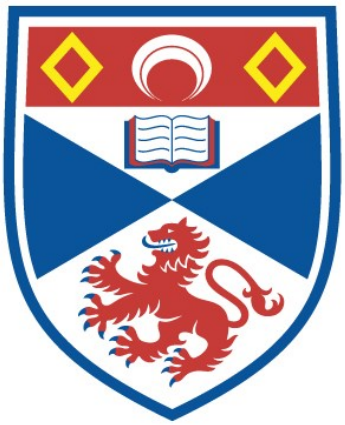
# SYNTHESIS AND ANTI-TUMOUR ACTIVITY OF SOME NEW DERIVATIVES OF FLAVONE - 8 - ACETIC ACID

David William Joseph Wilson

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1995

# Synthesis and Anti-tumour Activity of Some New Derivatives of Flavone-8-acetic Acid

by

DAVID WILLIAM JOSEPH WILSON

Thesis presented for the degree of
DOCTOR OF PHILOSOPHY

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mB776.

To Mum and Dad

"Now consider the tortoise and the eagle.

The tortoise is a ground-living creature. It is impossible to live nearer the ground without being under it. Its horizons are a few inches away. It has about as good a turn of speed as you need to hunt down a lettuce. It has survived while the rest of evolution flowed past it by being, on the whole, no threat to anyone and too much trouble to eat.

And then there is the eagle. A creature of the air and high places, whose horizons go all the way to the edge of the world. Eyesight keen enough to spot the rustle of some small and squeaky creature half a mile away. All power, all control. Lightning death on wings. Talons and claws enough to make a meal of anything smaller than it is and at least take a hurried snack of anything bigger.

And yet the eagle will sit for hours on the crag and survey the kingdoms of the world until it spots a distant movement and then it will focus, focus, *focus* on the small shell wobbling among the bushes down there on the desert. And it will *leap* ...

And a minute later the tortoise finds the world dropping away from it. And it sees the world for the first time, no longer one inch from the ground but five hundred feet above it, and it thinks: what a great friend I have in the eagle.

And then the eagle lets go.

And almost always the tortoise plunges to its death. Everyone knows why the tortoise does this. Gravity is a habit that is hard to break off. No one knows why the eagle does this. There's good eating on a tortoise but, considering the effort involved, there's much better eating on practically anything else. It's simply the delight of eagles to torment tortoises.

But of course, what the eagle does not realise is that it is participating in a very crude form of natural selection.

One day the tortoise will learn how to fly."

Small Gods by Terry Pratchett

# Declaration

I, David William Joseph Wilson, hereby certify that this thesis has been composed by myself, it is a record of my own work and has not been accepted in partial of complete fulfilment of any other degree or professional qualification

Signed ..... Date 12/5/95

I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12 on October 1st 1991 and as a candidate for the degree of Ph.D. on October 1st 1992.

Signed ..... Date 12/5/95

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the Degree of Ph.D.

Signature of supervisor . ..... Date 12th May 1995

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# Lecture Courses

The following is a statement of courses attended during the period of research; Organic Research Seminars (3 years attendance); Theoretical Aspects of Drug Design, Dr. C. Thompson (5 lectures); Molecular Rearrangements, Dr. J. C. Walton (5 lectures); Advanced NMR, Dr. F. Riddell (5 lectures); Organic Synthesis, Prof. D. Gani (5 lectures); Advanced Electrochemistry, Prof C. A. Vincent (2 seminars) and Clusters, Dr. C. Glidewell (5 lectures).

# Acknowledgements

I would like to thank Alan for his help and supervision through the project. It's not been easy but we got there in the end. Other collaborators on the project, Prof. Double and Dr. Bibby, deserve my appreciation.

Thanks also go to the technical staff of St. Andrews University for the excellent service that they provided; Sylvia, Melanja, Colin, and Marjory always gave service with a smile.

I would like to thank Gillian for support and companionship during these three years.

Past and present members of the lab receive my thanks for their useful input and also friendliness.

Finally I would like to thank the Association for International Cancer Research for giving me financial support over the three years and also giving me the opportunity to help in mankind's struggle to combat cancer.

# Abstract

A robust six-step synthesis of substituted flavone-8-acetic acid sodium salts has been developed and optimised to allow preparation of a wide variety of products for testing as anti-tumour agents. The condensation and cyclisation steps have been combined in an efficient one-pot procedure and efficient procedures for subsequent oxidative cleavage of an allyl group and salt formation have been developed.

Using this method a total of 18 derivatives bearing substituents on the 2-phenyl ring have been prepared. Based on encouraging activity from methoxy substituted compounds, attention has been concentrated on these and various mono-, di-, tri- and tetramethoxy compounds have been prepared, most for the first time. Activity of these against MAC15A, both *in vitro* and *in vivo* has been determined by collaborators at Bradford and several compounds have comparable or greater activity than the unsubstituted prototype. The presence of a 2'-methoxyl substituent appears to be particularly favourable to activity.

Five different heterocyclic analogues with furyl, thienyl and benzothienyl groups at the 2-position have been obtained and these also show good activity with the 3-methyl-2-thienyl compound showing the highest *in vivo* activity of any compound examined. Attempts to prepare pyridyl and quinolyl derivatives were unsuccessful.

The 2-benzyl and 2-diphenylmethyl compounds have also been prepared and while the former shows good activity *in vitro*, it is inactive *in vivo* probably due to metabolic breakdown. The latter compound is completely inactive probably due to a requirement for planarity in the 2-substituent.

An extended derivative has been obtained by an unexpected mode of reaction encountered in an attempt to prepare the 2-phenylethynyl compound.

Both this and a dimeric ether analogue show significant activity, thereby adding to our understanding of the structural requirements for activity.

Variation in the 8-acetic acid substituted has been examined by synthesis of three compounds with $CH_2C(O)CH_2OH$ at this position and, by a separate multi-stage synthesis, the compound with tetrazolylmethyl. The former compounds are too insoluble for activity to be measured, while the latter is inactive.

A comparison of electron density, as reflected by $^{1}H$ and $^{13}C$ NMR shifts, against activity *in vitro* has been made for all the compounds prepared, and while some trends may be discerned, the correlation is generally poor.

A suitable single crystal of flavone acetic acid was prepared and its X-ray structure was obtained by the SERC Crystallography Unit, Cardiff. The structure, which proved to be of the monohydrate showed the preferred orientation of the 2- and 8-substituents and provided accurate dimensions for future theoretical work.

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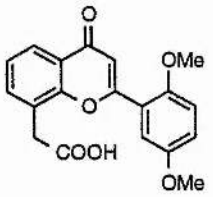
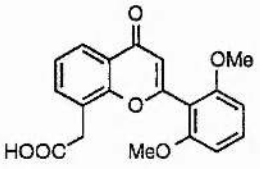
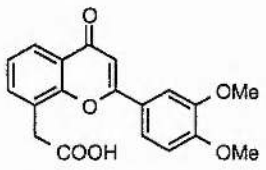
## EXPERIMENTAL



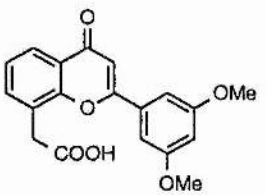


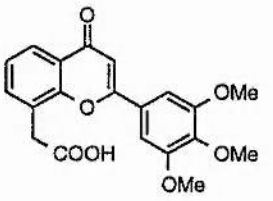





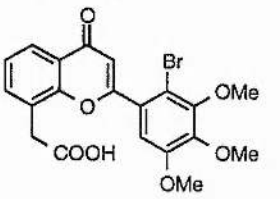





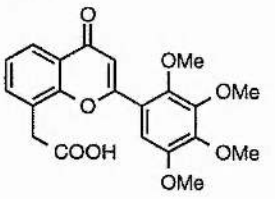



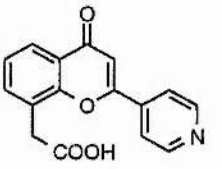




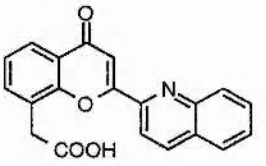





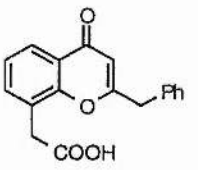









# INTRODUCTION

## A. Cancer

### 1. Classification

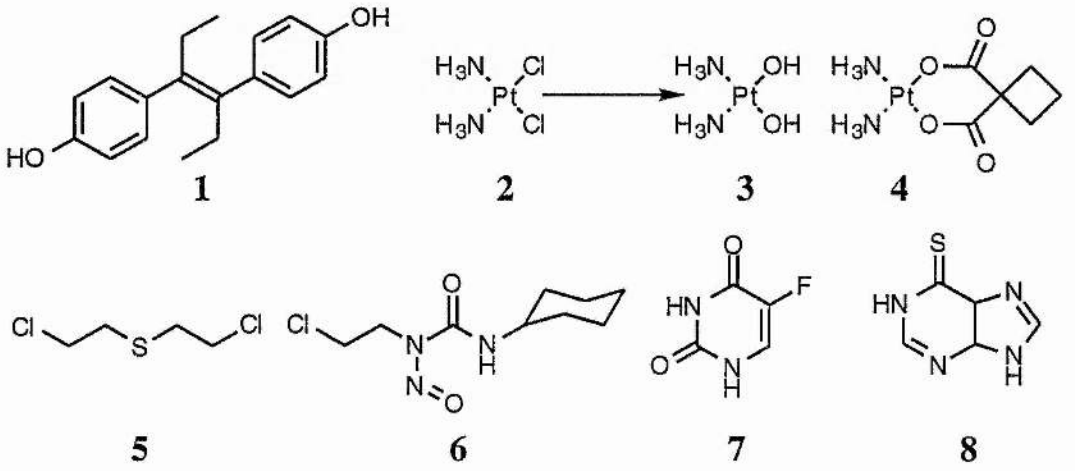
A chemically or genetically mutated cell will become cancerous if its own DNA causes it to replicate without control.[1] The mitotic replication rate of the tumour depends solely on the type of tissue affected with fast growing tumours normally less likely to be fatal for the patient as they can be inhibited to a certain extent by DNA intercalating drugs, which mimic nucleoside bases, leading to cessation of transcription. Slow growing solid tumours are harder to treat as they modify the surrounding body tissues to supply the tumour's needs. The diverting of host blood through the building of tumour vasculature, the change in plasma clotting times and changes in platelet and white blood cell counts are some ways that the cancer ensures its survival.

### 2. Therapeutics

#### a. General

Cancer chemotherapeutics can be split into several categories.[2] Antineoplastic agents that are steroidal in nature, resemble the female hormones, and are used in the treatment of prostate cancer. An example is diethylstilbestrol **1** and their mode of action is believed to be inhibition of protein synthesis. Cis-platin **2**, the square planar platinum complex drug, is converted to **3** in the body by replacing labile chlorines with hydroxyls and it is this activated form which interferes with the cell's DNA. Unfortunately it also attacks normal DNA replication associated with such functions as follicle hair production and so a new complex carboplatin **4** with fewer side effects was synthesised. Various toxic alkylating agents such as mustard gas **5** and nitrosoureas such as CCNU (lomustine **6**) modify cellular DNA by mismatching strands therefore interfering with cell division. Cell disrupting anti-metabolites interfere with cell processes, by imitating vital cytoplasmic compounds, binding irreversibly or

competitively with host structures - various anti-metabolites are antagonists for nucleoside bases e.g. 5-fluorouracil **7** and 6-mercaptopurine **8**, hence interfering with DNA replication.

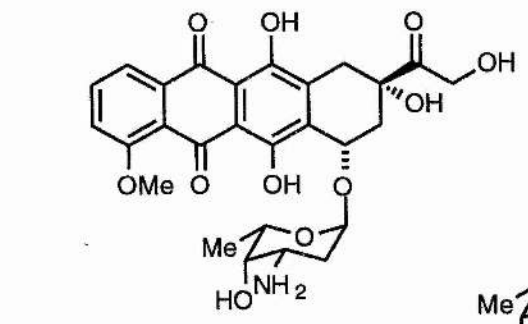



**1** **2** **3** **4**

**5** **6** **7** **8**

Antibiotics, such as adriamycin **9**, daunorubicin **10** and bleomycin $A_2$ **11** also have the capacity to kill cancer cells by blocking the synthesis of vital proteins.

Fragment **12** (daunosamine) falls off the active molecules in acid media. The anthracycline antibiotics **9** and **10** are based on anthraquinone structures which certainly intercalate with DNA. Bleomycins also cause DNA strand scission and have been shown to possess a type of oxygen transferase activity. Certain other drugs currently in use fall into a separate and fast growing area of research, that of phytochemistry.



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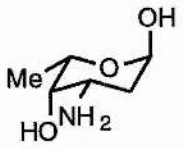

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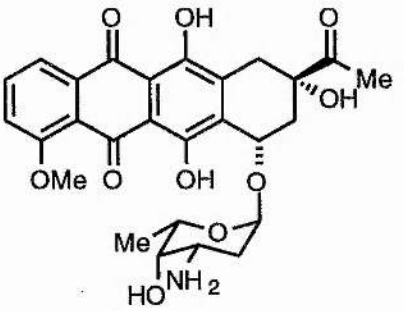

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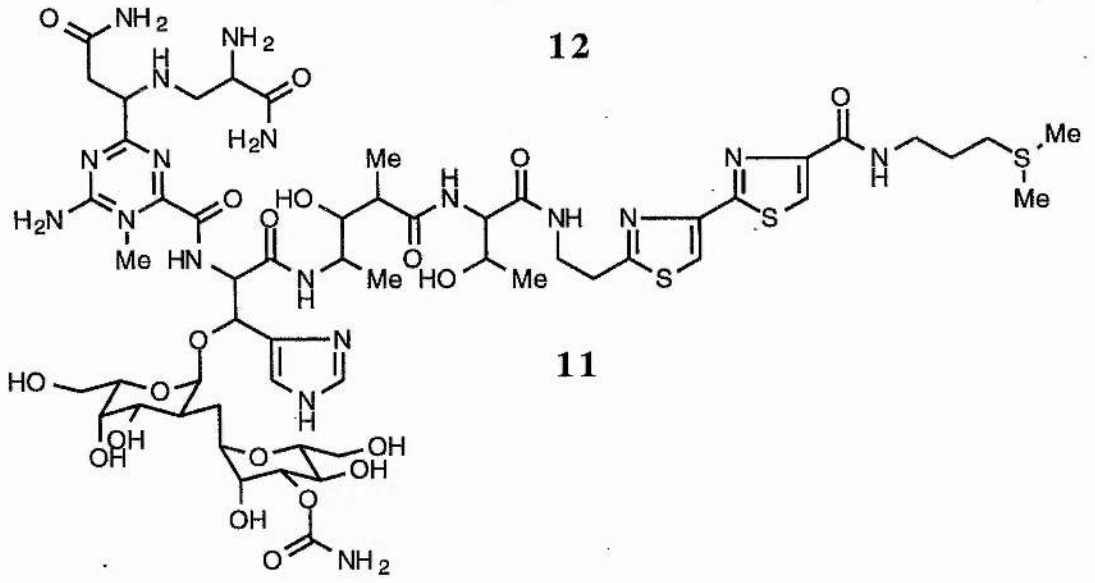
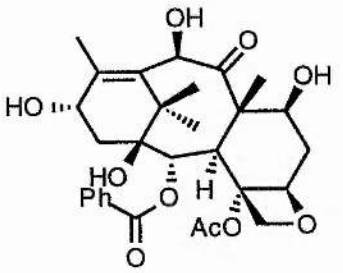


**11**

b. Phytochemistry

Phytochemistry, the chemistry of plants and the substances produced therefrom, has become the focus of a lot of academic attention within the last twenty years.[3,4] Tribal medicines, ancient rituals and folklore all use plants to a greater or lesser extent. The properties of these plants have been recognised by the pharmacist and by collaboration with other sciences especially chemistry, large scale syntheses of the plant drugs can be carried out. Taxol[5] **13**, the latest in a long line of anti-cancer plant extracts, originates from the bark of the Pacific Yew tree *Taxus brevifolia*. Although this tree is in danger of extinction, taxol's activity is so great that whole areas of forest are disappearing to accommodate the new-found interest.[6] Several groups are working on the total synthesis[7-9]

and a semi-synthetic route to taxol has already been achieved by several groups[10-13] from 10-deacetyl baccatin III **14**.



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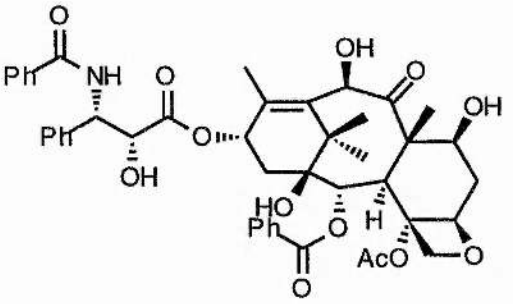
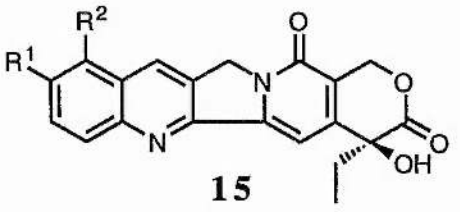


**13**

The mechanism of taxol's cytotoxicity stems from its ability to increase both rate and yield of microtubule assembly and it inhibits the tubulin disassembly process. This activity is opposite to the normal effect of mitotic inhibitors, with ovarian and breast cancers surprisingly susceptible to this treatment. As a result of phase II trials it is currently under investigation[14] by the U.S. Food and Drug Administration.

Wall and Wani, also discovered another drug camptothecin[15] **15**, a novel alkaloid with a quinoloindolizine structure, which similarly has cytotoxic properties. Discovered in 1966 and isolated from the stem wood of *Camptotheca acuminata* it inhibits topoisomerase I. Its toxicity,[16] however, has led to congeners such as topotecan, a dimethylaminomethyl derivative, which has recently entered phase II clinical trials.

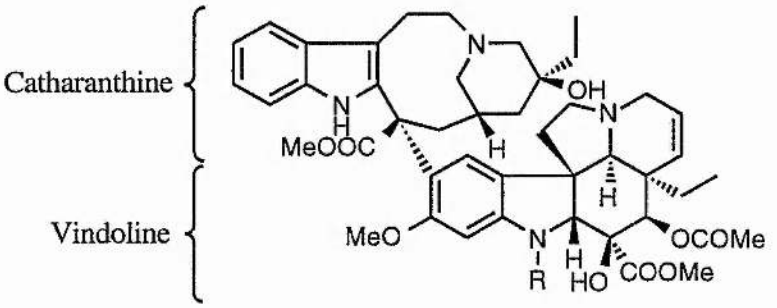


**15**

Camptothecin $R^1 = H$ $R^2 = H$

Topotecan $R^1 = OH$ $R^2 = (CH_3)_2NHCH_2$

Vincristine **16** and vinblastine **17**, currently used in cancer chemotherapy,[2] have interesting structures.




**16** Vinblastine R = Me

**17** Vincristine R = CHO

Both molecules were obtained from *Vinca rosea* extracts. The molecules act on cells by binding to tubulin and inhibiting microtubule activity which makes them more selective to proliferating tumour cells.[17]

## B. Flavone-8-acetic acid

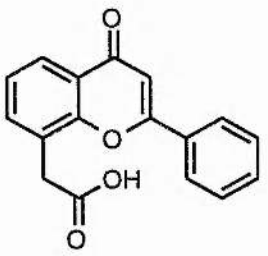
1. Cancer Screening

Early cancer screens from the National Cancer Institute (NCI) were based on leukaemia models.[18] Such fast growing tumours are susceptible to DNA intercalating drugs which not only interfere with the tumour cell cytology but also the host organism's cytology. In recent times, with increases in the knowledge of carcinogenesis, the NCI changed their *in vitro* screens of human cell lines to incorporate slow growing solid tumours. These highly vascularised tumours, like murine colon adenocarcinoma 38 (MAC38), were previously untreatable and hence usually fatal. With the inclusion of cancerous cell lines like MAC38 it was possible to test the whole spectrum of drugs against solid tumours.

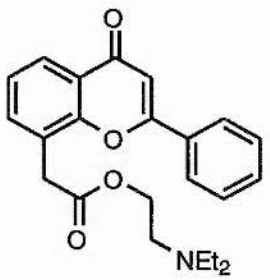
2. Discovery of flavone-8-acetic acid

A French company, Lyonnaise Industrielle Pharmaceutique (Lipha), was synthesising a lot of flavonoids when the above pre-clinical screen highlighted a flavone as active.[19,20] It was originally developed as an anti-inflammatory agent showing no anti-metabolite activity and having little or no DNA binding affinities.

From these studies flavone-8-acetic acid **18** (FAA, LM975, NSC347512) and its diethyl aminoethyl derivative **19** (LM985) came to the fore as agents which were less suitable at combating fast growing malignant cells but were almost universally effective, causing haemorrhagic necrosis, in slow growing cachectic tumours.




**18**




**19**

As hypoxic cancer cells may well constitute a significant subpopulation of a tumour and as they obtain their energy solely from a higher than normal glycolytic rate, it is reasonable to assume that any compound that interferes with glycolysis would be regarded as partially selective towards tumour cytotoxicity. Bioflavonoids are known[21] to inhibit enzymes in the glycolytic pathway, specifically $Na^+$-$K^+$-ATPase of the plasma membrane, by interfering with generation of adenosine diphosphate and inorganic phosphate.

FAA is an analogue of the natural plant pigments, hydroxylated flavones, which are found throughout the plant kingdom[3] where their pH dependence gives rise to a wide variety of colours. They are, however,

not found in animals in which they exhibit effects[4] ranging from toxic to beneficial.[22-24] The related family of coumarins and chromones have found wide ranging applications e.g. as herbicides, anti-fungal and anti-viral agents. Warfarin is a good example of the toxicity found in animals in that it interrupts the cascade effect of blood clotting in rats.

Due to the high activity the company rapidly synthesised a number of analogues and produced a patent.[25] In the light of disappointing clinical trial results, which will be explained later, they dropped the project and lost interest in the drug. The company did not publish most of the activity results and the two papers that did contain activity results[26,27] were only for simple derivatives and were not comprehensive.

3. *In vivo* activity and mechanism of action

a. *In vivo* activity

FAA treatment was effective in transplantable solid tumours, namely colon 51, 07, 10, 26, pancreatic adenocarcinomas 02 and 03, mammary adenocarcinoma 16/C/Adr, M5076 reticulum cell sarcoma and Glasgow's osteosarcoma. It was found[20] that high concentrations and long exposure times were necessary *in vitro* to achieve the same result as a single low dose *in vivo*. Kerr *et al.* showed[28] that LM985, the ethyl diaminoethyl derivative of FAA **19** was more efficacious but also more toxic to the mice due to the catabolism of the ester functionality.

Smith *et al.*[29] showed that the murine tumours were necrotic after only 4 hours of treatment with FAA and that after 24 hours the few tumours cells that were still viable could hardly be recognised. The authors claimed that this effect was remarkably similar to that of tumour necrosis factor-α (TNF-α).

b. Indirect mechanism of action

Studies of Bibby *et al.*[30] showed that the concentrations *in vivo* were too low to have a direct effect on the tumour and hypothesised that FAA must be acting by some indirect mechanism or as a response modifier.[31-36] Highly vascularised solid tumours, which either scavenged the host's own vascular system or, through tumour angiogenesis, created small knotted or meandering blood tubules, were more susceptible to FAA treatment than leukaemia models. An example of such a susceptible solid tumour is MAC16 which causes weight loss in animals without a reduction in food intake and is also highly vascularised. This normally drug-resistant tumour becomes necrotic quickly on FAA treatment which showed that some novel factor was responsible whose effectiveness was not only governed by vasculature but also tumour site.[30,37] This fact was further exemplified by MAC26TC tumour cells that were shown to be resistant to FAA when transplanted interperitoneally or intravenously but responded with 100% inhibition of growth when transplanted subcutaneously.

c. Vasculature Targeting

From evidence so far it seems that direct cytotoxicity is not the reason for activity *in vivo* but it is possibly due to some other factor unique to the host. Various groups[38-40] showed that FAA caused tumour regression via a vasculature shutdown process. Tumour blood flow was monitored[41] over a 24 hour period as a single peritoneal injection of FAA was administered. This caused a 90% inhibition of tumour volume and a 60% tumour blood flow reduction with necrotic changes as early as 2 hours after drug treatment related to the reduced vascular blood flow to the tumour. With certain anti-cancer agents (e.g. hydralazine) this reduction in tumour blood flow can happen in several ways.[40,42] The most important of these ways being the 'steal effect' where the rest of the body's blood vessels undergo vasodilation, hence lowering the volume of blood

available to the tumour. However it was found that at therapeutic doses FAA had no effect on systemic blood pressure.

d. Coagulation and interstitial pressure

The question raised by researchers was 'if FAA is not acting by the steal effect then what exactly is the mode of action?' Electron microscopy showed that FAA caused epithelial cell separation and stromal damage but with no obvious damage to endothelial cells.[43] Interesting studies[21,44] using EMT-6 spheroids showed that when they were allowed to vascularise and then treated with FAA the cells close to the blood supply died rapidly while the avascular portion of cells survived. However, FAA does not stop the formation of new tumour vessels.[39] When various other tissues were analysed by Honess and Bleehen[40] i.e. skin, muscle, lung, liver, spleen and kidney it was found that only blood flow in the spleen was affected. FAA was also found to break single stranded DNA in Glasgow's osteosarcoma by Bissery *et al.*[45] but this was found to be an effect rather than the cause of cell death. Reduction in tumour blood flow over a certain period correlated well with tumour regression.

