Structure and Synthesis of Bioactive Natural Products

Submitted in fulfilment of the requirements of the degree

Doctor of Philosophy

College of Agriculture, Engineering and Science

School of Chemistry and Physics

Pietermaritzburg

by

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Declaration

I hereby certify that this research is a result of my own investigation and has not already been submitted for any degree

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Abstract

Selected South African medicinal plants were screened *in vitro* for the anti-HIV activity using the HIV-RT colorimetric assay and XTT cell viability assay. In the cell-based assay the plant extracts screened exhibited no anti-HIV activity and most plant extracts were not highly toxic, with a few exceptions. In the HIV-RT assay extracts of *Harpephyllum caffrum, Combretum kraussii, Plumbago spp., Berkheya speciosa, Polygala fruticosa, Vernonia glabra, Lippia javanica, Smilax anceps,* and *Vernonia spp* showed inhibition greater than 50% at a concentration of 500 µg/mL. *Combretum kraussii s*tem extract inhibited 70% of the HIV-RT and the leaf extract inhibited 67%. Because of these results obtained for *Combretum kraussii*, the leaf extract was investigated further resulting in the isolation of three compounds, combretastatin B-1, combretastatin B-5, and combretastatin B-1 2-β-D-glucoside. These compounds were not investigated further because of the non-activity observed on the cell-based assay. The plant metabolites arzanol and lepidissipyrone were chosen for synthesis.

Arzanol, a prenylated α-pyrone-phloroglucinol, isolated from *Helichrysum italicum* ssp. *microphyllum*, exhibits antioxidant, anti-inflammatory and anti-HIV activities. Despite failure to complete its total synthesis, its two precursors, 2-(2-ethyl-1,3-dioxolan-2-yl)propanal and ethyl 3-(3-acetyl-2,4,6-tribenzoxyphenyl)propanoate were successfully synthesised. Lepidissipyrone, the α-pyrone flavanone structurally similar to arzanol, was isolated from *Helichrysum lepidissimum* and from *Helichrysum excisum*. Both species are endemic to South Africa. The first total synthesis of lepidissipyrone was successfully achieved by a multicomponent Carba-Betti strategy to couple 6-ethyl-4-hydroxy-5-methyl-α-pyrone and 7-*tert*-butyldimethylsilyloxy-5-hydroxyflavanone. During the last step of the total synthesis of lepidissipyrone, helipyrone was also synthesised.

Various structural analogues of α-pyrone, *i.e.* 4-hydroxy-5,6-dimethyl-α-pyrone and 4-hydroxy-5-methyl-6-propyl-α-pyrone and acylphloroglucinols, *i.e.* 2,4-bis(*tert*-butyldimethylsilyl)phloroacetophenone, 2,4-bis(*tert*-butyldimethylsilyl)-3-prenylphloroacetophenone, and 2,4-bis(*tert*-butyldimethylsilyl)- 3-prenylisobutyrophenone, were synthesised. These analogues could be used to synthesise arzanol derivatives

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List of Abbreviations

$(CH_2O)n$	Paraformaldehyde
AIDS	Acquired immune deficiency syndrome
AlBN	Azobisisobutyronitrile
ARVs	Antiretroviral drugs
AZT	Azidothymidine
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4
COSY	Correlation spectroscopy
CSA	Camphorsulfonic acid
CTCL	Cutaneous T-cell lymphoma
CXCR4	C-X-C chemokine receptor type 4
d	Doublets
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DEPT	Distortionless enhancement by polarization transfer
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess–Martin periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EC_{50}	Half maximal effective concentration
ELISA	Enzyme-linked immunosorbent assay
$Eu(fod)_3$	$Eu(OCC(CH_3)_3CHCOC_3F_7)_3$
FDA	United State Food and Drug Administration
gp	Glycoprotein
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple-bond correlation
HRESI	High-resolution electrospray ionisation
HRESIMS	High-resolution electrospray ionisation mass spectrometry
HSQC	Heteronuclear single-quantum correlation
$HTLV_{IIIB}$	Human T-lymphotropic virus type III
HWE	Horner–Wadsworth–Emmons reaction
IBX	2-Iodoxybenzoic acid

IC_{50}	Half maximal inhibitory concentration
IKK	IκB kinase
IR	Infrared spectroscopy
J	Coupling constant
LDA	Lithium diisopropylamide
m	Multiplet
MOM	Methoxymethyl
MOMCl	Chloromethyl methyl ether
MS	Mass spectrometry
MT-4	Metallothionein 4, human T cell line
MTT	3-{4,5-Dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide
n-BuLi	*n*-Butyllithium
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NMR	Nuclear magnetic resonance
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTIs	Nucleoside reverse transcriptase inhibitors
NtRTIs	Nucleotide reverse transcriptase inhibitors
OD	Optical density
PBS	Phosphate buffered saline
$Pd(CH_3CN)_2Cl_2$	Palladium(II) chloride diacetonitrile complex
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladium(0)
pH	Measure of the acidity or basicity
Ph	Phenyl
PI	Protease inhibitor
PPA	Polyphosphoric
p-TsOH	*p*-Toluenesulfonic acid
q	Quartet
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
RT	Reverse transcription
s	Singlet
t	Triplet
TB	Tuberculosis

TBDMS	*tert*-Butyldimethylsilyl
TCID50	50% Tissue culture infective dose
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TNF-α	Tumour necrosis factor alpha
UNAIDS	Joint United Nations Programme on HIV/AIDS
VERO cells	A line of cells derived from monkey kidney
WHO	World Health Organization
XTT	(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide)

CHAPTER 1

Introduction

1.1 DRUG DISCOVERY FROM MEDICINAL PLANTS

Nature has provided mankind with therapeutic agents for thousands of years, and a large fraction of modern marketed drugs are derived from natural products. Many of the plant-derived bioactive natural products were discovered based on the ethnopharmacological uses of the plants. Plants in particular have provided humans with cures to or alleviation of many infectious diseases and, even today, they continue to play an important role in primary health care as therapeutic agents in developing countries.[1, 2] Besides their medicinal use, plant products have also been used as poisons for use in warfare and hunting, as effective agents in euthanasia and capital punishment and as narcotics, hallucinogens, or stimulants to relieve the tedium or alleviate fatigue and hunger.[3] It has been shown that medicinal plants have a much higher chance of producing bioactive compounds and thus play a crucial role in drug discovery and development.[2]

Africa is an important producer of a great number of medicinal plants to the world's drug, food, and dietary supplement market. In Asian cultures like China and India medicinal plants usage has been documented for at least 3500 years.[4] However, because African knowledge has been transferred orally, much of ethnobotany in Africa has only been documented after the arrival of Arabic and later European botanists during the past millennium. Important products from Africa include the beverage rooibos from A*spalathus linearis* (Fabaceae),[5] the prostate remedy pygeum, also known as African prune from *Prunus africana* or *Pygeum africanum* (Rosaceae),[6] the appetite suppressant and dietary supplement Hoodia from *Hoodia gordonii* (Apocynaceae),[7] the cosmetic ingredient shea butter from *Vitellaria paradoxa* (Sapotaceae),[8] and the classic aphrodisiac herb used in psychiatry, yohimbe from *Pausinystalia johimbe* (Rubiaceae).[9] Also based on clinical trials umckaloaba, an extract of the roots of the South African *Pelargonium sidoides* (Geraniaceae)[10] is used in the treatment of tonsillitis, bronchitis, and other upper respiratory tract infections (Figure 1.1).[4]

Aspalathus linearis[11]

Prunus africana[12]

Hoodia gordonii[12]

Vitellaria paradoxa[13]

Pausinystalia johimbe[14]

Pelargonium sidoides[15]

FIGURE 1.1: African medicinal plants.

South Africa, especially the province of KwaZulu-Natal, has a rich heritage of knowledge on the uses of plant material. In South Africa there are an estimated 200 000 indigenous healers and 24 000 species with about 4 000 species of plants that are used as traditional medicines.[16] However, the chemistry and pharmacology of many of these plants have not yet been investigated. It is only recently that the South African health authorities acknowledged traditional

medicines. The South African government is now promoting the integration of traditional healing into the official health care system as a way of recognising the usefulness of traditional medicine.[17, 18]

The era in drug discovery from plants was marked by the isolation of the pure isoquinoline alkaloid morphine **(1.1)** from *Papaver somniferum* in 1805, however its structure was only elucidated in 1923.[19, 20] The 19th century marked the discovery of a number of plant-derived drugs such as atropine **(1.2)** from *Atropa belladonna*,[21] caffeine **(1.3)** from *Coffea arabica*,[22] cocaine **(1.4)** from *Erythroxylum coca*,[23] ephedrine **(1.5)** from *Ephedra* species,[24] codeine **(1.6)** from *Papaver somniferum*,[20] pilocarpine **(1.7)** from *Pilocarpus jaborandi*,[25] physostigmine **(1.8)** from *Physostigma venenosum*,[26] quinine **(1.9)** from *Cinchona cordifolia*,[27] salicin **(1.10)** from *Salix* species,[28] theobromine **(1.11**) from *Theobroma cacao*,[29] theophylline **(1.12**) from *Camellia sinensis*[30] and tubocurarine **(1.13)** from *Chondodendron tomentosum*[31] (Figure 1.2).[32]

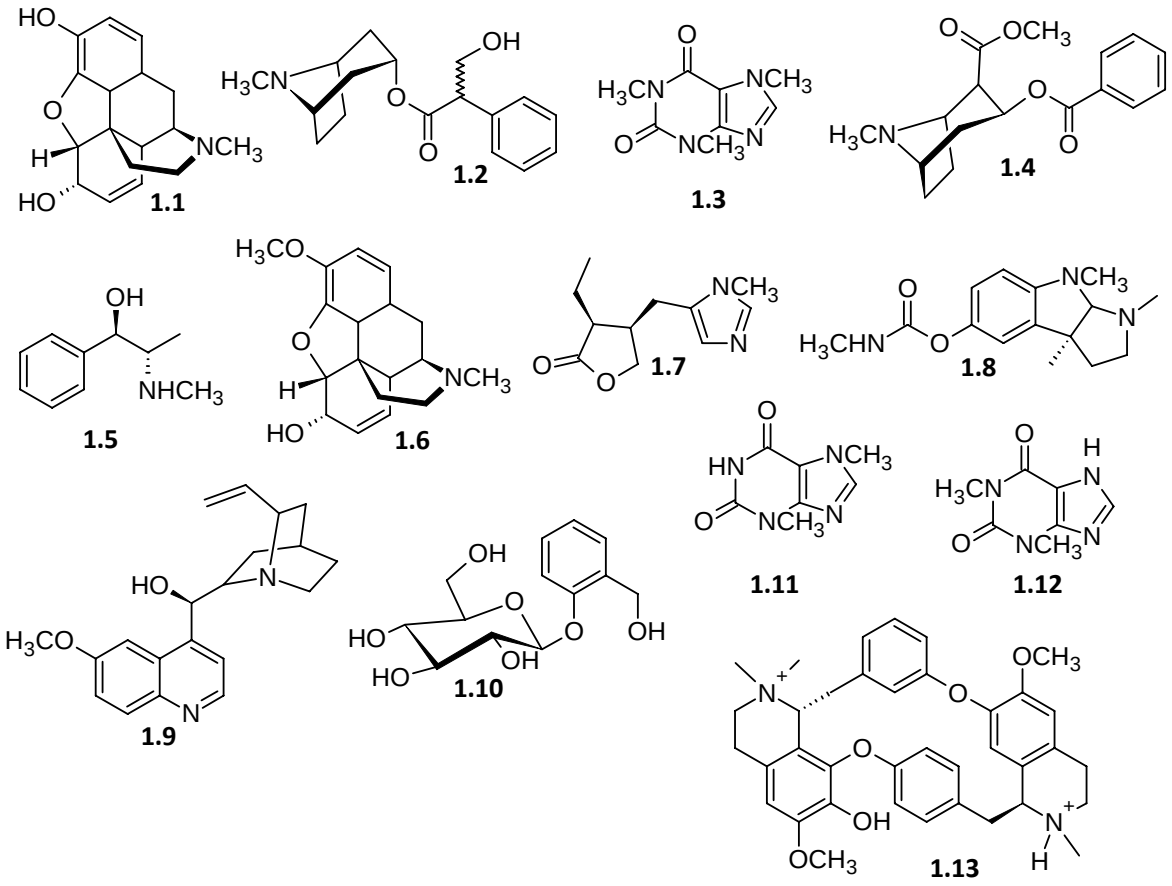
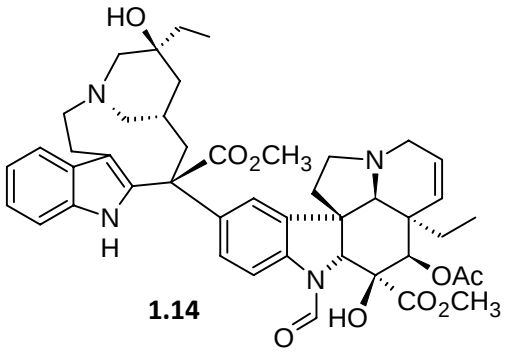


FIGURE 1.2: Early plant-derived drugs.

This was the beginning of a new era in medicine, where compounds were extracted and isolated from plants and administered as drugs in precise dosages, regardless of the source or age of the

plant material. Even today, plants continue to provide lead compounds for the development of drugs against various pharmacological targets. Medicinal plants are once again becoming more popular in the form of nutraceuticals, food supplements, and complementary and alternative medicine.[3, 33-35]

Over 50% of all currently marketed drugs are either derived from or inspired by natural products and several plant extracts and secondary metabolites are currently in clinical trials for the treatment of various diseases including cancer, HIV/AIDS, malaria, Alzheimer's and pain. [35-37] Besides the direct usage of secondary metabolites in their unmodified state as drugs, these compounds are also used as drug precursors, templates for synthetic modification and pharmacological probes. Vincristine **(1.14)** from *Catharanthus roseus* is used in cancer chemotherapy in its original form, diosgenin **(1.15)** from *Dioscorea floribunda* is used as a precursor for the synthesis of progesterone **(1.16)**, an oral contraceptive which is also a key intermediate in the synthesis of cortisone **(1.17)**, an important anti-inflammatory drug, and penicillin from *Penicillium notatum* and its synthetic analogues **(1.18)** are used in the treatment of bacterial infections. [3, 38]



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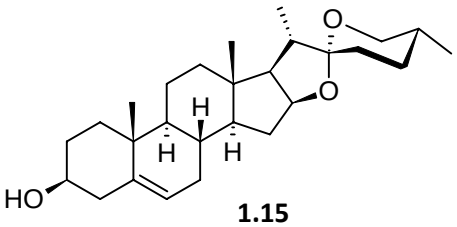

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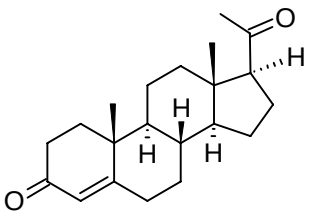

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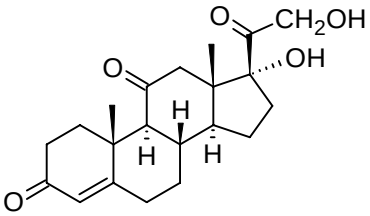

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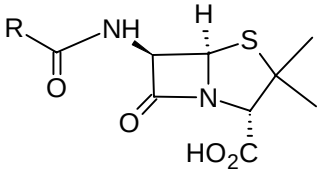
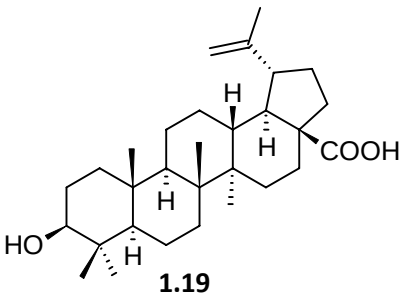


1.18

After more than three decades of battling with Acquired Immune Deficiency Syndrome (AIDS), and its causative agent Human Immunodeficiency Virus (HIV), HIV still remains one of the leading

infectious diseases globally. Attention has been focused on natural products, particularly plant-derived natural products, as sources of potential new lead compounds for anti-HIV agents. Plants have the ability to produce a vast number of secondary metabolites ranging in chemical complexity and biological activities.[39] Thus, the screening of plant extracts is of interest for the discovery of new drugs effective in the treatment of HIV. To date, no plant-derived drug has entered the pharmaceutical market for the treatment of HIV/AIDS; however a number of secondary metabolites have been reviewed as potential HIV inhibitors and drugs for AIDS treatments. Clinical trials have been conducted on some secondary metabolites and their synthetic analogues.

Betulinic acid **(1.19)** isolated from the leaves of *Syzigium claviflorum* and also from the London plane tree *Platanus acerifolia*, exhibited anti-HIV activity. Subsequent modification of betulinic acid by esterification of the C-3 hydroxy, successfully increased its anti-HIV potency and resulted in the discovery of a betulinic acid derivative, 3-*O*-(3',3'-dimethylsuccinyl)betulinic acid (also known as PA-457, YK-FH312, bevirimat®, BVM, DSB, and MPC-4326) **(1.20)**. Studies confirm a unique effect of PA-457 on viral budding/maturation, a completely novel mechanism of action amongst current HIV drugs. PA-457 is the first maturation inhibitor to have passed in phase II clinical trials.[40-42] However the clinical trials revealed a high baseline drug resistance.[40] The resistance posed serious limitations to further development of PA-457; as a result, analogues of PA-457 have been developed which may serve as new leads for development of anti-AIDS clinical trial candidates. The most promising analogue so far is *N*-[3β-*O*-(3',3'-dimethylsuccinyl)lup-20(29)-en-28-oyl]-5-piperazinepentanoic acid **(1.21)** with an IC_{50} value of 0.0059 µM compared to 0.087 µM for PA-457.[41] Other plant metabolites to have undergone clinical trials include (+)-calanolide A **(1.22)** isolated from the fruits and twigs of Malaysian tropical rainforest plant *Calophyllum lanigerum* in 1992.[43] (+)-Calanolide A is the first natural product identified as active against HIV reverse transcriptase and was investigated in phase II/III clinical trials. It also inhibits *Mycobacterium tuberculosis* and is thus a possible therapeutic agent for AIDS or TB or both. [34, 44-47]



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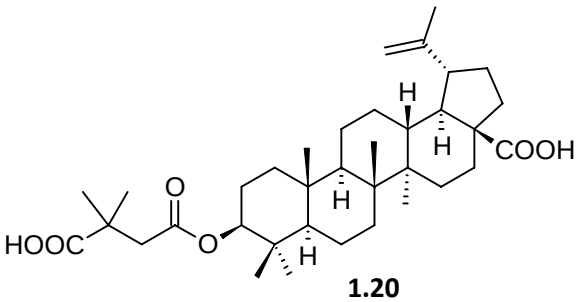

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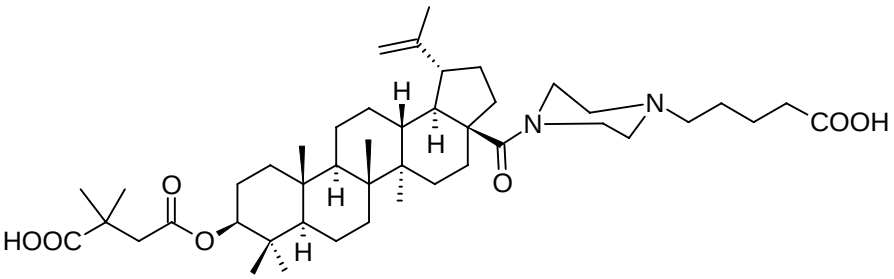

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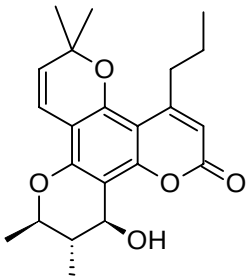


1.22

There is a renewed interest in discovery of novel and interesting chemical scaffolds from natural products research due to developments in the areas of separation and spectroscopic techniques. Current research strategies to drug discovery include: *in vitro* screening assay; extraction, isolation and identification of active lead compounds from natural sources; production of libraries of natural products; production of active compounds in cell or tissue culture, genetic manipulation, natural combinatorial chemistry; stronger focus on bioactivity; introduction of the concepts dereplication, chemical fingerprinting and metabolomics; and the selection of species based on ethnopharmacological information, indigenous knowledge and also those randomly selected.[38] Natural products will certainly continue to be viewed as one of the major sources of new drugs in the years to come because of incomparable structural diversity they offer, many of which that are relatively small and have drug-like properties (*i.e*. they can be absorbed and metabolized).[38, 48, 49]

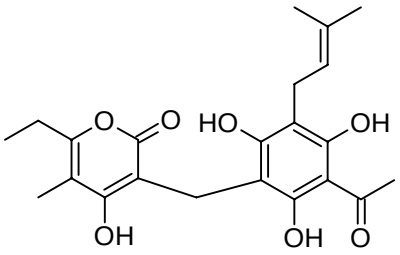
1.2 AIM OF THE STUDY

The study was aimed at combining natural products research and organic synthesis, with the main focus on anti-HIV activity of randomly selected South African medicinal plants and the development of a synthetic route for the preparation of bioactive compounds.

The aim of this study was to investigate natural products from South African plants for potential anti-HIV activity. The specific objectives were:

- To investigate the *in vitro* inhibition of HIV reverse transcriptase by selected South African medicinal plant extracts using a calorimetric assay.
- To investigate the *in vitro* anti-HIV activity of the plant extracts using a XTT cell viability assay.

- To isolate and characterize secondary metabolites from *Combretum kraussii*, a plant that showed significant inhibition of the HIV reverse transcriptase.
- To develop a method for the synthesis of an anti-HIV active compound, arzanol **(1.23)**, its acylphloroglucinol and α-pyrone derivatives **1.24** and a structurally similar flavanone, lepidissipyrone **(1.25)**.



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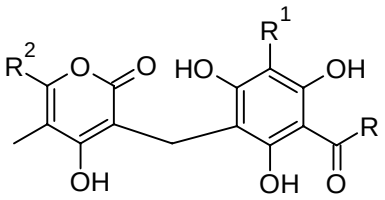
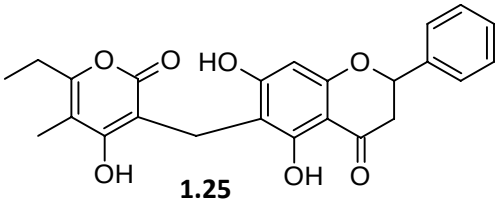


124 R = CH_3, $CH(CH_3)_2$

R^1 = H, $CH_2CH{=}C(CH_3)_2$

R^2 = CH_3, CH_2CH_3, $CH_2CH_2CH_3$



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Chapter 2 will focus on HIV and its impact, current HIV treatment and limitation, and a brief overview of plant metabolites with anti-HIV activity. Chapter 3 will discuss the techniques used and the results obtained from the *in vitro* anti-HIV screening of selected South African medicinal plant. Chapter 4 will focus on the phytochemical studies of *Combretum kraussii*. Chapter 5 will discuss the route toward the synthesis of arzanol, lepidissipyrone, and phloroglucinol and α-pyrone derivatives. Lastly, will be the conclusion and future work in chapter 6.

CHAPTER 2

Plant metabolites with HIV reverse transcriptase inhibitory activity

2.1 HIV/AIDS AND ITS IMPACT

Acquired ***I***mmune ***D***eficiency ***S***yndrome (AIDS) has become one of the most devastating infectious diseases to have emerged in recent history since it was first discovered among young homosexual men in the early 1980s.[50, 51] A retrovirus, ***H***uman ***I***mmunodeficiency ***V***irus (HIV) was subsequently identified as the causative agent. According to the World Health Organization (WHO) in 2012 HIV/AIDS was the leading cause of death in Africa and sixth in the world.[52] HIV progressively damages and eventually destroys the immune system resulting in the inability of the body to fight off viruses, fungi and bacteria. This makes one susceptible to fatal opportunistic infections such as tuberculosis, pneumonia, meningitis, certain cancers and other parasitic, fungal and viral infections.[53]

There are two strains of the virus; HIV-1 and HIV-2. HIV-1 was first reported in 1981 and HIV-2 in 1986. The strains are similar in many ways; they are transmitted from one person to another in the same way, *i.e.* through unsafe sexual intercourse with an infected person, contact with the blood of an infected person, blood transfusion, mother-to-child during pregnancy, labour, delivery or breastfeeding, and sharing injection needles with an infected person. HIV-1 is more common, by far the more virulent and more easily transmitted strain. It also spreads easily and therefore accounts for the vast majority of global HIV infections. HIV-2 occurs in different parts of the world; it is highly concentrated in West African countries such as Senegal, Nigeria, Ghana, and the Ivory Coast and those countries with strong ties to West Africa, Portugal, France, and Angola, to name three, but very few cases have been reported outside of the areas mentioned. HIV-1 is also known to mutate more efficiently than HIV-2 and generally progresses to AIDS at a significantly faster rate than HIV-2. HIV-2 has a longer incubation period and weakens the immune system more slowly than HIV-1 as it is less infectious in the early stages.[54] Without anti-HIV treatment, HIV infection can quickly progress to AIDS and eventually death.

According to the global estimates from UNAIDS/WHO Epidemic Update (published by WHO in December 2013), an estimated 36 million people have died of HIV/AIDS since it was first reported in early eighties.[52] Around 35 million people are now living with HIV globally (Figure 2.1) and 2013 alone saw 2.1 million newly infected people and 1.5 million deaths. Sub-Saharan Africa is more affected by HIV/AIDS than any other region in the world with nearly 24.7 million people living with HIV and accounting for about 71% of all people living with HIV worldwide. The factors contributing to such large numbers of HIV infection in South Africa include poverty, inequality and social instability, high levels of sexual transmitted infections, the low status of women, sexual violence, high mobility (particularly migrant labour), limited and uneven access to quality health care, and a history of poor leadership in the response to the epidemic.

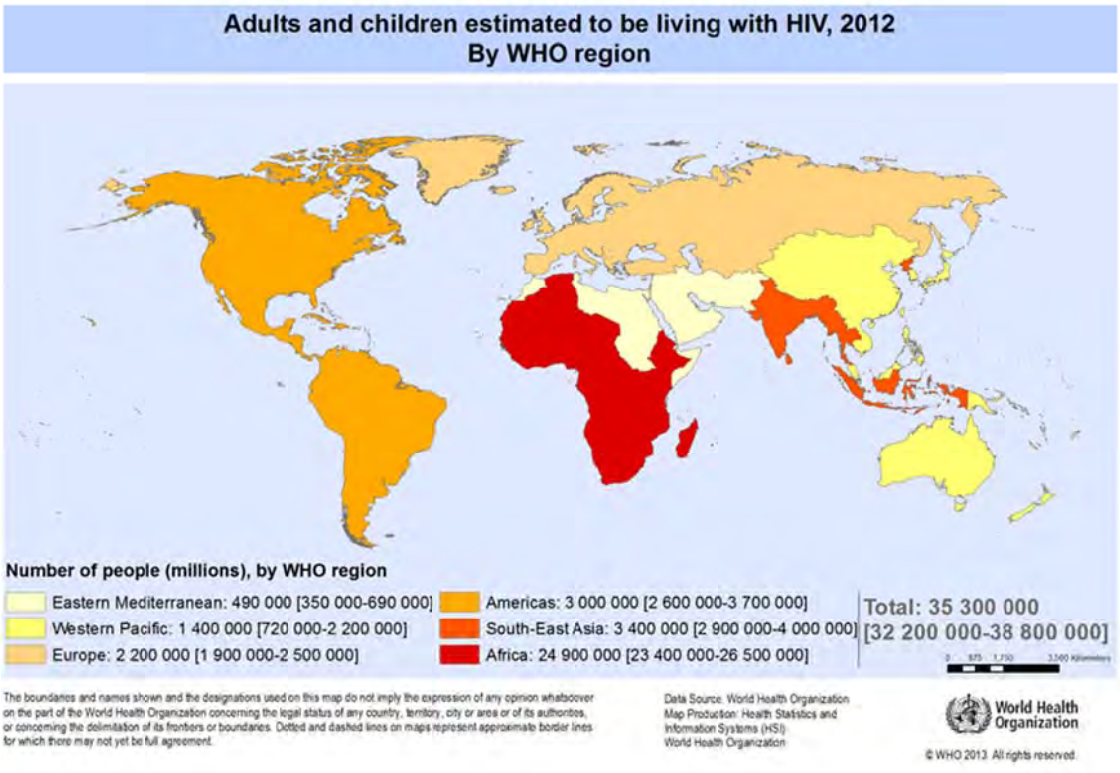


FIGURE 2.1: HIV prevalence by WHO region.[52]

2.2 HIV LIFE CYCLE

The HIV life cycle can be roughly divided into 8 different steps (Figure 2.2), *i.e.*, virus-cell adsorption, virus-cell fusion, reverse transcription, integration, DNA replication, translation, budding (assembly/release), and maturation.[53] HIV replication begins with the attachment of the gp120 glycoprotein to the CD4 receptor and one of the co-receptors (either CCR5 or CXCR4) on

the surface of the host CD4+ T-lymphocyte. T-lymphocytes are a type of white blood cell that detects and fight foreign invaders of the body. This is then followed by the fusion of the viral envelope with the cell membrane and this process is facilitated by gp41. The virus then releases its genetic material (RNA) into the host cell. Inside the cell the enzyme reverse transcriptase converts the single-stranded RNA to double-stranded DNA; the process is called reverse transcription. The viral DNA is transported to the cell nucleus where it is then integrated into the host cell's genome by the enzyme integrase; the process is called integration. The integrated DNA is called the provirus. The provirus may lie dormant for several years producing few or no new copies of HIV. The provirus is copied to shorter strands of RNA called messenger RNA (mRNA). These mRNAs are spliced into regulatory proteins Tat, Rev, and Nef, which encourage new virus production. The full length mRNA produces structural proteins Gag and Env. The viral core is formed by the assembly of these proteins, enzymes, and genomic RNA at the plasma membrane of the cells.[55] The HIV lifecycle affords multiple avenues of drug intervention.

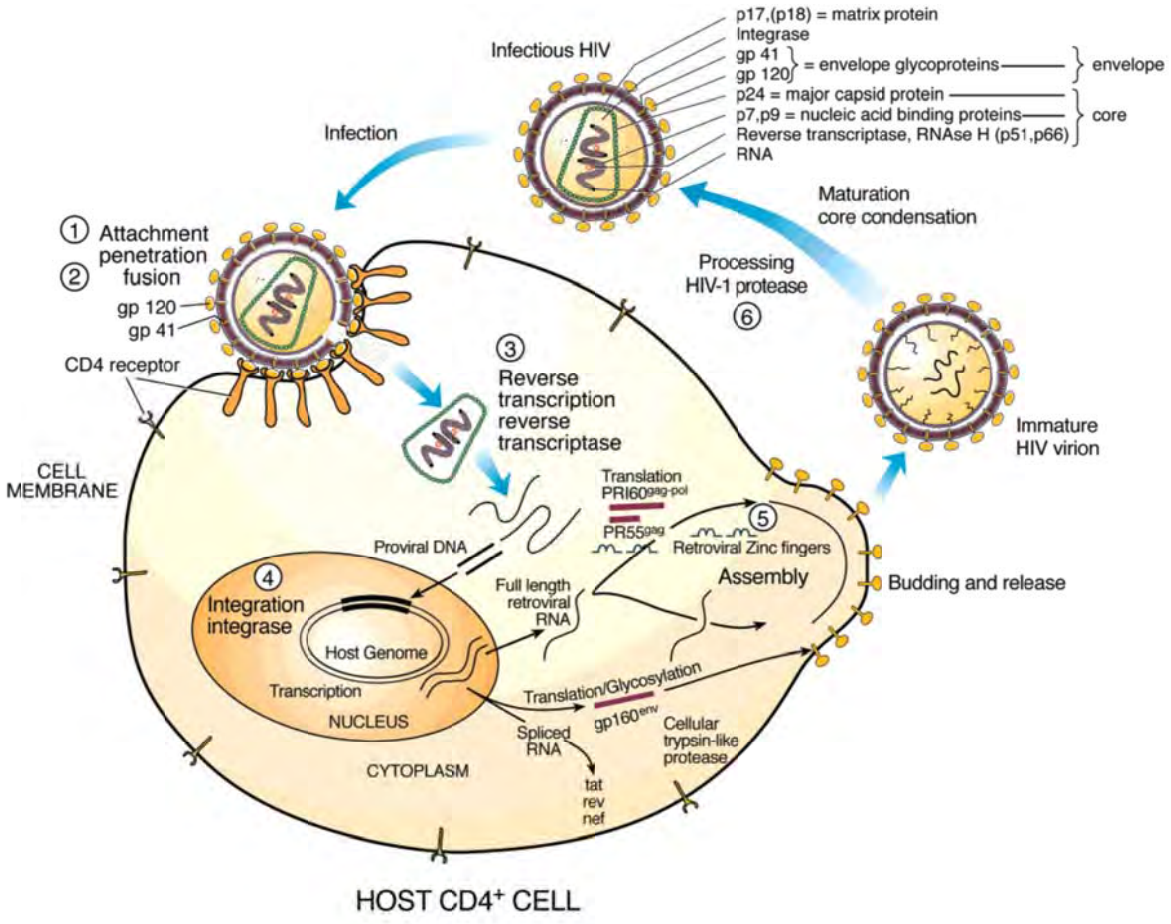


FIGURE 2.2: HIV life cycle.[55]

2.3 CURRENT HIV/AIDS TREATMENT AND LIMITATIONS

To date there is no cure for HIV/AIDS; however anti-HIV drugs, known as antiretroviral drugs (ARVs), have been approved by the United States Food and Drug Administration (FDA). For effective treatment, more than one antiretroviral drug has to be administered at a time, this is known as the Highly Active Antiretroviral Therapy (HAART). Early attempts at use of single-agent therapy resulted in viral resistance. Antiretroviral regimens today typically have three drugs, consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) with either a boosted protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI). Their goal of therapy is to suppress the HIV viral load, restore the immune function, prevent the HIV transmission, and prevent the resistance thus leading to a remarkable improvement in quality of life and increased life expectancy.[53, 56] ARVs have transformed HIV infection from being a near-certainly fatal affliction to a chronically manageable illness. Over 28 million people are eligible for antiretroviral therapy, under WHO 2013 consolidated ARV guidelines and 11.7 million had access to antiretroviral therapy in low and middle income countries. Despite the beneficial effects of ARVs in improving the quality of life of HIV/AIDS patients, they have been unable to eliminate HIV in a patient after infection and the emergence of resistant viruses, appreciable levels of toxicity, high cost, and above all the lack of a curative effect are their major short-comings. More patients are taking antiretroviral drugs (ARVs) for longer periods of time, which naturally results in more observed toxicity.[57] The side effects experienced by patients on HAART range in severity from skin rashes and gastrointestinal intolerance to coronary artery disease, nephrotoxicity, and bone marrow suppression. For the more severe conditions, discontinuation of the causative antiretroviral agent is recommended.[58]

While the global prevalence of HIV infection appears to have stabilized in recent years, the total number of people living with HIV is increasing because of on-going accumulation of new infections and longer survival times due to the beneficial impact of antiretroviral therapy, measured over a continuously growing general population. A combination of prevention and treatment modalities is still needed to successfully control the spread of the disease. Thus, the search for new and better anti-HIV drugs is still a global concern, to overcome the drawbacks of current drugs.[56] ARVs interfere with the different stages of the HIV-life cycle therefore slowing down the HIV replication, thus lowering mortality and morbidity in HIV-infected patients. One critical and unique step is the reverse transcription (RT) of the genetic material. In spite of more than 20 years of developments, RT remains an important target in antiretroviral therapy, as most FDA approved individual agents for the treatment of HIV-1 infection target its polymerization

active site.[59] The classes of anti-HIV drugs include the fusion/entry inhibitors, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors and maturation inhibitors.

2.4 HIV-REVERSE TRANSCRIPTASE INHIBITORS

HIV Reverse transcriptase inhibitors are responsible for inhibitory activity of the enzyme reverse transcriptase, responsible for the conversion of the single-stranded viral RNA into double-stranded DNA which is the most attractive target for anti-HIV drug development. Reverse transcriptase inhibitors are divided into three types, non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitor (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTIs)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are structurally diverse compounds as seen in Figure 2.3. However, their mode of action is the same; they interfere with the RT activity by binding noncompetitively with an allosteric site of the enzyme preventing the enzyme from converting the viral RNA to DNA. Thus HIV genetic material cannot be incorporated into the healthy genetic material of the cell and the cell cannot produce new virus. As of September 2014, there are five FDA approved NNRTIs; nevirapine (**2.1**, Sustiva®), etravirine (**2.2**, Entelence®), efavirenz (**2.3**, Viramune®) and delavirdine (**2.4**, Rescriptor®) and rilpivirine (**2.5**, Edurant®). Nevirapine was the first NNRTI, and was approved in 1996. NNRTIs are selective inhibitors of RT of only HIV-1 and not of HIV-2 or any other retrovirus (such as hepatitis and herpes viruses). However, there have been reports of quick development of drug resistance to single medicine NNRT treatment due to drug-specific mutations of the RT, therefore, they are used in combination with other inhibitors to reduce and prevent the development of resistance. NNRTIs are classified as non-competitive inhibitors.[60]

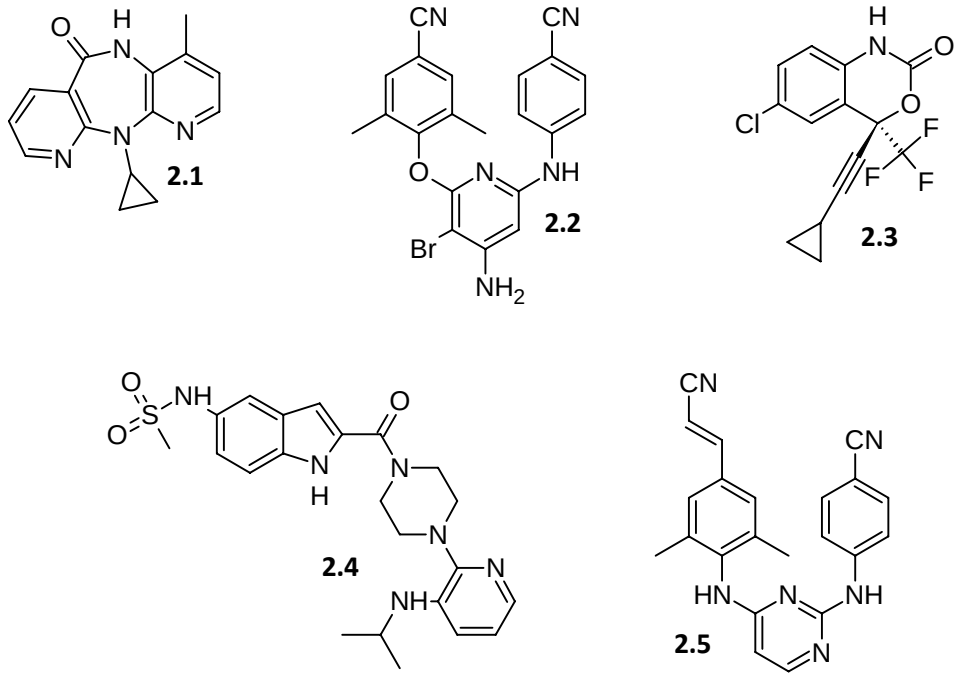


FIGURE 2.3: FDA approved non-nucleoside reverse transcriptase inhibitors.

NtRTIs contain faulty nucleotide (building blocks) used by the RT to convert RNA to DNA. When the faulty nucleotides are used, faulty DNA is created that cannot be integrated into the healthy generic material of the cell preventing a new virus from being created. Nucleoside analogues are converted to nucleotide analogues by the body for them to be active, therefore, taking nucleotide analogue allows the conversion step to be skipped. When NRTI and NtRTI are incorporated, the synthesis of viral DNA is terminated. Zidovudine (**2.6**, AZT, ZDV, azidothymidine, Retrovr®) was the first drug approved for the treatment of AIDS and HIV infection in 1987. It was initially intended for the treatment of cancer but was shelved as it proved to be ineffective against cancer. Other current NRTIs include abacavir (**2.7**, Ziagen®), didanosin (**2.8**, Videx®), stavudine (**2.9**, Zerit®), lamivudine (**2.10**, Epivir®) and emtricitanine (**2.11**, Emtriva®). The only approved NtRTI is tenofovir (**2.12**, Viread®) (Figure 2.4). NRTI and NtRTIs are classified as competitive inhibitors.[44, 61, 62]

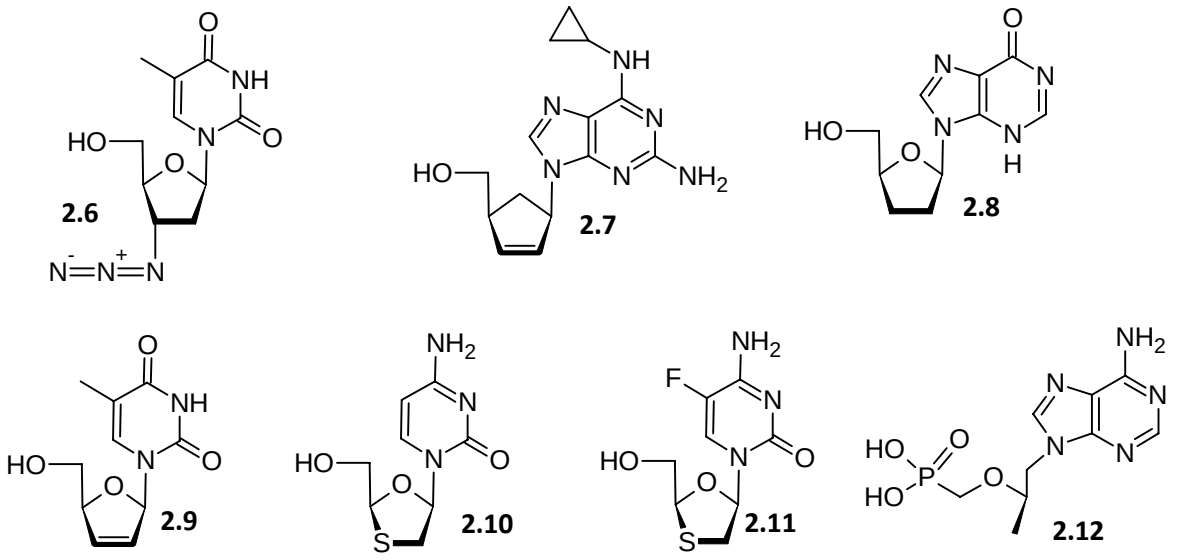


FIGURE 2.4: FDA approved nucleoside and nucleotide reverse transcriptase inhibitors.

2.5 HIV-PROTEASE INHIBITORS

Protease inhibitors (PIs) are the second biggest group of anti-HIV drugs after reverse transcriptase inhibitors. During HIV replication, long stands of HIV genetic material are created and the protease is the enzyme responsible for the cleavage of the viral Gag and Gag-Pol polyprotein precursors into mature enzymes and proteins, this process is called maturation.[63, 64] Protease inhibitors are used to prevent the formation of the mature infectious forms thus preventing HIV replication.[65] To date there are nine FDA approved protease inhibitors. The first approved PI, was saquinavir (**2.13**, SQV, Invirase®) in 1995.[66] Other PIs include ritonavir (**2.14**, RTV, Norvir®), nelfinavir (**2.15**, NFV, Viracept®), amprenavir (**2.16**, APV), indinavir (**2.17**, IDV, Crixivan®), fosamprenavir (**2.18**, FPV, Lexiva®), lopinavir(**2.19**, LPV/r, Kaletra®, Aluvia®), darunavir (**2.20**, DRV, Prezista®), tipranavir (**2.21**, TPV, Aptivus®) and atazanavir (**2.22**, ATV, Reyataz) (Figure 2.5). Amprenavir (**2.16)** was in the market in 1999 but withdrawn in 2006 when its derivative fosamprenavir (**2.18)** was found to be more effective.[67] Combination of protease inhibitors with reverse transcriptase inhibitors under HAART, for effective HIV treatment, resulted in the reduction in mortality and prolonged life in HIV positive patients.[64] However, the development of resistance through mutation still remains a challenge in HIV treatment.[68] Other shortcomings are toxicity, adherence, high costs and tolerability. Thus there is still a need for the development of better PIs with better pharmacokinetics, broad specification against PI-resistant HIV mutants, lower toxicity and simple dosage.[64]

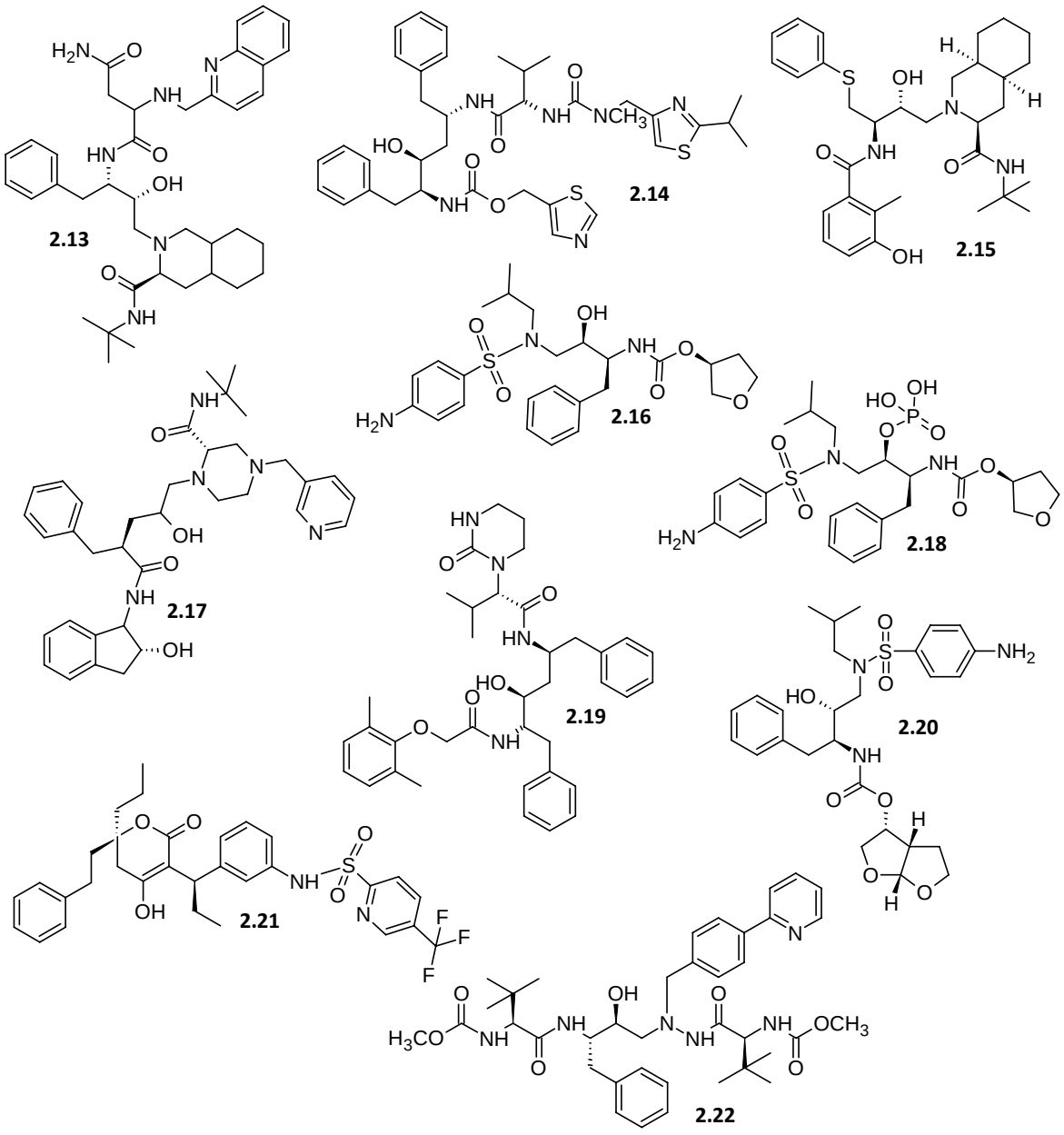


FIGURE 2.5: FDA approved protease inhibitors.

2.6 INTEGRASE INHIBITORS

Another target for HIV treatment is the enzyme integrase. The integrase is vital in the HIV viral replication because it is an enzyme responsible for the integration of the viral DNA into a host cell's genome.[69-71] The integration process involves two sequences, the 3'-processing or dinucleotide cleavage and strand transfer reactions. 3'-Processing involves the cleavage of the two nucleotides from each 3'-end of the double stranded viral DNA, produced by reverse transcription, resulting in a tailored viral DNA recessed by two nucleotides CAOH-3'. The strand

transfer reaction takes place in the nucleus and it involves the joining of each 3'-end of the recessed DNA to the 5'-end of the host DNA.[69-74] Integrase inhibitors block the integrase enzyme and prevent the integration of the viral DNA into the DNA of the host cells, thus preventing the virus from replicating. To date there are three FDA approved integrase inhibitors. Raltegravir (**2.23**, RAL, Isentress®) was the first HIV integrase inhibitor approved by the FDA in 2007;[75] others are elvitegravir (**2.24**, EVG, Viteka®) and dolutegravir (**2.25**, DTG, Tivicay®). MK-2048 **(2.26)** is still under development by Merck & Co. It has been found to have the inhibition of the integrase four times longer than raltegravir (**2.23**) (Figure 2.6).

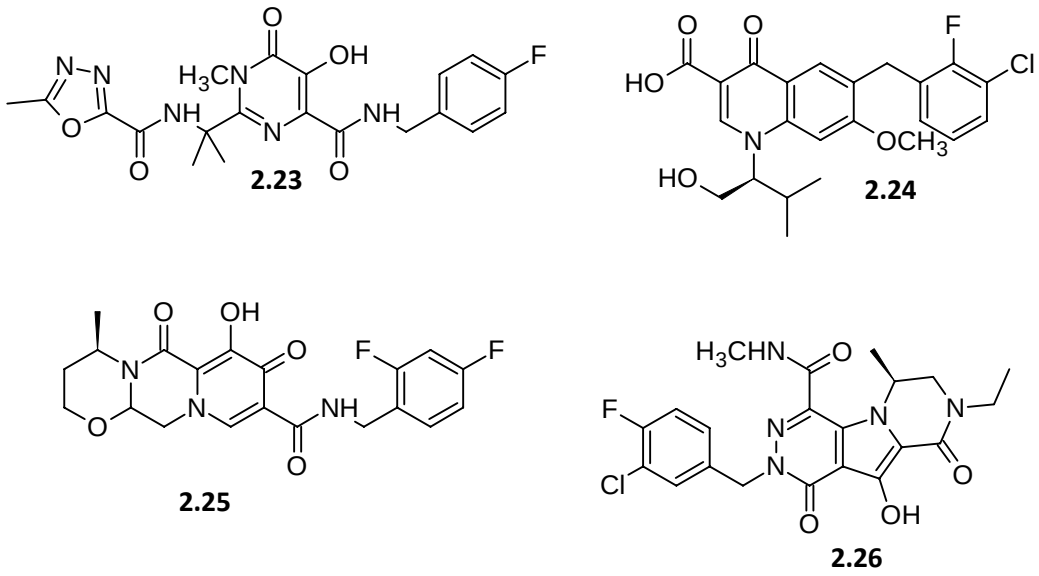


FIGURE 2.6: FDA approved integrase inhibitors.

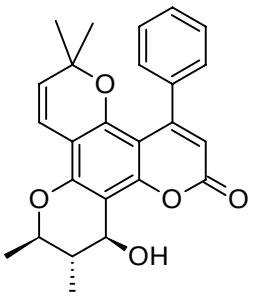
Natural products with antiviral and immunomodulation effects are viewed as possible sources of new compounds to inhibit HIV and treat AIDS.[76] Plant metabolites with anti-HIV activity have been reviewed by Mathee,[44] Jung,[62] Cowan,[77], Clercq,[78] Cos,[79] Lee,[80] and Singh.[81, 82] Plants produce an amazing variety of secondary metabolites that are gaining importance for their therapeutic and biotechnological applications. Many *in vitro* anti-HIV studies have been performed with plant metabolites, however, the mode of activity has not been defined for most of them. The next sections will review compounds that have shown activity against the specific three HIV enzymes, the reverse transcriptase, protease and integrase, non-specific anti-HIV and HIV-latency reversing metabolites

2.7 PLANT METABOLITES AS HIV-RT INHIBITORS

Many plant metabolites have been shown to be active as HIV-RT inhibitors. These compounds can be classified into a wide range of different groups according to their structural classes, *e.g.* coumarins, flavonoids, tannins, lignans, alkaloids, terpenoids, naphtha- and anthraquinones, polysaccharides, *etc*.[44]

2.7.1 COUMARINS

Coumarins are phenolic substances made of benzopyrone ring systems. They are responsible for the characteristic sweet odour of hay. They are known to have interesting biological activities such as antithrombotic, anti-inflammatory, and vasodilatory.[77] Coumarins are found in almost every plant family. Plants use them as growth inhibitors (anti-auxins) as well as defence compounds. (+)-Inophyllum B **(2.27)** was isolated from the Malaysian tree *Calophyllum inophyllum* and was found to be active against HIV-RT. Antibacterial, anti-inflammatory and phagocytosis stimulant activities were reported for this plant, which traditionally had been used for the treatment of eye diseases.[83] Also (+)-calanolide A **(1.22),** (already discussed in Chapter 1) and (+)-calanolide B **(2.28)** from *Calophyllum lanigerum* acted as HIV-RT inhibitors.[44]



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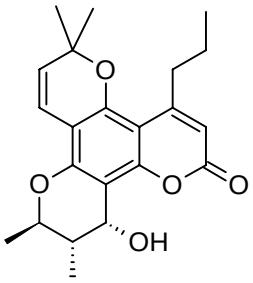

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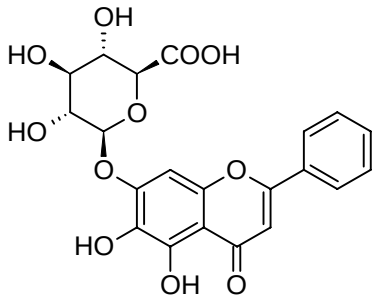
2.7.2 FLAVONOIDS

Flavonoids are hydroxylated polyphenolic substances and occur as a C6-C3 unit linked to an aromatic ring. These naturally occurring phenylchromones are present in most higher plants as they are known to be synthesised by plants in response to microbial infection, so it is not surprising that they have been found to be effective antimicrobial substances *in vitro* against a wide number of microorganisms. They are able to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Flavonoid compounds exhibit inhibitory activity against

multiple viruses.[77] Other activities include antiallergic, anti-inflammatory, antiviral and antioxidant. Baicalin **(2.29)**, isolated from the Chinese medicinal herb *Scutellaria baicalensis*, was reported as a non-competitive inhibitor of HIV-1 RT. Baicalin was also effective in inhibiting RT from Moloney murine leukaemia virus and Rous-associated virus type 2, and RT activity in cells infected with human T-cell leukaemia virus type.[44, 61] Quercitrin **(2.30)** and Quercetin **(2.31),** isolated from *the leaves of Alnus firma*, were reported to be an inhibitor of the HIV- RT.[84]



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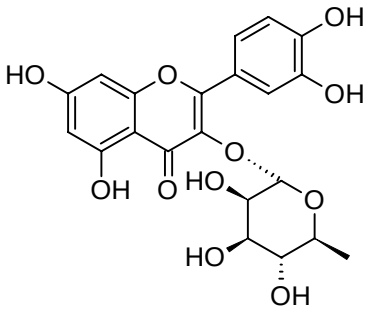

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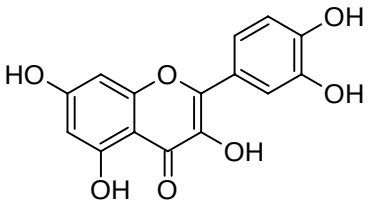
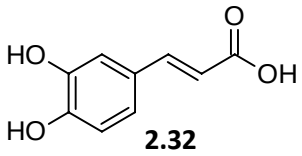


2.31

2.7.3 TANNINS

"Tannin" is a general descriptive name for a group of polyphenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Tannins are widely distributed in most plant species. This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of illnesses. Tannins in plants disrupt digestive events in ruminal animals and inhibit insect growth.[77] Most of the HIV inhibitory effect is because they are non-selective and inhibit a large range of enzymatic pathways, resulting in a positive result. Although they have anti-HIV activity, they are known to be suspected of causing liver damage, carcinogenic potential and anti-nutritional activity.[44, 85] Caffeic acid **(2.32)**, isolated from *Sanicula europaea* L. (Apiaceae), and digallic acid **(2.33**, isolated from *Pistacia lentiscus* are examples of tannins that inhibit HIV-RT.[44, 62, 77, 86] *Sanicula europaea* has been traditionally used for gastrointestinal, dermatological, and urinary system diseases, upper airway,

and chronic chest pains. The aqueous extracts of the plant were found to exhibit antimicrobial, haemolytic and antioxidant activities.[86]



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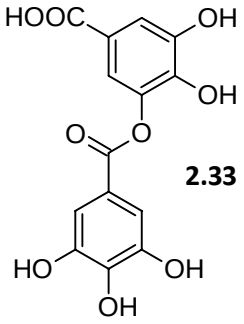

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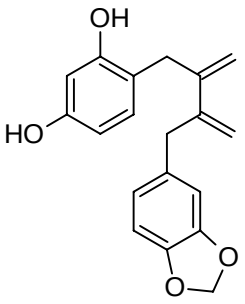
2.7.4 LIGNANS

Lignans are present in the roots, stems, bark, fruit and seeds of many plant species and are derived from dimerization of phenylpropanoid units at the central carbons of their side chains.[87] Lignans exhibit a wide range of biological activities, including antifungal, antimicrobial, antiviral, antitumorigenic and inhibitory activity of many enzymes systems.[88] Two dibenzylbutadiene lignans, anolignan A **(2.34)** and anolignan B **(2.35)** isolated from *Anogeissus acuminate* showed inhibitory activity against HIV-RT. The two lignans showed weak activity alone but better activity was observed when combined with each other because of their synergistic effects.[44, 62, 89] In addition, two aryl naphthalene lignans, phyllamiricin B **(2.36)** and retrojusticidin B **(2.37)** isolated from *Phyllanthus myrtifolius* possess strong inhibition activity against HIV-1 reverse transcriptase.[88]



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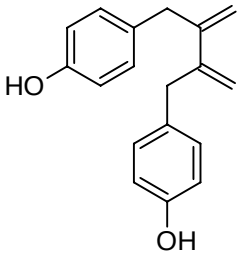

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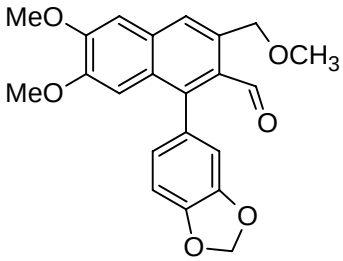

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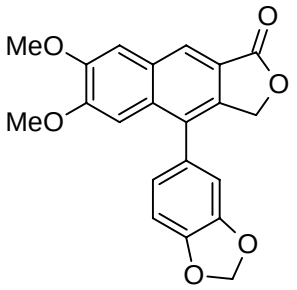

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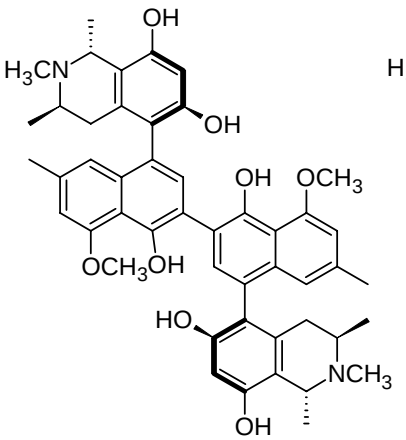
2.7.5 ALKALOIDS

The naphthylisoquinoline alkaloids michellamine A **(2.38)**, michellamine B **(2.39)** and michellamine C **(2.40)** were isolated from an extremely rare tropical rain forest plant of Cameroon called *Ancistrocladus korupensis*. Michellamine B, **(2.39)** the most abundant compound in the series and the most potent against HIV. It has undergone preclinical drug development by the U.S. National Cancer Institute, and preliminary pharmacokinetic studies have been reported. It was found to possess the inhibitory activity against HIV reverse transcriptase and fusion of HIV-infected cells from both HIV-1 and HIV-2.[44, 90-92]



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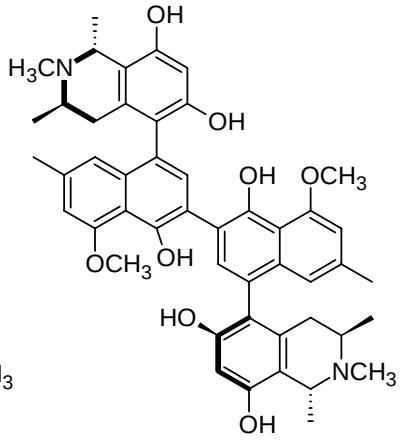

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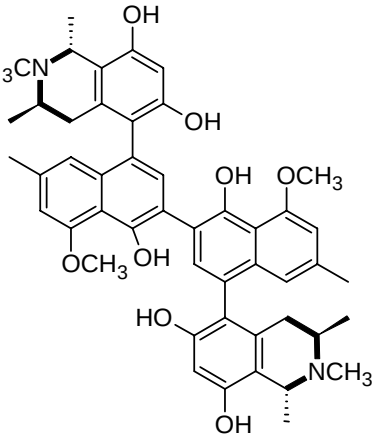

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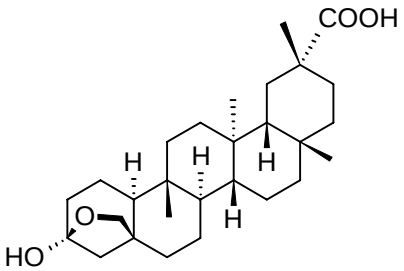
2.7.6 TERPENOIDS

The fragrance of plants originates from the so-called “quinta essential”, or essential oil fraction. The oil fractions contain secondary metabolites that are highly enriched in compounds based on an isoprene structure. These compounds are known as terpenes. Terpenes have a general chemical structure of $C_{10}H_{16}$, and they occur as diterpenes (C_{20}), triterpenes (C_{30}), and

tetraterpenes (C_{40}), as well as hemiterpenes (C_5) and sesquiterpenes (C_{15}). They sometimes contain additional elements, usually oxygen, these are termed terpenoids. Terpenoids share their origins with fatty acids as they are synthesised from acetate units. The difference they have to fatty acids is that they contain extensive branching and are cyclised.[77] The triterpenoid salaspermic acid **(2.41)** isolated from *Tripterygium wilfordii* showed activity against HIV-RT.[44] Nigranoic acid **(2.42,** isolated from *Schisandra sphaerandra*, a Southern China plant used for treatment of stomach disorders, inhibited HIV-RT.[93] Betulinic acid **(1.19)** and platonic acid **(2.43)** were isolated from *Syzigium claviflorum* and they were found to possess inhibitory activity against HIV-RT.[44, 62, 77] Betulinic acid was isolated from a South African medicinal plant *Peltophorum africanum* which had been traditionally used for the treatment of diarrhoea, dysentery, sore throats, wounds, HIV/AIDS, venereal diseases, and infertility.[94]



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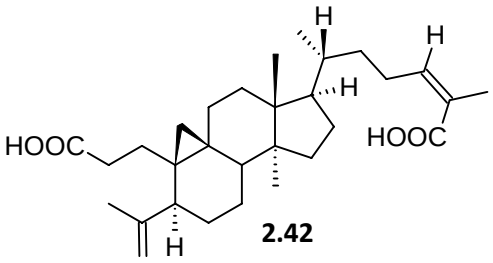

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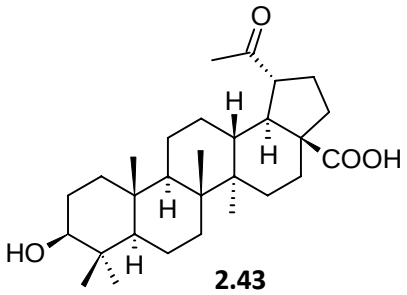

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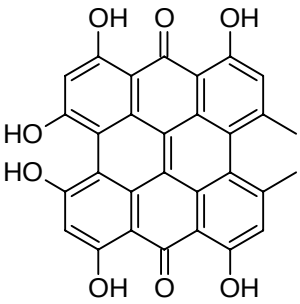
2.7.7 NAPHTHA- AND ANTHRAQUINONES

Isolated from the plant *Hypericum perforatum* (St. John's wort), hypericin **(2.44)** was found to be active against many viruses including HIV-RT. It has received attention as a natural antidepressant.[95] Phase I clinical trials were initiated, however, studies of the mechanism of action revealed that the virucidal effect was mainly caused by affecting the budding of the virus. An oral formulation of hypericin was investigated in further clinical trials.[44] It was found that almost all patients on hypericin experienced phototoxicity of grade 2 or higher (moderate or severe), resulting in discontinuation of its use.[95]



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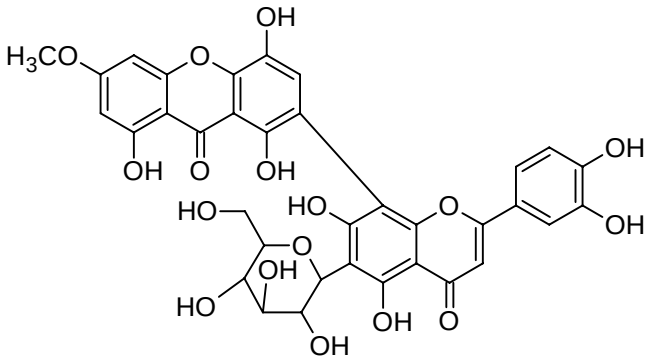
2.7.8 XANTHONES

Xanthones are polyphenols comprising a tricylic aromatic system (C6–C3–C6).[96] From the extract of Chinese medicinal plant *Swertia franchetiana*, swertifrancheside **(2.45)** was isolated and it was found to inhibit HIV-RT.[81] *Swertia franchetiana* has the ability to reduce fever, is used as an antidote, and is used for the treatment of haematogenous jaundice and cholecystitis.[97, 98]



2.45

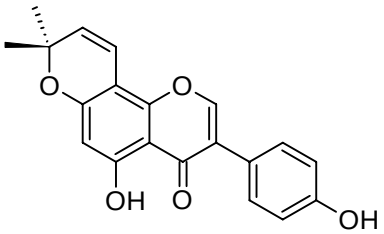
2.8 PLANT METABOLITES AS HIV-PROTEASE INHIBITORS

The protease is the second most targeted enzyme in the HIV cycle. Some plant-derived compounds have been found to exhibit HIV protease inhibitory activity.

