by-products of redox cycling.[28] Interestingly analogues **17** and **18,** which did not possess a polyprenyl side-chain, had the highest activity and were the only compounds to induce apoptosis of the cancer cells at 10 μM (Figure 18).

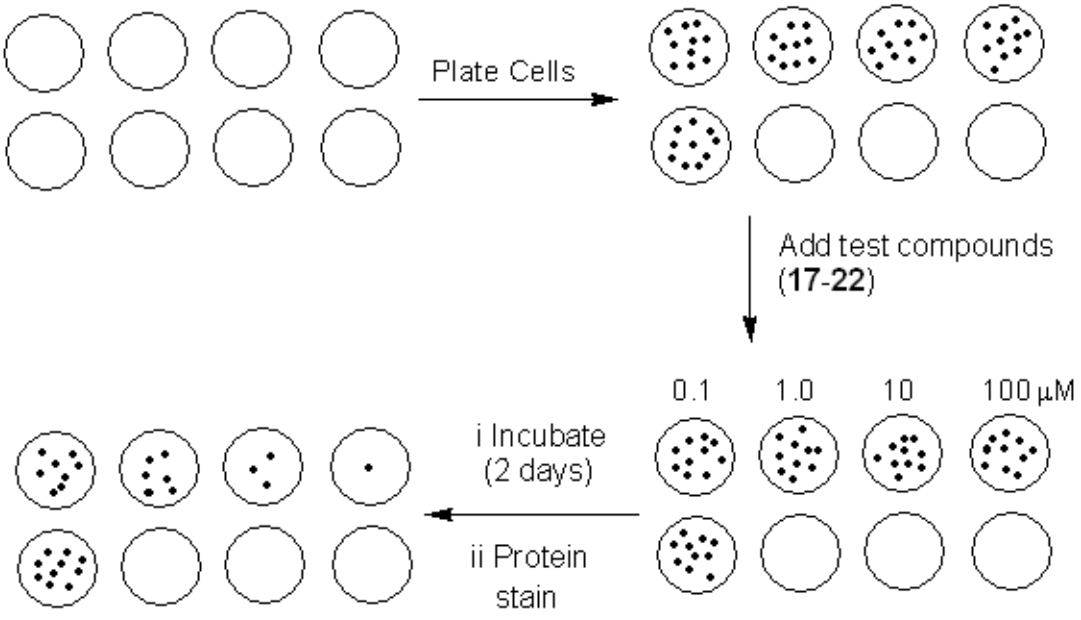


Figure 17: The crystal violet assay.

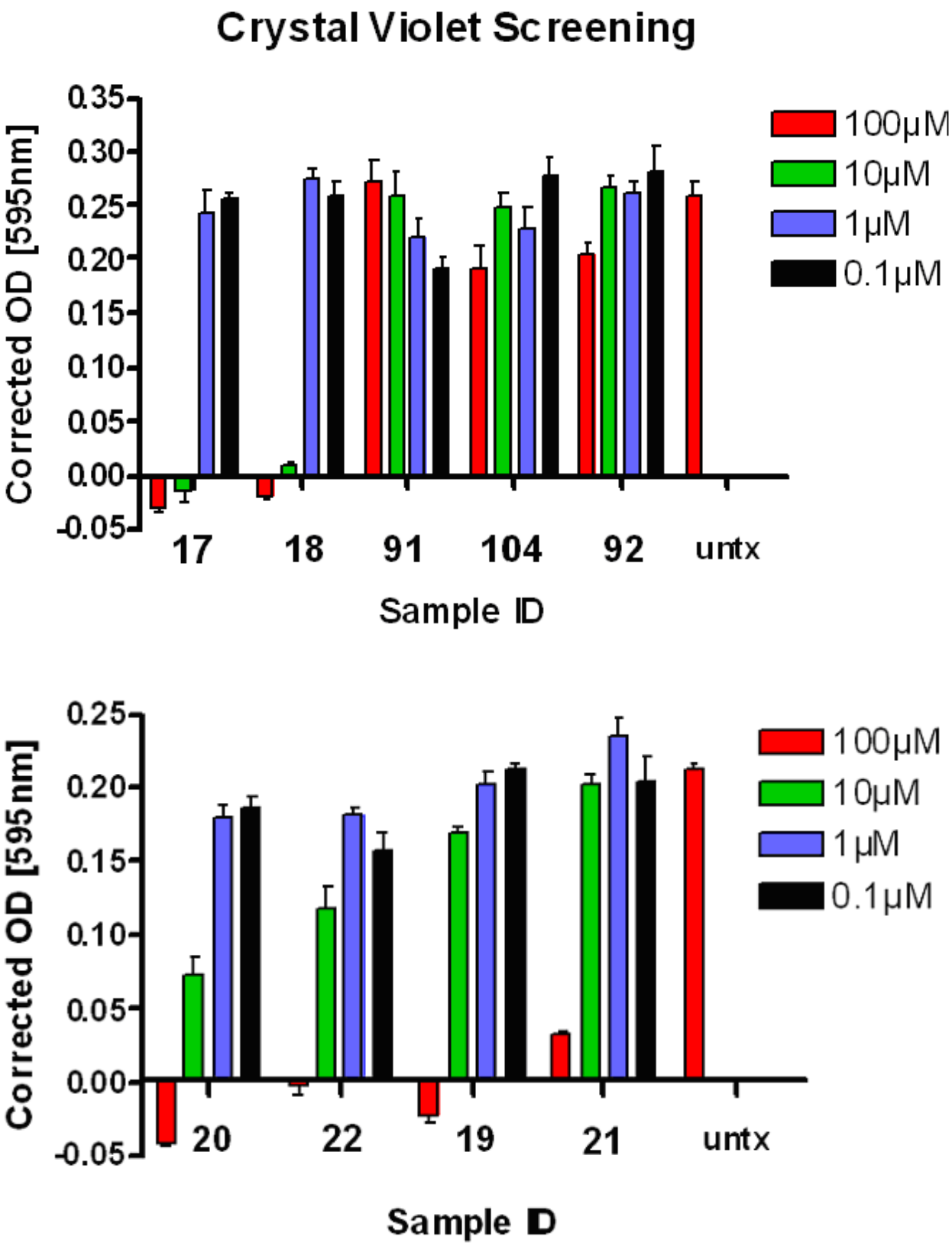
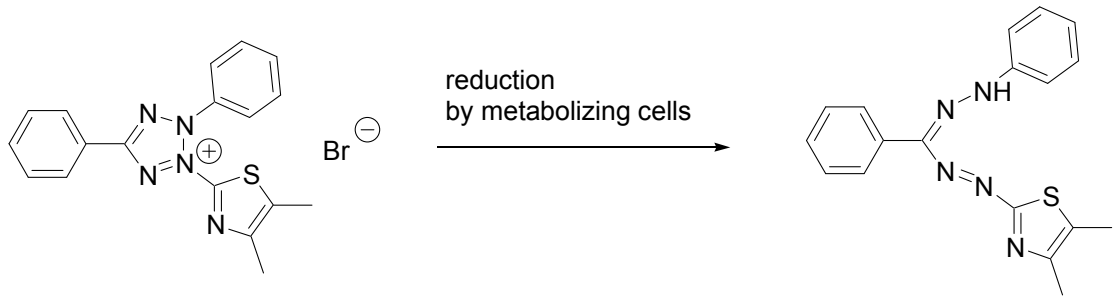


Figure 18: Crystal violet screening results for compounds **17**-**22**, **91**, **92** and **104**.

Following the initial promising results of the crystal violet assay, the cytotoxicity of analogues **17-22** were further investigated using the MTT assay to calculate their IC_{50} values. The methylated precursors **91**, **92** and **104** were not included in this assay because of the lack of activity exhibited by these compounds on the WHCO1 oesophageal cancer cell line during the crystal violet assay. The MTT assay is commonly used as an indication of cell viability (living metabolising cells) in that metabolising cells are able to reduce the yellow water-soluble tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the insoluble purple formazan precipitate (Scheme 26).[156] The advantage of this assay over the crystal violet assay, is that it can be used as a means of quantifying cell death and is thus commonly used to evaluate the cytotoxicity of various chemical compound in terms of an IC_{50} value.



Scheme 26: The conversion of the soluble yellow MTT salt to the insoluble purple formazan.

