Synthesis of Labelled Flavonoids and Novel Isoflavonoid Phytoestrogens

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by
Matthew R. Benton

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Above all, I would like to praise God for his guidance, tolerance and his patience over this period.

Abbreviations

DMTST.....Dimethyl(methylthio) sulfonium triflate

DPPE.....1,2-Bis(diphenylphosphino)ethane

EDTA.....Ethylenediaminetetraacetic acid

HMPA.....Hexamethylphosphoramide

HPLC..... High performance liquid chromatography

LC-MS..... Liquid chromatography mass spectrometry

LDA.....Lithium diisopropylamide

LHMDS..... Lithium hexamethyldisilazide

PBS.....Phosphate buffered saline

TBAB..... Tertiarybutylammonium bromide

TBDMSCl..... Tertiarybutyldimethylsilyl chloride

TMEDA..... . *N,N,N',N'*-Tetramethylethylenediamine

TMSOTf..... Trimethylsilyltriflate

Contents

Abstract

Studies have been carried out on the synthesis of ^{13}C-labelled flavones to produce derivatives suitable for use in LC-MS as internal standards and in metabolic studies. Methods have been developed that allow the incorporation of one or two ^{13}C-atoms into 2,4,6-trimethoxyacetophenone and a single ^{13}C-atom into *p*-methoxybenzoyl chloride. The optimum method for introduction of the ^{13}C-atoms into the 2,4,6-trimethoxyacetophenone was found to be via a Friedel-Crafts acylation of 2,4,6-trimethoxybenzene with ^{13}C-labelled acetyl chloride as the source of the isotopic label. The ^{13}C-labelled *p*-methoxybenzoyl chloride is prepared via a palladium catalysed cyanation reaction using potassium [^{13}C]cyanide. The best method for the assembly of the flavone from these two fragments involved a variation of the Baker-Venkateraman synthesis. Thus is it now possible to prepare flavones with up to three ^{13}C-atoms incorporated into the structure. The formal synthesis of [2-^{13}C]apigenin was then achieved using this method and as far as we are aware, is the first synthetic example of a ^{13}C-labelled flavone.

A new method for the efficient synthesis of isoflavone-7-*O*-glycosides has been established from the coupling of various isoflavone aglycones with glycosyl trichloroacetimidates using a boron trifluoride catalyst. This methodology proved successful for the formation of glucosides and glucuronides of formononetin and for glucosides of daidzein with coupling yields of between 43-47%.

The *in vivo* halogenation of isoflavones has been associated with an increased ability to prevent lipid oxidation and recently some new metabolites have been isolated and partially identified. A number of novel chlorinated derivatives of daidzein and genistein have now been unambiguously prepared. The antioxidant activity of 3'-chlorodaidzein has subsequently been compared to that of the non-chlorinated derivative. Results for the *in vitro* oxidation of low-density lipoprotein (LDL) confirm 3'-chlorodaidzein to be a more potent antioxidant and also that it reduces the rate of oxidation by 50%.

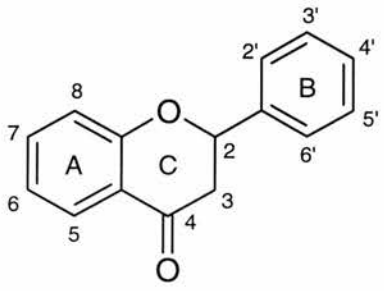
Chapter 1

Introduction

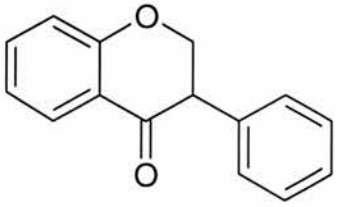
1.1: Structural diverstity of the flavonoids in nature

1.1.1: Introduction

Plant phenolic compounds of shikimate origin are a diverse class of secondary metabolites of which the flavonoids constitutes a large and distinctive group.[1] The isoflavonoids represent a particularly broad sub-class of the flavonoids that are biosynthetically related, sharing the same C_{15} phenylchroman skeleton. The isoflavonoids possess a phenyl ring at the *C*-3 position of the pyrone ring instead of at the *C*-2 in the conventional flavonoid structure.

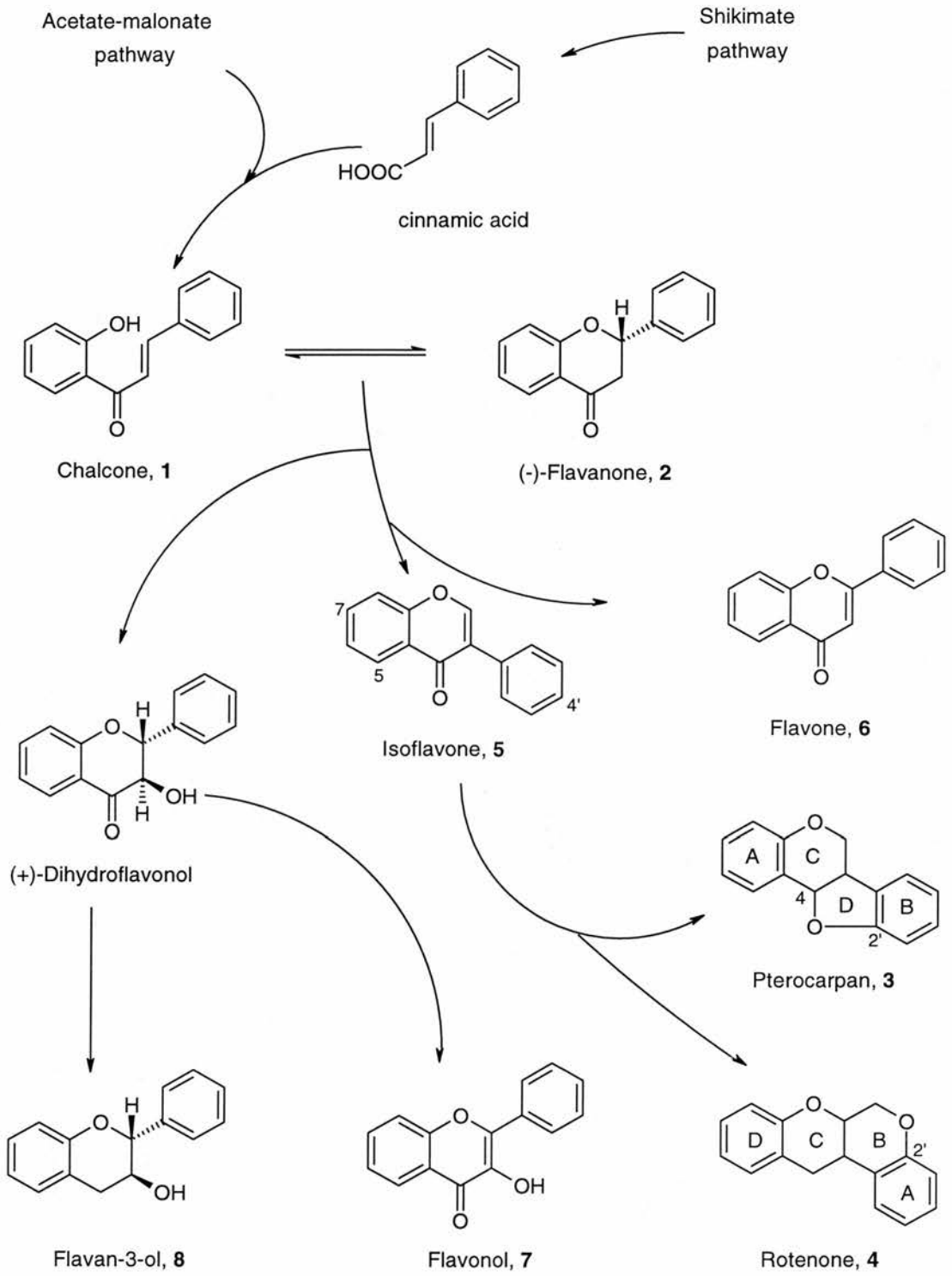


Flavonoid Skeleton



Isoflavonoid Skeleton

The accumulation of the flavonoid aglycones is often correlated with the existence of secretory structures and the production of other lipophilic natural products, mainly of terpenoid origin. As a result they are most commonly encountered on the plant surface as leaf wax and resins as a result of epidermal excretion and are observed in distinct families and genera scattered throughout the plant kingdom. These secreted mixtures appear to occur preferentially in plants of arid or semi-arid regions and appear to function as heat reductants and UV screens. They also exist as anti-microbial agents as well as insect-feeding deterrents. Because the flavonoids tend towards a lipophilic nature they often exist as water-soluble glycosides and this allows their storage within the aqueous environment of the cell vacuole.



Scheme 1: *Principle biosynthetic routes between the flavonoid classes*[2]

The biosynthesis of the flavonoids was originally thought to arise from the condensation of three acetate units from the acetate-malonate pathway with cinnamic acid or related compounds from the shikimate pathway.[3] This was indeed proven to be

the case from feeding experiments using radioactive precursors.[4] The biosynthetic inter-relationship between the various flavonoid sub-classes has subsequently been established and is shown in scheme 1.

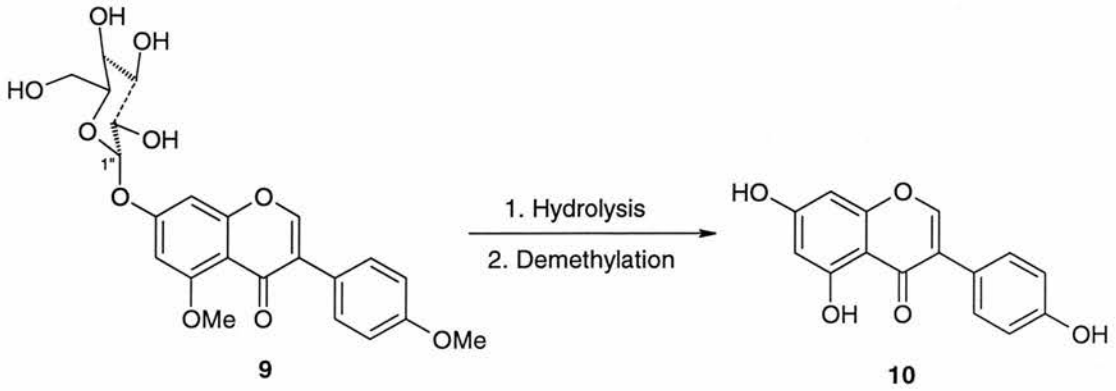
Formation of the chalcone condensation product (**1**) is initially afforded and this exists in equilibrium with the cyclized (-)-flavanone (**2**). From this equilibrium the major biosynthetic pathways allow further subdivision into various compound classes accorded to structural complexity and oxidation levels within the skeleton.

1.1.2 : The isoflavonoids

The isoflavonoids display a very limited distribution within the plant kingdom as a result of the scarcity of the enzyme chalcone isomerase and exist almost entirely in the subfamily Papilionoideae (Lotoidease) of the *leguminosae*. Both the pterocarpans (**3**) and the rotenoids (**4**) can be described as isoflavonoid sub-classes and are generally biosynthesized from the chalcone and (-)-flavanone equilibrium via the formation of isoflavones. The pterocarpans are the second largest sub-group of the isoflavonoids and contain an ether linkage between the 4 and 2' positions.[5] In nature they have found to be widely distributed as heartwood and bark constituents. The rotenoids are characterised by the inclusion of an extra carbon atom in an additional six-membered heterocyclic ring and are believed to form from the oxidation of 2'-methoxyisoflavones. Insecticidal activity is regarded as the most important phytochemical property of the rotenoids, however, a variety of these compounds isolated from Amorpha fruticosa have been shown to be active anti-tumor agents.[6]

The largest naturally occurring sub-class of isoflavonoids, however are the isoflavones (**5**). It is probable that the first isoflavone to be described was the glycoside ononin (table 1), isolated in 1855 by Hlasiwetz from the root constituents of *Ononis spinosa* L.[7] However, it was not until 1910 that an isoflavone was correctly identified from isolated extracts. Here, Finnemore described the isolation of a glycoside (**9**) from the bark of an (unspecified) species of *Prunus*, which upon hydrolysis and demethylation gave a substance he labelled prunetol.[8] Subsequent base hydrolysis yielded phloroglucinol and *p*-hydroxyphenylacetic acid, allowing the

structure of prunetol to be deduced as an isoflavone ($C_{15}H_{10}O_5$). This is now more commonly referred to as genistein (**10**).

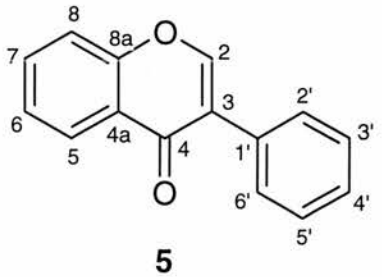


9 **10**

Scheme 2: *First isolation and identification of an isoflavone, genistein (**10**)*

However, the subsequent augmentation of the list of identified isoflavones was slow and erratic. The report by Warburton[9] in 1954 listed only fifteen isoflavones of undisputed structure, however by 1983, a checklist in *The Flavonoids* by J. B. Harborne[10] shows the number of known isoflavone aglycones to be 160. But more recently the 1993 version[11] of this review shows the number of known structures had increased dramatically to 364 reflecting what appears to be an increased level of interest in this area.

The sugar moiety in isoflavone glycosides is most commonly D-glucose. Other saccharides such as rhamnose, xylose and arabinose also occur, however and can be attached via either *O*- or *C*- linkages to the aglycone. These glycosides have been found to exist only in the β-anomeric configuration, referring to the arrangement of substituents around the *C*-1", or linking position in the saccharide. The α-anomeric configuration is not a naturally existing structure. The table below describes some of the more commonly occurring isoflavone aglycones and their 7-*O*-β-glucosides.



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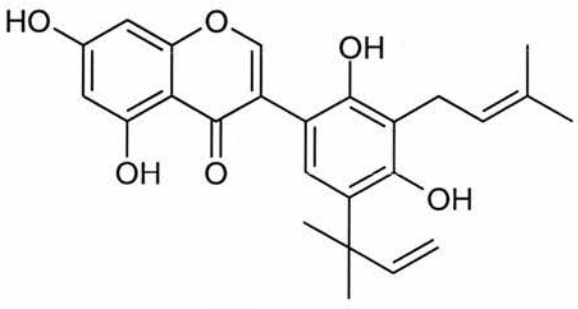
Name	Constitution	7	5	4'
Daidzein	7,4'-dihydroxyisoflavone	OH	H	OH
Daidzin	daidzein-7-glucosidc	O-glucosc	H	OH
Formononetin	7-hydroxy-4'-methoxyisoflavone	OH	H	OCH_3
Ononin	formononetin-7-glucoside	O-glucose	H	OCH_3
Genistein	5,7,4'-trihydroxyisoflavone	OH	OH	OH
Genistin	genistein-7-glucoside	O-glucose	OH	OH
Biochanin-A	5,7-dihydroxy-4'-methoxyisoflavone	OH	OH	OCH_3

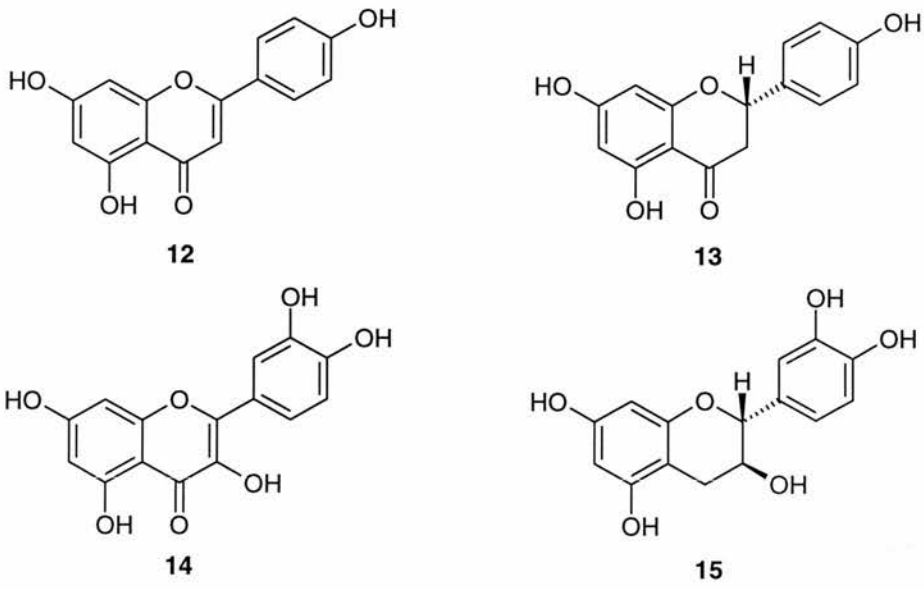
The majority of isolated examples include the basic 5, 7, 4'- or 7, 4'- oxygenation patterns associated with genistein and daidzein precursors, however further structural complexities often arise. This most commonly involves the addition of isoprenyl groups onto the ring system. These are more frequently encountered as the *C*-3,3-dimethylallyl group or as the *C*-1,1-dimethylallyl group. The isoflavone fremontone (**11**), [12] isolated from the roots of *Psorothamnus fremontii* contains both of these side-chains and can be seen in the structure below.



11

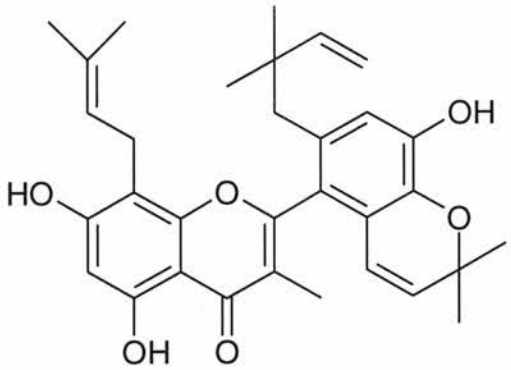
1.1.3 : Some other common flavonoids

Other principal biosynthetic routes that extend from the chalcone and (-)-flavanone equilibrium involve the formation of flavones as well as flavonols and flavan-3-ols via the (+)-dihydroflavonol structure. Flavones (**6**) are structural isomers of isoflavones with the aryl B ring present on the *C*-2 position instead of *C*-3. The flavone apigenin (**12**) shown below is analogous to genistein possessing hydroxyl groups at the 5, 7 and 4' positions. The flavanone structure it similar to that of the flavones but is saturated across the *C*-2, 3 bond. A common example is naringenin (**13**) and can be seen below in scheme 2. The flavonols (**7**), for example quercetin (**14**), are identical to the flavone skeleton but with an added hydroxyl group on the *C*-3 position. However, (+)-catechin (**15**), a flavan-3-ol (**8**), is lacking in carbonyl functionality at the *C*-4 position and is saturated across the *C*-2, 3 bond.



Scheme 3: *Some common flavonoids in nature*

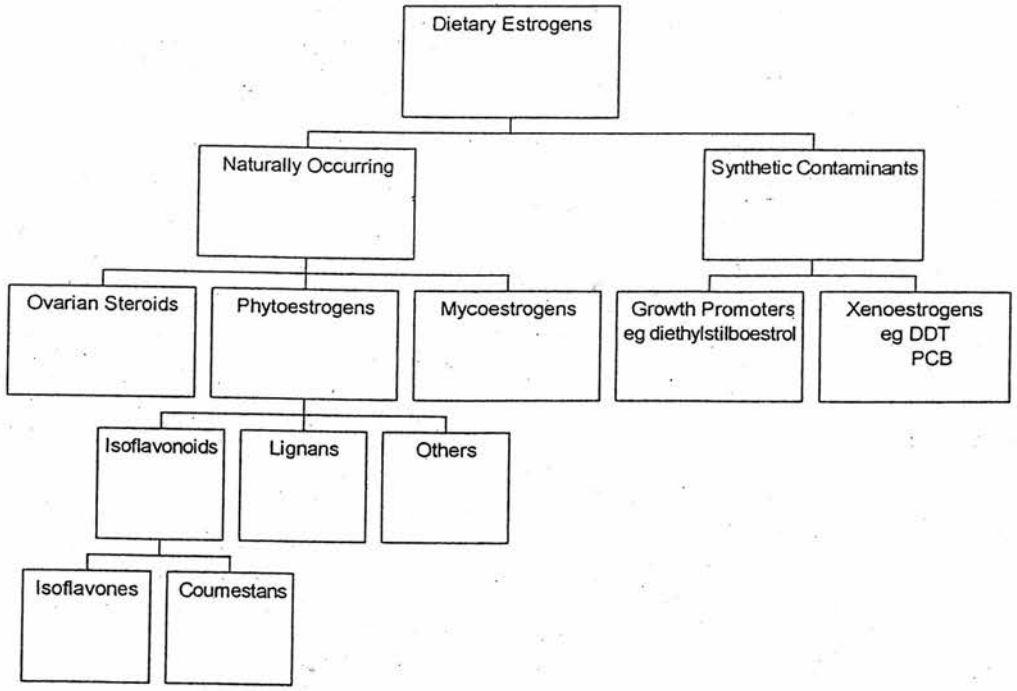
Many of the examples from these sub-classes are isolated as methyl ethers and display varied substitution patterns. The isoprenyl substituents are a common inclusion on the flavonoid skeleton, as are fused pyran or furan ring systems. Broussoflavonol D (**16**) is an example of a benzochromene, so called because of the pyran ring fused to the aryl B ring. [13]



16

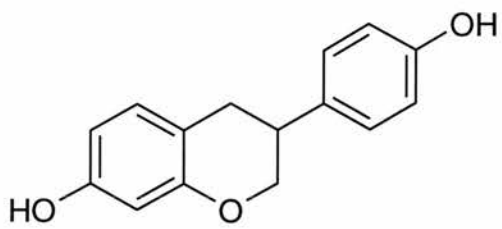
1.2: The flavonoids and estrogenicity

Plant extracts were first reported to exhibit estrogenic activity in 1926 following the Allen Doisy bioassay for estrogens,[14] however it was not until the 1940's that phytoestrogens assumed biological and economic importance. The outbreak of infertility in sheep in Western Australia was later attributed to the high levels of isoflavones present in their main food source, subterranean clover (*trifolium subterraneum*).[15] The isoflavones formononetin and daidzein were found to degrade to equol (**17**), a weakly estrogenic isoflavan in the rumen of the animal. Urinary levels of equol, indicating the presence of isoflavones in humans as a result of dietary intake, however, were not established[16] until the 1980's. The phytoestrogens constitute one group of naturally occuring dietary estrogens in humans and can themselves be subdivided into two main groups. Of these, the isoflavones are most commonly encountered.



Scheme 4 : *Classification of dietary phytoestrogens*[17]

The structural similarity of these phytoestrogens to the steroidal estrogen 17-ß-estradiol (**18**) would appear to be responsible for their weakly estrogenic properties. The comparison of the isoflavan equol with the steroid can be seen below and it is clear that the size of the molecules and distance between terminal hydroxyl groups is very similar.



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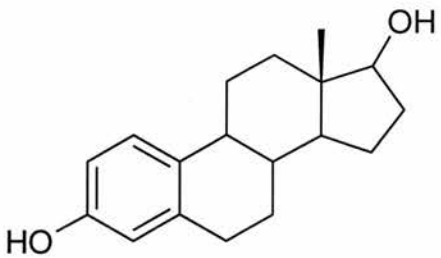

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These factors suggest an ability to act in an agonistic manner towards the estrogen receptor (ER), an intranuclear binding protein, in certain target cells in biological systems. Once bound in these sites, a conformational change in the protein occurs and this allows transcription of target genes. It has been found that the estrogen receptor exists as two separate sub-units in rats,[18] mice and humans, consequently termed ERα and ERβ and it is believed that they play different roles in gene regulation.

The tissue distribution of the ERα and β subtypes are different, with ERβ commonly found in the brain, bone, bladder, prostate and lungs. Furthermore, the relative binding affinities of phytoestrogens and environmental xenoestrogens have significantly higher affinities for ERβ rather than ERα suggesting it to be of importance to non-steroidal estrogens.[19]

Compound	RBA[a]		RBA[b]	
	ERα	ERβ	ERα	ERβ
17-β-estradiol	100	100	100	100
Genistein	4	87	0.7	13
Daidzein	0.1	0.5	0.2	1
Formononetin	<0.01	<0.01	N.D	N.D
Apigenin	0.3	6	N.D	2

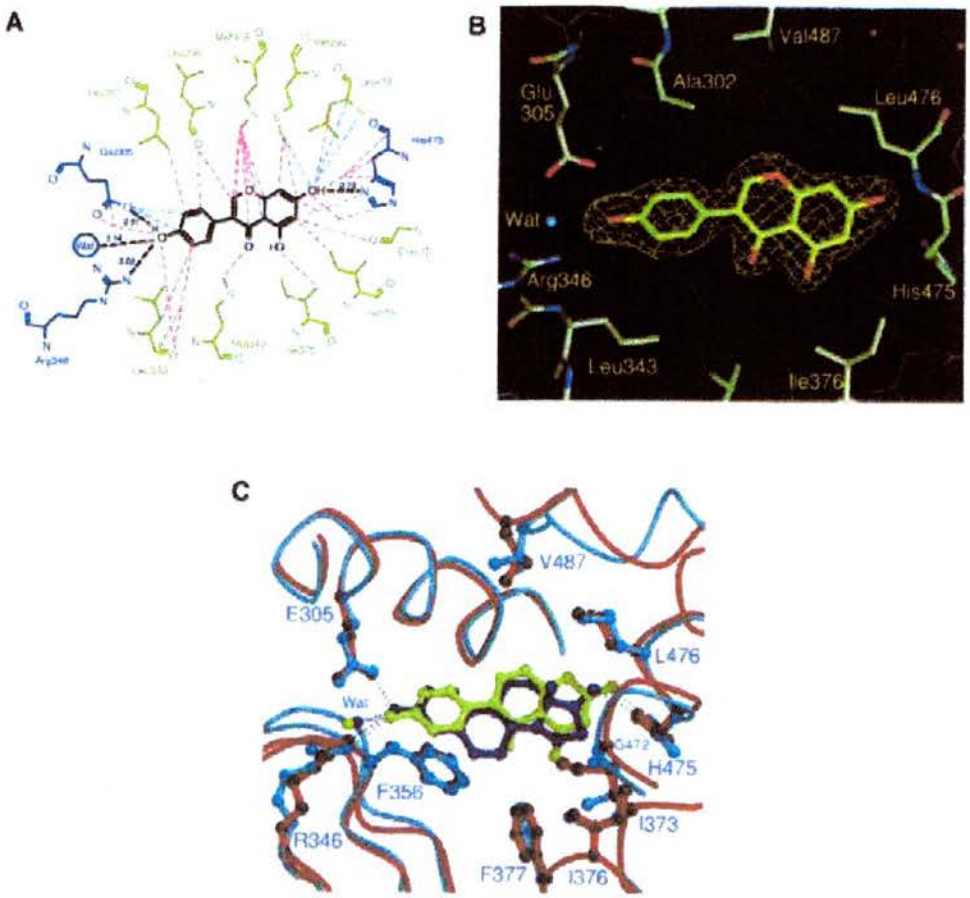
RBA[a] = relative binding affinity determined from solid phase (Scintisrip) competition experiments

RBA[b] = relative binding affinity determined from solubilized receptor competition experiments

ND = not determined

The above table compares the various binding affinities of various phytoestrogens with 17-β-estradiol (**18**) under both competitive and non-competitive conditions.[20] In the non-competitive solid phase ligand binding system, genistein (**10**) demonstrates an affinity for the ERβ sub-type that is almost comparable to that of estradiol. For the ERα sub-type, however, the affinity is over twenty times reduced and this is a general trend of non-steroidal estrogens. The role of the three hydroxyl groups in genistein appears to be an essential factor for binding and the removal of one such group as in the case of daidzein (**19**) is responsible for a large decrease in binding affinity. In the case of formononetin (**20**) where only one hydroxyl group is present a further reduction in the binding affinity is observed. The flavone apigenin (**12**) demonstrates a moderate affinity for both of the ER sub-types.

To more closely mimic physiological conditions, however, binding affinities of the phytoestrogens to ERα and β were determined in the presence of 17-β-estradiol under competitive conditions. As is to be expected, binding affinities for the phytoestrogens decrease, however, genistein does demonstrate a significant, although reduced level of affinity towards ERβ and has even lead Gustafsson to describe it as the genistein receptor. The values for daidzein and apigenin are almost comparable to those taken under non-competitive conditions.



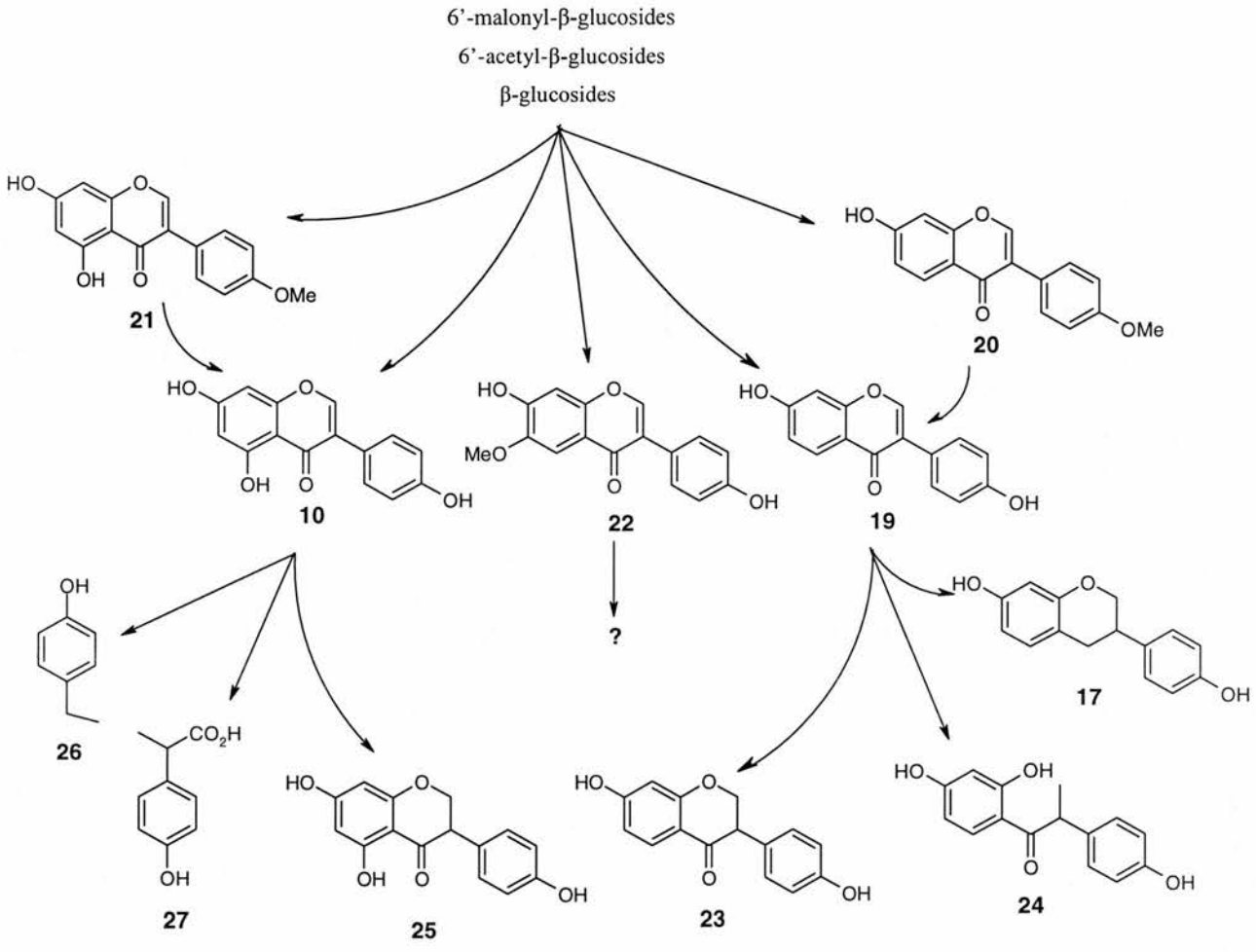
A: Schematic representation showing the interactions between genistein and the ligand binding domain of human ERβ. Residues in green make Van der Waals interaction while residues coloured blue make hydrogen bonds. B: Electron density map for the ligand binding cavity in the ERβ-genistein complex. C: A comparison of the ligand binding mode of genistein and 17-β-estradiol. Genistein (protein; light blue, ligand; green) and 17-β-estradiol (protein; red, ligand; purple).

The three-dimensional structure of the ERβ isoform has been determined[21] and as expected, is very similar to that previously reported for ERα.[22] The ligand binding cavity of ER is buried deep within the hydrophobic core of the ligand binding domain (LBD) and is lined with twenty-two mostly hydrophobic residues that interact with the bound ligands. It was shown that genistein binds across the cavity in a manner similar to 17-β-estradiol, ER's natural ligand, whereby the phenolic hydroxyl group is hydrogen bound to the side chains of Glu305, a Arg346 and a buried water molecule (structure A and B). The 7-hydroxy group of genistein is also hydrogen bound with

the imidazole side-chain of the His475 residue. The binding of genistein in the LBD is similar to that of 17-β-estradiol (structure C). The 'perfect fit' for genistein in the LBD, however, is probably hindered by its increase in length in comparison to 17-β-estradiol. The distance between the two terminal hydroxyl groups (12.1Å compared to 10.8Å) is responsible for the outward movement of the His475 residue and for the slight movement of genistein out of the cavity.

1.3: The metabolism of isoflavones

Dietary isoflavones from plant sources exist as either their β-glucosides, 6-malonyl-β-glucosides or 6-acetyl-β-glucosides.[23] Intestinal microflora are then responsible for the hydrolysis of these compounds, using glucosidases to release the free aglycones which can then be absorbed into the bloodstream or broken down into further metabolites.[24] The breakdown route of these glucosides has been found to be dependent on the sugar moiety. The simple, unesterified glucosides have been found to undergo hydrolysis in the small bowel and the isoflavones are subsequently absorbed into the bloodstream. The malonyl and acetyl glucosides are hydrolysed in the large bowel where further metabolism can occur prior to absorption. The breakdown products of the isoflavones can be seen in scheme 5. Following hydrolysis, both formononetin and biochanin A (**21**) undergo demethylation to give daidzein and genistein respectively. Further breakdown of daidzein gives dihydrodaidzein (**23**), equol and *O*-desmethylangolensin (**24**) while genistein forms dihydrogenistein (**25**), 4-ethylphenol (**26**) and 4-hydroxyphenyl-2-propionoic acid (**27**). The metabolites of glyceitin as yet are not fully understood. The isoflavones can be re-conjugated to form glucuronides and sulfate esters allowing transport to various tissues within the body as well as their excretion in the urine.[25]



Scheme 5: *Metabolites of dietary isoflavones*

1.4: The flavonoids and their effects towards human health

1.4.1: Introduction

In western society today, there is a turning away from nutrition deficiency diseases to chronic diseases associated with affluence.[26] Consequently, the pharmaceutical profession has made a noticeable shift into extending the knowledge of the utility of plant-derived foods, or phyto-nutrients into promoting health and preventing disease.

The isoflavones are one of three main classes of phytoestrogens that appear in either their plants or their seeds (scheme 4). When their biological activities were first

observed in the 1930's,[27] it was proposed that they constituted an essential requirement in the human diet and the term vitamin P was assigned. Although this was eventually dropped, the potential importance of these compounds still holds true, especially in the prevention or modulation of a range of chronic diseases.

Human food sources that contain significant levels of isoflavones are almost exclusively legumes such as lentils and chickpea. The most abundant source of isoflavones, however is the soybean. Soy-germ products are rich in isoflavones containing up to 20 mg/g,[28] while second generation soy-foods such as soymeal and tofu generally consist of only 0.1-3 mg/g. These processed foods are made by mixing soy lecithin and oils to a variety of manufactured ingredients and generally involve the removal of soy protein. As a result, this process is responsible for the loss of most of the isoflavone content.

Traditionally, the diet in Far Eastern countries such as Japan and China consists predominantly of fruit, vegetables, nuts and legumes as well as soy-germ products while the Western diet has become increasingly refined consisting more of animal fats and proteins and lacking in dietary fibre. Studies from Finland have shown that the traditional intake of dietary isoflavones from soy-derived products is very low (1 mg/day) when compared to that of countries such as China (20-50 mg/day) and is reflective of the relative overall phytoestrogen intake of these populations. As a result, incidences of hormone dependent cancers, coronary heart disease and osteoporosis are far higher in the west than for Far Eastern or Asian populations. It has also been shown that incidences of these diseases have recently become more comparable throughout the world.[29] Although the traditional rural diet remains, it is likely that the so-called Westernisation of Far Eastern countries, for example the introduction of fast food, may be responsible for this trend.

1.4.2: Dietary flavonoids and hormone-dependant cancers

In western countries, breast cancer is the most common cause of death in young women while prostate cancer is the second most common in men. However, incidences of these hormone dependent cancers in Asian and Far Eastern countries

have been shown to be much lower and this has been highlighted by various studies that have taken place over the last fifty years.[30] Breast cancer levels for women in western countries are known to between 4-5 times greater than their counterparts[31] while prostate cancer incidences was found to be 125 times greater in black U.S. men when compared to that of Japanese men.[32] Additional findings from this study showed that U.S. immigrants from Asia show similar incidence levels to the indigenous population after 1-2 generations, suggesting that dietary or lifestyle changes are responsible for this trend

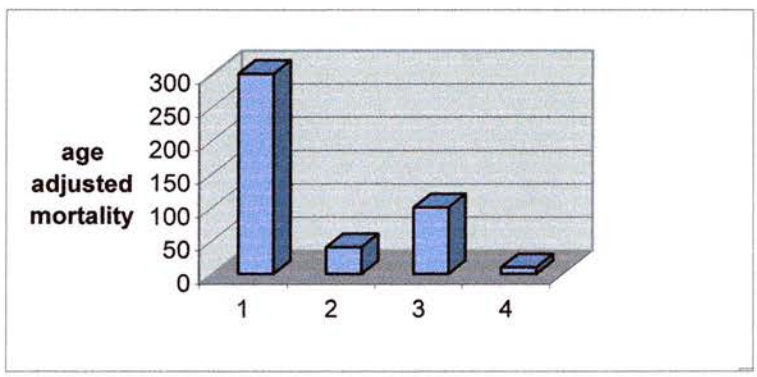
It has long been established that steroidal estrogens play an important role in the modulation of breast, colon and prostate cancers. Hormones are believed to be potentially responsible for promoting cancers by increasing cell proliferation causing cytokine release, free-radical formation and subsequent genomic damage.[33] Population groups that have lower incidences of these cancers have been found to have higher plasma and urinary levels of phytoestrogens, suggesting that plant-derived estrogens compete with steroidal estrogens for cell receptors in an antagonistic manner.

The effective reduction of plasma concentrations of steroidal estrogens by the indirect action of phytoestrogens is achieved in a number of different ways. Firstly, high levels of flavonoids, isoflavones and lignans have been shown to inhibit the action of the aromatase enzymes, catalysing the formation of estrogens from androgens.[34] Additionally, these phytoestrogens have been shown to stimulate the formation of sex-hormone binding globulin (SHBG), responsible for binding to testosterone and estradiol in plasma.[35] This results in lower estrogen levels and thus a reduction in cell proliferation.

It has also been shown that the isoflavone genistein is an inhibitor of the tyrosine protein kinases topoisomerase II and protein histidine kinase.[36] These enzymes are created by oncogenes and are responsible for activating the development of certain types of cancers. Although they play an important role in cell proliferation and transformation the mechanism of their action is still unclear.

1.4.3: Dietery flavonoids and cardiovascular protection

Evidence that dietary phytoestrogens protect against the development of coronary artery atherosclerosis (CAA) and coronary heart disease (CHD) can also be seen from a comparison of Western and Far Eastern populations. Age-standardised mortality rates among men and women (40-69 years of age) from CHD in Japan are extremely low in comparison to incidences in the United States and is believed to be as a result of a soy-protein rich diet. This can be seen in scheme 6 below and is adapted from the report by Beaglehole (1990).[37]



Scheme 6: *A comparison of mortality rates from coronary heart disease in the United States and Japan.*

Key; 1: U.S. males, 2: Japanese males, 3: U.S. females and 4: Japanese females.

Lipoprotein (a) is a cholesterol-carrying particle that is similar in structure to low density lipoprotein (LDL) and is an independent risk factor for coronary heart disease.[38]Where LDL levels are responsive to dietary changes and conventional pharmocological treatments, however, lipoprotein (a) remains unaffected. One of the few treatments for lowering lipoprotein (a) levels in post-menopausal women is estrogen replacement therapy suggesting the weak estrogenicity of lignans and isoflavonoids may be responsible for world-wide trends in CHD mortality. In clinical trials, it has been found that phytoestrogen intake in primates is responsible for the removal of low density lipoprotein (LDL) cholesterol and the increase in levels of high density lipoprotein (HDL) cholesterol.[39]

The major dietary antioxidant found used in the protection of LDL is α-tocopherol, however studies have shown that an increased intake of flavonoids from sources such as onions, apples, tea and red wine have cardioprotective properties.[40] Dietary flavonoids act as free-radical scavengers as a result of their ability to act as electron or hydrogen donating agents. The polyphenol ring structure is ideal for this type of chemistry and flavonoids have been shown to be more effective antioxidants than vitamins C and E *in vitro*.[41]

Catechin and epicatechin are the two main phytoestrogens found in black grapes and are believed to be responsible for the 'French paradox', where people in the South of France enjoy lower incidences of CHD in comparison to the North, despite a higher fat intake and smoking tendencies. The high consumption of red wine is thought to be the cause of this, with total antioxidant activities ranging from 12-14 mM for Pinot Noir through to 23 mM for red Bordeaux and Chianti. In comparison, the polyphenol content of white wines is only about 10-20% to that of red wine and antioxidant activity is reduced accordingly. Catechins have also been identified with antioxidant activity, lowering cardiovascular risks by reducing levels of serum cholesterol and an increasing level of HDL.[42]

High concentrations of dietary flavonoids and polyphenols in blood serum have been shown to reduce the oxidation of LDL's, an indication of the onset of athlerosclerosis. This process reduces the formation of lipid hydroperoxides and subsequent take up by macrophages to form the foam cells, the main contituent in athlerosclоerotic lesions.

1.4.4: Dietary flavonoids and thrombosis

Protein tyrosine kinase is responsible for the aggregation of red blood cell platelets, used for the purpose of repairing cellular damage. Platelet aggregation on artery walls can lead to athlerosclerosis, however, where flow diameter is reduced and blockages can occur. This thrombolytic effect can often be the cause of strokes and haemorrhaging. It has been demonstrated, however, that the role of genistein as a protein tyrosine kinase inhibitor is responsible for a decrease in tyrosine phosphorylation and thus a decrease in platelet activation.[43] As a result, a reduction in

platelet aggregation is observed, leading to a slowing in the progression of athlerosclerosis.

1.4.5: Dietary flavonoids and women's health

The production of estrogen hormones are crucial for the functioning and maintenance of a woman's reproductive system. From around fifty years of age, however, the menopause begins and is associated with the production of less and less of these estrogens. Menopausal women commonly experience symptoms known as "hot flashes", headaches, muscle aches, weight gain and mood swings which are traditionally treated by estrogen replacement therapy (ERT). However, some women complain of side-effects of ERT such as weight gain and worry that it may increase the risk of breast cancer.

It has been shown that the discomfort from menopausal symptoms is much less for women in Japan than for their counterparts in the U.S.[44] Few Japanese women complain of physical problems associated with the change and it has been suggested that the high intake of phytoestrogens from soy-protein may help to explain this.[45] Studies on phytoestrogens as an alternative to ERT were performed and it was shown that after a three-month diet containing soy flour hot flashes were reduced by 40 percent compared to a control.[46]

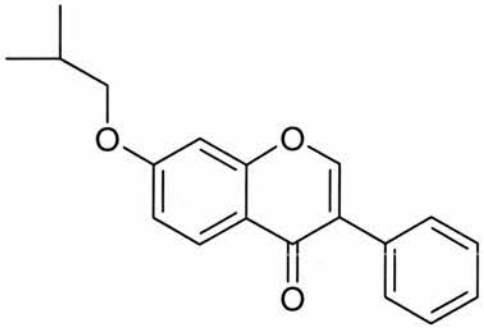
Osteoporosis is the loss of bone mineral density (BMD) and is a severe long-term problem that can lead to fractures and breakage and is related to ageing and hormone deficiency. It is a condition that can affect ageing men, however, it is more prevalent in post-menopausal women. Statistics from Hong Kong and the United States, however suggest another contributing factor is the diet as hip fracture incidences in the Far East for men and women are only a third those of the West.[47]

The relationship between the dietary intake of soy-protein, rich in isoflavones and the increase in BMD has only been studied over the last decade. However, no epidemiological reports have thus far been carried out to confirm this.

Animal tests have provided convincing data for the significant improvement of bone mass following feeding on a soy diet, containing significant levels of the isoflavones genistein and daidzein.[48] It was discovered that there was an optimal dosage of isoflavone intake for best effects on bone mass, while beyond this amount there appeared to be a deleterious effect.

Only one published report of soy isoflavone supplementation in humans has included bone measurements as end-points.[49] This involved two sets of subjects, one on a diet of soy-protein enriched in the isoflavones genistein and daidzein and the other on a diet of soy-protein alone (isoflavones removed). Subjects that received the isoflavone rich supplement experienced significant gains in BMD while those receiving the soy-protein alone experienced only insignificant gains. This suggests that the isoflavone content of dietary soy-protein plays a key role in the conservation of bone mass. The presence of vitamin K or other phyto-nutrients such as lignans may also be of importance in this area, although studies have not thus far been carried out to confirm this.

In addition to the effects on bone tissue, the consumption of soy-protein has effects on some aspects of calcium metabolism. It has been shown that the intake of soy-protein, rich in isoflavones results in a modest increase in urinary levels of calcium, while the consumption of lactalbumin, an animal protein, leads to vastly increased calcium loss over a twenty-four hour period.[50] Although this suggests that a constituent of the soy-protein, possibly the isoflavones is responsible for a calcium conserving effect, the issue has yet to be tested in any rigorous research study.



28

Treatment by estrogen-replacement therapy has proved to be successful in reducing the loss of bone mass and it has been speculated that dietary phytoestrogens may be a natural alternative to this procedure. Evidence for the benefits of soy isoflavones in treating this disease can be seen by the pharmocological use of ipriflavone (**28**). This synthetic isoflavone demonstrated significant improvements in promoting bone mass when administered in doses of 200-600 mg/day and has been approved as an alternative to HRT in preventing bone loss.[51] However, it is unlikely that such phytoestrogen concentrations could be achieved from dietary intake alone. This could suggest that lignans and isoflavones have a long-term role in the prevention of bone mineral density although further studies need to be carried out to fully understand their effects.

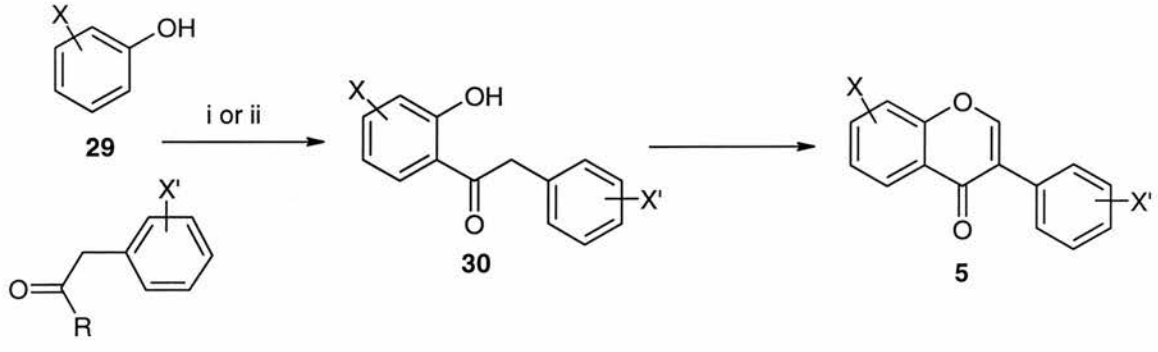
1.5 Methods for the syntheses of isoflavones

Literature concerning the preparation of isoflavones can be divided into three main synthetic areas.[52] The formylation of deoxybenzoins, oxidative rearrangement of chalcones and flavanones and the arylation of a pre-formed chromanone ring.

1.5.1: The formylation of deoxybenzoins

1.5.1.1: Synthesis of deoxybenzoins

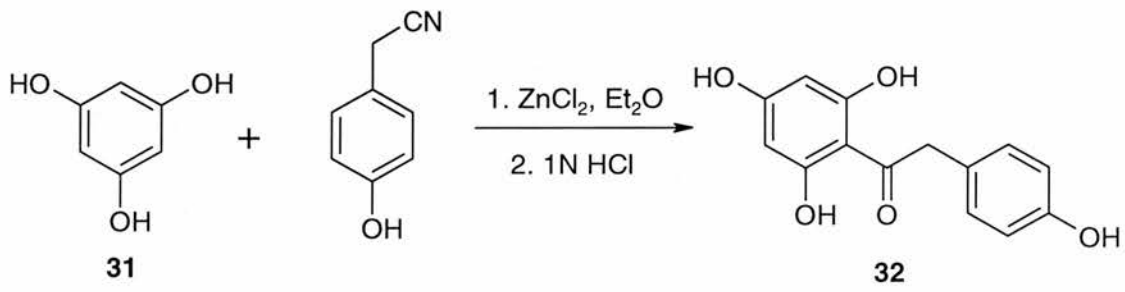
The preparation of phenolic ketones are traditionally carried out under Friedel-Crafts acylation conditions using a phenol (**29**) and phenylacetyl chloride with an $AlCl_3$ catalyst.[53] The deoxybenzoin (**30**) formation is now more commonly afforded by boron complexation of a phenol and phenylacetic acid using either boron trifluoride gas in chloroform[54] or boron trifluoride etherate as both catalyst and solvent (scheme 7).[55] This method has proved to give products in an efficient manner with high product yields and involves only a short reaction time. However, limitations to this procedure concern the number of oxygenated substituents on the aromatic rings. Deoxybenzoins such as 4'-hydroxybenzyl-2,4-dihydroxyphenyl ketone have been synthesised using this method in good yields from resorcinol (**29**, X = *m*-OH) and *p*-hydroxyphenylacetic acid, however as the number of oxygenated substituents increase the reaction yield tends to decrease. Subsequent formylation to give the isoflavone (**5**) can then be achieved using any of a number of reported methods.



i) R = Cl; $AlCl_3$ in ether, 80% ii) R = OH; $BF_3.Et_2O$ 80-90%

Scheme 7: *Deoxybenzoin preparation and formylation*

With the introduction of additional oxygenated substituents onto the phenol ring, as in the case of phloroglucinol (**31**), Baker and Robinson employed an extension of the Hoesch reaction[56] in their 1926 synthesis of genistein.[57] The formation of 2,4,6-trihydroxyphenyl-4'-hydroxybenzyl ketone (**32**) was carried out from phloroglucinol and 4'-hydroxyphenylacetonitrile with a $ZnCl_2$ catalyst in satisfactory yields.



Scheme 8*: Deoxybenzoin formation via the Hoesch reaction*

1.5.1.2: Formylation and cyclization

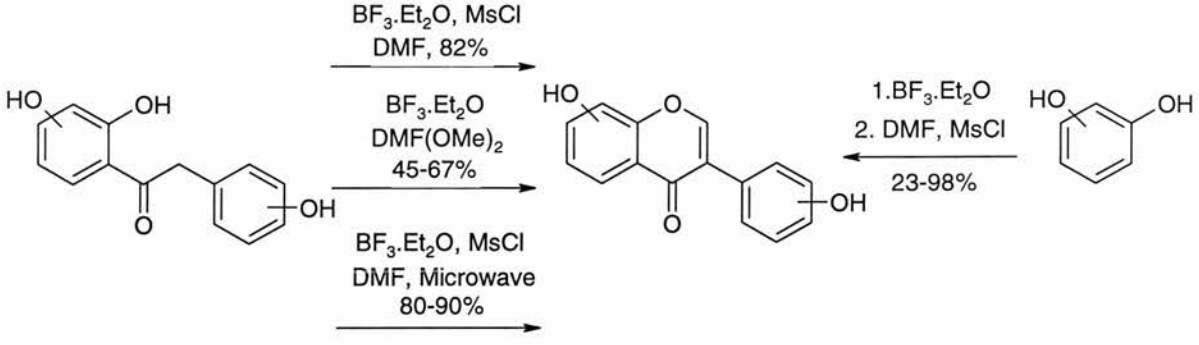
There are a wide range of literature methods concerning the formylation of deoxybenzoins, however many have proven to be sensitive to the number and nature of the substituents present on the aromatic rings. As a result, many methods are limited in scope so far as naturally occurring isoflavones are concerned because of their tendancy to possess the phloroglucinol or 1,2,3,5-oxygenated substitution pattern in the A ring. However, a number of reagents have been identified for the efficient preparation of hydroxyl protected isoflavones from their corresponding deoxybenzoins and are outlined below.

The use of sodium and ethyl formate was reported by Spath and Lederer[58] in 1933 and the reaction was later modified by Venkateraman[59] to give product yields of between 30-40%. Ethyl orthoformate was later shown to be an effective method of cyclization[60] and the use of sodium acetate in acetic anhydride was reported to afford isoflavones in satisfactory yields as far back as 1926.[26] The more efficient use of ethoxalyl chloride in pyridine was reported by Baker and Ollis *et al* in 1953.[61] This was considered the most convenient method for deoxybenzoin cyclization until the

1970's, although the preparation of polyhydroxyisoflavones still required multiple steps due to the necessity of hydroxyl protection and deprotection.

The single step formation of such compounds from deoxybenzoins was provided by the use of boron trufluoride etherate. In the presence of 2-hydroxyphenyl ketones it was suggested that a complex formed, deactivating the polysubstituted aromatic ring and preventing ring formylation and subsequent polymerization.[62] Simultaneously, it activated the methylene group to aid formylation. Bass then demonstrated the successful cyclization of deoxybenzoins using boron trifluoride etherate and methane sulfonyl chloride in dimethylformamide to give isoflavones in excellent yields.[63] Boron trifluoride etherate and dimethylformamide dimethyl acetal was also shown to be a useful and efficient alternative.[64] A one-pot synthesis of isoflavones has also been achieved by Hase and Wahala using boron trifluoride catalysis for deoxybenzoin formation and, in conjunction with methanesulfonyl chloride, for its cyclization.[55]

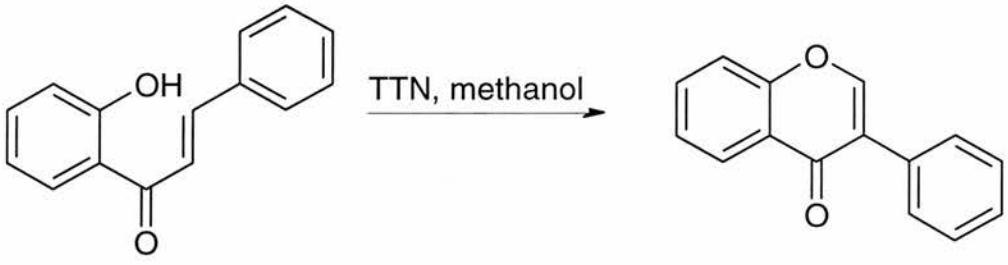
Other single step methods for the preparation of polyhydroxyisoflavones from their deoxybenzoins include dimethoxydimethylaminomethane in benzene,[65] however, yields varied on the aromatic substitution patterns. A more recent report outlines a quick and reliable microwave-mediated synthesis of isoflavones. A range of poly-oxygenated isoflavones were afforded in yields of over 80% after heating for two minutes under microwave conditions. This is shown, together with other common methods for boron-mediated isoflavone synthesis (**10**) in figure 9.[66]



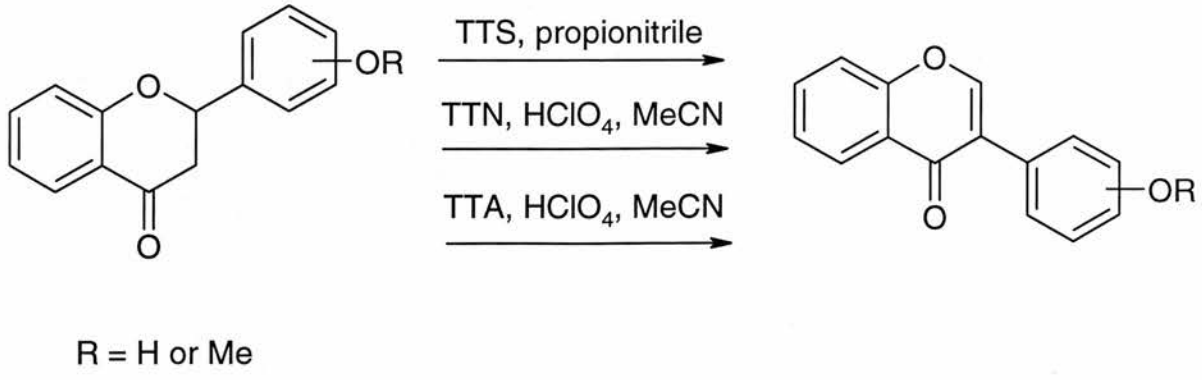
Scheme 9: *Boron-mediated one-step isoflavone syntheses*

1.5.2: Oxidative rearrangement of chalcones

Chalcones are more readily accessible than deoxybenzoins, particularly when the need for complex substitution patterns arise and are readily obtained by the condensation of acetophenones and aromatic aldehydes.[67] One of the more successful methods of isoflavone formation *via* the oxidative rearrangement of chalcones makes use of thallium (III) nitrate (TTN) in methanol.[68]



The oxidative rearrangement of flavanones has also been reported using thallium-based reagents. Isoflavones were prepared in quantitative yields using thallium (III) *p*-tolylsulfonic acid (TTS) from flavanones possessing electron donating substituents within the B ring.[69] Where nitro groups are present, a mixture of flavones and isoflavones was observed which leads to problems concerning their separation. Subsequent procedures show that isoflavones containing either electron withdrawing or donating substituents can be selectively synthesised using thallium (III) nitrate (TTN) or thallium (III) acetate (TTA) in the presence of perchloric acid.[70]



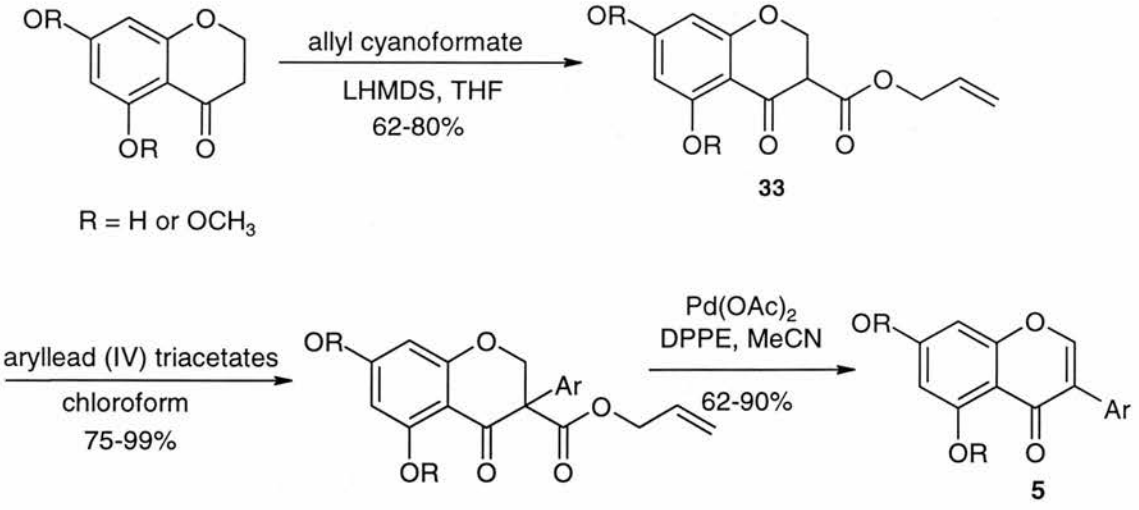
R = H or Me

Scheme 10: *Oxidative rearrangement of flavanones*

The requirement for stoichiometric amounts of toxic thallium (III) salts in oxidative rearrangements does limit the use of these reactions, however. As a result of the recent interest in isoflavones due to their biological activities, the synthesis of these compounds for use in biological testing or in metabolic studies that involve human consumption should be free from even trace amounts of thallium. Because of this, these procedures tend to be unsuitable in many instances.

1.5.3: Arylation of a chromanone ring

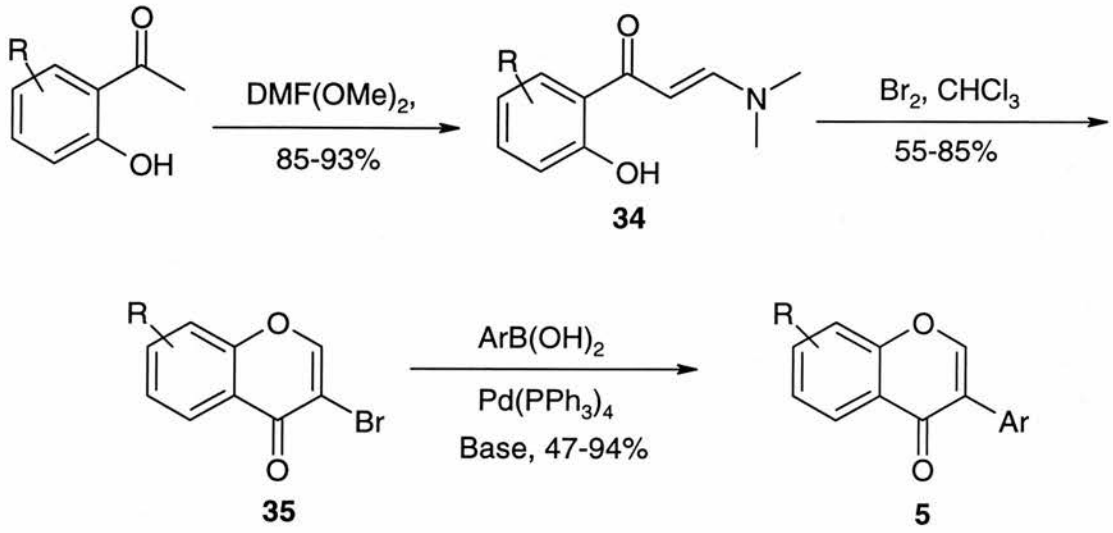
Arylation of chromanone rings to give isoflavones has been demonstrated using aryllead (IV) triacetates in chloroform.[71] In general, arylation of ketonic substrates with aryllead reagents requires their activation as β-diketo compounds or as β-keto esters. Donnelly *et al* have shown that 3-allyloxycarbonylchroman-4-ones (**33**), formed from chroman-4-ones will undergo arylation using aryllead (IV) triacetate in 75-99% yield (scheme 11). Isoflavone (**5**) formation was then afforded in 62-90% yield after reaction with palladium acetate and DPPE in acetonitrile.



