SYNTHESIS OF FLAVONOIDS
AND FLAVONOID-BASED
DESIGNED MULTIPLE LIGANDS
FOR HYPERTENSION

by

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I am the author of the thesis entitled

“Synthesis of Flavonoids and Flavonoid-Based Designed Multiple Ligands for Hypertension”

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Abbreviations

$^1$H NMR  Proton Nuclear Magnetic Resonance (Spectroscopy)
$^{13}$C NMR  Carbon-13 Nuclear Magnetic Resonance (Spectroscopy)
$\beta$-MHC  Beta-myosin heavy chain
$\delta$ (ppm)  Chemical shift
$\pi$  pi (bond)
Ac  acetyl
Ar  Aromatic
AR  Analytical Reagent
ACE  Angiotensin-Converting Enzyme
AcCl  Acetyl chloride
AcOH  Acetic acid
ANF  Atrial natriuretic factor
Bn  Benzyl
BnBr  Benzyl bromide
BnOH  Benzyl alcohol
BNP  Brain natriuretic peptide
Boc  tert-Butoxycarbonyl
br  broad peak
$n$Bu  normal-Butyl
Bu$_4$NOH  Tetrabutylammonium hydroxide
Cbz-  N-Carboxybenzyl
CNS  Central nervous system
d  Doublet
DAF-FM  4-Amino-5-methylamino-2',7'-dichlorofluorescein
dd  Doublet of doublets ($^1$H NMR peak)
DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene
Cy$_2$NMe  N,N-Dicyclohexylmethylamine
DIPA  Diisopropylamine
DMAP  4-Dimethylaminopyridine
DMDO  Dimethylidioxirane
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>DMEDA</td>
<td>N,N'-Dimethylethylenediamine</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DML</td>
<td>Designed Multiple Ligand</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dppe</td>
<td>1,2-Bis(diphenylphosphino)ethane</td>
</tr>
<tr>
<td>ECE</td>
<td>Endothelin-Converting Enzyme</td>
</tr>
<tr>
<td>EDCI</td>
<td>N-3-Dimethylaminopropyl-N'-ethyl carbodiimide hydrochloride</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FDC</td>
<td>Fixed dose combination</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-fluorenylmethyloxycarbonyl</td>
</tr>
<tr>
<td>g</td>
<td>grams</td>
</tr>
<tr>
<td>HEK cells</td>
<td>Human embryonic kidney (cell line)</td>
</tr>
<tr>
<td>HepG2</td>
<td>Hepatocellular carcinoma (cell line)</td>
</tr>
<tr>
<td>HOBt</td>
<td>1-Hydroxybenzotriazole hydrate</td>
</tr>
<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectrometry</td>
</tr>
<tr>
<td>hrs/hr</td>
<td>Hours/ Hour (reaction time)</td>
</tr>
<tr>
<td>HTS</td>
<td>High throughput screening</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
<td>IL</td>
<td>Ionic Liquid</td>
</tr>
<tr>
<td>Ile-Pro-Pro</td>
<td>Isoleucine-proline-proline</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared (frequency)</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
</tr>
<tr>
<td>L</td>
<td>litres</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>LiOH</td>
<td>Lithium hydroxide</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet (¹H NMR peak)</td>
</tr>
<tr>
<td>m-</td>
<td>Meta</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl (protecting group)</td>
</tr>
<tr>
<td>MeI</td>
<td>Methyl iodide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Singlet ($^1$H NMR peak)</td>
</tr>
<tr>
<td>SARs</td>
<td>Structure-Activity Relationships</td>
</tr>
<tr>
<td>$S_N$Ar</td>
<td>Nucleophilic aromatic substitution</td>
</tr>
<tr>
<td>t</td>
<td>Triplet ($^1$H NMR peak)</td>
</tr>
<tr>
<td>TBB</td>
<td>4,5,6,7-Tetrabromobenzimidazole</td>
</tr>
<tr>
<td>$t$Bu</td>
<td>tert-Butyl</td>
</tr>
<tr>
<td>T2D</td>
<td>Type 2 Diabetes</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet (light)</td>
</tr>
<tr>
<td>Val-Pro-Pro</td>
<td>Valine-proline-proline</td>
</tr>
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</table>
Publications

Parts of this work have been, or will be published (publication appears in its entirety in Appendix One)

**Accessing highly-halogenated flavanones using protic ionic liquids and microwave irradiation**
Megan T. Thornton, Luke C. Henderson, Nolene Byrne & Frederick M. Pfeffer

**Antihypertensive, flavonoid-based Designed Multiple Ligands (DMLs)**
Megan T. Thornton, Luke C. Henderson, Frederick M. Pfeffer
*In Preparation*
Abstract

The flavonoids are a family of compounds with a C$_6$-C$_3$-C$_6$ scaffold found ubiquitously in nature. They are well known for their various therapeutic effects, including antioxidant, anticancer, antidiabetic, and antihypertensive properties. The development of new synthetic methodologies, as well as the synthesis of a library of flavonoid compounds (including 2'-hydroxychalcones, flavanones, flavones and flavonols) was investigated during this research project. Cytotoxicity evaluations of a series of 2'-hydroxychalcones offered three non-cytotoxic compounds, which were submitted for further evaluation of their antibacterial, antidiabetic and anticancer properties.

A focus of this flavonoid library was the incorporation of halogen atoms onto the flavonoid scaffold, as halogen bonding has been shown in medicinal chemistry to strengthen drug to molecular target binding. Halogenated flavonoids were also used as substrates in the Heck reaction, so as to further functionalise the flavonoid scaffolds.

The synthesis of flavonoid-based Designed Multiple Ligands (DMLs) was then attempted, combining the antihypertensive flavonol 3',4'-dihydroxyflavonol with various peptidomimetic and peptidic ACE inhibitors. Attempts were made to join 3',4'-dihydroxyflavonol to peptidomimetic enalapril analogues in a ‘conjugate’ fashion (through a linker group), through the use of four different linkers.

The synthesis of ‘fused’ DMLs, joining 3',4'-dihydroxyflavonol and di- and tripeptides directly through an ester successfully provided a total of five ‘fused’ DMLs. These included three with Fmoc-protected dipeptide ligands, and two with tripeptide ligands. These five DML compounds were submitted for biochemical evaluation of their antihypertensive properties, which is to be conducted in 2013.
Plain English Summary

The flavonoids are a family of compounds found in nature, and are synonymous with therapeutic benefits. This thesis outlines attempts to synthesise a library of novel flavonoid compounds, such that new beneficial properties might result. A particular focus of this work was the attachment of a flavonoid with antioxidant and antihypertensive properties, 3’,4’-dihydroxyflavonol, to a number of known antihypertensive compounds, so as to provide Designed Multiple Ligands (DMLs), dual action compounds for the treatment of hypertension.
Chapter One:
Introduction
1.1 Chapter Overview

The flavonoids are a broad family of polyphenolic compounds found ubiquitously in nature. They are well known for their antioxidant and therapeutic effects, and are the subject of the synthetic efforts in this project. This research aims to further functionalise flavonoids for enhanced therapeutic activity against several afflictions including cardiovascular disease, and therefore, an introduction to the flavonoids, hypertension and ACE inhibition is provided.

1.2 The Flavonoids

1.2.1 Naming and Structure

The flavonoids are a family of compounds with a C$_6$-C$_3$-C$_6$ scaffold (Figure 1.1). This structural formula makes up three rings, labelled A, B and C, to give the ‘flavan’ scaffold 1 of the flavonoids (Figure 1.1). Rings A and B are those to the left and right of the structure respectively, and Ring C is the middle ring, as it is the last ring to be closed synthetically.\(^1\) The structure is numbered as shown in Figure 1.1, with Rings A and C numbered as single digits, and Ring B numbered using prime symbols.

![Figure 1.1. The ‘flavan’ scaffold 1 is made up of Rings A, C and B.](image)

There are three main classes of flavonoid compounds which depend on the positioning of Ring B; the flavonoids (or 2-phenylbenzopyrans) 1, the isoflavonoids 2, and the neoflavonoids 3 (Figure 1.2).\(^1\) Flavonoids incorporate their Ring B at the 2-position of Ring C, isoflavonoids at the 3-position, and neoflavonoids at the 4-position.

![Figure 1.2. The three main classes of flavonoids differ by the position of Ring B.](image)
The minor flavonoids, related compounds which also contain the C₆-C₃-C₆ backbone, include the chalcones 4 (which do not have a Ring C but are used synthetically to access numerous flavonoid structures) and the aurones 5 (Figure 1.3).¹ As this research focuses on compounds in the flavonoid class of structures 1, as well as the chalcones 4, the isoflavonoids and neoflavonoids will not be discussed further.

![Figure 1.3. The minor flavonoids, chalcones 4 and aurones 5, also contain the C₆-C₃-C₆ backbone.](image)

Within the flavonoid class, further structural subclasses exist. These subclasses are distinct from one another depending on the functionalities of the 2-, 3- and 4-positions of Ring C. One such subclass are the flavanones 6 (Figure 1.4), which incorporate a single bond between the 2- and 3-positions (corresponding to the –an– of flavanone), and a ketone functionality at the 4-position (corresponding to the –one of flavanone). Similarly, flavones 7 incorporate an alkene at the 2-position and a ketone at the 4-position (–one), and flavonols 8 are flavones with an additional hydroxyl group at the 3-position (flavonol) (Figure 1.4). Not all flavonoids follow this somewhat systematic naming system; for example, the anthocyanins 9 are charged compounds with an alkene between positions 2 and 3, and a hydroxyl group at the 3-position (Figure 1.4).¹

![Figure 1.4. Subclasses of flavonoids include the flavanones 6, flavones 7, flavonols 8, and anthocyanins 9.](image)

Many flavonols are fluorescent, often exhibiting orange or yellow fluorescence under UV light,² and other flavonoids are brightly coloured due to their high degree of conjugation. Anthocyanins, including pelargonidin 10, cyanidin 11, and delphinidin 12, are plant pigments in flowers responsible for their orange, red, blue, and violet colouring.³⁵ These flavonoids are important to plants as they assist in attracting insects to food, support pollination, and even protect against fungal parasites, pathogens, and oxidative cell injury.⁶
1.2.2 Natural Sources

The flavonoids are plant-specific secondary metabolites, in that their production in plants is not vital to the survival of their cells. They occur ubiquitously in plants, fruits and vegetables, with over 9000 structures discovered to date. Flavonoids exist in many of the foods and beverages we consume every day; from citrus fruits to licorice, onions to soy beans, and even red wine and chocolate.

The pathways through which flavonoids are synthesised in plants have been thoroughly examined (Scheme 1.1). There are numerous enzymes responsible for converting simple, hydroxylated starting materials into the various types of flavonoids, including flavanones, flavones and flavonols, as well as the colourful anthocyanins.
Scheme 1.1. Different types of flavonoids are synthesised by various enzymes in plants. Natural flavonoids are hydroxylated only at certain positions. These are the 5- and 7-positions of Ring A, and the 4'-/3',4'-/5',4',5'-positions of Ring B.

Quercetin, a flavonol (3-hydroxy) with hydroxyl groups at the 5-, 7-, 3'-, and 4'-positions, is the most prevalent flavonoid in nature (Figure 1.6). Methoxyl, sulfonl, and malonyl substitutions are also common in natural flavonoids.

CHS, chalcone synthase; CHKR, chalcone polyketide reductase; CHI, chalcone isomerase; FHT, flavanone 3β-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase; FGT, flavonoid glycosyltransferase; FNS, flavone synthase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidins reductase; IFS, isoflavone synthase; IFD, isoflavone dehydratase.
Flavonoids are found in both their glycosidic (attached to a sugar molecule) and aglycone (non-sugar) forms in natural sources. Quercitrin (3-O-rhamnose) and rutin (3-O-rutinose disaccharide) are two naturally-occurring glycosides of the flavonol quercetin (Figure 1.7).^{15, 16}

1.2.3 Flavonoids Throughout History

Flavonoids have long been recognised for their therapeutic benefits. Propolis, a substance that bees extract from plants to use as a type of ‘glue’ in their hives, is a concentrated form of flavonoids^{17, 18} which has been used for centuries for its antiseptic, antimycotic, bacteriostatic, astringent, choleric, spasmylytic, anti-inflammatory, anaesthetic and antioxidant properties.^{2, 6, 17} The Old Testament (propolis was known as ‘tzori’ in Old Hebrew) and the Koran have both referred to the use of propolis as a remedy, and it was also prescribed by Hippocrates (460-377 BC) to treat sores and ulcers.^{2, 19} The presence of flavonoids in many plants and foods used in traditional herbal remedies also demonstrates their capabilities as treatments for human ailments. These remedies include the use of licorice as a traditional Chinese medicine to treat tuberculosis,^{10} and Huangqin (a tree root rich in flavonoids) for the treatment of various inflammatory diseases, hepatitis, tumours and diarrhoea in East Asian countries.^{20}
Between the 1920’s and 1950’s, an extract from lemon juice named ‘citrin’ which contained the flavanone glycoside hesperidin \(16\) gave rise to the proclamation that flavonoids were a vitamin (due to its treatment of scurvy), and flavonoids were given the title of ‘Vitamin P’ (Figure 1.8).\(^{21, 22}\) This title was later removed as researchers were unsure which polyphenol was responsible for the therapeutic effect, and because earlier claims of reduction in haemorrhage and scurvy could not be substantiated in later research.\(^{23, 24}\)

![Figure 1.8. ‘Citrin’, a flavonoid extract from lemons which contained hesperidin 16, gave flavonoids the title of ‘Vitamin P’.](image)

While ‘vitamin’ status was taken from the flavonoids, they are often referred to as nutraceuticals, or ‘a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease’.\(^{25}\) While the intake of flavonoids by humans has been researched, much is still unknown.

### 1.2.4 Flavonoids in Food: Content and Intake

Flavonoids are present in many plants and foods, although the types of flavonoid and their quantities vary considerably between sources. For example, soybeans contain the isoflavone genistein \(17\) in a concentration of approximately 350 \(\mu\)g per gram of soybean.\(^{26}\) Meanwhile, onions contain up to 486 \(\mu\)g of quercetin \(13\) per gram of fresh edible part, and mushrooms, cucumber and radish contain less than 1 \(\mu\)g of quercetin per gram of vegetable (Figure 1.9).\(^{27}\) Flavonoid content in fruits and vegetables also differ between varieties; cherry tomatoes contain six times more quercetin than normal tomatoes,\(^{28, 29}\) and Granny Smith and Red Delicious contain more procyanidins than Golden Delicious apples.\(^{30}\) Concentrations even vary between seasons.\(^{27}\) These variations are the result of different flavonoid synthesis enzymes being available in different sources, and the storage of flavonoids in the skin of fruits and vegetables, leading to higher concentrations in smaller fruits like tomatoes.\(^{12}\) There are also discrepancies on the flavonoid content of foods between publications, due a lack of agreement on an appropriate analytical method.\(^{12}\)
Figure 1.9. Genistein 17 and quercetin 13 are found in high concentrations in soybeans and onions respectively.

The intake of flavonoids by humans is also subject to variance between publications, once again due to discrepancies between analytical methods. In 1976, Kühnau published that the average dietary flavonoid intake of people in the USA was approximately 1g per day. This data was based on the concentrations of flavonoids in foods as acquired using analytical methods of the time (ultraviolet and visible light spectroscopy, NMR spectroscopy, paper and thin layer chromatography, gas liquid chromatography), however such food analysis techniques are now obsolete.

In 1993, a study by Hertog utilised data on the flavonoid content of foods as determined by high-performance liquid chromatography, a far more reliable method. This research studied five particular flavonoids (the flavonols kaempferol 18, quercetin 13, and myricetin 19, and the flavones luteolin 20 and apigenin 21, Figure 1.10), as these were most commonly investigated in anticarcinogenesis studies at the time. It was determined that the average intake of these five flavonoids by people in the Netherlands was approximately 23 mg per day, a far cry from Kühnau’s 1g per day.

The fact that flavonoids are present in many of the foods we eat daily establishes a good safety profile. However, chronic pharmacological doses (greater than those of the estimated dietary intake of 23-170 mg per day) may cause side effects. Examples include cyanidanol 22 and sciadopitysin 23 (Figure 1.11), which cause acute renal failure (loss of kidney function), hepatitis (inflammation of the liver), fever, and skin reactions at extremely high doses (≥500 mg).
1.2.5 The Absorption and Bioavailability of Flavonoids

Whilst humans consume flavonoids on a daily basis from food and beverages, there are numerous problems regarding the ability of the body to metabolise them, and they are generally poorly bioavailable.\(^3^6\)

The absorption of flavonoids was originally thought to occur only through the large intestine, however the discovery of quercetin 13 in plasma by researchers suggested absorption also from the small intestine.\(^1^3\) Knowing that flavonoids are often in their glycosidic forms in plants, the presence of glucosidases in the small intestine suggested that flavonoid glycosides are cleaved in the lumen and cells of the gut prior to absorption.\(^3^6^-^3^9\) Deglycosylated flavonoids from celery are absorbed better than their glycoside counterparts, suggesting that deglycosylation increases absorption.\(^4^0\) However, anthocyanins and some quercetin glycosides have been found in their glycosidic forms when excreted in urine.\(^1^6, 4^1, 4^2\)

Following deglycosylation, flavonoids are often conjugated to form a more polar compound for rapid excretion: the formation of flavonoid glucuronides, sulfates, and even a mixture of the two (sulfoglucuronides) is common.\(^3^6, 3^9, 4^1^-^4^3\) Also, the methylation of hydroxyl groups in the liver and intestinal wall is possible, especially with quercetin 13.\(^4^4\) It is in these forms that flavonoids are often found in urine.

Conflicting data is rife regarding the presence of flavonoids in plasma; quercetin 13 is one such example. Hollman \textit{et. al.} found quercetin aglycone in plasma samples in concentrations of approximately 64 mg after ingestion of fried onions,\(^4^5\) and analysis of absorption of quercetin from its glycosidic and aglycone forms relied on blood plasma concentrations to determine that the aglycone was more quickly absorbed.\(^4^6, 4^7\) However, other studies have returned results whereby no quercetin aglycone could be found in plasma, even after consuming up to \textit{four grams} of the \textit{pure} flavonoid.\(^4^8, 4^9\)
As only general, and often conflicting, data is available regarding the absorption and destination of flavonoids in the body, it is no surprise that a thorough review in 2009 on the bioavailability of flavonoids stated that ‘much is still unknown about membrane transporters involved in the gastro-intestinal absorption and tissue distribution of flavonoids’. Nevertheless, research on the isotopic labelling of flavonoids has proven that structural differences and the different chemical properties of flavonoids (such as polarity) do have an effect on the route of their absorption.

The isotopic labelling of flavonoids using \(^2\text{H}\), \(^3\text{H}\), \(^{13}\text{C}\) and \(^{14}\text{C}\) has been conducted in the interests of determining absorption and distribution routes in the body (Figure 1.12).\(^{50-52}\) While a comprehensive review of the isotopic labelling of flavonoids is outside the scope of this thesis, a thorough review by Barron is recommended.\(^{52}\) Interestingly, when isotopically-labelled flavonoids were investigated in animal models, different flavonoids followed different routes of absorption, distribution and elimination, as well as different concentrations of uptake.\(^{52}\) This supports research suggesting that those flavonoids found most abundantly in food are not necessarily those found in the greatest concentration in the body.\(^{36}\)

Figure 1.12. \(^{13}\text{C}\)-labelled (±)-catechin 24 and \(^{13}\text{C}\)-labelled quercetin 3-glycoside 25 have been synthesised for transport and metabolism studies.

Research has also shown that flavonoid absorption varies between people. Two separate studies regarding the absorption and excretion of flavonoids from green tea showed interindividual differences, with some volunteers showing 100% absorption/excretion, and others less so.\(^{53,54}\)

One of the reasons for the low bioavailability of flavonoids is their lack of solubility in water.\(^{55,57}\) This is due to the planar conformation of flavonoids, which allows \(\pi-\pi\) stacking, an occurrence supported by crystal structures (Figure 1.13).\(^{58}\) This stacking has implications on flavonoid bioavailability; research regarding the accumulation of quercetin 13 in plasma after delivery in different solvent vehicles revealed reduced uptake when administered in water.\(^{57}\)
This lack of solubility plagues the use of flavonoids as pharmaceuticals, however numerous methods have been discovered to circumvent the problem. Examples have included the formation of flavonoid salts, complexation with cyclodextrin, co-administration with lipids and emulsifiers, and the formation of semisynthetic analogues incorporating acetate, propionate, and palmitol side chains. As the solubility issue can be addressed, there are no longer bioavailability issues regarding the use of flavonoids as therapeutic agents.

1.2.4 Confirmed Therapeutic Properties

As outlined in Chapter 1.2.3, the flavonoids have long been associated with the treatment of disease. Recent scientific research has confirmed the therapeutic benefits of the flavonoids against numerous diseases. In fact, a study of the flavonoid intake and health of over 10,000 men and women in Finland verified that a decreased risk of chronic diseases including cancer, Type 2 Diabetes (T2D), mortality from ischemic heart disease and even asthma, is associated with increased dietary flavonoid intake. For the sake of brevity, only four major therapeutic properties of the flavonoids will be discussed here; antioxidant, anticancer, antidiabetic, and cardiovascular activities. It is worth noting that flavonoids have also been associated with antiobesity, antiallergic, asthma, antimicrobial, neuroprotective, and antialcoholism properties amongst many others.

1.2.4.1 Antioxidant Properties

Oxygen-centred free radicals, also known as reactive oxygen species (ROS), cause damage to cells in the body by attacking lipids in cell membranes, proteins in tissues of enzymes, carbohydrates, and DNA, causing membrane and DNA damage, and protein modification (including enzymes). This damage plays a causative role in aging and several degenerative diseases associated with it, such as heart disease, cataracts, and cancer. ROS radicals include superoxide (O$_2^-$), peroxyl (ROO$^•$), alkoxy (RO$^•$), hydroxyl (HO$^•$), and nitric oxide (NO$^•$).
This oxidant damage can be avoided if other compounds are present to react with ROS, thereby terminating the radical process before it can affect the cells. The flavonoids are well known for their antioxidant properties, and many foods and beverages containing flavonoids are associated with, and marketed for, their antioxidant potential, especially teas and wine. This activity is strongly associated with the phenols present in natural flavonoids.

Flavonoids provide this antioxidant activity through a number of pathways. Flavonoids inhibit enzymes that produce superoxide anions, such as xanthine oxidase and protein kinase, and may also chelate to trace metals, preventing the metal’s ability to catalyse the formation of ROS such as the hydroxyl radical. As well as preventing the formation of ROS, flavonoids also scavenge ROS: flavonoids such as quercetin rapidly react with peroxyl radicals, forming a flavonoid radical, which then reacts with other free radicals to form a quinone and thus terminate the reaction (Scheme 1.2).

The relationship between the structures of natural flavonoids and their antioxidant potential has been intensively studied, to the point where the free radical scavenging activity of flavonoids can be predicted. Generally, more hydroxyl groups on a flavonoid correlates with an increase in antioxidant activity. More specifically, there are three key structural features that increase radical scavenging ability of flavonoids (Figure 1.14): (i) an ortho-dihydroxy structure on Ring B, shown in blue, which scavenges reactive radical species and also chelates metals which promote oxidation; (ii) a 2,3-double bond with a hydroxyl at the 3-position and a ketone at the 4-position of Ring C, shown in green, which provides a more stable flavonoid
radical through conjugation and electron delocalisation, and (iii) hydroxyl groups at the 5- and 7-positions of Ring A, shown in red, which also scavenge radical species and prevent lipid oxidation. The combination of these criteria, which result in the structure of quercetin, produce a Trolox Equivalent Antioxidant Capacity (TEAC) score almost five times greater than the antioxidant activity of Vitamin E. It is noted that flavonoid glycosides do not portray the antioxidant activity of their aglycones.

Figure 1.14. Antioxidant activity of the flavonoids can be attributed to three structural criteria.

Although it is mainly the natural flavonoids that have been examined for their antioxidant capacity, this does not mean that synthetic examples are unresearched; the synthetic flavanone 3'-hydroxyfarrerol (also known as IdB1031, Figure 1.15), which fulfils criteria i. and iii. for antioxidant activity of flavonoids, inhibits lipid peroxidation to approximately the same level as quercetin.

Figure 1.15. The synthetic flavanone 3'-hydroxyfarrerol has antioxidant properties.

1.2.4.2 Anticancer Properties

Cancer, the unregulated growth of abnormal cells in the body, is the second leading cause of death (after cardiovascular disorders). The flavonoids have been well documented in regards to anticancer activity. Treatment of many types of cancer (including colon, breast, and prostate cancers) has been undertaken, in vitro and in vivo, using pure flavonoids and foods high in flavonoid content, and for the treatment of both chemical- and UV-induced cancers.

Kuo published a thorough review regarding the cancer preventative activity of dietary flavonoids, and recognised three possible results from the treatment of cancer with flavonoids: (i) a decrease in tumour incidence; (ii) the inhibition of tumour progression; and (iii) the enhancement of the antiproliferative activity of other
chemotherapeutic agents when administered in combination. However, extreme doses of flavonoids (far greater than those found in foods) have been linked to an increase in cancer incidence. The link between intake of foods high in flavonoid content and anticancer activity has been well researched, with low incidences of lung cancer and breast cancer associated with increased flavonoid intake. Isolated natural flavonoids have also been extensively studied, and genistein has been noted for its antileukemic and antitumour (breast cancer) activity (Figure 1.16). Quercetin in particular has undergone Phase I Clinical trials for in vivo tyrosine kinase inhibition (tyrosine kinases are known to cause unregulated cell growth).

![Figure 1.16. Genistein 17 and quercetin 13 exhibit anticancer activity.](image)

Synthetic flavonoids have also been studied for their anticancer activities, however none to date have had the success of flavopiridol. Flavopiridol is structurally based on the natural product rohitukine from the plant Dysoxylum binectariferum (Figure 1.17), and has the drug name Alvocidib. It is a potent inhibitor of cyclin-dependent kinases (CDK), which when inhibited in cancer cells, prevent cancer cell growth. Flavopiridol has undergone Phase I and II trials for anticancer effects, including breast cancer, chronic lymphocytic leukaemia, and a range of other tumours, and is reportedly in Phase III trials under the pharmaceutical company Sanofi Aventis.

![Figure 1.17. Flavopiridol 29, based on the natural product rohitukine 30, is an anticancer agent.](image)
1.2.4.3 Antidiabetic Activity

Diabetes is a chronic condition currently affecting over 235 million people worldwide,\textsuperscript{153} with millions of people remaining undiagnosed.\textsuperscript{153, 154} This steadily growing epidemic is expected to escalate to over 439 million people by the year 2030.\textsuperscript{153} Alarmingly, over 3.2 million people die each year from complications associated with diabetes.\textsuperscript{155} Diabetes is clinically characterised by an excess of glucose in the blood, a condition known as hyperglycaemia,\textsuperscript{154, 156} which results from the impaired action of the hormone insulin.\textsuperscript{157}

There are many ways in which to treat diabetes, including the protection of the β-cells in the pancreas (which produce insulin), the stimulation of insulin production by the β-cells, and the stimulation of glucose uptake into cells to remove excess glucose from the blood (through the targeting of receptors such as the Peroxisome Proliferator-Activated Receptors).\textsuperscript{154} Due to the link between oxidative stress and diabetic complications,\textsuperscript{158} it is understandable that many antioxidant flavonoids have been trialled for antidiabetic activity.\textsuperscript{159-161}

A study of over 200,000 people in the United States with over 3.64 million person years of follow up found that higher consumption of anthocyanins and anthocyanin-rich fruit was associated with a lower risk of Type 2 Diabetes.\textsuperscript{162} Extracts from food sources high in flavonoid content, including jambolana seeds,\textsuperscript{163} licorice,\textsuperscript{70, 164} chamomile tea,\textsuperscript{165} and soy beans,\textsuperscript{166, 167} have also been analysed for antidiabetic activity.\textsuperscript{168, 169}

Natural isolated flavonoids have been examined for antidiabetic activity through \textit{in vivo} and \textit{in vitro} testing.\textsuperscript{69, 159, 170-178} The anthocyanins in particular exhibit a number of beneficial properties in terms of Type 2 Diabetes, including inhibition of α-Glucosidase, an enzyme which cleaves glycosidic bonds, resulting in higher glucose levels in blood (α-Glucosidase is the target of the current pharmaceutical acarbose)\textsuperscript{154}. Anthocyanins have also been shown to protect the β-cells which produce insulin, and increase their production of insulin.\textsuperscript{179, 180} Quercetin \textbf{13} has been shown to protect β-cells from damage in rat pancreas,\textsuperscript{181-183} enhance insulin release in rat pancreas cells,\textsuperscript{184} and improve the glucose uptake of cells (thereby reducing plasma glucose levels).\textsuperscript{160} Kaempferol \textbf{18}, which is structurally similar to quercetin,\textsuperscript{160, 185} and genistein \textbf{17}, an isoflavone from soy beans,\textsuperscript{183, 186, 187} also exhibit antidiabetic effects in \textit{in vivo} and \textit{in vitro} testing (Figure 1.18).
Figure 1.18. Quercetin 13, kaempferol 18 and genistein 17 all exhibit antidiabetic properties.

Novel synthetic flavonoids have also been examined for antidiabetic activity, including novel chalcones and isoflavones as agonists of PPARα and PPARγ.

1.2.4.4 Cardiovascular/ Heart Disease

Cardiovascular diseases are the leading cause of death worldwide. They are defined as those diseases which affect the heart and blood vessels, and include atherosclerosis (the blocking of arteries by fatty deposits), inflammation, coronary heart disease, and hypertension (increased blood pressure).

The flavonoids have been researched at length for their ability to prevent or reduce cardiovascular problems. The most significant study in regards to flavonoid intake and coronary heart disease is the Zutphen Elderly Study. The study examined the medical history and flavonoid intake levels of quercetin, kaempferol, myricetin, apigenin and luteolin, of 805 men between 65 and 84 years old in 1985, with medical follow up for five years. There was a significant inverse correlation between flavonoid intake (mainly from tea, onions and apples in the diet) and the risk of coronary heart disease. This correlation has been further supported by other short term studies which have concluded that people with low flavonoid intake have higher risks of coronary disease.

Figure 1.19. Naringenin 31 exhibits antiatherosclerotic effects, whilst taxifolin 32 has anti-inflammatory effects.

Citrus flavonoids including naringenin 31, and other natural flavonoids have been analysed for their antiatherosclerotic properties. The anti-inflammatory effects of some flavonoids have also been investigated, including the flavanol taxifolin 32, novel alkoxyflavonols, and others. The anthocyanins have
proven ideal in the treatment of many cardiovascular diseases, with a thorough review by Wallace describing their many effects.\textsuperscript{197}

Hypertension, which falls under the category of cardiovascular disease, is the therapeutic focus of this research and so will be addressed in more detail.

\subsection*{1.3 Hypertension}

Hypertension is the clinical term for blood pressure exceeding 140/90 mmHg.\textsuperscript{193} It occurs due to increased blood volume in the cardiovascular system, or the vasoconstriction (narrowing) of arterioles, and is the result of genetic predisposition or lifestyle.\textsuperscript{212} Hypertension is the leading cause of stroke,\textsuperscript{213, 214} and is estimated to cause 7.1 million premature deaths worldwide per year.\textsuperscript{212, 213} It also constitutes 4.5\% of the disease burden (64 million disability-adjusted life years, or DALYs), affecting over 800 million people worldwide.\textsuperscript{213, 215} This epidemic is only expected to become worse: hypertension is predicted to affect over 1.56 billion people by the year 2025 (Figure 1.20).\textsuperscript{214}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{hypertension_figure.png}
\caption{Frequency of hypertension for people over the age of 20 years, in 2000 and 2025.\textsuperscript{214}}
\end{figure}

The risk of developing hypertension increases with age, to the point where the probability of developing hypertension from 50 to 85 years of age is 90\%.\textsuperscript{216} For all its frequency, however, hypertension is dubbed the ‘silent killer’, often showing no symptoms until it is too late.\textsuperscript{215} Studies in the USA have shown that up to 30\% of American adults are unaware they have hypertension, 40\% of those with hypertension are not using any form of treatment, and a further two thirds of those with hypertension are unable to effectively manage their blood pressure through treatment.\textsuperscript{193} However,
with proper management and treatment (lifestyle, exercise, and/or pharmaceutical), hypertension is a manageable condition.

1.3.1 The History of Antihypertensive Agents

Hypertension has plagued humankind for centuries, dating back to 2600 BC when it was described as ‘hard pulse disease’. Early treatments included acupuncture, venesection, and bleeding by leeches. A traditional Chinese medicine using the tree root of the Mudan plant (which contains polyphenols) has also been used for at least 2000 years for the treatment of high blood pressure.

The discovery that hypertension was due to improper blood flow is credited to Richard Bright, who associated left ventricular hypertrophy (the thickening of the muscle of the left ventricle of the heart) with high blood pressure in 1836. This was quite impressive, as a method to measure blood pressure would not be discovered for another 50 years, by von Basch and von Recklinghausen in Germany in the 1880s. The term ‘hypertonic essential’ was then coined by Frank in 1925, which translated to essential (meaning ‘with unknown cause’) hypertension.

Until the 1950s, only the Smithwick splanchnicectomy (to remove a portion of the splanchnic nerves), and the Kempner low-salt rice diet existed in the way of treatments for hypertension, although neither had any real beneficial effect on patients. The first effective treatment was the ganglion-blocking drugs, including hexamethonium and pentolinium (Figure 1.21), which blocked the parasympathetic and sympathetic nervous system, but were later dismissed due to a number of side effects (including hypotension and severe heart palpitations) and the availability of more specific antihypertensive drugs.

Although lifestyle changes, including exercise, weight loss, and change of diet, are the first port of call in terms of hypertension treatment, these changes do not suit everyone. Therefore, numerous blood-pressure lowering pharmaceuticals have emerged, and can be categorised into five classes. These are the thiazide diuretics, beta-blockers, calcium channel blockers, Angiotensin II receptor antagonists, and the Angiotensin Converting Enzyme (ACE) inhibitors. As shown in Table 1.1, each has a
different mode of action, and unfortunately, each pharmaceutical comes with its own side effects.

<table>
<thead>
<tr>
<th>Pharmaceutical type</th>
<th>Structural example</th>
<th>Mode of action</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazide Diuretics</td>
<td>Chlorothiazide,</td>
<td>Promote excretion of water to decrease total blood volume</td>
<td>Hyperglycaemia, hyponatraemia, postural hypotension, gout, impotency, cardiac arrhythmias, increased thirst</td>
</tr>
<tr>
<td>(Chlorothiazide, Metolazone)</td>
<td>Metolazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-Blockers</td>
<td>Atenolol, Carvedilol</td>
<td>Inhibit β-adrenoreceptors to reduce heart rate</td>
<td>Dyspnea (laboured respiration), sleep disturbances and vivid nightmares, erectile dysfunction, bronchial restriction, lethargy</td>
</tr>
<tr>
<td>Calcium Channel Blockers</td>
<td>Verapamil, Diltiazem</td>
<td>Block Ca\textsuperscript{2+} channels to reduce cardiac muscle contraction</td>
<td>Constipation, headache, flushing, oedema, postural hypotension, abdominal pain</td>
</tr>
<tr>
<td>Angiotensin II Receptor Antagonists</td>
<td>Losartan, Candesartan</td>
<td>Block binding of angiotensin II to AT1 receptors, to prevent bradykinin destruction and cause vasodilation</td>
<td>Hyperkalaemia, postural hypotension</td>
</tr>
<tr>
<td>ACE Inhibitors</td>
<td>Captopril, Enalapril</td>
<td>Bind Angiotensin-Converting Enzyme (ACE) to cause vasodilation</td>
<td>Postural hypotension, cough, hyperkalaemia, hyponatraemia,</td>
</tr>
</tbody>
</table>

1.3.2 Angiotensin-Converting Enzyme (ACE)

Of the most interest to the research performed in this project are the Angiotensin-Converting Enzyme (ACE) inhibitors. ACE was first isolated from horse blood in 1956, and labelled a “hypertensin-converting enzyme”.\textsuperscript{231} This chloride-dependent metallopeptidase is a part of the Renin-Angiotensin-Aldosterone System (RAAS), and exists in the endothelium of the vascular bed, kidney, and lung.\textsuperscript{232}

The Renin-Angiotensin-Aldosterone System is responsible for the regulation of blood pressure (Figure 1.22).\textsuperscript{233} When blood volume is low, the kidneys secrete the
enzyme renin, which converts angiotensinogen (an \( \alpha \)-globulin protein produced by the liver) into the decapeptide Angiotensin I. The role of ACE is to cleave the decapeptide Angiotensin I to form the octapeptide Angiotensin II.\(^{233}\)

![Diagram of the Renin-Angiotensin-Aldosterone System](image)

Figure 1.22. The Renin-Angiotensin-Aldosterone System regulates blood pressure.\(^{233}\)

Angiotensin II ultimately affects blood pressure in four ways: increased thirst leading to increased blood volume, the degradation of bradykinin (a natural antihypertensive present in the body), systemic vasoconstriction, and the production of aldosterone and the antidiuretic hormone (ADH) vasopressin, which reabsorb water and sodium into the blood to increase blood volume.\(^{232, 234, 235}\) While the action of ACE is useful in terms of increasing blood volume and therefore blood pressure, it is detrimental to those already experiencing high blood pressure. The inhibition of ACE was therefore seen as a possible treatment for hypertension.

### 1.3.3 ACE Inhibitors

In the late 1960s, the first ACE inhibitor was discovered; a nonapeptide known as ‘teprotide’\(^{41}\) from snake venom (of *Bothrops jararaca*, a Brazilian pit viper), which was noted to cause a sudden drop in blood pressure when administered to humans.\(^{236-238}\) Further peptide-based research and Structure Activity Relationship studies by Ondetti and Cushman revealed that ACE contained three important ‘pockets’ for binding to inhibitors (Figure 1.23); one for binding a carboxyl group at the C-terminal (S2’), a second pocket with affinity for the C-terminal peptide bond (S1’), and a tightly bound
zinc ion that could co-ordinate with the carbonyl of the penultimate peptide bond (Zn$^{++}$).\textsuperscript{239}

This significant find lead to two different research approaches to ACE inhibition; peptidomimetic ACE inhibitors and peptidic ACE inhibitors.

### 1.3.3.1 Peptidomimetic ACE Inhibitors

Targeting three key binding pockets of ACE, captopril \textsuperscript{39} (Figure 1.24), a peptide-based structure with a sulfhydryl group to bind the zinc ion (Figure 1.23), was the first peptidomimetic ACE inhibitor to be synthesised.\textsuperscript{241}

It was soon discovered that captopril, whilst being an extremely potent competitive inhibitor of ACE (with an IC$_{50}$ of 2.0 × 10$^{-8}$ M),\textsuperscript{242} produced a number of side effects in patients. These included bone marrow suppression (a decrease in cells responsible for oxygen transport and blood clotting),\textsuperscript{243} nephrotic syndrome (kidney damage resulting in protein being leaked from blood to urine),\textsuperscript{244} rashes, and loss of taste amongst others, which were all associated with the sulfhydryl moiety of captopril.\textsuperscript{242, 245} Research began for a second generation of ACE inhibitors, and in 1980, enalaprilat \textsuperscript{42} became available, in which the sulfhydryl was replaced with a carboxylic acid to bind the zinc ion of ACE, and a phenyl group was incorporated to bind the
S1 pocket of ACE (Figure 1.25). Enalapril 42 was found to be even more potent than captopril (with an IC$_{50}$ of $1.2 \times 10^{-9}$ M), and avoided the sulfhydryl side effects.  

Unfortunately, enalapril 42 is poorly bioavailable when taken orally. To increase its bioavailability, enalapril is administered as its ethyl ester, known as enalapril 43 (Figure 1.26). Wyvratt and Patchett proposed that the installation of this ethyl ester assists in membrane transport in two ways: (i) by removing the charge of the carboxylate group and increasing lipophilicity, and (ii) by lowering the pK$_a$ of the NH group. The ethyl group makes enalapril a prodrug; prodrugs are inactive forms of a pharmaceutical compound (usually inactive to as to provide a benefit such as greater bioavailability) which \textit{in vivo}, by virtue of enzymatic and/or chemical lability, supply the active compound (in this case enalaprilat 42) to the body.

Many peptidomimetic ACE inhibitors have since been synthesised, including ramipril 44 and lisinopril 45. These ACE inhibitors contain the same structural pharmacophores as captopril 39 and enalapril 43 (Figure 1.27); proline or a structurally related compound to bind the S2’ pocket (blue), an amide bond to the proline with an alkyl side chain for the S1’ pocket (green), a chelator to bind the zinc pocket (red), and often a phenyl group to bind the S1 pocket (purple).
Figure 1.27. Peptidomimetic ACE inhibitors follow a general pharmacophoric structure.

Many other molecular structures have been investigated for their ACE inhibition. Hydrophobic ACE inhibitors have clinical superiority over hydrophilic ones, as while both are able to bind the C-terminal (S2') catalytic site, only hydrophobic structures are able to bind the N-terminal (S1) catalytic site.\textsuperscript{248} Antioxidant ACE inhibitors are also of particular interest in terms of treating hypertension, as hypertension and oxidative stress are interdependent.\textsuperscript{249-251} Examples have included selenium analogues of captopril (Figure 1.28),\textsuperscript{250, 252} as well as enalapril and captopril themselves.\textsuperscript{253-255} The Heart Outcomes Prevention Evaluation (HOPE) and Study to Evaluate Carotid Ultrasound changes in patients treated with Ramopril and Vitamin E (SECURE) studies have demonstrated that long term ACE inhibition reduces cardiovascular events, suggesting that the therapeutic effects of ACE inhibitors extend to the reduction of oxidative stress.\textsuperscript{256-259}

Figure 1.28. Selenium analogues of captopril 46, 47, and 48 exhibit antioxidant and ACE inhibiting effects.

As these peptidomimetic ACE inhibitors alter the peripheral action of hormones rather than the central nervous system, they avoid many of the side effects that plague other antihypertensive agents, such as drowsiness, impotence, depression and energy loss.\textsuperscript{245} However, this does not exclude ACE inhibitors from side effects (other than those specifically associated with captopril’s sulfhydryl group): hypotension (low blood pressure), a persistent cough, increased potassium levels and decreased sodium levels in the blood are commonly associated with ACE inhibitors.\textsuperscript{230}
1.3.3.2 Peptidic ACE Inhibitors

Following the lead from teprotide 41 (the nonapeptide from snake venom), extensive research has been conducted regarding the isolation of ACE-inhibiting peptide sequences from natural sources, such as salmon and krill,260-262 peanuts (IKP),263 garlic,264 algae265, 266 and eggs,267 amongst others.268-270 A number of peptides from fermented milk, including the tripeptides Isoleucine-Proline-Proline (Ile-Pro-Pro) 49 and Valine-Proline-Proline (Val-Pro-Pro) 50, have demonstrated ACE inhibiting activity in both animal270-272 and human trials (Figure 1.29).273, 274 Indeed, a product containing both Ile-Pro-Pro 49 and Val-Pro-Pro 50 has been commercialised.275

As with the peptidomimetic ACE inhibitors, the binding of peptidic ACE inhibitors is strongly influenced by the C-terminal tripeptide.232 Predictive modelling for ACE inhibitor peptides using databases has found that for tripeptides, the most favourable residues for the C-terminal were aromatic amino acids, while positively-charged amino acids were preferred for the middle position, and hydrophobic amino acids were preferred for the N-terminal.275

A major problem with peptidic ACE inhibitors is their lack of bioavailability, which is the result of their susceptibility to digestion (hydrolysis) during gastrointestinal passage absorption, and their low intestinal permeability.276 Many in vitro tests do not take into account the low bioavailability of peptides;232 consequently, only a minority of ACE-inhibiting peptides administered will actually reach the site of action.276 This problem can be overcome however, with the strict selection of amino acids: dipeptides with proline, serine, threonine and asparagine at the C-terminal and proline, glycine and asparagine at the N-terminal are more stable to peptide hydrolysis.276

1.3.4 Flavonoids in Hypertension

The flavonoids have been investigated in terms of their antihypertensive properties, especially as the antioxidant activity of flavonoids contributes to their cardioprotective activity.100, 199, 206, 277 Plants and foodstuffs rich in flavonoids, such as tea,195 grape juice,278 soybeans,279 propolis280 and wine281 all exert antihypertensive
effects, although the pathways of their activity is unknown. Natural isolated flavonoids, including xanthoangelol 51 and 4-hydroxyderricin 52 (isolated from the plant Angelica keiskei, Figure 1.30), 282 and the flavone apigenin also exert hypotensive actions. 283

Figure 1.30. Xanthoangelol 51 and 4-hydroxyderricin 52 have antihypertensive properties.

Some synthetic flavonoids also exhibit antihypertensive activity. Synthetic hydroxylated chalcones have been shown to bind ACE, with their free phenols binding the zinc pocket of ACE. 284 However it is the synthetic 3',4'-dihydroxyflavonol that has received the most attention for its antihypertensive activity.

1.3.4.1 3',4'-Dihydroxyflavonol

In 1993, Duarte et. al. investigated the vasorelaxant effects of seven structurally distinct flavonoids on rat aortic tissue, and found that flavonols exhibited a stronger vasorelaxant effect than flavones or flavanols. 285 Woodman et. al. performed further Structure-Activity Relationship (SAR) studies using both flavones and flavonols, 286 and identified three structural features which contributed to their vasorelaxant activity (Figure 1.31): the structure must be a flavonol (shown in red), lack substitution on Ring A (shown in green), and contain hydroxyl groups at the 3- and 4-positions of Ring B (shown in blue). These three criteria give 3',4'-dihydroxyflavonol 53. Interestingly, two of these three criteria are also relevant for the antioxidant activity of flavonoids: (i) an ortho-dihydroxy structure on Ring B, and (ii) a 2,3-double bond with a hydroxyl at the 3-position and a ketone at the 4-position of Ring C. 11, 97, 103, 113

Figure 1.31. 3',4'-dihydroxyflavonol 53 is a vasorelaxant flavonoid.

Since the identification of 3',4'-dihydroxyflavonol 53 as a vasorelaxant compound, Woodman and co-workers have thoroughly investigated its manner of action. Early studies in rat cells, live rats and sheep revealed that the flavonoid possesses antioxidant activity which, through its scavenging of O$_2^-$, increases the
vast relaxant effect of acetylcholine (a neurotransmitter that lowers heart rate) in the body.\textsuperscript{277, 287} Further research revealed that this flavonol can increase vasodilation in rats,\textsuperscript{288-290} and can improve vascular function after ischemia and reperfusion injury in rats and sheep,\textsuperscript{291-293} leading to its establishment as a cardioprotective agent.\textsuperscript{294} 3’,4’-Dihydroxyflavonol has also been shown to restore endothelial function and relaxation in rat aorta in the case of diabetes-induced endothelial dysfunction.\textsuperscript{295, 296}

While the exact pathway through which 3’,4’-dihydroxyflavonol \textsuperscript{53} produces its vasorelaxant activity is unknown, Woodman and co-workers have shown that it decreases vascular contraction at least in part by inhibition of the RhoA/Rho-kinase pathway in endothelium-denuded rat aorta.\textsuperscript{289} It also causes Ca\textsuperscript{2+} desensitisation in vascular smooth muscle, resulting in reduced vascular smooth muscle contraction.\textsuperscript{290}

As mentioned previously, a major problem with the use of flavonoids as therapeutic agents is their lack of solubility and bioavailability. However, this problem has been overcome in the case of 3’,4’-dihydroxyflavonol \textsuperscript{53}, with the installation of amine salts and carboxylic acid chains on Ring A,\textsuperscript{62} or the inclusion of phosphates and hemiadiptes at the 3-hydroxyl position (Figure 1.32, flavonols \textsuperscript{54} and \textsuperscript{55} respectively).\textsuperscript{61}

![Figure 1.32. Different functionalities (blue) have made 3’,4’-dihydroxyflavonol more bioavailable.](image)

While the antioxidant and vasorelaxant compound 3’,4’-dihydroxyflavonol \textsuperscript{53} suffers from a lack of bioavailability due to its lack of solubility, enalapril and other ACE inhibitors have the opposite problem, in that they are too water soluble. It was thought that by combining both of these compounds into a single structure could avoid the solubility problems, and provide a double-action antihypertensive. Pharmaceuticals of this type are known as Designed Multiple Ligands, and will be discussed in more detail in Chapter Five.

### 1.4 Project Aims

Given the established therapeutic potential of flavonoids, it was the purpose of this research to further investigate the therapeutic effects of the flavonoids, specifically...
flavonoids which were novel or had been further functionalised so as to improve their bioavailability. The synthesis of a library of novel halogenated and functionalised flavonoid compounds was the first aim within this research, as it enabled the establishment of synthetic methodology and also provided various flavonoid structures for preliminary biochemical evaluation, including cytotoxicity evaluations (Chapters Two and Three). It was envisaged that the compounds would then be evaluated for therapeutic effects, including antibacterial, anticancer, and antidiabetic evaluations.

The installation of halogens onto the flavonoid scaffolds was also an aim of this project. A number of current pharmaceuticals take advantage of the incorporation of halogens,297 as these provide not only steric bulk to occupy binding sites of molecular targets,297 but also form halogen bonds in drug-target interactions, which contribute to the stability of protein-ligand complexes.297, 298 Halogenated compounds provide this benefit as the halogen ‘X’ acts as a Lewis acid, and forms direct close contacts with electron donor moieties in binding sites.299 This interaction is driven by the σ-hole, a positively-charged region on the hind side of ‘X’, caused by an anisotropy of electron density on the halogen (Figure 1.33).

Figure 1.33. Halogen bonding between halogenated pharmaceuticals and molecular targets is driven by the σ-hole, shown as a red δ+.299

One halogenated example discussed already in this chapter is Flavopiridol 29, a chlorinated flavonol which has undergone Phase I and II trials for anticancer effects (Figure 1.34).143, 149-152 Other examples of halogenated therapeutic compounds include numerous natural products from marine sponges and seaweeds which incorporate bromine atoms. These brominated structures have been shown to exhibit antifungal activity,300 HIV protease inhibition,301 and even antifouling properties.302 Also, the brominated compound 4,5,6,7-tetrabromobenzimidazole 56 (TTB) is a potent protein kinase CK2 inhibitor, utilised for its antiviral activity (Figure 1.34).303-306
These halogenated flavonoid structures also provided an opportunity to investigate the Heck reaction (a palladium-mediated reaction) in order to incorporate other functionalities (Chapter Four); for example, to add ‘handles’ for further synthesis.

The final aim of this research was the synthesis of a series of Designed Multiple Ligand (DML) compounds, incorporating the ACE-inhibiting effect of specific peptides and the vasorelaxant 3’,4’-dihydroxyflavonol 53 into a single, highly bioavailable cardioprotective compound (Chapters Five and Six).

With these aims in mind, the synthesis of a series of 2’-hydroxychalcones, a synthetic stepping stone to many flavonoid structures including flavanones, flavones and flavonols, was investigated first.
Chapter Two:
Synthesis of 2′-Hydroxychalcones
2.1 Chapter Overview

As outlined in Chapter One, the flavonoids are commonly used as therapeutic agents; two common uses are as antihypertensive or anticancer agents. To synthetically access many different members of the flavonoid family, the 2′-hydroxychalcones are an ideal starting material, and these compounds also offer therapeutic benefits. This chapter will begin by looking at the Claisen-Schmidt condensation, the most common method used to synthesise chalcones. The traditional methodology, as well as modern techniques including the use of microwave technology and even solid and cellular supports, will be discussed.

In order to synthesise a library of 2′-hydroxychalcones, the optimisation of a microwave-assisted methodology was undertaken, and provided a number of highly-functionalised, and in particular highly-halogenated, 2′-hydroxychalcones. Some of the members of this small library were also evaluated for their cytotoxic properties, the results of which will be presented at the end of this chapter.

The methodology developed within was published in Current Organic Chemistry (2012, 16(1), 121-126) (see Appendix A).

2.2 The Claisen-Schmidt Condensation

The term ‘chalcone’ 4 is used to describe an aromatic enone (α,β-unsaturated ketone), whereby an aryl group is present on either end of the enone (Figure 2.1). The condensation reaction of a benzaldehyde and an acetophenone to form a chalcone was first reported in 1881, by two separate research groups. The first was published by Schmidt, who outlined the reaction using acetone, furfurol (furan-2-carbaldehyde) and bittermandelol (benzaldehyde).

![Figure 2.1. The most simple chalcone 4, also known as benzylideneacetophenone or 1,3-diphenyl-2-propene-1-one.](image-url)

In the following issue of Berichte der Deutschen Chemischen Gesellschaft, Claisen and Claparede published their work regarding the condensation of a ketone and an aldehyde. Both groups detailed the use of a base (sodium hydroxide) in order to access the desired product.

The reaction, named the Claisen-Schmidt condensation, is a form of the aldol condensation, whereby a ketone and an aldehyde react to form water and a new carbon-
carbon bond in the aldol compound. The mechanism of the Claisen-Schmidt condensation is as shown in Scheme 2.1. First, the base (B) removes an α-proton from the acetophenone 57, and the resultant enolate 58 attacks the electrophilic carbonyl carbon of benzaldehyde 59. This is followed by protonation of the negatively-charged oxygen 60 to form aldol 61. Finally, dehydration via an E1cb mechanism provides the trans-chalcone 4.310, 311

![Scheme 2.1. The mechanism of the Claisen-Schmidt condensation between acetophenone 57 and benzaldehyde 59.](image1)

The stereoselectivity of the reaction (where the trans- rather than cis- product is formed) arises during the dehydration step. Due to the presence of an unfavourable steric interaction between the two phenyl groups of the chalcone, the trans-product is favoured (Figure 2.2).312

![Figure 2.2. The trans-chalcone 4 is favoured in the Claisen-Schmidt condensation.](image2)

### 2.2.1 Conventional Claisen-Schmidt Condensation

Over the years, the Claisen-Schmidt condensation has been used to form chalcones from many functionalised acetophenones and aldehydes.77, 313-316 In more recent times, adaptations to make these Claisen-Schmidt condensations “green” have included solvent-free conditions, with the use of silica chloride (chlorinated silica gel),317 solid NaOH318 and KOH,319 solid supports,320 and even near-critical water.321 Ultrasonic irradiation with zeolites has also been applied to chalcone synthesis.322 Ionic
liquids have also been investigated as catalysts for Claisen-Schmidt reactions, including room temperature ionic liquids (RTILs), basic ionic liquids, and acyclic acidic ionic liquids.

One problematic side reaction of base-catalysed Claisen-Schmidt condensation reactions is the Cannizzaro reaction. This reaction occurs between two molecules of benzaldehyde and a hydroxide base to produce a benzyl alcohol and a carboxylic acid (Scheme 2.2). As these reagents are present in the Claisen-Schmidt condensation, it is understandable that there have been reports of the Cannizzaro reaction providing undesired benzyl alcohol and carboxylic acid in the synthesis of chalcones. However, the absence of the Cannizzaro product has been noted previously, in the case of ultrasound-accelerated Claisen-Schmidt condensation.

Research within this chapter focuses on the synthesis of 2′-hydroxychalcones, which are formed by reactions between functionalised 2′-hydroxyacetophenones and functionalised benzaldehydes. These particular chalcones were of interest as they provide a stepping stone from which to synthesise other members of the flavonoid family, including the flavanones, flavones, isoflavones, and flavonols.

The formation of 2′-hydroxychalcones was first reported by Pfeiffer in 1933, and since then, many functionalised forms have been synthesised. Generally, the traditional methods of 2′-hydroxychalcone synthesis, via a base-catalysed Claisen-Schmidt condensation of 2′-hydroxyacetophenones and benzaldehydes, employ long reaction times (around 24 hours), require strong bases, and yields range from poor to very good (16%-81%).

Yet another problem that must be avoided in 2′-hydroxychalcone synthesis is their conversion to the analogous flavanone, which can occur via an acid- or base-catalysed process (see Schemes 2.3 and 2.4 respectively).
Scheme 2.3. The acid-catalysed cyclisation of 2′-hydroxychalcone 66 to form flavanone 6.

This phenomenon means that same conditions used to synthesise the 2′-hydroxychalcones may also produce some of the cyclised analogue.\textsuperscript{329, 345, 346} This phenomenon has been reported numerous times,\textsuperscript{329, 330, 347} and once again, flavanone formation can be avoided through careful selection of reaction conditions, including reaction time and acid or base strength. Along with the careful choice of reaction conditions to increase yields and avoid isomerisation, microwave chemistry has also been embraced in chalcone synthesis.

Scheme 2.4. The base-catalysed cyclisation of 2′-hydroxychalcone 66 to form flavanone 6.