Similarly to the crystal violet assay, the MTT assay initially involves the seeding of approximately 1500 oesophageal cancer cells (WHCO1) per well in 96 well plates. DMSO solutions of each of the target analogues (**17-22**) were then plated in different concentrations and incubated for 48 h. The MTT solution was added to each well following the 48 h incubation period and the cells incubated once again for a further 4 h. It was during this time (4 h incubation period) that the remaining living cells metabolised the MTT to form the insoluble purple formazan. The amount of MTT metabolised in each well is thus directly proportional to the amount of living cells still present. The purple formazan precipitates were solubilized and the plates read at 595

nm on an Anthos microplate reader. The IC_{50} values for each of the target analogues **17-22** were calculated by plotting the log concentration [μM] for each analogue against their corrected optical density (OD) readings at 595 nm. The IC_{50} curve for **18** is shown as a representative example (Figure 19) along with the IC_{50} values for all the analogues (**17-22**) in Table 10. The results of the assay revealed that un-prenylated analgues **17** and **18** were the most active against WHCO1, followed closely by the prenylated toluquinones **20** and **22** and lastly by the prenylated toluhydroquinones **19** and **21**. The conclusion that can be drawn from the results of both assays (crystal violet and MTT assays) is that the polyprenyl side-chain plays a negligible role in the cytotoxicity of compounds such as **11** and **9** against the oesophageal cancer cell line WHCO1.

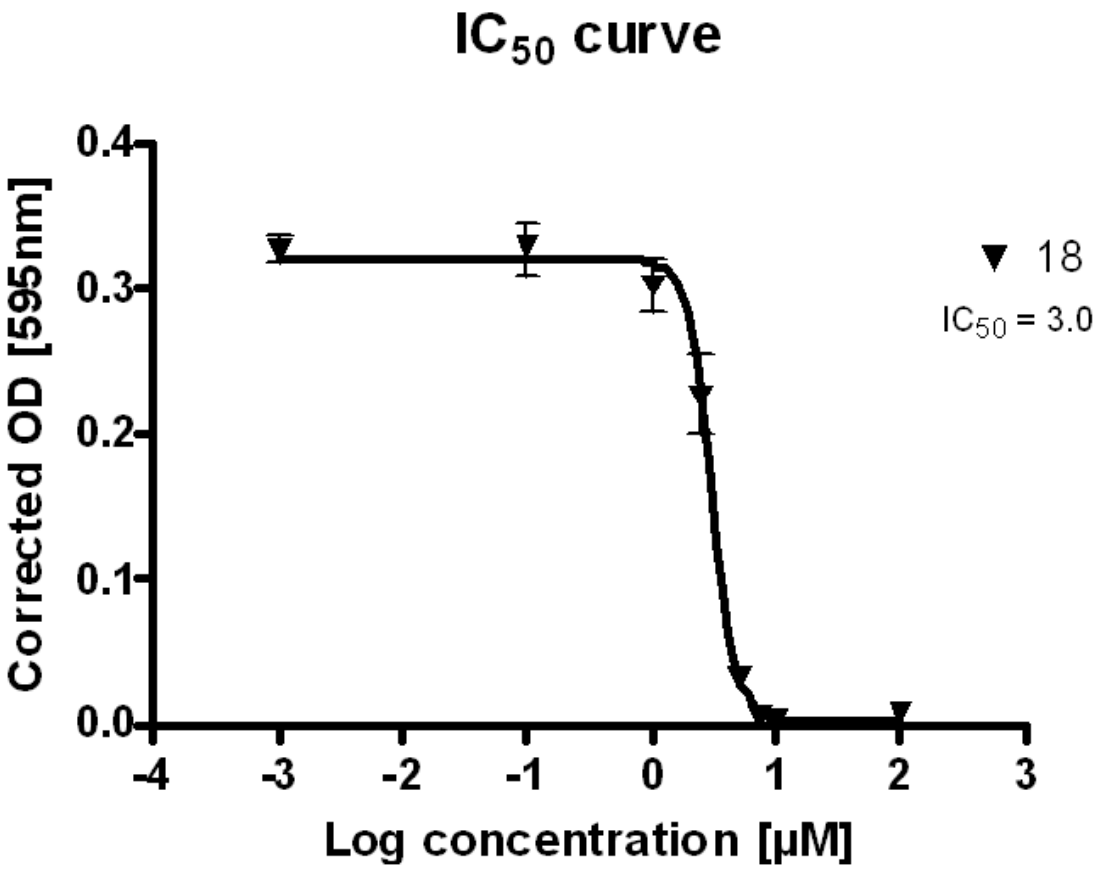


Figure 19: The determination of the IC_{50} value of **18** by plotting log concentration [μM] against the corrected optical density (OD) reading at 595 nm obtained from the MTT assay.

Table 10: The comparative IC_{50} values of analogues **17-22** against the oesophageal cancer cell line WHCO1.

Compound	IC_{50} [μM]
17	5.2
18	3.0
19	12.0
20	5.7
21	32.0
22	7.1

Chapter 5.

5 Experimental

All anhydrous reactions were conducted under an inert argon atmosphere with solvents dried using established procedures as described in Perrin *et al.*[142] and stored over an appropriate drying agent. The dry solvents were distilled under an inert atmosphere (nitrogen) and collected over 4 Å molecular sieves prior to being used for anhydrous reactions. THF, diethyl ether, hexane and dioxane were distilled from sodium metal/benzophenone ketal, acetonitrile from calcium hydride and acetone from 3 Å molecular sieves. Glassware for anhydrous reactions were dried at temperatures exceeding 150 ^{o}C either in an oven, using a Bunsen burner or heat gun under an inert atmosphere. Normal phase HPLC solvents were of analytical grade.

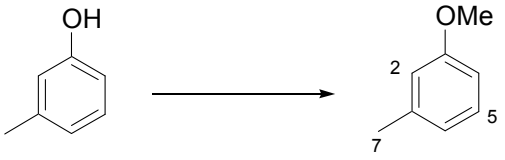
All reactions were monitored using normal phase thin layer chrmatography (TLC), 25 DC-Plastikfolien Kieselgel 60 on plastic sheets using general laboratory solvents distilled from glass. The TLC plates were viewed under a UV lamp at 254 nm and developed using an acid spray (10 % conc. sulphuric acid in methanol) followed by heating on a hotplate. Iodine staining was another method of development made use of where necessary *i.e.* if compounds possessed double bonds. Most reactions were purified using Merck 46271 silica gel 60 (0.040-0.063 mm) for column chromatography. Normal phase HPLC was performed using a Spectra-Physics SpectraSeries P100 pump, Whatman Partisil 10M/9-50 column, a Waters Associates R401 differential refractometer and a Rikadenki chart recorder.

NMR spectra were recorded in deuterated chloroform or methanol using a Bruker Avance 400 spectrometer. ^{1}H and ^{13}C NMR were recorded at 400 and 100 MHz respectively. The chemical shifts are reported in ppm and no internal standards were used. Coupling constants were calculated directly from the NMR spectra using a computer programme. IR spectra were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer with the compounds on sodium chloride (NaCl) discs. All UV spectra were obtained using a Cary 50 1E UV-visible spectrometer. GLC was performed using a Duraband DB-1 non-polar column and samples injected at a column temperature of 100 ^{o}C which was subsequently increased to 200 ^{o}C at a rate of 15 ^{o}C/min. Low resolution mass spectra were recorded using a Finnigan GCQ

5988A spectrometer at 70 eV whereas high resolution mass spectra were provided by Dr Louis Fourie at the University of the North West by using HRFABMS. The bioassays were performed at the University of Cape Town with the assistance of Catherine Whibley of the Department of Medical Biochemistry. Microscopy images of WHCO1 oesophageal cancer cells were taken using a Zeiss Axiovert 200 with an Axiocam camera and Axiovision 4.1 image software.

5.1 Synthetic procedures

5.1.1 The methylation of compound 79



79 **80**

Method 1

K_2CO_3 was finely crushed and dried in an oven at 150 ^oC for an hour prior to use. K_2CO_3 (103.0 mg, 0.73 mmol) was added to a solution of acetone (20 mL), *m*-cresol (**79**, 0.10 mL, 0.93 mmol), MeI (2 mL, 32 mmol) and refluxed for 5 h under nitrogen. TLC suggested that the reaction had been partially successful. The reaction mixture was accordingly filtered and partitioned between $CHCl_3$ (3 x 5 mL) and water. The organic layers were combined, dried over anhydrous magnesium sulphate ($MgSO_4$) and concentrated *in vacuo*. ^1H NMR analysis of the reaction mixture confirmed that **80** had been synthesized in < 20 % yield. The product was not purified further due to the low yield obtained.