Scheme 11: *Arylation with aryllead (IV) triacetates*

3-Bromochromones (**35**) have been shown to undergo arylation in the presence of an arylboronic acid and a palladium catalyst under Suzuki coupling conditions.[72] Preparation of the 3-bromochromones can be afforded from the reaction of an acetophenone and dimethylformamide dimethylacetal to give the enamino ketone species (**34**).[73] Subsequent reaction with bromine in chloroform gave the 3-

bromochromone. Isoflavones (**5**) were then prepared from Suzuki coupling reactions in yields of 47-94% and proved to be successful when using a wide range of substituents on the aryl B ring.



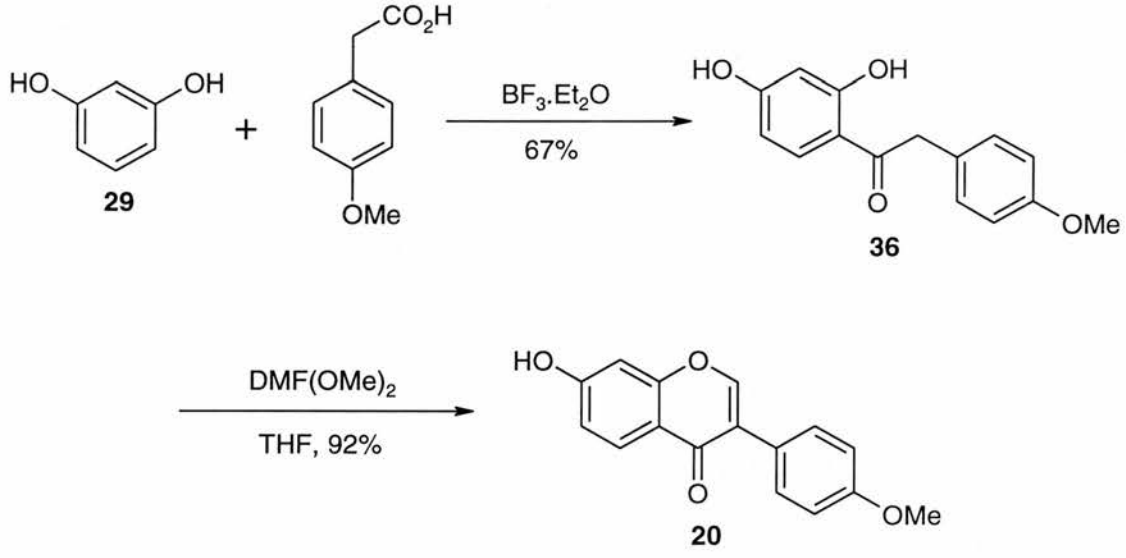
Scheme 12: *Isoflavone synthesis via Suzuki coupling*

Chapter 2

Studies on the Synthesis of Isoflavone-7-*O*-glucosides and glucuronides

2.1: Synthesis of isoflavone aglycones

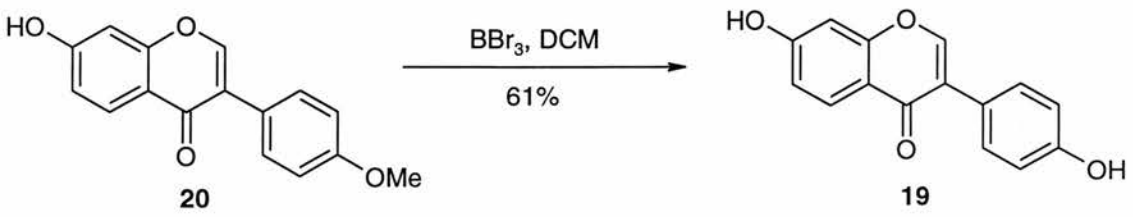
In order to examine the synthesis of glucosides and glucuronides the isoflavones first had to be synthesized. The isoflavones daidzein, formononetin and genistein were thus prepared using existing methodology via their deoxybenzoin intermediates. For formononetin, 4'-methoxybenzyl-2,4-dihydroxyphenyl ketone (**36**) was formed by the boron trifluoride catalysed condensation of resorcinol and *p*-methoxyphenylacetic acid.[55] After heating the reaction mixture at reflux for 20 minutes the deoxybenzoin was formed and isolated as an off-white powder in 67% yield. ^{13}C n.m.r. spectroscopy showed a new carbonyl resonance at 204.4 ppm and the mass spectrum gave the expected molecular ion at *m/z* 258. Formylation and cyclization were then afforded by reaction of **36** with dimethylformamide dimethyl acetal in tetrahydrofuran at reflux. Formononetin (**20**) was subsequently obtained as a white powder in 92% yield. The ^{13}C n.m.r. spectrum showed the new carbonyl carbon at 175.4 ppm and mass spectroscopy showed a molecular ion at *m/z* 268.



Scheme 13: *Preparation of formononetin (**20**)*

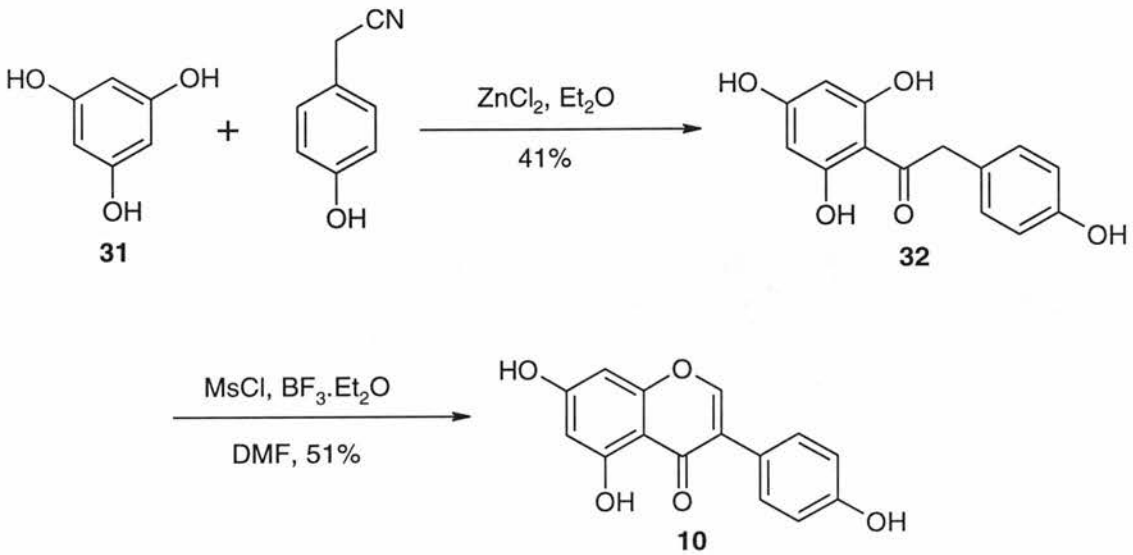
The synthesis of daidzein (**19**) has been carried out using a similar reaction sequence, however it was found that a more efficient route was via the demethylation of formononetin. This was achieved using boron tribromide in dichloromethane and gave

the isoflavone as a white powder in 61% yield (scheme 14). The mass spectrum clearly identified the new peak at *m/z* 254 and the methyl resonance at 3.81 ppm completely disappeared from the ^{1}H n.m.r. spectrum.



Scheme 14: *Preparation of daidzein (**19**)*

For the synthesis of genistein, 4'-hydroxybenzyl-2,4,6-trihydroxyphenyl ketone (**32**) was first prepared using Hoesch reaction conditions[57] from phloroglucinol (**31**) and *p*-hydroxyphenylacetonitrile. The mass spectrum showed a single peak at *m/z* 261 and the carbonyl ketone was observed in the ^{13}C n.m.r. spectrum at 205.4 ppm The formation of genistein (**10**) from its deoxybenzoin was then carried out under microwave conditions using boron trifluoride etherate and methanesulfonyl chloride in dimethylformamide.[66] The isoflavone was isolated in 51% yield as a yellow solid. The ^{13}C n.m.r. spectrum showed the new carbonyl peak at 180.4 ppm. All other spectral data were identical to those in the literature

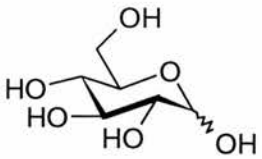


Scheme 15: *Preparation of genistein (**10**)*

2.2: The isoflavone-7-*O*-glycosides

2.2.1: Introduction

Isoflavone storage within the plant kingdom is afforded in the cell interior as water-soluble glycosides. The most common conjugate is D-glucose (**37**), forming a range of isoflavone-7-*O*-β-glucosides (for example, genistin **38**), however other glycosides with sugars such as xylose, arabinose and rhamnose have also been isolated.[1]



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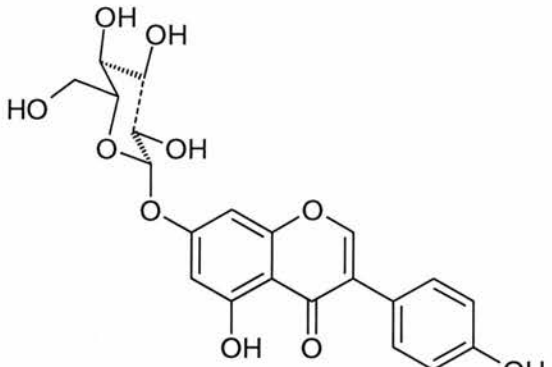

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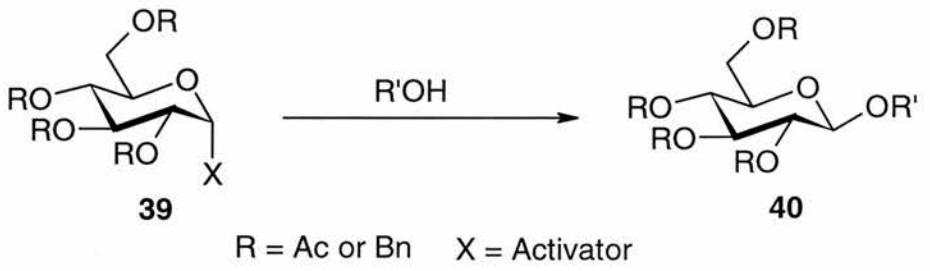
Dietary sources of isoflavone-7-*O*-β-glucosides constitute the bulk form of isoflavone intake, undergoing hydrolysis by bacterial enzymes in the gut to release the free aglycone.[24] Although they have created interest in both medical and dietary fields as a result of their biological activities, the existing synthetic routes to these compounds are limited and boast only poor yields, generally between 5 and 40%.

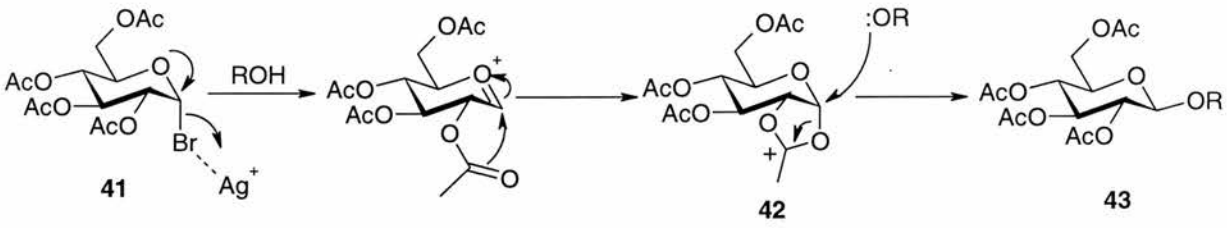
Consequently, it was intended to devise a new synthetic route to these compounds using the isoflavone aglycones formononetin, daidzein and genistein. The isoflavone-7-*O*-glucosides occur naturally in the ß-anomeric configuration and are commonly afforded the names ononin, daidzin and genistin, respectively.

The stereochemical outcome of the glycosylation reaction is an important consideration as only the β-anomeric configuration of the product is required. In order to avoid the production of anomeric mixtures a glycosyl donor (**39**) needs to be chosen to allow complete inversion of configuration from the α-anomer to the β-anomer in the product (**40,** scheme 16).



Scheme 16: *Formation of β-glucopyranosides*

The reported activators (X) that have been used as leaving groups at the anomeric (C-1) position in the formation of β-*O*-glycosides are quite extensive. These include methyl and ethylthio (-SMe, -SEt),[74] diphenyl phosphate [-OP(O)$(OPh)_2$],[75] P,P-diphenyl-N-(p-toluenesulfonyl)-phosphin-imidate [-O-P(=NTs)$(Ph)_2$],[76] trichloroacetimidate [-$OC(=NH)CCl_3$][77] and pent-4-en-1-yloxy [O-$(CH_2)_3$-$CH=CH_2$].[78] The *O*-acylglycosyl halides have also proven to be good synthetic intermediates due to the facile nucleophilic displacement of the halogen and their ease of preparation. Their order of reactivity is I > Br > Cl > F as a result of the increasing bond strength. The glycosyl iodides are generally unstable and the glycosyl fluorides are too unreactive under normal conditions, however the use of fluorophilic catalysts has been shown to turn them into effective glycosylating agents.[79]



Scheme 17: *The Koenigs-Knorr reaction*

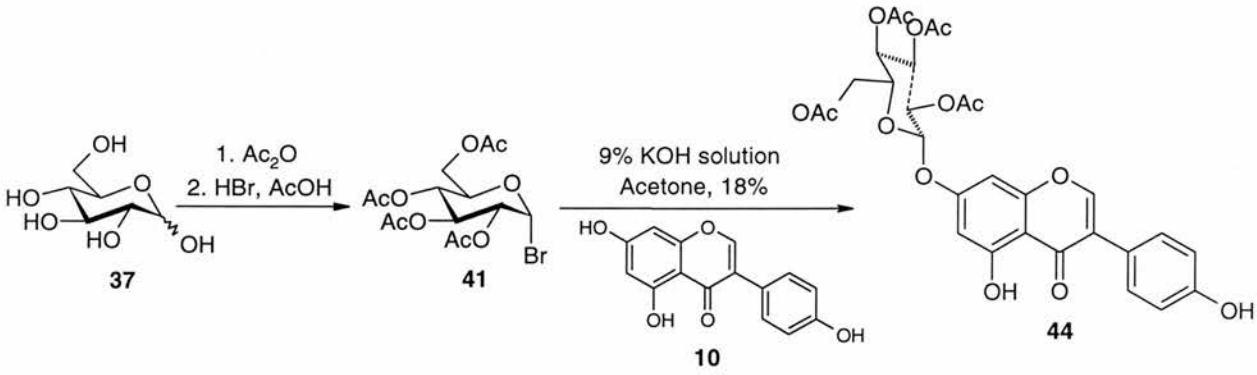
The bromides, however, combine the most useful reactivity with stability and the displacement of the glycosyl bromide with alcohols or phenols in the presence of silver oxide or silver carbonate is the basis for the Koenigs-Knorr reaction (scheme 17).[80] α-Acetobromoglucose (**41**) was shown to react with oxygen nucleophiles to form the β-glucopyranoside (**43**) in good yield with complete stereochemical inversion. The mechanism probably involves elimination of AgBr followed by the formation of a 1,2-acyloxonium ion (**42**) by the participation of the adjacent acetyl group. The oxygen nucleophile can then only attack from the β-face to give the β-*O*-glycoside in good yields for most simple alcohols. One distinct disadvantage, however, in using this procedure for isoflavone-*O*-glucoside preparation (R = isoflavone aglycone) is that lower yields are generally observed for substrates of increasing structural complexity.[81]

Upon successful glucosylation, the removal of acetyl protecting groups may be afforded under mild conditions in order to prevent the breakdown of the weak isoflavone-glucoside linkage. Suitable methods include MeONa/MeOH,[82] KCN/EtOH,[83] K_2CO_3/MeOH[84] and triethylamine in methanol.[85]

2.2.2: Literature syntheses

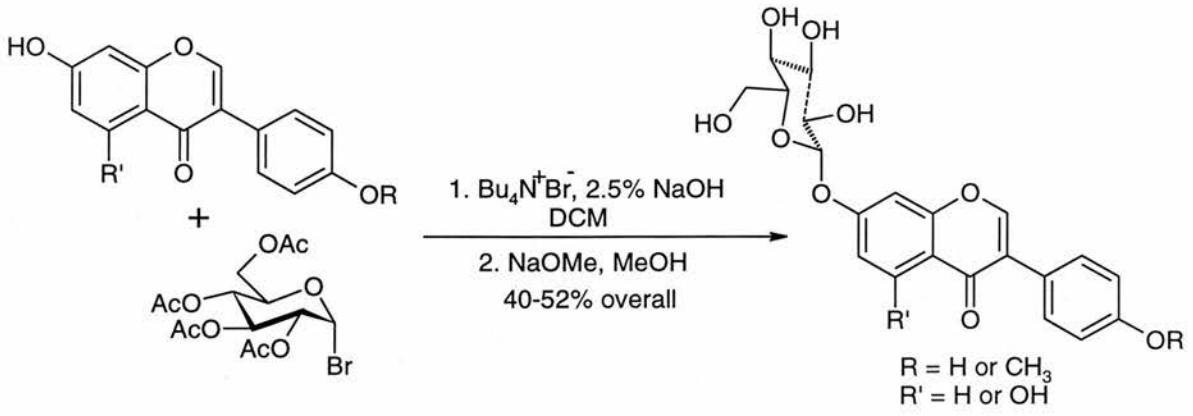
Reported methods for the glycosylation of isoflavone aglycones use α-acetobromoglucose as the glucoside donor and product yields tend to be poor. However, the preferred site of glycosylation in 4',7-dihydroxyisoflavones is at the 7-position as a

result of its greater acidity and the formation of isomeric mixtures does not easily occur. One of the earliest reported attempts to synthesise glucoside derivatives of isoflavones was Zemplen's 1943 base-catalysed reaction of α-acetobromoglucose (**41**) with unprotected hydroxyisoflavones in 9% potassium hydroxide solution.[86] The genistein-7-*O*-glucoside tetra-acetate (**44**) was formed from genistein (**10**) in 18% yield (scheme 18), however subsequent publications complain of isoflavone C-ring cleavage using this method.[82] Walz had also previously reported a similar problem with his 1931 attempt at isoflavone-7-*O*-glucoside synthesis using 5% potassium hydroxide solution.[28]



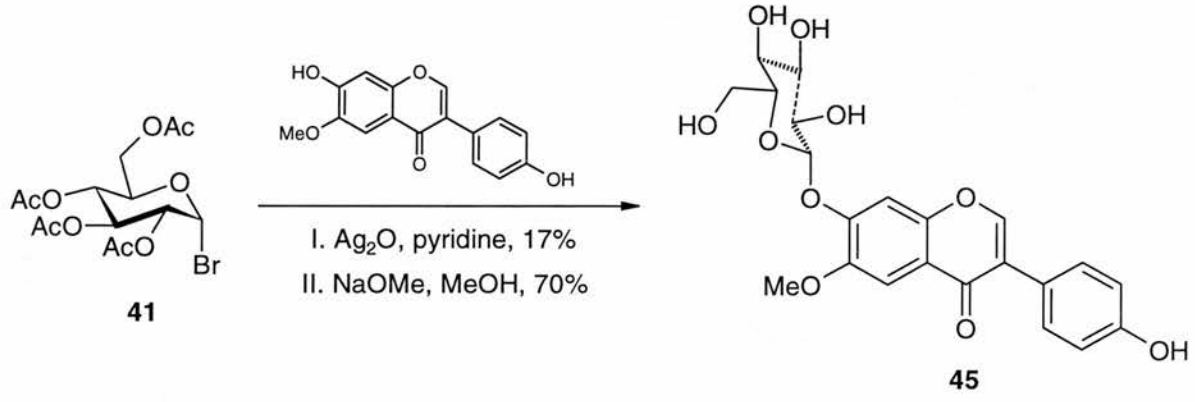
Scheme 18: *Base catalysed formation of genistein-7-O-glucoside tetra-acetate (**44**)*

A more recent 1998 study demonstrated the successful synthesis of a number of isoflavone glucosides in reasonable yields using phase transfer catalysis.[82] α-Acetobromoglucose was used in a two-phase system with aqueous sodium hydroxide and dichloromethane with TBAB as a catalyst. Subsequent removal of the acetyl protecting groups using sodium methoxide in methanol gave four isoflavone-7-*O*-glucosides in yields of between 40 and 52%.



Scheme 19: *Two-phase synthesis of isoflavone-7-O-β-glucosides*

Using a silver oxide catalyst under Koenigs-Knorr conditions, the isoflavone glycitein was shown to undergo glucosylation to form glycitein-7-*O*-glucoside tetra-acetate in 17% yield.[87] Subsequent removal of the acetate protecting groups was afforded by stirring in a mixture of sodium methoxide and methanol to yield glycitein-7-*O*-β-glucoside (glycitin, **45**) in 70% yield.



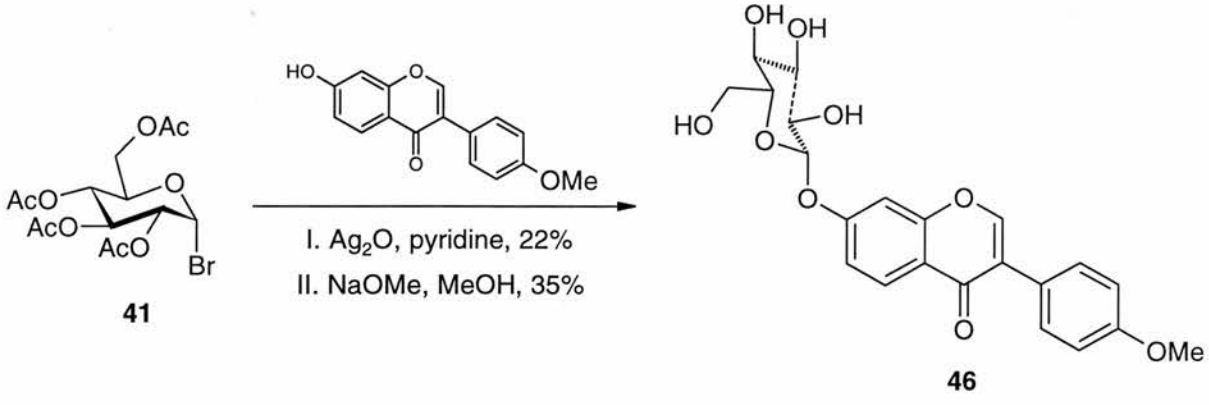
Scheme 20: *Synthesis of glycitin (**45**)*

2.2.3: Attempted synthesis using a two-phase system

Our own investigation of a similar method to that described in scheme 19, (section 2.2.1) was attempted using formononetin and α-acetobromoglucose in ethyl acetate. Sodium hydroxide was added and stirring was afforded at room temperature for 18 hours. The reaction was unsuccessful, however, most probably due to the insolubility of the isoflavone in the reaction media. In a separate reaction, dichloromethane was used as the solvent instead of ethyl acetate, however the only isolated product was the starting material after 50 hours stirring at room temperature.

2.2.4: Synthesis of ononin using Koenigs-Knorr conditions

Our own attempts to repeat the reaction using formononetin instead of glycitein were successful to give formononetin-7-*O*-glucoside tetra-acetate in 22% yield (scheme 21). Ononin (**46**) was then afforded in 35% yield by removal of the acetate protecting groups. The required α-acetobromoglucose was prepared by the addition of hydrogen bromide in acetic acid to a solution of D-glucose in acetic anhydride. Following stirring at room temperature for 48 hours, the product was obtained as a pale golden syrup in 86% yield (scheme 18).



Scheme 21: *Koenig-Knorr synthesis of Ononin (**46**)*

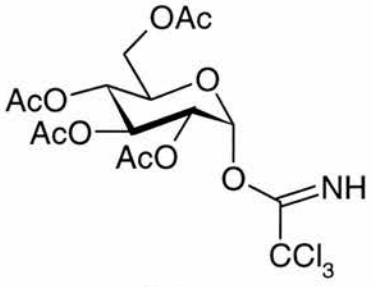
The yields for the coupling reaction are only fair. However, this was shown to be a reliable and reproduceable procedure. This is an especially useful quality considering future reactions may be performed at the end of long synthetic procedures for the formation of unusual or isotopically labelled isoflavonoids.

An alternative metal catalyst was then considered at this stage in an attempt to improve the reaction yields. The use of silver triflate in dichloromethane, was employed in a similar procedure, however analysis by t.l.c. revealed that only the isoflavone aglycone was present. However, as many of these glucosylation compounds may have future involvement in human metabolic studies consideration of toxic metal catalysts, particularly silver salts, should be limited.

2.2.5: Synthesis of a glucosyl trichloroacetimidate (**47**)

It has been shown that glycosyl imidates, particularly trichloroacetimidates are versatile glycosylation donors due to their high reactivity towards nucleophiles under mild conditions.[88] The reaction of α-trichloroacetimidates with oxygen nucleophiles was found to selectively form β-glucosides in the presence of either a boron trifluoride etherate catalyst or TMSOTf in propionitrile at –80 °C.[77]

Preparation of the glycosyl imidates from the 2,3,4,6-tetra-*O*-acetyl-α/β-D-glucopyranose (**50**) can be afforded using trichloroacetonitrile to give either of the two anomers, depending on the base used. In the presence of a weaker base such as potassium carbonate the β-anomer is allowed to form as the kinetically favoured product. However, because imidate formation is a reversible process, the use of sodium hydride as base produces the more thermodynamically favoured α-anomer as the single product.



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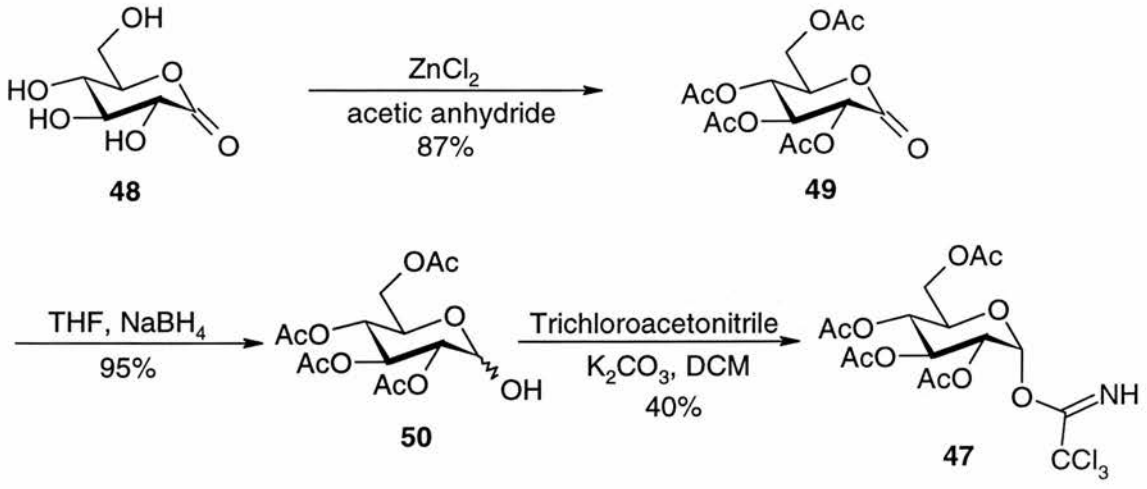
The glucosyl imidate (**47**) was thus prepared in three steps starting with the acetylation of D-glucono-γ-lactone (**48**). Stirring in acetic anhydride and zinc chloride gave 2,3,4,6-tetra-*O*-acetyl-D-glucono-γ-lactone (**49**) as a golden syrup in 87% yield. Subsequent reduction to the 2,3,4,6-tetra-*O*-acetyl-α/β-D-glucopyranose (**50**) was then afforded using sodium borohydride in tetrahydrofuran. This yielded a syrup in 95% yield. ^{13}C n.m.r. spectroscopy revealed the carbonyl resonance due to the C-1 position, previously at 165.1 ppm was now absent and instead two resonances were observed at 96.0 and 90.6 ppm. These corresponded to the C-1 carbon in the α and β anomers of the products.

Reaction of this alcohol with trichloroacetonitrile in dichloromethane was not initially successful. The base used in the reaction was sodium hydride and stirring was carried out for 20 minutes at room temperature. The crude product was purified by column chromatography, yielding the desired product in only 4% yield as well as the returned starting material. The reaction was then repeated using glowing hot potassium carbonate as an alternative to sodium hydride. After stirring at room temperature for 48 hours, the resultant crude product was purified by column chromatography to leave the desired product as a white solid in 40% yield. This was shown by ^{1}H n.m.r. spectroscopy to be predominately the α-anomeric product, displaying the H-1 resonance as a doublet at 6.55 ppm with a coupling constant, $J_{1,2}$ of 3.3 Hz. This can be distinguished from the β anomeric product where the $J_{1,2} = 7.0$ Hz. The melting point however was recorded at 68-71 °C, intermediate of the literature values of the pure α and β-anomers. The β-anomeric configuration melts at 154-155 °C where the α-anomer exists as an oil.

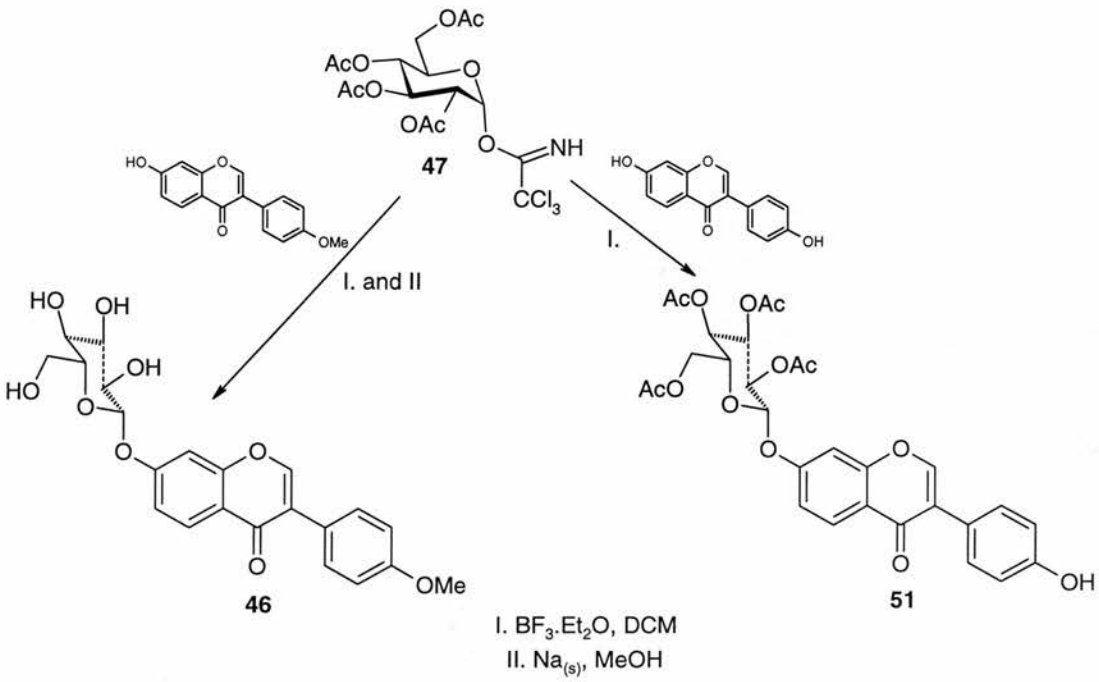


Scheme 22: *Preparation of glucosyl imidate (**47**)*

2.2.6: The synthesis of ononin (**46**) and daidzein-7-*O*-glucoside tetra-acetate (**51**)

The coupling reaction was then carried out using formononetin and the glucosyl trichloroacetimidate in dichloromethane. Boron trifluoride etherate was added turning the suspension to a light green solution and stirring was continued for 18 hours. Analysis of the crude product by t.l.c. revealed the presence of the desired product as well as the isoflavone aglycone. Column chromatography was attempted, however, the glucoside (**52**) could not be fully separated from its aglycone and a mixture of these species was isolated.

The mixture of the glucoside and isoflavone was reacted further with a solution of sodium methoxide in methanol to allow cleavage of the acetate groups. The sodium methoxide was formed *in situ* by the addition of a catalytic amount of sodium metal to methanol and the reaction was heated at 60 °C for 2 hours. The deprotected glucoside, ononin (**46**) was separated from minor reaction products by column chromatography. The solvent mixture used was 10% methanol in acetonitrile to remove the highly polar glucoside. However, microanalysis results showed significant errors and this was thought to be due to the inclusion of methanol soluble salts during the chromatography procedure.



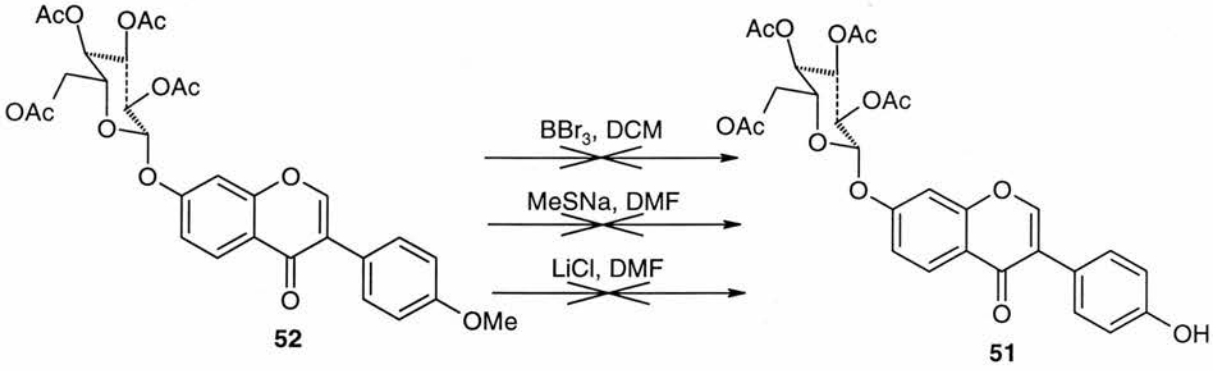
Scheme 23: *Preparation of isoflavone-7-O-glucosides using glycosyl imidates*

Ononin was obtained in a yield of 47% over two steps, however this takes into account the recovery of unreacted starting material. ^{1}H n.m.r. spectroscopy indicated a single anomer to be present with the appearance of a doublet at 5.16 ppm, $J_{1,\ 2}$ = 5.8 Hz, denoting the proton at the H-1 position. No resonance corresponding to the H-1 proton for the α anomer was observed. The positions of H-5, 6 and 8 had also changed to 8.08, 7.16 and 7.26 ppm from their positions of 7.99, 6.95 and 6.89 ppm respectively in the free aglycone. The melting point was recorded at 214-218 °C, close to that of the literature value of 213-214 °C.[89]

The electrospray mass spectrum of the ononin revealed the main peak to be at *m/z* 453, corresponding to the desired mass plus a sodium ion. The isoflavone aglycone was also present at 291 as a minor peak although this is probably due to partial degradation of the glucoside on the column.

Attempts were also made to improve the yield of the coupling reaction by varying the amounts of boron trifluoride etherate used. Initially, 4 equivalents of the catalyst were used and in subsequent reactions this was later raised to 8 and then 12 equivalents. However, observation of integral heights in the ^{1}H n.m.r. spectra revealed that the relative amounts of formononetin to ononin in the product mixture remained constant in each case.

The preparation of daidzein-7-*O*-glucoside (commonly termed daidzin tetra-acetate, **51**), was initially attempted by demethylation of the impure formononetin-7-*O*-glucoside tetra-acetate (**52**) under a variety of conditions. The use of boron tribromide in dichloromethane[90] proved too harsh and resulted in total cleavage of the glycoside linkage giving a mixture of formononetin and daidzein as products. Alternative conditions were also found to be unsuitable, as in all cases the glycoside linkage did not survive the reaction. The use of sodium methanethiolate in dimethylformamide[91] also yielded a mixture of formononetin and daidzein whereas the use of lithium chloride in dimethylformamide,[92] a far milder set of conditions, gave formononetin as the only reaction product.



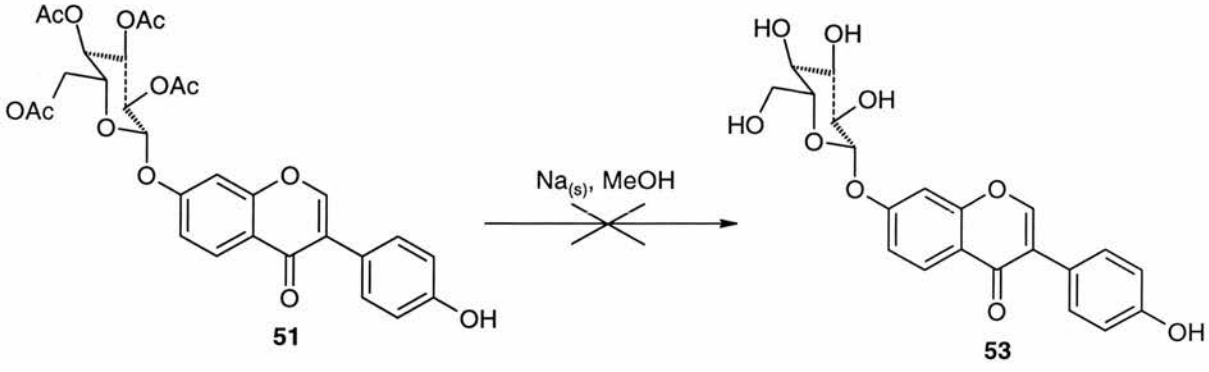
Scheme 24: *Attempted formation of daidzein-7-O-glucoside tetra-acetate (**51**)*

Preparation was then attempted using a similar procedure to ononin using the glucosyl trichloroacetimidate and daidzein. The coupling reaction and work up were performed as before but it was found that in this case separation of the glucoside from daidzein was

successful by column chromatography using normal phase silica gel. This gave the desired product as a white solid in 43% yield.

^{1}H n.m.r. spectroscopy again revealed changes to H-5, 6 and 8 positions moving from 7.83, 6.80 and 6.71 ppm respectively in daidzein to 7.99, 6.97 and 7.07 ppm in the glycoside (**51**). The single anomeric configuration is also present with the H-1α proton appearing as a doublet at 5.62 ppm, $J_{1,2} = 7.6$ Hz.

Electrospray mass spectrometry revealed two major peaks at *m/z* 607 and 623 corresponding to the desired mass plus a sodium ion and a potassium ion respectively. A minor peak at *m/z* 254 was also seen showing that some of the free aglycone was present. The attempted formation of daidzin (**53**) by removal of the acetyl protecting groups of the tetra-acetate (**51**) using similar conditions as for the formation of ononin was unsuccessful (scheme 25). ^{1}H n.m.r. spectroscopy of the crude reaction product showed that only the isoflavone aglycone was present and the glycoside bridging linkage had not survived the reaction. This was found to be a reproducible result over several reaction attempts.



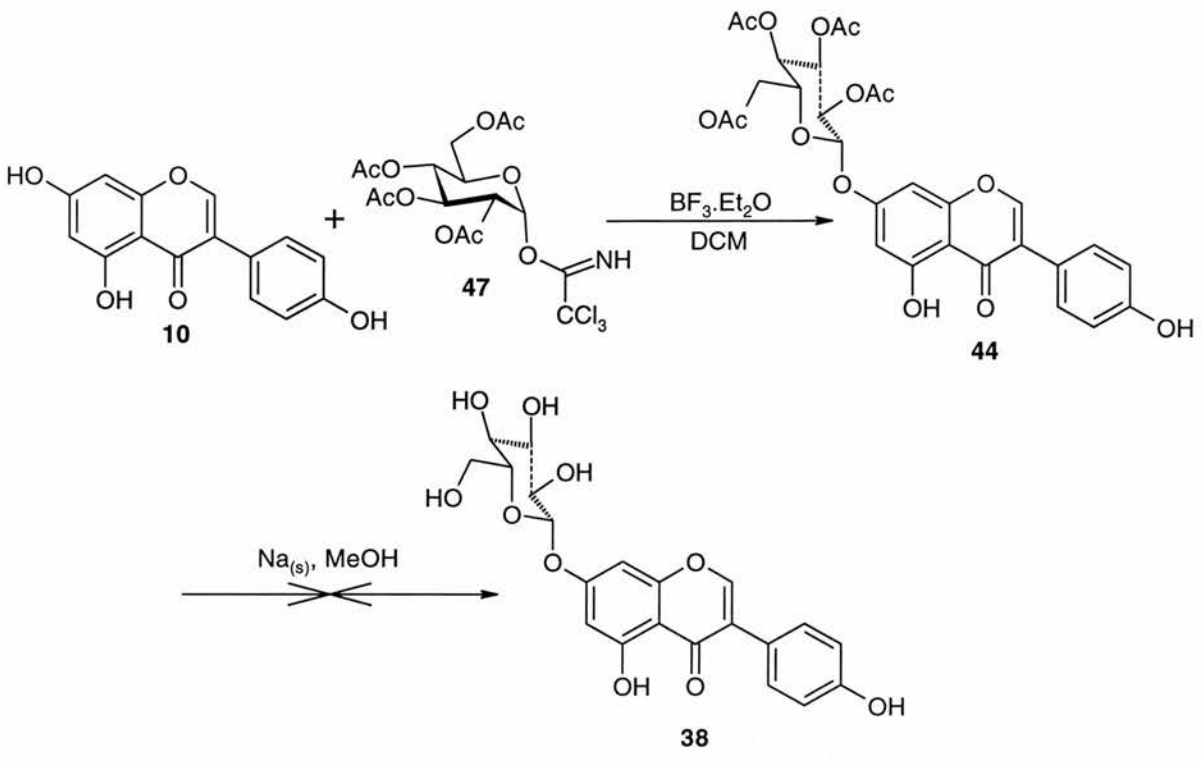
Scheme 25: *Attempted formation of daidzin (**53**)*

Further attempts to improve the yield of the coupling reaction involved the use of additional 0.3 equivalents of the sugar, added one hour after the reaction had commenced. However, analysis by t.l.c. showed no visible signs of improvement and upon work up the product was isolated in a similar yield.

2.2.7: Attempted preparation of genistin (**38**)

The synthesis of genistin (genistein-7-*O*-glucoside, **38**) has also been attempted using this route (scheme 26) and initial signs for the reaction were encouraging. Analysis of the reaction by t.l.c. after stirring at room temperature for 18 hours suggested that only a small amount of starting material remained. However, work up of the reaction involving the addition of sodium hydrogen carbonate solution seemed to be responsible for degradation of the product back to the starting material. Work up at lower temperatures and even immediate concentration of the reaction mixture at reduced pressure did not help to prevent this problem. Attempted separation of the genistein from the remaining glycosylation product by column chromatography eluted only the starting material. Analysis by 2D t.l.c. revealed that silica gel aided the degradation of the genistin due to its acidity and so alternative chromatographic supports were considered.

Column chromatography was performed using basic aluminium oxide (pH 9.3-9.7) however elution did not occur with the product mixture remaining bound to the column. Separation was also attempted using a column of lipophilic sephadex LH-20 using dimethylformamide as eluent.[93] In this case a small amount of genistein was isolated but again, the genistin remained bound to, or degraded on the column. The pore of the lipohilic beads should be large enough to allow even larger molecules such as isoflavone glucosides to maintain an adequate flow rate, however it is possible that aggregation and subsequent blockages occur.



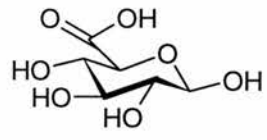
Scheme 26: *Attempted preparation of genistein-7-O-glucoside (**38**)*

As an alternative to glucoside separation the crude coupling reaction mixture was reacted with a mixture of sodium methoxide in methanol at 60-65 °C. This attempt to afford acetyl deprotection, however, gave genistein as the only isolated product. It is unclear why the glycoside linkage in acetyl protected genistin is unstable to these conditions where the analogues of daidzin and ononin have been prepared in reasonable yields. However, attempts to prepare genistin under Koenigs-Knorr conditions as previously described in section 2.2.4 using a silver oxide catalyst also proved to be unsuccessful.

2.3: The isoflavone-7-*O*-glucuronides

2.3.1: Introduction

The removal of toxic or unwanted xenobiotics and their metabolites in biological organisms is essential in the avoidance of genomic damage. Enzymatic conjugation to glucuronic acid (**54**) is one such biological method used to remove a wide variety of aglycones, including isoflavones and their metabolites, by increasing water solubility and allowing subsequent excretion in the urine. Glucuronic acid is structurally similar to β-D-glucose, possesing the same pyranose ring, however it contains a carboxylic acid group instead of the alcohol at the 5" position.



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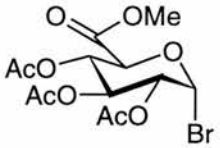

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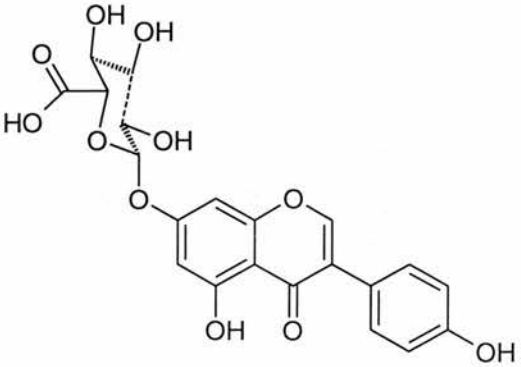

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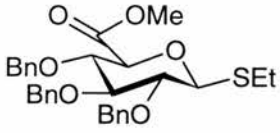

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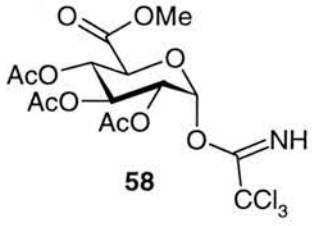

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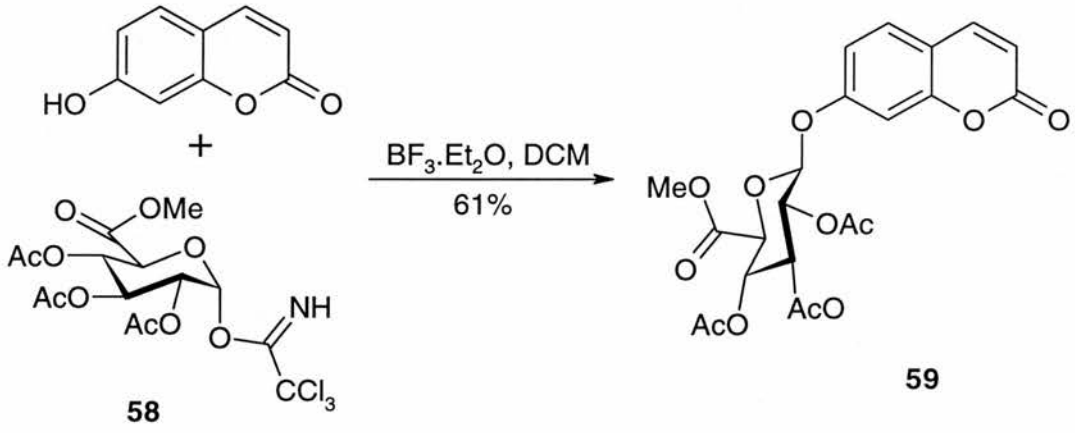
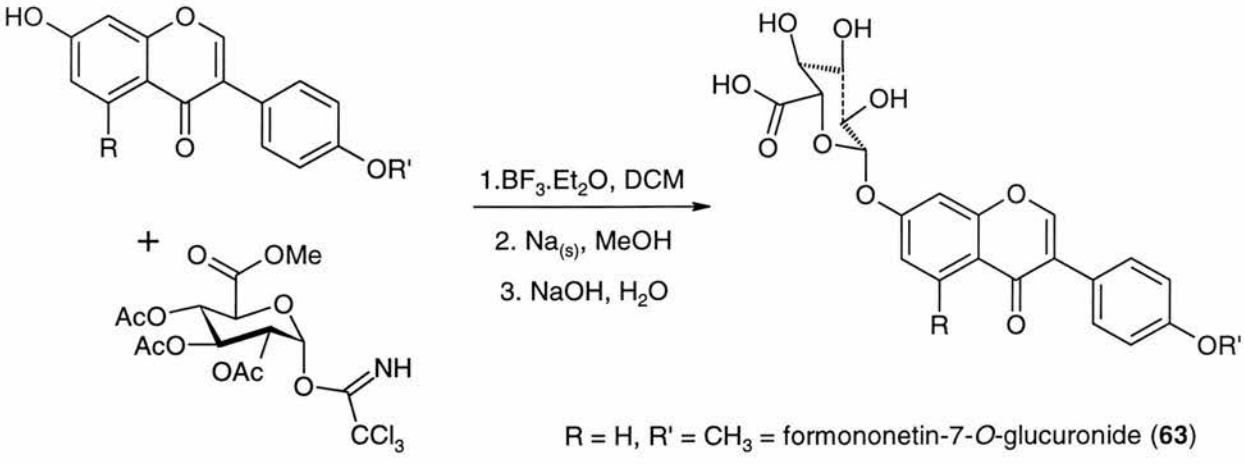
As far as we are aware, no previous chemical synthesis of isoflavone-7-*O*-β-glucuronides (for example genistein glucuronide, **55**) are known and it was our intention to prepare a range of these compounds from formononetin, daidzein and genistein aglycones. Existing methodology for the coupling of glucuronic acids to other aglycones mainly involve the use of the bromide (**56**)[94] in a Koenigs-Knorr type reaction similar to that previously discussed for glycosides. Alternatively, Tsou and Seligman have shown that catalytic oxidation of corresponding glycosides[95] can form glucuronic acid conjugates using gaseous oxygen and a palladium catalyst as in the procedure described by Marsh.[96] The thioglucuronides (**57**) have also been tested as donors in coupling reactions with unreactive alcohols using DMTST as a promoter to give glucuronide disaccharides in

good yields.[97] However, it has been previously reported that the glucuronyl trichloracetimidate (**58**) can be successfully coupled to 7-hydroxycoumarins using boron trifluoride etherate (scheme 27).[98] Stirring at ambient temperatures for 16 hours produced the coumarin glucuronide (**59**) in a very reasonable 61% yield. During the coupling procedure the glucuronic acid is protected as a methyl ester. Conversion back to the acid usually takes place at the end of the reaction sequence and this is achieved by hydrolysis under basic conditions.



Scheme 27: *Synthesis of a coumarin-7-O-β-glucuronide (**59**)*

It was thus proposed that the glucuronyl trichloroacetimidate could be used in an adapted reaction to allow preparation of isoflavone glucuronides in a method similar to that used for the glucosyl analogues.



R = H, R' = CH_3 = formononetin-7-*O*-glucuronide (**63**)

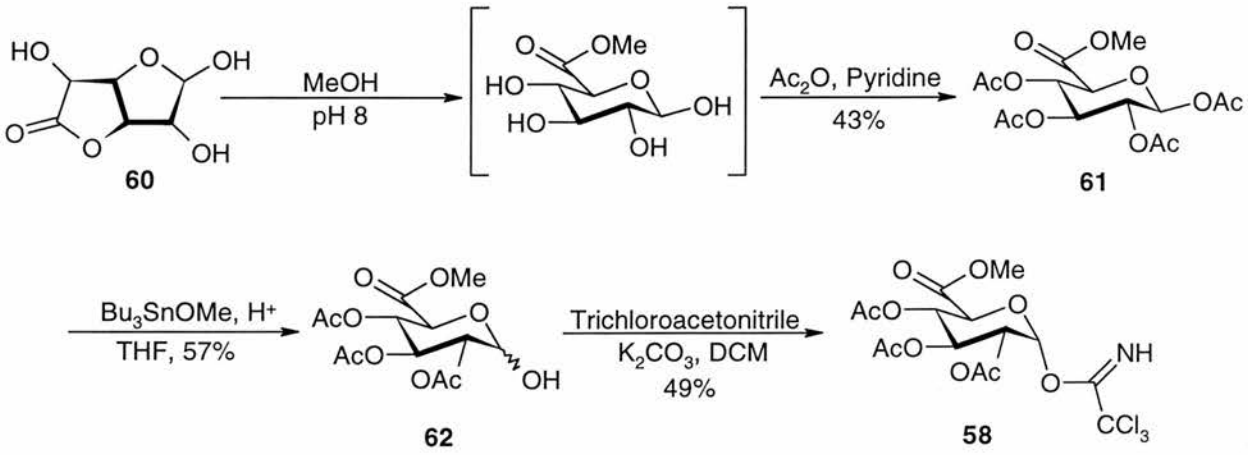
R = H, R' = H = daidzein-7-*O*-glucuronide (**64**)

R = OH, R' = H = genistein-7-*O*-glucuronide (**65**)

Scheme 28: *Preparation of isoflavone–7-O-β-glucuronides (**63-65**)*

2.3.2: Synthesis of a glucuronyl trichloroacetimidate (**58**)

Synthesis of the imidate (**58**) required an alternative approach to that of the glucosyl trichloroacetimidate. The route used can be seen in scheme 29 and involves the ring cleavage and rearrangement of D-glucurono-6,3-lactone (**60**) in methanol under mildly basic conditions to give the six membered ring. Acetyl protection of the free hydroxy groups was then afforded by the addition of acetic anhydride in pyridine and methyl 1,2,3,4-tetra-*O*-acetyl-α/β-D-glucopyranuronate (**61**) was subsequently isolated as a brown solid in 43% yield. The literature melting point of 178 °C[99] corresponded well with the value of 174-177 °C obtained and the ^{13}C n.m.r. spectroscopic data revealed the presence of four acetyl methyl resonances between 20.98 and 21.29 ppm in addition to the methyl ester at 53.8 ppm.



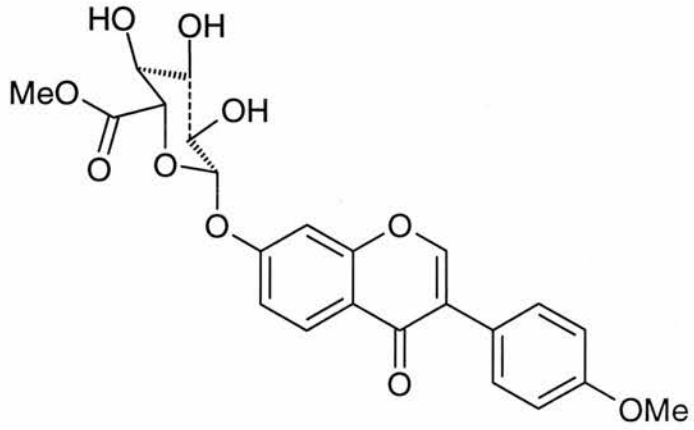
Scheme 29: *Synthesis of glucuronyl trichloroacetimidate (58)*

The formation of the uronate, **62** was successfully achieved using tributyltin methoxide under acidic conditions.[100] The crude compound was trituated from hexane at -78 °C to leave the desired alcohol as a white solid. ^{13}C n.m.r. spectroscopic data revealed the presence of only three acetyl carbon resonances between 20.96 and 21.00 ppm and the literature melting point of 116 °C[101] compared reasonably with the values obtained (101-103 °C). The subsequent reaction of the alcohol with trichloroacetonitrile was performed in an identical manner to that of the glucoside and purification of the crude product yielded compound **58** in 49% yield as a white solid. ^{1}H n.m.r. spectroscopy showed the presence of only one anomeric configuration with the H-1 proton appearing as a doublet at 5.13 ppm, $J_{1,2} = 6.6$ Hz. The isolated product possessed a melting point of 104-106 °C which corresponded favourably with the literature value of the α-anomer (108 °C).[102]

2.3.3: Synthesis of formononetin glucuronide methyl ester (**63**)

The coupling reaction with boron trifluoride etherate and the isoflavone formononetin was then carried out in an identical manner as before. Again, the reaction did not go to completion but stirring at room temperature for 18 hours gave a mixture of both the starting material and the desired isoflavone glucronide. T.l.c. values again proved to be too similar to allow their separation by column chromatagraphy and so the product

mixture was heated with sodium methoxide in methanol, affording the cleavage of the acetyl protecting groups. This was successful and the glucuronide (**63**) was isolated as its methyl ester by column chromatography using C-18 reverse phase silica gel as a white solid. The product yield was 46% combined for the two steps. ^{1}H n.m.r. spectroscopy showed the H-1 shift as a doublet at 5.38 ppm and the melting point was obtained as 166-168 °C.



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2.3.4: Attempted synthesis of daidzein and genistein glucuronides (**64** and **65**)

Repetition of the procedure for both daidzein and genistein, however proved to be less successful. In the case of daidzein-7-*O*-glucuronide (**64**), analysis by t.l.c. suggested that only a negligible amount of a new product had been formed at any stage of the 24 hour reaction period. The reaction work-up produced an oily solid. ^{1}H n.m.r. spectroscopy confirmed this to be almost entirely the isoflavone aglycone, daidzein.

The same reaction, but using genistein, initially appeared to be more successful. Analysis by t.l.c. over a similar reaction period as for daidzein showed that reasonable amounts of a new product had been formed and the crude product was isolated as a pale yellow solid. ^{1}H n.m.r. spectroscopy showed that the only isolated product was the isoflavone aglycone, however, and that the modest amount of genistein-7-*O*-glucuronide (**65**) that may have formed appeared to have degraded during the reaction work-up. Addition of the

sodium hydrogen carbonate solution at low temperature or direct transfer of the concentrated reaction mixture onto chromatographic supports were also carried out in an attempt to prevent the breakdown of the glucuronide. However, these modifications also failed to isolate any of the desired product. Both of these reactions were repeated several times and results proved to be reproducible.

2.4: Future work

It has been shown that the isoflavones formononetin and daidzein can be successfully glycosylated using glycosyl trichloroacetimidate methodology. However, it is disappointing that these conditions do not appear to be suitable for genistein, the isoflavone responsible for the greatest interest in the medical and pharmacological world. It is feasible that imidate methodology may be successful by using TMSOTf as an alternative catalyst to boron trifluoride etherate or by employing a different reaction work-up procedure and this is something that warrants further investigation. However, at this stage it was decided to concentrate on other more productive parts of the project.

Once a reliable glycosylation procedure has been established, it would then be possible to repeat the reactions using ^{13}C labelled isoflavone aglycones to give a range of [^{13}C]isoflavone–7-*O*-glycosides. It would also be possible to prepare these compounds with the isotopic labels in the sugar moiety as well as in both the sugar and aglycone areas of the molecule. These compounds could then be used in helping to determine more accurately the metabolic pathways of dietary isoflavones and as internal standards in LC-MS analysis.

Chapter 3

Synthesis of Chlorinated Isoflavones

3.1: Isoflavone metabolism as an enhancement to biological function

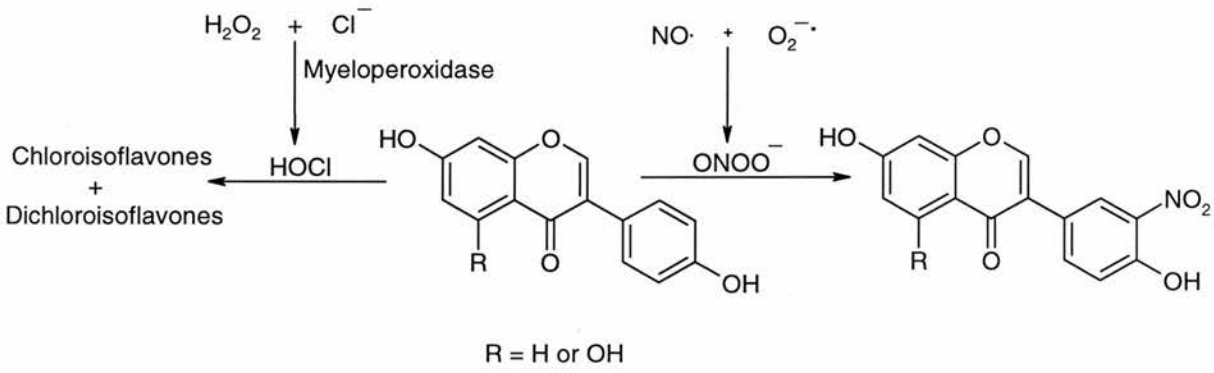
The consumption of dietary isoflavones, predominatly from soy products, has been shown to be associated with a lowered risk of several chronic inflammatory diseases. Amongst these are hormone dependent cancers, especially breast cancer and also with a reduction of LDL oxidation which can lead to athlerosclerosis. However, although they can prevent LDL oxidation *in vitro* it is unlikely that the nanomolar concentrations of the isoflavones found in the blood of soy consumers are adequate to explain the *in vivo* observations. As an alternative, it has been speculated that instead of acting as free radical scavengers, the isoflavones may undergo metabolic activation to generate compounds that may be more potent in the regulation of cellular disfunction.[103] The metabolism of these compounds, however, in particular genistein and daidzein at localized sites is at present poorly understood.

The analysis of blood and urine levels is important in understanding the biological effects of isoflavones. However, blood does not perfuse into the cells of the breast which are instead surrounded by an interstitial fluid. It was therefore crucial that the chemical nature of the isoflavones in this medium be determined and this has been achieved by examination of the composition of interstitial fluid in the breast.

It was speculated that isoflavones will react with oxidants produced by other cells that congregate around the tumour, particularly during inflammatory processes. Cell culture experiments that mimic strongly inflammatory conditions within the breast show that isoflavones are readily chlorinated and nitrated by reactions with the cellular oxidants hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$). Since isoflavones in the breast are correlated with a positive prognosis for breast cancer it has been suggested that the halogenation of the isoflavones in this medium enhances their biological functions and thus has a greater protective effect on surrounding cells.