2.2.2 Microwave Chemistry

In the electromagnetic radiation spectrum, microwaves (0.3 GHz–300 GHz) lie between radiowave (Rf) and infrared (IR) frequencies. Microwaves, a nonionizing radiation incapable of breaking bonds, are a form of energy rather than heat. However, this energy is manifested as heat through its interaction with materials wherein they can be reflected (metals), transmitted (good insulators that will not heat) or absorbed (decreasing the available microwave energy and rapidly heating the sample).\textsuperscript{348}
The heating of samples (including foodstuffs, solvents, and chemical reactions) using microwave irradiation involves first the alignment of dipoles and ions with an electric field.\textsuperscript{349, 350} As the field oscillates, the dipoles or ions attempt to continue to align with the electric field, resulting in the loss of energy through heat. The amount of heat produced is relative to the ability of the dipoles or ions to align with the field, and is dependent on the dielectric properties of the sample. As the microwave irradiation is able to penetrate the sample, unlike conventional heating methods, efficient and rapid internal heating is achieved.\textsuperscript{349, 350}

This rapid internal form of heating, as well as the use of increased pressure, allows for the superheating of solvents, whereby the solvent can be heated to temperatures greater than its boiling point.\textsuperscript{351} The superheating of chemical reactions has been shown to enhance reaction rates; Baghurst and co-workers note that superheating chemical reactions by 100 °C results in a rate enhancement of approximately 1000.\textsuperscript{352}

Microwave-assisted organic chemistry is a modern method in which microwave irradiation is applied to organic reactions, with this rapid heating leading to reduced reaction times. Microwave irradiation was first applied to organic reactions in 1986, using domestic microwaves.\textsuperscript{353, 354} As microwave chemistry increased in popularity, more opportunities were realised, including the use of ‘dry’ media including solid support and solvent-free reactions.\textsuperscript{355} However, domestic microwave reactors have since been deemed unreliable in a chemistry setting, as they do not offer reproducible results, reaction stirring is not possible, they offer little control over reaction conditions, and their use has even caused explosions.\textsuperscript{355}

In the 1990s, research into more specialised microwave reactors began, in order to allow controllable and safer reactions. Magnetic stirrers were incorporated to ensure uniform temperature through the reaction mixture, the ability to add chemicals and remove samples during heating was offered, and computer-controlled power input became possible. More importantly in terms of safety, these specialised reactors could provide concurrent heating and cooling, minimising any chance of explosion.\textsuperscript{355}

In the year 2000, the commercialisation of these specialised laboratory microwave reactors began.\textsuperscript{356} In addition to the advantages of the first specialised microwave reactors, these commercial microwave reactors offered far greater control over the parameters used: temperature, reaction time, microwave power, and cooling
methods can now all be regulated and strictly monitored, providing reproducibility between reactions.\textsuperscript{356} Commercial microwave reactors also influence reaction selectivity, provide cleaner transformations, and improve yields.\textsuperscript{356} Reduced solvent volumes, to the point of ‘neat’ reactions, and the use of ionic liquids\textsuperscript{357} or water as solvents,\textsuperscript{358} have also been realised. It is also noted that every 10 °C increase in microwave reaction temperature halves the reaction time;\textsuperscript{355} by this approximation, if a reaction is heated by conventional methods (oil bath or hot plate) at 80 °C for 16 hours, the same reaction could be carried out at 200 °C for 16 seconds with microwave irradiation.\textsuperscript{355}

Microwave chemistry is recognised as a ‘green’ chemistry approach, due to the reduced energy and solvent required. The application of microwave irradiation to organic reactions has been very broad;\textsuperscript{359-361} cycloaddition reactions,\textsuperscript{362} heterocycle synthesis\textsuperscript{363, 364} and nanomaterial synthesis\textsuperscript{361} have all enjoyed the benefits of microwave chemistry. The use of microwave irradiation has also extended to the synthesis of flavonoids and chalcones.

### 2.2.3 Microwave-Assisted Claisen-Schmidt Condensation

Microwave-assisted chalcone synthesis has been introduced over the last 15 years, and greatly-reduced reaction times have been realised (2-60 minutes).\textsuperscript{339, 365-372} The first report of microwave-assisted 2′-hydroxychalcone synthesis employed a domestic microwave reactor, and eight chalcones (varying only in the functionality of the benzaldehyde) were synthesised.\textsuperscript{339}

In the microwave, the use of solid support and heterogeneous catalysts in place of solvents to synthesise chalcones has also been explored, including montmorillonite K10 clay,\textsuperscript{366} Al\textsubscript{2}O\textsubscript{3},\textsuperscript{370} hydroxyapatite,\textsuperscript{371} and mesoporous zirconium phosphate,\textsuperscript{373} among others.\textsuperscript{374-377} However, only one of these microwave-assisted methods specifically address the formation of the 2′-hydroxychalcones.\textsuperscript{370}

More recently, functionalised cellulose supports have been successfully employed by Bowman \textit{et al.} for the SPOT-synthesis of chalcones, and excellent functional group tolerance was realised.\textsuperscript{367, 368} However, only two 2′-hydroxychalcones (which as mentioned earlier are valuable as precursors for other flavonoids) were synthesised in moderate yields (40% and 52%).\textsuperscript{368}

Whilst microwave-assisted chalcones synthesis has been reported, it is difficult to find methods specifically for 2′-hydroxychalcones. In 2002, Stoyanov and co-
workers\textsuperscript{339} also noted that previous microwave-assisted chalcone formation methods\textsuperscript{366, 372, 378} could not be applied to 2'-hydroxychalcones. They found that applying the earlier microwave-assisted methods lead to the formation of the flavanone rather than the 2'-hydroxychalcone, and that the additional step of hydroxyl protection was required.\textsuperscript{379} In order to overcome the need for hydroxyl protection, they developed and optimised a procedure which utilised KOH, EtOH, and a domestic microwave (which, as noted previously, lacks the precision and control that is guaranteed by a purpose-built microwave).\textsuperscript{356}

Given that only one literature method specifically addressed the synthesis of the 2'-hydroxychalcones via a microwave-assisted approach, and that this method utilised the unreliable domestic microwave, a study was undertaken to determine a reliable and robust method for the microwave-assisted synthesis of 2'-hydroxychalcones.

### 2.3 Results and Discussion

#### 2.3.1 The Traditional Claisen-Schmidt Condensation Conditions

In order to compare the use of the traditional method (a mineral hydroxide base in ethanol (EtOH), stirred at room temperature overnight) and the microwave-assisted method, a trial reaction was carried out using the traditional conditions.\textsuperscript{342} The condensation was attempted using 2'-hydroxyacetophenone 72 (2.9 mmol) and an excess of benzaldehyde 59 (4.4 mmol), with potassium hydroxide (KOH, 15.0 mmol) as the base, and EtOH (10 mL) as the solvent. To the solution of KOH in EtOH, the acetophenone was added, followed by the benzaldehyde. The reaction was stirred at room temperature for 16 hours, and acidic work up provided a crude product which was analysed by Proton Nuclear Magnetic Resonance (\textsuperscript{1}H NMR) spectroscopy (Figure 2.3).
The spectrum showed trace amounts of the acetophenone \( 72 \) (signified by the methyl peak at \( \delta \) 2.58 ppm) and benzaldehyde \( 59 \) (signified by the aldehyde peak at \( \delta \) 9.98 ppm) starting materials, as well as significant amounts of \( 2' \)-hydroxychalcone \( 66 \) (signified by the hydroxyl proton peak at \( \delta \) 12.84 ppm). The corresponding flavanone compound \( 6 \) was also present (by a multiplet at \( \delta \) 2.83-3.10 ppm, and a doublet of doublets centred at \( \delta \) 5.43 ppm, which had relative integrations of 2 and 1 respectively).

As mentioned in Chapter 2.2, this phenomenon is quite common due to the equilibrium between chalcones and their corresponding flavanones in the presence of either acid or base. A trace amount of the Cannizzaro product \( 64 \) was also present in the crude reaction material, as identified by a singlet at \( \delta \) 4.69 ppm, representative of the CH\(_2\) of the benzyl alcohol.

According to the \(^1\text{H}\) NMR spectrum, excellent conversion was realised, with the ratio of the acetophenone \( 72 \) to desired chalcone \( 66 \) to flavanone \( 6 \) as approximately 1:100:10. However, there was still room for improvement, due to the formation of the flavanone and Cannizzaro products, and the 16 hour reaction time.

As the aim of this research was to identify ideal conditions for the microwave-assisted reaction, no further optimisation was performed on the traditional bench
method. The results from this method were simply used for comparison to the microwave method.

2.3.2 Optimisation of the Microwave-Assisted Claisen-Schmidt Condensation

For the optimisation of the microwave-assisted formation of the functionalised 2'-hydroxychalcones, the parameters of base used, solvent, reaction time, and microwave temperature were all examined.

An important point that must be explained is the integration of the chalcone hydroxyl peak in $^1$H NMR spectra. When purified, the hydroxyl peak of 2'-hydroxychalcone at $\delta$ 12.84 ppm was observed as the only peak that represented the chalcone and was instantly distinguishable from the peaks of any other products or reactants in the reaction mixture (Figure 2.4). This peak was therefore the only peak that could be used for integration ratios (against the acetophenone CH$_3$ peak at $\delta$ 2.58 ppm).

The hydroxyl peak was consistently observed to have an integration of 0.85 rather than an integration of one, when compared to the integration of the aryl peaks (in particular those from $\delta$ 6.90-7.05 ppm, representing the two alkene CH peaks). This could be explained by the proton-deuterium exchange of the phenol proton resulting in a reduced integration value, however the assumption that the integration is always 0.85 could provide problems if this integration was not reproducible between experiments. For this reason, the deuterated chloroform used for NMR experiments was stored over molecular sieves and under nitrogen, to ensure that the presence of water in the chloroform would not distort the proportion of proton-deuterium exchange. Also, all reaction mixtures and purified products were dried for at least six hours under a high-vacuum system prior to performing spectroscopy, to remove any water. This method of ‘drying’ the chloroform and reaction material proved reproducible (the reaction was repeated numerous times) and the hydroxyl peak always provided an integration of 0.85.
With this in mind, optimisation could begin, using integration to determine the relative ratio of products and starting materials in each reaction.

### 2.3.2.1 Base

The first parameter investigated was the base used. Numerous bases, mainly hydroxides, have been used previously for chalcone formation in a conventional reaction setting. Therefore, many different types of bases were examined, including hydroxides, amines, and other bases.

Parameters of the microwave reactor were kept constant; a 50 °C reaction temperature for 15 minutes at 300 watts. Unsubstituted 2'-hydroxyacetophenone 72 and benzaldehyde 59 were reacted together in a 1:1 ratio on a 0.5 millimolar scale, and tetrahydrofuran (THF) was chosen as the solvent (2 mL), as it provided a polar aprotic medium in which to carry out the reaction.

It was found during the first reaction that one equivalent of benzaldehyde 59 was inadequate to achieve complete conversion. According to the $^1$H NMR spectrum, the reaction mixture contained the desired 2'-hydroxychalcone 66 and acetophenone reagent 72 (in a ratio of 2:1 chalcone 66 to acetophenone 72 respectively), and no benzaldehyde 59. This lack of benzaldehyde did not coincide with the appearance of the
peak representing the Cannizzaro product (at $\delta$ 4.69 ppm), and as the acetophenone and benzaldehyde react in a 1:1 ratio, the lack of benzaldehyde suggested that it may be evaporating (due to low vapour pressure) during the reaction and acidic workup. When the reaction was repeated using two equivalents of benzaldehyde, conversion to desired chalcone 66 increased, with a ratio of 100:1 for chalcone 66 to acetophenone 72 respectively. This increase in conversion occurred with no sign of the Cannizzaro or flavanone products. Therefore, all reactions using the unfunctionalised benzaldehyde were completed using a 2:1 ratio of benzaldehyde 59 to acetophenone 72.

Due to the use of excess benzaldehyde, conversion ratios in the following tables show only the relative amount of residual acetophenone 72 to chalcone 66. The chalcone hydroxyl proton at $\delta$ 12.84 ppm was integrated as 0.85, and the acetophenone methyl peak at $\delta$ 2.58 ppm was then integrated, and its total was divided by three as it represents three protons. This gave a relative conversion ratio by comparing the amount of starting material to product, and avoided the problem of benzaldehyde volatility giving inaccurate conversion rates. In any case where the conversion ratio to the flavanone was required, the methine (CH) peak at $\delta$ 5.43 ppm with an integration of one was used, and for the Cannizzaro product, the methylene (CH$_2$) peak at $\delta$ 4.69 ppm with an integration of two was used (Figure 2.5).

Due to the use of excess benzaldehyde, conversion ratios in the following tables show only the relative amount of residual acetophenone 72 to chalcone 66. The chalcone hydroxyl proton at $\delta$ 12.84 ppm was integrated as 0.85, and the acetophenone methyl peak at $\delta$ 2.58 ppm was then integrated, and its total was divided by three as it represents three protons. This gave a relative conversion ratio by comparing the amount of starting material to product, and avoided the problem of benzaldehyde volatility giving inaccurate conversion rates. In any case where the conversion ratio to the flavanone was required, the methine (CH) peak at $\delta$ 5.43 ppm with an integration of one was used, and for the Cannizzaro product, the methylene (CH$_2$) peak at $\delta$ 4.69 ppm with an integration of two was used (Figure 2.5).

It was also important to use an excess of base in these reactions. As shown in Scheme 2.2, one equivalent of base is needed to carry out the Claisen-Schmidt condensation with an unfunctionalised acetophenone. However, with the use of 2'-hydroxyacetophenone 72 as a reagent, an additional equivalent of base is required in order prevent any problems regarding the deprotonation of the hydroxyl rather than the desired $\alpha$-proton of the acetophenone. Theoretically, two equivalents of base may have been adequate for the reaction to be carried out, however it was noted that previous attempts at 2'-hydroxychalcone synthesis had used four or more equivalents of base, and so four equivalents of base were used. With these molar ratios of base and aldehyde determined, attention returned to the choice of base.
The microwave reaction using two equivalents of benzaldehyde 59 and four equivalents of KOH (Table 2.1, entry 1) resulted in complete conversion to the desired 2′-hydroxychalcone 66, as indicated in the 1H NMR spectrum by the complete loss of the acetophenone methyl peak (δ 2.58 ppm), and identical 1H NMR peaks to those previously published in the literature, including the chalcone hydroxyl peak (δ 12.84 ppm). Purification of the product by column chromatography (40% dichloromethane (CH2Cl2) in petroleum spirits (Pet)) isolated chalcone 66 in quantitative yield. There was no sign of the undesired Cannizzaro product or flavanone. Although this was an excellent result, the identification of other suitable bases was pursued to provide other options for the synthesis of more functionalised products.

Table 2.1. Choice of base for microwave-assisted 2′-hydroxychalcone 66 formation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>72:66&lt;sup&gt;a&lt;/sup&gt; (yield)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KOH</td>
<td>0:1 (quant.)</td>
</tr>
<tr>
<td>2</td>
<td>NaOH</td>
<td>0:1 (95%)</td>
</tr>
<tr>
<td>3</td>
<td>Ba(OH)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1:16 (92%)</td>
</tr>
<tr>
<td>4</td>
<td>LiOH</td>
<td>2:1</td>
</tr>
<tr>
<td>5</td>
<td>NaOAc</td>
<td>1:0</td>
</tr>
<tr>
<td>6</td>
<td>NEt&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1:0</td>
</tr>
<tr>
<td>7</td>
<td>DIPA</td>
<td>1:0</td>
</tr>
<tr>
<td>8</td>
<td>NaHMDS</td>
<td>1:0</td>
</tr>
<tr>
<td>9</td>
<td>DBU</td>
<td>1:2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>Bu&lt;sub&gt;4&lt;/sub&gt;NOH&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0:1 (81%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio determined by 1H NMR integration  
<sup>b</sup> Isolated yield (%) after column chromatography  
<sup>c</sup> Ratio of 72:66:66(flavanone) was 1:2:2  
<sup>d</sup> As a 1.0 M solution in MeOH

First, other mineral bases were examined. As expected, complete conversion was realised when sodium hydroxide (NaOH) was used (Table 2.1, entry 2), and an isolated yield of 95% was obtained upon purification of the 2′-hydroxychalcone 66. Barium hydroxide (Ba(OH)<sub>2</sub>) was also successfully applied to the reaction, with a very good ratio of 16:1 chalcone 66 to acetophenone 72, and an isolated yield of 92% achieved (Table 2.1, entry 3). Lithium hydroxide (LiOH) provided a reduced conversion ratio of 2:1 for acetophenone 72 to chalcone 66 respectively.

Sodium acetate, a weak base whose corresponding acid (acetic acid) has a pK<sub>a</sub> of 4.76 in water, was also tested in the reaction (Table 2.1, entry 5), as it has been
reported to convert the chalcone 66 via ring closure to the flavanone 6. However, even in an extreme excess of 14 equivalents, no conversion from acetophenone 72 to chalcone 66 was noted.

Amine bases were the next to be trialled. While their use in traditional chalcone formation is not common, they have been used previously in microwave-assisted chalcone formation. Triethylamine (NEt₃), diisopropylamine (DIPA), and sodium bis(trimethylsilyl)amide (NaHMDS) were all trialled (Table 2.1, entries 6, 7, and 8), however none provided desired chalcone 66.

Interestingly, when the amidine base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was used (Table 2.1, entry 9), the product included not only some of desired chalcone 66, but also flavanone 6. This reaction provided a 1:2:2 ratio of acetophenone 72 to chalcone 66 to flavanone 6 respectively. It has previously been reported that DBU can be used under microwave irradiation to convert chalcones to flavanones, which would explain the presence of flavanone 6. This result will be discussed further in Chapter 3.2.2.

Lastly, tetrabutylammonium hydroxide (Bu₄NOH, Table 2.1, entry 10) was used (as a one molar solution in methanol (MeOH)). This base provided the desired 2′-hydroxychalcone 66 with 100% conversion, however an isolated yield of only 81% was achieved. While this isolated yield was not as high as those achieved with the other hydroxide bases, this result was of note as, unlike KOH or NaOH, Bu₄NOH is soluble in organic solvents other than MeOH and EtOH (the most commonly used solvents for this reaction), and therefore more base/solvent combinations were potentially possible for the microwave-assisted reaction.

In conclusion, KOH and NaOH were identified as the best bases, which was in accordance with the previous 2′-hydroxychalcone synthesis publication, as well as the traditional methodology. In addition, 1.0 M Bu₄NOH in MeOH was established as an option, allowing more scope in terms of solvent choice. With this information in hand, attention turned to the selection of solvent.

2.3.2.2 Solvent

In order to identify an ideal solvent, the best base (KOH) was utilised for the reaction. The other parameters (reaction time, temperature and power) were also kept constant, with a reaction of 15 minutes at 50 °C and a power setting of 300 watts.
Traditionally, polar protic alcohols are the solvents of choice in Claisen Schmidt reactions, due to their ability to dissolve hydroxide bases. Therefore, MeOH and EtOH were examined first. Their use in the microwave-assisted method gave very good, but not complete conversion to the 2'-hydroxychalcone 66 (Table 2.2, entries 1 and 2), with a ratio of 19:1 of the desired 2'-hydroxychalcone 66 to acetophenone 72. However, when the polar aprotic solvents N,N-dimethylformamide (DMF) and tetrahydrofuran (THF) were tested, complete conversion to the desired product 66 was noted (Table 2.2, entry 3 and Table 2.1, entry 1 respectively). Understandably, the less polar solvents dioxane and toluene (Table 2.2, entries 5 and 6) did not facilitate the reaction, likely due to their inability to dissolve the hydroxide bases. Acetone was also trialled (Table 2.2, entry 4) as it was the solvent of choice (and reagent) in Schmidt’s original work, and gave an approximate ratio of 2:1, acetophenone 72 to 2'-hydroxychalcone 66 respectively.

Table 2.2. Optimisation of solvent used for microwave-assisted 2'-hydroxychalcone 66 formation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>72:66&lt;sup&gt;a&lt;/sup&gt; (yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH</td>
<td>1:19</td>
</tr>
<tr>
<td>2</td>
<td>EtOH</td>
<td>1:19</td>
</tr>
<tr>
<td>3</td>
<td>DMF</td>
<td>0:1</td>
</tr>
<tr>
<td>4</td>
<td>acetone</td>
<td>2:1</td>
</tr>
<tr>
<td>5</td>
<td>dioxane</td>
<td>1:0</td>
</tr>
<tr>
<td>6</td>
<td>PhMe</td>
<td>3:1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio determined by <sup>1</sup>H NMR integration

The ideal solvents identified for this microwave reaction were therefore THF and DMF, rather than the expected MeOH and EtOH. It was decided that THF would be utilised in future reactions, as DMF is more difficult to remove from reactions.

2.3.2.3 Microwave reaction time

Microwave reaction time is another parameter that affects the conversion of acetophenone to chalcone. With base (KOH) and solvent (THF) already determined, the microwave was set at 300 W power and a reaction temperature of 50 °C.
Table 2.3. Optimisation of reaction time for microwave-assisted 2′-hydroxychalcone 66 formation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction time</th>
<th>72:66*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 min</td>
<td>1:19</td>
</tr>
<tr>
<td>2</td>
<td>15 min</td>
<td>0:1</td>
</tr>
<tr>
<td>3</td>
<td>20 min</td>
<td>0:1</td>
</tr>
<tr>
<td>4</td>
<td>30 min</td>
<td>1:19</td>
</tr>
</tbody>
</table>

*a Ratio determined by 1H NMR integration

While faster reaction times than the traditional method (16 hours) were achieved through the use of the microwave reactor, it was found that there was a very fine balance regarding reaction time and conversion. While reaction times of 15 and 20 minutes ensured complete conversion to the desired 2′-hydroxychalcone 66 (Table 2.3, entries 2 and 3), it was noted that extending or reducing these reaction times resulted in very slightly reduced conversions (19:1 ratio of 2′-hydroxychalcone 66 to acetophenone 72, see Table 2.3, entries 1 and 4). The extension of reaction time resulting in decreased product formation may be attributed to the formation of water, which may then cause a retro-aldol reaction, in which the acetophenone and benzaldehyde starting materials are re-formed. As short reaction times were desired, a 15 minute reaction under microwave irradiation was deemed ideal.

2.3.2.4 Microwave temperature

The final parameter to be optimised for these reactions was microwave temperature. With the other three reaction parameters set (KOH, THF, 15 minutes) and a power setting of 300 W, the temperature of the microwave reaction was examined. As Luthman and co-workers had used 170 °C in their synthesis of flavanones from acetophenones and benzaldehydes, this was identified as an ideal starting point to pursue chalcone formation. However, this high temperature gave no conversion to the desired chalcone 66 (see Table 2.4, entry 1), and resulted in the loss of benzaldehyde 59, possibly due to evaporation. Therefore, lower temperatures were used.

Lowering the temperature to 120 °C and 100 °C (Table 2.4, Entries 2 and 3 respectively) also resulted in no conversion to the 2′-hydroxychalcone 66, with only acetophenone 72 present in 1H NMR spectra. When a reaction temperature of 100 °C
and reaction time of 5 minutes was tested, almost complete conversion to chalcone 66 was noted (Table 2.4, entry 4), however this increase in desired product was accompanied by the presence of the Cannizzaro product, in a ratio of 4:1 chalcone 66 to benzyl alcohol 64. These results lead to the conclusion that temperatures in excess of 100 °C were too high for chalcone formation in good yield and purity.

Table 2.4. Optimisation of reaction temperature for microwave-assisted 2′-hydroxychalcone 66 formation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>72:66a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>170</td>
<td>60</td>
<td>1:0</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>30</td>
<td>1:0</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>20</td>
<td>1:0</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>5</td>
<td>1:10b</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>10</td>
<td>1:10</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>15</td>
<td>0:1</td>
</tr>
</tbody>
</table>

a Ratio determined by 1H NMR integration  
b Also produced Cannizzaro product 64 in relative ratio of 2.5

When the lower temperature of 80 °C was employed, good conversion to the 2′-hydroxychalcone 66 was noted (Table 2.4, entry 5), by the presence of the δ 12.84 ppm peak representing the hydroxyl proton in the 1H NMR spectrum. As this reduction in temperature afforded great conversion without the occurrence of the Cannizzaro reaction, the temperature was again lowered to 50 °C (Table 2.4, entry 6), and found to provide 2′-hydroxychalcone with 100% conversion from the starting acetophenone, and none of the corresponding Cannizzaro product.

2.3.2.5 Optimisation Summary

The ideal conditions for the microwave-assisted 2′-hydroxychalcone formation were a reaction time of 15 minutes at 50 °C. Microwave power was not investigated, but kept constant at 300 watts. The best bases were the mineral bases KOH or NaOH, as well as Bu4NOH (as a 1.0 M solution in MeOH), in a ratio of 4:1 base to acetophenone, with THF as the solvent. These conditions produced the desired 2′-hydroxychalcone 66 in a far greater yield than the trialled traditional method, with the benefits of no side products (undesired flavanone and Cannizzaro products), and significantly reduced reaction time (15 minutes compared to 16 hours). Attention could now turn to the
conversion of more functionalised starting materials to their corresponding 2’-hydroxychalcones.

2.3.3 Synthesis of a Library of Functionalised 2’-Hydroxychalcones

The starting materials incorporated a number of different functionalities into their 2’-hydroxyacetophenone and benzaldehyde scaffolds. In particular, starting materials with halogens were favoured, as these were noted as ideal in medicinal chemistry scaffolds,297, 298, 389 provided the opportunity to investigate the effect of halogen bonding of flavonoid compounds with therapeutic targets,299 and also allowed for research into the use of the Heck reaction with flavonoid scaffolds (to be discussed in Chapter Four).390, 391

As the optimisation reactions identified both potassium hydroxide (KOH) and tetrabutylammonium hydroxide (Bu₄NOH) as ideal bases, each reaction was completed in duplicate; once each with these selected bases. In each case, only the reaction which provided greater conversion to the desired chalcone was purified. Each of the compounds was identified by comparison to literature ¹H and ¹³C NMR spectra in the case of previously synthesised compounds. Novel compounds were fully characterised by ¹H NMR spectroscopy, including their hydroxyl peak at approximately δ 12.20-12.90 ppm, as well as High Resolution Mass Spectrometry (HRMS) and ¹³C NMR spectroscopy. Note that the compounds underlined in the following tables were novel compounds.

2.3.3.1 Reaction of 2’-Hydroxyacetophenone with Substituted Benzaldehydes

The first series of 2’-hydroxychalcones were those synthesised using 2’-hydroxyacetophenone 72 and various substituted benzaldehydes. As the unsubstituted 2’-hydroxychalcone 66 had already been synthesised in the optimisation reactions (Table 2.1, entries 1 and 2), the next chalcone to be synthesised was the product of 2’-hydroxyacetophenone 66 and 4-bromobenzaldehyde 73 (chalcone 74, Table 2.5, entries 3 and 4). The greatest conversion was achieved using KOH, and the product was isolated as a yellow solid in excellent yield (96%) with the characteristic chalcone hydroxyl peak appearing at δ 12.76 ppm.392
Table 2.5. Microwave-assisted synthesis of functionalised 2′-hydroxychalcones.*

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Base</th>
<th>3:5&lt;sup&gt;b&lt;/sup&gt; (yield)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>H</td>
<td>H</td>
<td>KOH</td>
<td>0:1 (100)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>66</td>
<td>H</td>
<td>Bu₄NOH</td>
<td>0:1 (81)</td>
</tr>
<tr>
<td>3</td>
<td>74</td>
<td>4-Br</td>
<td>H</td>
<td>KOH</td>
<td>0:1 (96)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>74</td>
<td>4-Br</td>
<td>Bu₄NOH</td>
<td>1:2</td>
</tr>
<tr>
<td>5</td>
<td>76</td>
<td>3-Br</td>
<td>5-Br</td>
<td>KOH</td>
<td>1:0</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>76</td>
<td>3-Br</td>
<td>Bu₄NOH</td>
<td>1:5 (52)</td>
</tr>
<tr>
<td>7</td>
<td>78</td>
<td>3-OBn</td>
<td>H</td>
<td>KOH</td>
<td>1:10 (88)</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>78</td>
<td>3-OBn</td>
<td>Bu₄NOH</td>
<td>1:5</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>KOH</td>
<td>0:1 (90)</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>80</td>
<td>2,4-OMe</td>
<td>Bu₄NOH</td>
<td>1:2</td>
</tr>
<tr>
<td>11</td>
<td>82</td>
<td>4-F</td>
<td>H</td>
<td>KOH</td>
<td>1:17 (58)</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>82</td>
<td>4-F</td>
<td>Bu₄NOH</td>
<td>3:1</td>
</tr>
<tr>
<td>13</td>
<td>85</td>
<td>4-NO₂</td>
<td>H</td>
<td>KOH</td>
<td>1:1 (16)</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>85</td>
<td>4-NO₂</td>
<td>Bu₄NOH</td>
<td>1:0</td>
</tr>
<tr>
<td>15</td>
<td>87</td>
<td>2,4,5-MeO</td>
<td>H</td>
<td>KOH</td>
<td>1:0</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>87</td>
<td>2,4,5-MeO</td>
<td>Bu₄NOH</td>
<td>1:0</td>
</tr>
<tr>
<td>17</td>
<td>88</td>
<td>4-NHCOCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>H</td>
<td>KOH</td>
<td>1:0</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>88</td>
<td>4-NHCOCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Bu₄NOH</td>
<td>1:0</td>
</tr>
<tr>
<td>19</td>
<td>91</td>
<td>2-COOH</td>
<td>H</td>
<td>KOH</td>
<td>9:1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>91</td>
<td>2-COOH</td>
<td>Bu₄NOH</td>
<td>1:0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Underlined compounds are novel
<sup>b</sup> Ratio determined by 1H NMR integration
<sup>c</sup> Isolated yield (%) after column chromatography
<sup>d</sup> The corresponding flavanone 92 was also identified, in a ratio of 9:1:9 for 3:5:92 respectively
<sup>e</sup> The corresponding flavanone 92 was also identified, in a ratio of 1:0:2 for 3:5:92 respectively

As the reaction to form the mono-halogenated benzaldehyde had been successful, 3,5-dibromobenzaldehyde 75 was also predicted to react well. Greater conversion was achieved with the use of Bu₄NOH rather than KOH (Table 2.5, entries 6 and 5 respectively), and column chromatography (1:1 CH₃Cl₂:Pet) isolated the novel chalcone 76 in a moderate 52% yield. The compound was fully characterised using 1H NMR (including its hydroxyl peak at δ 12.60 ppm) and 13C NMR, as well as HRMS.

2′-Hydroxyacetophenone 72 was next reacted with 3-benzyloxybenzaldehyde 77 to produce chalcone 78 (Table 2.5, entries 7 and 8). This aldehyde was utilised as it provided a pathway to a hydroxylated chalcone, through benzyl deprotection under hydrogenation conditions.<sup>393</sup> Conversion was better when KOH was used, and the desired compound 78 was afforded as a bright yellow solid in an isolated yield of 78% as confirmed by comparison with the literature.<sup>394</sup>
Another novel compound was synthesised from the reagents 2’-hydroxyacetophenone 72 and 5-bromo-2,4-dimethoxybenzaldehyde 79. Using KOH as the base gave complete conversion to the desired novel chalcone 80 (Table 2.5, entry 9), which was isolated as a yellow solid in a yield of 90%. This compound was characterised by $^1$H and $^{13}$C NMR spectroscopy, as well as HRMS.

The reaction of a more electron-withdrawn benzaldehyde was then attempted, in the form of 4-fluorobenzaldehyde 81. Its reaction with 2’-hydroxyacetophenone 72 was attempted using both KOH and Bu$_4$NOH (Table 2.5, entries 11 and 12 respectively), with the greater conversion (1:17 acetophenone 72 to chalcone 81) occurring with KOH. Column chromatography provided the desired chalcone 82 as a bright yellow solid, in a yield of 58%.

![Figure 2.6. $^1$H NMR spectrum of crude reaction material of 2’-hydroxyacetophenone 72 and 4-fluorobenzaldehyde 81, showing the $S_{N}Ar$ aldehyde product 83 and acetophenone starting material 72 (CDCl$_3$).](image)

It was also interesting to note that the $^1$H NMR spectrum of the product from the Bu$_4$NOH reaction indicated the presence of a methoxy group, with the appearance of a singlet peak with an integration of three at $\delta$ 3.85 ppm (Figure 2.6). Also present was an aldehyde peak at $\delta$ 9.84 ppm with an integration of one. These peaks suggested that the aldehyde starting material 81 had undergone an nucleophilic aryl substitution ($S_{N}Ar$) reaction, in which the methoxy anion (produced from the Bu$_4$NOH and its methanol
solvent) attacks the para-fluoro position of the aldehyde to produce aldehyde 83 (Scheme 2.5).\textsuperscript{395}

Unfortunately, the SNAr product could not be isolated from the reaction mixture, and therefore its formation could not be confirmed. However, this ipso-substitution has been reported previously by Kumar \textit{et. al.}, who used benzyltrimethylammonium hydroxide (Triton B) in MeOH to effect the synthesis of aryl alkyl ethers from heteroaryl fluorides.\textsuperscript{396}

Another highly electron-withdrawn reagent, 4-nitrobenzaldehyde 84, was then reacted with 2ʹ-hydroxyacetophenone 72 (Table 2.5, entries 13 and 14). The conversion ratios for the KOH and Bu₄NOH reactions were much lower than all of the previously synthesised 2ʹ-hydroxychalcones, presumably due to the electron-withdrawing nature of the nitro group. Nevertheless, the desired chalcone 85 was isolated, albeit in a poor yield of 16%. As the nitro-substituted product was not a focus in this study, and other researchers had also experienced low yields with nitro-analogues, further optimisation was not attempted.

The reaction of 2,4,5-trimethoxybenzaldehyde 86 with 2ʹ-hydroxyacetophenone 72 provided no conversion to the desired chalcone 87 with either base (Table 2.5, entries 15 and 16), and only starting materials were observed in the \textsuperscript{1}H NMR spectrum. For this reason, 2,4,5-trimethoxybenzaldehyde was not utilised in any further chalcone formation reactions.

Synthesis of chalcone 88 was then attempted using 4-acetamidobenzaldehyde 89 and 2ʹ-hydroxyacetophenone 72 (Table 2.5, entries 17 and 18), however no conversion was effected with either base. This chalcone 88 has been synthesised previously by
Cabrera and co-workers using the traditional method (NaOH in EtOH, room temperature for 16 hours), however no yield was reported in that case. 141

Finally, 2'-hydroxyacetophenone 72 was reacted with 2-carboxybenzaldehyde 90 (Table 2.5, entries 19 and 20). The results from these reactions were quite interesting, as it was noted that the 1H NMR spectrum showed a ratio of 9:1:9 acetophenone 72 to chalcone 91 to flavanone 92 respectively. Purification provided the flavanone 92 in a yield of 46%, however the acetophenone and chalcone could not be separated. The reason for this increased formation of the cyclised product is unknown. As the aim of this section was the synthesis of a 2'-hydroxychalcone library, and not the corresponding flavanones, 2-carboxybenzaldehyde 90 was not utilised in any more reactions.

In total, seven 2'-hydroxychalcones were synthesised from 2'-hydroxyacetophenone and substituted benzaldehydes, including two novel compounds (76 and 80). Generally, less substituted benzaldehydes provided 2'-hydroxychalcones in greater yields, and KOH effected greater conversions than Bu 4NOH. It was also noted that benzaldehydes with electron-withdrawing substituents (including nitro, carboxylic acid, and acetamide groups) did not perform well, with little to none of the desired products isolated in these cases.

As the entire series of chalcones synthesised to this point used 2'-hydroxyacetophenone 72, attention turned to the synthesis of chalcones using multiply-substituted acetophenone starting materials. Note that due to poor results in the synthesis of chalcones 87, 88, and 91, 2,4,5-trimethoxybenzaldehyde 86, 4-acetamidobenzaldehyde 89 and 2-carboxybenzaldehyde 90 were not used.

2.3.3.2 Reaction of 2',4'-Dihydroxyacetophenone 93 and 2',4',6'-Trihydroxyacetophenone 94 with Substituted Benzaldehydes

The reactions of more hydroxylated acetophenones were examined next. This was of importance as natural flavonoids and chalcones are highly hydroxylated, and as established in Chapter One, it is these compounds (including quercetin 13 and kaempferol 18) that often produce the greatest therapeutic activity. 139, 279, 397-400

First, 2',4'-dihydroxyacetophenone 93 was reacted with benzaldehyde 59, with KOH and then Bu 4NOH (Table 2.6, entries 1 and 2 respectively). However, only the starting material 93 could be verified by 1H NMR spectroscopy in the case of the KOH-catalysed reaction, and the Bu 4NOH equivalent showed a mixture of products,
including the acetophenone 93, chalcone 94, flavanone 95, Cannizzaro benzyl alcohol 64 (in an approximate ratio of 1:10:10:6 respectively), and other unknown compounds. From this result, it appeared that the protection of hydroxyl groups was required.

To ensure that these results were due to the substitution of the acetophenone, 2',4'-dihydroxyacetophenone 93 was also reacted with 4-bromobenzaldehyde 73, 3,5-dibromobenzaldehyde 75 and 3-benzyloxybenzaldehyde 77. In all of these examples, and with both bases, analysis of the crude material revealed a mixture of products, with the desired chalcone in each case accounting for less than 50% of the total mass. For this reason, the similar 2',6'-dihydroxyacetophenone 96 was not applied to the reaction.

Table 2.6. Microwave-assisted synthesis of functionalised 2',4'-dihydroxychalcones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starting Material</th>
<th>R₁</th>
<th>Base</th>
<th>Acetophenone: Chalcone a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93</td>
<td>H</td>
<td>KOH</td>
<td>1:0</td>
</tr>
<tr>
<td>2</td>
<td>93</td>
<td>H</td>
<td>Bu₄NOH b</td>
<td>1:10 b</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
<td>6'-OH</td>
<td>KOH</td>
<td>1:0</td>
</tr>
<tr>
<td>4</td>
<td>97</td>
<td>6'-OH</td>
<td>Bu₄NOH</td>
<td>1:0</td>
</tr>
</tbody>
</table>

a Ratio determined by 'H NMR integration
b A relative ratio of 10 was found for the corresponding flavanone 95 and 6 for benzyl alcohol 64

Reacting 2',4',6'-trihydroxyacetophenone 97 with benzaldehyde 59 and each of the bases also proved unsuccessful (Table 2.6, entries 3 and 4), and, as expected, only starting materials were seen in the 'H NMR spectrum.

The obvious next step was the protection of the additional hydroxyl groups of 2',4'-dihydroxyacetophenone 93 and 2',6'-dihydroxyacetophenone 96, and their subsequent reaction with various substituted benzaldehydes.

2.3.3.3 Monoprotection of Dihydroxyacetophenones 93 and 96

Methyl protection was initially pursued, as the formation of methyl ethers is simple, and their small size would prevent any steric issues. A literature method for the methyl monoprotection of these dihydroxylated acetophenones was used, in which the acetophenone was reacted with one equivalent of potassium carbonate (K₂CO₃), followed by the slow addition of one equivalent of dimethyl sulfate (Me₂SO₄) with reflux in acetone for an hour.
This method was first applied to 2′,4′-dihydroxyacetophenone 93. After workup, a golden-orange oil was afforded, from which a crude NMR spectrum was obtained. An approximate 1:1 ratio of the 2′-hydroxy-4′-methoxychalcone 98 to the 4′-hydroxy-2′-methoxychalcone 99 was noted (Scheme 2.6). As it was desired that only the 4′-hydroxyl group of the acetophenone would be protected (as the 2′-hydroxyl group was required in future reactions for Ring C closure to other flavonoids), the protection of this compound for use in chalcone synthesis was abandoned.

Scheme 2.6. The mixture of products obtained by mono-methyl protection of 2′,4′-dihydroxyacetophenone 93.

The reaction was then applied to 2′,6′-dihydroxyacetophenone 96. Following workup, the desired product 2′-hydroxy-6′-methoxyacetophenone 100 was isolated as yellow needle-like crystals in a yield of 97%, as confirmed by comparison to literature data, including a singlet with an integration of three at δ 3.88 ppm in the $^1$H NMR spectrum. As the product was mono-protected, and protection of either the 2′- or 6′-hydroxyl still left a free hydroxyl group for Ring C closure to flavonoids (Figure 2.7), this protected acetophenone 100 was used in chalcone synthesis.

Figure 2.7 Mono-methyl protection of 2′,6′-dihydroxyacetophenone 96 gives one product.

2.3.3.4 Reaction of 2′-Hydroxy-6′-methoxyacetophenone 100 with Substituted Benzaldehydes

With 2′-hydroxy-6′-methoxyacetophenone 100 in hand, attention turned to its use in microwave-assisted 2′-hydroxychalcone formation. The first reaction of this series was that of the mono-protected acetophenone 100 with benzaldehyde 59 (Table 2.7, entries 1 and 2). While both KOH and Bu$_4$NOH effected good conversion ratios, the product from the KOH reaction was purified by column chromatography to
afford chalcone 101 as a yellow oil in a yield of 67%. Comparison to literature data confirmed the desired product.404

Next, the mono-protected acetophenone was reacted with 4-bromobenzaldehyde 73 (Table 2.7, entries 3 and 4). Once again, KOH was more effective than Bu₄NOH, and the desired chalcone 102 was isolated in a yield of 46%, and the structure confirmed by comparison to literature data.405

The dihalogenated 3,5-dibromobenzaldehyde 75 was the next reactant with 2′-hydroxy-6′-methoxyacetophenone 100 (Table 2.7, entries 5 and 6). Once again with this aldehyde, Bu₄NOH provided the greater conversion to the chalcone 103 (see also Table 2.5, Entry 6). However, conversion using this base was only 2:1 for the starting acetophenone 100 to desired 2′-hydroxychalcone 103. Due to such a poor conversion, and as a poor yield had already been experienced from this benzaldehyde (52%, Table 2.5, Entry 6), the yield for chalcone 103 was understandably low (26%). This novel structure was fully characterised by ¹H and ¹³C NMR Spectroscopy, as well as HRMS.

The microwave reaction of 2′-hydroxy-6′-methoxyacetophenone 100 with 3-benzyloxybenzaldehyde 77 was attempted next (Table 2.7, entries 7 and 8). Using KOH
as the base gave full conversion to the chalcone, with purification yielding chalcone 104 in 83%. Once again, comparison to literature data confirmed the structure as that of the desired.406

The next benzaldehyde to be trialled was 5-bromo-2,4-dimethoxybenzaldehyde 79 (Table 2.7, entries 9 and 10). Complete conversion was achieved using KOH as the base, and the synthesis of the novel chalcone 105 (52%) was fully characterised by 1H and 13C NMR spectroscopy, as well as HRMS.

The reaction of 2'-hydroxy-6'-methoxyacetophenone 100 with 4-fluorobenzaldehyde 81 was the next microwave reaction to be attempted (Table 2.7, entries 11 and 12). Conversion to the desired chalcone 106 did not occur with either base. However, the presence of an aldehyde peak at δ 9.80 ppm and a methoxyl peak at δ 3.80 ppm in the spectrum from the Bu4NOH reaction suggested that once again an SNAr reaction (seen previously in Table 2.5, entry 12) had occurred to substitute the para-fluoro group for a methoxyl group on the benzaldehyde (see previous Scheme 2.5).396

Although the 4-nitrobenzaldehyde 84 had proven to be a poor reactant previously (see Table 2.5, entries 13 and 14), it was thought that reaction with a more electron-donating substituent on the acetophenone (2'-hydroxy-6'-methoxyacetophenone) would be more successful. However, this was not the case, and no conversion to desired compound 107 could be seen when either base was used (Table 2.7, entries 13 and 14).

In summary, it was found that chalcones synthesised with 2'-hydroxy-6'-methoxyacetophenone 100 generally gave lower yields than those synthesised with 2'-hydroxyacetophenone 72. This may have simply been because of the added functionality complicating the electronics of the reaction. A total of five compounds were synthesised in this series, with two of those (chalcones 103 and 105) being novel compounds.

As the series of 2'-hydroxy-6'-methoxychalcones had now been examined, the microwave reaction of the halogenated starting material 5'-bromo-2'-hydroxyacetophenone 108 in the Claisen-Schmidt condensation was then investigated.
2.3.3.5 Reaction of 5′-Bromo-2′-hydroxyacetophenone 108 with Substituted Benzaldehydes

The next acetophenone to be investigated in the synthesis of the library of 2′-hydroxychalcones was 5′-bromo-2′-hydroxyacetophenone 108. As mentioned earlier, halogenated reagents were desired as they provided the benefit of halogen bonding in drug-target interactions, and also could allow the application of palladium-catalysed reactions (including the Heck reaction) to introduce various other functional groups to chalcone or flavonoid scaffolds by means of new carbon-carbon bond formation (see Chapter Four).

This halogenated acetophenone 108 was first reacted with benzaldehyde 59 under microwave irradiation using the optimal conditions (KOH or Bu₄NOH, THF, MW, 50 °C, 15 minutes). Both bases gave full conversion to the desired chalcone 109 (Table 2.8, entries 1 and 2), which was isolated in a yield of 86%, as confirmed by comparison to literature data.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R₁</th>
<th>R₂</th>
<th>Base</th>
<th>108:chalcone (yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109</td>
<td>H</td>
<td>H</td>
<td>KOH</td>
<td>0:1(86)</td>
</tr>
<tr>
<td>2</td>
<td>109</td>
<td>H</td>
<td>H</td>
<td>Bu₄NOH</td>
<td>0:1</td>
</tr>
<tr>
<td>3</td>
<td>110</td>
<td>4-Br</td>
<td>H</td>
<td>KOH</td>
<td>0:1(64)</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>4-Br</td>
<td>H</td>
<td>Bu₄NOH</td>
<td>1:5</td>
</tr>
<tr>
<td>5</td>
<td>111</td>
<td>3-OBn</td>
<td>H</td>
<td>KOH</td>
<td>1:10(80)</td>
</tr>
<tr>
<td>6</td>
<td>111</td>
<td>3-OBn</td>
<td>H</td>
<td>Bu₄NOH</td>
<td>3:10</td>
</tr>
<tr>
<td>7</td>
<td>112</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>KOH</td>
<td>4:1</td>
</tr>
<tr>
<td>8</td>
<td>112</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>Bu₄NOH</td>
<td>1:8(66)</td>
</tr>
<tr>
<td>9</td>
<td>113</td>
<td>4-F</td>
<td>H</td>
<td>KOH</td>
<td>0:1(87)</td>
</tr>
<tr>
<td>10</td>
<td>114</td>
<td>4-F</td>
<td>H</td>
<td>Bu₄NOH</td>
<td>0:1(88)</td>
</tr>
<tr>
<td>11</td>
<td>115</td>
<td>4-NO₂</td>
<td>H</td>
<td>KOH</td>
<td>1:0</td>
</tr>
<tr>
<td>12</td>
<td>115</td>
<td>4-NO₂</td>
<td>H</td>
<td>Bu₄NOH</td>
<td>1:0</td>
</tr>
</tbody>
</table>

* Underlined compounds are novel
* Ratio determined by 1H NMR integration
* Isolated yield (%) after column chromatography
* Isolated product had 4-MeO as R₁ in place of 4-F

The next benzaldehyde trialled was 4-bromobenzaldehyde 73 (Table 2.8, entries 3 and 4). Greater conversion was accomplished using KOH, and purification gave the
desired product 110 as a bright yellow solid in a yield of 64%, as confirmed by comparison to literature data.407

Following this, 3-benzyloxybenzaldehyde 77 was reacted with the 5′-bromo-2′-hydroxyacetophenone 108. Once again, KOH provided the greatest conversion (Table 2.8, entry 5), and the novel chalcone 111 was isolated as a bright yellow solid (80%), and fully characterised.

Another novel compound was then synthesised, by reacting 5′-bromo-2′-hydroxyacetophenone 108 with 5-bromo-2,4-dimethoxybenzaldehyde 79. Notably, the reaction using Bu₄NOH proved to be better than that of KOH (Table 2.8, entries 8 and 7 respectively), and purification by column chromatography isolated chalcone 112 as a bright orange solid in a yield of 66%. This structure was characterised by HRMS, and ¹H and ¹³C NMR spectroscopy.

Next, the polar 4-fluorobenzaldehyde 81 was reacted with 5′-bromo-2′-hydroxyacetophenone 108 to synthesise chalcone 113. When the reaction was conducted using KOH (Table 2.8, entry 9), complete conversion to chalcone 113 was noted, and purification gave the desired compound as a bright yellow solid in a yield of 87%.407

Scheme 2.7. S₅Ar chalcone 114 is formed from chalcone 113 in the presence of Bu₄NOH and MeOH.

However, when the 1.0 M Bu₄NOH in MeOH was used as a base (Table 2.8, Entry 10), evidence of an S₅Ar reaction was once again present in the ¹H NMR spectrum, with peaks representing a chalcone hydroxyl group (singlet at δ 12.87 ppm)
and a methoxyl group (at $\delta$ 3.84 ppm with an integration of three), and was predicted to occur as shown in Scheme 2.7. As with all $S_N$Ar reactions, a resonance-stabilised intermediate is formed. Purification provided the di-substituted 2'-hydroxychalcone in an excellent yield of 88%, as a bright yellow solid.

Finally, 4-nitrobenzaldehyde was reacted with 5'-bromo-2'-hydroxyacetophenone (Table 2.8, Entries 11 and 12). Previous experience with this aldehyde gave little hope for success, and this proved true as neither base provided the desired chalcone.

In conclusion, six chalcones were synthesised using 5'-bromo-2'-hydroxyacetophenone as the starting material. Of these, two were novel (chalcones 111 and 112), and full characterisation for these compounds was completed. Interestingly, using Bu$_4$NOH as a base produced greater conversion ratios than KOH in more instances with this acetophenone than any other.

As the use of an acetophenone with a single halogen substitution had been successfully employed in this microwave-assisted chalcone synthesis, attention turned to the use of the dihalogenated 3'-bromo-5'-chloro-2'-hydroxyacetophenone.

2.3.3.6 Reaction of 3'-Bromo-5'-chloro-2'-hydroxyacetophenone with Substituted Benzaldehydes

Introducing another halogen into the chalcone scaffold was achieved through the reaction of 3'-bromo-5'-chloro-2'-hydroxyacetophenone with various benzaldehydes under the microwave-assisted conditions. Once again, all known compounds were compared to literature data, and novel compounds were characterised by $^1$H and $^{13}$C NMR spectroscopy, as well as HRMS.

The first of this series of 2'-hydroxychalcones was synthesised though the reaction of acetophenone with 59 and KOH or Bu$_4$NOH under microwave irradiation. Greater conversion was achieved with KOH (Table 2.9, entry 1), and chalcone was isolated as an orange-yellow solid in excellent yield (86%), as confirmed by comparison to the literature data.

In the reaction of 3'-bromo-5'-chloro-2'-hydroxyacetophenone with 4-bromobenzaldehyde, yet another halogen could be introduced onto the scaffold (Table 2.9, entries 3 and 4). Once again, KOH provided the best conversion, and purification isolated the novel chalcone as a yellow solid in a yield of 80%.
Synthesising a tetrahalogenated 2′-hydroxychalcone proved difficult. After reacting 3′-bromo-5′-chlo-ro-2′-hydroxyacetophenone 116 and 3,5-dibromobenzaldehyde 75 under the optimised conditions (KOH or Bu4NOH, THF, MW, 50 °C, 15 minutes), none of the desired chalcone 119 was observed by 1H NMR Spectroscopy (Table 2.9, entries 5 and 6); only starting materials were identified. This result was anticipated, as the 3,5-dibromobenzaldehyde had proven to be a poor reagent in previous experiments (see also Table 2.5, entry 6 and Table 2.7, entry 6).

Table 2.9. Microwave-assisted synthesis of functionalized 3′-bromo-5′-chloro-2′-hydroxychalcones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R1</th>
<th>R2</th>
<th>Base</th>
<th>Y16:chalconeb</th>
<th>(yield)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>117</td>
<td>H</td>
<td>H</td>
<td>KOH</td>
<td>0:1 (86)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>117</td>
<td>H</td>
<td>H</td>
<td>Bu4NOH</td>
<td>0:1 (86)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>118</td>
<td>4-Br</td>
<td>H</td>
<td>KOH</td>
<td>0:1 (80)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>118</td>
<td>4-Br</td>
<td>H</td>
<td>Bu4NOH</td>
<td>0:1 (80)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>119</td>
<td>3-Br</td>
<td>5-Br</td>
<td>KOH</td>
<td>1:0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>119</td>
<td>3-Br</td>
<td>5-Br</td>
<td>Bu4NOH</td>
<td>1:0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>120</td>
<td>3-OBn</td>
<td>H</td>
<td>KOH</td>
<td>1:10 (78)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>120</td>
<td>3-OBn</td>
<td>H</td>
<td>Bu4NOH</td>
<td>1:10 (78)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>121</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>KOH</td>
<td>1:4 (71)</td>
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<td>10</td>
<td>121</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>Bu4NOH</td>
<td>1:4 (71)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>122</td>
<td>4-F</td>
<td>H</td>
<td>KOH</td>
<td>0:1(93)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>122</td>
<td>4-F</td>
<td>H</td>
<td>Bu4NOH</td>
<td>0:1(93)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>123</td>
<td>4-NO2</td>
<td>H</td>
<td>KOH</td>
<td>1:0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>123</td>
<td>4-NO2</td>
<td>H</td>
<td>Bu4NOH</td>
<td>1:0</td>
<td></td>
</tr>
</tbody>
</table>

* Underlined compounds are novel
b Ratio determined by 1H NMR integration
c Isolated yield (%) after column chromatography

After this, 3′-bromo-5′-chloro-2′-hydroxyacetophenone 116 and 3-benzyloxybenzaldehyde 77 were reacted (Table 2.9, entries 7 and 8). The best conversion was achieved with KOH, and chalcone 120 was isolated in a yield of 78%. This novel 2′-hydroxychalcone was fully characterised.

The next chalcone 121 was the product of 3′-bromo-5′-chloro-2′-hydroxyacetophenone 116 and 5-bromo-2,4-dimethoxybenzaldehyde 79 (Table 2.9, entries 9 and 10). A greater conversion ratio was achieved with KOH (1:4 acetophenone 116 to chalcone 121), and purification isolated the novel chalcone 121 as a bright yellow solid in a yield of 71%, which was fully characterised.
Following this, 4-fluorobenzaldehyde 81 was reacted with 3‘-bromo-5‘-chloro-2‘-hydroxyacetophenone 116. Complete conversion to the desired 2‘-hydroxychalcone 122 was seen in the KOH reaction (Table 2.9, entry 11), and purification isolated a yellow solid in a yield of 93%. No conversion to a chalcone product was noted in the Bu4NOH reaction (Table 2.9, entry 12), although the singlet benzaldehyde peak (δ 9.80 ppm) and singlet methoxy peak (δ 3.81 ppm) in the ¹H NMR spectrum indicated that nucleophilic aryl substitution (SNAr) had occurred (Scheme 2.5).

4-Nitrobenzaldehyde 84 was also utilised as the benzaldehyde in this series (Table 2.9, Entries 13 and 14), however none of the desired chalcone 123 could be identified in the ¹H NMR spectrum, and as this aldehyde has been proven to be unsuccessful in other cases, the synthesis of chalcone 123 was abandoned.

In conclusion for this series of compounds, five compounds were synthesised using 3‘-bromo-5‘-chloro-2‘-hydroxyacetophenone 116 and various substituted benzaldehydes, including three novel compounds (Table 2.9, chalcones 118, 120 and 121). Most of the compounds were achieved in greater conversions with the base KOH, and all were characterised by ¹H and ¹³C NMR spectroscopy, as well as HRMS.

2.3.3.7 Conclusion for 2‘-Hydroxychalcones

A total of 23 chalcones were synthesised using the optimised methodology, with the majority of these provided through the KOH-mediated reaction. Of these, nine chalcones were novel compounds, and have been fully characterised. With this library of chalcones in hand, a significant attempt towards the synthesis of a flavonoid library had been completed, in accordance with the first aim of this research. As the synthesis was progressing, thirteen of these 2‘-hydroxychalcones were evaluated for cytotoxicity using a colourimetric MTT Assay, as carried out by Doctor Sunil Ratnayake. These studies were completed in order to select ideal candidates to be submitted for further evaluation of possible therapeutic effects, yet another aim of this research.

2.4 Cytotoxicity Evaluation

The colourimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay can be used to examine the proliferation or cell toxicity of a number of different cell lines. This assay relies on the reduction of MTT 124, a yellow tetrazole (Figure 2.8), to its corresponding purple formazan, by mitochondrial succinate dehydrogenase. As the optical density of the dye at a particular wavelength is directly
correlated to the number of metabolically active cells, measurement of the absorption of
the dye using an ELISA reader (spectrophotometer) at a particular wavelength allows
for the determination of live cell numbers. Through the addition and incubation of
healthy cells with test compounds, the concentration of the compound at which 50% of
the cells are killed (LC50) can be determined, giving an indication of the compound’s
cell toxicity.

Figure 2.8. Colorimetric MTT assays rely on the reduction of the yellow tetrazole MTT.

In more recent times, water-soluble tetrazoles (WSTs) have been applied to the
MTT assay. These dyes make the assay much quicker and easier, as they remove the
need for any solubilisation steps of the dyes, give a more effective signal than MTT,
and are less toxic to the cells. The dye used for the cytotoxicity testing in this research
was the pale yellow 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-
tetrazolium, which is also known as WST-1. When reduced by the mitochondrial
succinate dehydrogenase of healthy cells, the corresponding formazan is produced
(Scheme 2.8), which is dark yellow in colour and its relative concentration can be
determined from its absorbance at 438 nm.