Method 2

NaH (96 mg, 4 mmol) was added to *m*-cresol (**79**, 0.21 mL, 2.0 mmol) in dry THF (15 mL) at 0 ^oC and the mixture stirred for approximately 2 h. MeI (0.12 mL, 0.002 mol) was added and the reaction allowed to warm slowly to room temperature (3 h). The

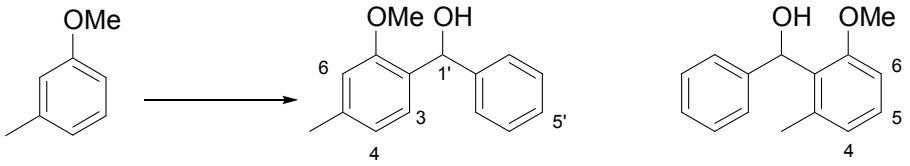
reaction mixture was quenched with water and extracted with $CHCl_3$ (3 x 5 mL). The combined $CHCl_3$ extracts were dried using anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield *m*-methoxytoluene (**80**, 109 mg, 45 %) as a pale yellow liquid; IR V_{max}: 3000, 2954, 2835, 1603, 1586, 1492, 1261, 1153, 1056, 1044 cm^{-1} ; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.19 (1H, dt, *J* = 7.7 ; 0.6 Hz, CH), 6.79 (1H, dquin, *J* = 7.5 ; 0.8 Hz, CH), 6.76 (1H, br dd, *J* =7.7 ; 2.5 Hz, CH), 6.75 (1H, br s, CH), 3.80 (3H, s, CH_3), 2.36 (2H, s, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 100MHz) δ 159.6 (C-1), 139.4 (C-3), 129.1 (C-5), 121.5 (C-4), 114.7 (C-2), 110.8 (C-6), 55.0 (OMe), 29.7 (C-7) ppm; EIMS (70 eV): 122 [M^+] (82.7), 91 (100).

Method 3

A three-necked round bottom flask was fitted with a separating funnel, a Liebig condenser and a stopper. *m*-Cresol (**79**, 0.97 mL, 9,25 mmol) was dissolved in a NaOH solution (0.37g, 9.25 mmol) and cooled to 0 ^oC with constant stirring before adding Me_2SO_4 (1,76 mL, 18,50 mmol) drop wise. The reaction mixture was then heated at approximately 80 ^oC for 1 h before being quenched with water and extracted with $CHCl_3$ (3 x 15 mL). The $CHCl_3$ extracts were washed twice with sodium hydroxide (10%), which yield fairly pure *m*-methoxytoluene (**80**, 1.0 g, 94 %). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20 (1H, t, *J* = 7.7 Hz, CH), 6.80 (1H, dquin, *J* = 7.5 ; 0.5 Hz, CH), 6.76 (1H, br s, CH), 6.73 (1H, br dd, *J* = 2.4 Hz, CH), 3.81 (3H, s, CH_3), 2.37 (2H, s, CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) 159.6 (C-1), 139.4 (C-3), 129.1 (C-5), 121.5 (C-4), 114.7 (C-2), 110.8 (C-6), 55.0 (OMe), 29.7 (C-7) ppm; EIMS (70 eV): 122 [M^+] (82.7), 91 (100).

5.1.2 Directed *ortho* Metalation

5.1.2.1 Attempted synthesis of compound 89.



80 **89** **90**

m-Methoxytoluene (**80**, 0.21 mL, 1.64 mmol) was dissolved in dry THF (3 mL) and the solution cooled to –78 ^{o}C before adding *n*-BuLi (1 mL, 1.64 mmol). The reaction mixture was stirred for approximately 1 h before adding pure distilled benzaldehyde (0.17 mL, 1.64 mmol) and left to slowly warm to room temperature. The reaction was quenched by the addition of water (5 mL) and the reaction products extracted with EtOAc (3 x 20 mL). The combined organic extracts were then dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Unfortunately, the ^{1}H NMR spectrum of the crude extract showed no resonance indicating that the coupling reaction had been successful.

5.1.2.2 Attempted synthesis of compound 89 using a similar protocol to that used by Harder *et al*[127] to prepare compound 85.

m-Methoxytoluene (**80**, 0.21 mL, 1.6 mmol) and TMEDA (0.5 mL, 3.3 mmol) were dissolved in dry hexane (4 mL) and cooled to –78 ^{o}C before adding *n*-BuLi (1 mL, 1.6 mmol). The dark yellow solution was allowed to stir for 0.5 h at –78 ^{o}C before adding pure distilled benzaldehyde (0.17 mL, 1.6 mmol) and the reaction mixture allowed to warm slowly to 4 ^{o}C. The reaction was quenched by the addition of water and extracted with $CHCl_3$ (3 x 20 mL). The combined $CHCl_3$ extracts were dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow oil. Once again the ^{1}H NMR spectrum showed no resonance implying that coupling had been successfully achieved.

5.1.2.3 Synthesis of compounds 89 and 90 using Shirley *et al's*. synthetic protocol.[125]

A solution of *m*-methoxytoluene (**80**, 100 mg, 0.82 mmol) was dissolved in dry ether (4 mL) and refluxed for 15 min under argon before adding *n*-BuLi (1 mL, 1.6 mmol). The solution was then allowed to reflux for a further 4 h before adding dry benzaldehyde (0.084 mL, 0.82 mmol). The reaction mixture was quenched with water (4 mL) and the products extracted with $CHCl_3$ (3 x 20 mL) in the usual manner. The combined $CHCl_3$ extracts were dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a pale yellow oil (226 mg) which after normal phase HPLC (10:1 ; hexane/EtOAc) yield **89** (49 mg, 0.21 mmol, 26%) and **90** (43 mg, 0.19 mmol, 23%) as pale yellow oils.

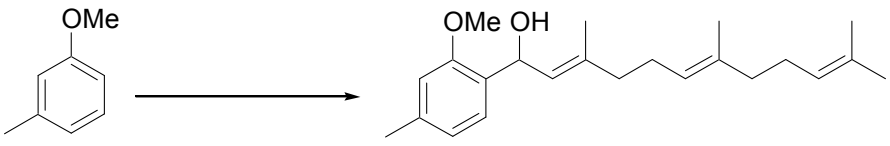
Compound **89** was isolated as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (2H, m, H-3' and 7'), 7.31 (2H, m, H-4' and 6'), 7.24 (1H, m, H-5'), 7.09 (1H, d, *J* = 7.6 Hz, H-3), 6.75 (1H, d, *J* = 7.8 Hz, H-4), 6.71 (1H, s, H-6), 6.02 (1H, d, *J* = 2.9 Hz, 1'), 3.80 (3H, s, OMe), 3.01 (1H, d, *J* = 4.2 Hz, OH), 2.34 (3H, s, H-7) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7 (C-1), 143.5 (C-2'), 138.8 (C-5), 129.1 (C-2), 128.1 (C-4' and 6'), 127.8 (C-3), 127.0 (C-5'), 126.5 (C-3' and 7'), 121.3 (C-4), 111.7 (C-6), 72.1 (C-1'), 55.3 (OMe), 21.5 (C-7); HRFABMS obsd. *m/z* 228.1151, calcd. for $C_{15}H_{16}O_2$ [M^+], 228.1150.

Compound **90** was isolated as a yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (2H, s, H-3' and 7'), 7.27 (2H, s, H-4' and 6'), 7.20 (1H, dd, *J* = 8.5, 7.60 Hz, H-5), 6.85 (1H, d, *J* = 7.6 Hz, H-4), 6.80 (1H, d, *J* = 8.3 Hz, H-6), 6.07 (1H, d, *J* = 10.7 Hz, H-1'), 4.16 (1H, d, *J* = 10.8 Hz, OH), 3.69 (3H, s, OMe), 2.35 (3H, s, H-7) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) 157.7 (C-1), 144.1 (C-2'), 137,1 (C-3), 129.5 (C-2), 128.2 (C-5'), 128.0 (C-4' and 6'), 126.7 (C-5), 125.8 (C-3' and 7'), 123.7 (C-4), 109.7 (C-6), 71.4 (C-1'), 55.6 (OMe), 19.8 (C-7) ppm; HRFABMS obsd. *m/z* 228.1150, calcd for $C_{15}H_{16}O_2$ [M^+], 228.1150.