Hypochlorous acid is the reaction product formed *in vivo* from H_2O_2 and chloride ion by the action of myeloperoxidase (scheme 30). The products of reaction between HOCl and

genistein were identified by HPLC-mass spectrometry.[104] The mass spectra indicated that both a monochlorinated (*m/z* 303 and 305) and a dichlorinated derivative (*m/z* 337, 339 and 341) were present. The analysis of mixtures from cell-culture experiments by ^{1}H n.m.r. spectroscopy revealed that chlorination of genistein occurs at the 6, 8 and 3' positions. However, due to the small amounts of these compounds being produced and the difficulty of their purification from the mixture it has not been possible to unambiguously determine the chlorination sites in each isomer. Daidzein has also been shown by ^{1}H n.m.r. spectroscopy to react in a similar manner to yield a mixture of 6, 8 and 3' chlorinated isomers.



Scheme 30: *Chlorination and nitration of isoflavones*

The reaction of genistein and daidzein with peroxynitrite, formed from the reaction between the cellular superoxide ion ($O_2^{-\cdot}$) and nitric oxide ($NO^{\cdot}$), resulted in single products for both of the isoflavones. Analysis by HPLC-mass spectrometry revealed that the products possessed an *m/z* of 314 and 298, corresponding to the mono-nitrated isomers. ^{1}H n.m.r. spectroscopy revealed the nitrate group had reacted at the 3' position to give 3'-nitrogenistein and 3'-nitrodaidzein.

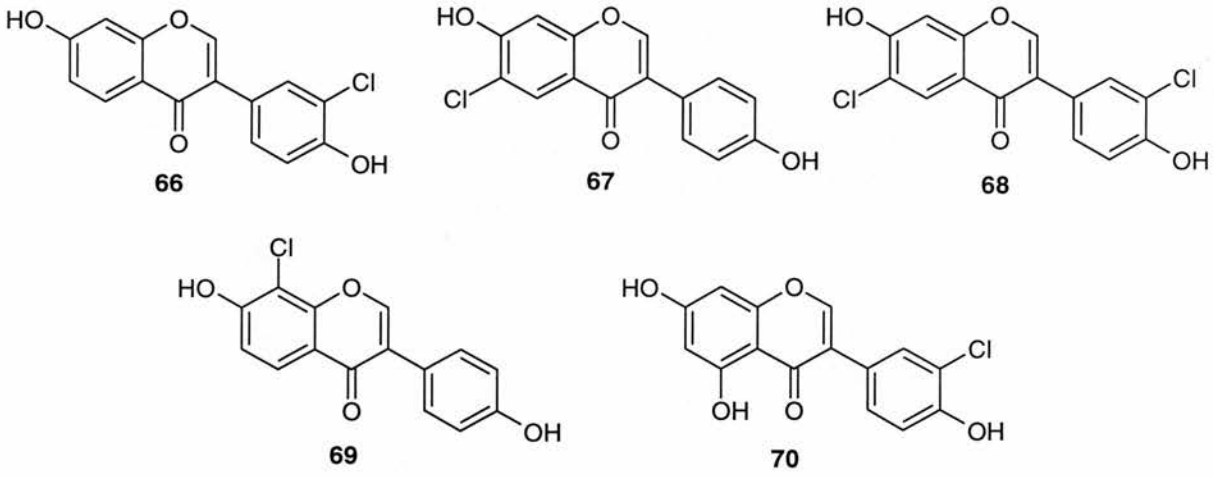
The aim of this work was therefore to synthesise the individual chlorinated isoflavones in order to develop parent ion-daughter ion mass spectrometry assays for quantitative analysis of each isomer. In addition, their biochemical and biological effects in cell

culture and other assay systems could be determined using only pure samples of material, to compare their activities with parent isoflavones.

3.2: Synthesis of chlorinated isoflavones

3.2.1: Introduction

The initial target was the preparation of a range of chlorinated derivatives of daidzein and genistein. The required mono and dichlorinated isomers of daidzein have thus been prepared. These are the 3'-chloro, 6-chloro, 3', 6-dichloro and 8-chloro derivatives (structures **66-69** respectively). In addition to this 3'-chlorogenistein (**70**) has also been prepared (scheme 31).



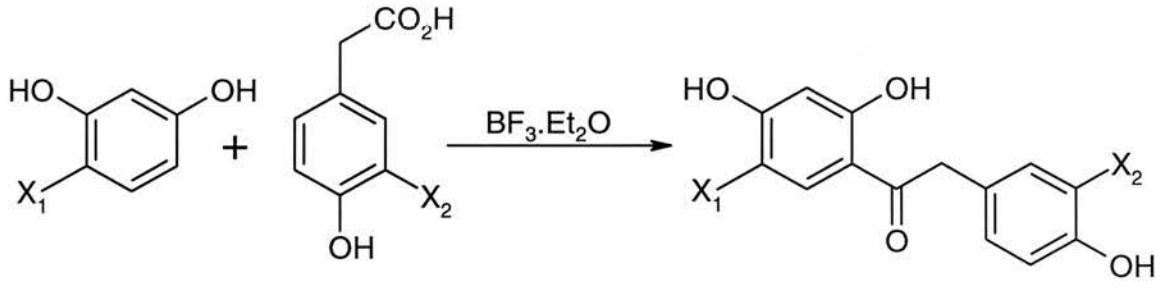
Scheme 31: *Structures of target chloroisoflavones*

All the isoflavones are likely to produce mixtures of compounds if chlorinated by chemical methods. This will result in the use of complicated and wasteful separation and purification methods. It was thus decided that it would be more advantageous to construct

the isoflavone skeleton using chlorinated starting materials. This would allow the production of a single chlorinated isomer.

3.2.2: Synthesis of 3'-chloro, 6-chloro and 3', 6-chlorodaidzeins

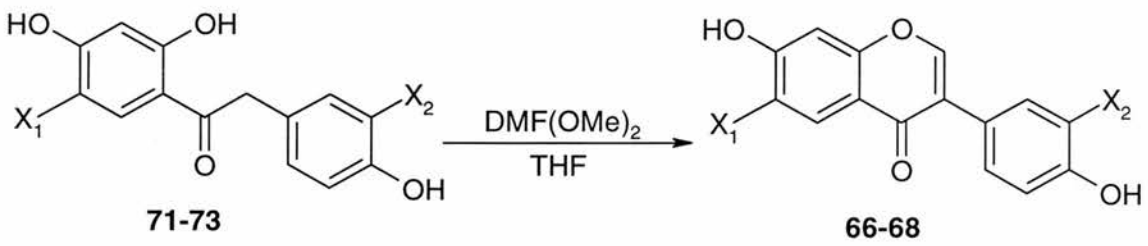
Using the strategy in scheme 32, 3'-chlorodaidzein (**66**) was prepared using a similar procedure to that employed in the formation of daidzein. 3-Chloro-4-hydroxybenzyl-2,4-dihydroxyphenyl ketone (**71**) was prepared from the condensation of resorcinol and 3-chloro-4-hydroxyphenylacetic acid in good yield using boron trifluoride etherate.[55] Analysis by ^{13}C n.m.r. spectroscopy clearly showed the carbonyl has now moved downfield to 203.9 ppm indicating the presence of a ketone rather than an acid. The mass spectrum gave an *m/z* of 279, 281 suggesting formation of the desired product. Subsequent cyclization was performed in the presence of dimethylformamide dimethyl acetal[55] and tetrahydrofuran to give impure 3'-chlorodaidzein. The isoflavone was then purified using column chromatography and by washing in aqueous methanol to give the product as a white powder. Analysis by ^{13}C n.m.r. spectroscopy revealed the carbonyl had again changed to 176.6 ppm and the mass spectrum gave molecular ions at *m/z* 289 and 291 as a result of the two isotopes of chlorine present in the isoflavone.



Scheme 32: *Preparation of chlorodeoxybenzoins (**71-73**)*

	X_1	X_2	Yield (%)	^{13}C n.m.r. C=O (ppm)	M.S. *m/z* (M^+)	m.p. (°C)
71	H	Cl	63	203.9	279, 281	192-195
72	Cl	H	77	204.1	279, 281	188-191
73	Cl	Cl	81	203.3	313, 315	208-211

4'-Hydroxybenzyl-2,4-dihydroxy-5-chlorophenyl ketone (**72**) was then prepared by a similar reaction sequence using 4-chlororesorcinol. 3'-Chloro-4'-hydroxybenzyl-2,4-dihydroxy-5-chlorophenyl ketone (**73**) was also prepared using both of the mono-chlorinated precursors. The deoxybenzoin formation and subsequent cyclization were shown to have worked using ^{13}C n.m.r. spectroscopy and mass spectrometry. Purification of the isoflavones also involved column chromatography however in the case of **67** and **68** a further column was required on C-18 reverse phase silica-gel.

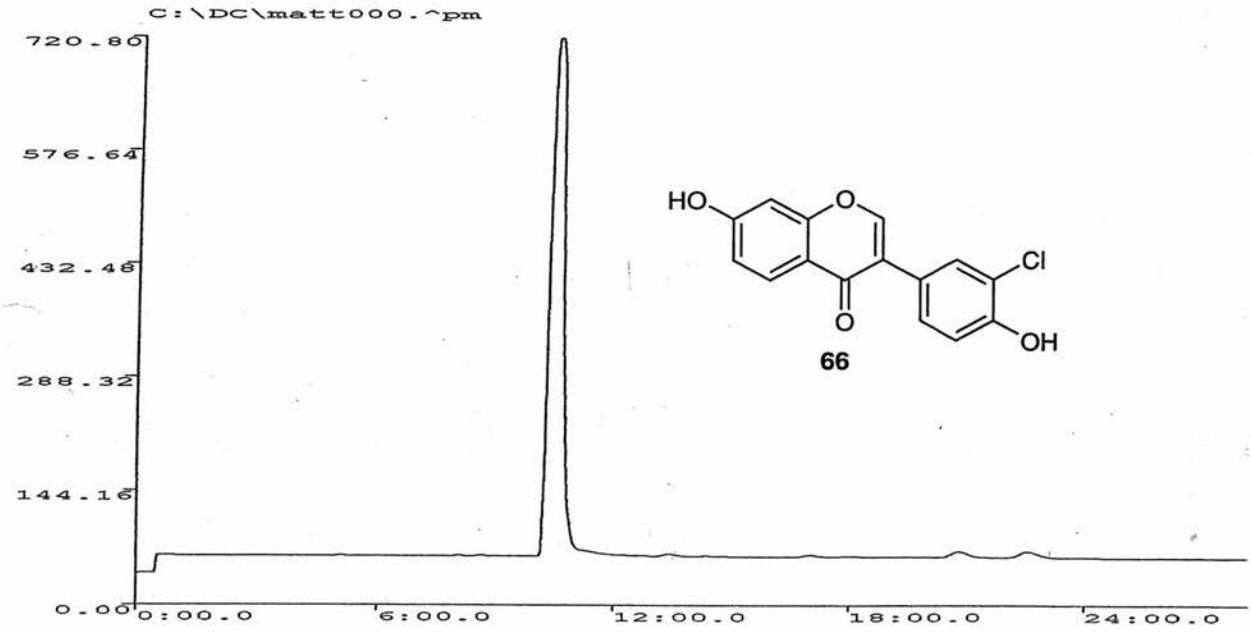


Scheme 34: *Preparation of isoflavones (**66-68**)*

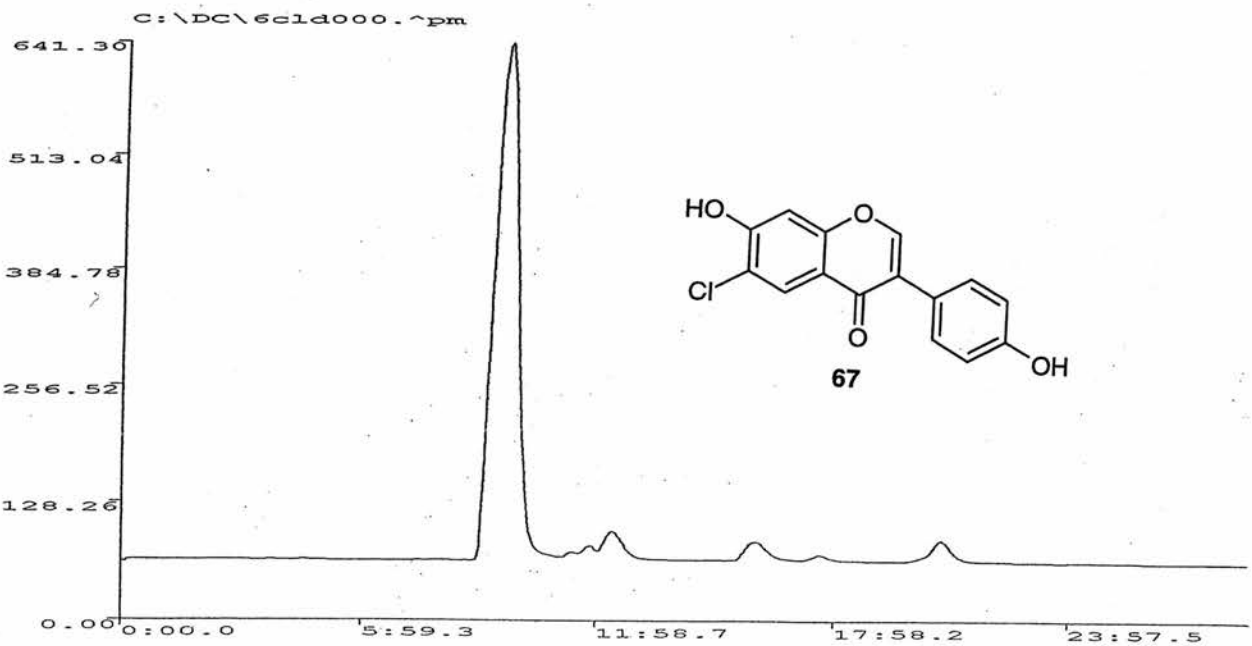
	X_1	X_2	Yield (%)	^{13}C n.m.r. C=O, (ppm)	M.S. *m/z* (M^+)	m.p.(°C)
66	H	Cl	25	176.6	289, 291	267-268
67	Cl	H	29	174.2	289, 291	318-320
68	Cl	Cl	44	173.5	323, 325	235-236

Analysis by HPLC was also performed using a luna 5 C-18 silica-gel (150 x 4.60 mm) column. The solvent mixture used for all the isoflavone reaction products was acetonitrile : water, 1 : 1 and the flow rate was set at 0.3 ml per minute for a period of 30 minutes.

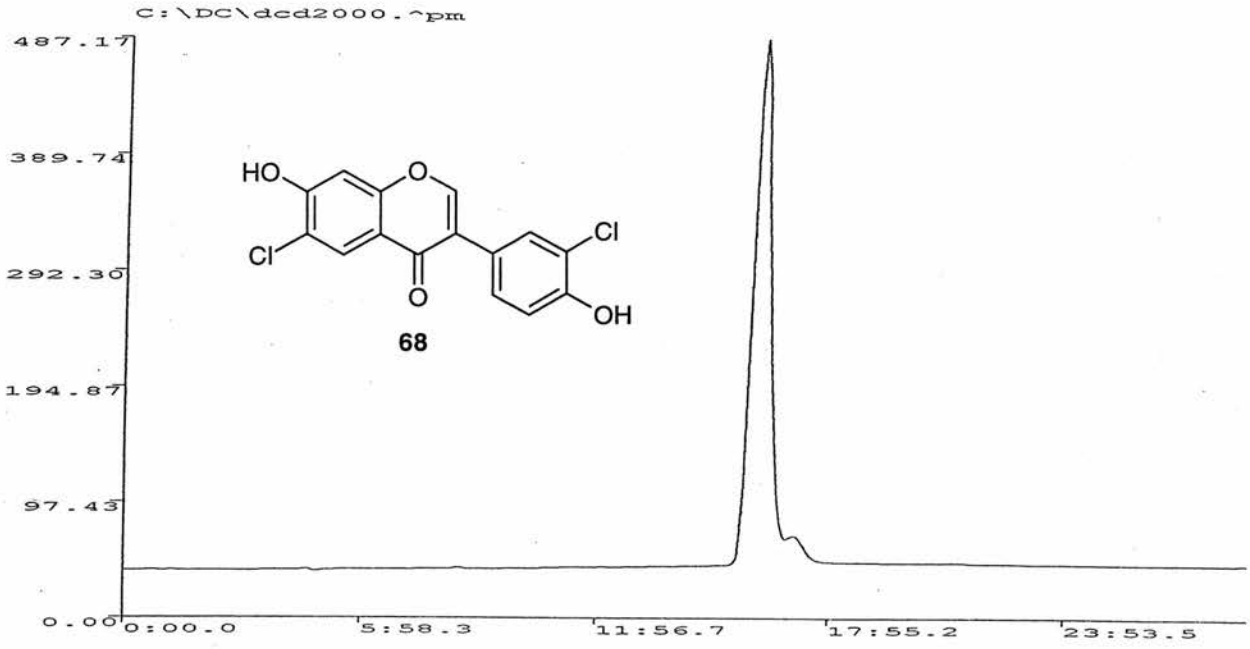
The HPLC traces for the 3'-chloro, 6-chloro and 3', 6-dichloro derivatives of daidzein are shown below in scheme 33. 3'-Chlorodaidzein shows a single peak with a retention time of approximately 10 minutes. No other trace peaks can be observed. The HPLC results for 6-chlorodaidzein showed minor impurities with retention times of 12, 15 and 19 minutes in addition to the isoflavone peak appearing at 9 minutes. 3', 6-Dichlorodaidzein showed no visible traces of impurity on the trace and displayed a retention time of 16 minutes.



66



67

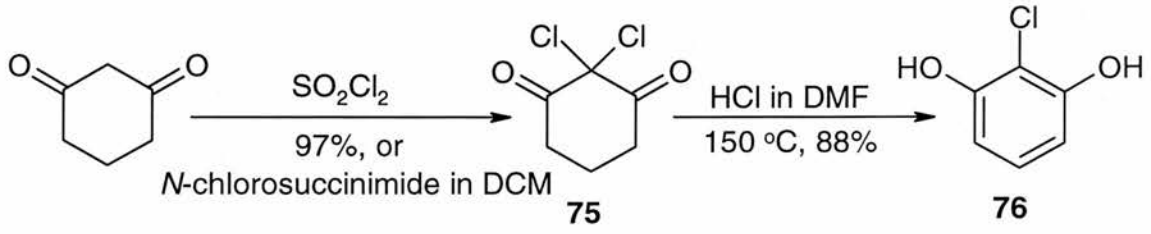


Scheme 33: *HPLC traces for 3'-chloro, 6-chloro and 3', 6-dichlorodaidzein*

3.2.3: Synthesis of 8-chlorodaidzein (**69**)

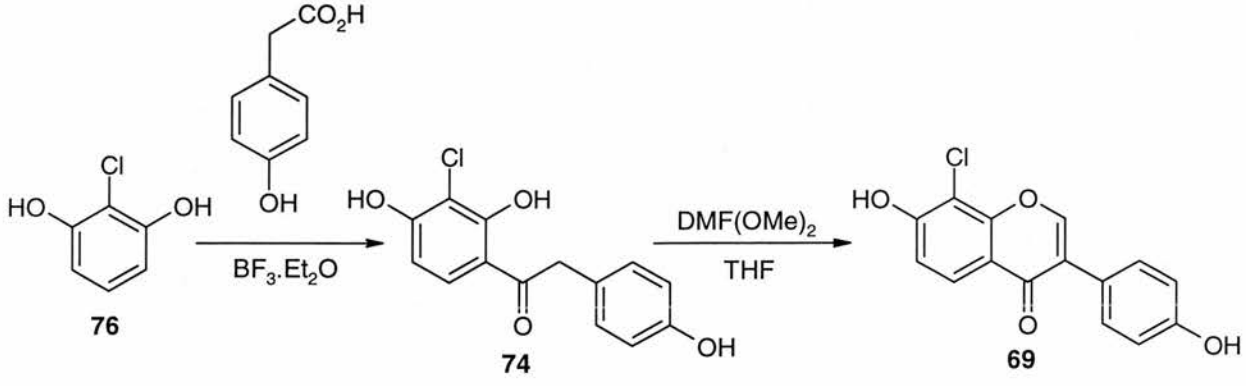
Formation of 8-chlorodaidzein proved to be less straightforward, however, first requiring the synthesis of 2-chlororesorcinol (**76**). It had been shown by Schamp that chlorination of 1,3-cyclohexanedione can be achieved using chlorine gas in chloroform to give 2,2-dichloro-1,3-cyclohexanedione in quantitative yields (**75**).[105] Aromatization was then afforded by heating in a 25% solution of hydrogen chloride in dimethylformamide to give 2-chlororesorcinol in 80% yield. The toxicity of chlorine gas, however, prompted us to search for solution based reagents and *N*-chlorosuccinimide in dichloromethane was initially used. The reaction proceeded to yield the desired product, as shown by the ^{1}H n.m.r. spectrum displaying a quintet at 2.0 ppm and a triplet at 3.0 ppm. It was discovered that the succinimide by-product was mixed with the crude reaction product, however, and the separation of these two compounds was not very easy. A more simple alternative strategy was to use sulfuryl chloride[106] instead of *N*-chlorosuccinimide. As sulfuryl chloride is a liquid it can also be used as the solvent for the reaction. On completion of the reaction, the solvent can then be removed at reduced pressure eliminating the need for

product purification. The sulfur dioxide by-product is also removed by evaporation. The reaction was indeed successful and gave the product as a white solid in 97% yield. The mass spectrum displayed three peaks due to the two isotopes of the two chlorine atoms present at *m/z* 181, 183 and 185. ^{1}H n.m.r. spectroscopy showed the H-5 proton as a quintet at 2.00 ppm and the H-4 and 6 present as a triplet at 3.00 ppm.



Scheme 35: *Preparation of 2-chlororesorcinol (**76**)*

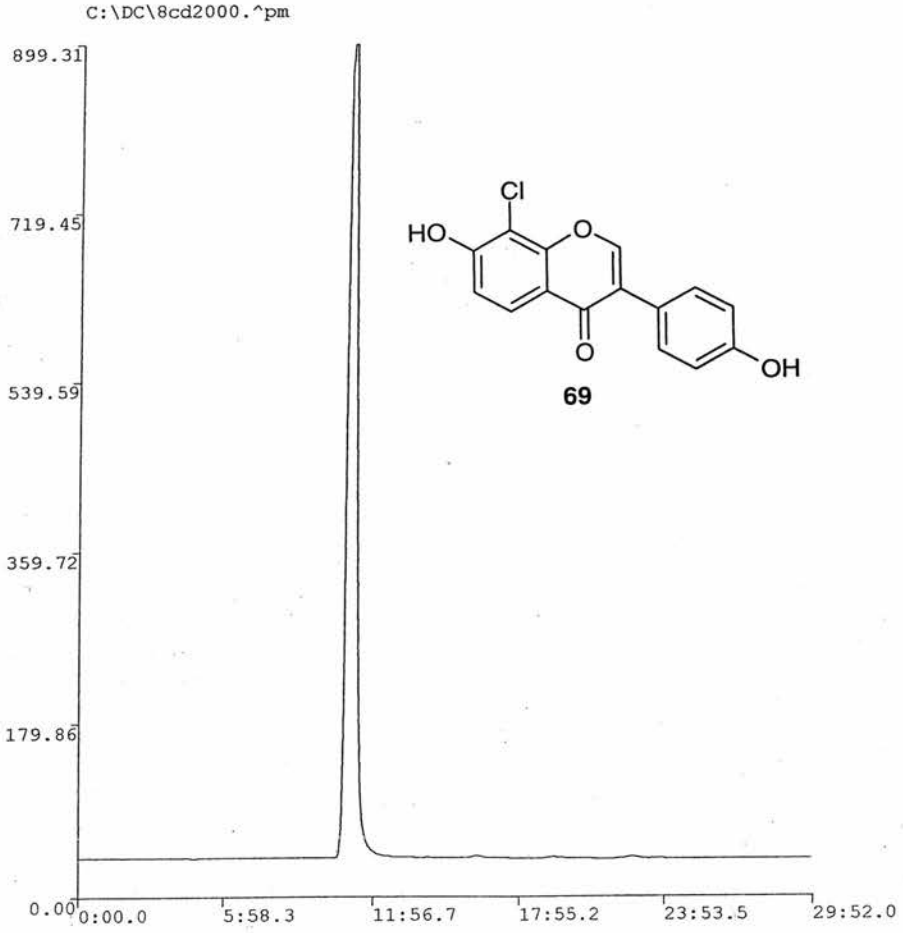
Aromatization of 2,2-dichloro-1,3-cyclohexanedione was then achieved using a saturated solution of HCl in dimethylformamide. The reaction was heated at 150 °C for 10 minutes and the desired product was obtained after purification by column chromatography as a white solid in 88% yield. The ^{1}H n.m.r. spectrum revealed that the starting material had indeed aromatized. The H-5, 4 and 6 positions previously at 2.00 and 3.00 ppm were observed in the aromatic range at 6.90 and 6.40 ppm respectively. The ^{13}C n.m.r. spectrum also displayed shifts in the aromatic range with C-1 and 3 now present at 156.0 ppm and not in the carbonyl region as before. Interestingly, the C-2, 4 and 6 resonances were co-incident at 109.0 ppm. This corresponded with calculated values for the shifts at these positions.



Scheme 36: *Preparation of 8-chlorodaidzein (**69**)*

	Yield (%)	^{13}C n.m.r. C=O, (ppm)	M.S. *m/z* (M^+)	m.p.(°C)
74	91	203.5	279, 281	208-212
69	63	174.4	289-291	316-318

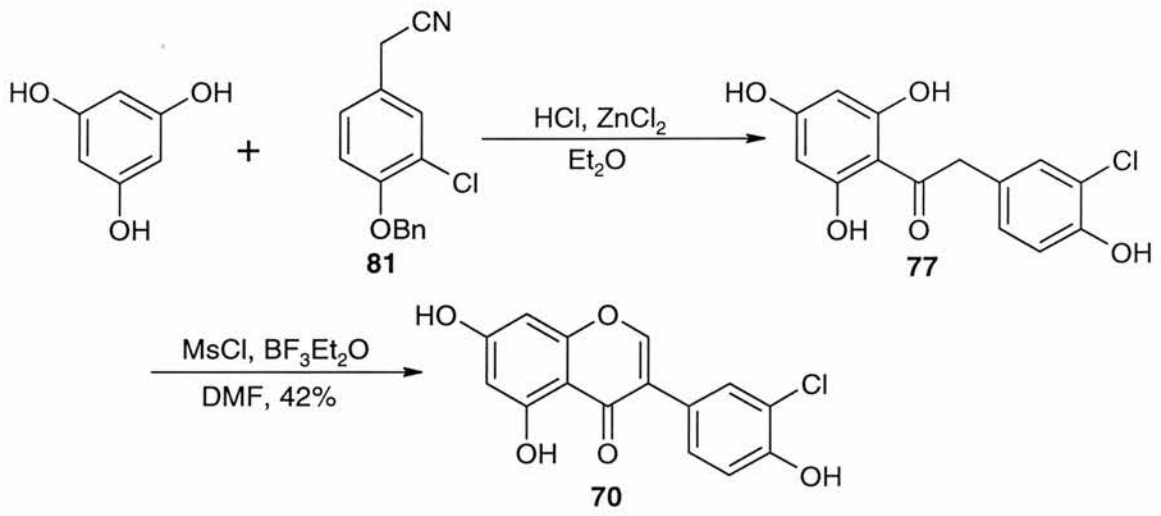
Formation of 4'-hydroxybenzyl-3'-chloro-2,4-dihydroxyphenylketone (**74**) from 2-chlororesorcinol and the phenylacetic acid was performed successfully in a manner similar to previous examples (scheme 36). Subsequent cyclization to 8-chlorodaidzein was then carried out using dimethylformamide dimethyl acetal. Purification of the isoflavone required column chromatography on silica gel as well as on C-18 reverse phase silica gel. The HPLC trace of the purified product, as seen in scheme 37, showed a single peak with a retention time of approximately 11 minutes, without the presence of any impurities.



Scheme 37: *HPLC trace for 8-chlorodaidzein*

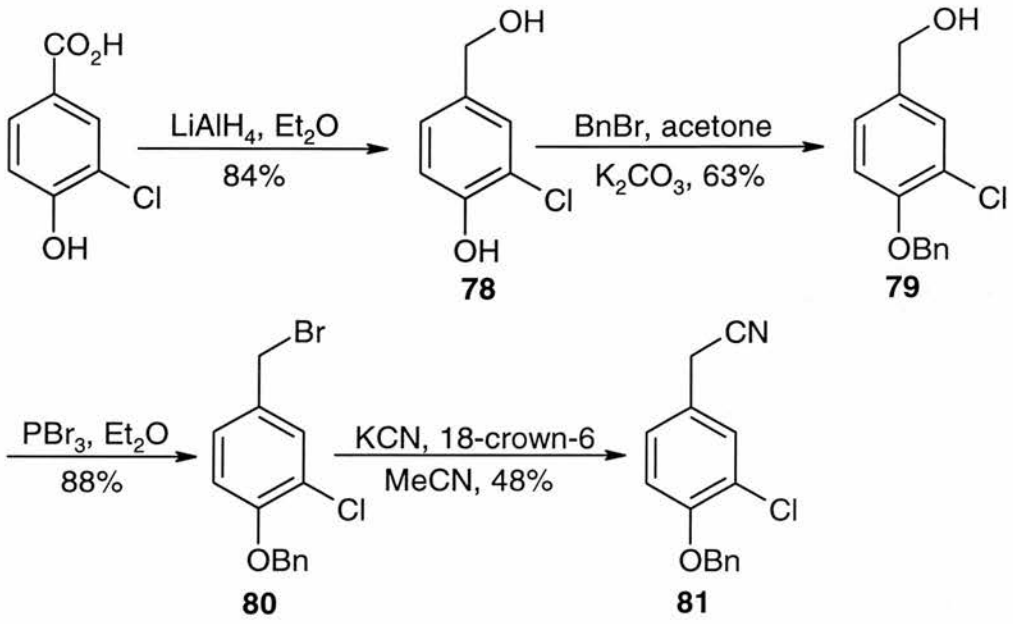
3.2.4: Synthesis of 3'-chlorogenistein (**70**)

Using similar methodology to that previously discussed in earlier chapters we have also devised a synthetic route to allow the preparation of 3'-chlorogenistein (**70**). This is shown in scheme 38 and involves the use of phloroglucinol and 3-chloro-4-benzyloxyphenylacetonitrile in a Hoesch type reaction.[56]



Scheme 38: *Formation of 3'-chlorogenistein (70)*

The 3-chloro-4-benzyloxyphenylacetonitrile itself was prepared from 3-chloro-4-hydroxyphenylacetic acid (scheme 39). Reduction to the benzyl alcohol (**78**) was achieved using lithium aluminium hydride in diethyl ether and the desired product was isolated as a white solid. The ^{13}C n.m.r. spectrum showed the absence of any carbonyl shift and a new methylene shift at 64.7 ppm. The ^{1}H n.m.r. spectrum also displayed methylene protons present at 4.48 ppm while the mass spectrum contained the expected peaks at *m/z* 158 and 160. At this stage the free hydroxy group was benzyl protected using benzyl bromide with potassium carbonate in acetone to give the alcohol (**79**). The mass spectrum revealed the desired peaks at *m/z* 248 and 251 while ^{1}H and ^{13}C n.m.r. spectroscopy showed the presence of additional methylene resonances at 5.14 and 71.4 ppm respectively.

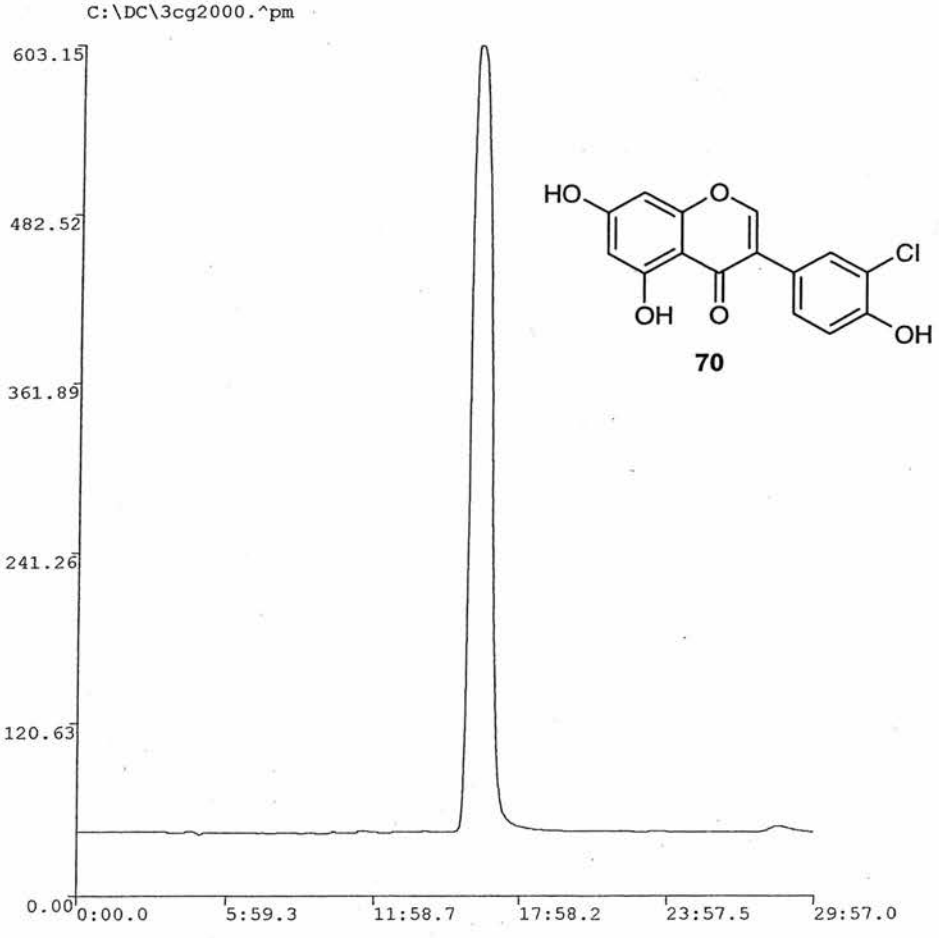


Scheme 39: *Preparation of 3-chloro-4-benzyloxyphenylacetonitrile (**81**)*

Subsequent reaction with phosphorous tribromide in diethyl ether gave the benzyl bromide (**80**) which was isolated in 88% yield. This was converted to the phenylacetonitrile (**81**) by reaction with potassium cyanide and 18-crown-6 in acetonitrile to give a pale coloured oil. The 18-crown-6 was removed by filtration through a bed of celite and purification by column chromatography eluted the product as white needles in 48% yield. The nitrile was clearly seen in the ^{13}C n.m.r. spectrum at 118.1 ppm while the mass spectrum revealed strong peaks at *m/z* 258 and 261. Microanalysis results confirmed the purity of the compound.

The Hoesch reaction, which involved the bubbling of HCl gas through a diethyl ether solution of phloroglucinol and phenylacetonitrile containing zinc chloride at 0 °C was successful. The resulting precipitate was washed in diethyl ether and heated at reflux for 3 hours. Purification by column chromatography and by washing in dichloromethane gave the deoxybenzoin (**77**) as an off-white powder. The structure was confirmed by the ^{13}C n.m.r. spectrum showing the ketone at 203.1 ppm. The mass spectrum showed peaks at *m/z* 295 and 297 corresponding to the deoxybenzoin. Cyclization to give 3'-

chlorogenistein was successfully achieved using methanesulfonyl chloride, boron trifluoride etherate and dimethylformamide under microwave conditions.[56] Purification was achieved by column chromatography initially using normal phase silica gel. This was followed by the use of C-18 reverse phase silica gel and by washing with 40% aqueous methanol. The mass spectrum displayed two strong peaks at *m/z* 304, 306 while ^{13}C n.m.r. spectroscopy showed the presence of a carbonyl group at 182.1 ppm. The HPLC trace can be seen below in scheme 40 and shows only a single peak with a retention time of approximately 16 minutes. No other minor peaks could be observed suggesting the absence of any organic impurities in the purified sample.



Scheme 40: *HPLC trace for 3'-chlorogenistein*

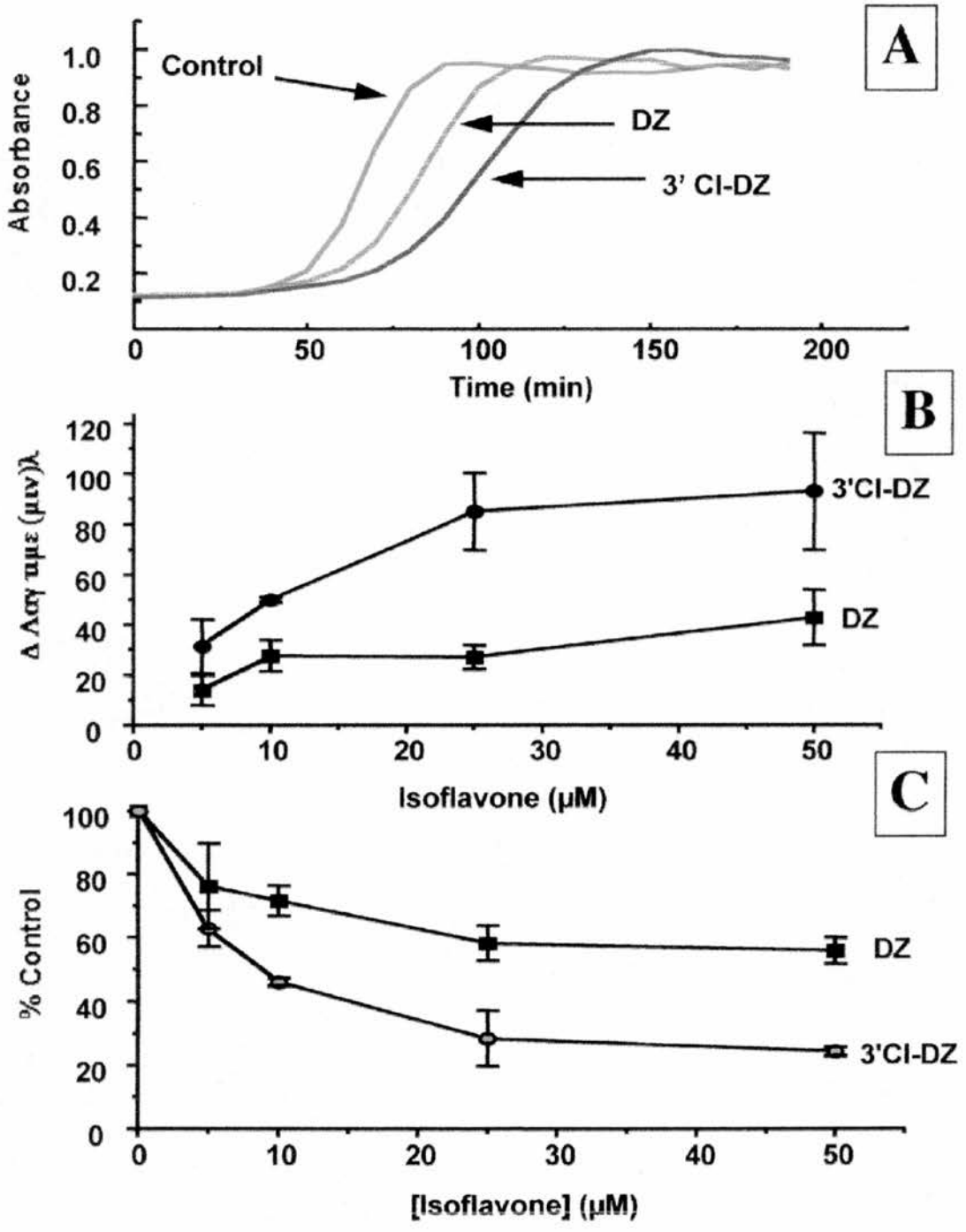
3.2: Antioxidant activity of daidzein vs 3'-chlorodaidzein

Following the synthesis of 3'-chlorodaidzein, its antioxidant properties were compared with daidzein using copper-mediated LDL oxidation. Testing was carried out by Brenda Boersma and Stephen Barnes of the University of Alabama at Birmingham in the United States.

LDL was isolated from plasma from individual donors by differential centrifugation and after dialysis against calcium and magnesium free PBS containing NaCl (140 mM), KCl (2.7mM), Na_2HPO_4 (8.13 mM), KH_2PO_4 (1.47 mM) and EDTA (10 μM) the LDA was sterilized by filtration through a 0.22 μm filter. Storage was then afforded at 4 °C until use. Samples of LDL (75 μg/ml) diluted with PBS were incubated at 37 °C in the presence and absence of daidzein and 3'-chlorodaidzein (0-50 μM). Oxidation was then initiated by the addition of $CuSO_4$ (5 μM) and was monitored continuously by the formation of conjugated dienes in the LDL particle at 234 nm, assuming an extinction coefficient of 25,000 M^{-1} cm^{-1}. This was achieved using a Beckman DU7000 diode array spectrophotometer. This assay determines the lag phase, a measure of the oxidizability of the LDL and the rate of oxidation. The addition of any antioxidants to this system shifts the curves to the right, indicating a change in the lag time while the slope of the curve indicates the rate of oxidation of LDL. This can be used to elucidate the possible mechanism of oxidation. If the rate of oxidation is altered to control, it may be that the antioxidant is interfering with the copper used to initiate the oxidation.

Upon the addition of either daidzein or 3'-chlorodaidzein to the LDL oxidation system, a change in lag time and rate of oxidation are observed (scheme 41A) demonstrating antioxidant characteristics. However, at the same concentrations the chlorinated derivative proved to be a more potent antioxidant showing an increase in lag time of 2-3 minutes when compared to daidzein (scheme 41B). Furthermore, the rate of oxidation of LDL over this concentration range is twice as quick when daidzein is present, compared to the chlorinated compound (scheme 41C). This suggests that the chlorinated daidzein is

a more potent antioxidant and that it may proceed through a different mechanism than daidzein.

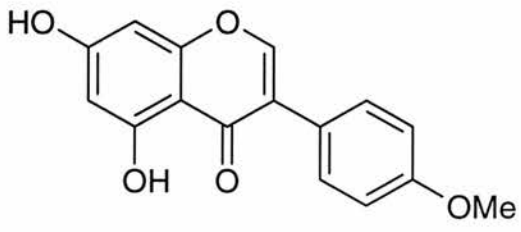


Scheme 41

3.3: Future work

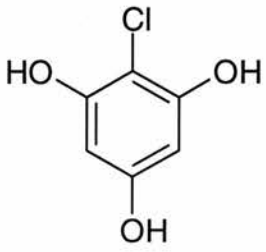
A range of mono and di-chlorinated derivatives of daidzein and genistein have been produced from this work and samples have been sent to the University of Alabama to assess their ability as antioxidants. Further work is currently underway in Dr. Botting's laboratory to prepare chlorinated derivatives for the isoflavones genistein, biochanin A and glycitein.

In the case of biochanin A (**21**), chlorination of the B ring could be achieved in the same way as for genistein in section 4.2.4, but using 3-chloro-4-methoxybenzoic acid as the starting reagent. This would allow the preparation of 3-chloro-4-methoxyphenylacetonitrile and subsequently the synthesis of 3'-chloro derivatives of the isoflavone.



21

In order to achieve chlorination in the A ring of genistein and biochanin A it will be necessary to prepare corresponding phloroglucinol derivatives. It has been shown in section 6.4.2 that iodophloroglucinol can be prepared under mild iodination conditions. It should therefore be possible to employ mild chlorination conditions to prepare chlorophloroglucinol (**82**) by using one equivalent of chlorine and dichlorophloroglucinol (**83**) by the use of two equivalents. In the case of phloroglucinol, however, subsequent Hoesch and cyclization reactions would give a mixture of both 6- and 3-chloroisoflavones as a result of the symmetrical nature of the phenol. Separation of these compounds, however, would be easier than for chlorination products of the isoflavone itself and would also allow the reaction to be carried out on a larger scale.



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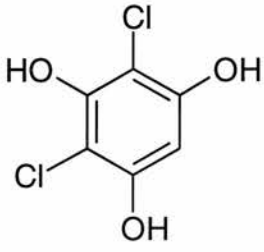

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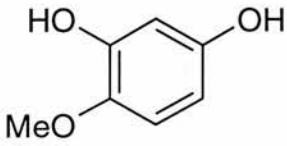
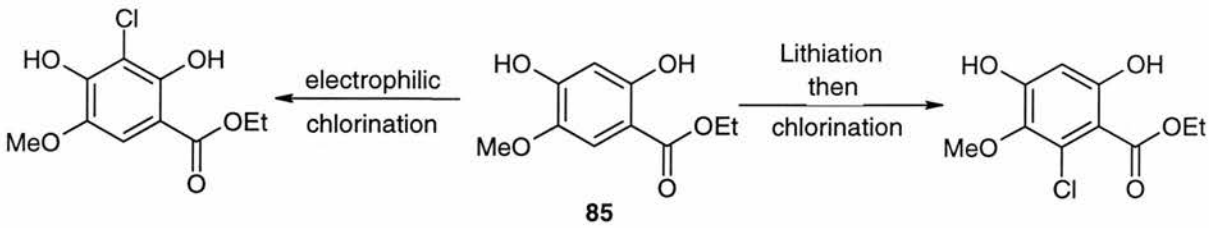


84

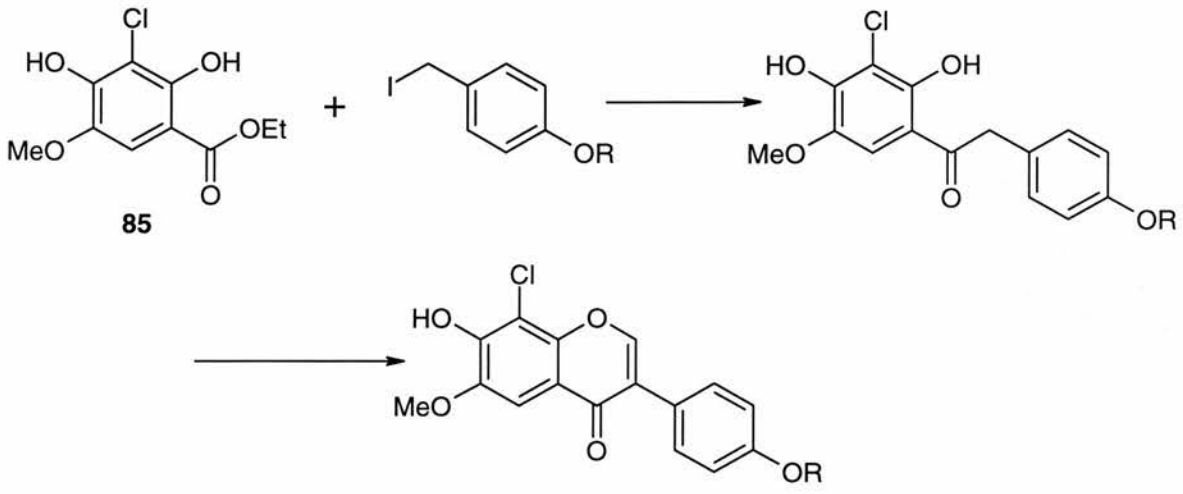
The phenol (**84**) is necessary for the synthesis of glycitein and can be prepared from 5-amino-2-methoxyphenol. This is commonly performed by diazotization followed by replacement with a hydroxyl group using sodium nitrite in aqueous sulfuric acid. In deoxybenzoin formation, the acylation of the phenol takes place at the 6 position and so it is likely that chlorination of the phenol would also occur at this site. An alternative to chlorination of the deoxybenzoin of isoflavone, which may lead to complex product mixtures, would be to build up the deoxybenzoin via the ethyl benzoate (**85**). The 2 and 5 positions would then be chemically differentiated with the 2 position more activated to electrophilic attack (scheme 42). The 5 position could be selectively chlorinated using lithiation methodology, however, in this case a more powerful ortho-metallation directing group such as a diethyl amide may be required instead of the carboxylate ester.



85

Scheme 42: *Chlorination sites of ethyl ester (85)*

The deoxybenzoin may then be prepared by reaction of the ethyl ester with the organo cuprate derived from the benzyl iodide (scheme 43). Cyclization to the isoflavone may then be achieved according to methods previously described.



Scheme 43: *Formation of chloroglycitein from the ethyl ester (**85**)*

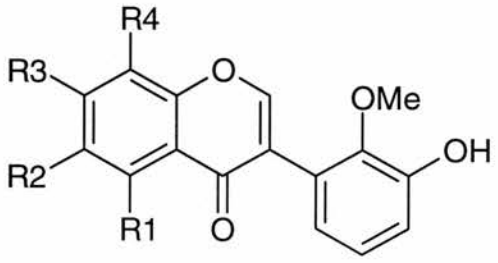
Recent evidence has also been obtained to suggest that isoflavones react with hypobromous acid (HOBr) in biological conditions to form a range of brominated derivatives.[99] Preparation of each of the individual isomers will also need to be carried out, however, there are no brominated phenols, phenylacetic acids or phenylacetonitriles commercially available. As a result, these compounds will need to be individually prepared to allow the subsequent formation of brominated isoflavones.

Chapter 4

Synthesis of Novel Isoflavones

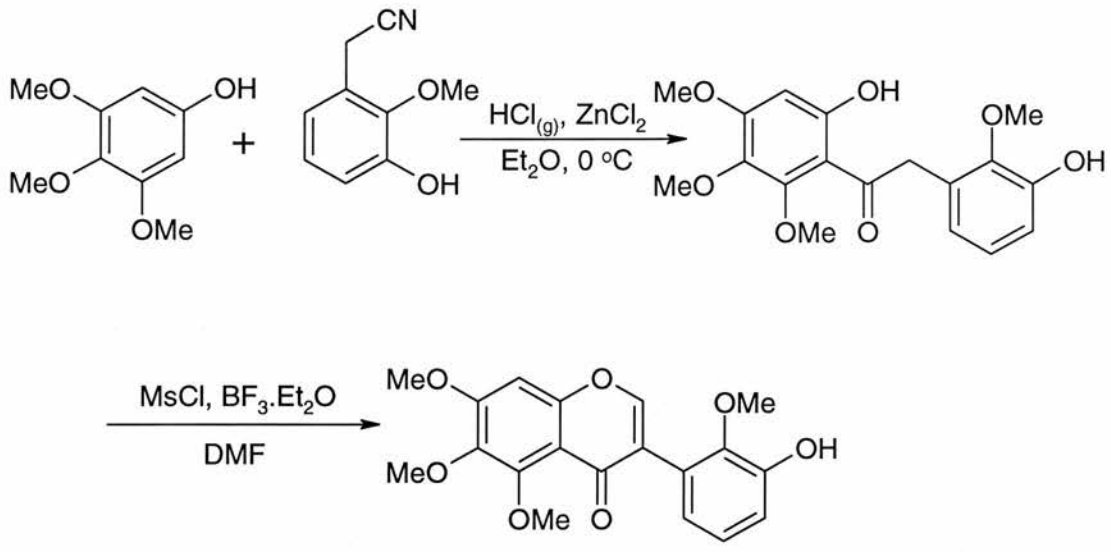
4.1: Introduction

Ethiopia has a wide variety of plants traditionally used as anthelmintics.[107] This is the ability to remove worms or other endo-parasites by the action of certain agents in the plant upon consumption. One of the most impressive medicinal plants used in the treatment of tape-worm is *Dima* (*salonsa somalensis*) which has recently been investigated by Abegaz and Woldu.[108] The roots are used as a tooth pick and the juice is swallowed. The parasite is then often expelled on the same day. Biological testing of the various root extracts showed ethyl acetate and chloroform residues to be very active against the test organism, *Taenia saginata.* The residue from these extracts yielded, in addition to known compounds, 12 novel isoflavonoids. These were of unusual structure, shown below, having no oxygenation at the 4' position on the B ring. Three of these isoflavonoids were isolated and tested for anthelmintic activity. Rigorous testing revealed only moderate activity, however the remaining novel isoflavonoids were not tested due to the lack of sufficient pure compound.



($R_1 = R_2 = R_3 = R_4$ = H, OH or OMe)

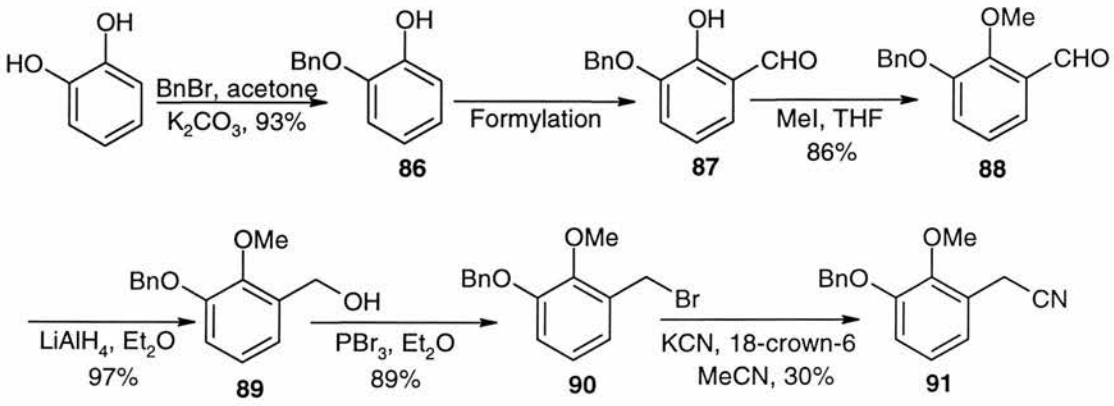
One of the isolated isoflavonoids was found to be 5,6,7,2'-tetramethoxy-3'-hydroxyisoflavone. Synthetic routes previously employed for the formation of genistein could be applied to the synthesis of this novel example from 3,4,5-trimethoxyphenol (scheme 44) via the Hoesch reaction. However, there is a lack of commercially avaliable phenylacetonitriles needed for the formation of the deoxybenzoin. Indeed there are no commercially available benzeneoid derivatives with the correct substitution pattern. Therefore further disconnections would need to be made to find a suitable synthetic route for the formation of the starting material.



Scheme 44: *Proposed synthesis of deoxybenzoin via Hoesch reaction and subsequent cyclization*

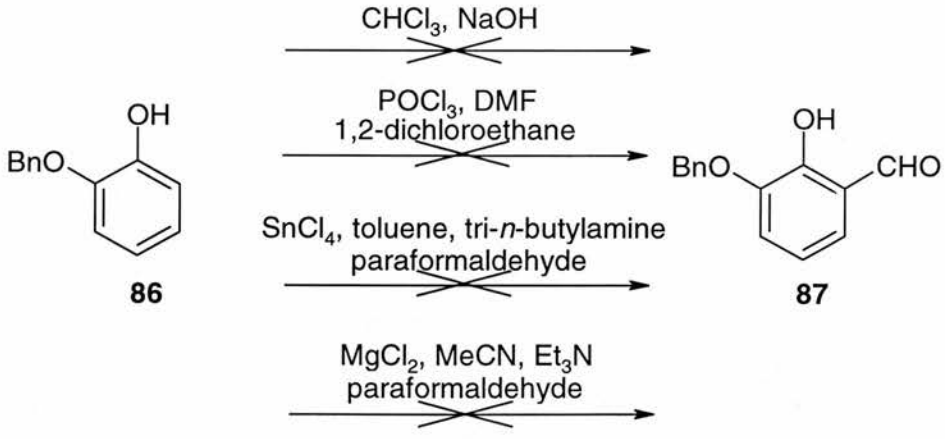
4.2: Synthesis of 2-methoxy-3-benzyloxyphenylacetonitrile (91)

The strategy initially considered involving the formylation of benzylcatechol (**86**) is shown in scheme 45. The initial protection of catechol was achieved using 1.0 equivalents of benzyl bromide in acetone and with potassium carbonate as base. Column chromatography of the crude product gave benzylcatechol in 93% yield as a pale coloured oil. The structure was confirmed by ^{1}H and ^{13}C n.m.r. spectroscopy which displayed the single methylene group with peaks at 5.14 and 71.58 ppm respectively. The mass spectrum showed the only *m/z* peak at 200, corresponding to the desired product in addition to the benzyl fragments at *m/z* 91 and 65. The dibenzylcatechol was also isolated in 1.5% yield in addition to 2% of the starting material.



Scheme 45: *Preparation of 2-methoxy-3-benzyloxyphenylacetonitrile (**91**)*

A number of methods were then examined for the desired ortho-formylation of benzylcatechol (scheme 46). Firstly, Riemer-Tieman conditions were used.[109] The benzylcatechol was heated at reflux for 12 hours in sodium hydroxide solution in the presence of chloroform. However, only starting material was obtained. Vilsmaier-Haack conditions[110] were then tried. However, the addition of phosphorus oxychloride to a solution of benzylcatechol in dimethylformamide failed to give any reaction. It was clear from the crude ^1H n.m.r. spectrum that no aldehyde proton was present in the region of 9-12 ppm.



Scheme 46: *Attempted procedures for ortho-formylation of benzylcatechol (**86**)*

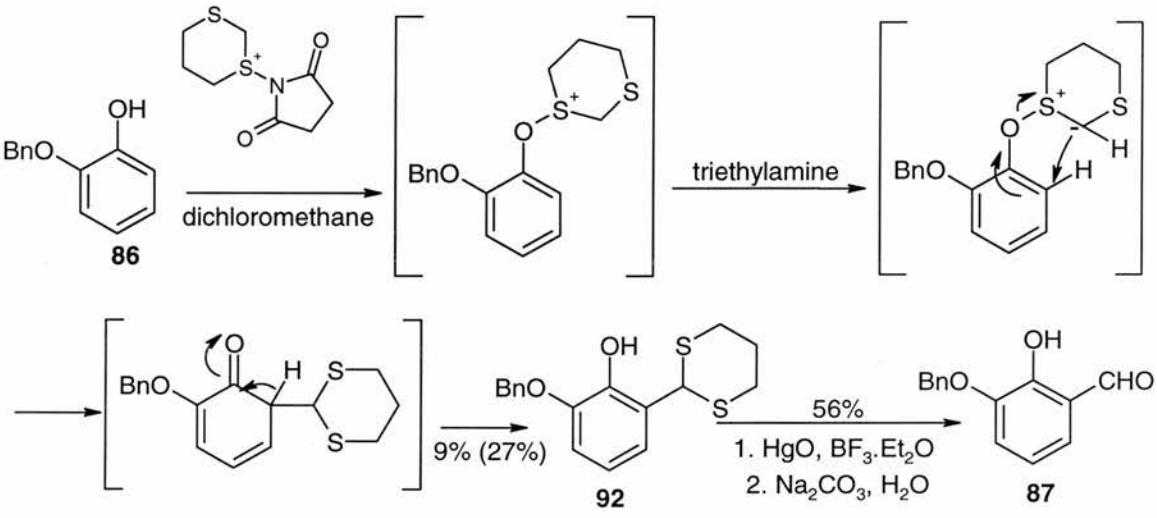
At this stage a review of the literature for other suitable procedures was carried out and it was found that reaction with either stannic chloride[111] or magnesium chloride[112] in the presence of paraformaldehyde had been shown to successfully formylate various phenol substrates. However, it was found that the reaction of benzylcatechol

in a solvent mixture of either toluene or acetonitrile in the presence of a base with either of the above metal chloride reagents and paraformaldehyde yielded none of the desired product. Heating at reflux for 8 hours, followed in both cases by analysis using ^{1}H n.m.r. spectroscopy did not reveal a shift that could correspond to an aldehyde proton. Also, t.l.c. examination of the crude product showed no change in their R_f value from that of the starting material.

A more novel approach did eventually give the desired product. This involved an extension of the Gassman ortho formylation method via a [2,3]-sigmatropic type rearrangement.[113] The active agent was readily available through the reaction of *N*-chlorosuccinimide with 1,3-dithiane in dichloromethane. Addition of the phenol (**86**) at –70 °C formed an intermediate which was not isolated, but was treated immediately with triethylamine. The resulting ylide spontaneously rearranged to give the dienone followed by re-aromatization to give the trimethylene mercaptal (**92**, scheme 47). The crude product mixture was separated by column chromatography to give the starting material and the desired product as an oily white solid. Recrystalization from carbon tetrachloride gave the pure product as a white powder in a yield of 9% (27% accounting for recovered starting material). The structure was confirmed by ^{1}H n.m.r. spectroscopy showing the C-1" proton as a singlet at 5.69 ppm. The H-3" and 5" protons were shifted as two doublets of triplets at 3.14 and 2.90 ppm. The ^{13}C n.m.r. spectrum also showed the C-1" and 4" shifts at 44.5 and 26.0 ppm in addition to the C-3" and 5" positions at 32.8 ppm. The mass spectrum displayed only the single strong peak at *m/z* 319 and microanalysis results showed an error of 0.06 and 0.11% in the carbon and hydrogen compositions from calculated values. Subsequent repeats of this procedure avoided re-crystallisation of the crude product and rather the by-products were fully separated after the subsequent trimethylene mercaptal hydrolysis. No improvement was observed in the reaction yield, however.

Hydrolysis of the trimethylene mercaptal to the aldehyde (**87**) was achieved according to a modification of the Vedejs-Fuchs procedure[114] for hydrolysis of dithiane derivatives. Stirring the trimethylene mercaptal in a solution of tetrahydrofuran and water in the presence of red mercuric oxide and boron trifluoride etherate for 24 hours gave a red coloured suspension. A yellow precipitate was formed on the addition of

sodium carbonate solution and was removed by filtration. Examination by t.l.c. showed that two new products were present and these were separated by column chromatography from the benzyl catechol still present. ^{1}H n.m.r. spectroscopy suggested these products were the desired product as well as 3-benzyloxy-4-hydroxybenzaldehyde. Both compounds displayed an aldehyde proton shift at 9.92 and 10.35 ppm respectively. The two isomers were identified by the resonances of the aromatic protons. In the 1,2,3- isomer the H-4 and 6 are present as double doublets at 7.21 and 7.14 ppm. The H-5 proton is seen as a triplet at 6.91 ppm. The 1,3,4- isomer displays two doublets and a double doublet for the 1,4 and 6 positions respectively. The mass spectra were also identical with a single strong peak at *m/z* 228.



Scheme 47: *Formation and hydrolysis of trimethylene mercaptal (**92**)*

The 2-hydroxy-3-benzyloxybenzaldehyde was then reacted with methyl iodide, initially using acetone as solvent in the presence of potassium carbonate as base. However, the isolated product showed no aldehyde shift in the ^{1}H n.m.r. spectum and one possible explanation is that the acetone had reacted with the starting material. Under basic conditions the enol form would be present and is likely that this could react at the carbonyl of the aldehyde to give an aldol condensation. However, this product was not characterised further and the reaction was instead repeated using tetrahydrofuran as the solvent. The desired product, 2-methoxy-3-benzyloxybenzaldehyde (**88**) was then isolated in 86% yield as an oil, turning to a white solid overnight at room temperature. The ^{1}H and ^{13}C n.m.r. spectra clearly showed the presence of the new methoxy group at 4.05 and 62.9 ppm respectively.