Scheme 2.8. The reduction of WST-1 to its corresponding dark yellow formazan.

Thirteen 2'-hydroxychalcones were chosen for cytotoxic evaluation, against
both human embryonic kidney (HEK) and hepatocellular carcinoma (HepG2) cell lines.
The general method used is described in the Experimental Chapter (See Chapter Eight),
and involved the incubation of the 2'-hydroxychalcones with the cells for a period of
24 hours, at concentrations of 1, 2, 4, 10, 20, 40, and 80 μg/mL. Positive (Triton (10%
v/v)) and negative controls (no compound added) were also analysed. Following this,
WST-1 was added, and after a further incubation period of 20 minutes, the cells were
viewed under the spectrophotometer at 440 nm. From these results, the LC$_{50}$ values were determined for each compound (Table 2.10) for both the HEK and HepG2 cell lines (See Appendix B for the cytotoxicity results in graph form).

In the results from the HEK cell line, it was found that most of the tested 2’-hydroxychalcones LC$_{50}$ values were at concentrations less than 20$\mu$g/mL (Table 2.10, entries 1-3, 6-9, 10 and 12). Interestingly, 2’-hydroxy-3-benzyloxychalcone 78 had an extremely low LC$_{50}$ value of 1.5 $\mu$g/mL. However the remaining three compounds 80, 118 and 121, two of which contained the 5-bromo-2,4-dimethoxy substitutions at R$_3$ and R$_4$, were not toxic at the highest concentration tested (80 $\mu$g/mL).

Table 2.10. Cytotoxicity LC$_{50}$ values of 2’-hydroxychalcones for HEK and HepG2 cells.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Chalcone</th>
<th>R$_1$, R$_2$</th>
<th>R$_3$, R$_4$</th>
<th>HEK LC$_{50}$ values (µg/ml)</th>
<th>HepG2 LC$_{50}$ values (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>H</td>
<td>H</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>H</td>
<td>Br (4)</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>H</td>
<td>Br (3,5)</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>H</td>
<td>OBn(3)</td>
<td>1.5</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>H</td>
<td>Br (5) MeO (2,4)</td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td>6</td>
<td>101</td>
<td>MeO (6’)</td>
<td>H</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>102</td>
<td>MeO (6’)</td>
<td>Br (4)</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>104</td>
<td>MeO (6’)</td>
<td>OBn(3)</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>105</td>
<td>MeO (6’)</td>
<td>Br (5) MeO (2,4)</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>117</td>
<td>Br (3’) Cl (5’)</td>
<td>H</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>11</td>
<td>118</td>
<td>Br (3’) Cl (5’)</td>
<td>Br (4)</td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
<tr>
<td>12</td>
<td>120</td>
<td>Br (3’) Cl (5’)</td>
<td>OBn (3)</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>13</td>
<td>121</td>
<td>Br (3’) Cl (5’)</td>
<td>Br (5) MeO (2,4)</td>
<td>&gt;80</td>
<td>&gt;80</td>
</tr>
</tbody>
</table>

The HepG2 cell line showed comparable results, in that once again chalcones 80, 118 and 121 (as shown in Table 2.10, entries 5, 11, and 13, Figure 2.9) were not toxic to cells at the maximum concentration tested (80 $\mu$g/mL). Chalcone 120 (Table 2.10, entry 12) also gave a relatively high LC$_{50}$ value of 70 $\mu$g/mL. The remainder of the chalcones showed LC$_{50}$ values of between 14 and 35 $\mu$g/mL.

Figure 2.9. Three 2’-hydroxychalcones, 80, 118 and 121, were not toxic at the highest concentration tested (80 $\mu$g/mL), in both liver and kidney cell lines.
From this information, it could be concluded that chalcones 80, 120, and 121 could serve as potential therapeutic agents due to their low cytotoxicity for both liver and kidney cell lines. For this reason, these three chalcones were sent for biochemical evaluation in terms of their antibacterial, anticancer (breast and prostate), and antidiabetic activity, and results are expected in the near future. Also, chalcone 78 could be ideal as an anticancer agent due to its high cytotoxicity for the kidney cell line, but not the liver cell line.

2.5 Conclusion

In conclusion, an optimised method for the microwave-assisted synthesis of 2’-hydroxychalcones from functionalised 2’-hydroxyacetophenones and benaldehydes using the Claisen-Schmidt condensation was developed. This method took advantage of microwave irradiation, with an optimised reaction time of 15 minutes and a temperature of 50 °C, and used THF as a solvent (Scheme 2.9).

Two bases, KOH and NaOH, were found to produce the highest yields, in accordance with the traditional method, and the base Bu4NOH, as a 1.0 M solution in MeOH, was also found for the first time to give good conversion ratios to the 2’-hydroxychalcones. These conditions avoided the side reactions of flavanone formation and Cannizzaro reaction, a quality not seen when the traditional method was trialled.

While KOH generally gave the higher conversion ratios in the synthesis of 2’-hydroxychalcones, there were a few exceptions. It was observed that these exceptions shared the fact that the starting materials and products contained multiple bromine substituents (see those reactions with 3,5-dibromobenzaldehyde 75, and also the reaction of 5’-bromo-2’-hydroxyacetophenone 108 with 5-bromo-2,4-dimethoxybenzaldehyde 79). However, this phenomenon could not be explained.

The application of the optimised methodology to numerous substituted 2’-hydroxyacetophenones and benaldehydes produced 23 2’-hydroxychalcones (Table 2.11). These included nine novel compounds, which are underlined in the table.
As mentioned earlier, this methodology, along with characterisation details for a number of the synthesised chalcones, was published in *Current Organic Chemistry* (2012, 16(1), 121-126) (see Appendix A).

Table 2.11. The microwave-assisted Claisen-Schmidt condensation produced 23 functionalised 2’-hydroxychalcones.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Base</th>
<th>Yield (%)²</th>
</tr>
</thead>
<tbody>
<tr>
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<td>66</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>KOH</td>
<td>&gt;99</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>H</td>
<td>H</td>
<td>4-Br</td>
<td>H</td>
<td>KOH</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>H</td>
<td>H</td>
<td>3-Br</td>
<td>5-Br</td>
<td>Bu₄NOH</td>
<td>52</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>H</td>
<td>H</td>
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<tr>
<td>5</td>
<td>80</td>
<td>H</td>
<td>H</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>KOH</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>82</td>
<td>H</td>
<td>H</td>
<td>4-F</td>
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<td>KOH</td>
<td>58</td>
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<tr>
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<td>85</td>
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<td>H</td>
<td>4-NO₂</td>
<td>H</td>
<td>KOH</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>101</td>
<td>6’-OMe</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>KOH</td>
<td>67</td>
</tr>
<tr>
<td>9</td>
<td>102</td>
<td>6’-OMe</td>
<td>H</td>
<td>4-Br</td>
<td>H</td>
<td>KOH</td>
<td>46</td>
</tr>
<tr>
<td>10</td>
<td>103</td>
<td>6’-OMe</td>
<td>H</td>
<td>3-Br</td>
<td>5-Br</td>
<td>Bu₄NOH</td>
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</tr>
<tr>
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<td>104</td>
<td>6’-OMe</td>
<td>H</td>
<td>3-OBn</td>
<td>H</td>
<td>KOH</td>
<td>83</td>
</tr>
<tr>
<td>12</td>
<td>105</td>
<td>6’-OMe</td>
<td>H</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>KOH</td>
<td>52</td>
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<tr>
<td>13</td>
<td>109</td>
<td>5’-Br</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>KOH</td>
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<td>110</td>
<td>5’-Br</td>
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<td>4-Br</td>
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<td>KOH</td>
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<td>16</td>
<td>112</td>
<td>5’-Br</td>
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<td>5-Br</td>
<td>Bu₄NOH</td>
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<td>4-F</td>
<td>H</td>
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<tr>
<td>20</td>
<td>118</td>
<td>3’-Br</td>
<td>5’-Cl</td>
<td>4-Br</td>
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<td>KOH</td>
<td>80</td>
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<tr>
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<td>121</td>
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<td>5’-Cl</td>
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<td>5-Br</td>
<td>KOH</td>
<td>71</td>
</tr>
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<td>23</td>
<td>122</td>
<td>3’-Br</td>
<td>5’-Cl</td>
<td>4-F</td>
<td>H</td>
<td>KOH</td>
<td>93</td>
</tr>
</tbody>
</table>

² Isolated yield (%) after column chromatography

³ Isolated product had 4-MeO as R₃ in place of 4-F

Thirteen substituted 2’-hydroxychalcones were tested for their cytotoxicity, and three of these were not toxic at the highest concentration tested (80 μg/mL, Figure 2.9), in both liver and kidney cell lines (see Appendix B).
Of these three nontoxic compounds, it is also noted that two of these (118 and 121) were novel compounds. This work provides a starting point for the development of new, nontoxic chalcone and flavonoid-based therapeutic agents.

![Chemical structures](image)

Figure 2.9. Three 2'-hydroxychalcones, 80, 118 and 121, were not toxic at the highest concentration tested (80 μg/mL), in both liver and kidney cell lines.

In conclusion, a simple and rapid microwave-assisted methodology for the synthesis of 2'-hydroxychalcones has been developed.
Chapter Three:
Synthesis of a Flavonoid Library
3.1 Chapter Overview

In accordance with the aim of synthesising a library of flavonoid compounds, attention turned to the synthesis of other members of the flavonoid family, utilising the previously synthesised 2ʹ-hydroxychalones as starting materials. These flavonoids were to be synthesised by existing or newly developed methods, so as to provide a library of novel, highly halogenated compounds for biochemical evaluations. The flavonoids synthesised and discussed in this chapter include flavanones (via an acid or base-catalysed cyclisation) and flavones (via an I₂-mediated cyclisation).

3.2 Flavanones

3.2.1 The Synthesis of Flavanones

The flavanone structure 6 is the ring-closed equivalent of 2ʹ-hydroxychalcone 66, and is defined by the presence of a single bond between positions 2 and 3 (corresponding to –an– in flavanone), and a ketone at position 4 (corresponding to –one in flavanone). As already explained in Chapter 1.2.1, the three rings of flavonoids are labelled A, C and B from left to right, and the flavanones are numbered as shown in Figure 3.1.

![Figure 3.1. The simplest flavanone 6 is also known as 2,3-dihydroflavone or 2-phenyl-2,3-dihydro-4H-chromen-4-one.](image)

As mentioned in Chapter One, flavanones are commonly found in plants and fruits as their glycosylated products; examples include naringin 127 found in grapefruit, and hesperidin 16 found in citrus fruits (Figure 3.2). In plants, flavanones are synthesised asymmetrically by chalcone isomerase (CHI) from 2ʹ-hydroxychalones.

![Figure 3.2. Naringin 127 and hesperidin 16 are two natural glycosylated flavanones.](image)
There are a number of methods available for the laboratory synthesis of the flavanones. The first of these is the acid- or base-catalysed cyclisation of 2ʹ-hydroxychalcones. These pathways have been discussed previously in Chapter 2.2, and occur via the mechanisms shown in Schemes 2.3 and 2.4. It is important to acknowledge once again that this isomerisation is reversible, and that 100% conversion to flavanones from chalcones is a significant challenge.

In 1904, Kostanecki was the first to accomplish the acid-catalysed cyclisation, using hydrochloric acid, although no yield was published (Scheme 3.1). Since then, many acids have been applied to the cyclisation, including H₂SO₄ and CF₃COOH. Base-catalysed cyclisations have utilised pyridine, Co(salpr) (a cobalt Schiff base complex) under O₂, KOH in MeOH (pH 10), and NaOAc heated in refluxing EtOH. However, as noted by Sagrera, basic conditions are less commonly used as they can lead to a retro-aldol reaction, in which the acetophenone and benzaldehyde starting materials are re-formed.

The recent focus on ‘green chemistry’ has ensured the investigation of microwave-assisted methods for the cyclisation, with Patonay using a microwave-assisted, solvent free methodology with DBU as a base, and Nie synthesising flavanone hydrazones from chalcones and hydrazides using 2-propanol as a solvent.

Other methods for the isomerisation of chalcones to flavanones include the use of chiral Brønsted acids and bases to form asymmetric flavanones, MgO powder in place of a base, and even a chiral thiourea catalyst amongst others.

Flavanones have also been synthesised directly from acetophenones and benzaldehydes. In such cases, the 2ʹ-hydroxychalcone intermediate is formed in solution, and reacts further to form the corresponding flavanone compound. Conditions reported to effect this two-step conversion include solvent-free synthesis over aminopropyl-functionalized SBA-15 (Scheme 3.2), L-Proline, and Li-Al layered double hydroxides amongst others.
3.2.1.1 Ionic Liquids

One approach that has not been thoroughly investigated for flavanone formation is the use of ionic liquids. Ionic liquids are simply salts that have melting points below 100 °C, making most of them liquids at room temperature.\textsuperscript{432} They are valued for their high thermal and chemical stability, low vapour pressure, and non-flammability.\textsuperscript{433} Due to these properties, they have emerged over the last 20 years as both catalysts and ideal solvents in chemical reactions.\textsuperscript{324, 431, 432, 434-445} There are two main types of ionic liquids, based on the ionic species present; aprotic and protic ionic liquids. The protic variety has an acidic proton on the cationic species, the aprotic does not.\textsuperscript{446}

Ionic liquids have also commonly been coupled with microwave reactors in synthetic methodologies. This provides a number of benefits, as the combination results in the need for little to no solvent, decreases reaction times, increases reaction efficiency, provides simple work-up procedures, allows for greater catalyst recovery, and has also been shown to increase yields.\textsuperscript{433, 447-452}

Protic Ionic Liquids (pILs) have emerged as an ideal means of carrying out acid-promoted reactions and rearrangements cleanly and rapidly.\textsuperscript{438, 439} pILs are made by mixing strictly equimolar amounts of Bronsted acids and bases, and are neutral as there is no free H\textsuperscript{+} in the reaction media.\textsuperscript{453} However, it is possible to ‘tune’ the acidity of the pIL, through the careful selection of the acid and base counter-ions.\textsuperscript{454} This selection gives rise to pILs with different proton activity values, as measured by pK\textsubscript{a}: pILs containing cations of low pK\textsubscript{a} values have higher proton activity.\textsuperscript{455, 456}

While different types of ionic liquids have been utilised in the formation of chalcones from acetophenones and benzaldehydes,\textsuperscript{325, 441, 442, 457} there are only two reports of ionic liquids in flavanone synthesis. The first describes the use of a Zn-Al hydrotalcite adhered with ionic liquid 1-(triethoxysilylpropyl)-3-methylimidazolium chloride (TESP-MImCl), to form flavanones directly from the corresponding 2'-hydroxyacetophenones and benzaldehydes (Scheme 3.3).\textsuperscript{431}
Scheme 3.3. The ionic liquid TESP-MImCl, adhered to a Zn-Al hydrotalcite-like clay (CHTlc), has been used for the synthesis of flavanones and chalcones from acetophenones and benzaldehydes.431

The second reports the use of the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF4]) as a solvent, with ytterbium triflate (Yb(OTf)3) as the catalyst to isomerise chalcones (Scheme 3.4).458 In both cases, few flavanones were produced (five and seven respectively), and products in which Ring A was substituted were not examined.

Scheme 3.4. The ionic liquid [bmim][BF4] has been used as a solvent with Yb(OTf)3 to synthesise flavanones.

The use of ionic liquids, in particular pILs, as dual reagent/solvent for 2′-hydroxychalcone isomerisation to the flavanone had not been investigated. Given their pseudo-acidic nature, and the fact that most flavanone syntheses occur under acidic conditions, an investigation into the pIL-mediated, microwave-assisted cyclisation of 2′-hydroxychalcones to flavanones was pursued.

3.2.2 Flavanones: Results and Discussion

3.2.2.1 Base-Mediated Flavanone Synthesis

As the aim of this synthesis was the development of a pIL-mediated methodology, it was first pertinent to examine a base-mediated method for comparison. This base-mediated method for flavanone synthesis took advantage of results from the optimisation of chalcone formation. Following the DBU-mediated, microwave-assisted reaction of 2′-hydroxyacetophenone 72 and benzaldehyde 59 (See Chapter Two, Table 2.1, entry 9), it was noted that the ¹H NMR spectrum of the crude material showed not only the desired chalcone 66, but also the corresponding flavanone 6 (Figure 3.3), in a ratio of 1:2:2 for the acetophenone to chalcone to flavanone respectively. This integration ratio was determined in accordance with those of Chapter Two; the
integrations of the chalcone hydroxyl (0.85 at δ 12.84 ppm), flavanone (1.0 at δ 5.33 ppm) and acetophenone (3.0 at δ 2.57 ppm) peaks were used.

Figure 3.3. ¹H NMR spectrum of the DBU-mediated, microwave-assisted synthesis of flavanone 6 and chalcone 66 (CDCl₃).

This result was reasonable, however the appearance of unknown peaks at approximately δ 2.90 ppm, δ 3.90 ppm and δ 11.92 ppm warranted further investigation (as outlined in blue in Figure 3.3). It was found that Patonay et. al. had established that a microwave-assisted, DBU-mediated flavanone synthesis from 2ʹ-hydroxychalcones produced not only the analogous flavanone, but also dimers incorporating the chalcone and flavanone compounds. These dimers corresponded to those unknown peaks in the ¹H NMR spectrum. This addition of complexity to the reaction mixture would complicate purification and reduce yields.

Whilst this conversion was not great, the purpose of this reaction was to identify any complications that may be experienced, and provide a point of reference between the base- and pIL-mediated reactions.

### 3.2.2.2 Optimisation of pIL-Mediated Microwave-Assisted Flavanone Synthesis

Attention turned to the use of the slightly acidic pILs in combination with microwave irradiation to synthesise flavanones from acetophenone and benzaldehyde starting materials. As explained previously, in such methods the 2ʹ-hydroxychalcone is
formed in situ, and then cyclises to the flavanone. In an attempt to achieve this two-step method, 2’-hydroxyacetophenone 72 (0.5 mmol) and benzaldehyde 59 (1.0 mmol) were reacted according to the microwave method optimised for chalcone synthesis in the previous chapter (MW, 50 °C, 15 minutes). However, in place of a base and solvent, the readily available pIL, TeaHSO₄ (triethylamine: sulphuric acid), was used (Scheme 3.5).

![Scheme 3.5. pIL-mediated flavanone synthesis from 2’-hydroxyacetophenone 72 and benzaldehyde 59 gave a 19:1:9 ratio of 72:66:6.](image)

Analysis of the crude product by ¹H NMR spectroscopy revealed a mixture of acetophenone 72, chalcone 66 and flavanone 6 in a ratio of 19:1:9 respectively. While full conversion from the acetophenone was not achieved, the 1:9 ratio of chalcone to flavanone was encouraging, as it suggested that this combination of pIL and microwave irradiation may be promoting cyclisation. Therefore, the conversion of chalcones synthesised in Chapter Two to flavanones via a pIL-mediated reaction was investigated.

For the optimisation of the pIL-mediated, microwave-assisted formation of flavanones from 2’-hydroxychalcones, 4-bromochalcone 74 was chosen as model substrate (Scheme 3.6). It was easily accessible (a yield of 96% was achieved using the microwave-assisted methodology from Chapter Two), and the resulting flavanone 130 is a well-documented compound. Also, literature examples suggest that para-substituted chalcones and acetophenones are typically easier to convert to their corresponding flavanones than those with substitutions at other positions. Conversion ratios were once again measured by comparison of the bromochalcone 74 2’-hydroxyl peak (a singlet peak at δ 12.74 ppm, with an integration of 0.85) and the CH peak of the corresponding 4’-bromoflavanone 130 (a doublet of doublets at δ 5.44 ppm, with an integration of 1).

![Scheme 3.6. 4-Bromochalcone 74 was the substrate chosen for the optimisation of pIL-mediated, microwave-assisted flavanone synthesis.](image)
Using four available pILs of varying proton activity (note once again that pILs containing cations of low pK\textsubscript{a} values have higher proton activity), each were trialled using different microwave reaction times and temperatures, so as to determine an ideal set of conditions for each pIL. In all reactions, the microwave power was set to an upper limit of 50 W, as the degradation of both reagents and ionic liquids had been noted in previous explorations when excess power was employed.

The first pIL examined was TeaFA (triethylamine: formic acid), which was reacted under microwave irradiation with bromochalcone 74 at 100 °C for 20 minutes. Analysis of the crude product by \textsuperscript{1}H NMR spectroscopy showed 42% conversion to flavanone 130 (Table 3.1, entry 1). This was an encouraging result, and further optimisation commenced.

<table>
<thead>
<tr>
<th>Entry</th>
<th>pIL</th>
<th>pK\textsubscript{a} of acid\textsuperscript{455, 456}</th>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>74:130\textsuperscript{a}</th>
</tr>
</thead>
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<td>TeaFA</td>
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<td>100</td>
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<td>58:42</td>
</tr>
<tr>
<td>2</td>
<td>TeaFA</td>
<td>3.75</td>
<td>130</td>
<td>20</td>
<td>26:65\textsuperscript{b}</td>
</tr>
<tr>
<td>3</td>
<td>TeaFA</td>
<td>3.75</td>
<td>100</td>
<td>40</td>
<td>29:69\textsuperscript{b}</td>
</tr>
<tr>
<td>4</td>
<td>TeaFA</td>
<td>3.75</td>
<td>100</td>
<td>60</td>
<td>29:68\textsuperscript{b}</td>
</tr>
<tr>
<td>5</td>
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<td>100</td>
<td>20</td>
<td>100:0</td>
</tr>
<tr>
<td>6</td>
<td>TeaMs</td>
<td>-1.9</td>
<td>100</td>
<td>20</td>
<td>62:38</td>
</tr>
<tr>
<td>7</td>
<td>TeaHSO\textsubscript{4}</td>
<td>-9</td>
<td>100</td>
<td>20</td>
<td>46:54</td>
</tr>
<tr>
<td>8</td>
<td>TeaHSO\textsubscript{4}</td>
<td>-9</td>
<td>130</td>
<td>20</td>
<td>34:66\textsuperscript{c}</td>
</tr>
<tr>
<td>9</td>
<td>TeaHSO\textsubscript{4}</td>
<td>-9</td>
<td>100</td>
<td>40</td>
<td>48:52</td>
</tr>
<tr>
<td>10</td>
<td>TeaHSO\textsubscript{4}</td>
<td>-9</td>
<td>100</td>
<td>10</td>
<td>59:41</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Ratio determined by \textsuperscript{1}H NMR spectroscopy
\textsuperscript{b} A trace amount (< 10%) of 2'-hydroxyacetophenone 74 was observed in the crude \textsuperscript{1}H NMR spectrum
\textsuperscript{c} An isolated yield of 60% was obtained in this instance

Increasing the reaction temperature to 130 °C (Table 3.1, entry 2), increased conversion to flavanone 130 (65%), however trace amounts of 2'-hydroxyacetophenone 72 and 4-bromobenzaldehyde 73 was observed in the \textsuperscript{1}H NMR spectrum of the crude material (as indicated by singlet peaks at δ 2.61 ppm and δ 9.95 ppm respectively). These presumably formed from a retro-aldol reaction facilitated by adventitious moisture (Scheme 3.7). As the presence of acetophenone and benzaldehyde complicate
purification, the optimal temperature for this conversion was established as 100 °C, and optimisation of reaction time was pursued.

Using TeaFA and a reaction temperature of 100 °C, extended reaction times of 40 and 60 minutes were trialled (Table 3.1, entries 3 and 4). These reactions gave similar conversion to entry 2, however trace amounts of 2′-hydroxyacetophenone 72 were again observed in the 1H NMR spectrum. Therefore, while good conversion was achieved using TeaFA, the formation of side products was undesirable and so other pIL alternatives were investigated.

When EAN (ethylamine: nitric acid, Table 3.1, entry 5) was trialled, no conversion to the desired flavanone 130 was observed with the microwave conditions of 100 °C and 15 minutes. Similarly, when another common pIL, TeaMs (triethylamine: methanesulfonic acid) was tested (Table 3.1, entry 6), poor conversion to flavanone 130 was noted (36%), and so no further optimisation was carried out with this pIL.

Finally, TeaHSO4 (triethylamine:sulfuric acid), a pIL with high ‘proton activity’ and a low cationic pKa value, was employed, and using the microwave conditions of 100 °C for 20 minutes with bromochalcone 74, a conversion of 54% (Table 3.1, entry 7) was achieved. Analysis of the product by 1H NMR spectroscopy showed no sign of the retro-aldol products, and so further optimisation using this pIL was performed.

Increasing the reaction temperature to 130 °C with a reaction time of 20 minutes (Table 3.1, entry 8) gave excellent conversion to flavanone 130 (66%),* and no trace of the side products was noted by 1H NMR spectroscopy. In this case, the desired product 130 was isolated by column chromatography (1:1 CH2Cl2:Pet) in good yield (60%).

* Note once again that the isomerisation of chalcones to form flavanones is reversible, and so 100% conversion to flavanones from chalcones is a significant challenge.
When the reaction time was increased (40 minutes, Table 3.1, entry 9) or decreased (10 minutes, Table 3.1, entry 10) at a temperature of 100 °C, conversions of 52% and 41% respectively were realised. These results were acceptable, but as the conditions of 130 °C and 20 minutes had produced the greatest conversion to flavanone 130 (66%, Table 3.1, entry 8), further changes to reaction time were not examined.

As the greatest conversion of 4-bromochalcone 74 to its corresponding flavanone 130 was achieved using TeaHSO₄, with microwave conditions of 50 W, 130 °C and 20 minutes, these conditions were then applied to a series of available 2'-hydroxychalcones, which had been previously synthesised (Chapter Two).

### 3.2.2.3 Synthesis of a Library of Functionalised Flavanones

It must be repeated at this point that due to the isomerisation of chalcones and flavanones, flavanone synthesis from 2'-hydroxychalcone substrates are relatively low yielding reactions. Many authors report the impressive conversion ratios of chalcone to flavanone, but isolated yields are rarely reported. Bromoflavanone 130, which has been synthesised seven times from 2'-hydroxychalcones according to the literature, has been isolated in isolated yields ranging from 30% to 59% whilst our pIL method produced bromoflavanone 130 in 65% yield (Table 3.2, entry 1). Given this information, it is noted once again that isolated yields of greater than 50% for flavanone synthesis from substituted 2'-hydroxychalcones are actually considered impressive. Also, many of the flavanones synthesised in this research were either novel, or had not been synthesised via chalcone isomerisation (but rather by halogenation of unsubstituted flavanones, or rhodium-catalyzed 1,4-addition of phenylboronic acids to chromenone).

Flavanones synthesised by method were isolated by column chromatography (1:1 CH₂Cl₂:Pet). Flavanones were identified in the ¹H NMR spectrum by the appearance of a multiplet at approximately δ 3.00 ppm representing the CH₂ and a doublet of doublets at approximately δ 5.50 ppm representing the CH of the flavanone. The spectra obtained for the known compounds synthesised were compared to literature data, and novel compounds were fully characterised by ¹H, ¹³C NMR spectroscopy, and HRMS.
Table 3.2. Synthesis of functionalised flavanones using the novel pIL-mediated, MW-assisted reaction.\(^a\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R(_1)</th>
<th>R(_2)</th>
<th>R(_3)</th>
<th>R(_4)</th>
<th>Yield (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>130</td>
<td>H</td>
<td>H</td>
<td>4'-Br</td>
<td>H</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>131</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>3'-OBn</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>132</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>4',5'-OMe</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>133</td>
<td>5-MeO</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>134</td>
<td>5-MeO</td>
<td>H</td>
<td>H</td>
<td>4'-Br</td>
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<tr>
<td>7</td>
<td>135</td>
<td>5-MeO</td>
<td>H</td>
<td>3'-OBn</td>
<td>H</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>136</td>
<td>5-MeO</td>
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<td>2',4'-OMe</td>
<td>5'-Br</td>
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<tr>
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<td>4'-Br</td>
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<td>8-Br</td>
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<tr>
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<td>6-Cl</td>
<td>8-Br</td>
<td>2',4'-OMe</td>
<td>5'-Br</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) underlined compounds are novel

\(^b\) isolated yields following column chromatography

When the optimal conditions (MW, TeaHSO\(_4\), 130 °C, 20 min) were applied to the unsubstituted 2'-hydroxychalcone 66, a good isolated yield of flavanone 6 (60%, Table 3.2, entry 2) was obtained (Figure 3.4). This unsubstituted flavanone has been synthesised previously in better yields (99%),\(^{463}\) however the method herein was ‘greener’ than those used previously, and was novel in its approach.
The effect of Ring B functionalisation on flavanone formation was investigated first (Table 3.2, entries 3 & 4, Scheme 3.8). The reaction of chalcone 78 under the optimised reaction conditions gave the corresponding flavanone 131 in a lower yield than that of the previous examples (36%, entry 3), whilst the reaction of the 5-bromo-2,4-dimethoxy variant 80 showed very little conversion to the desired flavanone 132 (14%, entry 4). These compounds were both novel.

The 2’-hydroxy-6’-methoxy chalcones were reacted next (Table 3.2, entries 5-8). Interestingly, none of the desired flavanones could be formed from this set of chalcones (Scheme 3.9). Data in the literature revealed that 2’-hydroxy-6’-methoxylchalcones are prone to aurone formation rather than flavanone formation under the same conditions, however as the desired flavanones had not been accessed, the products of these reactions were not analysed further. It is worth noting that of these desired
flavanones, only 5-methoxyflavanone 133 (entry 5) has been accessed previously, in a yield of 59% from the same starting material, through the use of a cobalt Schiff-base complex.\textsuperscript{416}

The reaction conditions were then applied to the 5'-bromo-2'-hydroxychalcones. These fared better than their alkoyxyl variants, with the formation of the unsubstituted Ring B flavanone 137 in a yield of 56% when reacted under the pIL-mediated, microwave-assisted method (Table 3.2, entry 9). The 4-bromo and 3-benzyloxy members (110 and 111) were also converted to their flavanones (138 and 139), in yields of 22% and 59% respectively (entries 10 and 11). This last result was quite interesting, as it was the only example in this study where a flavanone with a Ring B functionalisation was formed in a greater yield than the unsubstituted version. Also, flavanones 138 and 139 (entries 10 and 11) were novel compounds. When the 5-bromo-2,4-dimethoxy chalcone 140 was reacted, no conversion was noted (entry 12).

Finally, the conversion of the 3'-bromo-5'-chloro-2'-hydroxychalcones to their analogous flavanones was attempted. Chalcone 117, with an unsubstituted Ring B, was reacted first and the analogous flavanone 141 was produced in a yield of 54% (Table 3.2, entry 13). 3'-Bromo-5'-chloro-2'-hydroxychalcones with substitutions on Ring B were also reacted, and produced flavanones in lower yields to those previously synthesised (19% and 50% respectively for the 4-bromo and 3-benzyloxy chalcones, entries 14 and 15). Flavanones 142 and 143 (entries 14 and 15) were also novel compounds. Once again, the 5-bromo-2,4-dimethoxy version produced none of the desired flavanone 144 (entry 16), as indicated by the presence of only the starting material in the \textsuperscript{1}H NMR spectrum.

3.2.3 Flavanones: Conclusion

A new method for the formation of flavanones from analogous chalcones was developed, using the combination of protic ionic liquids and microwave irradiation to provide a rapid, efficient, and simple synthesis. Ten flavanones were produced in yields
of 14-65% (Scheme 3.10), including six novel compounds 131, 132, 138, 139, 142 and 143.

Scheme 3.10. The synthesis of ten flavanones, including six novel compounds, was accomplished using the novel pIL-mediated, MW-assisted methodology.

A number of trends were identified in these microwave-assisted pIL-mediated reactions. An increase in substitutions on Ring A resulted in decreased yields (Scheme 3.11). This trend was also generally seen in Ring B substitutions, to the point where those chalcones with 3 substituents on Ring B (5-bromo-2,4-dimethoxy) returned none of the desired flavanone. Methoxyl substituents on the 6'-position of chalcones also prevented cyclisation to the flavanones.

Scheme 3.11. Increasing Ring A substitution decreased yields in pIL-mediated, MW-assisted flavanone synthesis.

It was also noted that while Choudhary et al. found in their research that chalcones with para-withdrawing groups provided greater conversion to flavanones when using nanocrystalline MgO, this was not the case for the majority of the pIL-mediated conversions performed in this study.

With this handful of flavanone compounds synthesised, and a new synthetic method devised, the synthesis of another flavonoid family derivative, the flavones, was investigated.

### 3.3 Flavones

#### 3.3.1 The Synthesis of Flavones

The flavones 7, or 2-phenylchromones, are distinguished from other flavonoids by an alkene at the 2-position of Ring C, as well as a ketone at the 4-position (Figure 3.5).
Note that the flavones are not to be confused with flavonols 8, which also contain a hydroxyl group at the 3-position and will be discussed in Chapter Five.

![Flavone and Flavonol Structures](image)

**Figure 3.5.** The simplest flavone 7 is also known as 2-phenyl-4H-chromen-4-one, and must not be confused with flavonol 8.

Examples of natural flavones include apigenin 145 (found as glycosides in parsley and celery) 8, 12, 465 and luteolin 146 (found in alfalfa and celery), 12, 286, 465, 466 both of which have been identified as possessing anticancer, 467-471 antidiabetic,472 and antihypertensive properties (Figure 3.6). 286

![Apigenin and Luteolin Structures](image)

**Figure 3.6.** Apigenin 145 and luteolin 146 are two natural flavones found in plants.

### 3.3.1.1 Baker-Venkataraman Rearrangement

There are a number of methods available for the synthesis of the flavone scaffold, most commonly via the Baker-Venkataraman Rearrangement. 473, 474 This rearrangement involves the reaction of 2'-hydroxyacetophenone 72 and an aroyl chloride 147 in pyridine to form an ester 148, followed by treatment with base to form a 1,3-diketone 149 (Scheme 3.12).
From this 1,3-diketone 149, flavone 150 can be synthesised by treatment with strong acid, through the mechanism shown in Scheme 3.13.\textsuperscript{475}

Whilst the conditions used for the 1,3-diketone formation are fairly uniform throughout the literature, a number of different conditions have been used to convert 1,3-diketones to flavones. Li et. al. have published a thorough review on the acid-catalysed,\textsuperscript{473, 474} base-catalysed,\textsuperscript{476} solid supported, microwave-assisted, and other methods used to obtain flavones from 1,3-diketones.\textsuperscript{477} In more recent times, the application of microwave irradiation has provided reduced reaction times, with conditions including CuCl\textsubscript{2} in EtOH for 5 minutes,\textsuperscript{478} K\textsubscript{2}CO\textsubscript{3} and pyridine reacted for 10 minutes,\textsuperscript{479} as well as acetic acid and H\textsubscript{2}SO\textsubscript{4}.\textsuperscript{81, 480} Even pILs have been applied to
the microwave-assisted reaction, with [bmim]BF₄ and [EtNH₃]NO₃ employed by Pawar and co-workers.⁴³⁵, ⁴⁸¹

### 3.3.1.2 Oxidative Cyclisation by Iodine and DMSO

Another common method for the synthesis of flavones is the oxidative cyclisation of 2'-hydroxychalcones. An early paper on the subject demonstrated the SeO₂ oxidation of 2-hydroxy-5-acetaminochalcone to its corresponding flavone,⁴⁸² and recently, ionic liquids such as [bmim][NTf₂] have also been applied.⁴³⁶ The most common conditions to effect this transformation are reflux with iodine (I₂) in DMSO.⁴⁸³ The I₂-mediated mechanism is shown in Scheme 3.14. It is related to that of the Swern oxidation, and was proposed by Patonay and co-workers when they could find no evidence of nuclear iodination (the formation of an iodonium ion).⁴⁸⁴-⁴⁸⁶

![Scheme 3.14. Mechanism of oxidative cyclisation of chalcones by I₂.](image)

First, ring closure of the chalcone 151 to form chroman-4-one 152 occurs. The iodine reacts with the solvent to form I₂/DMSO reagent 154. When reagent 154 reacts with chroman-4-one 152, chroman-4-one sulfoxide 155 is formed and hydrogen iodide is lost. Removal of the proton at the 2-position of the chroman-4-one sulfoxide 155 by I⁻ is followed by the loss of hydrogen iodide and DMSO, to form flavone 156. DMSO and two HI molecules reform the catalytic iodine, along with dimethylsulfide and water.⁴⁸⁶ This method is commonly used as the formation of side products is rarely observed; the 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)-mediated formation of flavones from chalcones produces a mixture of flavanone, flavone and aurone products.⁴⁸⁷
While conventional heating with reflux for over 16 hours is the traditional method,\textsuperscript{141, 331, 417, 488, 489} microwave irradiation has been recently applied to the reaction,\textsuperscript{332} although with the use of conventional microwave, which as explained in Chapter 2.2.2 are generally unreliable and irreproducible. Solid supported iodine, in the form of I$_2$-Al$_2$O$_3$, has also been used to effect microwave-assisted flavone synthesis, with reaction times of less than five minutes.\textsuperscript{370, 490}

Many other methods for flavone synthesis are possible, including the oxidation of flavanones by manganese acetate, which has provided flavones in 90-96% yields.\textsuperscript{491} A microwave-assisted radical method synthesis using N-bromosuccinimide and azabisisobutyronitrile (AIBN) effects the same transformation.\textsuperscript{492} Ferrocenyl-substituted flavones have also been accessed using KCN, oxone, DCM/acetone and a carbonate buffer.\textsuperscript{493} Even an intramolecular photochemical Wittig reaction in water has been used to synthesise flavones.\textsuperscript{494}

When attention turned to the synthesis of substituted flavones, it was established that the I$_2$-mediated oxidation of 2'-hydroxychalcones was an ideal method, as a series of 2'-hydroxychalcones were already in hand (See Chapter Two).

### 3.3.2 Flavones: Results and Discussion

To synthesise flavones from the 2'-hydroxychalcones in hand, a microwave-assisted method was first attempted, based on the conditions used by Menezes (who used a conventional microwave).\textsuperscript{332} Chalcone 66 was reacted with I$_2$ in DMSO under microwave irradiation for 5 minutes at 180 °C. However, due to extreme pressure build-up, the microwave safety system turned the microwave off. Lower reaction temperatures were then trialled (100 °C and 120 °C, Scheme 3.15), however only starting material was returned. For this reason, attention turned to the traditional method (5% Iodine in DMSO under reflux for 16 hours), in accordance with Yamasaki \textit{et. al.}\textsuperscript{331} and Chimenti \textit{et. al.}\textsuperscript{417}

![Scheme 3.15. Microwave-assisted flavone formation of 2'-hydroxychalcone 66 did not proceed at lower temperatures.](image)

The conventional method was applied to 16 of the 2'-hydroxychalcones synthesised in Chapter Two, beginning with those chalcones with an unsubstituted Ring A (apart from the 2'-hydroxyl group). Note that novel flavones were fully characterised,
by $^1$H and $^{13}$C NMR spectroscopy, and HRMS. The $^1$H NMR spectroscopic analysis included the loss of the chalcone hydroxyl peak (approximately $\delta$ 12.50-13.00 ppm), the movement of the aromatic peaks, and also the appearance of the CH peak of the flavone at approximately $\delta$ 6.80 ppm in the $^1$H NMR spectrum (Figure 3.7). Known flavones were compared to literature data to confirm their structure.

The unfunctionalised chalcone 66 (Table 3.3, entry 1) was treated with 5% I$_2$ in DMSO, and refluxed for 16 hours. After cooling to room temperature, excess water was added until the solution became opaque. Extraction, followed by column chromatography (10% MeOH in CHCl$_3$) gave the desired flavone 7 in 61% yield (Figure 3.7).

![Figure 3.7. $^1$H NMR spectrum of flavone 7, as synthesised by the I$_2$/DMSO method (CDCl$_3$).](image)

Next, 4-bromochalcone 74 was reacted under the same conditions, and the desired flavone 157 was formed in a yield of 71% (Table 3.3, entry 2). The 3-benzyloxy, and 5-bromo-2,4-dimethoxy analogues were also successfully reacted (entries 3 and 4 respectively), and formed their corresponding flavones 158 and 159 in yields of 57% and 88% respectively. It was noted from these few reactions that the more substituents on Ring B, the greater the yield, the opposite trend to that seen in flavanone synthesis.

The 2′-hydroxy-6′-methoxychalcones were investigated next. Chalcone 101 was reacted first, and the corresponding flavone 160 was isolated in a yield of 66% (Table...
3.3, entry 5). This success was in complete contrast to earlier attempts at flavanone synthesis, wherein none of the 2’-hydroxy-6’-methoxychalcones could be converted to their corresponding flavanones. The 4-bromo analogue 102 was reacted next, and produced the desired novel flavone 161 in a yield of 59% (entry 6).

3-Benzyloxychalcone 104 was also reacted (entry 7), and produced the debenzylated flavone 162 in 14% yield, as confirmed by comparison to known spectral data in the literature, including the lack of a benzyl peak at approximately δ 5.10 ppm and the presence of only eight aromatic protons in the ¹H NMR spectrum (Figure 3.8). To validate this deprotection, the reaction was repeated and the same result was observed. Of note was that this deprotection did not occur with any of the other benzylated chalcones in the synthesis of flavones. A review of the literature revealed that Anuradha and co-workers also experienced removal of benzyl groups in the synthesis of flavones from 2’-hydroxychalcones (using I₂ in glycol), however no explanation or mechanism was provided.४९५

Finally, the 5-bromo-2,4-dimethoxy analogue 105 was also reacted, and produced the corresponding flavone 164 in a poor yield of 26% (entry 8). Once again, there were no trends in the reactivity of this series of chalcones.

Next, the 5’-bromo-2’-hydroxychalcones were reacted. Chalcone 109 with its unsubstituted Ring B was reacted first, and flavone 165 was isolated in a good yield of
72% (Table 3.3, entry 9). When the 4-bromo and 3-benzyloxy analogues (chalcones 110 and 111 respectively) were applied to the reaction conditions, lower isolated yields of the corresponding flavones were acquired (44% and 48%, entries 10 and 11 respectively). However, the novel 5-bromo-2,4-dimethoxyflavone 168 was isolated in very good yield (75%) when its analogous chalcone was reacted (entry 12).

Table 3.3. Synthesis of functionalised flavones from 2'-hydroxychalcones using the I2/DMSO method.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R\textsubscript{1}</th>
<th>R\textsubscript{2}</th>
<th>R\textsubscript{3}</th>
<th>R\textsubscript{4}</th>
<th>Isolated Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>157</td>
<td>H</td>
<td>H</td>
<td>4'-'Br</td>
<td>H</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>158</td>
<td>H</td>
<td>H</td>
<td>3'-'OBn</td>
<td>H</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>159</td>
<td>H</td>
<td>H</td>
<td>2',4'-OMe</td>
<td>5'-'Br</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>160</td>
<td>5-MeO</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>161</td>
<td>5-MeO</td>
<td>H</td>
<td>4'-'Br</td>
<td>H</td>
<td>59</td>
</tr>
<tr>
<td>7</td>
<td>162</td>
<td>5-MeO</td>
<td>H</td>
<td>3'-'OH</td>
<td>H</td>
<td>14\textsuperscript{b}</td>
</tr>
<tr>
<td>8</td>
<td>164</td>
<td>5-MeO</td>
<td>H</td>
<td>2',4'-OMe</td>
<td>5'-'Br</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>165</td>
<td>6-Br</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>72</td>
</tr>
<tr>
<td>10</td>
<td>166</td>
<td>6-Br</td>
<td>H</td>
<td>4'-'Br</td>
<td>H</td>
<td>44</td>
</tr>
<tr>
<td>11</td>
<td>167</td>
<td>6-Br</td>
<td>H</td>
<td>3'-'OBn</td>
<td>H</td>
<td>48</td>
</tr>
<tr>
<td>12</td>
<td>168</td>
<td>6-Br</td>
<td>H</td>
<td>2',4'-OMe</td>
<td>5'-'Br</td>
<td>75</td>
</tr>
<tr>
<td>13</td>
<td>169</td>
<td>6-Cl</td>
<td>8-Br</td>
<td>H</td>
<td>H</td>
<td>70</td>
</tr>
<tr>
<td>14</td>
<td>170</td>
<td>6-Cl</td>
<td>8-Br</td>
<td>4'-'Br</td>
<td>H</td>
<td>12</td>
</tr>
<tr>
<td>15</td>
<td>171</td>
<td>6-Cl</td>
<td>8-Br</td>
<td>3'-'OBn</td>
<td>H</td>
<td>38</td>
</tr>
<tr>
<td>16</td>
<td>172</td>
<td>6-Cl</td>
<td>8-Br</td>
<td>2',4'-OMe</td>
<td>5'-'Br</td>
<td>33</td>
</tr>
</tbody>
</table>

\textsuperscript{a} underlined compounds are novel
\textsuperscript{b} starting material chalcone 104 was benzylated (3-OBn)

Finally, the 3'-bromo-5'-chloro-2'-hydroxychalcones were reacted. The unsubstituted Ring B chalcone 117 was reacted to form the desired flavone 169 in a yield of 70% (Table 3.3, entry 13). However the remainder of this family of chalcones did not react as well, with the 4-bromo, 3-benzyloxy and 5-bromo-2,4-dimethoxy analogues all being formed in much lower yields (12%, 38% and 33% respectively, Table 3.3, entries 14-16). It is also noted that although produced in low yields, the latter two flavones, 171 and 172, were novel compounds.
3.3.3 Flavones: Conclusion

A series of flavones were synthesised from the 2’-hydroxychalcones synthesised in Chapter Two, using the traditional method of reflux with iodine in DMSO for 16 hours. A total of 16 flavones were synthesised in yields of 12-88% (Scheme 3.16), including seven novel compounds (Table 3.3, entries 3, 6, 8, 11, 12, 15 and 16).

While there appeared to be no reactivity trends in the synthesis of the flavones, their synthesis was accomplished in greater yields than those of the flavanones (which were synthesised from the same 2’-hydroxychalcones). This was noticed especially in the case of the 2’-hydroxy-6’-methoxychalcones, which were unreactive in the conditions used to form the flavanones.

3.4 Conclusion

The 2’-hydroxychalcones are an ideal stepping stone from which to access a number of flavonoid substructures, including their constitutional isomers the flavanones, and the flavones. It was the aim of this research to access a library of flavonoid-based compounds, and also to incorporate halogens into these structures, so as to take advantage of the Heck reaction (discussed in Chapter Four).

The research in this chapter outlined attempts to access flavanones and flavones. A new method was developed for the synthesis of flavanones from 2’-hydroxychalcones, using the combination of protic ionic liquids (pILs) and microwave irradiation (Scheme 3.17). This methodology allowed for the synthesis of ten flavanones in yields varying from 14-65%, including six novel compounds (Table 3.2, entries 3, 4, 10, 11, 14 and 15);

- 2-(3-benzyloxyphenyl)chroman-4-one 131,
- 2-(5-bromo-2,4-dimethoxyphenyl)chroman-4-one 132,
- 6-bromo-2-(4-bromophenyl)chroman-4-one 138,
- 6-bromo-2-(3-benzyloxyphenyl)chroman-4-one 139,
- 8-bromo-6-chloro-2-(4-bromophenyl)chroman-4-one 142, and
- 8-bromo-6-chloro-2-(3-benzyloxyphenyl)chroman-4-one 143.
This work was the basis of a publication in *Current Organic Chemistry* (2012, 16(1), 121-126), (Appendix A).

Scheme 3.17. The pIL-mediated, MW-assisted methodology provided ten flavanones.

To further increase this flavonoid library, the synthesis of flavones from their corresponding 2′-hydroxychalcones was performed using I₂ and DMSO (Scheme 3.18). Sixteen flavones were accessed, in yields varying from 12-88%, including seven novel compounds (Table 3.3, entries 3, 6, 8, 11, 12, 15 and 16);

- 2-(3-benzyloxyphenyl)chromen-4-one \(158\),
- 5-methoxy-2-(4-bromophenyl)chromen-4-one \(161\),
- 5-methoxy-2-(5-bromo-2,4-dimethoxyphenyl)chromen-4-one \(164\),
- 6-bromo-2-(3-benzyloxyphenyl)-4H-chromen-4-one \(167\),
- 6-bromo-2-(5-bromo-2,4-dimethoxyphenyl)chromen-4-one \(168\),
- 8-bromo-6-chloro-2-(3-benzyloxyphenyl)chromen-4-one \(171\), and
- 8-bromo-6-chloro-2-(5-bromo-2,4-dimethoxyphenyl)chromen-4-one \(172\).

Scheme 3.18. The synthesis of flavones from 2′-hydroxychalcones with I₂ and DMSO provided 16 flavones.

With a library of flavonoid structures now synthesised (a total of 49 including 2′-hydroxychalcones, flavanones and flavones), the first aim of this research was completed. As with the 2′-hydroxychalcones, these flavanones and flavones were submitted for biochemical evaluation of their cytotoxic effects, the results of which are expected in the near future. With these flavonoids in hand, many incorporating halogens, attention turned to the use of the Heck reaction for further functionalisation of these flavonoids, and the introduction of synthetic handles for use in the synthesis of DMLs (Chapter Five).
Chapter Four:
Functionalisation of Flavonoids:
The Heck Reaction
4.1 Chapter Overview
With a number of halogenated 2'-hydroxychalcones, flavanones and flavones in hand, attention turned to their functionalisation using the Heck reaction, in order to further functionalise the flavonoid scaffolds, and also to provide synthetic handles for use in the synthesis of Designed Multiple Ligands (Chapter Five).

4.2 Heck Reaction of Halogenated Flavonoids

4.2.1 Palladium Chemistry
The use of palladium in chemistry has become established as a convenient means to form carbon-carbon bonds. The importance of such ‘cross coupling’ reactions was recognised when in 2010, three of the pioneers in this field (R. F. Heck, E. Negishi, and A. Suzuki) were awarded a joint Nobel Prize in Chemistry "for palladium-catalyzed cross couplings in organic synthesis".496

Examples of palladium-catalysed cross coupling reactions include the Heck-Mizoroki reaction (commonly known as the Heck reaction),497, 498 Sonogashira coupling,499 Negishi coupling,500 Stille cross coupling,501 Suzuki-Miyaura reaction (commonly known as the Suzuki reaction),502 Hiyama coupling,503 Kumada reaction,504, 505 and the Buchwald-Hartwig amination reaction (Figure 4.1).506, 507

Figure 4.1. Numerous palladium-catalysed reactions are possible, and can introduce various functional groups.

These reactions all utilise an organohalide substrate and a palladium catalyst. Depending on the reaction conditions, reagents and other organometallic species used, a variety of functional groups can be installed.508, 509
Palladium-catalysed cross coupling reactions are ubiquitous in total syntheses (see Nicolaou et al. for a thorough review), but their application to flavonoids has been largely unresearched, likely due to flavonoid research largely revolving around hydroxylated rather than halogenated flavonoids. The Suzuki reaction has been applied to flavone substrates, and the Stille coupling applied to a coumarin, and a relatively thorough investigation into microwave-assisted Suzuki and Buchwald-Hartwig reactions to numerous flavone structures has been conducted. The most thorough investigation of palladium-based reactions with flavonoids to date applied the Stille, Heck, Suzuki, and Sonogashira reactions to 2-aryl/styryl-8-bromo-6-chloro-3-hydroxychromone scaffolds (Scheme 4.1).

![Scheme 4.1. A Sonogashira and a Stille coupling, as applied to halogenated flavonol 173 by Dahlén and co-workers.](image)

However, there has been very little investigation into the use of the Heck reaction with halogenated flavonoid substrates. With a number of halogenated acetophenones, benzaldehydes, chalcones, flavanones and flavones in hand, attention turned to functionalising these substrates using the Heck reaction.

### 4.2.2 The Heck Reaction

The Heck-Mizoroki reaction is a vinylic substitution reaction, in which a vinylic hydrogen is replaced with an aryl, benzyl or vinyl group (Scheme 4.2). It was discovered independently by the groups of Heck and Moritani in 1967 and 1968. Heck first detailed the use of palladium salts with substituted vinyls and arylmercuric salts to produce arylated vinyl products. However, these reactions used stoichiometric amounts of the palladium source, and involved toxic mercuric salts. Moritani produced the same vinylic products, but via organopalladium intermediates generated by the direct electrophilic palladation of arenes.
Two years later, Mizoroki et al. published modifications to Heck’s reaction, detailing the use of only a catalytic amount of the palladium source (PdCl$_2$), and showed that halogens, in particular iodides, could be used in place of the toxic mercuric reagents. Mizoroki also detailed the use of potassium acetate as a scavenger for the hydrogen iodide formed during the reaction.

The requirements for a Heck reaction are as follows: a palladium$^{(0)}$ or palladium$^{(II)}$ complex, a ligand (most commonly a phosphine ligand) to co-ordinate to the palladium, and a base, in addition to the organohalide (which is often an aryl halide) and a vinylic substrate. The generally accepted mechanism is shown in Scheme 4.3.

The Heck reaction begins with preactivation of the palladium complex by the ligand to form the active catalyst, a Pd$^{(0)}$ species $^{176}$. This undergoes oxidative addition with organohalide $^{177}$, ‘inserting’ the palladium-ligand species between the carbon and
halogen. Displacement of either a ligand or the halide allows for co-ordination to the alkene, forming a $\pi$-complex 180. Migratory insertion of the complex into the olefin, otherwise known as carbometallation, occurs next with syn stereochemistry. In this step, the alkene reactant 180 is incorporated via the less-substituted alkene carbon. Rotation around the carbon-carbon bond provides a $\beta$-hydrogen that is syn-periplanar to the palladium, allowing syn-$\beta$-hydride elimination to produce the thermodynamically stable (Z)- (or trans-) alkene 183. Finally, reductive elimination by the base re-forms the catalytic palladium species 176.

There are a number of factors which influence the success of the Heck reaction. First, aryl iodides are the most reactive to Heck reactions, followed by bromides and triflates. Chlorides are far less reactive, due to the strength of the carbon-chloride bond, however Heck reactions of aryl chlorides are still possible. In terms of the vinylic substrates, higher yields are generally associated with electron-poor vinyls (including acrylates). Also, the use of more substituted vinyl substrates effects slower reactions, as the steric hindrance obstructs access to the alkene for the formation of the $\pi$-complex.

The Heck reaction is widely used in organic chemistry, being used in the synthesis of natural products, novel polymers, and in domino reactions. It has even been used to synthesise flavonoid scaffolds, including the anthocyanidins, 2-arylchromenes, chalcones, and even flavones. However, the use of the Heck reaction on flavonoid substrates is not well explored. Few examples exist in the literature, including application to hydroxychromones and flavones with 1,3-dienes. Dahlèn and co-workers in particular successfully conducted the Heck reaction at the 6-chloro and 8-bromo positions of substituted flavonols, and yields of 64-85% and 80-87% respectively for the acrylate products were obtained (with a total of only six examples, Scheme 4.4).
At the time of our research, Akçok et. al. published an attempt to introduce a series of stilbenes to 3’-bromoflavonone 187 using the Heck reaction, although a low 43% yield was their only result, using palladium acetate (Pd(OAc)\(_2\)) and triethanolamine (Scheme 4.5). The use of other functionalised stilbenes resulted in inseparable mixtures of products, and indicated that the Heck reaction of flavanones was difficult.

When Akçok applied the Heck reaction to 3-bromobenzaldehyde 189, and used the stilbene product 190 in a Claisen-Schmidt condensation and then flavanone formation, a series of stilbene-functionalised flavanones including 193 were produced, with overall yields of approximately 40-50% (Scheme 4.6).
This review of the literature showed little precedence in the application of the Heck reaction to flavonoids, and so provided an avenue for this research. Brominated acetophenone, benzaldehyde, chalcone, flavanone and flavone substrates were all available for this research. While it was realised that the use of base in the Heck reaction may cause the isomerisation of the chalcone or flavanone substrates, it was envisioned that careful base selection could avoid or minimise this isomerisation.

The use of the Heck reaction with brominated flavonoids provided an avenue from which to introduce functional groups to attach other pharmacophores or therapeutic agents.† This was in line with the aims of functionalising flavonoids, as well as synthesising Designed Multiple Ligands for the treatment of diseases.

4.3 Heck Reaction: Results and Discussion

In the selection of substrates for the Heck reaction of flavonoids, 2’-hydroxychalcones, flavanones and flavones with bromine substituents were chosen. This included chalcones 74 and 109, flavanones 130 and 137, and also flavones 157 and 165, which incorporated bromine atoms into either Ring A or Ring B of the flavonoids. Research towards functionalisation of Ring B will be addressed first, followed by Ring A functionalisation.

4.3.1 Heck Reaction: Ring B

To trial the Heck reaction conditions, 4-bromobenzaldehyde 73 was used as the aryl halide (Scheme 4.7). Palladium acetate (Pd(OAc)₂) was chosen as the palladium catalyst, as it is relatively inexpensive, commonly used in such reactions, and stable in air. Triphenylphosphine (PPh₃) was selected as the phosphine ligand, and N,N-dimethylformamide (DMF) was chosen as the solvent, as it is the ideal solvent for this catalyst-ligand set, as it weakly co-ordinates to the palladium catalyst, increasing its stability.

Scheme 4.7. The optimisation of the Heck reaction was conducted using 4-bromobenzaldehyde 73.