Studies into blood clotting times showed that at 15–30 minutes after FAA administration of 300 mg/kg both tumour and non-tumour bearing animals showed a distinct reduction in clotting times.[29,46] This initial decrease was followed 4–6 hours later by an increase in clotting times, thrombin time, fibrin degradation level and platelet count. Conclusions from this study were that it was indeed the reduction in blood flow that caused extensive necrosis due to increased endothelial barrier permeability and increased pro-coagulant activity by tumour necrosis factor-α.

e. Involvement of nitric oxide and endotoxin

Nitrate/nitrite level was monitored in treated mice and the results showed that levels of both increased, reinforcing the presence of TNF-α. TNF-α

has been associated with the *in vivo* and *in vitro* killing of cancer cells.[47,48] Direct cytotoxicity was first discovered from the inoculation of mice with *Mycobacterium bovis* strain Calmette-Guérin (BCG). A clear relationship was demonstrated between nitrate/nitrite levels in the plasma and the ensuing tumour regression and growth delay caused by FAA. Now that their precursor nitric oxide (NO) was inferred as the cytotoxic agent various studies[49,50] were targeted at this radical.

Endotoxin is a heat-stable lipopolysaccharide protein. It is a biological modifier which causes haemorrhagic necrosis in transplanted murine tumours via stimulated production of nitric oxide by way of the arachidonic acid pathway. It induces both interferons in plasma and TNF-$\alpha$ and has therefore very similar action to FAA. A study[50] was carried out on endotoxin resistant mice ($C_3H$/HeJ) and normal mice ($C_3H$/HeN) to investigate the effect of FAA's and XAA's (see later) when one facet of the immune system is removed. Both strains of mice were injected with cancer cells at six weeks old and the cancers allowed to develop a blood vasculature before drug treatment. *In vitro* tests were conducted by examining nitrite produced and although a greater concentration of FAA (890μM) was required than 5,6-dimethyl XAA (80μM) for optimum activity the method of action is the same. It was found that endotoxin stimulates NO production only in the C3H/HeN (normal) mice and not in the resistant mutant whereas FAA and XAA analogues have similar dose-response curves for NO production in both variants. Peak nitrate levels[51,52] from the plasma of each of the mouse strains were taken 12 hours after drug infusion. It was found that FAA (1180μM) and 5,6-dimethyl XAA (100μM) gave similar values for haemorrhagic necrosis (within experimental error). A summary of the results obtained is found in the table below.

| | Haemorrhagic Necrosis | |
|---|---|---|
| Treatment | $C_3H$/HeN mice | $C_3H$/HeJ mice |
| No treatment | 12±4 | 18±2 |
| FAA (1180μmol/kg) | 81±11 | 78±9 |
| 5,6-diMeXAA(100μmol/kg) | 50±14 | 58±18 |
| Endotoxin (10μg/mouse) | 63±24 | 8±4 |
| Endotoxin (100μg/mouse) | 95±3 | 27±22 |
| Endotoxin (600μg/mouse) | Death | 50±3 |

As can be seen there is a marked contrast between the effect of endotoxin and FAA or XAA analogues in the $C_3H$/HeJ mice. Moreover endotoxin caused haemorrhagic necrosis in the $C_3H$/HeJ mice at a concentration that would have killed the normal mice. The conclusion from this study was that FAA or its relative XAA did not stimulate the endotoxin pathway in mice.

f. Immune Effects

NO was indeed proved to be the killing agent but unfortunately the mode of action was still unclear. Various studies[35,49,53,54] pinpointed the macrophages in the host as playing the role of NO producers. FAA stimulates not only these natural killer (NK) cells in the spleen in mice but also lymphokine activated killer cells (LAK) which are potent interferon (IFN) inducers of polyinosinic-polycytidylic acid. The highest macrophage activity occurs 24 hours after treatment, returning to normal levels after 6 days.

A preliminary study by Urba *et al.*[55] showed that FAA caused a significant increase in NK cell activity in three out of six patients with no induction of IFN-γ. The evidence that anti-IFNα/β antibodies inhibited FAA action at 4 hours suggests that FAA mediated its tumour response

indirectly by immunomodulation as well as directly by anti-proliferative or cytotoxic activity. Further evidence that it is macrophage and lymphocyte activity that causes tumour necrosis by NO came from Bibby and Double[56] using normal NMRI, thymectomised mice and nude mice strains. Normal mice have a fully functioning immune system, thymectomised mice have all of their immune system removed surgically and chemically and nude mice are genetically altered to be born immunodeficient. They found that the tumours were highly responsive to FAA only in the normal mice but the nude mice and thymectomised strain showed no tumour regression. This research, more than any other proves that FAA acts on some part of the immune system, perhaps through a cascade effect. Recently Futami *et al.*[57] showed that when murine splenic leukocytes were cultured with 100 μg/ml of FAA there was an increase in the cytokine mRNA expression which led to expression of TNF-α after 1 hour and maximal expression of TNF-α, IFN-α, IFN-γ mRNA after 3 hours. Unfortunately human peripheral blood leukocytes did not respond by cytokine expression.

Inhibition of platelet aggregation and adhesion by NO may be two of the reasons that affected vessels become leaky and divert host blood from the tumour site.[58] Direct cytotoxicity could be explained by the production of high concentrations of NO which would occur during cell-cell contact with the macrophage.

4. Clinical Trials

The concept of a pharmaceutical window for flavone acetic acid was presented by Zaharko *et al.*.[59] The maximum tolerated dose was found to be 600μg/ml in the plasma for short periods with delayed lethality resulting from long exposure (24hrs) at a range of doses.

From these results three conditions must be fulfilled for maximum anti-cancer effect:

- The concentration of the drug in the plasma must lie within the therapeutic window of activity.
- There must exist an adequate blood vasculature to the cancer.
- The immune system must be fully functioning.

Preclinical results[60] selected LM985 (ethyl diaminoethyl derivative of FAA) **19** as a good agent for cancer treatment due to the fact that it had few side effect and little or no myelosuppression or major organ toxicity. The drug was found, however, to break down into the parent acid **18** via a de-esterification enzyme and it was the parent FAA therefore that was finally chosen for full clinical trials.[61,62]

a. Metabolism

Cummings *et al.*[63,64] attempted to characterise the two major metabolites of FAA found in the plasma because the phase II clinical trials showed that drug concentrations were much lower in man than that found in mice. Urine was taken from patients and the metabolites purified by HPLC. Metabolite 1 (M1) was a glucuronide conjugated to the acetic acid group which was shown to be chemically labile and tended to rearrange under alkaline pH conditions. Metabolite 2 (M2) was also a glucuronide and was shown to be an isomer of M1 with both M1 and M2 being non-cytotoxic. As much as 80% of low doses were excreted in the urine as M1 and M2 but at high drug doses peak concentrations were reached which showed saturation of the glucuronidation pathway explaining low clearance rates. Studies in the UK and Italy have shown that tumour FAA levels in patients are similar to those obtained in mice reinforcing the fact that the lack of efficacy of FAA in humans is not due to a lack of penetration of the drug.

b. Plasma Concentration

Whereas plasma clearance of any drug is proportional to the body surface area and hence faster in small animals the converse is true of FAA, it being removed more readily from man and dog than in mouse models. Analysis of the plasma by Damia *et al.*[65] found that FAA clearance from mice was monoexponential and dose dependent. Highest drug concentrations were found in the liver and small intestine in humans with the lowest concentration found in the brain. Later studies confirmed that FAA was found in high concentrations in the gastrointestinal tract, mainly in the duodenum, which would suggest biliary excretion as the main excretory path for the drug.

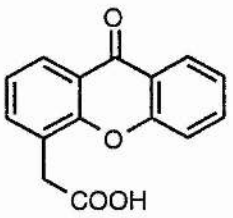
c. Toxicity *in vivo*

The phase I and II trials[28,61,62,66-69] showed six main areas of contraindication. These areas included temperature regulation, blood pressure changes, central nervous systems effects, gastrointestinal system effects, allergic effects at point of entry and myalgias. Severity of these effects encountered during the trials range from slight warming effects to dose limiting extreme hypotension with one fatal cardiac arrest.

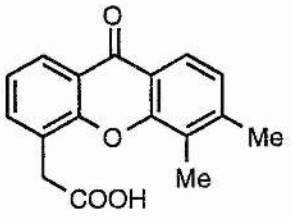
5. Developments since the discovery of FAA

a. Xanthenone-4-acetic acids

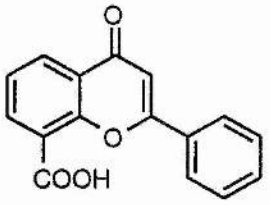
Several years after the first FAA patent appeared in the literature a group from New Zealand headed by Denny reported a homologue of FAA known as xanthenone-4-acetic acid (XAA)[27,70-73] **20**. They claimed not only that this compound was as non-toxic as FAA and that it was an effective anti-cancer drug but also that it was tenfold more active than FAA. More recently the 5,6-dimethyl XAA derivative **21** has been shown to be ten times more active than **20** and clinical trials using this are now in progress.[74] Their synthetic plan is shown in section C.




**20**




**21**




**22**

b. Flavone-8-carboxylic acid

The paper by Blanton *et al.*[75] outlining the synthesis of flavone-8-carboxylic acids **22** and their biological activity produced startling results. They discovered that the Diels-Alder cycloaddition reaction of substituted 3-cyanofurans with vinylketones yielded the corresponding 3-cyano-2-hydroxyacetophenones which they realised were useful starting materials for flavone synthesis. The biological activity for the series of functionalised flavone-8-carboxylic acids were disappointing showing no activity *in vitro* but comparable activity *in vivo* with flavone-8-acetic acid. The most active compound was **22**. Their synthetic plan is shown in section C.

## C. Synthetic Methods for Flavonoids

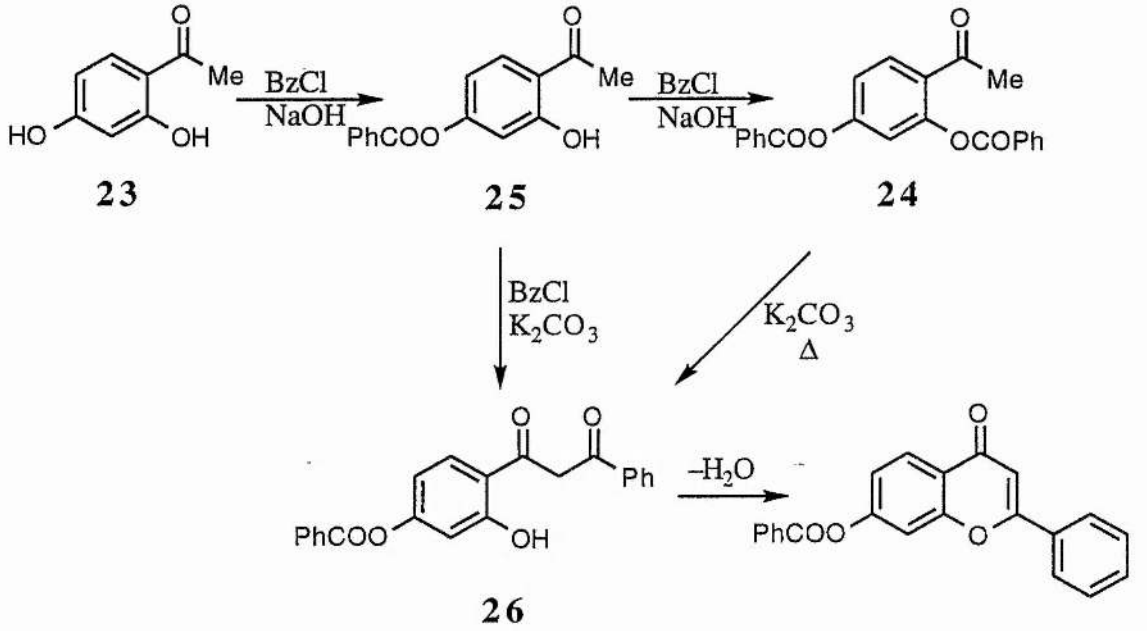
1. General

A large number of synthetic routes to flavonoids have been developed. A representative selection of these is shown here.

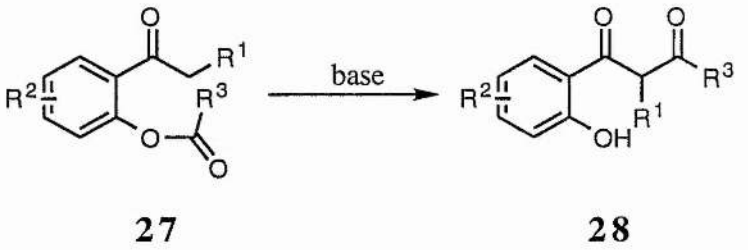
a. The Baker-Venkataraman Rearrangement

In 1933 Baker reported a method for the formation of flavones[76] (later known as the Baker-Venkataraman rearrangement) starting from resacetophenone **23**. This serendipitous discovery (actually starting out to make resacetophenone dibenzoate **24**) involved firstly the reaction with one equivalent of benzoyl chloride to form **25** followed by the

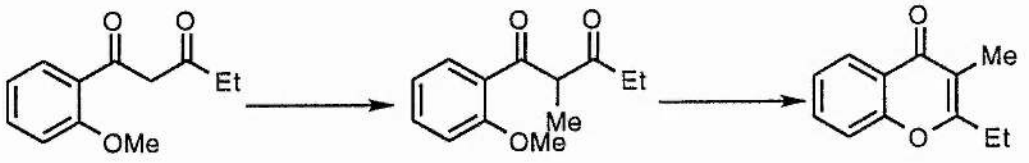
introduction and migration of a second benzoyl group to form **26**. The reaction does form **24**, however the conditions are such that the rearrangement occurs spontaneously with **24** never being isolated. The flavone itself is formed by dehydration. This reaction promoted great interest and further synthetic work began on functionalising the starting acetophenone.



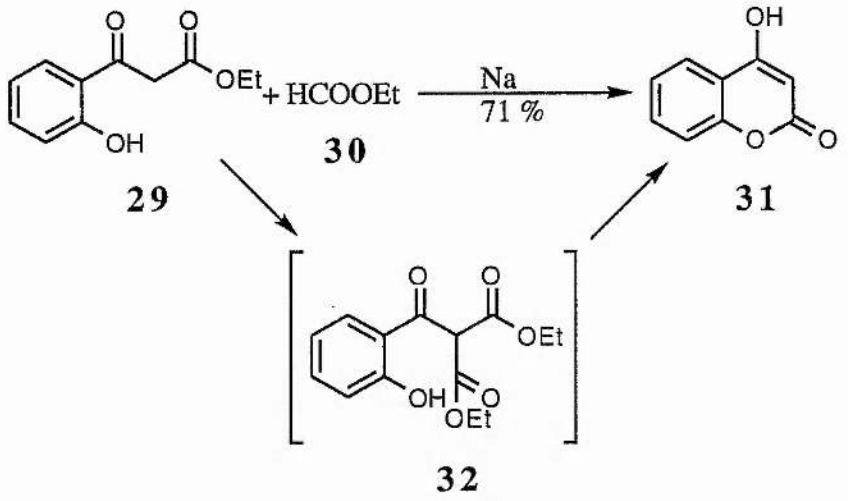

For the general reaction of **27** to **28** it was found that $R^3$ may be alkyl or aryl, hence flavones, and that $R^1$ could only be alkyl or hydrogen because of the reactivity of the ketone towards nucleophiles.




However it is possible to alkylate the β-diketone once formed, usually though, with low yields. This leads to a 3-substituted benzopyranones.

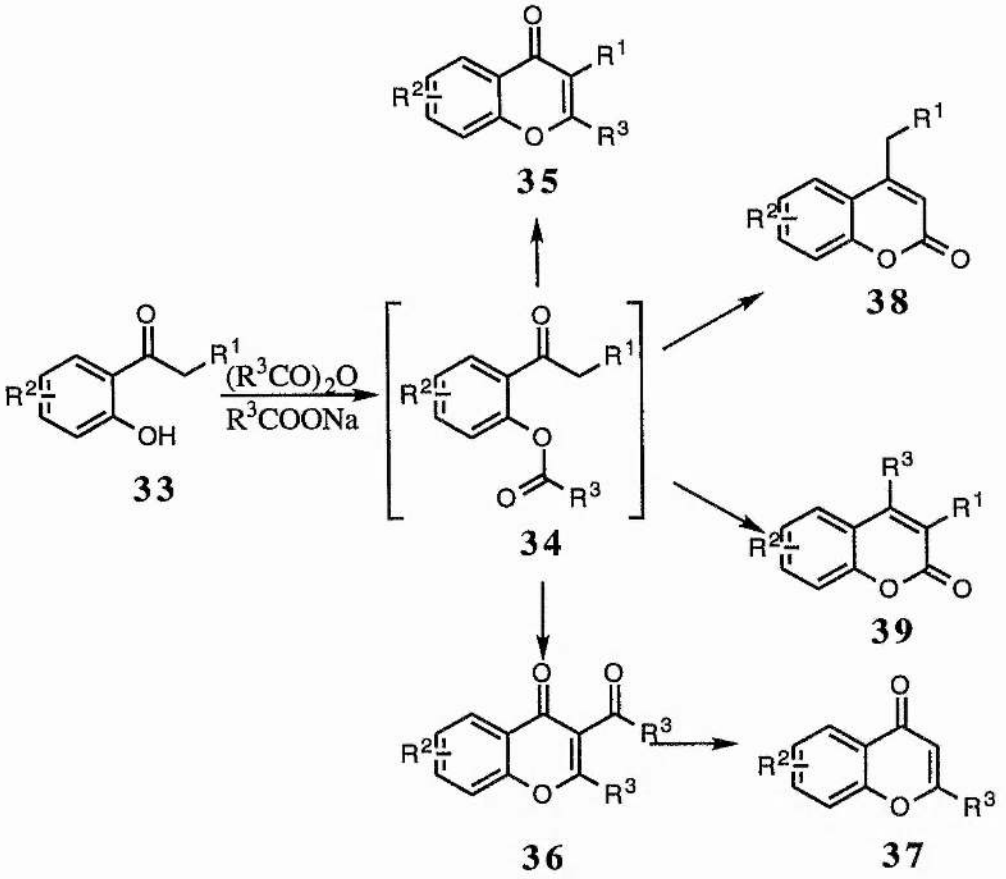


It was found that if $R^1$ in **27** was an ester group, reacting compounds such as **29** with ethyl formate **30** in the presence of sodium gave a 71% yield of the 4-hydroxycoumarin **31**. The intermediate **32** is never isolated.

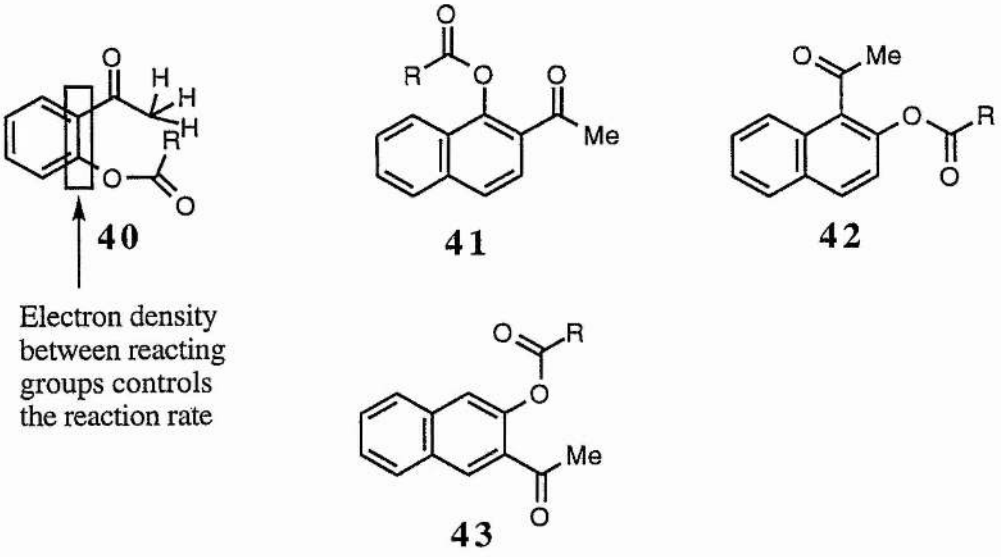


b. The Kostanecki Robinson reaction

An important synthetic route to 3-substituted flavones was exploited by Kostanecki and Robinson.[77,78] It was shown that the substituent affected the products obtained from the reaction. When an α-substituted hydroxyacetophenone **33** was reacted with any aliphatic or aromatic anhydride in the presence of the corresponding base of the anhydride, then several pathways existed for the initially formed acyloxyacetophenone **34**. It could undergo a Baker-Venkataraman rearrangement to yield the two products **35** and **36** (**37** can also be obtained) shown or if $R^1$ was a substituent other than hydrogen then the chromone **35** was formed. It was possible, however, that a further reaction could occur involving the intramolecular aldol condensation with elimination of water to form the coumarins **38** and **39**.

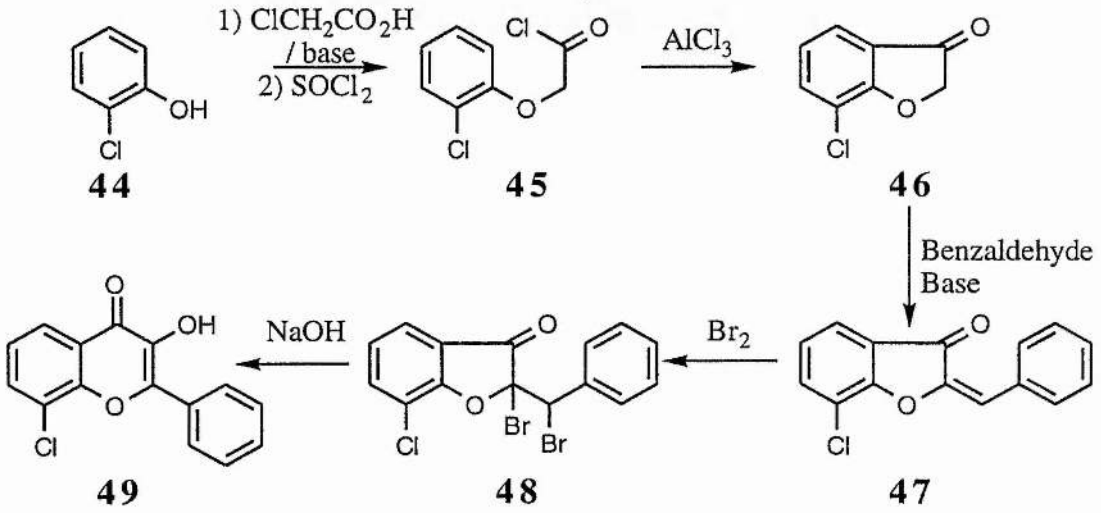



A study was undertaken by Nowlan *et al.*[79] to discover the reason for facile synthesis of chromones discovered by Baker. In their paper, which compared the reactivity of 2-(nitrobenzoyloxy)acetonaphthones, it was found that the rearrangement intermediate **40** was influenced by the position of the double bond between the two reacting groups. Isomeric napthones were prepared and their ease of transformation monitored. In general it seems that the high electron density of the π-electrons between the acetyl group and the ester group in **41** and **42** decreases the rate of rearrangement. Molecule **43**, however, rearranges under the same conditions in around one minute due to the lessened electron density between the reacting groups on the ring.



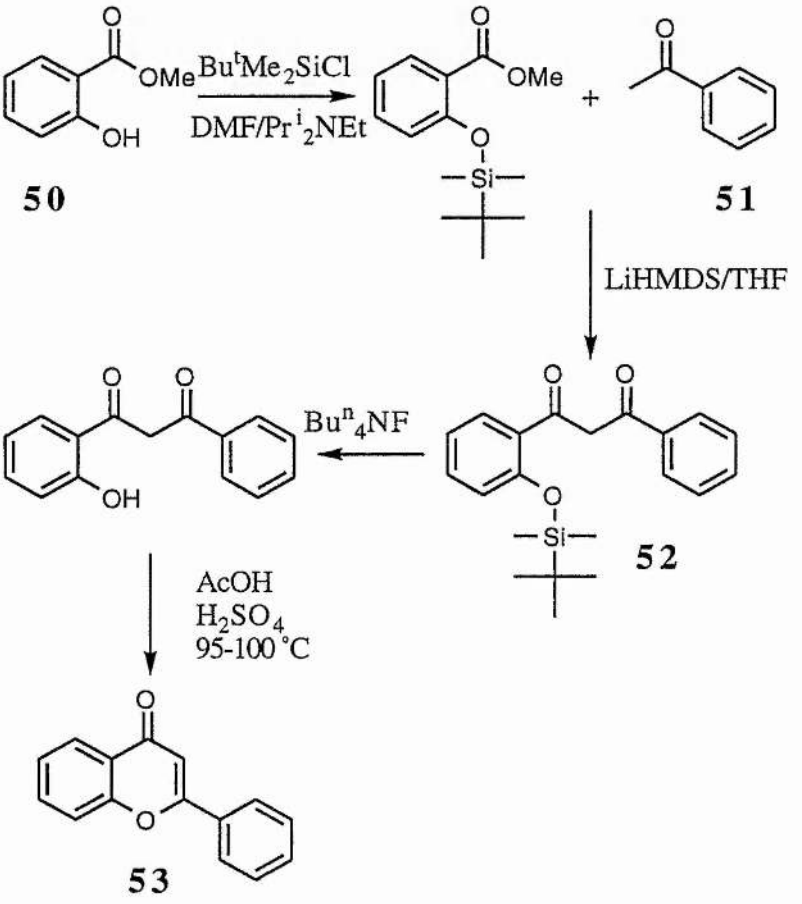

c. Via Coumaranones

In papers by Auwers[80] and later Minton,[81] flavones were prepared from benzofuranones. Minton's synthesis started from 2-chlorophenol **44** reacting with chloroacetic acid in base followed by the further formation of the acid chloride **45**. Cyclisation of this molecule using aluminium chloride as catalyst yielding **46** followed by condensation with benzaldehyde resulted in the formation of 7-chloro-2-benzylidenecoumaran-3-one **47** in good yield. Bromination across the double bond led to **48** which was then reacted with sodium hydroxide and dehydrated to form the 8-chloro-3-hydroxyflavone (8-chloroflavonol) **49**.