5.1.2.4 Oxidation of farnesol to farnesal

Manganese dioxide (2 g) was added to farnesol (200 mg, 0.90 mmole) and left to stir for 24 h in dry benzene (2 mL). The reaction mixture was then filtered using a PTFE filter (Millipore) attached to a syringe. The residue was washed a few times with dry benzene and also filtered through the PTFE filter. The resulting filtrate was concentrated *in vacuo* and analyzed using ^{1}H and ^{13}C NMR from which it was evident by the appearance of two aldehyde signals and inconsistencies in proton integration that some isomerisation had taken place. No attempt was made to purify this isomeric mixture; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.97 (1H, d, J = 8.0 Hz, CHO), 9.89 (1H, d, J = 8.0 Hz, CHO), 5.86 (2H, d, J = 7.4 Hz), 5.07 (4H, m); 2.30 (24H, m); 1.69 (6H, s); 1.61 (12H, s) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.2 (C-1), 163.8 (C-3), 136.6 (C-7), 131.5 (C-11), 127.4 (C-2), 124.1 (C-5), 122.4 (C-9), 40.6 (C-8), 39.6 (C-4), 26.6 (C-12), 25.7 (C-15x2), 17.7 (C-13), 17.6 (C-13'), 16.0 (C-14) ppm.

5.1.2.5 Attempted coupling of compound 80 with farnesal using Shirley *et al's.* synthetic protocol

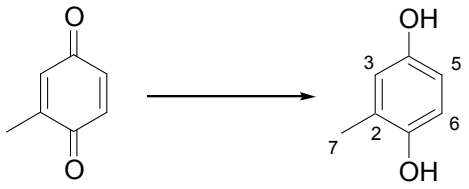


80

Shirley *et al's*. synthetic protocol used in the preparation of **89** and **90** above was repeated in an attempt to couple **80** with farnesal. Before repeating Shirley *et al's* synthetic protocol all glassware was thoroughly dried at > 200 ^{o}C in an oven overnight and the reaction performed under dry argon. The anhydrous conditions were carefully maintained for the duration of the reaction. The quantities of the reagents made use of are as follows: *m*-methoxytoluene (**80**, 100 mg, 0.82 mmol), *n*-BuLi (1 mL, 1.6 mmol), farnesal (180 mg, 0.82 mmol) in diethyl ether, reflux. ^{1}H NMR

spectroscopic analysis of the reaction products indicated that it comprised of only starting material (**80** and farnesal) with no resonance supporting the presence of the coupled product. This reaction was repeated with the addition of TMEDA (0.26 mL, 1.6 mmol) without any improvement in yield. Numerous further attempts to bring about this reaction, ensuring all the reagents were effectively dry, unfortunately proved unsuccessful.

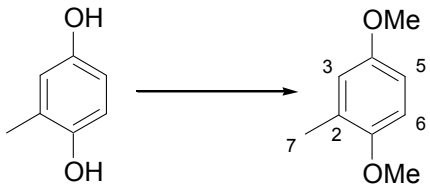
5.1.2.6 Preparation of compound 17



18 **17**

A mixture of 1-methyl-1,4-benzoquinone (**18**, 1 g, 8.19 mmol) with Zn (1 g, 15,3 mmol) and ZnCl (1 g, 7.34 mmol) was dissolved in dry ethanol (10 mL) and refluxed for approximately 4 h. The solution was then filtered through filter paper followed by a PTFE filter (Millipore) attached to a syringe to yield pure **17** as a yellow oil in quantitative yield; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.61 (1H, d, J = 8.5 Hz, H-6), 6.57 (1H, d, J = 2.7 Hz, H-3), 6.48 (1H, dd, J = 8.5; 2.9 Hz, H-5), 2.16 (3H, s, H-7) ppm; ^{13}C NMR (CD_3OD, 100 MHz) δ 151.29 (C-4), 149.62 (C-1), 126.81 (C-2), 118.68 (C-3), 116.66 (C-6), 114.16 (C-5), 16.66 (C-7) ppm.

5.1.2.7 Preparation of compound 91

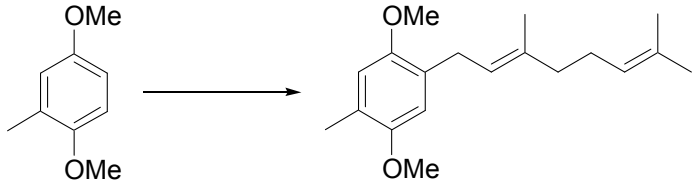


17 **91**

2-methylbenzene-1.4-diol (**17**, 625 mg, 5.08 mmol) was methylated with an aqueous solution of NaOH (336 mg, 8.4 mmol) and Me_2SO_4 (0.96 mL, 10.16 mmol) using method 3 (Section 5.1.1) described above. The reaction was quenched with water and

extracted with EtOAc (3 x 30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The reaction products were purified using column chromatography (1:5 ; EtOAc:Hex) to yield **91** (463 mg, 3 mmol, 60 %); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.74 (1H, d, *J* = 8.7 Hz, H-6), 6.73 (1H, d, *J* = 2.3 Hz, H-3), 6.68 (1H, dd, *J* = 3.1 ; 8.7 Hz, H-5), 3.78 (3H, s, OMe), 3.75 (3H, s, OMe), 2.21 (3H, s, H-7) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.4 (C-4), 152.1 (C-1), 127.8 (C-2), 117.1 (C-3), 110.9 (C-6), 110.7 (C-5), 55.9 (OMe), 55.7 (OMe), 16.4 (C-7).

5.1.2.8 Attempted preparation of compound 92 using the reaction protocol reported by Bouzbouz and Kirschleger.[128]

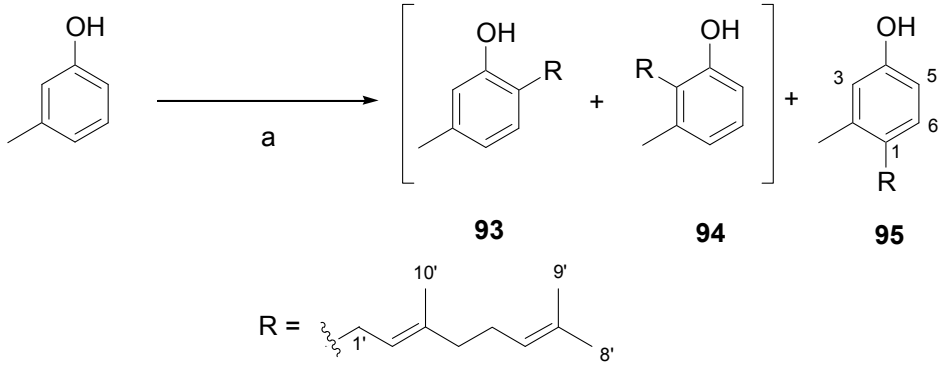


91 **92**

Compound **91** (100 mg, 0.66 mmol) was dried in a vacuum dessiccator for at least 1.5 h prior to being dissolved in dry THF (1 mL) under an argon atmosphere. The solution was cooled to 0 ^{o}C before adding *s*-BuLi (0.1 mL, 1.3 mmol) which resulted in the solution, turning dark yellow. The reaction mixture was allowed to stir for 2 h and then cooled to –70 ^{o}C before adding CuI (3 mg, 3 %) and geranyl bromide (0.086 mL, 0.45 mmol). The reaction was quenched by the addition of water and the products extracted with diethyl ether (3 x 15 mL). The combined organic extracts were subsequently washed with aq. NH_3, brine, aq. HCl, water, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. ^{1}H NMR analysis of the normal phase HPLC (10:1 ; Hex:EtOAc) fractions revealed only the presence of the two starting materials (**91** and geranyl bromide).