Reduction of the aldehyde to give the 2-methoxy-3-benzyloxybenzyl alcohol (**89**) was achieved using lithium aluminium hydride in diethyl ether. The product was isolated in 97% yield as a pale coloured oil, solidifying to a white solid overnight. The ^{1}H and ^{13}C n.m.r. spectra showed that the aldehyde resonances at 10.47 and 209.8 ppm were missing. The mass spectrum also showed one strong peak at *m/z* 244, corresponding to that of the desired product.

The benzyl bromide (**90**) was formed from the reaction of the alcohol with phosphorus tribromide in diethyl ether. The product was isolated as a clear oil in 89% yield and was used immediately in the reaction with potassium cyanide. Acetonitrile was used as the solvent with 18-crown-6 added to help solubilise the potassium cyanide. The reaction was complete after 18 hours under reflux. Purification by column chromatography yielded the 2-methoxy-3-benzyloxyphenyl acetonitrile (**91**) in 30% yield as an oil, again solidifying on standing overnight at room temperature. The structure was confirmed by the ^{13}C n.m.r. spectrum displaying the nitrile at 118.3 ppm. The ^{1}H n.m.r. spectrum showed that the methylene group had moved upfield and was now present at 3.73 ppm. The mass spectrum displayed one major peak at *m/z* 254.

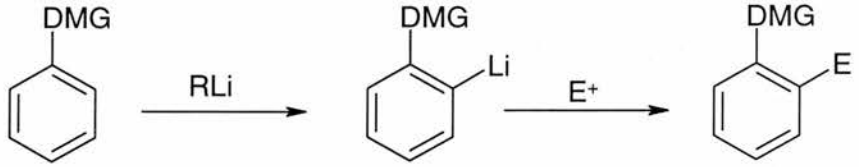
Subsequent reaction with 3,4,5-trimethoxyphenol under Hoesch conditions should allow formation of the desired deoxybenzoin, as shown in scheme 44. Cleavage of the acid-labile benzyl protecting group should also occur during this process to give the isoflavone.

4.3: Synthetic studies involving directed ortho metallation

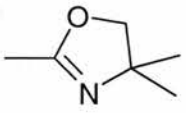
4.3.1: Introduction

Due to the poor yield of the formylation process in the previous reaction series it was decided to look for a more efficient synthesis of the desired phenylacetonitrile. One possible alternative involved the use of directed ortho-metalation.[115] This area of chemistry was originally shown to have great value in synthetic organic chemistry by Gilman[116, 117]and in more recent reviews such as those by Gschwend and Rodriguez.[118]

The directed ortho-metalation reaction involves deprotonation of a site ortho to a heteroatom containing directed metalating group (DMG). This is normally carried out using an alkyl-lithium reagent and leads to the formation of an ortho-lithiated species. Upon the addition of a suitable electrophile a 1,2-disubstituted product is formed, as shown in scheme 48.



Common DMG's

Carbon based : -$CONR_2$ 

Heteroatom based : -NCO_2R -OCH_2OMe

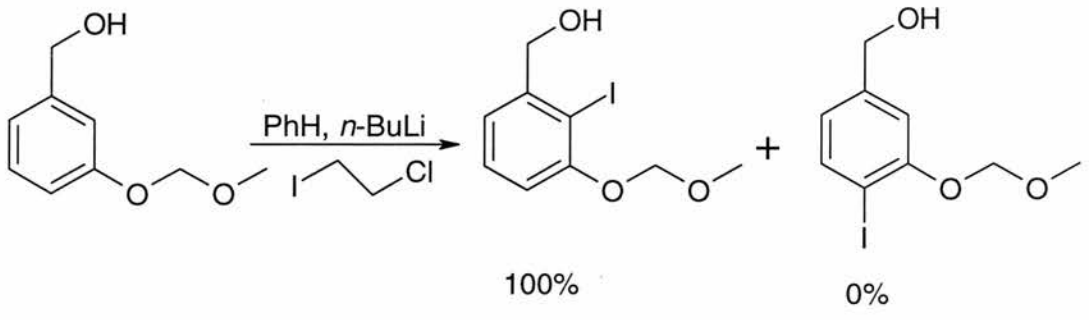
Scheme 48: *The directed ortho-metallation reaction*

For successful deprotonation to occur, a directed metalation group needs to be a good co-ordinating site for alkyl lithium as well as being a poor electrophilic site for attack by this strong base. DMG's can be either carbon or heteroatom based and many such

as the tertiary amide, oxazoline and methoxymethoxy are groups that are well-proven and have been extensively applied in synthesis.

4.3.2: Synthesis of 2-iodo-3-methoxymethoxybenzylalcohol (**95**)

It has been shown by Winkle and Ronald that the reaction of 3-(methoxymethoxy)benzyl alcohol and *n*-butyllithium in benzene followed by ethylene iodochloride yielded the 1,2,3-isomer of iodo(methoxymethoxy)benzyl alcohol exclusively instead of the 1,3,4-isomer (scheme 49).[119]

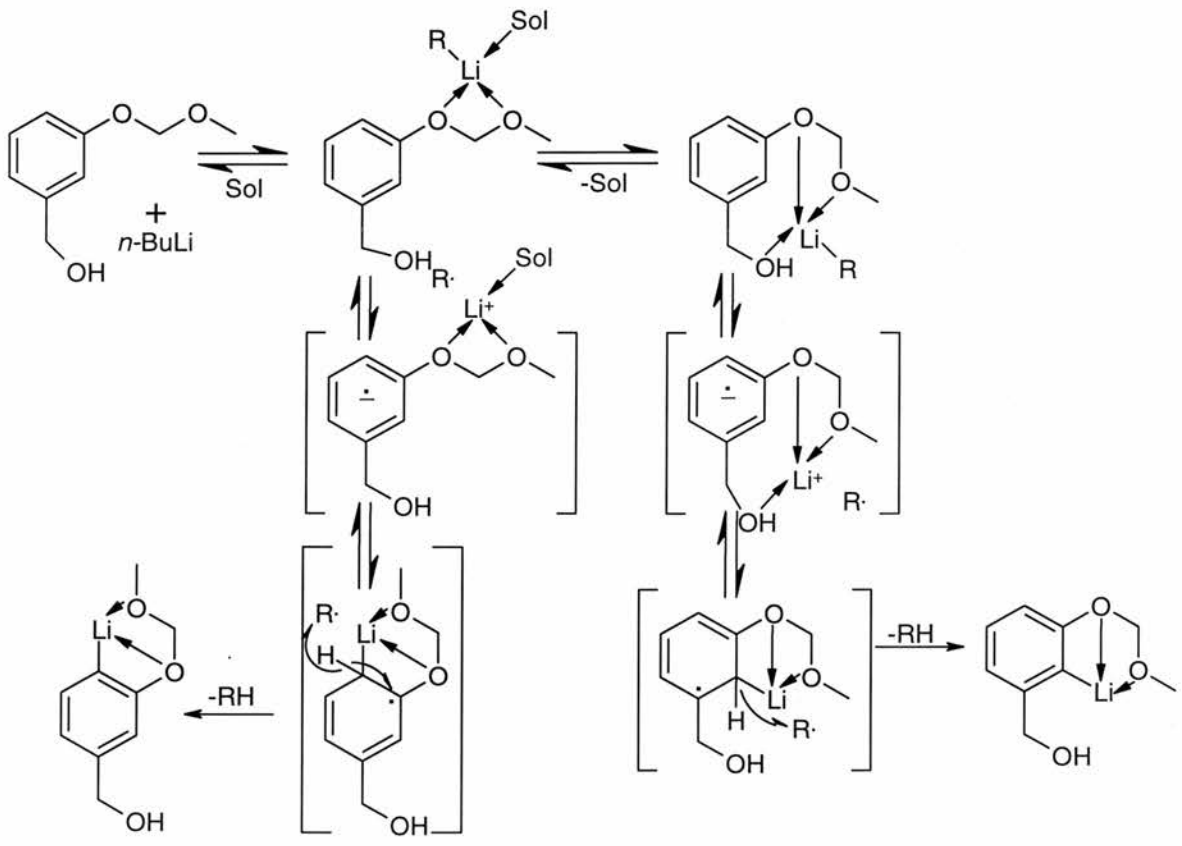


Scheme 49: *Electrophilic iodination by directed ortho-metallation*

It was suggested that butyllithium initially co-ordinated to the strongly ortho-directing methoxymethoxy group followed by electron transfer, as shown in scheme 50. The caged radical-radical anion pair then collapsed with hydrogen abstraction to give the product. In the presence of a non co-ordinating solvent such as benzene then the weakly ortho-directing CH_2O- group directed metalation between the two substituents. A strongly co-ordinating solvent such as TMEDA, however, would aid metalation to the least hindered site, and formation of the 1,3,4-isomer would be preferred.

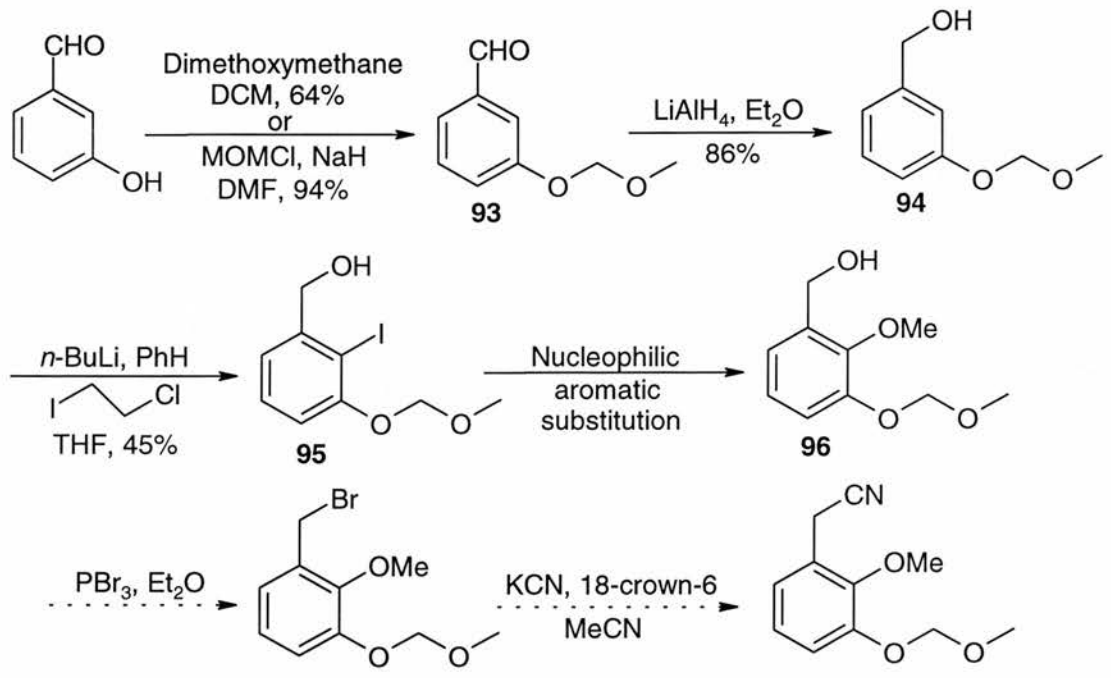
The plan was to follow the synthetic route outlined by Winkle and Ronald to form 2-iodo-3-(methoxymethoxy)benzyl alcohol. A nucleophilic aromatic substitution type reaction with an alkoxide species could then be employed to give the 2-methoxy-3-(methoxymethoxy)benzyl alcohol. If this key step proved to be successful only bromination of the alcohol and subsequent reaction with potassium cyanide would

need to be carried out. Both of these have proved to be high yielding reactions on similar substrates.



Scheme 50: *Metalation routes of 3-(methoxymethoxy)benzyl alcohol*

The reaction sequence, outlined below in scheme 51, involved protection of 3-hydroxybenzaldehyde to give 3-(methoxymethoxy)benzaldehyde (**93**). The reagents initially used for this procedure were chloromethyl methyl ether in a solvent mixture of diethyl ether and dimethylformamide.[120] Sodium hydride was used as a base. The desired product was isolated in 94% yield as a pale coloured oil. However, the carcinogenic nature of chloromethyl methyl ether prompted us to search for an alternative procedure. This method involved the use of dimethoxymethane with dichloromethane as the solvent.[121] *p*-Toluenesulfonic acid was added and the reaction heated at reflux. A Soxhlet apparatus containing 4Å molecular sieves was used to absorb the water produced from the reaction. This method gave the desired product in 64% yield

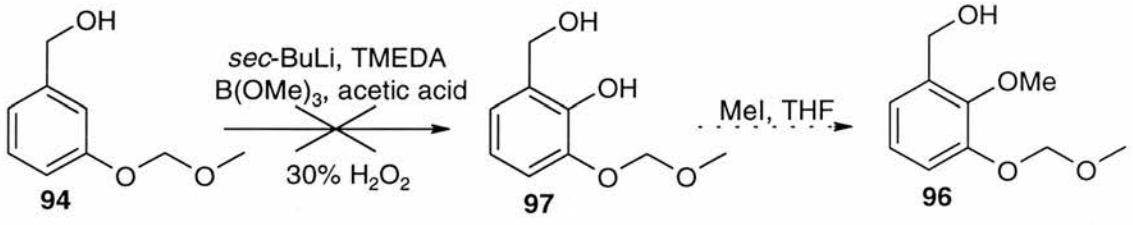


Scheme 51: *Attempted route to phenylacetonitrile utilising directed ortho-metallation*

Reduction of the aldehyde was then carried out using lithium aluminium hydride in diethyl ether to give the benzyl alcohol (**94**) in as a pale coloured oil in 86% yield. The ^{13}C n.m.r. spectrum revealed that the carbonyl peak was missing while the ^{1}H n.m.r. spectrum showed the presence of the new methylene protons at 4.64 ppm. The metalation reaction was then performed as previously described, using *n*-butyllithium in benzene at room temperature. The ethylene iodochloride in tetrahydrofuran was added after one hour and the reaction mixture stirred at room temperature. Purification by column chromatography yielded starting material in addition to the 2-iodo-3-(methoxymethoxy)benzyl alcohol (**95**) as a fluffy white solid in 45% yield. The mass spectrum displayed a strong peak at m/z 294, corresponding to that of the desired product. The ^{1}H n.m.r. spectrum showed the H-4 and H-6 protons to be present as doublets at 7.13 and 7.00 ppm respectively while the H-5 proton was a triplet at 7.30 ppm. No resonance was observed in the aromatic region for the H-2 proton.

4.3.3: Attempted phenol formation by boration-oxidation

At this stage we decided to attempt the metalation reaction with a different electrophile than previously used. It has been shown by Peak and Brown that the tertiary amide *N,N*-diethylbenzamide forms an ortho-lithiated species following treatment with *sec*-butyllithium in TMEDA.[122] Treatment with trimethyl borate followed by acetic acid and 30% hydrogen peroxide yielded *N,N*-diethylsalicylamide in 56% yield. Extending this reaction for use on the substrate 3-(methoxymethoxy)benzyl alcohol would allow formation of the phenol (**97**). Subsequent methyl ether protection would afford the alcohol (**96,** scheme 52), displaying the desired side-groups at positions 2 and 3 (analogous to compound **89**, scheme 45). Bromination and cyanation could then be afforded under the conditions displayed in the same scheme. However, when the reaction was attempted, no trace of the desired phenol was observed. The ^{1}H n.m.r. spectrum indicated the only isolated product to be starting material.



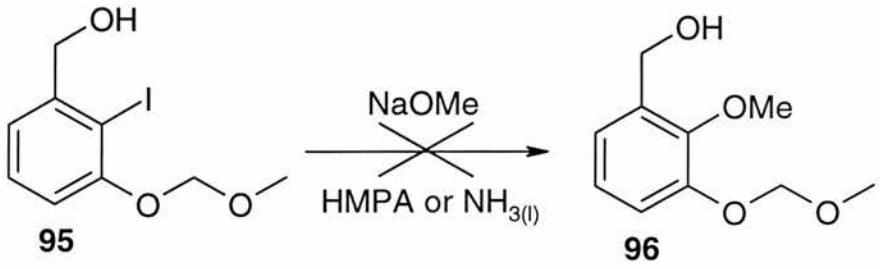
Scheme 52: *Attempted phenol formation by directed ortho-metalation and subsequent boration-oxidation.*

4.3.4: Attempted nucleophilic aromatic substitution reactions

4.3.4.1: The Williamson reaction

An extension of the Williamson reaction[123] has shown that unactivated aryl chlorides react with sodium methoxide in HMPA to give aryl methyl ethers in varying yields. The aryl iodide (**95**) containing the methoxymethyl sidechain would increase electron density into the aromatic ring preventing nucleophilic attack. Steric factors would also hinder any attacking species. However, the mean carbon-iodine bond strength of 52

Kcal/mol is less than that of the mean carbon-chlorine bond strength (79 Kcal/mol) and should promote the reaction if the pathway of the nucleophile is not impeded.



Scheme 53: *Attempted Williamson reaction*

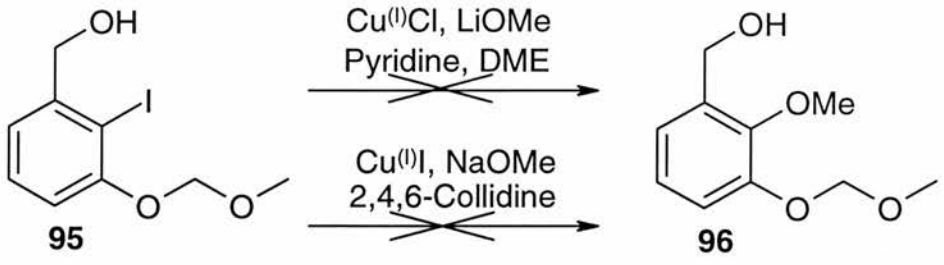
The reaction was thus attempted using pre-formed sodium methoxide and the aryl iodide (**93**) in HMPA as shown in scheme 53. Stirring at 50 °C for 15 hours yielded an array of new products, as shown by t.l.c. The ^{1}H n.m.r. spectrum of the crude product mixture displayed a weak signal at 3.35 ppm, possibly corresponding to a methoxide group. The number of products present, however dissuaded further purification attempts. Because of the carcinogenic nature of the solvent it was decided to attempt the reaction using liquid ammonia as an alternative. In this instance the reaction was carried out at -78 °C over 18 hours, but no reaction was observed.

4.3.4.2: The use of copper $^{(II)}$ methoxide reagents

It has also been shown by Bacon and Rennison that aryl halides can be converted into aryl alkyl ethers in the presence of cuprous iodide using 2,4,6-collidine as a solvent.[124] This method proved successful for substrates containing substituents having a positive inductive effect into the π ring system, namely 2-, 3- and 4-bromophenol. The yield for these examples was generally moderate (between 35-50%) however, the demonstrated applicability for such a wide of range of substrates prompted us to attempt the reaction as a method for forming 2-methoxy-3-(methoxymethoxy)benzyl alcohol (scheme 54).

The reaction involved the use of vacuum dried cuprous iodide and dry, re-distilled 2,4,6-collidine. Freshly cut sodium was added to methanol and when dissolution was complete 2,4,6-collidine was added. The aryl iodide (**95**) was then added followed by more collidine. After heating at reflux for 20 hours no reaction was observed and the ^{1}H n.m.r. spectrum showed only starting material to be present.

An extension of this reaction was attempted using cuprous chloride and lithium methoxide (formed from methanol and *n*-butyllithium).[125] The reaction was carried out using a pyridine and dimethoxyethane solvent mixture, however, heating at reflux for 12 hours produced only starting material.



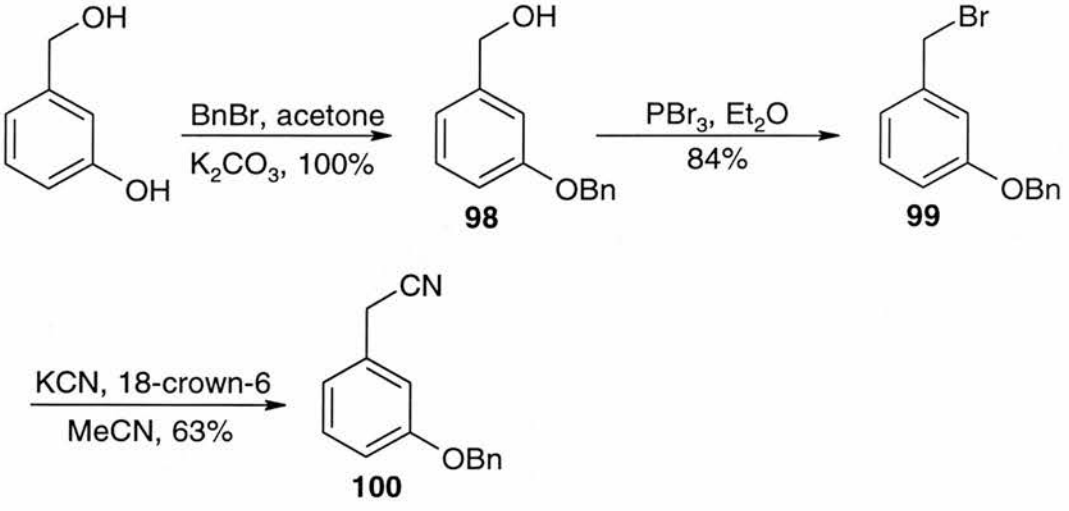
Scheme 54: *Attempted substitution using Cu$^{(I)}$ species*

4.4: Synthesis of 3-benzyloxyphenylacetonitrile (100)

The limited progression of the previous reaction sequence has prompted temporary abandonment of the metalation procedures. However, reliance on the initial sequence utilising phenol ortho-formylation dictates only small amounts of the required phenylacetonitrile (**91**) are present for subsequent Hoesch reaction attempts.

As a result, a structurally similar yet more easily obtainable model compound was synthesised to test the reliability of the final steps of the reaction series. 3-Benzyloxyphenylacetonitrile (**100**) may be prepared in a reliable three-step reaction sequence starting from 3-hydroxybenzyl alcohol (scheme 55).

Initial benzyl protection of the phenol group was achieved using benzyl bromide to give the benzyl alcohol (**98**) as an off-white solid in 100% yield. The ^{1}H n.m.r. spectrum clearly shows the presence of additional methylene protons at 5.08 ppm while the mass spectrum confirms the desired product has been formed with a strong peak at *m/z* 215. Conversion to the benzyl bromide (**99**) using phosphorus tribromide gave the product as a white solid (in 84% yield) and was followed by immediate reaction with potassium cyanide and 18-crown-6 as before. Filtration of the reaction mixture through a bed of silica preceded subsequent concentration of the filtrate at reduced pressure. This left the desired phenylacetonitrile (**100**) as a pale coloured oil in 63% yield without the need for further purification. Again, the structure was confirmed by ^{13}C n.m.r. spectroscopy with the nitrile signal present at 113.9 ppm. The mass spectrum suggested this to be the only structure present with a single strong peak at *m/z* 223.



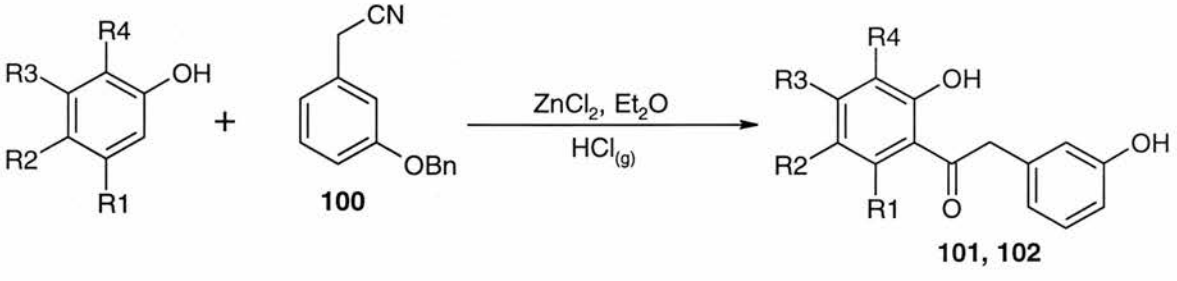
Scheme 55: *Preparation of 3-benzyloxyphenylacetonitrile (**100**)*

4.5: Attempted deoxybenzoin formation

4.5.1: Via the Hoesch reaction

The Hoesch reaction was then attempted using 3-benzyloxyphenylacetonitrile (**100**) with 3,4,5-trimethoxyphenol in diethyl ether as shown in scheme 56. Zinc chloride solution was added and HCl gas bubbled through the solution for 48 hours. However, the insolubility of the phenol in the ethereal solution during the reaction may explain the failure of a precipitate to emerge during addition of HCl. Subsequent hydrolysis in 1.0 M HCl solution was carried out, however, the only isolated products were starting materials. None of the derived deoxybenzoin (**101**) was observed.

However the substitution of this phenol for phloroglucinol produced more success when the reaction was repeated. Again a precipitate failed to emerge, however an orange oil was formed in the reaction vessel. Acid hydrolysis was followed by purification by column chromatography. An off-white solid was isolated and ^{13}C n.m.r spectroscopy demonstrated the formation of the desired deoxybenzoin (**102**). A signal at 204.9 ppm was present, corresponding to a carbonyl functionality. Both ^{1}H and ^{13}C n.m.r. spectra revealed the absence of the benzyl proton and carbon signals and the mass spectrum confirms this with a single peak at *m/z* 260. No peak at *m/z* 350 is observed and it seems the acidic reaction conditions were responsible for the cleavage of the benzyl group to give the deoxybenzoin.



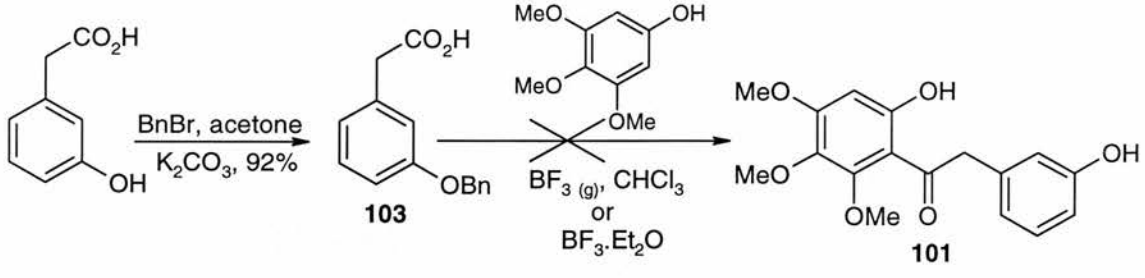
Scheme 56: *Deoxybenzoin formation via the Hoesch reaction*

Compound	R_1	R_2	R_3	R_4	Yield (%)
101	OMe	OMe	OMe	H	No reaction
102	OH	H	OH	H	84

4.5.2: Via boron trifluoride complexation

The method used for the preparation of daidzein deoxybenzoin intermediates was also considered as a route to these novel isoflavones (scheme 57). It has been shown that condensation of 3,4,5-trimethoxyphenol and *p*-methoxyphenylacetic acid has been achieved using boron trifluoride gas. It would be feasible to prepare the phenylacetic acid from 2-methoxy-3-benzyloxyphenylacetonitrile (**91**) by hydrolysis under basic conditions. This involves the addition of one extra step to the original reaction scheme.

However, the reaction was first attempted, as before, using a structurally similar model compound (**103**) prepared by the benzyl protection of 3-hydroxyphenylacetic acid.



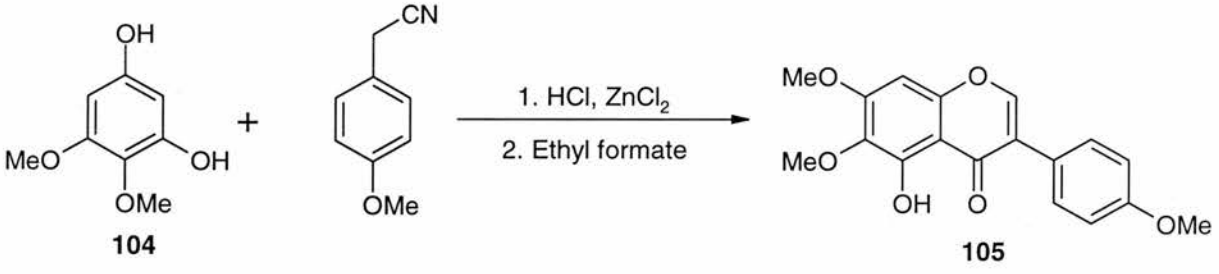
Scheme 57: *Attempted deoxybenzoin preparation by acid-boron complexation*

The reactants were stirred in chloroform, saturated with boron trifluoride gas at 0 °C for 12 hours. However, analysis by t.l.c. as well as ^{1}H and ^{13}C n.m.r. spectroscopy revealed only the presence of starting material. Heating at reflux for 2 hours using

boron trifluoride etherate gave a new product by t.l.c which was isolated by column chromatography to give a dark red coloured oil. ^{13}C n.m.r. spectroscopy showed no carbonyl resonance in the range 200-210 ppm and that the acid carbonyl shift at 171.6 ppm was still present. ^{1}H and ^{13}C n.m.r. spectra also revealed that the proton and carbon signals corresponding to the benzyl group were absent. This suggested that the acid-labile benzyl group had been removed during the reaction to re-form 3-hydroxyphenylacetic acid.

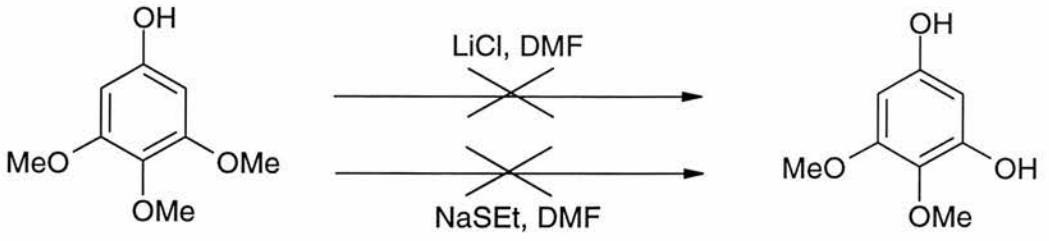
4.6: Attempted formation of 4,5-dimethoxyresorcinol (104)

The preparation of Tectoriginin dimethylether (**105**) has been achieved using 4,5-dimethoxyresorcinol (**104**) and *p*-methoxyphenylacetonitrile under Hoesch conditions (scheme 58).[126] The solubility of 4,5-dimethoxyresorcinol in diethyl ether persuaded us to consider this as an alternative to using 3,4,5-trimethoxyphenol, however, the route used for its synthesis was a complex and time consuming one. This initially involved the nitration of guiacol to give 4,6-dinitroguiacol. Conversion to 3,5-dinitroveratrole was followed by reduction to 3,5-diaminoveratrole. This then lead to the formation of 4,5-dimethoxyresorcinol, according to the direction of Baker and Robinson.[127]



Scheme 58: *Formation of tectorigenin trimethylether (**105**)*

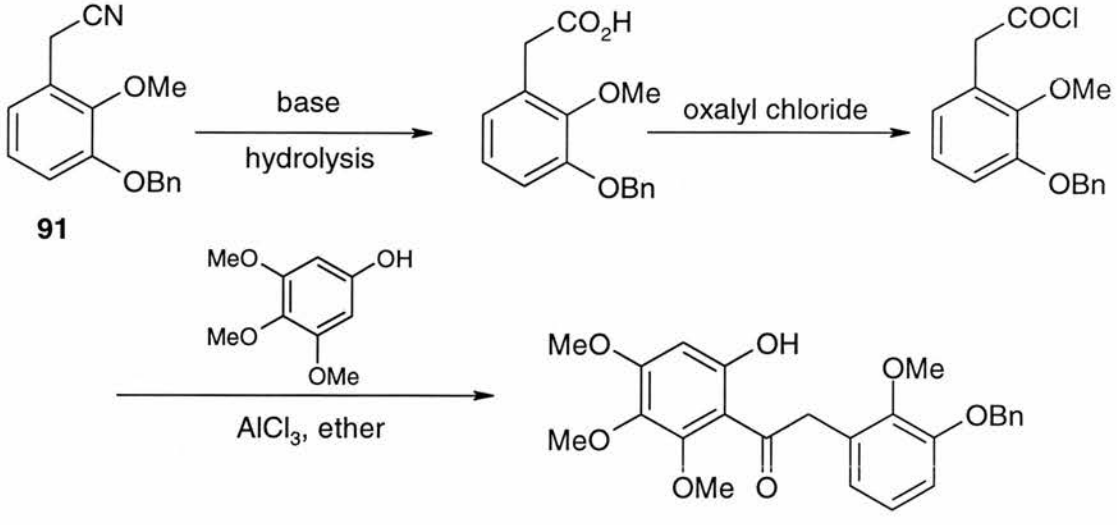
A more straight-forward alternative that we considered, however, was treating 3,4,5-trimethoxyphenol with a single equivalent of a demethylating agent. Both lithium chloride and sodium ethanethiolate were used, however, under both of these conditions no reaction was observed (scheme 59).



Scheme 59: *Attempted demethylation of 3,4,5-trimethoxyphenol*

4.7: Future work

The synthesis of the desired phenylacetonitrile precursor needed for the formation of 4,5,6,4'-tetramethoxyisoflavone has been achieved successfully. However, repeating the literature procedure outlined for deoxybenzoin formation using similar substitution patterns in the phenol has so far failed to yield any of the desired product. The modification of either the boron trifluoride reaction or the Hoesch condensation may be a possibility, however, it may also be a possibility to consider reacting *p*-methoxyphenylacetyl chloride with the phenol and an aluminium trichloride catalyst in a Friedel-Crafts acylation reaction. This would add only two more reaction steps to the synthesis, requiring hydrolysis of the nitrile (**91**) to give the acid and then reaction with oxalyl chloride to form the acid chloride (scheme 60). However, both of these steps are simple and typically demonstrate high product yields.



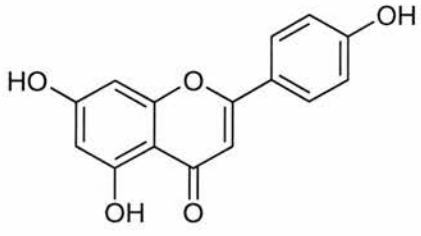
Scheme 60: *Proposed deoxybenzoin formation via Friedel-Crafts acylation*

Chapter 5

Synthesis of ^{13}C Labelled Flavones

5.1: Introduction

There is considerable interest in the biological activities of the flavone phytoestrogens.[128] In particular this includes compounds such as apigenin (**12**) and its reduced counterpart naringenin (**13**). However, accurate analysis of these compounds in foodstuffs and biological samples is hampered by the lack of isotopically labelled internal standards. For accurate LC-MS analysis it is necessary to have internal standards labelled to give a mass of at least 3 units greater than the compound of interest. The aim is thus to develop synthetic routes to allow the incorporation of three ^{13}C atoms into the target flavone to produce labelled derivatives suitable for use as internal standards.



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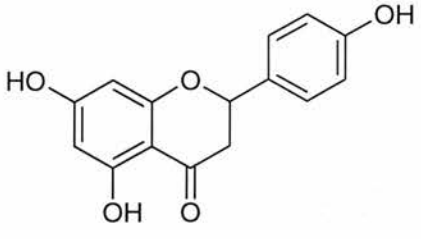
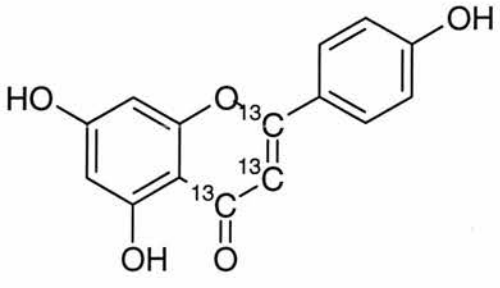


13

It is necessary to design synthetic routes to these compounds that involve commercially available ^{13}C labelled starting materials or reagents. Two cheap sources of ^{13}C are potassium [^{13}C]cyanide and [^{13}C]methyl iodide. These offer a number of possibilities for ^{13}C incorporation. The [^{13}C]methyl iodide can be used as a precursor for the formation of Grignard or other alkyl metal species. The [^{13}C] cyanide ion can be incorporated via nucleophilic attack and is readily converted to a number of functional groups, including a carboxylic acid via hydrolysis or an amine via reduction.

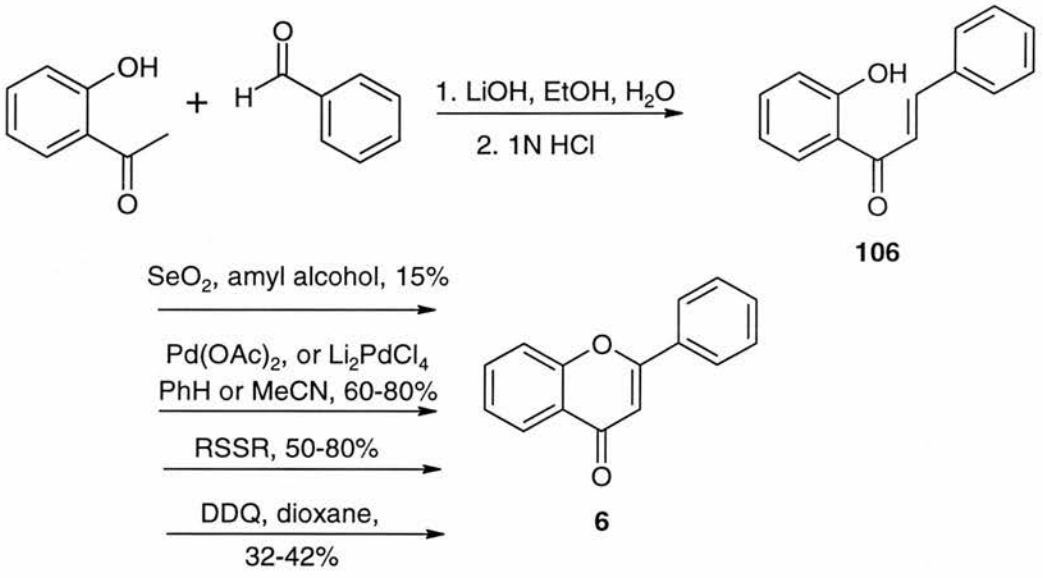


There is no reason why the three ^{13}C atoms cannot be localised into any of the fifteen carbon sites in the flavone structure. For ease of synthetic design however, incorporation into the positions shown in the structure above would avoid complex chemistry involving the breaking and re-formation of the aromatic ring systems.

5.2: A Review of common procedures used in flavone synthesis

5.2.1: Synthesis via 2-hydroxychalcone precursors

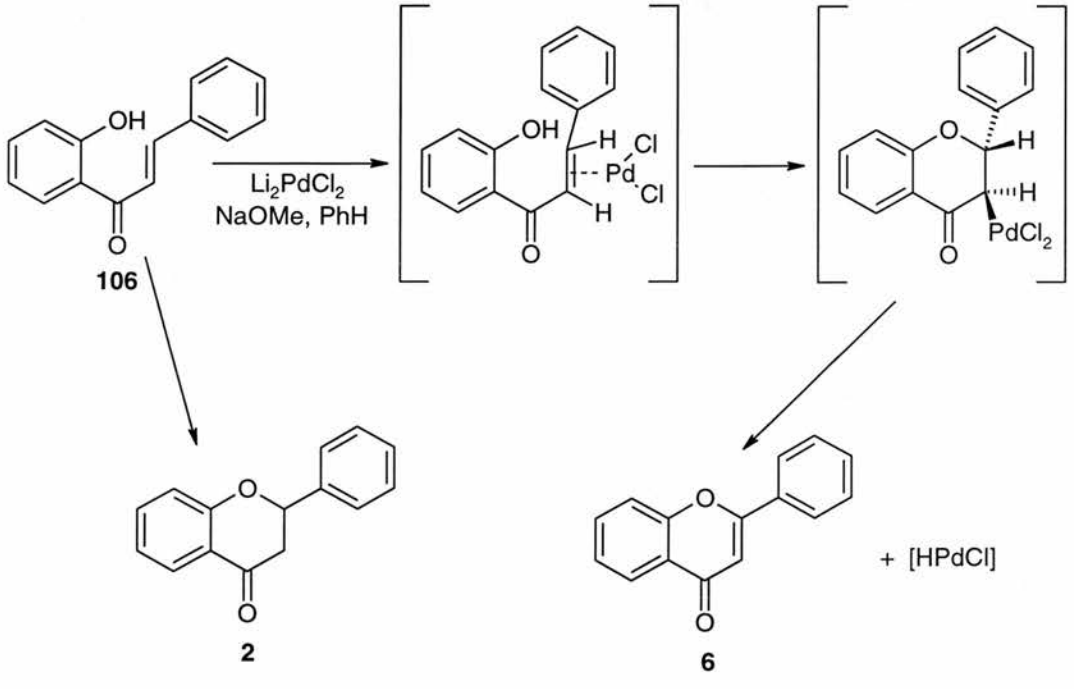
One of the more common synthetic procedures for the preparation of flavones (**6**) involves the formation and subsequent cyclization of 2-hydroxychalcones (**106**). These compounds are generally formed from base condensation of 2-hydroxyacetophenones with benzaldehydes as shown in scheme 61.



Scheme 61: *Preparation of 2-hydroxychalcones and their cyclization to flavones*

Existing literature procedures outlining the direct cyclization of chalcones to flavones are more numerable, displaying a wide range of chemistry and reaction yields. Selenium dioxide[129] was shown by Venkateraman in 1935 to form flavones from their 2-hydroxychalcones using amyl alcohol as solvent and form related flavanones in xylene. The reaction yields in both cases however were very low. In the presence of palladium (II) salts,[130] the formation of flavones has been afforded from chalcones

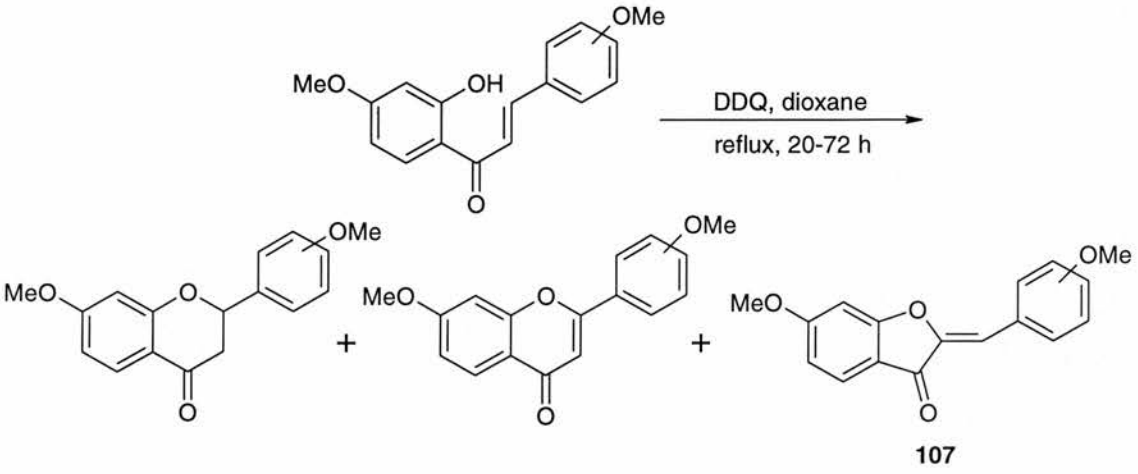
using solvents such as benzene or acetonitrile. Both palladium (II) acetate and lithium chloropalladite catalysed the formation of flavones via an intramolecular trans phenoxypalladation followed by a cis palladium-(II)-hydride elimination in yields of 60–80%.[131] The flavanone is also produced as a by-product via base-catalysed cyclization of chalcones in yields of between 5 and 20%. This can be seen in scheme 62. The involvement of palladium reagents in the formation of ^{13}C labelled flavonoids is undesirable, however, as a result of its toxicity. Metabolic studies in humans would require their consumption and residual heavy metal contamination is something that should be avoided.



Scheme 62: *Pd(II) catalysed formation of flavones from 2-hydroxychalcones*

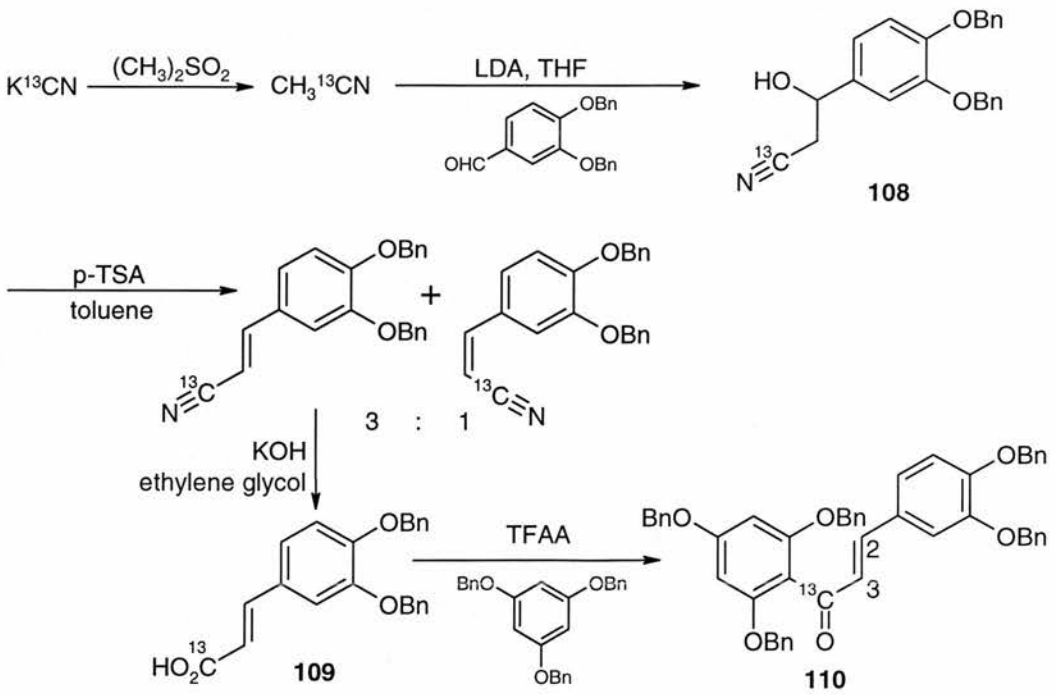
Alternative methods include the use of disulfides[132] or sulfides in dimethylformamide[133] to form flavones in yields of between 50-80%. However, reaction yields tend to be dependent on the substituents present on the aromatic rings. The presence of electron withdrawing groups reduces the efficiency of the reaction and in these cases yields of less than 20% are observed. DDQ has also been shown to be a useful reagent in chalcone cyclization[134, 135] although reaction yields are again dependent on the substituents present on the aromatic rings and side products tend to occur (scheme 63). Prolonged reaction in dioxane gave flavones in 28-38% in addition to aurones (**107**, 3-5%) and flavanones (8-12%). Although a small amount of

starting material was recovered, the reaction efficiency is too low to consider the involvement of ^{13}C labelled reagents using a similar type of reaction.



Scheme 63: *Flavone preparation using DDQ in dioxane*

A recent publication has reported a synthetic route to [4-^{13}C]2'-hydroxychalcone (**110**)[136] starting from a benzaldehyde and forming a mixture of *Z*- and *E*-cinnamonitrile by reaction with the nitrile (**108**) and *p*-toluenesulfonic acid. Hydrolysis of the nitrile under basic conditions gave the benzylcaffeic acid (**109**) and reaction with phloroglucinol tribenzylether gave the chalcone (scheme 64).

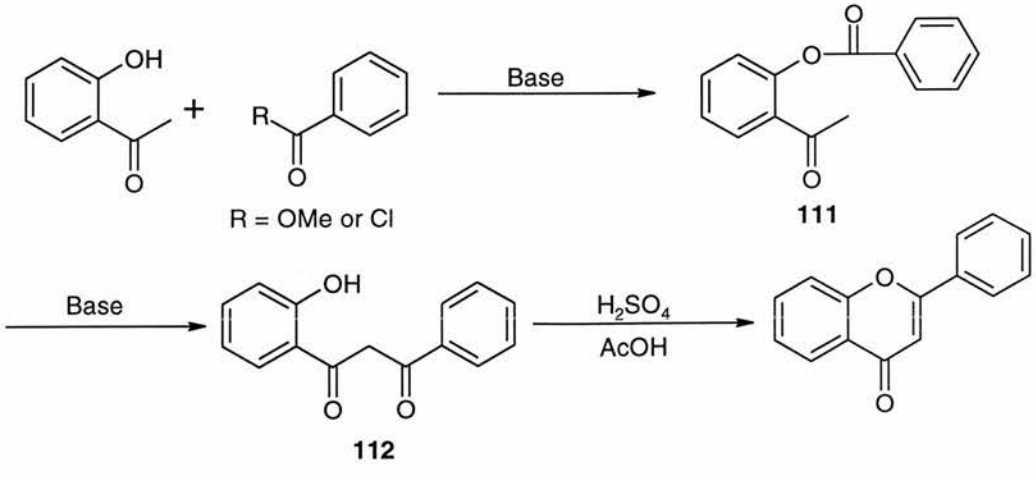


Scheme 64: *Synthesis of [4-^{13}C]2-hydroxychalcone (**110**)*

It would be possible to incorporate 3 x ^{13}C labels into the chalcone using this method by employing [1,2-$^{13}C_2$]acetonitrile and a [^{13}C]benzaldehyde. This would afford the chalcone with ^{13}C atoms at the 2, 3 and carbonyl positions. The reaction of an iodobenzene with potassium [^{13}C]cyanide using a palladium catalyst should afford the [^{13}C]benzonitrile and oxidation to the aldehyde would be possible using either stannous chloride or a Raney-Nickel alloy. The disadvantage is that [1,2-$^{13}C_2$]acetonitrile is a very expensive reagent, and also as only the *Z*- isomer of the cinnamonitrile would be required, a reasonable portion of this intermediate would be wasted.

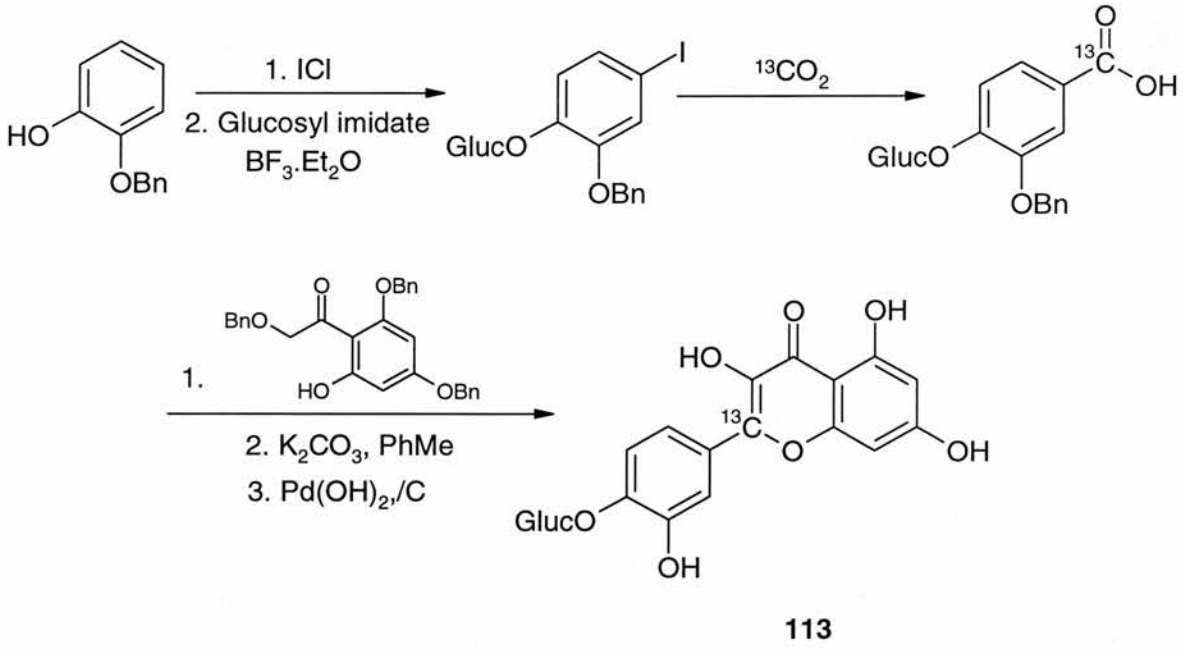
5.2.2: Synthesis via the Baker-Venkateraman rearrangement

The general reaction in scheme 65 is commonly referred to as the Baker-Venkateraman procedure and is probably the most widely used method of preparing flavones[137, 138] The 2'-hydroxyacetophenone is stirred in a strong base and the methyl benzoate (R = OCH_3) is added at low temperature.[139] This gives the ester (**111**) which, on addition of further base, undergoes rearrangement to give the diketone (**112**). Acid-catalyzed cyclodehydration to give the flavone is then commonly achieved by stirring in a mixture of acetic and sulfuric acids. Reported variations include the use of an acid chloride[140] instead of a methyl benzoate and the use of potassium hydroxide[141] or LDA[142] instead of LHMDS as a base. Side reactions do not tend to occur under these conditions and so unreacted starting materials can often be recovered. Formation of both the diketone and the flavone generally progress in excellent yields under many of the reported reaction conditions.



Scheme 65: *Conventional Baker-Venkateraman preparation of flavones*

The preparation of [2-^{13}C]quercetin-4'-O-ß-glucoside (**113**) has recently been achieved from monobenzyl catechol (scheme 66).[143] Iodination and glucosylation using a glucosyl imidate gave the iodophenol. Incorporation of the ^{13}C label was then achieved using $^{13}CO_2$ prepared from the acid-catalysed decomposition of [^{13}C]barium carbonate to give the [^{13}C]benzoic acid in good yields. Formation of the ester and the flavone was then achieved under basic conditions and finally benzyl deprotection over $Pd(OH)_2/C$.



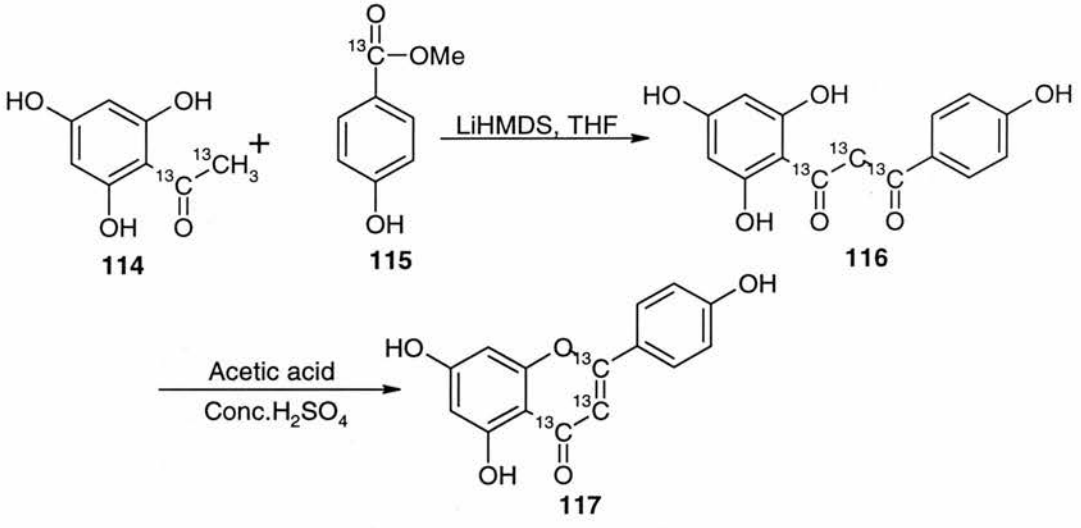
113

Scheme 66: *Formation of [^{13}C]quercetin-7-O-glucoside (**113**)*

5.3: Studies towards the synthesis of [2,3,4-^{13}C]apigenin (117)

5.3.1: Proposed synthetic route

The proposed synthesis of [2,3,4-^{13}C]apigenin (**117**) is outlined below in scheme 34. Formation of the [1,2,3-^{13}C]1,3-dioxo-1,3-diphenylpropane (**116**) intermediate would require coupling of [7,8-^{13}C]2,4,6-trihydroxyacetophenone (**114**) and [7-^{13}C]methyl 4-hydroxybenzoate (**115**) under basic conditions, followed by acid catalysed cyclization to yield the flavone (scheme 67).

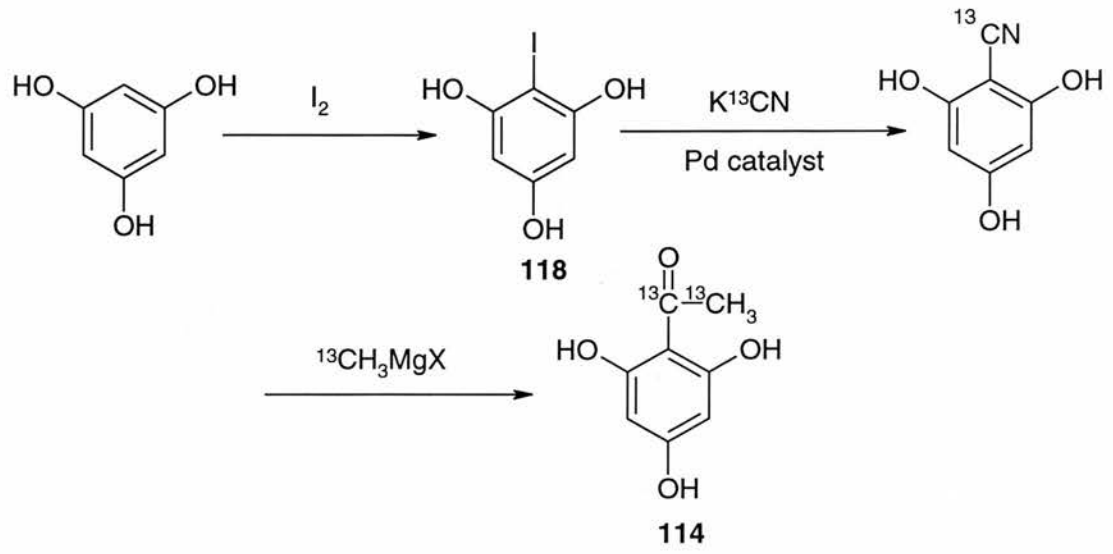


Scheme 67: *Synthesis of [2,3,4-^{13}C]apigenin (**117**)*

5.3.2: Studies towards the synthesis of [7,8-^{13}C]2,4,6-trimethoxyacetophenone (**114**)

5.3.2.1: Proposed synthetic route

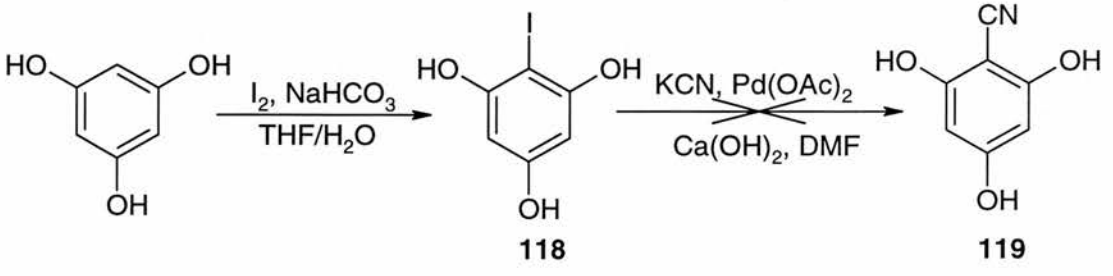
The proposed route for the preparation of [7,8-^{13}C] 2,4,6-trihydroxyacetophenone involved a three-step reaction sequence starting from phloroglucinol and is shown in scheme 68.



Scheme 68: *Proposed route to [7,8-^{13}C]2,4,6-trihydroxyacetophenone (**114**)*

5.3.2.2: Iodination and cyanation reactions

Reaction of phloroglucinol with iodine should afford the iodinated phenol (**118**) which can then be converted to the benzonitrile by use of potassium [^{13}C]cyanide. Subsequent reaction with a labelled Grignard species should then give the acetophenone (**114**). Although ^{13}C labelled reagents will eventually be required, the reactions outlined in this section use the unlabelled substrates. Only when reactions are optimised and reliable were the ^{13}C atoms to be introduced.

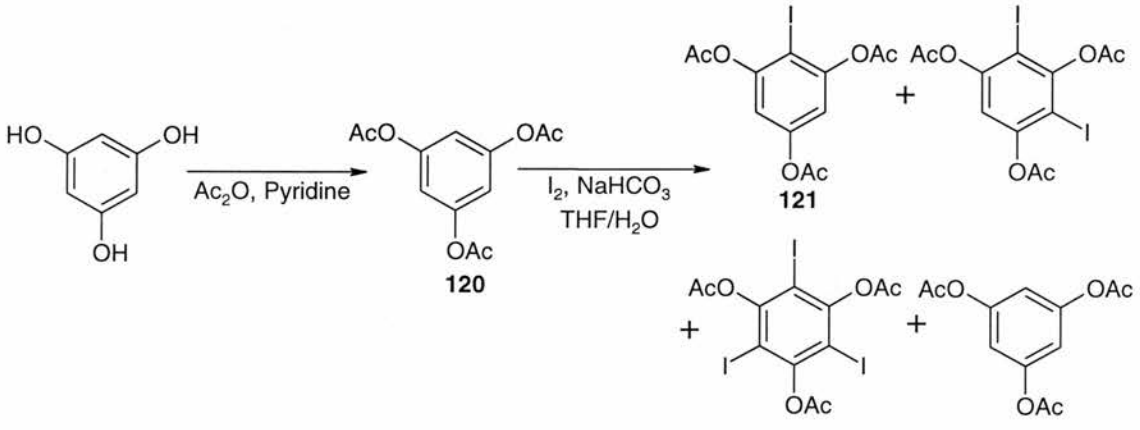


Scheme 69: *Attempted preparation of 2,4,6-trihydroxybenzonitrile (**119**)*

Iodination using a single equivalent of iodine and sodium hydrogen carbonate in tetrahydrofuran and water has been reported to give iodophloroglucinol in good yields.[144] This procedure was followed to give the desired iodination product in 90%

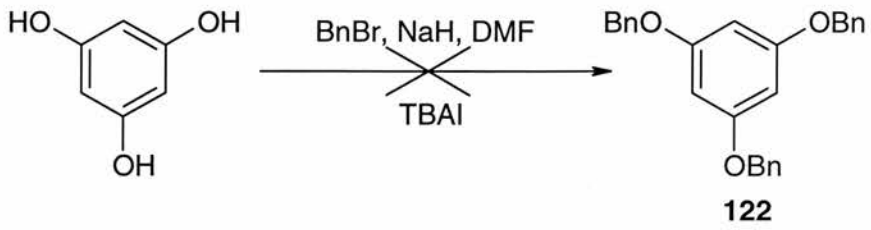
yield as a black solid. The aromatic protons were observed as a singlet at 5.85 ppm in the ^{1}H n.m.r. spectrum and the mass spectrum displayed a molecular ion at *m/z* 252. This is shown in scheme 69. The subsequent cyanation using a palladium catalyst, however did not yield any 2,4,6-trihydroxybenzonitrile even after the reaction had been heated at reflux for 24 hours. Instead only starting material was present and it was speculated from previous work carried out in our laboratory that protection of hydroxy groups was necessary for successful cyanation to occur.