2.8.1 FLAVONOIDS

From the Cameroonian medicinal plant *Erythrina senegalensis*, derrone **(2.46)**, and alpinumisoflavone **(2.47)** were isolated and they inhibited HIV protease.[82, 99, 100] The extracts of *Erythrina senegalensis* are used traditionally for the treatment of female infertility, stomach pains and gonorrhoea.[101, 102] *Erythrina senegalensis* possesses pharmacological activities such as

antiplasmodial, analgesic, and anti-inflammatory and phospholipase C inhibition.[103, 104] The chalcone hydroxypanduratin A **(2.48)**, isolated from the rhizomes of *Boesenbergia pandurata* showed anti-HIV-1 protease activity.[79, 105] The rhizomes of *Boesenbergia pandurate* in Thailand are traditionally used for dyspepsia.[106] It has been found to possess activities such as antibacterial, anti-inflammatory and antitumor.[107-109]



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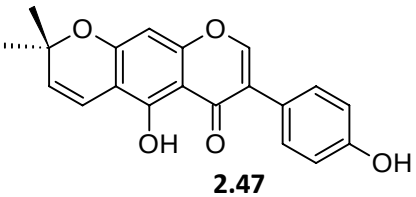

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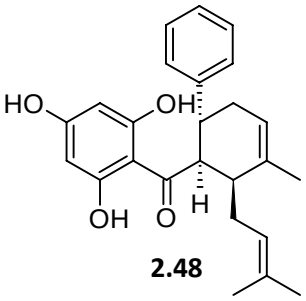
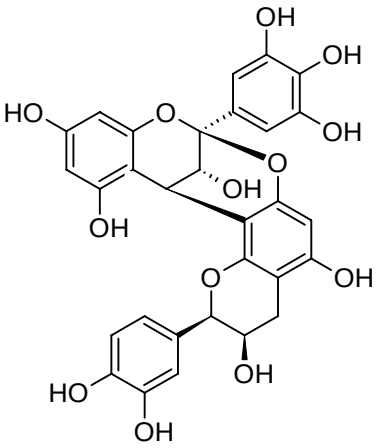


2.48

2.8.2. TANNINS

From the extract of *Cupressus sempervirens* epigallocatechin-(4β→8,2β→*O*-7)-epicatechin **(2.49)** and proanthocyanidin A_2 **(2.50)** were isolated. Both compounds inhibited HIV protease but **(2.49)** was more potent.[110, 111]



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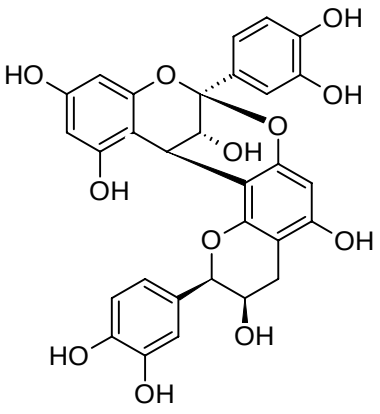
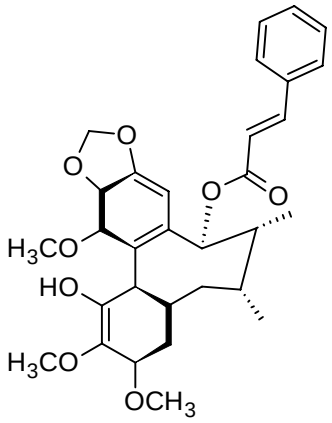


2.50

2.8.3 LIGNAN

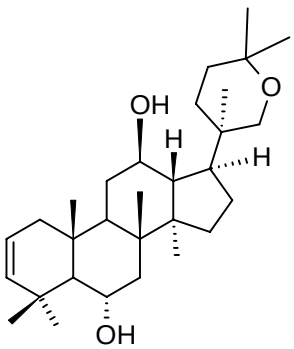
Longipedunins A **(2.51),** was isolated from the roots and stems of *Kadsura longipedunculata*, a plant endemic to Southern China, it exhibited anti-HIV protease activity.[82] *Kadsura longipedunculata* is traditionally used for the treatment of gastric ulcers, rheumatoid arthritis, menstrual irregularities and other feminine disorders.[112]



2.51

2.8.4 TERPENOIDS

The triterpenoids, (20*R*)-20,25-epoxydammaran-2-en-6α,12β-diol **(2.52)**, (20*R*)-20,25-epoxy-3-methyl-28-nordammaran-2-en-6α,12β-diol **(2.53)**, and isodehydroprotopanaxatriol **(2.54)** isolated from an acidic hydrolysate of the Chinese medicinal plant *Panax ginseng* showed HIV protease inhibitory activity.[82, 113] *Panax ginseng* is used for the physical strength promotion and disease resistance.[113, 114]



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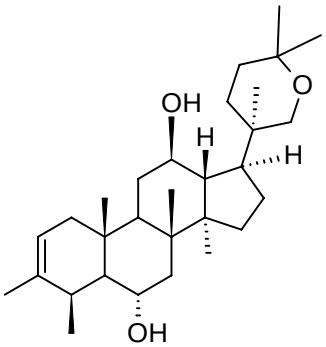

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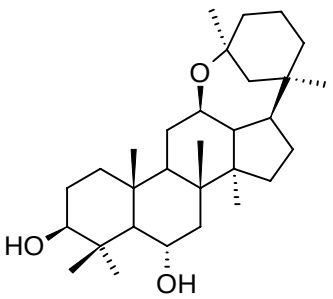


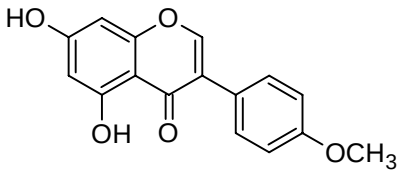
2.54

2.9 PLANT METABOLITES AS HIV-INTEGRASE INHIBITORS

As noted in the previous section of the FDA approved integrase inhibitors, less than five drugs have entered the market, the reason could be the complexity of the integration process. Some plant-derived compounds that have shown inhibitory activity of the HIV-integrase include flavonoids, lignans, terpenoids and phenols.

2.9.1 FLAVONOIDS

Orobol **(2.55)** and wedelolactone **(2.56)** isolated from *Eclipta prostrata* showed anti-HIV integrase activity.[115] *Eclipta prostrate* grows in Asia, and it is used in Thailand for the treatment of skin diseases and dying hair, blood tonic, anaemia, tuberculosis, amoebiasis, asthma, antibacterial agent, hepatoprotectant, and also exhibits immunomodulatory, anti-inflammatory antimicrobial activities.[115, 116] Other activities that wedelolactone possesses include snake antidote, liver disease treatment and IKK complex inhibition.[82, 117, 118] Thalassiolin A **(2.57)**, a flavone, isolated from the sea grass of the Caribbean, *Thalassia testudinum*, showed inhibition of HIV integrase, and showed no viral resistance after long periods exposure.[79, 119]



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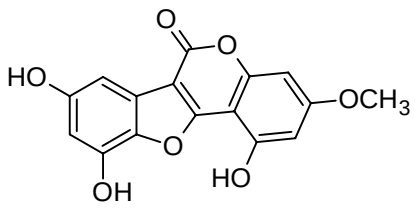

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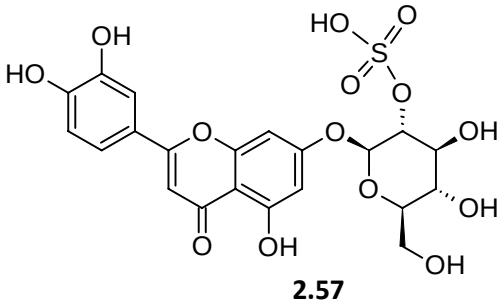
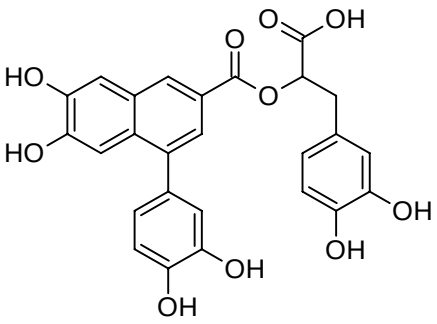


2.57

2.9.2 LIGNAN

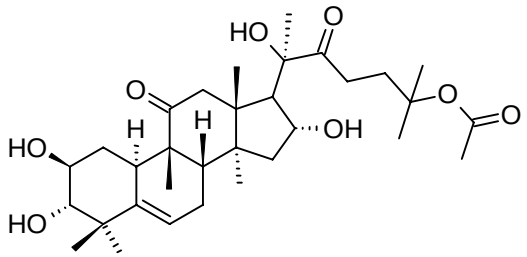
Globoidnan A **(2.58)** was isolated from *Eucalyptus globoidea* and was found to be active against the HIV integrase.[79, 120, 121]



2.58

2.9.3 TERPENOIDS

From the Chinese medicinal plants, *Hemsleya jinfushanensis*, two cucurbitacins, hemslecin A **(2.59)** and hemslecin B **(2.60)** were isolated. These compounds are known for the treatment of bacillary dysentery, bronchitis, tuberculosis, and inflammatory diseases such as enteritis, diarrhoea and bronchitis and showed anti-HIV protease activity.[82, 122]



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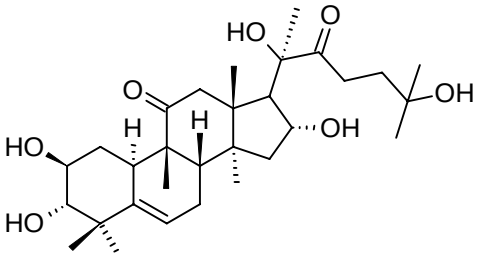
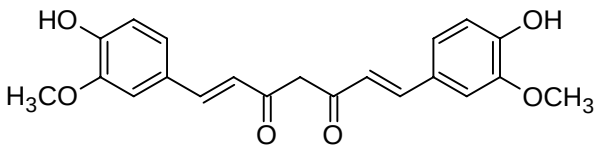


2.60

2.9.4 PHENOLICS

Curcuma longa (turmeric) is dried and finely ground to a powder which is widely used as a spice, food colouring and preservative, and medicinally for disorders.[79, 123] One of its constituents is curcumin **(2.61)** which has been found to inhibit HIV integrase.[78, 79] 3,5-Dicaffeoylquinic acid **(2.62)** and L-chicoric acid **(2.63)**, isolated from the wild herbs of *Asteraceae* family exhibited anti-HIV-1 integrase activity.[53, 78, 79, 124, 125]



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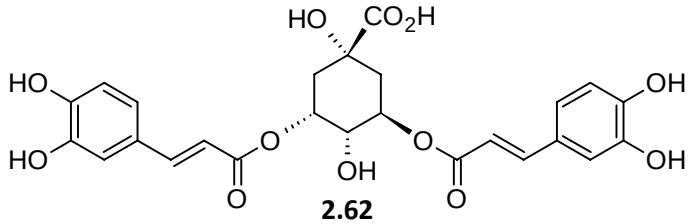

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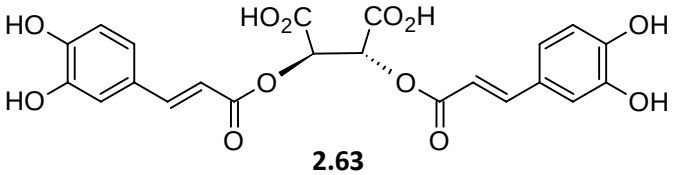
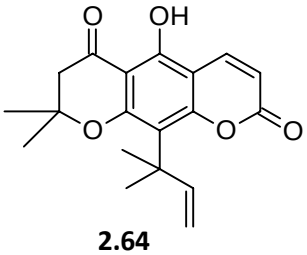


2.63

2.10 PLANT METABOLITES AS ANTI-HIV AGENTS

2.10.1 COUMARINS

A coumarin, clausenidin **(2.64)**, isolated from *Clausena excavate*, exhibited anti-HIV activity.[82, 126] Suksdorfin **(2.65)**, an anti-HIV active courmarin, was isolated from *Lomatium sukdorfsii*.[42] Structurally similar 3'*R*,4'*R*-di-*O*-(–)-camphanoyl-(+)-*cis*-khellolactone, DCK **(2.66)**, was selected for preclinical trials. Mesuol **(2.67)** was isolated from the Colombian tree *Marila pluricostata* and it also showed anti-HIV properties.[79, 127]



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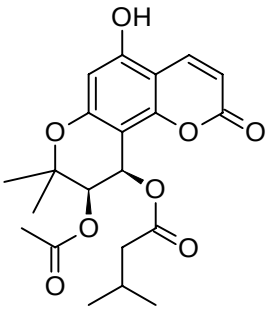

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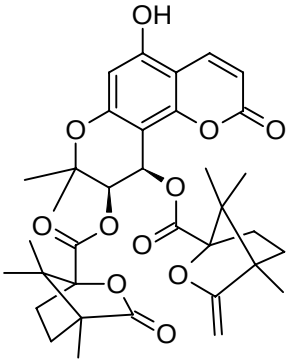

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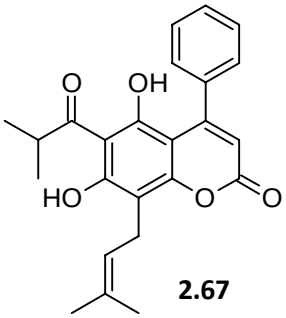
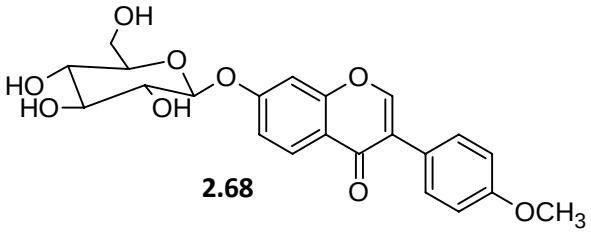


2.67

2.10.2 FLAVANOIDS

Calycosin-7-*O*-β-D-glucopyranoside **(2.68)**, isolated from the Chinese medicinal plant, *Astragalus membranaceus* var. *mongholicus*, possess anti-HIV activity. The plant is traditionally used for the treatment of diarrhoea, prolapse of the rectum, hematochezia and abnormal uterine bleeding, edema, anaemia, albuminuria in chronic nephritis, and diabetes.[82, 128] Aromadendrin, 3,5,7,4'-tetrahydroxyflavanone **(2.69)**, from *Cuscuta reflexa* exhibited anti-HIV activity.[129]



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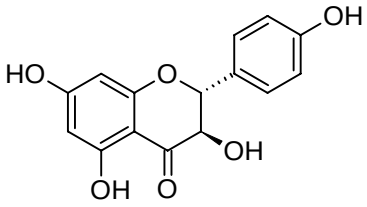
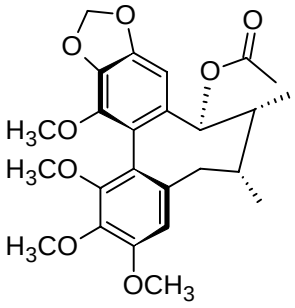


2.69

2.10.3 LIGNAN

Lignans kadsurin **(2.70)**, hetetoclitin **(2.71)**, and acetoxy oxokadsurane **(2.72)**, isolated from the stems of *Kadsura heteroclite* were found to possess anti-HIV activity.[82] *Kadsura heteroclite* is distributed in Southern China and is traditionally used for blood deficiencies and feminine disorders. Besides the anti-HIV activity, other pharmacological properties include antitumor, anti-hepatitis and anti-lipid peroxidative activities.[130-138]



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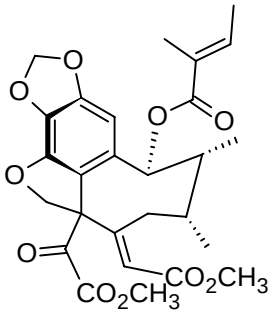

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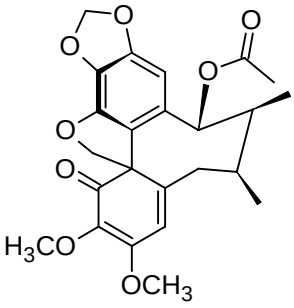
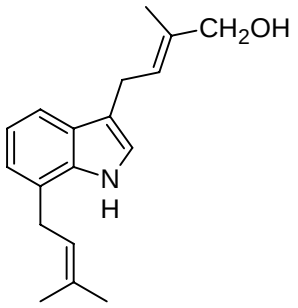


2.72

2.10.4 ALKALOIDS

(*E*)-3-(3-Hydroxymethyl-2-butenyl)-7-(3-methyl-2-butenyl)-1*H*-indole **(2.73)**, a diprenyl indole alkaloid isolated from the twig and leaves of the Chinese plant *Glycosmis montana*, with no known medicinal use, exhibited anti-HIV activity.[82, 139] It was suggested for further studies as a potential anti-HIV drug candidate. Drymaritin **(2.74)**, a canthin-4-one type alkaloid was isolated from *Drymaria cordata* subsp. *diandra* was found to possess anti-HIV properties.[140] Decarine **(2.75)**, γ-fagarine **(2.76)**, and (+)-tembamide **(2.77)**, were isolated from the bark of *Zanthoxylum ailanthoides*.[141] They were found to be the most potent anti-HIV metabolites isolated from the species.[82] *Zanthoxylum ailanthoides* is distributed in Korea, China, Taiwan, Japan and Philippines and is used traditionally for the treatment of cold, arthralgia, rheumatism, stasis, contusions, and snakebite, and to stimulate blood circulation.[141]



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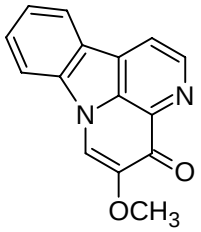

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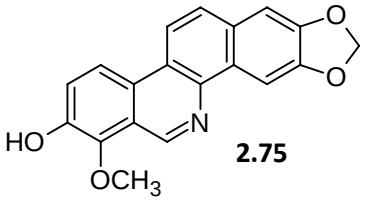

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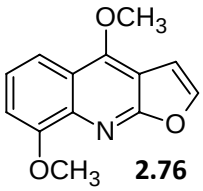

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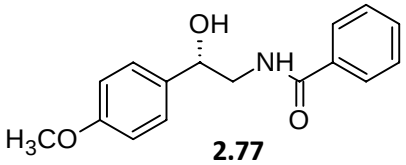
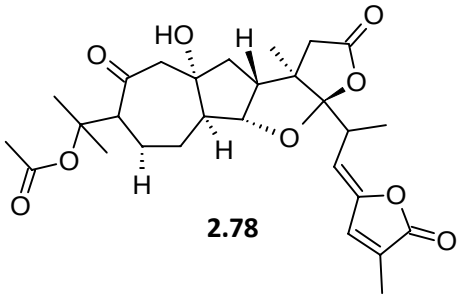


2.77

2.10.5 TERPENOIDS

The terpenoid, schilancidilactone A **(2.79)**, was isolated from *Schisandra lancifolia* and was found to exhibit anti-HIV activity.[82] *Schisandra lancifolia* is the Chinese medicinal plant used for the treatment of cuts, fractures, and eliminate stasis.[142] The diterpenes, forskolin **(2.80)**, the major constituent of *Coleus forskohlii* exhibited anti-HIV properties. Other known biological activities of forskolin include cardiotonic, antihypertensive, and adenylcyclase activator. Also isolated was a structurally similar compound 1-deoxyforskolin **(2.81)** which also exhibited anti-HIV activity.[82, 143]



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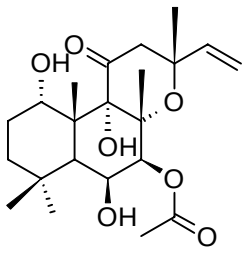

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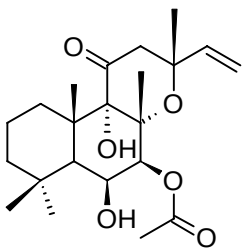
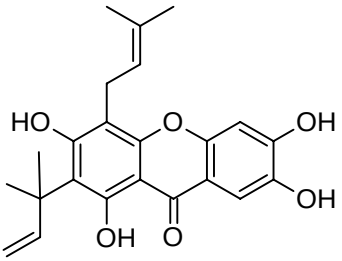


2.80

2.10.6 XANTHONES

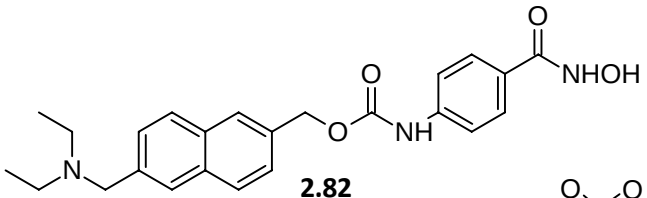
Macluraxanthone B **(2.81)**, a prenylated xanthone, isolated from *Maclura tinctoria* was found to exhibit anti-HIV activity. [144]



2.81

2.11 HIV LATENCY

The use of anti-HIV drugs (HAART) has led to the reduction of the viral load, increase in life expectancy and improved quality of life in HIV-positive patients. However, anti-HIV drugs do not cure or eliminate HIV, they only target the HIV replication and the establishment of viral latency in CD4 cells still remains a challenge for effective HIV treatment.[145-149] Thus, the combination of HAART with agents that target latent reservoirs of HIV can provide the solution in the HIV elimination. Agents such as givinostat **(2.82)**, used for treatment of arthritis, belinostat **(2.83)** used in the treatment of various cancers and romidepsin, for the treatment of CTCL (a type of cancer of the immune system), are already in clinical trials for potential HIV latency-reversing activities.[147] Some compounds isolated from plants have been found to target HIV latency.



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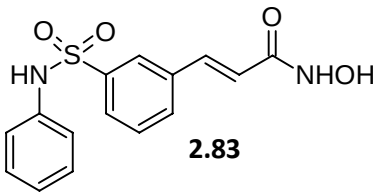
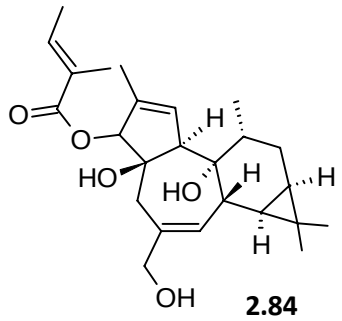


2.83

2.11.1 INGENOL

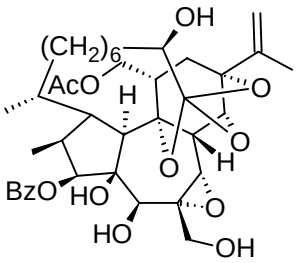
Ingenols were initially isolated from the sap of the tropical shrub *Euphorbia tirucalli*. Synthetic derivatives of ingenols, such as ingenol-3-angelate **(2.84)**, were found to activate latent HIV.[147, 148]



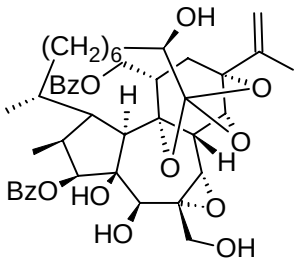
2.84

2.11.2 DAPHNANE DITERPENOIDS

The daphnane diterpenoids, first isolated from *Trigonostemon reidioides* and later from other species of the genus, have since attracted the attention of many chemists and biologists.[150] They have also been isolated from Thymelaeaceae (*Daphne, Wikstromia, Stellera, etc.)* and Euphorbiaceae (*Euphorbia, Croton, Excoecaria, etc*) families and exhibit a range of biological activities such as anticancer, neurotropic, anti-fertility, pesticidal, antihyperglycemic, anti-bladder-hyper-reflexia and anti-HIV activity.[150-154] Stelleralide A **(2.85**) and gnidimacrin **(2.86)** isolated from the toxic Chinese plant *Stellera chamaejasme,* whose roots are traditionally used as emulgent and dermatological agents, were found to exhibit HIV latency-reversing activity.[154, 155] Gnidimacrin exhibited activity at picomolar concentrations with low cytotoxicity.[145]



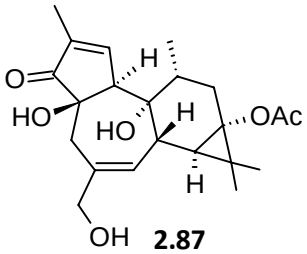
2.85



2.86

2.11.3 PROSTRATIN

Prostratin **(2.87)** was initially isolated from *Pimelea prostrata* in the 1970s and later isolated from the Samoan medicinal plant *Homalanthus nutas* in the 1990s.[149] It was found to exhibit inhibitory activity against various cancer cell lines and is a clinical lead candidate for HIV latency-reversing activity. Various synthetic analogues of prostatin have since been developed, showing hundred-fold more potency than postratin.[149] The activity observed for prostatin and ingenol derivatives on latent HIV has attracted a lot of interest and it is likely that one or more of these compounds may soon entire clinical trials. The availability of drugs to reverse HIV latency is likely to add a new dimension in the treatment of HIV-positive patients. In combination with HAART therapy, they have a potential to cure patients completely.



2.87

2.12 CONCLUSION

Compounds that are HIV reverse transcriptase inhibitors are widely distributed in nature and are from diverse structural classes but not much has been discovered in South Africa. Selected South African medicinal plants have been screened for the inhibition of HIV-RT based on their ethnomedicinal usage directly in HIV/AIDS, or for the treatment of symptoms, or conditions closely related to the disease such as persistent cough, chronic diarrhoea, progressive weight loss and skin infection.[156-158] The next chapter will discuss the results obtained from the screening of selected South African plants for anti-HIV activity.

CHAPTER 3

In vitro anti-HIV screening of selected South African plant extracts

3.1 INTRODUCTION

Prompted by the urgent need of anti-HIV drugs, researchers have turned to ethnomedicines for potential drug discovery and development. Already numerous secondary metabolites from plants have been shown to have anti-HIV inhibitory activity as described in Chapter 2. Many South African plants have ethnomedical uses for the treatment of illnesses that are associated with HIV positive patients. Thus, South African medicinal plants have the potential to contain secondary metabolites that can be used for the treatment of HIV/AIDS. *Sutherlandia frutescens* (Fabaceae), a medicinal plant indigenous to South Africa, has been used for the treatment of HIV. Traditionally it has been used for the treatment of stomach ailments, internal cancers, diabetes, piles, stress, fever, wounds, and backaches.[159, 160] However its antiviral activity is likely to be indirect because no evidence of activity has been found in its commercial phytochemical product; *i.e.* it could be the antimicrobial and antioxidant activities that results in the reduction of opportunistic infections and boosting of the immune system.[159] Health practitioners have reported that HIV-positive patients using a *Sutherlandia frutescens* extract have gained weight, improved CD4 count, decreased viral load, improved appetite and general improvement in mood.[85, 160] Phase I of clinical trials (safety studies) was successfully completed, but there is no documentation on phase II clinical trials.[159]

In vitro screening of plant extracts is very important for the validation of the ethnomedicinal use of the plant and for providing leads in the discovery of new drugs.[161] Screening is also essential in the preliminary stages of the discovery of new drugs. However the effect of a drug on humans cannot be explained and predicted a by simple biochemical assay; it is more complex and involves interaction at multiple levels. Colorimetric assays are an important, valuable and convenient tool for screening the biological activity and cytotoxicity studies of potential medicinal agents. The aim of this chapter is to report on anti-HIV activity of plant extracts by using two assays, an *in vitro* non-radioactive HIV-RT colorimetric ELISA assay commercially available from Roche[162] and a cell-based XTT assay.[163]

3.2 RESULTS AND DISCUSSION

Plants selected were from different families and species based on their ethnomedicinal uses and some were chosen randomly. Most plants were selected for conditions that are mostly associated with HIV positive patients ranging from: venereal disease, skin problems, rashes, wounds, sores, infections, respiratory tract complaints, chest problems, fevers, influenza, malaria, colds and coughs, TB, *etc,* and also based on availability of plant material. The plant species selected and their uses are summarised in Table 3.1.

TABLE 3.1: Plant investigated for the anti-HIV activity.

Plant species	Family	Usage
Acokanthera oppositifolia	Apocynaceae	Headaches, snakebites, abdominal pain, toothaches, colds, coughs, chest ailments, anthrax and tapeworm, urinary track treatment, and scarification.[17, 161, 164]
Artemisia afra	Asteraceae	Cold, cough, influenza, pneumonia, headaches, gout, measles, gumboils, earaches, toothaches, mumps, sprains, colic, malaria, intestinal worms, constipation, indigestion, fever, loss of appetite, clear blocked nasal passages, and sore throat.[17, 161, 164]
Berkheya speciosa	Asteraceae	Abdominal disorders, schistosomiasis, and sore eyes.[17]
Bulbine frutescens	Asphodelaceae	Fever, mosquito bites, cuts, and burns.[17]
Bulbine latifolius	Asphodelaceae	Eczema and similar itchy conditions, syphilis, vomiting and diarrhoea, urinary complaints, dysentery, rheumatism, and blood purifier.[17]
Chrysocoma ciliata	Asteraceae	Dysmenorrhoea, pain, fertility, and stomach disorders.[165]
Combretum kraussii	Combretaceae	To stimulate appetite, fever, inflammation, cleanse urinary system, and for wounds.[17, 161]
Conyza scabrida	Asteraceae	Colds, coughs, influenza, chest and heart complaints, and headaches.[17, 161]
Dioscorea dregeana	Dioscoreaceae	Cuts and sores, hysteria, convulsions and epilepsy, scabies, easy childbirth, and soporifics.[17, 164]
Euclea natalensis	Asteraceae	Toothaches, headaches, scrofulous swelling, blood purifier, stomach disorders, urinary tract infection, venereal diseases, susceptibility of sores,

		dysmenorrhoea, ague, ulcers, chest problems, coughs, and TB.[17, 161]
Ficus ingens	Moraceae	Anaemia.[17]
Gardenia thunbergia	Rubiaceae	Fever, biliousness, skin lesions, syphilis, purgative, and nausea.[17]
Harpephyllum caffrum	Anacardiaceae	Blood purifier or emetic, facial sauna and shin washes, acne and eczema, sprains and fractures, scarification, and rashes.[17, 164]
Helichrysum cephaloideum	Asteraceae	Poisonous to sheep.[166]
Helichrysum cymosum	Asteraceae	Cold, coughs, emetic and purgative, fever, and headaches.[166]
Helichrysum melanacme	Asteraceae	coughs, TB, fever, headache, colds, and chest pain.[161, 166]
Helichrysum nudifolium	Asteraceae	Colic, colds, coughs, TB, antiseptic, fast healing, headache, fever, bruises, infections of the respiratory tract, and wounds.[17, 161, 166]
Helichrysum odoratissimum	Asteraceae	Wounds, burns, headache, fever, colds, coughs, TB, pimples, dysmenorrhoea, eczema, colic, tonic for pregnant women, galactagogue, heartburn, flatulence, dehydration, abdominal pain.[17, 161, 166]
Helichrysum panduratum	Asteraceae	Antiseptic, fast healing, prevent inflammation, wounds, infections of the respiratory tract, and stomach ailments.[166]
Heteromorpha trifoliate	Apiaceae	Abdominal disorders, colic, scrofula, mental and nervous disease, worms, stomach and kidney cleanser, headaches, coughs, asthma, chest and back pain, fever, infertility, gonorrhoea, and shortness of breath.[17, 161]
Lasiospermum bipinnatum	Asteraceae	Chest infections, and disinfect sickrooms.[12]
Leonotis species	Lamiaceae	Colds, coughs, fever and headaches.[17]
Lippia javanica	Verbenaceae	Colds, coughs, fever, bronchitis, influenza, measles, rashes, diarrhoea, sprained joints, skin diseases, malaria, stomach problems, asthma, sore eyes, headaches, scabies, and lice.[17, 164]

Ochna serrulata	Ochnaceae	Gangrenous rectitis, bone diseases.[17]
Orthosiphon labiatus	Lamiaceae	-
Pentzia globose	Asteraceae	-
Plectranthus ambiguus	Lamiaceae	Colds and coughs.[17]
Plectranthus ecklonii	Lamiaceae	Skin infection.[12]
Plectranthus hadiensis	Lamiaceae	Enemas.[17]
Plectranthus madagascariensis	Lamiaceae	Cough, chest complaints, and scabies.[17, 161]
Plectranthus saccatus	Lamiaceae	Non-medicinal.[167]
Plectranthus verticillatus	Lamiaceae	Non-medicinal.[167]
Plectranthus zuluensis	Lamiaceae	Non-medicinal.[167]
Plumbago auriculata	Plumbaginaceae	Headaches, removal of warts, scarification, scrofula, oedema, treatment of fractures, malaria, and skin lesions.[17, 164]
Plumbago spp.	Plumbaginaceae	Headaches, influenza, fever, ulcers, and scabies[17]
Polygala fruticosa	Polygalaceae	Poor circulation, blood purifying, scrofula, dropsy, chronic ulcers, venereal diseases, TB, and sinusitis.[17, 161, 164]
Psoralea pinnata	Fabaceae	Mental disturbance.[17]
Rhus chirindensis	Anacardiaceae	Heart complaints, mental disturbance, body strengthening, stimulate circulation, and rheumatism.[17]
Rothmannia globosa	Rubiaceae	Leprosy[168]
Siphonochilus aethiopicus	Zingiberaceae	Catarrh, colds, cough, influenza, hysteria, pain, asthma, malaria, and dysmenorrhoea.[17, 161, 164]
Smilax anceps	Smilacaceae	Sore eyes, venereal diseases, coughs, abscesses, diuretics, promote fertility, neuralgia, eczema, ulcers, scabies, gout, diaphoretic, toothache, asthma, and varicose veins.[17]
Stangeria eriopus	Stangeriaceae	Headaches, congestion, high blood pressure, purgative, and pain in bones.[17, 164]
Strelitzia reginae	Strelitziaceae	Inflamed glands associated with venereal diseases.[17]
Tetraselago natalensis	Scrophulariaceae	Venereal diseases.[17]
Vernonia angulifolia	Asteraceae	Stomach ailments.[17]
Vernonia glabra	Asteraceae	Pneumonia and stomach ailments.[169]

Vernonia spp.	Asteraceae	Fever, malaria, and stomach-aches.[17]
Ziziphus mucronata	Rhamnaceae	Cough and chest problems, diarrhoea and dysentery, painful sore, boils and glandular swellings, toothaches, scrofula, infertility, measles and scarlet, fever, gonorrhoea, rheumatism, dysmenorrhoea, and urogenital complains.[17, 161, 164]

The extracts prepared are summarised in Table 3.2.

TABLE 3.2: The combined DCM and MeOH extracts prepared for anti-HIV screening.

Number	Extract code	Plant	Part used
1	NP 83a	*Combretum kraussii*	Stems
2	NP 83b	*Combretum kraussii*	Leaves
3	NP 84	*Vernonia glabra*	Leaves and stems
4	NP 85	*Tetraselago natalensis*	Leaves and stems
5	NP 86	*Smilax anceps*	Leaves and stems
6	NP 87	*Berkheya speciosa*	Leaves and stem
7	NP 88a	*Gardenia thunbergia*	stem/leaves
8	NP 88b	*Gardenia thunbergia*	Fruits
9	NP 89a	*Psoralea pinnata*	Leaves
10	NP 89b	*Psoralea pinnata*	Stem
11	NP 90	*Pentzia globose*	Leaves and stem
12	NP 91	*Plectranthus ecklonii*	Leaves and stems
13	NP 92	*Leonotis species*	Leaves and stems
14	NP 93	*Artemisia afra*	Leaves and stems
15	NP 94	*Plumbago auriculata*	Leaves and stems
16	NP 95	*Stangeria eriopus*	Leaves and stems
17	NP 96a	*Heteromorpha trifoliate*	Leaves
18	NP 96b	*Heteromorpha trifoliate*	Stems
19	NP 97	*Lippia javanica*	Leaves and stems
20	NP 98	*Lasiospermum bipinnatum*	Leaves and stems
21	NP 99	*Vernonia species*	Leaves and stems
22	NP 100a	*Dioscorea dregeana*	Stems
23	NP 100b	*Dioscorea dregeana*	Leaves

24	NP 101	*Streltzia reginae*	Leaves and stems
25	NP 102	*Bulbine frutescens*	Roots
26	NP 103	*Plumbago spp.*	Leaves and stems
27	NP 104	*Chrysocoma ciliate*	Leaves and stems
28	NP 105	*Conyza scabrida*	Leaves and stems
29	NP 106	*Plectranthus saccatus*	Leaves and stems
30	NP 107	*Plectranthus zuluensis*	Leaves and stems
31	NP 108	*Plectranthus ambiguous*	Leaves and stems
32	NP 109	*Plectranthus verticillatus*	Whole plant
33	NP 110	*Acokanthera oppositifolia*	Leaves and stems
34	NP 111	*Rhus chirindensis*	Stems
35	NP 112	*Rhus chirindensis*	Leaves
36	NP 113	*Polygala fruticosa*	Leaves
37	NP 114	*Polygala fruticosa*	Stem
38	NP 115	*Bulbine latifolius*	Roots
39	NP 116	*Bulbine latifolius*	Bulbs
40	NP 117	*Siphonochilus aethiopicus*	Leaves and stems
41	NP 806	*Plectranthus hadiensis*	Leaves
42	NP 817a	*Euclea natalensis*	Leaves
43	NP 817b	*Euclea natalensis*	Stem
44	NP 830a	*Harpephyllum caffrum*	Leaves
45	NP 830a-40	*Harpephyllum caffrum*	Leaves
46	NP 830a-60	*Harpephyllum caffrum*	Leaves
47	NP 830a-80	*Harpephyllum caffrum*	Leaves
48	NP 830b	*Harpephyllum caffrum*	Stems
49	NP 842	*Vernonia angulifolia*	Leaves
50	NP 852a	*Ficus ingens*	Leaves
51	NP 852b	*Ficus ingens*	Stems
52	NP 853a	*Ochna serrulata*	Leaves
53	NP 853b	*Ochna serrulata*	Stems
54	NP 878	*Plectranthus madagascariensis*	Leaves
55	NP 885 a	*Ziziphus mucronata*	Leaves
56	NP 885b	*Ziziphus mucronata*	Stems

57	NP 895	*Rothmannia globose*	Roots
58	NP 896	*Rothmannia globose*	Leaves
59	NP 897	*Helichrysum panduratum*	Whole plant
60	NP 898	*Helichrysum cymosum*	Roots and leaves
61	NP 899	*Orthosiphon labiatus*	Leaves
62	NP 900	*Helichrysum cephaloideum*	Leaves, stems, and flower
63	NP 901	*Helichrysum melanacme*	Leaves and stems
64	NP 902	*Helichrysum nudifolium*	Leaves, stems, and flower
65	NP 903	*Helichrysum odoratissimum*	Leaves and stems

3.2.1 INHIBITION OF HIV-REVERSE TRANSCRIPTASE

A commercial kit was used to screen for inhibition of HIV reverse transcriptase, the enzyme responsible for the viral replication, as a preliminary anti-HIV test. The kit is designed to determine the DNA polymerase activity. This enzyme immunoassay involves the synthesis of the DNA by RNA and a primer. The reverse-transcriptase (RT) activity is measured by the addition of enzyme conjugate which results in the development of a colour.[162, 170, 171] The absorbance measured is related to the RT activity.

The combined DCM and MeOH extracts from forty-nine South African plant species were screened for the inhibition of recombinant HIV-RT at a concentration of 500 µg/mL, the standard concentration used by other people. Twelve extracts from *Harpephyllum caffrum, Combretum kraussii, Plumbago spp., Berkheya speciosa, Polygala fruticosa, Vernonia glabra, Lippia javanica, Smilax anceps,* and *Vernonia spp* (Figure 3.1) showed inhibition greater than 50%. The assay was done in duplicates as shown in Table 3.3.

TABLE 3.3: Results from the recombinant HIV-RT screening assay.

Number	Extract code	Plant	% inhib. 1	% inhib. 2	Av.	SD
1	NP 83a	*Combretum kraussii*	70	70	70	0
2	NP 83b	*Combretum kraussii*	67	67	67	0
3	NP 84	*Vernonia glabra*	59	59	59	0
4	NP 85	*Tetraselago natalensis*	46	49	47	2.192
5	NP 86	*Smilax anceps*	53	53	53	0
6	NP 87	*Berkheya speciose*	62	62	62	0.2828

7	NP 88a	*Gardenia thunbergia*	43	34	39	6.7175
8	NP 88b	*Gardenia thunbergia*	4	16	10	8.2166
9	NP 89a	*Psoralea pinnata*	52	40	46	8.6267
10	NP 89b	*Psoralea pinnata*	26	24	25	1.0607
11	NP 90	*Pentzia globose*	40	33	36	5.374
12	NP 91	*Plectranthus ecklonii*	48	31	39	12.1622
13	NP 92	*Leonotis species*	42	41	41	0.6364
14	NP 93	*Artemisia afra*	43	45	44	1.6263
15	NP 94	*Plumbago auriculata*	44	41	43	2.3335
16	NP 95	*Stangeria eriopus*	42	45	44	1.9092
17	NP 96a	*Heteromorpha trifoliate*	33	42	38	6.2933
18	NP 96b	*Heteromorpha trifoliate*	30	45	37	10.3945
19	NP 97	*Lippia javanica*	52	58	55	3.9598
20	NP 98	*Lasiospermum bipinnatum*	42	30	36	8.9803
21	NP 99	*Vernonia species*	51	53	52	0.9899
22	NP 100a	*Dioscorea dregeana*	40	33	36	4.4548
23	NP 100b	*Dioscorea dregeana*	41	23	32	12.5865
24	NP 101	*Streltzia reginae*	42	33	37	6.4347
25	NP 102	*Bulbine frutescens*	47	45	46	0.7778
26	NP 103	*Plumbago spp.*	68	62	65	4.5255
27	NP 104	*Chrysocoma ciliate*	9	-5	2	9.9136
28	NP 105	*Conyza scabrida*	31	26	29	3.3941
29	NP 106	*Plectranthus saccatus*	13	32	23	13.0815
30	NP 107	*Plectranthus zuluensis*	30	38	34	5.3033
31	NP 108	*Plectranthus ambiguous*	31	15	23	11.5258
32	NP 109	*Plectranthus verticillatus*	38	38	38	0.1414
33	NP 110	*Acokanthera oppositifolia*	25	25	25	0.2828
34	NP 111	*Rhus chirindensis*	34	23	28	7.7782
35	NP 112	*Rhus chirindensis*	34	31	32	2.5456
36	NP 113	*Polygala fruticosa*	65	59	62	3.7477
37	NP 114	*Polygala fruticosa*	4	43	24	27.266
38	NP 115	*Bulbine latifolius*	7	10	8	1.895
39	NP 116	*Bulbine latifolius*	29	31	30	1.2021

40	NP 117	*Siphonochilus aethiopicus*	32	36	34	3.2527
41	NP 806	*Plectranthus hadiensis*	12	9	11	2.6163
42	NP 817a	*Euclea natalensis*	-1	4	1	3.1254
43	NP 817b	*Euclea natalensis*	19	19	19	0.2121
44	NP 830a	*Harpephyllum caffrum*	84	78	81	4.3134
45	NP 830a-40	*Harpephyllum caffrum*	80	78	79	1.4142
46	NP 830a-60	*Harpephyllum caffrum*	62	73	67	7.4246
47	NP 830a-80	*Harpephyllum caffrum*	40	47	44	4.879
48	NP 830b	*Harpephyllum caffrum*	-2	19	9	14.7078
49	NP 842	*Vernonia angulifolia*	37	35	36	1.3435
50	NP 878	*Ficus ingens*	36	37	37	0.3536
51	NP 852a	*Ficus ingens*	-12	8	-2	14.2128
52	NP 852b	*Ochna serrulata*	-10	18	4	19.8697
53	NP 853a	*Ochna serrulata*	34	35	34	0.9899
54	NP 853b	*Plectranthus madagascariensis*	34	38	36	3.0406
55	NP885 a	*Ziziphus mucronata*	17	12	14	3.5355
56	NP 885b	*Ziziphus mucronata*	19	27	23	5.9397
57	NP 895	*Rothmannia globose*	28	24	26	2.7577
58	NP 896	*Rothmannia globose*	23	20	21	2.4042
59	NP 897	*Helichrysum panduratum*	20	9	15	7.4246
60	NP 898	*Helichrysum cymosum*	5	1	3	2.5456
61	NP 899	*Orthosiphon labiatus*	-4	3	0	5.1619
62	NP 900	*Helichrysum cephaloideum*	-5	2	-2	5.0205
63	NP 901	*Helichrysum melanacme*	-5	13	4	12.9401
64	NP 902	*Helichrysum nudifolium*	7	11	9	3.0406
65	NP 903	*Helichrysum odoratissimum*	12	5	9	4.9497

The crude extract of *Harpephyllum caffrum* leaves (NP 830a) exhibited the highest percentage inhibition of the recombinant HIV-RT at an average of 81% while the stem extract only exhibited 9%. The second highest inhibition was from *Combretum kraussii* stem extract (70%), then *Combretum kraussii* leaf extract (67%). As a result of the activity observed by the *Combretum kraussii* leaf extract, the extract was investigated further and three compounds were isolated.

These results are described in Chapter 4. Figure 3.1 shows some of the plants species that showed significant inhibitory activity of the HIV-RT.

Harpephyllum caffrum[168]

Combretum kraussii[168]

Berkheya speciosa[172]

Polygala fruticosa[173]

Vernonia glabra[168]

Lippia javanica[168]

Smilax anceps[174]

Tetraselago natalensis[175]

FIGURE 3.1: Plants with significant HIV-RT inhibitory activity.

Figure 3.2 shows the results as average percentage inhibition of the HIV-RT by crude extracts. All extracts showed some inhibition of HIV-RT activity except for *Orthosiphon labiatus, Ficus ingens* and *Helichrysum cephaloideum* (NP 899, NP 852a and NP 900).

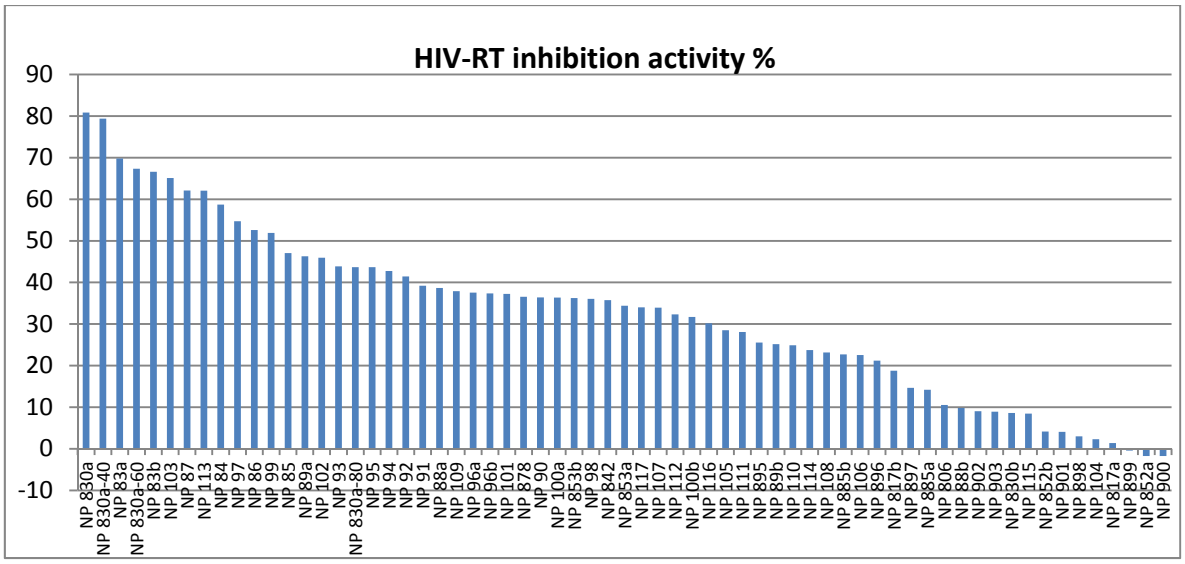


FIGURE 3.2: Percentage inhibition of HIV-RT by plant extracts.

3.2.2 ANTI-HIV CELL-BASED ASSAY

A second independent screening method, a cell-based assay, was used to determine the cytotoxicity and the anti-HIV activity of plant extracts. This assay is used to determine the effects of potential drugs on *in vitro* infections induced on HIV infected cells and uninfected cells.[176] Infection of cells by HIV results in cell death. On addition of plant extract with anti-HIV properties the cell death is reduced. The cell viability is measure by a tetrazolium salt. Tetrazolium salts assays such as MTT, 3-{4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide, and XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyloamino)carbonyl]-2*H*-tetrazolium hydroxide are widely used to determine the proliferation and viability of cell cultures. The colourless XTT is metabolically reduced by mitochondria enzymes in viable cells to a water-soluble orange formazan. The assay detects drug-induced suppression of viral cytopathic effects by the generation of the soluble formazan in surviving cells.[176] The formazan formed is proportional to the number of metabolically active cells and can be quantified photometrically by measuring the absorbance. Shown below are the chemical structures of tetrazolium salts (Figure 3.3).

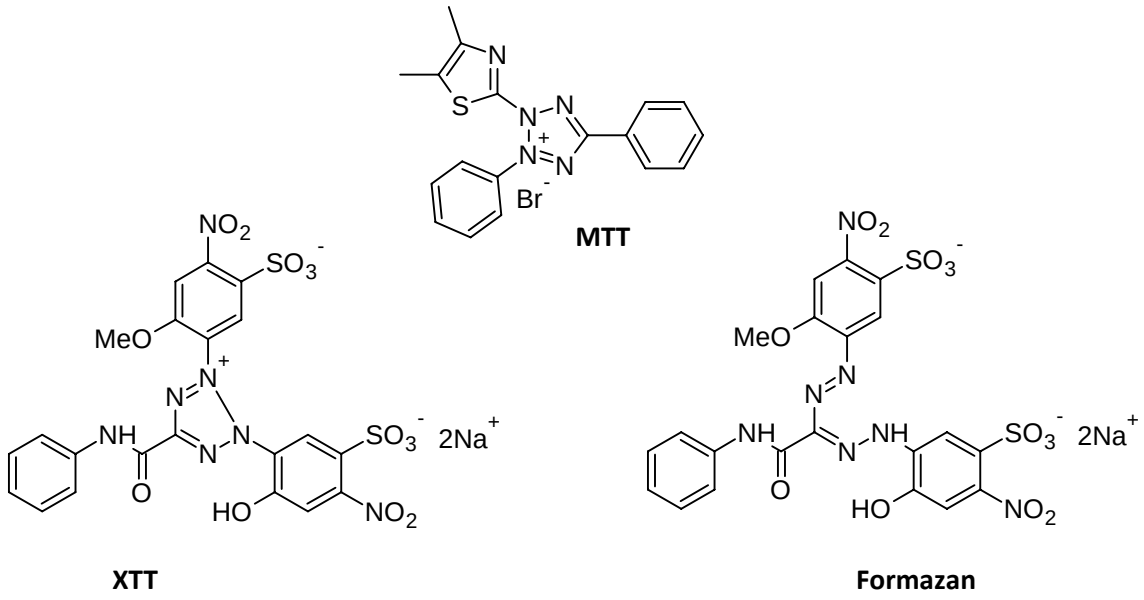


FIGURE 3.3: Structures of tetrazolium salts.

AZT, a known anti-HIV drug, was used as a positive control in the screening process for comparison of the results. It demonstrated the expected antiviral results with the IC_{50} of 583.69 µg/mL, EC_{50} of 0.347 µg/mL, and a selectivity index of 1666.67. The inhibitory concentration at 50% (IC_{50}) is the concentration of the plant extract that reduced the absorbance of the uninfected cell by 50% (cytotoxicity), the effective concentration at 50% (EC_{50}), is the concentration of the extract that achieved 50% protection in infected cells, and the selectivity index of the test compound is calculated as the ratio IC_{50}/ $EC_{50.}$ Table 3.4 shows the XTT assay results. Although many extracts showed the significant inhibition of the HIV-RT, no antiviral effect (not ≥ 50% protective effect) was observed, with a few exceptions, most plant extracts were not highly toxic on the uninfected cell at the concentrations tested.

TABLE 3.4: Results from the XTT anti-HIV screening assay.

Code	**Plant species**	**IC_{50} (µg/mL)**	**EC_{50} (µg/mL)**
NP 83	*Combretum kraussii*	98	No activity
NP 84	*Vernonia glabra*	70	No activity
NP 85	*Tetraselago natalensis*	909	No activity
NP 86	*Smilax anceps*	815	No activity
NP 87	*Berkheya speciosa*	682	No activity
NP 88	*Gardenia thunbergia*	1119	No activity

NP 89	*Psoralea pinnata*	36	No activity
NP 90	*Pentzia globose*	626	No activity
NP 91	*Plectranthus ecklonii*	176	No activity
NP 92	*Leonotis*	44	No activity
NP 93	*Artemisia afra*	91	No activity
NP 94	*Plumbago auriculata*	256	No activity
NP 95	*Stangeria eriopus*	174	No activity
NP 96	*Heteromorpha trifoliate*	143	No activity
NP 97	*Lippia javanica*	188	No activity
NP 98	*Lasiospermum bipinnatum*	184	No activity
NP 99	*Vernonia spp.*	18	No activity
NP 100	*Dioscorea dregeana*	188	No activity
NP 101	*Strelitzia reginae*	34	No activity
NP 102	*Bulbine frutescens*	165	No activity
NP 103	*Plumbago spp.*	196	No activity
NP 105	*Conyza scabrida*	281	572
NP 106	*Plectranthus saccatus*	111	No activity
NP 107	*Plectranthus zuluensis*	1150	No activity
NP 108	*Plectranthus ambiguous*	191	No activity
NP 109	*Plectranthus verticillatus*	311	No activity
NP 110	*Acokanthera oppositifolia*	2	No activity
NP 112	*Rhus chirindensis* (leaves)	40	No activity
NP 113	*Polygala fruticosa* (leaves)	873	No activity
NP 114	*Polygala fruticosa (stem)*	854	No activity
NP 116	*Bulbine latifolius* (fruit)	941	No activity
NP 117	*Siphonochilus aethiopicus*	196	No activity
NP 806	*Plectranthus hadiensis*	112	No activity
NP 817	*Euclea natalensis* (stem)	124	No activity
NP 830a	*Harpephyllum caffrum* (leaves)	38	835
NP 830b	*Harpephyllum caffrum* (stem)	186	No activity
NP 842	*Vernonia angulifolia*	36	No activity
NP 853	*Ochna serrulata (leaves)*	225	No activity
NP 853 b	*Ochna serrulata(stem)*	147	No activity

NP 878	*Plectranthus madagascariensis*	32	No activity
NP 885	*Ziziphus mucronata (leaves)*	32	No activity
NP 885 b	*Ziziphus mucronata(stem)*	81	No activity
NP 895	*Rothmannia globosa(leaves)*	180	No activity
NP 896	*Helichrysum panduratum*	158	No activity
NP 902	*Helichrysum nudifolium*	160	No activity
-AZT	AZT	584	0.347

The graphs below, Figure 3.4, represent the results of the XTT assay for AZT and *Plectranthus saccatus* treated HIV-infected and uninfected cells.