† This will be addressed further in Chapter Five.
The acrylate substrates were chosen based on availability (Figure 4.2), and included normal-butyl (CO$_2$nBu) and tert-butyl (CO$_2$tBu) acrylate. These esters, although similar, are cleaved using different methods (saponification and acid treatment respectively). Acrylonitrile (CN), acrylamide (CONH$_2$) and acrylic acid (COOH) were also available. Note that nBu acrylate was the acrylate substrate used for the trial reactions.

![Figure 4.2. The five acrylate substrates used in this research.](image)

Of particular interest for these trial reactions was the identification of numerous suitable bases, in order to avoid the undesired chalcone to flavanone isomerisation when using the Heck reaction. Sodium acetate (NaOAc), triethylamine (NEt$_3$) and sodium or potassium carbonate (Na$_2$CO$_3$ or K$_2$CO$_3$) are all noted as ideal bases for the Heck reaction. N-methylmorpholine is another commonly-used base, however as this was unavailable in our laboratory at the time, the similar amine base N,N-dicyclohexylmethylamine (Cy$_2$NMe) was also trialled. Strong hydroxide bases, including KOH, NaOH, and Bu$_4$NOH were not used, as these hydroxide bases facilitate chalcone to flavanone isomerisation; DBU was also avoided for this reason (see Chapters Two and Three).

To compare the success of these bases, the benzaldehyde peaks of 4-bromobenzaldehyde (δ 9.95 ppm) and the desired acrylate product (δ 10.01 ppm) were each given an integration of 1 to determine the ratio of reactant to product.

### 4.3.1.1 Heck Reaction: Trials

The following methodology was used; palladium acetate (Pd(OAc)$_2$, 0.02 mmol), triphenylphosphine (PPh$_3$, 0.02 mmol), and base (0.40 mmol) were stirred in DMF under an inert atmosphere for 20 minutes, followed by addition of benzaldehyde (0.13 mmol) and nBu acrylate (0.13 mmol). The reaction was refluxed for 16 hours, filtered through a celite plug, the solvent removed in vacuo, and analysis of the crude product was conducted using $^1$H NMR spectroscopy.

When NaOAc was trialled (Table 4.1, entry 1), analysis of the crude reaction product by $^1$H NMR spectroscopy revealed the presence of desired acrylate 194, as confirmed by comparison to literature data, including the appearance of a doublet...
representing the β-vinyl proton at δ 6.50 ppm and additional peaks between δ 7.40-8.00 ppm representing the α-vinyl proton. The benzaldehyde starting material 73 was also observed (δ 9.95 ppm), in a ratio of 1:1 with the desired product. With this reasonable conversion, other bases were trialled.

Dicyclohexyl-N-methylamine (Cy₂NMe) was examined next (Table 4.1, entry 2), and a ratio of 1:0.3 of starting material 73 to nBu acrylate product 194 was determined by ¹H NMR spectroscopy. This low conversion was somewhat expected, as Cy₂NMe is not commonly utilised in Heck reactions, and has previously shown to be low yielding when used with Pd(OAc)₃ in comparison to more specialised palladium catalysts.

| Entry | Base          | Equivalents of alkene | 73:194 (yield)  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaOAc</td>
<td>1</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>Cy₂NMe</td>
<td>1</td>
<td>1:0.3</td>
</tr>
<tr>
<td>3</td>
<td>NEt₃</td>
<td>1</td>
<td>1:1.5 (42%)</td>
</tr>
<tr>
<td>4</td>
<td>K₂CO₃</td>
<td>1</td>
<td>1:1.5 (50%)</td>
</tr>
<tr>
<td>5</td>
<td>NEt₃</td>
<td>4</td>
<td>1:1.4</td>
</tr>
<tr>
<td>6</td>
<td>K₂CO₃</td>
<td>4</td>
<td>1:5 (82%)</td>
</tr>
</tbody>
</table>

*a* Isolated yield (%) after column chromatography

Triethylamine (NEt₃) was the next base to be trialled (Table 4.1, entry 3). Analysis of the crude material by ¹H NMR spectroscopy showed the starting material 73 and product 194 in a ratio of 1:1.5 respectively. Purification by column chromatography (1:1 CH₂Cl₂:Pet) isolated the desired product in 42% yield.

Next, K₂CO₃ was used (Table 4.1, entry 4), and ¹H NMR analysis showed the starting material 73 and product 194 in a ratio of 1:1.5. Acrylate 194 was isolated by column chromatography (1:1 CH₂Cl₂:Pet) in 50% yield.

With only moderate conversions achieved using the four bases, attention turned to the use of increased equivalents of the acrylate. Using four equivalents of nBu acrylate with NEt₃ as the base (Table 4.1, entry 5) produced a slightly lower conversion of 1:1.4 for benzaldehyde 73 to acrylate 194 respectively.
However, when four equivalents of \( n\text{Bu} \) acrylate were used with \( K_2CO_3 \) as the base (Table 4.1, entry 6), very good conversion to the acrylate product was noted (1:5 for the starting material 73 to product 194 respectively). Purification by column chromatography (1:1 CH\(_2\)Cl\(_2\):Pet) isolated the desired acrylate 194 as a pale gold oil in a yield of 82% (Table 4.2, entry 1).

While the use of NEt\(_3\) with one equivalent of acrylate was one option, the \( K_2CO_3 \) methodology (\( K_2CO_3 \) as the base with four equivalents of the acrylate) had provided the greatest conversion, and as such this system was used for the reaction of other acrylates with 4-bromobenzaldehyde 73.

### 4.3.1.2 Heck Reaction of 4-Bromobenzaldehyde with Acrylates

The next acrylate used was \( t\text{Bu} \) acrylate (Table 4.2, entry 2). Purification by column chromatography isolated the desired acrylate 195 in 60% yield.

Acrylonitrile was trialled next (Table 4.2, entry 3). Analysis of the crude material by \(^1\)H NMR spectroscopy showed a complex mixture of products, as indicated by nine peaks in the aldehyde region (from \( \delta \) 9.96-10.12 ppm), and numerous sets of peaks representing the vinylic \( \beta \)-hydrogen (\( \delta \) 5.60-6.06 ppm). Analysis by thin layer chromatography (TLC) showed numerous barely separable spots, and literature revealed that the use of acrylonitrile in Heck reactions can be problematic;\(^{544}\) therefore, the product was not purified.

Acrylamide and acrylic acid were also trialled (Table 4.2, entries 4 and 5). Analysis of the crude reaction product by \(^1\)H NMR spectroscopy revealed only the benzaldehyde reactant, with no sign of the desired products.

#### Table 4.2. The Heck reaction of 4-bromobenzaldehyde 73.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acrylate</th>
<th>R</th>
<th>Product (yield)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( n\text{Bu} ) acrylate</td>
<td>CO(_2)Bu</td>
<td>194 (82%)</td>
</tr>
<tr>
<td>2</td>
<td>( t\text{Bu} ) acrylate</td>
<td>CO(_2)Bu</td>
<td>195 (60%)</td>
</tr>
<tr>
<td>3</td>
<td>Acrylonitrile</td>
<td>CN</td>
<td>mixture</td>
</tr>
<tr>
<td>4</td>
<td>Acrylamide</td>
<td>CONH(_2)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Acrylic acid</td>
<td>COOH</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) Isolated yield (%) after column chromatography
Overall, the only successful Heck reactions of 4-bromobenzaldehyde 73 were with nBu and tBu acrylate. Attention then turned to the use of a different aryl halide scaffold, in the hope that more functionalised products could be obtained.

Another benzaldehyde, 5-bromo-2,4-dimethoxybenzaldehyde 79, was also trialled, with nBu acrylate and the optimised conditions (Pd(OAc)$_2$, PPh$_3$, K$_2$CO$_3$ and DMF, Scheme 4.8), however only the benzaldehyde starting material was observed in the $^1$H NMR spectrum of the crude reaction material. No evidence of the successful use of this substrate in the Heck reaction could be found, and so the use of this substrate was discontinued and attention turned to the use of the halogenated flavonoids.

![Scheme 4.8. Heck reaction with 5-bromo-2,4-dimethoxybenzaldehyde 79 gave only the starting material.](image)

### 4.3.1.3 Heck Reaction of 4-Bromochalcone 74

The reaction of 4-bromochalcone 74 was then attempted using the same reaction conditions (Scheme 4.9). Analysis of the crude product by $^1$H NMR spectroscopy revealed a mixture of compounds, including a trace amount of the corresponding halogenated flavanone (verified by the appearance of multiplet peaks at $\delta$ 2.80-3.20 ppm and $\delta$ 5.40 ppm representing the CH$_2$ and CH of Ring C), and the chalcone 74 and acrylate starting materials.

![Scheme 4.9. Heck reaction of bromochalcone 74 with nBu acrylate did not proceed, but the crude reaction product revealed the starting material and a trace amount of bromoflavanone 130.](image)

With no sign of the desired acrylate product 197, attention turned to the Heck reaction of brominated flavonoids, $4'$-bromoflavanone 130 and $4'$-bromoflavone 157.
4.3.1.4 Heck Reaction of 4′-Bromoflavanone 130 and 4′-Bromoflavone 157

Using the established conditions (Pd(OAc)$_2$, PPh$_3$, K$_2$CO$_3$, DMF, 4′-bromoflavanone 130 and four equivalents of $n$Bu acrylate, see Scheme 4.10), $^1$H NMR analysis showed the presence of only the flavanone starting material, and none of the desired acrylate product 198. Of note was the fact that there was also no sign of the corresponding brominated chalcone isomer 74, indicating that K$_2$CO$_3$ did not cause isomerisation.

![Scheme 4.10](image)

Scheme 4.10. Heck reaction of bromoflavanone 130 with $n$Bu acrylate showed only the using K$_2$CO$_3$ was unsuccessful; only starting material was seen in the $^1$H NMR spectrum.

The reaction was then repeated, with NEt$_3$ as the base with only one equivalent of the acrylate (Scheme 4.11). However, the $^1$H NMR spectrum of the crude material in this case showed the presence of the flavanone starting material 130 and its corresponding chalcone 74 in a ratio of 3:2, indicating that the NEt$_3$ had caused the isomerisation of the flavanone starting material.

![Scheme 4.11](image)

Scheme 4.11. The Heck reaction of bromoflavanone 130 using NEt$_3$ produced a 3:2 mixture of the starting material and corresponding chalcone 74 respectively, with none of the desired acrylate 198.

While both of these reactions were unsuccessful in synthesising the Heck product, it was interesting that when K$_2$CO$_3$ was used as a base, none of the corresponding chalcone was detected, inferring that K$_2$CO$_3$ may be used in other base-mediated reactions and applied to this flavanone. Attention then turned to the use of the Heck reaction with 4′-bromoflavone 157; note that the flavones are not subject to isomerisation as the chalcones and flavanones are.

When the Heck reaction was attempted using 4′-bromoflavone 157 as a substrate (Scheme 4.12), analysis of the crude product by $^1$H NMR spectroscopy
revealed the presence of the corresponding acetophenone 72 and benzaldehyde 73, as exhibited by representative peaks at \( \delta \) 2.60 ppm and \( \delta \) 9.97 ppm respectively. These degradation products were present in a ratio of 3:3:1 with the flavone starting material 157 respectively, and there was no sign of the desired flavone acrylate 199. While others have previously used the Heck reaction with flavones, it must be noted they used 1,3-dienes rather than acrylates, as well as different reagents (5mol% Pd(dba)\(_2\), 5mol% dppe, 200mol% Ag\(_2\)CO\(_3\) in dioxane and H\(_2\)O at 100 °C for 24 hours) to form annulated products.\(^{539}\)

Scheme 4.12. The Heck reaction of 4'-bromoflavone 157 did not proceed.

Although the Heck reactions of the Ring-B substituted chalcone 74, flavanone 130 and flavone 157 were unsuccessful, the effective application to the corresponding benzaldehyde provided a means to access the flavonoid equivalents.

The Heck reaction of brominated Ring A substrates was then examined.

### 4.3.2 Heck Reaction: Ring A

#### 4.3.2.1 Heck Reaction of 5'-Bromo-2'-Hydroxyacetophenone 108 with Acrylates

Attempts were then made to access acrylate products from a brominated acetophenone, or flavonoid with a bromine atom on Ring A. The first substrate examined was 5'-bromo-2'-hydroxyacetophenone 108, using the optimised conditions (Pd(OAc)\(_2\), PPh\(_3\), K\(_2\)CO\(_3\), DMF, aryl halide and four equivalents of acrylate). Note that the synthesis of acrylate products from 5'-bromo-2'-hydroxyacetophenone 108 had not been attempted previously.

First, \( n \)Bu acrylate was trialled (Table 4.3, entry 1, and Scheme 4.13), and analysis of the crude product by \(^1\)H NMR spectroscopy revealed full conversion of the starting material and excess \( n \)Bu acrylate.
The Heck reaction of 5′-bromo-2′-hydroxyacetophenone 108 with nBu acrylate proceeded in 76% yield.

Purification by column chromatography (1:1 CH₂Cl₂:Pet) isolated acetophenone nBu acrylate 200 in a yield of 76% (Figure 4.3). Acrylate 200 was identified in the ¹H NMR spectrum by peaks representing the β-vinilic proton as a doublet at δ 6.31 ppm, and the 2′-hydroxyl proton as a singlet peak at δ 12.45 ppm. Also, the appearance of peaks representing the nBu group at δ 0.95 ppm, δ 1.30-1.80 ppm and δ 4.20 ppm further established the formation of the desired acetophenone 200. With this success, attention turned to the use of other acrylates.

Next, tBu acrylate was trialled (Table 4.3, entry 2), and following purification by column chromatography, acetophenone tBu acrylate 201 was isolated in 90% yield.

Table 4.3. The Heck reaction of 5′-bromo-2′-hydroxyacetophenone 108 with various acrylates.
Acrylonitrile, acrylamide and acrylic acid were also reacted with 5′-bromo-2′-hydroxyacetophenone 108 (Table 4.3, entries 3, 4 and 5). However, as with the Heck reactions of 4-bromobenzaldehyde 73 (see Table 4.2, entries 3, 4 and 5), analysis of the crude material showed only the acetophenone starting material, with no sign of the acrylate reagents or desired acrylate product. As these reagents had twice proven unsuccessful, these acrylates were examined no further.

The Heck reaction conditions were also applied to 3′-bromo-5′-chloro-2′-hydroxyacetophenone 116, using nBu acrylate (Scheme 4.14). The reaction was predicted to occur via the more reactive bromine rather than the chlorine substituent, but 1H NMR spectroscopy showed only peaks representing the starting material acetophenone, and so this was abandoned as a substrate.

Attention then turned to the use of 5′-bromochalcone as the aryl halide substrate.

### 4.3.2.2 Heck Reaction of 5′-Bromochalcone 109

When the Heck reaction was applied to 5′-bromochalcone 109 (Scheme 4.15), only the chalcone starting material, benzaldehyde 59 (formed from a retro-aldol reaction, as indicated by a singlet peak at δ 9.98 ppm), and a trace amount of the corresponding flavanone 137 were noted in the 1H NMR spectrum. The fact that the desired acrylate product 203 could not be seen in the 1H NMR spectrum, as well as the
formation of the undesired benzaldehyde, meant that no further reactions were carried out using bromochalcone 109 as a substrate. Again, only a trace amount of conversion between chalcone 109 and flavanone 137 occurred with the use of K₂CO₃.

Scheme 4.15. The Heck reaction did not proceed with bromochalcone 109, with only benzaldehyde 59, 5'-bromochalcone 109 and a trace amount of the corresponding flavanone 137 identified in the ¹H NMR spectrum.

4.3.2.3 Heck Reaction of 6-Bromoflavanone 137 and 6-Bromoflavone 165

Following application of the Heck reaction to brominated flavanone 137 (Scheme 4.16), analysis of the crude material showed only the flavanone and nBu acrylate starting materials. There was a trace amount of the corresponding chalcone 109 (in a ratio of 1:9 for chalcone 109 to flavanone 137 respectively), as indicated by a small broad singlet at δ 12.76 ppm representing the 2'-hydroxyl proton of the chalcone.

Scheme 4.16. The Heck reaction of 6-bromoflavanone 137 did not produce the desired acrylate 204.

The reaction conditions were also applied to 6-bromoflavone 165, however, as with 4'-bromoflavone 157, only starting material 165 and the corresponding acetophenone 108 and benzaldehyde 59 were identified in the ¹H NMR spectrum of the crude product material, in a ratio of 1:2:2 respectively (Scheme 4.17).

Scheme 4.17. The Heck reaction of 6-bromoflavone 165 also did not proceed.
4.3.3 Alternate Pathway to Flavonoid Acrylates

While the Heck reaction could not be successfully applied to Ring A or Ring B brominated chalcones, flavanones and flavones, an alternate method was trialled. It was thought that the use of acetophenone acrylate 200 in a Claisen-Schmidt condensation would allow access to these desired chalcone and flavonoid acrylates, and so acetophenone nBu acrylate was reacted under the optimal Claisen-Schmidt condensation conditions identified in Chapter Two (benzaldehyde 59, 2’-hydroxyacetophenone acrylate 200, KOH, THF, MW, 50 °C, 15 min, Scheme 4.18).

Analysis of the crude material by ¹H NMR spectroscopy (Figure 4.4) revealed complete conversion of the acetophenone starting material 200 (from the loss of the acetophenone CH₃ peak at δ 2.61 ppm), along with the presence of excess benzaldehyde 59 (the aldehyde peak at δ 9.99 ppm) and the desired chalcone acrylate 206 (by the ‘movement’ of aromatic peaks, and the appearance of a singlet at δ 13.10 ppm representing the chalcone hydroxyl). A small amount of the corresponding flavanone 207 was also present in the ¹H NMR spectrum, as two multiplets at δ 2.65-2.87 ppm and δ 5.12-5.22 ppm representing the CH₂ and CH respectively. The ratio of chalcone 206 to flavanone 207 was approximately 4:1, as determined from the integrations of the hydroxyl peak of the chalcone (integration of 0.85 at δ 13.10 ppm), and the CH peak of the flavanone (integration of 1 at δ 5.12 ppm).
It was also noted that the \( n \text{Bu} \) protecting group was still attached, by the presence of peaks at \( \delta \ 0.90\text{-}1.70 \ \text{ppm} \) and \( \delta \ 4.20 \ \text{ppm} \). Purification by column chromatography (1:1 CH\(_2\)Cl\(_2\):Pet) isolated the protected chalcone \( n \text{Bu} \) acrylate 206 in a yield of 87\%, which was then fully characterised. This result allowed access to chalcone and flavonoid acrylates, even though the direct Heck reaction with brominated chalcones and flavonoids had been unsuccessful.

### 4.4 Heck Reaction of Flavonoids: Conclusion

Trials of known Heck reaction conditions were carried out using 4-bromobenzaldehyde 73 as the aryl halide and \( n \text{Bu} \) acrylate as the acrylate substrate. While Pd(OAc)$_2$, PPh$_3$ and DMF were selected as the palladium source, ligand and solvent respectively, due to their common use in Heck reactions,\(^{390, 540} \) K$_2$CO$_3$ was found to be the base that gave the greatest yields, and NEt$_3$ was the next best. It was also determined that when using K$_2$CO$_3$, the acrylate should be used in a 4:1 ratio with the aryl bromide in order to maximise conversion. Five acrylates were reacted under the optimised conditions: \( n \text{Bu} \) acrylate, \( t \text{Bu} \) acrylate, acrylonitrile, acrylamide and acrylic acid; however with the brominated benzaldehyde and acetophenone substrates, only the two acrylic esters were successfully reacted.
The application of the Heck reaction conditions to chalcone, flavanone and flavone substrates brominated on either Ring A or Ring B was unsuccessful. However, such flavonoid acrylates could still be accessed: Scheme 4.19 shows that through the acetophenone nBu acrylate 200, chalcone and flavanone acrylates were able to be synthesised.

Scheme 4.19. Flavonoid acrylates could be accessed through the use of acetophenone nBu acrylate 200 in Claisen-Schmidt condensation and isomerisation or flavone formation reactions.

With this knowledge, attention turned to the synthesis of Designed Multiple Ligands (DMLs), which included the use of acetophenone acrylates 200 and 201.
Chapter Five:
‘Conjugate’ Designed Multiple Ligands: Functionalised 3’,4’-Dihydroxyflavonols and Peptidomimetic ACE Inhibitors
5.1 Chapter Overview

Designed Multiple Ligands, or DMLs, are therapeutic agents which incorporate two or more pharmacophores or pharmaceutical structures into a single compound. Such compounds offer a number of advantages, including synergistic effects leading to reduced doses, lower costs for patients and increased compliance to medications. This chapter will discuss attempts to synthesise a number of DML compounds for the treatment of hypertension, incorporating 3′,4′-dihydroxyflavonol compounds with various linker groups as one ligand, and enalapril analogues as another ligand.

5.2 Designed Multiple Ligands

Designed Multiple Ligands, or DMLs, are a relatively new development in pharmaceutical drug design. Their main premise is that they incorporate two or more known bioactives with similar therapeutic effects into a single compound, thus providing more than one beneficial effect in the treatment of a particular disease. There are a number of different names for DMLs in the literature, including codrugs, Multi-Target-Directed Ligands, hybrid drugs, bi- or multifunctional drugs, and multitarget drugs, however DML is the most commonly used title, and was coined by Morphy and Rankovic. DMLs fall under the topic of polypharmacology, or the development of therapies that modulate multiple targets simultaneously.

There are two approaches in the design and synthesis of DML compounds; the knowledge based approach and the screening approach (Figure 5.1). In both approaches, a lead generation phase accesses either two highly-selective ligands, one selective ligand (of two), or a non-selective ligand (with two other selective ligands). The processes of ‘designing in’ desirable structural features, ‘balancing’ of the ligands’ features to configure ligand selectivity, and ‘designing out’ undesirable features is used to access a DML lead candidate from the lead compound. Next is the lead optimisation phase, to balance the activity ratio of the ligands, optimise the selectivity of the DML to target sites and other receptors in the body, optimise the physicochemical properties, absorption, distribution, metabolism, excretion (ADME) and toxicological properties of the DML, so as to increase the compound’s bioavailability. This results in a DML drug candidate, ready for clinical trials.
5.2.1 Knowledge-Based Approach to the Discovery of DMLs

The knowledge-based approach to the discovery of DML compounds utilises marketed drugs with known targets, or compounds which are historically known to provide certain therapeutic effects.\textsuperscript{548} There are three different ways in which the ligands of DMLs may be joined. The first is the attachment of the ligands through a linker group, and such compounds are known as ‘conjugate’ DMLs (Figure 5.2a). This linker must be structurally different to the ligands themselves, and its length, strength and flexibility can be carefully selected to accommodate the ligands and their respective target sites.\textsuperscript{548} A commonly used liker is poly(ethylene glycol), as it can be simply used to install different linker lengths, and also confers good aqueous solubility.\textsuperscript{548}

![Diagram of knowledge-based approach to the discovery of DMLs](image)

Linkers may also be enzymatically cleavable, to allow the ligands to be cleaved from one another to reach different or distant active sites.\textsuperscript{545, 548, 556} Most of the cleavable conjugates reported in the literature contain an ester-based linker, which is designed to be cleaved by plasma esterases to release the two or more individual drugs that then act independently.\textsuperscript{548} One such example is the non-steroidal anti-inflammatory
drug (NSAID) DML 209, which links the NSAID ibuprofen through an ester linker group to an NO-donor (Figure 5.3). The ester is cleaved by plasma esterases to provide two bioactives, which act separately.

![Figure 5.3. The NSAID DML 209 links ibuprofen to an NO-donor through a cleavable linker group.](image)

As the size of this linker group decreases, it reaches a point where the ligands are essentially touching. Such compounds are known as ‘fused’ DMLs, and involve the direct linking of the ligands (Figure 5.2b). The non-peptide gastrin receptor 210, developed for the prevention of peptic ulcers caused by chemotherapy with histamine H2 receptor antagonists, is such an example, where a hydrophobic gastrin antagonist (shown in blue) and a hydrophilic histamine H2 pharmacophore (shown in green) are ‘fused’ to provide the DML.

![Figure 5.4. ‘Fused’ DML 210 was developed for the prevention of peptic ulcers in chemotherapy.](image)

Finally, the most common form of knowledge-based DMLs is ‘merged’ DMLs, which incorporate the pharmacophores of two or more different pharmaceuticals onto a single scaffold, by taking advantage of commonalities in the structures of the starting ligands (Figure 5.2c). Once such example is omapatrilat 211, which will be discussed in more detail under the topic of cardiovascular DMLs (Chapter 5.2.4).

Possibly the greatest advantage of the DMLs is that the design process eliminates many of their potential disadvantages. The ‘designing in’ of structural features that are associated with beneficial therapeutic effects, or the ‘designing out’ of structural features known to cause side effects, is of great value to the design process.

Following the development of a lead compound through this knowledge-based strategy, the lead optimisation phase can begin. This involved the balancing of the activity ratio, the optimisation of physicochemical properties (to promote
bioavailability, for example), and the optimisation of ADME properties, once again to promote bioavailability.548

5.2.2 Screening-Based Approach to the Discovery of DMLs

The screening approach to discover DMLs involves either high throughput screening (HTS), where numerous small molecules are tested against a number of targets, or focused screening, where compound classes that are already known to be active against one of the targets of interest are screened against another target.545, 548 The latter is more commonly utilised than HTS,548 potentially due to a lower probability of the screening of compounds delivering suitable combinations of activities, or the logistical complications and costs associated with conducting multiple screens.546

DMLs discovered through a screening approach also take advantage of the design process to eliminate potential disadvantages. Lead DML compounds identified through either screening process offer the advantage of possible further modification, through the ‘designing in’ of desired activities through the incorporation of certain functionalities, as well as the ‘designing out’ of specific structural features associated with detrimental effects.545, 548 The ‘balancing’ of these lead DML compounds through the modification of their structural features also helps to ensure that the compound has optimal affinity for all targets.548

As with the knowledge-based strategy, once a lead compound has been developed, the lead optimisation phase modifies the physicochemical properties and affinity for targets to optimise bioavailability of the compound.548

5.2.3 Advantages of DMLs

As well as the benefits associated with the design process of DMLs, there are a number of other advantages for DMLs. First and foremost, their different ligands allow for more than one target to be reached, providing more than one beneficial effect or pathway of action from a single pharmaceutical.545, 548 Whilst this advantage can also be assigned to the administration of multiple drugs (a ‘drug cocktail’) and ‘fixed dose combinations’ (FDCs, where two or more drugs are co-formulated into a single tablet),545 the latter approaches are associated with complications due to highly complex/ different pharmacokinetic and pharmacodynamic relationships.

DMLs are also known to exhibit ‘synergistic’ effects,545, 550, 553, 554 whereby the combination of ligands leads to a stronger response from the body than the
administration of the ligands separately. Once again, this effect does not apply to FDCs or drug cocktails.545 This synergistic effect allows for the administration of lower doses of the DMLs, and is therefore also beneficial in the case of side effects, which may be reduced or even removed with lower doses.

Another benefit is patient compliance; the simple fact that a pharmaceutical plan with one tablet is easier to follow than three tablets indicates that patients are more likely to comply with their medication. This leads to a significant reduction to healthcare costs associated with lack of medication.545 Also, the risks and costs of developing DMLs are in principle no different to the development of any other single entity, therefore DMLs should not be any more costly than single drugs.546

A possible disadvantage of DMLs is that each ligand incorporated into the structure may have different active concentrations. This may result in either an overdose or cytotoxic effects from one ligand, or insufficient response or effect from the other ligand. This is a common problem in the synthesis of DML compounds,545,548 and in most examples, the aim has been to obtain in vitro activities within an order of magnitude of each other in animal models, with the assumption that this will lead to similar levels of receptor occupancy in vivo.548 Ultimately, the results of these animal model studies help to identify the optimal ratio of the ligands to design a second generation of DML compounds.548

Another problem with the DMLs is that incorporating two or more compounds into a single structure often leads to large molecular weights,560 which are associated with poor drug absorption and cell permeation, in accordance with Lipinski’s Rule of Five (especially those with molecular weights greater than 500).561 This can be avoided, once again in the design process, through the use of smaller linker groups and ligands when ‘conjugate’ DMLs are concerned, and is less problematic in the case of ‘merged’ DMLs, where only a single scaffold is required and so molecular weights can be kept down.545, 547, 548

With the many advantages of DMLs, and the design process allowing for the removal of many of the potential disadvantages, there have been a number of diseases for which the DMLs have been designed. Of particular relevance to this research are those which have been developed for cardiovascular diseases.
5.2.4 Cardiovascular DMLs

DMLs have been developed for a number of different diseases, including malaria, cancer, and HIV, Alzheimer’s disease, diabetes, inflammation, infection, and diseases of the central nervous system (CNS, including depression and schizophrenia), amongst others. Of note to this research is the fact that no DMLs published to date incorporate a flavonoid scaffold; as mentioned in Chapter One, the flavonoids are associated with a large number of biological benefits.

A number of cardiovascular DMLs have been developed. Omapatrilat was one such ‘merged’ example (Figure 5.5), which inhibited both the Angiotensin-Converting Enzyme (ACE) and neutral endo-peptidase (NEP, an enzyme which cleaves a natural peptide known to reduce blood pressure). It incorporates features of compound SQ28603, an inhibitor of neutral endo-peptidase (NEP, an enzyme which cleaves a natural peptide known to reduce blood pressure), and captopril, an ACE inhibitor (Figure 5.5). Omapatrilat elicited cardiovascular effects greater than either component alone (a synergistic effect) and offered the advantage of a reduced risk of serious side effects (including death and hospitalisation) in comparison to enalapril. However, after reaching Phase III Clinical trials, omapatrilat was linked to angioedema (swelling of the skin), and was not approved by the FDA.

Following omapatrilat, the cardiovascular compound CGS35601 has advanced furthest in clinical trials. CGS35601 is a triple vasopeptidase inhibitor (VPI) of ACE, NEP and endothelin-converting enzyme (ECE, an enzyme which produces a vasoconstrictive peptide). It was developed from ECE inhibitor CGS30084 at Novartis and underwent further modification to produce the triple-action DML (Figure 5.6).
CGS35601 213 is potent at all three active sites, with IC\textsubscript{50} concentrations (half maximal inhibitory concentration) of 22 nM, 2 nM, and 119 nM for ACE, NEP and ECE respectively.\textsuperscript{570} Preclinical pharmaco-toxicological evaluations have also been performed using the prodrug of CGS35601 (known as CGS37808 215, Figure 5.7) in rats, and found that it presented no safety concerns and did not cause angioedema.\textsuperscript{570} Further testing and clinical trials are still required,\textsuperscript{567, 571} and as of February 2012, neither CGS35601 213 nor its prodrug 215 had entered the Novartis drug pipeline.\textsuperscript{563}

With the knowledge that no one had taken advantage of the antioxidant activity of the flavonoids, in particular the vasorelaxant and antioxidant compound 3',4'-dihydroxyflavonol 53, our attention turned to its use in the synthesis of DMLs combining this antihypertensive compound with peptidomimetic ACE inhibitors.

### 5.3 The Ligands of the ‘Conjugate’ DMLs

With the vasorelaxant and antioxidant 3',4'-dihydroxyflavonol 53 in mind, as well as analogues of the peptidomimetic ACE-inhibitor enalapril 43, it was thought that a series of ‘conjugate’ (joined through a linker group) DMLs could be synthesised and evaluated for their antihypertensive activity. The research herein describes attempts to synthesise a series of antihypertensive DMLs incorporating 3',4'-dihydroxyflavonol 53 and peptidomimetic (enalapril) analogues attached through a linker group. A number of linker groups were explored, and will be explained in turn. Note that following the
synthesis of the DMLs, biochemical analysis of the DML compounds as well as the individual ligands was to be performed, to compare both individual and combined effects, as well as identify any synergistic effects.

5.3.1 Ligand 1: 3’,4’-Dihydroxyflavonol 53

As explained in Chapter 1.3.4.1, 3’,4’-dihydroxyflavonol 53 is a vasorelaxant flavonoid. Three important features have been identified as responsible for its vasorelaxant activity (Figure 5.8); it is a flavonol (shown in red), it lacks substitution on Ring A (shown in green), and contains hydroxyl groups on both positions 3 and 4 of Ring B (shown in blue).286 Coincidentally, two of these features (shown in red and blue) are also linked to the antioxidant activity of flavonoids, and so it is no surprise that this flavonol also offers the benefit of antioxidant activity.277, 288

![Figure 5.8. 3’,4’-Dihydroxyflavonol 53 is a vasorelaxant, antioxidant flavonoid.](image)

While the exact pathway through which 3’,4’-dihydroxyflavonol 53 produces its vasorelaxant activity is unknown, Woodman and co-workers have shown that it decreases vascular contraction at least in part by inhibition of the RhoA/Rho-kinase pathway in endothelium-denuded rat aorta.289 It also causes Ca²⁺ desensitisation in vascular smooth muscle, resulting in reduced vascular smooth muscle contraction.290 Biochemical and biological evaluation has revealed other therapeutic effects, including antioxidant properties, the ability to reduce tissue death after ischaemia and reperfusion (in sheep hearts and rat brains),61, 277, 292 and the prevention of diabetes-induced endothelium dysfunction.295, 296

As the biological target of 3’,4’-dihydroxyflavonol 53 is unknown, so is its active concentration. This makes the ‘designing in’ and ‘designing out’ process to ensure affinity to the receptor target for the flavonol, as well as ACE for the dipeptides, as well as determine similar active concentrations, impossible at this stage.

As mentioned in Chapter 1.3.4.1, a problem in the use of 3’,4’-dihydroxyflavonol 53 as a pharmaceutical is its lack of solubility and therefore bioavailability. In fact, in order to conduct biochemical testing, Williams and co-
workers had to dissolve the compound in DMSO for administration to cells. However, this lack of solubility was overcome through the introduction of various functionalities. One of these in particular was succinamic acid, attached to the 6-position of the 3’,4’-dihydroxyflavonol (Figure 5.9).

![Figure 5.9. Woodman and co-workers synthesised the succinamic acid 54 (shown in blue) of 3’,4’-dihydroxyflavonol 53 to increase solubility.](image)

The sodium salt of the succinamic acid on Ring A has also been reported to greatly increase solubility in water (Figure 5.3 c); from less than $10^{-7}$ M for 3’,4’-dihydroxyflavonol to $10^{-1}$ M for the sodium salt. When the succinamic derivative 54 was applied to rat aorta in vasorelaxant studies, it exhibited none of the vasorelaxant activity of 3’,4’-dihydroxyflavonol 53, however maintained some of its antioxidant activity. However, no studies were completed regarding the enzymatic digestion of the compound, providing the possibility that enzymatic cleavage may produce the vasorelaxant flavonol to the body. This soluble flavonol was an ideal compound for the synthesis of the ‘conjugate’ DMLs, as it provided not only the antioxidant 3’,4’-dihydroxyflavonol 53 but also a linker group from which to attach the second ligand, a peptidomimetic ACE inhibitor.

Another possible linker group for the 3’,4’-dihydroxyflavonol ligand arose from the acetophenone acrylates synthesised using a Heck reaction in Chapter Four (Scheme 5.1). Acetophenone acrylates 200 and 201, which were successfully used in Claisen-Schmidt condensations to access their corresponding 2’-hydroxychalcones (Chapter 4.2.3.2), were ideal starting materials for the synthesis of 3’,4’-dihydroxyflavonol compounds 216 with an acrylic side chain on Ring A.

![Scheme 5.1. 3’,4’-Dihydroxyflavonol compound 216 with an acrylic acid side chain could be accessed from the acetophenone acrylates 200 and 201 synthesised in Chapter Four.](image)
As both the flavonol succinamic acid 54 and flavonol acrylic acid 216 provided a carboxylic acid linker group, it was ideal that the peptidomimetic ACE inhibitors, based on enalapril 43, would contain an amine for coupling to the carboxylic acid of the flavonols.

5.3.2 Ligand 2: Peptidomimetic ACE Inhibitors

For the second ligand of these DMLs, compounds were synthesised based on the peptidomimetic ACE inhibitor, enalapril 43 (Figure 5.10). Enalapril 43 is able to bind the pockets of ACE through its carboxylic acid (red), methyl substituent (green) and C-terminal proline unit (blue). These functionalities were used to produce a general scaffold for the enalapril analogues synthesised for the DMLs. These enalapril analogues also required an amine functionality (purple), so that they may be linked to the acid of the flavonols 54 and 216 through an amide bond. Lysine 217 was noted as an ideal functionality for these enalapril analogues, as it provided both a carboxylic acid and an amine group for linking, leaving the methyl substituent to be incorporated between the proline and lysine units.

As the two antihypertensive ligands had now been selected, attention first turned to the synthesis of the flavonol ligands. The synthesis of each of the 3′,4′-dihydroxyflavonol ligands 54 and 216 will be discussed in turn, followed by the synthesis of the enalapril analogues, and finally the coupling of the two ligands. The synthesis of the succinamic acid-3′,4′-dihydroxyflavonol 216 will be addressed first.

5.4 The Succinamic Acid-Linked DMLs

5.4.1 Retrosynthesis of Succinamic Acid-Linked DMLs

For the succinamic acid-linked DMLs, the following retrosynthesis was developed (Scheme 5.2).
Scheme 5.2. Retrosynthetic pathway for the synthesis of succinamic acid-linked DMLs 219.

This retrosynthetic method begins with the benzyl deprotection of the phenols of Ring B of flavonol 220, followed by the amide coupling of the amine of the enalapril analogue 218 to the carboxylic acid of the flavonol succinamic acid 221 (Scheme 5.1). The flavonol succinamic acid 221 could be accessed using the methodology of Woodman and co-workers, from \( p \)-anisidine 222 and 3,4-dihydroxybenzaldehyde 223.62

5.4.2 Synthesis of the Flavonol Succinamic Acid

To access flavonol succinamic acid 221 (Figure 5.11), acetophenone acetamide 224 had to be first synthesised, using a method from Bennett et. al.98 The protection of the 3,4-dihydroxybenzaldehyde 223 was also required. The chalcone and flavonol compounds could then be synthesised using the conditions provided by Woodman.62

Figure 5.11. The desired benzyl-protected flavonol succinamic acid 221.
5.4.2.1 Acetamide formation and Friedel-Crafts reaction

Acetophenone 224 was synthesised from para-anisidine 222 in two steps.\textsuperscript{98} First, p-anisidine 222 was stirred with acetic anhydride in CH\textsubscript{2}Cl\textsubscript{2} at room temperature for three hours (Scheme 5.3). Following work up, acetamide 225 was isolated as a purple solid in 98% yield.

![Scheme 5.3. The synthesis of acetophenone acetamide 224 over two steps from p-anisidine 222.](image)

Friedel-Crafts acylation of acetamide 225 using acetyl chloride (AcCl) and aluminium chloride (AlCl\textsubscript{3}) was performed in CH\textsubscript{2}Cl\textsubscript{2} under reflux for five hours. The crude product was recrystallised from EtOH to give acetophenone 224 in 47% yield as pale green crystals (Scheme 5.3). The structure was confirmed by comparison to literature data,\textsuperscript{98} including an acetamide CH\textsubscript{3} peak at $\delta$ 2.16 ppm, the acetoephene CH\textsubscript{3} peak at $\delta$ 2.60 ppm, and the acetophenone OH peak at $\delta$ 12.09 ppm in the $^{1}$H NMR spectrum (Figure 5.12). Note that the amide N-H proton was obscured by the aromatic peak of proton H\textsubscript{A} (Figure 5.10, $\delta$ 7.24-7.33 ppm). With acetophenone 224 in hand, the protection of 3,4-dihydroxybenzaldehyde 223 was investigated, to provide a protected benzaldehyde to react with the acetophenone acetamide 224 in a Claisen-Schmidt condensation.
5.4.2.2 Protecting 3,4-Dihydroxybenzaldehyde 223

The most common protecting group for catechols (ortho-dihydroxyl groups on an aromatic ring) is an acetal, and is relatively simple to introduce. However, the deprotection of acetals relies on strongly acidic conditions, which is known to cause problems in terms of chalcone-flavanone isomerisation (see Chapters 2.2.1 and 3.2 for our experiences with this isomerisation). For this reason, the protection of each phenol individually (as phenolic ethers) was attempted. Methyl and benzyl protection was selected, as these groups could be easily installed, and deprotected using methods that would not affect the flavonoid scaffold (BBr₃ demethylation and hydrogenation respectively).

First, dimethylation of 3,4-dihydroxybenzaldehyde 223 was attempted (Table 5.1, entry 1). Reflux of the benzaldehyde with K₂CO₃, dimethyl sulfate ((CH₃)₂SO₄) and acetone for two hours, followed by column chromatography (2:1 Pet:EtOAc) isolated 3,4-dimethoxybenzaldehyde 226 in 93% yield. While this reaction was successful, the use of (CH₃)₂SO₄ in large scale reactions is hazardous, and therefore benzyl protection was also investigated.
Table 5.1. Protection of 3,4-dihydroxybenzaldehyde 223.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product</th>
<th>R</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>226</td>
<td>Me</td>
<td>93%</td>
</tr>
<tr>
<td>2</td>
<td>227</td>
<td>Bn</td>
<td>74%</td>
</tr>
</tbody>
</table>

For the dibenzyl protection of 3,4-dihydroxybenzaldehyde 223, conditions from the work of Woodman and co-workers were used. The unprotected benzaldehyde 223 was stirred with benzyl bromide (BnBr, 2.2 equivalents) and K₂CO₃ (2.4 equivalents) in EtOH for 72 hours at room temperature (Table 5.1, entry 2). Recrystallisation of the crude material from EtOH isolated 3,4-dibenzyloxybenzaldehyde 227 in 74% yield, as confirmed by comparison to literature data, including the appearance of peaks corresponding to the benzyl (δ 5.21 ppm and δ 5.23 ppm) and aldehyde (δ 9.80 ppm) of the desired product (Figure 5.13). While benzyl bromide is also hazardous in large scale reactions, this protection was ideal as 3,4-dibenzyloxybenzaldehyde 227 had been successfully used in the synthesis of flavonols by Woodman and co-workers, whose methodologies were also used in the current synthesis.

Figure 5.13. ¹H NMR spectrum of the protected compound, 3,4-dibenzyloxybenzaldehyde 227 (CDCl₃).
With the protected 3,4-dibenzyloxybenzaldehyde 227 in hand, attention turned to the reaction of this aldehyde with acetophenone acetamide 224 to access the desired chalcone acetamide 228, using a Claisen-Schmidt condensation.

5.4.2.3 Claisen-Schmidt Condensation to Synthesise Chalcone Acetamide 228

To synthesise chalcone acetamide 228, a number of methods were trialled using acetophenone acetamide 224 and protected benzaldehyde 227 as starting materials (Scheme 5.4). These methods included the microwave-assisted Claisen-Schmidt methodology developed in Chapter Two, as well as that of Woodman and co-workers.62 Note that the conversion ratios were determined by the comparison of the integration values of the CH₃ peak of acetophenone 224 (δ 2.60 ppm with an integration of 3), and the hydroxyl peak of chalcone 228 (δ 12.75 ppm with an integration of 0.85, as explained in Chapter 2.3.2).

Scheme 5.4. The synthesis of chalcone acetamide 228 was attempted using a number of methods.

The first method attempted was the optimised microwave-assisted Claisen-Schmidt condensation developed in Chapter Two (Table 5.2, entry 1). Following work up, analysis by ¹H NMR spectroscopy revealed both the acetophenone 224 and desired chalcone 228, in a ratio of 1:3. Purification by recrystallisation (1:1 Pet:THF) isolated the desired chalcone acetamide 228 in 65% yield. The synthesis of the desired compound was confirmed by comparison to the literature data,62 including the appearance of the chalcone hydroxyl peak (δ 12.75 ppm) in the ¹H NMR spectrum (Figure 5.14). The success of this reaction validates the practical utility of the method developed herein, as Woodman and co-workers were unable to effect the same transformation without first protecting the acetophenone hydroxyl group, which required an additional two steps in the synthesis (p-methoxybenzyl (PMB) protection and deprotection).62
While chalcone acetamide 228 had been isolated in good yield, a number of other methods were also trialled in the hope of greater conversion and yields. When the reaction was repeated using microwave irradiation, with EtOH in place of THF as the solvent (EtOH is the solvent of choice for the conventional Claisen-Schmidt condensation method), decreased conversion (2:1 acetophenone 224 to chalcone 228) was noted (Table 5.2, entry 2).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Solvent</th>
<th>224:228 (yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MW 70 °C 30 min</td>
<td>THF</td>
<td>1:3 (65%)</td>
</tr>
<tr>
<td>2</td>
<td>MW 70 °C 30 min</td>
<td>EtOH</td>
<td>2:1</td>
</tr>
<tr>
<td>3</td>
<td>16 hrs rt</td>
<td>THF</td>
<td>2:1</td>
</tr>
<tr>
<td>4</td>
<td>40 hrs rt</td>
<td>THF</td>
<td>1:0</td>
</tr>
<tr>
<td>5</td>
<td>16 hrs 40 °C</td>
<td>EtOH</td>
<td>1:4 (79%)</td>
</tr>
</tbody>
</table>

The reaction was then repeated using the conventional methodology, KOH in THF at room temperature for 16 hours (Table 5.2, entry 3), and chalcone acetamide 228
was produced in a ratio of 1:2 with acetophenone 224. When these conditions were repeated with a reaction time of 40 hours (Table 5.2, entry 4), none of the desired chalcone 228 was produced; only starting materials were observed in the $^1$H NMR spectrum. This outcome suggested that increased reaction times could allow the retroaldol reaction (shown previously in Scheme 3.7).

The reaction was also carried out in EtOH for 16 hours at 40 °C, in accordance with Woodman’s Claisen-Schmidt conditions (Table 5.2, entry 5). This provided the best conversion (1:4 acetophenone 224 to chalcone 228). Recrystallisation from 1:1 Pet:THF afforded chalcone acetamide 228 in 79% yield, and its structure was confirmed by $^1$H NMR spectroscopy. With chalcone acetamide 228 in hand, the synthesis of the flavonol acetamide was attempted.

Before discussing the synthesis of the flavonol acetamide, a brief introduction to the synthesis of flavonols is required.

5.4.2.4 Flavonols

5.4.2.4.1 Flavonols: Synthesis

As mentioned in Chapter One, the flavonols 8 are structurally distinguishable from other flavonoids due to the alkene in the 2-position, hydroxyl in the 3-position, and ketone in the 4-position of Ring C (Figure 5.15). The flavonols are simply flavones with an added hydroxyl group, giving rise to another name, 3-hydroxyflavones.

Figure 5.15. The general structure of the flavonols 8, or 3-hydroxyflavones.

As also established in Chapter One, two well-known flavonols found in nature are quercetin 13,13 which is commonly found in its glycosidic forms in green and black teas,573 onion,574 and cranberries,575 and kaempferol 18, which can be found in many plant species (see the mini-review by Calderon-Montano et. al. for an extensive list),576 including tea573 and spinach574 (Figure 5.16). Quercetin 13 and kaempferol 18 both exhibit antileishmanial,91 antidiabetic,160, 185 and anticancer properties,131 and numerous other flavonol compounds exhibit these and other beneficial qualities.61, 84, 210, 577, 578 In plants, the flavonols are synthesised from flavanols (which lack the double bond between the 2- and 3-positions) by flavonol synthase. However, there are numerous
methods available for the laboratory synthesis of flavonols, from various starting materials.

Figure 5.16. Quercetin 13 and kaempferol 18 are two common flavonols found in nature.

5.4.2.4.2 The Auwers Synthesis

In 1908, the first synthetic methodology for the synthesis of flavonols, the Auwers Synthesis, was discovered. This method transforms 2-bromo-2-(α-bromobenzyl)coumarones 229 to flavonols 8 using hydroxides, (Scheme 5.5). The formation of oxonium 230 results from the loss of the α-bromine from dibromide 229. This is followed by attack of the activated α-position by the hydroxide, and then removal of a β-hydrogen to give chalcone-like structure 232. Finally, attack at the β-position of the alkene, and loss of the β-bromine gives the desired flavonol 8. This method is quite dated, and other syntheses with simpler starting materials have been developed in more recent years.

Scheme 5.5. Mechanism of the Auwers reaction for the synthesis of flavonol 8.

5.4.2.4.3 DMDO-mediated synthesis

Another method for the synthesis of flavonols uses dimethyldioxirane (DMDO) 234 as an epoxidising reagent with 2'-hydroxychalcones or flavones. DMDO 234 is an extremely unstable compound, and is generated in situ in quite low concentrations, typically 0.04-0.12 M solutions, from acetone, oxone, sodium bicarbonate (NaHCO₃), and H₂O (Scheme 5.6). Its concentration can be
determined by the NMR or GLC titration of the oxidation of thioanisole, which is oxidised to its sulfoxide and then sulfone.

![Scheme 5.6. DMDO is synthesised from acetone, NaHCO₃ and oxone.](image)

Whilst produced only in low concentrations, DMDO is made from extremely cheap reagents, and the by-product of its reaction with alkenes is the relatively safe acetone. Due to its instability, DMDO must be synthesised for immediate use, or can be stored under refrigeration for a maximum of three to four days. Disadvantages of its use in flavonol formation include long reaction times of up to 140 hours, and the use of excess reagent (up to 15 equivalents). The application of DMDO to 2′-hydroxychalcones leads to the formation of aurones as a side product, while the DMDO reaction of flavones does not (Scheme 5.7).

![Scheme 5.7. Mechanism of flavonol formation from flavone using DMDO.](image)

This DMDO-mediated synthesis was attempted in our hands, using flavone as a substrate (synthesised in Chapter 3.3.2). DMDO solution was synthesised according to a method by Wright, and using thioanisole, NMR titrations determined the concentration of DMDO as approximately 0.10 M in acetone, as also seen in Wright’s results. The solution was used immediately.

Treatment of flavone with fresh DMDO (Scheme 5.8) revealed only the starting material in the ¹H NMR spectrum. Even when the reaction was repeated with greater than ten equivalents of the DMDO solution and reaction times of up to 40 hours, flavonol could not be accessed.

![Scheme 5.8. The DMDO-mediated epoxidation of flavone did not proceed.](image)

As this DMDO-mediated method had proven unsuccessful, attention turned to another method, the Algar-Flynn-Oyamada reaction.
5.4.2.4.4 The Algar-Flynn-Oyamada Reaction

In 1934, the Algar-Flynn-Oyamada (AFO) reaction was developed for the synthesis of flavonols.\(^{334, 335}\) This method involves the oxidation of 2′-hydroxychalcones by hydrogen peroxide (H\(_2\)O\(_2\)) under basic conditions (usually NaOH or KOH) at 0 °C. There has been much debate over the years as to whether the mechanism proceeds via an epoxide derivative\(^{599, 600}\) or not.\(^{601, 602}\) The mechanism of the epoxide-generated flavonol involves the cis-Weitz-Scheffer epoxidation of the alkene 66 by H\(_2\)O\(_2\) (Scheme 5.9). The phenoxide of 235 then attacks the β-position to form the flavanol 236, followed by oxidation to form the desired flavonol 8.\(^{600, 603}\)

Scheme 5.9. The mechanism of the Algar-Flynn-Oyamada reaction via an epoxide intermediate.

On the other hand, the non-epoxide mechanism involves the attack of the alkene by the deprotonated oxygen, followed by re-protonation to give the hydroxyl group at the 3-position (Scheme 5.10). Finally, oxidation of flavanol 236 gives flavonol 8.\(^{602, 603}\)

Scheme 5.10. The non-epoxide mechanism of the AFO reaction.

A problem in the synthesis of flavonols by the AFO reaction is the formation of side products, namely the aurones 5, which form in a 5-exo-tet fashion, rather than the desired 6-exo-tet closure which leads to the flavonol.\(^{604}\) Both of these processes are favoured by Baldwin’s Rules.\(^{605}\) These compounds are formed when the deprotonated
oxygen of 235 attacks the α-position rather than the β-position, followed by dehydration to produce aurone 5 (Scheme 5.11). 600, 602

![Scheme 5.11. Aurones 5 are formed in a 5-exo-tet fashion, a side product in the AFO reaction.](image)

Whilst the formation of aurones provides evidence for the epoxide mechanism, aurones don’t form in all cases, and thus the topic is still open for debate. Certain structural features promote aurone formation in the AFO reaction; a methoxyl substituent at the 6'-position of 2'-hydroxychalcones, or a lack of the 6'-methoxyl group accompanied by hydroxyl groups at the 2'- and 4'-positions of the chalcone 93 produce a mixture of the aurone 5 and flavonol 8 (Scheme 5.12). 603, 606-608 Aurones can also be synthesised from 2'-hydroxychalcones using other reagents, including Hg(OAc)₂⁴⁹³, 609 and DDQ.⁴⁸⁷

![Scheme 5.12. The AFO reaction of 2',4'-Dihydroxychalcone 93 leads to a mixture of aurone 5 and flavonol 8 products.](image)

Even with the risk of side-product formation, the AFO reaction has been highly popular in the synthesis of flavonols from 2'-hydroxychalcone starting materials. 303, 407, 606, 610 Reactions have even been attempted with α-substituted chalcones. 607, 608

More recently, a ‘Modified’ AFO method has become popular, in which 2'-hydroxyacetophenones and benzaldehydes are reacted under Claisen-Schmidt Condensation conditions to afford a 2'-hydroxychalcone, which is then directly treated with AFO conditions (without isolation of the chalcone) to afford the flavonol. ²⁹¹, ⁵⁹⁹, ⁶¹¹, ⁶¹² Whilst this ‘Modified’ method appeared ideal for the synthesis of flavonols required in this project, the traditional, two-step AFO reaction was first trialled to access the desired flavonol acetamide.

### 5.4.2.4.5 Algar-Flynn-Oyamada Reaction of Chalcone Acetamide 238

The AFO method for flavonol formation from chalcone acetamide 228 was taken directly from that of Woodman and co-workers. ⁶² In our hands, chalcone 228 was stirred with 2M NaOH and EtOH, and cooled to 0 °C. Hydrogen peroxide (H₂O₂) was
added slowly, and the solution stirred for a further two hours at 0 °C before stirring for 16 hours at room temperature (Scheme 5.13).

Scheme 5.13. AFO reaction using Woodman’s conditions provided flavonol acetamide 238 in 91% yield.

Neutralisation, followed by filtration, provided the desired flavonol in a yield of 91%. The structure was confirmed through comparison to literature data, and included the loss of the chalcone hydroxyl peak (δ 12.75 ppm) and the slight downfield shifts of the acetamide CH₃ (δ 2.21 ppm) and benzyl CH₂ (δ 5.24 ppm) peaks in the ¹H NMR spectrum (Figure 5.17). Note that the peaks of the spectra are somewhat broad, likely due to the π-π stacking of the flavonol.⁵⁸

However, when this reaction was repeated (numerous times, using not only the same conditions but also longer reaction times and at larger and smaller scales), none of the desired flavonol was produced. Nevertheless, a limited quantity of the requisite flavonol 238 was available to be used in the formation of the amine.

Before advancing to the amine formation of the flavonol acetamide, the ‘Modified’ AFO reaction was examined for the synthesis of the flavonol acetamide 238.
from acetophenone 224 and benzaldehyde 227. The ‘Modified’ AFO reaction was successful in previous flavonol syntheses by Woodman and co-workers, and so their methodology was utilised. It was hoped that this method would provide the desired flavonol acetamide 238 in a reproducible manner, allowing access to larger quantities of the flavonol.

5.4.2.4.6 ‘Modified’ Algar-Flynn-Oyamada Reaction

Using acetophenone acetamide 224 and 3,4-dibenzyloxybenzaldehyde 227 as substrates, the ‘Modified’ AFO conditions were applied; stirring at room temperature with 40% KOH, EtOH and dioxane for five days, followed by treatment with 30% H₂O₂ and 2M NaOH in EtOH and dioxane at 0 °C for two hours, and then 16 hours at room temperature (Scheme 5.14). Following this two-step reaction, analysis of the crude product by ¹H NMR spectroscopy revealed a spectrum with broad peaks, including those of the benzaldehyde 227 (CHO at δ 9.80 ppm), Cannizzaro primary alcohol (δ 4.77 ppm, as produced by the intramolecular reaction of benzaldehydes with KOH, see Chapter 2.2.1), and broad aromatic peaks. Analysis of the reaction mixture by MS, revealed the presence of the desired flavonol, however purification by either column chromatography (2:1 Pet:EtOAc) or recrystallisation (EtOAc) failed to isolate the desired flavonol acetamide 238.

While the ‘Modified’ methodology could not be used to access the flavonol acetamide, the small amount of flavonol 238 accessed through the AFO reaction of the chalcone acetamide 228 was in hand to access the desired flavonol succinamic acid 221.

5.4.2.5 Amine Formation

The final step for the synthesis of the flavonol succinamic acid 221 involved two parts: the formation of the amine (as its hydrochloride salt) from the flavonol acetamide 238 under acidic conditions, followed by the reaction of this amine with succinic acid in pyridine to form flavonol succinamic acid 221 (Scheme 5.15).
Scheme 5.15. Formation of amine 239, followed by reaction with succinic anhydride, provides the desired flavonol succinamic acid 221.