5.1.3 Phenoxide carbon-alkylation

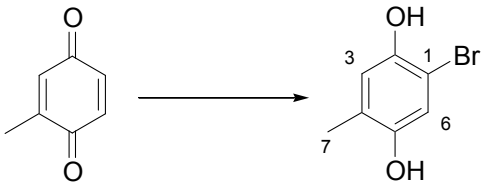
5.1.3.1 Preparation of compound 95 using the synthetic strategy reported by *Kornblum et al*[120]



m-Cresol (**79**, 0.48 mL, 4.6 mmol) was added to an aqueous solution of NaOH (2.7 mL, 4.6 mmol). The resulting solution was protected by a N_2 atmosphere before adding geranyl bromide (0.88 mL, 4.6 mmol) and stirred vigorously for 48 h. The products were then acidified (10 % HCl) and extracted with benzene (2 x 5 mL). The benzene extracts were combined dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. ^{1}H NMR analysis of the normal phase HPLC (7:1 ; Hex:EtOAc) fractions revealed a mixture of the two *ortho* isomers (**93** and **94**) which could not be separated, the *para* product (**95**) and un-reacted starting material. 2D NMR and HRFABMS was used to confirm the structure of **95**; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, d, *J* = 8.1 Hz, H-6), 6.63 (1H, d, *J* = 2.1 Hz, H-3), 6.59 (1H, dd, *J* = 8.0 ; 2.5 Hz, H-5), 5.21 (1H, t, *J* = 6.9 Hz, H-2'), 5.09 (1H, t, *J* = 6.6 Hz, H-6'), 3.22 (1H, d, *J* = 7.0 Hz, H-1'), 2.22 (3H, s, H-7), 2.08 (2 x 2H, m, H-4' ; H-5'), 1.69 (3H, s, H-10'), 1.67 (3H, s, H-8'), 1.59 (3H, s, H-9') ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.6 (C-4), 137.7 (C-2), 135.7 (C-3'), 132.0 (C-1), 131.4 (C-7'), 129.6 (C-6), 124.3 (C-6'), 122.9 (C-2'), 116.8 (C-3), 112.4 (C-5), 39.7 (C-4'), 31.2 (C-1'), 26.6 (C-5'), 25.7 (C-8'), 19.5 (C-7), 17.7 (C-9'), 16.0 (C-10') ppm; HRFABMS obsd. *m/z* 244.1827 calcd. for $C_{17}H_{24}O$ [M^+], 244.1827.

5.1.4 Metal halogen exchange

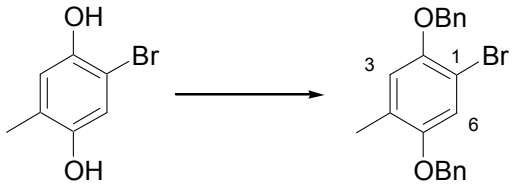
5.1.4.1 Preparation of 98 using the protocol outlined by Miller and Stewart[130]



18 **98**

1-methyl-1,4-benzoquinone (**18**, 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 acetonitrile (15 mL) and allowed to stir overnight at room temperature. The reaction was quenched by the addition of water (10 mL) and the products extracted with dichloromethane (3 x 30 mL). The organic layers were combined and rinsed with water (10 mL) followed by a saturated sodium chloride solution (10 mL). The resulting organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The products were purified using column chromatography (5:1 ; Hexane:EtOAc) which yielded pure **98** as a white amorphous solid (495 mg, 2.3 mmol, 61 %) IR V_{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 (2 x 1H, s, 2 x OH), 2.12 (1H, s, H-7) ppm; ^{13}C NMR (CD_3OD, 100 MHz) δ 150.4 (C-5), 148.1 (C-2), 126.7 (C-4), 119.6 (C-3), 119.5 (C-6), 107.3 (C-1), 16.3 (C-7) ppm; EIMS (70 eV): 204 (90), 203 [M^+] (23) 202 (100), 124 (29), 123 (59).

5.1.4.2 Preparation of 101

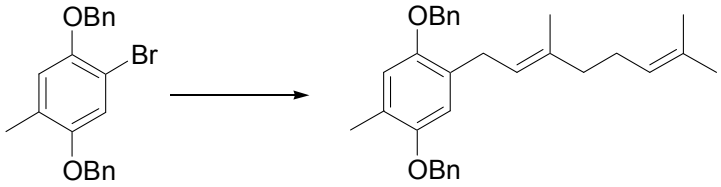


98 **101**

Compound **98** was dried in a desiccator overnight prior to use. Compound **98** (1 g, 5.28 mmol) was dissolved in dry acetone (15 mL) before the addition of benzyl

bromide (0.63 mL, 5.28 mmol), anhydrous K_2CO_3 (4.38 g, 31.68 mmol) and NaI (4.75 g, 31.68 mmol). After the reaction mixture was refluxed overnight, water was added and the products extracted with $CHCl_3$ (3 x 15 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give a dark brown amorphous solid. The product was purified using silica column chromatography to give **101** (1.6 g, 4.22 mmol, 80 %) as a white amorphous solid; IR V_{max}: 2916, 2870, 1501, 1382, 1203, 1011, 754 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.49-7.30 (10H, series of multiplets, Ar-H), 7.10 (1H, s, H-6), 6.82 (1H, s, H-3), 5.07 (2H, s, OCH_2Ph), 5.00 (2H, s, OCH_2Ph), 2.20 (3H, s, H-7) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.7, 149.2, 137.0, 136.9, 128.5, 128.4, 127.9, 127.8, 127.5, 127.2, 117.7, 117.0, 109.3, 72.0 (OCH_2Ph), 70.9 (OCH_2Ph), 16.4 (C-7) ppm; EIMS (70 eV): 383 [M^+] (29), 384 (24), 91 (100).

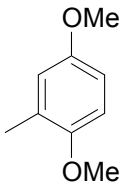
5.1.4.3 Attempted coupling of compound 101 with geranyl bromide using the reaction conditions reported by Odejinmi and Wiemer[133]



101

Before attempting the coupling reaction all apparatus and solvents (diethyl ether and benzene) were thoroughly died. The reaction was performed under an atmosphere of dry argon and the anhydrous conditions carefully maintained for the duration of the reaction. Compound **101** (200 mg, 0.52 mmol) was dissolved in anhydrous benzene (2 mL) and diethyl ether (4 mL) before adding *n*-BuLi (0.48 mL, 078 mmol). The reaction was allowed to stir for 5 min before adding CuBr.DMS (54 mg, 0.26 mmol) and another 30 min before adding the geranyl bromide (0.1 mL, 0.52 mmol). The reaction was then left to proceed for 5 h before quenching with a saturated aqueous NH_4Cl solution. The products were extracted with diethyl ether in the usual manner, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. ^1H NMR analysis of the crude products revealed a mixture of the starting products *viz.* **101** and geranyl bromide and no resonance indicating that coupling had been achieved.

5.1.4.4 Demethylation of compound 91



91

5.1.4.4.1 Attempted demethylation of 91 using *L*-Selectride following a protocol reported by Majetich *et al.*[135]

L-Selectride (0.23 mL, 1.30 mmol) was added to a solution of **91** (65 mg, 0.43 mmol) in dry THF (1 mL) and the reaction left to stir for 48 h. Water was added and the products extracted in $CHCl_3$ (3 x 5 mL). ^{1}H NMR analysis of the crude products only revealed resonance's corresponding to the starting material **91.** The two ^{1}H NMR resonance's at δ 3.82 and 3.77 which correspond to the two OMe protection groups could be seen clearly and each integrated perfectly for three protons with respect to the resonances at δ 6.74, 6.73 and 2.17 corresponding to H-3, H-6 and H-7 of **91** as described earlier.

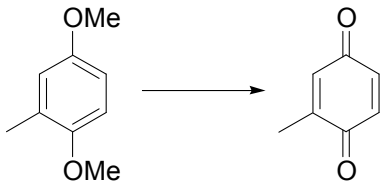
5.1.4.4.2 Attempted demethylation of 91 using Me_3SiI following a protocol reported by Jung and Lyster.[137]

The reaction was performed in a dry NMR tube under an argon atmosphere with a tightly sealed cap. Me_3SiI (0.08 mL, 0.56 mmol) was added to a solution of **91** (65 mg, 0.43 mmol) in $CDCl_3$ (0.5 mL) and the reaction monitored by ^{1}H NMR over 48 h. ^{1}H NMR integration of pertinent peaks revealed only partial demethylation of **91**. (The two ^{1}H NMR resonance's at δ 3.82 and 3.77 ppm corresponding to the two OMe protecting groups could be seen, however, the resonance at δ 3.77 ppm was greatly reduced in comparison with the resonance at δ 3.82 ppm. ^{1}H NMR integration of δ 3.77 ppm with the resonances at δ 6.74, 6.73 and 2.17 ppm corresponding to H-3, H-6 and H-7 of **91** confirmed that partial demethylation of **91** had taken place.)