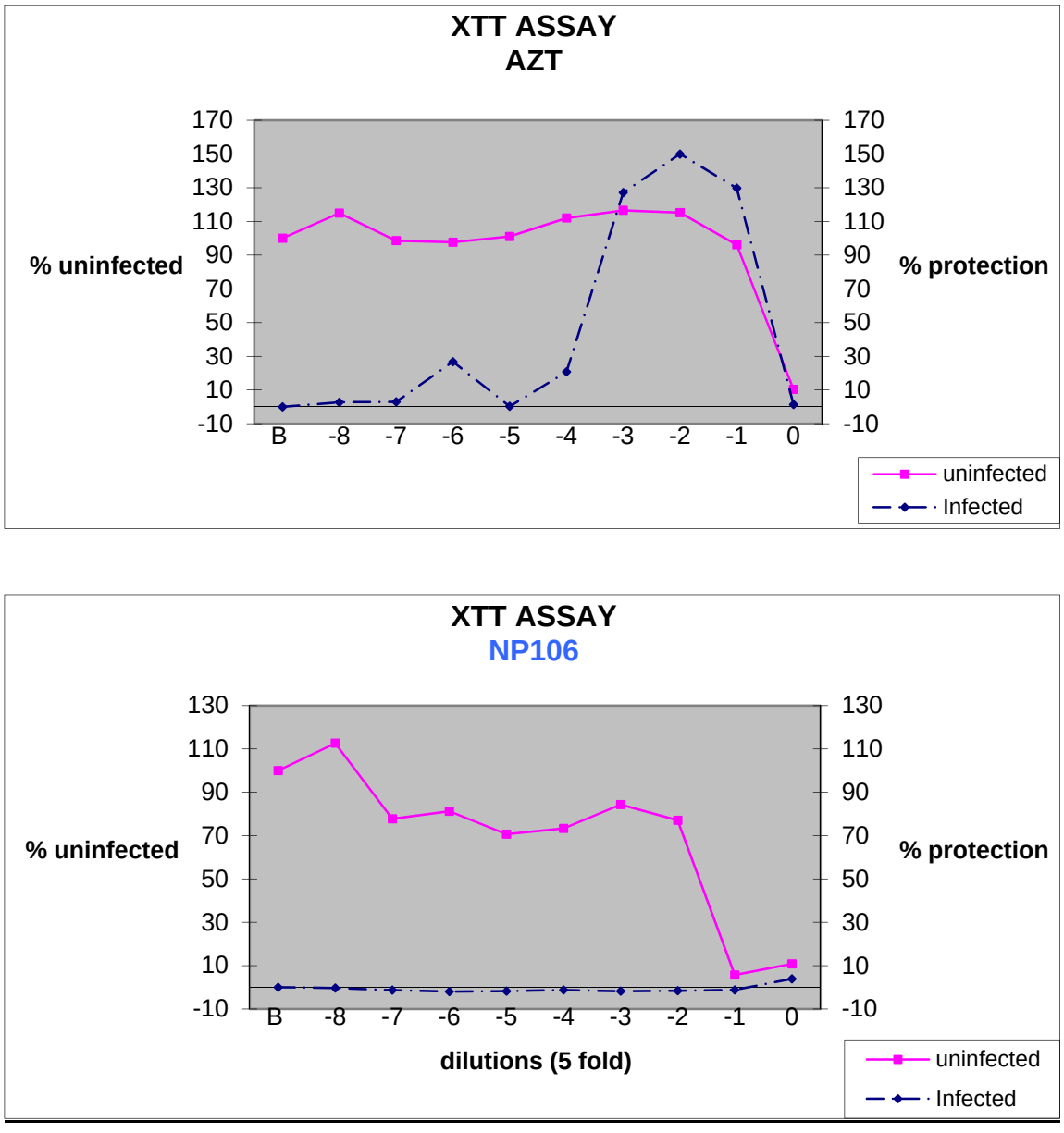


FIGURE 3.4: Anti-HIV activity of AZT and *Plectranthus saccatus*.

On the x-axis; B represents the blank, *i.e*. the culture medium (RPMI) without test substance, on the x-axis 0 represents the test substance at 1.5 mg/mL, and -1 to -8 represent the successive five-fold dilutions on a logarithmic scale, *i.e.* the concentration is increasing from left to right. The curves for AZT can be explained as follows: For the uninfected cells at low concentration AZT is non-toxic as shown by the straight line. At high concentrations AZT becomes toxic and the production of XTT formazan decrease. For the infected cells, at low concentrations no antiviral activity is observed, the cells die and no XTT formazan production is observed. As the concentration of AZT is increased, the protection of the cells increases and so is the production of XTT formazan. For the *Plectranthus saccatus* treated cell, the toxicity of the extract is observed as the concentration increases on uninfected cells. For the infected cells, no production of XTT formazan is observed, and thus no protection of the cells is observed.

The results of the HIV-RT assay and that of the XTT assay were not in agreement No pre-treatment of the crude extract was done to remove any compounds that might interfere with the HIV-RT screening results, *e.g.* tannins. Tannins are present in most plants and they inhibit a large number of enzymatic pathways including the RT which could have resulted in the positive HIV-RT inhibitory activity on the recombinant HIV-RT assay. Although they have shown antiviral properties they are known to cause serious health problems such as liver damage, and have carcinogenic potential and anti-nutritional activity.[85]

3.3 CONCLUSION

Several of the crude extracts inhibited the enzyme HIV-reverse transcriptase. Based on the preliminary results, *C. kraussi* was subjected to phytochemical studies, which are described in Chapter 4.

However, the cell-based *in vitro* anti-HIV assay did not show any activity for any of the extracts. The cytotoxicity against the MT-4 cells used was also determined. Some of the extracts were toxic to the cells, but the majority of the extracts had a low cytotoxicity towards the cells.

As a result of this disappointing result, the focus of the project changed to the synthesis of compounds with anti-HIV activity, as will be discussed in Chapter 5.

3.4 EXPERIMENTAL

3.4.1 GENERAL

Thin-layer chromatography (TLC) was performed on aluminium sheets or glass coated with Merck silica gel 60 P_{254}, with visualization of the compounds by inspection under UV (254 or 365 nm) and/or by staining with an anisaldehyde solution. Column chromatography was performed using Merck silica gel 60 (0.040-0.063 mm). For centrifugal chromatography on a chromatotron, Merck silica gel 60 P_{254} containing gypsum was coated on the glass plate. Hexane was distilled before use.

3.4.2 PLANT COLLECTION

Plants were collected from the KwaZulu-Natal National Botanical Garden or the University of KwaZulu-Natal Botanical Garden in Pietermaritzburg. Only the aerial parts of the plants were collected unless otherwise stated (Table 3.2). Plants collected in the KwaZulu-Natal National Botanical Garden were identified by Ms Isabel Johnson. Plants collected in the University of KwaZulu-Natal Botanical Garden were identified by Dr Allison Young.

3.4.3 PREPARATION OF PLANT EXTRACTS

The plants collected were allowed to dry in a closed ventilated room for 2 to 3 weeks at room temperature. Dried plant material was milled to a fine powder and stored in closed containers at room temperature until ready to be used. The milled material was weighed and extracted with two solvents, DCM and MeOH. The amount of plant material used to prepare extracts depended on the material present, the ratio of dried material to solvent was 10 g:150 mL. The plant material was first extracted at room temperature overnight with DCM. The DCM was filtered off and evaporated under reduced pressure to afford the DCM extract. The residue was then extracted again overnight with MeOH. The solvent was filtered off and evaporated under reduced pressure at temperatures not more than 60 °C to afford the MeOH extract. The DCM and MeOH extracts were weighed for each plant species. The crude extracts were analysed by TLC. The TLC showed that the DCM and MeOH extracts were similar, thus the extracts were combined.

The crude extract of *Harpephyllum caffrum* leaf showed significant inhibition and was fractionated using a reverse-phase C_{18} silica cartridge to determine the most active fraction.

Water was first run through the cartridge before the extract was loaded under vacuum. 200 mg of extract was dissolved in the minimum amount of 40% MeOH in water, the initial mobile phase. The step gradient (40%, 60%, 80% 100% MeOH in water) was used for the fractionation. Three fractions were obtained, the solvent was evaporated under reduced pressure and the aqueous remainder was freeze dried, fractions NP 830a-40, NP 830a-60 and 830a-80. The extracts prepared are indicated in Table 3.2.

3.4.4 REVERSE TRANSCRIPTASE ASSAY

The inhibition of HIV-1 reverse-transcriptase by plant extracts was determined by using a commercial kit from Roche. Recombinant HIV-1 reverse transcriptase was diluted with autoclaved water to a concentration of 2 ng/µL. This stock solution was further diluted with the lysis buffer to a concentration of 0.2 ng/µL. The crude extracts were dissolved in DMSO to an initial concentration of 15 mg/mL. This initial solution was further diluted by the lysis buffer to a concentration of 1.5 mg/mL. The testing was done in duplicates. The inhibition of HIV-1 RT was measured using a 20 µL of extract, 20 µL of recombinant HIV-1 RT (4 ng) and 20 µL of reaction mixture. The negative control contained 40 µL of the lysis buffer and 20 µL of reaction mixture. The positive control contained 20 µL 10% DMSO in lysis buffer, 20 µL of recombinant HIV-1 RT (4 ng) and 20 µL of reaction mixture. All this was added in wells of a microplate, covered with foil and incubated for one hour at 37 °C. After 1 hour the solution was removed and each well was washed 5 times with 250 µL of washing buffer for about thirty seconds and the buffer was carefully removed. 200 µL of anti-digoxigen peroxidase, (anti-DIG-POD), was added to each well, the wells were covered in foil and incubated for another one hour at 37 °C. The solution was removed again and washed 5 times with 250 µL of the washing buffer. Lastly 200 µL of substrate solution was added and the wells were left at room temperature for about 10-20 minutes until the developed green colour was sufficient for photometric analysis. The absorbance was measured at 405 nm and 492 nm (background) using an ELISA reader. The inhibition was calculated using the formula below.

$$\text{Percentage inhibition} = 100 - (((A_{405}-A_{490})_{EXTRACT} / (A_{405}-A_{492})_{+VE\ CONTROL\ AV.}) \times 100)$$

Where:

A_{405} = Absorbance at 405nm

A_{490} = Absorbance at 490nm

The results of this assay are summarised in Table 3.3.

3.4.5 CELL-BASED ASSAY

These experiments were conducted by Anneta Naidoo and Prof R. Parboosingin from the Department of Virology/National Health Laboratory services, Nelson R Mandela School of Medicine, University of KwaZulu-Natal Durban. MT-4 cells were suspended with RPMI-1640 at 6 X 10^5 cells/mL. 50 µL of cells were pipetted into each well. 50 µL of $HTLV_{IIIB}$ virus was added to infect the MT-4 cells at 100-300 TCID50 (50% tissue culture infective dose) or 50 µL of RPMI-1640 medium was added for mock cells (uninfected). Eight successive five-fold dilutions of plant extracts were added to the wells, with the initial concentration being 1.5 mg/mL in DMSO. AZT was used as a control drug. The RPMI-1640 medium without test substance was used as a blank measurement. The plate was incubated for five days at 37 °C in a CO_2 incubator. XTT salt with phosphate buffered saline (PBS) was made up in RPMI-1640 medium. After five days of incubation 20 µL of the XTT solution was added and the plate was incubated for four hours at 37 °C in a CO_2 incubator for the determination of cell viability. The absorbance was measured at 450 nm with the reference wavelength of 620 nm using an ELISA reader. The data was calculated using the average optical density of the wells. The dilutions were performed in triplicate. The effective Concentration at 50% (EC_{50}) was calculated according to the formula:

$$[(OD_T)_{HIV} - (OD_C)_{HIV}]/ [(OD_C)_{MOCK} - (OD_C)_{HIV}]$$

Where:

$(OD_T)_{HIV}$ is the optical density measured with a given concentration of the test compound in the HIV-infected cells.

$(OD_C)_{HIV}$ is the optical density measured for the control untreated HIV-infected cells.

$(OD_C)_{MOCK}$ is the optical density measured for the control untreated uninfected

The results of this assay are summarised in Table 3.4.

3.4.6 CELL LINE AND VIRUS

MT-4 cells were grown in RPMI 1640 medium containing sodium carbonate and L-glutamine, supplemented with 10% heat-inactive foetal calf serum and antibiotics and maintained at 37 °C in a 5% CO_2 incubator. The HIV virus ($HTLV_{IIIB}$) was obtained from the culture supernatant of HIV-1 infected MT-4 cell lines from Aids Research and Reference Reagent Program, Division of AIDS,

NIAID, NIH. The virus titer, the lowest concentration at which the virus infects cells, was determined in MT-4 cell. The virus stock was stored at -70 °C.

CHAPTER 4

Phytochemical studies of *Combretum kraussii*

4.1 *COMBRETUM* GENUS

This chapter reports the isolation of compounds from the species *Combretum kraussi* Hochst. (*Combretum* Loeft.). The genus *Combretum* Loeft. belongs to the Combretaceae family which has about 20 genera and 600 species.[177] *Combretum* and *Terminalia* are the largest genus within the *Combretaceae* family with approximately 370 and 200 species, respectively.[177-180] Other genera are *Anogeissus*, *Buchenavia*, *Bucida*, *Calopyxis*, *Calycopteris*, *Conocarpus, Dansiea*, *Guiera*, *Laguncularia*, *Lumnitzera*, *Macropteranthes*, *Melostemon*, *Pteleopsis*, *Quisqualis*, *Strephonema*, *Terminaliopsis* and *Thiloa*.[181] Combretaceae is known as a family of the tropics and subtropics as it occurs mostly in Africa and Brazil. The name *Combretum* was used by a Greek philosopher Pliny and means climbing/creeping plant in Latin.[168]

South Africa has about 30 species of *Combretum*, most are trees or shrubs, but a few are scramblers. In South Africa the members of the genus are widespread, except for the extreme southern and south-western areas.[181] The characteristic feature of the genus is the papery, four-winged fruit as seen for *C. microphyllum* and *C. imberbe* in Figure 4.1.[179] However, there is also *C. bracteosum*, which does not have the characteristic four-winged fruit. It is called hiccough-nut, hiccup nut or hiccough creeper in English. It is a true nut in that it has a hard outer shell and the fruit is almost spherical. It is said that it causes hiccups but another opinion is that it actually cures them.[168]

FIGURE 4.1: *C. microphyllum, C. imberbe* and *C. bracteosum*.[168]

4.1.1 TRADITIONAL USES OF *COMBRETUM*

Combretum species are widely used for medicinal purposes.[180, 181] The plant parts mostly used are the leaves and bark, as fruits were found to be toxic to humans.[182] *Combretum* species have many medical applications including treating abdominal disorders and pains, headaches, backache, toothache, earache, bacterial infections, bilharziasis, cancer, fever, colds, chest coughs, pneumonia, cleansing the urinary system, conjunctivitis, stomach and gastric problems, constipation, gallstones, diarrhoea, dysentery, dysmenorrhoea, gastric ulcers, general weakness, heart conditions, hookworm, blennorrhagia, hypertension, jaundice, leprosy, nosebleeds, oedema, skin diseases, sore throats, scorpion and snake bites, swelling caused by mumps, fattening babies, infertility in women, syphilis, gonorrhoea, venereal diseases.[17, 178, 181, 183-185]

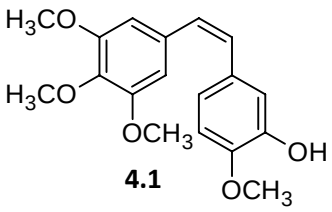
4.1.2 BIOLOGICAL ACTIVITIES OF *COMBRETUM*

A considerable number of studies have suggested that extracts or active compounds obtained from *Combretum* species have a broad spectrum of biological activities, including antibacterial, antiprotozoal, anticancer, cytotoxic, analgesic, anti-inflammatory, hepatoprotective and antiviral activities.[180, 182-184, 186-188]

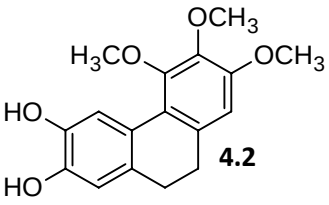
The extracts of the species mentioned below were evaluated for activity. *C. molle*, *C. petrophilum*, *C. moggii*, *C. padoides*, *C. paniculatum, C. mossambicense, C. zeyheri, C. erythrophyllum* and *C. woodii* exhibited antibacterial activity.[181, 182, 189, 190] *C. nelsonii*, *C. albopunctactum*, *C. imberbe*, *C. moggii* and *C. petrophilum* were found to have antifungal activity.[178] Antiprotozoal activity was observed from the extract of *C. molle*.[187] *C. caffrum*, *C. imberbe*, *C. hartmannianum* and *C. padoides* were found to have anticancer properties.[186, 191-193] *C. leprosum* has analgesic activity.[194] *C. quadrangulare* was found to have hepatoprotective activity.[183] *C. molle, C. paniculatum* and *C. hartmannianum* exhibited anti-HIV activity.[157, 181, 191, 195] Antioxidant activity was observed from the extracts of *C. erythrophyllum, C. woodii, C. collinum* ssp. *Taborense* and *C. hereroense*.[189] *C. micrantum*, *C. apiculatum*, *C. imberbe*, *C. molle* and *C. mossambicense* were found to have anti-inflammatory activity.[182, 196] *C. molle*, *C. paniculatum* and *C. petrophilum* showed anthelmintic activity.[182] *C. apiculatum C. hereroense* and *C. mossambicense* showed antischistosomal activity.[182] *C. apiculatum*, *C. hereroense* and *C. mossambicense* showed DNA-damaging activity.[182]

4.1.3 PHYTOCHEMISTRY OF *COMBRETUM*

Phytochemical studies on *Combretum* resulted in the isolation of many structural classes *e.g*. alkaloids, triterpenes, chalcones, flavones, flavanones, flavonoids, stilbenes, phenanthrenes, lignans, tannins and non-protein amino acids, among others.[177, 180, 181, 197-201] From the extracts of South African *Combretum* species, combretastatins, e.g. combretastatin A-4 (**4.1**) have been isolated from *C. caffrum* and *C. kraussi*.[181, 202] Combretastatin A-4 is a potent inhibitor of tubulin polymerization and an anticancer compound.[203-205] It is currently in clinical trials for anticancer therapy.[206] 9,10-Dihydrophenanthrene **(4.2)** was isolated from the extracts of *C. apiculatum*, *C. molle* and *C. caffrum*.[193] Triterpenoids and their glycosides, like arjungenin **(4.3)** and arjunglucoside **(4.4)**, were isolated from *C.* molle, *C. padoides* and *C. eleagnoides*.[207, 208] Cyclobutane dimer **4.5** was isolated from *C. apiculatum* and *C. albopunctatum*.[181, 209]



4.1



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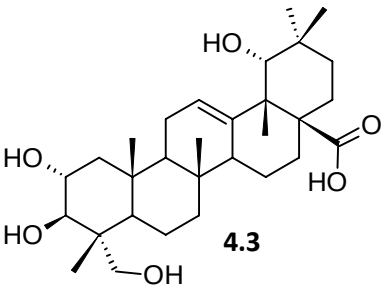

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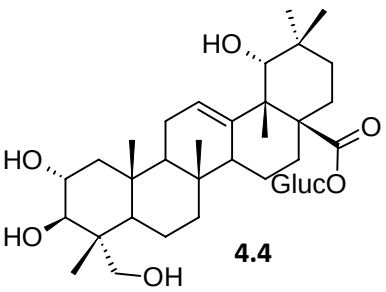

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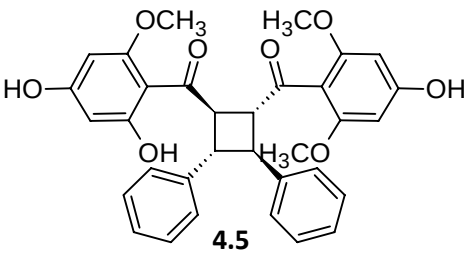


4.5

4.2 COMBRETUM KRAUSSII

C. kraussii is known as 'Forest Bush willow' in English, 'umdubu' in isiZulu, and 'Bosvaderlandswilg' in Afrikaans. It was named in honour of Dr F. Krauss from Stuttgart Museum in Germany who in 1837-1840 made a collecting trip to South Africa. *C. kraussii* occurs from the coast to the midlands in the eastern regions of South Africa (over the Eastern Cape, KwaZulu-Natal, Mpumalanga and Northern Province) and neighbouring Swaziland and Mozambique (Figure 4.2). *C. kraussii* occurs in evergreen forest and woodland habitats where there is high rainfall or ground water.

FIGURE 4.2: Geographical distribution of *Combretum kraussii*.[174]

It is usually a medium-sized deciduous tree with an upright habit and a dense canopy, but it can reach heights of 20 m. The leaves are oppositely arranged and can be up to 90 mm long and 50 mm wide. In winter the leaves turn bright red to purple and drop just before flowering. It has creamy-white flowers that are carried in dense heads. Some trees also have a showy flush of small white leaves in spring which either turn green or are replaced by the bright green leaves. Four-winged fruit are small, light to dark red and turn a brownish red when dry (Figure 4.3). The tree is cold resistant and recommended for shady areas in gardens with a mild to warm climate.[168]

FIGURE 4.3: *Combretum kraussii*.[168]

4.2.1 ETHNOPHARMACOLOGY

Powdered roots of *C. kraussii* are used as a tonic or to stimulate appetite. The plant is also given as an enema for the cleansing of the urinary tract and for strengthening of the spinal column.[17] Together with a *Terminalia* species, it is used for dressing of wounds.[17] The roots of *C. kraussii* are commonly used by Zulu and Xhosa women as 'isihlambezo', to promote a favourable course of pregnancy and a quick uncomplicated labour. Other parts from the plant are used to produce antidiuretics, lotions for eye infections, as well as antiseptics.[17, 200] It was found that aqueous extracts *of Clivia miniata*, *Agapanthus africanus*, *Pentanisia prunelloides*, *Gunnera perpensa* are also used as 'isihlambezo' by pregnant women, stimulated smooth muscle contraction in isolated rat uterine tissue.[200, 210, 211]

4.2.2 PHYTOCHEMICAL STUDIES OF *COMBRETUM KRAUSSII*

Compounds containing a hydroxybenzene moiety are widely spread in nature. Phytochemical studies on *C. kraussi* resulted in isolation of many compounds possessing this structural unit (Figure 4.4). Compounds already isolated are β-sitosterol **(4.5)**, ellagic acids: ellagic acid **(4.6)**, 3,3',4-tri-*O*-methylellagic acid **(4.7)**, and 3,3'-di-*O*-methylellagic acid **(4.8)**, combretastatins and their glucosides: combretastatin **(4.9)**, combretastatin B-1 **(4.10)**, combretastatin B-5 **(4.11)**, combretastatin B-1 glucoside **(4.12)** and combretastatin B-5 glucoside **(4.13)**. Also isolated were stilbenes and their glucosides: combretum A-1 **(4.14)**, and β-D-glucopyranosides **4.15** and **4.16**.[212]

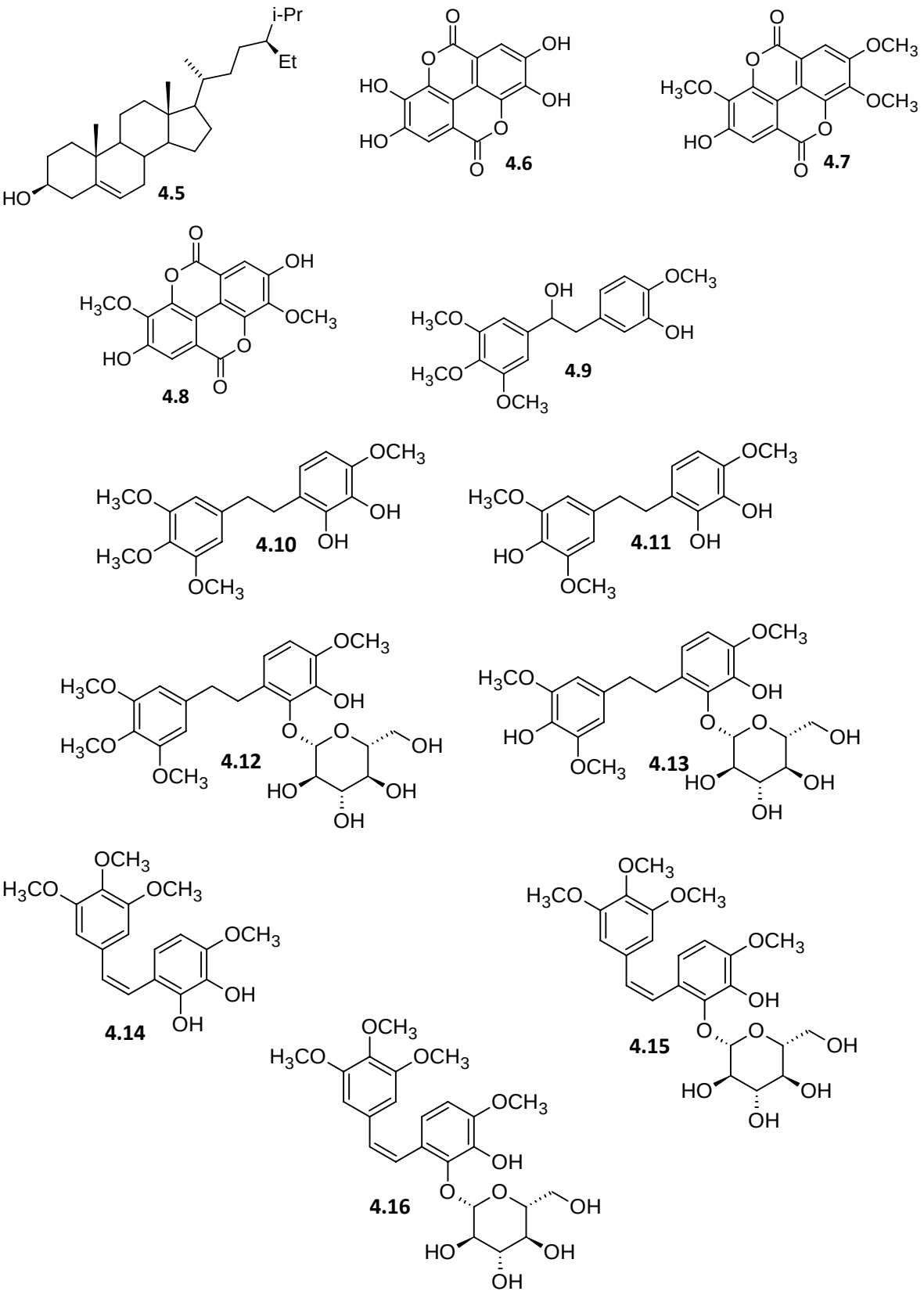


FIGURE 4.4: Compounds isolated from *Combretum kraussii.*

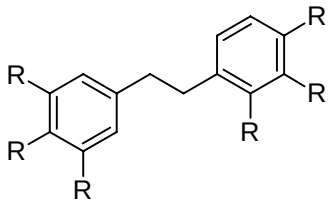
Biological studies of combretastatins showed that they are among the most cytotoxic agents tested so far against a series of cancer cell lines.[213, 214] The high potency together with their simple structures may offer a new approach to cancer treatment.[213, 215] Their anti-HIV potential has not been investigated.

4.3 ISOLATION OF COMPOUNDS FROM *COMBRETUM KRAUSSII*

4.3.1 STRUCTURAL ELUCIDATION OF COMBRETASTATIN B-1 (4.10)

Combretastatin B-1 was the first compound to be isolated from the extract. In the HRESI mass spectrum in a positive mode a peak with *m/z* 357.1313 $[M+Na]^+$ corresponding to a compound with a molecular formula $C_{18}H_{22}O_6$, was observed.

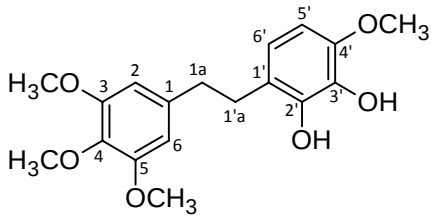
The ^1H NMR data showed three singlets at δ_H 6.43, 3.86 and 3.84, two doublets at δ_H 6.59 and 6.40 and a multiplet at δ_H 2.87 (Plate 1). The multiplets at δ_H 2.87 integrating for four protons, was typical of a benzyl group. The singlet at δ_H 6.43 integrating for two protons was typical for aromatic protons. The two doublets at δ_H 6.59 and 6.39 integrating for one proton each, with a coupling constant *J* = 8.4 Hz, were characteristic aromatic *ortho*-coupled protons. This information led to the sub-structure below.



The singlets at δ_H 3.84 and 3.86 integrating for nine and three protons, respectively can be assigned to four methoxy groups. From the formula obtained from the mass data, this accounts for all the carbons, less two hydrogens and two oxygens, meaning the remaining two unaccounted R-groups are two hydroxy groups.

In the DEPT spectrum the presence of four CH, two CH_2 as well as four CH_3 groups were confirmed. In the ^{13}C NMR spectrum, aromatic signals between δ_C 105.7 and 153.1 with four signals more downfield confirming their attachment to oxygen atoms, methoxy signals at δ_C 56.0 and 60.7 as well as the methylene signals at δ_C 31.7 and 36.6, were observed, suggesting that the compound was a indeed a combretastatin (Plate 2).

In the HMBC spectrum correlations were observed between the methylene proton and C-2, C-6 and C-2' and C-6'. HMBC correlations were also between H-6' and C-1a', C-2' C-3' and C-4', between H-5' and C-1', C-2' and C-3', and between H-2,6 with C-1a, C-3,5 and C-4. The structure was found to be combretastatin B-1 and confirmation of the structure of the compound was obtained by comparing the NMR data obtained with the literature data (Table 4.1).[202]



4.10

TABLE 4.1: The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of combretastatin B-1 **(4.10)** in $CDCl_3$.

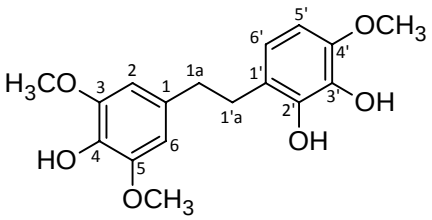
Experimental				**Literature**[202]	
Position	**δ_C**	**DEPT**	**δ_H** (400 MHz)	**δ_C**	**δ_H** (100 MHz)
1	138.1	C		138.2	
2	105.5	CH	6.43 (1H, *s*)	105.7	6.42 (1H, *s*)
3	153.0	C		153.1	
4	132.5	C		132.4	
5	153.0	C		153.1	
6	105.5	CH	6.43 (1H, *s*)	105.7	6.42 (1H, *s*)
1a	36.7	CH_2	2.87 (2H, *m*)	36.5	2.85 (2H, *m*)
1'a	31.8	CH_2	2.87 (2H, *m*)	31.8	2.85 (2H, *m*)
1'	121.4	C		121.6	
2'	142.1	C		142.2	
3'	136.1	C		136.2	
4'	145.3	C		145.4	
5'	102.4	CH	6.59 (1H, *d*, 8.4 Hz)	102.5	6.56 (1H, *d*, 8.4 Hz)
6'	120.0	CH	6.40 (1H, *d*, 8.4 Hz)	120.3	6.39 (1H, *d*, 8.4 Hz)
3,5-OCH_3	56.0	CH_3	3.84 (6H, *s*)	56.1	3.83 (6H, *s*)
4-OCH_3	60.8	CH_3	3.86 (3H, *s*)	60.2	3.86 (3H, *s*)
4'-OCH_3	56.1	CH_3	3.84 (3H, *s*)	56.2	3.83 (3H, *s*)

4.3.2 STRUCTURAL ELUCIDATION OF COMBRETASTATIN B-5 (4.11)

In the HRESI mass spectrum in a positive mode a peak with *m/z* 343.1157 $[M+Na]^+$ corresponding to a compound with a molecular formula $C_{17}H_{20}O_6$, was obtained.

The ^1H NMR spectrum of this compound was similar to that of the compound **4.10**. The only difference was the absence of one methoxy group. From the ^1H NMR spectrum two singlets, two doublets and a multiplet were observed. The singlet at δ_H 3.83 integrating for nine protons was assigned to the three methoxy groups (Plate 3). A multiplet at δ_H 2.75 was assigned to two methylene protons, the *ortho*-coupled protons were observed as doublets at δ_H 6.30 and 6.46 with a coupling constant of 8.4 Hz and at δ_H 6.34 a singlet which was assigned to two aromatic protons was observed.

Plate 4 shows the ^{13}C NMR spectrum of **4.11**. HMBC correlations were observed between the methylene protons at δ_H 2.75 and C-1, C-2, C-6, C-1', C-2' and C-6', between H-2,6 and C-1a, C-3,5 and C-4, between H-5' and C-1', C-2'and C-3', and between H-6' and C-1a', C-2', C-3' and C-4'. The structure was confirmed to be combretastatin-B5 **(4.11)** and the NMR data match the data reported (Table 4.2).[201]



4.11

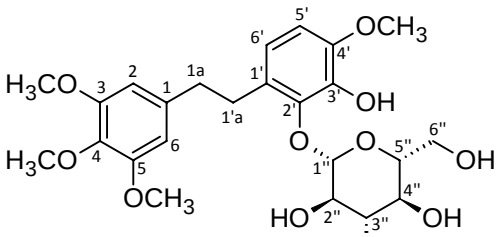
TABLE 4.2 : The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of combretastatin B-5 **(4.11)** in $CDCl_3$ and CD_3OD.

Experimental				**Literature**[201]	
Position	**δ_C**	**DEPT**	**δ_H** (400 MHz)	**δ_C**	**δ_H** (300 MHz)
1	133.4	C		133.4	
2	105.2	CH	6.34(1H, *s*)	105.1	6.50 (1H, *s*)
3	146.8	C		146.8	
4	132.6	C		132.4	
5	146.8	C		153.1	
6	105.2	CH	6.34 (1H, *s*)	105.7	6.50 (1H, *s*)
1a	36.3	CH_2	2.75 (2H, *m*)	36.5	2.82 (2H, *m*)
1'a	31.9	CH_2	2.75 (2H, *m*)	31.8	2.82 (2H, *m*)
1'	121.6	C		121.6	
2'	142.6	C		142.2	
3'	132.7	C		136.2	
4'	145.7	C		145.4	
5'	102.6	CH	6.46 (1H, *d*, 8.4 Hz)	102.5	6.50 (1H, *d*, 8.4 Hz)
6'	120.6	CH	6.40 (1H, *d*, 8.4 Hz)	120.3	6.36 (1H, *d*, 8.4 Hz)
3,5-OCH_3	56.2	CH_3	3.76 (6H, *s*)	56.1	3.84 (6H, *s*)
4'-OCH_3	60.8	CH_3	3.76 (3H, *s*)	60.2	3.84 (3H, *s*)

4.3.3 STRUCTURAL ELUCIDATION OF COMBRETASTATIN B-1 2-B-D-GLUCOSIDE (4.12)

The ^{1}H NMR data of this compound was similar to that of combretastatin B-1 **(4.10)** (Plate 5). However, it was clear that there was also a glucoside moiety present in this compound. The four methylene protons, aromatic singlet for two protons and two *ortho*-coupled protons were present, but there were additional signals, a characteristic H-1'' proton of a sugar moiety was observed as a doublet at δ_H 4.43. The signals for methoxy protons were overlapping with the CH protons of the sugar moiety.

In the HRESI mass spectrum in a positive mode, a peak with *m/z* 519.1831 $[M+Na]^+$ corresponding to a compound with a molecular formula $C_{24}H_{32}O_{11}$, was obtained, confirming the structural assignment of combretastatin B-1 2-β-D-glucoside **(4.12)** and the NMR data match the data reported (Table 4.3).[216]



4.12

TABLE 4.3: The ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of combretastatin B-1 2-β-D-glucoside **(4.12**) in $CDCl_3$.

Experimental				**Literature[216]**	
Position	**δ_C**	**DEPT**	**δ_H (500 MHz)**	**δ_C**	**δ_H (500 MHz)**
1	135.9	C		137.1	
2	105.5	CH	6.30 (1H, *s*)	107.0	6.47 (1H, *s*)
3	152.8	C		154.1	
4	132.7	C		134.0	
5	152.8	C		154.1	
6	105.5	CH	6.30 (1H, *s*)	107.0	6.47 (1H, *s*)
1a	36.8	CH_2	2.70 (2H, *m*)	38.4	2.84 (2H, *m*)
1'a	31.5	CH_2	2.81 (1H, *m*), 2.91 (1H, *m*)	32.8	2.92 (2H, *m*)
1'	128.0	C		129.7	
2'	143.7	C		145.2	
3'	139.0	C		140.7	
4'	146.9	C		148.5	
5'	108.6	CH	6.49 (1H, *d*, 8.6 Hz)	102.5	6.69 (1H, *d*, 8.5 Hz)
6'	119.9	CH	6.44 (1H, *d*, 8.6 Hz)	120.3	6.58 (1H, *d*, 8.5 Hz)
3,5-OCH_3	56.1	CH_3	3.51-3.87 (6H, *m*)	56.6	3.78 (6H, *s*)
4-OCH_3	55.9	CH_3	33.51-3.87 (6H, *m*)	56.1	3.71 (3H, *s*)
4'-OCH_3	56.1	CH_3	3.51-3.87 (6H, *m*)	56.8	3.80 (3H, *s*)
1''	105.7	CH	4.43 (1H, *d*, 7.6 Hz)	107.1	4.59 (1H, *d*, 7.9 Hz)
2''	73.9	CH	3.51-3.87 (*m*)	75.6	3.52 (1H, *dd,* 7.9, 9.1 Hz)
3''	69.6	CH	3.51-3.87 (*m*)	71.1	3.40 (1H, *dd,* 8.0, 9.1 Hz)
4''	76.2	CH	3.51-3.87 (*m*)	78.1	3.42 (1H, *dd,* 8.0, 7.0 Hz)
5''	76.5	CH	3.21 (1H, *m*)	78.5.	3.21 (1H, *m*)
6''	60.7	CH_2	3.51-3.87 (*m*)	62.4	3.65-3.90 (2H, *m*)

4.4 ANTI-HIV TESTING

All compounds isolated combretastatin B-1 (**4.10)**, combretastatin B-5 **(4.11)**, and combretastatin B-1 2-β-D-glucoside **(4.12)** were tested for the inhibition of HIV-1 replication at a single dose of 10 µM. They all showed significant inhibition, however toxicity precluded antiviral testing. The testing results are shown in Table 4.4.

Table 4.4: Results for antiviral inhibition at 10 µM single dose.

Compound	**CC_{50} ± Std Dev (µM)**	**% inhibition**	**EC_{50} [d] ± Std Dev (µM)**
4.10	21.85 ± 2.50	94.87	-
4.11	14.72 ± 1.85	94.99	-
4.12	11.40 ± 0.49	91.57	-

4.5 CONCLUSION

All compounds isolated combretastatin B-1 (**4.10)**, combretastatin B-5 **(4.11)**, and combretastatin B-1 2-β-D-glucoside **(4.12)** are known compounds that have been isolated from *C. kraussi* and other *Combretum* species. They are known to possess anti-cancer activity but their anti-HIV activity is not known. The isolation and identification of these compounds was prompted by inhibitory activity of the crude extract on the enzyme HIV-reverse transcriptase. The tests conducted on the isolated compounds showed inhibition of HIV-1 replication at a single dose of 10 µM, however, toxicity precluded antiviral testing and the compounds were not investigated further.

4.6 EXPERIMENTAL

4.6.1 GENERAL EXPERIMENTAL PROCEDURED

Thin-layer chromatography (TLC) was performed on aluminium sheets or glass plates coated with Merck silica gel 60 P_{254}, with visualization of the compounds by inspection under UV (254 or 365 nm) and/or by staining with an anisaldehyde solution. Column chromatography was performed using Merck silica gel 60 (0.040-0.063 mm). For centrifugal chromatography on a chromatotron, Merck silica gel 60 P_{254} containing gypsum was coated on the glass plate. Hexane was distilled before use.

The structures of the isolated compounds were analysed using ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, HMBC, IR and High resolution MS. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C at 100 MHz on a Bruker Avance III 400 spectrometer. Deuterated solvents, $CDCl_3$ and CD_3OD were used for NMR samples. Mass spectra were recorded on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization. IR spectra were recorded Bruker Alpha FT-IR.

4.6.2 ISOLATION OF COMBRETASTATINS (4.10, 4.11, 4.12)

The plant material was collected from the KwaZulu-Natal National Botanical Garden. A voucher specimen was deposited in the Warren Laboratory at University of KwaZulu-Natal in Pietermaritzburg. The dried, milled plant material (1.5 kg) was extracted with DCM-MeOH (1:1) to yield 65.0 g of extract. Chromatography on a short column was performed on the extract to remove very polar impurities (compounds on the baseline of the TLC plate), using hexane-ethyl acetate (1:1). The extract was further fractionated by column chromatography on silica gel using a step-gradient hexane-EtOAc 5:1 to 1:1, followed by EtOAc, resulting in the collection of 5 fractions. The fraction with purple spots on the TLC was repeatedly cleaned up by a number of columns and chromatotrons, resulting in the isolation of three compounds, combretastatin B-1 **(4.10)** (3.68 g), combretastatin B-5 **(4.11)** (320 mg) and combretastatin B-1 2-β-D-glucoside **(4.12)** (106 mg).

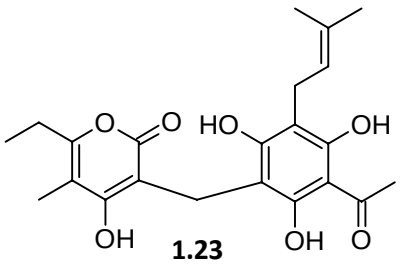
CHAPTER 5

Synthesis of lepidissipyrone

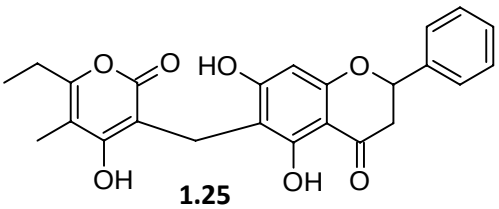
5.1 INTRODUCTION

In the investigations described in Chapters 3 and 4, no products with anti-HIV activity were isolated. Therefore, the focus was changed to natural products with known anti-HIV activity.

Secondary metabolites such as flavonoids, acetophenones, phloroglucinols, pyrones, triterpenoids and sesquiterpenes, have been isolated from several *Helichrysum* species and have shown remarkable antibacterial, antimicrobial, antiviral, antifungal, antioxidant, anti-inflammatory, anti-allergic, and anti-diabetic properties.[166, 217-223] Arzanol **(1.23)** was isolated from *Helichrysum italicum* ssp. *microphyllum*, a plant endemic to Sardinia.[224, 225] Arzanol contains 3-prenylphloroacetophenone and 6-ethyl-4-hydroxy-5-methyl-α-pyrone moieties linked to a methylene group. *In vitro* studies done on arzanol showed antioxidant, anti-inflammatory and anti-HIV activities.[224-227] Arzanol has antioxidant activity against linoleic acid and cholesterol oxidation, inhibition of HIV-1 replication and was nontoxic to VERO cells, a line of fibroblasts derived from monkey kidney. The interesting biological activities shown by arzanol, makes it an interesting target for synthesis.



1.23



1.25

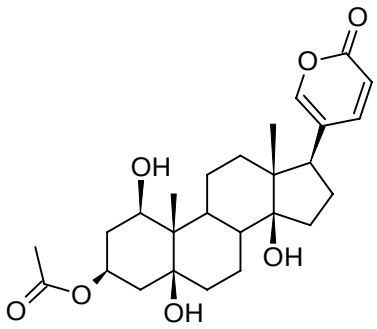
A structurally similar compound, lepidissipyrone **(1.25)** was isolated from *Helichrysum lepidissimum* in the late 1980s[228] and again isolated recently by our research group from *Helichrysum excisum*.[229] Lepidissipyrone **(1.25)** contains a 6-ethyl-4-hydroxy-5-methyl-α-pyrone moiety, like arzanol, and a flavanone linked to a methylene group. The synthesis of this compound has not been investigated. *H. lepidissimum* and *H. excisum* are both endemic to South Africa. The anti-HIV activity of arzanol (**1.23**) and the structural similarity between **1.23 and 1.25**, prompted

us to investigate the synthesis of both arzanol **(1.23)** and lepidissipyrone **(1.25)** with the major focus on lepidissipyrone. To date there is only one total synthesis of arzanol **(1.23)**,[230] reported in 2012. No synthesis has been reported for lepidissipyrone **(1.25)**. In the next section, a literature overview of naturally occurring 2-pyrones and their synthesis will be discussed. This will then be followed by our route towards the synthesis of arzanol **(1.23)** and lepidissipyrone **(1.25)**.

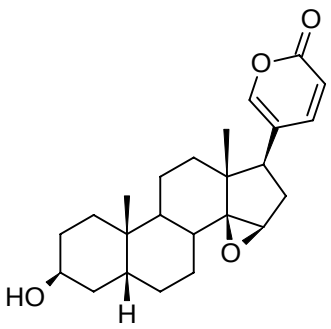
5.2 NATURALLY OCCURRING 2-PYRONES

2-Pyrone (α-pyrone), an unsaturated cyclic ester, is a moiety present in many naturally occurring pharmacologically active compounds. Some activities and uses it possesses include HIV-protease inhibition, treatment of Alzheimer's disease, treatment of high cholesterol, cancer treatment, rheumatoid arthritis treatment, anti-microbial, anti-fungal, cardiotonic, anticonvulsants, pheromones, and plant growth regulators,.[231-244] 2-Pyrones derived from fungi of various genera exhibit properties like cytotoxicity, neurotoxicity, and phytotoxicity. It was only after 1960 that the interest in 2-pyrones in organic synthesis was recognized.[232] Some classes of naturally occurring 2-pyrones include; bufadienenolides, 4-hydroxy-2-pyrones, gibepyrones, herbalins, 6-alkyl-2-pyrones, peripyrones, coumarins, and styryl-2-pyrone.[231]

Bufadienenolides contain a 2-pyrone moiety with a steroid moiety attached to position five. They are found in many plant and animal families and are traditionally used in the treatment of cancer, rheumatism, inflammation, central nervous system disorders, treatment of biliousness, leprosy, dysentery, asthma, leucoderma, emetic, acdysteriod agonist, and heart failure.[232] 3-*O*-Acetyldaigredorigenin **(5.1)**, from the plant species *Kalanchoe daigremontiana*, had an immunosuppressive effects in animal models.[232] Resibufogenin **(5.2)**, isolated from the skin extract of the Chinese toad inhibited growth of a leukaemia cell line.[231]

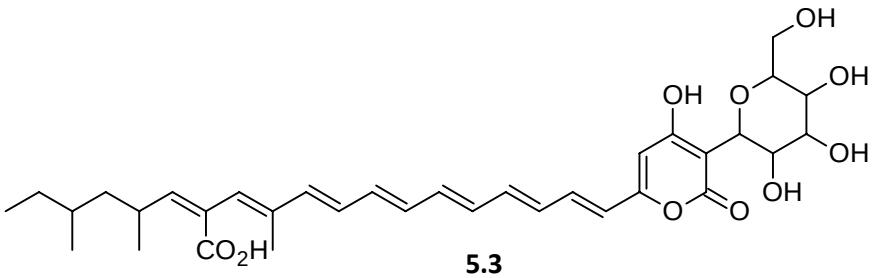


5.1



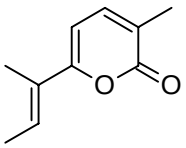
5.2

4-Hydroxy-2-pyrones are abundant in nature and are an important class of naturally occurring 2-pyrones. Their biological activity includes antibiotic, antibacterial, antifungal, and cytotoxicity. The conjugated 4-hydroxy-2-pyrone **(5.3)**, isolated from the culture broth of *Epicoccum purpurascens* exhibited telomerase inhibitory activity.[231, 232, 245]

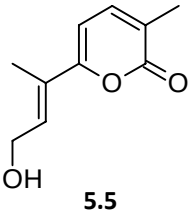


5.3

Gibepyrones are 6-substituted-3-methyl-2-pyrones and 6 metabolites, gibepyrone A-F, were isolated from *Gibberella fujikuroi*. Gibepyrone A **(5.4)** and B **(5.5)** exhibited growth inhibitory activity against the microorganism *Staphylococcus aureus, Bacillus subtilis, Candida albicans and Saccharomyces cerevisiae*.[231, 232]

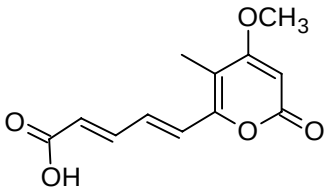


5.4

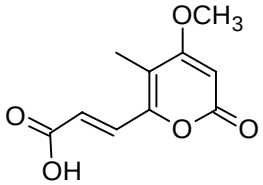


5.5

Herbalins are 2-pyrone isolated from the *Cladosporium herbarum* fungus strain associated with the sponges *Aplysina aerophoba* and *Callyspongia aerizusa*. Herbalin A **(5.6)** and B **(5.7)** exhibited activity against *Artemia salina*.



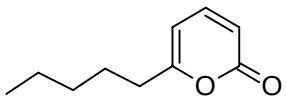
5.6



5.7

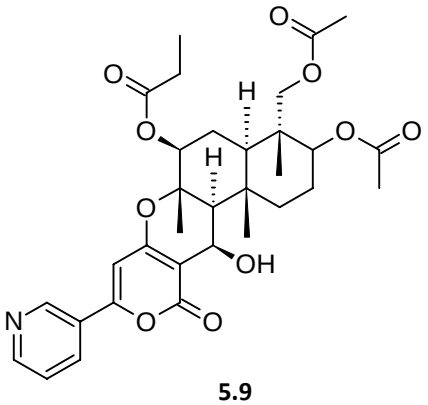
6-Alkyl/aryl-2-pyrones have attracted great interest in the food industry because of their flavourant properties and their biological properties such as tyrosinase inhibition, preventive and

therapeutic effect on hyperpigmentation disease, antibacterial, phytotoxic, anticancer, anti-inflammatory, anti-oxidant, antiaging, and immunomodulatory activities.[231, 232] 6-Pentyl-2-pyrone **(5.8)**, an antifungal product of *Trichoderma viride*, was also isolated from a marine algicolous fungus of the *Myrothecium* genus and exhibited tyrosinase inhibitory, antifungal, and phytotoxic activity as well as preventive and therapeutic effect on hyperpigmentation disease.[232]

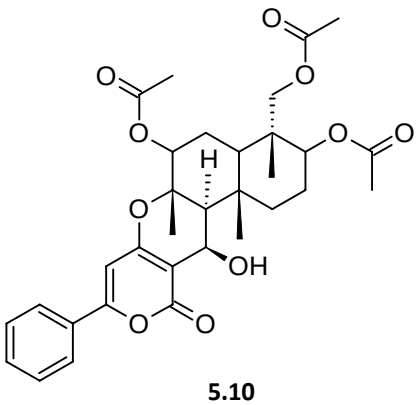


5.8

Peripyrones have been found to exhibit cholesterol acyltransferase inhibitory activity and treatment of Alzheimer's disease. Four peripyrones containing the pyridine, 2-pyrone and sesquiterpene moieties were isolated from culture broth of *Aspergillus fumigatus* isolated from Japanese soil.[231, 246] Pyripyropene C **(5.9)** was the most potent inhibitor of cholesterol acyltransferase. Structurally similar phenylpyropene A **(5.10)** isolated from the broth of *Penicillium griseofulvum*, also inhibited cholesterol acyltransferase.[231]

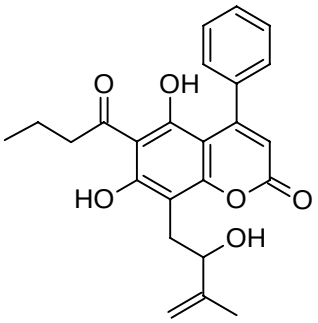


5.9

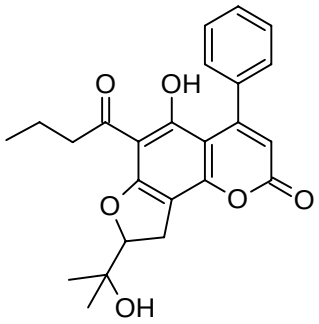


5.10

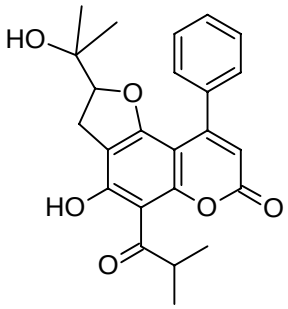
Racemisol **(5.11)** and mammea A/AC cyclo F **(5.12)** were isolated from the leaf extract of *Mesua racemosa* and achrocarpin E **(5.13)** was isolated from *Ochrocarpos punctatus*.[231] These coumarins were found to exhibit cytotoxicity against a human ovarian cancer line.



5.11

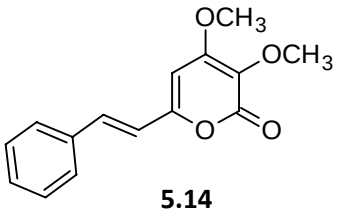


5.12



5.13

6-Styryl-2-pyranones show antioxidant and anticancer activities. 3,4-Dimethoxy-6-styryl-2-pyrone, **(5.14)** was isolated from the branches and leaves of a Chinese medicinal plant *Miliusa balansae*, which is traditionally used for gastropathy and glomerulonephropathy.[232]

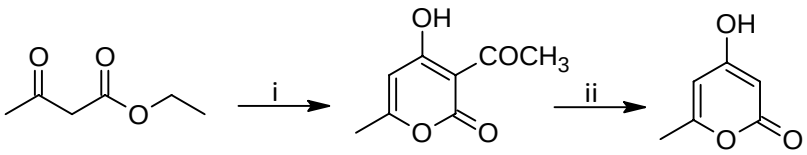


5.14

5.3 SYNTHESIS OF 2-PYRONES

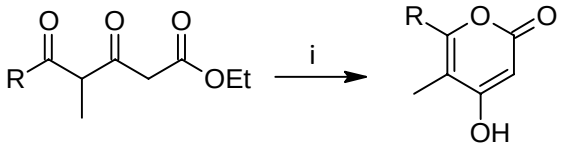
5.3.1 CONDENSATION-CYCLISATION REACTION

There are several routes for the synthesis of 2-pyrone, with the acid-catalysed condensation-cyclisation of the β-ketoesters being the most common route.[232] 2-Hydroxy-6-methyl-2-pyrone has been synthesised by the self-condensation of ethyl acetoacetate in the presence hydrochloric acid, followed by cyclisation then deacetylation in sulfuric acid (Scheme 5.1).[232] Other methods include the base-catalysed condensation of β-ketoesters, base-catalysed condensation of 1,3-diketones, acid-catalysed self-condensation of formylacetic acid, and heating of β,δ-diketoester under vacuum.[232, 247]



Scheme 5.1: *Reagents and conditions:* i) HCl (g) ii) H_2SO_4.

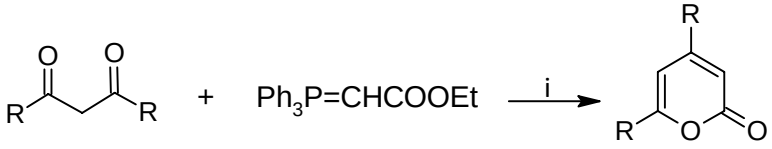
Condensation-cyclisation by heating of a 3,5-diketoester in polyphosphoric acid results in the formation of substituted-2-pyrones (Scheme 5.2).[230]



Scheme 5.2: *Reagents and conditions:* i) PPA, 120 °, 74%.

5.3.2 THE WITTIG REACTION

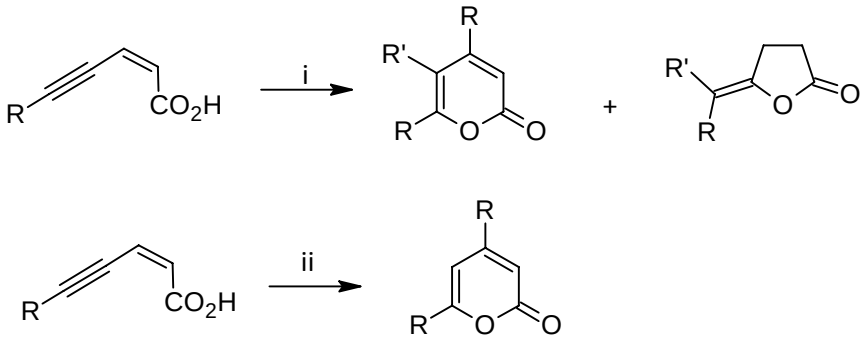
For the synthesis of a 4-6-disubstituted-2-pyrones, the Wittig reaction has been used by heating a 1,3-diketone with a phosphorane (Scheme 5.3).[232, 248]



Scheme 5.3: *Reagents and conditions:* i) heat.

5.3.3 FROM (*Z*)-5-ALKYL-2-ENE-4-YNOIC ACID

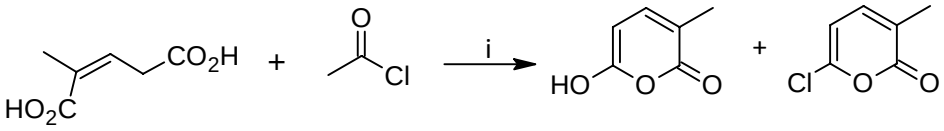
The reaction between aryl chlorides and (*Z*)-5-alkyl-2-ene-4-ynoic acid in the presence of potassium carbonate and a catalytic amount of $Pd(PPh_3)_4$ does not give satisfactory yields of the 2-pyrone and the improvement has been made by treatment of the (*Z*)-5-alkyl-2-ene-4-ynoic acid with $ZnBr_2$ (5-10 mol%) (Scheme 5.4).[249, 250]



Scheme 5.4: *Reagents and conditions:* i) R'Cl, $Pd(Ph_3P)_4$, K_2CO_3/CH_3CN ii) $ZnBr_2$ (5-10 mol%), THF, 25 °C.

5.3.4 CYLISATION OF GLUTACONIC ACID

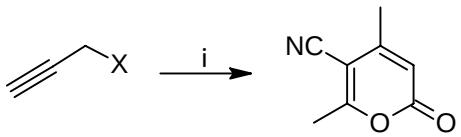
Cyclisation of glutaconic acid with acetyl chloride at 100 °C gives a mixture of 6-hydroxy-3-methyl-2-pyrone and the corresponding 6-chloro-3-methyl-2-pyrone (Scheme 5.5).[251]



Scheme 5.5: *Reagents and conditions:* i) 100 °C.