The amine formation step had previously been carried out on similar substrates by Woodman and co-workers using either 5M HCl in EtOH or 12M HCl in acetic acid (AcOH) under reflux for 2-3 hours, which was also reported to remove the benzyl protecting groups. Therefore, both of these methods were attempted (Table 5.3).

Table 5.3. Attempted synthesis of flavonol amine 239 from flavonol acetamide 238.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Solvent</th>
<th>238:239</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5M HCl, reflux 2hr</td>
<td>EtOH</td>
<td>1:0</td>
</tr>
<tr>
<td>2</td>
<td>12M HCl, reflux 3hr</td>
<td>AcOH</td>
<td>1:0a</td>
</tr>
</tbody>
</table>

*a only chalcone 228 was isolated*

First, flavonol acetamide 238 was reacted with 5M HCl in EtOH (Table 5.3, entry 1), and analysis of the crude material by \(^1\)H NMR spectroscopy revealed only the starting material. While Woodman and co-workers were able to use these conditions to not only access the amine, but also deprotect the benzyl groups, in our hands neither transformation occurred.

Flavonol acetamide 238 was then reacted with 12M HCl in AcOH (Table 5.3, entry 2), and unexpectedly, the corresponding chalcone 228 was produced. This structure was confirmed by the presence of the chalcone hydroxyl peak (\(\delta 12.76 \text{ ppm}\)), and the appropriate peaks and integrations for the benzyl (\(\delta 5.23 \text{ ppm}\)) and acetamide (\(\delta 2.21 \text{ ppm}\)) peaks in the \(^1\)H NMR spectrum. Once again, in our hands there was also no deprotection of the benzyl groups.\(^{62}\)
Without amine 239, the desired flavonol succinamic acid 221 could not be synthesised, and succinamic acid-linked DMLs were therefore not accessed. With this lack of access, attention turned to the use of acetophenone acrylates, which were accessed using the Heck reaction in Chapter Four.

5.5 The Acrylic Acid-Linked DMLs

Another opportunity to produce DMLs incorporating 3′,4′-dihydroxyflavonol 53 arose from the acetophenone acrylates 201 and 200 synthesised in Chapter Four (Scheme 5.16). These acetophenones were accessed using the Heck reaction with 5′-bromo-2′-hydroxyacetophenone 108 and tBu or nBu acrylate, and could provide a carboxylic acid to link the enalapril-derived ligand through the deprotection of their tBu or nBu groups respectively.

![Scheme 5.16. 3′,4′-Dihydroxyflavonol compound 216 with an acrylic acid side chain could be accessed from the acetophenone acrylates 201 and 200 synthesised in Chapter Four.](image)

5.5.1 Retrosynthesis of Acrylic Acid-Linked DMLs

For the acrylic acid-linked DMLs 240, retrosynthetic analysis was performed (Scheme 5.17). This retrosynthesis begins with the benzyl deprotection of the phenols of Ring B of the flavonol 240, followed by the amide coupling of the amine of enalapril analogue 218 to the carboxylic acid of flavonol acrylate 242. Deprotection of the tBu or nBu group would provide the carboxylic acid required for coupling.
The flavonol acrylates 243 and 244 could be accessed from 3,4-dibenzyloxybenzaldehyde and their corresponding acetophenone acrylates 201 and 200 respectively, using a Claisen-Schmidt condensation followed by an Algar-Flynn-Oyamada reaction.

### 5.5.2 Synthesis of Flavonol tBu Acrylate 243

The first flavonol to be synthesised was that of the acetophenone tBu acrylate 201 (Scheme 5.18).

The starting material, acetophenone tBu acrylate 201, was synthesised in Chapter 4.2.3.2, in 90% yield, using Pd(OAc)$_2$, PPh$_3$ and K$_2$CO$_3$ in toluene under reflux for 16 hours (Scheme 5.19).
Scheme 5.19. Acetophenone tBu acrylate 201 was synthesised in 90% yield using the Heck reaction.

The next step of this synthetic pathway was the formation of the corresponding 2'-hydroxychalcone, using the Claisen-Schmidt condensation with acetophenone tBu acrylate 201 and 3,4-dibenzyloxybenzaldehyde 227.

5.5.2.1 Claisen-Schmidt Condensation to Synthesise Chalcone tBu Acrylate 245

A number of methods were trialled in attempts to synthesise chalcone tBu acrylate 245. In order to compare the success of these methods, conversion ratios were determined using $^1$H NMR spectroscopy. The determination of conversion was accomplished by allocating the CH$_3$ peak of acetophenone tBu acrylate 201 at $\delta$ 2.60 ppm with an integration of 3, and the hydroxyl peak of chalcone 245 at $\delta$ 13.24 ppm was integrated as 0.85 (as explained in Chapter 2.3.2).

First, a literature method from Woodman et al. was applied to acetophenone tBu acrylate 201 and 3,4-dibenzyloxybenzaldehyde 227. The starting materials were stirred with NaOH in EtOH for 16 hours at 40 °C (Table 5.4, entry 1). However, following acidic work up, analysis of the reaction mixture by $^1$H NMR spectroscopy revealed none of the desired chalcone 245. Another literature method by Ko et al. which utilised Ba(OH)$_2$ was also unsuccessful (Table 5.4, entry 2), with only starting materials identified in the $^1$H NMR spectrum.

Next, the optimised microwave-assisted Claisen-Schmidt conditions established in Chapter Two (MW, 4.0 equivalents KOH, THF, 50 °C, 15 minutes) were applied to acetophenone tBu acrylate 201 and 3,4-dibenzyloxybenzaldehyde 227 (Table 5.4, entry 3). However, once again only starting materials were present in the crude material. Repeating the reaction for 30 minutes (Table 5.4, entry 4), also produced none of the desired chalcone.
Table 5.4. The synthesis of chalcone tBu acrylate 245 by Claisen-Schmidt condensation methods.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Conditions</th>
<th>201:245</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaOH</td>
<td>MeOH</td>
<td>16 hr 40 °C</td>
<td>1:0</td>
</tr>
<tr>
<td>2</td>
<td>Ba(OH)₂</td>
<td>MeOH</td>
<td>16 hr 40 °C</td>
<td>1:0</td>
</tr>
<tr>
<td>3</td>
<td>KOH</td>
<td>THF</td>
<td>MW 50 °C 15 min</td>
<td>1:0</td>
</tr>
<tr>
<td>4</td>
<td>KOH</td>
<td>THF</td>
<td>MW 50 °C 30 min</td>
<td>1:0</td>
</tr>
<tr>
<td>5</td>
<td>KOH</td>
<td>THF</td>
<td>MW 70 °C 30 min</td>
<td>1:2</td>
</tr>
<tr>
<td>6</td>
<td>KOH</td>
<td>THF</td>
<td>MW 100 °C 30 min</td>
<td>1:0</td>
</tr>
</tbody>
</table>

Increasing the microwave temperature to 70 °C for 30 minutes (Table 5.4, entry 5), analysis of the crude material by \(^1\)H NMR spectroscopy showed acetophenone 201 and desired chalcone 245 in a ratio of 1:2, with the tBu group visible as a singlet at \(\delta 1.56\) ppm. Attempts to isolate the chalcone using column chromatography (using a variety of solvent systems, including CH₂Cl₂/Pet, Pet/EtOAc, MeOH/CHCl₃ and ether/Pet in different ratios) were not successful as the products co-eluted.

![Figure 5.18. \(^1\)H NMR spectrum of the impure chalcone tBu acrylate 245, with acetophenone 201 in a ratio of 10:1 respectively (CDCl₃).]
Figure 5.18 shows the purest sample obtained by column chromatography (40% CH₂Cl₂ in Pet), in a ratio of approximately 10:1 desired chalcone tBu acrylate 245 to acetophenone tBu acrylate 201.

When the reaction was repeated at 100 °C (Table 5.4, entry 6), none of the desired chalcone was produced, and so the impure sample of chalcone 245 (10:1 chalcone tBu acrylate 245 to acetophenone tBu acrylate 201 respectively) was used for flavonol formation.

5.5.2.2 Algar-Flynn-Oyamada (AFO) Reaction

The Algar-Flynn-Oyamada reaction was then applied to the impure chalcone tBu acrylate 245. Using conditions from Woodman and co-workers (Scheme 5.20), impure chalcone acrylate 245 was dissolved in EtOH with 5% NaOH, and cooled to 0 °C. Then 15% H₂O₂ in H₂O was added, and the solution stirred at 0 °C for 2 hours. Further stirring at room temperature for 16 hours, followed by work up and purification by column chromatography (2:3 CH₂Cl₂:Pet) isolated flavonol 243 in a respectable yield of 43% over two steps (from acetophenone 201 to chalcone 245 to flavonol 243).

Scheme 5.20. The AFO reaction was used to synthesise flavonol tBu acrylate 243 from chalcone 245, in 43% yield from acetophenone 201.

The isolated compound 243 was characterised using ¹H NMR spectroscopy, including the loss of the chalcone and acetophenone peaks, the appearance of a broad singlet at δ 8.31 ppm indicative of the 3-hydroxyl group of the flavonol, and the movement of the aromatic peaks (Figure 5.19). The structure of this novel functionalised flavonol was further confirmed by ¹³C NMR spectroscopy and HRMS.
With flavonol tBu acrylate 243 in hand, the next synthetic step was the removal of the tBu group, to provide the carboxylic acid required for coupling to the enalapril analogue.

### 5.5.2.3 Removal of the tBu Group

The final step of this flavonol synthesis was the removal of the tBu group to provide the free carboxylic acid. Most deprotection methods for tBu esters involve acidic hydrolysis. One of the most common methods is treatment with trifluoroacetic acid (TFA) in CH₂Cl₂, and so flavonol 243 was reacted with 5% TFA in CH₂Cl₂ for 16 hours (Table 5.5, entry 1). However, analysis of the crude material revealed only the starting material. When the reaction was repeated for 48 hours, and also when the concentration was increased to 15% TFA in CH₂Cl₂ for 16 hours (Table 5.5, entries 2 and 3), the ¹H NMR spectra showed only the acetophenone 201 of the starting material, signified by a singlet peak at δ 2.68 ppm, and fewer aromatic peaks, indicating the degradation of the starting material.

Other acidic conditions were also trialled (Table 5.5 entries 4-7), including acetyl chloride (AcCl) in MeOH, HCl in acetic acid (AcOH), and the pIL TeaHSO₄ under microwave irradiation at 100 °C for 5 minutes. In all cases, the method was either
unable to remove the tBu group, or degraded the starting material to form an insoluble black tar.

Table 5.5. Deprotection of the flavonol tBu acrylate 243.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>243:242</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5% TFA/ CH₂Cl₂ 16 hr</td>
<td>1:0</td>
</tr>
<tr>
<td>2</td>
<td>5% TFA/ CH₂Cl₂ 48 hr</td>
<td>0:0a</td>
</tr>
<tr>
<td>3</td>
<td>15% TFA/CH₂Cl₂ 16 hr</td>
<td>0:0a</td>
</tr>
<tr>
<td>4</td>
<td>AcCl/MeOH 10 min</td>
<td>1:0</td>
</tr>
<tr>
<td>5</td>
<td>AcCl/MeOH 16 hrs</td>
<td>0:0b</td>
</tr>
<tr>
<td>6</td>
<td>HCl/ AcOH</td>
<td>0:0b</td>
</tr>
<tr>
<td>7</td>
<td>TeaHSO₄ MW 100 °C 5 min</td>
<td>1:0</td>
</tr>
<tr>
<td>8</td>
<td>HNO₃ CH₂Cl₂ rt 2 hrs⁶¹⁵</td>
<td>1:0</td>
</tr>
<tr>
<td>9</td>
<td>p-TSA MW 100 °C 10 min⁶¹⁶</td>
<td>1:0</td>
</tr>
<tr>
<td>10</td>
<td>NaH DMF 16 hrs⁶¹⁷</td>
<td>1:0</td>
</tr>
<tr>
<td>11</td>
<td>Silica MW 50W 50 °C 4 min⁶¹⁸</td>
<td>1:0</td>
</tr>
<tr>
<td>12</td>
<td>Silica MW 50W 120 °C 4 min</td>
<td>1:0</td>
</tr>
</tbody>
</table>

a only acetophenone 201 was seen in 'H NMR spectrum
b product was undissolvable black tar

When these common methods were unable to remove the tBu group, alternative methods were sought. Stirring the flavonol with nitric acid (HNO₃)⁶¹⁵ in CH₂Cl₂ for two hours at room temperature (Table 5.5, entry 8) provided only the deprotected acetophenone acrylate 246, and the use of p-toluenesulfonic acid under microwave irradiation (100 °C for 10 minutes, Table 5.5, entry 9) gave the same result.⁶¹⁶ Even a basic method was trialled, using sodium hydride in DMF for 16 hours at room temperature, however once again only starting material was recovered. Finally, a method from Park et. al. was applied to the flavonol tBu acrylate 243,⁶¹⁸ which involved treatment with silica under microwave irradiation at 50 °C for 4 minutes (Table 5.3, entry 11). When no deprotection occurred, the reaction was repeated at 120 °C, with the same result (Table 5.5, entry 12).

With nine different methods unsuccessful in the removal of the tBu group of flavonol acrylate 243, attention turned to the use of acetophenone nBu acrylate 200 to access the flavonol nBu acrylate, in the hope that the nBu group could be removed.
5.5.3 Synthesis of Flavonol \textit{n}Bu Acrylate 244

Once again following the retrosynthetic pathway in Scheme 5.18, flavonol acrylic acid 242 (Scheme 5.21), was to be synthesised from acetophenone \textit{n}Bu acrylate 200.

![Scheme 5.21. The desired benzyl-protected flavonol acrylic acid 242 was to be accessed from acetophenone 201.]

Acetophenone \textit{n}Bu acrylate 200 was previously synthesised in Chapter 4.2.3.2, in 76\% yield, using Pd(OAc)$_2$, PPh$_3$ and K$_2$CO$_3$ in toluene under reflux for 16 hours (Scheme 5.22).

![Scheme 5.22. Acetophenone \textit{n}Bu acrylate 201 was synthesised in 76\% yield using the Heck reaction.]

With this acetophenone in hand, as well as the protected 3,4-dibenzyloxybenzaldehyde 227, the synthesis of chalcone \textit{n}Bu acrylate 247 was attempted.

5.5.3.1 Claisen-Schmidt Condensation to Synthesise Chalcone \textit{n}Bu Acrylate 247

As both chalcone formation and the deprotection of esters occur with the use of strong hydroxide bases, it was reasoned that the removal of the \textit{n}Bu protecting group to produce chalcone 248 could also occur during Claisen-Schmidt condensation of acetophenone \textit{n}Bu acrylate 200 and 3,4-dibenzyloxybenzaldehyde 227. The resultant carboxylic acid was not expected to react under the Algar-Flynn-Oyamada reaction conditions, therefore deprotection of the ester at this stage was not considered a problem. In accordance with the previous determination of conversion ratios, the CH$_3$ peak of acetophenone 200 at $\delta$ 2.62 ppm was allocated an integration of 3, and the hydroxyl peak of the chalcone at $\delta$ 13.25 ppm was allocated an integration of 0.85 (as explained in Chapter 2.3.2).
The first method attempted utilised the microwave-assisted Claisen-Schmidt conditions from Chapter Two (MW, 4.0 equivalents KOH, THF, 50 °C, 15 minutes), with acetophenone $n$Bu acrylate 200 and 3,4-dibenzyloxybenzaldehyde 227 (Table 5.6, entry 1). Following acidic work up, analysis by $^1$H NMR spectroscopy revealed only the starting materials. Increasing microwave reaction times to 30 and 60 minutes (Table 5.6, entries 2 and 3 respectively) also produced none of the desired chalcone.

Table 5.6. Claisen-Schmidt condensation of acetophenone $n$Bu acrylate 200 and benzaldehyde 227.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Conditions</th>
<th>200:245</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KOH</td>
<td>THF</td>
<td>MW 50 °C 15 min</td>
<td>1:0</td>
</tr>
<tr>
<td>2</td>
<td>KOH</td>
<td>THF</td>
<td>MW 50 °C 30 min</td>
<td>1:0</td>
</tr>
<tr>
<td>3</td>
<td>KOH</td>
<td>THF</td>
<td>MW 50 °C 60 min</td>
<td>1:0</td>
</tr>
<tr>
<td>4</td>
<td>KOH</td>
<td>THF</td>
<td>MW 70 °C 30 min</td>
<td>1:30$^a$</td>
</tr>
<tr>
<td>5</td>
<td>NaOH</td>
<td>EtOH</td>
<td>16 hr rt</td>
<td>1:0</td>
</tr>
<tr>
<td>6</td>
<td>Ba(OH)$_2$</td>
<td>MeOH</td>
<td>16 hr 40 °C</td>
<td>10:1</td>
</tr>
<tr>
<td>7</td>
<td>Ba(OH)$_2$</td>
<td>MeOH</td>
<td>72 hr 40 °C</td>
<td>7:1</td>
</tr>
<tr>
<td>8</td>
<td>Ba(OH)$_2$</td>
<td>MeOH</td>
<td>MW 50 °C 30 min</td>
<td>2:1</td>
</tr>
</tbody>
</table>

$^a$ benzaldehyde 225 was also present in a ratio of 1:30:60 for 201:247:227

Increasing the reaction temperature to 70 °C for 30 minutes (Table 5.6, entry 4) produced some of the $n$Bu-protected chalcone 247, as indicated by the appearance of the chalcone hydroxyl peak at $\delta$ 13.25 ppm in the $^1$H NMR spectrum, and peaks at $\delta$ 0.99-1.56 ppm, and $\delta$ 4.25 ppm representing the $n$Bu group (Figure 5.15). A trace amount of the acetophenone was also present, as indicated by a small peak at $\delta$ 2.64 ppm, and a large amount of benzaldehyde, as indicated by the peak at $\delta$ 9.79 ppm. The ratio of benzaldehyde 227 to chalcone 247 was approximately 2:1 respectively (Figure 5.20). This lack of acetophenone 200 (in comparison to the large amount of benzaldehyde still present) suggested that degradation of the acetophenone starting material at this increased temperature was occurring, and an alternate Claisen-Schmidt condensation method was required.
Figure 5.20. Higher microwave temperatures resulted in the degradation of acetophenone 200 in the 70 °C, 30 minute Claisen-Schmidt condensation reaction (Table 5.6, entry 4).

The literature method from Woodman and co-workers\textsuperscript{291} was also applied to the reaction (NaOH, EtOH, rt for 16 hours, Table 5.6, entry 5), however only starting materials were returned.

Another literature method from Ko et al.\textsuperscript{613} was also applied to acetophenone \textit{n}Bu acrylate 200 and benzaldehyde 227 (Table 5.6, entry 6), providing acetophenone 200 and chalcone 247 (still \textit{n}Bu-protected) in a ratio of 10:1 respectively. In an attempt to increase conversion, the reaction was repeated for 72 hours (Table 5.6, entry 7), and resulted in a small increase in conversion to the chalcone (acetophenone 200 to chalcone 247, 7:1).

Using the base and solvent from the previous method (Ba(OH)\textsubscript{2} and MeOH), with microwave irradiation (50 °C, 30 min, Table 5.6, entry 8) greatly improved conversion to \textit{n}Bu-protected chalcone acrylate 247 (2:1 acetophenone to chalcone). However, when attempts were made to isolate the chalcone by column chromatography, it was found that once again the acetophenone co-eluted with the chalcone, making purification impossible. The purest sample was produced by column chromatography (1:1 CH\textsubscript{2}Cl\textsubscript{2}:Pet), in a ratio of 1:30 for acetophenone 200 to chalcone 247 respectively (Figure 5.21). It was also noted that the \textit{n}Bu group had not been removed, due to the presence of representative peaks at $\delta$ 0.99-1.56 ppm and $\delta$ 4.25 ppm.
Figure 5.21. Chalcone $n$Bu acrylate 247 co-eluted with its corresponding acetophenone 200, and so purification provided the chalcone in a ratio of approximately 30:1 with the acetophenone.

As per the previous reaction of impure chalcone $t$Bu acrylate 245 (Chapter 5.5.2.1), this slightly impure sample of the chalcone $n$Bu acrylate 247 was used in the Algar-Flynn-Oyamada reaction to synthesise the desired flavonol $n$Bu acrylate 249.

5.5.3.2 Algar-Flynn-Oyamada Reaction

Once again, the AFO methodology of Woodman and co-workers was used, in an attempt to synthesise flavonol acrylic acid 244 from its corresponding chalcone 247 (5% NaOH, 15% H$_2$O$_2$, EtOH for 2 hours at 0 °C, then 16 hours at rt, Scheme 5.23). Note that once again, the strong hydroxide used for this reaction was expected to cleave the $n$Bu group to provide flavonol acrylic acid 244.

Analysis of the crude material by $^1$H NMR spectroscopy showed the presence of the chalcone starting material 247 (hydroxyl peak at $\delta$ 13.25 ppm) as well as the acetophenone 200 (hydroxyl peak at $\delta$ 12.33 ppm), and appeared to show some other product, due to the integrations of the benzyl and aromatic peaks being far greater than
that of the chalcone alone. The peaks of the spectrum were quite broad, which is common with flavonol compounds due to $\pi-\pi$ stacking.\textsuperscript{58} However, when the sample was examined using Mass Spectrometry, neither the mass of the desired flavonol acrylic acid \textbf{244} or its butyl ester could be found.

The structure of this unknown product or products could not be determined, although it was thought that the alkene of the nBu acrylate side chain may be providing another position for epoxidation. It was reasoned therefore, that the removal of this alkene by hydrogenation (Pd/C, H\textsubscript{2} gas) could allow for the formation of the desired flavonol. A hydrogenation reaction after the Claisen-Schmidt condensation would remove the alkene of the chalcone and remove the benzyl-protection of Ring B of the flavonol, and after the Algar-Flynn-Oyamada step would also remove the benzyl-protection of Ring B. For this reason, the hydrogenation step had to be performed following the synthesis of the acetophenone nBu acrylate \textbf{200}. With this in mind, attention turned to the synthesis of flavonol propanoic acid \textbf{249}.

\subsection*{5.5.4 Synthesis of Flavonol Propanoic Acid 249}

Acetophenone nBu acrylate \textbf{200} was once again the starting material in the synthesis of flavonol propanoic acid \textbf{249} (Scheme 5.24).

![Scheme 5.24. The desired benzyl-protected flavonol propanoic acid 249 was to be accessed from acetophenone nBu acrylate 200.](image)

The first step of this synthesis was the hydrogenation of acetophenone nBu acrylate \textbf{200}, to remove the alkene functionality. Acetophenone nBu acrylate \textbf{200} was accessed previously in Chapter 4.2.3.2 in 76\% yield, using the Heck reaction.

\subsection*{5.5.4.1 Acetophenone nBu Ester 250}

With acetophenone nBu acrylate \textbf{200} in hand, hydrogenation was carried out using 10\% palladium on carbon (Pd/C), hydrogen gas (H\textsubscript{2}), and a solvent of 1:1 MeOH and toluene (Scheme 5.25).

![Scheme 5.25. Hydrogenation of acetophenone nBu acrylate 200 provided the acetophenone nBu ester 250.](image)
The desired acetophenone \textit{nBu} ester 250 was isolated in quantitative yield. The structure was confirmed by the loss of the alkene CH peaks (one at $\delta$ 6.29 ppm and the other within the aromatic peaks, $\delta$ 7.54-7.65 ppm, Figure 5.22), the appearance of the new ethyl CH$_2$ resonances as a triplet at $\delta$ 2.88 ppm and a multiplet between $\delta$ 2.58-2.61 ppm. The hydroxyl group also shifted to $\delta$ 12.10 ppm in the $^1$H NMR spectrum. Novel acetophenone 250 was fully characterised by $^{13}$C NMR spectroscopy and HRMS.

Figure 5.22. Hydrogenation of acetophenone \textit{nBu} acrylate 200 provided acetophenone \textit{nBu} ester 250 in quantitative yield.

With acetophenone \textit{nBu} ester 250 in hand, the next step of the synthesis was the Claisen-Schmidt condensation to synthesise chalcone propanoic acid 251.

5.5.4.2 Claisen-Schmidt Condensation to Synthesise Chalcone Propanoic Acid 251

As before, conversion ratios were determined using $^1$H NMR spectroscopy. Deprotection of the \textit{nBu} ester was once again expected under the reaction conditions, to provide chalcone propanoic acid 251.

The optimised microwave-assisted Claisen-Schmidt conditions from Chapter Two were the first to be applied to acetophenone \textit{nBu} ester 250 and 3,4-dibenzzyloxybenzaldehyde 227 (MW, 4.0 equivalents KOH, THF, 50 °C, 15 minutes, Table 5.7, entry 1), however analysis of the crude material by $^1$H NMR spectroscopy revealed only starting materials.
Table 5.7. Claisen-Schmidt condensation of acetophenone nBu ester 250 and benzaldehyde 227.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Scale</th>
<th>Conditions</th>
<th>250:251 (isolated yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>THF</td>
<td>0.3 mmol</td>
<td>MW 50 °C 15 min</td>
<td>1:0</td>
</tr>
<tr>
<td>2</td>
<td>THF</td>
<td>0.3 mmol</td>
<td>MW 50 °C 30 min</td>
<td>2:1</td>
</tr>
<tr>
<td>3</td>
<td>THF</td>
<td>0.3 mmol</td>
<td>MW 70 °C 30 min</td>
<td>2:1</td>
</tr>
<tr>
<td>4</td>
<td>EtOH</td>
<td>0.3 mmol</td>
<td>rt 16 hrs</td>
<td>1:4 (48%)</td>
</tr>
<tr>
<td>5</td>
<td>EtOH</td>
<td>1.1 mmol</td>
<td>rt 16 hrs</td>
<td>1:1</td>
</tr>
<tr>
<td>6</td>
<td>EtOH</td>
<td>1.1 mmol</td>
<td>rt 16 hrs</td>
<td>1:4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> dilute reaction with slow addition of benzaldehyde 227 reduced occurrence of Cannizzaro reaction

Increasing the microwave reaction time to 30 minutes resulted in some of the desired compound being formed, in a ratio of 2:1 of the acetophenone 250 to the desired chalcone 251 (Table 5.7, entry 2). In an attempt to increase formation of the desired chalcone 251, the reaction was also repeated at 70 °C (Table 5.7, entry 3), however no additional conversion was achieved.

The literature method of Woodman et. al. was then trialled, with KOH in EtOH on a 0.30 mmol scale (Table 5.7, entry 4). Analysis by <sup>1</sup>H NMR spectroscopy revealed the ratio of acetophenone 250 to chalcone 251 as 1:4 respectively, and following purification by column chromatography (2:1 Pet:EtOAc), chalcone 251 was isolated in 48% yield. This was confirmed by the loss of the acetophenone (δ 2.61 ppm) and benzaldehyde (δ 9.80 ppm) peaks and the appearance of the chalcone hydroxyl peak (δ 12.81 ppm) in the <sup>1</sup>H NMR spectrum (Figure 5.23), as well as <sup>13</sup>C NMR spectroscopy and HRMS. Also evident in the spectrum was the loss of the nBu group, by the absence of peaks between δ 0.90-1.20 ppm and δ 4.20 ppm. It was of interest to note that both acrylic esters 245 and 247 were not deprotected under these same conditions. While no further experiments were performed, it seemed that the inability to deprotect those acrylic esters was somehow linked to the conjugation of the starting materials.
When this reaction was repeated on a larger scale (1.1 mmol, Table 5.7, entry 5), chalcone 251 formation decreased to a ratio of 1:1 with acetophenone 250, with a relative ratio of 1 for benzaldehyde 227. This decrease in conversion coincided with the appearance of a singlet peak at δ 4.57 ppm, which suggested the formation of the benzyl alcohol Cannizzaro side product, as shown in Scheme 5.26 (see Chapter 2.3.1 for a review of the Cannizzaro reaction and its occurrence in Claisen-Schmidt condensations).

To confirm that this peak was due to Cannizzaro benzyl alcohol 252, benzaldehyde 227 was reduced to its primary alcohol using sodium borohydride (NaBH₄) in MeOH (Scheme 5.27). The ¹H NMR spectrum of the product exhibited a peak at δ 4.57 ppm (CH₂ of the benzyl alcohol), which corresponded to the unknown peak of the Claisen-Schmidt condensation. This product was therefore determined to be present in a ratio of 0.4:1:1 with the acetophenone and chalcone respectively (the peak at δ 4.57 ppm was allocated an integration of 2).
As the Cannizzaro products were undesired, and signified a decrease in the formation of the desired chalcone 251, a method to prevent this side reaction was investigated. The reaction was repeated in a dilute solution, and with slow addition of benzaldehyde 227 (Table 5.7, entry 6), so as to prevent the aldehyde molecules from reacting with themselves rather than acetophenone 250. The conversion ratio returned to 1:4 acetophenone to chalcone respectively, and only a trace amount of Cannizzaro benzyl alcohol 252 was present in the $^1$H NMR spectrum. Purification by column chromatography (2:1 Pet:EtOAc) isolated chalcone propanoic acid 251 in 46% yield.

With chalcone propanoic acid 251 in hand, attention turned to the formation of its corresponding flavonol 254, using the Algar-Flynn-Oyamada reaction.

### 5.5.4.3 Algar-Flynn-Oyamada Reaction to Synthesise Flavonol Propanoic Acid 254

The formation of flavonol 254 was then attempted using the Algar-Flynn-Oyamada reaction conditions of Woodman and co-workers (5% NaOH, 15% H$_2$O$_2$, EtOH for 2 hours at 0 °C, then 16 hours at rt, Scheme 5.28).$^{291}$

Analysis of the crude material by $^1$H NMR spectroscopy revealed broadened peaks, suggesting the presence of the desired flavonol 254 (Figure 5.24). However, the integration values for the benzyl CH$_2$ peaks were too large. The presence of the chalcone starting material 251 was also confirmed by a small peak at δ 12.78 ppm.

Attempts to purify the flavonol by recrystallisation were unsuccessful (EtOAc, 1:1 THF:Pet), and column chromatography (2:1 Pet:EtOAc, 5% MeOH in CHCl$_3$) could not separate chalcone 251 and flavonol 254. Although flavonol 254 could not be isolated, its presence was confirmed by MS (as well as chalcone propanoic acid 251). This impure sample, in a ratio of approximately 0.15:1 chalcone 251 to flavonol 254,
was kept in the hope that attempts at its coupling to the enalapril analogue of the DML might afford a product that would be more readily purified.

Attempts to repeat the reaction were also unsuccessful, and so attention turned to the use of the ‘Modified’ AFO reaction, to access pure flavonol propanoic acid \textit{254}.

5.5.4.4 ‘Modified’ Algar-Flynn-Oyamada Reaction to Synthesise Flavonol Propanoic Acid \textit{254}

Using the methodology from Woodman \textit{et. al.} (Scheme 5.29),\textsuperscript{201} the ‘Modified’ AFO reaction was applied to acetophenone \textit{nBu} ester \textit{250} and benzaldehyde \textit{227}.

Following acidic work up, analysis of the crude material by \textsuperscript{1}H NMR spectroscopy revealed none of the desired flavonol \textit{254}. The spectrum consisted of extremely broad peaks, however showed only the benzaldehyde peak at \(\delta\) 9.81 ppm, and many aromatic peaks (\(\delta\) 7.15-7.99 ppm). There was no sign of the acetophenone
The lack of flavonol propanoic acid 254 was confirmed by MS. This was disappointing, however the sample of impure flavonol propanoic acid 254 (synthesised through the Claisen-Schmidt condensation and then AFO reaction) in hand was able to be used to synthesise the desired DMLs.

### 5.6 Peptidomimetic ACE Inhibitor Ligands

As mentioned earlier in this chapter (Chapter 5.3.2), the second ligand chosen for these DMLs were analogues of the peptidomimetic ACE inhibitor enalapril 43 (Figure 5.25). These analogues were based on a scaffold which incorporated certain functional groups of enalapril responsible for its binding to the ACE pocket (Figure 5.25), including a carboxylic acid (red), methyl group (green) and C-terminal proline unit (blue), as well as an amine (purple) to couple to the carboxylic acid side chain of flavonol propanoic acid 254. Lysine was noted as an ideal functionality for these enalapril analogues 218, as it provided the required carboxylic acid and the amine group for linking, leaving the methyl substituent to be incorporated between the proline and lysine units.

Two strategies were developed to incorporate the desired functionalities into the enalapril analogue ligands; the first incorporated the methyl substituent (Strategy A), and the second did not (Strategy B). These strategies will be addressed in turn.

#### 5.6.1 Enalapril Analogue: Strategy A

##### 5.6.1.1 Retrosynthesis: Strategy A

Strategy A was based on the methodology used in the commercial synthesis of enalapril (Scheme 5.30).\(^ {619} \) Beginning with amine 255, deprotection of the amine provides the protected amine 256, which could be accessed using a reductive amination reaction with ketone 257 and diprotected lysine 258. It was noted that this step would give rise to diastereomers, a potential problem that is solved in the commercial synthesis of enalapril by fractional crystallography.\(^ {619} \) In this project, the formation of
diastereomers was not predicted to be problematic, as it is noted that both diastereomers of enalapril are active ACE inhibitors (with IC$_{50}$ values of $1.2 \times 10^{-9}$ M and $8.2 \times 10^{-7}$ M). Ketone 257 could be synthesised from the amide coupling of protected proline 259 and pyruvic acid 260, with the final retrosynthetic step being the protection of the carboxylic acid of proline 261.

![Scheme 5.30. Retrosynthesis of Strategy A.](image)

**5.6.1.2 Synthesis of Enalapril Analogue: Strategy A**

**5.6.1.2.1 Protection of L-Proline 261**

To benzyl-protect the C-terminal proline 261, benzyl alcohol (BnOH), thionyl chloride (SOCl$_2$) and L-Proline were reacted for 48 hours whilst warming from 0 °C to rt, as per the literature method of Ramachandran (Scheme 5.31).$^{620}$

![Scheme 5.31. Benzyl protection of L-proline 261 occurred in 95% yield.](image)

Following recrystallisation in ether, Pro-OBn 259 was isolated as a white solid in 95% yield as confirmed by comparison to literature data,$^{620}$ including the appearance of the benzyl CH$_2$ peak (multiplet, $\delta$ 5.13-5.26 ppm) and aromatic peaks ($\delta$ 7.33 ppm)

150
in the $^1$H NMR spectrum. With Pro-OBn 259 in hand, coupling of proline to pyruvic acid 260 was attempted.

5.6.1.2.2 Coupling Pro-OBn 259 to Pyruvic Acid 260

The methodology of Jou et. al. was used for this reaction, and utilised 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI.HCl) and 1-hydroxybenzotriazole (HOBt) in CH$_2$Cl$_2$ (Scheme 5.32).

Scheme 5.32. EDCI-mediated coupling of pyruvic acid 2560 to Pro-OBn 259 proceeded in 48% yield.

Following purification by column chromatography (30% EtOAc in Pet), the desired ketone 257 was isolated in 48% yield, and the $^1$H NMR spectrum identical to that of Jou et. al..

The next step was the coupling of ketone 257 to lysine, however lysine required protection to provide the desired free amine for reductive amination with ketone 257.

5.6.1.2.3 Synthesis of Lys(Boc)-OBn 258

Di-protected Lys(Boc)-OBn 258 was synthesised from N$\alpha$-9-fluorenylmethoxycarbonyl-N$\epsilon$-tert-butoxycarbonyl-L-lysine 262 (Fmoc-Lys(Boc)-OH) (Scheme 5.33). The free carboxylic acid was first benzyl protected (BnBr, NEt$_3$, EtOAc), as the benzyl group is orthogonal to the Fmoc- and Boc-protecting groups on lysine 262. Purification by column chromatography (30% EtOAc in Pet) isolated the desired tri-protected lysine 263 in quantitative yield.

Scheme 5.33. Benzyl protection of Fmoc-Lys(Boc)-OH 262 occurred in quantitative yield.

Fmoc-Lys(Boc)-OBn 263 was then stirred with 20% piperidine in DMF for two hours (Scheme 5.34), and purification by column chromatography (3% MeOH in CHCl$_3$), isolated desired Lys(Boc)-OBn 258 in 80% yield.
5.6.1.2.4 Reductive Amination of Ketone 257 and Lysine 258

Next, the coupling of ketone 257 and lysine 258 was attempted, using a reductive amination. Reductive aminations are conducted with an amine and a ketone or aldehyde substrate to produce an imine 264, which is then reduced \textit{in situ} to amine 256 by sodium cyanoborohydride (Scheme 5.35).

Using the literature method of traditional enalapril synthesis, sodium cyanoborohydride (NaCNBH₃), EtOH, and 4Å molecular sieves were stirred with lysine 258 and an excess of ketone 257 (1.3 equivalents) at room temperature for 16 hours. Following a careful workup, the crude material was analysed by ¹H NMR spectroscopy, which showed only the lysine starting material 258, and no sign of ketone 257, desired imine 264 or amine 256.

The reaction was then repeated using 1.6 equivalents of ketone 257, however the same result was obtained, and none of the desired amine 256 was observed. This reaction may have been unsuccessful due to the steric bulk of the benzyl-protected lysine preventing access to the carbonyl carbon of the ketone, and also the presence of a
second (although less reactive secondary) amine. As this reductive amination was unsuccessful, a second strategy (Strategy B) was derived.

5.6.2 Enalapril Analogue: Strategy B

5.6.2.1 Retrosynthesis: Strategy B

Strategy B did not incorporate the methyl substituent seen in enalapril, and required only SN2 reactions and amide bond formations in the retrosynthetic pathway (Scheme 5.36). The amine 265 required for coupling to the carboxylic acid of flavonol propanoic acid 254 was provided by the amine side chain of compound 266 (following Boc-deprotection). Compound 266 could be accessed by the SN2 reaction of the amine of di-protected lysine 258 and brominated compound 267. Finally, the bromo-acetyl-Pro-OBn 267 was to be synthesised by coupling bromoacetic acid 268 and Pro-OBn 259.

Although this retrosynthesis provided an enalapril analogue without the methyl substituent, the inclusion of the proline unit and carboxylic acid (seen in red and blue respectively, Scheme 5.40) should be adequate in their ability to bind to ACE. It must be noted once again that antihypertensive evaluations were to be conducted on not only the DML compounds, but also their individual ligands, to identify the activities and verify modes of action for the joined (DML) and separate (ligand) compounds. This would allow for the determination of activity depending on whether the compound may act as a joined DML, or in a prodrug approach, where the ligands are cleaved.
5.6.2.2 Synthesis of Enalapril Analogue: Strategy B

5.6.2.2.1 Protection of L-Proline 261

Once again, synthetic methodology began with Pro-OBn 261, as accessed previously in 95% yield (Scheme 5.29). The next step of this approach was the coupling of the free amine with bromoacetic acid, to provide bromoacetyl-Pro-OBn 267.

5.6.2.2.2 Coupling Pro-OBn 259 to Bromoacetic Acid 268

Benzyl proline 259 was stirred with bromoacetic acid 268, EDCI and HOBT in CH$_2$Cl$_2$ for 16 hours at room temperature. Following workup, solvent removal \textit{in vacuo}, and purification by column chromatography (2:1 Pet:EtOAc), bromoacetyl-Pro-OBn 267 was isolated in 48% yield (Scheme 5.37). This structure was confirmed using $^1$H NMR spectroscopy by the appearance of a multiplet at $\delta$ 3.99-4.10 ppm with an integration of two which was assigned to the CH$_2$ of the bromoacetate, as well as by $^{13}$C NMR spectroscopy and HRMS (Figure 5.26).

![Scheme 5.37. Amide coupling between Pro-OBn 259 and bromoacetic acid 268 provided bromoacetyl-Pro-OBn 267.](image)

![Figure 5.26. $^1$H NMR spectrum of bromoacetyl-Pro-OBn 267 (CDCl$_3$).](image)
5.6.2.2.3 $S_N2$ Reaction of Bromoacetyl-Pro-OBn 267 and Lys(Boc)-OBn 258

The next step of Strategy B was the coupling of bromoacetyl-Pro-OBn 267 to the free amine of previously synthesised Lys(Boc)-OBn 258 using an $S_N2$ reaction. With both compounds in hand, Lys(Boc)-OBn 258 and NaH were stirred in DMF at 0 °C for one hour, before addition of bromoacetyl-Pro-OBn 267, and further stirring for 16 hours at room temperature (Scheme 5.38). Following column chromatography (2:1 Pet:EtOAc), novel compound 266 was isolated in 68% yield. The compound was fully characterised by $^1$H NMR and $^{13}$C NMR spectroscopy, as well as HRMS.

Scheme 5.38. An $S_N2$ reaction coupled di-protected lysine 268 with bromoacetyl-Pro-OBn 267 in 68% yield.

5.6.2.2.4 Boc-Deprotection of Compound 266

The final step for the synthesis of the enalapril analogue ligand of the DML was deprotection of compound 266, to free the amine for coupling with the carboxylic acid of flavonol propanoic acid 254. Compound 266 was stirred in 20% TFA in CH$_2$Cl$_2$ for two hours at room temperature (Scheme 5.39) and following work up, the desired amine 265 was produced in quantitative yield.

The structure was confirmed using $^1$H NMR spectroscopy, by the complete loss of the $t$Bu peak at $\delta$ 1.34 ppm, and fully characterised by $^{13}$C NMR spectroscopy and HRMS. With this Strategy B enalapril analogue 265 in hand, attention turned to the coupling of this amine to the carboxylic acid of flavonol propanoic acid 254, using an amide coupling reaction.

5.7 Coupling of Flavonol Propanoic Acid 254 with Enalapril Analogue 265

The coupling of the amine of enalapril analogue 265 with the carboxylic acid of the semi-pure flavonol propanoic acid 254 to produce the desired DML compound 269 was then attempted, using EDCI, HOBT, NEt$_3$ and CH$_2$Cl$_2$ (Scheme 5.40). The reaction was stirred at room temperature for 16 hours, and following work up, the crude material was analysed by $^1$H NMR spectroscopy. The $^1$H NMR spectrum showed no sign of the chalcone 251 impurity ($\delta$ 12.87 ppm), however the broad peaks of the spectrum and the overlapping of the alkyl peaks of both the flavonol side chain and the enalapril analogue made the spectrum difficult to interpret. When the crude material was analysed by MS, the masses of desired DML 269 or its salts and dimers could not be found.

The reaction was repeated for 40 hours, and then again without the use of base, however in both cases, the desired DML 269 could not be detected using MS. While side reactions were possible, including the EDCI-mediated coupling of two flavonol propanoic acid 254 through the hydroxyl and carboxylic acid groups or coupling through the secondary amine of amine 265 rather than the primary amine, these products were not identified in the $^1$H NMR spectrum or by MS.
Unfortunately, this reaction used the last sample of the available flavonol propanoic acid 254, and the reaction could not be repeated. As mentioned in Chapter 5.5.4.3 and 5.5.4.4, attempts to re-synthesise the flavonol propanoic acid 254 were unsuccessful.

Unfortunately, none of the desired ‘conjugate’ DMLs were accessed. Nevertheless, a series of ‘fused’ DMLs were also the target of this project, and these will be discussed in Chapter Six.

5.8 ‘Conjugate’ DMLs: Conclusion

For the synthesis of ‘conjugate’ DMLs incorporating a 3’,4’-dihydroxyflavonol structure with a carboxylic acid side chain, and an enalapril analogue with an amine for coupling to the carboxylic acid, a number of synthetic methodologies were attempted.

First, the synthesis of flavonol succinamic acid 221 was attempted (Scheme 5.41). Using the methodology of Woodman and co-workers,62 flavonol acetamide 238 was synthesised. However, attempts to deprotect the acetamide to form the required amine 239 (before its coupling with succinic acid to provide the carboxylic acid required for coupling to enalapril analogues) were unsuccessful, and either no reaction, or the formation of the corresponding chalcone 228 was observed.

Following this, the synthesis of three flavonol acrylates was attempted. The first flavonol 243 was synthesised from acetophenone tBu acrylate 201 in two steps in an overall yield of 43% (Scheme 5.42). However, at this point the tBu group could not be cleaved to provide the desired carboxylic acid 242.
Scheme 5.42. Flavonol \(n\)Bu acrylate 243 could not be deprotected.

The next attempt to synthesise a flavonol acrylate used acetophenone \(n\)Bu acrylate 200 as a starting material (Scheme 5.43). The Claisen-Schmidt condensation of acetophenone 200 with 3,4-dibenzylxybenzaldehyde 227 provided 2'-hydroxychalcone 247, albeit slightly impure due to the co-elution of acetophenone 200 and chalcone 247 during column chromatography. However, application of the AFO reaction to this impure sample was unsuccessful.

Scheme 5.43. Flavonol \(n\)Bu acrylate 244 also could not be synthesised.

The final attempt to synthesise a flavonol with an acid side chain began with the hydrogenation of acetophenone \(n\)Bu acrylate 200 in quantitative yield (Scheme 5.44). This was followed by the Claisen-Schmidt condensation (48% yield) and then the Algar-Flynn-Oyamada reaction. While the flavonol 254 produced was slightly impure (in a ratio of approximately 0.15:1 chalcone 251 to flavonol 254, approximately 85% pure), it was this flavonol propanoic acid 254 that was used to attach to the enalapril analogues. Unfortunately, attempts to repeat the synthesis of the flavonol were unsuccessful, and quantities of flavonol propanoic acid 254 were limited.
Scheme 5.44. Flavonol propanoic acid 254 was accessed, although slightly impure.

Two strategies were devised for the synthesis of enalapril analogues. The first, Strategy A, had as its target an enalapril analogue that incorporated a methyl group (Scheme 5.45). However, reductive amination of compound 257 with Lys(Boc)-OBn 258 failed to produce the desired amine 256.

Scheme 5.45. The Strategy A enalapril analogue could not be synthesised, as the reductive amination step was unsuccessful.

Strategy B, in which the target contained no methyl group (Scheme 5.46), was successful and began with Pro-OBn 259, which was coupled to bromoacetic acid 268 to provide bromoacetyl-Pro-OBn 267 in 46% over two steps. With Lys(Boc)-OBn 258, the two were joined using an S_N2 reaction, in 68% yield. Finally, deprotection of 266 provided enalapril analogue 265 in quantitative yield.
Scheme 5.46. Strategy B enalapril analogue 265 was successfully synthesised in six steps.

Attempts were then made to couple enalapril analogue 265 to the slightly impure flavonol propanoic acid 254 (Scheme 5.47). However, these amide bond formations were unsuccessful, and used the remainder of flavonol propanoic acid 254, which unfortunately could not be resynthesised.

Scheme 5.47. Attempts to couple enalapril analogue 265 and flavonol propanoic acid 254 were unsuccessful.

Overall, the synthesis of 3’,4’-dihydroxyflavonols incorporating a Ring A side chain (through the use of acetophenones with acetamide and acrylate functionalities) was unreliable. The purification of the Claisen-Schmidt condensation products often proved problematic, as the acetophenone and chalcone analogues would co-elute in column chromatography. Also, the synthesis of flavonols from the corresponding chalcones by the AFO reaction proved highly unreliable. The few times that these
flavonols were synthesised, with either protected or unprotected carboxylic acid side chains, the reactions were not reproducible.

Although the synthesis of ‘conjugate’ DMLs incorporating 3’,4’-dihydroxyflavonol and an enalapril analogue was not achieved, the synthesis of ‘fused’ DMLs was also pursued in this project, once again combining the 3’,4’-dihydroxyflavonol structure, as well as peptidic ACE inhibitors. This more successful approach will be discussed in Chapter Six.
Chapter Six:
‘Fused’ Designed Multiple Ligands:
3’,4’-Dihydroxyflavonol and Peptidic ACE Inhibitors
6.1 Chapter Overview

As explored in Chapter Five, Designed Multiple Ligands, or DMLs, are therapeutic agents that incorporate two or more pharmacophores or pharmaceutical structures into a single compound. In this chapter, the ‘fused’ DMLs, where the two or more ligands of the DML are essentially touching, are the focus. This chapter will discuss attempts to synthesise a number of DML compounds for the treatment of hypertension, incorporating 3’,4’-dihydroxyflavonol 53 and di- or tripeptides as ligands.

6.2 The Ligands of the ‘Fused’ DMLs

With the vasorelaxant and antioxidant 3’,4’-dihydroxyflavonol 53 in mind, as well as ACE-inhibiting di- and tripeptides, it was thought that a series of ‘fused’ (directly joined) DMLs could be synthesised and evaluated for their antihypertensive activity. Herein, the synthesis of a series of completely nature-inspired, potentially antihypertensive DMLs incorporating 3’,4’-dihydroxyflavonol 53 and a series of di- and tripeptides is described. Once synthesised, biochemical evaluation of the DML compounds and the individual ligands is to be performed to identify any antihypertensive or synergistic effects.

6.2.1 Ligand 1: 3’,4’-Dihydroxyflavonol 53

As explained in Chapter 5.3.1, 3’,4’-dihydroxyflavonol 53 is a vasorelaxant and antioxidant flavonoid (Figure 6.1).

Research in Chapter Five, using functionalised forms of 3’,4’-dihydroxyflavonol were unsuccessful in attempts to synthesise a series of ‘conjugate’ DMLs. Following this failure, attention turned to the use of this flavonol in a ‘fused’ sense, in that the flavonol could be attached directly (without a linker group) to the other DML ligand. An ideal position from which to attach the second ligand was the 3-hydroxyl position of flavonol 53. The attachment of other functionalities to this 3-hydroxyl position had been completed previously by Woodman and co-workers to form hemiadipate 270 and

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Figure 6.1. 3’,4’-Dihydroxyflavonol 53 is a vasorelaxant, antioxidant flavonoid.
phosphate 271 prodrugs (Figure 6.2).\textsuperscript{61} This also provides an unfunctionalised Ring A, a feature associated with increased vasorelaxant activity of the flavonol.\textsuperscript{286}

![Figure 6.2. Woodman and co-workers have added functionalities (blue) to the 3-hydroxyl position of 3’,4’-dihydroxyflavonol 53, to increase its solubility and therefore bioavailability.](image)

Amino acids have also been previously attached to the 3-hydroxyl position of flavonols.\textsuperscript{625} Through carbodiimide-mediated ester formation, Boc- (tert-butoxycarbonyl) protected amino acids were coupled through their carboxylic acid to the 3-hydroxyl position of quercetin-based flavonol 272 (Scheme 6.1). This methodology was deemed suitable for the attachment of the peptidic ligands of these ‘fused’ DMLs to the 3-hydroxyl position of 3’,4’-dihydroxyflavonol.

![Scheme 6.1. The 3-hydroxyl position of quercetin-based flavonol 272 has been used to attach amino acids such as 273, using a coupling reaction.](image)

While in this section, the final compounds are discussed as DMLs, the compounds are subject to cleavage by the esterases of the body. An example of a DML which incorporates an ester linkage to join its ligands is the previously mentioned (NSAID) DML 209, which links the NSAID ibuprofen through an ester linker group to an NO-donor (Figure 6.3).\textsuperscript{557} The ester is cleaved by plasma esterases to provide two bioactives, which act separately.\textsuperscript{548} This is a prodrug-like approach, in that the two joined ligands provide a benefit such as increased bioavailability or decreased gastrotoxicity in the case of DML 209, and are cleaved \textit{in vivo} to provide the bioactive ligands.
In a similar fashion, if 3′,4′-dihydroxyflavonol was to be joined to the second peptidic ligand through an ester linkage, improved bioavailability of the \textit{joined} DML may result. Traditionally, peptide-based drugs suffer from a lack of bioavailability (due to hydrolysis during gastrointestinal absorption and poor intestinal permeability),\textsuperscript{276} and the flavonoids are also poorly bioavailable (due to solubility issues).\textsuperscript{55-57} Following cleavage of the \textit{joined} DML by plasma esterases, the two bioactive ligands can be provided to the body.

\subsection*{6.2.2 Ligand 2: Peptidic ACE Inhibitors}

For the second ligand of these DMLs, it was thought that peptidic, rather than peptidomimetic, ACE inhibitors would be ideal. This provided a wholly ‘nature-inspired’ approach, whereby both DML ligands could be sourced from nature. The use of peptidic ligands was also beneficial as such compounds are commercially available, rather than the peptidomimetic ligand 265 synthesised in Chapter Five.

A number of potentially antihypertensive dipeptides were chosen, taking inspiration from a number of ACE-inhibiting peptides from milk\textsuperscript{270-272} and other natural sources.\textsuperscript{261, 263} Many peptidomimetic (such as enalapril 43)\textsuperscript{240} and peptidic (such as Val-Pro-Pro 50, a tripeptide from fermented milk)\textsuperscript{273-275} ACE inhibitors terminate in a proline unit (Figure 6.4), as it is this proline unit that binds the S2′ pocket of ACE.\textsuperscript{232, 240, 275} For this reason, proline 261 was chosen as the C-terminal amino acid for the dipeptides. It is also noted that dipeptides with proline at the C-terminal are more stable to peptide hydrolysis,\textsuperscript{232, 276} which would help to avoid any problems in terms of lack of bioavailability during absorption and digestion.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6_4}
\caption{Enalapril 43 and Val-Pro-Pro 50 are two ACE inhibitors, and contain a C-terminal proline unit (blue).}
\end{figure}
For the synthesis of the dipeptides, the N-terminal amino acids to attach to the proline unit were selected from the literature. Alanine was chosen due to its position next to proline in enalapril \(^{43,619}\) and lysine was selected due to its position next to proline in the pharmaceutical ACE inhibitor lisinopril \(^ {45}\) (Figure 6.5).\(^ {240,263}\) Finally, glutamine and glutamic acid were also selected for the dipeptide, as they provided different functionalities on their side chains (an amide and a carboxylic acid respectively).

![Figure 6.5. Alanine (blue) and lysine (red) were chosen as N-terminal amino acids in the dipeptides due to their presence in enalapril 43 and lisinopril 45 respectively.](image)

Tripeptides in the form of isoleucine-proline-proline (Ile-Pro-Pro) \(^ {49}\) and valine-proline-proline (Val-Pro-Pro) \(^ {50}\) were also selected for use as ligands in these DMLs. These tripeptides have been shown as ACE inhibitors in animal \(^ {270-272}\) and human trials (Figure 1.28).\(^ {273,274}\) Indeed, a product containing both Ile-Pro-Pro \(^ {49}\) and Val-Pro-Pro \(^ {50}\) has been commercialised.\(^ {275}\) These two tripeptides were purchased with N-carboxybenzyl- (Cbz-) protection, in order to be coupled through the C-terminal proline unit (Figure 6.6).

![Figure 6.6. The Cbz-protected ACE-inhibiting tripeptides Ile-Pro-Pro 275 and Val-Pro-Pro 276 were used in the synthesis of ‘fused’ DMLs.](image)

### 6.3 Retrosynthesis

In order to synthesise these 3’,4’-dihydroxyflavonol DMLs, a retrosynthesis was first performed (Scheme 6.2). The desired DML compounds \(^ {277}\) could be obtained by deprotection of the peptide chain and 3’,4’-dihydroxyl positions. The coupling of the 3-hydroxyl position of flavonol \(^ {281}\) and the C-terminal carboxylic acid of dipeptides \(^ {279}\) could be completed using the aforementioned ester formation.\(^ {625}\)
To synthesise the dipeptides 279, protection and deprotection steps were required, including benzyl protection and deprotection of the proline unit 261. An EDCI-mediated coupling reaction could join the two amino acids 280 and 261.

The synthesis of flavonol 281 could be achieved using 2′-hydroxyacetophenone 72 and 3,4-dibenzylxybenzaldehyde 227, using an Algar-Flynn-Oyamada (AFO) reaction and Claisen-Schmidt condensation.

Scheme 6.2. Retrosynthesis of ‘fused’ DMLs incorporating 3′,4′-dihydroxyflavonol 281 and dipeptides 279.

With this retrosynthesis in mind, attention turned to the synthesis of 3′,4′-dihydroxyflavonol 281.

6.4 Synthesis of 3′,4′-Dihydroxyflavonol-Dipeptide DMLs

As the synthesis of the flavonol required the protection of the 3,4-dihydroxyl groups to prevent any undesired couplings, the first step of the synthesis was the benzyl protection of 3,4-dihydroxybenzaldehyde. This was previously completed in 74% yield (Scheme 6.3). 291

Scheme 6.3. Benzyl protection of 3,4-dihydroxybenzaldehyde 223.
With 3,4-dibenzylxyobenzaldehyde 227 in hand, attention turned to the use of this aldehyde with 2’-hydroxyacetophenone 72 to access the desired 2’-hydroxychalcone 282, using a Claisen-Schmidt condensation.

6.4.1 Claisen-Schmidt Condensation

As with previous chapters, the conversion ratio for this condensation was determined by comparing the integrations of relevant peaks in the ¹H NMR spectrum of the crude reaction product.

To synthesise the desired 3,4-dibenzylxyo-2’-hydroxychalcone 282, the optimised microwave-assisted Claisen-Schmidt Condensation conditions from Chapter Two (KOH, THF, MW, 50 °C, 15 minutes) were applied to the substrates 2’-hydroxyacetophenone 72 and 3,4-dibenzylxyobenzaldehyde 227 (Table 6.1, entry 1). However, analysis of the crude material by ¹H NMR spectroscopy revealed only starting materials.