Protection of the free hydroxy groups was initially carried out using acetic anhydride in pyridine. After stirring at room temperature for 18 hours triacetyl phloroglucinol (**120**) was isolated as a white powder (scheme 70). ^{13}C n.m.r. spectroscopy showed the new carbonyl carbons at 169.1 ppm.



Scheme 70: *Iodination products of triacetyl phloroglucinol (**120**)*

Subsequent reaction with iodine and sodium hydrogen carbonate in a solvent mixture of tetrahydrofuran and water yielded the crude product as a black solid. However, analysis by t.l.c. indicated an array of products had been formed while mass spectrometry indicated the presence of mono- (**121**), di-, and tri-iodo acetyl phloroglucinol with peaks at *m/z* 379, 506 and 631. Also present was a strong peak at *m/z* 253 indicating the presence of starting material. The R_f values of the species in the crude product were too close for separation by column chromatography and further purification of this compound was not carried out.

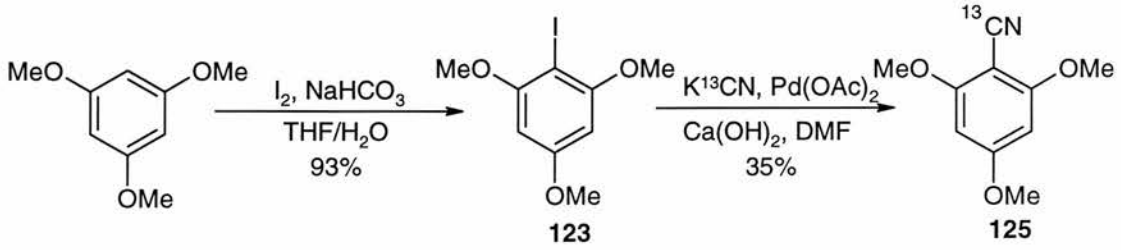


122

Scheme 71: *Attempted formation of phloroglucinol tribenzyl ether (**122**)*

Benzyl protection of phloroglucinol was also attempted using benzyl bromide and sodium hydride in dimethylformamide (scheme 71). *tert*-Butylammonium iodide was added and the reaction mixture heated at reflux. Analysis by t.l.c. revealed a range of products had been formed and that their separation would be not be possible by column chromatography. Phloroglucinol tribenzyl ether (**122**) may be prepared by the alternative method of benzyl bromide in acetone with potassium carbonate, although this has so far not been necessary.

At this stage we showed that the iodination of 2,4,6-trimethoxybenzene could be achieved under conditions used previously (scheme 72). Isolation of the crude product was immediately followed by column chromatography. This gave 2,4,6-trimethoxyiodobenzene (**123**) as black crystals in 93% yield. Their decomposition occurred at room temperature and so they were stored at -20 °C. The subsequent cyanation reaction was also successful. Thus reaction with potassium cyanide and palladium acetate in dimethylformamide gave 2,4,6-trimethoxybenzonitrile (**124**). Purification by column chromatography produced an off-white coloured solid in a yield of 35%. This yield is not ideal, especially where the use of a labelled reagent will be involved, however this is probably due to the instability of the iodobenzene as it may partially decompose before the reaction has fully occurred. The new nitrile carbon can be observed in the ^{13}C n.m.r. spectrum at 115.0 ppm and can be seen in the IR spectrum with a peak at 2230 cm^{-1} The mass spectrum gave a strong molecular ion at *m/z* 193. Repeating the experiment using potassium [^{13}C]cyanide gave the desired product (**125**) with an identical yield, showing a single, enhanced resonance at 115.0 ppm in the ^{13}C n.m.r. spectrum. The mass spectrum also gave the expected molecular ion at *m/z* 194.



Scheme 72: *Preparation of 2,4,6-trimethoxybenzo [^{13}C]nitrile (**125**)*

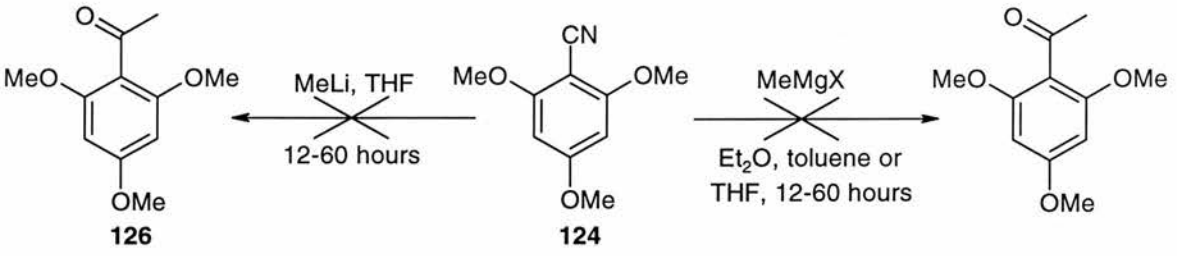
5.3.2.3: The action of MeMgX and MeLi species on 2,4,6-trimethoxybenzonitrile

Literature examples of Grignard attack on benzonitriles reveal that success is variable. Ashby *et al* demonstrated that benzonitrile reacted with methylmagnesium bromide to give acetophenone as the product in quantitative yields.[145] Although the presence of oxygenated substiuents on the ring has been shown to reduce the efficiency of the reaction 2,6-dimethoxybenzonitrile has been shown to form the corresponding phenyl isobutyl ketone in 75% yield using isobutylmagnesium bromide.[146] From both an electronic and steric perspective this example is similar to the reaction we wish to perform, however other reports suggest the inclusion of an additional methoxyl group appears to be responsible for a low reaction yield and the production of undesired by-products. Haller *et al* demonstrated that 3,4,5-trimethoxybenzonitrile will react with isobutylmagnesium bromide to give the desired ketone in 26% yield, however 3,5-dimethoxy-4-hydroxyphenyl isobutyl ketone is also formed in 27% yield in addition to minor amounts of what was thought to be a semicarbazone by-product.[147]

Initial attempts to form the acetophenone (**126**) from 2,4,6-trimethoxybenzonitrile (scheme 73) were performed using a commercial solution of methylmagnesium bromide in tetrahydrofuran. Reaction was carried out by heating at reflux for 12 hours, although analysis by t.l.c. showed that only starting material was present. ^{1}H and ^{13}C N.m.r. spectroscopy confirmed this with the absence of the expected methyl shift in the region 2-3 ppm and with the nitrile functionality still present at 115.0 ppm. The duration of the reaction was increased to 60 hours, however the same results were obtained. The Grignard species was also prepared in house from methyl iodide and magnesium turnings in tetrahydrofuran. The solvent was also changed to a mixture of

toluene and tetrahydrofuran and to diethyl ether in separate repeat experiments. However the formation of 2,4,6-trimethoxyacetophenone was not observed under any of these conditions.

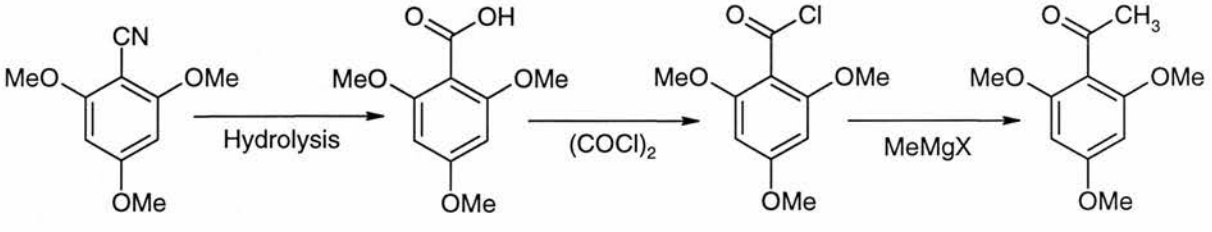
The same reaction was also attempted using a pre-formed methyllithium reagent instead of the Grignard species. Heating at reflux in tetrahydrofuran for up to 60 hours, however, gave no reaction.



Scheme 73: *Reaction of Grignard and methyllithium reagents with 2,4,6-trimethoxybenzonitrile (**124**)*

5.3.2.4: Preparation of 2,4,6-trimethoxybenzoyl chloride (**128**)

An alternative route to 2,4,6-trimethoxyacetophenone involved the formation of the benzoic acid followed by conversion to the benzoyl chloride. This species would be more susceptible to attack by a Grignard species, although the addition of two extra reaction steps would be required (scheme 74).

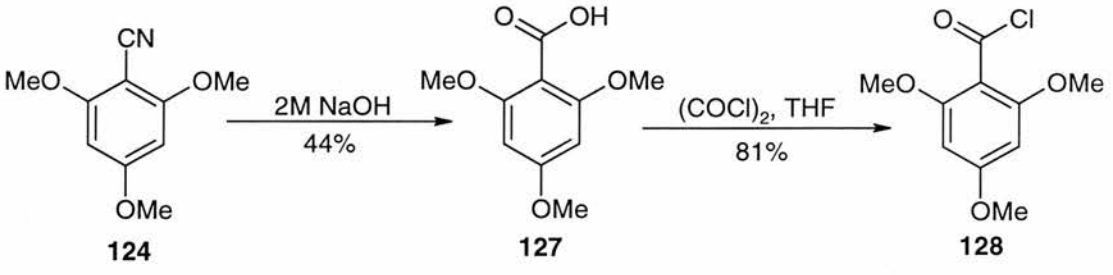


Scheme 74: *Proposed formation of 2,4,6-trimethoxyacetophenone via the corresponding benzoyl chloride (**128**)*

The formation of 2,4,6-trimethoxybenzoic acid (**127**) was attempted by hydrolysis under acidic conditions. The nitrile was suspended in 2M HCl and heated at reflux for

up to 48 hours, however analysis by t.l.c. showed that no reaction had occurred. Repeating the hydrolysis under basic conditions, using 2M sodium hydroxide was more successful. The suspension of 2,4,6-trimethoxybenzonitrile formed a solution on heating at reflux and analysis by t.l.c. showed that a new product had been formed. The mixture was acidified to pH 3 using 2M HCl, however extraction and separation of the product was not possible due to the insolubility of the product in organic solvents. Several different isolation techniques were explored, the first being column chromatography. Here, the aqueous solution was concentrated to dryness at reduced pressure to leave a mixture of the reaction products and inorganic salts. This was placed directly onto a column of silica gel, although on elution with solvent no organic products were collected. This may be caused by the product sticking to inorganic salts at the top of the column.

In an attempt to avoid this problem the reaction was repeated as before and the solution was neutralised and concentrated at reduced pressure to dryness as before. Tetrahydrofuran was now added and the undissolved salts removed by filtration. Concentration of the filtrate gave a pure white solid and was shown by ^{13}C n.m.r. spectroscopy to be 2,4,6-trimethoxybenzoic acid with a carbonyl resonance now present at 167.3 ppm. The mass spectrum also showed a single strong peak at *m/z* 212. The product was extracted twice from tetrahydrofuran to give only a moderate yield of 44% (scheme 75). No other organic matter was observed and in accounting for this it is assumed it must remain bound to the salts.



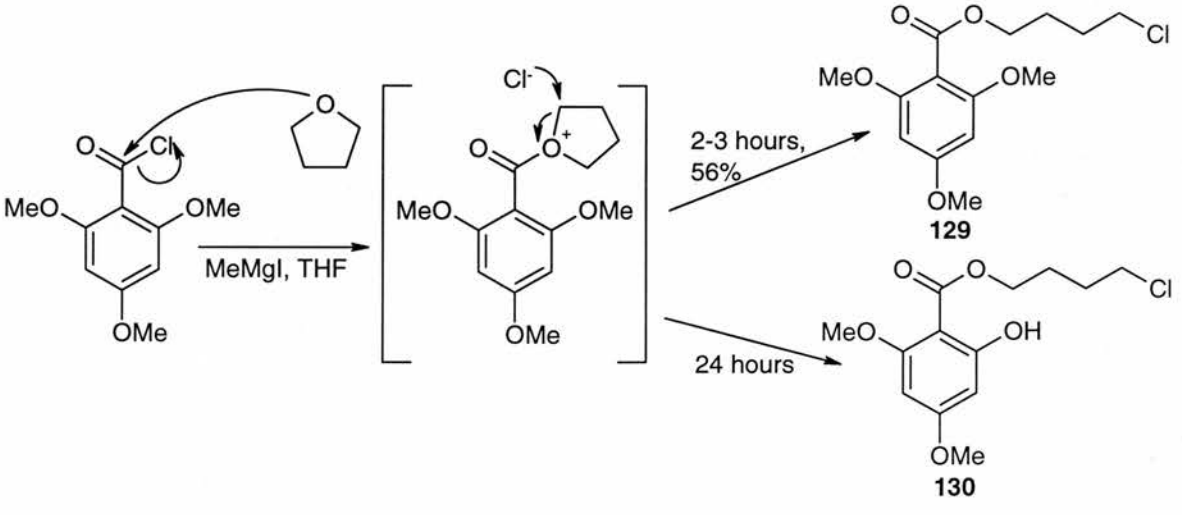
Scheme 75: *Formation of 2,4,6-trimethoxybenzoyl chloride (**128**)*

2,4,6-Trimethoxybenzoyl chloride (**128**) was prepared from the corresponding benzoic acid by stirring with oxalyl chloride in tetrahydrofuran at room temperature for 18 hours. The solvent was removed to leave the desired product as an off-white coloured solid in 81% yield. The product proved to be unstable, turning to a dark oil

upon storage for 24 hours under an inert atmosphere at 0 °C. Thus the compound was prepared and used in subsequent reactions immediately after it had been isolated.

5.3.2.5: Action of MeMgI on 2,4,6-trimethoxybenzoyl chloride in THF

Subsequent Grignard attack on the benzoyl chloride instead of the benzonitrile produced some interesting, if undesired products. A solution of 2,4,6-trimethoxybenzoyl chloride was added to a Grignard species formed from methyl iodide and magnesium turnings in tetrahydrofuran. After heating at reflux for 2-3 hours analysis by t.l.c. revealed a new product had been formed and this was isolated by column chromatography as a colourless oil. ^{1}H n.m.r. spectroscopy showed 2 quintets between 2.04-1.94 and 1.88-1.78 ppm as well as two triplets at 4.31 and 3.23 ppm. ^{13}C n.m.r. spectroscopy also showed 4 new peaks at 63.7, 30.1, 29.7 and 5.9 ppm and the mass spectrum showed a strong peak at *m/z* 195. It was suggested that the compound formed was 4'-chlorobutyl 2,4,6-trimethoxybenzoate (**129**) in 56% yield and implied reaction of the solvent with the benzoyl chloride. Mechanistically, it looks feasible that the oxygen in the tetrahydrofuran could attack the carbonyl functionality and release chloride. The chloride ion is then able to open the furan ring giving the product, shown in scheme 76.

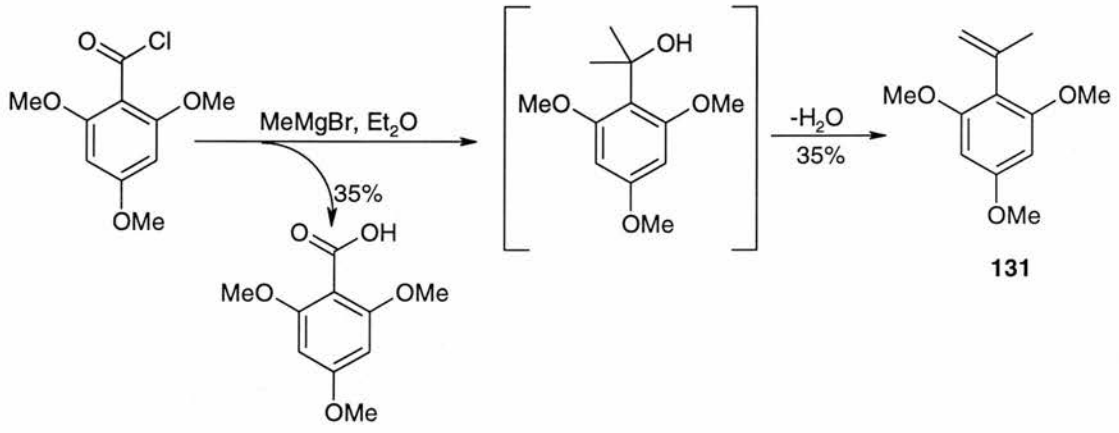


Scheme 76: *Action of MeMgI and tetrahydrofuran on 2,4,6-trimethoxybenzoyl chloride*

This reaction was also carried out at reflux for 24 hours. Analysis by t.l.c. revealed that a single new product had been formed and this was isolated as a pale red coloured oil. Purification by column chromatography was not carried out in this instance. ^{1}H n.m.r spectroscopy once again showed that quintets shifting between 2.08-2.00 and 1.93-1.83 ppm were also present and the singlet at 6.09 had now changed to two peaks at 6.02 ppm. The two triplets were again present at 4.37 and 3.27 ppm and the integral heights of the peaks at 56.0 and 55.4 ppm showed that only two methoxy groups were present. The mass spectrum now had one strong peak at *m/z* 180. This would suggest that the longer reaction time has simply allowed cleavage of one of the methyl ether groups by action of iodide to give 4'-chlorobutyl-2,4-dimethoxy-6-hydroxybenzoate (**130**).

5.3.2.6: Action of MeMgI on 2,4,6-trimethoxybenzoyl chloride in diethyl ether

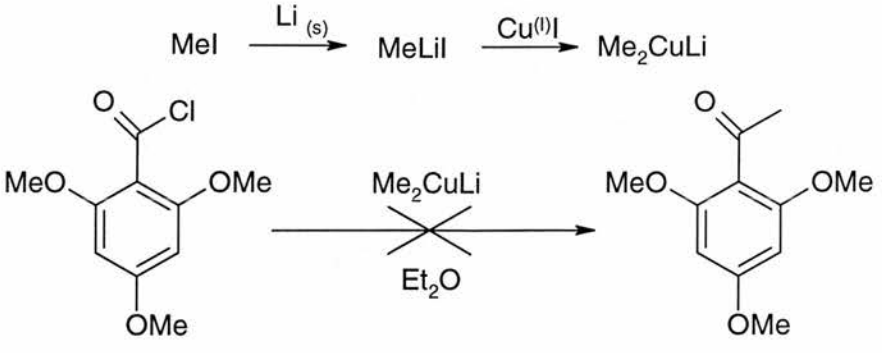
The use of diethyl ether instead of tetrahydrofuran as solvent should prevent solvent interaction and so the reaction was repeated using a pre-formed Grignard species in diethyl ether. After heating at reflux for 20 hours analysis by t.l.c. showed that a single new product had formed. The product was extracted from 1 M HCl and the aqueous layer was concentrated at reduced pressure to leave a white solid. ^{13}C n.m.r spectroscopy and mass spectrometry showed this to be 2,4,6-trimethoxybenzoic acid. The organic layer was concentrated and purified by column chromatography. This yielded a white solid with a melting point of 62-65 °C, too low to be the desired product. ^{1}H n.m.r. spectroscopy showed two new singlets at 5.32 and 4.87 ppm and the expected methyl protons were not observed in the region 2-3 ppm. ^{13}C n.m.r. spectroscopy showed the absence of any carbonyl group and the mass spectrum gave the major peak at *m/z* 209. The spectral data are consistent with a styrene derivative, 2,4,6-trimethoxy-α-methylstyrene. This suggested that the reaction of the Grignard species did not give the acetophenone. Instead subsequent additional attack on the more reactive ketone had taken place to give dimethylbenzyl alcohol. Dehydration would then account for the formation of 2,4,6-trimethoxy-α-methylstyrene (**131**), which fits with the spectral data. This was obtained in 35% yield (scheme 77).



Scheme 77: *Formation of 2,4,6-trimethoxy-α-methylstyrene (**131**)*

5.3.2.7: The use of alkyl cuprates

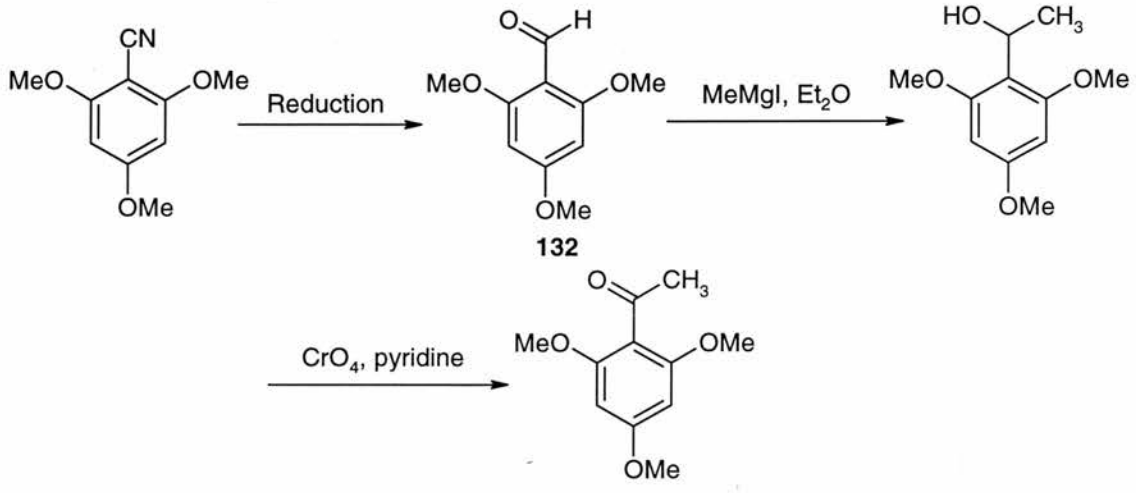
Prevention of this over-reaction may be achieved by the use of a higher order organo cuprate species, a less reactive nucleophile. Preparation required the initial formation of methyllithium, formed from heating a mixture of methyl iodide and lithium metal at reflux for two hours. Addition of 0.5 equivalents of cuprous iodide at 0 °C resulted in the formation of a yellow suspension and stirring was continued for thirty minutes. To this dimethylcuprate complex was added 2,4,6-trimethoxybenzoyl chloride at 0 °C and heating at reflux was carried out for a further 18 hours (scheme 78). ^{1}H n.m.r. spectroscopy of the crude product revealed that a new singlet at 2.53 ppm was present which was thought to correspond to the methyl protons in the desired product. Integration however showed this to be only a small fraction of the crude product and further purification was not attempted.



Scheme 78: *Formation and reaction of higher order alkyl cuprate species*

5.3.2.8: Attempted formation of 2,4,6-trimethoxybenzaldehyde (**132**)

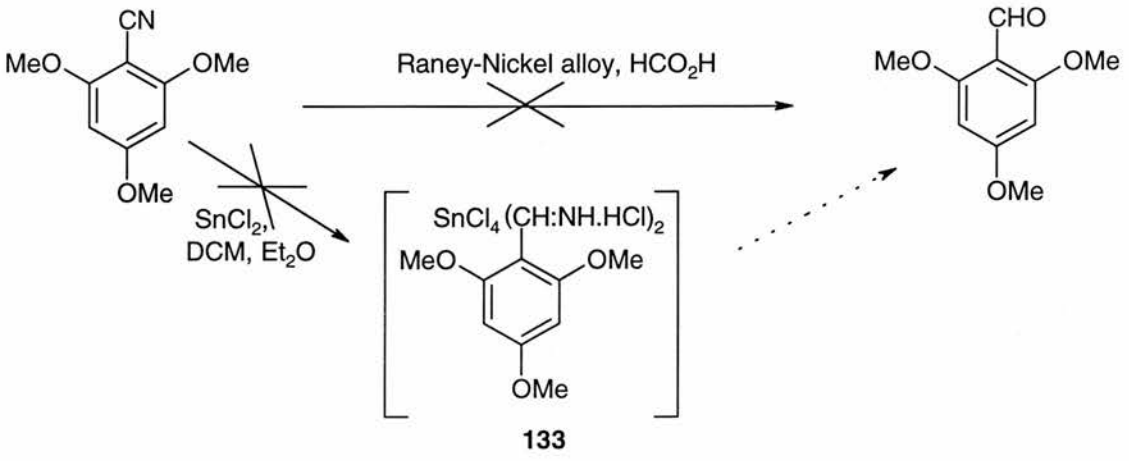
At this stage it was considered necessary to return to the 2,4,6-trimethoxybenzonitrile and to attempt direct conversion to the benzaldehyde (**132**). From this point, the use of a Grignard reagent would allow addition at the reactive carbonyl group to form the α-methylbenzyl alcohol. Standard oxidation procedures could then be implemented to afford the acetophenone. This is shown below in scheme 79.



Scheme 79: *Proposed Formation of 2,4,6-trimethoxyacetophenone via the corresponding aldehyde (**132**)*

Methods were then investigated for the reduction. The use of a Raney-Nickel alloy catalyst in formic acid[148] did not afford any of the desired product however (scheme 80). Analysis by t.l.c. after stirring for 18 hours at reflux showed that only the starting material remained. Stephen's method, utilising stannous chloride in diethyl ether and dichloromethane, was also investigated.[149] This involved the formation of an intermediate stannichloride complex (**133**). The 1925 publication suggested that 3,4,5-trimethoxybenzonitrile is converted to the benzaldehyde in 90% yield, however our attempts to duplicate the reaction with the 2,4,6-isomer failed to yield any product. An explanation for this may be found by considering the steric hindrance at the nitrile as a result of the two *o*-methoxy groups. This will also hinder nucleophilic attack at the nitrile in this reaction and indeed may explain why many of the reactions attempted on similar substrates in this section have either failed or are of low yield. The polarity

of the nitrile functionality will also be reduced as a result of the substantial positive inductive and mesomeric effects of the substituents on the benzene ring.

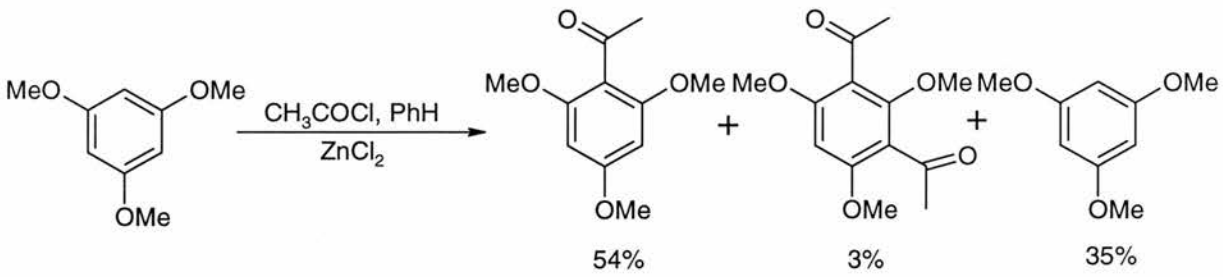


Scheme 80: *Attempted nitrile reduction to its corresponding aldehyde (**132**)*

5.3.2.9: Acetophenone formation via Friedel-Crafts acylation

Our studies had shown that the chemistry that could be performed on the 2,4,6-trimethoxybenzonitrile was quite limited. The option of preparing the [7,8-^{13}C]-2,4,6-trimethoxyacetophenone in a single step was becoming more attractive. Friedel-Crafts acylation has been shown to be successful on 2,4,6-trimethoxybenzene using acetyl chloride in benzene to give the acetophenone in 80% yield.[150] As [1,2-^{13}C]acetyl chloride is a commercially available reagent this reaction could be repeated to give the desired labelled product.

The reaction was first performed with unlabelled reagents (scheme 81) using a procedure similar to that reported, although just a single equivalent of acetyl chloride was used instead of 1.6 equivalents. This attempt to achieve a more frugal consumption of the labelled reagent resulted in the desired product being isolated in 54% yield (scheme 49). Also isolated was the starting material (35%) as well as a small amount of the double acylation product (3%).



Scheme 81: *Friedel-Crafts acylation of 2,4,6-trimethoxybenzene*

5.3.2.10: Conclusions

It has been shown in the previous section that the necessary 2,4,6-trimethoxyacetophenone can be potentially prepared with one or two ^{13}C-atoms incorporated using the Friedel-Crafts acylation method. However, the expense of ^{13}C-labelled acetyl chloride was such that it was decided not to carry out the labelled synthesis at this stage.

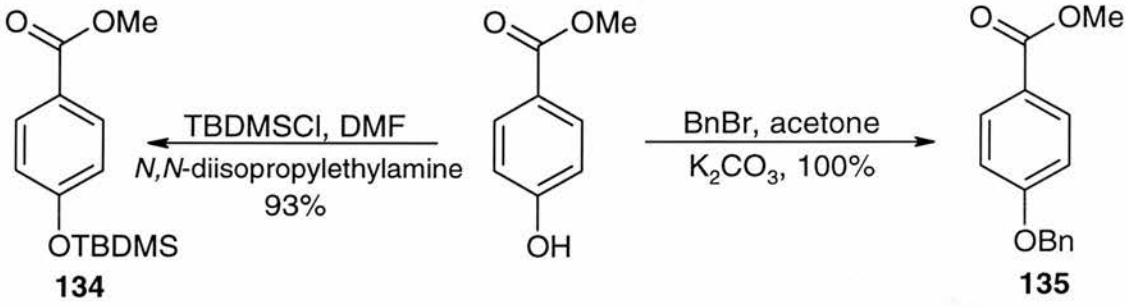
The third ^{13}C-atom of the three required, was to be incorporated into the 2 position using a suitably ^{13}C-labelled benzoic acid. In this case, the source of the ^{13}C-atom was ^{13}C-labelled potassium cyanide, a much cheaper starting material.

It was thus decided to confirm the utility of our procedures by synthesising [2-^{13}C]apigenin. If this could be prepared successfully, then it demonstrates that, using the ^{13}C-labelled acid chloride, the multiply ^{13}C-labelled apigenin would also be accessible, and could be prepared when required.

5.4: Synthetic attempts at flavone synthesis

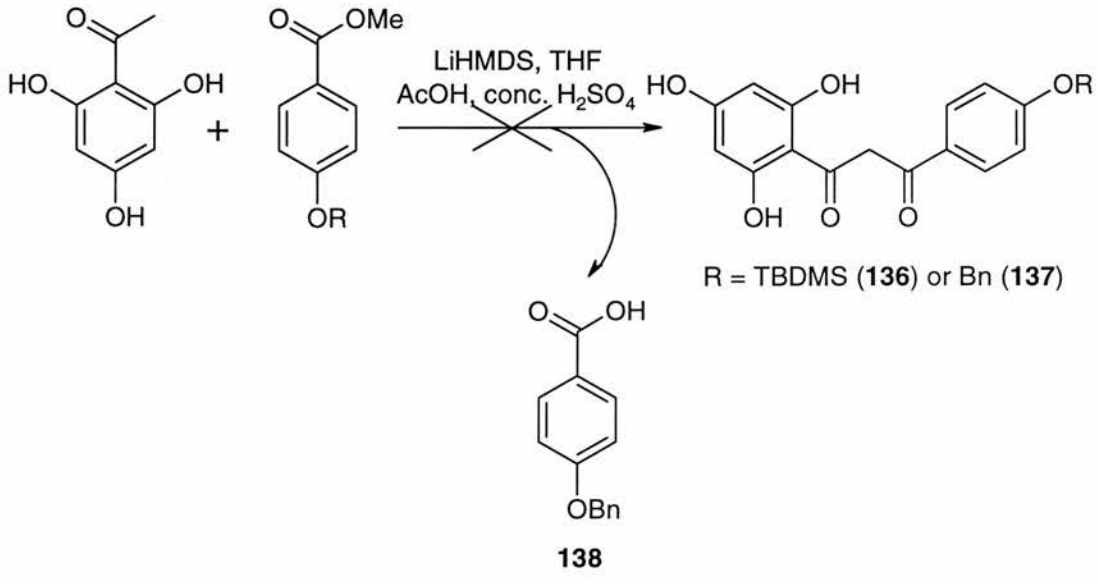
The wide range of reported variations on the Baker-Venkateraman procedure for flavone synthesis allowed us to explore a number of options. Although most traditional flavone syntheses require full protection of the hydroxyl groups, a recent procedure has reported successful coupling by using an excess of the base LHMDS. This involves the use of *O*-silyloxylated benzoates and 2,4,6-trihydroxyacetophenone.

Methyl 4-hydroxybenzoate was thus protected using TBDMSCl in dimethylformamide to give the desired *O*-silyloxyated benzoate (**134**). Protection as the benzyl ether using standard conditions was also carried out to yield the corresponding benzoate (**135,** scheme 82). Both of these were used in subsequent coupling procedures in attempt to form the diketone intermediate (**136**).



Scheme 82: *Protection of methyl 4-hydroxybenzoate*

Initial attempts at this novel coupling reaction involved methyl 4-benzyloxybenzoate (2) and commercially available 2,4,6-trihydroxyacetophenone (scheme 83). The acetophenone was stirred in a solution of LHMDS in tetrahydofuran for 1 hour at room temperature. The benzoate was then added at –78 °C and stirring was carried out initially for 1 hour and then at –10 °C for 2 hours. The crude product was isolated and acetic and sulphuric acids were added and the reaction mixture stirred at reflux for 3 hours in an attempt to afford acid catalysed cyclodehydration to the flavone. Water was added and the resulting precipitate was removed by filtration to leave an off-white coloured solid. Mass spectrometry showed a single strong peak at *m/z* 228 suggesting the formation of *p*-benzyloxybenzoic acid (**137**). The desired flavone was not obtained by this method.

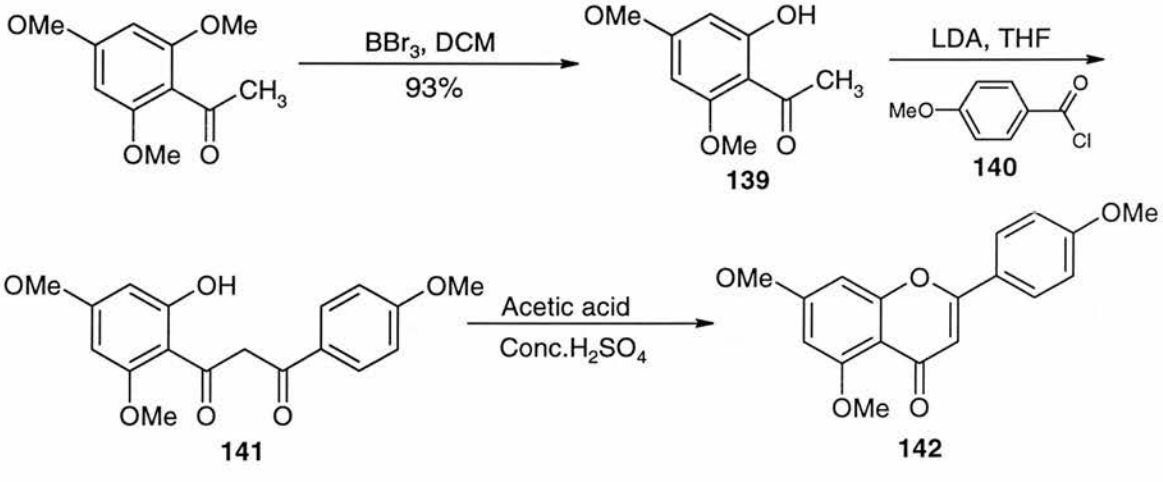


Scheme 83: *Attempted flavone preparation using a methyl benzoate*

Repeating the procedure using the methyl 4-*tert*-butyldimethylsilyloxybenzoate (**134**) gave similar results. The crude product from the coupling reaction was isolated and dried. Cyclization again failed to give any of the desired product. Concentration and subsequent analysis of the reaction mixture by ^{1}H n.m.r. spectroscopy showed two doublets at 7.96 and 6.86 ppm to be present, corresponding to the benzoate. Further signals were not observed in the aromatic range. ^{13}C n.m.r. spectroscopy revealed only the presence of the ester carbonyl at 167.1 ppm.

It was thus decided to use an acetophenone derivative where the 2 and 4 hydroxy groups were protected as their methyl ethers. It has been demonstrated by Banerji *et al* that 2,4-dimethoxy-6-hydroxyacetophenone reacts successfully with *p*-anisoyl chloride using LDA in tetrahydrofuran.[134] The presence of one *o*-hydroxy group is necessary in the acetophenone to allow the following cyclization to occur. The removal of one methyl ether group was selectively achieved using boron tribromide in dichloromethane. Stirring overnight at room temperature afforded a single product when analysed by t.l.c. and its isolation yielded the acetophenone (**139**) as a white powder in 93% yield. Mass spectrometry revealed a single peak at *m/z* 196 and ^{1}H n.m.r. spectroscopy showed that the original aromatic singlet had now become two doublets at 6.06 and 5.93 ppm.

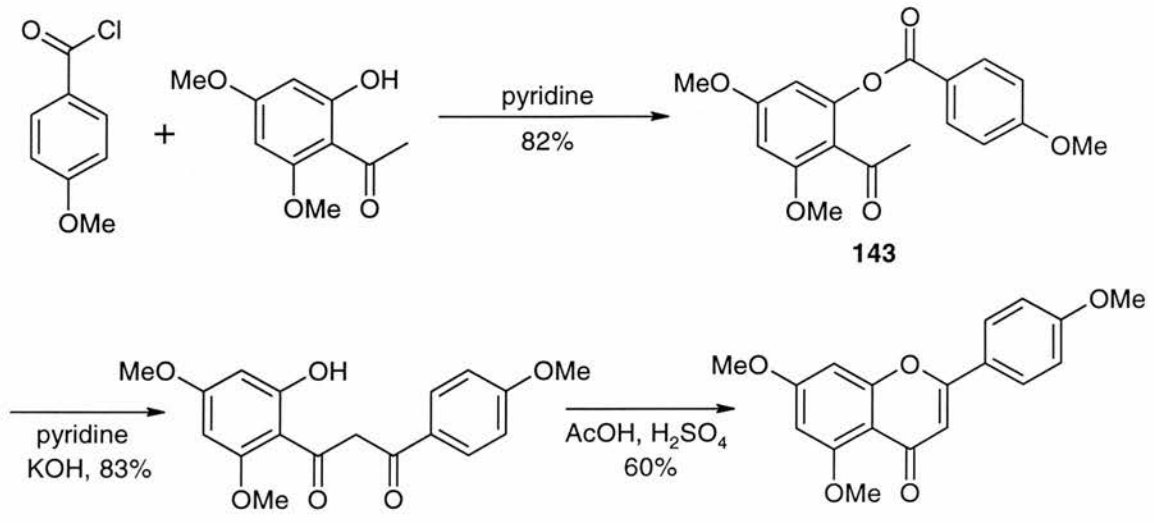
The 2,4-dimethoxy-6-hydroxyacetophenone was then stirred in tetrahydrofuran and LDA (scheme 84) was added at room temperature. The acid chloride (**140**) in tetrahydrofuran was then added at -78 °C and stirring was continued for 3 hours and then overnight at room temperature. Analysis by t.l.c. revealed that two new products were present with similar R_f values and ^{13}C n.m.r. spectroscopy of the crude mixture revealed the presence of the diketone (**141**) with a carbonyl group present at 200.37 ppm. Only one carbonyl was observed, however suggesting the enol tautomer is favoured. Column chromatography was not successful in separation of these products although other minor side products as well as unreacted acetophenone were isolated and removed. The mass spectrum of the two-product mixture suggested the presence of *p*-anisic acid with a strong peak at *m/z* 152 as well as the diketone, or tautomer of, at *m/z* 331. Acid-catalysed cyclodehydration was then carried out as before and the precipitate isolated by filtration to leave a brown solid. This was shown to be apigenin trimethyl ether (**142**) although it was only isolated in trace amounts. ^{1}H n.m.r. spectroscopy showed the H-3 proton as a singlet at 6.59 ppm and the H-6 and 8 protons as doublets at 6.75 and 6.50 ppm repsectively. ^{13}C n.m.r. spectroscopy showed the new carbonyl peak at 180.3 ppm and the mass spectrum showed a single peak at *m/z* 312.



Scheme 84: *Flavone preparation using an acid chloride and LDA*

Unfortunately, repeated attempts at this reaction failed to improve the product yield and a more traditional preparative method was used, involving the formation and isolation of an ester species before the diketone.[135] The reaction sequence is described

in scheme 85 and involves the use of pyridine and potassium hydroxide as bases. Acid catalysed cyclodehydration was again afforded using acetic acid and concentrated sulfuric acid.



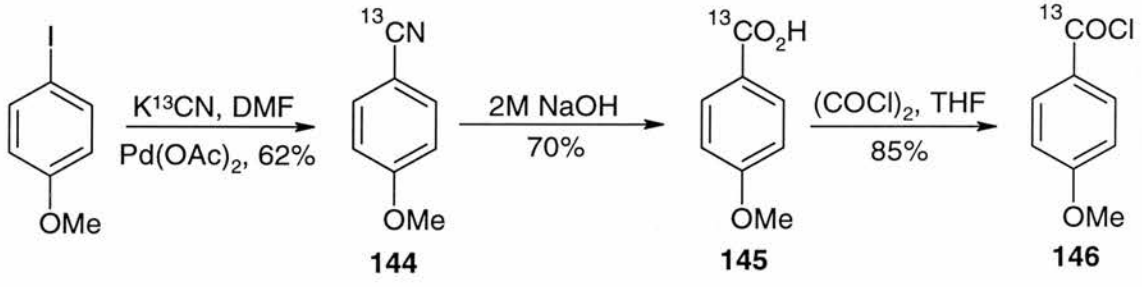
Scheme 85: *Flavone preparation using an acid chloride and pyridine*

The formation of the ester (**143**) was achieved in 82% yield from heating the *p*-anisoyl chloride and the acetophenone in pyridine for 10 minutes at 100 °C to give the product as a white solid. The ^{13}C n.m.r. spectrum clearly showed the new carbonyl resonance at 162.2 ppm while the mass spectrum contained a strong peak at *m/z* 331. The diketone was then formed by heating the ester in pyridine at 100 °C for 10 minutes in the presence of potassium hydroxide. The crude product was re-crystallised from ethanol to give a yellow powder in 83% yield. The ^{13}C n.m.r. spectrum showed that the product existed as the enol tautomeric form with the carbonyl at 197.9 ppm and the second enolized carbon at 193.9 ppm. The mass spectrum showed a peak at *m/z* 331 while the melting point of 132-134 °C was consistent with the literature value[135] of 132-133 °C.

Formation of apigenin trimethyl ether was then achieved by heating the diketone in the presence of acetic acid and 20% sulphuric acid in acetic acid for 10 minutes. Water was added to give a pale yellow gelatinous solid and collection by filtration gave the product as a brown solid in 60% yield. The ^{13}C n.m.r. and mass spectra clearly showed the product had been formed (as previous) and at this point it was decided to repeat the procedure using *p*-[^{13}C]anisoyl chloride.

5.5: Synthesis of *p*-[^{13}C]anisoyl chloride

The introduction of one [^{13}C] label into this flavone precursor involved the use of *p*-iodoanisole as starting material (scheme 86). Reaction with potassium [^{13}C]cyanide using a palladium acetate catalyst in a manner similar to that previously discussed yielded 4-methoxybenzo[^{13}C]nitrile (**144**) as a white solid in 62% yield. The new product has a melting point of 57-60 °C and ^{13}C n.m.r. spectroscopy showed an enhanced nitrile resonance at 119.3 ppm. Hydrolysis of the nitrile under basic conditions was carried out giving the [^{13}C]benzoic acid (**145**) in 70% yield as a white solid. ^{13}C n.m.r. spectroscopy highlighted the carbonyl peak at 167.3 ppm and the mass spectrum gave a single peak at *m/z* 153 as expected.



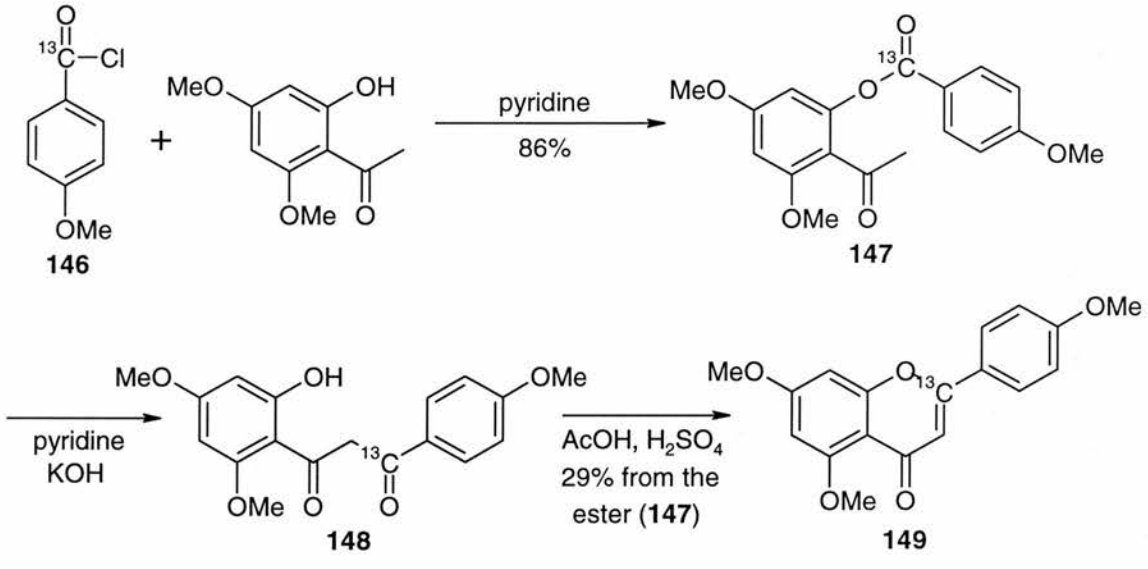
Scheme 86: *Preparation of p-[^{13}C]anisoyl chloride (**146**)*

Preparation of the benzoyl chloride (**146**) was then achieved by stirring in a solution of oxalyl chloride in tetrahydrofuran for 18 hours. The solvent was removed to leave the desired product as a pale oil. The mass spectrum confirmed the presence of the benzoyl chloride showing peaks at *m/z* 170 and 172.

5.6: Synthesis of [2-^{13}C]apigenin trimethyl ether (149)

The procedure outlined in scheme 87 was repeated using *p*-[^{13}C]anisoyl chloride to give [2-^{13}C]apigenin trimethyl ether (**149**) in three steps. The ^{13}C n.m.r. spectra showed the presence of the labelled carbon atom with enhanced peaks at 162.8 ppm for the ester carbonyl in **147** and 193.9 ppm for the 3 position in the diketone (**148**). The crude diketone was isolated as a yellow solid and cyclization to give the flavone

was performed without carrying out the wasteful recrystalization procedure. The ^{13}C n.m.r. spectrum of the flavone displayed an enhanced peak at 160.2 ppm for the 2 position. *p*-[^{13}C]Anisic acid was also observed as a minor impurity in these intermediates, seen in the ^{13}C n.m.r. spectra as an enhanced shift at 165.2 ppm in *d*-chloroform and at 167.2 ppm in d^6-DMSO. There are a number of well documented literature procedures for the demethylation to apigenin. Thus, this remains the first formal synthesis of a ^{13}C labelled flavone.



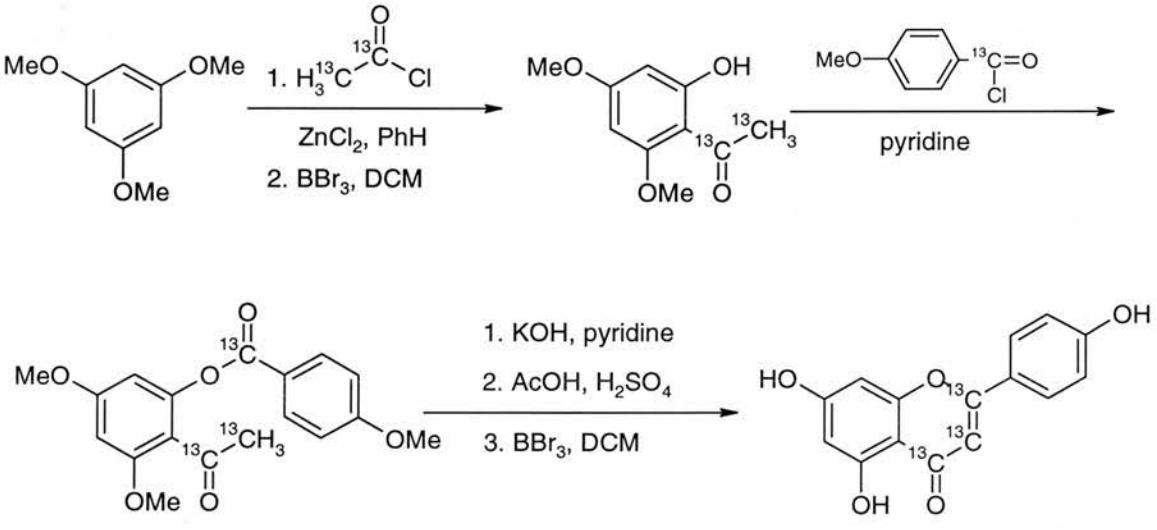
Scheme 87: *Preparation of [2-^{13}C]apigenin trimethyl ether (**149**)*

5.7: Conclusions and future work

This chapter has described the synthetic studies undertaken on potential routes for the preparation of multiply ^{13}C labelled flavones. The original route proposed to incorporate two ^{13}C labels into 2,4,6-trimethoxyacetophenone was shown not to be feasible. It was found that the reactivity of functional groups at the 1-position was severely reduced as a result of the flanking methoxy groups. This is mainly as a result of the steric hindrance that they cause. However, their ability to increase electron density into the aromatic ring and reduce the polarity, and hence reactivity, of functional groups on the 1-position may also be important. Eventually, the reaction of 2,4,6-trimethoxybenzene and [1,2-^{13}C]acetyl chloride, via a Friedel-Crafts acylation reaction provided an efficient method for its preparation, however. The third ^{13}C atom was more straightforwardly incorporated into *p*-anisoyl chloride.

Methods for the synthesis of flavones were examined and it was found that the use of an acid chloride in conjunction with LDA as a base was unreliable. This was also found to be the case using various hydroxyl protected methyl benzoates, in the presence of LHMDS as the base. A more traditional approach involving the formation and isolation of the ester, diketone and finally flavone species was found to be the most efficient synthetic route. This was then used in the first formal synthesis of a ^{13}C labelled flavone, [2-^{13}C]apigenin trimethyl ether. The final removal of the methyl groups may be achieved using standard procedures.

A procedure for the formation of flavones incorporating three ^{13}C labels has thus been established and validated (scheme 88). Such examples have not thus far been prepared due to the cost, however this route will allow access to a wide range of multiply labelled flavone and flavanone compounds.



Scheme 88: *Procedure for the formation of [2,3,4-$^{13}C_3$]apigenin (**117**)*

Chapter 6

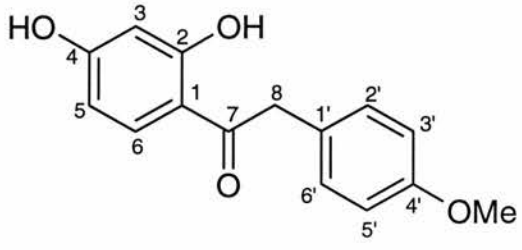
Experimental

6.1: Experimental details

NMR spectra were recorded on a Varian Gemini f.t. spectrometer (^{1}H, 300 MHz; ^{13}C, 74.76 MHz) and a Varian Gemini f.t. spectrometer (^{1}H, 200 MHz; ^{13}C, 50.31 MHz). ^{1}H NMR spectra were referenced on chloroform, TMS, DMSO or methanol. NMR spectra are described in parts per million downfield shift from TMS and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (s-singlet, d-doublet, t-triplet, q-quadruplet, m-multiplet, dd-doublet of doublets, dt-doublet of triplets and b-broad), coupling constant (Jx,y Hz if applicable) and assignment. I.R spectra were taken on a Perkin–Elmer series 1500 f.t. IR spectrometer. The samples were prepared as Nujol mulls or as thin films between sodium chloride discs. Absorption maxima are given in wavenumbers (cm^{-1}). Flash chromatography was performed according to the procedure of Stille[32] using Sorbisil C60 (40-60 mm) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (MN SIL G/UV_{254}) and compounds were visualized by UV fluoresence, bromocresol green or ninhydrin. Low resolution mass spectra (electrospray) were acquired using a VG platform with VG Masslynx software. Microwave reactions were carried out using a Panasonic 650 Watt machine set to "simmer". HPLC spectra were obtained from a Cecil CE1200 series chromatograph using Luna 5u C-18 silica-gel on a 150 x 4.60 mm 5 micron sized column. The solvent system used in all cases was a mixture of acetonitrile : water (1 : 1) at a run-rate of 0.3 ml per minute.

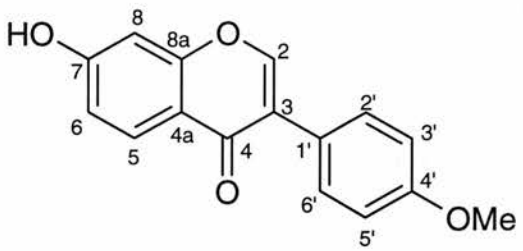
6.2: Isoflavone-7-*O*-glucosides and glucuronides

Synthesis of 4'-methoxybenzyl-2,4-dihydroxyphenyl ketone (**36**)[55]



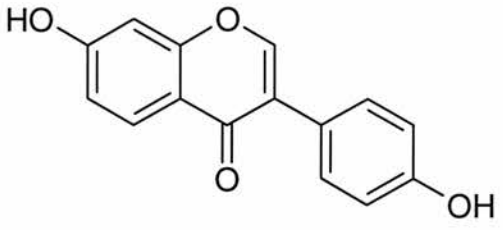
Boron trifluoride etherate (5 cm^3, 40.6 mmol) was added to a mixture of 4-methoxyphenyl acetic acid (2.2 g, 0.013 mmol) resorcinol (2.9 g, 0.026 moles) under a nitrogen atmosphere. The mixture was heated to reflux for 20 minutes, forming a dark red oil, then cooled to room temperature. Sodium acetate (30 cm^3 of a saturated solution), sodium hydrogen carbonate (15 cm^3 of a saturated solution) and diethyl ether (50 cm^3) were added sequentially. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an off-white powder. This was washed with dichloromethane to give the desired product (1.40 g, 67%) as a pure white powder (Found: M^+, 258.089841. $C_{15}H_{14}O_4$ requires *M*, 258.089209); m.p. 156-159 °C (Lit.,[55] 159-163 °C); ν_{max} (thin film)/cm^{-1} 3530 (OH) and 1650 (C=O); δ_H (200 MHz; CD_3OD) 7.86 (1H, d, $J_{5,6}$ = 8.2, H-6), 7.19 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-2' and 6'), 6.86 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-3'and 5'), 6.38 (1H, dd, $J_{3,5}$ = 2.4 and $J_{5,6}$ = 8.2, H-5), 6.28 (1H, d, $J_{3,5}$ = 2.4, H-3), 4.15 (2H, s, $C\underline{H}_2$), 3.77 (3H, s, $OC\underline{H}_3$); δ_C (50.31 MHz, CD_3OD) 204.4 ($\underline{C}$=O), 167.2 (C-4'), 166.0 (C-4), 160.3 (C-2), 134.7 (C-2' and 6'), 128.1 (C-6), 115.3 (C-3'and 5'), 113.0 (C-1), 108.5 (C-5), 104.1 (C-3), 56.0 ($O\underline{C}H_3$), 44.04 ($\underline{C}H_2$); *m/z* (EI) 258 (M^+, 15%), 137 ($[C_7H_5O_3]^+$, 100), 121 ($[C_8H_9O]^+$, 20).

Synthesis of 7-hydroxy-4'-methoxyisoflavone (formononetin, **20**)[64]



N,N-Dimethylformamide dimethyl acetal (1.36 cm^3, 1 mmol) was added dropwise to a stirred solution of 4'-methoxybenzyl-2,4-dihydroxyphenyl ketone (1.5 g, 5 mmol) in tetrahydrofuran (10 cm^3). Stirring was continued for 4 hours to reflux under a nitrogen atmosphere and then the reaction was cooled to room temperature. The reaction was stirred for a further 1.5 hours and methanol (10 cm^3) was added. Concentration of the reaction mixture at reduced pressure left an orange-red solid which was washed with methanol (100 cm^3). Removal of the solvent by filtration left the desired product (1.23 g, 92%) as a white powder (Found: M^+, 268.072945. $C_{16}H_{12}O_4$ requires *M*, 268.073559); m.p. 255-259 °C (Lit.,[151] 257 °C); ν_{max} (thin film)/cm^{-1} 3420 (OH) and 1625 (C=O); δ_H (200 MHz, d^6-DMSO) 8.37 (1H, s, H-2), 7.99 (1H, d, $J_{5,6}$ = 8.8, H-5), 7.53 (2H, d, $J_{2',3'}$ = $J_{5',6'}$ = 8.8, H-2' and 6'), 7.01 (2H, d, $J_{2',3'}$ = $J_{5',6'}$ = 8.8, H-3' and 5'), 6.95 (1H, dd, $J_{6,8}$ = 2.3 and $J_{5,6}$ = 8.7, H-6), 6.89 (1H, d, $J_{6,8}$ = 2.3, H-8), 3.81 (3H, s, OC$\underline{H}_3$); δ_C (50.31 MHz, d^6-DMSO) 175.4 (C-4), 162.8 (C-4'), 159.2 (C-8a), 157.7 (C-7), 153.5 (C-2), 130.3 (C-2' and 6'), 127.6 (C-5), 124.5 (C-3), 116.9 (C-4a), 115.5 (C-6), 113.8 (C-3' and 5'), 102.4 (C-8), 55.4 (O$\underline{C}H_3$); *m/z* (EI) 268 (M^+, 100%), 253 ($[M - CH_3]^+$, 14).

7, 4'-Dihydroxyisoflavone (Daidzein **19**)



Attempted synthesis using hydroiodic acid and phenol.[152]

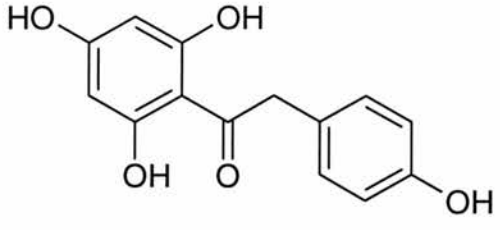
Phenol (12 mg, 0.13 mol) was added to a stirred solution of formononetin (120 mg, 0.45 mmol) in hydroiodic acid (5 cm^3 of a 57.5% solution) under a nitrogen atmosphere. Stirring was continued for 5 hours at 120 °C and then the solution cooled to room temperature. Potassium hydroxide (15 cm^3 of a 4.0 mol dm^{-3} solution) and acetic acid (10 cm^3) were added to adjust to pH 4-5. The insoluble material product was removed by filtration and washed with ethanol (60 cm^3 of a 50% aqueous solution) to leave a grey-white solid. ^1H n.m.r. spectroscopy and analysis by t.l.c. (hexane : ethyl acetate, 4 : 1) showed only starting material to be present.

Synthesis using boron tribromide in dichloromethane[90]

A solution of boron tribromide (0.46 cm^3, 4.93 mmol) in dichloromethane (20 cm^3) was added dropwise to a stirred solution of formononetin (201 mg, 0.75 mmol) in dichloromethane (10 cm^3) at 0 °C under a nitrogen atmosphere. Stirring was continued for 18 hours at room temperature and ice water (75 cm^3) was then added. A precipitate was formed which was collected by filtration and washed with ethanol (10 cm^3). This left the desired product (0.12 g, 61%) as a white powder (Found: M^+, 254.057743. $C_{15}H_{10}O_4$ requires *M*, 254.057909); m.p. 320-325 °C (Lit.,[28] 315-320 °C); ν_{max} (thin film)/cm^{-1}

3410 (OH) and 1630 (C=O); δ_H (200 MHz, d^6-DMSO) 10.68 (1H, s, OH), 9.44 (1H, s, OH), 8.19 (1H, s, H-2), 7.83 (1H, d, $J_{5,6}$ = 8.8, H-5), 7.25 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.4, H-2' and 6'), 6.80 (1H, dd, $J_{6,8}$ = 2.3 and $J_{5,6}$ = 8.8, H-6), 6.71 (1H, d, $J_{6,8}$ = 2.3, H-8), 6.66 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.4, H-3' and 5'); δ_C (50.31 MHz, d^6-DMSO) 175.0 (C-4), 162.8 (C-8a), 158.0 (C-7), 157.4 (C-4'), 153.1 (C-2), 130.3 (C-2' and 6'), 127.6 (C-5), 123.75 (C-3), 122.8 (C-1'), 116.9 (C-4a), 115.4 (C-6), 115.2 (C-3' and 5'), 102.8 (C-8); *m/z* (EI) 254 (M^+, 100%), 137 ($[C_7H_4O_3]^+$, 78).