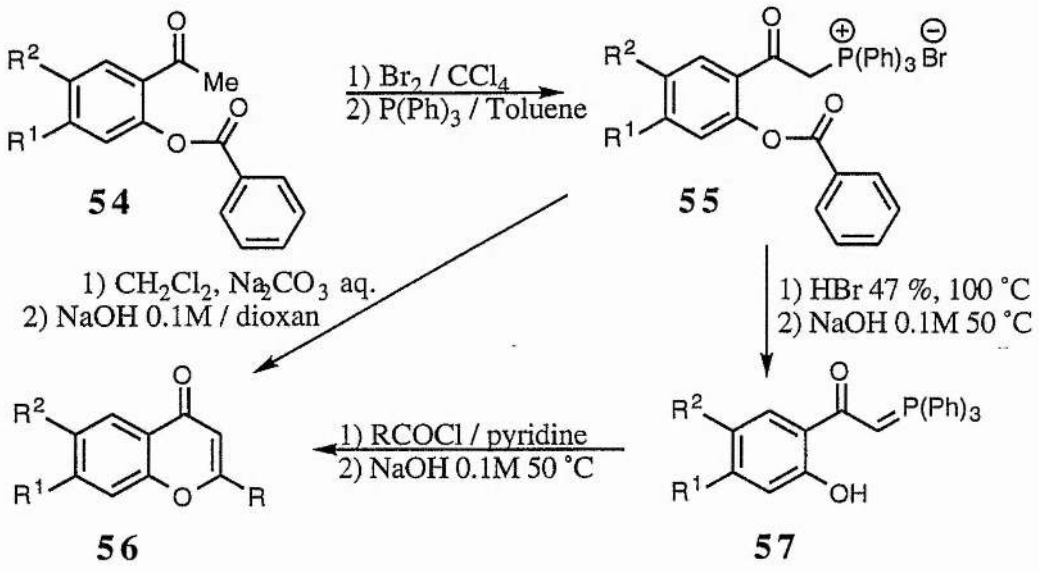
d. Condensation of protected salicylates

Much work has been done on naturally occurring flavones for example the synthesis of polyhydroxylated analogues by Cushman *et al.*.[82-84] Their work stems from an interest in the inhibition of retroviral reverse transcriptases, protein-tyrosine kinases and serine/threonine kinases related especially to ring A hydroxylated flavones. In their synthesis they protect the hydroxyl group of methyl salicylate with $Bu^tMe_2SiCl$ to avoid side reactions in the presence of base. When this compound is coupled with any aryl ketone **51** in the presence of base it forms a protected β-diketone **52** which can be deprotected using fluoride. The hydroxy β-diketone is then set for cyclisation under acid conditions to isolate the flavone **53** in good yield.



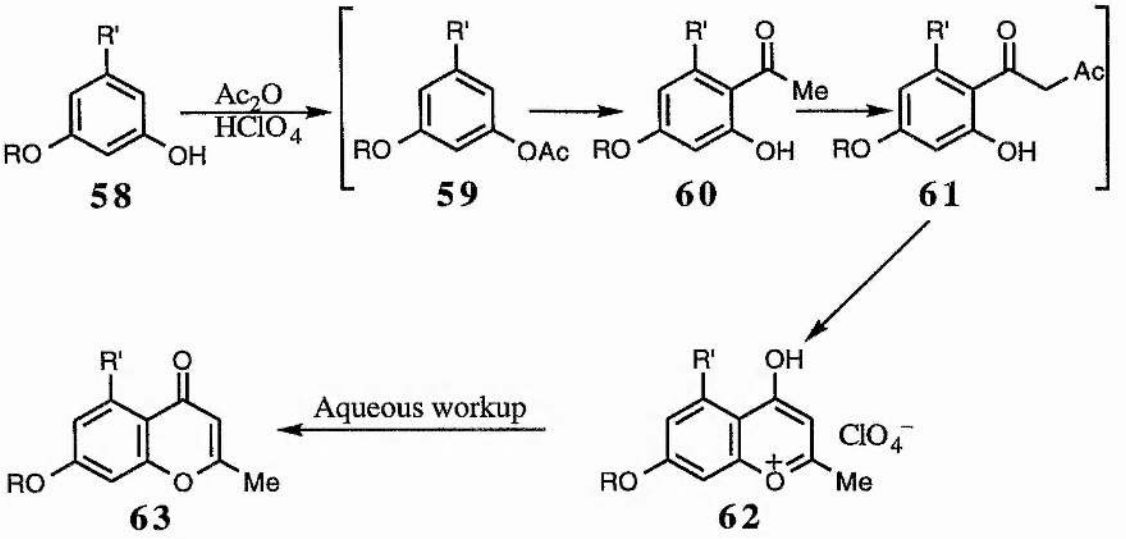
e. Using intramolecular Wittig reaction

High yields (83–94%) for the coupling reactions have been reported. Le Floc'h *et al.* based their synthesis[85] on the overall ylid formation followed by subsequent removal to form the flavone. Following the bromination of **54** and the addition of triphenylphosphine, the bromophosphonium salt **55** is formed. This phosphonium salt can either be pushed on directly to the flavone **56** or can be made into the ylid **57** which will then undergo an intramolecular Wittig reaction with the O-acyl group to form **56**.



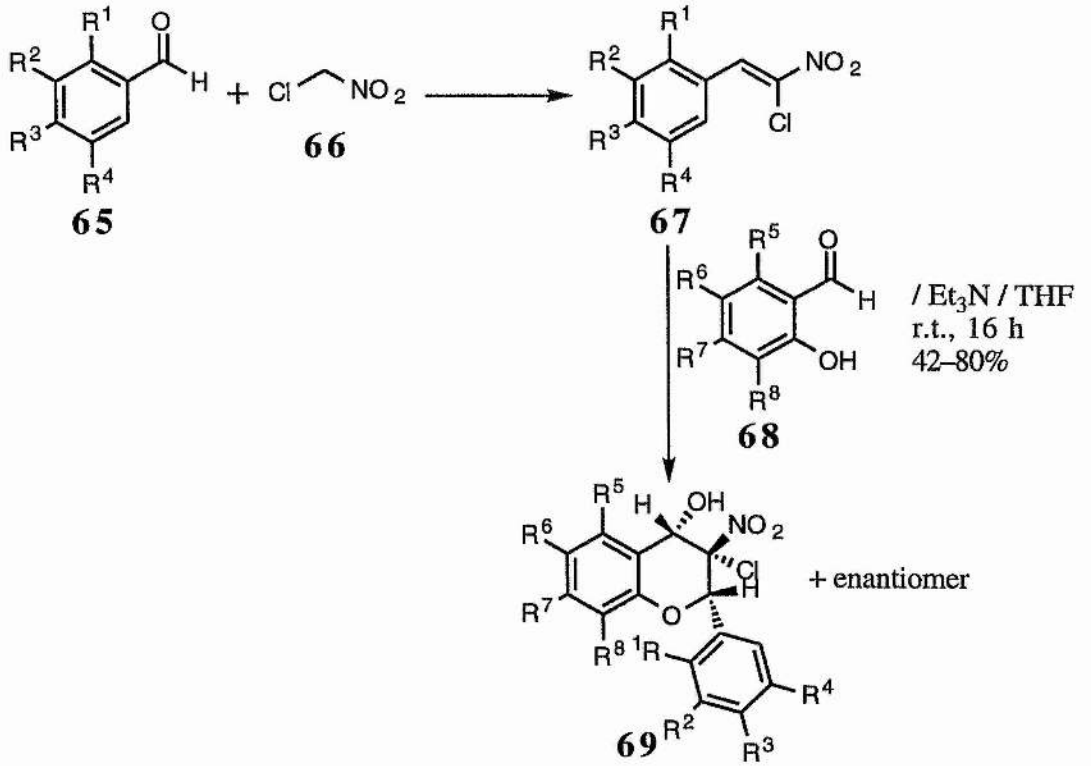

f. Acylation under acidic conditions

Chromones may also be formed under acidic conditions. The reaction pathway exploited by Dorofeenko and Tkachenko[86] in 1971 involves treating substituted phenols **58** with acetic anhydride and perchloric acid. The phenol undergoes successive O-acylation **59**, Fries rearrangement **60**, C-acetylation **61** and finally cyclisation to the benzopyrilium salt **62** which can be hydrolysed to the chromone **63**.

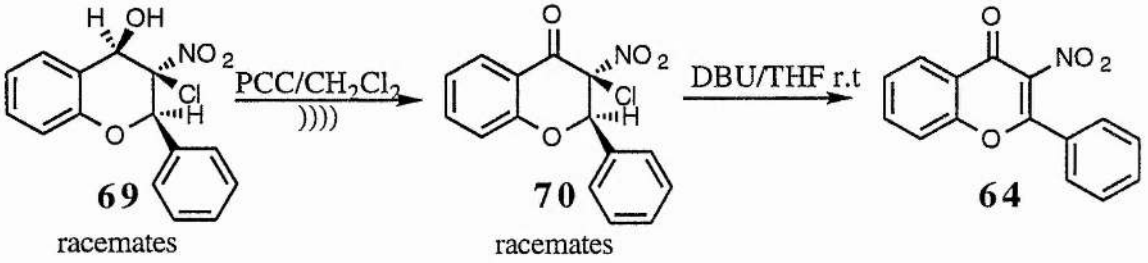



g. From flavanols

Flavanone involvement in the reactions has been exploited in the synthesis by Dauzonne *et al.*.[87,88] 3-Nitroflavone **64** and its derivatives have been postulated by them as having novel biological properties.

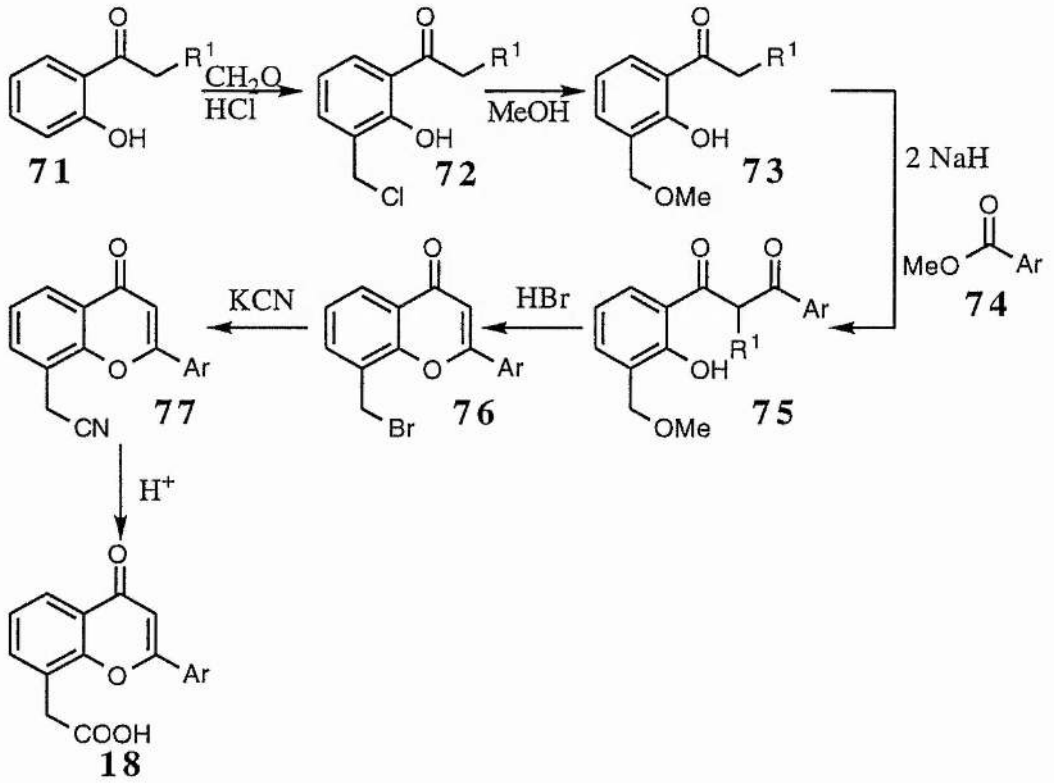


In their synthesis they take the aldehyde **65** and treat it with chloronitromethane **66** to form **67**. Reaction of this compound with the other aldehyde **68** forms an enantiomeric mixture of the diastereomers with the relative configuration 2R*, 3R*, 4R*. Their procedure towards the 3-nitroflavone target encompasses two relatively simple steps. Firstly sonication of the flavanol **69** in the presence of PCC oxidises the hydroxyl to the ketone **70**. On treatment of **70** with DBU in THF the target nitroflavone **64** is formed in reasonably good yield via HCl elimination.



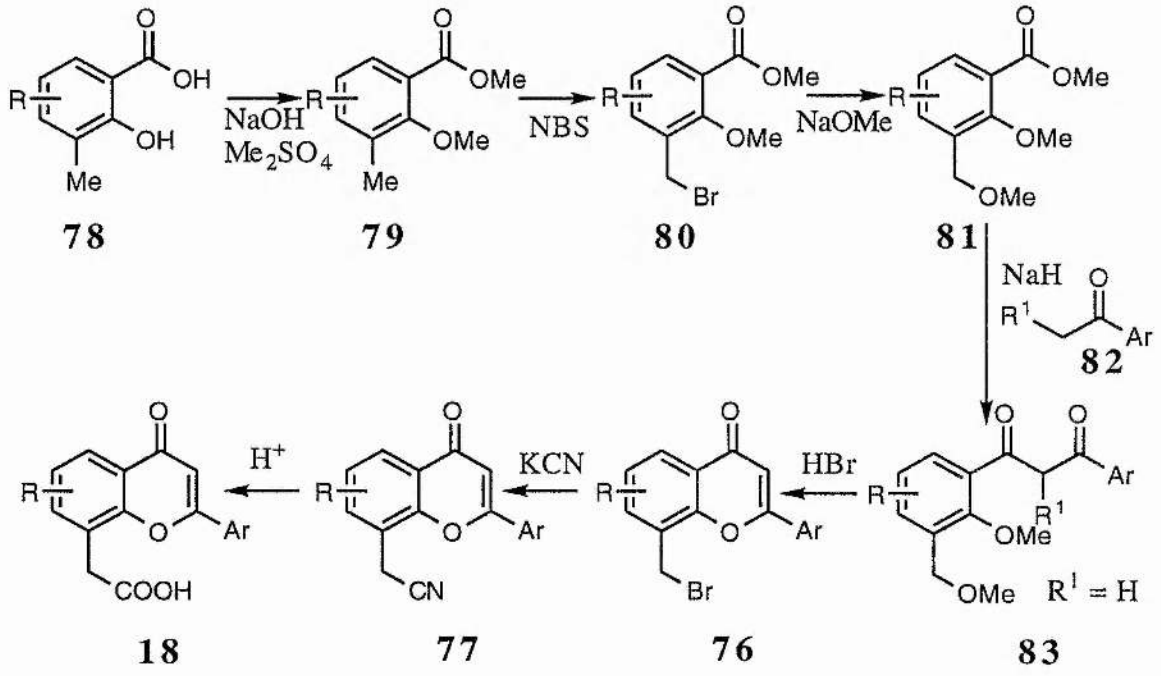

2. Syntheses used by Lipha

The company produced many derivatives by following the routes shown below.[25,89-91]



In the first route, starting from a substituted hydroxyacetophenone **71**, they chloromethylated the 3-position to give **72** and then replaced the chlorine with a methoxy group **73**. Standard condensation with any aryl ester **74** under basic conditions resulted in the β-diketone **75** which could be cyclised and brominated in one step. Treatment of **76** with potassium cyanide gave **77** and further acid hydrolysis gave the substituted flavone-8-acetic acid **18**. This route has problems in that the chloromethylation step occurs not only on the 3-position but also on the 5-position (see section 4).

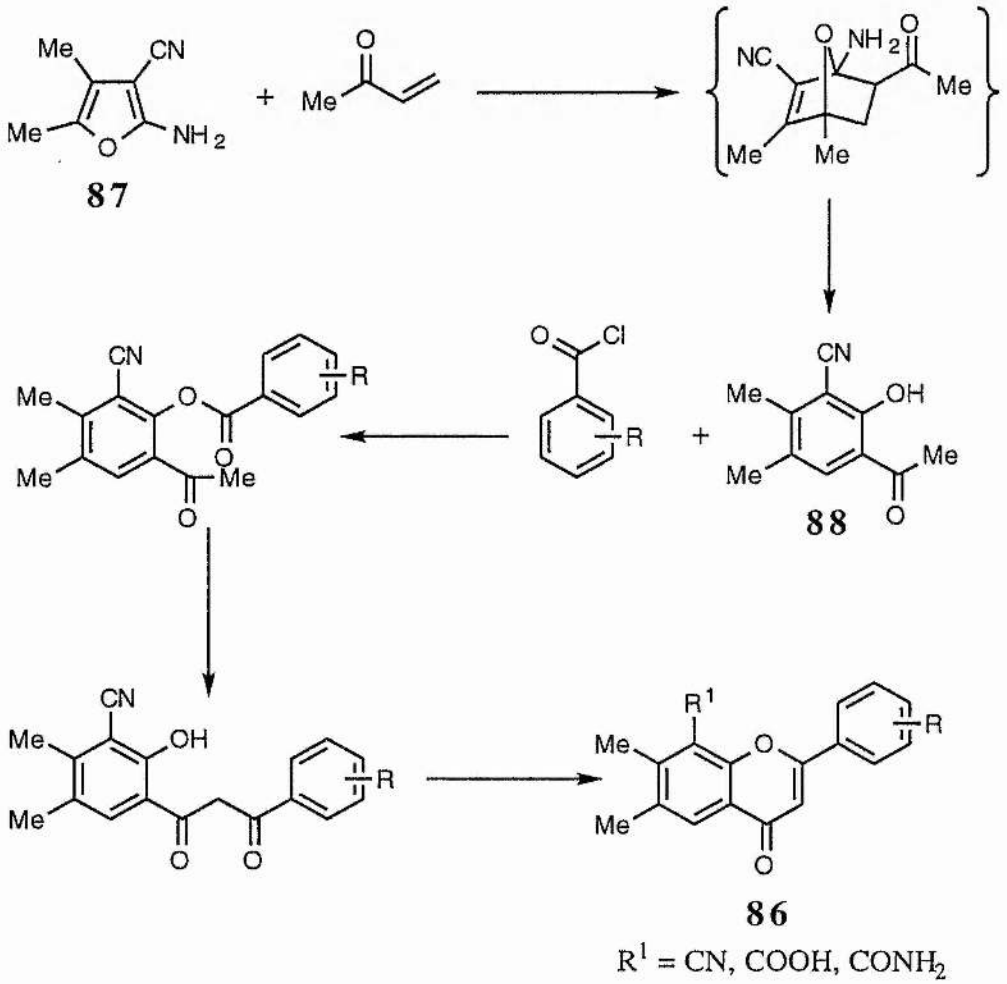
The other route was in essence a reversal of the more conventional first route. This time the company started from a methyl salicylic acid **78**, firstly producing the ester **79**, then brominating the 3-methyl substituted to give **80**. Conversion of this brominated compound to the methoxy compound **81** then allowed coupling to the complementary acetophenone **82** to form the β-diketone **83**. The rest of the synthesis of flavone-8-acetic acids **18** was the same as the above route.



3. Synthesis of related compound types

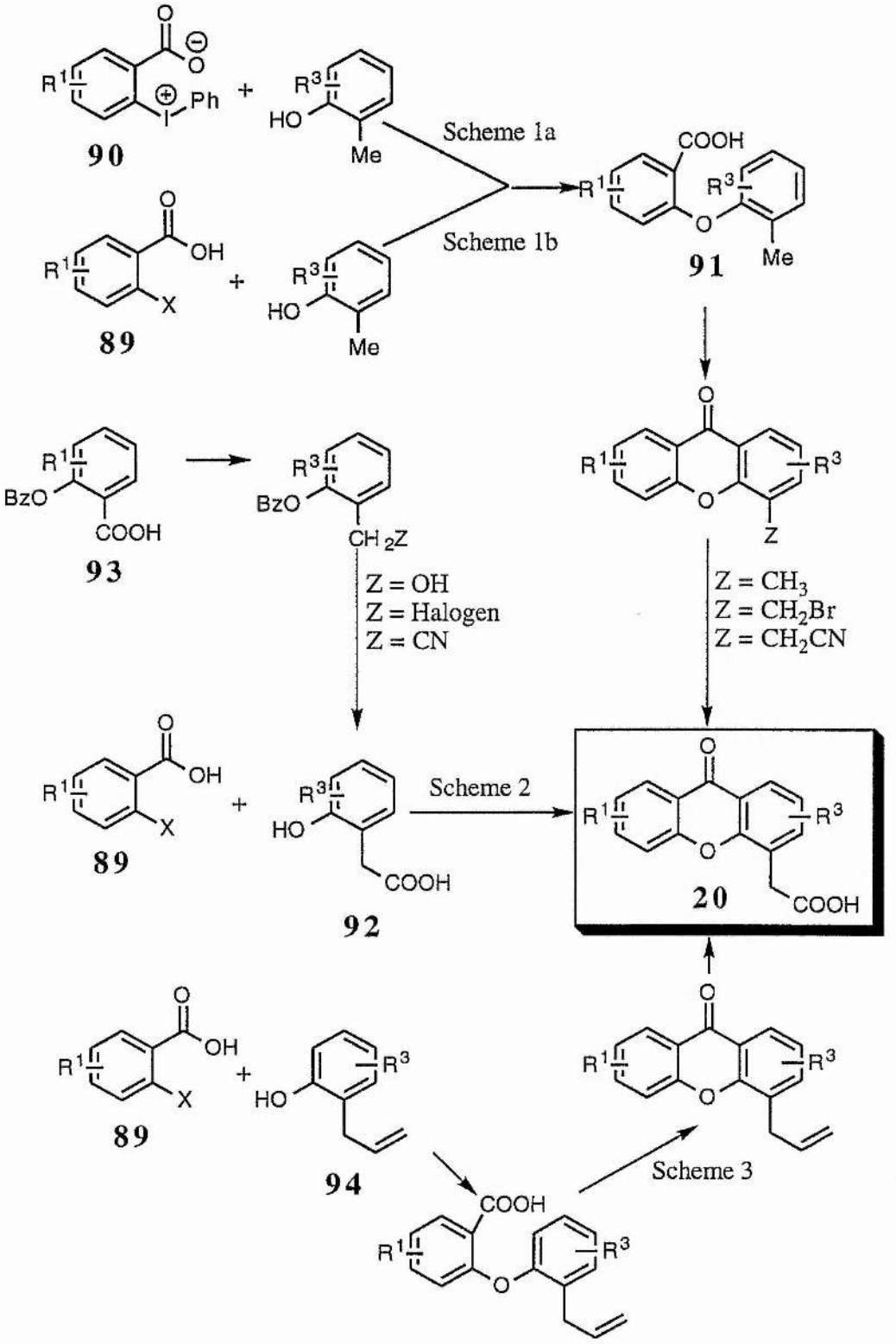
a. Flavone-8-carboxylic acids

Blanton *et al.*[75] used classical Baker-Venkataraman methodology finally cyclising the flavone and hydrolysing the cyano group to the carboxylic acid **86**. Starting from substituted 3-cyanofurans **87** and reacting with vinylketones, in a Diels Alder fashion, yielded the corresponding 3-cyano-2-hydroxyacetophenones **88**. They realised that these compounds were useful starting materials for flavone synthesis and reacted these acetophenones with substituted acid chlorides. This gave a molecule which could undergo classical Baker-Venkataraman rearrangement into a β-diketone which could be cyclised using acid conditions.



b. Xanthenone-4-acetic acids

The synthetic routes shown below have been developed by Denny and co-workers.[27,70-73]



The first involved the initial Ullmann coupling of either halobenzoic acids (X= Br or Cl) **89** or diphenyliodonium-2-carboxylates **90** with substituted phenols forming substituted 2-phenoxybenzoic acids **91** followed by cyclodehydration using a range of acids (including concentrated sulphuric acid, methanesulphonic acid and polyphosphoric acid). This reaction, which gave moderate yields, was carried out by heating to 140 °C in excess phenol or DMF for 5 to 10 hours.

The halobenzoic acids **89**, however, required a higher temperature, typically 180 °C, in more dipolar aprotic solvents with catalytic amounts of CuCl and TDA-1 (tris-[2-(2-methoxyethoxy)ethyl]amine). The methylxanthenones were then brominated by NBS with a radical initiator and thence converted to their cyano derivatives with inorganic cyanides. Subsequent acid hydrolysis gave the corresponding xanthenone-4-acetic acids in good yields.