![Table 6.1 Claisen-Schmidt condensation of 2’-hydroxyacetophenone 72 and 3,4-dibenzylxyobenzaldehyde 227.](image)

The reaction was then repeated using a higher temperature and longer reaction time (70 °C and 30 minutes, Table 6.1, entry 2), which provided 2’-hydroxyacetophenone 72 and chalcone 282 in a ratio of 2:1. Unfortunately, when purification was attempted using column chromatography (with a number of solvent combinations including 2:1 Pet:EtOAc, 5% MeOH in CHCl₃ and 40% CH₂Cl₂ in Pet), the acetophenone and chalcone would co-elute, making isolation of the desired chalcone 282 impossible.

In an attempt to effect full conversion to chalcone 282 and thereby negate the need for purification, conventional methodology was also trialled. Stirring acetophenone 72 and benzaldehyde 227 with KOH in EtOH for 16 hours (Table 6.1, entry 3) produced acetophenone 72 and chalcone 282 in a ratio of 1:2 respectively. As purification by column chromatography had proven unsuccessful in the previous
attempt, the crude material was purified by recrystallisation (1:1 THF:Pet), resulting in a product with a ratio of 1:30 acetophenone 72 to chalcone 282 respectively (Figure 6.7).

![Figure 6.7. 1H NMR spectrum of the impure chalcone 282 following recrystallisation, in a 30:1 ratio of chalcone 280 to acetophenone 72 respectively (CDCl3).](image)

The reaction was also repeated using THF as the solvent (Table 6.1, entry 4); a conversion ratio of 4:1 acetophenone 72 to chalcone 282 was noted. As such, the slightly impure chalcone 282 from Table 6.1, entry 3 was used in the Algar-Flynn-Oyamada reaction, as it was envisioned that purification following flavonol synthesis would remove the unwanted acetophenone.

As the purest sample of chalcone 282 was provided by the conventional methodology (the product of the reaction in Table 6.1, entry 3), this was used in the next synthetic step, the synthesis of the corresponding flavonol 281. This process of purification after a second reaction was successfully used previously in Chapter Five.

### 6.4.2 Algar-Flynn-Oyamada Reaction

The Algar-Flynn-Oyamada (AFO) methodology of Woodman and co-workers was used to synthesise flavonol 279, which involved the addition of 30% hydrogen peroxide (H₂O₂) in H₂O to the impure sample of chalcone 282 (1:30 acetophenone 72 to chalcone 282), with 2M NaOH in EtOH and 1,4-dioxane at 0 °C for two hours,
followed by stirring at room temperature for 16 hours (Scheme 6.4). Recrystallisation from EtOAc isolated the desired flavonol 281 in an extremely poor yield of 9%, as confirmed by comparison to literature data. Analysis of the mother liquor revealed only the presence of chalcone 282 and its corresponding acetophenone.

As flavonol 281 was afforded in such a small yield (8% over two steps), attention turned to the use of the ‘Modified’ Algar-Flynn-Oyamada reaction.

### 6.4.3 ‘Modified’ Algar-Flynn-Oyamada Reaction

In the ‘Modified’ methodology, the starting materials are reacted under Claisen-Schmidt Condensation conditions to form the 2′-hydroxychalcone, which is then directly treated with AFO conditions (without isolation of the chalcone) to afford the flavonol. While the ‘Modified’ AFO reaction had proven unsuccessful in our hands in the synthesis of flavonol acetamides and acrylates in Chapter Five, it had been successfully utilised by Woodman and co-workers in the synthesis of flavonols similar to 281, and so their methodology was utilised.

The starting materials 2′-hydroxyacetophenone 72 and 3,4-dibenzylxoybenzaldehyde 227 were reacted with 40% KOH in EtOH and dioxane for five days according to the methodology of Woodman, followed by treatment with 30% H₂O₂ in H₂O and 2M NaOH in EtOH and dioxane for 2 hours at 0 °C and then 16 hours at room temperature (Scheme 6.5). Following recrystallisation from EtOAc, the desired flavonol 281 was isolated in 53%, as confirmed by comparison to literature data. The ¹H NMR spectrum of
flavonol 281 shows the loss of the acetophenone CH$_3$ peak (δ 2.57 ppm), chalcone hydroxyl peak (δ 12.86 ppm), and benzaldehyde peak (δ 9.80 ppm) (Figure 6.8). Note that the broadness of the $^1$H NMR peaks is due to the π-π stacking configuration of the flavonol.$^{58}$ This ‘Modified’ AFO method provided the desired flavonol in a greater yield (53%) than the two-step Claisen-Schmidt condensation and AFO reaction (8%).

With 3’,4’-dibenzyloxyflavonol 281 in hand, attention turned to the synthesis of the dipeptides. As these dipeptides were to be linked to the flavonol through its 3-hydroxyl position, a late stage deprotection of the 3’,4’-dibenzyloxy ethers was required, and therefore flavonol 281 could be used in its protected form in the coupling reactions.

### 6.4.4 Synthesis of Dipeptides

As explained in Chapter 6.2.2, all dipeptides selected for use in these DMLs contained a C-terminal proline unit. For the second amino acid, alanine and lysine were chosen from the literature based on their appearance in other ACE inhibitors (enalapril $^{43}$ and lisinopril $^{45}$ respectively),$^{240, 263, 619}$ and glutamine and glutamic acid were also selected as they provided different functionalities on their side chains (an amide and a carboxylic acid respectively). Fmoc- (9-fluorenylmethyloxy-) protected amino acids were utilised in this synthesis as they prevented any undesired side couplings.
The amino acids used also had side chain protecting groups where required.

The protection of the C-terminal proline unit 261 was the first step of the dipeptide synthesis. Benzyl protection was ideal, as it could be removed under hydrogenation conditions, which were orthogonal to those of the Fmoc- and side group protections of the amino acids.393

6.4.4.1 Protection of L-Proline 261

This was completed previously in 95% yield (Scheme 6.6).620 The next step of the dipeptide synthesis was coupling to the second amino acid.

6.4.4.2 Coupling of Pro-OBn 259 to Second Amino Acid

The general method for the coupling of Pro-OBn 259 to the N-terminal amino acid of the dipeptide involved stirring the N-terminal amino acid in EDCI, HOBt and CH₂Cl₂ for 10 minutes, followed by the addition of Pro-OBn 259 (Scheme 6.7). The reaction was stirred for 16 hours at room temperature, and following work up and purification by column chromatography (3% MeOH in CHCl₃), each of the desired dipeptides were isolated in good yields (46-63%).
Scheme 6.7. Four dipeptides were synthesised using an EDCI-mediated coupling reaction.

With Fmoc-dipeptides 287-290 in hand, the next step was the deprotection of the benzyl group of the proline unit, to provide the C-terminal carboxylic acid for coupling to the 3-hydroxyl position of 3’,4’-dibenzyloxyflavonol 281.

### 6.4.4.3 Deprotection of the Benzyl Group of Fmoc-Dipeptides 287-290

Hydrogenation was used to deprotect the benzyl group Fmoc-dipeptides 287-290. The dipeptides were each stirred with 10% palladium on carbon (Pd/C) in MeOH and H₂ gas for 16 hours at room temperature (Scheme 6.8). Purification by column chromatography (3% MeOH in CHCl₃) isolated the desired deprotected dipeptides 291-294 in good to excellent yields (50-92%). The deprotected dipeptides were confirmed by the loss of the benzyl CH₂ peaks (approximately δ 5.20 ppm) and aromatic peaks (with an integration of five) in the ¹H NMR spectrum, and also confirmed by HRMS.

Scheme 6.8. Benzyl deprotection provided the four deprotected dipeptides 291-294 in good yields.

With these four dipeptides in hand, each with a carboxylic acid for coupling, the next step of the synthesis was the coupling of the dipeptides to the 3-hydroxyl position of 3’,4’-dibenzyloxyflavonol 281.
6.5 Coupling of 3’,4’-Dihydroxyflavonol 281 to Dipeptides 291-294

6.5.1 EDCI-Mediated Coupling of 3’,4’-Dihydroxyflavonol 281 to Dipeptides 291-294

As mentioned earlier, the coupling of similar substrates had been completed previously by Huang and co-workers, using DCC, DMAP and THF. With EDCI on hand in our laboratory, this was used in place of DCC, and CH2Cl2 in place of THF.

A trial reaction was conducted using 3’,4’-dibenzyloxyflavonol 281 and N-acetylglycine 295, to ensure that these conditions and reagents were suitable (Scheme 6.9). Following reaction for 16 hours, the compound 296 was purified by recrystallisation (EtOH) in 72% yield. With this success, attention turned to the coupling of the four synthesised dipeptides 291-294 to the 3-hydroxyl position of 3’,4’-dibenzyloxy flavonol 281.

![Scheme 6.9. The trial coupling of N-acetylglycine 295 to flavonol 281 succeeded in 72% yield.](image)

Dipeptides 291-294 were then coupled to 3’,4’-dibenzyloxyflavonol 281 (Scheme 6.10), and following workup and purification by column chromatography (2:1 Pet:EtOAc), were analysed by ¹H NMR and ¹³C NMR spectroscopy and HRMS. Three of the four DMLs, the alanine, lysine and glutamine derivatives, were successfully synthesised (protected DMLs 297, 298 and 299) in good yields (45%, 70% and 48% respectively).
However, it was noted during the addition of the glutamic acid dipeptide to the reaction mixture that it was not soluble. Following the reaction, only starting materials were noted in the $^1$H NMR spectrum. The reaction was then repeated with a small amount of DMF to aid solubility, however this also did not produce the desired flavonol-dipeptide 300. With the other three dipeptides successfully synthesised, the use of this glutamic acid dipeptide was abandoned. Attention then turned to the removal of the benzyl groups of the flavonol, to provide the 3,4-dihydroxyl groups of Ring B.

### 6.5.2 Removal of the Flavonol Benzyl Groups

Next, the deprotection of the flavonol benzyl groups of protected DMLs 297-299 was performed. Using a hydrogenation method from Huang *et. al.*, the DMLs 297, 298 and 299 were each stirred in 1,4-dioxane and EtOH with H$_2$ and Pd/C for 16 hours at room temperature (Scheme 6.11). Following filtration, the desired deprotected DMLs 301, 302 and 303 were isolated in quantitative yield, as established by the loss of the benzyl and aromatic peaks ($\delta$ 5.0-5.2 ppm and $\delta$ 7.0-8.0 ppm respectively) in the $^1$H NMR spectra, the observance of signals representing the desired compounds including the Fmoc group ($\delta$ 4.2-5.0 ppm and $\delta$ 7.0-8.0 ppm for the CH$_2$ and CH, and aromatic protons respectively), as well as confirmation by HRMS.
6.5.3 Removal of the Fmoc-Group of the Peptides

The final step for the synthesis of the flavonol-dipeptide DMLs was the deprotection of the Fmoc-group of the peptide. To trial the deprotection, a small scale reaction of Fmoc-Ala-Pro-flavonol 301 and 20% piperidine in DMF was stirred for 20 minutes at room temperature (Scheme 6.12). However, analysis of the crude material by \(^1\)H NMR spectroscopy revealed none of the desired deprotected product 304, nor any of the starting material. Analysis by MS also revealed neither the Fmoc-starting material nor the deprotected product.

It was possible that the use of the base piperidine caused the degradation of the starting material, and so a weaker piperidine solution was then trialled (5% piperidine in DMF) for 20 minutes. Once again, analysis of the crude product by \(^1\)H NMR spectroscopy and MS revealed neither the starting material 301, nor the desired deprotected amine product 304. As this degradation was believed to be the result of
using a base, and all other Fmoc-deprotection conditions utilised bases, (piperazine, morpholine, dicyclohexylamine, and tetrabutylammonium fluoride (TBAF),\textsuperscript{627} the deprotection of the Fmoc-DMLs was not attempted using these conditions. Huang and co-workers had also noted difficulties in deprotecting amines of similar flavonol compounds,\textsuperscript{625} albeit using acidic conditions to remove a Boc-protected amine and hydrogenation to remove benzyl groups.

It was also thought that the acidic nature of the phenols may be interfering with the use of base in the deprotection. To test this theory, the benzyl-protected flavonol 281 was coupled to Fmoc-Ala-OH 283 using the coupling conditions identified earlier (EDCI and DMAP in CH$_2$Cl$_2$ for 16 hours at room temperature, Scheme 6.13). This product 305 was then treated with 20% piperidine in DMF, and the crude product analysed by $^1$H NMR spectroscopy and HRMS, revealing once again the degradation of the flavonol-amino acid 305. This was unfortunate, as it meant that the Fmoc-group could not be removed.

![Scheme 6.13. Fmoc-deprotection of compound 305 degraded the starting material once again, proving that the use of piperidine was detrimental to the flavonol-dipeptide DMLs.](image)

The presence of the Fmoc-group during biochemical evaluation might prove problematic due to its hydrophobic nature, however compounds 301-303 have nevertheless been submitted for biochemical evaluation. Another strategy, involving the use of the milk-derived ACE-inhibiting tripeptides Ile-Pro-Pro 49 and Val-Pro-Pro 50 was then pursued.
6.6 Coupling of 3',4'-Dihydroxyflavonol 281 to Tripeptides 275 and 276

Two tripeptides were purchased for coupling to the 3-hydroxyl position of 3',4'-dibenzyloxyflavonol. These were carboxybenzyl (Cbz-) protected Ile-Pro-Pro (Cbz-Ile-Pro-Pro-OH) 275, and Val-Pro-Pro (Cbz-Val-Pro-Pro-OH) 276 (Figure 6.10). As mentioned earlier, these tripeptides exhibit ACE-inhibiting activity, and were Cbz-protected at the N-terminal amine; benzyl and Cbz- groups can both be deprotected under hydrogenation conditions. This provided a free C-terminal carboxylic acid for coupling to the 3-hydroxyl position of 3',4'-dibenzyloxyflavonol 281.

![Figure 6.10. Cbz-Ile-Pro-Pro-OH 275 and Cbz-Val-Pro-Pro-OH 276, with slightly different side chains (shown in blue) were purchased for coupling to 3',4'-dihydroxyflavonol 281.](image)

6.6.1 EDCI-Mediated Coupling of 3',4'-Dihydroxyflavonol 27981 to Tripeptides 275 and 276

The tripeptides Cbz-Ile-Pro-Pro-OH 275 and Cbz-Val-Pro-Pro-OH 276 were coupled to 3',4'-dihydroxyflavonol 281 using EDCI and DMAP in CH2Cl2 for 16 hours at room temperature (Scheme 6.14). Purification by column chromatography (5% MeOH in CH2Cl2) provided flavonol-Pro-Pro-Ile-Cbz 307 in 97% yield, and flavonol-Pro-Pro-Val-Cbz 308 in 81% yield.

![Scheme 6.14. Coupling of Cbz-Ile-Pro-Pro-OH 275 and Cbz-Val-Pro-Pro-OH 276 to 3',4'-dihydroxyflavonol 281 occurred in good yields.](image)

The structure of these novel compounds was confirmed by ¹H and ¹³C NMR spectroscopy, as well as HRMS. In the ¹H NMR spectrum, the compounds (Figure 6.11...
shows that of flavonol-Pro-Pro-Ile-Cbz 307) were confirmed by the presence of the benzyl (δ 5.24 and 5.34 ppm) and Cbz- (δ 5.08 ppm) groups in the correct integrations (four and two respectively), and the presence of appropriate aromatic peaks (δ 7.03-8.22 ppm) with an integration of 22 for the aromatic protons.

Figure 6.11. 1H NMR spectrum of the Cbz-Ile-Pro-Pro-flavonol compound 307, highlighting the benzyl, Cbz- and aromatic peaks (CDCl3).

6.6.2 Global Deprotection of the Benzyl and Cbz- Groups

Following these successful couplings, the final step for these flavonol-tripeptide DMLs was the global deprotection of benzyl and Cbz- groups. This was performed using hydrogenation conditions (H2 and Pd/C in 1,4-dioxane and EtOH for 16 hours at rt, Scheme 6.15), and the desired 3’,4’-dihydroxyflavonol-tripeptide DMLs 309 and 310 were produced in quantitative yields.
Scheme 6.15. Deprotection of the Cbz- and Bn groups using hydrogenation conditions provided the desired flavonol-tripeptide DMLs 309 and 310.

These compounds were identified in the $^1$H NMR spectrum by the complete loss of the benzyl ($\delta$ 5.24 and 5.34 ppm) and Cbz- ($\delta$ 5.08 ppm) groups, however the extremely broad peaks of the spectrum were difficult to interpret. Further confirmation was provided by $^{13}$C NMR spectroscopy, as well as HRMS. These two 3',4'-dihydroxyflavonol-tripeptide DMLs 307 and 308 have been submitted for evaluation of their antihypertensive activity.

6.7 Antihypertensive Analysis

To analyse the antihypertensive activity of the synthesised DMLs 301, 302, 303, 309 and 310, a number of biochemical assays are to be performed by Dr Kylie Venardos. Once again it should be noted that these analyses are to be performed on not only the DML compounds, but also the individual ligands, so as to identify the modes of action of the individual ligands, as well as any synergistic effects.

The biochemical assays are to be performed on isolated mouse heart cells (cardiomyocytes) which have been stressed using various neurohormones, including endothelin, angiotensin II, aldosterone and noradrenaline. These particular neurohormones are elevated and circulate during hypertension in humans, and contribute to cardiac complications such as hypertrophy (where the pathological growth of cells causes an increase in organ size and a decrease in contractile function/cardiac output). Once the cells have been stressed, they are to be treated with and without the DML compounds, and then utilised in the biochemical assays listed below. As well as the measurements involved in the following assays, cell size will also be measured.
using light microscopy. Ribonucleic acid (RNA) will also be collected for real-time polymerase chain reaction (PCR) of messenger RNA (mRNA) markers of hypertrophy, such as β-myosin heavy chain (β-MHC), brain natriuretic peptide (BNP) and atrial natriuretic factor (ANF). The measurement of cell size and presence of mRNA markers will help to determine if the DML compounds have direct antihypertrophic effects on heart cells.

The compounds will be examined for their ability to stimulate nitric oxide (NO) production in the stressed cardiomyocytes. NO plays a pivotal part in the modulation of vascular tone and vascular smooth muscle proliferation, and its production is reduced in hypertensive patients. When oxidised by NO, [DAF-FM] forms 2′,7′-dichlorofluorescein, a fluorescent compound which can be measured on a fluorescence microplate reader at 495 nm excitation and 538 nm emission. The measurement of fluorescence allows the determination of the amount of NO produced by the cells following treatment with the DML and ligand compounds. Endothelial cells will also be treated with the DML compounds to see if they directly cause NO production by the endothelium. This will be done under normal conditions as well as following neurohormone stress and oxidative stress (with H₂O₂ and pyrogallol). All of these stress conditions are associated with hypertension and reduced endothelial bioavailability.

The DMLs and ligands will also be tested for their ability to relieve oxidative stress (which is increased in hypertension) in cardiomyocytes, through the measurement of Reactive Oxygen Species (ROS). The cells will be stressed with the neurohormones or treated with the ROS generator, pyrogallol, and then treated with and without the DML and ligand compounds. The cells are then loaded with the fluorescent probe 2′,7′-dichlorofluorescein diacetate. The ROS generated by the cells oxidises the 2′,7′-dichlorofluorescein diacetate to form the fluorescent compound 2′,7′-dichlorofluorescein, which can be measured a fluorescence microplate reader at 495 nm excitation and 538 nm emission. H₂O₂ treated cells will also be used as a positive control in the assay. The measurement of ROS in these cells will help to determine if oxidative stress is reduced in the presence of these compounds.
The compounds will also be analysed for their ability to reduce cellular necrosis in isolated mouse cardiomyocytes, through the measurement of lactate dehydrogenase (LDH). LDH is released by cells as their membranes are disrupted during cellular necrosis, thus the measurement of its activity reflects the amount of cell death. To measure LDH activity, culture media is collected from the heart cells (following neurohormone stress with and without the DML compounds) and added to a mixture of NADH, NaH₂PO₄, and sodium pyruvate. The LDH released from the necrotic cell into the media reduces the pyruvate to lactate using NADH, which is in turn oxidised to NAD⁺ and no longer produces light. Spectrophotometric measurement of the rate of decrease in absorbance at 340 nm over two minutes allows for the determination of the percentage of cellular necrosis caused by the compounds.

In conclusion, the measurements of cell size, and the analysis of mRNA markers will help to determine if the compounds have a direct antihypertrophic effects on heart cells. The ROS assay will help to identify the effect of the DML compounds on removing oxidative stress (which is increased during hypertension), and the NO assay will determine if the DML compounds have an effect on NO production (which is decreased during hypertension). Finally, if there are changes in the rate of cell death following treatment with the DML compounds (as compared to without treatment), then these other assays will allow for the determination of how the DML compounds are acting within the cells, and also whether or not they are cardioprotective and/or antihypertensive.

6.8 ‘Fused’ 3’,4’-Dihydroxyflavonol-Peptidic DMLs: Conclusion

The synthesis of several 3’,4’-dihydroxyflavonol-peptidic DMLs was successfully completed. Three dipeptide (301, 302 and 303) and two tripeptide (309 and 310) ‘fused’ DMLs were accessed.

The 3’,4’-dihydroxyflavonol ligand 281 of the DMLs was synthesised using a ‘Modified’ AFO reaction in good yield (53%, Scheme 6.16).
Four dipeptides were also synthesised, using an EDCI-mediated coupling reaction as well as protections and deprotection steps; Fmoc-Ala-Pro-OH 291, Fmoc-Lys(Boc)-Pro-OH 292, Fmoc-Gln-Pro-OH 293, and Fmoc-Glu(tBu)-Pro-OH 294 (Scheme 6.17).

The first three of these dipeptides were then successfully coupled to the 3',4'-dihydroxyflavonol ligand 281, and then hydrogenated to remove the benzyl groups of the flavonol (Figure 6.12). Unfortunately, the Fmoc group could not be deprotected. These compounds were submitted for biochemical evaluation of their antihypertensive properties, and results are still pending at the time of submission of this thesis.
Two known ACE-inhibiting tripeptides were also purchased for coupling to 3',4'-dihydroxyflavonol 281; Cbz-Ile-Pro-Pro-OH 275 and Cbz-Val-Pro-Pro-OH 276. These were used to access the two flavonol-tripeptide DMLs 309 and 310, in total yields of 97% and 81% respectively over two steps (Figure 6.13). These compounds were also submitted for biochemical evaluation of their antihypertensive properties, and results are still pending at the time of submission of this thesis.

Figure 6.13. Two flavonol-tripeptide DMLs 309 and 310 were also successfully synthesised, and submitted for evaluation of their antihypertensive activity.

A total of five ‘fused’ (whereby the ligands are joined directly) DMLs were synthesised, which incorporated both the vasorelaxant and antioxidant activity of 3',4'-dihydroxyflavonol and the ACE-inhibiting activity of various peptides.
Chapter Seven:
Conclusions and Future Work
7.1 Thesis Conclusions

In conclusion, this thesis details the synthesis of a number of novel flavonoids. Both halogenated and peptide-functionalised flavonoids were synthesised, using a number of novel and established methodologies. A number of these compounds have submitted for evaluation of their bioactivity and therapeutic effects, including cytotoxic, antibacterial, anticancer, antidiabetic and antihypertensive properties.

The incorporation of halogens onto the flavonoid scaffold was of importance, as halogen bonding has been shown in medicinal and biomedical chemistry to strengthen drug-target interactions. It will be interesting to see how the halogenated flavonoids perform in assays compared to the non-halogenated analogues.

Attempts were also made to further functionalise the halogenated flavonoids using the Heck reaction, and while brominated benzaldehydes and acetophenones were successfully utilised in the Heck reaction, chalcone, flavanone and flavone substrates could not be used to access acrylate products.

Finally, using both the methods developed and the compounds synthesised throughout Chapters Two to Four, the synthesis of a number of antihypertensive Designed Multiple Ligands was attempted. The synthesis of ‘conjugate’ DMLs incorporating the vasorelaxant compound 3’,4’-dihydroxyflavonol and peptidomimetic ACE-inhibiting enalapril-derived compounds attached through a linker group was not successful. Of four different functionalised 3’,4’-dihydroxyflavonols, only one was accessed (albeit slightly impure), and of two enalapril derivatives, only one was accessed. However, the flavonol and enalapril derivative could not be coupled.

A series of ‘fused’ DMLs were then synthesised, joining 3’,4’-dihydroxyflavonol and potentially ACE-inhibiting di- and tripeptides through an ester linkage. These DMLs may be subject to cleavage by plasma esterases in the body, in which case the two ligands will be provided to the body and act separately (in a prodrug-like approach). Five DML compounds were successfully synthesised, and have been submitted for biochemical evaluation of their antihypertensive activity.

7.2 Future Work

There are a number of opportunities for future work. In terms of the 2’-hydroxychalcones, the use of other functionalised starting materials, particularly more hydroxylated compounds, would provide more functionalised 2’-hydroxychalcones, which could therefore be used to identify further trends within the microwave-assisted
synthetic methodology. Also, once the biochemical evaluation results of 2′-hydroxychalcones 80, 118 and 121 are received, results will provide direction for further development. These evaluations will also help to identify whether or not flavonoids containing halogens offer the benefit of halogen-bonding with molecular targets, in comparison to non-halogenated flavonoids.

There is also the option of applying the pIL-mediated, microwave-assisted flavanone synthetic methodology to a wider variety of chalcone substrates, in particular those incorporating hydroxyl substitutions. This too could provide more information in terms of Structure-Activity Relationships.

A future aim for this research is the application of the Algar-Flynn-Oyamada reaction to 2′-hydroxychalcone substrates to access a library of functionalised flavonol compounds, and for those compounds to be evaluated for therapeutic properties.

Finally, in terms of the Designed Multiple Ligands, a future aim is to obtain other Cbz-protected dipeptides and tripeptides to attach to 3′,4′-dihydroxyflavonol. This protecting group should remove any problems concerning solubility that were associated with the inability to deprotect the Fmoc-group, and provide more bioavailable DML compounds. Results regarding the antihypertensive activity of the five original DML compounds will allow for the development of a second, more active generation of DML compounds for the treatment of hypertension.

There is also the opportunity to attach the peptidomimetic ACE inhibitor enalaprilat 42 to 3′,4′-dihydroxyflavonol 53, so as to examine the benefit of a flavonol-ester prodrug, rather than the ethyl ester utilised in enalapril.

Figure 7.1. Future work includes the synthesis of the ‘flavonol-ester’ prodrug 311.
Chapter Eight:
Characterisation
8.1 General Experimental

8.1.1 Instrumentation

Microwave reactions were conducted using a CM Discover S-Class Explorer 48 Microwave Reactor, operating on a frequency of 50/60 Hz and continuous irradiation power from 0 to 200 W. All reactions were performed in 10mL septa vials with snap caps, with the following conditions: pressure (17 bar); power max (on); and stirring (high).

All \(^1\)H and \(^{13}\)C NMR spectra were recorded on a JEOL JNM-EX 270 MHz FT-NMR spectrometer or JEOL JNM-ECP 400 MHz FT-NMR spectrometer as indicated. Samples were dissolved in deuterated chloroform (CDCl\(_3\)), with the residual solvent peak used as an internal reference (CDCl\(_3\) – \(\delta_H 7.26\) ppm and \(\delta_C 77.10\) ppm). Proton spectra are reported as follows: chemical shift \(\delta\) (ppm), (integral, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet), coupling constant \(J\) (Hz), assignment). Carbon spectra are reported as chemical shift \(\delta\) (ppm).

Mass Spectra (MS), including High Resolution Mass Spectra (HRMS), were recorded on a 6210 MSD TOF mass spectrometer (Agilent Technologies, Australia) with the following conditions: drying gas nitrogen (7.0 L/min, 325 °C); nebuliser gas nitrogen (15 psi); capillary voltage 3.0 kV; vaporiser temperature 29 °C; and cone voltage 40 V. Acetonitrile was used as the mobile phase. Samples were dissolved in acetonitrile, with less than 1 mg sample per mL solvent.

All melting points were obtained using a Bibby Stuart Scientific SMP3 melting point apparatus, version 5.0.

8.1.2 Chromatography

Thin Layer Chromatography (TLC) was performed using aluminium-backed Merck TLC Silica gel 60 F254 plates, and samples were visualised using 254 nm ultraviolet (UV) light, and potassium permanganate/potassium carbonate oxidising dip (1:1:100 KMnO\(_4\):K\(_2\)CO\(_3\):H\(_2\)O w/w).

Column Chromatography was performed using silica gel 60 (70-230 mesh). All solvents used were AR grade. Petroleum spirits refers to the fraction boiling between 40-60 °C.
8.1.3 Dry solvents
THF, CHCl₃ and DMF were dried using a Pure Solv (Innovative Technologies) solvent drying system. Solvents are degassed, and passed through two drying chambers of alumina and collected under a positive pressure of nitrogen gas.

8.1.4 Reagents
All general reagents were analytical grade and used as supplied unless otherwise stated. Purification and/or drying of general reagents was performed according to Perrin et. al. Specialist reagents such as peptide coupling reagents were supplied by Aldrich Chemical Co., A.K Scientific Inc. or Auspep Pty. Ltd. and used without further purification.

8.1.5 Example of Compound Ratio Calculation from ¹H NMR Spectra

In the case of reactions involving chalcones, as reagents or products, the following calculations were conducted. The chalcone peak (approximately δ 12.5-13.5 ppm) was allocated an integration of 0.85. The acetophenone CH₃ peak (approximately δ 2.5-2.7 ppm) was then integrated, and its integration value divided by three (for each of the three protons). For the spectrum below, this calculation provides the ratio of chalcone 66 to acetophenone 72 as 1:0.45, or approximately 2:1. Note that when the
ratio involved comparison to flavanone compounds, that the integration of the methyne (CH) peak (approximately δ 5.3-5.7 ppm) was used, with an integration of one.

Chalcone OH (highlighted in blue) integration = 0.85
Acetophenone CH₃ (highlighted in green) integration = 1.35/CH₃, or 0.45/hydrogen
∴ Normalised ratio of 66:72 = 1:0.45 ≈ 2:1

8.2 Specific Experimental

The following sections detail the specific experimental conditions for each of the compounds successfully synthesised during this research project. The compounds are listed in chronological order according to the chapter in which they first appear.

8.3 Chapter Two Experimental

8.3.1 General Procedure for 2’-Hydroxychalcones

Representative experimental procedure: Substituted 2’-hydroxyacetophenone (0.5 mmol) was added to a solution of base (2.0 mmol) in THF (2.0 mL) in a microwave vial. Substituted benzaldehyde (0.5 mmol) was added, the vial capped, and the solution heated to 50 °C for 15 minutes using microwave irradiation. Solvent was evaporated in vacuo, and 10% HCl solution (20 mL) added. The product was transferred to a separating funnel, extracted with EtOAc (3 × 10 mL), washed with H₂O (10 mL) and brine (10 mL), dried over MgSO₄, filtered, and the solvent removed under reduced pressure to give a resin. Purification of the crude material by column chromatography (1:1 CH₂Cl₂:Pet) gave the desired chalcone

8.3.2 Compound Characterisation Data

1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one 66

2’-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), KOH (112 mg, 2.0 mmol) and benzaldehyde 59 (0.101 mL, 1.0 mmol) were reacted according to the general procedure. The desired compound was obtained as a pale-yellow solid (112 mg, 100%, Rf = 0.61 (1:1 CH₂Cl₂:Pet)); mp 86.0-88.2 °C (lit. 88 °C) ; δH NMR (270 MHz, CDCl₃): 6.90-6.97 (1H, m, CH), 7.03 (1H, dd, J 8.4, J 0.9, CH), 7.41-7.52 (4H, m, ArH), 7.62-7.67 (3H, m, ArH), 7.88-7.94 (2H, m, ArH), 12.84 (1H, s, OH); MS (ESI m/z): Calcd. for [C₁₅H₁₂O₂+H]⁺ 225.09, found 225.09.
3-(4-bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 74\textsuperscript{392, 407}

2′-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-bromobenzaldehyde 73 (139 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright-yellow solid (145 mg, 96%, \(R_f = 0.61\) (1:1 CH\(_2\)Cl\(_2\):Pet)); mp 146.4-149.1 °C (lit. 138-140 °C)\textsuperscript{392}; \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 6.89-6.96 (1H, m, CH), 7.01 (1H, dd, \(J = 8.4, J = 0.9\), CH), 7.46-7.90 (8H, m, ArH), 12.74 (1H, s, OH); MS (ESI m/z): Calcd. for [C\(_{15}\)H\(_{11}\)BrO\(_2\)-H]\(^-\) 300.99, found 300.99.

3-(3,5-dibromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 76

2′-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), Bu\(_4\)NOH (1.0 M in MeOH, 2.0 mL, 2.0 mmol) and 3,5-dibromobenzaldehyde 75 (198 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright-yellow solid (99 mg, 52%, \(R_f = 0.5\) (1:1 CH\(_2\)Cl\(_2\):Pet)); mp 168.7-174.8 °C; \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 6.93-6.99 (1H, m, CH), 7.03 (1H, dd, \(J = 8.4, J = 0.9\), CH), 7.49-7.55 (1H, m, ArH), 7.58-7.76 (5H, m, ArH), 7.90 (1H, dd, \(J = 7.9, J = 1.4\), ArH), 12.60 (1H, s, OH); \(\delta_C\) NMR (67.5 MHz, CDCl\(_3\)): 118.9, 119.1, 119.8, 122.8, 123.7, 129.8, 130.0, 135.8, 137.0, 138.2, 142.0, 163.8, 193.0; HRMS (ESI m/z): Calcd. for [C\(_{15}\)H\(_{10}\)Br\(_2\)O\(_2\)+Na\(^+\)] 404.89248, found 404.89040.

3-(3-(benzyloxy)phenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 78\textsuperscript{394}

2′-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 3-benzyloxybenzaldehyde 77 (106 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright-yellow solid (146 mg, 78%, \(R_f = 0.57\) (1:1 CH\(_2\)Cl\(_2\):Pet)); mp 122.8-128.3 °C; \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 5.12 (2H, s, CH\(_2\)), 6.91-7.07 (3H, m, ArH), 7.25-7.63 (10H, m, ArH), 7.84-7.92 (2H, m, ArH), 12.80 (1H, s, OH); MS (ESI m/z): Calcd. for [C\(_{22}\)H\(_{18}\)O\(_3\)-H]\(^-\) 329.12, found 329.12.

3-(5-bromo-2,4-dimethoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 80

2′-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 5-bromo-2,4-dimethoxybenzaldehyde 79 (123 mg, 0.5 mmol) were reacted according to the general
procedure. The desired compound was obtained as a yellow solid (163 mg, 90%, \(R_f = 0.35\) (1:1 CH\(_2\)Cl\(_2\):Pet)); mp 186.1-187.7 °C; \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 3.93 (3H, s, CH\(_3\)), 3.94 (3H, s, CH\(_3\)), 6.45 (1H, s, ArH), 6.45 (1H, s, ArH), 6.90-7.01 (2H, m, ArH), 7.43-7.61 (2H, m, ArH), 7.79 (1H, s, ArH), 7.88-7.98 (1H, m, ArH), 8.07-8.13 (1H, m, ArH), 12.96 (1H, s, OH); \(\delta_C\) NMR (67.5 MHz, CDCl\(_3\)): 56.1, 56.5, 96.1, 102.9, 117.8, 118.6, 118.8, 118.9, 120.2, 129.7, 133.0, 136.2, 139.3, 159.0, 159.9, 163.6, 193.9; HRMS (ESI m/z): Calcd. for [C\(_{17}\)H\(_{15}\)BrO\(_4\)+H\(^+\)] 363.02320, found 363.02540.

**3-(4-fluorophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 82**

2'-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-fluorobenzaldehyde 81 (0.053 mL, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright-yellow solid (70 mg, 58%, \(R_f = 0.52\) (1:1 CH\(_2\)Cl\(_2\):Pet)); mp 118.6-120.4 °C (lit. 118-120 °C); \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 6.91-7.16 (4H, m, ArH), 7.46-7.68 (4H, m, ArH), 7.84-7.92 (2H, m, ArH), 12.78 (1H, s, OH); MS (ESI m/z): Calcd. for [C\(_{15}\)H\(_{11}\)FO\(_2\)+H\(^+\)] 243.08, found 243.08.

**1-(2-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one 85**

2'-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-nitrobenzaldehyde 84 (50.4 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as an orange solid (21 mg, 16%, \(R_f = 0.39\) (1:1 CH\(_2\)Cl\(_2\):Pet)); mp 204.8-206.6 °C (lit. 205-206 °C); \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 6.97 (1H, t, \(J = 7.2\), CH), 7.05 (1H, d, \(J = 8.5\), CH), 7.49-7.57 (1H, m, ArH), 7.72-7.94 (5H, m, ArH), 8.20-8.31 (2H, m, ArH), 12.57 (1H, s, OH); MS (ESI m/z): Calcd. for [C\(_{15}\)H\(_{11}\)NO\(_4\)+H\(^+\)] 270.08, found 270.08.

**2-(4-oxochroman-2-yl)benzoic acid 92**

2'-Hydroxyacetophenone 72 (0.06 mL, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 2-carboxybenzaldehyde 90 (75.7 mg, 0.5 mmol) were reacted according to the general procedure. The flavanone compound was obtained as a pale brown solid (61.7 mg, 46%, \(R_f = 0.05\) (1:1 CH\(_2\)Cl\(_2\):Pet)); mp 133.4-147.3 °C; \(\delta_H\) NMR (270 MHz, CDCl\(_3\)): 3.44 (1H, dd, \(J = 17.7\), J 6.6, CH\(_{2a}\)), 3.75 (1H, dd, \(J = 17.7\), J 6.3, CH\(_{2b}\)), 6.14 (1H, t, \(J = 6.4\), CH), 6.85-7.01 (2H, m, ArH), 7.45-7.70 (5H, m, ArH), 7.89-7.92 (1H, m, ArH), 193.9.
11.99 (1H, s, CO₂H); δC NMR (67.5 MHz, CDCl₃): 43.4, 69.7, 118.9, 119.2, 119.4, 122.7, 126.0, 129.7, 129.9, 134.1, 134.5, 137.3, 149.4, 162.7, 170.0, 201.8; HRMS (ESI m/z): Calcd. for [C₁₆H₁₂O₄+H]+ 269.08084, found 269.08212.

2-hydroxy-6-methoxyacetophenone 100

2',6'-Dihydroxyacetophenone 96 (441.2 mg, 2.9 mmol), K₂CO₃ (409.6 mg, 2.9 mmol) and acetone (15 mL) were added to a round bottom flask. Dimethyl sulfide (Me₂SO₄, 0.277 mL, 2.9 mmol) was then added dropwise, and the solution refluxed for one hour. The solution was quenched with H₂O, and extracted with EtOAc ×3. The organic phase was then washed with H₂O and brine, and then dried over MgSO₄. Purification by column chromatography (1:1 CH₂Cl₂:Pet) provided the desired acetophenone as a pale yellow solid (439.7 mg, 91%, Rf = 0.66 (1:1 CH₂Cl₂:Pet)); mp 61.2-62.0 °C (lit. 60 °C); δH NMR (270 MHz, CDCl₃): 2.65 (3H, s, COCH₃), 3.88 (3H, s, OCH₃), 6.73 (1H, d, J 8.1, ArH), 6.55 (1H, d, J 8.4, ArH), 7.33 (1H, t, J 8.4, ArH), 13.31 (1H, s, OH).

1-(2-hydroxy-6-methoxyphenyl)-3-phenylprop-2-en-1-one 101

2'-Hydroxy-6'-methoxyacetophenone 100 (83 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and benzaldehyde 59 (0.101 mL, 1.0 mmol) were reacted according to the general procedure. The desired compound was obtained as a yellow oil (86 mg, 67%, Rf = 0.5 (1:1 CH₂Cl₂:Pet)); δH NMR (270 MHz, CDCl₃): 3.93 (3H, s, CH₃), 6.42 (1H, d, J 8.4, CH₃), 6.61 (1H, dd, J 8.4, J 0.9, ArH), 7.31-7.45 (4H, m, ArH), 7.58-7.63 (2H, m, ArH), 7.76-7.90 (2H, m, ArH), 13.14 (1H, s, OH); δC NMR (67.5 MHz, CDCl₃): 56.1, 101.7, 111.0, 112.1, 127.7, 128.6, 129.0, 130.4, 135.4, 136.1, 143.0, 161.1, 165.0, 194.6; MS (ESI m/z): Calcd. for [C₁₆H₁₄O₃-H]- 253.09, found 253.09.

3-(4-bromophenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one 102

2'-Hydroxy-6'-methoxyacetophenone 100 (83 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-bromobenzaldehyde 73 (93 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as an orange solid (77 mg, 46%, Rf = 0.44 (1:1 CH₂Cl₂:Pet)); mp 119.8-124.0 °C (lit. 125-127 °C); δH NMR (270 MHz, CDCl₃): 3.92 (3H, s, CH₃), 6.41 (1H, d, J 8.4, ArH), 6.60 (1H, dd, J 8.5, J 0.9, ArH), 7.34 (1H, t, J 8.4, ArH), 7.42-7.53 (4H, m, ArH), 7.66-7.85 (2H, m,
ArH), 13.07 (1H, s, OH); MS (ESI m/z): Calcd. for [C_{16}H_{13}BrO_{3}\cdot H]^- 330.99, found 330.99.

3-(3,5-dibromophenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one 103

2'-Hydroxy-6'-methoxyacetophenone 100 (83 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 3,5-dibromobenzaldehyde 75 (132 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a yellow solid (53.1 mg, 26%, R_f = 0.37 (1:1 CH2Cl2:Pet)); mp 159.4-160.6 °C; δ_H NMR (270 MHz, CDCl3): 3.94 (3H, s, CH3), 6.41 (1H, d, J 8.3, ArH), 6.61 (1H, d, J 8.4, ArH), 7.37 (1H, t, J 8.3, ArH), 7.54-7.67 (4H, m, ArH), 7.78 (1H, d, J 15.6, ArH), 12.92 (1H, s, OH); δ_C NMR (67.5 MHz, CDCl3): 56.2, 101.7, 111.1, 111.9, 123.5, 129.9(2×C), 130.3, 135.2, 136.5, 139.1, 139.2, 161.1, 165.0, 193.9; HRMS (ESI m/z): Calcd. for [C_{16}H_{12}Br_{2}O_{3}+H]^+ 410.92260, found 410.92075.

3-(3-(benzyloxy)phenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one 104

2'-Hydroxy-6'-methoxyacetophenone 100 (83 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 3-benzyloxybenzaldehyde 77 (98 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as an orange-yellow oil (142 mg, 76%, R_f = 0.40 (1:1 CH2Cl2:Pet)); δ_H NMR (270 MHz, CDCl3): 3.91 (3H, s, CH3), 5.11 (2H, s, CH2), 6.42 (1H, dd, J 8.4, J 0.9, ArH), 6.61 (1H, dd, J 8.4, J 0.9, ArH), 7.00-7.04 (1H, m, ArH), 7.20-7.47 (9H, m, ArH), 7.72-7.85 (2H, m, ArH), 13.10 (1H, s, OH); δ_C NMR (67.5 MHz, CDCl3): 56.1, 70.2, 101.7, 111.0, 112.1, 114.8, 116.9, 121.5, 127.6, 128.0, 128.2, 128.8, 130.0, 136.1, 136.8, 136.9, 142.8, 159.2, 161.1, 165.0, 194.6; HRMS (ESI m/z): Calcd. for [C_{23}H_{20}O_{4}+H]^+ 359.12833, found 359.13010.

3-(5-bromo-2,4-dimethoxyphenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one 105

2'-Hydroxy-6'-methoxyacetophenone 100 (83 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 5-bromo-2,4-dimethoxybenzaldehyde 79 (123 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as an orange-yellow solid (163 mg, 83%, R_f = 0.39 (1:1 CH2Cl2:Pet)); mp 133.8-139.8 °C; δ_H NMR (270 MHz, CDCl3): 3.89 (3H, s,
1-(5-bromo-2-hydroxyphenyl)-3-phenylprop-2-en-1-one 109

5′-Bromo-2′-hydroxyacetophenone 108 (108 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and benzaldehyde 59 (0.101 mL, 1.0 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright yellow solid (131 mg, 86%, $R_f = 0.59$ (2:3 CH$_2$Cl$_2$:Pet)); mp 109.4-112.3 °C (lit. 109-110 °C) $\delta$H NMR (270 MHz, CDCl$_3$): 6.91 (1H, d, $J = 8.9$, ArH), 7.41-7.46 (3H, m, ArH), 7.50-7.56 (2H, m, ArH), 7.63-7.69 (2H, m, ArH), 7.89-7.98 (2H, m, ArH), 12.75 (1H, s, O$_2$H); MS (ESI m/z): Calcd. for [C$_{15}$H$_{11}$BrO$_2$+H]$^+$ 303.00, found 303.00.

3-(4-bromophenyl)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 110

5′-Bromo-2′-hydroxyacetophenone 108 (108 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-bromobenzaldehyde 73 (93 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright yellow solid (123 mg, 64%, $R_f = 0.36$ (2:3 CH$_2$Cl$_2$:Pet)); mp 178.7-179.4 °C (lit. 180-182 °C) $\delta$H NMR (270 MHz, CDCl$_3$): 6.93 (1H, d, $J = 8.9$, ArH), 7.50-7.60 (6H, m, ArH), 7.83-7.89 (1H, m, ArH), 7.97 (1H, d, $J = 3.3$, ArH), 12.67 (1H, s, O$_2$H); MS (ESI m/z): Calcd. for [C$_{15}$H$_{10}$Br$_2$O$_2$+H]$^+$ 380.91, found 380.91.

3-(3-benzyloxyphenyl)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 111

5′-Bromo-2′-hydroxyacetophenone 108 (108 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 3-benzyloxybenzaldehyde 77 (98 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright yellow solid (165 mg, 80%, $R_f = 0.37$ (2:3 CH$_2$Cl$_2$:Pet)); mp 104.0-106.3 °C; $\delta$H NMR (270 MHz, CDCl$_3$): 5.12 (2H, s, CH$_2$), 6.92 (1H, d, $J = 8.9$, ArH), 7.05-7.08 (1H, m, ArH), 7.24-7.57 (10H, m, ArH), 7.85-7.96 (2H, m, ArH), 12.76 (1H, s, O$_2$H); $\delta$C NMR (67.5 MHz, CDCl$_3$): 70.3, 110.6, 114.8, 117.9, 119.7, 120.8, 121.3, 122.0, 127.6, 128.3, 128.8, 130.3, 131.9, 135.8, 136.7,
3-(5-bromo-2,4-dimethoxyphenyl)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 112

5′-Bromo-2′-hydroxyacetophenone 108 (108 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 5-bromo-2,4-dimethoxybenzaldehyde 79 (123 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright orange solid (145.4 mg, 66%, R_f = 0.17 (3:1 Pet:CH_2Cl_2)); mp 224.4-228.3 °C; δ_H NMR (270 MHz, CDCl_3): 3.95 (3H, s, OCH_3), 3.96 (3H, m, OCH_3), 6.47 (1H, s, ArH), 6.91 (1H, d, J 8.9, ArH), 7.46-7.55 (2H, m, ArH), 7.83 (1H, s, ArH), 7.99 (1H, d, J 2.6, ArH), 8.14 (1H, d, J 15.4, ArH), 12.90 (1H, s, OH); δ_C NMR (67.5 MHz, CDCl_3): 56.1, 56.5, 96.1, 110.4, 114.1, 117.6, 118.1, 120.6, 131.9, 133.2, 138.7, 140.5, 160.1, 162.5, 192.9; HRMS (ESI m/z): Calcd. for [C_{17}H_{14}Br_2O_4+H]^+ 440.93316, found 440.93169.

3-(4-fluoro)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 113^407

5′-Bromo-2′-hydroxyacetophenone 108 (108 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-fluorobenzaldehyde 81 (0.053 mL, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright yellow solid (139.0 mg, 87%, R_f = 0.75 (1:1 Pet:CH_2Cl_2)); mp 178.6-179.9 °C (lit. 162-164 °C)^407; δ_H NMR (270 MHz, CDCl_3): 6.92 (1H, d, J 8.9, ArH), 7.09-7.17 (2H, m, ArH), 7.43-7.70 (4H, m, ArH), 7.86-7.97 (2H, m, ArH), 12.70 (1H, s, OH); MS (ESI m/z): Calcd. for [C_{15}H_{10}BrFO_2+H]^+ 320.99, found 320.99.

3-(4-methoxy)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 114^394, 638

5′-Bromo-2′-hydroxyacetophenone 108 (108 mg, 0.5 mmol), Bu_4NOH (2.0 M in MeOH, 2.0 mmol) and 4-fluorobenzaldehyde 81 (0.053 mL, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright yellow solid (146.0 mg, 88%, R_f = 0.43 (1:1 Pet:CH_2Cl_2)); mp 102.6-105.6 °C (lit. 102-104 °C)^638; δ_H NMR (270 MHz, CDCl_3): 3.84 (3H, s, CH_3), 6.88-6.96 (3H, m, ArH), 7.36-7.63 (4H, m, ArH), 7.85-7.96 (2H, m, 197
Ar\textit{H}), 12.87 (1H, s, OH); MS (ESI \textit{m/z}): Calcd. for [C_{16}H_{13}BrO_{3}+H]^+ 333.01, found 333.01.

1-(3-bromo-5-chloro-2-hydroxyphenyl)-3-phenylprop-2-en-1-one 117

3'-Bromo-5'-chloro-2'-hydroxyacetophenone 116 (125 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and benzaldehyde 59 (0.101 mL, 1.0 mmol) were reacted according to the general procedure. The desired compound was obtained as an orange-yellow solid (146 mg, 86%, \textit{R}_f = 0.74 (1:1 CH\textsubscript{2}Cl\textsubscript{2}:Pet)); \textit{mp} 136.7-140.8 °C (lit. 128-129 °C); \textit{\delta}\textsubscript{H} NMR (270 MHz, CDCl\textsubscript{3}): 7.39-7.52 (4H, m, Ar\textit{H}), 7.61-7.71 (3H, m, Ar\textit{H}), 7.81 (1H, d, \textit{J} 2.6, Ar\textit{H}), 7.92-7.97 (1H, m, Ar\textit{H}), 13.47 (1H, s, OH); MS (ESI \textit{m/z}): Calcd. for [C\textsubscript{15}H\textsubscript{10}BrClO\textsubscript{2}-H] - 334.95, found 334.95.

1-(3-bromo-5-chloro-2-hydroxyphenyl)-3-(4-bromophenyl)prop-2-en-1-one 118

3'-Bromo-5'-chloro-2'-hydroxyacetophenone 116 (125 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-bromobenzaldehyde 73 (139 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a yellow solid (166 mg, 80%, \textit{R}_f = 0.74 (1:1 CH\textsubscript{2}Cl\textsubscript{2}:Pet)); \textit{mp} 202.2-207.1 °C; \textit{\delta}\textsubscript{H} NMR (270 MHz, CDCl\textsubscript{3}): 7.50-7.61 (5H, m, Ar\textit{H}), 7.75-7.93 (3H, m, Ar\textit{H}), 13.40 (1H, s, OH); \textit{\delta}\textsubscript{C} NMR (67.5 MHz, CDCl\textsubscript{3}): 113.3, 119.4, 120.9, 123.9, 126.2, 128.2, 130.3, 132.6, 133.0, 139.0, 146.2, 158.9, 192.3; HRMS (ESI \textit{m/z}): Calcd. for [C\textsubscript{15}H\textsubscript{9}Br\textsubscript{2}ClO\textsubscript{2}-H] - 414.85591, found 414.85560.

3-(3-(benzyloxy)phenyl)-1-(3-bromo-5-chloro-2-hydroxyphenyl)prop-2-en-1-one 119

3'-Bromo-5'-chloro-2'-hydroxyacetophenone 116 (125 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 3-benzyloxybenzaldehyde 77 (106 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a yellow solid (173 mg, 78%, \textit{R}_f = 0.54 (1:1 CH\textsubscript{2}Cl\textsubscript{2}:Pet)); \textit{mp} 121.5-125.5 °C; \textit{\delta}\textsubscript{H} NMR (270 MHz, CDCl\textsubscript{3}): 5.11 (2H, s, CH\textsubscript{2}), 7.06-7.09 (1H, m, Ar\textit{H}), 7.23-7.48 (9H, m, Ar\textit{H}), 7.81 (1H, d, \textit{J} 2.6, Ar\textit{H}), 7.92-7.97 (1H, m, Ar\textit{H}), 13.47 (1H, s, OH); \textit{\delta}\textsubscript{C} NMR (67.5 MHz, CDCl\textsubscript{3}): 70.4, 113.2, 115.0, 118.2, 119.2, 120.9, 122.1, 123.8, 127.6, 128.2, 128.3, 128.8, 129.3, 130.3, 135.5, 136.6, 138.8, 139.0,
147.5, 158.9, 159.3, 192.4; HRMS (ESI m/z): Calcd. for [C_{22}H_{16}BrClO_3-H]^- 440.98931, found 440.9876.

**3-(5-bromo-2,4-dimethoxyphenyl)-1-(3-bromo-5-chloro-2-hydroxyphenyl)prop-2-en-1-one 121**

3'-Bromo-5'-chloro-2'-hydroxyacetophenone 116 (125 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 5-bromo-2,4-dimethoxybenzaldehyde 79 (123 mg, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a bright yellow solid (169 mg, 71%, \( R_f = 0.47 \) (1:1 CH_2Cl_2:Pet)); mp 227.5-232.1 °C; \( \delta_H \) NMR (270 MHz, CDCl_3): 3.96 (3H, s, CH_3), 3.97 (3H, s, CH_3), 6.47 (1H, s, ArH), 7.45-7.84 (4H, m, ArH), 8.15-8.21 (1H, m, ArH), 13.70 (1H, s, OH); \( \delta_C \) NMR (67.5 MHz, CDCl_3): 56.1, 56.6, 96.1, 103.1, 113.0, 117.4, 121.2, 123.7, 128.2, 128.7, 133.4, 138.5, 141.5, 158.9, 159.6, 160.3, 192.6; HRMS (ESI m/z): Calcd. for [C_{17}H_{13}Br_2ClO_4-H]^- 474.87704, found 474.8722.

**1-(3-bromo-5-chloro-2-hydroxyphenyl)-3-(4-fluorophenyl)prop-2-en-1-one 122**

3'-Bromo-5'-chloro-2'-hydroxyacetophenone 116 (125 mg, 0.5 mmol), KOH (112 mg, 2.0 mmol) and 4-fluorobenzaldehyde 81 (0.053 mL, 0.5 mmol) were reacted according to the general procedure. The desired compound was obtained as a yellow solid (166 mg, 93%, \( R_f = 0.77 \) (1:1 CH_2Cl_2:Pet)); mp 198.4-200.6 °C (lit. 178 °C)^639; \( \delta_H \) NMR (270 MHz, CDCl_3): 7.13-7.23 (2H, m, ArH), 7.42-7.48 (1H, m, ArH), 7.67-7.96 (5H, m, ArH), 13.44 (1H, s, OH); MS (ESI m/z): Calcd. for [C_{15}H_{9}BrClFO_2+H]^+ 354.95, found 354.95.

**8.3.3 Typical temperature, pressure and power profile for microwave reaction**

Temperature profile over 15 minutes.
Note: as the reaction is carried out at a temperature lower than the boiling point of THF, no increase in pressure was observed. The blue line indicated the upper safety pressure limit of the microwave reactor.

8.4 Chapter 3 Experimental

8.4.1 Preparation of Ionic Liquids
The acid portion of the ionic liquid was added dropwise to an equimolar amount of neat triethylamine cooled to -78 °C (acetone/CO_2(s) slurry). The resulting solution was stirred for one hour at -78 °C then allowed to warm to room temperature followed by stirring for 5 hours. In cases where an aqueous acid solution, such as in the case of H_2SO_4, was used, water was removed by heating at 60 °C under vacuum overnight.

8.4.2 General Procedure for Flavanone Synthesis
The pIL TeaHSO_4 (0.25 mL) was added to chalcone in a 10 mL microwave tube. The solution was heated to 130 °C at 50 W for 20 minutes under microwave irradiation. Thin Layer Chromatography was carried out on the crude sample, and the solution was then filtered through a silica plug with a CH_2Cl_2 wash. The solvent was evaporated in vacuo, and purification of the crude material by Column Chromatography (1:1CH_2Cl_2: Petroleum Spirits) gave the desired flavanone.
2-(4-bromophenyl)chroman-4-one 130

3-(4-bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 74

(100 mg, 0.33 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale yellow solid (64.7 mg, 65%, R<sub>f</sub> = 0.19 (1:1 CH<sub>2</sub>Cl<sub>2</sub>:Pet)); mp 115.5-120.1 °C (lit. 115.8-119.0 °C)<sup>141</sup>; δ<sub>H</sub> NMR (270 MHz, CDCl<sub>3</sub>): 2.82-3.08 (2H, m, \( \text{C}_2\text{H}_2 \)), 5.44 (1H, dd, \( J = 12.9, J = 3.3, \text{CH} \)), 7.02-7.08 (2H, m, ArH), 7.34-7.37 (2H, m, ArH), 7.47-7.58 (3H, m, ArH), 7.90-7.93 (1H, m, ArH).