5.3.5 FROM PROPARGYL CHLORIDE/ALCOHOL

Propargyl chloride/alcohol is reacted with carbon monoxide and potassium cyanide in the presence of $Ni(CN)_2$, in basic aqueous medium to give 4,6-dimethyl-5-cyano-2-pyrone (Scheme 5.6).[252]



X = OH or Cl

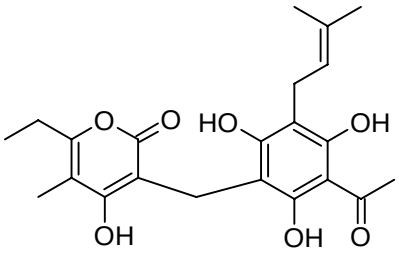
Scheme 5.6: *Reagents and conditions:* i) $Ni(CN)_2$, KCN, CO, $NaOH/H_2O$.

Other methods for the synthesis of 2-pyrone include acylation of the γ-position of α,β-unsaturated enones by diethyl oxalate, nucleophilic attack of malonic esters on electron deficient allenes, and [4+2]cycloaddition of silylketenes with electron-rich 1,3-dienes.[232]

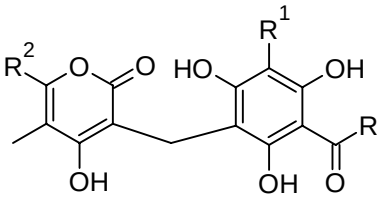
5.4 RESULTS AND DISCUSSION

In this study the aim was to develop a method for the synthesis of arzanol **(1.23)**, its derivatives **1.24**, and lepidissipyrone **(1.25)**. Arzanol has been isolated in satisfactory yields.[225] However, its structure-activity relationship is unknown because functionalization restricts possibilities of chemical modification.[230] The challenge with the synthesis of arzanol is that it possesses the phloroglucinol and pyrone moieties and both monomeric phloroglucinol and monomeric pyrone tend to form homodimeric adducts with carbonyl compounds as a linker molecule, thus controlling the conditions is important so that only heterodimeric adducts are formed.[230, 253] Another challenge is the presence of the acylphloroglucinol which tends to over alkylate when

reacted with carbon electrophiles as it possesses multiple nucleophilic sites.[253, 254] Arzanol being a mixture of tautomers and rotamers tends to be unstable when exposed to heat and change in pH, limiting the experimental conditions.[230]



1.23



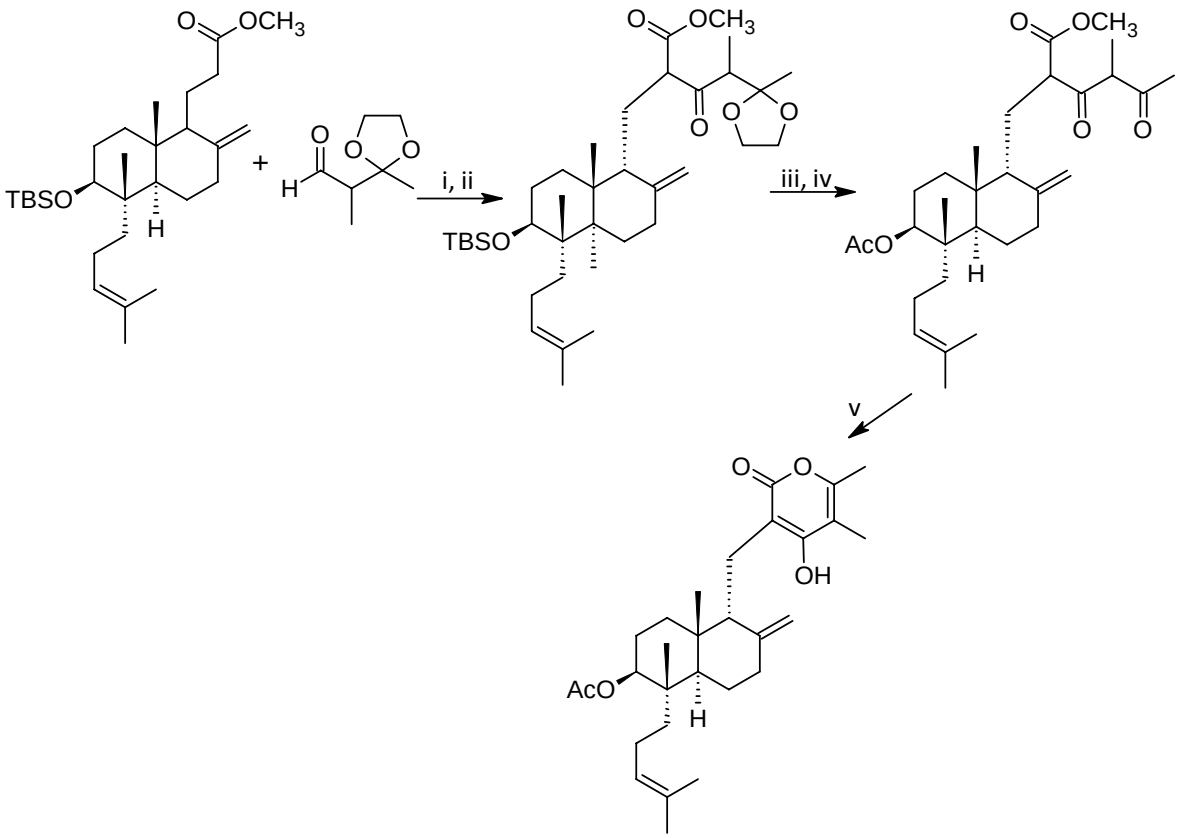
124 R = CH_3, $CH(CH_3)_2$

R^1 = H, $CH_2CH{=}C(CH_3)_2$

R^2 = CH_3, CH_2CH_3, $CH_2CH_2CH_3$

5.4.1 RETROSYNTHETIC ANALYSIS OF ARZANOL

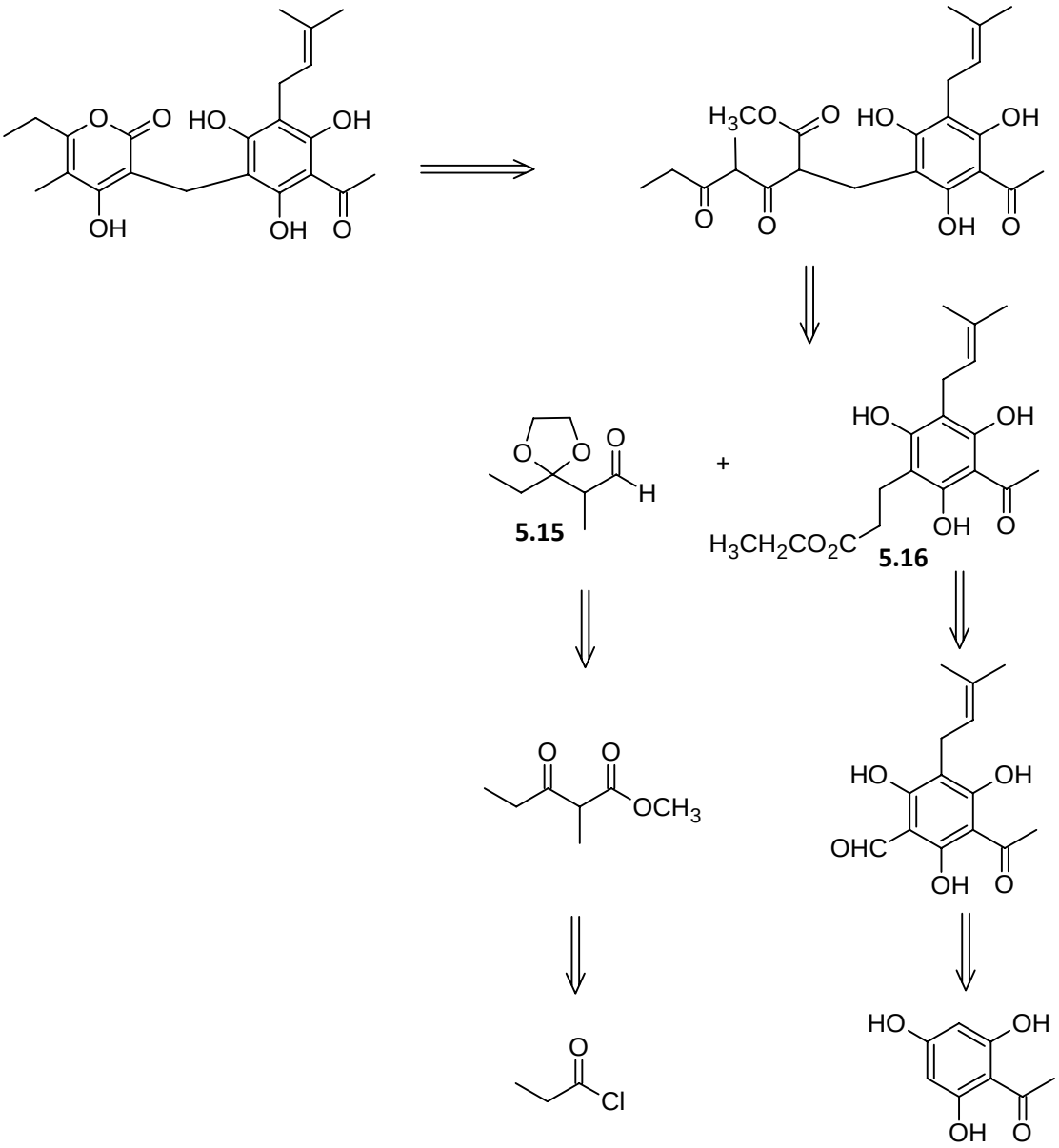
Zhang and Danishefsky reported the synthesis of a heavily substituted 2-pyrone moiety in their total synthesis of sesquicillin as shown in Scheme 5.7. In their synthesis the ester was coupled to an aldehyde using LDA to form an alcohol. Oxidation of the alcohol with DMP gave a ketone in quantitative yields. The ketal was deprotected using a palladium complex to give the 3,5-diketoester. The final cyclisation step was achieved by refluxing the 3,5-diketoester with DBU.[255]



sesquicillin

Scheme 5.7: Synthesis of sesquicillin. i) LDA, THF, -78 °C, 62% ii) DMP, DCM, 100% iii) a) HF/CH_3CN, Ac_2O, Et_3N, DMAP, DCM, 83% iv) [$Pd(CH_3CN)_2Cl_2$], acetone, 97% v) DBU benzene, 61%.

We were hoping to synthesis a similar aldehyde, couple it with an ester, and follow their synthetic route in the total synthesis of arzanol. The ester, ethyl 3-[3-acetyl-2,4,6-trihydroxy-5-(3-methylbut-2-en-1-yl)phenyl]propanoate (**5.16)** could be prepared from 2,4,6-trihydroxyacetophenone and the aldehyde, 2-(2-ethyl-1,3-dioxolan-2-yl)propanal (**5.15)** could be prepared from propionyl chloride (Scheme 5.8).

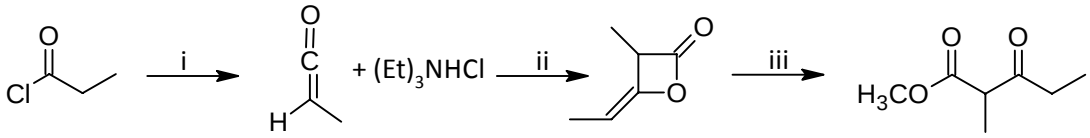


Scheme 5.8: Retrosynthesis of arzanol **(1.23)**.

5.4.2 PREPARATION OF 2-(2-ETHYL-1,3-DIOXOLAN-2-YL)PROPANAL (5.15)

The β-keto ester **5.19** was synthesised using an efficient and convenient method developed by Sung and Wu.[256] In our first attempt, commercial propionyl chloride was not available; it was therefore prepared by refluxing propionic acid and thionyl chloride. Propionyl chloride was reacted with triethylamine to afford the unstable ketene **5.17** with a thick precipitate of triethylammonium chloride. The ketene was not isolated and dimerised to the β-lactone **5.18** after two days. Refluxing of the lactone in excess MeOH and a catalytic amount of sodium acetate afforded the β-keto ester, methyl 2-methyl-3-oxopentanoate (**5.19)** (Scheme 5.9). In the ^{1}H NMR spectrum the formation of the β-keto ester was confirmed by appearance of a singlet at δ_H 3.63

corresponding to a methoxy group. Also present was a triplet and a quartet at δ_H 0.93 and 3.43 respectively, corresponding to the CH_2CH_3 group, a doublet at δ_H 1.24 corresponding to a CH_3 adjacent to a CH, and a multiplet at δ_H 2.5 for a methine proton. In the ^{13}C NMR spectrum the two carbonyl signals, for an ester and a ketone, at δ_C 171.2 and 206.3, respectively, were observed.

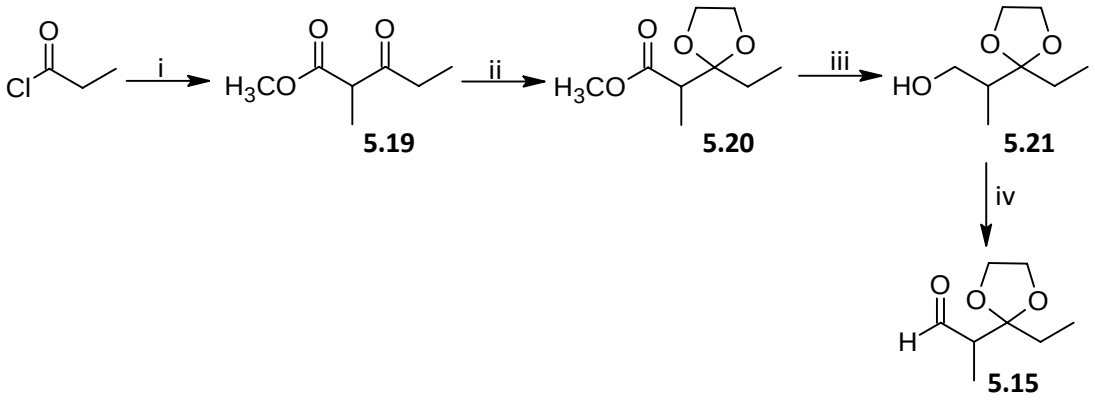


5.17 **5.18** **5.19**

Scheme 5.9: Synthesis of methyl 2-methyl-3-oxopentanoate (**5.19)**. i) Et_3N, ether, room temperature, 2 days ii) NaOAc, MeOH, reflux, overnight, 60%.

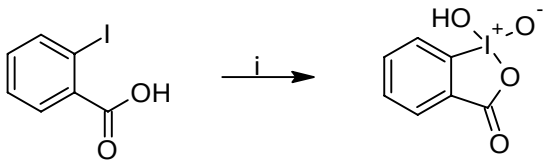
Literature precedence for the synthesis of the 2-(2-ethyl-1,3-dioxolan-2-yl)propanal (**5.15)** from methyl 2-methyl-3-oxopentanoate (**5.19)** was found in a publication by Klein; therefore no problems were anticipated (Scheme 5.10).[257] The ketone **5.19** was protected using ethylene glycol and a catalytic amount of *p*-toluenesulfonic acid monohydrate to afford the ketal, methyl 2-(2-ethyl-1,3-dioxolan-2-yl)propanoate (**5.20)**. ^1H NMR spectroscopic evidence for the formation of the ketal was the presence of a new multiplet at δ_H 3.90 corresponding to the four CH_2CH_2 protons. The disappearance of one carbonyl signal in the ^{13}C NMR spectrum and appearance of two deshielded CH_2 signals at δ_C 65.8 and 65.9 was further evidence for the formation of the ketal from the β-keto ester.

Reduction of the ester with lithium aluminium hydride afforded the alcohol, 2-(2-ethyl-1,3-dioxolan-2-yl)propan-1-ol **(5.21)**. In the ^1H NMR spectrum the evidence for the reduction of an ester to a primary alcohol was observed by the disappearance of the methoxy singlet at δ_H 3.6 and the appearance of the two multiplets integrating for one proton each, corresponding to the CH_2 protons of the primary alcohol, at δ_H 3.39 and 3.55. In the ^{13}C NMR spectrum, no carbonyl peak was present and a new peak at δ_C 64.8 was observed. In the IR spectrum the broad OH band at 3408 cm^{-1} was further evidence of the reduction of an ester to a primary alcohol. No carbonyl band was observed around 1700 cm^{-1}.



Scheme 5.10: Synthesis of 2-(2-ethyl-1,3-dioxolan-2-yl)propanal **(5.15)**. i) a) Et_3N, ether, room temperature, 2 days b) NaOAc, MeOH, reflux, overnight, 60% ii) *p*-TsOH, 1,2-ethanediol, toluene, reflux, overnight, 68% iii) $LiAlH_4$, ether, reflux, 30 min, 90% (iv) IBX, EtOAc-acetone 2:1, 80 °C, overnight, 85%.

The alcohol **5.21** was then oxidized with IBX to give the aldehyde, 2-(2-ethyl-1,3-dioxolan-2-yl)propanal **(5.15)**, in 85% yield. IBX (2-iodoxybenzoic acid) was synthesised by the slow addition of 2-iodobenzoic acid to a solution of potassium bromate in sulfuric acid at 60 °C (Scheme 5.11).[258] IBX was chosen because the method is simple and it results in insoluble by-products at room temperature thus after filtration no further purification is required.[259]



Scheme 5.11: i) $KBrO_3$, H_2SO_4, 50%.

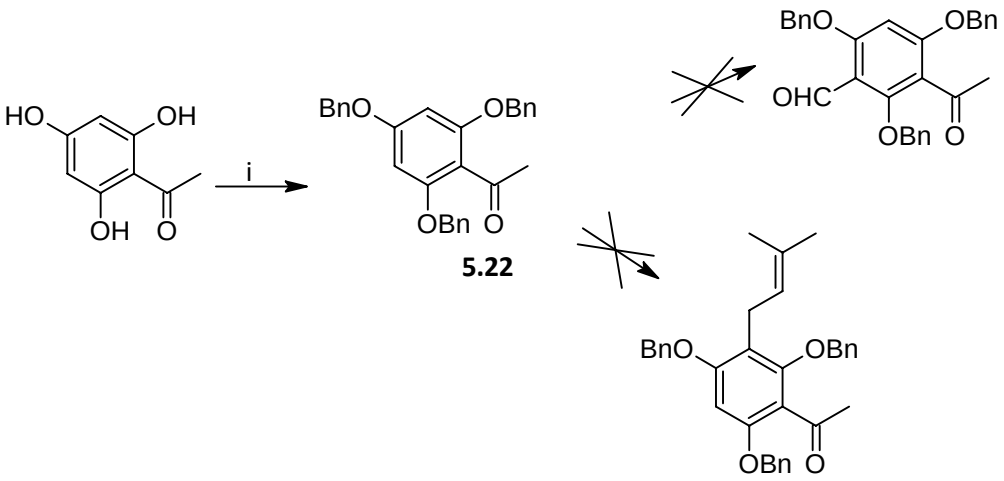
Oxidation of the primary alcohol to an aldehyde was confirmed by the appearance of a new aldehyde downfield proton peak at δ_H 9.70 and the disappearance of the CH_2 multiplets. In the ^{13}C NMR spectrum a new carbonyl carbon peak at δ_C 211.9 was observed and also a carbonyl band at 1722 cm^{-1} in the IR spectrum. Having synthesised the aldehyde **5.15**, the challenge was now to synthesise the phloroglucinol moiety **5.16**.

5.4.3 SYNTHESIS OF ETHYL 3-[3-ACETYL-2,4,6-TRIHYDROXY-5-(3-METHYLBUT-2-EN-1-YL)PHENYL]PROPANOATE (5.16) FROM 2,4,6-TRIHYDROXYACETOPHENONE

The aromatic moiety of arzanol was a more problematic synthetic target than the pyrone moiety. Phloroglucinols are notoriously difficult to work with. These molecules are much more electron rich than less-substituted benzene rings and the outcome of reactions with phloroglucinol starting material cannot always be predicted. Furthermore the aromatic moiety of arzanol is a fully substituted benzene and steric interactions may play a crucial role.

The first approach to **5.16,** was intended to introduce the formyl and prenyl groups sequentially on 2,4,6-trihydroxyacetophenone. Phloroglucinol derivatives are highly reactive and to prevent diformylation or diprenylation of the starting material, it was decided to protect the hydroxy groups. In the first approach to the synthesis of **5.16**, the tribenzylated derivative **5.22** was identified as a precursor (Scheme 5.12).

The desired tribenzylation of 2,4,6-trihydroxyacetophenone was achieved using benzyl chloride and potassium carbonate to afford 2,4,6-tribenzyloxyacetophenone **(5.22)** in 70% yield. The methyl group appeared as a singlet at δ_H 2.48 in the ^{1}H NMR and at δ_C 32.6 in the ^{13}C NMR spectra. A singlet at δ_H 6.26 integrating for the two aromatic protons of the acetophenone was proof that only *O*-benzylation took place. In the ^{1}H NMR spectrum, the deshielded methylene protons of the benzyl group were observed at δ_H 5.00 (2H, *s*, OCH_2Ph) and δ_H 5.03 (4H, *s*, OCH_2Ph) and appeared at δ_C 70.4 and 70.8 in the ^{13}C NMR spectrum. Aromatic protons integrating for fifteen protons were observed as a multiplet between δ_H 7.03 and 7.39. However, attempted formylation of the tribenzyloxyacetophenone proved to be problematic because no reaction took place. Prenylation was attempted on 2,4,6-tribenzyloxyacetophenone (**5.22)** using 3,3-dimethylallyl bromide (prenyl bromide) and different bases and different solvents, but no product was obtained, only the starting material was recovered (Scheme 5.12). The electron-withdrawing acetyl group and the protecting group might have reduced the electron density on the aromatic ring so that it is no longer a good enough nucleophile to react with the prenyl bromide.



Scheme 5.12: Synthesis of 2,4,6-tribenzyloxyacetophenone (**5.22)**. i) BnCl, K_2CO_3, DMF, 70%.

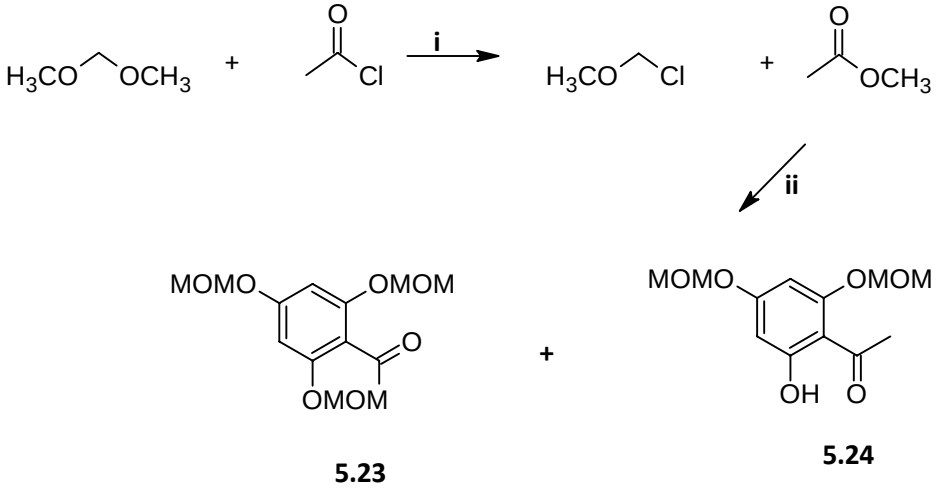
The benzyl group was initially chosen for the protection of 2,4,6-trihydroxyacetophenone because of its stability. However, all attempted reactions on the benzyl protected product were unsuccessful and therefore methoxymethyl (MOM) protection was considered. It was concluded that the benzyl groups are too bulky to allow the prenylation and formylation reactions on the ring.

Berline and Belecki reported the synthesis of MOMCl and the subsequent *in situ* protection of an alcohol; with their method there is no handling of MOMCl and any unreacted MOMCl is decomposed with NH_4Cl, thus avoiding contact with the toxic MOMCl.[260] The synthesis of MOM protected starting material is given in Scheme 5.13. MOMCl was synthesised using dimethoxymethane, acetyl chloride and zinc bromide as a catalyst in toluene. The MOMCl solution was cooled down to 0 °C, sodium hydride was then added followed by the solution of 2,4,6-trihydroxyacetophenone in DMF. The expected product, 2,4,6-trimethoxymethoxyacetophenone (**5.23)** was obtained in 73% yield (Scheme 5.13). The ^{1}H NMR spectrum showed four singlets at δ_H 2.49, 3.46, 5.14, and 6.51, which were assigned to the three protons of the methyl group attached to the ketone, the methoxy groups integrating for nine protons, the methylene groups integrating for six protons and the two aromatic protons, respectively. In the ^{13}C NMR spectrum the methoxy carbons resonated at δ_C 56.3 and the methylene carbons resonated at δ_C 94.6.

Also obtained was a small amount of 6-hydroxy-2,4-dimethoxymethoxyacetophenone **(5.24)**, its presence was confirmed by the hydrogen bonding between the free hydroxy group and the

carbonyl and in the ^{1}H NMR spectrum the peak corresponding to one proton was observed at δ_H 13.7. There were two methoxy sets of protons as two singlets at δ_H 3.48 and 3.53, and two methylene sets of protons as two singlets at δ_H 5.18 and 5.26. A characteristic OH band at 3314 cm^{-1} was observed in the IR spectrum.

The attempted formylation of both 2,4,6-trimethoxymethoxyacetophenone **(5.23)** and 6-hydroxy-2,4-dimethoxymethoxyacetophenone (**5.24)** using DMF and phosphoryl chloride was unsuccessful and instead deprotection of the MOM group took place. Attempted prenylation of 2,4,6-trimethoxymethoxyacetophenone **(5.23)** using *n*-BuLi and prenyl bromide in THF and potassium carbonate and prenyl bromide in acetone were also unsuccessful.



5.23 **5.24**

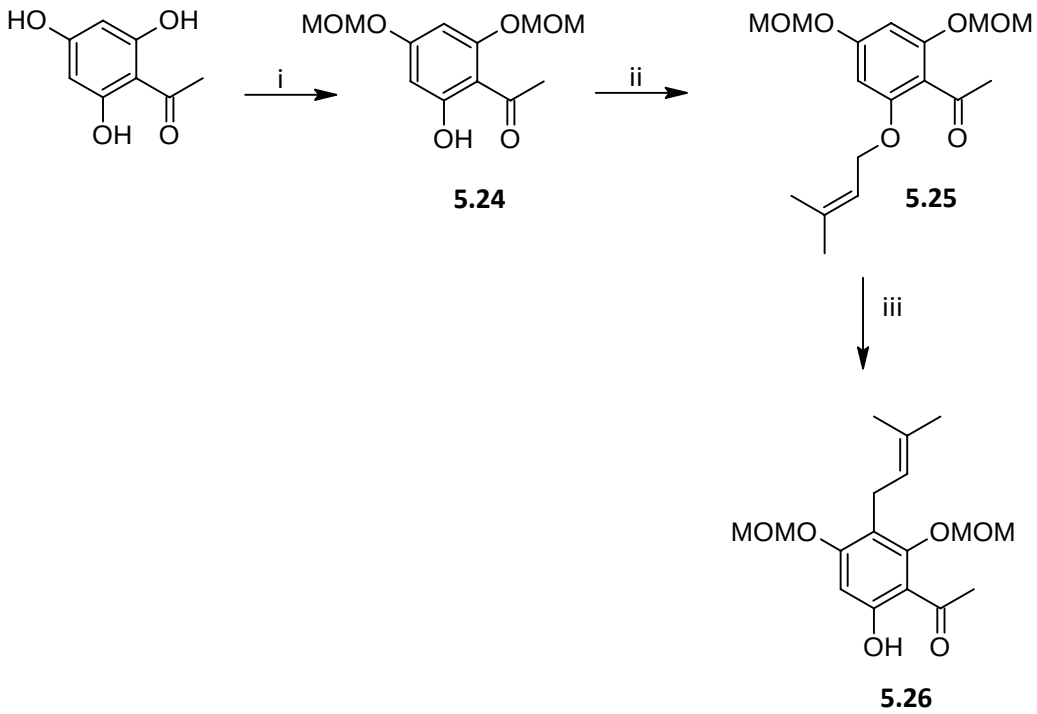
Scheme 5.13: Synthesis of MOMCl and the subsequent *in situ* protection of 2,4,6-trihydroxyacetophenone. i) $ZnBr_2$, toluene ii) 2,4,6-trihydroxyacetophenone, NaH, DMF, 73%.

Direct *C*-prenylation of 6-hydroxy-2,4-dimethoxymethoxyacetophenone **(5.24)** using sodium hydride and 3,3-dimethylallyl bromide (prenyl bromide) in DMF was unsuccessful. An alternative route (Scheme 5.14) was then chosen for the *C*-prenylation of 2,4,6-trihydroxyacetophenone using 6-hydroxy-2,4-dimethoxymethyloxyacetophenone (**5.24)**. This route would prevent the problem of both *O*- and *C*-alkylation with phloroglucinols. Two more steps are required, but better yields are obtained than with direct C-prenylation.[261] Selective MOM diprotection of 2,4,6-trihydroxyacetophenone was achieved best when potassium carbonate and chloromethyl methyl ether (MOMCl) in DMF were used.

6-Hydroxy-2,4-dimethoxymethoxyacetophenone **(5.24)** was treated with potassium carbonate and prenyl bromide in acetone to afford the *O*-prenylated product, 2,4-dimethoxymethoxy-6-prenyloxyacetophenone (**5.25)** in 51% yield. The ^{1}H NMR spectrum of 2,4-dimethoxymethoxy-6-prenyloxyacetophenone **(5.25)** showed two singlets at δ_H 1.70 and 1.74 integrating for three protons each for the two methyls on the prenyl group. A doublet at δ_H 4.48 (2H, *d*, *J* = 6.6 Hz, CHC<u>H</u>$_2$) and a triplet at δ_H 5.39 (1H, *t*, *J* = 6.6 Hz, C<u>H</u>CH$_2$) were observed for the methylene and methine protons, respectively, of the prenyl group. Further evidence that *O*- and not *C*-prenylation had taken place was the disappearance of the OH proton at δ_H 13.7 and both aromatic protons were still present at δ_H 6.30 and 6.42. In the ^{13}C NMR spectrum the two methyl carbons of prenyl group were observed at δ_C 18.1 and 29.2, the methylene carbon at δ_C 65.7, the methine carbon at δ_C 119.4 and the quaternary carbon at δ_C 137.8. No OH band was observed in the IR spectrum.

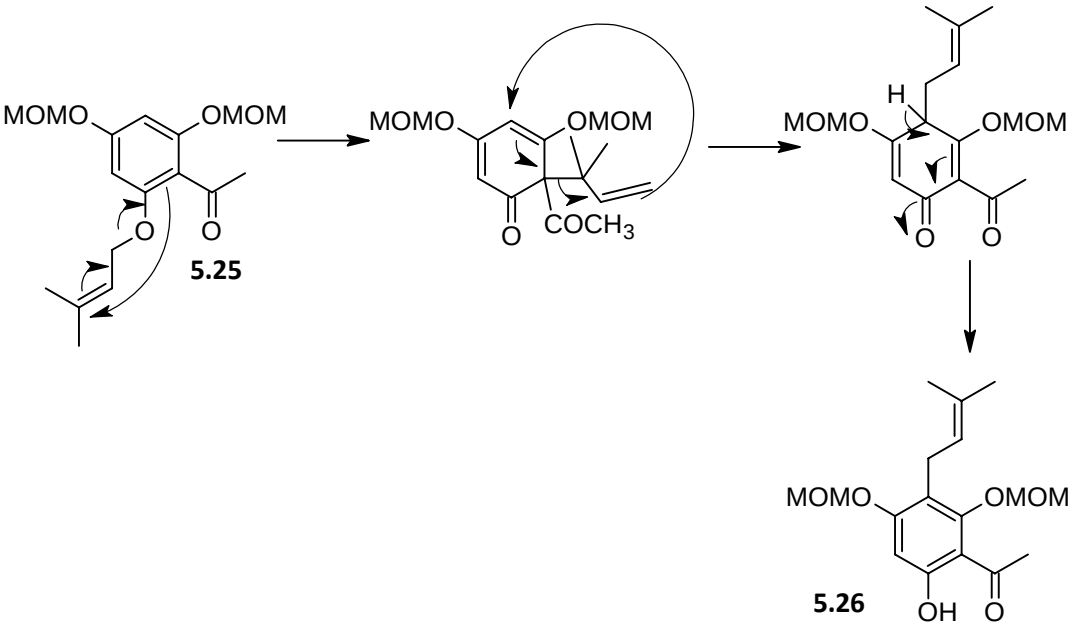
After the successful synthesis of the *O*-prenyl acetophenone **5.25**, the next reaction was the Claisen-Cope rearrangement of the prenyl group. Two routes were investigated: a) refluxing in *N,N*-diethylaniline and b) heating the reaction in toluene in the presence of Eu(fod)$_3$ catalyst.[262-264] Both routes gave the desired product. However the yields were lower when Eu(fod)$_3$ was used as compared to *N,N*-diethylaniline, making *N,N*-diethylaniline the best reagent. It has been reported that decomposition occurs when *N,N*-dimethylaniline is used in tandem Claisen-Cope rearrangement.[262] The *O*-prenylated product **5.25** was successfully converted to a 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)** using *N,N*-diethylaniline (Scheme 5.14), although a yield of only 50% was obtained.

The ^{1}H NMR spectrum still showed the presence of the prenyl group by the presence of the two singlets for the two methyl groups and a doublet and a triplet for the methylene and methine protons, respectively. That the prenyl group was now attached to carbon rather than oxygen was evident from the ^{1}H NMR spectrum where the methylene protons signal was observed more upfield at δ_H 3.32 instead of at δ_H 4.48 in the *O*-prenylated product **5.25**. One aromatic proton was present at δ_H 6.48 and an OH proton at δ_H 12.90. In the ^{13}C NMR spectrum the methylene carbon was now at δ_C 23.1 instead of δ_C 65.7. An OH band at 3381 cm^{-1} was observed in the IR spectrum.



Scheme 5.14: Synthesis of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)**. i) K_2CO_3, MOMCl, DMF, rt, 30 min, 81% ii) K_2CO_3, 3,3-dimethylallyl bromide, acetone, reflux, 24 h, 51% iii) *N,N*-diethylaniline, reflux, 3 h, 50%.

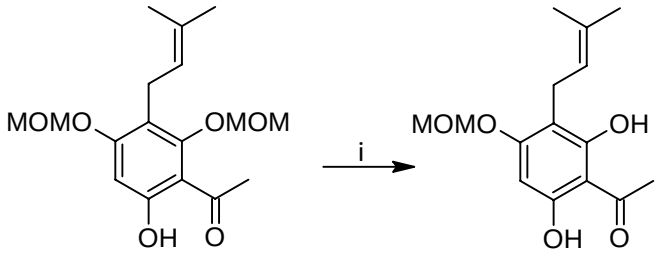
The Claisen-Cope rearrangement reaction involves the Claisen rearrangement to the *ortho*-position. However, because this position is substituted, aromatisation cannot take place. The compound then undergoes a Cope rearrangement and tautomerisation to regain aromaticity (Scheme 5.15).



Scheme 5.15: Claisen- Cope rearrangement.

After having successfully prenylated phloroacetophenone, the next step was to remove the MOM protecting group and then add the formyl group (Scheme 5.16). Selective methods for removing protecting groups in the presence of other functional groups are often required when synthesizing complex natural products.[265] In an attempt to remove the MOM groups, the diprotected acetophenone was treated with HCl. After stirring the reaction for 48 hours at room temperature, deprotection of only one MOM group was achieved, resulting in the formation of 2,6-dihydroxy-4-methoxymethoxy-3-prenylacetophenone **(5.27)** (Scheme 5.16). Changing the conditions to MeOH and the catalytic amount of iodine as a catalyst gave the same results.[262] Using camphorsulfonic acid (CSA) in MeOH resulted in the recovery of the starting material.[266]

In the ^{1}H NMR spectrum of **5.27** the methoxy and methylene protons for the MOM group were observed at δ_H 3.48 and 5.20, respectively. Further confirmation of the structure was observed with HRESIMS where a molecular ion peak at *m/z* 303.1205 $[M+Na]^+$ was observed, which was in agreement with the calculated value for $C_{15}H_{20}O_5Na$.

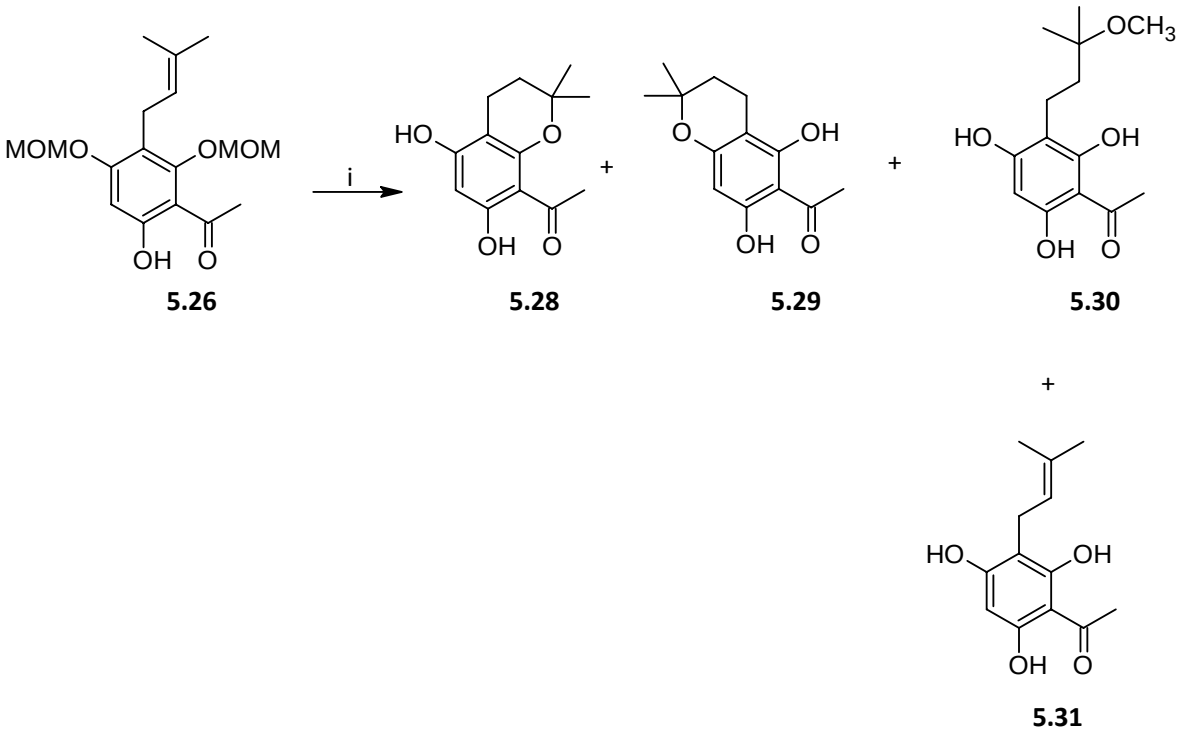


5.26 **5.27**

Scheme 5.16: Attempted MOM deprotection of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)**. i) MeOH, HCl, heat.

Different experimental conditions were attempted to deprotect the MOM groups. The presence of the large quantities of HCl in the reaction mixture resulted in undesired ring closure of the prenyl group (Scheme 5.17). Compounds 1-(5,7-dihydroxy-2,2-dimethyl-chroman-8-yl)ethanone (**5.28)** (30%), 1-(5,7-dihydroxy-2,2-dimethylchroman-6-yl)ethanone (**5.29)** (17%) and 1-(2,4,6-trihydroxy-3-(3-methoxy-3-methylbutyl)phenyl)ethanone (**5.30)** (18%) were obtained as crystals and their structures were confirmed by ^{1}H NMR, ^{13}C NMR, IR, MS, and X-ray crystallography. In compounds **5.28** and **5.29**, the ethers were obtained by the cyclisation of the prenyl group with the adjacent hydroxyl groups and in compound **5.30,** acid-catalysed ether formation resulted from the addition of MeOH to the double bond.

MOM-deprotection had taken place because there were no methoxy and methylene singlets around δ_H 3.5 and 5.2, respectively. The signals of the two methyls and the methylene of the prenyl moiety were still present in both the ^{1}H and ^{13}C NMR spectra of all three compounds, **5.28**, **5.29** and **5.30**. The triplet around δ_H 5.4 correspond to CH adjacent to CH_2 on the prenyl group was absent and so was the olefinic carbons for =CH and =C at δ_C 119.4 and 137.8 in the spectrum of **5.26**, suggesting that a reaction had taken place on the double bond.



5.26 **5.28** **5.29** **5.30** **5.31**

Scheme 5.17: MOM deprotection of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)**. i) MeOH, HCl, reflux, 1 hour.

Compounds 1-(5,7-dihydroxy-2,2-dimethyl-chroman-8-yl)-ethanone **(5.28)** and 1-(5,7-dihydroxy-2,2-dimethylchroman-6-yl)ethanone **(5.29)** showed new two triplets around δ_H 1.9 and 2.6 for a CH_2CH_2 group, while the other acetophenone signals remained unchanged. The NMR spectra of **5.28** and **5.29** were similar except that the ^{1}H NMR spectrum of **5. 28** showed a downfield singlet at δ_H 13.78 attributed to a hydroxy proton that is hydrogen bonded to the carbonyl oxygen. HRESIMS data showed a molecular ion peak at *m/z* 259.0947 $[M+Na]^+$ for compound **5.28** and at *m/z* 259.0945 $[M+Na]^+$ for compound **2.29,** which were both in agreement with the calculated value for $C_{13}H_{16}O_4Na$. The exact structures of compounds **5.28** and **5.29** were confirmed by X-ray crystallography.

In 1-(2,4,6-trihydroxy-3-(3-methoxy-3-methylbutyl)phenyl)ethanone **(5.30),** all the acetophenone peaks were present in the NMR data, there was also a CH_2CH_2, but no signals for vinylic carbons or vinylic proton for the prenyl group were observed in the NMR spectra. A methoxy singlet at δ_H 3.27 in the ^{1}H NMR spectrum and at δ_H 47.8 on the ^{13}C NMR spectrum was observed. HRESIMS showed a molecular ion peak at *m/z* 291.1208 $[M+Na]^+$ was observed, which was in agreement with the calculated value for $C_{14}H_{20}O_5$. The exact structure was further confirmed by X-ray diffraction analysis.

The X-ray structures of compound **5.28**, **5.29** and **5.30** are shown in Figures 5.1, 5.4, and 5.7 respectively.

Compound **5.28** crystallised in the monoclinic crystal system in the space group *P* 21/c (Figure 5.1). Asymmetric unit contains two chrystallographically independent molecules. Compound **5.28** exhibits intermolecular and intramolecular hydrogen bonding. The intramolecular O2—H2···O1 hydrogen bond is between the oxygen of the ketone and phenolic hydrogen. The same ketone oxygen atom is also involved in an intermolecular O2—H2···O1 hydrogen bond with the phenolic hydrogen atom of an adjacent molecule, the same phenolic hydrogen atom exhibits intermolecular hydrogen O7—H7···O2 bonding with the phenolic oxygen atom of a different molecule (a bifurcated hydrogen bond). Table 5.1 shows the geometric parameters of both the intramolecular and the intermolecular hydrogen-bonds.

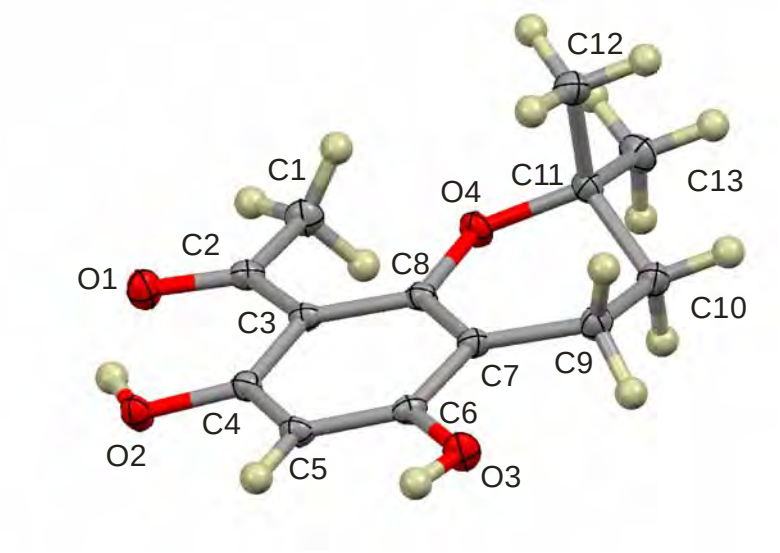


FIGURE 5.1: X-ray structure of compound **5.28.**

TABLE 5.1: Hydrogen-bond parameters of compound **5.28.**

Bond	***D*—H**	**H···*A***	***D*···*A***	***D*—H···*A***
O7—H7···O2	0.84	1.97	2.804(1)	174
O2—H2···O1	0.84	3.01(2)	2.437	126
O2—H2···O1	0.84	1.74	2.479 (1)	145

This hydrogen bonding motif leads to a two dimensional supramolecular structure (Figure 5.2 and 5.3). The ^{1}H NMR data suggests that this same hydrogen bonding pattern exists in solution as well as the solid state.

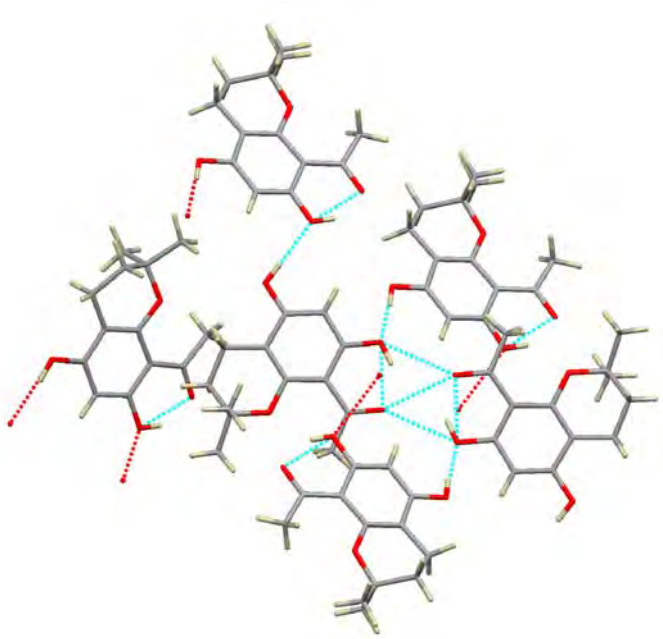


FIGURE 5.2: Intramolecular H-bonding of **5.28** showing strong hydrogen bonding.

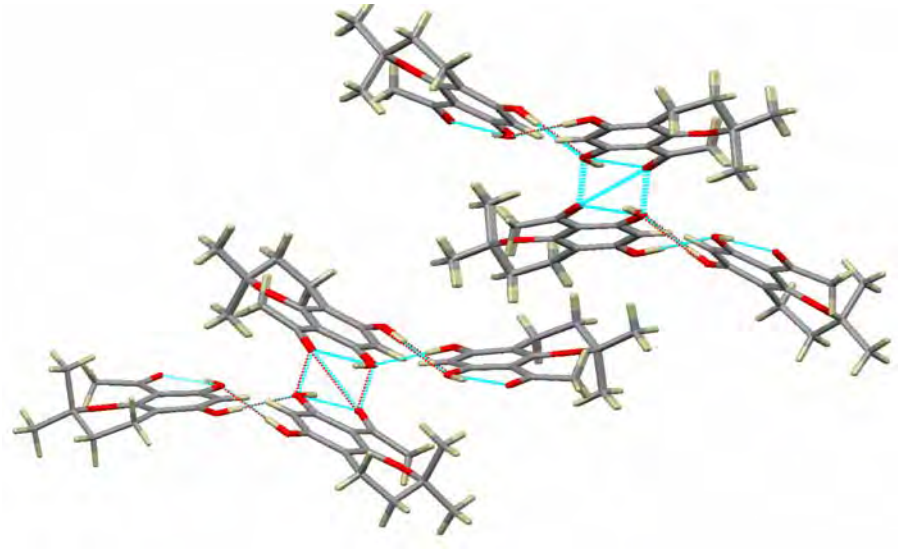


FIGURE 5.3: H-bonding of **5.29** viewed down the *c*-axis.

Compound **5.29** crystallised in the tetragonal crystal system in a space group $P\,4_12_12$ (Figure 5.4). Asymmetric unit contains one independent molecule. The presence of a ketone is indicated by a short bond length of 1.254 Å between carbon and oxygen and a bond angle of 116.9°. Compound **5.29** exhibits both intermolecular and intramolecular hydrogen bonding. The intramolecular O3—H103···O4 hydrogen bond is between the oxygen of the ketone and phenolic hydrogen. The same ketone oxygen atom is also involved in an intermolecular O2—H102···O4 hydrogen bond with a phenolic H atom of an adjacent molecule. Table 5.2 shows the geometric parameters of both the

intramolecular and the intermolecular hydrogen-bonds. The hydrogen bonds are both significantly shorter than the sum of the van der Waals radii, indicating an attractive force. Although hydrogen bond length does not necessarily correlate linearly to bond strength due to packing constraints in the lattice, the very short bond length coupled with the near ideal bond angle (180 deg) suggests these interactions are likely to be moderately strong.

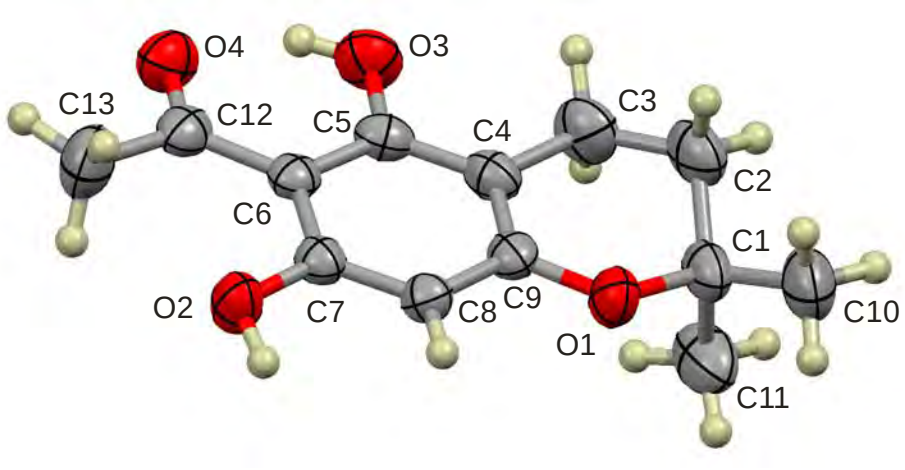


FIGURE 5.4: X-ray structure of compound **5.29.**

TABLE 5.2: Hydrogen-bond parameters of compound **5.29.**

Bond	***D*—H**	**H···*A***	***D*···*A***	***D*—H···*A***
O2—H102···O4	0.97 (2)	1.77 (2)	2.737 (2)	179 (1)
O3—H103···O4	0.86 (2)	1.71 (2)	2.501 (2)	151 (2)

The intermolecular hydrogen bonding links the molecules into a zigzag chain pattern as shown in Figure 5.5. When viewed down the c-axis (Figure 5.6) the 4-fold symmetry (the essential symmetry for the tetragonal crystal system) of the supramolecular structure is clearly evident.

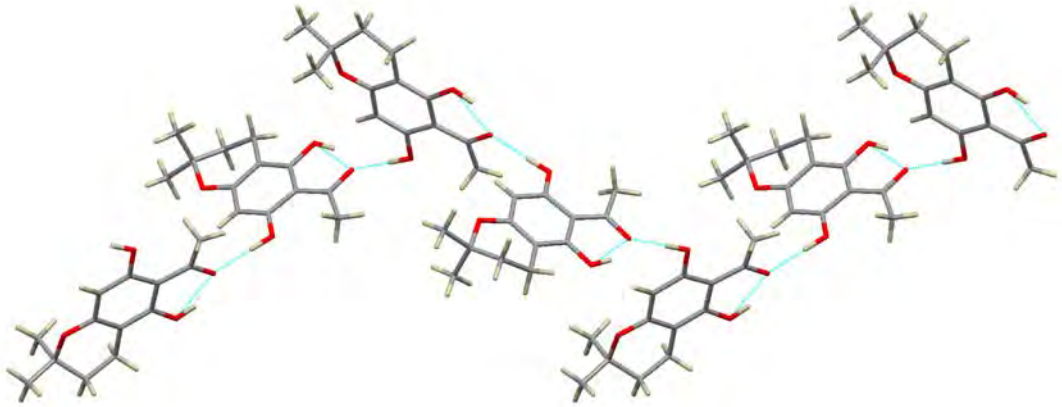


FIGURE 5.5: Intramolecular H-bonding of **5.29** viewed down the *b*-axis showing a zigzag pattern.

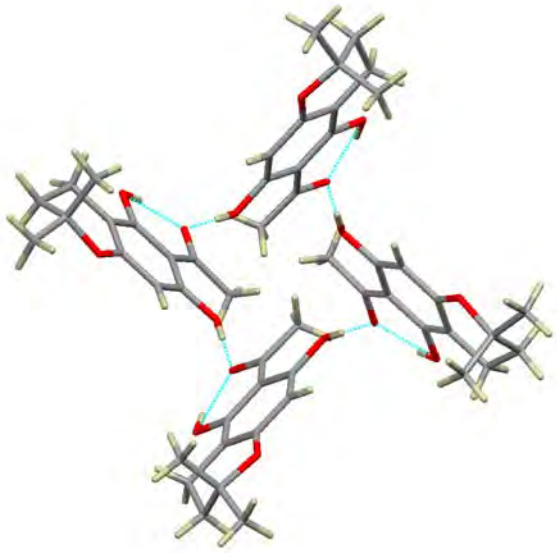


FIGURE 5.6: Intramolecular H-bonding of **5.29** viewed down the *c*-axis.

Compound **5.30** crystallised in the orthorhombic crystal system in the space group *Pbca* (Figure 5.7). Asymmetric unit contains one independent molecule. Compound **5.30** exhibits intermolecular and intramolecular hydrogen bonding. The intramolecular O003—H003···O1 hydrogen bond is between the oxygen atom of the ketone and phenolic hydrogen atom. The same ketone oxygen atom is also involved in an intermolecular O002—H002···O1 hydrogen bond with the phenolic hydrogen atom of an adjacent molecule. Also present is the intermolecular O001—H001···O2 hydrogen bond between the oxygen atom of the methoxy group and the phenolic hydrogen atom of an adjacent molecule. Table 5.3 shows the geometric parameters of both the intramolecular and the intermolecular hydrogen-bonds.

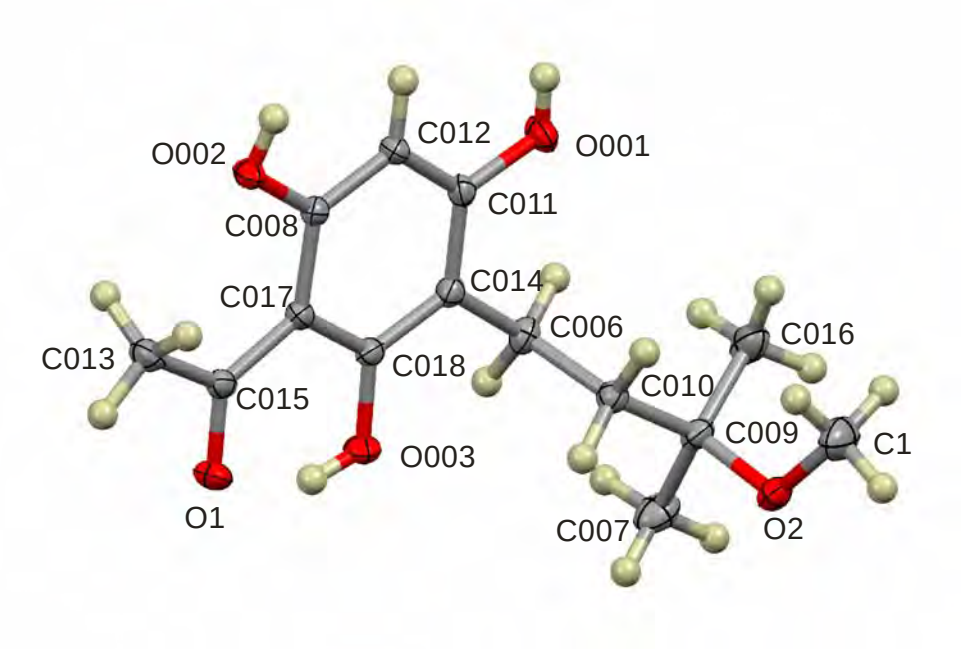


FIGURE 5.7: X-ray structure of compound **5.30.**

TABLE 5.3: Hydrogen-bond parameters of compound **5.30.**

Bond	***D*—H**	**H···*A***	***D*···*A***	***D*—H···*A***
O002—H002···O1	0.84	1.82	2.650 (1)	167
O001—H001···O2	0.84	1.85	2.682 (1)	171
O003—H003···O1	0.84	1.75	2.504 (1)	148

The data in Table 5.3 show that the bond lengths are again significantly shorter than the sum of the van der Waals radii of the interacting atoms, suggesting strong interactions. The intramolecular hydrogen bond shows a significant deviation from ideality and this is attributed to the inflexible geometry of the interacting atoms (Figure 5.8).

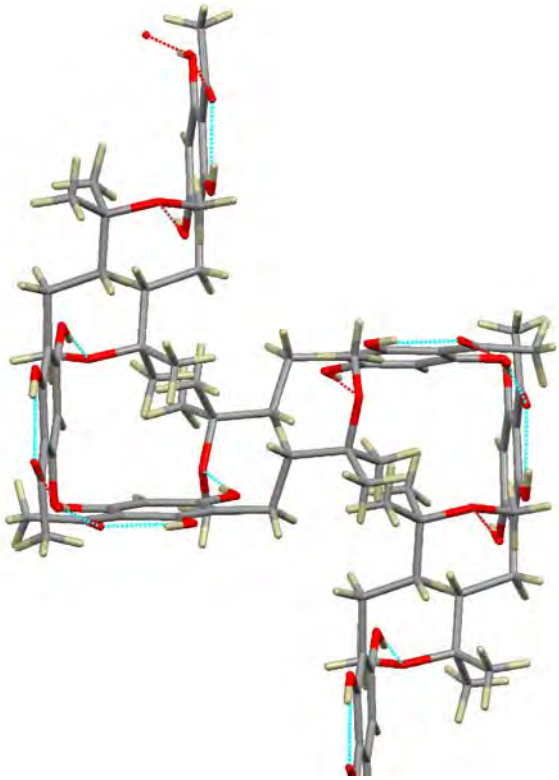


FIGURE 5.8: Supramolecular structure of **5.30** viewed down the *c*-axis.

Table 5.4 shows the crystal data and structure refinement of 1-(5,7-dihydroxy-2,2-dimethyl-chroman-8-yl)-ethanone **5.28**, 1-(5,7-dihydroxy-2,2-dimethylchroman-6-yl)ethanone **5.29** and 1-(2,4,6-trihydroxy-3-(3-methoxy-3-methylbutyl)phenyl)ethanone **5.30**.

TABLE 5.4: Crystal data and structure refinement of compounds **5.28, 5.29** and **5.30**.