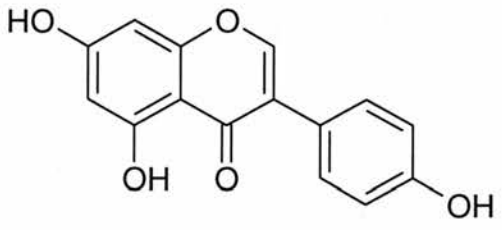
Synthesis of 4'-hydroxybenzyl-2,4,6-trihydroxyphenylketone (**32**)[57]



Zinc chloride (25 cm^3 of a 1.0 mol dm^{-3} solution, 25 mmol) was added to a stirred solution of phloroglucinol (3.0 g, 24 mmol) and 4-hydroxyphenylacetonitrile (2.2 g, 16 mmol) in diethyl ether (15 cm^3). This was saturated with a stream of dry HCl gas at 0 °C and stirred for 48 hours until a yellow precipitate had fully formed. The orange solution was decanted off and the yellow precipitate washed with ice-cold diethyl ether (50 cm^3). HCl (20 cm^3 of a 0.4 mol dm^{-3} solution) was added and the solution heated to reflux for 3 hours. The reaction was then cooled to room temperature and diethyl ether (50 cm^3) was added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave the desired product (1.71 g, 41%) as a pale yellow powder (Found: C, 64.17; H, 4.64%. $C_{14}H_{12}O_5$ requires C, 64.61; H, 4.65); m.p. 276-280 °C (Lit.,[61] 259 °C);

ν_{max} (thin film)/cm^{-1} 3540 (OH) and 1645 (C=O); δ_H (200 MHz, CD_3OD) 7.06 (2H, d, $J_{2',3'} = J_{5',6'} = 8.5$, H-2' and 6'), 6.71 (2H, d, $J_{2',3'} = J_{5',6'} = 8.5$, H-3' and 5'), 5.81 (2H, s, H-3 and 5) 4.28 (2H, s, C$\underline{H}_2$); δ_C (50.31 MHz, CD_3OD) 205.4 ($\underline{C}$=O), 166.6 (C-4), 166.2 (C-2 and 6), 157.2 (C-4'), 132.1 (C-2' and 6'), 128.5 (C-1'), 116.3 (C-3' and 5'), 104.4 (C-1), 96.1 (C-3 and 5) 44.0 ($\underline{C}H_2$); *m/z* (CI) 261 ([MH]$^+$, 100%), 127 ([$C_6H_6O_3$]$^+$,11).

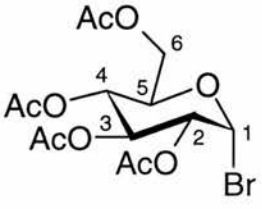
Synthesis of 5, 7, 4'-trihydroxyisoflavone (Genistein, **10**)[66]



Boron trifluoride etherate (6 cm^3, 48.7 mmol) was added to a solution of 4'-hydroxybenzyl-2,4,6-trihydroxyphenyl ketone (0.3 g, 1.5 mmol) in dimethylformamide (12 cm^3). This was heated using microwave conditions for 3 x 15 second intervals with swirling. Mesyl chloride (6 cm^3) was then added with swirling and heating continued for 2 x 30 seconds. Water (200 cm^3) was then added to give a brown oily mixture, turning to a pale yellow precipitate after stirring on a hotplate for 2-3 hours. The precipitate was collected by filtration to give the desired product (0.16 g, 51%) as a pale yellow powder (Found: M$^+$, 270.052519. $C_{15}H_{10}O_5$ requires *M*, 270.052824); m.p. 299-302 °C (Lit.,[153] 296-298 °C); ν_{max} (thin film)/cm^{-1} 3380 (OH), 1632 (C=O); δ_H (200 MHz, d^6-DMSO) 8.34 (1H, s, H-2), 7.39 (2H, d, $J_{2',3'} = J_{5',6'} = 8.2$, H-2', 6'), 6.81 (2H, d, $J_{2',3'} = J_{5',6'} = 8.2$, H-3', 5'), 6.41 (1H, d, $J_{6,8} = 2.0$, H-6), 6.25 (1H, d, $J_{6,8} = 2.0$, H-8); δ_C (50.31 MHz, d^6-DMSO) 180.4 (C-4), 164.63 (C-7), 162.25 (C-8a), 157.7 (C-5), 157.5 (C-4'), 154.1

(C-2), 130.3 (C-2' and 6'), 122.4 (C-1'), 121.4 (C-3), 115.8 (C-3' and 5'), 104.63 (C-4a), 99.1 (C-6), 94.0 (C-8); *m/z* (EI) 270 (M^+, 100%), 153 ($[C_7H_5O_4]^+$, 41).

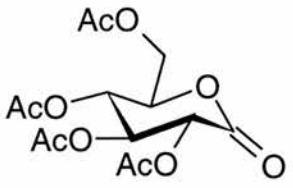
Synthesis of 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-glucopyranose (**41**)[154]



Acetic anhydride (1.0 cm^3) followed by hydrogen bromide (1.0 cm^3 of a 30% w/v solution in acetic acid, 2.70 mmol) were added to a stirred solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (0.50 g, 1.28 mmol) in dichloromethane (10 cm^3) under a nitrogen atmosphere. Stirring was continued at room temperature for 48 hours until the reaction had been shown to be complete by t.l.c. (ethyl acetate : hexane, 3 : 2). Toluene (40 cm^3) was then added and the resulting solution concentrated at reduced pressure. A further portion of toluene (20 cm^3) was added and the solution was again concentrated at reduced pressure to remove excess acetic acid. The residue was dissolved in dichloromethane (50 cm^3) and the solution washed with sodium hydrogen carbonate (2 x 50 cm^3 of a 1.0 mol dm^{-3} solution) and water (50 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to give pure 2,3,4,6-tetra-*O*-acetyl-1-bromo-α-D-glucopyranose as a pale gold syrup (0.45 g, 86%) which overnight turned to white solid, m.p. 86-7 °C (lit.,[155] 88-9 °C); $[\alpha]_D$ +195.9 ° (c 2.42 in $CHCl_3$) (lit.,[156] +197.84 ° (c 2.42 in $CHCl_3$)); ν_{max} (nujol)/cm^{-1} 1730 (CO); δ_H (200 MHz, $CDCl_3$) 6.61 (1H, d, $J_{1,2}$ = 3.5, H-1), 5.55 (1H, t, $J_{2,3} = J_{3,4}$ = 9.6, H-3), 5.16 (1H, t, $J_{3,4} = J_{4,5}$ = 9.6, H-4), 4.82 (1H, dd, $J_{1,2}$ = 3.1 and $J_{2,3}$ = 9.6, H-2), 4.30 (2H, m, H-6a,6b), 4.11 (1H, m, H-5), 1.91-2.21 (12H, 4s, 4 x $C\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 170,9, 170.3, 170.2 and 169.9 (4 x

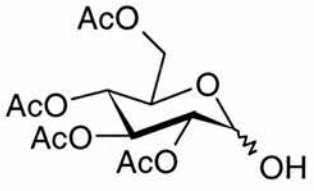
C=O), 86.6 (C-1), 72,6 (C-3), 71.0 (C-5), 70.6 (C-2), 67.6 (C-4), 61.4 (C-6), 21.1 and 21.0 (4 x $\underline{C}H_3$); *m/z* (CI) 430 and 428 ($[MH + NH_4]^+$,18%), 331 ($[MH - Br]^+$, 8) and 213 ($[MH - Br - C_4H_6O_4)]^+$, 29).

Synthesis of 2,3,4,6-tetra-*O*-acetyl-D-glucono-γ-lactone (**49**)[157]



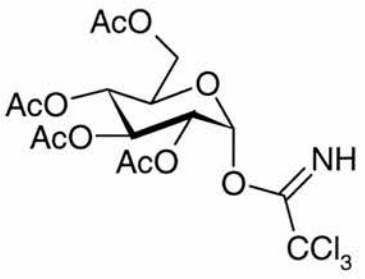
D-Glucono-γ-lactone (20.0 g, 112.2 mmol) was added to a stirred solution of zinc chloride (10.0 g, 73.4 mmol) in acetic anhydride (75 cm^3) under a nitrogen atmosphere. Stirring was continued at room temperature for 3 hours and then the reaction mixture was poured onto crushed ice (400 cm^3). Dichloromethane (2 x 250 cm^3) was added and the reaction mixture washed with further ice cold water (175 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to give the desired product (33.60 g, 87%) as a golden syrup $[\alpha]_D$ +74.4 ° (*c* 2.0 in $CHCl_3$), (Lit.,[157] +79.7 ° (*c* 2.0 in $CHCl_3$)); ν_{max} (thin film)/ cm^{-1} 2950 (CH), 1715 (CO); δ_H (200 MHz, $CDCl_3$) 5.52 (1H, t, $J_{3,4}$ = 9.0, H-3), 5.34 (1H, t, $J_{3,4}$ = 9.0, H-4), 5.09 (1H, d, $J_{2,3}$ = 9.5, H-2), 4.59 (1H, m, $J_{4,5}$ = 8.7, H-5), 4.36 (1H, dd, $J_{5,6b}$ = 3.7, $J_{6a,6b}$ = 12.6, H-6b), 4.21 (1H, dd, $J_{5,6a}$ = 2.9, $J_{6a,6b}$ = 12.6, H-6a), 2.05-2.31 (12H, s, 4 x $C\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 170.8, 170.5, 170.1, 169.7 (4 x $\underline{C}$=O), 165.1 (C-1), 76.2 (C-2), 70.73 (C-3 and 5), 66.8 (C-4), 61.8 (C-6), 21.1, 21.0, 21.0, 20.8 (4 x $\underline{C}H_3$); *m/z* (EI) 347 (M^+, 22%), 304 ($[M - C_2H_3O]^+$, 6), 287 ($[M - C_2H_3O_2]^+$, 14), 244 ($[M - C_4H_6O_3]^+$, 6), 202 ($[M - C_6H_9O_4]^+$, 6), 184 ($[M - C_6H_9O_5]^+$, 20).

Synthesis of 2,3,4,6-tetra-*O*-acetyl-α/β-D-glucopyranose (**50**)[95]



2,3,4,6-Tetra-*O*-acetyl-D-glucono-γ-lactone (33.60 g, 97.4 mmol) was dissolved in tetrahydrofuran (175 cm^3) and the solution cooled to 0 °C. A cold solution of sodium borohyride (1.84 g, 48.25 cm^3) in water (14 cm^3) was then added dropwise. Stirring was continued at 0 °C for 2 hours and DOWEX-50W H^+ ion exchange resin (35 g) was then added. The insoluble material was removed by filtration and the filtrate was concentrated at reduced pressure to leave an oily solid. This was washed with methanol (50 cm^3), to leave the desired product (32.02 g, 95%) as a colourless syrup $[\alpha]_D$ +11.2 ° (*c* 1.0 in $CHCl_3$) (Lit.,[158] +9.8 ° (*c* 1.0 in $CHCl_3$)); ν_{max} (nujol)/cm^{-1} 3320 (OH), 1730 (C=O); δ_H (300 MHz, $CDCl_3$) 4.75-5.74 (4H, m, H-1, 2, 3 and 4), 4.15 (2H, m, C$\underline{H}_2$-6), 3.71 (1H, m, H-5), 1.90-2.21 (12H, 4 x s, 4 x C$\underline{H}_3$); δ_C (74.76 MHz, $CDCl_3$) 171.3, 170.7, 170.7, 170.1 (4 x $\underline{C}$=O), 96.0 (C-1β), 90.6 (C-1α), 72.1 (C-3β), 71.6 (C-3α), 71.2 (C-5β), 70.3 (C-5α), 70.0 (C-2β), 68,9 (C-2α), 68.8 (C-4β), 67.6 (C-4α), 65.2 (C-6β), 62.5 (C-6α), 21.4, 21.3, 21.2, 21.0 (4 x $\underline{C}H_3$); *m/z* (CI) 366 ($[MH + NH_4]^+$, 95%), 331 ($[MH - OH]^+$, 100), 271 ($[MH - C_2H_5O_3]^+$, 8).

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**47**)



Synthesis using sodium hydride and trichloroacetonitrile[159]

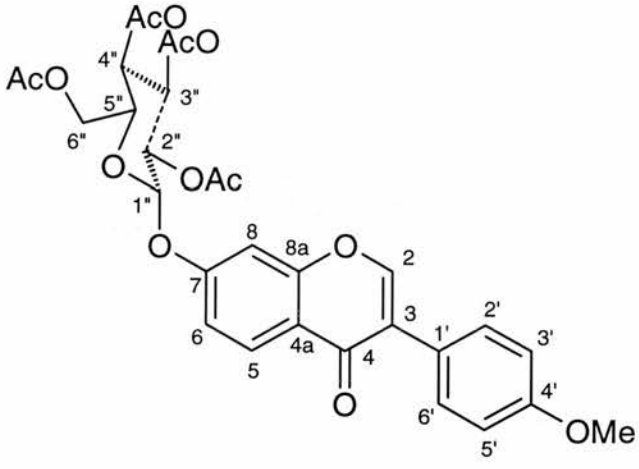
Sodium hydride (0.1 g, 4.2 mmol) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (0.61 g, 1.75 mmol) and trichloroacetonitrile (0.7 cm^3) in dichloromethane (3.5 cm^3). The solution was stirred for 30 minutes at room temperature and water (50 cm^3) was added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a dark oil, purified by column chromatograpy (silica; ethyl acetate : hexane, 1 : 1). This eluted the desired product (35 mg, 4%) as a pale oil (spectral data as below).

Synthesis using potassium carbonate and trichloroacetonitrile[160]

Fresh, glowing hot potassium carbonate (7.9 g) was added to a stirred solution of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (11.335 g, 32.6 mmol) and trichloroacetonitrile (11 cm^3) in dichloromethane (70 cm^3). The reaction was stirred for 48 hours at room temperature and water (50 cm^3) was added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. Purification by column chromatography (silica; ethyl acetate : petroleum ether 1 : 1) eluted the desired product (6.21 g, 40%) as a white solid m.p. 68-71 °C (Lit., (α) oil, (β)[160] 154-155 °C); ν_{max} (nujol)/cm^{-1} 3320 (OH), 1730 (C=O), 1650 (C=N); $[\alpha]_D$ +44.7 ° (*c* 1.0 in $CHCl_3$) (Lit., (α)[159] +61.5 ° (*c* 1.0 in $CHCl_3$), (β)[160]

+8.3 ° (*c* 1.0 in $CHCl_3$)); δ_H (200 MHz, $CDCl_3$) 8.69 (1H, s, NH), 6.55 (1H, d, $J_{1,2}$ = 3.3, H-1), 5.53 (1H, dd, $J_{1,2}$ = 3.3 and $J_{2,3}$ = 9.9, H-2), 5.16 (1H, dd, $J_{6a,6b}$ = 13.4 and $J_{5,6b}$ = 2.9, H-6b), 5.10 (1H, dd, $J_{6a,6b}$ = 13.4 and $J_{5,6a}$ = 2.9, H-6a), 4.08-4.23 (3H, m, H-3, 4, 5), 2.16-2.01 (12H, 4 x s, C<u>H</u>$_3$); δ_C (50.31 MHz, $CDCl_3$) 171.3, 170.8, 170.3, 170.1 (4 x <u>C</u>=O), 93.4 (C-1), 70.3 (C-3), 70.1 (C-5), 70.0 (C-2), 68.2 (C-4), 62.0 (C-6), 21.1, 20.9, 20.8, 20.5 (4 x <u>C</u>H$_3$).

<u>4'-Methoxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (**52**)</u>



<u>Synthesis using α-acetobromoglucose and a silver oxide catalyst[87]</u>

Silver oxide (500 mg, 2.16 mmol) was added to a stirred solution of formononetin (270 mg, 1 mmol) in pyridine (15 cm^3). Stirring was continued for 15 minutes at room temperature and α-acetobromoglucose (440 mg, 1 mmol) was added to the reaction mixture. Stirring was continued for a further 3 hours and the solution was then diluted with dichloromethane (30 cm^3) and with sulphuric acid (30 cm^3 of a 1.0 mol dm^{-3}

solution). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a pale solid. Purification by column chromatography (silica; toluene : ethyl acetate, 6 ; 1) gave the desired product (0.13 g, 22%) as a white solid (Found: C, 60.33; H, 4.91%. $C_{30}H_{30}O_{13}$ requires C, 60.20; H, 5.13); m.p. 197-198 °C (Lit.,[161] 183-184.5°C); ν_{max} (nujol)/cm^{-1} 3420 (OH), 1730 and 1625 (C=O); $[\alpha]_D$ +17.9 ° (*c* 1.0 in pyridine); δ_H (200 MHz, d^6-DMSO) 8.40 (1H, s, H-2), 8.11 (1H, d, $J_{5,6}$ =10.9, H-5), 7.55 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-2' and 6'), 7.27 (1H, d, $J_{6,8}$ = 2.3, H-8), 7.14 (1H, dd, $J_{5,6}$ = 10.9 and $J_{6,8}$ = 2.28, H-6), 7.03 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-3' and 5'), 5.86 (1H, d, $J_{1'',2''}$ = 8.0, H-1"), 5.44 (1H, dd, $J_{1'',2''}$ = 8.0 and $J_{2'',3''}$ = 9.7, H-2"), 5.20-5.02 (2H, m, H-3" and 4"), 4.41-4.09 (3H, m, H-5" and C<u>H</u>$_2$-6), 3.80 (3H, s, OC<u>H</u>$_3$), 2.13-1.95 (12H, 4 x s, C<u>H</u>$_3$); δ_C (74.76 MHz, $CDCl_3$), 175.5 (C-4), 170.6, 170.3, 169.5, 169.4 (4 x <u>C</u>=O), 160 7 (C-4'), 157 5 (C-8a), 153.0 (C-2), 150.8 (C-7), 130.1 (C-2' and 6'), 129.5 (C-1'), 124.8 (C-3), 120.3 (C-4a), 115.5 (C-6), 114.2 (C-3' and 5'), 104.4 (C-8), 98.4 (C-1"), 72.5 (C-5"), 72.4 (C-3"), 71.0 (C-2"), 68.1 (C-4"), 61.9 (C-6"), 20.5 (4 x <u>C</u>H$_3$); *m/z* (EI) 598 (M^+, 53%), 296 ($[C_{17}H_{12}O_5]^+$, 10), 268 ($[C_{16}H_{12}O_4]^+$, 31).

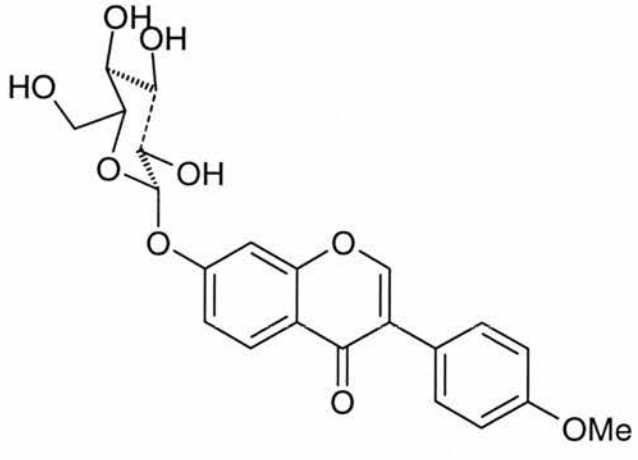
<u>Attempted synthesis using α-acetobromoglucose and a silver trifluoromethanesulfonate catalyst</u>

Silver trifluoromethanesulfonate (555 mg, 2.16 mmol) was added to a stirred solution of fomononetin (270 mg, 1 mmol) in pyridine (15 cm^3). Stirring was continued for 15 minutes at room temperature and α-acetobromoglucose (440 mg, 1 mmol) was added to the reaction mixture. Stirring was further continued for 18 hours and the solution was then diluted with dichloromethane (30 cm^3) and with sulphuric acid (30 cm^3 of a 1.0 mol dm^{-3} solution). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a pale solid. Examination by t.l.c. (toluene : ethyl acetate, 6 : 1) indicated only starting material to be present.

Attempted synthesis using tetrabutylammonium bromide

α-Acetobromoglucose (400 mg, 1 mmol) was added to a stirred solution of phenol (101 mg, 1.08 mmol) and tetrabutylammonium bromide (377 mg, 2.5 mmol) in ethyl acetate (4 cm^3) and sodium hydroxide (4 cm^3 of a 1.0 mol dm^{-3} solution). Formononetin (261mg, 0.97 mmol) was added and stirring was continued at room temperature for 18 hours. The reaction was monitored by t.l.c. (hexane : ethyl acetate, 3 : 2) showing only starting materials were present.

Synthesis of 4'-methoxy-7-(2,3,4,6-tetra-*O*-hydroxy-β-D-glucopyranosyl)-isoflavone (Ononin, **46**)[82]



Synthesis using 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl-tricloroacetimidate.

Boron trifluoride etherate (3 cm^3) was added to a stirred suspension of formononetin (0.97 g, 3.55 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl-trichloroacetimidate (1.78 g, 3.55 mmol) in dichloromethane (25 cm^3) at –15 °C. Stiring was continued at

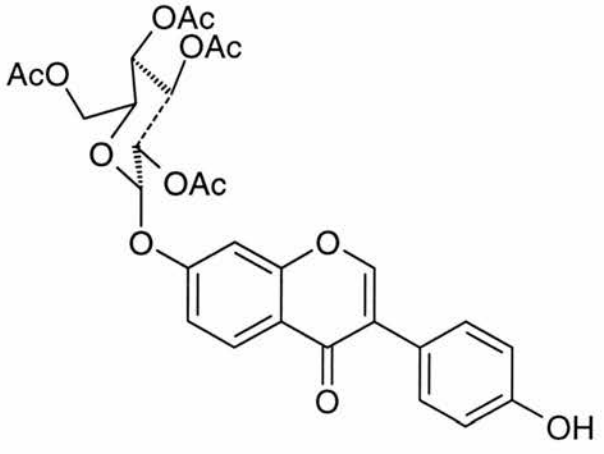
room temperature for 18 hours, the reaction mixture turning to a light green solution. Examination by t.l.c. (silica; ethyl acetate : hexane, 1 : 1) at this stage revealed a new product had been formed and sodium hydrogen carbonate (50 cm^3 of a 1.0 mol dm^{-3} solution) and ethyl acetate (50 cm^3) were added slowly. The organic layer was extracted, dried ($MgSO_4$) and concentrated at reduced pressure to leave an oily solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) yielded a mixture of the desired product and formononetin (946 mg) as a white solid. The mixture was dissolved in methanol (50 cm^3) and a catalytic amount of sodium was added. The reaction mixture was then heated to reflux for 2 hours under a nitrogen atmosphere. After this time the reaction was shown by t.l.c. to be complete (dichloromethane : methanol, 3 : 2) and DOWEX 50WXB-100 ion-exchange resin (30 mg) was added. The solution was concentrated at reduced pressure and the residue was recrystallized from ethanol to give the desired product (0.73 g, 47% from formononetin) as a white solid (Found: C, 61.39; H, 5.15. $C_{22}H_{22}O_9$ requires C, 61.17; H, 5.16%); m.p. 214-218 °C (Lit.,[89] 213-214 °C); ν_{max} (nujol)/cm^{-1} 3420 (OH), 1625 (C=O); $[\alpha]_D$ –14.4 ° (*c* 1.0 in pyridine) (Lit.,[82] -24.2 ° (*c* 1.0 in pyridine)); δ_H (200 MHz, d^6-DMSO) 8.46 (1H, s, H-2), 8.08 (1H, d, $J_{5,6}$ = 8.9, H-5), 7.52 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-2' and 6'), 7.26 (1H, d, $J_{6,8}$ = 2.6, H-8), 7.16 (1H, dd, $J_{6,8}$ = 2.6 and $J_{5,6}$ = 8.9, H-6), 7.01 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-3' and 5'), 5.18 (1H, d, $J_{1'',2}$ = 5.8, H-1''), 3.82 (3H, s, $OC\underline{H}_3$), 3.80-3.10 (6H, m, H-2'', 3'', 4'', 5'' and $C\underline{H}_2$-6); δ_C (50.31 MHz, d^6-DMSO) 175.0 (C-4), 161.7 (C-4'), 158.2 (C-8a), 157.3 (C-7), 153.9 (C-2), 130.3 (C-2' and 6'), 127.4 (C-5), 124.2 (C-1'), 123.8 (C-3), 118.7 (C-4a), 115.0 (C-6), 113.0 (C-3' and 5'), 103.1 (C-8), 100.3 (C-1''), 77.5 (C-5''), 76.7 (C-3''), 73.3 (C-2''), 69.8 (C-4''), 60.9 (C-6''), 55.4 ($O\underline{C}H_3$); *m/z* (ES) 453 ($[M + Na]^+$, 100%), 291 ($[C_{16}H_{12}O_4Na]^+$, 23).

Synthesis from 4'-Methoxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone

A catalytic amount of sodium was added to a stirred suspension of 4'-methoxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (70 mg, 0,12 mol) in methanol (20 cm^3). The reaction mixture was then heated to reflux for 2 hours under a nitrogen

atmosphere. After this time the reaction was shown by t.l.c. to be complete (dichloromethane : methanol, 3 : 2) and DOWEX 50WXB-100 ion-exchange resin (30 mg) was added. The solution was concentrated at reduced pressure and the residue was recrystallized from ethanol to give the desired product (0.18 g, 35%) as a white solid.

4'-Hydroxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (**51**)



Synthesis using tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate

Boron trifluoride etherate (0.8 cm^3, 6.5 mmol) was added to a stirred suspension of daidzein (400 mg, 1.57 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (760 mg, 1.59 mmol) in dichloromethane (15 cm^3) at –15 °C. The reaction was stirred for 4 hours at room temperature. The temperature was reduced back to –15 °C and further 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (200 mg, 0.42 mmol) was added. This was followed by the addition of boron trifluoride etherate (0.40 cm^3, 3.25 mmol) and stirring was continued for 2 hours. At this stage, t.l.c. (silica; ethyl acetate : hexane; 1 : 1) revealed no starting material remained and ethyl acetate (30 cm^3) was added and washed with sodium hydrogen carbonate (25 cm^3 of a saturated solution) and sodium acetate (20 cm^3 of a saturated solution). The organic layer

was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an off-white solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1), yielding the desired product (392 mg, 43%) as a white solid (Found: C, 58.80, H, 4.85. $C^{29}H^{28}O^{13}$ requires C, 59.59, H, 4.83), m.p 224-227 °C; ν_{max} (nujol)/cm^{-1} 3410 (OH), 1730 and 1630 (C=O); $[\alpha]_D$ +26.9 ° (*c* 1.0 in pyridine); δ_H (200 MHz, d^6-DMSO) 8.39 (1H, s, H-2), 7.99 (1H, d, $J_{5,6}$ = 8.8, H-5), 7.55 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-2' and 6'), 7.07 (1H, d, $J_{6,8}$ = 2.2, H-8), 6.97 (1H, dd, $J_{6,8}$ = 2.2 and $J_{5,6}$ = 8.8, H-6), 6.89 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-3' and 5'), 5.68 (1H, d, $J_{1,2}$ = 3.4, H-1"), 4.60-5.50 (3H, m, H-2", 3" and 4"), 3.90-4.40 (2H, m, $C\underline{H}_2$-6), 2.04 (12H, s, 4 x $COC\underline{H}_3$); δ_C (74.76 MHz, $CDCl_3$) 175.5 (C-4), 170.7, 170.3, 169.5, 169.4 (4 x $\underline{C}$=O), 160.7 (C-4'), 157.5 (C-8a), 153.0 (C-7), 150.8 (C-2), 130.1 (C-2' and 6'), 129.5 (C-1'), 124.8 (C-5), 120.3 (C-3), 115.5 (C-3' and 5'), 115.1 (C-6), 104.4 (C-8), 98.4 (C-1"), 72,5 (C-5"), 72.4 (C-3"), 71.0 (C-2"), 68.1 (C-4"), 61.9 (C-6"), 20.5 (4 x $\underline{C}H_3$); *m/z* (ES) 645 ($[M + K + Na]^+$, 11%), 623 ($[M + K]^+$, 90), 607 ($[M + Na]^+$, 100), 585 ($[M + H]^+$, 25), 254 ($[C_{15}H_{10}O_4]^+$, 12).

Attempted synthesis using boron tribromide in dichloromethane[90]

Boron tribromide (0.21 cm^3, 2.2 mmol) was added dropwise to a suspension of 4'-methoxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (200 mg, 0.334 mmol) in dichloromethane (15 cm^3) under a nitrogen atmosphere. The suspension turned yellow and stirring was continued for 48 hours. On addition of ice-cold water (60 cm^3) a yellow precipitate was formed and was collected by filtration to yield a yellow solid. Analysis by t.l.c. (ethyl acetate : hexane, 2 : 1) and the ^1H n.m.r. spectrum showed the product to be a mixture of formononetin and daidzein.

Attempted synthesis using lithium chloride in dimethylformamide[92]

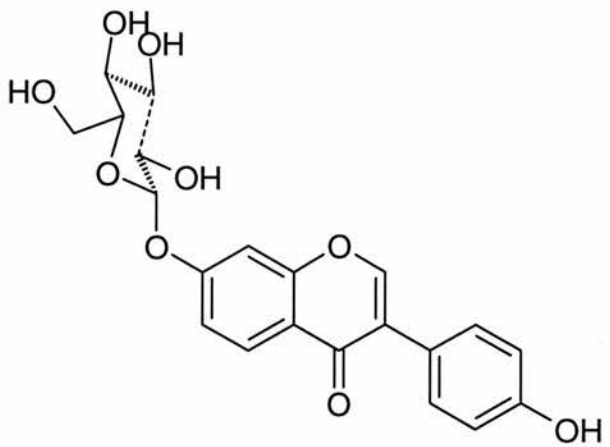
Lithium chloride (15 mg, 0.35 mmol) was added to a stirred solution of 4'-methoxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (50 mg, 0.12 mmol) in

dimethylformamide (5 cm^3). Stirring was continued to reflux for 18 hours at which point t.l.c. (ethyl acetate : hexane, 3 : 2) showed that a reaction had taken place. Sodium hydroxide (20 cm^3 of a 1.0 mol dm^{-3} solution), HCl (25 cm^3 of a 1.0 mol dm^{-3} solution) and diethyl ether (40 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a dark solid. ^1H n.m.r. spectroscopy revealed formononetin to be the single reaction product.

Attempted synthesis using sodium methanethiolate in dimethylformamide[91]

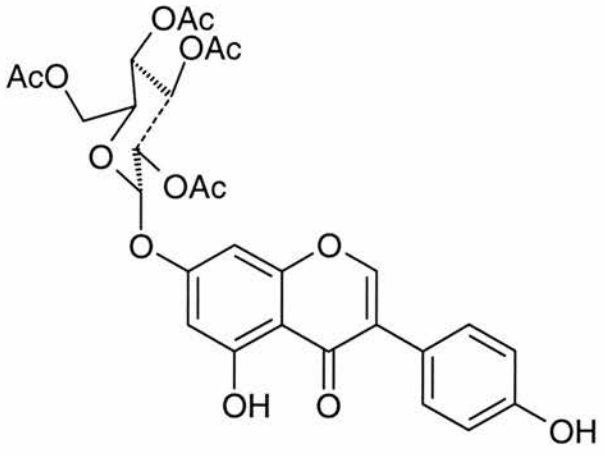
Sodium methanethiolate (42 mg, 0.6 mmol) was added to a stirred solution of 4'-methoxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (50 mg, 0.12 mmol) in dimethylformamide (5 cm^3) and the reaction mixture heated to reflux for 5 hours. At this stage the t.l.c. (ethyl acetate : hexane, 3 : 2) showed no remaining starting material and the reaction was cooled to room temperature. Acetic acid (1 cm^3), HCl (25 cm^3 of a 1.0 mol dm^{-3} solution) and diethyl ether (25 cm^3) were then added sequentially and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. This yielded a dark solid and was shown by ^1H n.m.r. to be a mixture of formononetin and daidzein.

Attempted synthesis of 4'-hydroxy-7-(2,3,4,6-tetra-*O*-hydroxy-β-D-glucopyranosyl)-isoflavone (daidzin)[82]



A catalytic amount of sodium was added to a stirred suspension of 4'-hydroxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (100 mg, 0.17 mmol) in methanol (20 cm^3). The reaction mixture was then heated to reflux for 2 hours under a nitrogen atmosphere. After this time the reaction was shown by t.l.c. to be complete (dichloromethane : methanol, 3 : 2) and DOWEX 50WXB-100 ion-exchange resin (30 mg) was added. The solution was concentrated at reduced pressure and the residue was recrystallized from ethanol to give the product as a pale yellow solid, insoluble in d^6-DMSO.

5,4'-Dihydroxy-7-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-isoflavone (**38**)



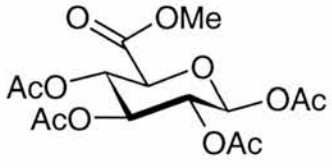
Attempted synthesis using tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate

Boron trifluoride etherate (0.4 cm^3, 3.75 mmol) was added to a stirred suspension of genistein (200 mg, 0.74 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (355 mg, 0.75 mmol) in dichloromethane (10 cm^3) at –15 °C. The reaction was stirred for 4 hours at room temperature. The temperature was reduced back to –15 °C and further 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (200 mg, 0.42 mmol) was added. This was followed by the addition of boron trifluoride etherate (0.40 cm^3, 3.25 mmol) and stirring was continued for 2 hours. At this stage, t.l.c. (silica; ethyl acetate : hexane; 1 : 1) revealed only a small amount of starting material remained and ethyl acetate (30 cm^3) was added. The reaction mixture was then quenched by the addition of sodium hydrogen carbonate (25 cm^3 of a saturated solution) and sodium acetate (20 cm^3 of a saturated solution). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an off-white solid. ^1H n.m.r. spectroscopy of the crude product and analysis by t.l.c (silica; ethyl acetate : hexane, 1 : 1) showed that only starting material was present and product decomposition had taken place at some stage during the reaction work up.

Attempted synthesis using α-acetobromoglucose and a silver oxide catalyst[87]

Silver oxide (370 mg, 1.60 mmol) was added to a stirred solution of genistein (200 mg, 0.74 mmol) in pyridine (10 cm^3). Stirring was continued for 15 minutes at room temperature and α-acetobromoglucose (325 mg, 0.74 mmol) was added to the reaction mixture. Stirring was continued for a further 3 hours and the solution was then diluted with dichloromethane (20 cm^3) and with sulphuric acid (20 cm^3 of a 1.0 mol dm^{-3} solution). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a pale solid. ^1H n.m.r. spectroscopy and analysis by t.l.c. (ethyl acetate : hexanes, 1 : 1) showed that only the starting material was present.

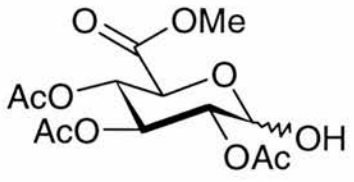
Synthesis of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**61**)[94]



Sodium hydroxide (0.3 g, 7.5 mmol), was dissolved in methanol (800 cm^3) and D-glucurono-6,3-lactone (107 g, 0.6075 moles) was added (considerable swirling was required to form the solution). Further sodium hydroxide was added (0.1 g) to achieve pH 8-9 and the reaction mixture was stirred at room temperature for 1 hour. The reaction mixture was then concentrated at reduced pressure to leave a viscous oil. Acetic anhydride (250 cm^3) was added to form a solution (dissolution required mild heat and constant swirling over 2-3 hours) and this was followed by the addition of acetic anhydride (350 ml) in pyridine (270 ml). The addition was performed at a rate that allowed the reaction temperature to be kept below 40 °C and on completion the reaction vessel was cooled to 4 °C for 12 hours. Concentration at reduced pressure yielded the crude product which was washed with diethyl ether (300 cm^3) to leave the desired product (98.0 g, 43%) as a light brown solid m.p. 174-177 °C (Lit.,[99] 178 °C); ν_{max}

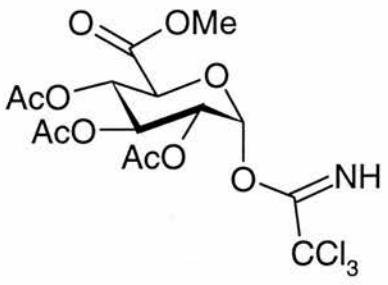
(nujol)/cm^{-1} 1730 (C=O); $[\alpha]_D$ +9.5 ° (*c* 1.0 in $CHCl_3$), (Lit.,[99] +8.7 ° (*c* 1.0 in $CHCl_3$)); δ_H (200 MHz, $CDCl_3$) 5.76 (1H, d, $J_{1,2}$ = 7.7, H-1), 5.10-5.37 (3H, m, H-2, 3 and 4), 4.19 (1H, d, $J_{4,5}$ = 9.4, H-5), 3.74 (3H, s, $OC\underline{H}_3$), 2.03 (12H, s, $C\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$), 169.9 ($\underline{C}$=O), 91.8 (C-3), 73.4 (C-1), 72.3 (C-2), 70.6 (C-4), 69.4 (C-5), 53.8 ($O\underline{C}H_3$), 21.0-21.3 (3 x $\underline{C}H_3$); *m/z* (CI) 317 ([MH - $C_2H_3O_2$]$^+$, 100%), 257 ([MH - $C_4H_6O_4$]$^+$, 77), 215 ([MH - $C_6H_8O_5$]$^+$, 20), 199 ([MH - $C_6H_8O_6$]$^+$, 22), 155 ([MH- $C_8H_{10}O_7$]$^+$, 43).

Synthesis of methyl 2,3,4-tri-*O*-acetyl-α/β-D-glucopyranuronate (**62**)[100]



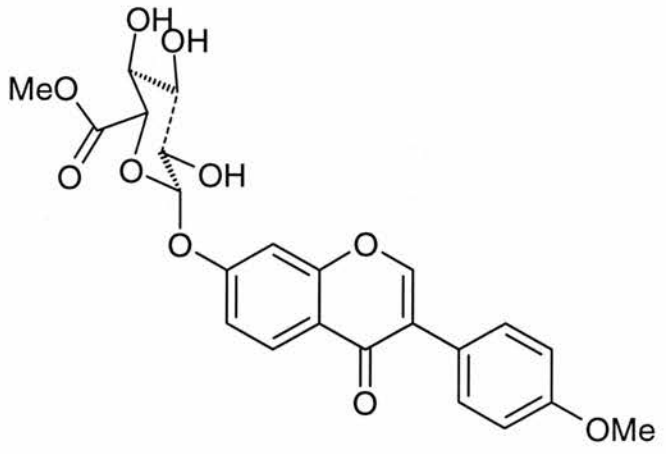
Trimethyltin methoxide (17.02 g, 53 mmol) was added to a stirred solution of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (20.0 g, 53.3 mmol) in tetrahydrofuran (90 cm^3) and the solution heated under reflux for 3 hours. The reaction was cooled to room temperature and concentrated at reduced pressure to leave a white solid. This was triturated twice, using hexane, at -78 °C to leave the desired product (10.07 g, 57%) as a pure white solid m.p. 101-103 °C (Lit.,[101] 116 °C); ν_{max} (nujol)/cm^{-1} 3300 (OH), 1730 (C=O); $[\alpha]_D$ +76 ° (*c* 1.0 in $CHCl_3$), (Lit.,[101] +81 ° (*c* 1.0 in $CHCl_3$); δ_H (200 MHz, $CDCl_3$) 5.55-4.84 (4H, m, H-2, 3, 4 and 5), 4.56 (1H, d, $J_{1\alpha,2}$ = 3.6, H-1α), 4.09 (1H, d, $J_{1\beta,2}$ = 9.9, H-1β), 3.71 (3H, s, $OC\underline{H}_3$), 2.05-1.83 (9H, s, $C\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 170.7, 170.6, 170.3, 169.1 ($\underline{C}$=O), 96.0 (C-1β), 90.6 (C-1α), 73.2 (C-3β), 72.1 (C-3α), 71.3 (C-5β), 70.0 (C-5α), 69 9 (C-2β), 69.6 (C-2α), 68.4 (C-4β), 67.9 (C-4α), 53.5 and 53.6 ($O\underline{C}H_3$), 21.3-21.0 (6 x $\underline{C}H_3$).

Synthesis of methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetylimidoyl)-α-D-glucopyranuronate (**58**)[98]



Fresh, glowing potassium carbonate (7.0 g) was added to a stirred solution of methyl 2,3,4-tri-*O*-acetyl-α/β-D-glucopyranuronate (10.0 g, 0.029 moles) and trichloroacetonitrile (9.0 cm^3) in dichloromethane (60 cm^3). Stirring was continued at room temperature for 18 hours after which time water (100 cm^3) was added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure. The residue was purified using column chromatography (silica; ethyl acetate : hexane, 1 : 1) yielding the desired product (6.92 g, 49%) as a white solid m.p. 104-106 °C (Lit.,[102] 108 °C); ν_{max} (nujol)/cm^{-1} 3320 (OH), 1730 (C=O), 1650 (C=N); $[\alpha]_D$ +85 ° (*c* 1.9 in $CHCl_3$), (Lit.,[102] +91.6° (*c* 1.9 in $CHCl_3$)); δ_H (200 MHz, $CDCl_3$) 8.73 (1H, s, NH), 6.61 (1H, d, $J_{1,2}$ = 3.5, H-1), 5.60 (1H, dd $J_{3,4}$ = 9.3 and $J_{4,5}$ = 9.8, H-4), 5.24 (1H, dd, $J_{2,3}$ = 9.8 and $J_{3,4}$ = 9.3, H-3), 5.12 (1H, dd, $J_{2,3}$ = 9.8 and $J_{1,2}$ = 3.5, H-2), 4.47 (1H, d, $J_{4,5}$ = 9.8, H-5), 3.73 (3H, s, OCH_3), 2.13-1.99 (9H, s, CH_3); δ_C (50.31 MHz, $CDCl_3$) 170.2, 170.2, 169.9, 167.6 (C=O), 93.1 (C-1β), 93.0 (C-1α), 70.9 (C-3), 70.9 (C-5), 69.9 (C-2), 69.5 (C-4), 53.5 (OCH_3), 21.1, 21.0, 20.9 (CH_3); *m/z* (CI) 478 ($[MH]^+$, 41%), 317 ($[MH - C_2HNOCl_3]^+$, 23), 257 ($[MH - C_2HNOCl_3 - C_2H_3O]^+$, 52), 217 ($[MH - C_2HNOCl_3 – C_4H_6O_3]^+$, 12), 197 ($[MH - C_2HNOCl_3 - C_4H_6O_4]^+$, 24), 155 ($[MH - C_2HNOCl_3 – C_6H_9O_4]^+$, 100).

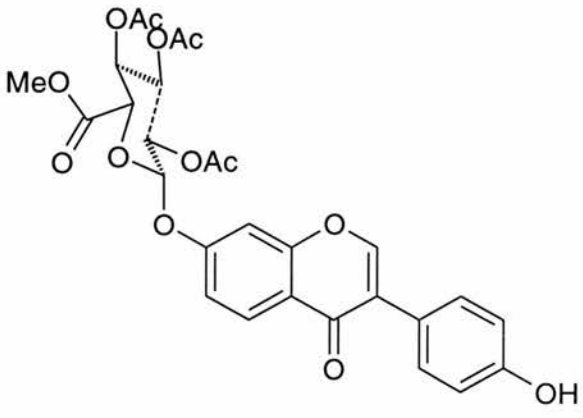
Synthesis of 4'-methoxy-7-(methyl-2,3,4-tri-*O*-hydroxy-β-D-glucopyranuronate)-isoflavone (**63**)



Boron trifluoride etherate (0.25 cm^3, 2.03 mmol) was added to a stirred suspension of formononetin (0.3 g, 1.12 mmol) and methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetylimidoyl)-α-D-glucopyranuronate (540 g, 1.13 mmol) in dichloromethane (20 cm^3) at –15 °C. The suspension turned to a light green solution and was stirred for a further 18 hours. Analysis by t.l.c. (silica; ethyl acetate : hexane, 1 : 1) at this stage revealed little formononetin remaining and sodium hydrogen carbonate (50 cm^3 of a 1.0 mol dm^{-3} solution) and ethyl acetate (50 cm^3) were added slowly. The organic phase was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an oily solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) yielded the impure product as an off-white solid (0.57 g). Methanol (30 cm^3) and sodium (catalytic amount) were added and the solution stirred to 65 °C for 2 hours. The reaction mixture was cooled to room temperature and concentrated at reduced pressure. Purification by column chromatography (C-18 reverse phase silica; methanol : water, 1 : 1) eluted the desired product (220 mg, 46% over 2 steps) as a white solid m.p. 166-168 °C; ν_{max} (nujol)/cm^{-1} 3420 (OH), 1730 and 1625 (C=O); $[\alpha]_D$ +37.6 ° (*c* 1.0 in $CHCl_3$); δ_H (300 MHz, CD_3OD) 8.42 (1H, s, H-2), 8.32 (1H, d, $J_{5,6}$ = 8.8, H-5), 7.67 (2H, d, $J_{2',3'} = J_{5',6'}$ = 7.0, H-2' and 6'), 7.46 (1H, d, $J_{6,8}$ = 2.1, H-8), 7.41 (1H, dd, $J_{6,8}$ = 2.1 and $J_{5,6}$ = 8.8, H-6), 7.18 (2H, d, $J_{2',3'} = J_{5',6'}$ = 7.0, H-3' and 5'), 4.02 (3H, s, C<u>H</u>$_3$), 4.16-3.61 (5H, m, H-1", 2", 3", 4", 5"), 3.95 (3H, s, OC<u>H</u>$_3$); δ_C (75.4 MHz, CD_3OD) 180.4 (C-4), 178.1 (C-6"),

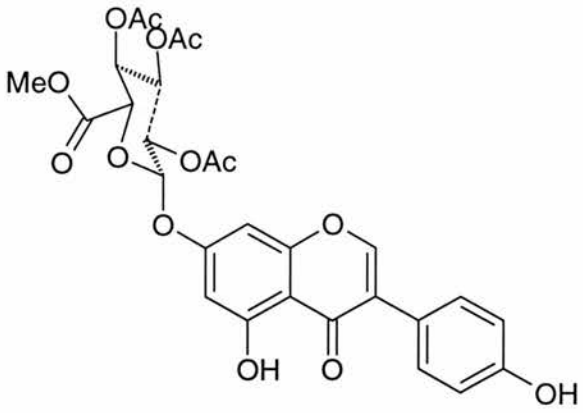
163.7 (C-4'), 161.4 (C-8a), 159.4 (C-7), 155.5 (C-2), 131.6 (C-2' and 6'), 128.5 (C-5), 125.6 (C-3), 120.5 (C-4a), 117.5 (C-6), 115.2 (C-3' and 5'), 105.3 (C-8), 83.7 (C-1''), 78.9 (C-3''), 78.4 (C-2''), 74.7 (C-4''), 73.6 (C-5''), 56.1 ($O\underline{C}H_3$), 53.1 ($\underline{C}H_3$).

Attempted synthesis of 4'-hydroxy-7-(methyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate)-isoflavone (**64**)



Boron trifluoride etherate (0.4 cm^3, 3.25 mmol) was added to a stirred suspension of daidzein (200 mg, 0.79 mmol) and methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetylimidoyl)-α-D-glucopyranuronate (380 g, 0.79 mmol) in dichloromethane (20 cm^3) at –15 °C. The suspension turned to a green solution and was stirred for a further 24 hours with monitoring by t.l.c. (ethyl acetate : hexanes, 1 : 1) every 2-3 hours. This suggested that only negligible amounts of a new product had formed during the reaction period. Sodium hydrogen carbonate (50 cm^3 of a 1.0 mol dm^{-3} solution) and ethyl acetate (50 cm^3) were added slowly. The organic phase was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an oily solid. ^1H n.m.r. spectroscopy and analysis by t.l.c. of the crude product showed that only negligible amounts of the desired product to be present.

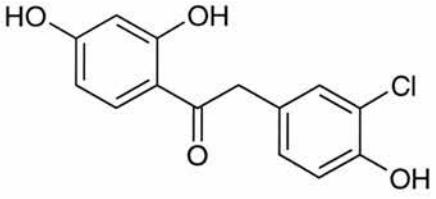
Attempted synthesis of 5,4'-dihydroxy-7-(methyl-2,3,4-tri-*O*-acetyl -β-D-glucopyranuronate)-isoflavone (**65**)



Boron trifluoride etherate (0.8 cm^3, 6.50 mmol) was added to a stirred suspension of genistein (200 mg, 0.74 mmol) and methyl 2,3,4-tri-*O*-acetyl-1-*O*-(trichloroacetylimidoyl)-α-D-glucopyranuronate (354 g, 0.74 mmol) in dichloromethane (20 cm^3) at –15 °C. The suspension turned to a green solution and was stirred for a further 24 hours with analysis by t.l.c. (ethyl acetate : hexane, 1 : 1) every 2-3 hours. This suggested that from 2-3 hours onwards a reasonable amount of a new product had been formed. Sodium hydrogen carbonate (50 cm^3 of a 1.0 mol dm^{-3} solution) and ethyl acetate (50 cm^3) were added slowly. The organic phase was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a pale yellow solid. ^1H n.m.r. spectroscopy and analysis by t.l.c. showed that only negligible amounts of the reaction product had survived the reaction work-up procedure. Modifications to this procedure were the addition of sodium hydrogen carbonate solution at –78 °C over a 5-10 minute period and alternatively, the concentration of the reaction mixture at reduced pressure prior to the reaction work-up. A dark coloured residue was then isolated and purified by column chromatography (silica; ethyl acetate : hexanes, 1 : 1). In this instance the only isolated product was crude genistein (340 mg).

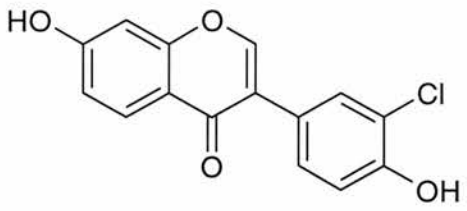
6.3: Chlorinated isoflavones

Synthesis of 3'-chloro-4'-hydroxybenzyl-2,6-dihydroxyphenyl ketone (**71**)[55]



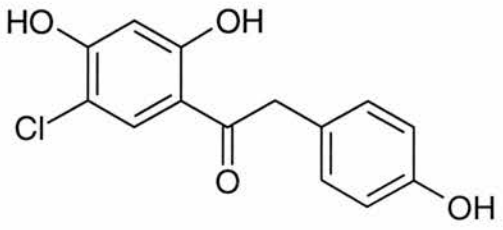
Boron trifluoride etherate (3.7 cm^3, 30 mmol) was added to a mixture of 3-chloro-4-hydroxyphenylacetic acid (2.0 g, 10.72 mmol) and resorcinol (2.36 g, 21.44 mmol) under a nitrogen atmosphere. The mixture was stirred to reflux for 15 minutes, cooled to room temperature and sodium acetate (30 cm^3 of a saturated solution) and sodium hydrogen carbonate (15 cm^3 of a saturated solution) were added sequentially. Diethyl ether (50 cm^3) was added and the organic layer was separated, dried ($MgSO_4$) and concentrated to leave a dark red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) gave a pale red solid which was washed in dichloromethane (25 cm^3) to leave the desired product (2.2 g, 63%) as a pure white powder (Found: C, 60.60; H, 4.07%; M^+, 278.033959. $C_{14}H_{11}ClO_4$ requires C, 60.34; H, 3.98; $C_{14}H_{11}{}^{35}ClO_4$ requires *M*, 278.034587); m.p. 192-195 °C; ν_{max} (nujol)/cm^{-1} 3600 (OH), 1680 (C=O); δ_H (200 MHz, CD_3OD) 7.82 (1H, d, $J_{5,6}$ = 8.9, H-6), 7.21 (1H, d, $J_{2',6'}$ = 2.1, H-2'), 7.02 (1H, dd, $J_{2',6'}$ = 2.1 and $J_{5',6'}$ = 8.3, H-6'), 6.84 (1H, d, $J_{5',6'}$ = 8.3, H-5'), 6.37 (1H, dd, $J_{5,6}$ = 8.9 and $J_{3,5}$ = 2.3, H-5), 6.26 (1H, d, $J_{3,5}$ = 2.3, H-3), 4.10 (2H, s, C$\underline{H}_2$); δ_C (50.31 MHz, CD_3OD) 203. 9 ($\underline{C}$=O), 167.1 (C-4), 166.9 (C-2), 153.5 (C-4'), 134.6 (C-6), 132.1 (C-2'), 130.3 (C-6'), 128.8 (C-1'), 121.8 (C-3'), 117.9 (C-5'), 114.0 (C-1'), 109.6 (C-5), 104.0 (C-3), 44.4 ($\underline{C}H_2$); *m/z* (EI) 280, 278 (M^+, 2, 8%), 137 ($[C_7H_5O_3]^+$, 100), 81 (8).

Synthesis of 3'-chloro-4',7-dihydroxyisoflavone (**66**)[64]



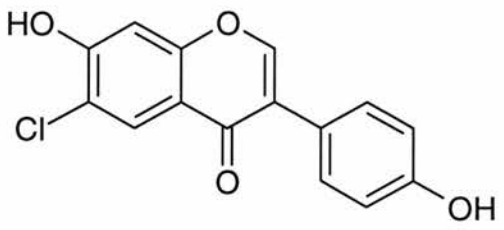
N,N-Dimethylformamide dimethylacetal (1.26 cm^3, 0.93 mmol) was added dropwise to a stirred solution of 3'-chloro-4'-hydroxybenzyl-2,6-dihydroxyphenyl ketone (1.5 g, 8.4 mmol) in tetrahydrofuran (10 cm^3). Stirring was continued to reflux for 5 hours under a nitrogen atmosphere. Methanol (10 cm^3) was added and the reaction mixture concentrated at reduced pressure to leave a dark red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) eluted the product as an off-white solid. Washing in methanol (10 cm^3 of a 40% aqueous solution) yielded the desired product (600 mg, 25%) as a pure white powder (Found: C, 62.00; H, 3.31%. M^+, 288.018266. $C_{15}H_9ClO_4$ requires C, 62.41; H, 3.14; $C_{15}H_9{}^{35}ClO_4$ requires *M*, 288.018937); m.p 267-268 °C; ν_{max} (nujol)/cm^{-1} 3600 (OH), 1680 (C=O); δ_H (300 MHz, CD_3OD) 8.15 (1H, s, H-2), 8.04 (1H, d, $J_{5,6}$ = 8.8, H-5), 7.53 (1H, d, $J_{2',6'}$ = 2.2, H-2'), 7.29 (1H, dd, $J_{5',6'}$ = 8.5 and $J_{2',6'}$ = 2.2, H-6'), 6.95 (1H, d, $J_{5',6'}$ = 8.5, H-5), 6.93 (1H, dd, $J_{6,8}$ = 2.2 and $J_{5,6}$ = 8.8, H-6), 6.83 (1H, d, $J_{6,8}$ = 2.2, H-8); δ_C (75.4 MHz, CD_3OD) 176.6 (C-4), 163.5 (C-8a), 158.5 (C-7), 153.8 (C-2), 153.1 (C-4'), 130.4 (C-5), 128.4 (C-2'), 127.2 (C-6'), 124.3 (C-1'), 123.4 (C-3), 120.3 (C-3'), 116.8 (C-4a), 116.1 (C-5'), 115.2 (C-6), 102.0 (C-8); *m/z* (CI) 289, 291 ($[MH]^+$, 100, 45%), 255 ($[MH - Cl]^+$, 52).

Synthesis of 4'-hydroxybenzyl-2,4-dihydroxy -5-chlorophenyl ketone (**72**)[55]



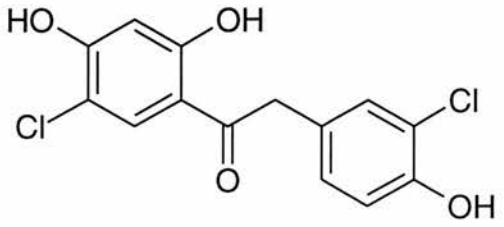
Boron trifluoride etherate (6.5 cm^3, 52.8 mmol) was added to a mixture of 4-hydroxyphenylacetic acid (4.4 g, 28.9 mmol) and 4-chlororesorcinol (7.62 g, 52.7 mmol) under a nitrogen atmosphere. The mixture was stirred for 15 minutes to reflux, cooled to room temperature and sodium acetate (30 cm^3 of a saturated solution) and sodium hydrogen carbonate (15 cm^3 of a saturated solution) were added. Diethyl ether (50 cm^3) was added and the organic layer was extracted, dried ($MgSO_4$) and concentrated to leave a dark red solid. Purification by column chromatography (silica; ethyl acetate : toluene, 2 : 3) gave a pale red solid which was washed in dichloromethane (25 cm^3) to leave the desired product (6.52 g, 77%) as an off-white powder (Found: C, 60.12; H, 3.68%; M^+, 279.041699. $C_{14}H_{12}ClO_4$ requires C, 60.34; H, 3.98; $C_{14}H_{12}{}^{35}ClO_4$ requires *M*, 279.042412); m.p. 188-191 °C; ν_{max} (nujol)/cm^{-1} 3600 (OH), 1680 (C=O); δ_H (200 MHz, CD_3OD) 7.93 (1H, s, H-6), 7.10 (2H, dd, $J_{2',3'} = J_{5',6'} = 8.2$ and $J_{2',6'} = J_{3',5'} = 1.7$, H-2' and 6'), 6.75 (2H, dd, $J_{2',3'} = J_{5',6'} = 8.2$ and $J_{2',6'} = J_{3',5'} = 1.7$, H-3' and 5'), 6.40 (1H, s, H-3), 4.12 (2H, s, $C\underline{H}_2$); δ_C (50.31 MHz, CD_3OD) 204.1 ($\underline{C}$=O), 165.1 (C-4), 161.7 (C-4'), 157.6 (C-2), 133.6 (C-6), 131.9 (C-2' and 6'), 127.0 (C-1), 116.8 (C-3' and 5'), 114.4 (C-1), 113.6 (C-5), 105.2 (C-3), 45.1 ($\underline{C}H_2$); *m/z* (CI) 279, 281 ($[MH]^+$, 75, 23%), 245 ($[MH - Cl]^+$, 46).

Synthesis of 6-chloro-4',7-dihydroxyisoflavone (**67**)[64]



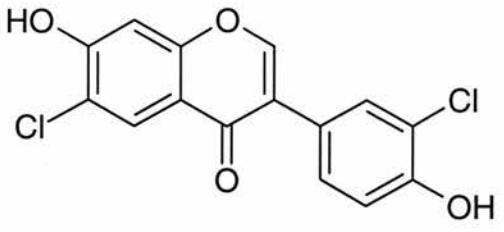
N,N-Dimethylformamide dimethylacetal (0.44 cm^3, 3.7 mmol) was added dropwise to a stirred solution of 4'-hydroxybenzyl-2,4-dihydroxy-5-chlorophenyl ketone (0.3 g, 1.1 mmol) in tetrahydrofuran (5 cm^3). Stirring was continued to reflux for 5 hours under a nitrogen atmosphere. Methanol (10 cm^3) was added and the solvents removed at reduced pressure to leave a dark red solid. Dichloromethane (20 cm^3) was added and the solution stirred at 0 °C under a nitrogen atmosphere. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) gave an off-white solid. This was purified further by column chromatography using reverse phase C-18 silica (acetonitrile : water, 1 : 1) which gave the product as a white powder. Washing in methanol (10 cm^3 of a 40% aqueous solution) yielded the desired product (90 mg, 29%) as a pure white powder (Found: M^+, 289.026054. $C_{15}H_9{}^{35}ClO_4$ requires *M*, 289.026762); m.p. 318-320 °C; ν_{max} (nujol)/cm^{-1} 3600 (OH), 1680 (C=O); δ_H (200 MHz, d^6-DMSO) 8.26 (1H, s, H-2), 8.00 (1H, s, H-5), 7.45 (2H, d, $J_{2',3'} = J_{5',6'} = 8.0$, H-2' and 6'), 7.14 (1H, s, H-8), 6.87 (2H, d, $J_{2',3'} = J_{5',6'} = 8.0$, H-3' and 5'); δ_C (50.31 MHz, d^6-DMSO) 174.2 (C-4), 158.3 (C-7), 157.6 (C-8a), 155.9 (C-4'), 153.4 (C-2), 130.3 (C-2' and 6'), 126.4 (C-5), 123.7 (C-3), 122.4 (C-1'), 120.5 (C-4a), 117.3 (C-6), 115.3 (C-3' and 5'), 103.9 (C-8); *m/z* (CI) 289, 291 ($[MH]^+$, 22, 10), 255 ($[MH - Cl]^+$, 16), 223 ($[MH - Cl - 2(OH)]^+$, 8).

Synthesis of 3'-chloro-4'-hydroxybenzyl-2,4-dihydroxy-5-chlorophenyl ketone (**73**)[55]



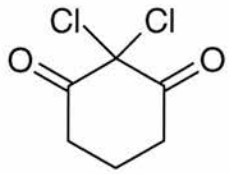
Boron trifluoride etherate (6.5 cm^3, 52.8 mmol) was added to a mixture of 3-chloro-4-hydroxyphenylacetic acid (5.39 g, 28.9 mmol) and 4-chlororesorcinol (7.62 g, 52.7 mmol) under a nitrogen atmosphere. The mixture was stirred for 15 minutes to reflux, cooled to room temperature and sodium acetate (30 cm^3 of a saturated solution) and sodium hydrogen carbonate (15 cm^3 of a saturated solution) were added sequentially. Diethyl ether (50 cm^3) was added and the organic layer was extracted, dried ($MgSO_4$) and concentrated to leave a dark red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 4) gave a pale red solid which was washed in dichloromethane (25 cm^3) to leave the desired product (4.97 g, 55%) as a white powder (Found: C, 54.04; H, 2.84%; M^+, 313.003039. $C_{14}H_{10}Cl_2O_4$ requires C, 53.70; H, 3.22; $C_{14}H_{10}{}^{35}Cl_2O_4$ requires *M*, 313.003439); m.p. 207-211 °C; ν_{max} (nujol)/cm^{-1} 3600 (OH), 1680 (C=O); δ_H (200 MHz, CD_3OD) 7.93 (1H, s, H-6), 7.21 (1H, d, $J_{2',6'}$ = 2.1, H-2'), 7.01 (1H, dd, $J_{2',6'}$ = 2.1 and $J_{5',6'}$ = 8.3, H-6'), 6.86 (1H, d, $J_{5',6'}$ = 8.3, H-5'), 6.40 (1H, s, H-3), 4.14 (2H, s, $C\underline{H}_2$); δ_C (50.31 MHz, CD_3OD) 203.3 ($\underline{C}$=O), 165.9 (C-4), 161.9 (C-4'), 153.5 (C-2), 133.4 (C-6), 132.3 (C-2'), 130.5 (C-6'), 128.3 (C-1'), 121.9 (C-3'), 117.9 (C-5'), 114.4 (C-1), 113.7 (C-5), 105.1 (C-3), 44.5 ($\underline{C}H_2$); *m/z* (CI) 313, 315 ($[MH]^+$, 100, 67%), 279, 281 ($[MH - Cl]^+$, 16, 5), 245 ($[MH - Cl_2]^+$, 4).