5.1.4.4.3 Preparation of AgO[140]

NaOH (36 g, 0.9 mol) was dissolved in water (500 mL) at a temperature of approximately 85 ^{o}C. An aqueous slurry of potassium peroxydisulfate (37.5 g, 0.14 mol) and silver(I) nitrate (25.5 g, 0.15 mol) was then added to the stirred alkaline solution (NaOH solution) and the temperature increased to 90 ^{o}C for 15 min. The solution turned dark grey as AgO precipitated. The AgO precipitate was filtered and washed with a slightly alkaline solution to remove any residual sulfate ions. The product was allowed to air-dry overnight to give AgO (16 g, 86 %) as a dark grey powder.

5.1.4.4.4 Preparation of 18 using Snyder and Rapoport's[139] AgO oxidative demethylation procedure

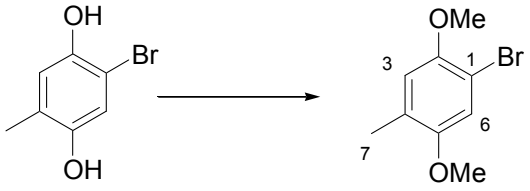


91 **18**

Compound **91** (30 mg, 0.2 mmol) was dissolved in dry dioxane (2 mL) followed by the addition of AgO (100 mg, 0.8 mmol). The mixture was sonicated to achieve a uniform dispersal of AgO (oxidant). The oxidation was then initiated by addition of 6 N HNO_3 (0.2 mL) and the reaction allowed to proceed until most of the AgO had been consumed (no longer than 2 min). The reaction was quenched by the addition of $CHCl_3$: H_2O (4:1 ; 10 mL) and the products extracted with $CHCl_3$ (3 x 8 mL). The combined organic extracts were washed with water (3 x 4 mL) to remove any excess acid, dried over anhydrous Na_2SO_4 and reduced *in vacuo*. The reaction was monitored using GLC. An aliquot of the reaction mixture and three standards (**18**, **91**, **17**) were compared using a Duraband DB-1 non-polar column to determine the degree of demethylation. The column temperature was increased from 100 to 200 ^{o}C at a rate of 15 ^{o}C/min and the standards **18**, **91** and **17** had R_T of 2.59, 5.28 and 6.17 min respectively. The aliquot of the reaction mixture revealed two peaks with R_T's of 2.49

min (major peak, 90 %) and 5.16 (minor peak, 10 %) corresponding to **18** and **91** respectively.

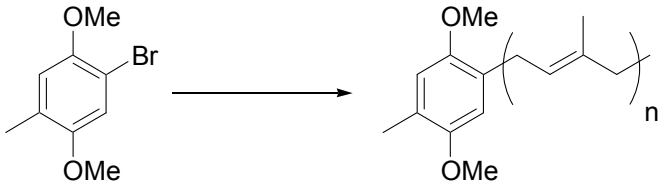
5.1.4.5 Preparation of compound 102



98 **102**

Compound **98** (586 mg, 2.89 mmol) was methylated with aqueous NaOH (170 mg, 4.20 mmol), and Me_2SO_4 (0.55 mL, 5.77 mmol) as described previously (method 3 ; Section 5.1.1) . The reaction mixture was quenched with water and extracted with EtOAc (3 x 15 mL). The EtOAc extracts combined, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The reaction products were purified using silica column chromatography (1:5 ; EtOAc:Hex) to yield **102 (**456 mg, 1.97 mmol, 68 %) as an amorphous white solid; IR V_{max}: 2945, 2833, 1505, 1217, 1037, 866, 788, 714 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, s, H-6), 6.73 (1H, s, H-3), 3.83 (3H, s, OMe), 3.77 (3H, s, OMe), 2.17 (3H, s, H-7) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 152.2 (C-5), 149.7 (C-2), 126.8 (C-4), 115.4 (CH), 115.3 (CH), 108.1 (C-1), 57.0 (OMe), 56.1 (OMe), 16.3 (C-7) ppm; EIMS (70 eV): 232 (100), 231 [M^+] (17), 230 (95), 217 (62), 216 (7), 215 (43); HRFABMS obsd. *m/z* 229.9943 calcd. for $C_9H_{11}O_2Br$ [M^+], 229.9942.

5.1.4.6 Attempted preparation of compound 92



102 **92** n = 2

1-bromo-2,5-dimethoxy-4-methylbenzene (**102**, 51.5 mg, 0.22 mmol) was dissolved in dry THF (2 mL) and cooled to –78 ^{o}C before adding *n*-BuLi (0.28 mL, 0.45 mmol). The reaction mixture was allowed to stir at –78 ^{o}C for 15 min before adding geranyl bromide (0.042 mL, 0.22 mmol) after which it was left to slowly warm to room temperature. The reaction was then quenched with saturated aqueous NH_4Cl (5 mL) and the products extracted with $CHCl_3$ (3 x 15 mL). The combined $CHCl_3$ extracts were dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. ^{1}H NMR analysis of the products revealed a mixture of **102**, **91** and geranyl bromide and no resonance indicating that coupling had been achieved.

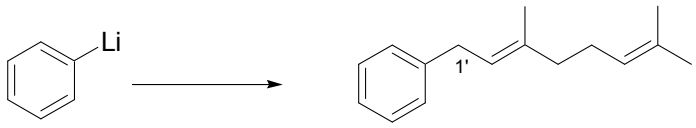
The same reaction protocol was repeated varying the type of alkylithium base (*n*-BuLi and *t*-BuLi), temperature (-78 ^{o}C and -55 ^{o}C), solvent (THF and diethyl ether) and additives (TMEDA, CuI). All glassware was oven dried at a temperature > 200 ^{o}C and the anhydrous reaction conditions carefully maintained for the duration of each experiment. Each experiment was also repeated at least twice without any evidence of coupling on the ^{1}H NMR spectrum. The details of the changes to the reaction conditions are provided as follows:

i. Compound **102** (50 mg, 0.22 mmol), *n*-BuLi (0.14 mL, 0.23 mmol), CuI (14.7 mg, 0.077 mmol) and geranyl bromide (0.042 mL, 0.22 mmol). The reaction was carried out in dry THF at approximately –78 ^{o}C.

ii. Compound **102** (100 mg, 0.43 mmol), *t*-BuLi (0.34 mL, 0.80 mmol) and geranyl bromide (0.082 mL, 0.43 mmol). The reaction was carried out in dry diethyl ether at approximately –55 ^{o}C.

iii. Compound **102** (100 mg, 0.43 mmol), *n*-BuLi (0.54 mL, 0.86 mmol), TMEDA (0.26 mL, 1.72 mmol), geranyl bromide (0.082 mL, 0.43 mmol). The reaction was carried out in dry diethyl ether at approximately –78 ^{o}C.

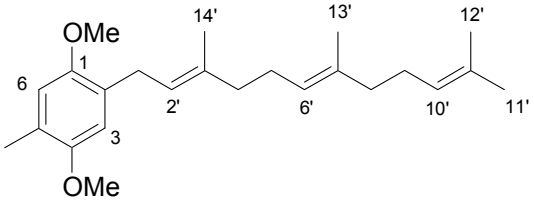
5.1.4.7 Preparation of compound 103



103

Geranyl bromide (0.23 mL, 1.19 mmol) was added to a solution of phenyllithium (0.12 mL, 1.19 mmol) in THF (2 mL) at –78 ^{o}C and left to slowly warm to room temperature. The reaction was quenched with saturated NH_4Cl (5 mL) and extracted with diethyl ether, dried using anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. ^{1}H NMR analysis revealed no evidence of coupling. The reaction was repeated at 0 and 25 ^{o}C in dry ether and monitored with ^{1}H NMR spectroscopy. Coupling could only be observed at 0 ^{o}C in diethyl ether; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28-7.14 (5H, Ar-H), 5.33 (1H, t, *J* = 7.1 Hz, H-2'), 5.09 (1H, t, *J* = 6.7 Hz, H-6'), 3.35 (2H, d, *J* = 7.3 Hz, H-1'), 2.13-2.02 (4H, m, H-4' and H-5'),), 1.70 (3H, s, H-10'), 1.67 (3H, s, H-8'), 1.59 (3H, s, H-9') ppm; HRFABMS obsd. *m/z* 214.1721 calcd. for $C_{16}H_{22}$ [M^{+}], 214.1724.