Crystal Data	**Compound 2.28**	**Compound 2.29**	**Compound 2.30**
Chemical formula	$C_{13}H_{16}O_4$	$C_{13}H_{16}O_4$	$C_{14}H_{20}O_5$
Mr	236.26	236.26	268.31
Crystal system	Monoclinic	Tetragonal	Orthorhombic
Space group	P $2_{1/c}$	P 4_12_12	Pbca
Temperature (K)	100	298	100
a, b, c (Å)	8.927 (5), 16.689 (5), 15.729 (5)	10.5677 (2), 10.5677 (2), 21.4244 (5)	11.313 (5), 12.501 (5), 20.463 (5)
α, β, γ (°)	90, 90.601, 90	90, 90, 90	90, 90, 90
V ($Å^3$)	2343.2 (17)	2392.60 (8)	2894.0 (19)
Z	4	16	8
Radiation type	MoK\α	MoK\α	MoK\α
μ (mm^{-1})	0.10	0.10	0.09
Diffractometer	Oxford calibre 2	Oxford calibre 2	Oxford Excalibur 2
Absorption correction	Multi-scan	Multi-scan	Multi-scan
No. of measured, independent and observed [I > 2σ(I)] reflections	24209, 7617, 5523	26011, 2366, 2046	27852, 4755, 3602
R_{int}	0.039	0.047	0.039
(sin θ/λ)max (Å−1)	0.755	0.617	0.746
R[F2 > 2σ(F2)], wR(F2), S	0.042, 0.114, 0.95	0.032, 0.091, 1.08	0.040, 0.115, 1.04
No. of reflections	7617	2366	4755
No. of parameters	317	166	179
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement		
Δρmax, Δρmin (e Å−3)	0.33, −0.32	0.12, −0.11	0.34, −0.31
Absolute structure parameter		0.7 (11)	

The desired product, 2,4-6-trihydroxy-3-prenylacetophenone **(5.31)** was also obtained in the reaction above in a yield of 15%. For the 2,4-6-trihydroxyacetophenone moiety only one aromatic proton was observed at δ_H 6.18 in the ^{1}H NMR spectrum. For the prenyl group the two methyl singlets, the methylene doublet and the methine triplet were observed at δ_H 1.75, 1.82, 3.35, and 5.20, respectively. A much higher yield of this compound was obtained when the solution of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)** in MeOH-THF (1:1) was refluxed in the presence of 5% HCl.[267] Table 5.5 shows the results for the deprotection of **5.26** to **5.31** using different solvent ratios.

TABLE 5.5: Deprotection of **5.26** to **5.31**.

Run	Solvent	Time (hours)	Temperature	Yield (%) **5.31**
1	5% HCl/THF-MeOH (1:1)	1	Reflux	15
2	1% HCl/MeOH	1	Reflux	70

A Vilsmeier-Haack formylation of 2,4-6-trihydroxy-3-prenylacetophenone **(5.31)** was attempted using DMF and $POCl_3$ in acetonitrile but it was unsuccessful.[268] Alternative methods were attempted by changing the solvent to EtOAc as described in the method by Bharate but the desired product was not obtained and instead an undesired ring closure occurred.[269] These results show that HCl was being generated in the reaction. We then decided to explore an alternative route to obtain **5.16** starting with 1,3,5-trihydroxybenzene. The electron-rich phloroglucinol seemed to be difficult to work with. The summary of results for the formylation of **5.31**, are tabulated in Table 5.4.

TABLE 5.6: Formylation of **5.31**.

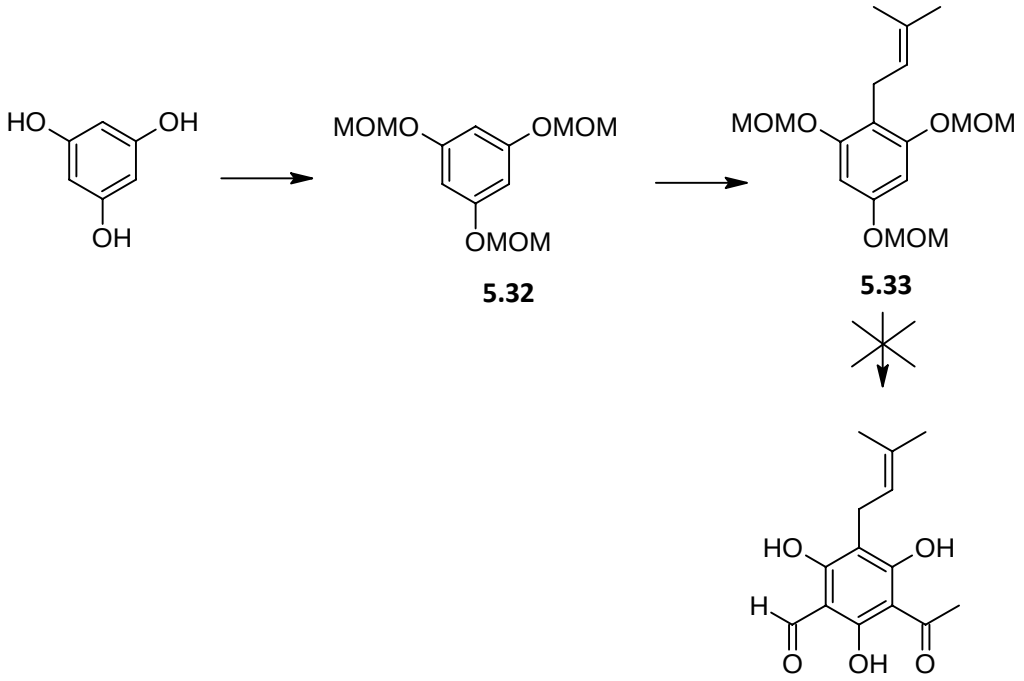
Run	Reagent	Solvent	Yield (%) **5.31**
1	$DMF/POCl_3$	CH_3CN	0
2	$DMF/POCl_3$1	DMF	0

5.4.4 ATTEMPTED SYNTHESIS OF ETHYL 3-[3-ACETYL-2,4,6-TRIHYDROXY-5-(3-METHYLBUT-2-EN-1-YL)PHENYL]PROPANOATE (5.16) FROM 1,3,5-TRIHYDROXYBENZENE

In the second approach to **5.16**, it was attempted to introduce the prenyl group first followed by the formyl and the acyl groups. Direct *C*-prenylation of 1,3,5-trihydroxybenzene was unsuccessful

thus protection of the OH groups was introduced. 1,3,5-Trihydroxybenzene was triprotected with MOM chloride to afford 1,3,5-trimethoxymethoxybenzene **(5.32)**. The ^{1}H NMR spectrum showed three singlets at δ_H 3.49, 5.14, and 6.43 which were assigned to the three methyl groups, the three equivalent methylene groups and the aromatic protons, respectively. In the ^{13}C NMR spectrum three signals were observed, methoxy carbons resonated at δ_C 56.0, the methylene carbons at δ_C 94.5, and the methane carbons at δ_C 98.5, and the aromatic carbons at δ_C 159.0. From the HRESIMS data, a molecular ion peak at *m/z* 281.1005 $[M+Na]^+$ was observed, in agreement with the molecular formula of $C_{12}H_{18}O_6Na$.

Prenylation of 1,3,5-trimethoxymethoxybenzene **(5.32)** was obtained using *n*-BuLi and 3,3-dimethylallyl bromide in THF to afford 1,3,5-trimethoxymethoxy-2-prenylbenzene **(5.33)** in 80% yield (Scheme 5.18).[270, 271] The ^{1}H NMR spectrum of **5.33** showed two singlets at 1.68 and 1.80 integrating for three protons each for the two methyl groups on the prenyl group. Also observed were a doublet at δ_H 3.34 (2H, *d*, *J* = 7.2 Hz, CHC$\underline{H}_2$) and a triplet at δ_H 5.18 (1H, *t*, *J* = 7.2 Hz, C$\underline{H}$CH_2) for the methylene and methine protons of the prenyl group, respectively as well as the proton signals of the MOM group and the aromatic nucleus. The ^{13}C NMR spectrum confirmed the presence of the prenyl group on 1,3,5-trimethoxymethyloxybenzene, the two methyl carbons were observed at δ_C 17.7 and 25.7, the methylene carbon at δ_C 22.3, methine carbon at δ_C 123.3 and the quaternary carbon at δ_C 130.6 . However, attempted acylation and formylation of prenylated phloroglucinol **5.33** proved to be unsuccessful. An alternative route starting with the benzaldehyde was attempted. The presence of the protecting groups might have reduced the electron-density and reactivity of phloroglucinol.



Scheme 5.18: Synthesis of 1,3,5-trimethoxymethoxy-2-prenylbenzene **(5.33)** i) MOMCl, NaH, DMF, 2 h, rt, 90% ii) *n*-BuLi, 3,3-dimethylallyl bromide, THF, 0 °C to rt, 80%.

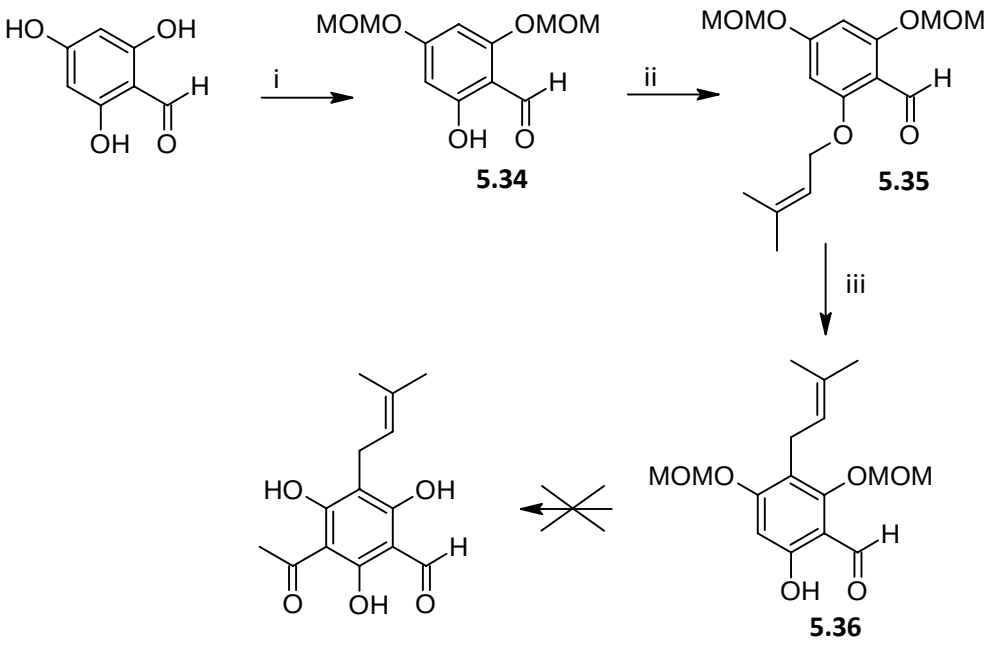
5.4.5 ATTEMPTED SYNTHESIS OF ETHYL 3-[3-ACETYL-2,4,6-TRIHYDROXY-5-(3-METHYLBUT-2-EN-1-YL)PHENYL]PROPANOATE (5.16) FROM 2,4,6-TRIHYDROXYBENZALDEHYDE

In the third approach to **5.16** we attempted to introduce the prenyl group first followed by the acyl group (Scheme 5.19). *C*-Prenylation of 2,4,6-trihydroxybenzaldehyde was achieved using the method previously described for 2,4,6-trihydroxyacetophenone. 2,4,6-Trihydroxybenzaldehyde was first di-MOM protected, then *O*-prenylated and lastly the Claisen-Cope rearrangement of the prenyl group yielded the *C*-prenylated **5.36**. MOM diprotection of 2,4,6-trihydroxybenzaldehyde was successfully achieved in 65% yield by the addition of potassium carbonate and the solution of 2,4,6-trihydroxybenzaldehyde in DMF to the crude solution of methoxymethyl chloride. In the ^{1}H NMR spectrum the methoxy and the methylene groups were observed as four singlets at δ_H 3.48 (3H, *s*, OCH_3), 3.52 (3H, *s*, $OC\underline{H}_3$), 5.18 (2H, *s*, $OC\underline{H}_2$), and 5.24 (2H, *s*, $OC\underline{H}_2$). The two aromatic protons were observed as doublets at δ_H 6.24 and 6.26. Further downfield were the aldehyde and the hydroxy protons at δ_H 10.17 and 12.91, respectively. The IR spectrum showed an OH band at 3295 cm^{-1}. In the ^{13}C NMR spectrum the methoxy carbons resonated at δ_C 56.5 and 56.6 and the methylene carbons resonated at δ_C 94.1 and 94.7.

O-Prenylation of 6-hydroxy-2,4-dimethoxymethoxybenzaldehyde **(5.34)** was achieved by treatment with potassium carbonate and 3,3-dimethylallyl bromide in acetone to afford the 2,4-dimethoxymethoxy-6-prenyloxybenzaldehyde **(5.35)** in 51% yield. The ^{1}H NMR spectrum of 2,4-dimethoxymethoxy-6-prenyloxybenzaldehyde (**5.35)** showed the two methyl groups of the prenyl group as two singlets at δ_H 1.74 and 1.78. Also observed in the spectrum were two signals, a more downfield doublet for the methylene group at δ_H 4.60 (2H, *d*, *J* = 6.7 Hz, CHC$\underline{H}_2$) and a triplet for the methine group at δ_H 5.47 (1H, *t*, *J* = 6.7 Hz, C$\underline{H}$CH_2). The absence of a resonance for the hydroxy proton further downfield was further evidence for the *O*-alkylation.

In the ^{13}C NMR spectrum, the signals of the prenyl group were obtained as two methyl carbons at δ_C 18.1 and 29.2, the methylene carbon at δ_C 65.7, the methine carbon at δ_C 119.4 and the quaternary carbon at δ_C 137.8. The HRESIMS data showed the molecular ion peak at 333.1310 [M + Na]$^+$, in agreement with the value calculated for $C_{16}H_{22}O_6Na$. The OH band was missing in the IR spectrum.

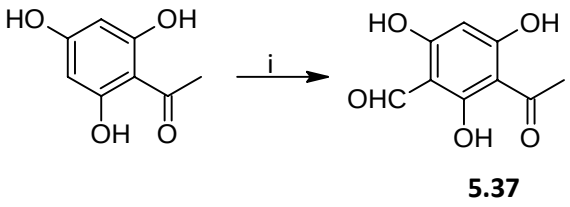
2,4-Dimethoxymethoxy-6-prenyloxybenzaldehyde **(5.35)** was refluxed in *N,N*-diethylaniline to afford 6-hydroxy-2,4-dimethoxymethoxy-3-prenylbenzaldehyde **(5.36)** in 50% yield. In the ^{1}H NMR spectrum the methylene protons were now observed more upfield at δ_H 3.29 (2H, *d, J* = 6.6 Hz, CHC$\underline{H}_2$) compared to δ_H 4.60 of the *O*-prenylated product. Also observed was the free OH at δ_H 12.09. In the ^{13}C NMR spectrum the methylene carbon shifted to δ_C 22.8 compared from δ_C 65.7 of 2,4-dimethoxymethoxy-6-prenyloxybenzaldehyde **(5.35)**. However, attempted acylation of 6-hydroxy-2,4-dimethoxymethoxy-3-prenylbenzaldehyde **(5.36)** proved to be unsuccessful. Again the fully substituted benzene ring could not be synthesised because of possible steric hindrance, the electron-withdrawing aldehyde and the presence of MOM-protecting groups. A decision was made to try another different route by first formylating acetophenone before prenylation.



Scheme 5.19: Synthesis of 6-hydroxy-2,4-dimethoxymethoxy-3-prenylbenzaldehyde **(5.36)**. i) K_2CO_3, MOMCl, DMF ii) K_2CO_3, acetone, reflux, 24 h, 51% iii) *N,N*,-diethylaniline, reflux, 3 h, 50%.

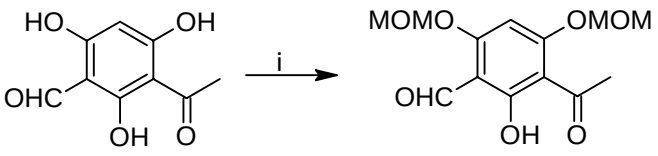
5.4.6. ATTEMPTED SYNTHESIS OF ETHYL 3-[3-ACETYL-2,4,6-TRIHYDROXY-5-(3-METHYLBUT-2-EN-1-YL)PHENYL]PROPANOATE (5.16) DERIVATIVE

In the fourth approach, the formyl group was introduced first followed by the prenyl group. The Vilsmeier-Haack formylation of the 2,4,6-trihydroxyacetophenone was successfully achieved using phosphoryl chloride and DMF to afford the product, 3-acetyl-2,4,6-trihydroxybenzaldehyde **(5.37)** in 80% yield (Scheme 5.20).[268, 272] The ^{1}H NMR spectrum showed three singlets, for the methyl group, aromatic proton and aldehyde proton at δ_H 2.63, 5.79 and 10.03, respectively. In the ^{13}C NMR spectrum, two carbonyl carbons were observed at δ_C 191.9 and 201.6 corresponding to the aldehyde and ketone, respectively.



Scheme 5.20: Synthesis of 3-acetyl-2,4,6-trihydroxybenzaldehyde **(5.37)**. i) $POCl_3$, DMF, reflux, 1.5 hours, 80%.

Prenylation was attempted on 3-acetyl-2,4,6-trihydroxybenzaldehyde **(5.37)** using sodium hydride and prenyl bromide in dioxane, sodium hydroxide and prenyl bromide in dioxane, potassium carbonate and prenyl bromide in acetone, and potassium hydroxide and prenyl bromide in MeOH, however the reaction was unsuccessful. To prevent *O*-alkylation, 3-acetyl-2,4,6-trihydroxybenzaldehyde **(5.37)** was MOM protected using 3.5 equivalents of MOM chloride resulting in a diprotected product, 3-acetyl-2-hydroxy-4,6-dimethoxymethoxybenzaldehyde **(5.38)** (Scheme 5.21). The ^{1}H NMR spectrum showed five singlets at δ_H 2.48, 3.43, 5.12, 6.52, and 10.03 corresponding to the methyl protons α to the ketone, the methoxy protons, the methylene protons, the aromatic proton and the aldehyde proton, respectively. Attempted *C*-prenylation of **5.38** was unsuccessful and *O*-prenylation did not take place, possibly because of the hydrogen bonding between the phenol hydrogen and the two carbonyl oxygen atoms. The protecting group was then changed to the benzyl group.



5.37 **5.38**

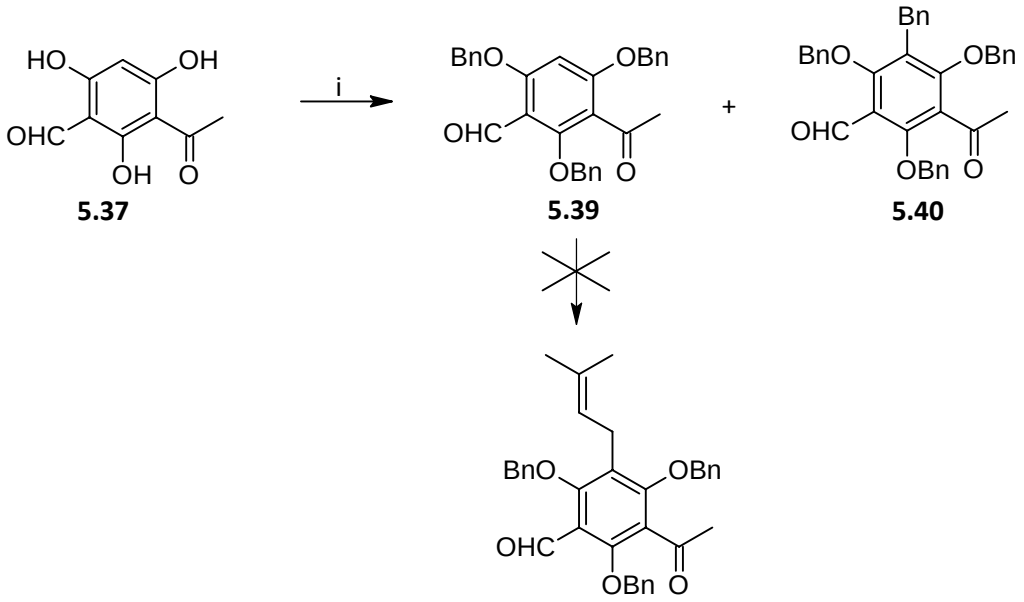
Scheme 5.21: MOM protection of 3-acetyl-2,4,6-trihydroxybenzaldehyde **(5.37)**. i) K_2CO_3, MOMCl, DMF, 62%.

The formylated 2,4,6-trihydroxyacetophenone **5.37** was then protected using benzyl chloride to afford 3-acetyl-2,4,6-tribenzyloxybenzaldehyde **(5.39)** in 60% yield alongside with the *C*-benzylated 3-acetyl-2,4,6-tribenzyloxy-5-benzylbenzaldehyde **(5.40)** in 18 % yield (Scheme 5.22).

In the ^{1}H NMR spectrum of 3-acetyl-2,4,6-tribenzyloxybenzaldehyde **(5.39)**, the three methylene groups were observed as three singlets at δ_H 5.00, 5.01 and 5.06 . The multiplet in the aromatic region at δ_H 7.35 integrated for 15 protons. The singlet for the methine proton in the benzene ring of acetophenone was still present at δ_H 6.40.

In the ^{1}H NMR spectrum of 3-acetyl-2,4,6-tribenzyloxy-5-benzylbenzaldehyde **(5.40)**, the four methylene groups were observed as three downfield singlets at δ_H 4.82, 4.88 and 5.08 and a fourth one at 4.02. The multiplet in the aromatic region from δ_H 7.28 integrated for 20 protons. No signal of the electron-rich phloroglucinol moiety was observed. The singlet for the methine

proton in the benzene ring of acetophenone at δ_H 6.40 was absent. Further evidence that *O*-benzylation of the three hydroxy groups as well as the *C*-benzylation had taken place was from the mass spectrometric data that showed a molecular ion peak at *m/z* 555.2177 $[M-H]^+$, in agreement with the calculated value for $C_{37}H_{31}O_5$. *C*-benzylation is more favoured than *C*-prenylation because the benzyl carbocation formed is stabilised by the benzene ring.



5.37 **5.39** **5.40**

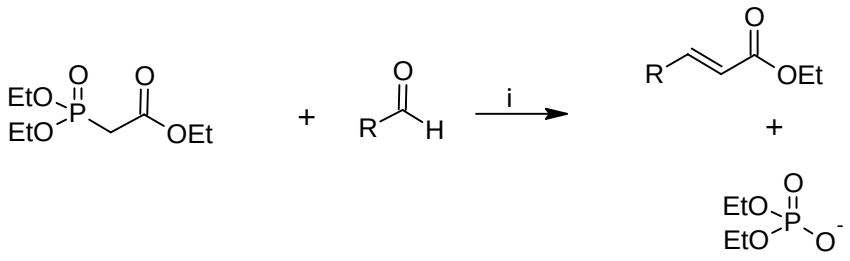
Scheme 5.22: Benzyl protection of 3-acetyl-2,4,6-trihydroxybenzaldehyde **(5.37)**. i) BnCl, K_2CO_3, DMF, rt, overnight.

The attempted prenylation of 3-acetyl-2,4,6-tribenzyloxybenzaldehyde **(5.39)** using potassium carbonate and prenyl bromide in acetone or DMF, and sodium hydride and prenyl bromide in dioxane proved to be unsuccessful. The synthesis was then continued without the prenyl substituent as a model study.

5.4.7 SYNTHESIS OF ETHYL 3-(3-ACETYL-2,4,6-TRIHYDROXYPHENYL)PROPANOATE (5.42)

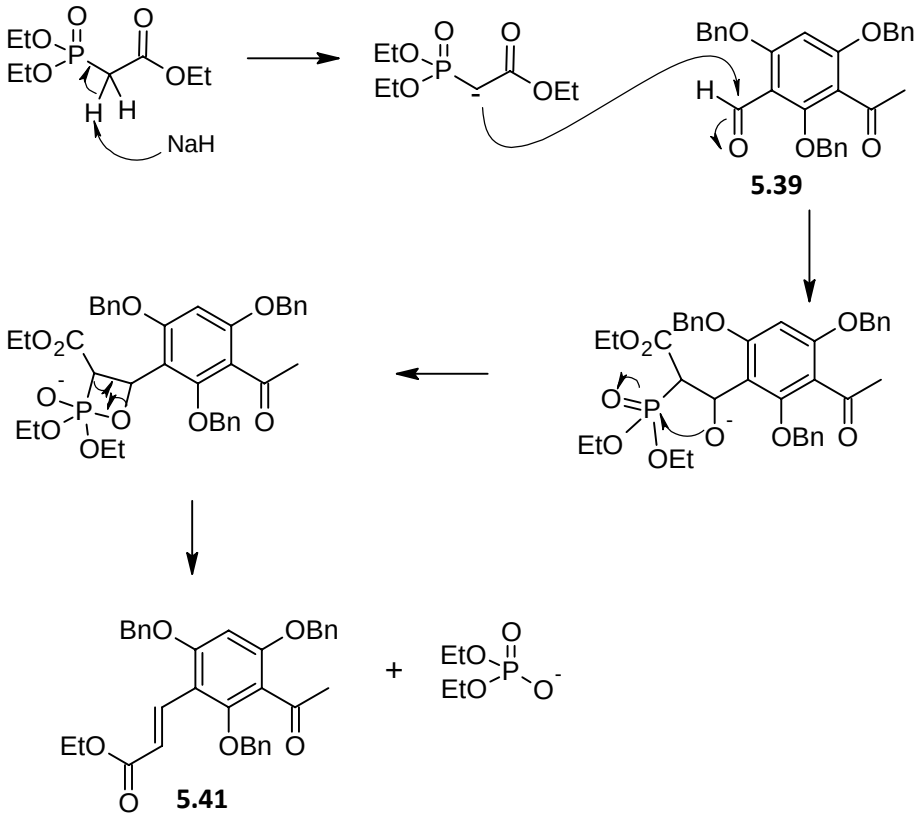
The Horner-Wadsworth-Emmons olefination reaction (HWE) is used in organic synthesis for carbon-carbon bond formation. It is a modified Wittig reaction and one of the most efficient methods used in the stereoselective preparation of α,β-unsaturated esters.[273] The phosphonate-stabilised carbanion reacts with the aldehyde or ketone to form the corresponding *E*-alkene. Various bases such as butyl lithium, sodium hydride and sodium ethoxide are used to form the

phosphonate carbanions. Unlike the Wittig reaction which produces triphenylphosphine oxide as the by-product, the HWE reaction produces the water-soluble phosphate ester (Scheme 5.23).



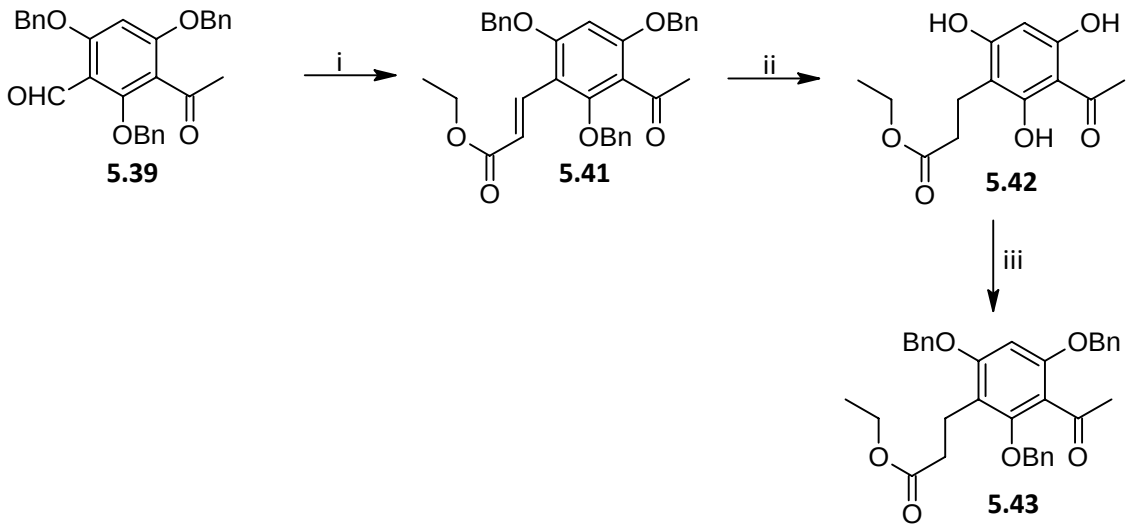
Scheme 5.23: Horner-Wadsworth-Emmons reaction.i) base.

The HWE olefination reaction of acetyl-2,4,6-tribenzyloxy-5-benzylbenzaldehyde **(5.39)** with ethyl diethylphosphonoacetate in the presence of sodium hydride in THF afforded the corresponding α,β-unsaturated esters, ethyl (2*E*)-3-(3-acetyl-2,4,6-tribenzyloxyphenyl)prop-2-enoate **(5.41)** in 74% yield (Scheme 5.24). The first step is the deprotonation of ethyl diethylphosphonoacetate using sodium hydride to form the phosphonate carbanion, which then reacted with the aldehyde **5.39** to form a betaine intermediate. The betaine forms a four-membered cyclic intermediate which subsequently opens to form an alkene and a phosphate ester.



Scheme 5.24: Mechanism of Horner-Wadsworth-Emmons reaction of acetyl-2,4,6-tribenzyloxy-5-benzylbenzaldehyde **(5.39)**.

In the ^{1}H NMR spectrum no aldehyde proton (expected at δ_H 10) was observed. The two doublets observed at δ_H 6.64 (1H, *d*, *J* = 16.1 Hz, α-H), and δ_H 8.03 (1H, *d*, *J* = 16.1 Hz, β-H) were evidence of the *trans*-alkene. Also observed were a quartet and a triplet for the CH_3CH_2 group of an ester at δ_H 0.98 and 4.81, respectively.

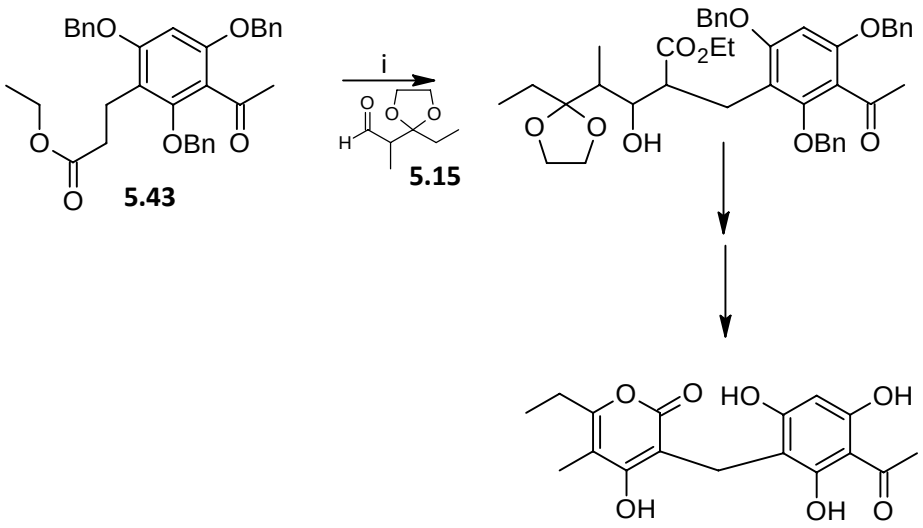


Scheme 5.25: Synthesis of ethyl 3-(3-acetyl-2,4,6-tribenzyloxyphenyl)propanoate **(5.43)**. i) NaH, ethyl diethylphosphonoacetate, THF, 5 hours, 0 °C, 80% ii) H_2, Pd/C THF/MeOH, rt, 6 hours, 98% iii) BnCl, K_2CO_3, DMF, rt, overnight, 75%.

Catalytic hydrogenation of **5.41** in the presence of the benzyloxy group resulted in the expected reduction of an alkene to an alkane and subsequent debenzylation to give ethyl 3-(3-acetyl-2,4,6-trihydroxyphenyl)propanoate **(5.42)** (Scheme 5.25). The disappearance of the two doublets at δ_H 6.64 and 8.03, integrating for one proton each and the appearance of two triplets, integrating for two protons each at δ_H 2.42 and 2.79 was further evidence of the hydrogenation of the alkene**.** The saturated ester **(5.42)** was benzyl protected with benzyl chloride to give ethyl 3-(3-acetyl-2,4,6-tribenzyloxyphenyl)propanoate **(5.43)**.

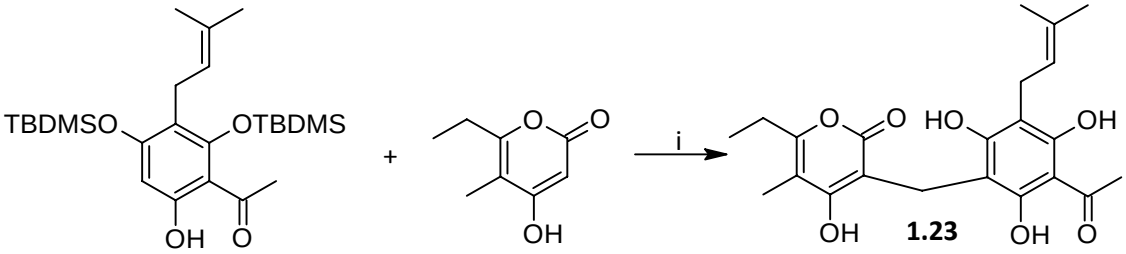
5.4.8 ATTEMPTED COUPLING OF THE ESTER 5.43 WITH AN ALDEHYDE 5.15

Having successfully synthesised **5.43**, the next step was to couple it with 2-(2-ethyl-1,3-dioxolan-2-yl)propanal **(5.15)**. Attempted coupling of the ester **5.43** and the aldehyde **5.15** as planned using LDA was unsuccessful. The product of this reaction would have undergone ring closure to form the deprenyl arzanol derivative (Scheme 5.26). When the total synthesis of arzanol failed, we then decided to synthesise lepidissipyrone **(1.23)**. The next section will discuss the route for the total synthesis of lepidissipyrone.



Scheme 5 26: Attempted coupling of the ester **5.43** and the aldehyde **5.15**. i) LDA, THF, -78 °C.

When this investigation started, there was no reported total synthesis of arzanol. Our attempted routes had failed due to the difficult nature of the electron-rich phloroacetophenone. In 2012 Minassi and co-workers reported the first total synthesis of arzanol using the multicomponent carba version of the Betti reaction (Scheme 5.27).[230] In their synthesis they also observed that unprotected 2,4-6-trihydroxy-3-prenylacetophenone **(5.31)** was inactive in neutral medium. They succeeded in forming arzanol by starting with the TBDMS protected phloroglucinol derivative. By the addition of TBAF, indirect deprotonated of the phloroacetophenone took place without compromising the stability of arzanol.



Scheme 5 27: Reported synthesis of arzanol **(1.23)**. i) $(CH_2)n$, $CHCl_3$, TBAF, 40 °C, 16h hours, 50%.

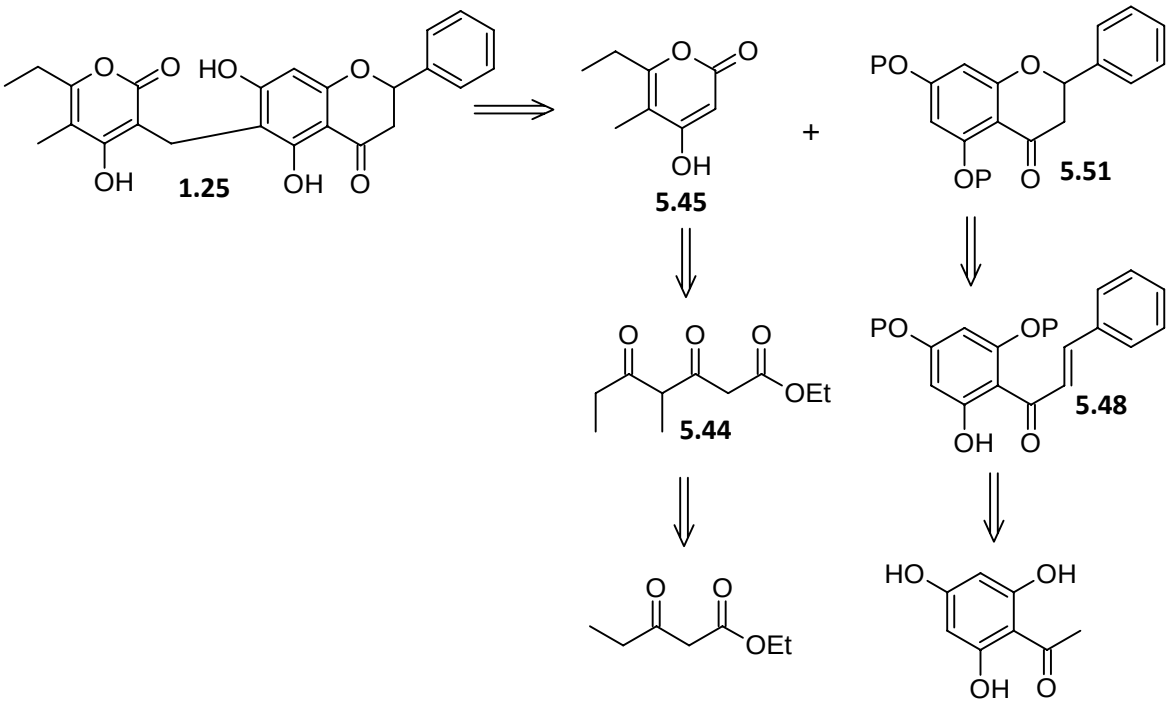
In the synthesis of arzanol by Manassi and co-workers,[230] paraformaldehyde was used in the presence of TBAF to couple the TBDMS protected phloroacetophenone moiety with α-pyrone moiety and at the same time removing the TBDMS protecting group. The 2-pyrone was synthesised by the cyclodehydration of 3,5-diketoester, ethyl 4-methyl-3,5-dioxoheptanoate,

using polyphosphoric acid (Scheme 5.29). Based on their synthetic route, we envisioned that a flavanone and a pyrone can serve as precursors in the total synthesis of lepidissipyrone.

5.4.9 TOTAL SYNTHESIS OF LEPIDISSIPYRONE (1.25)

Lepidissipyrone **(1.25)** is structurally similar to arzanol **(1.23)**; it possesses the same α-pyrone moiety and a flavanone instead of an acylphloroglucinol. Thus the challenges with the synthesis of lepidissipyrone will be the α-pyrone which tends to form homodimeric adducts as in arzanol.[230, 253] The present of the flavanone moiety poses another challenge because flavanones convert to chalcone under acidic, basic and thermal conditions.[274, 275] There is no reported total synthesis of lepidissipyrone. The synthesis of both, the flavanone and 2-pyrone, have been reported thus another challenge will be the coupling of the two moieties using CH_2 and the selective attachment of the CH_2 at the C-6 position not the C-8 of the flavanone.

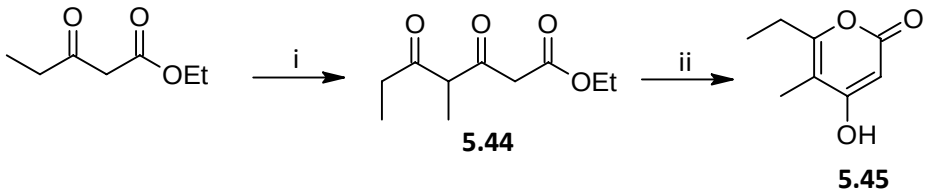
The proposed retrosynthetic analysis of lepidissipyrone is outlined in Scheme 5.28. The flavanone **(5.51)** will be prepared from 2,4,6-trihydroxyacetophenone and the α-pyrone **(5.45**) will be prepared from ethyl 3-oxopentanoate.



Scheme 5 28: Retrosynthesis of lepidissipyrone **(1.25)**.

5.4.9.1 SYNTHESIS OF 6-ETHYL-4-HYDROXY-5-METHYL-2-PYRONE (5.45)

The synthesis of ethyl 4-methyl-3,5-dioxoheptanoate **(5.44)** was successfully achieved using a method reported by Solladié and co-workers (Scheme 5.29).[276] Ethyl 3-oxopentanoate was acylated with ethyl propionate using sodium hydride and *n*-BuLi to afford ethyl 4-methyl-3,5-dioxoheptanoate **(5.44)** in 60% yield. Because condensation product has a more acidic hydrogen than the α-hydrogen of ethyl 3-oxopentanoate, ethyl propionate was added initially as 0.5 equivalents to the dianion of ethyl 3-oxopentanoate followed by the addition of 0.5 equivalents of the base to regenerate the dianion then finally 0.5 equiv. of ethyl propionate to afford the product **5.44**.[276]



5.44 **5.45**

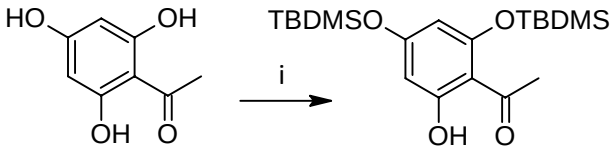
Scheme 5.29: Synthesis of 6-ethyl-4-hydroxy-5-methyl-α-pyrone **(5.45)**. i) 1. NaH, THF, 0 °C 2. *n*-BuLi 3. $CH_3CH_2CO_2Et$, 1 hour, 60% ii) PPA, 120 °C, 1 hour, 70%.

In the ^1H NMR spectrum, two sets of triplets and quartets were observed for the two ethyl groups, a doublet at δ_H 1.29 for the methyl adjacent to the methine, the singlet at δ_H 3.38 for the methylene between the two carbonyls and a quartet at δ_H 3.83 for the methine. In the ^{13}C NMR spectrum three carbonyl carbons were observed at δ_C 167.0, 200.0, and 207.5.

Ethyl 4-methyl-3,5-dioxoheptanoate **(5.44)** was then cyclodehydrated by heating it in polyphosphoric acid to afford 6-ethyl-4-hydroxy-5-methyl-α-pyrone **(5.45)**. The IR spectrum showed the OH absorption at 3318 cm^{-1} and 1738 cm^{-1} for the carbonyl. From the HRESIMS $[M+Na]^+$ at *m/z* 177.0535, the molecular formula of the pyrone was confirmed as $C_8H_{10}O_3$. The ^1H NMR showed a triplet and a quartet for the CH_2CH_3 group at δ_H 1.22 (3H, *t*, *J* = 7.3 Hz, $CH_2C\underline{H}_3$) and at δ_H 2.57 (2H, *q*, *J* = 7.3 Hz, $C\underline{H}_2CH_3$). Also observed were two singlets for a CH_3 at δ_H 1.96 and a methine proton at δ_H 5.71 (Plate 6). In the ^{13}C NMR only one carbonyl peak was observed at δ_C 172.8 (Plate 7). Having successfully synthesised the pyrone moiety, the challenge was now to synthesize the flavanone moiety and the subsequent CH_2 linkage to complete the total the synthesis. The next section will discuss the synthesis of the flavanone moiety.

5.4.9.2 SYNTHESIS OF 7-*TERT*-BUTYLDIMETHYLSILYLOXY-5-HYDROXYFLAVANONE (5.51)

An attempted Claisen-Schmidt condensation of 2,4,6-trihydroxyacetophenone with benzaldehyde was unsuccessful thus selective diprotection was investigated. 2,4,6-Trihydroxyacetophenone was selectively diprotected with *tert*-butyldimethylsilyl chloride (TBDMSCl) using triethyl amine in DCM to afford 2,4-bis(*tert*-butyldimethylsilyl)phloroacetophenone **(5.46)** in 74% yield (Scheme 5.30). The product was confirmed by the appearance of four singlets at δ_H 0.24 (6H, *s*, Si(C$\underline{H}_3$)$_2$), δ_H 0.35 (6H, *s*, Si(C$\underline{H}_3$)$_2$), δ_H 0.98 (9H, *s*, C(C$\underline{H}_3$)$_3$), and δ_H 1.01 (9 H, *s*, C(C$\underline{H}_3$)$_3$) in the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum the two dimethylsilyl carbons were observed at δ_C -4.3 and -3.5, the quaternary carbons of *tert*-butyl at δ_C 18.2 and 18.9 and methyl carbons at δ_C 25.5 and 26.1. The attempted Claisen-Schmidt condensation of 2,4-bis(*tert*-butyldimethylsilyl)phloroacetophenone **(5.46)** with the benzaldehyde was also unsuccessful. Unreacted starting material was recovered.



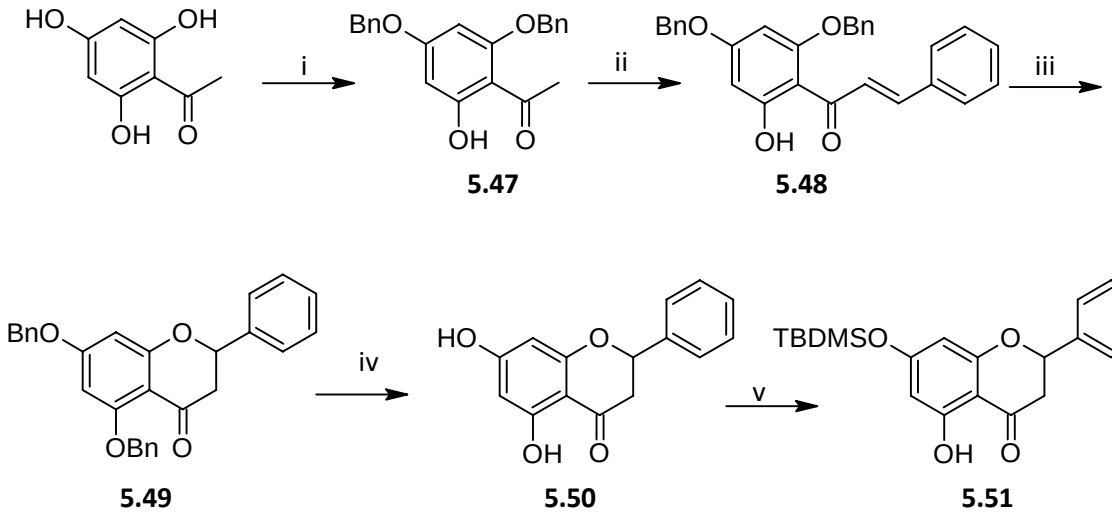
5.46

Scheme 5.30: TBDMS protection of 2,4,6-trihydroxyacetophenone. i) Et_3N, TBDMSCl, DCM, rt, 90 min, 74%.

The protecting group was then changed to benzyl (Scheme 5.31). 2,4,6-Trihydroxyacetophenone was dibenzylated by heating it at 80 °C with K_2CO_3 and benzyl chloride in DMF for 2 h. The dibenzylated product 2,4-bis-benzyloxy-6-hydroxyphloroacetophenone **(5.47)** was obtained in 79% yield. In the ^{1}H NMR spectrum the benzylic protons were observed as two singlets integrating for two protons each at δ_H 5.08 and 5.09, and the aromatic protons integrating for ten protons were observed as a multiplet at δ_H 7.42. In the ^{13}C NMR spectrum the two benzylic carbons at δ_C 70.3 and 71.3, and aromatic carbons were further evidence of the dibenzylation of 2,4,6-trihydroxyacetophenone.

The Claisen-Schmidt condensation of 2,4-bis-benzyloxy-6-hydroxyphloroacetophenone **(5.47)** with benzaldehyde using NaH in DMF led to the formation of the 2,4-bis-benzyloxy-6-hydroxychalcone **(5.48)** in quantitative yields. In the ^{1}H NMR spectrum, the OH proton appeared as a singlet at δ_H 14.2. The characteristic α,β-olefinic protons of a chalcone appeared as doublets at δ_H 7.76 (1H, *d*,

J = 15.6 Hz, β-H) and δ_H 7.98 (1H, *d*, *J* =15.6 Hz, α-H), with the coupling constant of 15.5 Hz confirming the *trans* geometry of the alkene. The aromatic protons resonating at δ_H 7.30 integrated for 15 hydrogens confirming the addition of another benzene ring. The ^{13}C NMR spectrum showed the α-C and β-C at δ_C 129.8 and 142.8, respectively, further confirming the chalcone structure.



Scheme 5.31: Synthesis of 7-*tert*-butyldimethylsilyloxy-5-hydroxyflavanone **(5.51)**. i) K_2CO_3, BnCl, DMF, 80 °C, 2 h, 79 % ii) NaH, benzaldehyde, DMF, 0 °C, 45 min, 96%, iii) DBU, DCM, rt, overnight, 46% iv) H_2, Pd/C, EtOAc/DCM, rt, overnight, 94% v) Et_3N, TBDMSCl, DCM, rt, 90 min, 85%.

Flavanones are a very important class of organic compounds, they are found in many synthetic and naturally occurring biological active compounds. They are also precursors for the synthesis of flavones, flavonols, isoflavones and dihydroflavonols.[277] Different methods have been reported for synthesis of flavanones but the most common is the cyclisation of 2-hydroxychalcones.[277, 278] Chalcone are cyclised to flavanone using acid reagents[279] such as H_2SO_4, CF_3COOH,[279] CH_3COOH,[276] CH_3SO_3H,[277] KF-celite,[280] and polyphosphoric acid, basic reagents[281] such as pyridine, DBU,[229, 282] and KOH,[283] heat,[284] silica gel,[285] electrolysis,[286] microwave,[287] zeolites,[288] L-proline,[289] and cobalt (II) Schiff-base complexes.[290] However, the yields are often moderate to low because there is an equilibrium between the chalcone and the flavanone.[291] The flavanone **5.49** was synthesised by a base-mediated reaction with DBU, and almost 50 % of the chalcone was recovered.

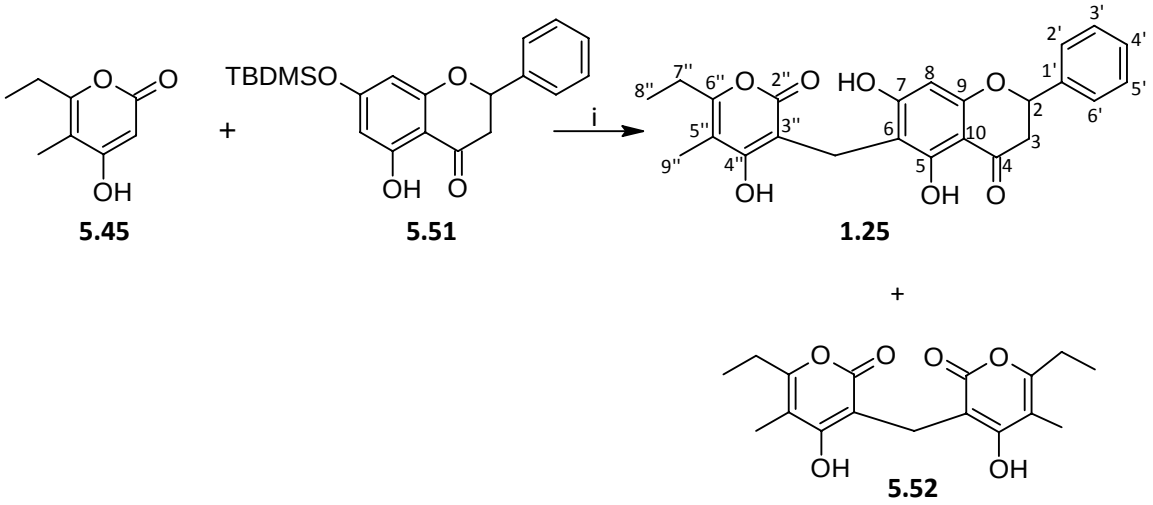
In the ^1H NMR spectrum of 5,7-bis-benzyloxyflavanone **(5.49)**, the OH singlet of the chalcone at δ_H 14.2 as well as the α and β-olefinic doublets were no longer present. The new typical flavanone

signals were observed at δ_H 2.84 (1H, *dd*, *J* =16.6 Hz, 3.1 Hz, H-3_{eq}), δ_H 3.07 (1H, *dd*, *J* =16.6 Hz, 13.3 Hz, H-3_{ax}), and δ_H 5.43 (1H, *dd*, *J* =13.3 Hz, 3.1 Hz, C<u>H</u>CH_2) and δ_C 42.8 and 78.9 in the ^{13}C NMR spectrum. From the HRESIMS $[M-H]^+$ observed at *m/z* 255.0658 $[M-H]^+$, the molecular formula of the chalcone was confirmed as $C_{15}H_{12}O_4$.

An attempt to couple the dibenzylated flavanone **5.49** with the pyrone **5.45** was unsuccessful. The flavanone was then deprotected using hydrogen gas at 200 kPa over Pd/C catalyst to afford 5,7-dihydroxyflavanone **(5.50)**. In the ^{1}H NMR spectrum the OCH_2 signals had disappeared and the aromatic protons only integrated for 5 protons. The flavanone was then protected using *tert*-butyldimethylsilyl chloride and triethyl amine in DCM. Under these mild conditions, only one OH group was protected, even on addition of two equivalents of *tert*-butyldimethylsilyl chloride and triethyl amine. In the ^{1}H NMR spectrum, the synthesis of 7-*tert*-butyldimethylsilyloxy-5-hydroxyflavanone **(5.51)** was confirmed by the presence of the two singlets at δ_H 0.27 integrating for 6 protons (dimethyl) and at δ_H 0.99 integrating for 9 protons (*t*-butyl) (Plate 8). In the ^{13}C NMR spectrum new signals at δ_C -4.4 (Si(<u>C</u>$H_3)_2$), -4.3 (Si(<u>C</u>$H_3)_2$), 18.2 (<u>C</u>$(CH_3)_3$), and 25.4 (C(<u>C</u>$H_3)_3$) were observed (Plate 9).

5.4.9.3 COUPLING OF 6-ETHYL-4-HYDROXY-5-METHYL-2-PYRONE (5.45) AND 7-*TERT*-BUTYLDIMETHYLSILYLOXY-5-HYDROXYFLAVANONE (5.51)

Having synthesised 6-ethyl-4-hydroxy-5-methyl-α-pyrone **(5.45)** and 7-*tert*-butyldimethylsilyloxy-5-hydroxyflavanone **(5.51)**, the two moieties were coupled using tertrabutylammonium fluoride (TBAF), and paraformaldehyde in chloroform at 40 °C to afford lepidissipyrone **(1.25)** (Scheme 5.32). These conditions allowed for the introduction of the CH_2 linkage and the subsequent *tert*-butyldimethylsilyl deprotection. The total synthesis of lepidissipyrone **(1.25)** was successfully completed and the structure was confirmed by ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HSQC, HMBC, IR and MS. Residual amounts of helipyrone **(5.52)** were also produced. The IR spectrum of lepidissipyrone **(1.25)** showed the OH absorption at 3368 cm^{-1} and the carbonyl absorption band at 1658 and 1623 cm^{-1}. From the HRESIMS $[M+Na]^+$ at *m/z* 445.1260 $[M+Na]^+$, the molecular formula of the product was confirmed as $C_{24}H_{22}O_7Na$, corresponding to lepidissipyrone **(1.25)**.



Scheme 5.32: Synthesis of lepidissipyrone **(1.25)**. i) $(CH_2O)n$, $CHCl_3$, TBAF, 40 °C, overnight, 50%.

In the ^1H NMR spectrum of lepidissipyrone **(1.25)**, the ethyl group was observed as a triplet and a quartet at δ_H 1.20 and 2.54 respectively, the methyl singlet at δ_H 1.88, the methylene protons as a singlet at δ_H 3.59, the three typical flavanone characteristic doublet of doublets at δ_H 2.86, 3.29 and 5.55, the singlet for the aromatic proton at δ_H 6.23 and the multiplet integrating for five protons at δ_H 7.53 (Plate 10). In the ^{13}C NMR spectrum the methylene carbon was observed at δ_C 99.7 (Plate 11). Also present were two carbonyl carbons, the ketone and the lactone resonating at δ_C 198.8 and 169.0, respectively. HMBC correlations were observed between the methylene protons connecting the pyrone and flavanone moieties and C-2'', C-4'', and C-3'', and C-6, and also between H-8 and C-7, C-9, C-6, C-10, C-4 and the methylene carbon. The spectroscopic data of lepidissipyrone **(1.25)** was in agreement with that of the natural product.[176]

Having successfully synthesised lepidissipyrone **(1.25)** we also synthesised some α-pyrone and some prenylated acylphoroglucinol derivatives. These motifs are found in many biological active natural products. They could be coupled with formaldehyde to form arzanol derivatives **1.24**, which can be tested for biological activity.

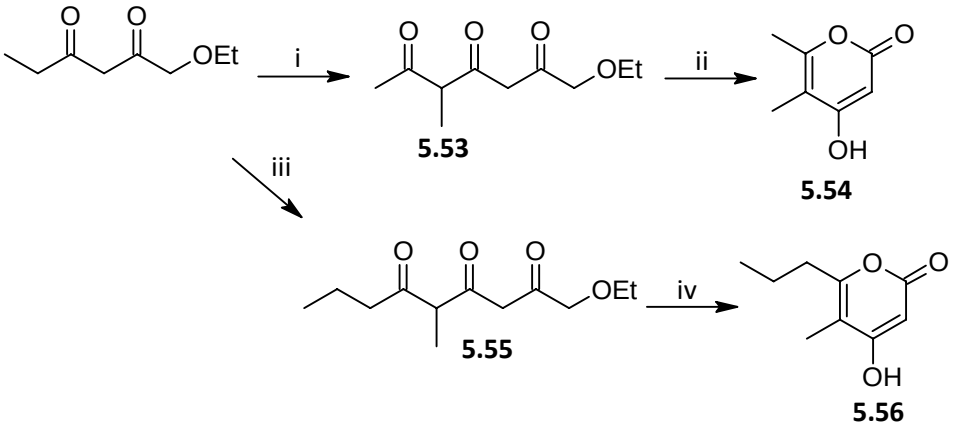
5.4.10 SYNTHESIS OF PYRONE DERIVATIVES

Because of the reported synthesis of arzanol **(1.23**) and our successful first total synthesis of lepidissipyrone **(1.25)**, we decided to synthesise their derivatives. The focus was on varying the alkyl groups on the hydroxypyrone moiety as well as varying the alkyl and acetyl groups on the phloroglucinol moiety. The method that was used for the synthesis of 6-ethyl-4-hydroxy-5-

methyl-α-pyrone **(5.45)**, was used to successfully synthesise two other pyrone derivatives, 4-hydroxy-5,6-dimethyl-α-pyrone **(5.54)** and 4-hydroxy-5-methyl-6-propyl-α-pyrone **(5.56)** (Scheme 5.33).

For the synthesis of the diketoester, ethyl 4-methyl-3,5-dioxohexanoate (**5.53)**, ethyl acetate was condensed with ethyl 3-oxopentanoate, and for the synthesis of ethyl 4-methyl-3,5-dioxooctanoate (**5.55)**, ethyl butyrate was condensed with ethyl 3-oxopentanoate. The ^{1}H NMR spectrum of ethyl 4-methyl-3,5-dioxohexanoate **(5.53)** showed a deshielded singlet for a methyl group at δ_H 2.16 and a quartet and a doublet at δ_H 3.81 and 1.28 for the $CHCH_3$ group. The ^{13}C NMR spectrum showed a methyl carbon next to a carbonyl at δ_C 28.3 and also the three carbonyl peaks at δ_H 166.7, 184.3 and 204.6. The ^{1}H NMR spectrum of ethyl 4-methyl-3,5-dioxooctanoate **(5.55)** showed the triplet and two multiplets at δ_H 0.87, 1.58 and 2.45 for the methyl and the two methylenes of the propyl group. The ^{13}C NMR spectrum showed three carbonyl peaks for an ester and two ketones at δ_C 167.7, 199.7 and 207.3, respectively.

The two diketoesters were cyclised by heating in polyphosphoric acid at 120 °C for 1.5 hours to yield 4-hydroxy-5,6-dimethyl-α-pyrone **(5.54)** and 4-hydroxy-5-methyl-6-propyl-α-pyrone **(5.56).** The ^{1}H NMR spectrum of 4-hydroxy-5,6-dimethyl-α-pyrone **(5.54)**, showed three singlets at δ_H 1.94, 2.17 and 5.72 for the two methyl groups and the methine proton. The ^{13}C NMR spectrum showed seven signals as expected and only one carbonyl was observed at δ_C 172.7. In the IR spectrum, the OH band was observed at 3424 cm^{-1} and the carbonyl band at 1695 cm^{-1}.

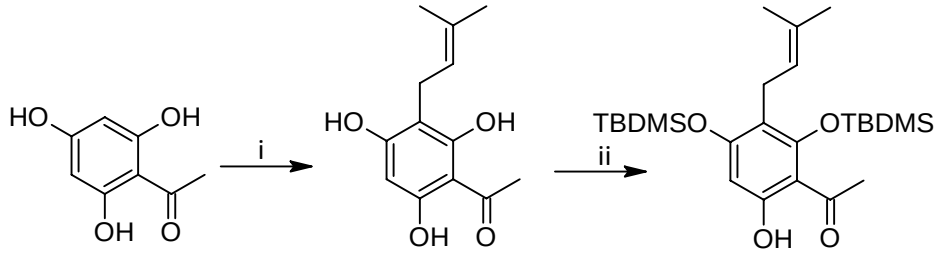


Scheme 5.33: Synthesis of 6-alkyl-4-hydroxy-5-methyl-α-pyrones, **5.54** and **5.56**. i) 1. NaH, THF, 0 °C 2. *n*-BuLi 3. CH_3CO_2Et, 1 hour, 54% ii) PPA, 120 °C, 1 hour, 68% iii) 1. NaH, THF, 0 °C 2. *n*-BuLi 3. $CH_3CH_2CH_2CO_2Et$, 1 hour, 55% iv) PPA, 120 °C, 1 hour, 65%.