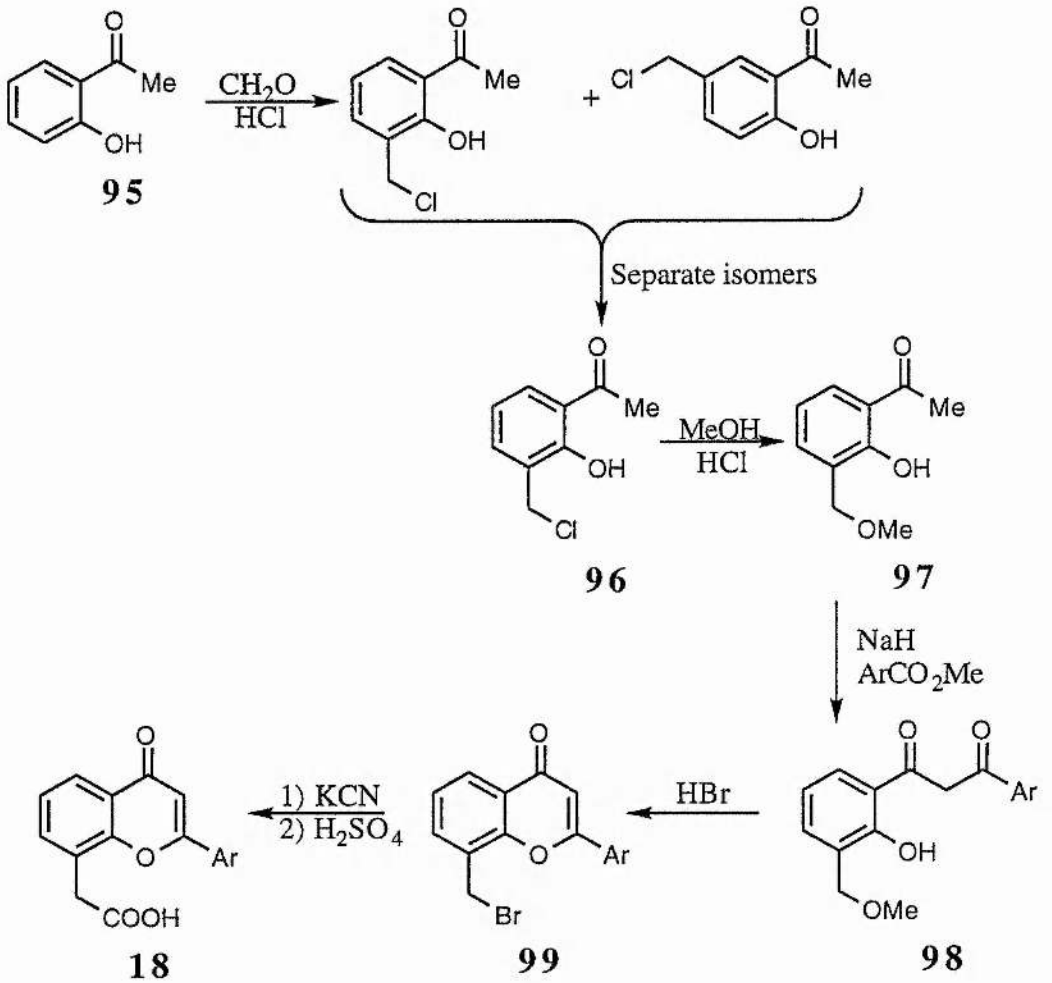
Scheme 2 shows a variation of the above reactions by having the acetic acid moiety already in place. The 2-hydroxyphenylacetic acids **92** were prepared as shown by the reaction of benzylated salicylic acids **93** under phase-transfer catalysis. Firstly the acid chloride is produced and after reduction, with sodium borohydride, the corresponding protected acid is obtained. On conversion to the halogen derivative and subsequent cyanation and alkaline deprotection the required product **92** is available to enter the coupling reaction.

Allylphenols **94** and halobenzoic acids **89** also form the xanthenone target when coupled together and cyclised, as shown in scheme 3, followed by careful permanganate oxidation in low to moderate yields.

4. Previous work in this laboratory

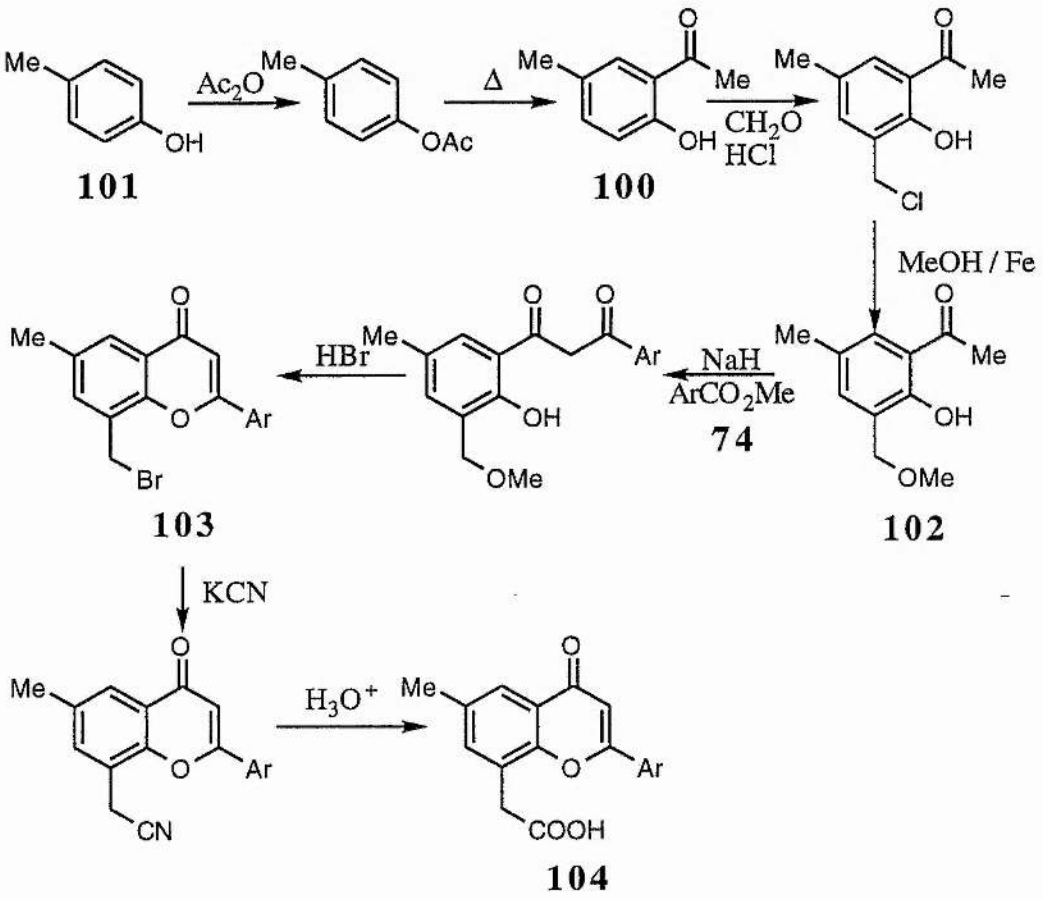
Work started in this group to use the first synthesis reported[89] by Lipha and described in section 2 above to prepare a range of substituted derivatives for anti-tumour evaluation. The synthesis began with the

reaction of methyl salicylate **95** and paraformaldehyde under acidic conditions. This step, however, produced two isomers which proved difficult to isolate. The correct isomer **96** was then pushed on in the scheme substituting the pendant chlorine with a methoxy group. This compound **97** could then be reacted in the standard fashion with any aryl ketone **30** to form the 2-hydroxy-3-methoxymethyl β-diketone **98**. Cyclisation of **98** with hydrobromic acid not only forms the flavone **99** but also replaces the methoxy group with bromine. Cyanation of this compound and subsequent acid hydrolysis gives **18** in reasonable yield.




It did not prove possible however to separate the isomeric chloromethyl compounds[92] and so an attempt was made to delay the separation until the next stage with the methoxy group replacing the chlorine. This proved

not to be any more of an advantage and thus it was decided to prevent formation of the unwanted isomer by blocking the site *para* to the hydroxy group. The rest of the synthesis was still viable with only the starting material needing to be changed.




To overcome separation problems 2-hydroxy-5-methylacetophenone **100** was chosen as the starting material. Acetylation and Fries rearrangement of *p*-cresol **101** yielded the product **100** that was protected at the *para* position. This could then be chloromethylated and reacted with methanol to give the main reactant for all of the further syntheses. This compound, 2-hydroxy-3-methoxymethyl-5-methylacetophenone **102**, could be coupled to any alkyl or aryl ester **74** under the influence of sodium hydride in THF as a solvent. The coupled molecule could then be cyclised using acetic acid and hydrobromic acid simultaneously replacing the

methoxy group with bromine. The bromine on **103** could then be displaced with cyanide and ultimately hydrolysed off under aqueous acid conditions to produce the 6-methyl flavone acetic acid **104**. Using this method a number of 6-methyl analogues were prepared and evaluated for anti-tumour activity *in vitro* and *in vivo*.[93] However these proved to be less effective as anti-cancer agents than the unsubstituted FAA itself. The reason for the 6-methyl FAA's lack of efficacy can be in part inferred from the fate of benzene versus toluene *in vivo*. The extra methyl group provided a useful handle for the enzymes of the body to dispose of toluene easier than benzene. It seems therefore that 6-methyl FAA's would be as cytotoxic to cancer cells if only they survived the transport to the cells.

## D. Programme of Research

As described in section B, FAA initially seemed a promising candidate for the treatment of solid tumours. The disappointing results with human patients contrasted with the encouraging preliminary results in the mouse models. Although a great deal of biological work has now been done into the mode of a action of this compound, we do not yet have a full understanding of its mechanism of action.[94]

The biological work as well as the clinical trials have concentrated almost exclusively on FAA itself. There was clearly a need to broaden the study by evaluation of a range of analogues with more diverse structures. A large number of these have indeed been prepared, but these are mostly described in patents,[25,89-91] and activity results have only been reported in two papers.[26,27]

We therefore set out to prepare a range of FAA analogues with differing substitution on the 2-phenyl group and the 8-acetic acid group for *in vitro* and *in vivo* anti tumour evaluation in collaboration with Prof. J. A. Double and Dr. M. C. Bibby at the Clinical Oncology Unit, University of Bradford. The compounds were required in gram quantities and so a robust straightforward synthesis had to be developed which allowed ready incorporation of the desired substitutents. When a range of compounds were in hand their electronic structure could be examined by NMR and any correlation between this and the structure-activity relationships discovered might lead to some understanding of their mechanism of action and allow design of new, more active analogues.

# EXPERIMENTAL

## A. Symbols and Abbreviations

| | |
|---|---|
| mmol | millimoles |
| M | mol $dm^{-3}$ |
| h, min | hours, minutes |
| GC-MS | gas chromatography-mass spectrometry |
| tlc | thin layer chromatography |
| NMR | nuclear magnetic resonance |
| $\delta$ | chemical shift in ppm |
| ppm | parts per million |
| Hz | hertz |
| *J* | spin-spin coupling constant in Hz |
| s, d, t, q, m | singlet, doublet, triplet, quartet, multiplet |
| $\nu_{max}$ | infrared absorption frequency in $cm^{-1}$ |
| *m/z* | mass to charge ratio |
| $M^+$ | mass of molecular ion |
| FAB | fast atom bombardment |
| NOBA | nitrobenzylalcohol matrix |
| m.p. | melting point |
| b.p. | boiling point |
| eq. | equivalent |
| 1 mmHg | 133.32 Pa |

## B. Instrumentation and General Techniques

### 1. N.M.R. Spectroscopy

#### a) $^{1}$H NMR

All routine spectra were recorded at 200 MHz on a Varian Gemini 200 instrument by the author while those of new compounds were recorded by Mrs M. Smith on a 300MHz Bruker AM-300 spectrometer.

#### b) $^{13}$C NMR

All routine spectra were recorded by the author on a Varian Gemini 200 instrument operating at 50 MHz while spectra of new compounds were recorded by Mrs M. Smith on a Bruker AM-300 running at 75.5 MHz.

All $^{1}$H and $^{13}$C NMR spectra were obtained from solutions in deuteriochloroform, except where the compound was a carboxylic acid or its sodium salt in which case deuteriodimethyl sulphoxide or deuterium oxide were used. Chemical shifts for both $^{1}$H and $^{13}$C are expressed in parts per million to high frequency of internal tetramethylsilane or, in the case of $D_2O$ solutions internal 3-(trimethylsilyl)-1-propanesulphonic acid sodium salt.

### 2. Infrared Spectroscopy

Spectra were recorded using a Perkin-Elmer 1420 ratio recording spectrophotometer or Perkin-Elmer 1710 Fourier transform spectrophotometer. The spectra were run on sodium chloride plates as a nujol mull for solids or as a thin film for liquids. The spectra were calibrated with the polystyrene peak at 1603 $cm^{-1}$.

3. Mass Spectrometry

Mass spectra were obtained on a Finnigan Incos 50 mass spectrometer or a Fisons VG Autospec by Mr C. Millar.

4. Elemental Analysis

Microanalysis for carbon, hydrogen and nitrogen were carried out by Mrs S. Smith using a Carlo-Erba 1106 elemental analyser.

5. Melting points

Routine melting points were determined on an Electrothermal melting point machine while accurate melting points of new compounds were determined on a Reichert hot-stage microscope.

6. Thin Layer Chromatography

Aluminium sheets coated with 0.2 mm of silica (Merck, Kieselgel $60F_{254}$) were used and the components observed under ultraviolet light.

7. Column Chromatography

This was carried out using Fisons silica gel (60-120 mesh).

8. Drying and Evaporation of Organic Solutions

All organic solutions were dried by adding appropriate amounts of anhydrous magnesium sulphate. This was filtered off and the filtrate evaporated under reduced pressure on a Büchi rotary evaporator.

9. Drying and Purification of Organic Solvents

Methanol

Following a procedure by Lund and Bjerrum[95] warmed clean dry magnesium turnings (5 g) and iodine (0.5 g) was added to 50–75 ml of

analar methanol until the iodine disappeared and all the magnesium was converted to methoxide. More methanol (1 l) was added and the mixture heated under reflux for 2 h. The methanol was then distilled and stored over molecular sieve (4Å)

Tetrahydrofuran

Dry THF was prepared by distillation from potassium benzophenone ketyl under $N_2$.

Dimethylformamide

DMF was stored over molecular sieve (4Å).

## C. Preparation of Flavone-8-acetic Acid and Monosubstituted Derivatives

### 1. Flavone-8-acetic Acid

**a.** Preparation of 2-allyloxyacetophenone **108**

Anhydrous potassium carbonate (41.5 g, 0.3 mol) was added to a solution of 2-hydroxyacetophenone **95** (40.9 g, 0.3 mol) and allyl bromide (36.3 g, 0.3 mol) in A.R. acetone (250 ml). The mixture was then heated under reflux with stirring for 4 h after which it was filtered and the filtrate poured into water (300 ml). The mixture was extracted with ether (3 x 25 ml) and the combined extracts washed with 2M sodium hydroxide (100 ml), dried over potassium carbonate and evaporated. The resulting yellow oil was distilled *in vacuo* to afford 2-allyloxyacetophenone (44.0 g, 83%) as a colourless liquid which solidified on storage, m.p. 19–21 °C; b.p. 263–265 °C (lit.,[96] 263 °C); $\nu_{max}$ (melt) 1667 (C=O), 1595, 1292, 1235, 1162, 1123 and 758 $cm^{-1}$; $\delta_H$ 7.70 (1 H, d, *J* 8), 7.38 (1 H, m), 6.90 (2 H, m), 6.02 (1 H, m), 5.45 (1 H, d, *J* 16), 5.26 (1 H, d, *J* 8), 4.59 (2 H, d, *J* 5) and 2.61 (3 H, s); $\delta_C$ 199.5 ($4^{ry}$), 157.9 ($4^{ry}$), 133.6 (CH), 132.6 (CH), 130.3 (CH), 128.5 ($4^{ry}$), 120.7 (CH), 118.1 ($CH_2$), 112.8 (CH), 69.3 ($CH_2$) and 32.0 ($CH_3$); *m/z* 176 ($M^+$, 20%), 161 (29), 147 (11), 133 (31), 121 (100), 105 (37), 91 (15) and 78 (25).

**b.** Preparation of 3-allyl-2-hydroxyacetophenone **109**

2-allyloxyacetophenone (20 g, 0.11 mol) was heated under reflux in a nitrogen atmosphere for 5 h. The flask was cooled and the mixture distilled between 140–160 °C at 16 mmHg to afford 3-allyl-2-hydroxyacetophenone (13.2 g, 66%) as a light yellow liquid, b.p. 110 °C at 0.3 mmHg (lit.,[96] 258 °C); $\nu_{max}$ 3380, 2979, 1637, 1317, 1246, 1126,

983, 917, 768 and 751 $cm^{-1}$; $\delta_H$ 12.61 (1 H, s), 7.58 (1 H, d, *J* 7), 7.32 (1 H, d, *J* 7), 6.80 (1 H, t, *J* 7), 6.08–5.89 (1 H, m), 5.11–5.02 (2 H, m), 3.37 (2 H, d, *J* 7) and 2.57 (3 H, s); $\delta_C$ 204.7 ($4^{ry}$), 160.4 ($4^{ry}$), 136.4 (CH), 136.1 (CH), 129.3 ($4^{ry}$), 128.8 (CH), 119.2 ($4^{ry}$), 118.4 (CH), 116.0 ($CH_2$), 33.4 ($CH_2$) and 26.7 ($CH_3$); *m/z* 176 ($M^+$, 86%), 161 (100), 143 (11), 133 (28), 115 (13), 105 (17), 84 (25) and 77 (26).

c. Preparation of 1-(3-allyl-2-hydroxyphenyl)-3-phenylpropane-1,3-dione **112**

Sodium hydride (60% dispersion in oil, 0.88 g, 22 mmol) was washed with petroleum (b.p. 40–60 °C) and the petroleum decanted. A solution of methyl benzoate (1.50 g, 11 mmol) in dry THF (50 ml) was added and the mixture was heated under reflux while 3-allyl-2-hydroxyacetophenone **109** (1.94 g, 11 mmol) in dry THF (50 ml) was added dropwise. After heating under reflux for 5 h the mixture was allowed to cool and dry methanol (200 ml) was added cautiously followed by dropwise addition of concentrated sulphuric acid until pH4 was reached. The mixture was evaporated and water (50 ml) was added to the residue which was extracted with methylene chloride (2 x 50 ml). The extracts were dried and evaporated to give a yellow solid which was recrystallised from ethanol to give 1-(3-allyl-2-hydroxyphenyl)-3-phenylpropane-1,3-dione (2.68 g, 87%) as yellow plates, m.p. 84–86 °C (Found: $M^+$, 280.1099. $C_{18}H_{16}O_3$ requires *M*, 280.1099); $\nu_{max}$ 1695, 1606, 1296, 1235, 1186, 1067, 913, 762 and 684 $cm^{-1}$; $\delta_H$ 15.50 (1 H, s), 12.43 (1 H, s), 7.95 (2 H, m), 7.67 (1 H, dd, *J* 8, 2), 7.61–7.42 (3 H, m), 7.38 (1 H, dd, *J* 8, 2), 6.85 (2 H, m), 6.10–5.90 (1 H, m), 5.11–5.00 (2 H, m) and 3.42 (2 H, m); $\delta_C$ 196.0 ($4^{ry}$), 177.2 ($4^{ry}$), 160.5 ($4^{ry}$), 136.2 (CH), 135.9 (CH), 133.7 ($4^{ry}$), 132.3 (CH), 129.7 ($4^{ry}$), 128.8 (2 x CH), 126.8 (2 x CH), 126.7 (CH), 118.6 (CH), 118.5 ($4^{ry}$), 116.0 ($CH_2$), 92.5 (CH) and

33.6 ($CH_2$); *m/z* 280 ($M^+$, 63%), 263 (5), 161 (29), 132 (17), 105 (100), 91 (5) and 77 (62).

**d.** Preparation of 8-allylflavone **123**

A solution of 1-(3-allyl-2-hydroxyphenyl)-3-phenylpropane-1,3-dione (2.50 g, 8.92 mmol) in methanol was heated under reflux for 10 min then 5 drops of concentrated sulphuric acid were added and the mixture heated under reflux for a further 4 h. The solvent was removed and the resulting cream solid recrystallised from ethanol to give 8-allylflavone (1.87 g, 80%) as colourless needles, m.p. 138 °C (Found: C, 82.6; H, 5.4. $C_{18}H_{14}O_2$ requires C, 82.4; H, 5.4%); $\nu_{max}$ 1637, 1596, 1585, 1498, 1213, 1140 and 1044 $cm^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 8, 2, H-5), 7.91 (2 H, m, H-2',6'), 7.53 (4 H, m, H-7,3',4',5'), 7.32 (1 H, t, *J* 8, H-6), 6.83 (1 H, s, H-3), 6.17–6.00 (1 H, m), 5.17 (2 H, m) and 3.75 (2 H, d, *J* 8); $\delta_C$ 178.7 ($4^{ry}$), 163.0 ($4^{ry}$), 154.2 ($4^{ry}$), 135.3 (CH), 134.1 (CH), 132.1 ($4^{ry}$), 131.6 (CH), 129.5 ($4^{ry}$), 129.1 (2 x CH), 126.3 (2 x CH), 125.0 (CH), 124.0 ($4^{ry}$), 123.9 (CH), 117.0 ($CH_2$), 107.4 (CH) and 34.0 ($CH_2$); *m/z* 262 ($M^+$, 100%), 234 (6), 160 (19), 145 (11), 131 (53), 115 (11), 89 (7) and 77 (33).

**e.** One-pot preparation of 8-allylflavone **123**

Sodium hydride (60% dispersion in oil, 0.88 g, 22 mmol) was washed with petroleum (b.p. 40–60 °C) and the petroleum decanted. A solution of methyl benzoate (1.50 g, 11 mmol) in dry THF (50 ml) was added and the mixture was heated under reflux while 3-allyl-2-hydroxyacetophenone **109** (1.94 g, 11 mmol) in dry THF (50 ml) was added dropwise. After heating under reflux for 5 h the mixture was allowed to cool and dry methanol (200 ml) was added cautiously followed by dropwise addition of concentrated sulphuric acid until pH 4 was reached. The mixture was once again heated under reflux for 3 h. Water (20 ml) was added and the

solution partly evaporated. Water (100 ml) was added and the mixture extracted with dichloromethane (3 x 50 ml). The extracts were washed with 2M sodium hydroxide to remove starting materials, dried and evaporated. The resulting solid was recrystallised from ethanol to afford 8-allylflavone (2.14 g, 74%) as colourless needles, m.p. 138 °C, identical to that obtained in **d.** above.

**f.** Preparation of flavone-8-acetic acid **129**

A solution of 8-allylflavone **123** (5.2 g, 20 mmol), acetone (200 ml), glacial acetic acid (200 ml) and water (100 ml) was stirred with cooling to below 5 °C during the addition of potassium permanganate (16.6 g, 110 mmol) over a period of 6 h. Saturated aqueous sodium metabisulphate was added dropwise until the solution turned a cream colour. The solution was evaporated and added to water (200 ml). The precipitate was then filtered off, washed with water and dissolved in a saturated solution of sodium bicarbonate (20 ml). The resulting suspension was filtered and the filtrate carefully acidified to pH 4. The precipitate that formed was filtered off, washed with water and recrystallised from glacial acetic acid and water (2:1) to afford flavone-8-acetic acid (2.86 g, 51%) as colourless needles, m.p. 238–240 °C (lit.,[25] 234 °C) (Found: C, 73.1; H, 4.4. Calc. for $C_{17}H_{12}O_4$ C, 72.9; H, 4.3%); $\nu_{max}$ 1716, 1628, 1601, 1583, 1273, 1186 and 1171, 988 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.0–12.0 (1 H, br s, OH), 8.10 (2 H, dd, *J* 8, 2, H-2',6'), 8.00 (1 H, dd, *J* 8, 2, H-5) 7.78 (1 H, dd, *J* 8, 2, H-7), 7.61 (3 H, m, H-3',4',5'), 7.45 (1 H, t, *J* 8, H-6), 7.03 (1 H, s, H-3) and 4.03 (2 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.6 ($4^{ry}$), 162.3 ($4^{ry}$), 154.0 ($4^{ry}$), 135.6 (CH), 131.7 (CH), 131.3 ($4^{ry}$), 129.1 (2 x CH), 126.3 (2 x CH), 125.6 ($4^{ry}$), 124.9 (CH), 123.6 (CH), 123.3 ($4^{ry}$), 106.9 (CH) and 35.5 ($CH_2$); *m/z* 280 ($M^+$, 100%), 235 (42), 208 (7), 133 (65), 107 (30) and 77 (35).

## 2. 2'-Methoxyflavone-8-acetic Acid

**a.** Preparation of methyl 2-methoxybenzoate **118**

A solution of 2-methoxybenzoic acid (5.0 g, 33 mmol) in methanol (100 ml) was heated under reflux for 10 min. Five drops of concentrated sulphuric acid were added and heating was continued for a further 8 h. The solution was evaporated and the residue taken up in dichloromethane (200 ml). This was washed twice with 2M sodium hydroxide (50 ml), dried and evaporated to afford methyl 2-methoxybenzoate (2.13 g, 39%) as a colourless liquid which was used without further purification, b.p. (oven temp.) 230 °C (lit.,[97] 228 °C); $\delta_H$ 7.60 (1 H, dd, *J* 9, 2, H-6), 7.30 (1 H, dd, *J* 9, 2, H-3), 7.02 (1 H, t, *J* 9, H-4), 3.91 (3 H, s) and 3.85 (3 H, s).

**b.** Alternative preparation of methyl 2-methoxybenzoate **118**

A mixture of 2-methoxybenzoic acid (5.0 g, 33 mmol) and thionyl chloride (50 ml) was heated under reflux for 15 min and then evaporated. A.R. methanol (100 ml) was then added to the residue dropwise with caution and the mixture was heated under reflux for 1 h. The solvent was evaporated leaving a yellow oil which was Kugelrohr distilled to afford methyl 2-methoxybenzoate (4.76 g, 87%) as a colourless liquid, b.p. (oven temp.) 230 °C (lit.,[97] 228 °C); $\delta_H$ 7.62 (1 H, dd, *J* 9, 2, H-6), 7.34 (1 H, dd, *J* 9, 2, H-3), 7.05 (1 H, t, *J* 9, H-4), 3.91 (3 H, s) and 3.83 (3 H, s).