Synthesis of 3',6-dichloro-4',7-dihydroxyisoflavone (**68**)[64]



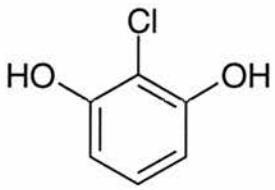
N,N-Dimethylformamide dimethylacetal (1.94 cm^3, 16.3 mmol) was added dropwise to a stirred solution of 3'-chloro-4'-hydroxybenzyl-2,4-dihydroxy-5-chlorophenylketone (2.6 g, 8.31 mmol) in tetrahydrofuran (15 cm^3). Stirring was continued to reflux for 5 hours under a nitrogen atmosphere. Methanol (10 cm^3) was added and the solvents removed at reduced pressure to leave a dark red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) gave an off-white powder. This was purified further by column chromatography using reverse phase C-18 silica (acetonitrile : water, 1 : 1) which gave the desired product (1.18 g, 44%) as a pure white powder (Found: M^+, 322.987167. $C_{15}H_9{}^{35}Cl_2O_4$ requires *M*, 322.987789); m.p. 235-236 °C; ν_{max} (nujol)/cm^{-1} 3600 (OH), 1680 (C=O); δ_H (300 MHz, d^6-DMSO) 8.49 (1H, s, H-2), 8.11 (1H, s, H-5), 7.70 (1H, d, $J_{2',6'}$ = 2.1, H-2'), 7.48 (1H, dd, $J_{2',6'}$ = 2.1 and $J_{5',6'}$ = 8.6, H-6'), 7.18 (1H, s, H-8), 7.13 (1H, d, $J_{5',6'}$ = 8.6, H-5'); δ_C (75.4 MHz, d^6-DMSO) 173.5 (C-4), 158.1 (C-7), 155.5 (C-8a), 153.5 (C-4'), 152.8 (C-2), 130.0 (C-5), 128.4 (C-2'), 126.0 (C-6'), 123.5 (C-1'), 122.1 (C-3), 119.8 (C-3'), 119.4 (C-4a), 116.9 (C-6), 116.3 (C-5'), 103.6 (C-8); *m/z* (CI) 323, 325 ($[MH]^+$, 20, 12%), 289, 291 ($[MH - Cl]^+$, 22, 8), 255 ($[MH - Cl_2]^+$, 8).

Synthesis of 2,2-dichloro-1,3-cyclohexanedione (**75**)[106]



1,3-Cyclohexanedione (5g, 44.6 mmol) was dissolved in sulfuryl chloride (25 cm^3) and stirred for 2 hours at room temperature under a nitrogen atmosphere. The reaction mixture was concentrated at reduced pressure yielding the desired product (7.8 g, 97%) as an off-white solid m.p. 66-67 °C (Lit.,[105] 68 °C); ν_{max} (nujol/cm^{-1}) 1720 (C=O); δ_H (300 MHz, $CDCl_3$) 3.00 (4H, t, $J_{4,5} = J_{5,6} = 8.0$, C$\underline{H}_2$-4 and 6), 2.00 (2H, quintet, $J_{4,5} = J_{5,6} = 8.0$, C$\underline{H}_2$-5); δ_C (75.4 MHz, $CDCl_3$) 192 (C-1 and 3), 77 (C-2), 35 (C-4 and 6), 17 (C-5); *m/z* (CI) 181, 183, 185 ($[MH]^+$, 100, 66, 11%), 147, 149 ($[MH - Cl]^+$, 54, 17).

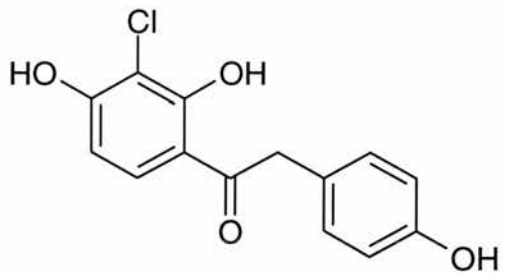
Synthesis of 2-chlororesorcinol (**76**)[105]



A saturated solution of HCl in dimethylformamide (10 cm^3) was added to a stirred solution of 2,2-dichlorocyclohexanedione (4.9 g, 27.0 mmol) in dimethylformamide (10 cm^3) at room temperature. Stirring was continued at 150-160 °C for 10 minutes and the reaction mixture cooled to room temperature. Concentration of the reaction mixture at reduced pressure was followed by the addition of sodium hydroxide (30 cm^3 of a 2 mol dm^{-3} solution), water (50 cm^3) and concentrated HCl (2 cm^3). Diethyl ether (3 x 100 cm^3) was added and the combined organic layers were separated, dried ($MgSO_4$) and

concentrated at reduced pressure to leave a brown solid. Purification by column chromatography (silica; ethyl acetate : toluene, 2 : 5) eluted the desired product (1.67 g, 43%) as white needles m.p. 76-78 °C (Lit.,[105] 97-98 °C); ν_{max} (nujol)/cm^{-1} 3600 (OH); δ_H (200 MHz, CD_3OD) 6.90 (1H, t, $J_{4,5} = J_{5,6} = 8.0$, H-5), 6.40 (2H, d, $J_{4,5} = J_{5,6} = 8.0$, H-4, 6); δ_C (50.31 MHz, CD_3OD) 156 (C-1 and 3), 129 (C-5), 109 (C-2, 4 and 6); *m/z* (CI) 145, 147 ([MH]$^+$, 100, 32%).

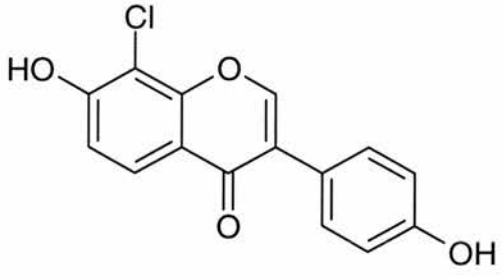
Synthesis of 4'-hydroxybenzyl-2,4-dihydroxy-3-chlorophenyl ketone (**74**)[55]



Boron trifluoride etherate (0.9 cm^3, 7.31 mmol) was added to a mixture of 3-chloro-4-hydroxyphenylacetic acid (0.6 g, 3.9 mmol) and 4-chlororesorcinol (1.14 g, 7.8 mmol) under a nitrogen atmosphere. The mixture was stirred for 15 minutes to reflux, cooled to room temperature and sodium acetate (30 cm^3 of a saturated solution) and sodium hydrogen carbonate (15 cm^3 of a saturated solution) were added sequentially. Diethyl ether (50 cm^3) was added and the organic layer was extracted, dried ($MgSO_4$) and concentrated to leave a dark red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 4) gave a pale red solid which was washed in dichloromethane (25 cm^3) to leave the desired product (1.0 g, 91%) as an off-white solid m.p. 208-212 °C;

ν_{max} (nujol)/cm^{-1} 3600 (OH), 1680 (C=O); δ_H (200 MHz, CD_3OD) 7.85 (1H, d, $J_{5,6}$ = 8.0, H-6), 7.10 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.0 and $J_{2',6'} = J_{3',5'}$ = , H-2' and 6'), 6.75 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.0 and $J_{2',6'} = J_{3',5'}$ = , H-3' and 5'), 6.50 (1H, d, $J_{5,6}$ = 8.0, H-5); δ_C (50.31 MHz, CD_3OD) 203.5 (C=O), 160.4 (C-4), 156.2 (C-2), 154.2 (C-4'), 130.1 (C-2' and 6'), 126.8 (C-6), 125.5 (C-1'), 115.1 (C-3' and 5'), 112.6 (C-1), 107.3 (C-5), 107.0 (C-3), 43.4 ($\underline{C}H_2$); *m/z* (CI) 279, 281 ([MH]$^+$, 100, 32%), 245 ([MH - Cl]$^+$, 13), 171, 173 ([MH - C_7H_7O]$^+$, 8, 3), 107 ([C_7H_7O]$^+$, 5).

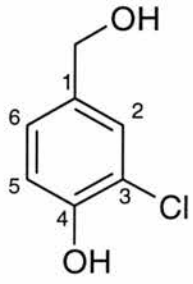
Synthesis of 8-chloro-4',7-dihydroxyisoflavone (**69**)[64]



N,N-Dimethylformamide dimethylacetal (1.02 cm^3, 8.54 mmol) was added dropwise to a stirred solution of 4'-hydroxybenzyl-2,4-dihydroxy-3-chlorophenylketone (0.8 g, 2.87 mmol) in tetrahydrofuran (10 cm^3). Stirring was continued to reflux for 5 hours under a nitrogen atmosphere. Methanol (10 cm^3) was added and the solvents removed at reduced pressure to leave a dark red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) gave an off-white coloured powder. Further purification by column chromatography using reverse phase C-18 silica (acetonitrile : water, 1 : 1) gave the desired product (0.52 g, 63%) as a pure white powder (Found: M$^+$, 289.026245. $C_{15}H_{10}{}^{35}ClO_4$ requires *M*, 289.026762); m.p. 316-318 °C; ν_{max} (nujol)/cm^{-1} 3600 (OH),

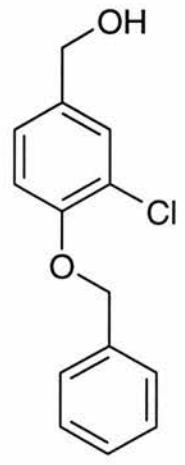
1680 (C=O); δ_H (300 MHz, d^6-DMSO) 8.52 (1H, s, H-2), 8.05 (1H, d, $J_{5,6}$ = 8.9, H-5), 7.52 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.6 and $J_{2',6'} = J_{3',5'}$ = 2.1, H-2' and 6'), 7.25 (1H, d, $J_{5,6}$ = 8.9, H-6), 6.94 (2H, dd, $J_{2',3'} = J_{5',6'}$ = 8.6 and $J_{2',6'} = J_{3',5'}$ = 2.1, H-3' and 5'); δ_C (75.4 MHz, d^6-DMSO) 174.4 (C-4), 158.3 (C-7), 157.3 (C-8a), 153.0 (C-4'), 152.7 (C-2), 130.0 (C-2' and 6'), 124.8 (C-5), 123.7 (C-3), 122.0 (C-1'), 117.4 (C-4a), 115.0 (C-3' and 5'), 114.6 (C-6), 106.6 (C-8); *m/z* (CI) 289, 291 ([MH]$^+$, 96, 34%), 255 ([M - Cl]$^+$, 98).

Synthesis of 3-chloro-4-hydroxybenzyl alcohol (**78**)[159]



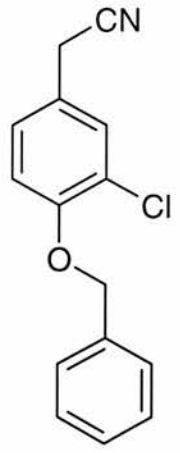
Lithium aluminium hydride (770 mg, 0.02 mol) was added to a stirred solution of 3-chloro-4-hydroxybenzoic acid (3.5 g, 0.2 mol) in diethyl ether (70 cm^3) over 5-10 minutes. Stirring was continued at room temperature for 24 hours after which time t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the absence of starting material. HCl (40 cm^3 of a 1.0 mol dm^{-3} solution) was added slowly followed by diethyl ether (30 cm^3) and water (30 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave the desired product (2.65 g, 84%) as a white solid (Found: M$^+$, 158.013146. $C_7H_7{}^{35}ClO_2$ requires *M*, 158.013457); m.p. 112-113 °C (Lit.,[162] 123 °C); ν_{max} (nujol)/cm^{-1} 3250 and 3320 (OH); δ_H (200 MHz, CD_3OD) 7.28 (1H, d, $J_{2,6}$ = 2.1, H-2), 7.10 (1H, d, $J_{5,6}$ = 6.3, H-5), 6.87 (1H, dd, $J_{5,6}$ = 6.3 and $J_{2,6}$ = 2.1, H-6), 4.48 (2H, s, C$\underline{H}_2$); δ_C (50.31 MHz, CD_3OD) 153.8 (C-4), 133.2 (C-1), 130.2 (C-2), 128.2 (C-6), 121.7 (C-3), 117.7 (C-5), 64.7 (C$\underline{H}_2$); *m/z* (CI) 158, 160 ([MH]$^+$, 17, 5%), 141, 143 ([MH - OH]$^+$, 100, 40).

Synthesis of 3-chloro-4-benzyloxybenzyl alcohol (**79**)



Potassium carbonate (3.5 g) was added to a stirred solution of 3-chloro-4-hydroxybenzyl alcohol (2.60 g, 0.016 mol) and benzyl bromide (2.81 g, 1.96 ml, 0.016 mol) in acetone (40 cm^3). Stirring was continued to reflux for 5 hours and at this stage t.l.c. (ethyl acetate : hexane, 1 : 1) showed the reaction had gone to completion. The potassium carbonate was removed by filtration and the filtrate concentrated at reduced pressure. Diethyl ether (50 cm^3) and water (50 cm^3) were added and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 3) eluted the desired product (2.54 g, 63%) as a colourless oil (Found: M^+, 248.059947. $C_{14}H_{13}{}^{35}ClO_2$ requires *M*, 248.060408); ν_{max} (nujol)/cm^{-1} 3300 (OH); δ_H (200 MHz, $CDCl_3$) 7.50-7.32 (5H, m, H-1', 2', 3', 4' and 5'), 7.43 (1H, d, $J_{2,6}$ = 2.1, H-2), 7.12 (1H, dd, $J_{2,6}$ = 2.1 and $J_{5,6}$ = 8.3, H-6), 6.92 (1H, d, $J_{5,6}$ = 8.3, H-5), 5.14 (2H, s, C$\underline{H_2}$Bn), 4.52 (2H, s, C$\underline{H_2}$OH); δ_C (50.31 MHz, $CDCl_3$) 154.0 (C-4), 137.0 (C-1'), 135.8 (C-1), 129.7 (C-2), 129.1 (C-2' and 6'), 128.5 (C-4'), 127.6 (C-3' and 5'), 126.9 (C-6), 123.7 (C-3), 114.5 (C-5), 71.4 ($\underline{C}H_2$Bn), 64.5 ($\underline{C}H_2$OH); *m/z* (CI) 248, 251 ($[MH]^+$, 12, 3%), 231, 233 ($[MH - OH]^+$, 100, 31), 141, 143 ($[MH - C_7H_7 - OH]^+$, 44, 12), 91 ($[C_7H_7]^+$, 39).

Synthesis of 3-chloro-4-benzyloxyphenylacetonitrile (**81**)

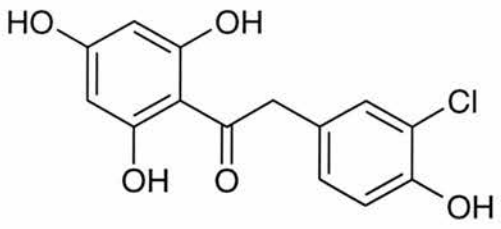


Phosphorus tribromide (0.95 cm^3, 0.01 mol) was added to a stirred solution of 3-chloro-4-benzyloxybenzyl alcohol (2.50 g, 0.01 mol) in diethyl ether (40 cm^3) under a nitrogen atmosphere. Stirring was continued for 2 hours at room temperature after which time t.l.c. (ethyl acetate : hexane, 1 : 3) revealed that no starting material remained. Water (60 cm^3) was added over 5-10 minutes and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. This yielded 3-chloro-4-benzyloxybenzyl bromide (2.72 g, 88%) as a colourless oil. Analysis by t.l.c. (ethyl acetate : hexane, 1 : 1) showed that no starting material remained and the product was used without further purification.

Potassium cyanide (570 mg, 8.45 mmol) was added to a stirred solution of 3-chloro-4-benzyloxybenzyl bromide (2.72 g, 8.45 mmol) and 18-crown-6 (2.25 g, 8.45 mmol) in acetonitrile (50 cm^3). Stirring was continued to reflux for 18 hours by which time t.l.c. (ethyl acetate : hexane, 1 : 1) showed that no starting material remained. The reaction mixture was cooled and concentrated at reduced pressure. Diethyl ether (50 cm^3) was added and the solution filtered through a bed of silica gel. The filtrate was concentrated at reduced pressure to leave a dark coloured oil. Purification by column chromatography (silica; ethyl acetate : hexane, 2 : 7) eluted the desired product (1.05 g, 48%) as pure white needles (Found: C, 69.94; H, 4.84; N, 5.41%; M^+, 258.067887. $C_{15}H_{12}ClNO$ requires C, 69.91; H, 4.69; N, 5.43; $C_{15}H_{12}{}^{35}ClNO$ requires *M*, 258.068080); m.p. 40-42 °C; ν_{max} (nujol)/cm^{-1} 2260 (CN); δ_H (200 MHz, $CDCl_3$) 7.47 (1H, d, $J_{2,6}$ = 2.2, H-2),

7.44-7.40 (4H, m, H-2', 3', 5', 6'), 7.14 (1H, d, $J_{5,6}$ = 8.4, H-5), 6.94 (1H, dd, $J_{2,6}$ = 2.2 and $J_{5,6}$ = 8.4, H-6), 5.16 (2H, s, C$\underline{H}_2$Bn), 3.64 (2H, s, C$\underline{H}_2$CN); δ_C (50.31 MHz, $CDCl_3$) 154.4 (C-4), 136.6 (C-1'), 130.4 (C-2), 129.2 (C-2' and 6'), 129.0 (C-4'), 127.8 (C-3' and 5'), 127.6 (C-6), 124.3 (C-1), 123.6 (C-3), 118.1 (<u>C</u>N), 114.8 (C-5), 71.4 (<u>C</u>H_2Bn), 23.1 (<u>C</u>H_2CN); *m/z* (CI) 258, 261 ($[MH]^+$, 100, 40%), 222 ($[MH\text{-}Cl]^+$, 14), 91 ($[C_7H_7]^+$, 85).

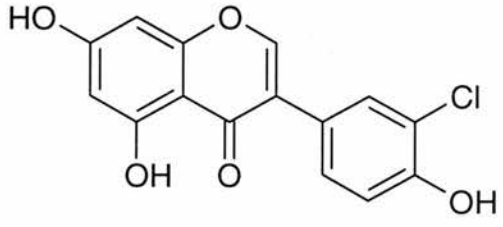
Synthesis of 3'-chloro-4'-hydroxybenzyl-2,4,6-trihydroxyphenyl ketone (**77**)[57]



Zinc chloride (3.0 cm^3 of a 1.0 mol dm^{-3} solution in diethyl ether, 3.0 mmol) was added to a stirred solution of phloroglucinol (385 mg, 2.93 mmol) and 3-chloro-4-benzyloxyphenylacetonitrile (500 mg, 1.95 mmol) in diethyl ether (8 cm^3). The solution was saturated with a stream of dry HCl gas for 2-3 days at 0 °C until a yellow precipitate had formed. The precipitate was washed in ice-cold diethyl ether (20 cm^3), HCl (30 cm^3 of a 0.5 mol dm^{-3} solution) was added and the suspension was heated for 3 hours under reflux. The reaction mixture was cooled to room temperature and brine (30 cm^3), water (30 cm^3) and diethyl ether (50 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 2) eluted an off-white solid,

which was washed in dichloromethane to give the desired product (360 mg, 42%) as a pure white powder (Found: C, 56.56; H, 4.00%; M^+, 295.037859. $C_{14}H_{11}ClO_5$ requires C, 57.06; H, 3.13; $C_{14}H_{11}{}^{35}ClO_5$ requires *M*, 295.037326); m.p. 230-233 °C; ν_{max} (nujol)/cm^{-1} 3625 (OH), 1690 (C=O); δ_H (300 MHz, CD_3OD) 7.18 (1H, d, $J_{2',6'}$ = 2.0, H-2'), 6.98 (1H, dd, $J_{2',6'}$ = 2.0 and $J_{5',6'}$ = 8.2, H-6'), 6.82 (1H, d, $J_{5',6'}$ = 8.2, H-5'), 5.83 (1H, d, $J_{3,5}$ = 1.7, H-3), 5.80 (1H, d, $J_{3,5}$ = 1.7, H-5), 4.25 (2H, s, C$\underline{H_2}$); δ_C (75.4 MHz, CD_3OD) 203.1 ($\underline{C}$=O), 165.2 (C-2), 164.7 (C-6), 158.9 (C-4), 151.6 (C-4'), 130.8 (C-2'), 128.9 (C-6'), 128.4 (C-1'), 119.6 (C-3'), 116.0 (C-5'), 103.9 (C-1), 94.5 (C-3), 94.2 (C-5), 47.7 ($\underline{C}H_2$); *m/z* (CI) 295, 297 ($[MH]^+$, 20, 7%), 261 ($[MH - Cl]^+$, 16), 127 ($[C_6H_7O_3]^+$, 100).

Synthesis of 3'-chloro-4,7,4'-trihydroxyisoflavone (**70**)[66]

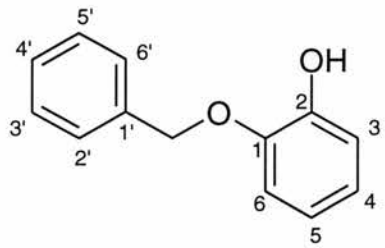


Boron trifluoride etherate (6.5 cm^3, 52.7 mmol) was added to a solution of 3'-chloro-4'-hydroxybenzyl-2,4,6-trihydroxyphenylketone (360 mg, 1.22 mmol) in dimethylformamide (13 cm^3). Heating was achieved under microwave conditions (2 x 15 seconds) and was followed by the addition of methanesulfonyl chloride (6.5 cm^3, 0.084 mol). Further heating was carried out (3 x 20 seconds) and water (300 cm^3) was added. The suspension was stirred at room temperature for 3 hours and the resulting precipitate was collected by filtration. Purification by column chromatography (silica; ethyl acetate ; hexane, 2 : 3), eluted an off-white solid. This was purified further by column

chromatography using C-18 reverse phase silica (acetonitrile : water, 3 : 2) and this eluted the desired product (120 mg, 32%) as a white powder (Found: M^+, 304.014164. $C_{15}H_9{}^{35}ClO_5$ requires *M*, 304.013851); m.p. 235-237 °C; ν_{max} (nujol)/cm^{-1} 3620 (OH), 1685 (C=O); δ_H (200 MHz, CD_3OD) 8.07 (1H, s, H-2), 7.53 (1H, d, $J_{2',6'}$ = 2.0, H-2'), 7.30 (1H, dd, $J_{5',6'}$ = 8.4 and $J_{2',6'}$ = 2.0, H-6'), 6.96 (1H, d, $J_{5',6'}$ = 8.4, H-5'), 6.32 (1H, d, $J_{3,5}$ = 2.1, H-8), 6.21 (1H, d, $J_{3,5}$ = 2.1, H-6); δ_C (50.31 MHz, CD_3OD) 182.1 (C-4), 166.3 (C-7), 164.1 (C-8a), 159.9 (C-5), 155.5 (C-2), 155.4 (C-4'), 131.9 (C-2'), 129.9 (C-6'), 124.9 (C-1'), 123.7 (C-3), 121.8 (C-3'), 117.9 (C-5'), 106.2 (C-4a), 100.5 (C-6), 95.1 (C-8); *m/z* (EI) 305, 307 (M^+, 100, 37%), 152 ($[C_7H_4O_4]^+$, 56).

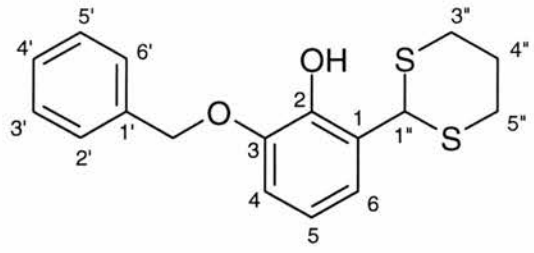
6.4: Precursors to 5,6,7,2'-tetramethoxy-3'-hydroxyisoflavone

Synthesis of 1-benzyloxy-2-hydroxy benzene (**86**)



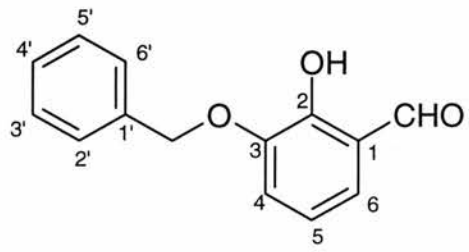
Potassium carbonate (25 g) was added to a stirred solution of catechol (15.0 g, 136.5 mmol) and benzyl bromide (16.2 cm^3, 136.5 mmol) in acetone (60 cm^3). Stirring was continued to reflux for 5 hours after which time t.l.c. (ethyl acetate : hexane, 1 : 7) revealed the absence of starting material and the reaction mixture was cooled to room temperature. The potassium carbonate was removed by filtration and the filtrate was concentrated at reduced pressure. Water (150 cm^3) and diethyl ether (150 cm^3) were added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an oil. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 7) gave dibenzyloxybenzene (550 mg, 1.5%) and the desired product (25.4 g, 93%) as a clear oil (Found: C, 77.40; H, 6.09%; M^+, 200.083031. $C_{13}H_{12}O_2$ requires C, 78.02; H, 6.04; *M*, 200.083730); ν_{max} (nujol)/cm^{-1} 3350 (OH); δ_H (200 MHz, $CDCl_3$) 7.49-7.40 (5H, m, H-2', 3', 4', 5' and 6'), 7.06-6.86 (4H, m, H-3, 4, 5 and 6), 5.14 (2H, s, $C\underline{H}_2$); δ_C (50.31 MHz, $CDCl_3$) 146.5, (C-2), 146.4 (C-1), 136.9 (C-6), 129.3 (C-2' and 6'), 129.0 (C-4'), 128.4 (C-3' and 5'), 122.4 (C-1'), 120.7 (C-3), 115.4 (C-5), 71.6 ($\underline{C}H_2$); *m/z* (EI) 200 (M^+, 14%), 91 ($[C_7H_7]^+$, 100), 65 ($[C_5H_5]^+$, 10).

Synthesis of 2-benzyloxy salicylaldehyde trimethylene mercaptal (**92**)[113]



A solution of 1,3-dithiane (600 mg, 5 mmol) in dichloromethane (5 cm^3) was added to a stirred slurry of *N*-chlorosuccinimide (665 mg, 5 mmol) in dichloromethane (25 cm^3) at -70 °C over a 5 minute period. After an additional 15 minutes a solution of 1-benzyloxy-2-hydroxy benzene (1.7 g, 8.5 mmol) in dichloromethane (5 cm^3) was added dropwise. After 15 minutes triethylamine (530 mg, 5.5 mmol) was added at a rate that maintained the temperature below –60 °C resulting in the formation of a deep red solution. The cooling bath was removed and the reaction mixture was allowed to warm gradually to room temperature. Water (75 cm^3) was added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a red oil. Examination by t.l.c. (silica; ethyl acetate : hexane, 1 : 5) showed the presence of a new product as well as starting material. Column chromatography (silica; ethyl acetate : hexane, 1 : 5) gave recovered starting material (1.148 g, 47%) and the desired product as an off-white solid. Recrystallisation from carbon tetrachloride yielded a pure white solid (248 mg, 9%) (Found: C, 64.06; H, 5.81%; M^+, 319.081418. $C_{17}H_{18}S_2O_2$ requires C, 64.12; H, 5.70; *M*, 319.082649); m.p. 128-131 °C; ν_{max} (nujol)/cm^{-1} 3350 (OH); δ_H (200 MHz, $CDCl_3$) 7.55-7.27 (5H, m, H-2', 3', 4', '5' and 6'), 7.07-6.71 (3H, m, H-3, 4 and 5), 5.69 (1H, s, C-1"), 5.10 (2H, s, C$\underline{H}_2$), 3.14 (2H, dt, $J_{3"a,\ 3"b} = J_{5"a,\ 5"b}$ = 13.9 and $J_{3"b,\ 4"} = J_{5"b,\ 4"}$ = 2.6, H-3"b, 5"b), 2.90 (2H, dt, $J_{3"a,\ 3"b} = J_{5"a,\ 5"b}$ = 13.9 and $J_{3"a,\ 4"} = J_{4",\ 5"a}$ = 3.9, H-3"a, 5"a), 2.22-1.90 (2H, m, C$\underline{H}_2$-4"); δ_C (74.76 MHz, $CDCl_3$) 149.8 (C-2), 142.8 (C-3), 139.1 (C-1'), 129.3 (C-2' and 6'), 129.0 (C-4'), 128.3 (C-3' and 5'), 126.8 (C-1), 121.7 (C-5), 120.7 (C-4), 112.3 (C-6), 71.8 (C-7), 44.5 (C-1"), 32.8 (C-3" and 5"), 26.0 (C-4"); *m/z* (CI) 319 (MH^+, 100), 119 ($[C_4H_7S_2]^+$, 5).

2-Hydroxy-3-benzyloxybenzaldehyde (**87**)



Synthesis by hydrolysis of 3-benzyloxysalicylaldehyde trimethylene mercaptal[114]

A solution of 3-benzyloxysalicylaldehyde trimethylene mercaptal (50 mg, 0.16 mmol) in tetrahydrofuran (1.0 cm^3) was added to a stirred suspension of mercuric oxide (105 mg, 0.47 mmol) and boron trifluoride dietherate (0.47 cm^3, 3.8 mmol) in water (2 cm^3). The reaction was stirred for 24 hours at room temperature. Sodium carbonate (35 cm^3 of a 0.5 mol dm^{-3} solution) was added and the resulting yellow precipitate was removed by filtration through a bed of celite. The filtrate was neutralized using HCl (12 cm^3 of a 2.0 mol dm^{-3} solution). Ethyl acetate (40 cm^3) and brine (25 cm^3) were added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an off-white solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 11) yielded 3-benzyloxy-4-hydroxybenzaldehyde (10 mg, 28%) in addition to the desired product (20 mg, 56%) as a white solid (Found: M^+, 228.079219. $C_{14}H_{12}O_3$ requires *M*, 228.07844); m.p. 79-81 °C; ν_{max} (nujol)/cm^{-1} 3350 (OH), 1670 (C=O); δ_H (200 MHz, $CDCl_3$) 11.13 (1H, s, O<u>H</u>), 9.92 (1H, s, C<u>H</u>O), 7.47-7.32 (5H, m, H-2', 3', 4' 5' and 6'), 7.21 (1H, dd, $J_{4,5}$ = 7.9 and $J_{4,6}$ = 1.2, H-4), 7.14 (1H, dd, $J_{5,6}$ = 7.9 and $J_{4,6}$ = 1.2, H-6), 6.91 (1H, t, $J_{4,5}$ = $J_{5,6}$ = 7.9, H-5), 5.20 (2H, s, C<u>H</u>$_2$); δ_C (74.76 MHz, $CDCl_3$) 196.5 (<u>C</u>=O), 152.3 (C-2), 147.2 (C-3), 136.5 (C-1'), 128.6 (C-2' and 6'), 128.1 (C-4'), 127.4 (C-3' and 5'), 125.3 (C-6), 121.1 (C-1), 121.0 (C-5), 119.4 (C-4), 71.4 (<u>C</u>H$_2$); *m/z* (EI) 228 (M^+, 16%), 91 ($[C_7H_7]^+$, 100), 65 ($[C_5H_5]^+$, 15).

Attempted *o*-formylation of 1-benzyloxy-2-hydroxybenzene using Reimer-Tiemann conditions[109]

1-Benzyloxy-2-hydroxybenzene (1.0 g, 5 mmol) was added to a mechanically stirred solution of sodium hydroxide (8.0 cm^3 of a 25 mol dm^{-3} solution) at room temperature. The reaction temperature was set to 60-65 °C and chloroform (9.0 cm^3, 10 mmol) was then added in 3 portions down the condenser with rapid stirring (to prevent the crystallization of sodium phenoxide). The reaction temperature was then raised to 100 °C and stirring was continued for 12 hours after which time the reaction had changed to a pinky-purple coloured solution. Chloroform was removed by steam distillation and the oily residue was acidified to pH 3 using dilute sulfuric acid (30 cm^3 of a 1.0 mol dm^{-3} solution). Diethyl ether (40 cm^3) was added and the organic layer was separated, dried ($MgSO_4$) and concentrated. Analysis by t.l.c. (ethyl acetate : hexane, 1 : 1) showed 2 products were present and these were separated by column chromatography (silica; ethyl acetate : hexane, 1 : 10). ^1H n.m.r. spectroscopy and mass spectrometry indicated them to be 1-benzyloxy-2-hydroxybenzene and the impurity 1,2-dibenzyloxybenzene.

Attempted *o*-formylation of 1-benzyloxy-2-hydroxybenzene using Vilsmaier-Haack conditions[110]

Phosphorus oxychloride (10.0 g, 6 cm^3, 6.3 mmol) was added to a stirred solution of 1-benzyloxy-2-hydroxybenzene (1.0 g, 5 mmol) in dimethylformamide (5 cm^3, 6.3 mmol) and 1,2-dichloroethane (15 cm^3) at 0 °C over 5 minutes. Stirring was continued to reflux for 2 hours after which time the reaction mixture was cooled and poured onto crushed ice (100 cm^3). Sodium acetate monohydrate (3.2 g) and diethyl ether (2 x 40 cm^3) were added and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an orange oil. ^1H n.m.r spectroscopy and t.l.c. (ethyl acetate : hexane, 1 : 2) of the crude product revealed the presence of unreacted 1-benzyloxy-2-hydroxybenzene only.

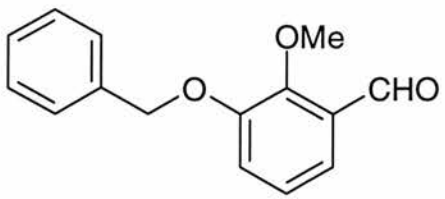
Attempted *o*-formylation of 1-benzyloxy-2-hydroxybenzene using stannic chloride[111]

1-Benzyloxy-2-hydroxybenzene (1.1.g, 5.5 mol) was added to a mechanically stirred suspension of tin (IV) chloride (143 mg, 0.55 mmol) in tri-*n*-butylamine (297 mg, 2.2 mmol) and toluene (20 cm^3) under a nitrogen atmosphere. Stirring was continued for 20 minutes at room temperature. This was followed by the addition of paraformaldehyde (363 mg, 12 mmol) and the resulting solution was heated for 48 hours at 100 °C. The solution was cooled to room temperature and water (75 cm^3), diethyl ether (60 cm^3) and brine (30 cm^3) were added. HCl (25 cm^3 of a 2.0 mol dm^{-3} solution) was added to acidify the solution to pH 2 and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a dark brown oil. ^1H n.m.r. spectroscopy and t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the presence of 1-benzyloxy-2-hydroxybenzene only.

Attempted *o*-formylation of 1-benzyloxy-2-hydroxybenzene using magnesium chloride[112]

Dry paraformaldehyde (0.51g, 16.9 mmol) was added to a mixture of 1-benzyloxy-2-hydroxybenzene (0.5.g, 2.5 mol), anhydrous magnesium chloride (0.29g, 3 mmol) and triethylamine (1.35 cm^3) in acetonitrile (15 cm^3). The mixture was heated under reflux for 4 hours and then cooled to room temperature. HCl (50 cm^3 of a 0.5 dm^{-3} solution) was added followed by diethyl ether (50 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a coloured oil (405 mg). Mass spectrometry and t.l.c. (ethyl acetate : hexanes, 1 : 1) revealed the presence of 1-benzyloxy-2-hydroxybenzene only.

2-Methoxy-3-benzyloxybenzene (**88**)



Attempted methylation using methyl iodide in acetone[163]

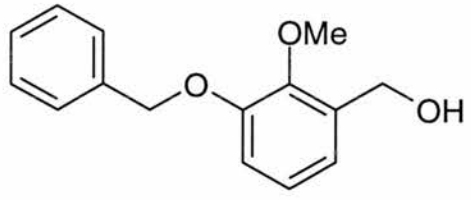
Potassium carbonate (4.0 g) was added to a stirred solution of 2-hydroxy-3-benzyloxybenzaldehyde (300 mg, 1.32 mmol) in acetone (20 cm^3) under a nitrogen atmosphere. Methyl iodide (0.09 cm^3, 1.38 mmol) was added and stirring was continued with heating to reflux for 3 hours. The reaction was cooled to room temperature and the potassium carbonate removed by filtration. The filtrate was concentrated at reduced pressure and diethyl ether (30 cm^3) and water (30 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a black oil (488 mg). ^1H n.m.r spectroscopy revealed the absence of the desired product and no further characterization was performed.

Synthesis using methyl iodide in tetrahydrofuran

Potassium carbonate (3.5 g) was added to a stirred solution of 2-hydroxy-3-benzyloxybenzaldehyde (130 mg, 0.57 mmol) in tetrahydrofuran (25 cm^3) under a nitrogen atmosphere. Methyl iodide (0.05 cm^3, 0.74 mmol) was then added and stirring was continued with heating to reflux for 3 hours. The reaction was cooled to room temperature and the potassium carbonate removed by filtration. The filtrate was concentrated at reduced pressure ethyl acetate (30 cm^3) and water (30 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to

leave the desired product (116 mg, 86%) as a clear oil, solidifying at room temperature (Found: M^+, 242.093472. $C_{15}H_{14}O_3$ requires *M*, 242.094294); m.p. 70-72 °C; ν_{max} (nujol)/cm^{-1} 1670 (C=O); δ_H (200 MHz, $CDCl_3$) 10.47 (1H, s, C<u>H</u>O), 7.48-7.35 (6H, m, H-6, 2', 3', 4', 5' and 6'), 7.21 (1H, dd, $J_{4,5}$ = 7.7 and $J_{4,6}$ = 1.1, H-4), 7.10 (1H, t, $J_{4,5}$ = $J_{5,6}$ = 7.7, H-5), 5.18 (2H, s, C<u>H</u>$_2$), 4.05 (3H, s, OC<u>H</u>$_3$); δ_C (50.31 MHz, $CDCl_3$) 190.8 (<u>C</u>=O), 152.7 (C-2), 136.9 (C-3), 130.5 (C-1'), 129.2 (C-2' and 6'), 128.7 (C-4'), 127.9 (C-3' and 5'), 124.6 (C-6), 120.5 (C-5), 120.2 (C-4), 71.6 (C-7), 62.9 (O<u>C</u>H$_3$); *m/z* (EI) 242 (M^+, 22%), 91 ($[C_7H_7]^+$, 100), 65 ($[C_5H_5]^+$, 9).

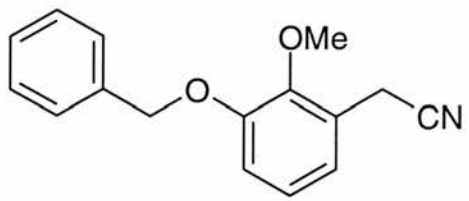
<u>Synthesis of 2-methoxy-3-benzyloxybenzyl alcohol (**89**)</u>



Lithium aluminum hydride (5 mg) was added to a stirred solution of 2-methoxy-3-benzyloxybenzaldehyde (15 mg, 0.062 mmol) in diethyl ether (5 cm^3) over 2-3 minutes. Stirring was continued for 18 hours at room temperature. Sodium sulfate (25 cm^3 of a saturated solution) was added and the organic layer extracted, dried ($MgSO_4$) and concentrated at reduced pressure to leave the desired product (15 mg, 97%) as a pale oil, solidifying to a white solid at room temperature (Found: M^+, 244.109196. $C_{15}H_{16}O_3$ requires *M*, 244.109945); m.p. 46-48 °C; ν_{max} (nujol/cm^{-1}) 3250 (OH); δ_H (200 MHz, $CDCl_3$) 7.54-7.34 (5H, m, H-2', 3', 4', 5' and 6'), 7.07-6.92 (3H, m, H-4, 5 and 6), 5.16

(2H, s, C<u>H</u>$_2$Bn), 4.71 (2H, s, C<u>H</u>$_2$OH), 3.96 (3H, s, O<u>C</u>H$_3$); δ_C (74.76 MHz, $CDCl_3$) 151.8 (C-2), 147.6 (C-3), 137.2 (C-1'), 135.0 (C-1), 128.7 (C-2' and 6'), 128.1 (C-4'), 127.5 (C-3' and 5'), 124.3 (C-4), 121.3 (C-5), 114.3 (C-6), 70.9 (<u>C</u>H$_2$Bn), 61.4 (<u>C</u>H$_2$OH), 61.0 (O<u>C</u>H$_3$); *m/z* (EI) 244 (M^+, 15%), 136 ($[M - C_7H_7O]^+$, 19), 91 ($[C_7H_7]^+$, 100), 65 ($[C_5H_5]^+$, 12).

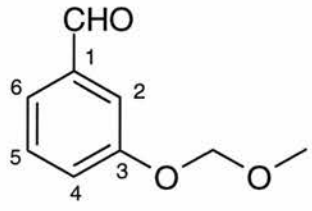
<u>Synthesis of 2-methoxy-3-benzyloxybenzonitrile (**91**)</u>



Phosphorus tribromide (380 mg, 1.4 mmol) was added to a stirred solution of 2-methoxy-3-benzyloxybenzyl alcohol (250 mg, 1.03 mmol) in diethyl ether (25 cm^3). Stirring was continued at room temperature for 2 hours at which point t.l.c. (ethyl acetate : hexane, 1 : 6) showed the reaction had gone to completion. Water (50 cm^3) was added slowly and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave 2-methoxy-3-benzyloxybenzyl bromide as a colourless oil (280 mg, 89%). Analysis by t.l.c. (ethyl acetate : hexanes, 1 : 1) showed the reaction had gone to completion and the product was used without further purification.

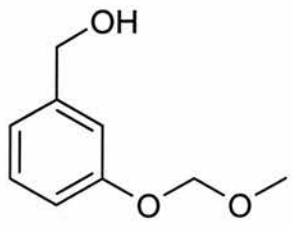
Potassium cyanide (77 mg, 1.18 mmol) was added to a stirred solution of 2-methoxy-3-benzyloxybenzyl bromide (280 mg, 0.912 mmol) and 18-crown-6 (241 mg, 0.912 mmol) in acetonitrile (25 cm^3). Stirring was continued for 24 hours to reflux after which time t.l.c. (ethyl acetate : hexane, 1 : 6) showed that the reaction had gone to completion. The reaction mixture was cooled to room temperature and insoluble materials were removed by filtration through a bed of silica gel. The filtrate was concentrated at reduced pressure and the residue was added to water (30 cm^3) and diethyl ether (30 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 6) yielded the desired product (67 mg, 30%) as a pale yellow solid (Found: M^+, 254.118410. $C_{16}H_{16}NO_2$ requires *M*, 254.118104); m.p. 44-45 °C; ν_{max} (nujol/cm^{-1}) 2250 (CN); δ_H (300 MHz, $CDCl_3$) 7.44-7.26 (5H, m, H-2', 3', 4', 5' and 6'), 7.06-6.95 (3H, m, H-4, 5 and 6), 5.13 (2H, s, C$\underline{H}_2$Bn), 3.96 (3H, s, OC$\underline{H}_3$), 3.73 (2H, s, C$\underline{H}_2$CN); δ_C (75.4 MHz, $CDCl_3$) 152.0 (C-2), 147.4 (C-3), 136.8 (C-1'), 128.8 (C-2' and 6'), 128.2 (C-4'), 127.5 (C-3' and 5'), 124.5 (C-1), 124.3 (C-6), 121.6 (C-5), 118.3 (<u>C</u>N), 114.7 (<u>C</u>-4), 70.9 (<u>C</u>H_2Bn), 60.7 (<u>C</u>H_2CN), 18.6 (O<u>C</u>H_3); *m/z* (CI) 254 ($[MH]^+$, 100), 164 ($[MH - C_7H_7]^+$, 7), 91 ($[C_7H_7]^+$, 65).

Synthesis of 3-methoxymethoxy-benzaldehyde (**93**)[120]



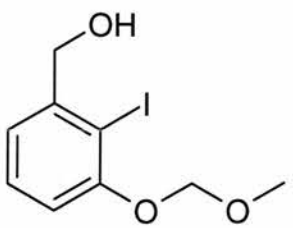
A solution of 3-hydroxybenzaldehyde (5.0 g, 41 mmol) in diethyl ether (25 cm^3) was added to a solution of sodium hydride (1.08 g, 45 mmol) in diethyl ether (100 cm^3) and dimethylformamide (20 cm^3) over 10 minutes under a nitrogen atmosphere. Stirring was continued for an additional 15 minutes, followed by the addition of a solution of chloromethyl methyl ether (3.75 cm^3, 49 mmol) in diethyl ether (25 cm^3) over a 10 minute period. Stirring was continued for 2 hours at room temperature at which stage t.l.c. (ethyl acetate : hexane, 1 : 1) showed the absence of starting material. Water (150 cm^3), sodium hydroxide (40 cm^3 of a 1.0 mol dm^{-3} solution), brine (40 cm^3) and diethyl ether (3 x 30 cm^3) were added to the reaction mixture. The combined organic layers were separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave the desired product (6.36 g, 94%) as a pale coloured oil ν_{max} (nujol)/cm^{-1} 1680 (C=O); δ_H (200 MHz, $CDCl_3$) 9.98 (1H, s, C<u>H</u>O), 7.55-6.98 (4H, m, H-2, 4, 5 and 6), 5.22 (2H, s, C<u>H</u>$_2$), 3.49 (3H, s, OC<u>H</u>$_3$); δ_C (50.31 MHz, $CDCl_3$) 192.5 (<u>C</u>HO), 158.2 (C-3), 138.3 (C-1), 130.6 (C-2), 124.3 (C-4), 123.3 (C-6), 116.4 (C-5), 65.6 (<u>C</u>H$_2$), 56.7 (O<u>C</u>H$_3$); *m/z* (EI) 166 (M^+, 26%), 77 ($[C_6H_5]^+$, 7), 45 ($[C_2H_5O]^+$, 100).

Synthesis of 3-methoxymethoxy-benzyl alcohol (**94**)



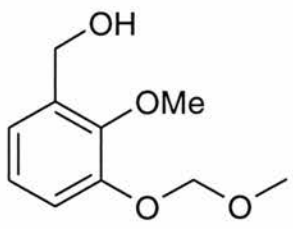
Lithium aluminium hydride (1.52 g, 40 mmol) was added to a solution of 3-methoxymethoxy-benzaldehyde (6.30 g, 38 mmol) in diethyl ether (50 cm^3) over 5 minutes under a nitrogen atmosphere. Stirring was continued for 18 hours at room temperature then the reaction was quenched with sodium sulfate (50 cm^3 of a saturated solution). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave the desired product (7.50 g, 86%) as a coloured oil; ν_{max} (nujol)/cm^{-1} 3300 (OH); δ_H (200 MHz, $CDCl_3$) 7.27 (1H, t, $J_{4,5} = J_{5,6} = 7.7$, H-5), 7.05-6.93 (3H, m, H-2, 4 and 6), 5.17 (2H, s, $OC\underline{H}_2O$), 4.64 (2H, s, -$C\underline{H}_2OH$), 3.47 (3H, s, $OC\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 158.0 (C-3), 143.5 (C-1), 130.2 (C-2), 120.9 (C-4), 116.0 (C-6), 115.1 (C-5), 74.8 (C-7), 65.6 ($O\underline{C}H_2O$), 56.5 ($O\underline{C}H_3$); *m/z* (EI) 168 (M^+, 43%), 138 ([M - CH_2O]$^+$, 16), 89 ([M - $C_2H_5O_2$ - OH]$^+$, 6), 77 ([C_6H_5]$^+$, 9), 45 ([C_2H_5O]$^+$, 100).

Synthesis of 2-iodo-3-methoxymethoxy-benzyl alcohol (**95**)[119]



n-Butyllithium (6.1 cm^3 of a 1.6 mol dm^{-3} solution in hexanes, 9.65 mmol) was added to a stirred solution of 3-methoxymethoxy-benzyl alcohol (750 mg, 4.46 mmol) in benzene (15 cm^3) at room temperature under a nitrogen atmosphere. Stirring was continued for 1 hour and was followed by the addition of a solution of ethylene iodochloride (1.24 g, 6.44 mmol) in tetrahydrofuran (2.0 cm^3) over 5 minutes. Stirring was continued for a further 1 hour, after which time t.l.c. (diethyl ether : hexane, 3 : 2) revealed a new product had been formed. Water (40 cm^3) was added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure. Purification by column chromatography (silica; diethyl ether : hexane, 1 : 1) eluted 3-methoxymethoxy-benzyl alcohol (208 mg) in addition to the desired product (590 mg, 45 %) as a fluffy white solid m.p. 74-77 °C (Lit.,[114] 88-91 °C); ν_{max} (nujol)/cm^{-1} 3300 (OH); δ_H (200 MHz, $CDCl_3$) 7.30 (1H, t, $J_{4,5} = J_{5,6} = 7.7$, H-5), 7.13 (1H, d, $J_{4,5} = 7.7$, H-6), 7.00 (1H, d, $J_{5,6} = 7.7$, H-4), 5.27 (2H, s, $OC\underline{H}_2O$), 4.72 (2H, s, $C\underline{H}_2OH$), 3.52 (3H, s, $OC\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 145.1 (C-3), 129.9 (C-1), 122.5 (C-4), 114.4 (C-6), 95.5 (C-2), 71.1 (C-7), 70.2 ($O\underline{C}H_2O$), 57.0 ($O\underline{C}H_3$); *m/z* (EI) 294 (M^+, 41%), 264 ($[M - CH_2O]^+$, 9), 77 ($[C_6H_5O]^+$, 14), 45 ($[C_2H_5O]^+$, 100).

2-Methoxy-3-methoxymethoxy-benzyl alcohol (**96**)



Attempted synthesis using sodium methoxide in hexamethylphosphoramide[123]

Sodium methoxide (40 mg, 9.15 mmol) was added to a stirred solution of 2-iodo-3-methoxymethoxy-benzyl alcohol (50 mg, 0.17 mmol) in hexamethylphosphoramide (10 cm^3) under a nitrogen atmosphere. Stirring was continued for 15 hours at 50 °C after which time t.l.c. (ethyl acetate : hexane, 1 : 1) revealed a large number of products to be present. The reaction was cooled and HCl (10 cm^3 of a 2.0 mol dm^{-3} solution), water (40 cm^3) and diethyl ether (20 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a crude oil (52 mg). ^1H n.m.r. spectroscopy gave no evidence for the desired product and further purification was not attempted.

Attempted synthesis using sodium methoxide in liquid ammonia

Sodium methoxide (610 mg, 9.4 mmol) was added to a stirred solution of 2-iodo-3-methoxymethoxy-benzyl alcohol (900 mg, 3.1 mmol) in liquid ammonia (50 cm^3). Stirring was continued for 18 hours at –78 °C after which time t.l.c. (ethyl acetate : hexane, 1 : 1) suggested that no reaction had occurred. The reaction mixture was warmed to room temperature to allow the ammonia to evaporate. Water (30 cm^3) and diethyl ether (30 cm^3) were then added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a dark coloured solid. ^1H n.m.r. spectroscopy revealed this to be starting material.

Attempted synthesis using cuprous methoxide in pyridine[125]

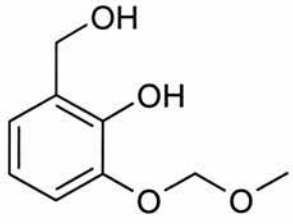
A mixture of methanol (0.44 cm^3, 5.43 mmol) and *n*-butyllithium (1.45 cm^3 of a 3.6 mol dm^{-3} solution in hexanes, 5.2 mmol) was stirred for 15 minutes under a nitrogen atmosphere. Dimethoxyethane (5 cm^3) was then added and stirring continued for 5

minutes. The contents were cannulated into a separate reaction vessel containing cuprous chloride (362 mg, 3.62 mmol) under a nitrogen atmosphere. Pyridine (18 cm^3) was added followed by 2-iodo-3-methoxymethoxy-benzyl alcohol (400 mg, 1.36 mmol) turning the reaction mixture to a yellow and then to a green colour. Stirring was then continued to reflux for 12 hours. At this stage t.l.c. (ethyl acetate : hexane, 1 : 1) suggested only starting material was present and the reaction was cooled to room temperature. Water (120 cm^3) and diethyl ether (50 cm^3) were added and the organic layer was separated, dried ($MgSO_4$) and concentrated at room temperature to leave a dark coloured oil. ^1H n.m.r. spectroscopy showed this to be starting material.

Attempted synthesis using cuprous methoxide in 2,4,6-collidine[124]

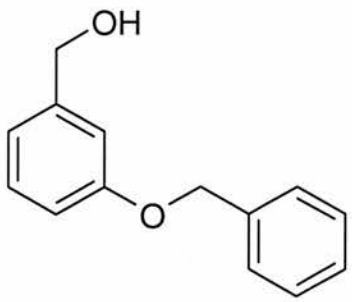
Freshly cut sodium metal (233 mg, 0.01 mol) was added to methanol (5 cm^3) under a nitrogen atmosphere. Upon dissolution of the sodium, 2,4,6-collidine (10 cm3) was added followed by cuprous iodide. 2-Iodo-3-methoxymethoxy-benzyl alcohol (100 mg, 0.34 mmol) and further 2,4,6-collidine (20 cm^3) were then added sequentially. The reaction mixture was heated to reflux for 20 hours. Analysis by t.l.c. (ethyl acetate : hexanes, 1 : 1) showed that only starting material was present and cooling was afforded to room temperature. The copper species was removed by filtration and the filtrate concentrated at reduced pressure. ^1H n.m.r. spectroscopy showed the reaction products to be starting material and 3-methoxymethoxy-benzyl alcohol.

Attempted synthesis of 2-hydroxy-3-methoxymethoxy-benzyl alcohol (**97**)[122]



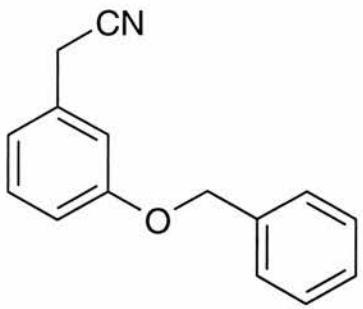
n-Butyllithium (17.0 cm^3 of a 2.5 mol dm^{-3} solution in hexanes, 42.5 mmol) was added to a stirred solution of 3-methoxymethoxy-benzyl alcohol (2.5 g, 14.9 mmol) in benzene (20 cm^3) under a nitrogen atmosphere. Stirring was continued for 1 hour at room temperature. The reaction mixture was then cannulated into a separate reaction vessel containing a solution of trimethyl borate (2.0 cm^3, 16.8 mmol) in tetrahydrofuran (25 cm^3) at -78 °C. After completion stirring was continued at 0 °C for 1 hour. Addition of acetic acid (1.35 cm^3) was followed immediately by addition of 30 % H_2O_2 (3.35 cm^3) and the resulting solution stirred for 18 hours at room temperature. Diethyl ether (40 cm^3) and potassium hydroxide (25 cm^3 of a 0.5 mol dm^{-3} solution) were washed in water (40 cm^3), ammonium ferric sulfate (20 cm^3 of a 1.0 mol dm^{-3} solution) and brine (25 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a pale oil. This was shown by ^1H n.m.r. spectroscopy and t.l.c. (ethyl acetate : hexane, 1 : 1) to be starting material.

Synthesis of 3-benzyloxybenzyl alcohol (**98**)



Potassium carbonate (3.5 g) was added to a stirred solution of 3-hydroxybenzyl alcohol (3.0 g, 0.024 mol) and benzyl bromide (2.88 cm^3, 0.024 mol) in acetone (50 cm^3). Stirring was continued to reflux for 5 hours and at this stage t.l.c. (ethyl acetate : hexane, 1 : 1) showed the reaction had gone to completion. Potassium carbonate was removed by filtration and the filtrate concentrated at reduced pressure. Diethyl ether (60 cm^3) and water (60 cm^3) were added, the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. This left the desired product (5.27 g, 100%) as an off-white solid (Found: M^+, 215.107728. $C_{14}H_{14}O_2$ requires *M*, 215.107205); m.p. 42-44 °C; ν_{max} (nujol)/cm^{-1} 3300 (OH); δ_H (300 MHz, $CDCl_3$) 7.45-7.37 (5H, m, H-2', 3', 4', 5' and 6'), 7.29 (1H, t, $J_{4,5} = J_{5,6} = 8.1$, H-5), 7.03-6.94 (3H, m, H-2, 4 and 6), 5.08 (2H, s, C$\underline{H}_2$Bn), 4.65 (2H, s, C$\underline{H}_2$OH); δ_C (75.4 MHz, $CDCl_3$) 159.3 (C-3), 142.8 (C-1), 137.2 (C-1'), 129.8 (C-5), 128.7 (C-2' and 6'), 128.1 (C-4'), 127.6 (C-3' and 5'), 119.5 (C-6), 114.2 (C-4), 113.4 (C-2), 70.0 ($\underline{C}H_2$Bn), 65.2 ($\underline{C}H_2$OH); *m/z* (CI) 253 ($[MH + K]^+$, 13%), 215 ($[MH]^+$, 46), 197 ($[MH - OH]^+$, 100), 91 ($[C_7H_7]^+$, 36).

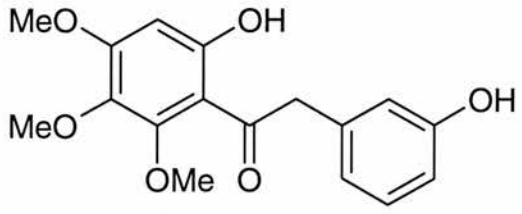
Synthesis of 3-benzyloxyphenylacetonitrile (**100**)



Phosphorus tribromide (1.86 cm^3, 18 mmol) was added to a stirred solution of 3-benzyloxybenzyl alcohol (3.0 g, 18 mmol) in diethyl ether (40 cm^3) under a nitrogen atmosphere. Stirring was continued for 2 hours at room temperature by which time t.l.c. (ethyl acetate : hexane, 1 : 3) revealed no starting material was present. Water (60 cm^3) was added over 5-10 minutes and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. This yielded 3-benzyloxybenzyl bromide (3.43 g, 84%) as a colourless oil. Analysis by t.l.c. (ethyl acetate : hexanes, 1 : 1) showed that the none of the starting material remained and the product was used without further purification.

Potassium cyanide (950 mg, 14.5 mmol) was added to a stirred solution of 3-benzyloxybenzyl bromide (3.40 g, 14.5 mmol) and 18-crown-6 (3.22 g, 14.5 mmol) in acetonitrile (50 cm^3). Stirring was continued to reflux for 18 hours by which time t.l.c. (ethyl acetate : hexane, 1 : 3) showed that no starting material was present. The reaction mixture was cooled and concentrated at reduced pressure. Diethyl ether (50 cm^3) was added and filtered through a bed of silica gel. The filtrate was concentrated at reduced pressure to leave the desired product (1.74 g, 63%) as a pale coloured oil (Found: M^+, 223.100197. $C_{15}H_{13}NO$ requires *M*, 223.099714); ν_{max} (nujol)/cm^{-1} 2250 (CN); δ_H (200 MHz, $CDCl_3$) 7.46-7.27 (6H, m, H-5, 2', 3', 4', 5' and 6'), 6.98-6.92 (3H, m, H-2, 4 and 6), 5.09 (2H, s, $C\underline{H}_2Bn$), 3.71 (2H, s, $C\underline{H}_2CN$); δ_C (50.31 MHz, $CDCl_3$) 159.8 (C-3), 137.1 (C-1'), 132.0 (C-1), 130.8 (C-5), 128.7 (C-2' and 6'), 128.2 (C-4'), 127.5 (C-3' and 5'), 121.1 (C-6), 115.2 (C-4), 114.9 (C-2), 113.9 ($\underline{C}N$), 70.3 ($\underline{C}H_2Bn$), 24.0 ($\underline{C}H_2CN$); *m/z* (EI) 223 (M^+, 14%), 117 ($[M - C_7H_7O]^+$, 10), 91 ($[C_7H_7]^+$, 100).

3'-Benzyloxybenzyl-2-hydroxy-4,5,6-trimethoxyphenyl ketone (**101**)



Attempted synthesis from acid-boron trifluoride etherate complexation[55]

Boron trifluoride etherate (1.6 cm^3, 13 mmol) was added to a mixture of 3-benzyloxyphenylacetic acid (120 mg, 0.65 mmol) and 3,4,5-trimethoxyphenol (160 mg, 0.65 mmol). The mixture was stirred to reflux for 2 hours, forming a dark red oil. The reaction mixture was cooled to room temperature and sodium acetate (30 cm^3 of a saturated solution), sodium hydrogen carbonate (25 cm^3 of a saturated solution) and diethyl ether (40 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to give the product as a dark red oil. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) eluted a new product identified by ^1H and ^{13}C n.m.r spectroscopy to be 3-hydroxyphenylacetic acid.

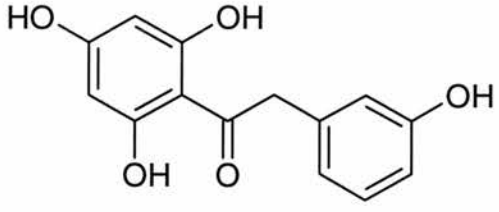
Attempted synthesis from acid-boron trifluoride (gas) complexation[54]

3-Benzyloxyphenylacetic acid (500 mg, 2.07 mmol) was dissolved in chloroform (10 cm^3), the solution cooled in an ice-bath and saturated with boron trifluoride gas. 3,4,5-Trimethoxyphenol (175 mg, 0.94 mmol) was then added and the reaction mixture was stirred at room temperature for 18 hours. Diethyl ether (40 cm^3) and water (40 cm^3) were added. The organic layer was separated, dried (MgSO4) and concentrated at reduced pressure to leave an oily solid. ^1H and ^{13}C n.m.r. spectroscopy and mass spectrometry showed that only starting material was present.

Attempted synthesis using Hoesch conditions[57]

Zinc chloride (0.34 cm^3 of a 1.0 mol dm^{-3} solution in diethyl ether, 0.34 mmol) was added to a stirred suspension of 3'-benzyloxybenzonitrile (75 mg, 0.34 mmol) and 3,4,5-trimethoxyphenol (93 mg, 0.54 mmol) in diethyl ether (5 cm^3). HCl gas was bubbled through for 24 hours with continuous stirring at 0 °C forming a yellow solid suspended in a yellow solution. The etheral solution was decanted off, HCl (20 cm^3 of a 1.0 mol dm^{-3} solution) was added and the suspension heated under reflux for 3 hours. On standing for 18 hours, white needles precipitated which were collected by filtration and washed with water (5 cm^3). ^1H n.m.r. spectroscopy and mass spectrometry showed that only 3,4,5-trimethoxyphenol was present.