2-phenyl-2,3-dihydrochromen-4-one 6<sup>460</sup>

1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one 66 (96.6 mg, 0.43 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale yellow solid (58.0 mg, 60%, R<sub>f</sub> = 0.29 (1:1 CH<sub>2</sub>Cl<sub>2</sub>:Pet)); mp 76.5-79.5 °C (lit. 76-77 °C)<sup>460</sup>; δ<sub>H</sub> NMR (270 MHz, CDCl<sub>3</sub>): 2.85-3.14 (2H, m, \( \text{C}_2\text{H}_2 \)), 5.45-5.51 (1H, dd, \( J = 13.1, J = 2.8, \text{CH} \)), 7.02-7.08 (2H, m, ArH), 7.38-7.50 (6H, m, ArH), 7.91-7.95 (1H, m, ArH); δ<sub>C</sub> NMR (67.5 MHz, CDCl<sub>3</sub>): 44.8, 79.7, 118.2, 121.0, 121.7, 126.3, 127.2, 128.9, 129.0, 136.3, 138.8, 161.7, 192.1.

2-(3-benzyloxyphenyl)chroman-4-one 131

3-(3-benzyloxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 78 (77.0 mg, 0.23 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale-yellow oil (27.9 mg, 36%, R<sub>f</sub> = 0.06 (1:1 CH<sub>2</sub>Cl<sub>2</sub>:Pet)); δ<sub>H</sub> NMR (400 MHz, CDCl<sub>3</sub>): 2.86-3.11 (2H, m, \( \text{CH}_2 \)), 5.10 (2H, s, OCH<sub>2</sub>Ar), 5.45 (1H, dd, \( J = 13.2, J = 2.8, \text{CH} \)), 6.98-7.14 (5H, m, ArH), 7.33-7.53 (7H, m, ArH), 7.93 (1H, dd, \( J = 8.3, J = 2.2, \text{ArH} \)); δ<sub>C</sub> NMR (100 MHz, CDCl<sub>3</sub>): 44.8, 70.2, 79.5, 113.0, 115.0, 118.2, 118.7, 121.8, 127.2, 127.6, 128.2, 128.7, 130.1, 136.3, 140.4, 159.3, 161.6, 192.0; HRMS (ESI m/z): Calcd. for [C<sub>22</sub>H<sub>18</sub>O<sub>3</sub> + H]<sup>+</sup> 331.13342, found 331.13219.

2-(5-bromo-2,4-dimethoxyphenyl)chroman-4-one 132

3-(5-bromo-2,4-dimethoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 80 (80.7 mg, 0.22 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale-yellow solid (11.5 mg, 14%, R<sub>f</sub> = 0.05 (1:1
202

CH₂Cl₂:Pet); mp 289.9 °C (dec.); δH NMR (400 MHz, CDCl₃): 2.88-2.90 (2H, m, CH₂), 3.85 (3H, s, CH₃), 3.93 (3H, s, CH₃), 5.73 (1H, t, J 8.0, CH), 6.49 (1H, s, ArH), 7.03-7.07 (2H, m, ArH), 7.48-7.53 (1H, m, ArH), 7.76 (1H, s, ArH), 7.91-7.93 (1H, m, ArH); δC NMR (100 MHz, CDCl₃): 43.9, 55.8, 56.5, 74.0, 96.4, 118.2, 121.0, 121.2, 121.6, 127.1, 130.9, 136.2, 156.4, 182.3, 192.6; HRMS (ESI m/z): Calcd. for [C₁₇H₁₅BrO₄+H]+ 363.02320, found 363.02108.

6-bromo-2-phenylchroman-4-one 137⁴⁶¹

3-phenyl-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 109 (30.7 mg, 0.10 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale-yellow solid (17.1 mg, 56%, Rf = 0.40 (2:3 CH₂Cl₂:Pet)); mp 113.9-116.1 °C (lit. 118-119 °C)⁴⁶¹; δH NMR (270 MHz, CDCl₃): 2.86-3.13 (2H, m, CH₂), 5.43-5.49 (1H, dd, J 13.1, J 3.0, CH), 6.95 (1H, d, J 8.7, ArH), 7.38-7.47 (5H, m, ArH), 7.55-7.59 (1H, m, ArH), 8.03 (1H, d, J 2.3, ArH); δC NMR (67.5 MHz, CDCl₃): 43.3, 79.9, 114.4, 120.3, 122.3, 126.2, 129.0, 129.1, 129.6, 138.3, 138.9, 160.5, 190.8.

6-bromo-2-(4-bromophenyl)chroman-4-one 138

3-(4-bromophenyl)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 110 (39.5 mg, 0.10 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale brown solid (8.6 mg, 22%, Rf = 0.30 (1:1 CH₂Cl₂:Pet)); δH NMR (270 MHz, CDCl₃): 2.84-3.07 (2H, m, CH₂), 5.40-5.46 (1H, dd, J 12.8, J 3.5, CH), 6.93-6.96 (1H, d, J 8.9, ArH), 7.32-7.35 (1H, m, ArH), 7.54-7.57 (3H, m, ArH), 8.02 (1H, d, J 2.6, ArH); δC NMR (100 MHz, CDCl₃): 44.2, 79.1, 114.6, 120.3, 123.0, 127.9, 129.7, 130.3, 132.2, 132.5, 137.3, 139.2, 145.2, 160.2, 190.3; Calcd. for [C₁₅H₁₀Br₂O₂+H]+ 382.91053, found 382.91164.

6-bromo-2-(3-benzyloxyphenyl)chroman-4-one 139

3-(3-benzyloxyphenyl)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 111 (145.0 mg, 0.36 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale yellow solid (85.0 mg, 59%, Rf = 0.63 (1:1 CH₂Cl₂:Pet)); mp 135.9-138.4 °C; δH NMR (270 MHz, CDCl₃): 2.87-2.92 (1H, m, CH₂), 3.01-3.09 (1H, m, CH₂), 5.09 (2H, s, CH₂O), 5.41-5.46 (1H, dd, J 13.2, J 3.1, CH), 6.95-7.11 (4H, m, ArH), 7.33-7.45
(6H, m, ArH), 7.56-7.59 (1H, dd, J 8.7, J 2.4, ArH), 8.03 (1H, d, J 2.8, ArH); δ<sub>C</sub> NMR (100 MHz, CDCl<sub>3</sub>): 44.3, 70.2, 79.7, 113.0, 114.5, 115.1, 118.7, 120.3, 122.3, 127.6(2×C), 128.2, 128.8(2×C), 129.6, 130.2, 136.7, 138.9, 139.9, 159.3, 160.4, 190.7; HRMS (ESI m/z): Calcd. for [C<sub>22</sub>H<sub>17</sub>BrO<sub>3</sub>]+ 409.04338, found 409.04272.

8-bromo-6-chloro-2-phenylchroman-4-one 141<sup>640</sup>

![Chemical structure](image)

3-phenyl-1-(8-bromo-6-chloro-2-hydroxyphenyl)prop-2-en-1-one 117 (99.7 mg, 0.30 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale-yellow solid (53.6 mg, 54%, R<sub>f</sub> = 0.40 (1:1 CH<sub>2</sub>Cl<sub>2</sub>:Pet)); mp 105.2-108.6 °C (lit. 98-100 °C)<sup>640</sup>; δ<sub>H</sub> NMR (270 MHz, CDCl<sub>3</sub>): 2.98-3.13 (2H, m, CH<sub>2</sub>), 5.59 (1H, dd, J 12.1, J 3.8, CH), 7.39-7.50 (5H, m, ArH), 7.73 (1H, d, J 2.4, ArH), 7.84 (1H, d, J 2.4, ArH); δ<sub>C</sub> NMR (67.5 MHz, CDCl<sub>3</sub>): 43.7, 80.0, 113.0, 122.4, 125.9, 126.0, 127.4, 129.0, 137.8, 138.7, 156.6, 190.1.

8-bromo-6-chloro-2-(4-bromophenyl)chroman-4-one 142

![Chemical structure](image)

3-(4-bromophenyl)-1-(8-bromo-6-chloro-2-hydroxyphenyl)prop-2-en-1-one 118 (70.0 mg, 0.17 mmol) was reacted according to the general procedure. The desired compound was obtained as a creamy-white solid (16.1 mg, 19%, R<sub>f</sub> = 0.7 (1:1 CH<sub>2</sub>Cl<sub>2</sub>:Pet)); mp 142.2-145.3 °C; δ<sub>H</sub> NMR (270 MHz, CDCl<sub>3</sub>): 2.95-3.10 (2H, m, CH<sub>2</sub>), 5.54 (1H, dd, J 10.7, J 5.1, CH), 7.34-7.38 (5H, m, ArH), 7.50-7.61 (3H, m, ArH), 7.73-7.73 (1H, m, ArH), 7.82-7.83 (1H, m, ArH); δ<sub>C</sub> NMR (67.5 MHz, CDCl<sub>3</sub>): 43.5, 79.3, 113.0, 122.4, 123.1, 125.9, 127.7, 130.4, 132.2, 136.8, 138.8, 156.4, 189.6; HRMS (ESI m/z): Calcd. for [C<sub>15</sub>H<sub>9</sub>Br<sub>2</sub>ClO<sub>2</sub>-H]- 414.85591, found 414.85601.

8-bromo-6-chloro-2-(3-benzyloxyphenyl)chroman-4-one 143

![Chemical structure](image)

3-(3-benzyloxyphenyl)-1-(8-bromo-6-chloro-2-hydroxyphenyl)prop-2-en-1-one 120 (157.8 mg, 0.36 mmol) was reacted according to the general procedure. The desired compound was obtained as a pale yellow solid (78.9 mg, 50%, R<sub>f</sub> = 0.27 (1:1 CH<sub>2</sub>Cl<sub>2</sub>:Pet)); mp 134.1-137.1 °C; δ<sub>H</sub> NMR (400 MHz, CDCl<sub>3</sub>): 2.98-3.10 (2H, m, CH<sub>2</sub>), 5.10 (2H, s, OCH<sub>3</sub>), 5.55 (1H, d, J 10.1, CH), 6.98-7.14 (3H, m, ArH), 7.35-7.45 (6H, m, ArH), 7.73 (1H, br s, ArH), 7.83 (1H, br s, ArH); δ<sub>C</sub> NMR (100 MHz, CDCl<sub>3</sub>): 43.6, 70.2, 79.7, 112.7, 113.0, 115.3,
118.4, 122.5, 125.9, 127.4, 127.6(2×C), 128.2, 128.7(2×C), 130.1, 136.8, 138.7, 139.4, 156.5, 159.3, 190.0; HRMS (ESI m/z): Calcd. for [C$_{22}$H$_{16}$BrClO$_3$+H]$^+$ 443.00441, found 443.00328.

8.4.3 General Procedure for Flavones
Substituted 2′-hydroxychalcone in DMSO was added to a round bottom flask. Catalytic iodine was added, and the solution refluxed overnight. The solution was cooled to rt, and H$_2$O added until a precipitate formed. The solution was transferred to a separating funnel, extracted with 3×EtOAc (20 mL), and the organic layer washed with 1.0 M HCl (20 mL), H$_2$O (20 mL), Na$_2$S$_2$O$_3$ (20 mL), and brine (20 mL). The organic phase was dried (MgSO$_4$), filtered, and the solvent removed in vacuo. Purification was conducted by column chromatography (40% CH$_2$Cl$_2$ in Pet).

**2-phenylchromen-4-one** 74

2′-hydroxychalcone 66 (87.7 mg, 0.39 mmol), I$_2$ (6.2 mg, 0.024 mmol) and DMSO. Purification gave the desired product as a pale brown solid (52.6 mg, 61%, R$_f$ = 0.22 (10% MeOH in CHCl$_3$)); mp 95.3-98.8 °C (lit. 97-97 °C)$^{641}$; δ$_H$ NMR (270 MHz, CDCl$_3$): 6.81 (1H, s, CH), 7.37-7.43 (1H, m, ArH), 7.48-7.57 (4H, m, ArH), 7.65-7.71 (1H, m, ArH), 7.89-7.94 (2H, m, ArH), 8.21 (1h, dd, J 8.0, J 1.6, ArH); δ$_C$ NMR (67.5 MHz, CDCl$_3$): 107.7, 118.2, 124.0, 125.3, 125.8, 126.4, 129.1, 131.7, 131.9, 133.9, 156.4, 163.5, 178.6.

**2-(4-bromophenyl)chromen-4-one** 157

3-(4-bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 78 (115.0 mg, 0.38 mmol), I$_2$ (6.0 mg, 0.02 mmol), and DMSO. Purification gave the desired as a white solid (90.0 mg, 79%, R$_f$ =0.15 (10% EtOAc in CHCl$_3$)); mp 179.1-179.7 °C (lit. 164-166 °C)$^{642}$; δ$_H$ NMR (270 MHz, CDCl$_3$): 6.76 (1H, s, CH), 7.36-7.42 (1H, m, ArH), 7.50-7.53 (1H, m, ArH), 7.59-7.77 (5H, m, ArH), 8.18 (1H, dd, J 8.0, J 1.6, ArH); δ$_C$ NMR (67.5 MHz, CDCl$_3$): 107.7, 118.1, 124.0, 125.5, 125.8, 126.4, 127.7, 130.7, 132.4, 134.0, 156.2, 162.3, 178.3.

**2-(3-benzyloxyphenyl)chromen-4-one** 158

3-(4-bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 78 (140.1 mg, 0.38 mmol), I$_2$ (6.0 mg, 0.02 mmol), and DMSO. Purification gave the desired as a cream-coloured solid (80.6 mg, 57%, R$_f$ =0.40 (2:1 Pet:EtOAc) mp 114.2-117.1 °C; δ$_H$ NMR (400 MHz, CDCl$_3$): 6.76 (1H, s, CH), 7.37-7.42 (1H, m, ArH), 7.50-7.53 (1H, m, ArH), 7.59-7.77 (5H, m, ArH), 8.18 (1H, dd, J 8.0, J 1.6, ArH); δ$_C$ NMR (67.5 MHz, CDCl$_3$): 107.7, 118.1, 124.0, 125.5, 125.8, 126.4, 127.7, 130.7, 132.4, 134.0, 156.2, 162.3, 178.3.
205 MHz, CDCl\textsubscript{3}): 5.13 (2H, s, O\textsubscript{CH\textsubscript{2}}Ph), 6.79 (1H, s, CH), 7.11-7.14 (1H, m, ArH), 7.33-7.55 (11H, m, ArH), 7.66-7.70 (1H, m, ArH), 8.21-8.23 (1H, dd, J 8.0, J 1.6, ArH); \text{\delta}_{C} NMR (100 MHz, CDCl\textsubscript{3}): 70.4, 107.9, 113.0, 118.0, 118.2, 119.1, 124.1, 125.3, 125.8, 127.6(2\times C), 128.3, 128.8(2\times C), 130.3, 133.3, 133.9, 136.5, 156.3, 159.3, 163.2, 178.5; HRMS (ESI m/z): Calcd. for [C\textsubscript{22}H\textsubscript{16}O\textsubscript{3}+H\textsuperscript{+}] 329.11722, found 329.11721.

2-(5-bromo-2,4-dimethoxyphenyl)chromen-4-one 159

3-(5-bromo-2,4-dimethoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one 80 (120.9 mg, 0.27 mmol), I\textsubscript{2} (4.2 mg, 0.017 mmol), and DMSO. Purification gave the desired product as a pale brown solid (105.8 mg, 88%, R\textsubscript{f} = 0.89 (10% MeOH in CHCl\textsubscript{3}); mp 200.0-203.5 °C (lit. 196 °C)

5-methoxy-2-phenyl-4H-chromen-4-one 160

1-(2-hydroxy-6-methoxyphenyl)-3-phenylprop-2-en-1-one 101 (150.0 mg, 0.5 mmol), I\textsubscript{2} (10.0 mg, 0.04 mmol), and DMSO. Purification isolated the product as pale brown crystals (83.7 mg, 66%, R\textsubscript{f} = 0.05 (10% EtOAc in CHCl\textsubscript{3}); mp 114.0-117.3 °C (lit. 130 °C)

5-methoxy-2-(4-bromophenyl)chromen-4-one 161

3-(4-bromophenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one 102 (160.0 mg, 0.48 mmol), I\textsubscript{2} (10.0 mg, 0.040 mmol), and DMSO. Purification isolated the product as an off-white solid (94.7 mg, 58%, R\textsubscript{f} = 0.05 (10% EtOAc in CHCl\textsubscript{3}); mp 171.6-174.8 °C; \text{\delta}_{H} NMR (400 MHz, CDCl\textsubscript{3}): 3.96 (3H, s, CH\textsubscript{3}), 6.65 (1H, s, CH\textsubscript{3}), 6.78 (1H, d, J 8.3, ArH), 7.06 (1H, d, J 8.7, ArH), 7.50-7.59 (3H, m, ArH), 7.68-7.70 (2H,
m, ArH); δc NMR (67.5 MHz, CDCl₃): 56.6, 106.6, 109.2, 110.1, 114.5, 126.1, 127.5, 130.4, 132.3, 134.0, 158.2, 159.8, 160.0, 178.2; HRMS (ESI m/z): Calcd. for [C₁₆H₁₁BrO₃+H]⁺ 330.99643, found 330.99305.

5-methoxy-2-(3-hydroxy)chromen-4-one 162

![Structure of 5-methoxy-2-(3-hydroxy)chromen-4-one](image)

3-(3-(benzyloxy)phenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one 104 (166.6 mg, 0.50 mmol), I₂ (8.0 mg, 0.032 mmol), and DMSO. Purification isolated the product as a pale brown solid (110.9 mg, 67%, Rf = 0.55 (10% EtOAc in CHCl₃); mp 236.4-239.9 °C (lit. 237-238.5 °C)⁴⁰⁶; δH NMR (270 MHz, CDCl₃): 3.96 (3H, s, CH₃), 6.67 (1H, s, CH), 6.79 (1H, d, J 8.2, ArH), 7.08 (1H, d, J 8.4, ArH), 7.51-7.61 (3H, m, ArH), 7.69-7.72 (2H, m, ArH); δc NMR (67.5 MHz, CDCl₃): 56.6, 106.7, 109.2, 110.2, 126.1, 127.6, 130.4, 132.3, 134.1, 158.2, 159.8, 160.1, 178.3.

5-methoxy-2-(5-bromo-2,4-dimethoxyphenyl)chromen-4-one 164

3-(5-bromo-2,4-dimethoxyphenyl)-1-(2-hydroxy-6-methoxyphenyl)prop-2-en-1-one 105 (165.5 mg, 0.42 mmol), I₂ (9.0 mg, 0.033 mmol), and DMSO. Purification isolated the product as a pale brown solid (43.6 mg, 26%, Rf = 0.15 (10% EtOAc in CHCl₃); mp 205.5-207.7 °C; δH NMR (270 MHz, CDCl₃): 3.94 (6H, s, 2×CH₃), 3.96 (3H, s, CH₃), 6.50 (1H, s, CH), 7.67 (1H, d, J 8.2, ArH), 7.02 (1H, s, ArH), 7.07-7.10 (1H, m, ArH), 7.52 (1H, t, J 8.2, ArH), 8.04 (1H, s, ArH); δc NMR (67.5 MHz, CDCl₃): 56.0, 56.5, 96.4, 102.6, 106.2, 110.2, 113.2, 132.8, 133.7, 157.1, 158.7, 159.1, 159.7, 179.0; HRMS (ESI m/z): Calcd. for [C₁₈H₁₅BrO₅+H]⁺ 391.01756, found 391.01778.

6-bromo-2-phenyl-4H-chromen-4-one 165

3-phenyl-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 109 (107.0 mg, 0.35 mmol), I₂ (6.0 mg, 0.02 mmol), and DMSO. Purification isolated the product as white needle-like crystals (91.9 mg, 87%, Rf = 0.29 (10% EtOAc in CHCl₃); mp 191.6-193.6 °C (lit. 190-191 °C)⁶⁴⁶; δH NMR (270 MHz, CDCl₃): 6.80 (1H, s, CH), 7.42-7.54 (4H, m, ArH), 7.72-7.77 (1H, m, ArH), 7.86-7.89 (2H, m, ArH), 8.31 (1H, d, J 2.6, ArH); δc NMR (67.5 MHz, CDCl₃): 107.6, 118.8, 120.1, 125.3, 126.4, 128.4, 129.2, 131.4, 132.0, 136.8, 155.1, 163.7, 177.1.

206
6-bromo-2-(4-bromophenyl)chromen-4-one 166

3-(4-bromo)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 110 (84.3 mg, 0.22 mmol), I₂ (4.0 mg, 0.016 mmol), and DMSO. Purification isolated the product as a white solid (37.1 mg, 44%, R_f = 0.37 (10% EtOAc in CHCl₃); mp 243.9-245.5 °C (lit. 253 °C)); δH NMR (270 MHz, CDCl₃): 6.79 (1H, s, CH), 7.44 (1H, d, J 8.9, ArH), 7.63-7.77 (4H, m, ArH), 8.33 (1H, d, J 1.9, ArH); δC NMR (67.5 MHz, CDCl₃): 107.8, 119.0, 120.1, 125.3, 126.7, 127.8, 128.5, 130.4, 132.5, 137.0, 155.0, 162.7, 176.9.

6-bromo-2-(3-benzyloxyphenyl)-4H-chromen-4-one 167

3-(3-benzyloxyphenyl)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 111 (166.0 mg, 0.41 mmol), I₂ (7.0 mg, 0.03 mmol), and DMSO. Purification isolated the product as an off-white solid (80.0 mg, 48%, R_f = 0.23 (10% EtOAc in CHCl₃); mp 147.2-148.1 °C; δH NMR (270 MHz, CDCl₃): 5.12 (1H, s, CH₂), 6.77 (1H, s, CH), 7.10-7.14 (1H, m, ArH), 7.33-7.47 (9H, m, ArH), 7.71-7.75 (1H, m, ArH), 8.30 (1H, d, J 2.4, ArH); δC NMR (67.5 MHz, CDCl₃): 70.4, 107.8, 113.0, 118.2, 118.8, 119.1, 120.1, 125.3, 127.6, 128.3, 128.4, 128.8, 130.3, 132.8, 136.4, 136.8, 155.0, 159.3, 163.4, 177.1; HRMS (ESI m/z): Calcd. for [C₂₂H₁₅BrO₃+H]+ 407.02773, found 407.02457.

6-bromo-2-(5-bromo-2,4-dimethoxyphenyl)chromen-4-one 168

3-(4-bromo)-1-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one 112 (87.3 mg, 0.20 mmol), I₂ (4.0 mg, 0.016 mmol), and DMSO. Purification isolated the product as a pale yellow solid (66.3 mg, 75%, R_f = 0.06 (10% EtOAc in CHCl₃); mp 236.2-237.6 °C; δH NMR (270 MHz, CDCl₃): 3.95 (3H, s, OCH₃), 3.96 (3H, s, OCH₃), 6.52 (1H, s, CH), 7.14 (1H, s, ArH), 7.42 (1H, d, J 9.0, ArH), 7.73 (1H, dd, J 8.9, J 2.6, ArH), 8.07 (1H, s, ArH), 8.30 (1H, d, J 2.3, ArH); δC NMR (67.5 MHz, CDCl₃): 56.1, 56.5, 96.4, 102.8, 111.7, 113.8, 118.4, 120.0, 125.1, 128.3, 132.9, 136.6, 155.0, 159.3, 177.5; HRMS (ESI m/z): Calcd. for [C₁₇H₁₂Br₂O₄+H]⁺ 438.91751, found 438.91818.
8-bromo-6-chloro-2-phenyl-4H-chromen-4-one 169

1-(3-bromo-5-chloro-2-hydroxyphenyl)-3-phenylprop-2-en-1-one 117 (115.7 mg, 0.34 mmol), I₂ (5.0 mg, 0.020 mmol), and DMSO. Purification isolated the product as off-white crystals (79.7 mg, 70%, R_f = 0.10 (10% EtOAc in CHCl₃); mp 178.4-179.2 °C (lit. 178-179 °C); δH NMR (400 MHz, CDCl₃): 6.84 (1H, s, CH), 7.50-7.58 (4H, m, ArH), 7.87 (1H, d, J 2.8, ArH), 7.97 (2H, dd, J 7.8, J 1.4, ArH), 8.11 (1H, d, J 2.4, ArH); δC NMR (67.5 MHz, CDCl₃): 107.3, 113.0, 124.7, 125.7, 126.6 (2×C), 129.3(2×C), 131.0, 131.4, 132.3, 136.9, 151.5, 163.7, 176.7.

8-bromo-6-chloro-2-(4-bromophenyl)chromen-4-one 170

3-(4-bromo)-1-(3-bromo-5-chloro-2-hydroxyphenyl)prop-2-en-1-one 118 (53.1 mg, 0.11 mmol), I₂ (3.0 mg, 0.011 mmol), and DMSO. Purification isolated the product as a pale yellow solid (28.5 mg, 12%, R_f = 0.34 (10% EtOAc in CHCl₃); mp 253.5-254.6 °C; δH NMR (270 MHz, CDCl₃): 6.83 (1H, s, CH), 7.65-7.71 (2H, m, ArH), 7.82-7.87 (2H, m, ArH), 7.90 (1H, d, J 2.6, ArH), 8.13 (1H, d, J 2.3, ArH); δC NMR (67.5 MHz, CDCl₃): 107.3, 118.8, 124.8, 126.3, 126.9, 128.9(2×C), 130.6, 132.1(2×C), 133.4, 135.9, 151.4, 164.0, 178.0; HRMS (ESI m/z): Calcd. for [C₁₅H₇Br₂ClO₂+H]^+ 333.93907, found 333.94135.

8-bromo-6-chloro-2-(3-benzyloxyphenyl)chromen-4-one 171

3-(3-(benzyloxy)phenyl)-1-(3-bromo-5-chloro-2-hydroxyphenyl)prop-2-en-1-one 120 (160.6 mg, 0.36 mmol), I₂ (9.0 mg, 0.033 mmol), and DMSO. Purification isolated the product as an off-white solid (60.4 mg, 38%, R_f = 0.33 (10% EtOAc in CHCl₃); mp 144.6-148.2 °C; δH NMR (270 MHz, CDCl₃): 5.16 (2H, s, CH₂), 6.93 (1H, s, CH), 7.14-7.23 (1H, m, ArH), 7.33-7.46 (5H, m, ArH), 7.55-7.58 (1H, m, ArH), 7.70 (1H, s, ArH), 7.86-7.88 (1H, m, ArH), 8.13 (1H, d, J 2.13, ArH); δC NMR (67.5 MHz, CDCl₃): 70.3, 107.3, 112.7, 115.5, 119.1, 119.3, 121.2, 123.0, 124.7, 125.7, 127.5, 128.8, 129.7, 130.5, 131.5, 132.3, 136.9, 159.3, 163.5, 171.3, 176.7; HRMS (ESI m/z): Calcd. for [C₂₂H₁₄BrClO₂H]^+ 440.98876, found 440.98487.
8-bromo-6-chloro-2-(5-bromo-2,4-dimethoxyphenyl)chromen-4-one 172

3-(5-bromo-2,4-dimethoxyphenyl)-1-(3-bromo-5-chloro-2-hydroxyphenyl)prop-2-en-1-one 121 (53.1 mg, 0.11 mmol), I2 (3.0 mg, 0.011 mmol), and DMSO. Purification isolated the product as a pale yellow solid (17.3 mg, 33%, Rf = 0.05 (10% EtOAc in CHCl3); mp 234.4-237.4 °C; δH NMR (270 MHz, CDCl3): 3.98 (6H, s, 2×OC6H3), 6.53 (1H, s, CH), 7.23 (1H, s, ArH), 7.86 (1H, d, J 2.6, ArH), 8.09 (1H, d, J 2.6, ArH), 8.30 (1H, s, ArH); δC NMR (67.5 MHz, CDCl3): 56.2, 56.6, 96.3, 103.1, 111.2, 112.8, 113.3, 124.5, 131.0, 133.6, 136.6, 151.4, 154.2, 159.6, 177.2; HRMS (ESI m/z): Calcd. for [C17H11Br2ClO4+H]+ 472.87854, found 472.87410.

8.5 Chapter 4 Experimental

8.5.1 General Procedure for Heck Reaction
Pd(OAc)2 (14 mg, 0.06 mmol), PPh3 (19.6 mg, 0.06 mmol), K2CO3 (224 mg, 1.62 mmol) and DMF (10 mL) were stirred for 10 minutes in a round bottomed flask. The aryl bromide (0.54 mmol) was then added, followed by the acrylate (2.16 mmol), and the solution was refluxed for 16 hrs. The solvent was removed in vacuo, and EtOAc (20 mL) added. Following filtration through a celite plug, the solution was dried over MgSO4, filtered, solvent removed in vacuo, resulting in the crude product which was purified by column chromatography (20% ether in Pet) to provide the acrylate product.

8.5.2 Compound Characterisation Data
(E)-butyl 3-(4-formylphenyl)acrylate (benzaldehyde nBu acrylate) 194

Pd(OAc)2 (14 mg, 0.06 mmol), PPh3 (19.6 mg, 0.06 mmol), K2CO3 (224 mg, 1.62 mmol), DMF (10 mL), 4-bromobenzaldehyde 73 (100 mg, 0.54 mmol), and nBu acrylate (0.310 mL, 2.16 mmol). Purification by column chromatography (20% Ether in Pet) provided the desired acrylate as a pale brown oil (102.9 mg, 82%, Rf = 0.82); δH NMR (400 MHz, CDCl3): 0.94 (3H, t, J 7.3, H14), 1.38-1.47 (2H, m, H13), 1.65-1.72 (2H, m, H12), 4.20 (2H, t, J 6.6, H11), 6.53 (1H, d, J 15.9, H9), 7.65-7.75 (3H, m, H3, H5 & H8), 7.87-7.89 (2H, d, J 8.4, H2 & H6), 10.00 (1H, s, CHO); δC NMR (100 MHz, CDCl3): 13.8, 19.3, 30.8, 64.8, 121.6, 128.6(2×C), 130.2(2×C), 137.2 140.2, 142.9, 166.5, 191.5.
**(E)-butyl 3-(4-formylphenyl)acrylate (benzaldehyde tBu acrylate)**

Pd(OAc)$_2$ (14 mg, 0.06 mmol), PPh$_3$ (19.6 mg, 0.06 mmol), K$_2$CO$_3$ (224 mg, 1.62 mmol), DMF (10 mL), 4-bromobenzaldehyde 73 (100 mg, 0.54 mmol), and tBu acrylate (0.316 mL, 2.16 mmol). Purification by column chromatography (20% Ether in Pet) provided the desired acrylate as a pale brown oil (75.3 mg, 60%); $\delta_H$ NMR (400 MHz, CDCl$_3$): 1.51 (9H, s, H$_{12}$), 6.46 (1H, d, $J$ 16.3, H$_9$), 7.44-7.71 (3H, m, H$_3$, H$_5$, H$_8$), 7.85-7.87 (2H, d, $J$ 8.0, H$_2$ & H$_6$), 9.99 (1H, s, CHO); $\delta_C$ NMR (100 MHz, CDCl$_3$): 28.2, 81.1, 123.5, 128.5(2×C), 130.2(2×C), 132.5, 137.0, 141.9, 165.7, 191.6.

**((E)-butyl 3-(3-acetyl-4-hydroxyphenyl)acrylate (acetophenone nBu acrylate)**

Pd(OAc)$_2$ (14 mg, 0.06 mmol), PPh$_3$ (19.6 mg, 0.06 mmol), K$_2$CO$_3$ (224 mg, 1.62 mmol), DMF (10 mL), 5′-bromo-2′-hydroxyacetophenone 108 (116 mg, 0.54 mmol), and nBu acrylate (0.23 mL, 2.16 mmol). Purification by column chromatography (20% Ether in Pet) provided the desired acetophenone as a pale brown oil (107.6 mg, 76%, $R_f$=0.19); $\delta_H$ NMR (270 MHz, CDCl$_3$): 0.92 (3H, t, $J$ 7.5, H$_{10}$), 1.33-1.46 (2H, m, H$_8$), 2.62 (3H, s, H$_1$), 4.16 (2H, t, $J$ 6.6, H$_7$), 6.29 (1H, d, $J$ 15.9, H$_6$), 6.94 (1H, d, $J$ 8.7, H$_4$), 7.54-7.65 (2H, m, H$_2$, H$_5$), 7.81 (1H, d, $J$ 2.1, H$_3$), 12.43 (1H, s, OH); $\delta_C$ NMR (67.5 MHz, CDCl$_3$): 13.8, 19.3, 26.7, 30.9, 64.6, 116.9, 119.4, 119.7, 125.7, 131.5, 135.0, 143.1, 164.1, 167.1, 204.4; HRMS (ESI $m/z$): Calcd. for [C$_{13}$H$_{18}$O$_4$+H]$^+$ 263.12779, found 263.12866.

**((E)-t-butyl 3-(3-acetyl-4-hydroxyphenyl)acrylate (acetophenone tBu acrylate)**

Pd(OAc)$_2$ (28 mg, 0.12 mmol), PPh$_3$ (39.2 mg, 0.12 mmol), K$_2$CO$_3$ (448 mg, 3.24 mmol), DMF (10 mL), 5′-bromo-2′-hydroxyacetophenone 108 (232 mg, 1.08 mmol) and tBu acrylate (0.732 mL, 4.32 mmol). The desired product was obtained by column chromatography (1:1 CH$_2$Cl$_2$:Pet) as a yellow oil (245.3 mg, 90%, $R_f$=0.06); $\delta_H$ NMR (270 MHz, CDCl$_3$): 1.48 (9H, s, H$_{11′}$), 2.60 (3H, s, H$_2$), 6.20 (1H, d, $J$ 15.9, H$_8$), 6.90 (1H, d, $J$ 8.7, H$_3′$), 7.45 (1H, d, $J$ 15.9, H$_7′$), 7.58 (1H, dd, $J$ 8.8, J 2.3, H$_6′$), 7.76 (1H, d, $J$ 2.3, H$_4′$), 12.39 (1H, s, OH); $\delta_C$ NMR (67.5 MHz, CDCl$_3$): 26.7, 28.3, 80.6, 118.9, 119.3, 128.0, 128.9, 131.2, 135.0, 142.1,
163.8, 166.3, 204.4; HRMS (ESI m/z): Calcd. for [C_{15}H_{18}O_{4}+K]^+ 301.08367, found 301.08444.

(E)-butyl 3-(3-cinnamoyl-4-hydroxyphenyl)acrylate (chalcone nBu acrylate) 206

To a microwave vial, KOH (992 mg, 1.64 mmol) and THF (2 mL) were added. Acetophenone 201 (107.2 mg, 0.41 mmol) and benzaldehyde 59 (0.083 mL, 0.82 mmol) were then added to the vial, and the reaction stirred under microwave irradiation at 200W for 15 minutes at 50 °C. Solvent was then removed in vacuo, and 1.0 M HCl (20 mL) added and the solution transferred to a separating funnel. Following extraction with 3 × EtOAc (20 mL), and washing of the combined organic phase with H_2O (20 mL) and brine (20 mL), the organic phase was dried over MgSO_4, filtered, and the solvent was evaporated in vacuo. The desired product was obtained by column chromatography (1:1 CH_2Cl_2:Pet) as a yellow solid (124.7 mg, 87%, R_f =0.06); mp 82.5-88.2 °C; δ_H NMR (400 MHz, CDCl_3): 0.97 (3H, t, J 7.4, H_{15}), 1.39-1.49 (2H, m, H_{14}), 1.66-1.72 (2H, m, H_{13}), 4.21 (2H, t, J 6.8, H_{12}), 6.36 (1H, d, J 16.0, H_{10}), 7.03 (1H, d, J 8.7, H_{5}), 7.42-7.46 (3H, m, H_{8}, H_{9}, H'_{4}), 7.62-7.71 (5H, m, H_{3}, H_{2}', H_{3}', H_{5}', H_{6}'), 7.94 (1H, d, J 15.6, H_{7}), 8.01 (1H, d, J 2.1, H_{2}), 13.09 (1H, s, OH); δ_C NMR (100 MHz, CDCl_3): 13.9, 19.3, 30.9, 64.6, 116.8, 119.5, 119.6, 120.0, 125.6, 129.0(2×C), 129.2(2×C), 130.5, 131.4, 134.4, 134.9, 143.3, 146.5, 165.3, 167.2, 193.5; HRMS (ESI m/z): Calcd. for [C_{22}H_{22}O_{4}+H]^+ 351.15909, found 351.16099.

8.6 Chapter 5 Experimental

8.6.1 Flavonol Succinamic Acid Synthesis

N-(4-Methoxyphenyl) acetamide 225

To a round bottom flask, 4-anisidine 222 (6.036 g, 49 mmol) was dissolved in CH_2Cl_2 (20mL). Acetic anhydride (5 mL, 53 mmol) was added slowly over 1 hour, and the solution stirred at rt for 1 hour. The solution was poured into hexane (60 mL) and stirred for 1 hour. The resultant precipitate was collected by filtration, and washed with cold hexane, to give the desired acetamide as a pale shimmery-purple solid (7.93 g, 98%, R_f=0.56 (EtOAc)); mp 129.6-130.5 °C (lit. 129-130 °C); δ_H NMR (270 MHz, CDCl_3): 2.11 (3H, s, COCH_3), 3.76 (3H, s, OCH_3), 6.80-6.83 (2H, m, H3), 7.34-7.38 (2H, m, H2), 7.56 (1H, br s, NH).
N-(3-acetyl-4-hydroxy-phenyl)-acetamide (acetophenone acetamide) \(^{224}\)

To a round bottom flask, \(N\)-(4-methoxyphenyl)acetamide \(^{225}\) (1.00 g, 6.04 mmol), acetyl chloride (1.25 mL, 17.6 mmol), and \(\text{CH}_2\text{Cl}_2\) (20 mL) were added. To this solution, \(\text{AlCl}_3\) (2.75 g, 20.6 mmol) was added in portions over 1 hour, and the solution refluxed for 5 hours. The mixture was then cooled for 16 hours with stirring, and cold \(\text{H}_2\text{O}\) added. The solution was extracted with \(3 \times \text{CH}_2\text{Cl}_2\), the organic phase dried over \(\text{MgSO}_4\), and solvent evaporated. Recrystallisation by EtOH gave the desired product as a pale green solid (476.4 mg, 41%, \(R_f=0.10\) (2:1 Pet:EtOAc)); mp 169.0-171.3 °C; \(\delta^1\text{H}\) NMR (270 MHz, \(\text{CDCl}_3\): 2.16 (3H, s, H\(_8\)), 2.58 (3H, s, H\(\text{I}\)), 6.91 (1H, d, \(J=8.9\), H\(\text{3}\)), 7.26 (1H, br s, NH), 7.29-7.34 (1H, m, H\(\text{4}\)), 8.15 (1H, d, \(J=2.3\), H\(\text{6}\)), 12.09 (1H, s, OH).

3,4-Dimethoxybenzaldehyde \(^{226}\)

To a round bottom flask, 3,4-dihydroxybenzaldehyde \(^{223}\) (69 mg, 0.5 mmol), \(\text{K}_2\text{CO}_3\) (173 mg, 1.25 mmol), and acetone (10mL) were added, and a reflux condenser attached. The solution was refluxed for 1 hour, before the slow addition of \(\text{Me}_2\text{SO}_4\) (0.142 mmol, 1.5 mmol) over an hour, and further reflux for an hour. The solution was then quenched with \(\text{H}_2\text{O}\). The solution was extracted with \(3 \times \text{EtOAc}\), and the organic phase washed with \(\text{H}_2\text{O}\) and brine. The organic phase was then dried over \(\text{MgSO}_4\), and solvent removed \textit{in vacuo}. The desired product was obtained after column chromatography (2:1 Pet:EtOAc) as a pale yellow oil (77.0 mg, 93%, \(R_f=0.39\)); \(\delta^1\text{H}\) NMR (270 MHz, \(\text{CDCl}_3\): 3.89 (3H, s, H\(\text{6}\)), 3.92 (3H, s, H\(\text{5}\)), 6.93 (1H, d, \(J=8.2\), H\(\text{3}\)), 7.35-7.43 (2H, m, H\(\text{2}\), H\(\text{4}\)), 9.80 (1H, s, CHO); \(\delta^1\text{C}\) NMR (67.5 MHz, \(\text{CDCl}_3\): 56.1, 56.2, 108.9, 110.4, 127.0, 130.2, 149.6, 154.5, 191.0; MS (ESI \(m/z\)): Calcd. for [\(\text{C}_9\text{H}_{10}\text{O}_3+\text{H}\)]\(^+\) 167.07, found 167.07.

3,4-Dibenzyloxybenzaldehyde \(^{227}\)

To a round bottom flask, 3,4-dihydroxybenzaldehyde \(^{223}\) (554 mg, 4.0 mmol), \(\text{K}_2\text{CO}_3\) (1.328 g, 9.6 mmol) and EtOH (10 mL) were added. Benzyl bromide (1.048 mL, 8.8 mmol) was added, and the solution stirred at rt for 72 hours. Solvent was removed \textit{in vacuo}, and the solid dissolved in \(\text{CH}_2\text{Cl}_2\). The organic phase was washed with 5% \(\text{NaOH}\) solution, and dried over \(\text{MgSO}_4\), before evaporation of the solvent. Recrystallisation in EtOH gave the desired product as a pale brown solid (945.2 mg, 74%, \(R_f=0.76\) (2:1 Pet:EtOAc));
mp 95.0-96.5 °C; δH NMR (270 MHz, CDCl3): 5.21 (2H, s, H7), 5.25 (2H, s, H7), 7.00 (1H, d, J 8.2, H5), 7.30-7.52 (12H, m, H2, H6, H9, H10, H11), 9.80 (1H, s, CHO).

3-(3,4-dibenzyloxyphenyl)-1-(2-hydroxy-5-acetamidophenyl)prop-2-en-1-one (chalcone acetamide) 228

To a round-bottom flask, KOH (112 mg, 2.0 mmol) and EtOH (10 mL) were added and stirred to dissolve the base. Acetophenone acetamide 224 (96.6 mg, 0.5 mmol) was added and stirred, followed by the addition of 3,4-dibenzyloxybenzaldehyde 227 (159.2 mg, 0.5 mmol). The solution was then stirred at 40 °C for 16 hours, and the solvent was removed. The dry red powder was acidified with 1M HCl, and extracted with 3 × EtOAc (20 mL). The organic solution was transferred to a separating funnel, then washed with H2O (20 mL) and brine (20 mL), and dried over MgSO4 and filtered. The desired product was obtained by recrystallisation (1:1 THF:Pet) as a bright yellow, fluffy solid (194.9 mg, 79%, Rf =0.28 (EtOAc)); mp 202.6- 204.7 °C; δH NMR (270 MHz, CDCl3): 2.18 (3H, br s, H11'), 5.20 (4H, br s, H7), 6.90-6.96 (2H, br s, H5, H3'), 7.13 (1H, br s, H2), 7.23-7.46 (14H, br m, H4', H8', H9', H6, H9, H10, H11), 7.76-7.81 (1H, br m, H6'), 8.28 (1H, br s, NH), 12.74 (1H, br s, OH); δC NMR (67.5 MHz, CDCl3): 24.5, 71.0, 71.6, 114.3, 115.1, 118.0, 118.9, 119.7, 121.6, 123.8, 127.3, 127.6, 128.0, 128.1, 128.7, 129.1, 136.6, 136.9, 146.0, 149.0, 151.9, 160.6, 168.5, 193.5; HRMS (ESI m/z): Calcd. for [C31H27NO5+H]+ 494.19620, found 494.19625.

Dimethyldioxirane (DMDO) 234

Water (250 mL), acetone (190 mL) and NaHCO3 (58 g, were vigorously stirred in a 1L round bottomed flask at rt. Oxone (120 g) was then added in three portions over 20 minutes. Stirring was continued for 20 minutes at which time a vacuum of 80-100 torr was applied, and the distillate was collected in a cold trap (-78 °C). The trapped solution was dried (K2CO3), filtered, and collected to yield DMDO at a concentration of approximately 0.1 M in acetone, as determined by 1H NMR titration with thioanisole.
6-acetamido-2-(3′,4′-bis(benzyloxy)phenyl)-3-hydroxy-4H-chromen-4-one (flavonol acetamide) 238

Chalcone acetamide 228 (121.9 mg, 0.25 mmol) was dissolved in EtOH in a round bottom flask, and cooled to 0 °C. NaOH solution (5%, 2mL), was added and stirred for 10 minutes. H₂O₂ solution (15% in H₂O, 2 mL) was added, and the solution stirred at 0 °C for two hours. The reaction was then stirred for 16 hours at rt, and carefully neutralised using 0.1M HCl. The resultant precipitate was collected by filtration, and dried under vacuum to give the desired flavonol as a pale yellow solid (114.7 mg, 91%); mp 228.6-230.2 °C (lit. 229-230 °C) 62; δ_H NMR (270 MHz, CDCl₃): 2.22 (3H, br s, H10), 5.25 (4H, br s, H7ʹ), 6.89 (1H, v br s, NH), 7.03 (1H, d, J 8.2, H5'), 7.27-7.51 (11H, m, H8, H6', H2', H9', H10'c, H10'd, H11'), 7.67 (1H, br s, OH), 7.80-7.95 (3H, m, H5, H10'a, H10'b), 8.24 (1H, br d, J 7.7, H7); δ_C NMR (67.5 MHz, CDCl₃): 24.6, 71.0, 71.6, 114.0, 114.2, 114.5, 119.0, 120.7, 122.1, 124.0, 126.7, 127.3, 127.6, 128.1, 128.6, 128.7, 135.0, 136.7, 137.1, 137.6, 145.4, 148.6. 150.8, 151.9, 168.7, 172.7; MS (ESI m/z): Calcd. for [C₃₁H₂₅NO₆+H]⁺ 508.17, found 508.17.

8.6.2 Flavonol tBu Acrylate Synthesis

(E)-tert-butyl 3-(2-(3′,4′-bis(benzyloxy)phenyl)-3-hydroxy-4-oxo-4H-chromen-6-yl)acrylate (flavonol tBu acrylate) 243

Impure chalcone tBu acrylate 245 (approximately 1.1 g, synthesised from 1.95 mmol acetophenone 202) was dissolved in EtOH, and cooled to 0 °C. To the solution, 5% NaOH was added (10 mL), and then 15% H₂O₂ in H₂O (10 mL). The solution was then stirred for 16 hours at room temperature, neutralised (1.0 M HCl), transferred to a separating funnel and extracted (3 × EtOAc). The organic phase was washed (H₂O and brine, 20 mL each), then dried over MgSO₄ and filtered, and the solvent evaporated in vacuo. Purification by column chromatography (4:1 CH₂Cl₂:Pet) isolated the desired flavonol in 43% from the acetophenone (479.4 mg, 0.83 mmol, R_f=0.06); δ_H NMR (270 MHz, CDCl₃): 1.55 (9H,
8.6.3 Flavonol Propanoic Acid Synthesis

**Butyl 3-(3-acetyl-4-hydroxyphenyl)propanoate (acetophenone nBu ester)** 250

Acetophenone nBu acrylate 201 (156.7 mg, 0.60 mmol) was added to a round bottom flask with Pd/C (20 mg), MeOH (5mL) and toluene (3 mL). A hydrogen-filled gas bladder was equipped, and the solution stirred for 16 hours at room temperature. The solution was then filtered through celite, and the solvent removed to give the desired product as a brown oil (158.3 mg, 100 %, Rf =0.72 (2:1 Pet: EtOAc)); δH NMR (270 MHz, CDCl₃): 0.88 (3H, t, J 7.4, H₁₃), 1.22-1.37 (2H, m, H₁₂), 1.49-1.60 (2H, m, H₁₁'), 2.58-2.61 (5H, m, H₂ & H₂'), 2.88 (2H, t, J 7.6, H₇'), 4.03 (2H, t, J 6.6, H₁₀'), 6.87 (1H, d, J 6.6, H₃'), 7.27-7.31 (1H, d, J 2.3, H₆'), 12.10 (1H, s, OH); δC NMR (67.5 MHz, CDCl₃): 13.8, 19.2, 26.7, 30.1, 30.7, 36.1, 64.5, 118.5, 119.5, 130.2, 131.0, 136.8, 161.0, 172.8, 204.5; HRMS (ESI m/z): Calcd. for [C₁₅H₂₀O₄+H]+ 265.14344, found 265.14317.

(E)-3-(3-(3,4-bis(benzyloxy)phenyl)acryloyl)-4-hydroxyphenyl)propanoic acid (chalcone propanoic acid) 251

To a round bottom flask, KOH (67.3 mg, 1.20 mmol) and EtOH (10 mL) were added and stirred to dissolve the base. Acetophenone nBu ester 250 (90.0 mg, 0.34 mmol) was added and stirred, followed by the addition of benzaldehyde 227 (106 mg, 0.34 mmol). The solution was stirred for 16 hours, after which time the solvent was removed in vacuo. The solution was acidified with 1.0 M HCl (20 mL), and extracted with 3 × EtOAc (20 mL). The organic phase was then washed with H₂O (20 mL) and brine (20 mL), and then dried (MgSO₄) and filtered. Evaporation of the solvent in vacuo, followed by column chromatography (2:1 Pet: EtOAc) gave the desired chalcone as a
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bright yellow solid (82.3 mg, 48%, \( R_f = 0.06 \) (2:1 Pet:EtOAc)); mp 130-132.3 °C; \( \delta_H \) NMR (270 MHz, CDCl\(_3\)): 2.64 (2H, t, \( J = 7.5 \), H8'), 2.92 (2H, t, \( J = 7.4 \), H7'), 5.19 (2H, s, H10), 5.21 (2H, s, H10), 6.88-6.94 (2H, m, H3' & H6), 7.20-7.48 (15H, m, H4', H6', H5, H9, H12, H13, H14, OH), 7.62-7.81 (2H, m, H2 & H3); \( \delta_C \) NMR (67.5 MHz, CDCl\(_3\)): 30.0, 35.9, 71.0, 71.6, 114.2, 114.6, 118.0, 118.9, 120.0, 124.1, 127.3, 127.5, 128.1, 129.0, 130.3, 136.4, 136.6, 137.0, 145.7, 149.0, 151.8, 162.2, 178.6, 193.5; HRMS (ESI m/z): Calcd. for [C\(_{32}\)H\(_{28}\)O\(_6\)+H]+ 509.19587, found 509.19314.

8.6.4 Enalapril Derivative Synthesis: Strategy A

**Pro-OBn 259\(^{620}\)**

Benzyl alcohol (3.0 mL) and SOCl\(_2\) (0.5 mL) were added to an RBF at 0 °C. L-Proline 261 (228 mg, 2.0 mmol) was then added, and the solution stirred for 48 hours whilst warming to room temperature. HCl was removed *in vacuo*, and ether added until the solution became opaque. The RBF was cooled on ice, and the white crystalline material was filtered, washed with cold ether, and dried on vacuum to give the desired protected proline as a white solid (382.9 mg, 95%, \( R_f = 0.0(3:1 \text{ EtOAc:Pet}) \)); mp 125.1-128.5 °C; \( \delta_H \) NMR (270 MHz, CDCl\(_3\)): 1.98-2.15 (3H, m, H2, H3a), 2.32-2.42 (1H, m, H3b), 3.52 (2H, br s, H1), 4.49 (1H, br s, H4), 5.13-5.26 (2H, m, H5), 7.33 (5H, br s, H6, H7, H8), 9.10 (1H, br s, NH), 10.74 (1H, br s, NH).

**Pyr-Pro-OBn 257\(^{621}\)**

Pro-OBn 259 (300 mg, 1.46 mmol), pyruvic acid 260 (0.114 mL, 1.60 mmol), EDCI (425.7 mg, 2.22 mmol) and HOBt (40.5 mg, 0.30 mmol) were stirred in CH\(_2\)Cl\(_2\) for 16 hours. Additional CH\(_2\)Cl\(_2\) was added, and the organic washed with brine and H\(_2\)O, and dried over MgSO\(_4\). Filtration and removal of the solvent *in vacuo*, followed by column chromatography (2:1 Pet: EtOAc) gave the desired compound as an off-white solid (193.1 mg, 48%, \( R_f = 0.37(30\% \text{ EtOAc:Pet}) \)); mp 43.8-47.4°C (lit.43-47 °C)\(^{621}\); \( \delta_H \) NMR (270 MHz, CDCl\(_3\)): 1.69-2.31 (4H, m, H2 & H3), 2.35 (2H, s, H9), 2.42 (1H, s, H9) 3.50-3.83 (2H, m, H1), 4.52-4.56 (0.33H, m, H4), 4.88-4.93 (0.66H, m, H4), 5.07-5.23 (2H, m, H5), 7.33 (5H, br s, H6, H7 & H8).
Fmoc-Lys(Boc)-OBn 263

Fmoc-Lys(Boc)-OH 262 (393.6 mg, 0.84 mmol) and NEt₃ (0.156 mL, 1.12 mmol) were added to an RBF and stirred in EtOAc. Benzyl bromide (0.124 mL, 1.04 mmol) was added, and the reaction stirred for 5 hours at 78 °C. Solvent was evaporated in vacuo, H₂O added (20 mL), and the solution acidified to neutral pH using 0.1M HCl. The compound was transferred to a separating funnel, extracted (3 × EtOAc, 20 mL) and washed (H₂O, brine, 20 mL of each), and the organic phase dried over MgSO₄ and filtered. The solvent was removed under reduced pressure, and purified by column chromatography (1:2 EtOAc:Pet), to give the tri-protected lysine as a clear oil (469.0 mg, 100%, Rᵥ=0.86); δH NMR (270 MHz, CDCl₃): 1.15-1.42 (13H, m, H₉, H₅, H₄), 1.65-1.85 (2H, m, H₃), 3.04 (2H, br s, H₆), 4.17-4.23 (1H, m, H₂), 4.32-4.56 (4H, m, H₁₄, H₁₅, H₇), 5.09-5.18 (2H, m, H₁₀), 5.47 (1H, br s, NH), 7.25-7.40 (9H, m, H₁₁, H₁₂, H₁₃, H₁₅, H₁₇, H₁₈), 7.58-7.60 (2H, m, H₁₆), 7.74-7.78 (3H, m, H₁₉, NH).

Lys(Boc)-OBn 258

Fmoc-Lys(Boc)-OBn 263 (469.0 mg, 0.84 mmol), was dissolved in 20% piperidine in DMF (8 mL), and stirred for 2 hours at rt. The solvent was removed in vacuo, the solid redissolved in CH₂Cl₂ (20 mL), transferred to a separating funnel, washed with H₂O (20 mL), and the organic phase dried over MgSO₄ and filtered. Solvent removal in vacuo, followed by column chromatography (3% MeOH in CHCl₃), gave the desired product as a clear oil (224.8 mg, 80%, Rᵥ=0.12); δH NMR (270 MHz, CDCl₃): 1.23-1.35 (11H, m, H₉, H₄), 1.51-1.74 (2H, m, H₅), 1.80 (2H, br s, NH₂), 2.11 (2H, br s, H₃), 3.02-3.08 (2H, m, H₆), 3.50 (1H, br s, H₂), 4.53 (1H, br s, H₇), 5.13 (2H, s, H₁₀), 7.31 (5H, s, H₁₁, H₁₂, H₁₃); δC NMR (67.5 MHz, CDCl₃): 22.8, 28.5, 34.3, 40.3, 54.4, 66.7, 79.1, 120.0, 128.4, 128.5, 128.7, 135.7, 156.1, 175.7; MS (ESI m/z): Calcd. for [C₁₉H₂₈N₂O₄+H]⁺ 337.21, found 337.21.
8.6.5 Enalapril Derivative Synthesis: Strategy B

**Bromoacetyl-Pro-OBn 267**

Pro-OBn 259 (300 mg, 1.46 mmol), bromoacetic acid 268 (223 mg, 1.59 mmol), HOBT (40.5 mg, 0.3 mmol), EDCI (425.7 mg, 2.22 mmol) and CH₂Cl₂ were stirred overnight in an RBF. The solution was transferred to a separating funnel, additional CH₂Cl₂ was added (20 mL), and the solution was washed twice with brine (20 mL). The organic phase was separated, dried (MgSO₄) and filtered, and solvent evaporated in vacuo. Purification by column chromatography (2:1 Pet:EtOAc) gave the desired compound as a clear oil (228.6 mg, 48%, R_f = 0.18); δ_H NMR (270 MHz, CDCl₃): 1.92-2.08 (3H, m, H2 & H3a), 2.11-2.26 (1H, m, H3b), 3.55-3.72 (2H, m, H1), 4.04 (2H, m, H9), 4.52-4.59 (1H, m, H4), 5.09-5.18 (2H, m, H5), 7.28-7.37 (5H, m, H6, H7 & H8); δ_C NMR (67.5 MHz, CDCl₃): 22.4, 24.9, 29.1, 31.4, 41.9, 47.2, 59.4, 67.0, 128.2, 128.4, 128.7, 135.6, 165.2, 171.6; MS (ESI m/z): Calcd. for [C₁₄H₆BrNO₃+H]+ 326.04, found 326.04.