**c.** Preparation of 8-allyl-2'-methoxyflavone **124**

The method of C1e was followed using methyl 2-methoxybenzoate (1.83 g, 1.58 ml, 11 mmol) to afford 8-allyl-2'-methoxyflavone (1.84 g, 52%) as colourless crystals, m.p. 88–89 °C (Found: C, 77.9; H, 5.4. $C_{19}H_{16}O_3$ requires C, 78.1; H, 5.5%); $\nu_{max}$ 1732, 1637, 1601, 1584, 1249, 1183, 1165, 1130, 1086, 1051, 917, 832 and 706 $cm^{-1}$; $\delta_H$ 8.10 (1 H,

dd, *J* 8, 2, H-5), 7.88 (1 H, dd, *J* 8, 2, H-7), 7.52 (1 H, d, *J* 6, H-3'), 7.44 (1 H, t, *J* 6, H-5'), 7.33 (1 H, t, *J* 8, H-6), 7.16 (1 H, s, H-3), 7.09 (1 H, t, *J* 6, H-4'), 7.03 (1 H, d, *J* 6, H-6'), 6.13–6.00 (1 H, m), 5.16–5.10 (2 H, m), 3.93 (3 H, s) and 3.70 (2 H, d, *J* 6); $\delta_C$ 179.2 ($4^{ry}$), 160.6 ($4^{ry}$), 158.0 ($4^{ry}$), 154.4 ($4^{ry}$), 135.5 (CH), 133.8 (CH), 132.4 (CH), 129.4 ($4^{ry}$), 129.2 (CH), 124.6 (CH), 123.70 (CH), 123.69 ($4^{ry}$), 120.9 ($4^{ry}$), 120.8 (CH), 116.8 ($CH_2$), 112.4 (CH), 111.8 (CH), 55.6 ($OCH_3$) and 34.0 ($CH_2$); *m/z* 292 ($M^+$, 35%), 161 (100), 145 (10), 131 (95), 103 (30), 89 (27) and 77 (40).

**d.** Preparation of 2'-methoxyflavone-8-acetic acid **130**

The method of C1f was followed using 8-allyl-2'-methoxyflavone (5.84 g, 20 mmol) to afford 2'-methoxyflavone-8-acetic acid (3.23 g, 52%) as colourless crystals, m.p. 203–205 °C (lit.,[91] 203–205 °C); $\nu_{max}$ 1694, 1622, 1566, 1253, 1189, 1073, 982, 886 and 683 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.50 (1 H, br s, OH), 7.98 (1 H, dd, *J* 8, 2, H-5), 7.89 (1 H, d, *J* 6, H-6'), 7.73 (1 H, dd, *J* 8, 2, H-7), 7.57 (1 H, t, *J* 6, H-4'), 7.43 (1 H, t, *J* 8, H-6), 7.23 (1 H, d, *J* 6, H-3'), 7.14 (1 H, t, *J* 6, H-5'), 6.94 (1 H, s, H-3), 3.95 (2 H, s) and 3.95 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.6 ($4^{ry}$), 160.2 ($4^{ry}$), 157.7 ($4^{ry}$), 154.3 ($4^{ry}$), 135.5 (CH), 132.8 (CH), 129.0 (CH), 125.4 ($4^{ry}$), 124.7 (CH), 123.5 (CH), 123.1 ($4^{ry}$), 120.7 (CH), 120.0 ($4^{ry}$), 112.6 (CH), 111.4 (CH), 55.9 ($OCH_3$) and 35.2 ($CH_2$); *m/z* 310 ($M^+$, 96%), 282 (18), 266 (27), 179 (27), 161 (38), 147 (38), 135 (78), 133 (100), 106 (37), 89 (38) and 77 (55).

**e.** Preparation of sodium 2'-methoxyflavone-8-acetate **136**

The acid (0.93 g, 3.0 mmol) and sodium hydroxide (0.12 g, 3.0 mmol) were added to 5 ml of water. The mixture was then heated with stirring to 70 °C, filtered and the filtrate evaporated to dryness. The brown residue was not further purified. This method did not give the required

purity for the biological testing and therefore it was abandoned in favour of the method below.

**f.** Improved preparation of sodium 2'-methoxyflavone-8-acetate **136**

A mixture of 2'-methoxyflavone-8-acetic acid (1.0 g, 3.2 mmol) and water (10 ml) was heated to 70 °C. Sodium bicarbonate (0.25 g, 3.0 mmol) was added very slowly. After the effervescence had subsided the mixture was heated to 70 °C again with stirring and then allowed to cool to 30 °C and filtered. The filtrate was then added dropwise to acetone (400 ml) and the white precipitate which formed was filtered off and washed with acetone to yield the sodium 2'-methoxyflavone-8-acetate (1.06 g, ~100%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1625, 1578, 1249, 1162, 1124, 1014, 864, 756 and 744 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.09 (1 H, dd, *J* 8, 2, H-5), 7.84 (1 H, dd, *J* 8, 2, H-7), 7.62 (1 H, d, *J* 8, H-6'), 7.54 (1 H, t, *J* 8, H-5'), 7.33 (1 H, t, *J* 8, H-6), 7.23 (1 H, d, *J* 8, H-3'), 7.14 (1 H, t, *J* 8, H-4'), 6.95 (1 H, s, H-3), 3.94 (3 H, s) and 3.60 (2 H, s); $\delta_H$ ($D_2O$) 7.57 (2 H, d, *J* 8), 7.46 (1 H, d, *J* 8), 7.26–7.10 (2 H, m), 6.89–6.82 (2 H, m), 6.63 (1 H, d, *J* 8), 3.63 (3 H, s) and 3.61 (2 H, s); $\delta_C$ ($D_2O$) 183.8 ($4^{ry}$), 181.3 ($4^{ry}$), 164.0 ($4^{ry}$), 160.7 ($4^{ry}$), 156.8 ($4^{ry}$), 138.8 (CH), 136.1 (CH), 131.2 (CH), 129.9 ($4^{ry}$), 127.9 (CH), 125.4 (CH), 124.3 ($4^{ry}$), 123.4 (CH), 120.6 ($4^{ry}$), 114.4 (CH), 112.6 (CH), 57.8 ($OCH_3$) and 41.3 ($CH_2$); *m/z* (FAB, glycerol) 687 ($[2M+Na]^+$, 31%), 355 ($[M+Na]^+$, 100), 333 ($[M+H]^+$, 26), 311 ($[M+H-Na]^+$, 31), 288 ($[M-CO_2]^+$, 14), 267 (46), 199 (14), 176 (64) and 154 (12).

### 3. 3'-Methoxyflavone-8-acetic Acid

**a.** Preparation of methyl 3-methoxybenzoate **119**

The method of C2a was followed using 3-methoxybenzoic acid (20.0 g, 132 mmol) to yield methyl 3-methoxybenzoate (15.3 g, 70%) as a colourless liquid, b.p. 88–89 °C at 1.0 mmHg (lit.,[98] 236–238 °C).

**b.** Preparation of 3'-methoxy-8-allylflavone **125**

The method of C1e was followed using methyl 3-methoxybenzoate (1.83 g, 11 mmol) to afford 8-allyl-3'-methoxyflavone (2.99 g, 93%) as colourless crystals, m.p. 109–110 °C (Found: C, 78.0; H, 5.6. $C_{19}H_{16}O_3$ requires C, 78.1; H, 5.5%); $\nu_{max}$ 1735, 1655, 1602, 1297, 1273, 1075 and 879 $cm^{-1}$; $\delta_H$ 8.07 (1 H, dd, *J* 8, 2, H-5), 7.52 (1 H, dd, *J* 8, 2, H-7), 7.47–7.34 (3 H, m, H-2',5',6'), 7.32 (1 H, t, *J* 8, H-6), 7.05 (1 H, d, *J* 8, H-4'), 6.77 (1 H, s, H-3), 6.14–6.00 (1 H, m), 5.18–5.11 (2 H, m), 3.85 (3 H, s) and 3.71 (2 H, d, *J* 6); $\delta_C$ 178.6 ($4^{ry}$), 162.7 ($4^{ry}$), 160.0 ($4^{ry}$), 154.1 ($4^{ry}$), 135.2 (CH), 134.1 (CH), 133.2 ($4^{ry}$), 130.1 (CH), 129.5 ($4^{ry}$), 124.9 (CH), 123.9 ($4^{ry}$), 123.8 (CH), 118.5 (CH), 117.1 (CH), 117.0 ($CH_2$), 111.6 (CH), 107.4 (CH), 55.4 ($CH_3$) and 33.9 ($CH_2$); *m/z* 292 ($M^+$, 100%), 264 (6), 160 (17), 145 (11), 132 (51), 115 (9), 103 (16), 89 (13) and 77 (20).

**c.** Preparation of 3'-methoxyflavone-8-acetic acid **131**

The method of C1f was followed using 8-allyl-3'-methoxyflavone (5.85 g, 20 mmol) to afford 3'-methoxyflavone-8-acetic acid (4.84 g, 78%) as colourless crystals, m.p. 253–257 °C (lit.,[25] 238–241 °C) (Found: C, 69.7; H, 4.4. Calc. for $C_{18}H_{14}O_5$ C, 69.7; H, 4.5%); $\nu_{max}$ 1713, 1627, 1583, 1283, 1171, 1080, 973 and 872 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.5–12.0 (1 H, br s, OH), 7.98 (1 H, dd, *J* 8, 2, H-5), 7.75 (1 H, dd, *J* 8, 2, H-7), 7.64 (1 H, d, *J* 8, H-6'), 7.58 (1 H, d, *J* 2, H-2'), 7.46 (2 H, m, H-6,5'), 7.15 (1 H, dd, *J* 8, 2, H-4'), 7.03 (1 H, s, H-3), 4.01 (2 H, s) and 3.88 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.2 ($4^{ry}$), 171.7 ($4^{ry}$), 162.1 ($4^{ry}$), 159.8 ($4^{ry}$), 154.1 ($4^{ry}$), 135.7 (CH), 132.6 ($4^{ry}$), 130.3 (CH), 125.5 ($4^{ry}$), 125.0 (CH), 123.7 (CH), 123.3 ($4^{ry}$), 118.6 (CH), 118.0 (CH), 111.0 (CH), 107.1 (CH), 55.4 ($OCH_3$) and 35.6 ($CH_2$); *m/z* 310 ($M^+$, 100%), 265 (25), 133 (65), 106 (20) and 77 (20).

**d.** Preparation of sodium 3'-methoxyflavone-8-acetate **137**

The method of C2f was followed using 3'-methoxyflavone-8-acetic acid (1.0 g, 3.2 mmol) to produce sodium 3'-methoxyflavone-8-acetate (1.04 g, 98%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1648, 1632, 1272, 1136, 1056, 790 and 701 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.71 (1 H, d, *J* 8), 7.55 (1 H, d, *J* 8), 7.32 (2 H, t, *J* 8), 7.23 (1 H, t, *J* 8), 7.11 (1 H, s), 6.86 (1 H, d, *J* 8), 6.58 (1 H, s), 3.72 (3 H, s) and 3.67 (2 H, s); $\delta_C$ ($D_2O$) 183.5 ($4^{ry}$), 181.1 ($4^{ry}$), 166.0 ($4^{ry}$), 161.5 ($4^{ry}$), 156.8 ($4^{ry}$), 139.1 (CH), 134.0 ($4^{ry}$), 133.0 (CH), 130.1 ($4^{ry}$), 128.3 (CH), 125.6 (CH), 124.8 ($4^{ry}$), 121.7 (CH), 120.9 (CH), 113.0 (CH), 108.1 (CH), 58.0 ($OCH_3$) and 41.5 ($CH_2$); *m/z* (FAB, glycerol) 623 ([(2 x M)–$CO_2$+Na]$^+$, 26%), 355 ([M+Na]$^+$, 56), 289 (50), 267 (64), 137 (62), 115 (100), 91 (9) and 77 (5).

## 4. 4'-Methoxyflavone-8-acetic Acid

**a.** Preparation of methyl 4-methoxybenzoate **120**

The method of C2a was followed using 4-methoxybenzoic acid (20.0 g, 132 mmol) to afford methyl 4-methoxybenzoate (16.5 g, 75%) as a white powder, m.p. 48–49 °C (lit.,[99] 49–51 °C); $\delta_H$ 7.99 and 6.91 (4 H, AB pattern, *J* 6), 3.91 (3 H, s) and 3.87 (3 H, s).

**b.** Preparation of 8-allyl-4'-methoxyflavone **126**

The method of C1e was followed using methyl 4-methoxybenzoate (1.83 g, 11 mmol) to afford 8-allyl-4'-methoxyflavone (2.83 g, 88%) as colourless crystals, m.p. 113–115 °C (Found: C, 77.7; H, 5.2. $C_{19}H_{16}O_3$ requires C, 78.1; H, 5.5%); $\nu_{max}$ 1718, 1637, 1513, 1282, 1169, 917, 833, 772 and 697 $cm^{-1}$; $\delta_H$ 8.12 (1 H, dd, *J* 8, 2, H-5), 7.93 and 7.04 (4 H, AB pattern, H-2',3',5',6'), 7.53 (1 H, dd, *J* 8, 2, H-7), 7.35 (1 H, t, *J* 8, H-6), 6.83 (1 H, s, H-3), 6.13 (1 H, m), 5.16 (2 H, m), 3.89 (3 H, s) and 3.76 (2 H, d, *J* 8); $\delta_C$ 178.6 ($4^{ry}$), 163.4 ($4^{ry}$), 162.5 ($4^{ry}$), 154.2 ($4^{ry}$),

135.3 (CH), 134.1 (CH), 129.4 ($4^{ry}$), 128.1 (2 x CH), 124.9 (CH), 124.1 ($4^{ry}$), 123.9 (CH), 123.7 ($4^{ry}$), 117.0 ($CH_2$), 114.6 (2 x CH), 105.8 (CH), 55.5 ($OCH_3$) and 34.0 ($CH_2$); *m/z* 292 ($M^+$, 100%), 161 (42), 152 (20), 132 (66), 115 (13), 103 (15), 89 (17) and 77 (35).

**c.** Preparation of 4'-methoxyflavone-8-acetic acid **132**

The method of C1f was followed using 8-allyl-4'-methoxyflavone (5.85 g, 20 mmol) to afford 4'-methoxyflavone-8-acetic acid (3.10 g, 50%) as colourless crystals, m.p. 252–254 °C (lit.,[25] 228–232 °C) (Found: C, 69.9; H, 4.5. Calc. for $C_{18}H_{14}O_5$ C, 69.7; H, 4.5%); $\nu_{max}$ 1718, 1634, 1604, 1586, 1182, 1078, 879 and 760 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.51 (1 H, br s, OH), 8.03 (2 H, d, *J* 9, H-2',6'), 7.96 (1 H, dd, *J* 8, 2, H-5), 7.72 (1 H, dd, *J* 8, 2, H-7), 7.42 (1 H, t, *J* 8, H-6), 7.12 (2 H, d, *J* 9, H-3',5'), 6.90 (1 H, s, H-3), 3.99 (2 H, s) and 3.86 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 176.9 ($4^{ry}$), 171.7 ($4^{ry}$), 162.3 ($4^{ry}$), 162.2 ($4^{ry}$), 153.9 ($4^{ry}$), 135.4 (CH), 128.1 (2 x CH), 125.4 ($4^{ry}$), 124.7 (CH), 123.6 (CH), 123.4 ($4^{ry}$), 123.3 ($4^{ry}$), 114.6 (2 x CH), 105.4 (CH), 55.5 ($OCH_3$) and 35.5 ($CH_2$); *m/z* 310 ($M^+$, 39%), 266 (100), 132 (83), 106 (23), 89 (13) and 77 (30).

**d.** Preparation of sodium 4'-methoxyflavone-8-acetate **138**

The method of C2f was followed using 4'-methoxyflavone-8-acetic acid (1.0 g, 3.2 mmol) to yield sodium 4'-methoxyflavone-8-acetate (1.0 g, 94%) as a white powder, m.p. 334–336 °C; $\nu_{max}$ 1635, 1580, 1245, 1144, 1026, 885, 851 and 763 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.55 (1 H, d, *J* 8, H-5), 7.49 (1 H, d, *J* 8, H-7), 7.40 and 6.45 (4 H, AB pattern, d, *J* 9), 7.21 (1 H, t, *J* 8, H-6), 6.24 (1 H, s, H-3), 3.61 (2 H, s) and 3.47 (3 H, s); $\delta_C$ ($D_2O$) 183.0 ($4^{ry}$), 181.3 ($4^{ry}$), 166.3 ($4^{ry}$), 164.2 ($4^{ry}$), 156.5 ($4^{ry}$), 138.8 (CH), 130.4 (2 x CH), 129.9 ($4^{ry}$), 128.0 (CH), 125.4 (CH), 124.9 ($4^{ry}$), 124.6 ($4^{ry}$), 116.6 (2 x CH), 106.3 (CH), 57.8 ($OCH_3$) and 41.5 ($CH_2$); *m/z*

(FAB, glycerol) 687 ([2M+Na]$^+$, 26%), 355 ([M+Na]$^+$, 74), 333 ([M+H]$^+$, 49), 288 (14), 267 (14), 199 (14), 176 (100) and 154 (31).

## 5. 4'-Methylflavone-8-acetic Acid

**a.** Preparation of methyl 4-methylbenzoate **121**

The method of C2a was followed using 4-methylbenzoic acid (5.0 g, 37 mmol) to afford methyl 4-methylbenzoate (4.05 g, 73.5%) as a low melting solid, m.p. 35–36 °C (lit.,[100] 32 °C); $\delta_H$ 7.91 and 7.22 (4 H, AB pattern, *J* 7), 3.87 (3 H, s) and 2.37 (3 H, s).

**b.** Preparation of 8-allyl-4'-methylflavone **127**

The method of C1e was followed using methyl 4-methylbenzoate (1.65 g, 11 mmol) to afford 8-allyl-4'-methylflavone (1.91 g, 63%) as colourless needles, m.p. 108–110 °C (Found: C, 82.6; H, 5.6. $C_{19}H_{16}O_2$ requires C, 82.6; H, 5.8%); $\nu_{max}$ 1637, 1212, 1142, 1039, 923, 824, 754 and 613 cm$^{-1}$; $\delta_H$ 8.06 (1 H, dd, *J* 8, 2, H-5), 7.74 (2 H, half AB pattern, *J* 8, H-2',6'), 7.50 (1 H, dd, *J* 8, 2, H-7), 7.33–7.24 (3 H, m, H-6,3',5'), 6.74 (1 H, s, H-3), 6.12–5.99 (1 H, m), 5.19–5.09 (2 H, m), 3.70 (2 H, d, *J* 6) and 2.37 (3 H, s); $\delta_C$ 178.6 (4$^{ry}$), 163.0 (4$^{ry}$), 154.1 (4$^{ry}$), 142.2 (4$^{ry}$), 135.3 (CH), 133.9 (CH), 129.8 (2 x CH), 129.6 (4$^{ry}$), 129.4 (4$^{ry}$), 126.1 (2 x CH), 124.8 (CH), 123.9 (4$^{ry}$), 123.8 (CH), 117.0 ($CH_2$), 106.6 (CH), 34.0 ($CH_2$) and 21.5 ($CH_3$); *m/z* 276 (M$^+$, 41%), 261 (5), 248 (6), 160 (13), 145 (8), 131 (43), 115 (100), 103 (32), 89 (22) and 77 (38).

**c.** Preparation of 4'-methylflavone-8-acetic acid **133**

The method of C1f was followed using 8-allyl-4'-methylflavone (5.52 g, 20 mmol) to afford 4'-methylflavone-8-acetic acid (2.18 g, 37%) as a white powder, m.p. 250–252 °C (lit.,[25] 250–252 °C); $\nu_{max}$ 1724, 1635, 1600, 1510, 1220, 1075, 1040, 836 and 758 cm$^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.3 (1 H ,br, OH), 7.95 (3 H, m, H-5,2',6'), 7.74 (1 H, dd, *J* 8, 2, H-7),

7.47–7.33 (3 H, m, H-6,3',5'), 6.99 (1 H, s, H-3), 4.01 (2 H, s) and 2.38 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.0 ($4^{ry}$), 172.0 ($4^{ry}$), 162.3 ($4^{ry}$), 153.9 ($4^{ry}$), 142.0 ($4^{ry}$), 135.6 (CH), 129.6 (2 x CH), 128.4 ($4^{ry}$), 126.2 (2 x CH), 125.5 ($4^{ry}$), 124.8 (CH), 123.6 (CH), 123.2 ($4^{ry}$), 106.1 (CH), 35.5 ($CH_2$) and 21.0 ($CH_3$); *m/z* 294 ($M^+$, 100%), 279 (10), 266 (10), 249 (20), 221 (3), 133 (19), 115 (10), 106 (7) and 77 (8).

**d.** Preparation of sodium 4'-methylflavone-8-acetate **139**

The method of C2f was followed using 4'-methylflavone-8-acetic acid (1.0 g, 3.4 mmol) to afford sodium 4'-methylflavone-8-acetate (0.99 g, 92%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1631, 1590, 1250, 1120, 870 and 751 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.56 (1 H, dd, *J* 8, 2, H-5), 7.50 and 7.32 (4 H, AB pattern, d, *J* 8, H-2',3',5',6'), 7.48 (1 H, dd, *J* 8, 2, H-7), 7.21 (1 H, t, *J* 8, H-6), 6.27 (1 H, s, H-3), 3.60 (2 H, s) and 2.14 (3 H, s); $\delta_C$ ($D_2O$) 183.1 ($4^{ry}$), 181.2 ($4^{ry}$), 166.0 ($4^{ry}$), 156.6 ($4^{ry}$), 139.0 (CH), 130.0 (2 x CH), 129.8 ($4^{ry}$), 128.0 (CH), 127.6 ($4^{ry}$), 126.2 (2 x CH), 125.74 (CH), 125.71 ($4^{ry}$), 124.6 ($4^{ry}$), 108.3 (CH), 41.6 ($CH_2$) and 21.6 ($CH_3$).

## 6. 4'-Chloroflavone-8-acetic Acid

**a.** Preparation of methyl 4-chlorobenzoate **122**

The method of C2a was followed using 4-chlorobenzoic acid (20.0 g, 128 mmol) to afford methyl 4-chlorobenzoate (21.4 g, 98%) as colourless plates, m.p. 40–43 °C (lit.,[101] 42–43 °C); $\delta_H$ 8.03 and 7.44 (4 H, AB pattern, *J* 8) and 3.94 (3 H, s).

**b.** Preparation of 4'-chloro-8-allylflavone **128**

The method of C1e was followed using methyl 4-chlorobenzoate (1.88 g, 11 mmol) to afford 4'-chloro-8-allylflavone (2.71 g, 83%) as colourless crystals, m.p. 133–135 °C (Found: C, 72.9; H, 4.1. $C_{18}H_{13}ClO_2$ requires

C, 72.9; H, 4.4%) $\nu_{max}$ 1726, 1637, 1597, 1277, 1194, 1092, 1016, 917, 851 and 761 $cm^{-1}$; $\delta_H$ 8.07 (1 H, dd, *J* 8, 2, H-5), 7.82 (2 H, half AB pattern, *J* 8, H-2',6'), 7.54–7.47 (3 H, m, H-7,3',5'), 7.33 (1 H, t, *J* 8, H-6), 6.77 (1 H, s, H-3), 6.13–5.99 (1 H, m), 5.19–5.09 (2 H, m) and 3.72 (2 H, d, *J* 6); $\delta_C$ 178.5 ($4^{ry}$), 161.8 ($4^{ry}$), 154.1 ($4^{ry}$), 137.8 ($4^{ry}$), 135.1 (CH), 134.3 (CH), 130.3 ($4^{ry}$), 129.4 (2 x CH), 129.4 ($4^{ry}$), 127.4 (2 x CH), 125.1 (CH), 123.9 (CH), 123.8 ($4^{ry}$), 117.1 ($CH_2$), 107.4 (CH) and 34.0 ($CH_2$); *m/z* 296 ($^{35}Cl$-$M^+$, 29%), 176 (100), 161 (100), 133 (64), 105 (34) and 77 (51).

**c.** Preparation of 4'-chloroflavone-8-acetic acid **134**

The method of C1f was followed using 8-allyl-4'-chloroflavone (5.93 g, 20 mmol) to afford 4'-chloroflavone-8-acetic acid (3.15 g, 50%) as colourless crystals, m.p. 234–237 °C (lit.,[91] 238–242 °C); $\nu_{max}$ 1726, 1688, 1624, 1586, 1269, 1114, 1093, 1075, 996, 813 and 721 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.12 (1 H, br s, OH), 8.09 and 7.63 (4 H, AB pattern, *J* 8, H-2',3',5',6'), 7.96 (1 H, dd, *J* 8, 2, H-5), 7.75 (1 H, dd, *J* 8, 2, H-7), 7.45 (1 H, t, *J* 8, H-6), 7.04 (1 H, s, H-3) and 4.00 (2 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.0 ($4^{ry}$), 171.7 ($4^{ry}$), 161.0 ($4^{ry}$), 153.9 ($4^{ry}$), 136.6 ($4^{ry}$), 135.6 (CH), 130.1 ($4^{ry}$), 129.1 (2 x CH), 128.0 (2 x CH), 125.5 ($4^{ry}$), 125.0 (CH), 123.7 (CH), 123.3 ($4^{ry}$), 107.2 (CH) and 35.5 ($CH_2$); *m/z* 314 ($^{35}Cl$-$M^+$, 100%), 300 (24), 296 (32), 134 (96), 106 (40), 83 (40) and 77 (32).