5.1.4.8 Preparation of compound 104

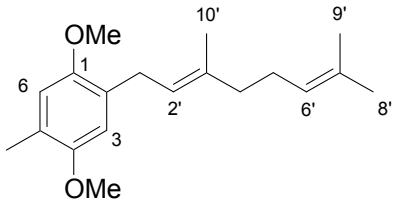


104

1-Bromo-2,5-dimethoxy-4-methylbenzene (**102**, 0.309 g, 1.34 mmol) was dissolved in dry ether (2 mL) under argon and 4 Å molecular sieves were added. The resulting solution was cooled to 0 ^{o}C before adding TMEDA (0.30 mL, 2.0 mmol) followed by *n*-BuLi (1.2 mL, 2.0 mmol) and allowed to stir for 15 min before adding farnesyl

bromide (0.36 mL, 1.34 mmol). The reaction was left to stir overnight and quenched the following day, using saturated aqueous NH_4Cl (5 mL). The aqueous solution was extracted with diethyl ether (3 x 5 mL). The ether extracts combined, washed with brine and dried over anhydrous $MgSO_4$. The ether extracts were filtered and concentrated *in vacuo*. The crude extract was purified using normal phase HPLC (10: 1 ; hexane:EtOAc) to yield **104** (0.129 g, 40 %) as a yellow oil; IR V_{max}: 2923, 2841, 1506, 1465, 1395, 1211, 1053 cm^{-1}; UV (MeOH) λ_{max} 290 (ε 2487), 230 (ε 3305) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 6.67 (1H, s, H-6), 6.66 (1H, s, H-3), 5.31 (1H, t, *J* = 7.2, H-2'), 5.13 (1H, t, *J* = 7.0 Hz, H-6'), 5.09 (1H, t, *J* = 7.4 Hz, H-10'), 3.77 (3H, s, OMe), 3.76 (3H, s, OMe), 3.30 (2H, d, *J* = 7.2 Hz, H-1'), 2.20 (3H, s, H-7), 2.05 (4 x 2H, m, H-4', H-8', H-5' and H-9'), 1.71 (3H, s, H-15'), 1.67 (3H, s, H-12'), 1.60 (2 x 3H, s, H-13' and H-14') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.6 (C-4), 151.0 (C-1), 136.1 (C-3'), 135.0 (C-7'), 131.2 (C-11'), 128.0 (C-2), 124.4 (C-6' and C-10'), 124.2 (C-5), 122.7 (C-2'), 114.1 (C-6), 112.4 (C-3), 56.3 (OMe), 56.1 (OMe), 39.8 (C-4'), 39.7 (C-8'), 28.2 (C-1'), 26.8 (C-5' and C-9'), 25.7 (C-12'), 17.7 (C-13'), 16.1 (C-15' and C-14'), 16.0 (C-7) ppm; HRFABMS obsd. *m/z* 356.2714 calcd. for $C_{24}H_{36}O_2$ [M^+], 356.2715.

5.1.4.9 Preparation of compound 92

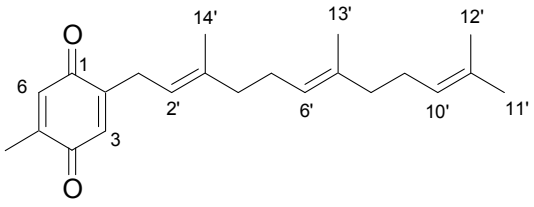


92

The same reaction conditions used for the preparation of **104** were used in the preparation of **92.** 1-Bromo-2,5-dimethoxy-4-methylbenzene (**102**, 1 g, 4.32 mmole) was dissolved in dry ether (8 mL) under argon and 4 Å molecular sieves were added. The resulting solution was cooled to 0 ^oC before adding TMEDA (0.98 mL, 6.5 mmol) followed by *n*-BuLi (4.1 mL, 6.5 mmol) and then allowed to stir for 15 min before adding geranyl bromide (0.82 mL, 4.32 mmol). The reaction was worked up as described previously for **104** to yield pure **92** (0.50 g, 1.7 mmol, 40 %) as a pale

yellow oil. IR V_{max}: 2915, 2849, 1506, 1400, 1211, 1051, 857, 651 cm^{-1}; UV (MeOH) λ_{max} 295 (ε 2972), 230 (ε 3140) nm; ^1H NMR (400 MHz, $CDCl_3$) δ 6.67 (1H, s, H-6), 6.66 (1H, s, H-3), 5.30 (1H, t, *J* = 7.3 Hz, H-2'), 5.11 (1H, t, *J* = 6.6 Hz, H-6'), 3.77 (3H, s, OMe), 3.76 (3H, s, OMe), 3.30 (2H, d, *J* = 7.2 Hz, H-1'), 2.19 (3H, s, H-7), 2.10 (2 x 2H, m, H-4' and H-5'), 1.70 (3H, s, H-10'), 1.66 (3H, s, H-8'), 1.59 (3H, s, H-9') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.6 (C-4), 151.0 (C-1), 136.1 (C-3'), 131.3 (C-7'), 128.0 (C-2), 124.4 (C-6'), 124.3 (C-5), 122.7 (C-2'), 114.1 (C-6), 112.4 (C-3), 56.3 (OMe), 56.1 (OMe), 39.8 (C-4'), 28.2 (C-1'), 26.8 (C-5'), 25.7 (C-8'), 17.7 (C-9'), 16.1 (C-10'), 16.1 (C-7) ppm; HRFABMS obsd. *m/z* 288.2090 calcd. for $C_{19}H_{28}O_2$ [M^+], 288.2089.

5.1.4.10 Preparation of compound 22

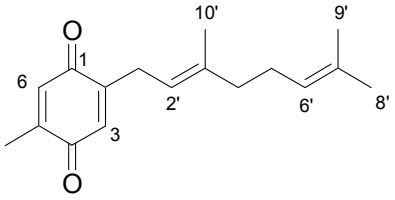


22

Compound **104** (0.19 g, 0.54 mmol), AgO (26.5 mg, 2,14 mmol) and dry dioxane (2 mL) were combined and the mixture briefly sonicated to obtain a uniform distribution of the oxidant (AgO). 6 N HNO_3 (0.2 mL) was then added and the reaction allowed to proceed until most of the AgO was consumed (approx 2 min). The reaction was quenched by addition to $CHCl_3$ - H_2O (4:1 ; 10 mL). The $CHCl_3$ layer was separated washed three times with H_2O (3 x 4 mL) to remove excess acid and dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Normal phase HPLC (10: 1 ; hexane:EtOAc) was used to isolate compound **22** (0.158 g, 0.49 mmol, 90 %) as a yellow oil; IR V_{max}: 2922, 1658, 1614, 1444, 1376, 1346, 1238, 1133, 909 cm^{-1}; UV (MeOH) λ_{max} 240 (ε 2968); ^1H NMR (400 MHz, $CDCl_3$) δ 6.57 (1H, dd, *J* = 2.9, 1.5 Hz, H-6), 6.49 (1H, t, *J* = 1.6 Hz, H-3), 5.14 (1H, t, *J* = 7.3 Hz, H-2'), 5.08 (2 x 1H, m, H-6' and H-10'), 3.09 (2H, d, *J* = 7.1 Hz, H-1'), 2.05 (4 x 2H, m, H-4', H-5', H-8' and H-9'), 2.01 (3H, d, *J* = 1.3 Hz, H-7), 1.66 (3H, s, H-12'), 1.61 (3H, s, H-15'), 1.58 (2 x 3H, s, H-13' and H-14') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.3 (C-4), 187.8 (C-1), 148.4 (C-2), 145.6 (C-5), 139.9 (C-3'), 135.4 (C-7'), 133.5 (C-6), 132.3

(C-3), 131.2 (C-11'), 124.3 (C-10'), 123.8 (6'), 117.9 (2'), 39.6 (C-4' and C-8'), 27.1 (C-1'), 26.7 (C-5'), 26.4 (C-9'), 25.7 (C-12'), 17.7 (C-13'), 16.1 (C-15'), 16.0 (C-14'), 15.4 (C-7) ppm; HRFABMS obsd. *m/z* 327.2323 calcd. for $C_{22}H_{31}O_2$ [M^+], 327.2324.