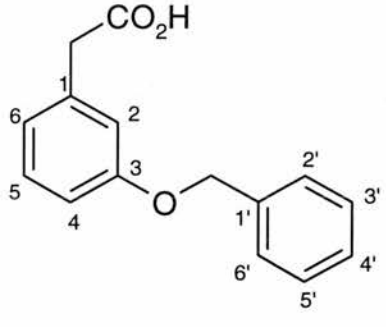
Synthesis of 3-hydroxybenzyl-2,4,6-trihydroxyphenylketone (**102**)[57]



Zinc chloride (1.30 cm^3 of a 1.0 mol dm^{-3} solution in diethyl ether, 1.30 mmol) was added to a stirred suspension of 3'-benzyloxybenzonitrile (320 mg, 1.01 mmol) and 3-benzyloxyphenylacetonitrile (350 mg, 0.67 mmol) in diethyl ether (5 cm^3). HCl gas was bubbled through for 24 hours with continuous stirring at 0 °C forming a pale oil suspended in an orange solution. The etheral solution was decanted off, HCl (40 cm^3 of a 1.0 mol dm^{-3} solution) was added and the suspension heated under reflux for 3 hours. The reaction mixture was cooled and diethyl ether (50 cm^3) was added. The organic layer was

separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an oil. Purification by column chromatography (silica; ethyl acetate : hexanes, 2 : 3) gave the desired product as a white powder (178 mg, 84%) (Found: M^+, 260.067541. $C_{14}H_{13}O_5$ requires *M*, 260.068474); m.p. 193-196 °C (Lit.,[164] 233-234 °C); ν_{max} (thin film)/cm^{-1} 3420 (OH), 1610 (C=O); δ_H (300 MHz, CD_3OD) 7.34-7.05 (2H, m, H-5' and 6'), 6.79-6.63 (2H, m, H-2' and 4'), 5.86 (2H, s, H-3 and 5), 4.34 (2H, s, $C\underline{H}_2$); δ_C (75.4 MHz, CD_3OD) 204.3 (C=O), 166.9 (C-3'), 160.4 (C-2 and 6), 158.3 (C-4), 139.2 (C-1'), 130.8 (C-6'), 130.4 (C-5'), 122.2 (C-4'), 117.9 (C-2'), 114.9 (C-1), 95.9 (C-3 and 5), 50.8 ($\underline{C}H_2$); *m/z* (EI) 260 (M^+, 8%), 243 ($[M - OH]^+$, 11), 153 ($[C_7H_5O_4]^+$, 100), 126 ($[C_6H_6O_3]^+$, 34).

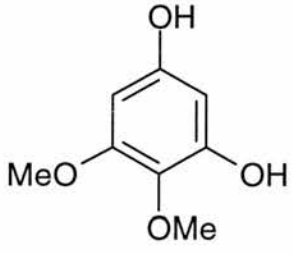
Synthesis of 3-benzyloxyphenylacetic acid (**103**)



Potassium carbonate (3.5 g) was added to a stirred solution of 3-hydroxyphenylacetic acid (2.0 g, 0.013 mol) and benzyl bromide (1.8 cm^3, 0.015 mol) in acetone (30 cm^3). Stirring was continued to reflux for 5 hours and at this stage t.l.c. (ethyl acetate : hexane, 1 : 1) showed the reaction had gone to completion. Potassium carbonate was removed by filtration and the filtrate concentrated at reduced pressure. Diethyl ether (50 cm^3) and water (50 cm^3) were added, the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. This left the desired product (2.90 g, 92%) as a colourless oil (Found: M^+, 243.101261. $C_{15}H_{14}O_3$ requires *M*, 243.102120); ν_{max} (nujol)/cm^{-1} 3550 (OH) 1690 (C=O); δ_H (300 MHz, $CDCl_3$) 7.45-7.36 (5H, m, H-2', 3', 4',

5' and 6'), 7.26 (1H, t, $J_{4,5} = J_{5,6} = 8.0$, H-5), 7.00-6.91 (3H, m, H-2, 4 and 6), 5.17 (2H, s, $C\underline{H}_2CO_2H$), 5.06 (2H, s, $C\underline{H}_2Bn$); δ_C (75.4 MHz, $CDCl_3$) 171.6 ($\underline{C}$=O), 159.2 (C-3), 137.2 (C-1'), 135.5 (C-1), 129.9 (C-5), 128.7 (C-2' and 6'), 128.4 (C-4'), 127.7 (C-3' and 5'), 122.1 (C-6), 115.9 (C-4), 113.8 (C-2), 70.0 ($\underline{C}H_2Bn$), 41.1 ($\underline{C}H_2CO_2H$); *m/z* (CI) 243 ($[MH]^+$, 17%), 153 ($[MH - C_7H_7]^+$, 20), 91 ($[C_7H_7]^+$, 63).

3,4-Dimethoxy-5-hydroxyphenol (**104**)



Attempted demethylation of 3,4,5-trimethoxyphenol using sodium ethanethiolate[91]

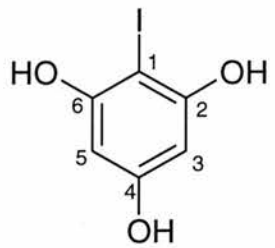
Sodium ethanethiolate (230 mg, 3.3 mmol) was added to a stirred solution of 3,4,5-trimethoxyphenol (300 mg, 1.63 mmol) in dimethylformamide (25 cm^3). Stirring was continued to reflux for 4 hours under a nitrogen atmosphere and the reaction mixture was cooled to room temperature. Water (130 cm^3) and diethyl ether (40 cm^3) were added and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a brown solid. ^1H n.m.r. spectroscopy and mass spectrometry showed only the starting material was present.

Attempted demethylation of 3,4,5-trimethoxyphenol using lithium chloride[92]

Lithium chloride (115 mg, 2.72 mmol) was added to a stirred solution of 3,4,5-trimethoxyphenol (500 mg, 2.72 mmol) in dimethylformamide (30 cm^3). Stirring was continued to reflux for 16 hours and the reaction was cooled to room temperature. Sodium hydroxide (100 cm^3 of a 1.0 mol dm^{-3} solution), diethyl ether (50 cm^3) and HCl (120 cm^3 of a 1.0 mol dm^{-3} solution) were added sequentially and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an off-white solid. ^1H n.m.r. spectroscopy and mass spectrometry showed only the starting material was present.

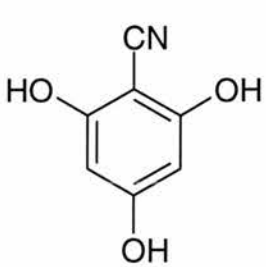
6.5: Unlabelled and [^{13}C] labelled flavones

Synthesis of iodophloroglucinol (**118**)[144]



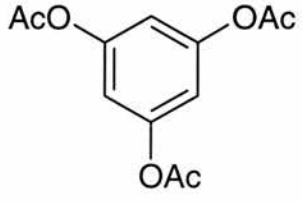
To a stirred solution of phloroglucinol (4.86 g, 30 mmol) in tetrahydrofuran (30 ml) and water (30 cm^3) was added a mixture of iodine (7.7 g, 30 mmol) and sodium hydrogen carbonate (2.7 g, 30 mmol) at room temperature in one portion. The iodination proceeded with strong evolution of CO_2 and was complete within 30 minutes. The solution was diluted with water (40 cm^3) and diethyl ether (40 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave the desired crude product (8.76 g, 90%) as a black solid ν_{max} (nujol)/cm^{-1} 3350 (OH); δ_H (200 MHz, $CDCl_3$) 5.85 (2H, s, H-3 and 5); δ_C (50.31 MHz, $CDCl_3$) 159.0 (C-2 and 6), 157.6 (C-4), 95.4 (C-3 and 5), 68.5 (C-1); *m/z* (EI) 252 (M^+, 100), 127 (I^+, 19).

Attempted synthesis of 2,4,6-trihydroxybenzonitrile (**119**)



Potassium cyanide (262 mg, 3.97 mmol) was added to a stirred solution of iodophloroglucinol (1.0 g, 3.97 mmol), calcium hydroxide (0.15 g, 2 mmol) and palladium acetate (0.15 g, 0.625 mmol) in dimethylformamide (50 cm^3). Stirring was continued to reflux for 18 hours under a nitrogen atmosphere. The reaction mixture was cooled, concentrated at reduced pressure and water (30 cm^3) and diethyl ether (30 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a black solid. This was shown by t.l.c. (ethyl acetate : hexane, 1 : 1) and ^{13}C n.m.r. spectroscopy to be starting material.

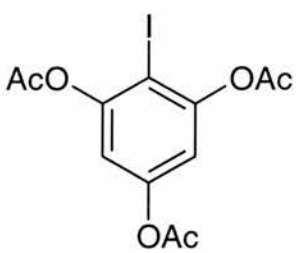
Synthesis of triacetylphloroglucinol (**120**)[165]



Acetic anhydride (26.95 g, 24.9 cm^3, 263 mmol) was added to a stirred solution of phloroglucinol (10.0 g, 80 mmol) in pyridine (70 cm^3) at 0 °C. Stirring was continued for 3 days at room temperature after which time the reaction mixture was concentrated at reduced pressure. Ethyl acetate (50 cm^3) was added and the reaction mixture washed with water (40 cm^3) and brine (30 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave the desired product (14.06 g, 71%) as a white solid m.p. 96-98 °C (Lit.,[166] 103-104 °C); ν_{max} (nujol)/cm^{-1} 1690 (C=O); δ_H (200 MHz, $CDCl_3$) 6.84 (3H, s, H-1, 3 and 5), 2.28 (9H, s, $C\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 169.1

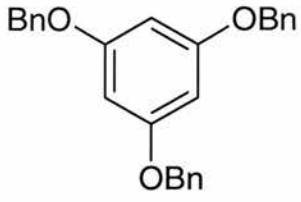
(C=O), 151.6 (C-2, 4 and 6), 113.3 (C-1, 3 and 5), 21.6 ($\underline{C}H_3$); *m/z* (EI) 252 (M^+, 8%), 210 ($[M - C_2H_3O]^+$, 20), 168 ($[M - C_4H_6O_2]^+$, 34), 126 ($[M - C_6H_9O_3]^+$, 100).

Attempted synthesis of iodotriacetylphloroglucinol (**121**)[144]



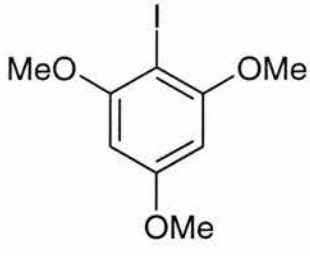
To a solution of triacetylphloroglucinol (1.0 g, 3.97 mmol) in tetrahydrofuran (20 cm^3) and water (20 cm^3) was added a mixture of iodine (1.01 g, 3.97 mmol) and sodium hydrogen carbonate (280 mg, 3.97 mmol). The reaction mixture was stirred at room temperature for 30 minutes and diluted with water (40 cm^3) and diethyl ether (40 cm^3). The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave the crude product (1.0 g) as a black solid. Examination by t.l.c. (ethyl acetate : hexane, 1 : 1) suggested starting material was present as well as several new products. The ^1H and ^{13}C n.m.r. spectra and mass spectrum suggest the presence of mono-, di-, and tri-iodotriacetylphloroglucinol in addition to starting material *m/z* (CI) 631 ($[MH + I_2]^+$, 1%), 589 ($[MH + I_2 - C_2H_3O]^+$, 2), 548 ($[MH + I_2 - C_4H_6O_2]^+$, 5), 506 ($[MH + I]^+$, 9), 463 ($MH + I - C_2H_3O]^+$, 19), 421 ($[MH + I - C_4H_6O_2]^+$, 17), 378 ($[MH]^+$, 15), 337 ($[MH - C_2H_3O]^+$, 10), 295 ($[MH - C_4H_6O_2]^+$, 72), 253 ($[MH - I]^+$, 98), 211 ($[MH - I - C_2H_3O]^+$, 100), 169 ($[MH - I - C_4H_6O_2]^+$, 31), 126 ($[MH - I - C_6H_9O_3]^+$, 32).

Attempted synthesis of phloroglucinol tribenzyl ether (**122**)[167]



Sodium hydride (7.50 g, 312 mmol) was added to a stirred solution of phloroglucinol (10.0 g, 80 mmol), benzyl bromide (28.6 cm^3, 312 mmol) and *t*-butylammonium iodide (270 mg) in dimethylformamide (60 cm^3). Stirring was continued for 3 days at room temperature and t.l.c. at this stage (ethyl acetate : hexane, 1 : 1) suggested many products were present. The reaction mixture was concentrated at reduced pressure and diethyl ether (50 cm^3) and water (50 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a dark oil which was not purified further.

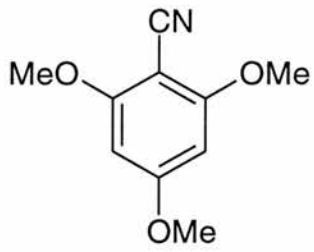
Synthesis of 2,4,6-trimethoxyiodobenzene (**123**)[144]



To a solution of phloroglucinol trimethyl ether (2.5 g, 14.9 mmol) in tetrahydrofuran (12 cm^3) and water (12 cm^3) was added a mixture of iodine (3.78 g, 14.9 mmol) and sodium hydrogen carbonate (1.325 g, 14.9 mmol). The reaction mixture was stirred at room temperature for 30 minutes with strong evolution of carbon dioxide. Water (40 cm^3) and diethyl ether (40 cm^3) were added and the organic layer separated, dried ($MgSO_4$) and

concentrated at reduced pressure to leave a black solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1: 1) eluted the desired product (3.90 g, 89%) as black crystals, stored below 0 °C; δ_H (200 MHz, $CDCl_3$) 6.14 (2H, s, H-3 and 5), 3.86 (9H, s, $OC\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 162.65 (C-2 and 6), 160.26 (C-4), 93.35 (C-1), 91.67 (C-3 and 5), 56.96, 56.03 ($O\underline{C}H_3$).

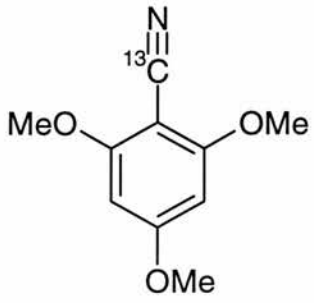
Synthesis of 2,4,6-trimethoxybenzonitrile (**124**)



Potassium cyanide (220 mg, 3.32 mmol) was added to a solution of 2,4,6-trimethoxyiodobenzene (1.0 g, 3.32 mmol), palladium acetate (124 mg, 0.55 mmol) and calcium hydroxide (130 mg, 1.71 mmol) in dimethylformamide (25 cm^3). The reaction mixture was stirred to reflux for 12 hours, after which t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the absence of starting material. The reaction mixture was cooled and insoluble materials were removed by filtration. The dimethylformamide was removed at reduced pressure and diethyl ether (30 cm^3) and water (30 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a brown solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 2) yielded the desired product (227 mg, 35%) as an off-white solid m.p. 141-2 °C, (Lit.,[168] 140 °C); ν_{max} (nujol)/cm^{-1} 2230 (CN); δ_H (200 MHz, $CDCl_3$) 6.07 (2H, s, H-3 and 5), 3.88 (9H, s, 3 x $OC\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 166.0 (C-4), 164.2 (C-2 and 6), 115.0

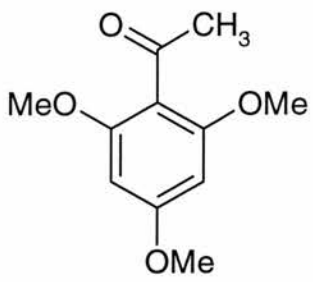
(C̲N) 90.8 (C-3 and 5), 80.1 (C-1); *m/z* (CI) 234 ($[MH + K]^+$, 13%), 194 ($[MH]^+$, 100), 169 ($[MH - CN]^+$, 65).

Synthesis of [^{13}C] 2,4,6-trimethoxybenzonitrile (**125**)



Potassium [^{13}C]cyanide (515 mg, 7.77 mmol) was added to a solution of 2,4,6-trimethoxyiodobenzene (2.27 g, 7.77 mmol), palladium acetate (282 mg, 1.24 mmol) and calcium hydroxide (295 mg, 3.90 mmol) in dimethylformamide (35 cm^3). The reaction mixture was stirred to reflux for 12 hours, after which t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the absence of starting material. The reaction mixture was cooled and insoluble materials were removed by filtration. The dimethylformamide was removed at reduced pressure and diethyl ether (30 cm^3) and water (30 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a brown solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 2) yielded the desired product (535 mg, 35%) as an off-white solid (Found: C, 62.66; H, 5.81; N, 6.53%; M^+, 194.076752. $^{12}C_9{}^{13}C_1H_{11}NO_3$ requires C, 62.36; H, 5.71; N, 7.21; *M*, 194.077248); m.p. 147-8 °C (Lit.,[169] 140 °C); ν_{max} (nujol)/cm^{-1} 2230 (CN); δ_H (200 MHz, $CDCl_3$) 6.07 (2H, s, H-3 and 5), 3.88 (9H, s, 3 x OC̲H_3); δ_C (50.31 MHz, $CDCl_3$) 115.0 (^{13}C̲N, enhanced); *m/z* (EI) 194 (M^+, 100%), 135 ($[M - {}^{13}CN - CH_3O]^+$, 7), 105 ($[M - {}^{13}CN - C_2H_6O_2]^+$, 7).

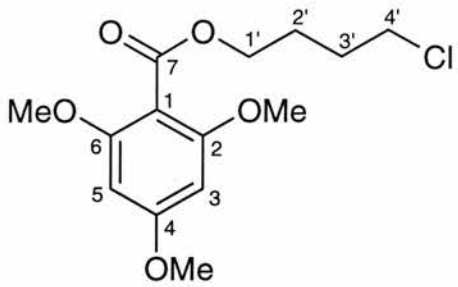
2,4,6-Trimethoxyacetophenone (**126**)



Attempted synthesis from 2,4,6-trimethoxybenzonitrile using methylmagnesium iodide

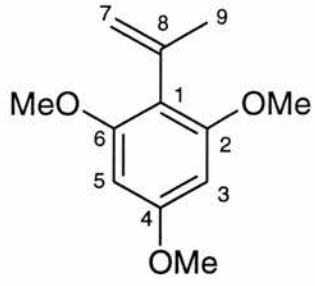
Methyl iodide (0.16 cm^3, 2.58 mmol) was added to a stirred suspension of magnesium (156 mg, 6.44 mmol) in diethyl ether (8 cm^3). Stirring was continued for 12 hours at room temperature allowing formation of the Grignard reagent. To this was added a solution of 2,4,6-trimethoxybenzonitrile (500 mg, 2.58 mmol) in diethyl ether (20 cm^3) and the reaction heated under reflux for 72 hours at which time t.l.c.(ethyl acetate : hexane, 1 : 1) suggested only starting material to be present. The reaction was cooled, HCl (40 cm^3 of a 1.0 mol dm^{-3} solution) was added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a brown solid. ^1H and ^{13}C n.m.r. spectroscopy revealed 2,4,6-trimethoxybenzonitrile as the only product.

Attempted synthesis from 2,4,6-trimethoxybenzoylchloride using methylmagnesium iodide. (Formation of 4'-chlorobutyl 2,4,6-trimethoxybenzoate (**129**))



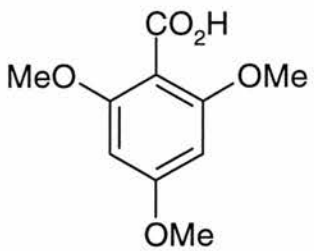
Methyl iodide (0.19 cm^3, 2.94 mmol) was added to a stirred suspension of magnesium (71 mg) in tetrahydrofuran (10 cm^3). Stirring was continued at room temperature for 12 hours allowing formation of the Grignard reagent. A solution of 2,4,6-trimethoxybenzoyl chloride (565 mg, 2.45 mmol) in tetrahydrofuran (20 cm^3) was added dropwise over a 5 minute period and the reaction heated to reflux for 3 hours. At this stage t.l.c. (ethyl acetate : hexane, 1 : 1) suggested the absence of starting material and the reaction was cooled to room temperature. HCl (70 cm^3 of a 1.0 mol dm^{-3} solution), water (80 cm^3) and ethyl acetate (40 cm^3) were added and the organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a red oil. Purification by column chromatography (silica, ethyl acetate : hexane, 1 : 4) yielded the product as a pale oil (415 mg, 56%); ν_{max} (nujol)/cm^{-1} 1750 (C=O); δ_H (300 MHz, $CDCl_3$) 6.09 (2H, s, H-3 and 5), 4.31 (2H, t, $J_{1,2}$ = 7.0, C$\underline{H}_2$-1'), 3.83 (3H, s, O$\underline{C}H_3$), 3.79 (6H, s, OC$\underline{H}_3$), 3.23 (2H, t, $J_{3,4}$ = 7.0, C$\underline{H}_2$-4'), 2.04-1.94 (2H, quintet, $J_{1,2} = J_{2,3}$ = 7.0, C$\underline{H}_2$-2'), 1.88-1.78 (2H, quintet, $J_{2,3} = J_{3,4}$ = 7.0, C$\underline{H}_2$-3'); δ_C (75.4 MHz, $CDCl_3$) 166.6 ($\underline{C}$=O), 162.7 (C-4), 158.8 (C-2 and 6), 106.2 (C-1), 90,7 (C-3 and 5), 63.7 (C-1'), 56.0 and 55.4 (3 x O$\underline{C}H_3$), 30.1 (C-4'), 29.7 (C-2'), 5.9 (C-3'); *m/z* (EI) 302 (M^+, 3%), 267 ([M - Cl]$^+$, 4), 195 ([M - C_4H_8OCl]$^+$, 100), 168 ([M - C_4H_8OCl - CH_3O]$^+$, 24), 137 ([M - C_4H_8OCl - CH_3O_2]$^+$, 10).

Attempted synthesis from 2,4,6-trimethoxybenzoylchloride using methylmagnesium bromide. (Formation of 2,4,6-trimethoxy-α-methylstyrene (**131**))



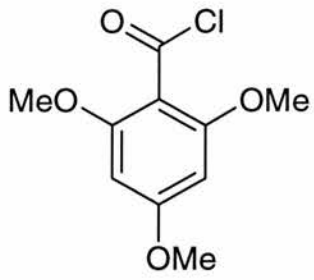
A solution of methylmagnesium bromide (0.43 cm^3 of a 3.0 mol dm^{-3} solution, 1.3 mmol) was added dropwise to a stirred solution of 2,4,6-trimethoxybenzoyl chloride (250 mg, 1.09 mmol) in diethyl ether (15 cm^3) over 5 minutes at room temperature. The reaction mixture was heated to reflux for 10 hours after which time t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the absence of starting material. The reaction was cooled to room temperature and HCl (50 cm^3 of a 1.0 mol dm^{-3} solution) and water (30 cm^3) were added sequentially. The aqueous layer was separated and concentrated at reduced pressure to leave a white solid. This was shown to be 2,4,6-trimethoxybenzoic acid (75 mg) by ^{13}C n.m.r. spectroscopy and mass spectrometry. The organic layer was dried ($MgSO_4$), concentrated at reduced pressure and the solid residue purified by column chromatography (silica, ethyl acetate : hexane, 1 : 3). This eluted a product (75 mg, 35%) as a white solid m.p. 62-65 °C; δ_H (200 MHz, $CDCl_3$) 6.17 (2H, s, H-3 and 5), 5.32 (1H, b, H-7a) 4.87 (1H, b, H-7b), 3.83 and 3.80 (3 x $OC\underline{H}_3$), 2.01 (3H, s, $C\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 160.5 (C-4), 158.3 (C-2 and 6), 139.6 (C-8), 116.5 (C-7), 114.6 (C-1), 91.2 (C-3 and 5), 56.4 and 55.7 ($O\underline{C}H_3$), 24.2 (C-9); *m/z* (CI) 209 ($[MH]^+$, 100%), 195 ($[C_{10}H_{11}O_4]^+$, 11).

Synthesis of 2,4,6-trimethoxybenzoic acid (**127**)



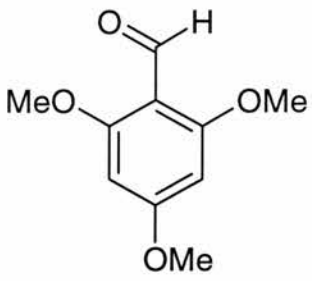
2,4,6-Trimethoxybenzonitrile (400 mg, 1.3 mmol) was suspended in aqueous sodium hydroxide (75 cm^3 of a 2.0 mol dm^{-3} solution) and stirred to reflux for 24 hours. At this stage, t.l.c. (ethyl acetate : hexane, 1 :1) suggested that starting material was absent and the reaction was cooled to room temperature. HCl (80 cm^3 of a 2.0 mol dm^{-3} solution, to pH 1-2) was added and the solvent was removed at reduced pressure to leave a mixture of salts and organic products. Tetrahydrofuran (40 cm^3) was added and the insoluble material removed by filtration. Concentration at reduced pressure yielded the desired product (190 mg, 43%) as a white solid m.p. 182-5 °C (Lit.,[169] 151 °C); ν_{max} (nujol)/cm^{-1} 3150 (br, OH), 1680 (br, C=O); δ_H (200 MHz, d^6-DMSO) 6.23 (2H, s, H-3, 5), 3.80 (3H, s, $OC\underline{H}_3$), 3.74 (6H, s, $OC\underline{H}_3$); δ_C (50.31 MHz, d^6-DMSO) 167.3 ($\underline{C}$=O), 162.2 (C-4), 157.9 (C-2 and 6), 107.8 (C-1), 91.3 (C-3 and 5), 56.3, 56.0 ($O\underline{C}H_3$); *m/z* (CI) 212 ($[MH]^+$, 100%), 195 ($[MH - OH]^+$, 7).

Synthesis of 2,4,6-trimethoxybenzoyl chloride (**128**)[170]



Oxalyl chloride (1.0 cm^3, mmol) was added to a stirred solution of 2,4,6-trimethoxybenzoic acid (400 mg, 1.89 mmol) in tetrahydrofuran (40 cm^3) in a nitrogen atmosphere. Stirring was continued at room temperature for 18 hours and at this stage t.l.c. (ethyl acetate : hexane, 1 : 1) suggested the absence of starting material. The reaction mixture was concentrated at reduced pressure to leave the desired product (350 mg, 81%) which was used without further purification ν_{max} (nujol)/cm^{-1} 1890 (C=O), 880 (COCl); δ_H (200 MHz, d^6-DMSO); 6.21 (2H, s, H-3 and 5), 3.76 (3H, s, $OC\underline{H}_3$), 3.71 (6H, s, $OC\underline{H}_3$); δ_C (50.31 MHz, d^6-DMSO); 167.2 ($\underline{C}$=O), 162.2 (C-4), 157.9 (C-2 and 6), 107.8 (C-1), 91.3 (C-3 and 5) 56.2, 55.9 ($O\underline{C}H_3$); *m/z* (CI) 230, 232 ($[MH]^+$, 26, 65%), 195 ($[MH - Cl]^+$, 100).

Attempted synthesis of 2,4,6-trimethoxybenzaldehyde (**132**)



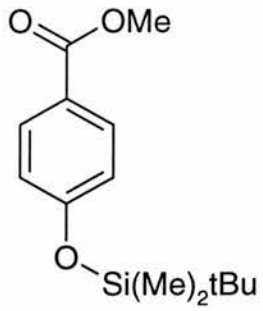
Using Raney Ni-Al alloy in formic acid[148]

Raney Ni-Al alloy (380 mg, 2.6 mmol) was added to stirred solution of 2,4,6-trimethoxybenzonitrile (500 mg, 2.6 mmol) in formic acid (15 cm^3 of a 75% solution). The mixture was heated at reflux for 18 hours and was then cooled to room temperature. Removal of the solid was performed over a bed of celite and the filtrate was added to water (60 cm^3) and ethyl acetate (40 cm^3). The organic layer was separated, dried (MgSO4) and concentrated at resuced pressure to leave a solid. T.l.c. (ethyl acetate : hexanes, 1 : 1) showed that only starting material was present.

Using stannous chloride in diethyl ether[149]

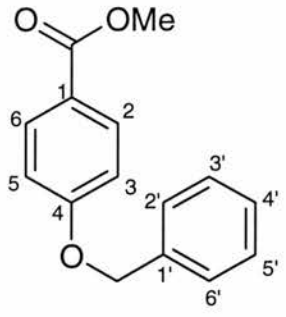
A solution of anhydrous stannous chloride (500 mg, 2.55 mmol) in diethyl ether (12cm^3) was saturated with a stream of HCl gas. At this stage a solution of 2,4,6-trimethoxybenzonitrile (500 mg, 2.6 mmol) in dichloromethane (3 cm^3) was added and the mixture stirred at room temperature for one hour. A precipitate formed and was removed by filtration, hydrolyzed by boiling water and added to dichloromethane (10 cm^3). The organic layer was removed, dried ($MgSO_4$) and concentrated at reduced pressure to leave an off-white solid. ^{13}C n.m.r. spectroscopy showed that only the starting material was present.

Synthesis of methyl 4-[(*tert*-butyldimethylsilyl)oxy]benzoate (**134**)[171]



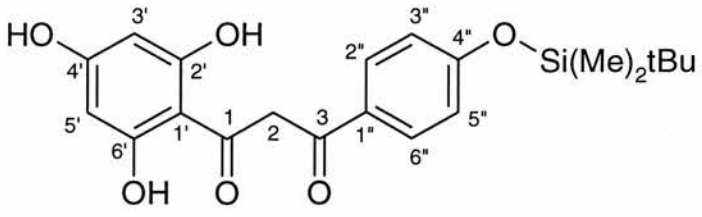
N,N-Diisopropylethylamine (6.50 g, 50 mmol) was added dropwise to a stirred solution of methyl 4-hydroxy benzoate (3.80 g, 25 mmol) in dimethylformamide (60 cm^3) at 0 °C over 5 minutes. *tert*-Butyldimethylsilyl chloride (4.22 g, 28 mmol) was added over 20 minutes at 0 °C and stirring was continued at room temperature for 4 hours. At this stage t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the absence of starting material and the mixture was poured onto ice (100 cm^3). Diethyl ether (60 cm^3) was added the organic layer was separated, dried ($MgSO_4$) and concentrated to leave the desired product (6.20 g, 93%) as a pale oil ν_{max} (nujol)/cm^{-1} 1730 (C=O); δ_H (300 MHz, $CDCl_3$) 7.96 (2H, d, $J_{2,3} = J_{5,6}$ = 8.6, H-2 and 6), 6.86 (2H, d, $J_{2,3} = J_{5,6}$ = 8.6, H-3 and 5), 3.88 (3H, s, $OC\underline{H}_3$), 0.99 (9H, s, $C(C\underline{H}_3)_3$), 0.23 (6H, s, $C\underline{H}_3$); δ_C (75.4 MHz, $CDCl_3$) 167.11 ($\underline{C}$=O), 160.28 (C-4), 131.76 (C-2 and 6), 123.47 (C-1), 120.06 (C-3 and 5), 52.04 ($O\underline{C}H_3$), 25.80 ($C(\underline{C}H_3)_3$), 18.43 ($\underline{C}(CH_3)_3$), -4.20 ($Si(\underline{C}H_3)_2$); *m/z* (EI) 266 (M^+, 100%).

Synthesis of methyl 4-benzyoxy benzoate (**135**)



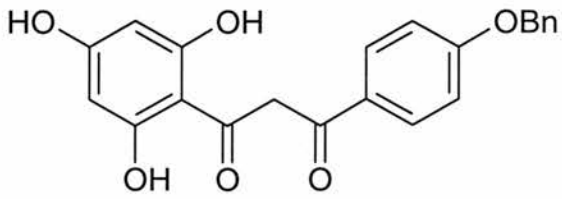
Potassium carbonate (20 g) was added to a stirred solution of methyl 4-hydroxy benzoate (5.0 g, 33 mmol) and benzyl bromide (6.2 g, 4.31 cm^3, 36.3 mmol) in acetone (50 cm^3). Stirring was continued to reflux for 5 hours after which time t.l.c. (ethyl acetate : hexane, 1 : 1) showed the reaction had gone to completion. The reaction mixture was cooled to room temperature and the potassium carbonate was removed by filtration. The reaction mixture was concentrated at reduced pressure and water (50 cm^3) and diethyl ether (50 ml) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave a white solid (8.04 g, 100%); (Found: M^+, 242.095205. $C_{15}H_{14}O_3$ requires *M*, 242.094294); m.p. 96-100 °C (Lit.,[172] 99 °C); ν_{max} (nujol)/cm^{-1} 1730 (C=O); δ_H (200 MHz, $CDCl_3$) 8.00 (2H, d, $J_{2,3} = J_{5,6}$ = 8.8, H-2 and 6), 7.46-7.36 (5H, m, H-2', 3', 4', 5' and 6'), 7.00 (2H, d, $J_{2,3} = J_{5,6}$ = 8.8, H-3 and 5), 5.12 (2H, s, C$\underline{H}_2$), 3.89 (3H, s, OC$\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 167.30 (C-7), 162.97 (C-4), 136.75 (C-1'), 132.10 (C-2, 6), 129.16 (C-3' and 5'), 128.69 (C-4'), 127.98 (C-2' and 6'), 123.33 (C-1), 114.95 (C-3, 5), 70.58 (C-7'), 52.35 (O$\underline{C}H_3$); *m/z* (EI) 242 (M^+, 12%), 91 ($[C_7H_7]^+$, 100), 65 ($[C_5H_5]^+$, 12).

Attempted synthesis of 1,3-dioxo-1-(2',4',6'-trihydroxy)-3-(4"-[*t*-butyldimethylsilyl]oxyphenyl)-propane (**136**)[139]



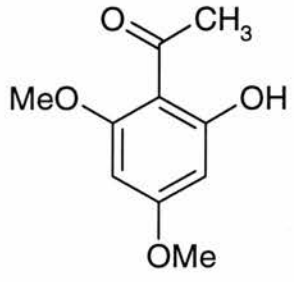
A solution of LiHMDS (6.75 cm^3 of a 1.0 mol dm^{-3} solution in tetrahydrofuran, 6.75 mmol) was added dropwise to a stirred solution of 2,4,6-trihydroxyacetophenone (250 mg, 1.35 mmol) in tetrahydrofuran (12 cm^3) at -78 °C. Stirring was continued for 1 hour at this temperature and then for 2 hours at -10 °C. At this stage the temperature was lowered to -78 °C and a solution of methyl 4-[*t*-butyldimethylsilyl]oxybenzoate (330 mg, 1.35 mmol) in tetrahydrofuran (5 cm^3) was added dropwise and the resulting solution stirred for 18 hours at room temperature. Concentrated HCl (0.4 cm^3), ice (150 g) and dichloromethane (40 cm^3) were added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure. Examination by t.l.c (ethyl acetate : hexane, 1 : 1) revealed only starting materials to be present.

Attempted synthesis of 1,3-dioxo-1-(2',4',6'-trihydroxy)-3-(4''-benzyloxyphenyl)propane (**137**)[139] Synthesis of *p*-benzyloxybenzoic acid (**138**)



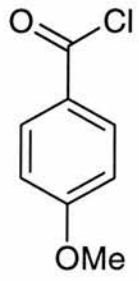
A solution of LiHMDS (13.5 cm^3 of a 1.0 mol dm^{-3} solution in tetrahydrofuran, 13.5 mmol) was added dropwise to a stirred solution of 2,4,6-trihydroxyacetophenone (500 mg, 2.7 mmol) in tetrahydrofuran (20 cm^3) at -78 °C. Stirring was continued for 1 hour at this temperature and then for 2 hours at -10 °C. At this stage the temperature was lowered to -78 °C and a solution of methyl 4-benzyloxybenzoate (653 mg, 2.7 mmol) in tetrahydrofuran (5 cm^3) was added dropwise over 5 minutes. The resulting solution was stirred for 18 hours at room temperature and concentrated HCl (0.4 cm^3), ice (150 g) and ethyl acetate (40 cm^3) were added and the organic layer separated, dried ($MgSO_4$) and concentrated at reduced pressure. The residue was dried over P_2O_5 at reduced pressure for 12 hours and the crude solid purified by column chromatography (silica; ethyl acetate : hexane, 1 : 1). This gave an off-white coloured powder (152 mg), identified as 4-benzyloxybenzoic acid as the only product m.p. 238-241°C (Lit.,[173] 193-195 °C); ν_{max} (nujol)/cm^{-1} 1710 (OH); δ_H (200 MHz, d^6-DMSO) 7.91 (2H, d, $J_{2,3} = J_{5,6} = 8.3$, H-2 and 6), 7.47-7.40 (5H, m, H-1', 2', 3', 4' and 5'), 7.12 (2H, d, $J_{2,3} = J_{5,6} = 8.3$, H-3 and 5); δ_C (50.31 MHz, d^6-DMSO) 167.2 (C=O), 162.2 (C-4), 136.8 (C-1'), 131.6 (C-2 and 6), 128.8 (C-2' and 6'), 128.3 (C-4'), 128.1 (C-3' and 5'), 123.2 (C-1), 114.9 (C-3 and 5), 69.7 (CH_2); *m/z* (EI) 228 (M^+, 19%), 91 ($[C_7H_7]^+$, 100), 65 ($[C_5H_5]^+$, 13).

Synthesis of 2,4-dimethoxy-6-hydroxyacetophenone (**139**)[90]



A solution of boron tribromide (10.95 cm^3 of a 0.1 mol dm^{-3} solution in dichloromethane, 1.095 mmol,) was added to a stirred solution of 2,4,6-trimethoxyacetophenone (200 mg, 0.95 mmol) in dichloromethane (15 cm^3) at 0 °C over 10 minutes. Stirring was continued for 15 hours at room temperature after which time t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the reaction had gone to completion. Ice (50 cm^3) was added and the organic layer separated, dried ($MgSO_4$) and concentrated to leave a red solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 3) yielded the desired product (174 mg, 93 %) as a white solid (Found: C, 61.49; H, 6.24%; M^+, 196.073332. $C_{10}H_{12}O_4$ requires C, 61.22; H, 6.16; *M*, 196.073559); m.p. 77-78 °C (Lit.,[174] 83 °C); ν_{max} (nujol)/cm^{-1} 3270 (OH); δ_H (300 MHz, $CDCl_3$) 14.02 (1H, s, OH), 6.06 (1H, d, $J_{3,5}$ = 2.47, H-5), 5.93 (1H, d, $J_{3,5}$ = 2.47, H-3), 3.86 (3H, s, OCH_3), 3.83 (3H, s, $OC\underline{H}_3$), 2.61 (3H, s, $C\underline{H}_3$); δ_C (75.4 MHz, $CDCl_3$) 203.6 ($\underline{C}$=O), 168.0 (C-2), 166.5 (C-6), 163.3 (C-4), 106.3 (C-1), 93.7 (C-3), 91.0 (C-5), 55.7, 55.6 ($O\underline{C}H_3$), 33.0 ($\underline{C}H_3$); *m/z* (CI) 197 ($[MH]^+$, 100%), 181 ($[MH - CH_3]^+$, 6).

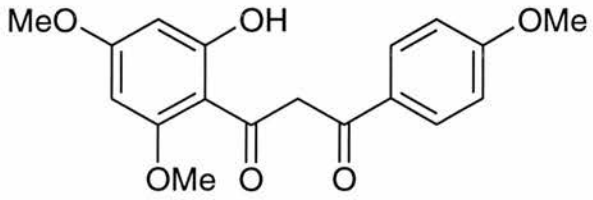
Synthesis of *p*-methoxybenzoyl chloride (**140**)



Oxalyl chloride (0.70 cm^3, 8 mmol) was added to a stirred solution of *p*-methoxybenzoic acid (600 mg, 3.94 mmol) in tetrahydrofuran (20 cm^3) at room temperature. Stirring was continued for 18 hours and t.l.c. (ethyl acetate, 1 : 1) at this stage showed the reaction had gone to completion. The reaction mixture was concentrated at reduced pressure to leave *p*-methoxybenzoyl chloride as a pale oil ν_{max} (nujol)/cm^{-1} 1820 (C=O), 870 (COCl); δ_H (200 MHz, $CDCl_3$) 8.04 (2H, d, $J_{2,3} = J_{5,6} = 8.8$, H-2 and 6), 6.95 (2H, d, $J_{2,3} = J_{5,6} = 8.8$, H-3 and 5), 3.90 (3H, s, $OC\underline{H}_3$); δ_C (50.31 MHz, $CDCl_3$) 167.7 ($\underline{C}$=O), 165.3 (C-4), 134.2 (C-2 and 6), 136.0 (C-1), 114.1 (C-3 and 5), 57.3 ($O\underline{C}H_3$); *m/z* (CI) 170, 172 ($[MH]^+$, 15, 10%), 135 ($[MH - Cl]^+$, 100), 77 ($[C_6H_5]^+$, 17), 65 ($[C_5H_5]^+$, 10).

Synthesis of 1,3-dioxo-1-(2'-hydroxy-4',6'-dimethoxy)-3-(4"-methoxyphenyl)-propane

(141)



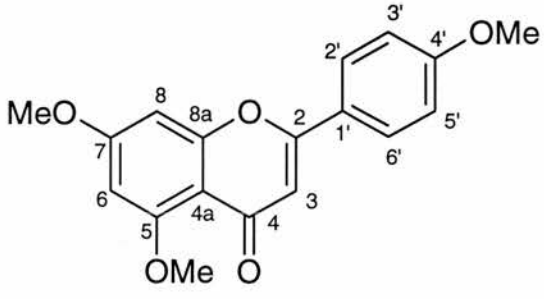
Using LDA as base and tetrahydrofuran[140]

LDA (1.8 cm^3 of a 2.0 mol dm^{-3} solution in tetrahydrofuran, 3.6 mmol) was added to a stirred solution of 2-hydroxy-4,6-dimethoxyacetophenone (320 mg, 1.6 mmol) in tetrahydrofuran (10 cm^3) at room temperature. Stirring was continued for one hour and the reaction mixture was then cooled to -78 °C. A solution of *p*-anisoyl chloride (400 mg, 1.76 mmol) was added in one portion and stirring continued for 3 hours followed by 2 hours at room temperature. The solution was re-cooled to -78 °C and further LDA (1.2 cm^3, 2.4 mmol) was added. Stirring was then continued for 12 hours at room temperature and t.l.c. (ethyl acetate : hexane, 1 : 1) revealed the absence of starting material. Ethyl acetate (50 cm^3), water (60 cm^3) and HCl (15 cm^3 of a 1.0 mol dm^{-3} solution, to pH 3) were added sequentially. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 2) yielded a yellow solid which, upon analysis by t.l.c. and ^1H and ^{13}C n.m.r. spectroscopy, proved to be a mixture of *p*-methoxybenzoic acid in addition to the desired product.

Using potassium hydroxide as base in pyridine[141]

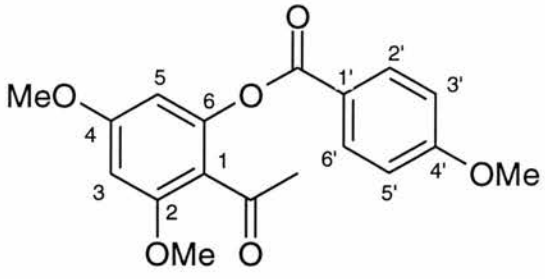
To a solution of 4,6-dimethoxy-2-(4'-methoxybenzyloxy)acetophenone (400 mg, 1.21 mmol) in pyridine (2 cm^3) was added added powdered potassium hydroxide (169 mg, 2.02 mmol) and the solution was heated to 100 °C in oil bath with magnetic stirring for 10 minutes. The mixture was cooled to room temperature and treated with glacial acetic acid (0.7 cm^3) which gave a thick yellow paste. The mixture was diluted with ethanol (1.5 cm^3) and water (1.5cm^3) to give a homogenous solution. After cooling to 0 °C, the product crystallized as yellow prisms. The crystals were collected by filtration and were rinsed with 50% ethanol (2 cm^3) to leave the pure product (330 mg, 83%) as a yellow powder (Found: M^+, 331.118618. $C_{18}H_{19}O_6$ requires *M*, 331.118164); m.p. 132-134 °C (Lit.,[141] 132-133 °C); δ_H (300 MHz, $CDCl_3$) 7.17 (2H, d, $J_{2',3'}$, = $J_{5',6'}$ = 8.6, H-2' and 6'), 6.82 (2H, d, $J_{2',3'} = J_{5',6'} = 8.6$, H-3' and 5'), 6.32 (1H, d, $J_{3,5} = 2.2$, H-5), 6.28 (1H, d, $J_{3,5}$ = 2.2, H-3); δ_C (75.4 MHz, $CDCl_3$) δ_C (50.4 MHz, $CDCl_3$) 199.5 (C=O), 193.9 (COCHCOH), 168.4 (C-4'), 167.1 (C-6'), 164.1 (C-4''), 162.5 (C-2'), 130.9 (C-2'' and 6''), 130.7 (C-1'') 114.4 (C-3'' and 5''), 106.2 (C-1'), 94.3 (C-3), 91.4 (C-5), 56.0, 55.4, 54.9 (9H, 3 x OCH_3); *m/z* (CI) 331 ($[MH]^+$, 66%), 181 ($[C_9H_8O_4]^+$, 12), 135 ($[C_8H_7O_2]^+$, 6).

Synthesis of apigenin trimethyl ether (**142**)[141]



A suspension of 1,3-dioxo-1-(2'-hydroxy-4',6'-dimethoxy)-3-(4''-methoxyphenyl)-propane (150 mg) in acetic acid (1.6 cm^3) was heated to 100 °C with magnetic stirring in an oil bath. To this suspension was added 20% sulfuric acid in glacial acetic acid (0.35 cm^3) and the mixture was stirred at 100 °C for 10 minutes. The mixture was poured onto water (8 cm^3) giving a pale yellow gelatinous solid. The solid was collected by filtration to leave a brown solid (84 mg, 60%) (Found: M^+, 313.108248. $C_{18}H_{17}O_5$ requires *M*, 313.107599); 151-153 °C (Lit.,[175] 156 °C); ν_{max} (thin film)/cm^{-1} 3410 (OH), 1620 (C=O); δ_H (300 MHz, d^6-DMSO) 8.09 (2H, d, $J_{2',3'} = J_{5',6'} = 8.9$, H-2' and 6'), 7.21 (2H, d, $J_{2',3'} = J_{5',6'} = 8.9$, H-3' and 5'), 6.94 (1H, d, $J_{6,8} = 2.3$, H-6), 6.73 (1H, s, H-3), 6.61 (1H, d, $J_{6,8} = 2.3$, H-8), 4.03, 3.98, 3.96 ($OC\underline{H}_3$); δ_C (75.4 MHz, d^6-DMSO) 175.6 (C-4), 163.8 (C-7), 161.8 (C-4'), 160.3 (C-2), 159.8 (C-8a), 159.1 (C-5), 127.7 (C-2' and 6'), 123.1 (C-1'), 114.5 (C-3' and 5'), 106.8 (C-4a), 96.3 (C-6), 93.5 (C-8), 56.1, 55.9, 55.5 ($O\underline{C}H_3$); *m/z* (CI) 313 ($[MH]^+$, 100%).

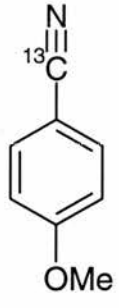
Synthesis of 4,6-dimethoxy-2-(4'-methoxybenzyloxy)acetophenone (**143**)[141]



To a solution of 2,4-dimethoxy-6-hydroxyacetophenone (250 mg, 1.27 mmol) in pyridine (3 cm^3) was added *p*-anisoyl chloride (216 mg, 1.27 mmol) and the solution heated at 100 °C for 10 minutes with magnetic stirring in an oil bath. The solution was cooled to room temperature and diluted with ethanol (4 cm^3) and water (4 cm^3). The mixture was cooled

to 0 °C in an ice bath and after scratching with a glass rod produced white plates. These were collected on a Büchner funnel and rinsed with cold 50% ethanol to leave the pure product (345 mg, 82%) (Found: M^+, 331.120151. $C_{18}H_{19}O_6$ requires *M*, 331.118164); m.p. 88-89 °C (Lit.,[141] 97-98 °C); δ_H ($CDCl_3$, 200 MHz) 8.08 (2H, d, $J_{2',3'} = J_{5',6'} = 8.3$, H-2 and 6), 6.95 (2H, d, $J_{2',3'} = J_{5',6'}$ = H-3 and 5), 6.38 (1H, s, H-3), 6.35 (1H, s, H-5), 3.86, 3.84 and 3.81 (9H, s, -OC$\underline{H}_3$), 2.46 (3H, s, -C$\underline{H}_3$); δ_C ($CDCl_3$, 50.31 MHz) 200.0 ($\underline{C}OCH_3$), 165.2 (C-4), 164.5 (C-4'), 162.6 (O$\underline{C}$OPh), 159.5 (C-2), 150.3 (C-6), 132.9 (C-2' and 6'), 121.9 (C-1'), 114.3 (C-3' and 5'), 100.6 (C-3), 97.0 (C-5), 93.9 (C-1), 56.4, 56.3 and 56.1 (3 x -OCH_3), 32.5 (-CH_3); *m/z* (CI) 331 ($[MH]^+$, 31%), 197 ($[C_{10}H_{13}O_4]^+$, 100), 153 ($[C_8H_9O_3]^+$, 20), 135 ($[C_8H_7O_2]^+$, 85).

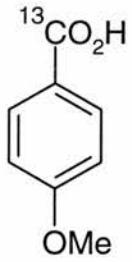
Synthesis of 4-methoxybenzo[^{13}C]nitrile (**144**)



Potassium [^{13}C]cyanide (1.0 g, 15 mmol) was added to a stirred solution of *p*-iodoanisole (3.54 g, 15 mmol), calcium hydroxide (600 mg, 7.5 mmol) and palladium acetate (560 mg, 2.4 mmol) in dimethylformamide (75 cm^3). Stirring was continued for 8 hours to reflux after which time t.l.c. (ethyl aceate : hexane, 4 : 1) revealed the absence of starting material. The reaction mixture was cooled to room temperature and filtered through a bed

of celite to leave a pale solution. The solvent was removed at reduced pressure and water (60 cm^3) and diethyl ether (50 cm^3) were added. The organic layer was separated, dried ($MgSO_4$) and concentrated at reduced pressure to leave an off-white solid. Purification by column chromatography (silica; ethyl acetate : hexane, 1 : 1) yielded the desired product (1.30 g, 62%) as a white solid (Found: C, 71.92; H, 5.28; N, 10.41%; M^+, 134.056507. $^{12}C_7{}^{13}CH_7NO$ requires C, 71.62; H, 5.23; N, 10.44; *M*, 134.056119); m.p. 57-60 °C (Lit.,[176] 60 °C); ν_{max} (nujol)/cm^{-1} 2240 (CN); δ_H (300 MHz, $CDCl_3$) 7.57 (2H, dd, $J_{2,3} = J_{5,6} = 8.8$ and $J = 6.4$ (^{13}C-^1H coupling), H-2, 6), 6.93 (2H, d, $J_{2,3} = J_{5,6} = 8.8$, H-3, 5), 3.84 (3H, s, $OC\underline{H}_3$); δ_C (75.4 MHz, $CDCl_3$) 163.1 (C-4), 134.1 (C-2 and 6), 119.3 (enhanced, $\underline{C}N$), 114.9 (C-3 and 5), 104.6 (C-1), 55.6 ($O\underline{C}H_3$); *m/z* (EI) 134 (M^+, 100%), 119 ($[M - CH_3]^+$, 7), 104 ($[M - CH_3O]^+$, 24), 91 ($[C_7H_7]^+$, 36), 65 ($[C_5H_5]^+$,10).

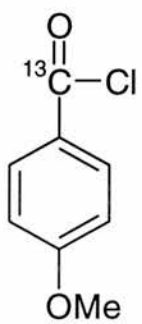
Synthesis of *p*-methoxy[^{13}C]benzoic acid (**145**)



A solution of 4-methoxybenzo[^{13}C]nitrile (1.0 g, 7.5 mmol) in sodium hydroxide (75 cm^3 of a 2.0 mol dm^{-3} solution) was stirred to reflux for 4 hours. The reaction mixture was cooled to room temperature and t.l.c. (ethyl acetate : hexane, 1 : 1) revealed no starting material to be present. HCl (80 cm^3 of a 2.0 mol dm^{-3} solution) and diethyl ether (2 x 50 cm^3) were added sequentially and the organic layers were separated and dried ($MgSO_4$). The combined extracts were concentrated to leave the desired product (790 mg, 70%) as

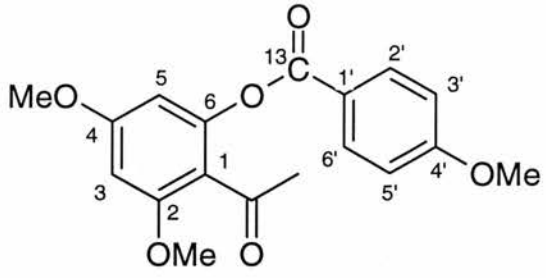
a white solid (Found: M^+, 153.051106. $^{12}C_7{}^{13}CH_8O_3$ requires *M*, 153.050699); m.p. 186-187 °C (Lit.,[177] 180-185 °C); ν_{max} (nujol)/cm^{-1} 3250 (OH) 1690 (C=O); δ_H (300 MHz, d^6-DMSO) 7.89 (2H, dd, $J_{2,3} = J_{5,6} = 8.9$ and $J = 3.9$ (^{13}C-^1H coupling), H-2 and 6), 7.00 (2H, d, $J_{2,3} = J_{5,6} = 8.9$, H-3 and 5), 3.81 (3H, s, O$\underline{C}H_3$); δ_C (75.4 MHz, d^6-DMSO) 167.3 (enhanced, $\underline{C}$=O), 163.0 (C-4), 131.4 (C-2 and 6), 113,9 (C-3 and 5), 113.85 (C-1), 55.4 (O$\underline{C}H_3$); *m/z* (EI) 153 (M^+, 100%), 136 ([M - OH]$^+$, 96), 107 ([M - $^{13}CO_2H]^+$, 10), 92 ([M - $^{13}CO_2H$ - $CH_3]^+$, 11), 77 ([M - $^{13}CO_2H$ - $CH_3O]^+$,17).

Synthesis of *p*-methoxy[^{13}C]benzoyl chloride (**146**)



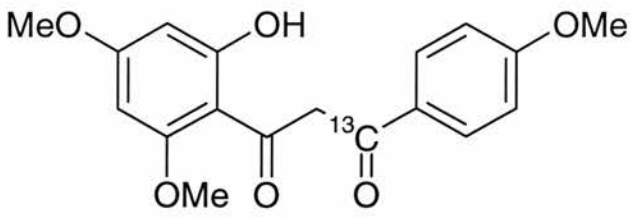
Oxalyl chloride (0.21 cm^3, 2.7 mmol) was added to a stirred solution of *p*-methoxy[^{13}C]benzoic acid (375 mg, 2.45 mmol) in tetrahydrofuran (20 cm^3) at room temperature. Stirring was continued for 18 hours and t.l.c. (ethyl acetate, 1 : 1) at this stage showed the reaction had gone to completion. The reaction mixture was concentrated at reduced pressure to leave *p*-methoxy[^{13}C]benzoyl chloride as a pale oil (380 mg, 91%) and was used without further purification.

Synthesis of 4,6-dimethoxy-2-(4'-methoxy [^{13}C]benzyloxy)acetophenone (**147**)[141]



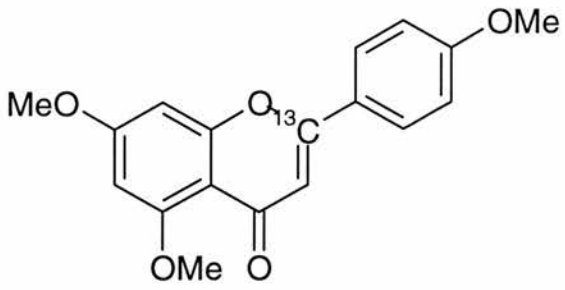
To a solution of 2,4-dimethoxy-6-hydroxyacetophenone (825 mg, 4.21 mmol) in pyridine (2.5 cm^3) was added *p*-[^{13}C]anisoyl chloride (720 mg, 4.21 mmol) and the solution heated at 100 °C for 10 minutes with magnetic stirring in an oil bath. The solution was cooled to room temperature and diluted with ethanol (4 cm^3) and water (4 cm^3). The mixture was cooled to 0 °C in an ice bath and after scratching with a glass rod produced white plates. These were collected on a Büchner funnel and rinsed with cold 50% ethanol to leave only a small amount of solid material. The solvent was removed at reduced pressure and the solid was purified by column chromatography (silica; ethyl acetate : hexanes, 1 : 2). This gave an the desired product (1.20 g, 86%) as a white powder (Found: M^+, 332.122168. $^{12}C_{17}{}^{13}C_1H_{19}O_6$ requires *M*, 332.121518); δ_H ($CDCl_3$, 200 MHz) 8.08 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.3, H-2 and 6), 6.95 (2H, d, $J_{2',3'} = J_{5',6'}$ = H-3 and 5), 6.38 (1H, s, H-3), 6.35 (1H, s, H-5), 3.86, 3.84 and 3.81 (9H, s, -OC<u>H</u>$_3$), 2.46 (3H, s, -C<u>H</u>$_3$); δ_C ($CDCl_3$, 50.31 MHz) 200.0 (<u>C</u>OCH$_3$), 165.2 (C-4), 164.5 (C-4'), 162.6 (O<u>C</u>OPh, enhanced), 159.5 (C-2), 150.3 (C-6), 132.9 (C-2' and 6'), 121.9 (C-1'), 114.3 (C-3' and 5'), 100.6 (C-3), 97.0 (C-5), 93.9 (C-1), 56.4, 56.3 and 56.1 (3 x -O<u>C</u>H$_3$), 32.5 (-<u>C</u>H$_3$); *m/z* (CI) 332 ([MH]$^+$, 53%), 197 ([$C_{10}H_{13}O_4$]$^+$, 55), 154 ([$C_8H_{10}O_3$]$^+$, 19), 136 ([$C_8H_8O_2$]$^+$, 100).

Synthesis of 1,3-dioxo-1-(2'-hydroxy-4',6'-dimethoxy)-3-(4''-methoxyphenyl)-[3-^{13}C]propane (**148**)[141]



To a solution of 4,6-dimethoxy-2-(4'-methoxy [^{13}C]benzyloxy)acetophenone (890 mg, 2.69 mmol) in pyridine (2.5 cm^3) was added powdered potassium hydroxide (380 mg, 6.77 mmol) and the solution was heated to 100 °C in oil bath with magnetic stirring for 10 minutes. The mixture was cooled to room temperature and treated with glacial acetic acid (0.7 cm^3) which gave a thick yellow paste. The mixture was diluted with ethanol (1.5 cm^3) and water (1.5 cm^3) to give a homogenous solution. After cooling to 0 °C, the solvent was removed at reduced pressure. The residue was dried (P_2O_5) for 16 hours, giving the crude product (1.89 g) as a yellow powder δ_H (200 MHz, $CDCl_3$) 7.94 (2H, d, $J_{2',3'}$, = $J_{5',6'}$ = 8.8, H-2' and 6'), 6.97 (2H, d, $J_{2',3'} = J_{5',6'}$ = 8.8, H-3' and 5'), 6.08 (1H, d, $J_{3,5}$ = 2.1, H-5), 5.83 (1H, d, $J_{3,5}$ = 2.1, H-3), 3.89, 3.86, 3.80 (9H, 3 x s, –OC<u>H</u>$_3$), 3.46 (1H, s, COC<u>H</u>COH); δ_C (50.4 MHz, $CDCl_3$) 199.5 (<u>C</u>=O), 193.9 (COCH<u>C</u>OH, enhanced), 168.4 (C-4'), 167.1 (C-6'), 164.1 (C-4''), 162.5 (C-2'), 130.9 (C-2'' and 6''), 130.7 (C-1'') 114.4 (C-3'' and 5''), 106.2 (C-1'), 94.3 (C-3), 91.4 (C-5), 56.0, 55.4, 54.9 (9H, 3 x OC<u>H</u>$_3$); *m/z* (CI) 332 ($[MH]^+$, 2%), 154 ($[C_8H_{10}O_3]^+$, 100), 136 ($[C_8H_8O_2]^+$, 11).

Synthesis of [2-^{13}C]apigenin trimethyl ether (**149**)[141]



A suspension of 1,3-dioxo-1-(2'-hydroxy-4',6'-dimethoxy)-3-(4"-methoxyphenyl)-[3-^{13}C]propane (1.55 g) in acetic acid (6.3 cm^3) was heated to 100 °C with magnetic stirring in an oil bath. To this suspension was added 20% sulfuric acid in glacial acetic acid (1.4 cm^3) and the mixture was stirred at 100 °C for 10 minutes. The solvents were removed at reduced pressure to leave a gummy green solid. Acetone (40 cm^3) was then added and the insoluble product isolated by filtration giving a pale yellow gelatinous solid. This was then washed in water (20 cm^3) to leave a dark brown solid. Recrystalization from acetone (40 cm^3) left the desired product (240 mg, 29% from the ester **145**) as a light brown solid (Found: M^+, 314.110183. $^{12}C_{17}{}^{13}C_1H_{17}O_5$ requires *M*, 314.110954); m.p. 121-123 °C (Lit.,[175] 156°C); δ_H (300 MHz, d^6-DMSO) 8.09 (2H, d, $J_{2',3'} = J_{5',6'} = 8.9$, H-2' and 6'), 7.21 (2H, d, $J_{2',3'} = J_{5',6'} = 8.9$, H-3' and 5'), 6.94 (1H, d, $J_{6,8} = 2.3$, H-6), 6.73 (1H, s, H-3), 6.61 (1H, d, $J_{6,8} = 2.3$, H-8), 4.03, 3.98, 3.96 (OC$\underline{H}_3$); δ_C (75.4 MHz, d^6-DMSO) 175.6 (C-4), 163.8 (C-7), 161.8 (C-4'), 160.3 (C-2, enhanced), 159.8 (C-8a), 159.1 (C-5), 127.7 (C-2' and 6'), 123.1 (C-1'), 114.5 (C-3' and 5'), 106.8 (C-4a), 96.3 (C-6), 93.5 (C-8), 56.1, 55.9, 55.5 (O$\underline{C}H_3$); *m/z* (CI) 314 ($[MH]^+$, 100%).

Chapter 7

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