**d.** Preparation of sodium 4'-chloroflavone-8-acetate **140**

The method of C2f was followed using 4'-chloroflavone-8-acetic acid (1.0 g, 3.2 mmol) to give sodium 4'-chloroflavone-8-acetate (1.03 g, 96%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1550, 1250, 1129, 1042, 1005, 911, 832 and 750 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.58 (1 H, dd, *J* 8, 2, H-5), 7.72 and 7.34 (4 H, AB pattern, d, *J* 9), 7.46 (1 H, dd, *J* 8, 2, H-7), 7.20

(1 H, t, *J* 8, H-6), 6.30 (1 H, s, H-3) and 3.58 (2 H, s); $\delta_C$ ($D_2O$) 183.2 (4ry), 181.1 (4ry), 165.3 (4ry), 156.6 (4ry), 140.7 (4ry), 139.4 (CH), 133.2 (2 CH), 132.0 (CH), 131.1 (2 CH), 129.9 (CH), 128.5 (4ry), 125.8 (4ry), 124.7 (4ry), 108.0 (CH) and 41.7 ($CH_2$); *m/z* (FAB, glycerol) 695 ([(2 x M)+Na]$^+$, 15%), 359 ([M+Na]$^+$, 44), 337 ($M^+$, 40), 315 (10), 176 (100), 154 (36), 133 (45) and 95 (99).

## 7. 4'-Nitroflavone-8-acetic Acid

**a.** Preparation of methyl 4-nitrobenzoate **141**

The method of C2a was followed using 4-nitrobenzoic acid (20.0 g, 120 mmol) to yield methyl 4-nitrobenzoate (13.2 g, 61%) as cream plates, m.p. 93–96 °C (lit.,[102] 94–96 °C); $\delta_H$ 8.31 and 8.20 (4 H, AB pattern, *J* 10, H-2,3,5,6) and 3.96 (3 H, s).

**b.** Attempted preparation of 8-allyl-4'-nitroflavone **142**

The method of C1e was followed using methyl 4-nitrobenzoate (1.99 g, 11 mmol) to afford a brown oil which, on analysis by $^1H$ and $^{13}C$ NMR, showed unreacted 3-allyl-2-hydroxyacetophenone and methyl 4-nitrobenzoate.

## D. Preparation of Polymethoxy Substituted Flavone-8-acetic Acids

### 1. 2',3'-Dimethoxyflavone-8-acetic Acid

a. Preparation of methyl 2,3-dimethoxybenzoate **143**

The method of C2a was followed using 2,3-dimethoxybenzoic acid (20.0 g, 110 mmol) to yield methyl 2,3-dimethoxybenzoate (16.4 g, 76%) as colourless cubes, m.p. 56–57 °C (lit.,[103] 57.5 °C); $\delta_H$ 7.29 (1 H, m, H-5), 7.05 (2 H, m, H-4,6), 3.90 (3 H, s), 3.89 (3 H, s) and 3.86 (3 H, s).

b. Preparation of 8-allyl-2',3'-dimethoxyflavone **147**

The method of C1e was followed using methyl 2,3-dimethoxybenzoate (2.16 g, 11 mmol) to yield 8-allyl-2',3'-dimethoxyflavone (2.80 g, 79%) as yellow plates, m.p. 87–88 °C (Found: C, 74.7; H, 5.4. $C_{20}H_{18}O_4$ requires C, 74.5; H, 5.6%); $\nu_{max}$ 1636, 1583, 1265, 1101, 1031, 997, and 749 $cm^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 8, 2, H-5), 7.50 (1 H, dd, *J* 8, 2, H-7), 7.40–7.27 (2 H, m, H-6,6'), 7.17 (1 H, t, *J* 8, H-5'), 7.04 (2 H, m, H-3,4'), 6.10–6.00 (1 H, m), 5.14–5.06 (2 H, m), 3.90 (3 H, s), 3.85 (3 H, s) and 3.69 (2 H, d, *J* 6); $\delta_C$ 179.0 ($4^{ry}$), 160.9 ($4^{ry}$), 154.4 ($4^{ry}$), 153.4 ($4^{ry}$), 148.1 ($4^{ry}$), 135.4 (CH), 133.9 (CH), 129.5 ($4^{ry}$), 126.4 ($4^{ry}$), 124.8 (CH), 124.3 (CH), 123.81 ($4^{ry}$), 123.80 (CH), 120.6 (CH), 117.0 ($CH_2$), 114.9 (CH), 112.2 (CH), 60.9 ($OCH_3$), 56.0 ($OCH_3$) and 33.8 ($CH_2$); *m/z* 332 ($M^+$, 56%) 239 (22), 199 (18), 185 (15), 161 (100), 149 (23), 131 (32), 115 (26), 97 (48), 81 (90) and 77 (33).

c. Preparation of 2',3'-dimethoxyflavone-8-acetic acid **151**

The method of C1f was followed using 2',3'-dimethoxy-8-(3-hydroxy-2-oxopropyl)flavone (see section G1b) (7.08 g, 20 mmol) and potassium permanganate (9.48 g, 60 mmol) to afford 2',3'-dimethoxyflavone-8-

acetic acid (3.06 g, 45%) as a white powder, m.p. 188–190 °C (Found: C, 67.4; H, 4.5. $C_{19}H_{16}O_6$ requires C, 67.1; H, 4.7%); $\nu_{max}$ 1729, 1633, 1575, 1285, 1105, 1034, 995, 863 and 797 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.0–11.0 (1 H, br s, OH), 8.00 (1 H, dd, *J* 8, 2, H-5), 7.76 (1 H, dd, *J* 8, 2, H-7), 7.48–7.42 (2 H, m, H-6,5'), 7.31–7.26 (2 H, m, H-4',6'), 6.87 (1 H, s, H-3), 3.96 (2 H, s), 3.90 (3 H, s) and 3.83 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.2 ($4^{ry}$), 171.7 ($4^{ry}$), 160.6 ($4^{ry}$), 154.4 ($4^{ry}$), 153.1 ($4^{ry}$), 147.5 ($4^{ry}$), 135.7 (CH), 125.5 (2 C, $4^{ry}$), 124.9 (CH), 124.5 (CH), 123.6 (CH), 123.2 ($4^{ry}$), 120.4 (CH), 116.1 (CH), 111.4 (CH), 60.5 ($OCH_3$), 56.1 ($OCH_3$) and 35.2 ($CH_2$); *m/z* 340 ($M^+$, 100%), 326 (41), 311 (6), 294 (14), 280 (23), 265 (20), 253 (14), 237 (17), 209 (13), 179 (30), 161 (69), 147 (66), 133 (82), 120 (16), 106 (34), 91 (34) and 77 (42).

**d.** Preparation of sodium 2',3'-dimethoxyflavone-8-acetate **154**

The method of C2f was followed using 2',3'-dimethoxyflavone-8-acetic acid (1.0 g, 2.9 mmol) to afford sodium 2',3'-dimethoxyflavone-8-acetate (0.88 g, 87%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1562, 1247, 1086, 1000, 855 and 752 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.69 (1 H, dd, *J* 8, 2, H-5), 7.57 (1 H, dd, *J* 8, 2, H-7), 7.32 (1 H, t, *J* 8, H-6), 7.22 (1 H, dd, *J* 9, 2, H-6'), 6.96 (1 H, t, *J* 9, H-5'), 6.86 (2 H, m, H-3,4'), 3.71 (3 H, s), 3.68 (3 H, s) and 3.62 (2 H, s); $\delta_C$ ($D_2O$) 183.9 ($4^{ry}$), 181.3 ($4^{ry}$), 164.0 ($4^{ry}$), 157.0 ($4^{ry}$), 155.1 ($4^{ry}$), 150.0 ($4^{ry}$), 139.2 (CH), 130.1 ($4^{ry}$), 128.3 (CH), 127.8 (CH), 126.5 ($4^{ry}$), 125.6 (CH), 124.6 ($4^{ry}$), 123.0 (CH), 118.6 (CH), 112.6 (CH), 63.1 ($OCH_3$), 58.3 ($OCH_3$) and 41.3 ($CH_2$).

## 2. 2',4'-Dimethoxyflavone-8-acetic Acid

**a.** Preparation of methyl 2,4-dimethoxybenzoate **144**

The method of C2b was followed using 2,4-dimethoxybenzoic acid (20.0 g, 110 mmol) to afford methyl 2,4-dimethoxybenzoate (16.3 g,

76%) as a yellow liquid, b.p. (oven temp.) 154 °C at 1.5 mmHg (lit.,[104] 294–296 °C); $\delta_H$ 7.83 (1 H, d, *J* 10, H-6), 6.43 (2 H, m, H-3,5), 3.87 (3 H, s), 3.82 (3 H, s) and 3.81 (3 H, s).

**b.** Preparation of 8-allyl-2',4'-dimethoxyflavone **148**

The method of C1e was followed using methyl 2,4-dimethoxybenzoate (2.16 g, 11 mmol) to yield 8-allyl-2',4'-dimethoxyflavone (1.91 g, 54%) as a yellow solid, m.p. 113–114 °C (Found: C, 74.4; H, 6.0. $C_{20}H_{18}O_4$ requires C, 74.5; H, 5.6%); $\nu_{max}$ 1628, 1564, 1256, 1215, 1023, 843 and 817 $cm^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 8, 2, H-5), 7.88 (1 H, d, *J* 6, H-6'), 7.50 (1 H, dd, *J* 8, 2, H-7), 7.30 (1 H, t, *J* 8, H-6), 7.15 (1 H, s, H-3), 6.63 (1 H, dd, *J* 6, 2, H-5'), 6.53 (1 H, d, *J* 2, H-3'), 6.15–6.01 (1 H, m), 5.18–5.09 (2 H, m), 3.93 (3 H, s), 3.88 (3 H, s) and 3.70 (2 H, d, *J* 6); $\delta_C$ 179.2 ($4^{ry}$), 163.2 ($4^{ry}$), 160.5 ($4^{ry}$), 159.7 ($4^{ry}$), 154.3 ($4^{ry}$), 135.5 (CH), 133.6 (CH), 130.3 (CH), 129.2 ($4^{ry}$), 124.4 (CH), 123.7 (CH), 116.8 ($CH_2$), 113.7 ($4^{ry}$), 112.3 ($4^{ry}$), 111.1 (CH), 105.3 (CH), 98.9 (CH), 55.6 ($OCH_3$), 55.5 ($OCH_3$) and 34.0 ($CH_2$); *m/z* 322 ($M^+$, 100%), 282 (15), 199 (3), 161 (67), 132 (12), 119 (7), 103 (9), 91 (7) and 77 (12).

**c.** Preparation of 2',4'-dimethoxyflavone-8-acetic acid **152**

The method of C1f was followed using 8-allyl-2',4'-dimethoxyflavone (6.45 g, 20 mmol) to afford 2',4'-dimethoxyflavone-8-acetic acid (4.83 g, 71%) as colourless crystals, m.p. 225–228 °C (lit.,[91] 225–227 °C) (Found: C, 65.7; H, 4.8. Calc. for $C_{19}H_{16}O_6$ + 0.5 $H_2O$ C, 65.3; H, 4.9%); $\nu_{max}$ 1709, 1620, 1555, 1260, 1216, 1172, 1155, 1025, 839 and 759 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.0–12.6 (1 H, br s, OH), 7.97 (1 H, dd, *J* 8, 2, H-5), 7.91 (1 H, d, *J* 8, H-6'), 7.72 (1 H, dd, *J* 8, 2, H-7), 7.42 (1 H, t, *J* 6, H-6), 6.98 (1 H, s, H-3), 6.79–6.71 (2 H, m, H-3',5'), 3.98 (2 H, s), 3.96 (3 H, s) and 3.90 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.2 ($4^{ry}$), 172.0 ($4^{ry}$), 163.3 ($4^{ry}$), 160.2 ($4^{ry}$), 159.6 ($4^{ry}$), 154.2 ($4^{ry}$), 135.4 (CH),

130.2 (CH), 125.4 ($4^{ry}$), 124.6 (CH), 123.6 (CH), 123.1 ($4^{ry}$), 112.4 ($4^{ry}$), 110.0 (CH), 106.2 (CH), 99.1 (CH), 56.1 ($OCH_3$), 55.7 ($OCH_3$) and 35.5 ($CH_2$); *m/z* 340 ($M^+$, 90%), 300 (7), 277 (15), 251 (14), 162 (100), 152 (30), 135 (80), 119 (50), 97 (61) and 77 (19).

**d.** Preparation of sodium 2',4'-dimethoxyflavone-8-acetate **155**

The method of C2f was followed using 2',4'-dimethoxyflavone-8-acetic acid (1.0 g, 2.9 mmol) to afford sodium 2',4'-dimethoxyflavone-8-acetate (0.83 g, 82%) as a cream powder, m.p. >350 °C; $\nu_{max}$ 1600, 1561, 1262, 1199, 1081, 1032, 920, 898 and 804 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.52 (1 H, dd, *J* 8, 2, H-5), 7.44 (2 H, m, H-7,5'), 7.20 (1 H, t, *J* 8, H-6), 6.67 (1 H, s, H-3), 6.13 (1 H, d, *J* 8, H-6'), 5.76 (1 H, s, H-3'), 3.57 (6 H, s) and 3.42 (2 H, s); $\delta_C$ ($D_2O$) 183.4 ($4^{ry}$), 181.2 ($4^{ry}$), 165.2 ($4^{ry}$), 163.8 ($4^{ry}$), 162.4 ($4^{ry}$), 156.4 ($4^{ry}$), 138.3 (CH), 132.4 ($4^{ry}$), 129.8 (CH), 127.6 (CH), 125.1 (CH), 124.2 ($4^{ry}$), 113.4 ($4^{ry}$), 110.9 (CH), 108.6 (CH), 100.0 (CH), 57.7 (2 x $OCH_3$) and 41.3 ($CH_2$).

### 3. 2',5'-Dimethoxyflavone-8-acetic Acid

**a.** Preparation of 2,5-dimethoxybenzoate **145**

The method of C2b was followed using 2,5-dimethoxybenzoic acid (20.0 g, 110 mmol) to yield methyl 2,5-dimethoxybenzoate (17.2 g, 80%) as a colourless liquid, b.p. (oven temp.) 150 °C at 0.1 mmHg (lit.,[105] 95–98 °C at 1 mmHg); $\delta_H$ 7.31 (1 H, d, *J* 3, H-6), 7.02 (1 H, dd, *J* 10, H-4), 6.79 (1 H, d, *J* 10, H-3), 3.89 (3 H, s), 3.85 (3 H, s) and 3.75 (3 H, s).

**b.** Preparation of 8-allyl-2',5'-dimethoxyflavone **149**

The method of C1e was followed using methyl 2,5-dimethoxybenzoate (2.16 g, 11 mmol) to yield 8-allyl-2',5'-dimethoxyflavone as colourless needles (2.73 g, 77%), m.p. 96–98 °C (Found: C, 74.6; H, 5.4. $C_{20}H_{18}O_4$

requires C, 74.5; H, 5.6%); $\nu_{max}$ 1580, 1550, 1274, 1222, 1033, 931, 843, 801 and 742 $cm^{-1}$; $\delta_H$ 8.11 (1 H, dd, *J* 8, 2, H-5), 7.53 (1 H, dd, *J* 8, 2, H-7), 7.46 (1 H, d, *J* 2, H-6'), 7.34 (1 H, t, *J* 8, H-6), 7.20 (1 H, s, H-3), 7.10–6.95 (2 H, m, H-3',4'), 6.16–6.02 (1 H, m), 5.18–5.06 (2 H, m), 3.90 (3 H, s), 3.84 (3 H, s) and 3.72 (2 H, d, *J* 6); $\delta_C$ 179.2 ($4^{ry}$), 160.1 ($4^{ry}$), 154.4 ($4^{ry}$), 153.4 ($4^{ry}$), 152.4 ($4^{ry}$), 135.4 (CH), 134.0 (CH), 129.3 ($4^{ry}$), 124.7 (CH), 123.81 (CH), 123.79 ($4^{ry}$), 121.3 ($4^{ry}$), 117.7 (CH), 116.8 ($CH_2$), 114.1 (CH), 113.0 (CH), 112.6 (CH), 56.1 ($OCH_3$), 55.8 ($OCH_3$) and 34.0 ($CH_2$); *m/z* 322 ($M^+$, 64%), 307 (9), 196 (34), 181 (71), 161 (100), 147 (40), 131 (19), 119 (16), 107 (20) and 92 (10).

**c.** Preparation of 2',5'-dimethoxyflavone-8-acetic acid **153**

The method of C1f was followed using 2',5'-dimethoxy-8-(3-hydroxy-2-oxopropyl)flavone (see section G1c) (7.08 g, 20 mmol) and potassium permanganate (9.48 g, 60 mmol) to afford 2',5'-dimethoxyflavone-8-acetic acid (4.28 g, 63%) as a white powder, m.p. 182–184 °C (Found: C, 65.3; H, 4.5. $C_{19}H_{16}O_6 + 0.5\ H_2O$ requires C, 65.3; H, 4.8%); $\nu_{max}$ 1707, 1620, 1569, 1271, 1239, 1044, 821 and 748 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.5–12.0 (1 H, br s, OH), 7.96 (1 H, dd, *J* 8, 2, H-5), 7.74 (1 H, d, *J* 8, 2, H-7), 7.46 (1 H, d, *J* 8, H-6'), 7.43 (1 H, t, *J* 8, H-6), 7.22–7.11 (2 H, m, H-3',4'), 7.01 (1 H, s, H-3), 3.91 (2 H ,s), 3.89 (3 H, s) and 3.82 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.2 ($4^{ry}$), 171.6 ($4^{ry}$), 159.6 ($4^{ry}$), 154.2 ($4^{ry}$), 153.2 ($4^{ry}$), 152.0 ($4^{ry}$), 135.6 (CH), 125.5 ($4^{ry}$), 124.7 (CH), 123.8 (CH), 123.1 ($4^{ry}$), 120.2 ($4^{ry}$), 118.9 (CH), 114.1 (CH), 113.1 (CH), 111.5 (CH), 56.3 ($OCH_3$), 55.6 ($OCH_3$) and 35.5 ($CH_2$); *m/z* 340 ($M^+$, 17%), 296 (54), 253 (55), 162 (100), 133 (41), 105 (21), 91 (21) and 77 (67).

**d.** Preparation of sodium 2',5'-dimethoxyflavone-8-acetate **156**

The method of C2f was followed using 2',5'-dimethoxyflavone-8-acetic acid (1.0 g, 2.9 mmol) to yield sodium 2',5'-dimethoxyflavone-8-acetate

(0.83 g, 82%) as a white powder, m.p. 304–306 °C; $\nu_{max}$ 1571, 1229, 1173, 1115, 1031, 853, 801 and 738 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.63 (1 H, d, *J* 8, H-5), 7.49 (1 H, d, *J* 8, H-7), 7.27 (1 H, t, *J* 8, H-6), 6.93 (2 H, m, H-3, 6'), 6.58 (2 H, m, H-3',4'), 3.64 (3 H, s), 3.61 (2 H, s) and 3.57 (3 H, s); $\delta_C$ ($D_2O$) 183.7 ($4^{ry}$), 180.6 ($4^{ry}$), 163.0 ($4^{ry}$), 156.6 ($4^{ry}$), 155.6 ($4^{ry}$), 154.5 ($4^{ry}$), 138.9 (CH), 129.8 (CH), 128.0 ($4^{ry}$), 125.4 ($4^{ry}$), 124.3 (CH), 122.0 ($4^{ry}$), 120.5 (CH), 115.8 (CH), 114.1 (CH), 112.8 (CH), 58.1 ($OCH_3$), 58.0 ($OCH_3$) and 41.1 ($CH_2$).

### 4. 2',6'-Dimethoxyflavone-8-acetic Acid

**a.** Preparation of methyl 2,6-dimethoxybenzoate **146**

The method of C2a was followed using 2,6-dimethoxybenzoic acid (15.0 g, 82 mmol) to yield methyl 2,6-dimethoxybenzoate (7.61 g, 47%) as colourless crystals, m.p. 87 °C (lit.,[106] 88 °C); $\delta_H$ 7.29 (1 H, t, *J* 9, H-4), 7.53 (2 H, d, *J* 9, H-3,5), 3.91 (3 H, s) and 3.79 (6 H, s).

**b.** Attempted preparation of 8-allyl-2',6'-dimethoxyflavone **150**

The general method of C1e was followed using methyl 2,6-dimethoxybenzoate (2.16 g, 11 mmol) to produce a brown tar which was shown spectroscopically to be the unreacted ester and the 3-allyl-2-hydroxyacetophenone.

### 5. 3',4'-Dimethoxyflavone-8-acetic Acid

**a.** Preparation of methyl 3,4-dimethoxybenzoate **157**

The method of C2a was followed using 3,4-dimethoxybenzoic acid (20.0 g, 110 mmol) to afford methyl 3,4-dimethoxybenzoate (14.3 g, 66%) as colourless needles, m.p. 60–61 °C (lit.,[107] 62 °C); $\delta_H$ 7.71 (1 H, dd, *J* 6, 2, H-6), 7.54 (1 H, d, *J* 2, H-2), 6.89 (1 H, d, *J* 6, H-5), 3.93 (3 H, s), 3.92 (3 H, s) and 3.86 (3 H, s).

**b.** Preparation of 8-allyl-3',4'-dimethoxyflavone **160**

The method of C1e was followed using methyl 3,4-dimethoxybenzoate (2.16 g, 11 mmol) to afford 8-allyl-3',4'-dimethoxyflavone (2.76 g, 78%) as colourless crystals, m.p. 141–142 °C (Found: C, 74.8; H, 5.6. $C_{20}H_{18}O_4$ requires C, 74.5; H, 5.6%); $\nu_{max}$ 1645, 1598, 1518, 1148, 1025, 880 and 724 $cm^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 8, 2, H-5), 7.55 (2 H, m, H-7,6'), 7.39 (1 H, d, *J* 2, H-2'), 7.32 (1 H, t, *J* 8, H-6), 6.99 (1 H, d, *J* 8, H-5'), 6.77 (1 H, s, H-3), 6.19–6.00 (1 H, m), 5.17–5.09 (2 H, m), 3.97 (3 H, s), 3.96 (3 H, s) and 3.75 (2 H, d, *J* 8); $\delta_C$ 178.7 ($4^{ry}$), 163.0 ($4^{ry}$), 154.2 ($4^{ry}$), 152.1 ($4^{ry}$), 149.3 ($4^{ry}$), 135.3 (CH), 134.1 (CH), 129.2 ($4^{ry}$), 124.9 (CH), 124.5 ($4^{ry}$), 123.95 (CH), 123.94 ($4^{ry}$), 119.9 (CH), 116.9 ($CH_2$), 111.3 (CH), 108.8 (CH), 106.2 (CH), 56.1 ($OCH_3$), 56.0 ($OCH_3$) and 34.1 ($CH_2$); *m/z* 322 ($M^+$, 100%), 307 (10), 279 (70), 162 (32), 147 (16), 131 (16), 103 (13), 91 (17) and 77 (16).

**c.** Preparation of 3',4'-dimethoxyflavone-8-acetic acid **163**

The method of C1f was followed using 8-allyl-3',4'-dimethoxyflavone (6.45 g, 20 mmol) to afford 3',4'-dimethoxyflavone-8-acetic acid (2.14 g, 31%) as colourless crystals, m.p. 254–256 °C (lit.,[25] 250–254 °C); $\nu_{max}$ 1710, 1624, 1582, 1279, 1051, 872 and 762 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.49 (1 H, br s, OH), 8.01 (1 H, dd, *J* 8, 2, H-5), 7.74–7.70 (2 H, m, H-7,6'), 7.61 (1 H, d, *J* 2, H-2'), 7.42 (1 H, t, *J* 8, H-6), 7.11 (1 H, d, *J* 8, H-5'), 7.06 (1 H, s, H-3), 4.02 (2 H, s), 3.93 (3 H, s) and 3.90 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 176.9 ($4^{ry}$), 171.9 ($4^{ry}$), 162.3 ($4^{ry}$), 153.9 ($4^{ry}$), 151.8 ($4^{ry}$), 148.9 ($4^{ry}$), 135.4 (CH), 125.4 ($4^{ry}$), 124.7 (CH), 123.6 (CH), 123.4 ($4^{ry}$), 123.2 ($4^{ry}$), 119.9 (CH), 111.9 (CH), 108.9 (CH), 105.6 (CH), 55.8 ($OCH_3$), 55.7 ($OCH_3$) and 35.6 ($CH_2$); *m/z* 340 ($M^+$, 13%), 296 (71), 253 (23), 162 (100), 133 (42), 105 (15), 91 (20) and 77 (15).

**d.** Preparation of sodium 3',4'-dimethoxyflavone-8-acetate **166**

The method of C2f was followed using 3',4'-dimethoxyflavone-8-acetic acid (1.0 g, 2.9 mmol) to yield sodium 3',4'-dimethoxyflavone-8-acetate (0.96 g, 90%) as a white powder, m.p. 160–161 °C; $\nu_{max}$ 1550, 1250, 1129, 1006, 831 and 751 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.45 (2 H, m, H-5,7), 7.18 (1 H, t, *J* 8, H-6), 7.03 (1 H, d, *J* 9, H-6'), 6.65 (1 H, d, *J* 2, H-2'), 6.36 (1 H, d, *J* 9, H-5'), 6.18 (1 H, s, H-3), 3.57 (2 H, s), 3.53 (3 H, s) and 3.44 (3 H, s); $\delta_C$ ($D_2O$) 182.6 ($4^{ry}$), 180.8 ($4^{ry}$), 165.8 ($4^{ry}$), 156.2 ($4^{ry}$), 153.6 ($4^{ry}$), 150.1 ($4^{ry}$), 138.6 (CH), 129.7 (CH), 127.9 ($4^{ry}$), 125.2 (CH), 124.6 ($4^{ry}$), 124.3 (CH), 122.8 ($4^{ry}$), 113.5 (CH), 109.7 (CH), 106.0 (CH), 57.9 ($OCH_3$), 57.1 ($OCH_3$) and 41.5 ($CH_2$); *m/z* (FAB, glycerol) 749 ($[2M+Na]^+$, 5%), 683 ($2[M-CO_2]^+$, 11), 385 ($[M+Na]^+$, 34), 363 ($M^+$, 7), 319 (15), 296 (11), 223 (11), 165 (10), 137 (76) and 115 (100).