The ^{1}H NMR spectrum of hydroxy-5,6-dimethyl-α-pyrone **(5.56)**, showed a singlet at δ_H 1.96 for a CH_3 attached to C-4, a singlet at δ_H 5.71 for a C-3 proton, and two triplets and a sextet for the propyl group, at δ_H 0.97 (3H, *t*, *J*=7.4 Hz, $CH_2CH_2C\underline{H}_3$), 1.69 (2H, *sextet*, *J*=7.4 Hz, $CH_2C\underline{H}_2CH_3$), and 2.53 (2H, *t*, *J*=7.4 Hz, $C\underline{H}_2CH_2CH_3$). The ^{13}C NMR spectrum showed nine signals as expected, including the carbonyl at δ_C 172.7. In the IR spectrum, the OH band was observed at 3135 cm^{-1} and the carbonyl band at 1697 cm^{-1}.

5.4.11 SYNTHESIS OF PHLOROGLUCINOL DERIVATIVES

Direct *C*-prenylation of 2,4,6-trihydroxyacetophenone using prenyl bromide and either potassium carbonate or potassium hydroxide gives low yields of between 30 and 34%. Lee reported the prenylation using 3,3-dimethylallyl bromide and DBU in THF in 40% yield.[292] Initially this route was not considered because of the low percentage yield. This method was used and we were able to produce 3-prenylphloroacetophenone **(5.31)** in 35% yield (Scheme 5.34). 3-Prenylphloroacetophenone was then diprotected using *tert*-butyldimethylsilyl chloride and triethyl amine in DCM and 2,4-bis(*tert*-butyldimethylsilyl)-3-prenylphloroacetophenone **(5.57)** was obtained in 70%. From both the ^{1}H and ^{13}C spectrum the *tert*-butyldimethyl and the prenyl peaks were present. Also observed in the ^{1}H NMR spectrum was the OH proton at δ_H 12.91.

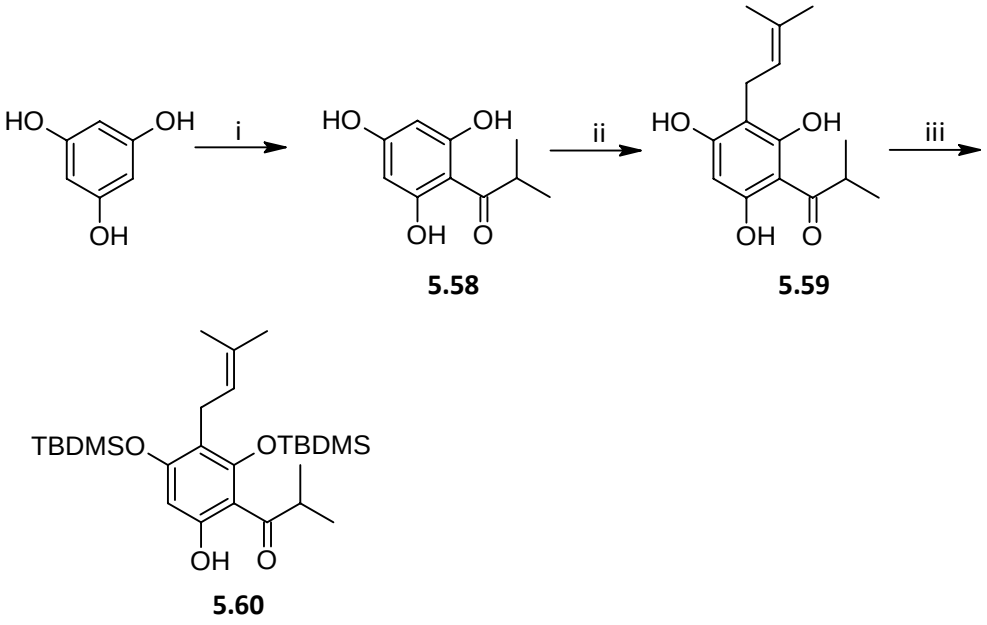


5.31 **5.57**

Scheme 5.34: Synthesis of 2,4-bis(*tert*-butyldimethylsilyl)-3-prenylphloroacetophenone **(5.57)**. i) DBU, 3,3-dimethylallyl bromide , THF, rt, overnight, 35% ii) Et_3N, TBDMSCl, DCM, rt, 90 min, 70%.

Friedel-Craft acylation of phloroglucinol using $AlCl_3$ and isobutyryl chloride in nitrobenzene yielded 2,4,6-trihydroxyisobutyrophenone **(5.58)** (Scheme 5.35)**.** In the ^{1}H NMR spectrum, the isopropyl group was observed as a multiplet at δ_H 1.18 and a septet at δ_H 3.93 for the methyl and the methine protons, respectively. The aromatic protons were observed as a singlet at δ_H 5.92.

2,4,6-Trihydroxyisobutyrophenone was prenylated using DBU and prenyl bromide in THF to yield 2,4,6-trihydroxy-3-prenylisobutyrophenone (**5.59)**. 2,4,6-Trihydroxy-3-prenylisobutyrophenone **(5.59)** was diprotected using *tert*-butyldimethylsilyl chloride and triethyl amine in DCM affording 2,4-bis(*tert*-butyldimethylsilyl)- 3-prenylisobutyrophenone **(5.60)** (Scheme 5.35).



5.58 **5.59** **5.60**

Scheme 5.35: Synthesis of 2,4-bis(*tert*-butyldimethylsilyl)- 3-prenylisobutyrophenone **(5.60)**. i) $AlCl_3$, isobutyryl chloride, $PhNO_2$, 80 °C, 1.5 hours, 70% ii) DBU, 3,3-dimethylallyl bromide , THF, rt, overnight, 40% iii) Et_3N, TBDMSCl, DCM, rt, 90 min, 79%.

5.5 ANTI-HIV ACTIVITY OF SELECTED SYNTHESISED COMPOUNDS

Selected synthesised compound were tested for the inhibition of HIV-1 replication at a single dose of 10 µM. Only 2,4-bis-benzyloxy-6-hydroxychalcone **(5.48)** showed significant inhibition of 67%. The testing results are shown in Table 5.7.

Table 5.7: Results from inhibition of HIV-1 replication by selected synthesised.

Compound	CC_{50} ± Std Dev (μM)	% inhibition	EC_{50} [d] ± Std Dev (μM)
1.25	71.55 ± 2.33	1.33	
5.31	≥ 100	5.06	
5.48	54.94 ± 184	67.44 ± 3.96	10.87 ± 2.05
5.49	≥ 200	2.74	
5.51	≥ 200	38.25	
5.56	75.61 ± 6.08	10.45	

5.6 CONCLUSION

The initial aim was to synthesise arzanol **(1.25)** by coupling the ester of a substituted phloroacetophenone with an aldehyde followed by the ring closure. The aldehyde was successfully synthesised but we failed to synthesise the correctly substituted phloroacetophenone ester. Prenylation of formylated phloroacetophenone and formylation of prenylated phloroacetophenone both proved to be difficult to achieve. We decided to synthesise a deprenyl derivative of arzanol. The phloroacetophenone derivative was successfully synthesised, however, attempted coupling of this derivative with the aldehyde failed. We then decided on a second target, lepidissipyrone **(1.25).** Lepidissipyrone **(1.25)** was successfully synthesised by the coupling of a flavanone with α-pyrone using formaldehyde and TBAF in chloroform. Some α-pyrone and prenylated acylphoroglucinol derivatives were synthesised which could be coupled to form arzanol derivatives. From the anti-HIV testing of selected synthesised compounds, only 2,4-bis-benzyloxy-6-hydroxychalcone **(5.48)** showed significant inhibition of 67%.

5.7 EXPERIMENTAL

Dry solvents were stored over molecular sieves and stored under nitrogen. THF, diethyl ether, DCM and acetonitrile were dried over molecular sieves using the Pure Solv MD-7 solvent purification system. Toluene was distilled from sodium wire. MeOH was distilled from magnesium. Hexane used for column chromatography was distilled prior to use. Air- and moisture-sensitive reactions were done in dried glassware under nitrogen. Column chromatography was performed using Merck silica gel 60. For centrifugal chromatography on a chromatotron, Merck silica gel 60 P_{254} containing gypsum was coated on a glass plate. Thin-layer chromatography (TLC) was performed on aluminium sheets coated with Merck silica gel 60 P_{254} or

glass-precoated plates, with visualization of the compounds by inspection under UV (254 or 365 nm) and/or by exposure to iodine vapour and/or by staining with an anisaldehyde solution. Anisaldehyde solution (500 mL) was prepared by the addition of *p*-anisaldehyde (13 mL), glacial acetic acid (5 mL) and concentrated sulphuric acid (17 mL) to absolute ethanol (465 mL) at 0 °C and the solution was stored in a fridge.

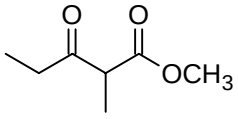
^{1}H NMR spectra were recorded at 400 MHz and ^{13}C at 100 MHz on a Bruker Avance III 400 spectrometer. $CDCl_3$ or CD_3OD were used as solvents for NMR samples. All chemical shifts (δ) are given in parts per million (ppm) and coupling constants are given in Hertz (Hz).

Mass spectra were recorded on a Thermo Finnigan PolarisQ Ion trap with Trace GC/MS or on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionization. IR spectra were recorded with a Perkin Elmer Spectrum One Series FT-IR spectrometer as thin films between NaCl discs for oils and KBr discs for solids or with a Bruker Alpha FT-IR. The absorptions are reported in wavenumber (cm^{-1}) scale, in the range 400-4000 cm^{-1}.

X-ray diffraction data were recorded on an Oxford Diffraction Xcalibur2 CCD 4-circle diffractometer equipped with an Oxford Instruments Cryojet operating at 100(2) K, unless otherwise specified. The data were collected with Mo Ka (l = 0.71073 Å) radiation at a crystal-to-detector distance of 50 mm using omega scans at θ = 29.389 with 5-25 s exposures taken at 2.00 kW X-ray power with 0.75° frame widths. The data were reduced with the programme CrysAlis RED Version 170[293] using outlier rejection, scan speed scaling, as well as standard Lorentz and polarisation correction factors. Unless otherwise stated, direct methods (SHELXS-97, WinGX32)[294, 295] were used to solve the structures. All non-hydrogen atoms were located in the E-map and refined anisotropically with SHELXL-97.[294] All hydrogen atoms in each of the structures were included as idealised contributors in the least squares process with standard SHELXL-9724 parameters, unless otherwise stated. All diagrams were generated using Mercury 3.5.[296]

5.6.1 Experimental procedures

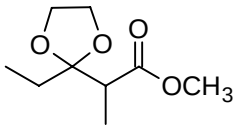
Methyl 2-methyl-3-oxopentanoate (5.19)



Propionyl chloride was prepared by refluxing propionic acid (5.0 mL, 67.0 mmol) and thionyl chloride (4.9 mL, 67.0 mmol) for 2 hours and was used without further purification. In a second reaction, commercial propionyl chloride was used. To the crude solution of propionyl chloride in anhydrous diethyl ether (20 mL) at 0 °C, was added dropwise a solution of triethylamine (10 mL, 71.9 mmol) in anhydrous diethyl ether (150 mL) over a period of 4 hours. The reaction mixture was stirred for 2 days at room temperature. The solvent was evaporated, the residue was dissolved in MeOH and sodium acetate (8 mg) was added. The reaction mixture was refluxed for 1 more day. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (15:1) as eluent to give methyl 2-methyl-3-oxopentanoate[256] **(5.19)** as a colourless oil (5.8 g, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.98 (3H, *t*, *J* = 7.3 Hz, $C\underline{H}_3CH_2$), 1.24 (3H, *d*, *J* = 7.2 Hz, $CH_3C\underline{H}$), 2.38-2.58 (2H, *m,* $CH_3C\underline{H}_2$), 3.46 (1H, *q*, *J* = 7.2 Hz, CH), 3.63 (3H, *s*, $OC\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	7.7 ($\underline{C}H_3CH_2$), 12.9 ($\underline{C}H_3CH$), 34.7 ($CH_3\underline{C}H_2$), 52.3 (CH), 52.4 ($O\underline{C}H_3$), 171.2 ($\underline{C}O_2CH_2CH_3$), 206.3 (CO).
HRESIMS (positive ionization mode):	*m/z* 167.0689 $[M + Na]^+$ (calc. for $C_7H_{12}O_3Na$ 167.0684).
ν_{max} (cm^{-1}):	1738, 1712, 1309, 1225, 1153, 1021.

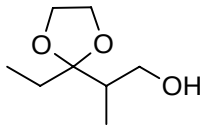
Methyl 2-(2-ethyl-1,3-dioxolan-2-yl)propanoate (5.20)



A solution of methyl 2-methyl-3-oxopentanoate **(5.19)** (2.0 g, 13.9 mmol), ethylene glycol (1.6 mL, 28.0 mmol), and *p*-toluenesulfonic acid monohydrate (20 mg) in toluene was refluxed with a Dean-Stark apparatus overnight. After cooling, the reaction mixture was washed with a saturated aqueous sodium hydrogen carbonate solution. The organic layer was washed with water, saturated brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product purified by column chromatography using hexane-EtOAc (15:1) as eluent to afford methyl 2-(2-ethyl-1,3-dioxolan-2-yl)propanoate **(5.20)** as a colourless oil (1.8 g, 68%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.84 (3H, *t*, *J* = 7.4 Hz, $C\underline{H_3}CH_2$), 1.14 (3H, *d*, *J* = 7.2 Hz, $C\underline{H_3}CH$), 1.66-1.73 (2H, *m*, $CH_3C\underline{H_2}$), 2.80 (1H, *q*, *J* = 7.2 Hz, CH), 3.63 (3H, *s*, $OC\underline{H_3}$), 3.89-3.94 (4H, *m*, $OC\underline{H_2}C\underline{H_2}O$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	7.4 ($\underline{C}H_3CH_2$), 12.7 ($\underline{C}H_3CH$), 28.1 ($CH_3\underline{C}H_2$), 46.8 (CH), 51.9 ($O\underline{C}H_3$), 65.8, 65.9 ($O\underline{C}H_2\underline{C}H_2O$), 111.7 ($\underline{C}CH_2CH_3$), 174.1 ($\underline{C}O_2CH_3$).
HRESIMS (positive ionization mode):	*m/z* 211.0948 $[M + Na]^+$ (calc. for $C_9H_{16}O_4Na$ 211.0946).
ν_{max} (cm^{-1}):	1736, 1362, 1274, 1107, 1077, 945.

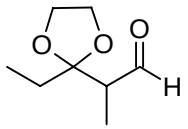
2-(2-Ethyl-1,3-dioxolan-2-yl)propan-1-ol (5.21)



A solution of the methyl 2-(2-ethyl-1,3-dioxolan-2-yl)propanoate **(5.20)** (100 mg, 0.53 mmol) in diethyl ether (10 mL) was added dropwise to a suspension of lithium aluminium hydride (22 mg, 0.58 mmol) in ether (20 mL). The reaction mixture was heated under reflux for half an hour. After cooling, water (133 µL) and 15% sodium hydroxide (50 µL) were added, and the reaction mixture was stirred for half an hour. The resulting mixture was filtered, washed with diethyl ether and the filtrate was dried over magnesium sulfate. Evaporation of the solvent quantitatively afforded 2-(2-ethyl-1,3-dioxolan-2-yl)propan-1-ol **(5.21)** as a colourless oil (77 mg, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) $\delta_{H:}$	0.79 (3H, *t*, *J* = 7.4 Hz, $C\underline{H_3}CH_2$), 0.84 (3H, *d*, *J* = 7.2 Hz, $C\underline{H_3}CH$), 1.56 (2H, *q*, *J* = 7.4 Hz, $CH_3C\underline{H_2}$), 1.91-1.99 (1H, *m*, CH), 3.07 (1H, *s*, $O\underline{H}$), 3.36-3.42, 3.53-3.58 (2 x 1H, *m*, $C\underline{H_2}OH$) 3.87 (4H, *s*, $OC\underline{H_2}C\underline{H_2}O$).
^{13}C NMR (100 MHz, $CDCl_3$) $\delta_{C:}$	7.5 ($\underline{C}H_3CH_2$), 12.4 ($\underline{C}H_3CH$), 26.7 ($CH_3\underline{C}H_2$), 40.7 ($\underline{C}H$), 64.8 ($\underline{C}H_2OH$), 65.1, 65.2 ($O\underline{C}H_2\underline{C}H_2O$), 114.4 (C).
HRESIMS (positive ionization mode):	*m/z* 183.0995 $[M+Na]^+$ (calc. for $C_8H_{16}O_3Na$ 183.0997).
ν_{max} (cm^{-1}):	3408, 1461, 1304, 1255, 1160, 1042, 982.

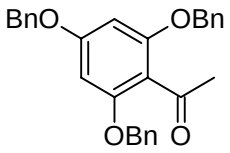
2-(2-Ethyl-1,3-dioxolan-2-yl)propanal (5.15)



2-Iodoxybenzoic acid (IBX) (2.2 g, 7.9 mmol) was added to the solution of the alcohol 2-(2-ethyl-1,3-dioxolan-2-yl)propan-1-ol **(5.21)** (850 mg, 5.3 mmol) in an 20 mL EtOAc-acetone (2:1) mixture. The resulting suspension was heated at 80 °C overnight. After cooling, the reaction mixture was filtered and washed with EtOAc. The solvent was evaporated to yield pure 2-(2-ethyl-1,3-dioxolan-2-yl)propanal **(5.15)** as a light yellow oil (713 mg, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.84 (3H, *t*, *J* = 7.4 Hz, $C\underline{H}_3CH_2$), 1.04 (3H, *d*, *J* = 7.2 Hz, $CH_3C\underline{H}$), 1.66-1.73 (2H, *m*, $CH_3C\underline{H}_2$), 2.63 (1H, *q*, *J* = 7.2 Hz, CH), 3.91 (4H, *m*, $OC\underline{H}_2C\underline{H}_2O$), 9.70 (1H, *s*, $C\underline{H}O$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	7.4 ($\underline{C}H_3CH_2$), 11.7 ($\underline{C}H_3CH$), 36.1 ($CH_3\underline{C}H_2$), 50.1 (CH), 64.9, 65.1 ($O\underline{C}H_2\underline{C}H_2O$), 104.9 ($\underline{C}CH_2CH_3$), 211.9 ($\underline{C}HO$).
HRESIMS (positive ionization mode):	*m/z* 181.0847 $[M+Na]^+$ (calc. for $C_8H_{14}O_3Na$ 181.0840).
ν_{max} (cm^{-1}):	1722, 1298, 1262, 1039.

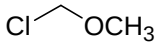
2,4,6-Tribenzyloxyacetophenone (5.22)



Benzyl chloride (2.2 mL, 19.1 mmol) in DMF (10 mL) was added dropwise to the stirred solution of potassium carbonate (4 g, 29.8 mmol) and oven dried 2,4,6-trihydroxyacetophenone (1 g, 5.9 mmol) in DMF (20 mL). The reaction mixture was refluxed for 24 hours. After cooling, the reaction mixture was acidified by addition of 1 M HCl and diluted with DCM. The layers were separated and the aqueous layer was extracted with DCM. The combined organic extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified with column chromatography using hexane-EtOAc (9:1) as eluent to give the 2,4,6-tribenzyloxyacetophenone **(5.22)** as a yellow oil (1.8 g, 70%).

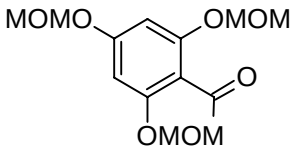
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.48 (3H, s, COC<u>H</u>$_3$), 5.00 (2H, *s*, OC<u>H</u>$_2$Ph), 5.03 (4H, *s*, OC<u>H</u>$_2$Ph), 6.26 (2H, *s*, 2 x H-3,5), 7.03 –7.39 (15H, *m*, Ar<u>H</u>).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	32.6 (CO<u>C</u>H$_3$), 70.4 (O<u>C</u>H$_2$Ph), 70.8 (2 x O<u>C</u>H$_2$Ph), 93.7 (2 x C-3,5), 110.5 (C-1), 126.9, 127.5, 128.0, 128.4, 128.6, 128.7 (Ar<u>C</u>), 136.5, 136.6 (CH$_2$<u>C</u>Ar), 157.3 (2 x <u>C</u>OCH$_2$Ar) 161.30 (<u>C</u>OCH$_2$Ar), 201.6 (<u>C</u>OCH$_3$).
HRESIMS (positive ionization mode):	*m/z* 461.1729 $[M+Na]^+$ (calc. for $C_{29}H_{26}O_4Na$ 461.1729).
IR (KBr) ν_{max} (cm^{-1}):	1689, 1583, 1602, 1371, 1164, 1121.

Chloromethyl methyl ether (MOM chloride)



Acetyl chloride (0.63 mL, 8.85 mmol) was added dropwise to a solution of dimethoxymethane (0.78 mL, 8.85 mmol) and zinc bromide (1mg) in toluene (10 mL). The temperature self-heated slowly to 40–45 °C and cooled down to room temperature and the reaction mixture was stirred for 12 hours. The clear, colourless solution of chloromethyl methyl ether (MOM chloride) in toluene was used directly in the subsequent protection step without further purification.

2,4,6-Trimethoxymethoxyacetophenone (5.23)

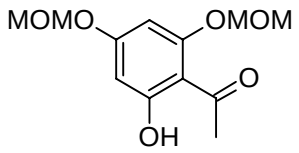


The reaction flask containing a solution of chloromethyl methyl ether (8.85 mmol) in toluene prepared above was cooled to 0 °C followed by the addition of sodium hydride and the solution of 2,4,6-trihydroxyacetophenone (333 mg, 1.96 mmol) in DMF (5 mL). The reaction mixture was stirred for 12 hours at room temperature. A saturated aqueous solution of NH_4Cl (10 mL) was added and the mixture stirred vigorously for five minutes to ensure all residual chloromethyl methyl ether was decomposed The layers were separated and the organic layer was washed once with water (20 mL), once with brine (20 mL) and dried with anhydrous $MgSO_4$.The solvent was evaporated and the resulting product was purified by column chromatography using hexane-

EtOAc (9:1) as eluent to give the 2,4,6-trimethoxymethoxyacetophenone **(5.23)** as a colourless oil (0.43 g, 73%) and 2-hydroxy-4,6-dimethoxymethoxyacetophenone **(5.24)** (50 mg, 10%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.49 (3H, s, COC<u>H</u>$_3$), 3.46 (9H, *s*, OC<u>H</u>$_3$), 5.14 (6H, *s*, OC<u>H</u>$_2$), 6.51 (2H, *s*, 2 x H-3,5).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	32.5 (CO<u>C</u>H$_3$), 56.3 (3 x O<u>C</u>H$_3$), 94.6 (3 x O<u>C</u>H$_2$), 97.2 (2 x C-3,5), 117.0 (C-1), 155.2, 159.5 (C-2, C-4, C-6), 201.3 (<u>C</u>OCH$_3$).
HRESIMS (positive ionization mode):	*m/z* 323.1107 $[M+Na]^+$ (calc. for $C_{14}H_{20}O_7Na$ 323.1107).
ν_{max} (cm^{-1}):	1706, 1592, 1147, 1110, 1010, 905.

2-Hydroxy-4,6-dimethoxymethoxyacetophenone (5.24)

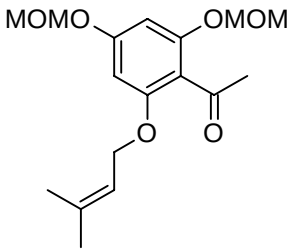


Potassium carbonate (12.0 g, 86.8 mmol) and the solution of 2,4,6-trihydroxyacetophenone (3.00 g, 17.8 mmol) in DMF (5 mL) were added to the crude solution of MOMCl (45 mmol; prepared from acetyl chloride (4.0 mL, 45 mmol) and dimethoxymethane (3.2 mL, 45 mmol)) at 0 °C. The reaction mixture was stirred for 30 min at room temperature. EtOAc (20 mL) and a saturated aqueous NH_4Cl solution (10 mL) were added and the mixture stirred vigorously for 30 minutes. The layers were separated and the organic layer was washed once with water (20 mL), once with brine (20 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (6:1) as eluent to give the 2-hydroxy-4,6-dimethoxymethoxyacetophenone[261] **(5.24)** as a colourless oil (3.7 g, 81%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.67 (3H, s, COC<u>H</u>$_3$), 3.48 (3H, *s*, OC<u>H</u>$_3$), 3.53 (3H, *s*, OC<u>H</u>$_3$), 5.18 (2H, *s*, OC<u>H</u>$_2$O), 5.26 (2H, *s*, OC<u>H</u>$_2$O), 6.26 (1H, *d*, *J* = 2.1 Hz, H-3 or 5), 6.27 (1H, *d*, *J* = 2.1 Hz, H-3 or 5), 12.91 (1H, *s*, O<u>H</u>).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	32.8 ($CO\underline{C}H_3$), 56.5 (2 x $O\underline{C}H_3$), 94.2 (2 x $O\underline{C}H_2$), 94.6 (C-3 or 5), 97.1 (C-3 or 5), 109.7 (C-1), 160.1, 162.9, 167.0 (C-2, 4, 6), 203.2 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 279.0840 $[M+Na]^+$ (calc. for $C_{12}H_{16}O_6Na$ 279.0845).
ν_{max} (cm^{-1}):	3314, 1591, 1261, 1146, 1055.

2,4-Dimethoxymethoxy-6-prenyloxy-acetophenone (5.25)

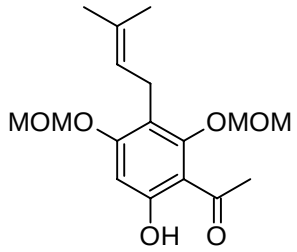


Potassium carbonate (280 mg, 2.03 mmol) was added to the solution of 2-hydroxy-4,6-dimethoxymethoxyacetophenone **(5.24)** (130 mg, 0.51 mmol) in acetone (10 mL) followed by the dropwise addition of 3,3-dimethylallyl bromide (89 µL, 0.76 mmol). The reaction mixture was refluxed for 24 hours. After cooling the mixture was filtered and the solvent was evaporated. The resulting product was purified by column chromatography using hexane-EtOAc (8:1) as eluent to give 2,4-dimethoxymethoxy-6-prenyloxy-acetophenone[261] **(5.25)** (108 mg, 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.70 (3H, *s*, $C(C\underline{H}_3)_2$), 1.74 (3H, *s*, $C(C\underline{H}_3)_2$), 2.45 (3H, *s*, $COC\underline{H}_3$), 3.44 (3H, *s*, $OC\underline{H}_3$), 3.46 (3 H, *s*, $OC\underline{H}_3$), 4.48 (2H, *d, J* = 6.6 Hz, $CHC\underline{H}_2$), 5.11 (2H, *s*, $OC\underline{H}_2$), 5.13 (2H, *s*, $OC\underline{H}_2$), 5.39 (1H, *t, J* = 6.6 Hz, $C\underline{H}CH_2$), 6.30 (1H, *d*, *J* = 2.1 Hz, H-3 or 5), 6.42 (1H, *d*, *J* = 2.1 Hz, H-3 or 5).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	18.14 ($C(\underline{C}H_3)_2$), 29.2 ($C(\underline{C}H_3)_2$), 32.4 ($COC\underline{H}_3$), 56.1 ($O\underline{C}H_3$), 56.2 ($O\underline{C}H_3$), 65.7 ($CH\underline{C}H_2$), 94.5 ($O\underline{C}H_2$), 94.9 (C-3 or 5), 95.2 (C3 or 5), 96.1 ($O\underline{C}H_2$), 116.5 (C-1), 119.4 ($\underline{C}HCH_2$), 137.8 ($\underline{C}(CH_3)_2$), 155.2, 157.2, 159.5 (C-2,4,6), 201.4 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 347.1465 $[M+Na]^+$ (calc. for $C_{17}H_{24}O_6Na$ 347.1471).
ν_{max} (cm^{-1}):	1697, 1587, 1144, 1067, 1019, 920.

2,4-Dimethoxymethoxy-6-hydroxy-3-prenyl-acetophenone (5.26)

Method A:



2,4-Dimethoxymethoxy-6-prenyloxy-acetophenone **(5.25)** (50 mg, 0.15 mmol) was dissolved in *N,N* diethylaniline (10 mL) and refluxed under nitrogen for 3 hours. After cooling, EtOAc was added and the reaction mixture was washed with 1M HCl (5 mL). The organic layer was dried over anhydrous magnesium sulfate, the solvent was evaporated and the resulting product purified by column chromatography using hexane-EtOAc (15:1) to afford 2,4-dimethoxymethoxy-6-hydroxy-3-prenyl-acetophenone[261] **(5.26)** as a colourless oil (31 g, 62%).

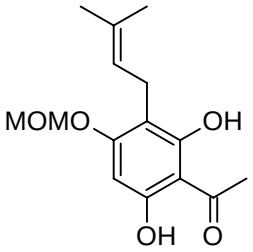
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.70 (3H, *s*, C($C\underline{H}_3$)$_2$), 1.78 (3H, *s*, C($C\underline{H}_3$)$_2$), 2.71 (3H, *s*, CO$C\underline{H}_3$), 3.32 (2H, *d, J* = 6.6 Hz, CHC$\underline{H}_2$), 3.47 (3H, *s*, O$C\underline{H}_3$), 3.50 (3 H, *s*, O$C\underline{H}_3$), 4.97 (2H, *s*, O$C\underline{H}_2$), 5.17 (1H, *t, J* = 6.6 Hz, C$\underline{H}$CH_2), 5.23 (2H, *s*, O$C\underline{H}_2$), 6.48 (1H, *s*, H-3), 12.90 (1H, *s*, O$\underline{H}$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	17.9 (C($\underline{C}H_3$)$_2$), 23.1 (CH$\underline{C}H_2$), 25.7 (C($\underline{C}H_3$)$_2$, 31.4 (CO$\underline{C}H_3$), 56.3 (O$\underline{C}H_3$), 58.3 (O$\underline{C}H_3$), 94.0 (O$\underline{C}H_2$), 98.9 (O$\underline{C}H_2$), 101.4 (C-3), 111.1 (C-1), 116.2 (C-5), 123.0 ($\underline{C}$HCH_2), 131.6 ($\underline{C}$(CH_3)$_2$), 157.2, 159.5, 163,5 (C-2,4,6), 201.4 ($\underline{C}$OCH_3).
HRESIMS (positive ionization mode):	*m/z* 347.1468 $[M+Na]^+$ (calc. for $C_{17}H_{24}O_6Na$ 347.1471).
ν_{max} (cm^{-1}):	3381, 1700, 1459, 1084, 1039.

Method B:

To a stirred solution of 2,4-dimethoxymethyloxy-6-prenyloxy-acetophenone **(5.25)** (140 mg, 0.43 mmol) in toluene (10 mL), Eu(fod)$_3$ (90 mg, 0.087 mmol) was added, and the resulting solution was refluxed overnight. The solvent was evaporated and the resulting product purified by column

chromatography using hexane-EtOAc (15:1) to afford the desired product 2,4-dimethoxymethoxy-6-hydroxy-3-prenyl-acetophenone **(5.26)** as a colourless oil (67 mg, 48%).

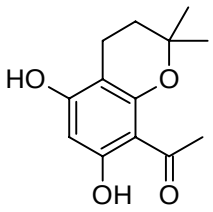
2,6-Dihydroxy-4-methoxymethoxy-3-prenylacetophenone (5.27)



To a solution of 2,4-dimethoxymethoxy-6-hydroxy-3-prenyl-acetophenone **(5.26)** (1.00 g, 3.08 mmol) in 50 mL MeOH-water (9:1), was added 1.0 M HCl until the pH was less than 1. The reaction mixture was stirred at room temperature for 48 hours. The reaction mixture was extracted with EtOAc (2 x 50 mL). The combined organic layers were dried over anhydrous magnesium sulfate. The solvent was evaporated and the product purified with column chromatography using hexane-EtOAc 5:1 as eluent to afford 2,6-dihydroxy-4-methoxymethoxy-3-prenylacetophenone **(5.27)** as yellow crystals (780 mg, 90%).

Mp:	97-99 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.75 (3H, *s*, C($C\underline{H}_3$)$_2$), 1.82 (3H, *s*, C($C\underline{H}_3$)$_2$), 2.68 (3H, *s*, CO$C\underline{H}_3$), 3.35 (2H, *d, J* = 6.6 Hz, CH$C\underline{H}_2$), 3.48 (3H, *s*, O$C\underline{H}_3$), 5.20 (1H, *t, J* = 6.6 Hz, $C\underline{H}CH_2$), 5.20 (2H, *s*, O$C\underline{H}_2$), 6.18 (1H, *s*, H-5), 12.91 (1H, *s*, O$\underline{H}$).
^{13}C NMR (125 MHz, $CDCl_3$) δ_C:	17.8 (C($\underline{C}H_3$)$_2$), 21.7 (CH$\underline{C}H_2$), 25.8 (C($\underline{C}H_3$)$_2$, 32.9 (CO$\underline{C}H_3$), 56.3 (O$\underline{C}H_3$), 94.0 (O$\underline{C}H_2$), 94.9 (C-5) 105.8 (C-1), 107.9 (C-3), 121.9 ($\underline{C}HCH_2$), 134.3 ($\underline{C}(CH_3)_2$), 160.6, 160.7, 161.5 (C-2, 4, 6), 203.8 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 303.1205 $[M+Na]^+$ (calc. for $C_{15}H_{20}O_5Na$ 303.1208).
ν_{max} (cm^{-1}):	3307, 1602, 1451, 1279, 1143, 1041.

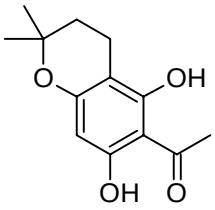
1-(5,7-Dihydroxy-2,2-dimethyl-chroman-8-yl)-ethanone (5.28)



To a solution of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)** (80 mg, 0.25 mmol) in MeOH (20 mL) was added 1.0 M HCl (6 mL). The reaction mixture was refluxed for 1 hour and then cooled down. The solvent was evaporated and the residue purified with column chromatography using hexane-EtOAc (2:1) to afford 1-(5,7-dihydroxy-2,2-dimethylchroman-8-yl)ethanone **(5.28)** as yellow crystals (18 mg, 30%).

Mp:	144-145 °C.
^{1}H NMR (400 MHz, CD_3OD) δ_H:	1.41 (2 x 3H, *s*, C(C$\underline{H}_3$)$_2$), 1.86 (2H, *t, J* = 6.6 Hz, C$\underline{H}_2$), 2.60 (2H, *t, J* = 6.6 Hz, C$\underline{H}_2$), 2.63 (3H, *s*, COC$\underline{H}_3$), 5.95 (1H, *s*, Ar$\underline{H}$), 13.78 (1H, *s*, OH).
^{13}C NMR (100 MHz, CD_3OD) δ_C:	17.9 (C($\underline{C}$H$_3$)$_2$), 26.80 (2 × $\underline{C}$H$_2$), 31.5 (C($\underline{C}$H$_3$)$_2$), 33.27 (CO$\underline{C}$H$_3$), 76.1 ($\underline{C}$(CH$_3$)$_2$, 95.2 (C-5), 99.5 (C-1), 106.3 (C-3), 157.5, 160.55, 164.8 (C-2,4,6), 203.5 ($\underline{C}$OCH$_3$).
HRESIMS (positive ionization mode):	*m/z* 259.0947 $[M+Na]^+$ (calc. for $C_{13}H_{16}O_4Na$ 259.0946).
IR (KBr) ν_{max} (cm^{-1}):	3429, 2967, 2923, 2875, 1665, 1609, 1489, 1154.

1-(5,7-Dihydroxy-2,2-dimethylchroman-6-yl)ethanone (5.29)

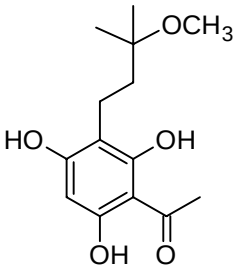


To a solution of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)** (80 mg, 0.25 mmol) in MeOH (20 mL) was added 1.0 M HCl (6 mL). The reaction mixture was refluxed for 1 hour and then cooled down. The solvent was evaporated and the residue purified with column

chromatography using hexane-EtOAc (2:1) to afford 1-(5,7-dihydroxy-2,2-dimethylchroman-6-yl)ethanone **(5.29)** as yellow crystals (10 mg, 17%).

Mp:	228-229 °C.
^{1}H NMR (400 MHz, CD_3OD) δ_H:	1.31 (2 x 3H, *s*, C($C\underline{H}_3$)$_2$), 1.78 (2H, *t, J* = 6.7 Hz, $C\underline{H}_2$), 2.55 (2H, *t, J* = 6.7 Hz, $C\underline{H}_2$), 2.62 (3H, *s*, $COC\underline{H}_3$), 5.77 (1H, *s*, Ar$\underline{H}$).
^{13}C NMR (125 MHz, CD_3OD) δ_C:	15.6 (C($\underline{C}H_3$)$_2$), 25.6 (2 × $\underline{C}H_2$), 31.3 (C($\underline{C}H_3$)$_2$), 31.8 ($CO\underline{C}H_3$), 75.3 ($\underline{C}(CH_3)_2$, 94.5 (C-5), 99.9 (C-1), 104.2 (C-3), 160.0, 160.9, 163.3 (C-2,4,6), 203.4 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 259.0945 $[M+Na]^+$ (calc. for $C_{13}H_{16}O_4Na$ 259.0946).
IR (KBr) ν_{max} (cm^{-1}):	2961, 2918, 2872, 1654, 1611, 1433, 1159.

1-(2,4,6-Trihydroxy-3-(3-methoxy-3-methylbutyl)phenyl)ethanone (5.30)



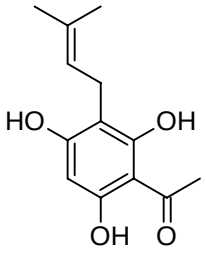
To a solution of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)** (80 mg, 0.25 mmol) in MeOH (20 mL) was added 1.0 M HCl (6 mL). The reaction mixture was refluxed for 1 hour and then cooled down. The solvent was evaporated and the residue purified with column chromatography using hexane-EtOAc: 2:1 to afford 1-(2,4,6-trihydroxy-3-(3-methoxy-3-methylbutyl)phenyl)ethanone **(5.30)** as yellow crystals (12 mg, 18%).

Mp:	240-242 °C.
^{1}H NMR (400 MHz, CD_3OD) δ_H:	1.22 (2 x 3H, *s*, C($C\underline{H}_3$)$_2$), 1.63 (2H, *m,* $C\underline{H}_2$), 2.53 (2H, *m*, $C\underline{H}_2$), 2.61 (3H, *s*, $COC\underline{H}_3$),), 3.27 (3H, *s*, $OC\underline{H}_3$), 5.91 (1H, *s*, Ar$\underline{H}$).
^{13}C NMR (100 MHz, CD_3OD) δ_C:	16.2 (C($\underline{C}H_3$)$_2$), 21.1 (2×CH_2) 31.4(C($\underline{C}H_3$)$_2$), 37.3 ($CO\underline{C}H_3$), 47.8 ($O\underline{C}H_3$), 75.5 ($\underline{C}(CH_3)_2$, 93.5 (C-5), 104.2 (C-1), 107.0 (C-3), 160.4, 162.6, 160.4 (C-2,4,6), 203.4 ($\underline{C}OCH_3$).

HRESIMS (positive ionization mode: *m/z* 291.1208 $[M+Na]^+$ (calc. for $C_{14}H_{20}O_5Na$ 291.1208).

IR (KBr) ν_{max} (cm^{-1}): 3476, 1645, 1605, 1472, 1105.

3-Prenyl-2,4,6-trihydroxyacetophenone (Mom-deprotection) (5.31)



To a solution of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone **(5.26)** (80 mg, 0.25 mmol) in MeOH (20 mL) was added 1.0 M HCl (6 mL). The reaction mixture was refluxed for 1 hour and then cooled down. The solvent was evaporated and the residue purified with column chromatography using hexane-EtOAc (2:1) to afford 3-prenyl-2,4,6-trihydroxyacetophenone[292] **(5.31)** as colourless crystals (8 mg, 15%).

Mp: 167-169 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.75 (3H, *s*, $C(C\underline{H}_3)_2$), 1.82 (3H, *s*, $C(C\underline{H}_3)_2$), 2.68 (3H, *s*, $COC\underline{H}_3$), 3.35 (2H, *d, J* = 6.6 Hz, $CHC\underline{H}_2$), 5.20 (1H, *t, J* = 6.6 Hz, $C\underline{H}CH_2$), 6.18 (1H, *s*, H-5), 12.91 (1H, *s*, OH).

^{13}C NMR (100 MHz, CDCl3) δ_C: 17.9 ($C(\underline{C}H_3)_2$), 22.0 ($CH\underline{C}H_2$), 25.6 ($C(\underline{C}H_3)_2$), 32.9 ($CO\underline{C}H_3$), 100.8 (C-5) 108.9 (C-1), 113.4 (C-3), 123.1 ($\underline{C}HCH_2$), 131.4 ($\underline{C}(CH_3)_2$), 161.3, 161.5 (C-2, 4, 6), 201.9 ($\underline{C}OCH_3$).

HRESIMS (positive ionization mode): *m/z* 259.0950 $[M^+ + Na]^+$ (calc. for $C_{13}H_{16}O_4Na$ 259.0946)

ν_{max} (cm^{-1}): 3358, 1640, 1390, 1260, 1152, 1065 cm^{-1}.

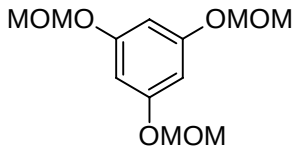
Alternative method 1

The solution of 6-hydroxy-2,4-dimethoxymethoxy-3-prenylacetophenone **(5.26)** (100 mg, 0.31 mmol) in 10 mL of 5% HCl in MeOH-THF (1: 1) was reflux for one hour. The reaction mixture was cooled down and the solvent was evaporated. The product was purified by column chromatography using hexane-EtOAc 4:1 as eluent to afford 3-prenyl-2,4,6-trihydroxyacetophenone **(5.31)** as colourless crystals (61 mg, 70%).

Alternative method 2 (direct prenylation)

DBU (0.453 g, 2.97 mmol) 2,4,6-trihydroxyacetophenone (0.500 g, 2.97 mmol), and 3,3-dimethylallyl bromide (0.346 mL, 2.97 mmol) were added to dry THF (20 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was then acidified with 2 M HCl and extracted with EtOAc. The combined organic extracts were washed with brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue purified with column chromatography using hexane-EtOAc: 5:1 to afford 3-prenylphloroacetophenone **(5.31)** as colour crystals (245 mg, 35%).

1,3,5-Trimethoxymethoxybenzene (5.32)

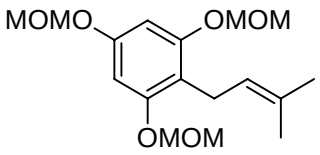


Acetyl chloride (1.6 mL, 18 mmol) was added dropwise to a solution of dimethoxymethane (1.3 mL, 18 mmol) and zinc bromide (1 mg) in toluene (10 mL). The temperature self-heated slowly to 40–45 °C and cooled down to room temperature and the reaction mixture was stirred for 2 hours. The resulting solution of chloromethyl methyl ether (18 mmol) in toluene was cooled to 0 °C followed by the addition of sodium hydride (360 mg, 15 mmol) and the solution of phloroglucinol (0.5 g, 4.0 mmol) in DMF (50 mL). The reaction mixture was stirred for 30 min at room temperature. EtOAc (50 mL) and a saturated aqueous NH_4Cl solution (20 mL) were added and the mixture stirred vigorously for five minutes. The layers were separated and the organic layer was washed with water (50 mL), brine (50 mL) and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (9:1) as eluent to give the trimethoxymethoxybenzene (5.32) as a colourless oil (0.93 g, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	3.49 (9H, *s*, 3 x OC<u>H</u>$_3$), 5.14 (6H, *s*, 3 x OC<u>H</u>$_2$), 6.43 (3H, *s*, 3 x H-1,3,5).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	56.0 (3 x O<u>C</u>H$_3$), 94.5 (3 x O<u>C</u>H$_2$), 98.5 (3 x C-1, 3, 5), 159.0 (C-2, 4, 6).
HRESIMS (positive ionization mode):	*m/z* 281.1005 $[M+Na]^+$ (calc. for $C_{12}H_{18}O_6Na$ 281.1001).

ν_{max} (cm^{-1}): 1602, 1467, 1380, 1140, 1075, 1030, 921, 840.

2-Prenyl-1,3,5-trimethoxymethoxybenzene (5.33)



A solution of *n*-BuLi (1.6 M in hexane, 2.0 mL, 3.2 mmol) was added to the solution of trimethoxymethoxybenzene **(5.32)** (200 mg, 0.77 mmol) in THF (15 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 hour and placed again at 0 °C for 30 minutes. 3,3-dimethylallyl bromide (0.14 mL, 1.2 mmol) was added dropwise and the reaction was stirred for additional 20 minutes at room temperature. EtOAc (20 mL) and a saturated solution of ammonium chloride (20 mL) were added. The layers were separated and the organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (15:1) as eluent to 2-prenyl-1,3,5-trimethoxymethoxybenzene **(5.33)** (200 mg, 80%).

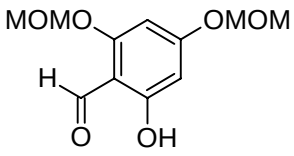
^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.68 (3H, *s*, C($C\underline{H}_3$)$_2$), 1.80 (3H, *s*, C($C\underline{H}_3$)$_2$), 3.34 (2H, *d, J* = 7.2 Hz, CHC$\underline{H}_2$), 3.49 (9H, *s*, O$C\underline{H}_3$), 5.14 (2H, *s*, O$C\underline{H}_2$), 5.18 (4H, *s*, O$C\underline{H}_2$), 5.18 (1H, *t, J* = 7.2 Hz, C$\underline{H}$CH$_2$), 6.52 (1H, *s*, H-6).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 17.7 (C($\underline{C}H_3$)$_2$), 22.3 (CH$\underline{C}$H$_2$), 25.7(C($\underline{C}H_3$)$_2$), 56.0 (3 x O$\underline{C}H_3$), 94.6 (2 x O$\underline{C}$H$_2$), 94.8 (O$\underline{C}$H$_2$), 97.1 (C-4,6), 114.0 (C-2), 116.2 (C-5), 123.3 ($\underline{C}$HCH$_2$), 130.6 ($\underline{C}$(CH$_3$)$_2$), 156.1 (C-1,3), 201.4 ($\underline{C}$OCH$_3$).

HRESIMS (positive ionization mode): *m/z* 349.1620 $[M+Na]^+$ (calc. for $C_{17}H_{26}O_6Na$ 349.1627).

ν_{max} (cm^{-1}): 1595, 1205, 1147, 1043, 920.

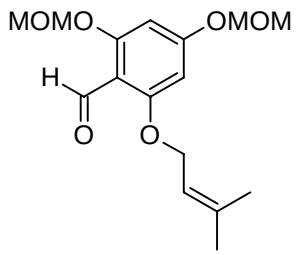
2,4-Dimethoxymethyloxy-6-hydroxybenzaldehyde (5.34)



Potassium carbonate (1.80 g, 13.0 mmol) and the solution of 2,4,6-trihydroxybenzaldehyde (500 mg, 3.24 mmol) in DMF (5 mL) were added to the crude solution of MOM chloride (8.0 mmol; prepared from acetyl chloride (0.70 mL, 8.0 mmol) and dimethoxymethane (0.57 mL, 8.0 mmol)) at 0 °C. The reaction mixture was stirred for 3 hours at room temperature. It was then filtered and the solvent was evaporated. The resulting product was purified by column chromatography using hexane-EtOAc (7:1) as eluent to give the 2,4-dimethoxymethyloxy-6-hydroxybenzaldehyde **(5.34)** as white crystals .

Mp:	247-249 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	3.48 (3H, *s*, OCH_3), 3.52 (3H, *s*, $OC\underline{H}_3$), 5.18 (2H, *s*, $OC\underline{H}_2$), 5.24 (2H, *s*, $OC\underline{H}_2$), 6.24 (1H, *d*, *J* = 2.1 Hz, H-3 or 5), 6.26 (1H, *d*, *J* = 2.1 Hz, H-3 or 5); 10.17 (1H, *s*, C<u>H</u>O), 12.91 (1H, *s*, OH).
^{13}C NMR (100 MHz, CDCl3) δ_C:	56.5 ($O\underline{C}H_3$), 56.6 ($O\underline{C}H_3$), 94.1 ($O\underline{C}H_2$), 94.2 (C-3, 5), 94.7 ($O\underline{C}H_2$), 96.7 (C-3,5), 107.0 (C-1), 161.3, 165.5, 165.7 (C-2, 4, 6), 192.1 (<u>C</u>HO).
HRESIMS (positive ionization mode):	*m/z* 265.0689 $[M^+ + Na]^+$ (calc. for $C_{11}H_{14}O_6Na$ 265.0688).
ν_{max} (cm^{-1}):	3295, 1612, 1449, 1271, 1138.

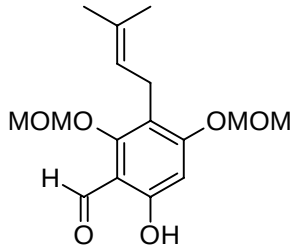
2,4-Dimethoxymethoxy-6-prenyloxybenzaldehyde (5.35)



Potassium carbonate (456 mg, 3.31 mmol) was added to the solution of 2,4-dimethoxymethyloxy-6-hydroxybenzaldehyde **(5.34)** (200 mg, 0.826 mmol) in acetone (30 mL) followed by the dropwise addition of 3,3-dimethylallyl bromide (143 µL, 1.25 mmol). The reaction mixture was refluxed for 24 hours. After cooling the mixture was filtered and the solvent was evaporated. The resulting product was purified by column chromatography using hexane-EtOAc (6:1) as eluent to give the 2,4-dimethoxymethoxy-6-prenyloxybenzaldehyde **(5.35)** (130 mg, 51%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.74 (3H, *s*, C(C<u>H</u>$_3$)$_2$), 1.78 (3H, *s*, C(C<u>H</u>$_3$)$_2$), 3.49 (3H, *s*, OC<u>H</u>$_3$), 3.50 (3 H, *s*, OC<u>H</u>$_3$), 4.60 (2H, *d, J* = 6.7 Hz, CHC<u>H</u>$_2$), 5.19 (2H, *s*, OC<u>H</u>$_2$), 5.23 (2H, *s*, OC<u>H</u>$_2$), 5.47 (1H, *t, J* = 6.7 Hz, C<u>H</u>CH$_2$), 6.31 (1H, *d*, *J* = 2.1 Hz, H-3 or 5), 6.43 (1H, *d*, *J* = 2.1 Hz, H-3 or 5), 10.39 (C<u>H</u>O).
^{13}C NMR (100 MHz, CDCl3) δ_C:	18.3 (C(<u>C</u>H$_3$)$_2$), 25.7 (C(<u>C</u>H$_3$)$_2$), 56.4 (O<u>C</u>H$_3$), 56.5 (O<u>C</u>H$_3$), 65.9 (CH<u>C</u>H$_2$), 94.2 (O<u>C</u>H$_2$), 94.6 (C-3 or 5), 94.9 (O<u>C</u>H$_2$), 95.8 (C3 or 5), 110.7 (C-1), 119.1 (<u>C</u>HCH$_2$), 138.3 (<u>C</u>(CH$_3$)$_2$), 160.9, 163.0, 163.4 (C-2, 4, 6), 187.8 (<u>C</u>HO).
HRESIMS (positive ionization mode):	*m/z* 333.1310 $[M+Na]^+$ (calc. for $C_{16}H_{22}O_6Na$ 333.1314).
ν_{max} (cm^{-1}):	1619, 1590, 1249, 1134, 1057.

6-Hydroxy-2,4-dimethoxymethyloxy-3-prenylbenzaldehyde (5.36)

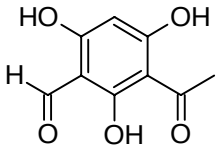


2,4-Dimethoxymethoxy-6-prenyloxybenzaldehyde **(5.35)** (50 mg, 0.16 mmol) was dissolved in *N,N*-diethylaniline (10 mL) and refluxed under nitrogen for 3 hours. After cooling, *N,N*-diethylaniline was distilled under vacuum. The resulting product was purified by column chromatography using hexane-EtOAc (15:1) to afford the desired product 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylbenzaldehyde **(5.36)** as a yellow oil (25 mg, 50%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.70 (3H, *s*, C(C<u>H</u>$_3$)$_2$), 1.78 (3H, *s*, C(C<u>H</u>$_3$)$_2$), 3.29 (2H, *d, J* = 6.6 Hz, CHC<u>H</u>$_2$), 3.47 (3H, *s*, OC<u>H</u>$_3$), 3.50 (3 H, *s*, OC<u>H</u>$_3$), 5.04 (2H, *s*, OC<u>H</u>$_2$), 5.13 (1H, *t, J* = 6.6 Hz, C<u>H</u>CH$_2$), 5.24 (2H, *s*, OC<u>H</u>$_2$), 6.47 (1H, *s*, H-3), 10.07 (C<u>H</u>O), 12.09 (1H, *s*, O<u>H</u>).
^{13}C NMR (100 MHz, CDCl3) δ_C:	17.9 (C(<u>C</u>H$_3$)$_2$), 22.8 (CH<u>C</u>H$_2$), 25.7 (C(<u>C</u>H$_3$)$_2$, 56.4 (O<u>C</u>H$_3$), 58.0 (O<u>C</u>H$_3$), 94.10 (O<u>C</u>H$_2$), 98.8 (O<u>C</u>H$_2$), 101.4 (C-5), 110.5

	(C-1), 115.7 (C-3), 123.2 (<u>C</u>HCH$_2$), 131.3 (<u>C</u>(CH$_3$)$_2$), 159.0, 159.4, 163,4 (C-2,4,6), 187.8 (<u>C</u>HO).
HRESIMS (positive ionization mode):	*m/z* 333.1312 $[M+Na]^+$ (calc. for $C_{16}H_{22}O_6Na$ 333.1314)
ν_{max} (cm^{-1}):	1617, 1151, 1136, 1032, 952.

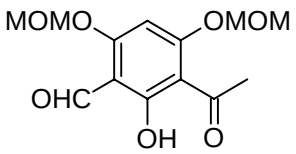
3-Acetyl-2,4,6-trihydroxybenzaldehyde (5.37)



To a stirred solution of 2,4,6-trihydroxyacetophenone (500 mg, 3.0 mmol) in EtOAc (50 mL) was added DMF (0.23 mL, 3.0 mmol) and phosphoryl chloride (0.28 mL, 3.3 mmol). The resulting solution was refluxed for 1.5 hour. Water and EtOAc were added and the layers were separated. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (11:4) as eluent to give 3-acetyl-2,4,6-trihydroxybenzaldehyde **(5.37)** as red crystals (519 mg, 89%).

Mp:	182-184 °C.
^{1}H NMR (400 MHz, CD_3OD) δ_H:	2.62 (3H, s, COC<u>H</u>$_3$), 5.79 (1H, *s*, H-5), 10.03 (1H, *s*, C<u>H</u>O).
^{13}C NMR (100 MHz, CD_3OD) δ_C:	32.6 (CO<u>C</u>H$_3$), 97.4 (C-5), 109.1, 115.8 (C-1, 3), 158.0, 159.2, 163.1 (C-2, 4, 6), 191.9 (<u>C</u>HO), 201.6 (<u>C</u>OCH$_3$).
HRESIMS (positive ionization mode):	*m/z* 219.0264 $[M+Na]^+$ (calc. for $C_9H_8O_5Na$ 219.0269).
ν_{max} (cm^{-1}):	3460, 1703, 1676, 1286, 1171, 1067.

3-Acetyl-2-hydroxy-4,6-dimethoxymethoxybenzaldehyde (5.38)

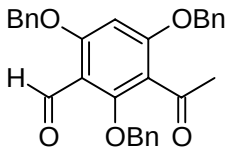


Acetyl chloride (0.44 mL, 6.2 mmol) was added dropwise to a solution of dimethoxymethane (0.55 mL, 6.2 mmol) and zinc bromide (1 mg) in toluene (5 mL). The temperature self-heated slowly to

40–45 °C and cooled down to room temperature and the reaction mixture was stirred for 2 hours. The resulting solution of chloromethyl methyl ether (6.2 mmol) in toluene was cooled to 0 °C followed by the addition of potassium carbonate (1.2 g, 8.7 mmol) and the solution of 3-acetyl-2,4,6-trihydroxybenzaldehyde (350 mg, 1.78 mmol) in DMF (10 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was filtered and EtOAc (20 mL) and water (20 mL) were added. The layers were separated and the organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (9:2) as eluent to give 3-acetyl-2-hydroxy-4,6-dimethoxymethoxybenzaldehyde **(5.38)** as a yellow oil (314 mg , 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.48 (3H, *s*, $COC\underline{H}_3$), 3.43 (6H, *s*, $OC\underline{H}_3$), 5.12 (4H, *s*, $OC\underline{H}_2$), 6.52 (1H, *s*, H-5), 10.03 (1H, *s*, $C\underline{H}O$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	32.5 ($CO\underline{C}H_3$), 56.2 ($O\underline{C}H_3$), 56.9 ($O\underline{C}H_3$), 94.5 ($O\underline{C}H_2$), 95.1 ($O\underline{C}H_2$), 98.2 (C-5), 110.1, 117.8 (C-1, 3), 159.4, 161.7 165.2 (C-2, 4,6), 194.6 ($\underline{C}HO$), 200.7 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 307.0794 $[M^+ + Na]^+$ (calc. for $C_{13}H_{16}O_7Na$ 307.0794).
ν_{max} (cm^{-1}):	1626, 1158, 1041, 942, 812.

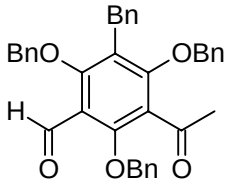
3-Acetyl-2,4,6-tribenzyloxybenzaldehyde (5.39)



Benzyl bromide (0.22 mL, 1.8 mmol) was added dropwise to the solution of potassium carbonate (0.28 g, 2.0 mmol) and 3-acetyl-2,4,6-trihydroxyacetophenone **(5.38)** (0.10 g, 0.51 mmol) in DMF (5 mL). The reaction mixture was stirred overnight at room temperature. Water (10 mL) and EtOAc (10 mL) were added and the layers were separated. The aqueous layer was acidified with 1 M HCl and extracted twice with EtOAc (15 mL). The combined organic extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified with column chromatography using hexane-EtOAc (10:1) as eluent to give the 3-acetyl-2,4,6-tribenzyloxybenzaldehyde **(5.39)** (143 mg, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.42 (3H, s, $COCH_3$), 5.00 (2H, *s*, OCH_2Ph), 5.01 (2H, *s*, OCH_2Ph), 5.06 (2H, *s*, OCH_2Ph), 6.40 (1H, *s*, H-5), 7.27 – 7.51 (15H, *m*, ArH), 10.42 (1H, *s*, CHO).
^{13}C NMR (100 MHz, CDCl3) δ_C:	δ_C 32.5 ($COCH_3$), 70.8 (OCH_2Ph), 71.2 (OCH_2Ph), 79.0 (OCH_2Ph), 94.4 (C-5), 113.5 (C-3), 120.8 (C-1), 127.1 (4 x C-2'',2''', 6'',6'''), 128.4 (2 x C-2',6'), 128.5 (2 x C-4'',4'''), 128.5 (C-4'), 128.8 (4 x C-3'',3''',5'',5'''), 129.0 (C-3',5'), 135.3,135.5,136.3 (C-1',1'',1'''), 158.9 160.9, 163.7 (C-2, 4, 6) , 187.2 (CHO), 201.6 ($COCH_3$).
HRESIMS (negative ionization mode):	*m/z* 465.1708 $[M-H]^+$ (calc. for $C_{30}H_{25}O_5$ 465.1702).
ν_{max} (cm^{-1}):	1701, 1580, 1369, 1162, 1090, 949.