**BnO-Pro-COCH₂-Lys(Boc)-OBn 266**

To a round bottom flask, Lys(Boc)-OBn 258 (52.6 mg, 0.16 mmol) was added and dissolved in DMF (5 mL), and the solution cooled to 0 °C. To this cooled solution, NaH (3.8 mg, 0.16 mmol) was added and stirred for 1 hour. Bromoacetyl-Pro-OBn 267 (51.0 mg, 0.16 mmol) was added, and the solution warmed to room temperature with stirring for 16 hours. The solvent was evaporated in vacuo, CH₂Cl₂ added (20 mL) and the solution filtered. The solvent was removed in vacuo to afford the desired compound as a pale yellow oil (61.7 mg, 68%, R_f =0.05); δ_H NMR (270 MHz, CDCl₃): 1.34-1.41 (13H, m, H4, H5, H9), 1.62-1.72 (2H, m, H3),1.84-2.00 (3H, m, H4', H5'a), 2.07-2.20 (1H, m, H5'b), 3.01-3.09 (2H, m, H6), 3.23-3.69 (5H, m, H1', H3', H2'), 4.46-4.58 (1H, m, H6'), 5.09-5.16 (4H, m, H8', H1''), 7.29-7.34 (10H, m, H10', H11', H12', H3'', H4'', H5''); δ_C NMR (67.5 MHz, CDCl₃): 24.7, 25.0, 28.5(3×C), 32.3, 40.3, 46.1, 46.7, 47.2, 49.2, 59.0, 60.8, 66.8, 67.0, 79.1, 127.1, 127.6, 128.1, 128.2, 128.3, 128.4, 128.6, 128.6, 128.7, 128.7, 135.7, 135.8,
162.4, 169.4, 171.9, 172.2; HRMS (ESI m/z): Calcd. for \([C_{32}H_{43}N_3O_7+H]^+\) 582.31738, found 582.31753.

**BnO-Pro-COCH\_2-Lys-OBn 265**

BnO-Pro-COCH\_2-Lys(Boc)-OBn 266 120.0 mg, 0.206 mmol) was stirred in 20% TFA in CH\_2Cl\_2 (6 mL) for two hours. Then, 2\times CH\_2Cl\_2 (10 mL), toluene (10 mL), and CHCl\_3 (10 mL) were added and then evaporated *in vacuo* to remove unwanted side products and give the desired amine as a pale yellow oil (99.2 mg, quant., \(R_f = 0.00\) (3:1 EtOAc:Pet); \(\delta_H\) NMR (270 MHz, CDCl\_3): 1.59-2.34 (10H, m, H\_3, H\_4, H\_5, H\_4\text{''}, H\_5\text{''}), 2.89-2.97 (2H, m, H\_6), 3.25-3.74 (4H, m, H\_1\text{'} & H\_3\text{''}), 4.03 (1H, br s, H\_2), 4.51 (1H, br s, H\_6\text{'}), 4.99-5.25 (4H, m, H\_8 & H\_1\text{''}), 7.30 (10H, br s, H\_10', H\_11', H\_12', H\_3'', H\_4'' & H\_5''), 7.79 ppm (1H, br s, NH), 9.63 (2H, br s, NH\_2); HRMS (ESI m/z): Calcd. for \([C_{27}H_{35}N_3O_5+H]^+\) 482.26495, found 482.26334.

### 8.7 Chapter Six Experimental

#### 8.7.1 Flavonol-Dipeptides

3',4'-Dibenzyloxyflavonol 281 291, 626

To a solution of ethanol (8 mL) and 1,4-dioxane (4 mL) was added 2'-hydroxyacetophenone 72 (280 µL, 2.32 mmol) and 3,4-dibenzyloxybenzaldehyde 227 (728 mg, 2.29 mmol). KOH solution (40%, 2 mL) was then added dropwise, and the reaction stirred for five days. The solution was diluted with CH\_2Cl\_2 (20 mL), transferred to a separating funnel and the organic washed with H\_2O (20 mL), dried (MgSO\_4), and filtered. The solvent was evaporated *in vacuo*, and the residue dissolved in 1,4-dioxane (8 mL) and ethanol (20 mL). NaOH was added as a 2 M solution (6 mL), and the reaction mixture cooled to 0 °C. Hydrogen peroxide (30%, 2 mL) was then added dropwise, and the reaction stirred at 0 °C for 2hrs. The reaction was left to stir overnight at rt, and then acidified with 2 M HCl. The resultant suspension was filtered, and washed with ethanol (20 mL).
Recrystallisation from hot EtOAc gave the desired flavonol as a pale yellow, fibrous solid (541.2 mg, 53%, R<sub>f</sub>=0.63 (2:1 Pet:EtOAc)); mp 148.5-151.8 °C (lit. 145.8-146.8 °C); δ<sub>H</sub> NMR (400 MHz, CDCl<sub>3</sub>): 5.21-5.26 (4H, m, H<sub>7</sub>ʹ), 6.97 (1H, br s, OH), 7.05 (1H, d, J 8.7, H2'), 7.30-7.53 (12H, m, H5', H6', H9', H10', H11'), 7.68 (1H, td, J 7.8, J 1.4, H6), 7.84 (1H, dd, J 8.5, J 1.8, H8), 7.93 (1H, d, J 2, H7), 8.22 (1H, dd, J 8.0, J 1.4, H5); δ<sub>C</sub> NMR (100 MHz, CDCl<sub>3</sub>): 71.0, 71.6, 114.2, 114.6, 118.2, 122.1, 124.5, 125.5, 127.3(2×C), 127.6(2×C), 128.1(2×C), 128.6(2×C), 128.7(2×C), 133.5, 136.8, 137.2, 137.9, 145.0, 146.7, 150.8, 155.3, 173.2; MS (ESI m/z): Calcd. for [C<sub>29</sub>H<sub>22</sub>O<sub>5</sub>+H]<sup>+</sup> 451.15, found 451.15.

**Fmoc-Ala-Pro-OBn 287**

Fmoc-Ala-OH 283 (155.7 mg, 0.50 mmol), EDCI (191.7 mg, 1.0 mmol), HOBt (15.3 mg, 0.1 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL) were stirred in a round bottom flask under N<sub>2</sub> for 10 minutes, before addition of Pro-OBn 259 (100 mg, 0.49 mmol). The solution was stirred at room temperature for 16 hours, then CH<sub>2</sub>Cl<sub>2</sub> added (20 mL), the solution transferred to a separating funnel, and the organic phase washed with 0.1 M HCl (20 mL), H<sub>2</sub>O (20 mL) and brine (2×20 mL). The organic phase was dried over MgSO<sub>4</sub>, filtered, and solvent removed in vacuo. Purification by column chromatography (2:1 Pet:EtOAc) isolated the dipeptide as a viscous oil (131.1 mg, 54%, R<sub>f</sub>=0.22); δ<sub>H</sub> NMR (400 MHz, CDCl<sub>3</sub>): 1.36 (3H, d, J 6.4, H3'), 1.90-2.10 (3H, m, H3, H4a), 2.20-2.24 (1H, m, H4b), 3.60-3.69 (2H, m, H2), 4.21-4.35 (3H, m, H5, H2', H3"), 4.53-4.62 (2H, m, H3"), 5.11-5.23 (2H, dd, J 17.0, J 8.4, H7), 5.88 (1H, br s, NH), 7.31-7.76 (13H, m, H9, H10, H11, H5", H6", H7", H8"; δ<sub>C</sub> NMR (67.5 MHz, CDCl<sub>3</sub>): 18.4, 25.0, 29.0, 46.9, 47.2, 48.4, 59.0, 67.1, 120.0(2×C), 125.3(2×C), 127.1(2×C), 127.8(2×C), 128.3(2×C), 128.4(4×C), 128.7(2×C), 135.6, 141.4, 144.0, 155.8, 171.4, 172.7; MS (ESI m/z): Calcd. for [C<sub>30</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub>+H]<sup>+</sup> 499.22, found 499.22.

**Fmoc-Lys(Boc)-Pro-OBn 288**

Fmoc-Lys(Boc)-OH 284 (237.3 mg, 0.50 mmol), EDCI (191.7 mg, 1.0 mmol), HOBt (15.3 mg, 0.1 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL) were stirred in an RBF under N<sub>2</sub> for 10 minutes, before addition of Pro-OBn 259 (100 mg, 0.49 mmol). The solution was stirred at room temperature for 16 hours, then CH<sub>2</sub>Cl<sub>2</sub> added (20 mL), the solution
transferred to a separating funnel, and the organic phase washed with 0.1 M HCl (20 mL), H₂O (20 mL) and brine (2×20 mL). The organic phase was dried over MgSO₄, filtered, and solvent removed in vacuo. Purification by column chromatography (2:1 Pet:EtOAc) isolated the dipeptide as a viscous oil (206.1 mg, 63%, Rₚ =0.22 (2% MeOH/CHCl₃)); δH NMR (400 MHz, CDCl₃): 1.25-1.42 (13H, m, H₄, H₅, H₉), 1.63-2.21 (5H, m, H₃, H₂, NH), 2.97-3.19 (2H, m, H₄), 3.45-3.71 (2H, m, H₆'), 4.18-4.81 (6H, m, H₃', H₅, H₂', H₂''), 5.10-5.23 (2H, m, H₇), 5.68 (1H, br s, H₃''), 7.25-7.76 (14H, m, H₉, H₁₀, H₁₁, H₅'', H₆'', H₇'', H₈'', NH); HRMS (ESI m/z): Calcd. for [C₂₄H₃₃N₃O₇+H]+ 476.23913, found 476.24002.

Fmoc-Gln-Pro-OBn 289

Fmoc-Gln-OH 285 (368.4 mg, 1.0 mmol), EDCI (383.4 mg, 2.0 mmol), HOBT (30.6 mg, 0.2 mmol) and CH₂Cl₂ (10 mL) were stirred in a round bottom flask under N₂ for 10 minute, before addition of Pro-OBn 259 (200 mg, 0.98 mmol). The solution was stirred at room temperature for 16 hours, then CH₂Cl₂ added, the solution transferred to a separating funnel, and the organic phase washed with 0.1 M HCl (20 mL), H₂O (20 mL) and 2×brine (20 mL). The organic phase was dried over MgSO₄, filtered, and solvent removed in vacuo. Purification by column chromatography (2:1 Pet:EtOAc) isolated the dipeptide as a viscous oil (298.6 mg, 46%, Rₚ =0.02 (3% MeOH/CHCl₃)); δH NMR (270 MHz, CDCl₃): 1.86-2.68 (8H, m, H₃, H₄, H₃', H₄'), 3.68 (2H, br s, NH₂), 4.14-4.64 (4H, m, H₂, H₅, C₂'), 5.07-5.21 (2H, m, H₇), 6.03-6.27 (3H, m, H₂', H₃'), 7.26-7.96 (13H, m, H₉, H₁₀, H₁₁, H₅'', H₆'', H₇'', H₈''), 9.60 (1H, br s, NH); δC NMR (67.5 MHz, CDCl₃): 25.1, 28.6, 29.3, 30.9, 47.3, 51.5, 53.7, 59.2, 63.0, 67.1, 67.2, 119.2, 120.1, 125.1, 125.2, 126.0, 127.1(2×C), 127.3(2×C), 127.8(2×C), 128.6(2×C), 128.8(2×C), 136.7, 141.4, 143.8, 156.4, 164.7, 170.6, 172.0; HRMS (ESI m/z): Calcd. for [C₃₂H₃₃N₃O₆+H]+ 556.24421, found 556.24413.
Fmoc-Glu(OtBu)-Pro-OH 286 (425.5 mg, 1.0 mmol), EDCI (383.4 mg, 2.0 mmol), HOBt (30.6 mg, 0.2 mmol) and CH₂Cl₂ (10 mL) were stirred in a round bottom flask under N₂ for 10 minute, before addition of Pro-OBn 259 (200 mg, 0.98 mmol). The solution was stirred at room temperature for 16 hours, then CH₂Cl₂ added, the solution transferred to a separating funnel, and the organic phase washed with 0.1 M HCl (20 mL), H₂O (20 mL) and 2×brine (20 mL). The organic phase was dried (MgSO₄), filtered, and solvent removed in vacuo. Purification by column chromatography (5% MeOH/CHCl₃) isolated the dipeptide as a viscous oil (327.6 mg, 55%, Rᶠ=0.53 (5% MeOH/CHCl₃)); δH NMR (270 MHz, CDCl₃): 1.46 (9H, br s, H7ʹ), 1.75-2.41 (8H, m, H3, H4, H3ʹ, H4ʹ), 3.67-3.73 (2H, m, H2), 4.16-4.21 (1H, br s, NH), 4.31-4.34 (2H, H5, H2ʹ), 4.58-4.65 (2H, m, H2ʺ), 5.10-5.22 (2H, m, H7), 5.90 (1H, br s, H3ʺ), 7.22-7.74 (13H, m, H9, H10, H11, H5ʺ, H6ʺ, H7ʺ, H8ʺ); δC NMR (100 MHz, CDCl₃): 25.0, 27.9, 28.2, 29.1, 30.8, 47.3, 50.7, 51.7, 59.1, 67.0, 80.6, 120.1, 125.3, 127.2(2×C), 127.8, 128.3(2×C), 128.4, 128.7(2×C), 135.7, 141.4, 143.9, 156.3, 170.8, 171.6, 172.3; 25.0, 27.9, 28.2, 29.1, 30.8, 47.3, 50.7, 51.7, 59.1, 67.0, 80.6, 120.1, 125.3, 127.2(2×C), 127.6(4×C), 127.8(2×C), 128.3(2×C), 128.4(2×C), 128.7(4×C), 137.7, 141.4, 143.9, 156.3, 170.8, 171.6, 172.3; HRMS (ESI m/z): Calcd. for [C₃₆H₄₀N₂O₇+H]⁺ 613.29083, found 613.28880.

Fmoc-Ala-Pro-OH 291

Fmoc-Ala-Pro-OH 291 (248.4 mg, 0.498 mmol) was added to a solution of Pd/C (25 mg) in MeOH (10 mL) under N₂. A hydrogen (H₂) balloon was added, and the reaction was stirred for 16 hours at room temperature. The solution was then filtered through a celite plug, and purification by column chromatography (5% MeOH/ CHCl₃) isolated the deprotected dipeptide as a viscous oil (186.8 mg, 92%, Rᶠ=0.02 (3%MeOH/CHCl₃)); δH NMR (270 MHz, CDCl₃): 1.34 (3H, d, J 6.4, H3ʹ), 1.95-2.07 (4H, m, H3, H4), 3.58-3.67 (2H, m, H2), 4.14-4.57 (5H, m, H5,
H2', H2", NH), 6.24 (1H, br s, H3"), 7.24-7.74 (11H, m, H5", H6", H7", H8"); δC NMR (67.5 MHz, CDCl3): 18.4, 25.0, 29.0, 46.9, 47.2, 48.4, 59.0, 120.0(2xC), 125.3(2xC), 127.1(2xC), 128.3, 128.4(2xC), 128.7(2xC), 141.4, 144.0, 155.8, 171.4, 172.7; HRMS (ESI m/z): Calcd. for [C23H24N2O5+H]+ 409.17635, found 409.17553.

**Fmoc-Lys(Boc)-Pro-OH 292**

Fmoc-Lys(Boc)-Pro-OBn 288 (192.9 mg, 0.288 mmol) was added to a solution of Pd/C (41 mg) in MeOH (10 mL) under N2. A hydrogen (H2) balloon was added, and the reaction was stirred for 16 hours at room temperature. The solution was then filtered through a celite plug, and purification by column chromatography (5% MeOH/CHCl3) isolated the deprotected dipeptide as a viscous oil (96.0 mg, 59%, Rf =0.02(5% MeOH/CHCl3); δH NMR (270 MHz, CDCl3): 1.40-2.19 (18H, m, H3, H2', H4', H5', H9', NH), 2.92-3.14 (2H, m, H4), 3.60-3.76 (2H, m, H6'), 4.12-4.55 (6H, m, H5, H2', H3', H2"), 6.19 (1H, br s, H3"), 6.86-7.72 (9H, m, H5", H6", H7", H8", NH); δC NMR (67.5 MHz, CDCl3); HRMS (ESI m/z): Calcd. for [C31H39N3O7+H]+ 565.27825, found 565.28167.

**Fmoc-Gln-Pro-OH 293**

Fmoc-Gln-Pro-OBn 289 (198.6 mg, 0.537 mmol) was added to a solution of Pd/C (30 mg) in MeOH (10 mL) under N2. A hydrogen (H2) balloon was added, and the reaction was stirred for 16 hours at room temperature. The solution was then filtered through a celite plug, and purification by column chromatography (5% MeOH/CHCl3) isolated the deprotected dipeptide as a viscous oil (127.1 mg, 50%, Rf =0.05(5% MeOH/CHCl3)); δH NMR (270 MHz, CDCl3): 1.72-2.41 (10H, m, H3, H4, H3', H4', NH2), 3.62-3.73 (2H, m, H2), 4.10-4.58 (5H, m, H2', H3', H2"), 5.65 (1H, br s, NH), 6.64-6.76(1H, m, H3"), 7.18-7.71 (8H, m, H5", H6", H7", H8"); δC NMR (67.5 MHz, CDCl3): 25.1, 28.6, 29.3, 30.9, 47.3, 51.5, 53.7, 59.2, 67.1, 119.2, 120.1, 125.1, 125.2, 126.0, 127.3, 127.8 (2xC), 128.6(2xC), 141.4, 143.8, 156.4, 164.7, 170.6, 175.0; HRMS (ESI m/z): Calcd. for [C25H27N3O6+H]+ 466.19726, found 466.19990.
Fmoc-Glu(OtBu)-Pro-OH 294

Fmoc-Glu(OtBu)-Pro-OBn 290 (304.7 mg, 0.537 mmol) was added to a solution of Pd/C (30 mg) in MeOH (10 mL) under N₂. A hydrogen (H₂) balloon was added, and the reaction was stirred for 16 hours at room temperature. The solution was then filtered through a celite plug, and purification by column chromatography (5% MeOH/CHCl₃) isolated the deprotected dipeptide as a viscous oil (204.5 mg, 79%, R_f =0.04 (5% MeOH/CHCl₃)); δ_H NMR (270 MHz, CDCl₃): 1.42 (9H, s, H₇ʹ), 1.83-2.44 (8H, m, H₃, H₄, H₃ʹ, H₄ʹ), 3.75 (2H, m, H₂), 4.16-4.62 (5H, m, H₅, H₂ʹ, H₂ʺ, NH) 6.19 (1H, m, H₃ʺ), 7.24-7.74 (8H, m, H₅ʺ, H₆ʺ, H₇ʺ, H₈ʺ), 10.82 (1H, br s, COOH); δ_C NMR (67.5 MHz, CDCl₃): 25.0, 27.9, 28.2, 29.1, 30.8, 47.3, 50.7, 51.7, 59.1, 80.6, 120.1, 125.3, 127.2(2×C), 127.6(2×C), 127.8(2×C), 128.3, 128.7(4×C), 141.4, 143.9, 156.3, 170.8, 171.6, 172.3; HRMS (ESI m/z): Calcd. for [C₂₉H₃₄N₂O₇+H]+ 523.24388, found 523.24476.

2-(3,4-bis(benzyloxy)phenyl)-4-oxo-4H-chromen-3-yl 2-acetamidoacetate 296

In a round bottom flask, 3ʹ,4ʹ-dibenzyloxyflavonol 281 (100 mg, 0.22 mmol), EDCI (71.4 mg, 0.32 mmol), HOBt (4.2 mg, 0.032 mmol) and DMAP (4 mg, 0.032 mmol) in CH₂Cl₂ (10 mL) were added. The solution was stirred for 10 minutes, and then N-acetylglycine 295 (30 mg, 0.22 mmol) was added. The solution was stirred at room temperature for 16 hours, CH₂Cl₂ (20 mL) was added, and the solution transferred to a separating funnel. The organic phase was washed (H₂O and brine, 20 mL of each), dried (MgSO₄), filtered, and the solvent removed in vacuo. Recrystallisation in EtOH provided the compound as a pale yellow amorphous solid (88.2 mg, 72%, R_f =0.05 (2:1 Pet:EtOAc)); δ_H NMR (400 MHz, CDCl₃): 2.04 (3H, s, H₁₂), 3.46-3.47 (2H, d, J 4.8, H₁₀), 5.24 (4H, br s, H₁ʺ), 6.04 (1H, br s, NH), 7.05 (1H, d, J 8.0, H₂'), 7.31-7.51 (14H, m, H₆, H₈, H₅', H₆', H₃", H₄", H₅"), 7.69-7.73 (1H, m, H₇), 8.21 (1H, dd, J 8.0, J 1.6, H₅); δ_C NMR (100 MHz,
CDCl₃): 23.0, 41.5, 71.0, 71.6, 114.2, 114.8, 118.2, 120.7, 122.1, 123.1, 124.5, 125.5, 126.1, 127.3(2×C), 127.4(2×C), 127.6(2×C), 128.7(2×C), 128.8, 133.5, 134.1, 136.5, 137.9, 145.0, 148.8, 152.1, 156.2, 167.9, 171.7, 173.2; HRMS (ESI m/z): Calcd. for [C₃₃H₂₇NO₇+H]+ 550.18603, found 550.18467.

2-(3,4-bis(benzyloxy)phenyl)-4-oxo-4H-chromen-3-yl 1-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoyl)pyrrolidine-2-carboxylate (Fmoc-Ala-Pro-diOBn-flavonol) 297

Fmoc-Ala-Pro-OH 291 (186.8 mg, 0.46 mmol), 3′,4′-dibenzoyloxyflavonol 281 (225.3 mg, 0.50 mmol), EDCI.HCl (157.7 mg, 0.82 mmol), DMAP (10 mg, 0.08 mmol) and CH₂Cl₂ (10 mL) were added to a round bottomed flask, and stirred at rt for 16 hrs. The solution was diluted with CH₂Cl₂ (20 mL), transferred to a separating funnel, and the organic phase washed with H₂O (3×20 mL) and brine (1×20 mL), and dried (MgSO₄) and filtered. Following purification by column chromatography (2:1 Pet:EtOAc), the desired compound was isolated as a pale yellow amorphous solid (172.4 mg, 45%, Rf=0.03); δH NMR (400 MHz, CDCl₃): 1.22 (3H, m, H15), 1.70-2.71 (4H, m, H11, H12), 3.67-3.72 (2H, m, H13), 4.19-4.90 (4H, m, H10, H14, H16 & H17), 5.24-5.33 (4H, br s, H1"), 7.03-7.94 (25H, m, H6, H7, H8, H2′, H5′, H6′, H3″, H4″, H5″, H18, H19, H20, H21, NH), 8.20 (1H, d, J 5.1, H5); δC NMR (100 MHz, CDCl₃): 18.4, 25.0, 29.0, 46.9, 47.2, 48.4, 59.0, 71.0, 71.6, 114.2, 114.8, 118.2, 120.0(2×C), 120.7, 122.1, 123.1, 124.5, 125.3(2×C), 125.5, 126.1, 127.1(2×C), 127.3(2×C), 127.4(2×C), 127.6(2×C), 128.7(2×C), 128.3, 128.4(2×C), 128.7(2×C), 128.8, 133.5, 134.1, 136.5, 137.9, 141.4, 144.0, 145.0, 148.8, 152.1, 155.8, 156.2, 171.4, 172.7, 173.2; HRMS (ESI m/z): Calcd. for [C₅₂H₄₄N₂O₉+H]+ 841.31196, found 841.31330.

2-(3,4-bis(benzyloxy)phenyl)-4-oxo-4H-chromen-3-yl 1-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoyl)pyrrolidine-2-carboxylate (Fmoc-Lys(Boc)-Pro-diOBn-flavonol) 298
Fmoc-Lys(Boc)-Pro-OH 290 (188.3 mg, 0.333 mmol), 3’,4’-dibenzoyloxyflavonol 281 (153.2 mg, 0.34 mmol), EDCI.HCl (115.0 mg, 0.60 mmol), DMAP (6.7 mg, 0.06 mmol) and CH2Cl2 (10 mL) were added to a round bottomed flask, and stirred at rt for 16 hrs. The solution was diluted with CH2Cl2 (20 mL), the solution transferred to a separating funnel, and the organic phase washed with H2O (3×20 mL) and brine (1×20 mL), dried (MgSO4) and filtered. Following purification by column chromatography (2:1 Pet:EtOAc), the desired compound was isolated as a pale yellow amorphous solid (223.0 mg, 70%, Rf = 0.04); δH NMR (270 MHz, CDCl3): 1.40-2.08 (16H, m, H12a, H15, H16, H17 & H21), 2.17-2.63 (3H, m, H11, H12b), 2.96-3.42 (4H, m, H18 & H13), 4.08-4.89 (4H, m, H10, H14, H22, H23), 5.22-5.42 (4H, m, H1″), 6.60-6.63 (1H, m, H2″), 7.21-7.84 (25H, m, H6, H7, H8, H24, H25, H26, H27, H5″, H6″, H3″, H4″, H5″, 2×NH), 8.11 (1H, d, J 7.8, H5); δC NMR (67.5 MHz, CDCl3): 18.1, 22.0, 24.9, 27.9(3×C), 31.6, 40.0, 45.0, 47.3, 52.6, 57.0, 59.0, 66.5, 70.5, 70.9, 77.6, 114.0, 114.2, 118.5, 120.0(2×C), 122.3, 123.0, 123.2, 124.8, 125.4 (2×C), 127.2 (2×C), 127.6 (3×C), 127.8 (3×C), 127.9 (2×C), 128.0 (2×C), 128.4 (3×C), 128.5 (3×C), 133.1, 134.3, 137.2, 137.7, 141.3, 144.3, 148.8, 151.9, 155.4, 155.9, 156.2, 165.9, 169.8, 171.3; HRMS (ESI m/z): Calcd. for [C60H59N3O11+H]+ 998.42224, found 998.42071.

2-(3,4-bis(benzyloxy)phenyl)-4-oxo-4H-chromen-3-yl 1-(2-((((9H-fluoren-9-yl) methoxy)carbonyl)amino)-5-amino-5-oxopentanoyl)pyrrolidine-2-carboxylate (Fmoc-Gln-Pro-diOBn-flavonol) 299

Fmoc-Gln-Pro-OH 293 (127.1 mg, 0.27 mmol), 3’,4’-dibenzoyloxyflavonol 281 (126.1 mg, 0.28 mmol), EDCI.HCl (93.9 mg, 0.49 mmol), DMAP (5.6 mg, 0.05 mmol) and CH2Cl2 (10 mL) were added to a round bottomed flask, and stirred at rt for 16 hrs. The solution was diluted with CH2Cl2 (10 mL), transferred to a
separating funnel, and the organic phase was washed with H2O (3×20 mL) and brine (1×20 mL), and then dried (MgSO4) and filtered. Following purification by column chromatography (2:1 Pet:EtOAc), the desired compound was isolated as a pale yellow amorphous solid (116.2 mg, 48%, Rf =0.04); δH NMR (270 MHz, CDCl3): 1.83-2.64 (8H, m, H11, H12, H15 & H16), 3.75-4.08 (4H, m, H13 & NH2), 4.19-4.87 (4H, m, H10, H14, H22, H23), 5.34 (4H, m, H1ʺ), 7.08 (1H, d, J 8.6, H2ʺ), 7.29-7.76 (24H, m, H6, H7, H8, H5ʹ, H6ʹ, H3ʺ, H4ʺ, H5ʺ & NH), 8.18 (1H, d, J 7.6, H5); δC NMR (67.5 MHz, CDCl3): 25.1, 28.6, 29.3, 30.9, 47.3, 51.5, 53.7, 59.2, 67.1, 70.9, 71.6, 114.0, 114.8, 118.2, 120.1, 123.1, 123.4, 125.1, 125.4, 126.0, 127.8 (2×C), 127.3(3×C), 127.6(3×C), 127.8, 127.9, 128.1, 128.6(3×C), 128.7(3×C), 133.0, 134.1, 136.6, 137.2, 141.4, 143.8, 144.0, 148.8, 152.0, 155.5, 156.4, 164.7, 170.6, 171.8, 175.0; HRMS (ESI m/z): Calcd. for [C54H47N3O10+H]+ 898.33342, found 898.33259.

(S)-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromen-3-yl 1-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoyl)pyrrolidine-2-carboxylate (Flavonol-Pro-Ala-Fmoc) 301

Fmoc-Ala-Pro-diOBn-flavonol 297 (172.0 mg, 0.205 mmol) was stirred with Pd/C (20 mg), in 1,4-dioxane (7 mL) and EtOH (3 mL), under H2 gas for 16 hrs at rt. The solution was then filtered through a celite plug, and the solvent removed in vacuo to provide the deprotected product as a dark green solid (135.4 mg, quant., Rf =0.04 (5% MeOH in CHCl3); δH NMR (270 MHz, CDCl3): 1.19 (3H, m, H15), 1.96-2.70 (6H, m, H11, H12 & 2×OH), 3.64-3.73 (2H, m, H13), 4.06-4.86 (4H, m, H10, H14, H16 & H17), 7.00-7.75 (15H, m, H6, H7, H8, H2ʹ, H5ʹ, H6ʹ, H18, H19, H20, H21, NH), 8.17 (1H, d, J 5.1, H5); δC NMR (67.5 MHz, CDCl3): 22.8, 25.0, 29.8, 45.5, 47.2, 48.5, 50.8, 59.4, 67.3, 115.8, 116.8, 118.2, 120.1(2×C), 120.7, 120.9, 123.4, 125.0, 125.2(2×C), 125.8(2×C), 127.0, 127.2(2×C), 127.8(2×C), 132.1, 133.9, 141.4(2×C), 143.8(2×C), 143.9, 155.5, 156.2, 157.3, 172.0; HRMS (ESI m/z): Calcd. for [C38H32N2O9-H]- 659.20350, found 659.20139.
(S)-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromen-3-yl 1-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoyl)pyrrolidine-2-carboxylate (Flavonol-Pro-Lys(Boc)-Fmoc) 302

Fmoc-Lys(Boc)-Pro-diOBn-flavonol 298 (223.0 mg, 0.22 mmol) was stirred with Pd/C (25 mg), in 1,4-dioxane (9 mL) and EtOH (4 mL), under H2 gas for 16 hrs at rt. The solution was then filtered through a celite plug, and the solvent removed in vacuo to provide the deprotected product as a dark green solid (179.9 mg, quant., Rf = 0.03 (5% MeOH in CHCl3); δH NMR (270 MHz, CDCl3): 1.20-1.52 (16H, m, H12a, H15, H16, H17 & H21), 2.12-3.02 (3H, m, H11, H12b), 3.53-4.88 (10H, m, H18, H13, H10, H14, H22, H23 & 2×OH), 6.60-6.63 (1H, m, H2'), 7.01-7.73 (15H, m, H6, H7, H8, H24, H25, H26, H27, H5', H6', 2×NH), 8.17 (1H, d, J = 7.8, H5); δC NMR (67.5 MHz, CDCl3): 18.2, 20.0, 22.7, 25.0, 28.5(3×C), 29.8, 39.8, 42.5, 45.4, 47.2, 50.8, 59.2, 67.3, 79.4, 115.8, 116.8, 118.2, 120.1(2×C), 121.0, 123.5, 124.1, 125.2(2×C), 127.0(2×C), 127.2(2×C), 127.8(2×C), 134.0, 141.4(2×C), 143.8, 143.9(2×C)148.5, 149.1, 155.5, 156.5, 156.6, 165.8, 170.6, 172.1; HRMS (ESI m/z): Calcd. for [C46H47N3O11+H]+ 818.32834, found 818.32804.

(S)-2-(3,4-dihydroxyphenyl)-4-oxo-4H-chromen-3-yl 1-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-amino-5-oxopentanoyl)pyrrolidine-2-carboxylate (Flavonol-Pro-Gln-Fmoc) 303

Fmoc-Gln-Pro-diOBn-flavonol 299 (116.2 mg, 0.13 mmol) was stirred with Pd/C (18 mg), in 1,4-dioxane (5 mL) and EtOH (3 mL), under H2 gas for 16 hrs at rt. The solution was then filtered through a celite plug, and the solvent removed in vacuo to provide the deprotected product as a dark green solid (92.6 mg, quant., Rf = 0.02 (5% MeOH in CHCl3)); δH NMR (270 MHz, CDCl3): 1.41-2.61 (8H, m, H11,
H12, H15 & H16), 3.52-4.85 (10H, m, H10, H13, H14, H22, H23, NH2 & 2×OH), 7.01-7.75 (15H, m, H6, H7, H8, H2', H5', H6', & NH), 8.18 (1H, d, J 7.7, H5); δc NMR (67.5 MHz, CDCl3): 13.5, 14.2, 18.3, 18.4, 22.7, 25.0, 26.0, 28.2, 29.8, 32.0, 42.5, 45.6, 47.2, 58.4, 59.2, 67.2, 118.3, 119.9(2×C), 120.1(2×C), 123.4, 124.1, 125.2, 125.8, 127.0(2×C), 127.2(2×C), 127.9(2×C), 134.1, 140.6, 141.4, 143.8, 149.1, 155.5, 156.6, 164.8, 171.3, 172.0; HRMS (ESI m/z): Calcd. for [C₄₀H₄₅N₃O₁₀+H]^+ 718.23652, found 718.24062.

8.7.2 Flavonol-Tripeptides

Flavonol-diOBn-Pro-Pro-Ile-Cbz 307

To a round bottom flask, 3',4'-dibenzylxyflavonol 281 (180.2 mg, 0.40 mmol), Cbz-Ile-Pro-Pro-OH 275 (160.8 mg, 0.35 mmol), EDCI (138.0 mg, 0.72 mmol), DMAP (8.8 mg, 0.072 mmol) and CH₂Cl₂ (10 mL) were added. The solution was stirred at room temperature for 16 hours, CH₂Cl₂ (20 mL) added, and the solution transferred to a separating funnel. The organic phase was washed with 2×H₂O (20 mL), and brine (20 mL), and then dried (MgSO₄) and filtered. The solvent was removed in vacuo, and purification by column chromatography (5% MeOH in CHCl₃) provided the flavonol-tripeptide as a yellow oil (303.0 mg, 97%, Rf=0.30); δH NMR (270 MHz, CDCl₃): 0.83-1.02 (6H, m, H27, H29), 1.54-2.68 (11H, m, H11, H12, H15, H16, H26, H28), 3.55-3.87 (4H, m, H13, H17), 4.27-4.90 (3H, m, H10, H14, H19), 5.01-5.48 (6H, m, H21 & H1"), 7.03 (1H, d, J 8.6, H2'), 7.19-7.68 (21H, m, H6, H7, H8, H23, H24, H25, H5', H6', H3", H4", H5" & NH), 8.17 (1H, dd, J 8.0, J 1.6, H5); δc NMR (67.5 MHz, CDCl₃): 11.2, 15.4, 24.7, 24.8, 25.1, 28.2, 29.3, 38.0, 46.9, 47.7, 56.8, 58.1, 58.8, 66.9, 70.9, 71.2, 114.0, 114.5, 118.1, 122.4, 123.2, 123.4, 125.2, 125.9, 127.2(2×C), 127.5(2×C), 127.9, 128.1(2×C), 128.2, 128.5(3×C), 128.6(3×C), 128.7(2×C), 133.2, 134.0, 136.5, 136.7, 137.3, 148.7, 151.7, 155.5, 156.4, 170.1, 170.3, 171.0, 171.9; HRMS (ESI m/z): Calcd. for [C₅₃H₅₂N₃O₁₀+H]^+ 892.38037, found 892.38442.
Flavonol-diOBn-Pro-Pro-Val-Cbz 308

To a round bottom flask, 3',4'-dibenzoyloxyflavonol 281 (180.2 mg, 0.40 mmol), Cbz-Val-Pro-Pro-OH 276 (155.9 mg, 0.35 mmol), EDCI (138.0 mg, 0.72 mmol), DMAP (8.8 mg, 0.072 mmol) and CH₂Cl₂ (10 mL) were added. The solution was stirred at room temperature for 16 hours, then CH₂Cl₂ (20 mL) added and the solution transferred to a separating funnel. The organic phase was washed with 2×H₂O (20 mL), and brine (20 mL), and then dried (MgSO₄) and filtered. The solvent was removed in vacuo, and purification by column chromatography (5% MeOH in CHCl₃) provided the flavonol-tripeptide as a yellow oil (249.6 mg, 81%, Rₛ=0.33); δ_H NMR (270 MHz, CDCl₃): 0.91 (3H, d, J₄.₃, H27), 1.02 (3H, d, J₃.₀, H28), 1.82-2.36 (8H, m, H11, H12, H15, H16), 2.69 (1H, br s, C26), 3.53-3.83 (4H, m, H13 & H17), 4.29-4.89 (3H, m, H10, H14 & H19), 5.04-5.51 (6H, m, H21, H1”), 7.03 (1H, d, J₅.₇, H2’), 7.28-7.68 (21H, m, H6, H7, H8, H23, H24, H25, H5’, H6’, H3”, H4”, H5” & NH), 8.18 (1H, d, J₅.₁, H5); δ_C NMR (67.5 MHz, CDCl₃): 17.8, 19.5, 24.9, 25.1, 28.1, 29.2, 31.4, 46.8, 47.6, 57.6, 58.0, 58.8, 66.9, 70.9, 71.2, 114.0, 114.6, 118.1, 122.4, 123.2, 123.4, 125.2, 125.9, 127.2(2×C), 127.5(2×C), 127.7, 128.1(2×C), 128.1, 128.2, 128.65(3×C), 128.6(3×C), 128.7(2×C), 133.2, 134.0, 136.5, 136.7, 137.3, 148.7, 151.7, 155.5, 156.5, 170.3, 170.6, 171.9; HRMS (ESI m/z): Calcd. for [C₅₂H₅₁N₃O₁₀+H]⁺ 878.36472, found 878.36626.

Flavonol-Pro-Pro-Ile-OH 309

Flavonol-diOBn-Pro-Pro-Ile-Cbz 307 (303.0 mg, 0.34 mmol) was stirred with Pd/C (30 mg) in EtOH (7 mL) and 1,4-dioxane (3 mL) at rt for 16 hours. The solution was then filtered through a celite plug, and the solvent removed in vacuo, and purification by column chromatography (5% MeOH in CHCl₃) provided the deprotected flavonol-tripeptide as a dark green solid (196.2 mg, quant., Rₛ=0.15); δ_H NMR (270 MHz, CDCl₃): 0.83-1.23 (6H, m, H27, H29),
1.40-2.59 (13H, m, H11, H12, H15, H16, H26, H28 & 2×OH), 3.67-3.86 (4H, m, H13 & H17), 4.26-4.98 (3H, m, H10, H14, H19), 7.03 (1H, br s, H2'), 7.19-7.68 (6H, m, H6, H7, H8, H5', H6' & NH2), 8.15 (1H, br s, H5); δC NMR (67.5 MHz, CDCl3): 11.1, 15.4, 24.6, 24.8, 25.0, 28.1, 29.2, 38.0, 47.0, 47.7, 54.8, 56.7, 58.1, 114.0, 114.5, 118.1, 122.4, 123.2, 123.4, 125.9, 127.9, 128.2, 133.1, 133.9, 145.7, 146.0, 155.5, 170.1, 170.3, 171.0, 171.9; HRMS (ESI m/z): Calcd. for [C31H35N3O8+H]+ 578.24969, found 578.24969.

Flavonol-Pro-Pro-Val-OH 310

Flavonol-diOBn-Pro-Pro-Val-Cbz 308 (249.6 mg 0.28 mmol) was stirred with Pd/C (30 mg) in EtOH (7 mL) and 1,4-dioxane (3 mL) at rt for 16 hours. The solution was then filtered through a celite plug, and the solvent removed in vacuo, and purification by column chromatography (5% MeOH in CHCl3) provided the deprotected flavonol-tripeptide as a dark green solid (157.8 mg, quant., Rf = 0.14); δH NMR (270 MHz, CDCl3): 0.91-1.22 (6H, br m, H27 & H28), 1.62-2.59 (9H, m, H11, H12, H15, H16, H26), 3.36-3.85 (6H, m, H13, H17 & NH2), 4.65-4.59 (3H, m, H10, H14 & H19), 6.95-8.18 (7H, m, H5, H6, H7, H8, H2', H5' & H6'); δC NMR (67.5 MHz, CDCl3): 17.8, 19.5, 24.9, 25.1, 28.1, 29.2, 31.4, 46.8, 47.6, 57.6, 58.0, 58.8, 114.0, 114.6, 118.1, 122.4, 123.2, 123.4, 125.9, 127.7, 128.1, 133.2, 134.0, 148.7, 151.7, 155.5, 156.5, 170.3, 170.6, 171.9; HRMS (ESI m/z): Calcd. for [C30H35N3O8+H]+ 564.23400, found 564.23319.
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Appendix A:
Publication
Accessing Highly-Halogenated Flavanones Using Protic Ionic Liquids and Microwave Irradiation

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Abstract: A series of highly-functionalized 2'-hydroxychalcones have been synthesized using a microwave-assisted Claisen-Schmidt condensation. Conversion of these 2'-hydroxychalcones to their corresponding flavanones was then performed utilizing proptic ionic liquids (pILs) and microwave irradiation. This methodology drastically reduces reaction time to 15 minutes compared to typical thermal methods (24 hrs) and is tolerant to a broad range of functional groups. Several chalcones reported bear four and five substituents — a degree of substitution rarely reported in the literature.

Keywords: Chalcone, Claisen-Schmidt, Flavanone, Hydroxyacetophenone, Microwave, Proptic Ionic Liquid.

INTRODUCTION

The 1,3-diaryl-2-propene (chalcone 1) and the phenylchromene (flavanone 2) scaffolds of the flavonoid family of compounds are of great interest to medicinal chemists [1]. 1,3-Diaryl-2-propenones (e.g. chalcone 1) exhibit a suite of biological activities including: anticancer [2], antioxidant [3], and antimicrobial [4] amongst others [5]. The flavanones also have a well known bioactivity profile, including but not limited to anticancer and anti-diabetic effects [6]. Their synthesis, via the Claisen Schmidt condensation of 2'-hydroxyacetophenones with benzaldehydes [7,8], and subsequent acid- or base-catalyzed ring closure [9], has evolved greatly over the years. This has eventually led to microwave-assisted methods, which provide products in high purity, in excellent yields and in reduced reaction times [10-13].

Microwave-assisted chalcone synthesis using Claisen-Schmidt condensation has been reported by several groups (reaction times ranging from 2–60 minutes) [10, 11]. The microwave-assisted, direct formation of flavanones (via 2'-hydroxychalcones) from 2'-hydroxyacetophenones and benzaldehydes has also been researched [12], as well as the simple ring closure of 2'-hydroxychalcones to flavanones [13].

![Fig. 1](image)

Fig. (1). The structures of 2'-hydroxychalcone (chalcone, 1) and phenylchromone (flavanone, 2).

Proptic ionic liquids (pILs) have recently received attention as a means of carrying out acid-promoted reactions/rearrangements rapidly and cleanly [14], and as such, appeared ideally suited for the conversion of 2'-hydroxychalcones to flavanones. Proptic ionic liquids are made by mixing strictly equimolar amounts of Brønsted acids and bases, and are neutral as there is no free H\(^+\) in the reaction media [15]. Nevertheless, their acidic character can be tuned by careful selection of acid and base counter-ions, and this parameter is often the variable of choice when investigating pIL suitability [16]. Furthermore, the high dielectric constant of pILs makes them ideal for microwave-assisted transformations.

To complement existing methods for chalcone synthesis, herein we report a modified microwave-assisted synthesis of highly-functionalized chalcones, resulting in the synthesis of five new, highly-halogenated chalcones. The production of halogenated 2'-hydroxychalcones is particularly advantageous as these, and their corresponding flavanones, are envisaged as excellent substrates for palladium-mediated further functionalization [7]. We also report the attempted use of proptic ionic liquids (pILs) for chalcone synthesis, and our successful use of pILs for the microwave-assisted conversion of 2'-hydroxychalcones to their corresponding flavanones, which produced three novel compounds.

MATERIALS AND METHODS

For detailed experimental details and compound characterisation please refer to the electronic supplementary information. All materials were obtained from Sigma-Aldrich and were used as received.

All \(^1\)H and \(^13\)C NMR spectra were recorded on a Jeol JNM-EX 270 MHz as indicated; 270 MHz for \(^1\)H and 67.5 MHz for \(^13\)C measurements with residual CDCl\(_3\) peak used as an internal standard. Thin layer chromatography (TLC) was performed using aluminium-backed Merck TLC Silica gel 60 F254 plates, and samples were visualised using 254 nm ultraviolet (UV) light, and potassium permanganate/potassium carbonate oxidising dip (1:1:100 KMnO\(_4\):K\(_2\)CO\(_3\):H\(_2\)O w/w).

Column Chromatography was performed using silica gel 60 (70-230 mesh). All solvents used were AR grade. Reagents were obtained from Sigma-Aldrich Chemical Company and used without further purification. Petroleum spirits refers to the fraction boiling between 40-60 °C.

Microwave reactions were conducted using a CEM Discover S-Class Explorer 48 Microwave Reactor, operating at a frequency of 50/60 Hz and continuous irradiation power from 0 to 200 W or 50
W where specified. All reactions were performed in 10 mL vials with snap caps, using the following conditions: pressure (17 bar); power max (off); and stirring (high).

**General Procedure for the Synthesis of 2'-Hydroxychalcone 5**

2'-Hydroxyacetophenone (0.5 mmol) was added to a solution of base (2.0 mmol) in THF (2.0 mL) in a 10 mL microwave tube. Benzaldehyde (0.5 mmol) was added, and the solution was heated to 50 °C for 15 minutes using microwave irradiation. Solvent was evaporated in vacuo, and 10% HCl solution (20 mL) added. The product was extracted with EtOAc (3 × 10 mL), washed with H2O (3 × 10 mL) and brine (3 × 10 mL), dried over MgSO4 and solvent was removed under reduced pressure to give a resin. Purification of the crude material by column chromatography (1:1 CH2Cl2:petroleum spirits) gave the desired chalcone.

**General Procedure for the Synthesis of Flavanone 6 and 7**

pIL (0.25 mL) was added to chalcone (0.1-0.4 mmol) in a 10 mL microwave tube. The solution was heated to 130 °C (maximum 50 W) for 20 minutes using microwave irradiation. Thin layer chromatography was carried out on the crude sample to confirm loss of starting material, and the solution was then filtered through silica and the silica plug flushed with CH2Cl2. The solvent was evaporated in vacuo, and purification of the crude material by column chromatography (1:1CH2Cl2:petroleum spirits) gave the desired flavanone.

**RESULTS AND DISCUSSION**

Whilst a number of methodologies have been previously reported regarding the microwave-assisted synthesis of chalcones, in our hands a subtly modified method (utilizing KOH or Bu4NOH as the base in THF, under microwave irradiation at 50 °C for 15 min) gave better yields and allowed for the discovery of an ideal, organic-soluble hydroxide source in the form of tetrabutylammonium hydroxide (Bu4NOH). The pIL, TeaH2SO4, was also trialed for this reaction, but chalcone formation was insignificant in this instance. The modified method was then applied to a number of substituted benzaldehydes and 2'-hydroxyacetophenones, and provided 14 2'-hydroxychalcones (see Table 1), varying greatly in the degree and type of functionalization. Included in the products are five novel compounds (5h, 5e, 5g, 5h, and 5i).

With a range of 2'-hydroxychalcones in hand, our attention turned to conversion of their corresponding flavanones. We were inclined to use the acidic conditions provided by protic acidic liquids to facilitate this ring closure. It should also be noted at this point that further advantages associated with the use of pILs include the ease of reaction set-up (the substrate in this case is simply dissolved in the pIL), reaction work-up (filtration through a silica plug), and the avoidance of strong mineral acids.

Again with an eye to halogenated products, bromochalcone 5e was chosen as model substrate, as it was easily accessible (a yield of 96% was achieved by the previous method), and the resulting flavanone 6 is a well documented compound. Initially, pILs of varying 'proton activity' were trialed as a suitable medium to effect this microwave-assisted transformation. In all cases, the microwave wattage was set to an upper limit of 50 W as degradation of both reagents and ionic liquids have been noted in previous explorations if excess power is employed. We began by using TeaFA (triethylammonium formate) at 100 °C for 20 minutes, for which a comforting 42% conversion to 6 was observed (entry 1, Table 2). Increasing the reaction temperature to promote ring closure (entry 2, Table 2) gave improved conversion (65%).

The formation of flavanones from 2'-hydroxychalcones is a reversible process [17]; as such it is difficult to isolate high yields (>70%) of flavanones, and typical yields vary from 30-70% [10]. It has been shown that higher yields of flavanones can be realized if an electron-withdrawing group (e.g. CO2Bu) is installed α to the ketone of the 2'-hydroxychalcone [18], but this strategy employed different starting materials to those presented here, and as such was not pursued.

Extending reaction times to 40 and 60 minutes (entries 3 and 4, Table 2) gave similar conversions as entry 2, thus 20 minutes was determined as the optimal reaction time for TeaFA. Note that while higher temperatures and extended reaction times were employed, trace amounts of 2'-hydroxyacetophenone were observed in the crude 1H NMR spectrum. The acetophenone presumably forms from a retro-aldol reaction facilitated by adventitious moisture; the presence of acetophenone is extremely undesirable as it complicates chromatographic purification.

When EAN ( ethylenimine:nitric acid, entry 5, Table 2) was used, no conversion to the desired flavanone was observed. Similarly, when another common pIL, TeaMs (triethylamine:methanesulfonic acid) was tested (entry 6, Table 2), poor conversion to the flavanone 6 was noted. Finally, TeaH2SO4 (triethylamine:sulfuric acid)—a pIL possessing very high 'proton activity'—was employed, and after heating at 100 °C for 20 minutes, 54% conversion (entry 7, Table 2) was noted. Increased temperature (130 °C, entry 8, Table 2) gave excellent conversion to flavanone 6, with no trace of 2'-hydroxyacetophenone, and the desired product was isolated in relatively good yield (60%). On the other hand, increased and reduced reaction times (entries 9 and 10, Table 2) did not improve conversion. Additionally, we carried out the reaction in the absence of protic ionic liquid (entry 11, Table 2) which returned only starting material, thus highlighting the critical role of the pIL in the formation of the flavone.

When these conditions (MW, 130 °C) were applied to the unsubstituted 2'-hydroxychalcone, a good isolated yield of flavanone 7a (60%, entry 1, Table 3) was obtained. Thus our attention turned to the synthesis of a range of more highly-substituted flavanones. Initially we varied the position, electronic nature and number of B ring substituents, but a dramatic drop in yield was observed when alkyl groups were incorporated (entries 2 and 3, Table 3). Furthermore, when chalcones with alkyl groups on ring A were used (entries 4 and 5, Table 3), no conversion to the desired flavanone was observed.

Halogenated 2'-hydroxychalcones gave unusual results, whereby the presence of one or more halogens on ring A with no ring B substitution (entries 6 and 8 respectively, Table 3) gave good isolated yields of the desired flavanones. However, the introduction of halogens on ring B resulted in poor yield (entries 7 and 9, Table 3). These varying results suggest that a delicate balance of electronic effects contribute to the reaction performance.

**CONCLUSION**

In conclusion, we have developed a modified microwave-assisted method for the synthesis of highly-functionalized 2'-hydroxychalcones. The method provides good yields of the desired products, and is tolerant to a range of functional groups. Of particular note are five novel, highly-halogenated chalcones (5h, 5e, 5g, 5h, and 5i). Additionally, we have converted eight 2'-hydroxy-
### Table 1. Synthesis of Functionalized 2'-Hydroxychalcones

![Reaction Scheme]

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<th></th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Base</th>
<th>Ratio 3:5(^*) (Yield)(^a)</th>
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<tr>
<td>a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>KOH</td>
<td>0:1 (100)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tetrH₂SO₄</td>
<td>19:1(^b)</td>
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<tr>
<td>b</td>
<td>H</td>
<td>H</td>
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<td>5-Br</td>
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<td></td>
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<td></td>
<td>Bu₄NOH</td>
<td>1:1 (52)</td>
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<tr>
<td>c</td>
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<td>H</td>
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<td>Bu₄NOH</td>
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<tr>
<td>d</td>
<td>H</td>
<td>H</td>
<td>3-Obn</td>
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<td>KOH</td>
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<td>e</td>
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<td>f</td>
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<td>5'-Cl</td>
<td>H</td>
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<td>KOH</td>
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<td>5'-Cl</td>
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<td>Bu₄NOH</td>
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<td>H</td>
<td>KOH</td>
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<td>Bu₄NOH</td>
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<td>6'-MeO</td>
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<td>H</td>
<td>KOH</td>
<td>0:1 (46)</td>
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<td>1:3</td>
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<td>l</td>
<td>6'-MeO</td>
<td>H</td>
<td>3-Obn</td>
<td>H</td>
<td>KOH</td>
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<td></td>
<td>Bu₄NOH</td>
<td>1:1</td>
</tr>
<tr>
<td>m</td>
<td>6'-MeO</td>
<td>H</td>
<td>2,4-OMe</td>
<td>5-Br</td>
<td>KOH</td>
<td>0:1 (52)</td>
</tr>
</tbody>
</table>

\(^a\) Ratio determined by \(^1\)H NMR integration (see ESI for example)

\(^b\) Isolated yield after column chromatography

\(^c\) Total conversion of < 5% to chalcone
Table 2. pHl. Screening and Optimization

<table>
<thead>
<tr>
<th>Entry</th>
<th>pHl</th>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>Ratio 6:5&lt;sup&gt;+&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>1</td>
<td>TeaFA</td>
<td>100</td>
<td>20</td>
<td>58:42</td>
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<tr>
<td>2</td>
<td>TeaFA</td>
<td>130</td>
<td>20</td>
<td>29:69&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>TeaFA</td>
<td>100</td>
<td>40</td>
<td>29:68&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>62:38</td>
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<tr>
<td>6</td>
<td>TeaMs</td>
<td>100</td>
<td>20</td>
<td>46:54</td>
</tr>
<tr>
<td>7</td>
<td>TeaH&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100</td>
<td>20</td>
<td>34:66&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>TeaH&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>130</td>
<td>40</td>
<td>48:52</td>
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<tr>
<td>9</td>
<td>TeaH&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>100</td>
<td>10</td>
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<tr>
<td>11</td>
<td>-</td>
<td>130</td>
<td>20</td>
<td>62:38</td>
</tr>
</tbody>
</table>

<sup>+</sup> Conversion determined by <sup>1</sup>H NMR spectroscopy (ESI).  
<sup>+</sup> A trace amount (< 10%) of 4-hydroxyxanthone was observed in the crude <sup>1</sup>H NMR spectrum.  
<sup>+</sup> An isolated yield of 60% was obtained in this instance.

Table 3. Reaction Scope of pHl-Mediated Flavanone Formation

<table>
<thead>
<tr>
<th>Entry</th>
<th>7</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>R&lt;sub&gt;3&lt;/sub&gt;</th>
<th>R&lt;sub&gt;4&lt;/sub&gt;</th>
<th>Yield&lt;sup&gt;+&lt;/sup&gt;</th>
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<tbody>
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<td>7a</td>
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<td>H</td>
<td>H</td>
<td>H</td>
<td>60</td>
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<td>7b</td>
<td>H</td>
<td>H</td>
<td>3-OBn</td>
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<td>22</td>
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<tr>
<td>3</td>
<td>7c</td>
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<td>H</td>
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<td>14</td>
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<td>6-OMe</td>
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<tr>
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<td>7g</td>
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<td>H</td>
<td>4-Br</td>
<td>H</td>
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<tr>
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<td>H</td>
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<td>4-Br</td>
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<sup>+</sup> Isolated yield  
<sup>+</sup>H NMR spectra showed no trace of desired flavanone

Chalcones to their corresponding flavanones—one of which (7b, 7c, and 7i) are novel—utilizing the protic ionic liquid TeaH<sub>2</sub>SO<sub>4</sub>. This research further confirms the role of microwave technology in the synthesis of chalcones, and clearly illustrates the suitability of pHlS to effect rapid and extremely clean conversion of 2'-hydroxychalcones to their corresponding flavanones using microwave irradiation.
ACKNOWLEDGEMENTS

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REFERENCES


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Appendix B:
Cytotoxicity LC\textsubscript{50} Results for 2'-Hydroxychalcones

Cytotoxicity evaluations were conducted on 13 functionalised 2'-hydroxychalcone compounds. These evaluations were performed by Dr Sunil Ratnayake, on both Human Embryonic Kidney (HEK) cells, and (HepG2) cells. Each compound was tested at concentrations between 1-80 \( \mu \text{mL} \) in triplicate, alongside both positive (Triton (10% v/v)) and negative controls (no compound added). From his results, Dr Ratnayake also determined the LC\textsubscript{50} values of the compounds and produced the graphs within this Appendix. These graphs are a visual interpretation of the percentage of dead cells versus the concentration of the compound tested.

Note that sample S8 is not included in these results as it was not a compound from our project.

### B.1. Structures of 2'-Hydroxychalcones Analysed

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**B.2 Graphs of LC₅₀ Values for 2’-Hydroxychalcone Compounds S1-S7 and S9-S14**

![LC₅₀ Graph](image)
LC₅₀ for S5-S7 (HepG2 Cells)
- S5: >80 μg/ml
- S6: 28 μg/ml
- S7: >80 μg/ml

LC₅₀ for S9-S12 (HepG2 Cells)
- S9: 70 μg/ml
- S10: >80 μg/ml
- S11: 18 μg/ml
- S12: 14 μg/ml