## 6. 3',5'-Dimethoxyflavone-8-acetic Acid

**a.** Preparation of methyl 3,5-dimethoxybenzoate **158**

The method of C2a was followed using 3,5-dimethoxybenzoic acid (20.0 g, 110 mmol) to afford methyl 3,5-dimethoxybenzoate (19.3 g, 90%) as a low melting solid, m.p. 40–42 °C (lit.,[108] 42–44 °C); $\delta_H$ 7.18 (2 H, m, H-2,6), 6.62 (1 H, t, *J* 2, H-4), 3.90 (3 H, s) and 3.81 (6 H, s).

**b.** Preparation of 8-allyl-3',5'-dimethoxyflavone **161**

The method of C1e was followed using methyl 3,5-dimethoxybenzoate (2.16 g, 11 mmol) to afford 8-allyl-3',5'-dimethoxyflavone as colourless crystals (2.27 g, 64%), m.p. 137–138 °C (Found: C, 74.3; H, 5.7. $C_{20}H_{18}O_4$ requires C, 74.5; H, 5.6%); $\nu_{max}$ 1732, 1644, 1602, 1588, 1573, 1300 and 1288 $cm^{-1}$; $\delta_H$ 8.05 (1 H, dd, *J* 8, 2, H-5), 7.50 (1 H, dd, *J* 8, 2, H-7), 7.38 (1 H, t, *J* 8, H-6), 7.07 (2 H, d, *J* 2, H-2',6'), 6.83 (1 H, s, H-3), 6.65 (1 H, t, *J* 2, H-4'), 6.20–6.00 (1 H, m), 5.20–5.10 (2 H, m), 3.89 (6 H, s) and 3.71 (2 H, d, *J* 8); $\delta_C$ 178.5 ($4^{ry}$), 162.6 ($4^{ry}$), 161.1

(2 C, $4^{ry}$), 154.1 ($4^{ry}$), 135.2 (CH), 134.1 (CH), 133.7 ($4^{ry}$), 129.4 ($4^{ry}$), 124.9 (CH), 123.9 ($4^{ry}$), 123.8 (CH), 117.0 ($CH_2$), 107.5 (CH), 104.3 (2 x CH), 103.4 (CH), 55.5 (2 x $OCH_3$) and 34.0 ($CH_2$); *m/z* 322 ($M^+$, 100%), 162 (15), 132 (24), 115 (7), 103 (11), 89 (6) and 77 (14).

**c.** Preparation of 3',5'-dimethoxyflavone-8-acetic acid **164**

The method of C1f was followed using 8-allyl-3',5'-dimethoxyflavone (6.45 g, 20 mmol) to afford 3',5'-dimethoxyflavone-8-acetic acid (3.03 g, 44%) as colourless crystals, m.p. 267–269 °C (lit.,[91] 261–263 °C); $\nu_{max}$ 1713, 1627, 1597, 1582, 1251, 1172 and 1065 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.50 (1 H, br s, OH), 7.97 (1 H, dd, *J* 8, 2, H-5), 7.74 (1 H, dd, *J* 8, 2, H-7), 7.45 (1 H, t, *J* 8, H-6), 7.22 (2 H, d, *J* 2, H-2',6'), 7.09 (1 H, s, H-3), 6.70 (1 H, t, *J* 2, H-4'), 3.99 (2 H, s) and 3.85 (6 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.6 ($4^{ry}$), 161.8 ($4^{ry}$), 160.9 (2 C, $4^{ry}$), 154.0 ($4^{ry}$), 135.7 (CH), 133.2 ($4^{ry}$), 125.5 ($4^{ry}$), 124.9 (CH), 123.6 (CH), 123.3 ($4^{ry}$), 107.2 (CH), 104.2 (CH), 104.1 (2 x CH), 55.5 (2 x $OCH_3$) and 35.6 ($CH_2$); *m/z* 340 ($M^+$, 7%), 296 (100), 268 (2), 152 (34), 134 (14), 106 (10), 89 (4) and 77 (8).

**d.** Preparation of sodium 3',5'-dimethoxyflavone-8-acetate **167**

The method of C2f was followed using 3',5'-dimethoxyflavone-8-acetic acid (1.0 g, 2.9 mmol) to yield sodium 3',5'-dimethoxyflavone-8-acetate (0.96 g, 92%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1632, 1206, 1157, 1063, 926, 838 and 723 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.75 (1 H, d, *J* 8), 7.60 (1 H, d, *J* 8), 7.37 (1 H, t, *J* 8), 6.87 (2 H, s), 6.71 (1 H, s), 6.42 (1 H, s), 3.76 (6 H, s) and 3.70 (2 H, s); $\delta_C$ ($D_2O$) 183.0 ($4^{ry}$), 180.8 ($4^{ry}$), 165.0 ($4^{ry}$), 162.4 (2 x C, $4^{ry}$), 156.4 ($4^{ry}$), 139.0 (CH), 134.2 ($4^{ry}$), 129.9 (CH), 128.2 (CH), 125.5 (CH), 124.6 ($4^{ry}$), 107.9 ($4^{ry}$), 106.1 (2 x CH), 58.0 (2 x $OCH_3$) and 41.3 ($CH_2$); *m/z* (FAB, glycerol) 747 ([(2 x M)+Na]$^+$, 22%),

385 ([M+Na]$^+$, 100), 363 ([M]$^+$, 25), 319 (40), 297 (46), 199 (17), 176 (93) and 154 (22).

**7. 3',4'-Methylenedioxyflavone-8-acetic Acid**

a. Preparation of methyl 3,4-methylenedioxybenzoate **159**

The method of C2a was followed using 3,4-methylenedioxybenzoic acid (20.0 g, 120 mmol) to afford methyl 3,4-methylenedioxybenzoate (10.3 g, 47%) as a white powder, m.p. 53 °C (lit.,[109] 53 °C); $\delta_H$ 7.67 (1 H, dd, *J* 9, 2, H-6), 7.52 (1 H, d, *J* 2, H-2), 6.88 (1 H, d, *J* 9, H-5), 3.94 (6 H, s) and 3.89 (3 H, s).

b. Preparation of 8-allyl-3',4'-methylenedioxyflavone **162**

The method of C1e was followed using methyl 3,4-methylenedioxybenzoate (1.98 g, 11 mmol) to afford 8-allyl-3',4'-methylenedioxyflavone (1.96 g, 58%) as colourless needles, m.p. 178–179 °C (Found: C, 74.1; H, 4.5. $C_{19}H_{14}O_4$ requires C, 74.5; H, 4.6%); $\nu_{max}$ 1732, 1645, 1595, 1506, 1252, 1165, 945, 864 and 725 cm$^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 8, 2, H-5), 7.55–7.49 (2 H, m, H-7,6'), 7.36–7.33 (2 H, m, H-6,2'), 6.92 (1 H, d, *J* 8, H-5'), 6.69 (1 H, s, H-3), 6.18–6.00 (3 H, m), 5.20–5.15 (2 H, m) and 3.73 (2 H, d, *J* 8); $\delta_C$ 178.5 (4$^{ry}$), 162.6 (4$^{ry}$), 154.1 (4$^{ry}$), 150.6 (4$^{ry}$), 148.5 (4$^{ry}$), 135.3 (CH), 134.0 (CH), 129.3 (4$^{ry}$), 125.9 (4$^{ry}$), 124.9 (CH), 123.91 (4$^{ry}$), 123.89 (CH), 121.3 (CH), 117.0 ($CH_2$), 112.3 (CH), 108.8 (CH), 106.4 (CH), 101.9 ($CH_2$) and 34.0 ($CH_2$); *m/z* 306 (M$^+$, 100%), 278 (6), 160 (11), 146 (75), 131 (32), 103 (13), 88 (11) and 77 (19).

c. Preparation of 3',4'-methylenedioxyflavone-8-acetic acid **165**

The method of C1f was followed using 8-allyl-3',4'-methylenedioxyflavone (6.13 g, 20 mmol) to yield 3',4'-methylenedioxyflavone-8-acetic acid (2.79 g, 43%) as a white powder,

m.p. 235–237 °C (Found: C, 63.9; H, 4.15. $C_{18}H_{12}O_6$ + 0.75 $H_2O$ requires C, 64.0; H, 4.0%); $\nu_{max}$ 1718, 1642, 1595, 1256, 1029, 913, 858 and 761 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.7 (1 H, br s, OH), 7.95 (1 H, dd, *J* 8, 2, H-5), 7.76–7.68 (2 H, m, H-7,6'), 7.61 (1 H, s, H-2'), 7.42 (1 H, t, *J* 8, H-6), 7.11 (1 H, d, *J* 8, H-5'), 6.98 (1 H, s, H-3), 6.17 (2 H, s) and 4.00 (2 H, s); $\delta_C$ ($CD_3SOCD_3$) 176.9 ($4^{ry}$), 171.9 ($4^{ry}$), 162.0 ($4^{ry}$), 154.0 ($4^{ry}$), 150.3 ($4^{ry}$), 149.1 ($4^{ry}$), 135.5 (CH), 125.4 ($4^{ry}$), 125.0 ($4^{ry}$), 124.8 (CH), 123.5 (CH), 123.1 ($4^{ry}$), 121.5 (CH), 109.6 (CH), 106.1 (CH), 105.8 (CH), 102.0 ($CH_2$) and 35.5 ($CH_2$); *m/z* 324 ($M^+$, 100%), 310 (11), 279 (10), 146 (52), 133 (22), 105 (10), 88 (6) and 77 (12).

**d.** Preparation of sodium 3',4'-methylenedioxyflavone-8-acetate **168**

The method of C2f was followed using 3',4'-methylenedioxyflavone-8-acetic acid (1.0 g, 3.1 mmol) to yield sodium 3',4'-methylenedioxyflavone-8-acetate (0.79 g, 73%) as a cream powder, m.p. >350 °C; $\nu_{max}$ 1645, 1586, 1301, 1251, 1212, 1111, 1028, 864 and 754 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.56 (2 H, m, H-5,7), 7.27 (1 H, t, *J* 8, H-6), 7.10 (1 H, dd, *J* 9, 2, H-5'), 6.89 (1 H, s, H-3), 6.44 (1 H, d, *J* 9, H-6'), 6.26 (1 H, d, *J* 2, H-2'), 5.77 (2 H, s) and 3.64 (2 H, s); $\delta_C$ ($D_2O$) 183.0 ($4^{ry}$), 181.2 ($4^{ry}$), 165.9 ($4^{ry}$), 156.5 ($4^{ry}$), 152.9 ($4^{ry}$), 150.2 ($4^{ry}$), 138.9 (CH), 137.6 ($4^{ry}$), 131.6 ($4^{ry}$), 130.0 (CH), 128.1 (CH), 126.3 ($4^{ry}$), 125.4 (CH), 124.6 (CH), 111.2 (CH), 108.1 (CH), 104.8 ($CH_2$) and 41.5 ($CH_2$); *m/z* (FAB, glycerol) 713 ($[2M+Na]^+$, 6%), 405 (40), 313 (93), 207 (20) and 115 (100).

## 8. 4'-Chloro-2'-methoxyflavone-8-acetic Acid

**a.** Preparation of methyl 4-chloro-2-methoxybenzoate **169**

The method of C2b was followed using 4-chloro-2-methoxybenzoic acid (20.0 g, 117 mmol) to afford an brown oil which was distilled at 143 °C at 0.2 mmHg to give methyl 4-chloro-2-methoxybenzoate (19.9 g, 93%)

as cream needles, m.p. 36 °C (lit.,[110] 36 °C); $\delta_H$ 7.71 (1 H, d, *J* 10, H-6), 6.91 (2 H, m, H-3,5), 3.82 (3 H, s) and 3.81 (3 H, s).

**b.** Preparation of 8-allyl-4'-chloro-2'-methoxyflavone **171**

The method of C1e was followed using methyl 4-chloro-2-methoxybenzoate (2.21 g, 11 mmol) to afford 8-allyl-4'-chloro-2'-methoxyflavone as yellow plates (2.08 g, 58%), m.p. 140–142 °C (Found: C, 70.1; H, 4.8. $C_{19}H_{15}ClO_3$ requires C, 69.8; H, 4.6%); $\nu_{max}$ 1635, 1590, 1108, 868 and 733 $cm^{-1}$; $\delta_H$ 8.09 (1 H, dd, *J* 8, 2, H-5), 7.82 (1 H, d, *J* 8, H-6'), 7.52 (1 H, dd, *J* 8, 2, H-7), 7.33 (1 H, t, *J* 8, H-6), 7.13 (1 H, s, H-3), 7.10 (1 H, dd, *J* 8, 2, H-5'), 7.02 (1 H, d, *J* 2, H-3'), 6.12–5.98 (1 H, m), 5.17–5.10 (2 H, m), 3.94 (3 H, s) and 3.68 (2 H, d, *J* 8); $\delta_C$ 179.0 ($4^{ry}$), 159.5 ($4^{ry}$), 158.5 ($4^{ry}$), 154.3 ($4^{ry}$), 138.2 ($4^{ry}$), 135.4 (CH), 134.0 (CH), 130.0 (CH), 129.3 ($4^{ry}$), 124.7 (CH), 123.8 (CH), 123.7 ($4^{ry}$), 121.1 (CH), 119.5 ($4^{ry}$), 116.9 ($CH_2$), 112.5 (CH), 112.4 (CH), 56.0 ($OCH_3$) and 34.0 ($CH_2$); *m/z* 326 ($^{35}Cl$-$M^+$, 56%), 161 (100), 131 (71), 103 (37) and 77 (35).

**c.** Preparation of 4'-chloro-2'-methoxyflavone-8-acetic acid **173**

The method of C1f was followed using 8-allyl-4'-chloro-2'-methoxyflavone (6.53 g, 20 mmol) to afford 4'-chloro-2'-methoxyflavone-8-acetic acid (4.00 g, 58%) as colourless crystals, m.p. 260–262 °C (Found: C, 62.7; H, 4.0. $C_{18}H_{13}ClO_5$ requires C, 62.7; H, 3.8%); $\nu_{max}$ 1729, 1621, 1579, 1556, 1161, 1144, 1109, 1017, 885 and 758 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.0–12.0 (1 H, br s, OH), 7.92 (1 H, dd, *J* 8, 2, H-5), 7.87 (1 H, dd, *J* 7, 2, H-5'), 7.73 (1 H, dd, *J* 8, 2, H-7), 7.43 (1 H, t, *J* 8, H-6), 7.33 (1 H, d, *J* 2, H-3'), 7.18 (1 H, d, *J* 7, H-6'), 6.93 (1 H, s, H-3), 3.96 (3 H, s) and 3.95 (2 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.0 ($4^{ry}$), 171.8 ($4^{ry}$), 159.1 ($4^{ry}$), 158.3 ($4^{ry}$), 154.1 ($4^{ry}$), 137.3 ($4^{ry}$), 135.6 (CH), 130.2 (CH), 125.4 ($4^{ry}$), 124.8 (CH), 123.5 (CH), 122.9 ($4^{ry}$),

120.7 (CH), 118.8 (4ry), 113.0 (CH), 111.4 (CH), 56.5 ($OCH_3$) and 35.2 ($CH_2$); *m/z* 344 ($^{35}Cl$-$M^+$, 100%), 300 (57), 179 (16), 165 (81), 147 (64), 133 (85), 106 (32) and 77 (48).

**d.** Preparation of sodium 4'-chloro-2'-methoxyflavone-8-acetate **175**

The method of C2f was followed using 4'-chloro-2'-methoxyflavone-8-acetic acid (1.0 g, 2.9 mmol) to afford sodium 4'-chloro-2'-methoxyflavone-8-acetate (0.96 g, 90%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1712, 1623, 1588, 1248, 1145, 1022, 891, 837, 808 and 753 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.27 (3 H, m, H-5,7,6'), 6.97 (1 H, t, *J* 8, H-6), 6.46 (2 H, m, H-3,5'), 6.20 (1 H, d, *J* 1, H-3') and 3.30 (5 H, s); $\delta_C$ ($D_2O$) 183.3 (4ry), 181.0 (4ry), 162.6 (4ry), 160.8 (4ry), 156.4 (4ry), 141.3 (CH), 138.9 (4ry), 131.8 (CH), 129.6 (CH), 128.0 (4ry), 125.4 (4ry), 124.0 (CH), 123.6 (CH), 118.9 (CH), 114.8 (4ry), 112.4 (CH), 58.3 ($OCH_3$) and 41.3 ($CH_2$); m/z (FAB, glycerol) 366 ($M^+$, 15%), 345 (35), 302 (10), 176 (23), 154 (23), 131 (28), 119 (51), 109 (63), 95 (100) and 91 (21).

## 9. 5'-Chloro-2'-methoxyflavone-8-acetic Acid

**a.** Preparation of methyl 5-chloro-2-methoxybenzoate **170**

The method of C2b was followed using 5-chloro-2-methoxybenzoic acid (20.0 g, 117 mmol) to afford methyl 5-chloro-2-methoxybenzoate (18.3 g, 85%) as a colourless liquid, b.p. (oven temp.) 240 °C (lit.,[111] 235–240 °C); $\delta_H$ 7.71 (1 H, d, *J* 3, H-6), 7.40 (1 H, dd, *J* 10, 3, H-4), 6.90 (1 H, d, *J* 10, H-3) and 3.90 (6 H, s).

**b.** Preparation of 8-allyl-5'-chloro-2'-methoxyflavone **172**

The method of C1e was followed using methyl 5-chloro-2-methoxybenzoate (2.21 g, 11 mmol) to afford 8-allyl-5'-chloro-2'-methoxyflavone as yellow plates (1.40 g, 39%), m.p. 108–110 °C (Found:

C, 70.0; H, 4.7. $C_{19}H_{15}ClO_3$ requires C, 69.8; H, 4.6%); $\nu_{max}$ 1637, 1594, 1395, 1283, 1146, 1029, 919 and 858 $cm^{-1}$; $\delta_H$ 8.08 (1 H, dd, *J* 8, 2, H-5), 7.87 (1 H, d, *J* 2, H-6'), 7.53 (1 H, dd, *J* 8, 2, H-7), 7.38 (1 H, dd, *J* 8, 2, H-4'), 7.33 (1 H, t, *J* 8, H-6), 7.12 (1 H, s), 6.95 (1 H, d, *J* 8, H-3'), 6.11–5.98 (1 H, m), 5.20 (2 H, m), 3.93 (3 H, s) and 3.70 (2 H, d, *J* 8); $\delta_C$ 179.0 ($4^{ry}$), 158.8 ($4^{ry}$), 156.6 ($4^{ry}$), 154.4 ($4^{ry}$), 135.4 (CH) 134.2 (CH), 131.8 (CH), 129.4 ($4^{ry}$), 128.3 (CH), 126.0 ($4^{ry}$), 124.8 (CH), 123.80 (CH), 123.79 ($4^{ry}$), 122.1 ($4^{ry}$), 117.0 ($CH_2$), 113.1 (CH), 112.8 (CH), 56.0 ($CH_3$) and 34.2 ($CH_2$); *m/z* 326 ($^{35}Cl$-$M^+$, 57%), 161 (100), 131 (52), 103 (30) and 77 (31).

**c.** Preparation of 5'-chloro-2'-methoxyflavone-8-acetic acid **174**

The method of C1f was followed using 8-allyl-5'-chloro-2'-methoxyflavone (6.53 g, 20 mmol) to afford 5'-chloro-2'-methoxyflavone-8-acetic acid (3.17 g, 46%) as colourless needles, m.p. 247–249 °C (Found: C, 62.7; H, 3.4. $C_{18}H_{13}ClO_5$ requires C, 62.7; H, 3.8%); $\nu_{max}$ 1725, 1622, 1585, 1257, 1184, 1033, 1015 and 816 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.50 (1 H, br s, OH), 7.97–7.88 (2 H, m, H-5,6'), 7.74 (1 H, d, *J* 8, H-7), 7.60 (1 H, dd, *J* 9, 2, H-4'), 7.44 (1 H, t, *J* 8, H-6), 7.29 (1 H, d, *J* 9, H-3'), 6.98 (1 H, s, H-3), 3.98 (2 H, s) and 3.95 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.6 ($4^{ry}$), 158.6 ($4^{ry}$), 156.5 ($4^{ry}$), 154.2 ($4^{ry}$), 135.6 (CH), 132.2 (CH), 128.3 (CH), 125.5 ($4^{ry}$), 124.91 ($4^{ry}$), 124.90 (CH), 123.5 (CH), 123.0 ($4^{ry}$), 121.4 ($4^{ry}$), 114.5 (CH), 111.9 (CH), 56.4 ($OCH_3$) and 35.3 ($CH_2$); *m/z* 344 ($^{35}Cl$-$M^+$, 0.9%), 300 (8), 240 (5), 166 (7), 135 (17), 120 (8) and 91 (100).

**d.** Preparation of sodium 5'-chloro-2'-methoxyflavone-8-acetate **176**

The method of C2f was followed using 5'-chloro-2'-methoxyflavone-8-acetic acid (1.0 g, 2.9 mmol) to afford sodium 5'-chloro-2'-methoxyflavone-8-acetate (0.86 g, 81%) as a white powder, m.p. 200–

202 °C; $\nu_{max}$ 1733, 1582, 1255, 1181, 1145, 1017, 975, 911, 807 and 723 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.54 (1 H, dd, *J* 8, 2, H-5), 7.46 (2 H, m, H-7, 4'), 7.26 (1 H, t, *J* 8, H-6), 6.97 (1 H, s, H-3), 6.81 (1 H, d, *J* 8, H-3'), 6.61 (1 H, s, H-6'), 3.63 (3 H, s) and 3.61 (2 H, s); $\delta_C$ ($D_2O$) 183.8 ($4^{ry}$), 181.1 ($4^{ry}$), 162.5 ($4^{ry}$), 159.5 ($4^{ry}$), 156.8 ($4^{ry}$), 139.1 (CH), 135.5 (CH), 130.4 (CH), 130.1 ($4^{ry}$), 128.2 (CH), 128.1 ($4^{ry}$), 125.6 (CH), 124.4 ($4^{ry}$), 121.9 ($4^{ry}$), 116.3 (CH), 113.2 (CH), 58.4 ($OCH_3$) and 41.6 ($CH_2$); *m/z* (FAB, NOBA) 389 ($[M+Na]^+$, 11%), 367 (35), 345 (76), 329 (16), 301 (30), 289 (11), 176 (76), 154 (100), 136 (82), 123 (17) and 107 (35).