3-Acetyl-2,4,6-tribenzyloxy-5-benzylbenzaldehyde (5.40)

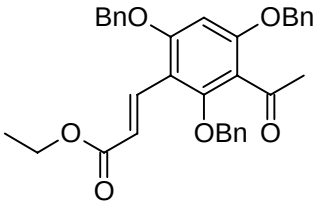


Benzyl bromide (0.22 mL, 1.8 mmol) was added dropwise to the solution of potassium carbonate (0.28 g, 2.0 mmol) and 3-acetyl-2,4,6-trihydroxyacetophenone **(5.38)** (0.10 g, 0.51 mmol) in DMF (5 mL). The reaction mixture was stirred overnight at room temperature. Water (10 mL) and EtOAc (10 mL) were added and the layers were separated. The aqueous layer was acidified with 1 M HCl and extracted twice with EtOAc (15 mL). The combined organic extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified with column chromatography using hexane-EtOAc (10:1) as eluent to give the 3-acetyl-5-benzyl-2,4,6-tribenzyloxybenzaldehyde **(5.40)** (50 mg, 18%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.53 (3H, s, $COCH_3$), 4.02 (2H, *s*, CCH_2Ph), 4.82 (2H, *s*, OCH_2Ph), 4.88 (2H, *s*, OCH_2Ph), 5.08 (2H, *s*, OCH_2Ph), 7.16-7.49 (20H, *m*, ArH), 10.32 (1H, *s*, CHO).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	^{13}C NMR (125 MHz, $CDCl_3$); δ_C 29.7 (CCH_2Ph) 32.8 ($COCH_3$), 77.8 (OCH_2Ph), 78.5 (OCH_2Ph), 79.9 (OCH_2Ph), 126.2-128.9 (ArC), 135.8, 135.9, 136.0 (C-1',1'',1'''), 140.1 (C-

	1’’’’), 157.4 159.8, 161.5 (C-2, 4, 6) , 187.9 (CHO), 202.0 ($COCH_3$).
HRESIMS (negative ionization mode):	*m/z* 555.2177 $[M-H]^+$ (calc. for $C_{37}H_{31}O_5$ 555.2172).
ν_{max} (cm^{-1}):	1686, 1600, 1266, 1358, 1084, 695.

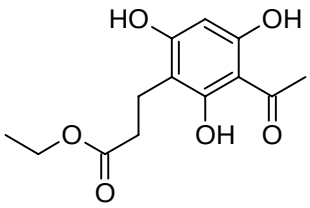
Ethyl (2*E*)-3-(3-acetyl-2,4,6-tribenzyloxyphenyl)prop-2-enoate (5.41)



To a suspension of NaH (60% dispersion in mineral oil, 10 mg, 0.25 mmol) in dry THF (10 mL) at 0 °C was slowly added ethyl diethylphosphonoacetate (49 µL, 0.25 mmol). The reaction mixture was stirred at 0 °C for 30 minutes and then 3-acetyl-2,4,6-tribenzyloxybenzaldehyde **(5.39)** (100 mg, 0.21 mmol) dissolved in dry THF (5 mL) was added to it slowly. After being stirred for 5 hours under nitrogen at 0 °C, the reaction mixture was quenched slowly with water (15 mL) at 0 °C and extracted with EtOAc, washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the product purified with column chromatography using hexane-EtOAc 5:1 as eluent to afford ethyl (2*E*)-3-(3-acetyl-2,4,6-tribenzyloxyphenyl)prop-2-enoate **(5.41)** as a colourless oil (83 mg, 74%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.98 (3H, *t*, *J* = 7.3 Hz, $C\underline{H}_3CH_2$), 2.42 (3H, s, $COC\underline{H}_3$), 4.81 (2H, *q*, *J* = 7.3 Hz, $CH_3C\underline{H}_2$), 5.00 (2H, *s*, $OC\underline{H}_2Ph$), 5.01 (2H, *s*, $OC\underline{H}_2Ph$), 5.06 (2H, *s*, $OC\underline{H}_2Ph$), 6.40 (1H, *s*, H-5), 6.64 (1H, *d*, *J* = 16.1 Hz, α-H), 7.27 –7.51 (15H, *m*, ArH), 8.03 (1H, *d*, *J* = 16.1 Hz, β-H).
^{13}C NMR (100 MHz, $CDCl_3$); δ_C:	14.4 ($CH_2\underline{C}H_3$), 32.6 ($CO\underline{C}H_3$), 60.1 ($\underline{C}H_2CH_3$), 70.7 ($O\underline{C}H_2Ph$), 70.9 ($O\underline{C}H_2Ph$), 78.5 ($O\underline{C}H_2Ph$), 94.9 (C-5), 111.5 (C-1,) 120.6, 120.8 (α-C, C-3), 120, 127.1-129.1 (ArC), 135.8, 135.9, 136.1 (C-1’,1’’,1’’’), 134.7 (β-C), 157.6, 160.3 (C-2, 4, 6), 168.1 ($\underline{C}OCH_2CH_3$), 201.4 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 559.2107 $[M+Na]^+$ (calc. for $C_{34}H_{32}O_6Na$ 559.2097).
ν_{max} (cm^{-1}):	1695, 1591, 1259, 1163, 1088, 743.

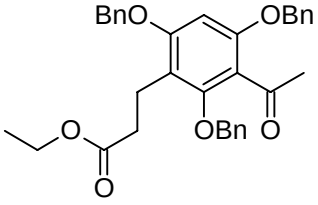
Ethyl 3-(3-acetyl-2,4,6-trihydroxyphenyl)propanoate (5.42)



A mixture of ethyl (2*E*)-3-(3-acetyl-2,4,6-tribenzyloxyphenyl)prop-2-enoate **(5.41)** (130 mg, 0.24 mmol) and 10% Pd/C (5 mg) in 20 mL of THF-MeOH (1:9), was placed under an atmosphere of H_2 (200 KPa) and stirred at room temperature for 6 hours. The reaction mixture was filtered and concentrated *in vacuo*. The crude product was purified by column chromatography using hexane-EtOAc (5:1) as eluent to afford ethyl 3-(3-acetyl-2,4,6-trihydroxyphenyl)propanoate **(5.42)** (52 mg, 81 %) as light brown crystals.

Mp:	174-176 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.20 (3H, *t*, *J* = 7.2 Hz, $C\underline{H}_3CH_2$), 2.42 (2H, *t*, *J* = 8.1 Hz, $CH_2C\underline{H}_2$), 2.58 (3H, s, $COC\underline{H}_3$), 2.79 (2H, *t*, *J* = 8.1 Hz, $C\underline{H}_2CH_2$), 4.07 (2H, *q*, *J* = 7.2 Hz, $CH_3C\underline{H}_2$), 5.88 (1H, *s*, H-5).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	14.8 ($CH_2\underline{C}H_3$), 19.4 ($C\underline{H}_2CH_2$), 33.1 ($CO\underline{C}H_3$), 34.8 ($CH_2C\underline{H}_2$), 61.8 ($\underline{C}H_2CH_3$), 95.5 (C-5), 105.8, 106.7 (C-1, 3), 162.6, 164.3, 165.3 (C-2, 4, 6), 176.3 ($\underline{C}O_2CH_2CH_3$), 205.0 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 291.0851 $[M^+ + Na]^+$ (calc. for $C_{13}H_{16}O_6Na$ 291.0851).
ν_{max} (cm^{-1}):	3250, 1688, 1607, 1294, 1059, 806.

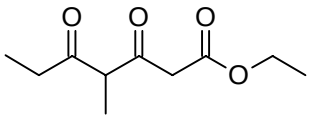
Ethyl 3-(3-acetyl-2,4,6-tribenzyloxyphenyl)propanoate (5.43)



Benzyl bromide (0.22 mL, 1.8 mmol) was added dropwise to the solution of potassium carbonate (0.28 g, 2.0 mmol) and ethyl 3-(3-acetyl-2,4,6-trihydroxyphenyl)propanoate (5.42) (0.10 g, 0.51 mmol) in DMF (5 mL). The reaction mixture was stirred overnight at room temperature. Water (10 mL) and EtOAc (10 mL) were added and the layers were separated. The aqueous layer was acidified with 1 M HCl and extracted twice with EtOAc (15 mL). The combined organic extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified with column chromatography using hexane-EtOAc (10:1) as eluent to give the ethyl 3-(3-acetyl-2,4,6-tribenzyloxyphenyl)propanoate (5.43) (136 mg, 68%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.21 (3H, *t*, *J* = 7.3 Hz, $C\underline{H}_3CH_2$), 2.43 (2H, *t*, *J* = 7.4 Hz, $CH_2\underline{CH}_2$), 2.62 (3H, s, $COC\underline{H}_3$), 2.81 (2H, *t*, *J* = 7.4 Hz, $CH_2C\underline{H}_2$), 4.12 (2H, *q*, *J* = 7.3 Hz, $CH_3C\underline{H}_2$), 4.85 (2H, *s*, $OC\underline{H}_2Ph$), 5.01 (2H, *s*, $OC\underline{H}_2Ph$), 5.18 (2H, *s*, $OC\underline{H}_2Ph$), 5.88 (1H, *s*, H-5), 7.29 –7.53 (15H, *m*, ArH).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	14.2 ($CH_2\underline{C}H_3$), 29.1 ($C\underline{H}_2CH_2$), 31.6 ($CO\underline{C}H_3$), 34.2 ($CH_2C\underline{H}_2$), 60.4 ($\underline{C}H_2CH_3$), 70.5 (2 x $O\underline{C}H_2Ph$), 76.1 ($O\underline{C}H_2Ph$), 95.8 (C-5), 110.2 (C-1,) 111.6 (C-3), 127.4, 127.7, 127.9, 128.5, 128.7, (ArC), 135.9, 136.1, 136.3 (C-1',1'',1'''), 158.7, 159.1, 162.4 (C-2, 4, 6), 168.0 ($\underline{C}OCH_2CH_3$), 203.9 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 561.2249 $[M+Na]^+$ (calc. for $C_{34}H_{34}O_6Na$ 561.2253).
ν_{max} (cm^{-1}):	1704, 1590, 1255, 1162, 1060, 752.

Ethyl 4-methyl-3,5-dioxoheptanoate (5.44)

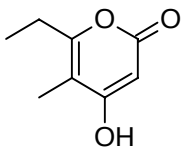


Ethyl 3-oxopentanoate (1.00 mL, 7.02 mmol) was added dropwise to a suspension of sodium hydride (60% dispersion in mineral oil, 560 mg, 14.0 mmol) in THF (30 mL). The reaction mixture was stirred at 0 °C for 10 minutes then *n*-BuLi (1.6 M in hexane, 4.40 mL, 7.04 mmol) was added dropwise. After stirring for 10 minutes, ethyl propionate (405 µL, 3.51 mmol) was added and the reaction was stirred for further 15 minutes. A second portion of *n*-BuLi (1.6 M in hexane, 2.85 mL,

4.56 mmol) was added followed after 15 minutes by another potion of ethyl propionate (405 µL, 3.51 mmol). After 15 min of stirring, the reaction was quenched by addition of conc. HCl (4.00 mL), diluted with water (20 mL) and extracted with diethyl ether. The combined organic extracts were washed with $NaHCO_3$ and dried over anhydrous magnesium sulfate. After filtration the solvent was evaporated and the residue was purified by column chromatography using hexane-EtOAc (19:1) as eluent to give ethyl 4-methyl-3,5-dioxoheptanoate **(5.44)** as a colourless oil (844 mg, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.00 (3H, *t*, *J* = 7.3 Hz, $COCH_2C\underline{H}_3$), 1.22 (3H, *t*, *J* = 7.3 Hz, $OCH_2C\underline{H}_3$), 1.29 (3H, *d*, *J* = 7.2 Hz, $CHC\underline{H}_3$), 2.50 (2H, *m*, $COC\underline{H}_2CH_3$), 3.38 (2H, *s*, $COC\underline{H}_2CO$), 3.83 (1H, *q*, *J* = 7.2 Hz, C<u>H</u>), 4.08 (2H, *q*, *J* = 7.3 Hz, $OC\underline{H}_2CH_3$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	7.4 ($COCH_2\underline{C}H_3$), 12.6 ($CH\underline{C}H_3$), 14.0 ($OCH_2\underline{C}H_3$), 34.7 ($CO\underline{C}H_2CH_3$), 47.7 ($CO\underline{C}H_2CO$), 59.9 (<u>C</u>H), 61.4 ($O\underline{C}H_2CH_3$), 167.0 ($\underline{C}O_2CH_2CH_3$), 200.0 ($CH\underline{C}OCH_2$), 207.5 ($COCH_2CH_3$).
HRESIMS (positive ionization mode):	*m/z* 223.0940 $[M+Na]^+$ (calc. for $C_{10}H_{16}O_4Na$ 223.0946).
ν_{max} (cm^{-1}):	3305, 1746, 1717, 1255, 1060, 817.

6-Ethyl-4-hydroxy-5-methyl-α-pyrone (5.45)



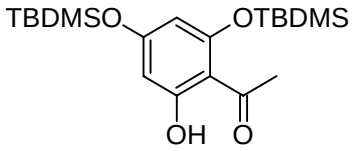
A solution of ethyl 4-methyl-3,5-dioxoheptanoate **(5.44)** (1.00 g, 4.99 mmol) in polyphosphoric acid (3.10 g) was heated at 120 °C for 2 hours and poured in ice. The mixture was filtered and the residue was washed with water to give 6-ethyl-4-hydroxy-5-methyl-α-pyrone **(5.45)** as a yellow solid (538 mg, 70%).

Mp:	110-112 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.22 (3H, *t*, *J* = 7.3 Hz, $CH_2C\underline{H}_3$), 1.96 (3H, *s*, $CC\underline{H}_3$), 2.57 (2H, *q*, *J* = 7.3 Hz, $C\underline{H}_2CH_3$), 5.71(1H, *s*, C<u>H</u>).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	9.0 ($C\underline{C}H_3$), 11.4 ($CH_2\underline{C}H_3$), 24.5 ($\underline{C}H_2CH_3$), 89.7 (<u>C</u>H), 108.3 ($\underline{C}CH_3$), 163.7 ($\underline{C}CH_2CH_3$), 167.7(COH), 172.8 (C=O).

HRESIMS (positive ionization mode): *m/z* 177.0535 $[M+Na]^+$ (calc. for $C_8H_{10}O_3Na$ 177.0528).

IR ν_{max} (cm^{-1}): 3318, 1738, 1650, 1265, 1055, 816.

2,4-Bis(*tert*-butyldimethylsilyl)phloroacetophenone (5.46)



Triethylamine (5.48 mL, 39.2 mmol) was added dropwise to a stirred suspension of 2,4,6-trihydroxyacetophenone (2.00 g, 11.9 mmol) in DCM (40 mL). To the resulting solution, TBDMSCl (3.94 g, 26.3 mmol) was added and the reaction mixture was stirred at room temperature for 90 min. The reaction was acidified with 2 M H_2SO_4 and the organic components were extracted with DCM. The layers were separated and the organic layer was dried over anhydrous $MgSO_4$. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (20:1) as eluent to give 2,4-bis(*tert*-butyldimethylsilyl)phloroacetophenone[264] **(5.46)** as a colourless oil (3.52 g, 74%).

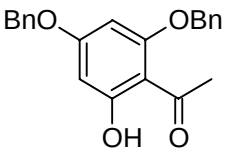
^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 0.24 (6H, *s*, Si($C\underline{H}_3$)$_2$), 0.35 (6H, *s*, Si($C\underline{H}_3$)$_2$), 0.98 (9H, *s*, C($C\underline{H}_3$)$_3$), 1.01 (9 H, *s*, C($C\underline{H}_3$)$_3$), 2.64 (3H, s, CO$C\underline{H}_3$), 5.85 (1H, *d*, *J* = 2.1 Hz, H-3,5), 6.03 (1H, *d*, *J* = 2.1 Hz, H-3,5), 13.50 (1H, *s*, OH).

^{13}C NMR (100 MHz, CDCl3) δ_C: -4.3 (Si($\underline{C}H_3$)$_2$), -3.5 (Si($\underline{C}H_3$)$_2$), 18.2 ($\underline{C}$(CH_3)$_3$), 18.9 ($\underline{C}$(CH_3)$_3$), 25.5 (C($\underline{C}H_3$)$_3$), 26.1(C($\underline{C}H_3$)$_3$), 32.8 (CO$\underline{C}H_3$), 101.7, 102.7 (C-3,5), 109.3 (C-1), 159.6, 162.7, 167.5 (C-2, 4,6), 201.3 ($\underline{C}OCH_3$).

HRESIMS (positive ionization mode): *m/z* 397.2244 $[M+H]^+$ (calc. for $C_{20}H_{37}O_4Si_2$ 397.1471).

ν_{max} (cm^{-1}): 3319, 1615, 1591, 1270, 1168, 832.

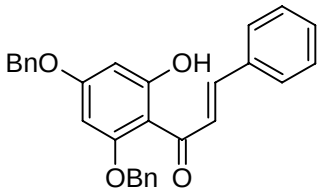
2,4-Bis-benzyloxy-6-hydroxyphloroacetophenone (5.47)



Benzyl chloride (2.90 mL, 25.2 mmol) in DMF (3 mL) was added dropwise to the stirred solution of potassium carbonate (8.00 g, 57.9 mmol) and 2,4,6-trihydroxyacetophenone (2.00 g, 11.9 mmol) in DMF (20 mL). The reaction mixture was stirred at 80 °C for 2 hours. After cooling, the reaction mixture was acidified with 1 M HCl and diluted with DCM. The layers were separated and the aqueous layer was further extracted with DCM. The combined organic extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (9:1) as eluent to give 2,4-bis-benzyloxy-6-hydroxyphloroacetophenone[297] **(5.47)** as a white solid (3.27 g, 79%)**.**

Mp:	100-102 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.58 (3H, s, COC<u>H</u>$_3$), 5.08 (2H, *s*, OC<u>H</u>$_2$Ph), 5.09 (2H, *s*, OC<u>H</u>$_2$Ph), 6.12 (1H, *d*, *J* = 2.4 Hz, H-3 or 5), 6.19 (1H, *d*, *J* = 2.4 Hz, H-3 or 5), 7.41-7.44 (10H, *m*, Ar<u>H</u>), 14.0 (1H, *s*, OH).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	34.2 (CO<u>C</u>H$_3$), 70.3 (O<u>C</u>H$_2$Ph), 71.3 (O<u>C</u>H$_2$Ph), 92.4, 94.9 (2 x C-3, 5), 106.5(C-1), 127.6, 128.0, 128.4, 128.7, 128.8 (Ar<u>C</u>), 135.9, 135.7 (CH$_2$<u>C</u>Ar), 161.9 (C-2), 165.1 (C-4), 167.7 (<u>C</u>OH), 203.3 (<u>C</u>OCH$_3$).
HRESIMS (positive ionization mode):	*m/z* 371.1261 $[M+Na]^+$ (calc. for $C_{22}H_{20}O_4Na$ 371.1259).
IR ν_{max} (cm^{-1}):	1615, 1591, 1269, 1170, 1099.

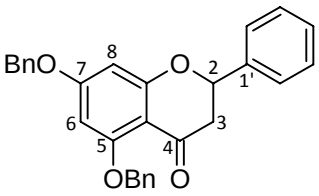
2,4-Bis-benzyloxy-6-hydroxychalcone (5.48)



Sodium hydride (60% dispersion in mineral oil, 515 mg, 12.9 mmol) was slowly added to a solution of 2,4-bis-benzyloxy-6-hydroxyphloroacetophenone **(5.47)** (1.50 g, 4.31 mmol) in DMF (10 mL) at 0 °C. Benzaldehyde (0.500 mL, 4.90 mmol) dissolved in DMF (1 mL) was then added dropwise. The reaction was stirred for a further 45 minutes at 0 °C. It was quenched by the addition of ice and acidified with 1 M HCl. The reaction mixture was filtered and 2,4-bis-benzyloxy-6-hydroxychalcone[298] **(5.48)** was obtained as orange crystals (1.80 g, 96%).

Mp:	120-122 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	5.09 (2H, *s*, OC<u>H</u>$_2$Ph), 5.18 (2H, *s*, OC<u>H</u>$_2$Ph), 6.15 (1H, *d*, *J* = 2.3 Hz, H-3), 6.21 (1H, *d*, *J* =2.3 Hz, H-5), 7.13-7.42 (15H, *m*, Ar<u>H</u>), 7.76 (1H, *d*, *J* = 15.6 Hz, H-β), 7.98 (1H, *d*, *J* =15.6 Hz, H-α), 14.2 (1H, *s*, OH).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	70.3 (OC<u>H</u>$_2$Ph), 71.5 (OC<u>H</u>$_2$Ph), 92.6, 95.1 (<u>C</u>H, 3,5), 106.4(C-1), 127.5, 127.7, 128.4, 128.6, 128.7, 128.7, 128.8, 129.0 (Ar<u>C</u>H), 129.8 (C-α) 135.3 (CH<u>C</u>Ar) 135.4, 135.9 (CH_2<u>C</u>Ar), 142.8 (C-β) 161.8, 165.3, 168.8 (C-2,4,6), 192.7 (C, C-4).
HRESIMS (positive ionization mode):	*m/z* 459.1570 $[M+Na]^+$ (calc. for $C_{29}H_{24}O_4Na$ 459.1572).
IR ν_{max} (cm^{-1}):	1602, 1552, 1205, 1097, 695.

5,7-Bis-benzyloxyflavanone (5.49)



DBU (0.960 mL, 6.43 mmol) was added dropwise to a stirred solution of 2,4-bis-benzyloxy-6-hydroxychalcone **(5.48)** (2.80 g, 6.41 mmol) in DCM (20 mL). The reaction was stirred overnight and quenched by acidifying with 1 M HCl. The layers were separated and the aqueous layer was further extracted with DCM. The combined organic extracts were washed with water, brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (14:1) as eluent to afford 5,7-bis-benzyloxyflavanone[298] as white needles **(5.49)** (1.29 g, 46%) with the recovery of 1.0 g of the chalcone**(5.48)**.

Mp:	120-122 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	2.84 (1H, *dd*, *J* =16.6 Hz, 3.1 Hz, H-3$_{eq}$), 3.07 (1H, *dd*, *J* =16.6 Hz, 13.3 Hz, H-3$_{ax}$), 5.05 (2H, *s*, OC<u>H</u>$_2$Ph), 5.17 (2H, *s*, OC<u>H</u>$_2$Ph), 5.43(1H, *dd*, *J* =13.3 Hz, 3.1 Hz, C<u>H</u>CH_2), 6.19(1H,

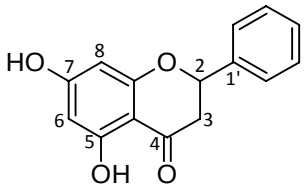
d, *J* =2.3 Hz, H-6 or 8), 6.26 (1H, *d*, *J* =2.3 Hz, H-6 or 8), 7.21-7.50 (15H, *m*, Ar<u>H</u>).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 45.0 (<u>C</u>H_2CH), 70.3 (O<u>C</u>H_2Ph), 70.4 (O<u>C</u>H_2Ph), 79.2(CH_2<u>C</u>H), 94.8, 95.2 (2 x C-6,8), 106.5(C-10), 126,1, 126.5, 127.5, 127.6, 128.3, 128.5, 128.7,129.8 (Ar<u>C</u>H), 134.3 (CH<u>C</u>Ar) 135.0 (CH_2<u>C</u>Ar), 138.8 (CH_2<u>C</u>Ar), 161.5, 164.9 (C-5,7,9), 188.8 (C, C-4).

HRESIMS (positive ionization mode): *m/z* 459.1568 $[M+Na]^+$ (calc. for $C_{29}H_{24}O_4Na$ 459.1572),

IR ν_{max} (cm^{-1}): 1675, 1571, 1157, 1103, 693.

5,7-Dihydroxyflavanone (5.50)



A mixture of the 5,7-bis-benzyloxyflavanone **(5.49)** (1.29 g, 2.96 mmol) and 10% Pd/C (60 mg) in EtOAc: DCM 1:1 (10 mL) was stirred overnight at room temperature under an atmosphere of hydrogen (200 kPa). The catalyst was filtered, the solvent was evaporated and the residue purified by column chromatography using hexane-EtOAc (9:1) as eluent to afford 5,7-dihydroxyflavanone **(5.50)** as white crystals (712 mg, 94%).

Mp: 192-194 °C.

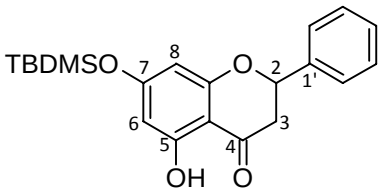
^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 2.85 (1H, *dd*, *J* =16.8 Hz, 3.1 Hz, H-3_{eq}), 3.09 (1H, *dd*, *J* = 16.8 Hz, 13.4 Hz, H-3_{ax}), 5.43 (1H, *dd*, *J* = 13.4 Hz, 3.1 Hz, C<u>H</u>CH_2), 6.19 (1H, *d*, *J* =2.3 Hz, H-6,8), 6.26 (1H, *d*, *J* =2.3 Hz, H-6,8), 6.98-7.46 (5H, *m*, Ar<u>H</u>), 12.05 (1H, *s*, ArO<u>H</u>).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 42.8 (<u>C</u>H_2CH), 78.9 (CH_2<u>C</u>H), 95.6, 96.4 (C-6,8), 102.2(C-10), 125.9, 128.6 (Ar<u>C</u>), 138.2 (C-1'), 162.0, 163.7, 168.7 (C-5,7,9), 198.5 (<u>C</u>=O).

HRESIMS (negative ionization mode): *m/z* 255.0658 $[M-H]^+$ (calc. for $C_{15}H_{11}O_4$ 255.0659);

IR ν_{max} (cm^{-1}): 3353, 1625, 1599, 1291, 1166, 701.

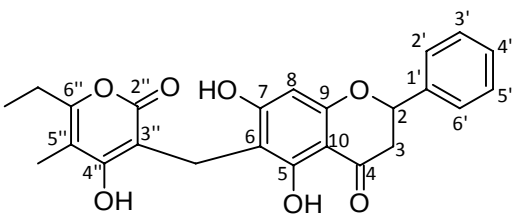
7-*Tert*-butyldimethylsilyloxy-5-hydroxyflavanone (5.51)



Triethylamine (1.35 mL, 9.69 mmol) was added dropwise to a stirred suspension of 5,7-dihydroxyflavanone (1.50 g, 5.85 mmol) in DCM (15 mL). To the resulting solution, *tert*-butyldimethylsilyl chloride (1.00 g, 6.63 mmol) was added and the reaction mixture was stirred at room temperature for 90 minutes. The reaction diluted with DCM and acidified with 2 M H_2SO_4. The layers were separated and the organic layer was dried over anhydrous $MgSO_4$. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (15:1) as eluent to give 7-*tert*-butyldimethylsilyloxy-5-hydroxyflavanone as a colourless oil (1.84 g, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.27 (6H, *s*, Si(C<u>H</u>$_3$)$_2$), 0.99 (9H, *s*, C(C<u>H</u>$_3$)$_3$), 2.84 (1H, *dd*, *J* = 17.2 Hz, 3.1 Hz, H-3$_{eq}$), 3.11 (1H, *dd*, *J* = 17.2 Hz, 13.1 Hz, H-3$_{ax}$), 5.44 (1H, *dd*, *J* = 13.1 Hz, 3.1 Hz, C<u>H</u>CH$_2$), 6.04 (2H, *dd*, *J* = 4.8 Hz, H-6,8), 7.37-7.50 (5H, *m*, Ar<u>H</u>), 11.96 (1H, *s*, O<u>H</u>).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	-4.4 (Si(<u>C</u>H$_3$)$_2$), -4.3 (Si(<u>C</u>H$_3$)$_2$), 18.2 (<u>C</u>(CH$_3$)$_3$), 25.4 (C(<u>C</u>H$_3$)$_3$), 43.5 (<u>C</u>H$_2$CH), 79.1(CH$_2$<u>C</u>H), 99.9, 101.3, 103.7 (C-6,8,10), 126.0, 128.6 (Ar<u>C</u>), 138.5 (C-1'), 161.5, 164.9 (C-5,7,9), 188.8 (<u>C</u>=O).
HRESIMS (positive ionization mode):	*m/z* 393.1261 $[M+Na]^+$ (calc. for $C_{21}H_{26}O_4NaSi$ 393.1498).
IR ν_{max} (cm^{-1}):	1628, 1301, 1177, 1161, 890, 780.

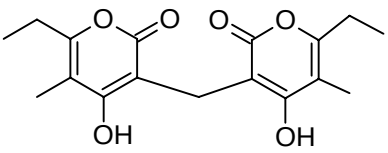
Lepidissipyrone (1.25)



To a stirred of 6-ethyl-4-hydroxy-5-methyl-α-pyrone **(5.45)** (30 mg, 0.19 mol) in chloroform (3 mL) were added paraformaldehyde (6 mg, 0.19 mmol), 7-*tert*-butyldimethylsilyloxy-5-hydroxyflavanone **(5.51)** (70 mg, 0.19 mmol), and TBAF (100mg, 0.38 mmol). The reaction mixture was stirred at 40 °C for 2 days. The reaction was quenched by the addition of H_2SO_4 and extracted with DCM. The combined organic extracts were dried over anhydrous magnesium sulfate and filtered. The solvent was evaporated and the resulting product was purified by column chromatography using hexane acidified with acetic acid (5% to 10%) as eluent to give lepidissipyrone[228, 229] **(1.25)** as a cream-white solid (40 mg, 50%).

Mp:	164-166 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.20 (3H, *t*, *J* = 7.6 Hz, $CH_2C\underline{H}_3$) 1.88 (3H, *s*, $CC\underline{H}_3$) 2.54 (2H, *q*, *J* = 7.6 Hz, $C\underline{H}_2CH_3$), 2.86 (1H, *dd*, *J* = 17.2 Hz, 2.9 Hz, $CHC\underline{H}_2$), 3.29 (1H, *dd*, *J* = 17.2 Hz, 13.2 Hz, $CHC\underline{H}_2$), 3.59 (2H, *s*, $C\underline{H}_2Ar$), 5.55 (1H, *dd*, *J* = 13.2 Hz, 2.9 Hz, $C\underline{H}CH_2$), 6.23 (1H, *s*, H-8), 7.52-7.55 (5H, *m*, Ar<u>H</u>) 8.10 (1H, *s*, ArO<u>H</u>), 10.61 (1H, *s*, ArO<u>H</u>); 11.90 (1H, *s*, ArO<u>H</u>).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	9.2 ($C\underline{C}H_3$), 11.6 ($CH_2\underline{C}H_3$), 17.5 ($\underline{C}H_2Ar$), 24.3 ($\underline{C}H_2CH_3$), 42.8 ($\underline{C}H_2CH$), 81.5 ($CH_2\underline{C}H$), 99.7 (C-8), 101.2(C-3''), 102.3(C-10) 104.9 (C-6), 107.6 (C-5''), 127.0 (C-2',6'), 129.3 (C-3',5'), 130.1 (C-4'), 136.5 (C-1'), 157, 7 (C-5), 161.8($\underline{C}CH_2CH_3$), 163.2 (C-7), 165.9 (C-9), 169.0 (C-2''), 198.8 (C-4).
HRESIMS (positive ionization mode):	*m/z* 445.1260 $[M+Na]^+$ (calc. for $C_{24}H_{22}O_7Na$ 445.1263).
IR ν_{max} (cm^{-1}):	3368, 1658, 1623, 1272, 765.

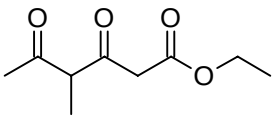
Helipyrone (5.52)



From the synthesis of lepidissipyrone (1.25), small amounts of helipyrone **(5.52)** were obtained after purification by column chromatography. Helipyrone[299] **(5.52)** was obtained as a white solid (6 mg, 10 %).

Mp:	189-200 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.21 (6H, *t*, *J* = 7.3 Hz, 2 x $CH_2\underline{C}H_3$), 1.95 (6H, *s*, 2 x $C\underline{C}H_3$), 2.56 (4H, *q*, *J* = 7.3 Hz, 2 x $C\underline{H}_2CH_3$), 3.57 (2H, *s*, $C\underline{H}_2Ar$), 11.20 (2H, s, 2 x OH).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	9.1 ($C\underline{C}H_3$), 11.5 ($CH_2\underline{C}H_3$), 18.5 ($\underline{C}H_2Ar$), 24.3 ($\underline{C}H_2CH_3$), 101.4, ($CH_2\underline{C}$), 108.6 ($\underline{C}CH_3$), 162.4 ($\underline{C}CH_2CH_3$), 167.9(COH), 171.3 (C=O).
HRESIMS (negative ionization mode):	*m/z* 319.1184 $[M-H]^+$ (calc. for $C_{17}H_{19}O_6$ 319.1182).
IR ν_{max} (cm^{-1}):	1658, 1623, 1340, 1285, 760.

Ethyl 4-methyl-3,5-dioxohexanoate (5.53)



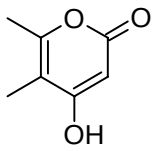
Ethyl 3-oxopentanoate (1.00 mL, 7.02 mmol) was added dropwise to a suspension of sodium hydride (60% dispersion in mineral oil, 560 mg, 14.0 mmol) in THF (30 mL). The reaction mixture was stirred at 0 °C for 10 minutes then *n*-BuLi (1.6 M in hexane, 4.40 mL, 7.04 mmol) was added dropwise. After stirring for 10 minutes, EtOAc (350 µl, 3.51 mmol) was added and the reaction was stirred for further 15 minutes. A second portion of *n*-BuLi (1.6 M in hexane, 2.85 mL, 4.56 mmol) was added followed after 15 minutes by another potion of EtOAc (350 µL, 3.51 mmol). After 15 min of stirring, the reaction was quenched by addition of conc. HCl (4.00 mL), diluted with water (20 mL) and extracted with diethyl ether. The combined organic extracts were washed with $NaHCO_3$ and dried over anhydrous magnesium sulfate. After filtration the solvent was evaporated and the residue was purified by column chromatography using hexane-EtOAc (19:1) as eluent to give ethyl 4-methyl-3,5-dioxohexanoate **(5.53)** as a colourless oil (706 mg, 54%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.20 (3H, *t*, *J* = 7.3 Hz, $OCH_2C\underline{H}_3$), 1.28 (3H, *d*, *J* = 7.3 Hz, $CHC\underline{H}_3$), 2.16 (3H, *s*, $COC\underline{H}_3$), 3.47 (2H, *s*, $COC\underline{H}_2CO$), 3.81 (1H, *q*, *J* = 7.3 Hz, C<u>H</u>), 4.12 (2H, *q*, *J* = 7.3 Hz, $OC\underline{H}_2CH_3$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	12.3 ($CH\underline{C}H_3$), 13.8 ($OCH_2\underline{C}H_3$), 28.3 ($CO\underline{C}H_3$), 47.7 ($CO\underline{C}H_2CO$), 59.9 (<u>C</u>H), 61.1 ($O\underline{C}H_2CH_3$), 166.7 ($\underline{C}O_2CH_2CH_3$), 184.3 ($CH\underline{C}OCH_2$), 204.6 ($\underline{C}OCH_3$).

HRESIMS (positive ionization mode): *m/z* 209.0792 $[M+Na]^+$ (calc. for $C_9H_{14}O_4Na$ 209.0790).

IR ν_{max} (cm^{-1}): 3312, 1750, 1709, 1250, 1059, 820.

4-Hydroxy-5,6-dimethyl-α-pyrone (5.54)



A solution of ethyl 4-methyl-3,5-dioxoheptanoate **(5.53)** (0.472 g, 2.56 mmol) in polyphosphoric acid (1.60 g) was heated at 120 °C for 2 hours and poured in ice. The mixture was filtered and the residue was washed with water to give 4-hydroxy-5,6-dimethyl-α-pyrone **(5.54)** as a light yellow solid (244 mg, 68%).

Mp: 196-198 °C.

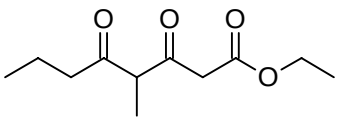
^1H NMR (400 MHz, $CDCl_3$) δ_H: 1.94 (3H, *s*, $CC\underline{H}_3$), 2.27 (3H, *s*, $OCC\underline{H}_3$), 5.72 (1H, *s*, $C\underline{H}$).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 13.0 ($C\underline{C}H_3$), 21.0 ($OC\underline{C}H_3$), 88.7 ($\underline{C}H$), 112.2 ($\underline{C}CH_3$), 162.8 ($O\underline{C}CH_3$), 171.0 (COH), 175.9 (C=O).

HRESIMS (negative ionization mode): *m/z* 199.0390 $[M-H]^+$ (calc. for $C_7H_7O_3$ 139.0395).

IR ν_{max} (cm^{-1}): 3424, 1695, 1598, 1438, 1227, 1094.

Ethyl 4-methyl-3,5-dioxooctanoate (5.55)

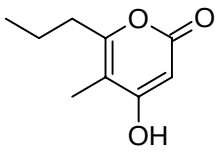


Ethyl 3-oxopentanoate (1.00 mL, 7.02 mmol) was added dropwise to a suspension of sodium hydride (60% dispersion in mineral oil, 560 mg, 14.0 mmol) in THF (30 mL). The reaction mixture was stirred at 0 °C for 10 minutes then *n*-BuLi (1.6 M in hexane, 4.40 mL, 7.04 mmol) was added dropwise. After stirring for 10 minutes, ethyl butyrate (460 µL, 3.51 mmol) was added and the reaction was stirred for further 15 minutes. A second portion of *n*-BuLi (1.6 M in hexane, 2.85 mL, 4.56 mmol) was added followed after 15 minutes by another potion of ethyl butyrate (460 µL, 3.51 mmol). After 15 min of stirring, the reaction was quenched by addition of conc. HCl (4.00 mL), diluted with water (20 mL) and extracted with diethyl ether. The combined organic extracts

were washed with $NaHCO_3$ and dried over anhydrous magnesium sulfate. After filtration the solvent was evaporated and the residue was purified by column chromatography using hexane-EtOAc (19:1) as eluent to give ethyl 4-methyl-3,5-dioxooctanoate **(5.55)** as a colourless oil (827, 55%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.87 (3H, *t*, *J* = 7.1 Hz, $CH_2CH_2C\underline{H}_3$), 1.24 (3H, *t*, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$), 1.30 (3H, *d*, *J* = 7.1 Hz, $CHC\underline{H}_3$), 1.58 (2H, *m*, $C\underline{H}_2CH_2CH_3$), 2.45 (2H, *m*, $CH_2C\underline{H}_2CH_3$), 3.48 (2H, *s*, $COC\underline{H}_2CO$), 3.84 (1H, *q*, *J* = 7.1Hz, C<u>H</u>), 4.16 (2H, *q*, *J* = 7.2 Hz, $OC\underline{H}_2CH_3$).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	12.2 ($CH\underline{C}H_3$), 13.5 ($CH_2CH_2\underline{C}H_3$), 14.0 ($OCH_2\underline{C}H_3$), 17.0 ($CH_2\underline{C}H_2CH_3$), 37.8 ($CO\underline{C}H_2CH_2CH_3$), 47.9 ($CO\underline{C}H_2CO$), 60.0 (<u>C</u>H), 61.4 ($O\underline{C}H_2CH_3$), 167.7 ($\underline{C}O_2CH_2CH_3$), 199.7 ($CH\underline{C}OCH_2$), 207.3 ($COCH_2CH_3$).
HRESIMS (positive ionization mode):	*m/z* 237.1110 $[M+Na]^+$ (calc. for $C_9H_{14}O_4Na$ 223.1103).
IR ν_{max} (cm^{-1}):	3315, 1751, 1720, 1250, 1055, 820.

4-Hydroxy-5-methyl-6-propyl-α-pyrone (5.56)

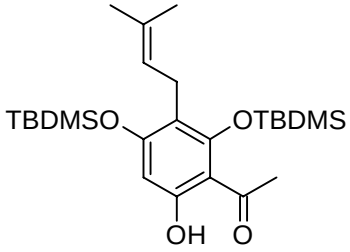


A solution of ethyl 4-methyl-3,5-dioxooctanoate **(5.55)** (0.452 g, 2.11 mmol) in polyphosphoric acid (1.30 g) was heated at 120 °C for 2 hours and poured in ice. The mixture was filtered and the residue was washed with water to give 6-propyl-4-hydroxy-5-methyl-α-pyrone **(5.56)** as a light brown solid (230 mg, 65%).

Mp:	120-122 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.97 (3H, *t*, *J*=7.4 Hz, $CH_2CH_2C\underline{H}_3$), 1.69 (2H, *sextet*, *J*=7.4 Hz, $CH_2C\underline{H}_2C\underline{H}_3$) 1.96 (3H, *s*, $CC\underline{H}_3$), 2.53 (2H, *t*, *J*=7.4 Hz, $C\underline{H}_2CH_2CH_3$), 5.71(1H, *s*, C<u>H</u>).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	9.2 ($C\underline{C}H_3$), 13.6 ($CH_2\underline{C}H_3$), 20.6 ($\underline{C}H_2CH_3$), 32.8 ($\underline{C}H_2CH_2CH_3$), 89.8 ($\underline{C}H$), 109.0 ($\underline{C}CH_3$), 162.7($\underline{C}CH_2CH_2CH_3$), 167.8($\underline{C}OH$), 172.7 (C=O).
HRESIMS (positive ionization mode):	*m/z* 191.0678 $[M+Na]^+$ (calc. for $C_9H_{12}O_3Na$ 191.0684).
IR ν_{max} (cm^{-1}):	3125, 1697, 1598, 1438, 1252, 1121.

2,4-Bis(*tert*-butyldimethylsilyl)-3-prenylphloroacetophenone (5.57)



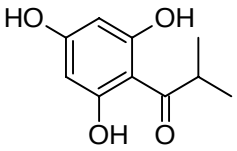
Triethylamine (5.48 mL, 39.2 mmol) was added dropwise to a stirred suspension of 3-prenyl phloroacetophenone (2.00 g, 11.9 mmol) in DCM (40 mL). To the resulting solution, TBDMSCl (3.94 g, 26.3 mmol) was added and the reaction mixture was stirred at room temperature for 90 min. The reaction was quenched with 2 M H_2SO_4 and the organic components extracted with DCM. The layers were separated and the organic layer was dried over anhydrous $MgSO_4$. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (20:1) as eluent to give 2,4-bis(*tert*-butyldimethylsilyl)phloroacetophenone[264] **(5.57)** as colourless oil (3.52 g, 70%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.20 (6H, *s*, $Si(CH_3)_2$), 0.32 (6H, *s*, $Si(CH_3)_2$), 0.95 (18H, *s*, 2 x $SiC(CH_3)_3$), 1.75 (3H, *s*, $C(CH_3)_2$), 1.82 (3H, *s*, $C(CH_3)_2$), 2.68 (3H, *s*, $COCH_3$), 3.35 (2H, *d, J* = 6.6 Hz, $CHCH_2$), 5.20 (1H, *t, J* = 6.6 Hz, $CHCH_2$), 6.18 (1H, *s*, H-5), 12.91 (1H, *s*, OH).
^{13}C NMR (100 MHz, CDCl3) δ_C:	-4.3 ($Si(\underline{C}H_3)_2$), -3.5 ($Si(\underline{C}H_3)_2$), 17.9 ($\underline{C}(CH_3)_2$), 18.3 ($\underline{C}(CH_3)_3$), 18.8 ($\underline{C}(CH_3)_3$), 22.0 ($CH\underline{C}H_2$), 25.6 ($C(\underline{C}H_3)_3$), 25.7 ($\underline{C}(CH_3)_2$), 26.1 ($C(\underline{C}H_3)_3$), 32.9 ($COCH_3$), 100.8 (C-5) 108.9 (C-1), 113.4 (C-3), 123.1 ($\underline{C}HCH_2$), 131.4 ($\underline{C}(CH_3)_2$), 161.3, 161.5 (C-2, 4, 6), 201.9 ($\underline{C}OCH_3$).

HRESIMS (positive ionization mode): *m/z* 465.2461 $[M^+ + H]^+$ (calc. for $C_{25}H_{45}O_4Si_2$ 465.2856).

IR ν_{max} (cm^{-1}): 3195, 1620, 1604, 1422, 1287, 1207, 1071.

2,4,6-Trihydroxyisobutyrophenone (5.58)



$AlCl_3$ (4.10 g, 30.7 mmol) was added to a solution of phloroglucinol (1.00 g, 7.93 mmol) in nitrobenzene (20 mL). The reaction mixture was stirred at room temperature for 30 min. Isobutyryl chloride (1.10 mL, 10.5 mmol) was slowly added and the reaction was stirred at 80 °C for 1 hour. The reaction was quenched by acidifying with 1M HCl, addition of water (20 mL), and extracted with EtOAc. The combined organic extracts were washed with $NaHCO_3$, water, brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated and the resulting product was purified by column chromatography using acidified hexane (5% acetic acid) as eluent to give 2,4,6-trihydroxyisobutyrophenone[300] **(5.58)** as white solid (1.09 g, 70 %).

Mp: 136-138 °C.

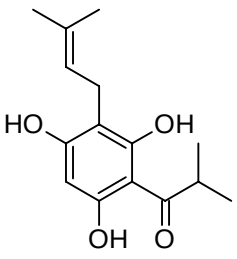
^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 1.18 (6H, *d*, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$), 3.93 (1H, *septet, J* = 6.8 Hz, $C\underline{H}(CH_3)_2$), 5.92 (2H, *s*, H-3,5).

^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 20.1 ($CH(\underline{C}H_3)_2$), 40.2 ($\underline{C}H(CH_3)_2$), 95.8 (C-3, 5), 104.9 (C-1), 163.7 (C-4), 163.9 (C-2, 6), 210.6 (COCH).

HRESIMS (negative ionization mode): *m/z* 195.0298 $[M-H]^+$ (calc. for $C_{10}H_{11}O_4$ 195.0293).

IR ν_{max} (cm^{-1}): 3320, 1615, 1500, 1269, 1140, 980.

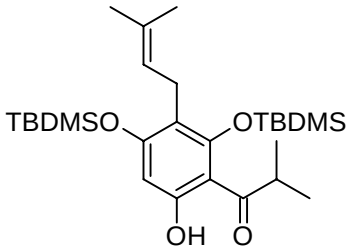
2,4,6-Trihydroxy-3-prenylisobutyrophenone (5.59)



DBU (0.233 g, 1.53 mmol) 2,4,6-trihydroxyisobutyrophenone **(5.58)** (0.300 g, 1.53 mmol) 3,3-dimethylallyl bromide (0.178 mL, 1.53 mmol) were added to dry THF (20 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was then acidified with 2 M HCl and extracted with EtOAc. The combined organic extracts were washed with brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue purified with column chromatography using hexane-EtOAc: 5:1 to afford 2,4,6-trihydroxy-3-prenylisobutyrophenone[298] **(5.59)** as colour crystals (162 mg, 40%).

Mp:	165-167 °C.
^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	1.09 (6H, *d*, *J* = 6.7 Hz, CH(C<u>H</u>$_3$)$_2$), 1.63 (3H, *s*, C(C<u>H</u>$_3$)$_2$), 1.71 (3H, *s*, C(C<u>H</u>$_3$)$_2$), 3.20 (2H, *d, J* = 6.8 Hz, CHC<u>H</u>$_2$), 3.92 (2H, *m,* C<u>H</u>(CH$_3$)$_2$, 5.17 (1H, *t, J* = 6.8 Hz, C<u>H</u>CH$_2$), 5.77 (1H, *s*, H-5).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	17.8 (C(<u>C</u>H$_3$)$_2$), 19.7 (CH(<u>C</u>H$_3$)$_2$), 22.0 (CH<u>C</u>H$_2$), 25.6 (C(<u>C</u>H$_3$)$_2$), 40.3 (<u>C</u>H(CH$_3$)$_2$), 98.8 (C-5), 103.6 (C-1), 108.4 (C-3), 123.5 (CHCH$_2$), 131.0 (<u>C</u>(CH$_3$)$_2$), 161.4, 163.5, 165.1 (C-2, 4, 6), 211.3 (COCH$_3$).
HRESIMS negative ionization mode):	*m/z* 263.1286 $[M-H]^+$ (calc. for $C_{15}H_{19}O_4$ 263.1286).
IR ν_{max} (cm^{-1}):	3424, 1700, 1599, 1227, 1093.

2,4-Bis(*tert*-butyldimethylsilyl)- 3-prenylisobutyrophenone (5.60)



Triethylamine (0.90 mL, 6.45 mmol) was added dropwise to a stirred suspension of 2,4,6-trihydroxy-3-prenylisobutyrophenone (0.500 g, 1.89 mmol) in DCM (40 mL). To the resulting solution, TBDMSCl (0.630 g, 4.18 mmol) was added and the reaction mixture was stirred at room temperature for 90 minutes. The reaction was quenched with 2 M H_2SO_4 and the organic components extracted with DCM. The layers were separated and the organic layer was dried over

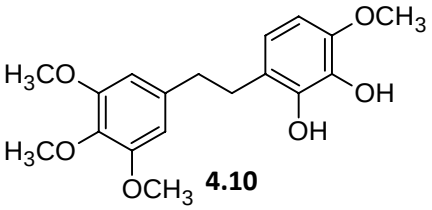
anhydrous $MgSO_4$. The solvent was evaporated and the resulting product was purified by column chromatography using hexane-EtOAc (20:1) as eluent to give as colourless oil (0.736 g, 79%).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H:	0.27 (6H, *s*, $Si(CH_3)_2$), 0.33 (6H, *s*, $Si(C\underline{H}_3)_2$), 1.00 (9H, *s*, $SiC(C\underline{H}_3)_3$), 1.01(9H, *s*, $SiC(C\underline{H}_3)_3$), 1.14 (6H, *d*, *J* = 6.8 Hz, $CH(C\underline{H}_3)_2$), 1.67 (3H, *s*, $C(C\underline{H}_3)_2$), 1.75 (3H, *s*, $C(C\underline{H}_3)_2$), 3.26 (2H, *d, J* = 6.7 Hz, $CHC\underline{H}_2$), 5.15 (1H, *t, J* = 6.7 Hz, $C\underline{H}CH_2$), 5.83 (1H, *s*, H-5), 13.64 (1H, *s*, OH).
^{13}C NMR (100 MHz, $CDCl_3$) δ_C:	-4.0 ($Si(\underline{C}H_3)_2$), -3.7 ($Si(\underline{C}H_3)_2$), 18.0 ($\underline{C}(CH_3)_2$), 18.2 ($\underline{C}(CH_3)_2$, 18.9 ($C(\underline{C}H_3)_2$), 19.4 (2 x $CH(\underline{C}H_2)$, 22.1 ($CHC\underline{H}_2$), $(CH_3)_2$), 25.6 ($\underline{C}(CH_3)_3$) 25.7 ($C(\underline{C}H_3)_2$), 26.1 ($\underline{C}(CH_3)_3$), 38.6 ($\underline{C}H(CH_3)_2$), 101.2 (C-5) 107.5 (C-1), 113.5 (C-3), 123.1 ($\underline{C}HCH_2$), 131.2 ($\underline{C}(CH_3)_2$), 156.3, 159.1, 164.3 (C-2, 4, 6), 210.9 ($\underline{C}OCH_3$).
HRESIMS (positive ionization mode):	*m/z* 493.3177 $[M+H]^+$ (calc. for $C_{27}H_{49}O_4Si_2$ 493.3169).
IR ν_{max} (cm^{-1}):	3179, 1625, 1601, 1430, 1268, 1170.

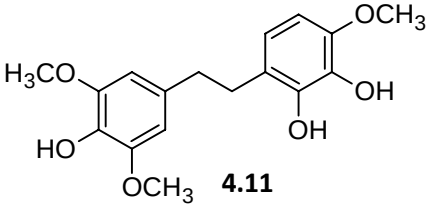
CHAPTER 6

Conclusion

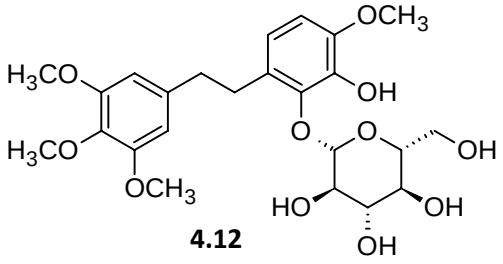
Preliminary *in vitro* screening of selected South African medicinal plants for the inhibition of HIV reverse transcriptase resulted in the selection of *Combretum kraussi* for further studies as it showed significant inhibition of the enzyme. However, an XTT cell viability assay of HIV infected cells used to screen for anti-HIV activity of plant extracts showed that none of the extracts assayed inhibited HIV and with a few exceptions, most plant extracts were not highly toxic to the cell line used. Phytochemical studies on *Combretum kraussi* resulted in the isolation of three secondary metabolites, combretastatin B-1 **(4.10)**, combretastatin B-5 **(4.11)** and combretastatin B-1 2-β-D glucoside **(4.12)**. The three compounds have been isolated before from *C. kraussi* and other *Combretum* species and their biological study revealed their cytotoxic activity against a number of cancer cell lines.[181, 200, 202, 213] All three isolated compounds showed inhibition of HIV-1 replication at a single dose of 10 µM, however, toxicity precluded antiviral testing. These results showed that obtaining an active antiviral agent from plant extracts is no easy task.



4.10

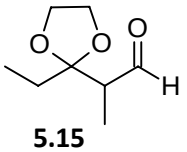


4.11

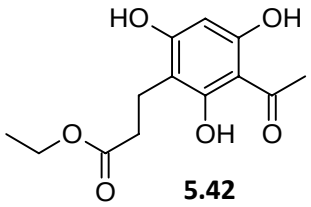


4.12

The bioactive compounds, arzanol and lepidissipyrone were then chosen from literature for synthesis. The preparation of the fully substituted benzene ring was problematic. Regardless of not being able to complete the total synthesis of arzanol, the two precursors, 2-(2-ethyl-1,3-dioxolan-2-yl)propanal **(5.15)** and ethyl 3-(3-acetyl-2,4,6-tribenzyloxyphenyl)propanoate **(5.42)** were successfully synthesised.

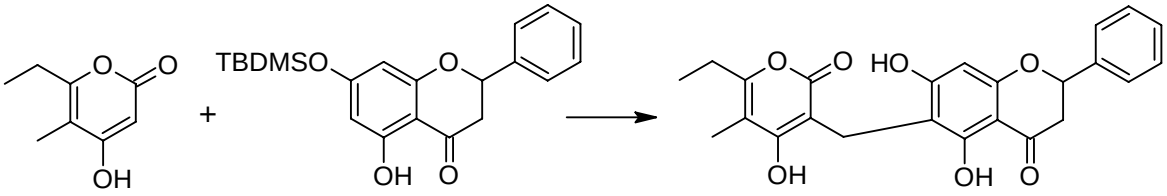


5.15



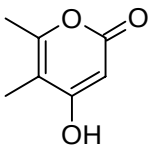
5.42

The flavanone moiety **5.51** was successfully synthesised from 2,4,6-trihydroxyacetophenone. The pyrone moiety **5.45** was synthesised from ethyl 3-oxopentanoate. The two moieties were coupled with a methylene linkage using paraformaldehyde and TBAF by applying the carba version of the Betti reaction to afford lepidissipyrone.

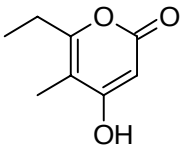


5.45 **5.51** **lepidissipyrone**

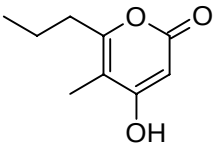
Acylphloroglucinol and α-pyrone are structural features present in many bioactive natural products. Various derivatives of phloroglucinol and α-pyrone were successfully synthesised. The various α-pyrone synthesised, varying the alkyl groups, are shown below. As illustrated earlier, the pyrone **(5.45)** is presence in both lepidissipyrone and arzanol.



5.54

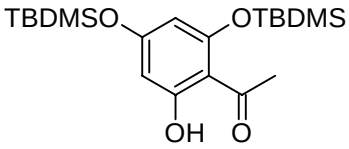


5.45

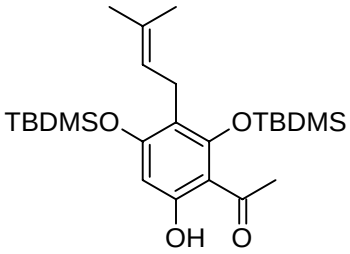


5.56

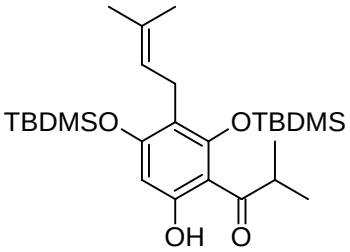
Various *tert*-butyldimethylsilyl protected acylphloroglucinol derivatives were successfully synthesised.



5.46

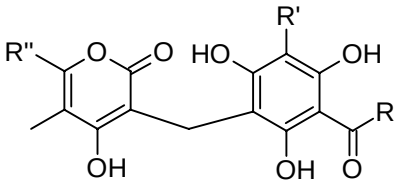


5.57



5.60

Future work includes using the strategy for linking the flavanone and the pyrone moiety of lepidissipyrone, to link the acylphloroglucinol and α-pyrone derivatives synthesised to complete the total synthesis of arzanol derivatives shown below. Some of the compounds synthesised were tested for inhibition of HIV-1 replication at a single dose of 10 µM, only 2,4-bis-benzyloxy-6-hydroxychalcone **(5.48)** showed significant inhibition of 67%.