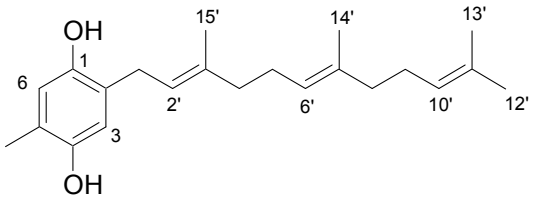
5.1.4.11 Preparation of compound 20



20

The same reaction conditions used for the preparation of **22** were used to prepare **20.** Compound **92** (0.15 g, 0.53 mmol), AgO (26.5 mg, 2,12 mmol) and dry dioxane (2 mL) were added together. The mixture briefly sonicated to obtain a uniform distribution of the oxidant (AgO). 6 N HNO_3 (0.4 mL) was then added and the reaction allowed to proceed until most of the AgO was consumed (approx 2 min). The reaction was worked up as described previously for **22**. Normal phase HPLC (10: 1 ; hexane:EtOAc) resulted in the isolation of pure **20** (0.12 g, 0.48 mmol, 90 %) as a yellow oil; IR V_{max}: 2924, 1657, 1377, 1239, 1134, 909 cm^{-1}; UV (MeOH) λ_{max} 240 (ε 3546); ^{1}H NMR (400MHz, $CDCl_3$) δ 6.57 (1H, td, *J* = 2.9, 1.5 Hz, H-6), 6.48 (1H, t, *J* = 1.8 Hz, H-3), 5.14 (1H, dt, *J* = 7.3, 7.3, 1.2 Hz, H-2'), 5.07 (1H, m, H-6'), 3.10 (2H, d, *J* = 7.1 Hz, H-1'), 2.06 (2 x 2H, m, H-4', H-5'), 2.02 (3H, d, *J* = 1.6, H-7), 1.68 (3H, s, H-8'), 1.60 (3H, s, H-10'), 1.59 (3H, s, H-9') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.3 (C-4), 187.9 (C-1), 148.5 (C-2), 145.6 (C-5), 139.8 (C-3'), 133.5 (C-6), 132.3 (C-3), 131.8 (C-7'), 123.9 (C-6'), 117.9 (C-7'), 39.6 (C-4'), 27.1 (C-1'), 26.4 (C-5'), 25.7 (C-8'), 17.7 (C-9'), 16.1 (C-10'), 15.4 (C-7) ppm; HRFABMS obsd. *m/z* 259.1698 calcd. for $C_{17}H_{23}O_2$ [M^+], 259.1697.

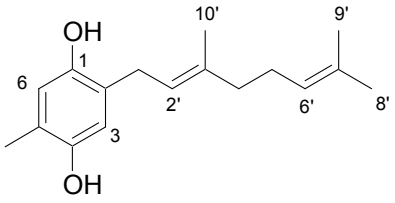
5.1.4.12 Preparation of compound 21



21

Compound **22** (87 mg, 0.27 mmol) was dissolved in $CHCl_3$ - ether (1:3) and shaken with a freshly prepared solution of sodium dithionite (0.33 g, 1.89 mmol) in water (4 mL). The organic layer was washed with brine and dried over anhydrous $MgSO_4$ before filtering and concentrating under reduced pressure to give **21** (93 mg, quantitative yield) as a dark brown oil (no further purification was done); IR V_{max}: 3395, 2926, 1647, 1513, 1376, 1192, 1044 cm^{-1}; UV (MeOH) λ_{max} 285 (ε 2260) and 245 (ε 2478); ^1H NMR (400 MHz, $CDCl_3$) δ 6.57 (1H, s, H-6), 6.53 (1H, s, H-3), 5.28 (1H, t, *J* = 7.0 Hz, H-2'), 5.08 (2 x 1H, m, H-6' and H-10'), 3.26 (2H, d, *J* = 7.1 Hz, H-1'), 2.16 (3H, s, H-7), 2.00 (4 x 2H, m, H-4, H-5', H-8' and H-9'), 1.74 (3H, s, H-15'), 1.67 (3H, s, H-12'), 1.59 (2 x 3H, s, H-13' and H-14') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8 (C-1), 147.4 (C-4), 138.3 (C-3'), 135.5 (C-7'), 131.3 (C-11'), 125.3 (C-2), 124.4 (C-10'), 123.7 (C-6'), 122.5 (C-5), 121.6 (C-2'), 118.1 (C-6), 116.2 (C-3), 39.7 (C-4' and C-8'), 29.3 (C-1'), 26.7 (C-5'), 26.4 (C-9'), 25.7 (C-12'), 17.7 (C-13'), 16.2 (C-15'), 16.0 (C-14'), 15.4 (C-7) ppm; HRFABMS obsd. *m/z* 328.2403 calcd. for $C_{22}H_{31}O_2$ $[M^+]$, 328.2402.

5.1.4.13 Preparation of compound 19



19

The same reaction conditions used for the preparation of **21** were used to prepare **19**. The details of reaction conditions are provided as follows: Compound **20** (81 mg, 0.32 mmol), sodium dithionite (0.39 g, 2.24 mmol). The reaction was worked up as

previously described for **21** and yield **19** (77 mg, 0.30 mmol, 93 %); IR V_{max}: 3392, 2928, 1191, 871 cm^{-1}; UV (MeOH) λ_{max} 295 (ε 3091) and 240 (ε 3343.8); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58 (1H, s, H-6), 6.53 (1H, s, H-3), 5.28 (1H, t, *J* = 6.6 Hz, H-2'), 5.07 (1H, t, *J* = 5.2 Hz, H-6'), 3.26 (2H, d, *J* = 7.0 Hz, H-1'), 2.16 (3H, s, H-7), 2.09 (2 x 2H, m, H-4', H-5'), 1.73 (3H, s, H-10'), 1.68 (3H, s, H-8'), 1.60 (3H, s, H-9') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8 (C-4), 147.4 (C-1), 138.2 (C-3'), 131.9 (C-7'), 125.4 (C-2), 123.9 (C-6'), 122.5 (C-5), 121.7 (C-2'), 118.1 (C-6), 116.2 (C-3), 39.7 (C-4'), 29.3 (C-1'), 26.4 (C-5'), 25.7 (C-8'), 17.7 (C-9'), 16.1 (C-10'), 15.4 (C-7); HRFABMS obsd. *m/z* 260.1776 calcd. for $C_{17}H_{24}O_2$ [M^+], 260.1775.

5.2 The anti-oesophageal cancer bioaasays (performed in at UCT)

5.2.1 Cell culture

Cells were maintained in a humidified, 37 °C, 5 % CO_2 incubator. WHCO1 oesophageal cancer cells were maintained in DMEM supplemented with 10 % fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin.

5.2.2 The crystal violet bioassay

1500 oesophageal cancer cells (WHCO1) were seeded per well in 96 well plates and incubated at 37 ^{o}C (24 h). DMSO solutions of the test compounds (**17-22, 91**, **92** and **104**) each in varying concentrations (100, 10, 1 and 0.1 μM) were added to the oesophageal cancer cells and incubated once again (48 h). The culture media from each of the wells was discarded and methanol (100 μL) added to fix the cells. After 10 min the methanol was replaced with the crystal violet solution (100 μL). The crystal violet solution was removed after 20 min and the plates rinsed thoroughly with distilled water before adding 50 % acetic acid (100 μL) to solubilise the protein dye. The plates were read at 595 nm on an Anthos microplate reader.

5.2.3 The MTT bioassay used to determine IC_{50} values

1500 oesophageal cancer cells (WHCO1) were seeded per well in 96 well plates. DMSO solutions of each of the target analogues (**17**-**22**) were plated in different concentrations and incubated at 37 ^{o}C (48 h). After 48 h the MTT (10 μL) solution was added to each well and incubated once more (4 h). Solubilisation solution (100 μL) was added to each well and incubated (37 ^{o}C) overnight before reading the plates at 595 nm on an Anthos microplate reader.

Chapter 6.

6 References

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