R = CH_3, $CH(CH_3)_2$

R’ = H, $CH_2CH=C(CH_3)_2$

R’’ = CH_3, CH_2CH_3, $CH_2CH_2CH_3$

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Appendix 1

NMR data of selected compounds

Plate 1: ^{1}H NMR spectrum of combretastatin B-1 (4.10)

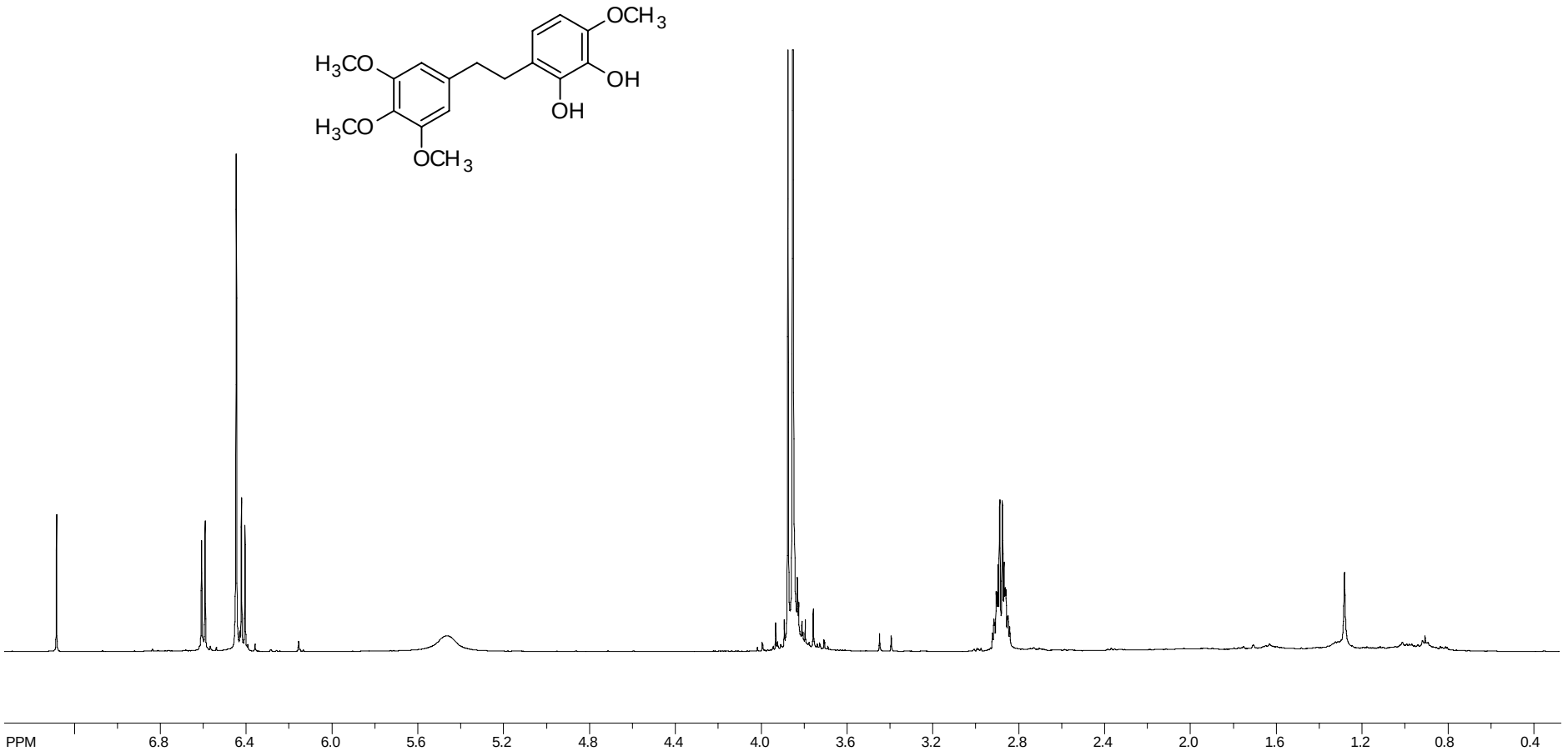


Plate 2: ^{13}C NMR spectrum of combretastatin B-1 (4.10)

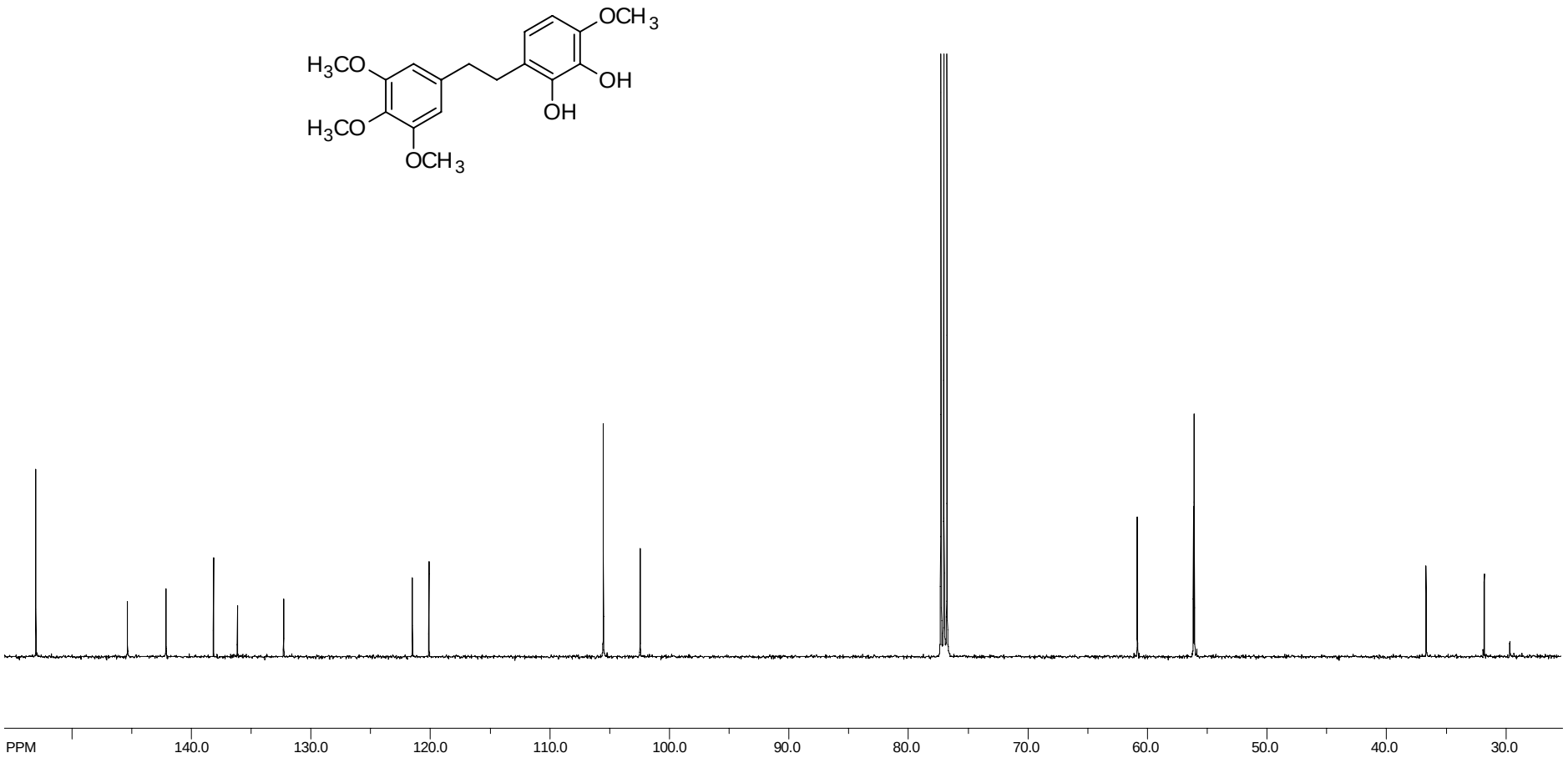


Plate 3: ^{1}H NMR spectrum of combretastatin B-5 (4.11)

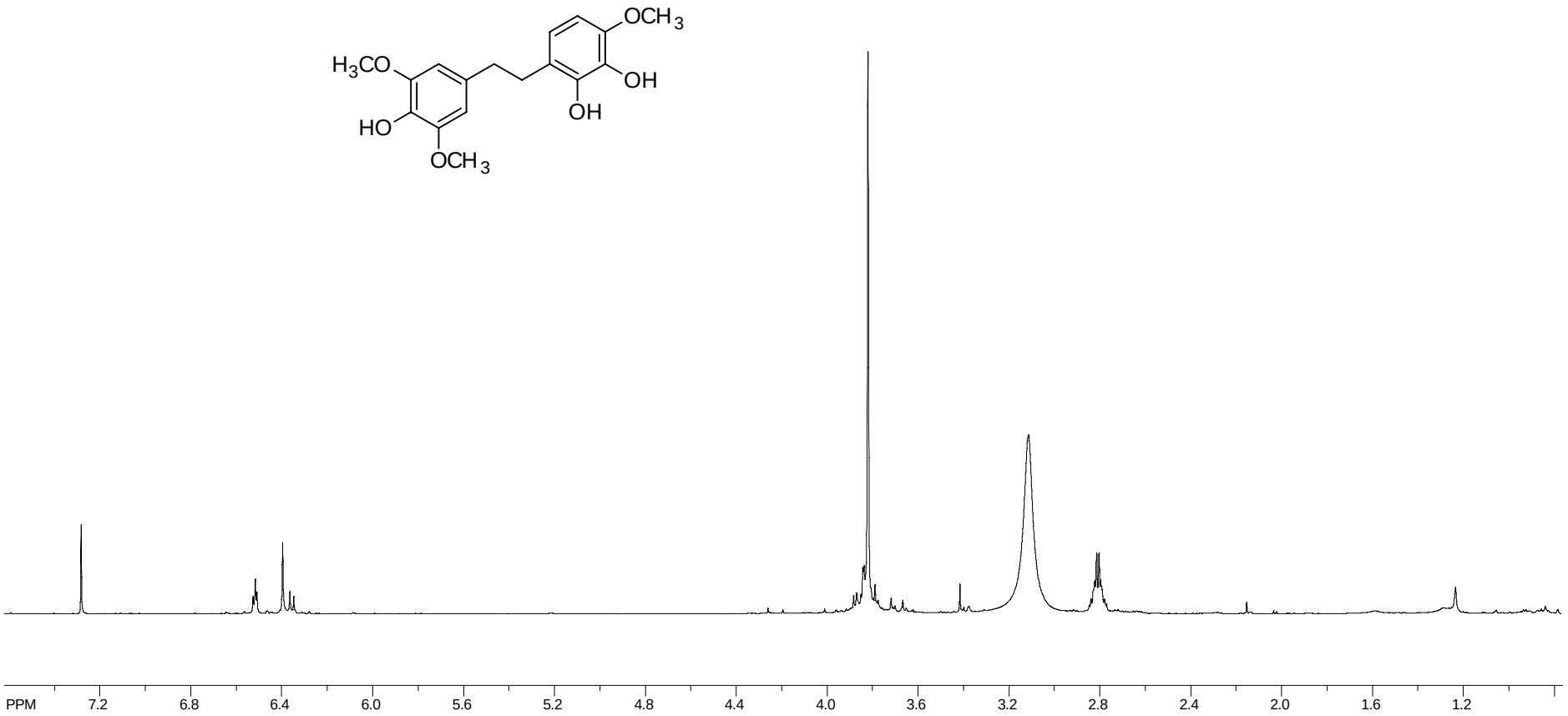


Plate 4: ^{13}C NMR spectrum of combretastatin B-5 (4.11)

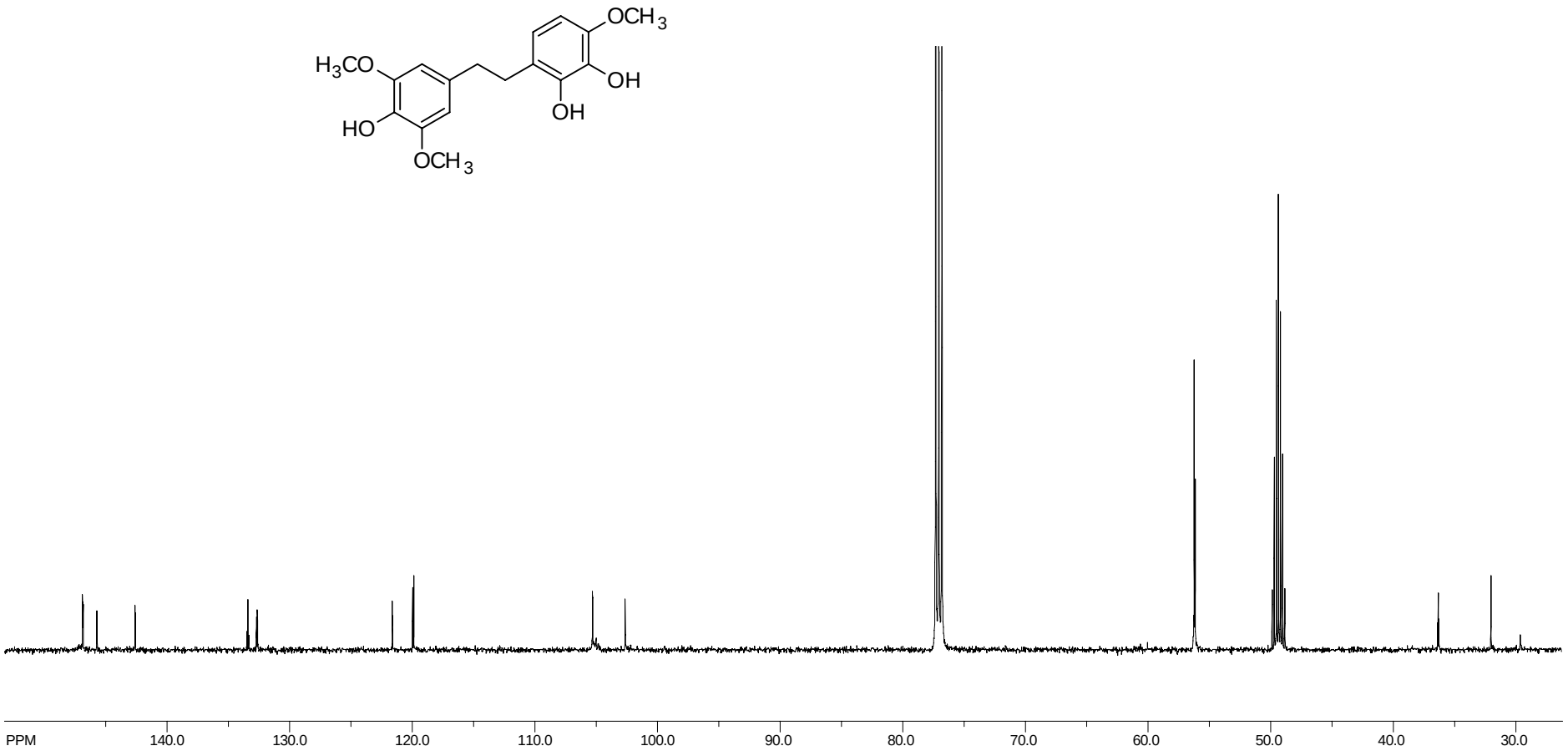


Plate 5: ^{1}H NMR spectrum of combretastatin B-5 glucoside 4.12

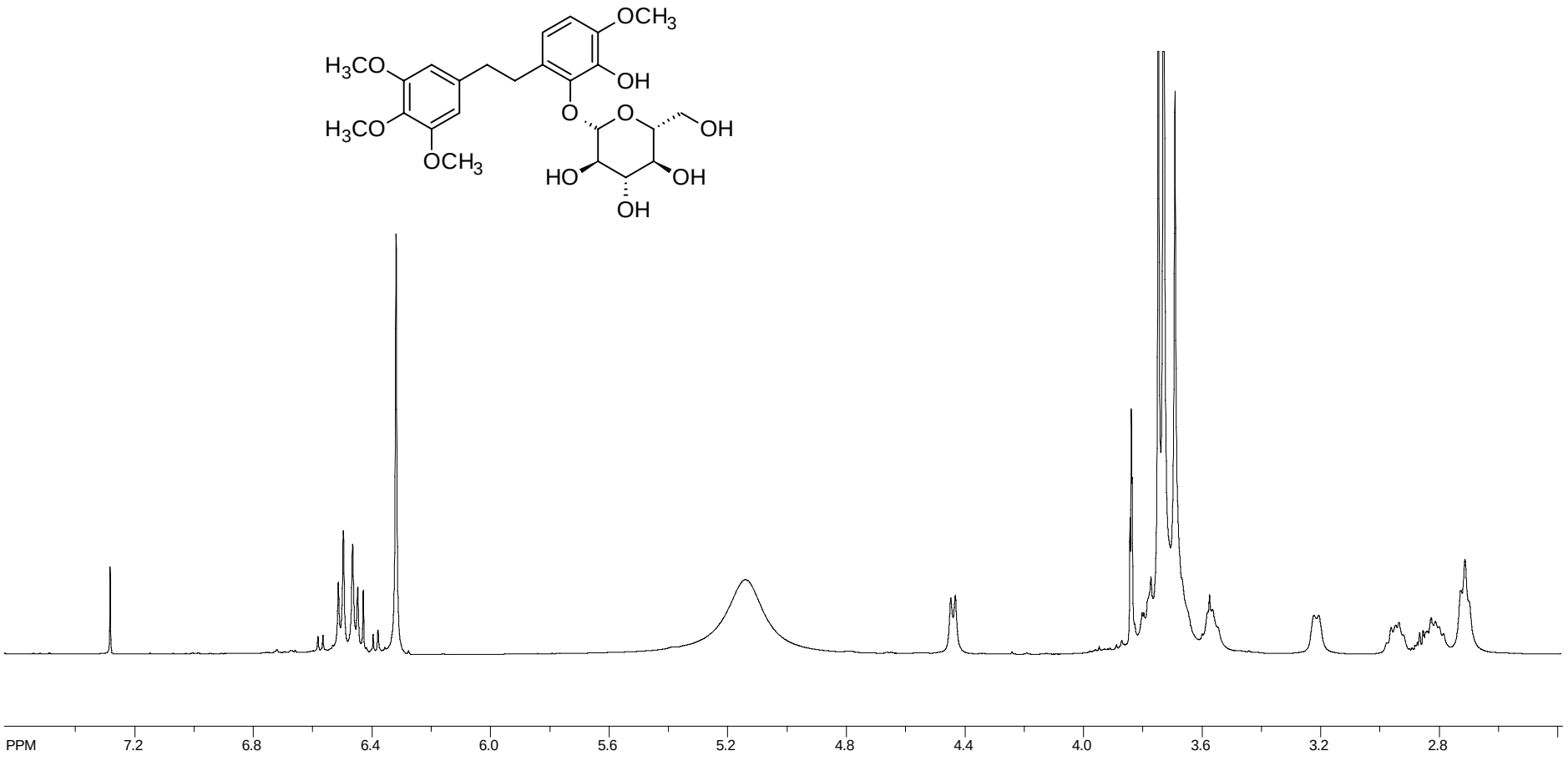
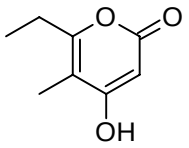


Plate 6: ^{1}H NMR spectrum of 6-ethyl-4-hydroxy-5-methyl-α-pyrone (5.45).



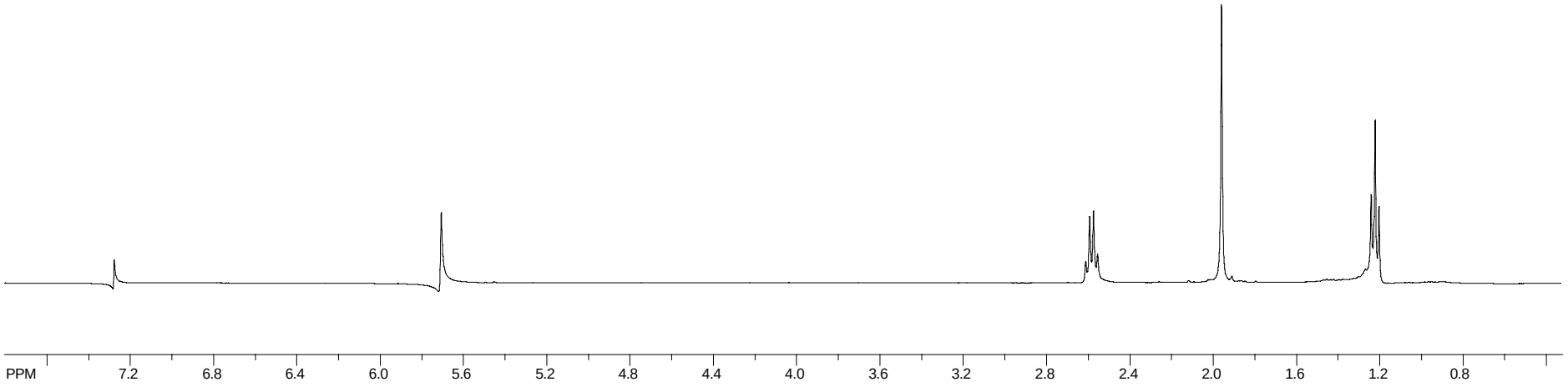


Plate 7: ^{13}C NMR spectrum of 6-ethyl-4-hydroxy-5-methyl-α-pyrone (5.45)

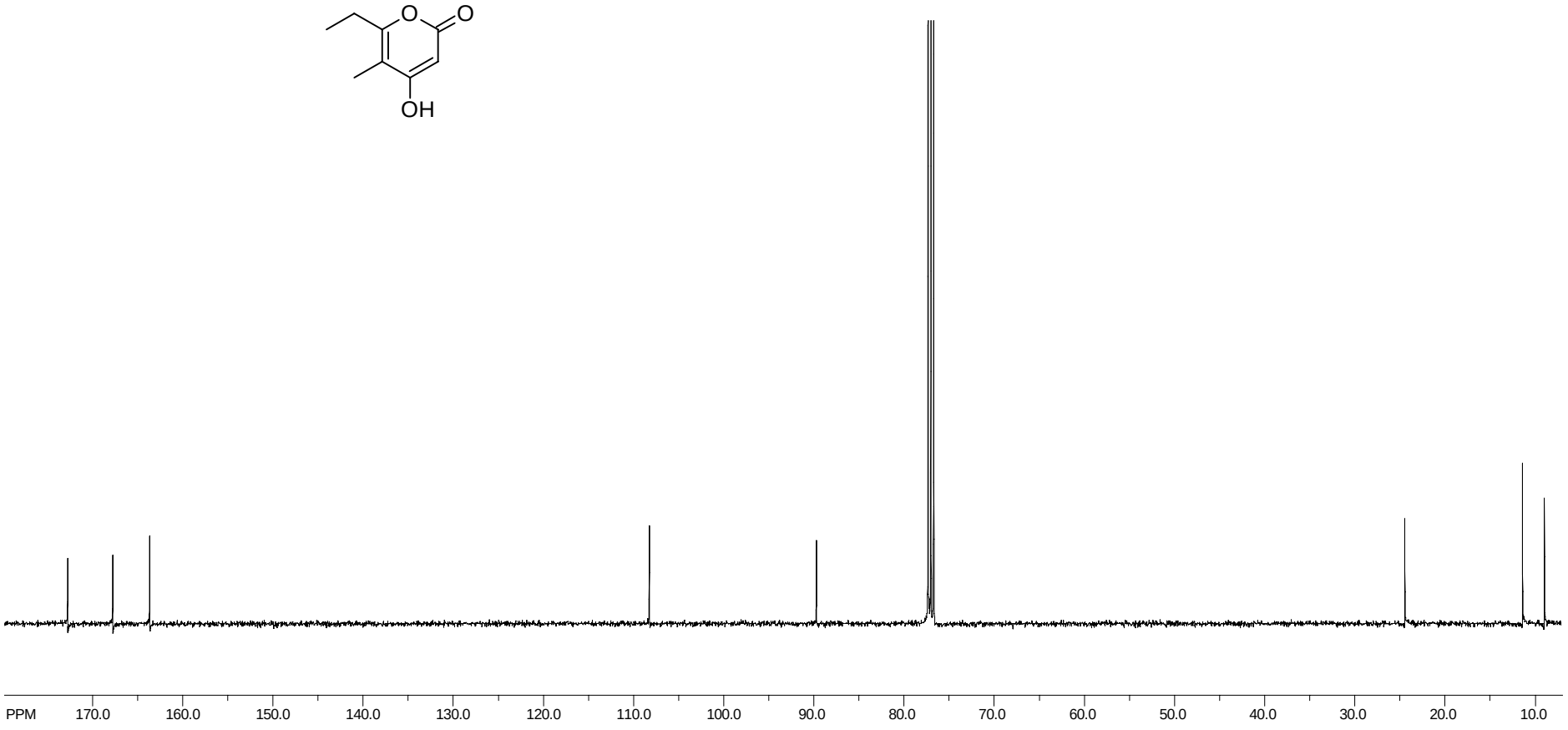


Plate 8: ^{1}H NMR spectrum of 5-hydroxy-7-*tert*-butyldimethylsilyloxyflavanone (5.51).

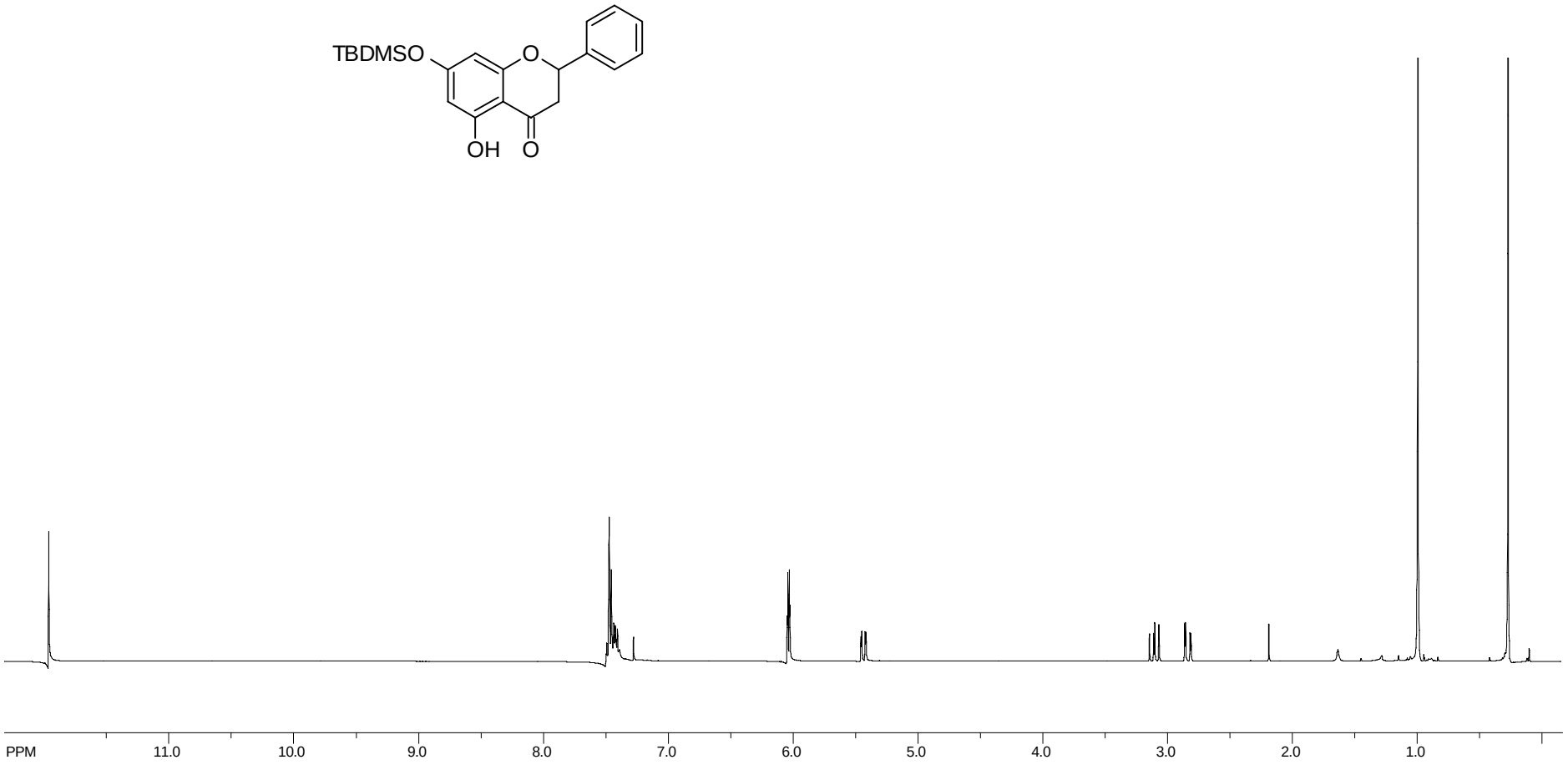


Plate 9: ^{13}C NMR spectrum of 5-hydroxy-7-*tert*-butyldimethylsilyloxyflavanone (5.51).

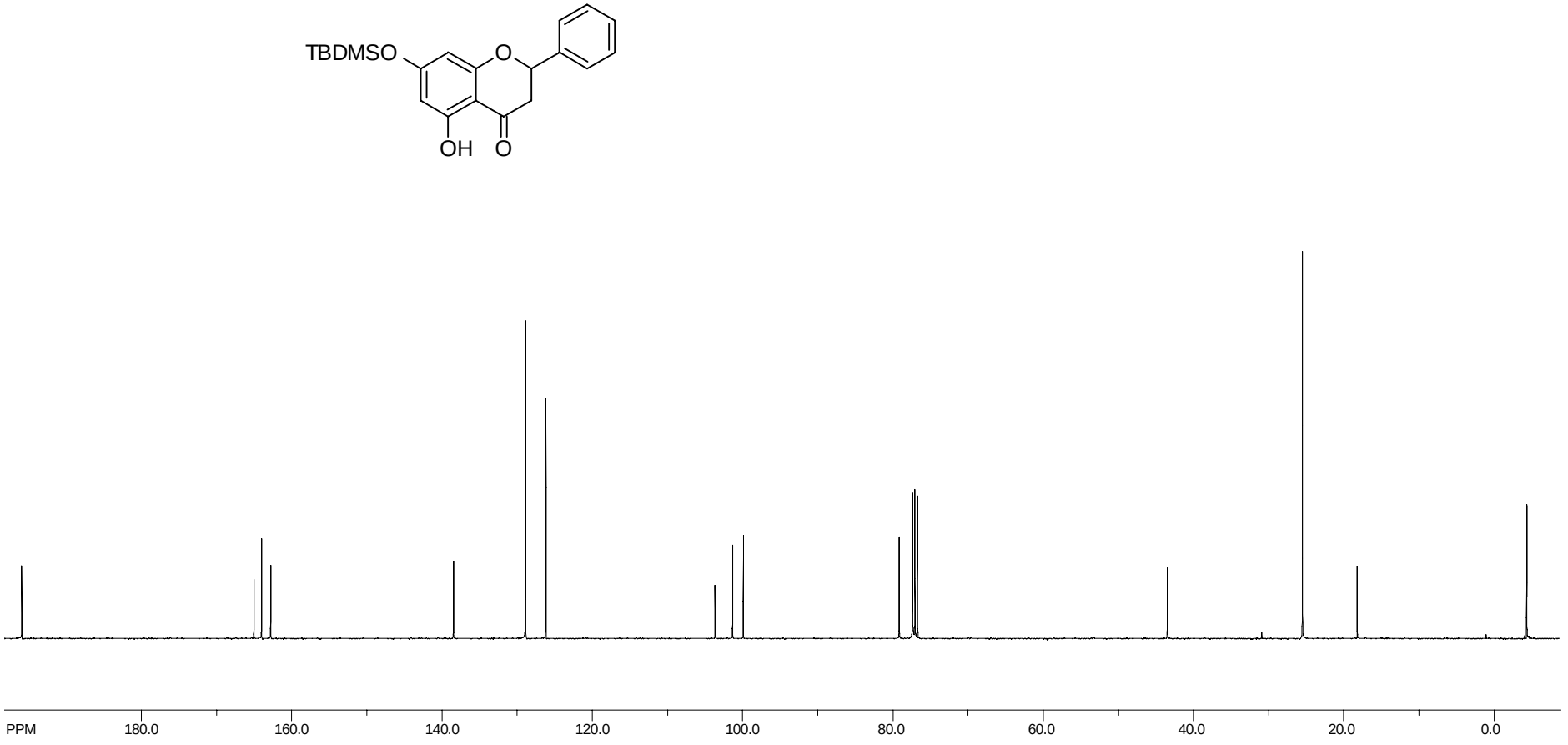


Plate 10: ^{1}H NMR spectrum of lepidissipyrone (1.25).

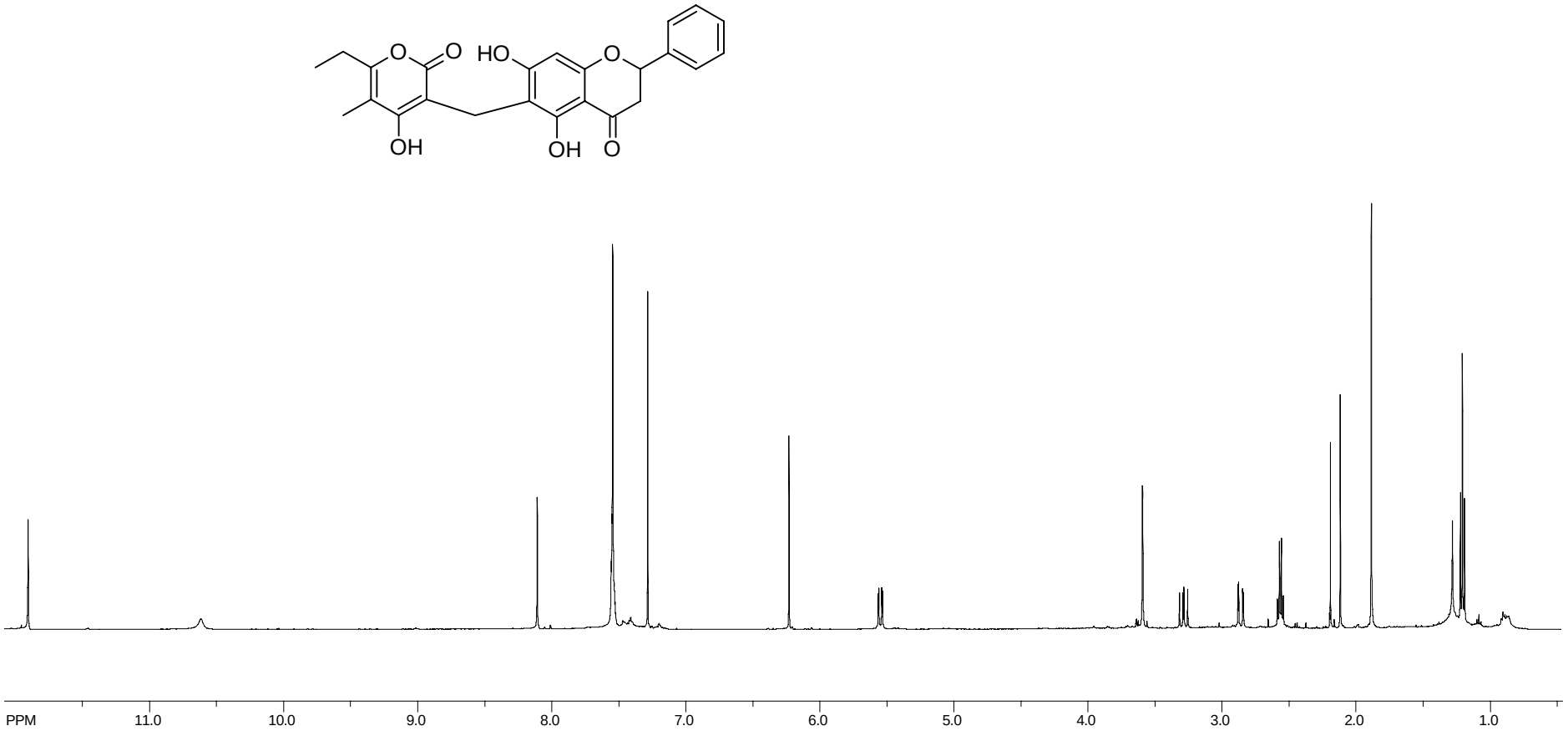
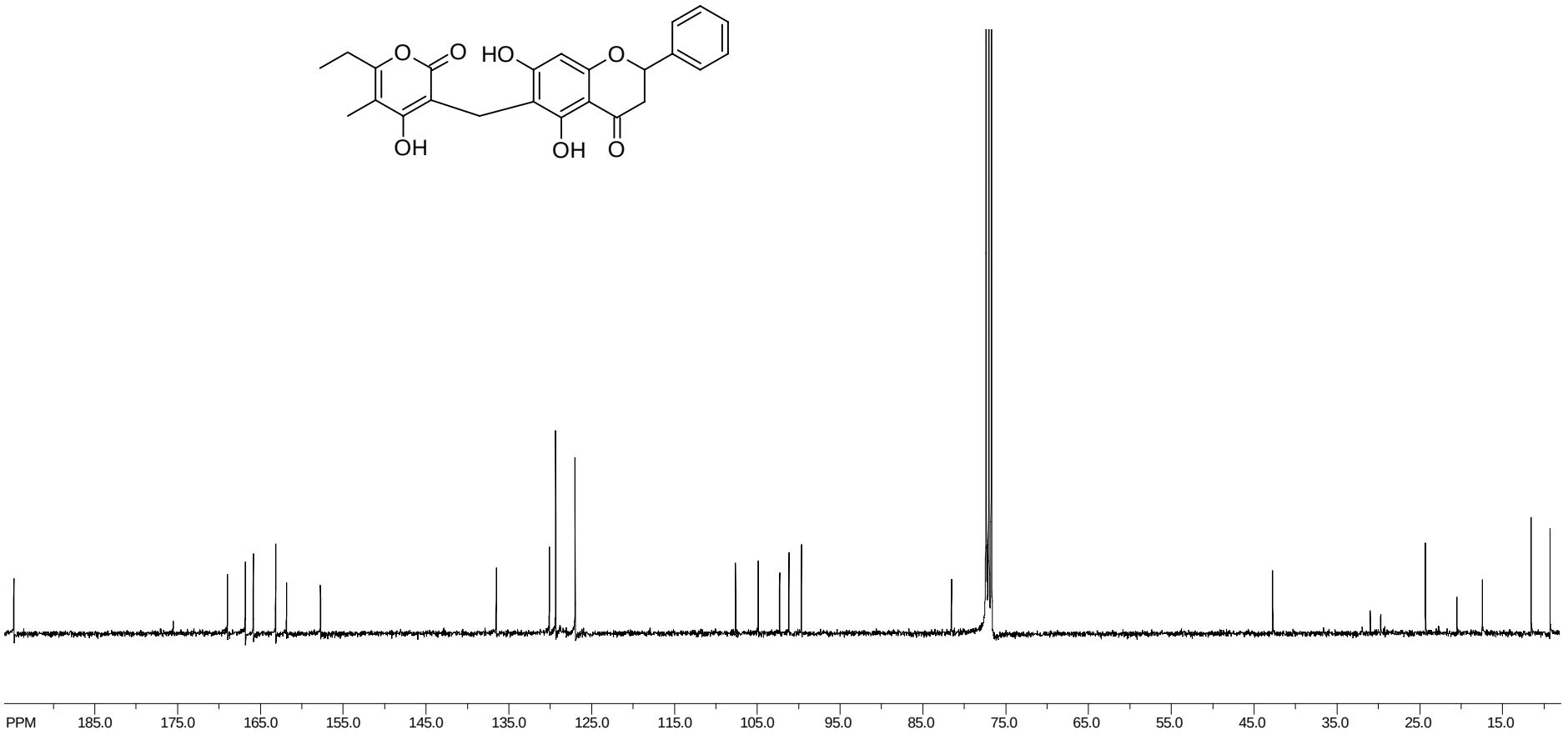


Plate 11: ^{13}C NMR spectrum of lepidissipyrone (1.25).



Appendix 2

Conference presentation

2010 11th Frank Warren Organic Chemistry Conference

(17 -21 January 2010, University of KwaZulu-Natal, Pietermaritzburg)

Poster Presentation: Approaches towards the total synthesis of arzanol

Appendix 3

Publication

Acta Crystallographica Section E
Structure Reports Online
ISSN 1600-5368

1-(5,7-Dihydroxy-2,2-dimethylchroman-6-yl)ethanone

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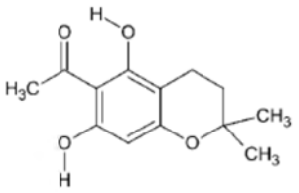
Key indicators: single-crystal X-ray study; T = 298 K; mean σ(C–C) = 0.002 Å; R factor = 0.032; wR factor = 0.091; data-to-parameter ratio = 14.3.

In the title molecule, $C_{13}H_{16}O_4$, the pyran ring is in a half-chair conformation. There is an intramolecular hydrogen bond involving the ketone O atom and an H atom of a phenol group which forms an S(6) ring. The ketone O atom is also involved in an intermolecular hydrogen bond with a different phenolic H atom of a symmetry-related molecule, forming C(6) chains along the c-axis direction.

Related literature

For applications of the title compound, see: Kraus *et al.* (2011); Basabe *et al.* (2010). For hydrogen-bond motifs, see: Bernstein *et al.* (1995). For a related structure, see: Chakkaravarthi *et al.* (2007).



Experimental

Crystal data

$C_{13}H_{16}O_4$
M_r = 236.26
Tetragonal, $P4_12_12$
a = 10.5677 (2) Å
c = 21.4244 (5) Å
V = 2392.6 (1) Å^3
Z = 8
Mo $K\alpha$ radiation
μ = 0.10 mm^{-1}
T = 298 K
0.6 × 0.4 × 0.4 mm

Data collection

Oxford Diffraction Xcalibur 2 CCD diffractometer
Absorption correction: multi-scan (*SORTAV*; Blessing, 1995)
T_{min} = 0.955, T_{max} = 0.962
26011 measured reflections
2366 independent reflections
2046 reflections with $I > 2\sigma(I)$
R_{int} = 0.047

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.032
$wR(F^2)$ = 0.091
S = 1.08
2366 reflections
166 parameters
H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$ = 0.12 e Å^{-3}
$\Delta\rho_{min}$ = −0.11 e Å^{-3}
Absolute structure: Flack (1983), 931 Friedel pairs
Flack parameter: 0.7 (11)

Table 1
Hydrogen-bond geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
O2—H102···O4^{i}	0.97 (2)	1.77 (2)	2.737 (2)	179 (1)
O3—H103···O4	0.86 (2)	1.71 (2)	2.501 (2)	151 (2)

Symmetry code: (i) $y+\frac{1}{2}, -x+\frac{1}{2}, z-\frac{1}{2}$.

Data collection: *CrysAlis CCD* (Oxford Diffraction, 2008); cell refinement: *CrysAlis CCD*; data reduction: *CrysAlis RED* (Oxford Diffraction, 2008); program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *WinGX* (Farrugia, 1999); software used to prepare material for publication: *publCIF* (Westrip, 2010).

We would like to thank the University of KwaZulu-Natal for their facilities and Kirsty Stewart for the data collection. We also wish to acknowledge the National Research Foundation of South Africa for their financial support.

Supplementary data and figures for this paper are available from the IUCr electronic archives (Reference: LH5372).

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supplementary materials

Acta Cryst. (2011). E**67**, o3412 [**doi:10.1107/S1600536811047982**]

1-(5,7-Dihydroxy-2,2-dimethylchroman-6-yl)ethanone

M. P. Akerman, Z. Mkhize and F. R. van Heerden

Comment

The title compound was synthesized as an intermediate in the preparation of flavonoids or other phenolic derivatives and an intermediate for an anti-HIV chromanone (Kraus *et al.*, 2011). It has also been obtained as a side product in the preparation of prenylated flavonoids with antitumour activity (Basabe *et al.*, 2010). The molecular structure of the title compound is shown in Fig. 1. The pyran ring is in a half-chair conformation. There are two types of hydrogen bonds, one intramolecular and one intermolecular. The intramolecular O3—H103···O4 hydrogen bond forms an S(6) ring motif (Bernstein *et al.*, 1995). This hydrogen bond motif is common to molecules which contain derivatized (2-hydroxyphenyl)ethanone structures (Chakkaravarthi *et al.*, 2007). In addition to the intramolecular hydrogen bonding, there is an intermolecular hydrogen bond between the phenolic group and the ketone O atom of an adjacent molecule. This O2—H102···O4^{i} (see Table 1 for symmetry code) hydrogen bond links the molecules to form infinite one-dimensional C(6) chains parallel to the *c* axis (base vector [0 0 1]). The same ketone oxygen atom therefore accepts two hydrogen bonds, one intermolecular and one intramolecular. The hydrogen bond lengths and bond angles are summarized in Table 1. Fig.2 depicts both the intermolecular and intramolecular hydrogen bonds. The length of intermolecular hydrogen bond is 0.303 Å shorter than the sum of the van der Waals radii. Although the length of hydrogen bonds does not necessarily correlate linearly with bond strength, due to packing constraints in the lattice, it is probable that this very short bond is moderate to strong. This is especially likely considering that the bond angle very closely approaches ideality.

Experimental

To a solution of 6-hydroxy-2,4-dimethoxymethyloxy-3-prenylacetophenone (80 mg, 0.25 mmol) in methanol (20 ml) was added 1.0 *M* HCl (6 ml). The reaction mixture was refluxed for 1 h before cooling. The solvent was evaporated and the residue purified by column chromatography using hexane:ethyl acetate: 2:1 to afford 1-(5,7-dihydroxy-2,2-dimethylchroman-6-yl)ethanone as yellow crystals (10 mg, 17%): mp 501–502 K;

Refinement

The positions of all hydrogen atoms bonded to C atoms were calculated using the standard riding model of *SHELXL97* (Sheldrick, 2008) with C—H(aromatic) and C—H (methylene) distances of 0.93 Å and U_{iso} = 1.2 U_{eq}, and *C*—*H*(methyl) distances of 0.96 Å and U_{iso} = 1.5U_{eq}. The phenolic hydrogen atoms were located in the difference Fourier map and allowed to refine isotropically.

Figures

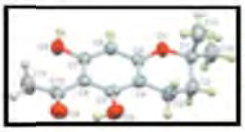

Fig. 1. The molecular structure of the title compound with 50% probability ellipsoids. Hydrogen atoms have been rendered as spheres of arbitrary radius.

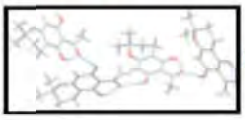

Fig. 2. Part of a hydrogen bonded (dashed lines) chain along [001].

1-(5,7-Dihydroxy-2,2-dimethylchroman-6-yl)ethanone

Crystal data

$C_{13}H_{16}O_4$	$D_x = 1.312$ Mg m^{-3}
$M_r = 236.26$	Mo $K\alpha$ radiation, $\lambda = 0.71073$ Å
Tetragonal, $P4_12_12$	Cell parameters from 2366 reflections
Hall symbol: P 4abw 2nw	$\theta = 2.9$–$26.0°$
$a = 10.5677$ (2) Å	$\mu = 0.10$ mm^{-1}
$c = 21.4244$ (5) Å	$T = 298$ K
$V = 2392.6$ (1) Å^3	Needle, colourless
$Z = 8$	0.6 × 0.4 × 0.4 mm
$F(000) = 1008$	

Data collection

Oxford Diffraction Xcalibur 2 CCD diffractometer	2366 independent reflections
Radiation source: fine-focus sealed tube	2046 reflections with $I > 2\sigma(I)$
graphite	$R_{int} = 0.047$
Detector resolution: 8.4190 pixels mm^{-1}	$\theta_{max} = 26.0°$, $\theta_{min} = 2.9°$
ω scans at fixed θ angles	$h = -13 \rightarrow 13$
Absorption correction: multi-scan (*SORTAV*; Blessing, 1995)	$k = -13 \rightarrow 13$
$T_{min} = 0.955$, $T_{max} = 0.962$	$l = -26 \rightarrow 26$
26011 measured reflections	

Refinement

Refinement on F^2	Hydrogen site location: inferred from neighbouring sites
Least-squares matrix: full	H atoms treated by a mixture of independent and constrained refinement
$R[F^2 > 2\sigma(F^2)] = 0.032$	$w = 1/[\sigma^2(F_o^2) + (0.0593P)^2 + 0.0612P]$ where $P = (F_o^2 + 2F_c^2)/3$
$wR(F^2) = 0.091$	$(\Delta/\sigma)_{max} = 0.001$

S = 1.08	$\Delta\rho_{max}$ = 0.12 e Å^{-3}
2366 reflections	$\Delta\rho_{min}$ = −0.11 e Å^{-3}
166 parameters	Extinction correction: *SHELXL*, $Fc^*=kFc[1+0.001xFc^2\lambda^3/\sin(2\theta)]^{-1/4}$
0 restraints	Extinction coefficient: 0.0094 (18)
Primary atom site location: structure-invariant direct methods	Absolute structure: Flack (1983), 931 Friedel pairs
Secondary atom site location: difference Fourier map	Flack parameter: 0.7 (11)

Special details

Experimental. ^{1}H NMR (400 MHz, CD_3OD) 1.31 (2*x* 3H, *s*, $C(CH_3)_2$), 1.78 (2*H*, *t*, *J* = 6.7 Hz, CH_2), 2.55 (2*H*, *t*, *J* = 6.7 Hz, CH_2), 2.62 (3*H*, *s*, $COCH_3$), 5.77 (1*H*, *s*, ArH); ^{13}C NMR 15.6 ($C(CH_3)_2$), 25.6 (2 × CH_2), 31.3 ($C(CH_3)_2$), 31.8 ($COCH_3$), 75.3 ($C(CH_3)_2$, 94.5 (C-5), 99.9 (C-1), 104.2 (C-3), 160.0, 160.9, 163.3 (C-2,4,6), 203.4 ($COCH_3$); ESITOFMS, *m/z* 259.0945 $[M+Na]^+$ (calc. for $C_{13}H_{16}NaO_4$ 259.0946); IR (KBr) υ 2961 2918 2872 1654 1611 1433 1159 cm^{-1}.

Geometry. All s.u.'s (except the s.u. in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell s.u.'s are taken into account individually in the estimation of s.u.'s in distances, angles and torsion angles; correlations between s.u.'s in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell s.u.'s is used for estimating s.u.'s involving l.s. planes.

Refinement. Refinement of F^2 against ALL reflections. The weighted *R*-factor *wR* and goodness of fit *S* are based on F^2, conventional *R*-factors *R* are based on *F*, with *F* set to zero for negative F^2. The threshold expression of $F^2 > 2\sigma(F^2)$ is used only for calculating *R*-factors(gt) *etc*. and is not relevant to the choice of reflections for refinement. *R*-factors based on F^2 are statistically about twice as large as those based on *F*, and *R*- factors based on ALL data will be even larger.

Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (Å^2)

	x	*y*	*z*	U_{iso}*/U_{eq}
H102	1.1657 (18)	0.6538 (18)	0.1786 (9)	0.064 (5)*
H103	0.794 (2)	0.877 (2)	0.3250 (10)	0.075 (7)*
O1	0.94129 (10)	0.98280 (11)	0.09038 (5)	0.0540 (3)
O2	1.11831 (12)	0.66838 (11)	0.21655 (5)	0.0563 (3)
C9	0.93675 (14)	0.92341 (14)	0.14598 (6)	0.0410 (3)
O3	0.77624 (12)	0.92148 (12)	0.29235 (6)	0.0619 (3)
C7	1.03346 (13)	0.76254 (13)	0.20884 (7)	0.0390 (3)
O4	0.87361 (12)	0.75511 (12)	0.36008 (5)	0.0611 (4)
C8	1.02635 (13)	0.82754 (14)	0.15389 (7)	0.0403 (3)
H8	1.0815	0.8079	0.1215	0.048*
C4	0.85141 (14)	0.95417 (14)	0.19248 (7)	0.0452 (3)
C6	0.95010 (13)	0.79073 (13)	0.25928 (6)	0.0397 (3)
C12	0.95429 (15)	0.72843 (14)	0.31906 (7)	0.0468 (4)
C5	0.86024 (13)	0.88832 (13)	0.24823 (7)	0.0427 (3)
C1	0.83994 (17)	1.07167 (15)	0.07346 (8)	0.0553 (4)
C2	0.79788 (18)	1.14369 (17)	0.12997 (8)	0.0657 (5)
H2A	0.7282	1.1988	0.1186	0.079*
H2B	0.8670	1.1966	0.1444	0.079*
C3	0.75608 (18)	1.05776 (18)	0.18283 (9)	0.0670 (5)

H3A	0.7474	1.1067	0.2209	0.080*
H3B	0.6743	1.0211	0.1730	0.080*
C13	1.0503 (2)	0.6319 (2)	0.33569 (9)	0.0717 (6)
H13A	1.0384	0.6062	0.3783	0.108*
H13B	1.1334	0.6671	0.3308	0.108*
H13C	1.0412	0.5598	0.3088	0.108*
C10	0.9031 (2)	1.15856 (19)	0.02626 (9)	0.0774 (6)
H10A	0.9746	1.1991	0.0453	0.116*
H10B	0.8437	1.2216	0.0127	0.116*
H10C	0.9307	1.1098	−0.0090	0.116*
C11	0.7364 (2)	0.9942 (2)	0.04359 (11)	0.0801 (6)
H11A	0.7687	0.9537	0.0068	0.120*
H11B	0.6672	1.0485	0.0325	0.120*
H11C	0.7076	0.9311	0.0725	0.120*

Atomic displacement parameters (Å²)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
O1	0.0605 (7)	0.0550 (7)	0.0466 (6)	0.0166 (5)	−0.0064 (5)	0.0060 (5)
O2	0.0642 (7)	0.0578 (7)	0.0469 (6)	0.0220 (6)	0.0037 (5)	0.0064 (5)
C9	0.0420 (8)	0.0391 (8)	0.0418 (7)	0.0004 (6)	−0.0102 (6)	−0.0035 (6)
O3	0.0626 (7)	0.0610 (8)	0.0621 (8)	0.0078 (6)	0.0188 (7)	−0.0060 (6)
C7	0.0381 (7)	0.0363 (7)	0.0427 (7)	0.0005 (6)	−0.0055 (6)	−0.0035 (6)
O4	0.0725 (8)	0.0614 (7)	0.0495 (6)	−0.0088 (6)	0.0142 (6)	0.0021 (5)
C8	0.0384 (7)	0.0433 (8)	0.0393 (7)	0.0037 (6)	−0.0016 (5)	−0.0030 (6)
C4	0.0408 (8)	0.0400 (8)	0.0548 (8)	0.0043 (6)	−0.0045 (7)	−0.0072 (6)
C6	0.0425 (8)	0.0359 (7)	0.0406 (7)	−0.0070 (6)	−0.0024 (6)	−0.0052 (6)
C12	0.0533 (9)	0.0437 (8)	0.0433 (8)	−0.0129 (7)	−0.0015 (7)	−0.0023 (7)
C5	0.0388 (8)	0.0403 (7)	0.0492 (8)	−0.0038 (6)	0.0028 (6)	−0.0100 (6)
C1	0.0624 (10)	0.0412 (8)	0.0622 (10)	0.0110 (7)	−0.0207 (8)	0.0042 (7)
C2	0.0660 (11)	0.0489 (9)	0.0822 (12)	0.0191 (9)	−0.0169 (10)	−0.0018 (9)
C3	0.0609 (11)	0.0643 (11)	0.0758 (12)	0.0237 (9)	−0.0025 (9)	−0.0044 (9)
C13	0.0795 (13)	0.0825 (14)	0.0532 (10)	0.0105 (10)	0.0037 (9)	0.0208 (9)
C10	0.0952 (15)	0.0560 (11)	0.0811 (13)	0.0109 (11)	−0.0145 (11)	0.0178 (10)
C11	0.0806 (14)	0.0621 (11)	0.0975 (15)	0.0070 (11)	−0.0408 (12)	−0.0017 (11)

Geometric parameters (Å, °)

O1—C9	1.3471 (18)	C1—C2	1.497 (2)
O1—C1	1.4699 (18)	C1—C11	1.509 (3)
O2—C7	1.3496 (17)	C1—C10	1.520 (3)
O2—H102	0.97 (2)	C2—C3	1.517 (3)
C9—C4	1.383 (2)	C2—H2A	0.9700
C9—C8	1.397 (2)	C2—H2B	0.9700
O3—C5	1.3432 (18)	C3—H3A	0.9700
O3—H103	0.86 (2)	C3—H3B	0.9700
C7—C8	1.365 (2)	C13—H13A	0.9600
C7—C6	1.426 (2)	C13—H13B	0.9600
O4—C12	1.2566 (19)	C13—H13C	0.9600

C8—H8	0.9300	C10—H10A	0.9600
C4—C5	1.386 (2)	C10—H10B	0.9600
C4—C3	1.502 (2)	C10—H10C	0.9600
C6—C5	1.422 (2)	C11—H11A	0.9600
C6—C12	1.441 (2)	C11—H11B	0.9600
C12—C13	1.482 (2)	C11—H11C	0.9600
C9—O1—C1	119.33 (12)	C1—C2—C3	112.68 (15)
C7—O2—H102	111.0 (11)	C1—C2—H2A	109.1
O1—C9—C4	123.42 (14)	C3—C2—H2A	109.1
O1—C9—C8	114.90 (12)	C1—C2—H2B	109.1
C4—C9—C8	121.68 (13)	C3—C2—H2B	109.1
C5—O3—H103	106.8 (14)	H2A—C2—H2B	107.8
O2—C7—C8	120.88 (13)	C4—C3—C2	110.10 (15)
O2—C7—C6	118.15 (13)	C4—C3—H3A	109.6
C8—C7—C6	120.97 (13)	C2—C3—H3A	109.6
C7—C8—C9	120.46 (13)	C4—C3—H3B	109.6
C7—C8—H8	119.8	C2—C3—H3B	109.6
C9—C8—H8	119.8	H3A—C3—H3B	108.2
C9—C4—C5	117.34 (13)	C12—C13—H13A	109.5
C9—C4—C3	120.64 (15)	C12—C13—H13B	109.5
C5—C4—C3	121.99 (14)	H13A—C13—H13B	109.5
C5—C6—C7	115.98 (13)	C12—C13—H13C	109.5
C5—C6—C12	120.00 (13)	H13A—C13—H13C	109.5
C7—C6—C12	124.01 (13)	H13B—C13—H13C	109.5
O4—C12—C6	119.89 (15)	C1—C10—H10A	109.5
O4—C12—C13	116.79 (14)	C1—C10—H10B	109.5
C6—C12—C13	123.32 (14)	H10A—C10—H10B	109.5
O3—C5—C4	115.54 (14)	C1—C10—H10C	109.5
O3—C5—C6	120.90 (14)	H10A—C10—H10C	109.5
C4—C5—C6	123.56 (13)	H10B—C10—H10C	109.5
O1—C1—C2	109.98 (12)	C1—C11—H11A	109.5
O1—C1—C11	106.62 (13)	C1—C11—H11B	109.5
C2—C1—C11	113.80 (18)	H11A—C11—H11B	109.5
O1—C1—C10	103.32 (15)	C1—C11—H11C	109.5
C2—C1—C10	111.17 (15)	H11A—C11—H11C	109.5
C11—C1—C10	111.33 (16)	H11B—C11—H11C	109.5
C1—O1—C9—C4	−9.6 (2)	C9—C4—C5—O3	179.59 (12)
C1—O1—C9—C8	170.94 (13)	C3—C4—C5—O3	1.4 (2)
O2—C7—C8—C9	178.90 (13)	C9—C4—C5—C6	−1.1 (2)
C6—C7—C8—C9	0.0 (2)	C3—C4—C5—C6	−179.29 (14)
O1—C9—C8—C7	178.31 (12)	C7—C6—C5—O3	179.24 (13)
C4—C9—C8—C7	−1.2 (2)	C12—C6—C5—O3	−1.8 (2)
O1—C9—C4—C5	−177.76 (13)	C7—C6—C5—C4	0.0 (2)
C8—C9—C4—C5	1.7 (2)	C12—C6—C5—C4	178.93 (13)
O1—C9—C4—C3	0.5 (2)	C9—O1—C1—C2	36.82 (19)
C8—C9—C4—C3	179.93 (15)	C9—O1—C1—C11	−87.00 (18)
O2—C7—C6—C5	−178.34 (12)	C9—O1—C1—C10	155.56 (14)
C8—C7—C6—C5	0.6 (2)	O1—C1—C2—C3	−55.9 (2)

O2—C7—C6—C12	2.7 (2)	C11—C1—C2—C3	63.6 (2)
C8—C7—C6—C12	−178.32 (13)	C10—C1—C2—C3	−169.70 (14)
C5—C6—C12—O4	4.3 (2)	C9—C4—C3—C2	−19.4 (2)
C7—C6—C12—O4	−176.84 (14)	C5—C4—C3—C2	158.76 (15)
C5—C6—C12—C13	−175.88 (16)	C1—C2—C3—C4	47.0 (2)
C7—C6—C12—C13	3.0 (2)		

Hydrogen-bond geometry (Å, °)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O2—H102···O4^{i}	0.97 (2)	1.77 (2)	2.737 (2)	179 (1)
O3—H103···O4	0.86 (2)	1.71 (2)	2.501 (2)	151 (2)

Symmetry codes: (i) $y+1/2, -x+3/2, z-1/4$.

Fig. 1

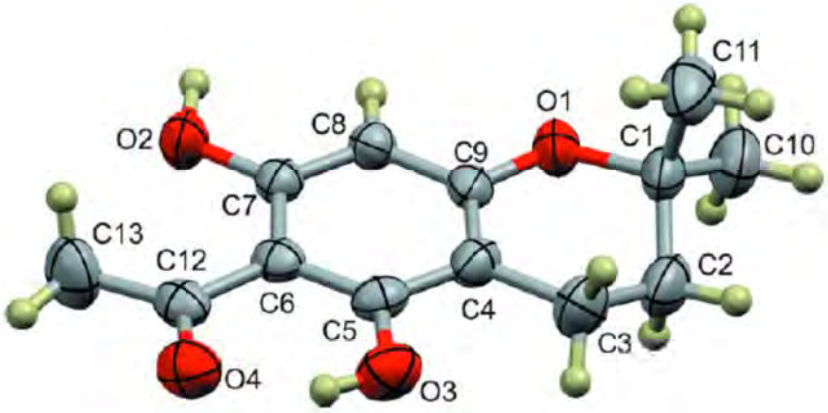

Fig. 2