**10. 2',3',4'-Trimethoxyflavone-8-acetic Acid**

a. Preparation of methyl 2,3,4-trimethoxybenzoate **180**

The method of C2a was followed using 2,3,4-trimethoxybenzoic acid (20.0 g, 94 mmol) to yield methyl 2,3,4-trimethoxybenzoate (19.1 g, 90%) as a colourless oil, b.p. (oven temp.) 100–102 °C at 1.0 mmHg (lit.,[112] 281 °C); $\delta_H$ 7.51 (1 H, d, *J* 8), 7.62 (1 H, d, *J* 8) 3.88 (3 H, s), 3.83 (3 H, s) and 3.82 (6 H, s).

b. Preparation of 8-allyl-2',3',4'-trimethoxyflavone **177**

The method of C1e was followed using methyl 2,3,4-trimethoxybenzoate (2.49 g, 11 mmol) to afford 8-allyl-2',3',4'-trimethoxyflavone (2.44 g, 63%) as cream needles, m.p. 115–117 °C (Found: C, 71.7; H, 5.7. $C_{21}H_{20}O_5$ requires C, 71.6; H, 5.7%); $\nu_{max}$ 1634, 1287, 1167, 1005, 917, 796 and 761 $cm^{-1}$; $\delta_H$ 8.12 (1 H, dd, *J* 8, 2, H-5), 7.61 (1 H, d, *J* 8, H-6'), 7.53 (1 H, dd, *J* 8, 2, H-7), 7.38 (1 H, t, *J* 8, H-6), 7.12 (1 H, s, H-3), 6.83 (1 H, d, *J* 8, H-5'), 6.10–6.00 (1 H, m), 5.19–5.14 (2 H, m), 3.97 (9 H, s) and 3.71 (2 H, d, *J* 6); $\delta_C$ 179.1 ($4^{ry}$), 160.6 ($4^{ry}$), 156.2 ($4^{ry}$), 154.1 ($4^{ry}$), 153.2 ($4^{ry}$), 142.6 ($4^{ry}$), 135.5 (CH), 133.8 (CH), 129.3 ($4^{ry}$), 124.6 (CH), 124.0 (CH), 123.82 (CH), 123.81 ($4^{ry}$), 119.0 ($4^{ry}$), 116.9 ($CH_2$), 111.0 (CH), 107.4 (CH), 61.1 ($OCH_3$), 61.0 ($OCH_3$), 56.1

($OCH_3$) and 33.9 ($CH_2$); *m/z* 352 ($M^+$, 84%), 192 (66), 177 (21), 162 (22), 161 (100), 131 (13), 103 (8) and 77 (7).

c. Preparation of 2',3',4'-trimethoxyflavone-8-acetic acid **188**

The method of C1f was followed using 8-allyl-2',3',4'-trimethoxyflavone (7.05 g, 20 mmol) to afford 2',3',4'-trimethoxyflavone-8-acetic acid (3.44 g, 49%) as colourless crystals, m.p. 185–186 °C (Found: C, 64.7; H, 5.0. $C_{20}H_{18}O_7$ requires C, 64.9; H, 4.9%); $\nu_{max}$ 1718, 1618, 1577, 1418, 1293, 1114, 1012, 807, 766, 687 and 623 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.6 (1 H, br s, OH), 7.97 (1 H, dd, *J* 8, 2, H-5), 7.73 (1 H, dd, *J* 8, 2, H-7), 7.65 (1 H, d, *J* 9, H-6'), 7.44 (1 H, t, *J* 8, H-6), 7.01 (1 H, d, *J* 9, H-5'), 6.98 (1 H, s, H-3), 3.97 (2 H, s), 3.92 (3 H, s), 3.89 (3 H, s) and 3.83 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 172.0 ($4^{ry}$), 160.1 ($4^{ry}$), 156.2 ($4^{ry}$), 154.2 ($4^{ry}$), 152.4 ($4^{ry}$), 142.2 ($4^{ry}$), 135.6 (CH), 125.4 ($4^{ry}$), 124.8 (CH), 123.9 (CH), 123.6 (CH), 123.1 ($4^{ry}$), 117.7 ($4^{ry}$), 110.0 (CH), 109.2 (CH), 61.0 ($OCH_3$), 60.5 ($OCH_3$), 56.1 ($OCH_3$) and 35.3 ($CH_2$); *m/z* 370 ($M^+$, 100%), 356 (10), 325 (8), 309 (11), 295 (16), 281 (6), 267 (8), 192 (31), 179 (23), 161 (29), 149 (13), 133 (42), 119 (6), 105 (16), 91 (6) and 77 (16).

d. Preparation of sodium 2',3',4'-trimethoxyflavone-8-acetate **185**

The method of C2f was followed using 2',3',4'-trimethoxyflavone-8-acetic acid (1.0 g, 2.7 mmol) to yield sodium 2',3',4'-trimethoxyflavone-8-acetate (0.83 g, 86%) as a light pink powder, m.p. >350 °C; $\nu_{max}$ 1561, 1236, 1160, 1100, 990, 853 and 745 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.30 (2 H, m, H-5,7), 7.04 (1 H, t, *J* 8, H-7), 6.95 (1 H, d, *J* 8, H-6'), 6.42 (1 H, s, H-3), 6.26 (1 H, d, *J* 8, H-5'), 3.46 (6 H, s), 3.42 (2 H, s) and 3.38 (3 H, s); $\delta_C$ ($D_2O$) 183.2 ($4^{ry}$), 181.1 ($4^{ry}$), 163.7 ($4^{ry}$), 158.5 ($4^{ry}$), 156.6 ($4^{ry}$), 154.8 ($4^{ry}$), 143.6 ($4^{ry}$), 138.8 (CH), 129.9 (CH), 128.0 (CH), 127.1 ($4^{ry}$),

125.4 ($4^{ry}$), 124.3 (CH), 118.8 (CH), 110.9 (CH), 110.7 (CH), 63.5 ($OCH_3$), 58.4 (2 x $OCH_3$) and 41.2 ($CH_2$).

## 11. 2',4',5'-Trimethoxyflavone-8-acetic Acid

**a.** Preparation of methyl 2,4,5-trimethoxybenzoate **181**

The method of C2b was followed using 2,4,5-trimethoxybenzoic acid (20.0 g, 94 mmol) to afford methyl 2,4,5-trimethoxybenzoate (16.8 g, 79%) as colourless needles, m.p. 91–93 °C (lit.,[113] 92.5 °C); $\delta_H$ 7.39 (1 H, s, H-6), 6.51 (1 H, s, H-3), 3.90 (3 H, s), 3.88 (3 H, s) and 3.84 (6 H, s).

**b.** Preparation of 8-allyl-2',4',5'-trimethoxyflavone **178**

The method of C1e was followed using methyl 2,4,5-trimethoxybenzoate (2.49 g, 11 mmol) to yield 8-allyl-2',4',5'-trimethoxyflavone (2.13 g, 55%) as colourless needles, m.p. 125–128 °C (Found: C, 71.6; H, 5.9. $C_{21}H_{20}O_5$ requires C, 71.6; H, 5.7%); $\nu_{max}$ 1630, 1564, 1516, 1269, 1229, 1214, 1159, 1027, 908, 863, 817 and 763 $cm^{-1}$; $\delta_H$ 8.08 (1 H, dd, *J* 8, 2, H-5), 7.49 (1 H, dd, *J* 8, 2, H-7), 7.45 (1 H, s, H-6'), 7.30 (1 H, t, *J* 8, H-6), 7.19 (1 H, s, H-3), 6.58 (1 H, s, H-3'), 6.17–6.04 (1 H, m), 5.15–5.05 (2 H, m), 3.96 (6 H, s), 3.91 (3 H, s) and 3.70 (2 H, d, *J* 6); $\delta_C$ 179.0 ($4^{ry}$), 160.2 ($4^{ry}$), 154.2 ($4^{ry}$), 154.0 ($4^{ry}$), 152.4 ($4^{ry}$), 143.2 ($4^{ry}$), 135.4 (CH), 134.0 (CH), 128.9 ($4^{ry}$), 124.6 (CH), 123.8 (CH), 123.7 ($4^{ry}$), 116.7 ($CH_2$), 111.8 ($4^{ry}$), 111.5 (CH), 111.2 (CH), 97.1 (CH), 56.5 ($OCH_3$), 56.2 ($OCH_3$), 56.1 ($OCH_3$) and 34.1 ($CH_2$); *m/z* 352 ($M^+$, 100%), 337 (16), 322 (9), 309 (6), 295 (12), 192 (10), 177 (22), 161 (24), 149 (14), 132 (24), 103 (16) and 77 (14).

**c.** Preparation of 2',4',5'-trimethoxyflavone-8-acetic acid **189**

The method of C1f was followed using 8-allyl-2',4'5'-trimethoxyflavone (7.05 g, 20 mmol) to afford 2',4',5'-trimethoxyflavone-8-acetic acid

(2.29 g, 31%) as yellow needles, m.p. 262–263 °C (Found: C, 64.6; H, 4.6. $C_{20}H_{18}O_7$ requires C, 64.9; H, 4.9%); $\nu_{max}$ 1715, 1616, 1558, 1271, 1212, 1159, 1044, 1017, 858 and 758 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.0–12.0 (1 H, br s, OH), 7.94 (1 H, dd, *J* 8, 2, H-5), 7.70 (1 H, dd, *J* 6, 2, H-7), 7.48 (1 H, s, H-6'), 7.40 (1 H, t, *J* 8, H-6), 7.00 (1 H, s, H-3), 6.84 (1 H, s, H-3'), 3.98 (3 H, s), 3.95 (2 H, s), 3.91 (3 H, s) and 3.84 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.6 ($4^{ry}$), 159.8 ($4^{ry}$), 154.1 ($4^{ry}$), 153.7 ($4^{ry}$), 152.7 ($4^{ry}$), 143.0 ($4^{ry}$), 135.3 (CH), 125.2 ($4^{ry}$), 124.5 (CH), 123.6 (CH), 123.5 ($4^{ry}$), 111.3 (CH), 110.4 ($4^{ry}$), 110.0 (CH), 98.4 (CH), 56.5 ($OCH_3$), 56.0 ($OCH_3$), 55.9 ($OCH_3$) and 35.5 ($CH_2$); *m/z* 370 ($M^+$, 35%), 341 (16), 309 (17), 295 (17), 213 (6), 192 (7), 161 (10), 149 (25), 129 (24), 111 (26), 97 (51) and 77 (100).

**d.** Preparation of sodium 2',4',5'-trimethoxyflavone-8-acetate **186**

The method of C2f was followed using 2',4',5'-trimethoxyflavone-8-acetic acid (1.0 g, 2.7 mmol) to afford sodium 2',4',5'-trimethoxyflavone-8-acetate as a white powder, m.p. >350 °C; $\nu_{max}$ 1558, 1268, 1215, 1156, 1021, 852 and 755 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.71 (1 H, d, *J* 8, H-5), 7.24 (1 H, d, *J* 8, H-7), 7.16 (1 H, s, H-3), 6.94 (1 H, t, *J* 8, H-6), 6.52 (1 H, s, H-3'), 6.46 (1 H, s, H-6'), 3.47 (6 H, s), 3.34 (2 H, s) and 3.33 (3 H, s); $\delta_C$ ($D_2O$) 183.4 ($4^{ry}$), 180.7 ($4^{ry}$), 163.4 ($4^{ry}$), 157.7 ($4^{ry}$), 156.6 ($4^{ry}$), 154.8 ($4^{ry}$), 144.0 (CH), 138.8 ($4^{ry}$), 129.8 (CH), 127.9 ($4^{ry}$), 125.5 ($4^{ry}$), 124.6 (CH), 111.4 (2 x CH), 111.3 (CH), 98.7 ($4^{ry}$), 58.3 ($OCH_3$), 58.1 (2 x $OCH_3$) and 41.4 ($CH_2$).

## 12. 2',4',6'-Trimethoxyflavone-8-acetic Acid

**a.** Preparation of methyl 2,4,6-trimethoxybenzoate **184**

The method of C2a was followed using 2,4,6-trimethoxybenzoic acid (20.0g, 94 mmol) to afford methyl 2,4,6-trimethoxybenzoate (16.9 g,

70%) as colourless needles, m.p. 65–68 °C (lit.,[114] 67–70 °C); $\delta_H$ 6.12 (2 H, s, H-3,5), 3.90 (3 H, s) and 3.82 (9 H, s).

**b.** Attempted preparation of 8-allyl-2',4',6'-trimethoxyflavone **183**

The method of C1e was followed using methyl 2,4,6-trimethoxybenzoate (2.82 g, 11 mmol) to afford a brown oil which on analysis by $^1$H NMR was shown to be the unreacted ester and starting acetophenone.

## 13. 3',4',5'-Trimethoxyflavone-8-acetic Acid

**a.** Preparation of methyl 3,4,5-trimethoxybenzoate **182**

The method of C2a was followed using 3,4,5-trimethoxybenzoic acid (20.0 g, 94 mmol) to yield methyl 3,4,5-trimethoxybenzoate (13.2 g, 62%) as colourless needles, m.p. 81–82 °C (lit.,[112] 81 °C); $\delta_H$ 7.30 (2 H, d, *J* 1, H-2,6) and 3.90 (12 H, s).

**b.** Preparation of 8-allyl-3',4',5'-trimethoxyflavone **179**

The method of C1e was followed using methyl 3,4,5-trimethoxybenzoate (2.49 g, 11 mmol) to afford 8-allyl-3',4',5'-trimethoxyflavone (3.61 g, 93%) as yellow needles, m.p. 179–181 °C (Found: C, 71.3; H, 5.5. $C_{21}H_{20}O_5$ requires C, 71.6; H, 5.7%); $\nu_{max}$ 1602, 1551, 1239, 1107, 982, 862 and 745 cm$^{-1}$; $\delta_H$ 8.09 (1 H, dd, *J* 8, 2, H-5), 7.53 (1 H, dd, *J* 8, 2, H-7), 7.34 (1 H, t, *J* 8, H-6), 7.13 (2 H, d, *J* 2, H-2',6'), 6.76 (1 H, s, H-3), 6.19–6.01 (1 H, m), 5.18–5.07 (2 H, m), 3.94 (6 H, s), 3.92 (3 H, s) and 3.73 (2 H, d, *J* 6); $\delta_C$ 178.6 (4$^{ry}$), 162.7 (4$^{ry}$), 154.2 (4$^{ry}$), 153.5 (2 C, 4$^{ry}$), 141.0 (4$^{ry}$), 135.3 (CH), 134.3 (CH), 129.1 (4$^{ry}$), 127.1 (4$^{ry}$), 125.0 (CH), 124.0 (CH), 123.9 (4$^{ry}$), 116.8 ($CH_2$), 107.1 (CH), 103.5 (2 CH), 61.1 ($OCH_3$), 56.2 (2 x $OCH_3$) and 34.2 ($CH_2$); *m/z* 352 ($M^+$, 100%), 337 (41), 309 (19), 281 (10), 251 (9), 195 (9), 177 (14), 161 (23), 149 (13), 133 (17), 103 (14), 89 (11) and 77 (26).

**c.** Preparation of 3',4',5'-trimethoxyflavone-8-acetic acid **190**

The method of C1f was followed using 8-allyl-3',4',5'-trimethoxyflavone (7.05 g, 20 mmol) to afford 3',4',5'-trimethoxyflavone-8-acetic acid (5.47 g, 74%) as colourless needles, m.p. 233–235 °C (Found: C, 64.5; H, 4.6. $C_{20}H_{18}O_7$ requires C, 64.9; H, 4.9%); $\nu_{max}$ 1695, 1610, 1563, 1243, 1119, 983, 843 and 742 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.3–12.0 (1 H, br s, OH), 7.95 (1 H, dd, *J* 8, 2, H-5), 7.73 (1 H, dd, *J* 8, 2, H-7), 7.42 (1 H, t, *J* 8, H-6), 7.35 (2 H, s), 7.12 (1 H, s), 4.01 (2 H, s), 3.92 (6 H, s) and 3.79 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 (4ry), 171.8 (4ry), 161.8 (4ry), 154.0 (4ry), 153.3 (2 C, 4ry), 140.7 (4ry), 135.6 (CH), 125.5 (4ry), 124.9 (CH), 123.6 (CH), 123.2 (4ry), 106.5 (CH), 104.3 (4ry), 103.9 (2 x CH), 60.2 ($OCH_3$), 56.2 (2 x $OCH_3$) and 35.8 ($CH_2$); *m/z* 370 ($M^+$, 1%), 316 (87), 285 (14), 270 (11), 255 (10), 242 (10), 217 (8), 161 (14), 138 (100), 133 (52), 123 (63), 105 (32) and 77 (52).

**d.** Preparation of sodium 3',4',5'-trimethoxyflavone-8-acetate **187**

The method of C2f was followed using 3',4',5'-trimethoxyflavone-8-acetic acid (1.0 g, 2.6 mmol) to afford sodium 3',4',5'-trimethoxyflavone-8-acetate (0.86 g, 84%) as a white powder, m.p. 193–195 °C; $\nu_{max}$ 1563, 1240, 1161, 1109, 1046, 979, 842 and 739 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.46 (2 H, m, H-5,7), 7.15 (1 H, t, *J* 8, H-6), 6.58 (1 H, s, H-3), 6.35 (2 H, s, H-2',6'), 3.66 (9 H, s) and 3.61 (2 H, s); $\delta_C$ ($D_2O$) 182.7 (4ry), 180.5 (4ry), 164.8 (4ry), 162.3 (4ry), 156.3 (4ry), 154.6 (2 C, 4ry), 138.2 (CH), 134.5 (4ry), 129.7 (CH), 127.9 (4ry), 125.2 (CH), 118.9 (4ry), 106.2 (CH), 105.2 (2 x CH), 61.2 ($OCH_3$), 58.4 (2 x $OCH_3$) and 41.4 ($CH_2$); *m/z* (FAB, glycerol) 415 ($[M+Na]^+$, 6%), 393 ($[M+H]^+$, 14), 371 (8), 176 (36), 154 (22), 133 (28), 119 (32), 109 (48), 95 (82) and 81 (100).

## 14. 2'-Bromo-3',4',5'-trimethoxyflavone-8-acetic Acid

a. Preparation of 2-bromo-3,4,5-trimethoxybenzoic acid **192**

Following the procedure of Mayer and Fikentscher[115] a solution of 3,4,5-trimethoxybenzoic acid **191** (50.0 g, 236 mmol) in chloroform (500 ml) containing water (5 ml) was heated under reflux while a solution of bromine (12.2 ml, 238 mmol) in chloroform (100 ml) was added dropwise over a period of 2 h. After heating for a further 2 h evolution of HBr had ceased and the solution was evaporated to give a cream solid which was recrystallised from water/acetone (20:1) to give 2-bromo-3,4,5-trimethoxybenzoic acid (54.5 g, 79%) as colourless needles, m.p. 146–148 °C (lit.,[115] 149–150 °C); $\delta_H$ 9.40 (1 H, br s, OH), 7.40 (1 H, s, H-6), 3.97 (3 H, s) and 3.91 (6 H, s).

b. Preparation of methyl 2-bromo-3,4,5-trimethoxybenzoate **194**

The method of C2b was followed using 2-bromo-3,4,5-trimethoxybenzoic acid (20.0 g, 68 mmol) to afford methyl 2-bromo-3,4,5-trimethoxybenzoate (14.1 g, 67%) as a colourless liquid, b.p. 200 °C at 15 mmHg (lit.,[116] 202 °C at 16 mmHg); $\delta_H$ 7.15 (1 H, s, H-6), 3.93 (3 H, s), 3.92 (3 H, s), 3.89 (3 H, s) and 3.86 (3 H, s).

c. Preparation of 8-allyl-2'-bromo-3',4',5'-trimethoxyflavone **196**

The method of C1e was followed using methyl 2-bromo-3,4,5-trimethoxybenzoate (3.37 g, 11 mmol) to afford 8-allyl-2'-bromo-3',4',5'-trimethoxyflavone (1.85 g, 39%) as colourless needles, m.p. 146–147 °C (Found: C, 58.1; H, 4.4. $C_{21}H_{19}BrO_5$ requires C, 58.5; H, 4.4%); $\nu_{max}$ 1632, 1566, 1196, 1099, 988, 919, 899, 834 and 737 $cm^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 8, 2, H-5), 7.52 (1 H, dd, *J* 8, 2, H-7), 7.35 (1 H, t, *J* 8, H-6), 6.91 (1 H, s), 6.55 (1 H, s), 6.12–5.92 (1 H, m), 5.13–5.02 (2 H, m), 4.00 (3 H, s), 3.99 (3 H, s), 3.91 (3 H, s) and 3.67 (2 H, d, *J* 6); $\delta_C$ 178.4 ($4^{ry}$), 163.7 ($4^{ry}$), 154.4 ($4^{ry}$), 152.9 ($4^{ry}$), 151.6 ($4^{ry}$), 145.1

(4ry), 135.5 (CH), 134.2 (CH), 129.7 (4ry), 129.4 (4ry), 125.0 (CH), 123.9 (4ry), 123.8 (CH), 116.8 ($CH_2$), 112.6 (CH), 109.9 (CH), 109.1 (4ry), 61.2 ($OCH_3$), 61.1 ($OCH_3$), 56.4 ($OCH_3$) and 33.6 ($CH_2$); *m/z* 430 ($^{79}$Br-$M^+$, 100%), 351 (24), 321 (6), 272 (14), 212 (6), 161 (13), 133 (19), 103 (11) and 77 (19).

**d.** Preparation of 2'-bromo-3',4',5'-trimethoxyflavone-8-acetic acid **198**

The method of C1f was followed using 8-allyl-2'-bromo-3',4',5'-trimethoxyflavone (8.62 g, 20 mmol) to afford 2'-bromo-3',4',5'-trimethoxyflavone-8-acetic acid (4.06 g, 45%) as a white powder, m.p. 233–236 °C (Found: C, 53.8; H, 3.8. $C_{20}H_{17}BrO_7$ requires C, 53.5; H, 3.8%); $\nu_{max}$ 1713, 1592, 1237, 1167, 1121, 1079, 1043, 862 and 754 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.04 (1 H, dd, *J* 8, 2, H-5), 7.79 (1 H, dd, *J* 8, 2, H-7), 7.51 (1 H, t, *J* 8, H-6), 7.25 (1 H, s), 6.69 (1 H, s), 3.91 (2 H, s), 3.88 (3 H, s), 3.87 (3 H, s) and 3.85 (3 H, s) (acid proton not apparent); $\delta_C$ ($CD_3SOCD_3$) 177.1 (4ry), 171.9 (4ry), 163.1 (4ry), 154.6 (4ry), 153.1 (4ry), 151.1 (4ry), 144.7 (4ry), 136.3 (CH), 128.7 (4ry), 125.8 (CH), 125.5 (CH), 124.0 (4ry), 123.4 (4ry), 112.4 (CH), 111.0 (CH), 108.1 (4ry), 61.2 ($OCH_3$), 61.1 ($OCH_3$), 56.6 ($OCH_3$) and 35.2 ($CH_2$); *m/z* 450 ($^{79}$Br-$M^+$, 10%), 400 (48), 375 (30), 281 (46), 231 (24), 181 (56), 149 (76), 119 (68), 97 (43) and 77 (100).

**e.** Preparation of sodium 2'-bromo-3',4',5'-trimethoxyflavone-8-acetate **200**

The method of C2f was followed using 2'-bromo-3',4',5'-trimethoxyflavone-8-acetic acid (1.0 g, 2.2 mmol) to afford sodium 2'-bromo-3',4',5'-trimethoxyflavone-8-acetate (0.95 g, 91%) as a grey powder, m.p. >350 °C; $\nu_{max}$ 1560, 1198, 1161, 1096, 989, 911, 854, 821 and 746 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.69 (1 H, dd, *J* 8, 2, H-5), 7.61 (1 H, dd, *J* 8, 2, H-7), 7.40 (1 H, t, *J* 8, H-6), 6.95 (1 H, s, H-3), 6.59 (1 H, s, H-

6'), 3.89 (6 H, s), 3.84 (2 H, s) and 3.78 (3 H, s); $\delta_C$ ($D_2O$) 182.5 ($4^{ry}$), 180.9 ($4^{ry}$), 166.3 ($4^{ry}$), 157.0 ($4^{ry}$), 154.6 ($4^{ry}$), 152.9 ($4^{ry}$), 146.9 ($4^{ry}$), 139.2 (CH), 130.4 ($4^{ry}$), 129.9 ($4^{ry}$), 128.5 (CH), 125.8 (CH), 124.6 (CH), 113.9 ($4^{ry}$), 113.0 ($4^{ry}$), 111.1 (CH), 63.9 (2 x $OCH_3$), 58.9 ($OCH_3$) and 40.9 ($CH_2$).

## 15. 2',3',4',5'-Tetramethoxyflavone-8-acetic Acid

**a.** Preparation of 2,3,4,5-tetramethoxybenzoic acid **193**

Following the procedure of Mayer and Fikentscher,[115] 2-bromo-3,4,5-trimethoxybenzoic acid (20.4 g, 70 mmol) was dissolved in methanol (250 ml) containing freshly dissolved sodium metal (4.8 g, 210 mmol) and copper bronze powder (2.0 g). The mixture was stirred vigorously and heated under reflux for 17 h, filtered through celite which was washed with methanol and the combined filtrates evaporated. The residue was dissolved in water (200 ml) and the solution acidified carefully with concentrated hydrochloric acid until pH 4 was reached. The mixture was extracted with methylene chloride (200 ml) which was dried and evaporated to give a white solid. This was recrystallised from hexane/ethyl acetate (5:1) to yield 2,3,4,5-tetramethoxybenzoic acid (13.6 g, 80%) as colourless needles, m.p. 85–87 °C (lit.,[115] 87–88 °C); $\delta_H$ 10.0–8.0 (1 H, br s, OH), 7.41 (1 H, s, H-6), 4.08 (3 H, s), 4.00 (3 H, s), 3.94 (3 H, s) and 3.90 (3 H, s).

**b.** Preparation of methyl 2,3,4,5-tetramethoxybenzoate **195**

The method of C2b was followed using 2,3,4,5-tetramethoxybenzoic acid (12.9 g, 53 mmol) to afford methyl 2,3,4,5-tetramethoxybenzoate (12.7 g, 93%) as a colourless liquid, b.p. (oven temp.) 120 °C at 1 mmHg (lit.,[117] 125–126 °C at 1–2 mmHg); $\delta_H$ 7.11 (1 H, s, H-6), 3.97 (3 H, s), 3.92 (3 H, s), 3.91 (3 H, s), 3.87 (3 H, s) and 3.85 (3 H, s).

**c.** Preparation of 8-allyl-2',3',4',5'-tetramethoxyflavone **197**

The method of C1e was followed using methyl 2,3,4,5-tetramethoxybenzoate (2.82 g, 11 mmol) to afford 8-allyl-2',3',4',5'-tetramethoxyflavone (1.13 g, 27%) as cream needles, m.p. 95–97 °C (Found: C, 69.4; H, 6.1. $C_{22}H_{22}O_6$ requires C, 69.1; H, 5.8%); $\nu_{max}$ 1581, 1551, 1222, 1092, 1037, 984, 900, 831 and 740 $cm^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 7, 2, H-5), 7.53 (1 H, dd, *J* 7, 2, H-7), 7.34 (1 H, t, *J* 7, H-6), 6.91 (1 H, s), 6.56 (1 H, s), 6.10–5.97 (1 H, m), 5.11–5.03 (2 H, m), 3.95 (6 H, s), 3.94 (3 H, s), 3.88 (3 H, s) and 3.65 (2 H, d, *J* 8); $\delta_C$ 178.4 ($4^{ry}$), 163.7 ($4^{ry}$), 154.5 ($4^{ry}$), 153.0 ($4^{ry}$), 151.7 ($4^{ry}$), 145.2 ($4^{ry}$), 135.5 (CH), 134.2 (CH), 129.8 ($4^{ry}$), 129.4 ($4^{ry}$), 125.1 (CH), 123.9 ($4^{ry}$), 123.9 (CH), 116.8 (CH), 112.6 (CH), 109.9 (CH), 109.1 ($4^{ry}$), 61.2 ($OCH_3$), 61.2 ($OCH_3$), 56.4 (2 x $OCH_3$) and 33.6 ($CH_2$); *m/z* 382 ($M^+$, 100%), 367 (24), 336 (9), 309 (5), 281 (7), 253 (10), 222 (13), 183 (10), 161 (42), 133 (24), 103 (18) and 77 (36).

**d.** Preparation of 2',3',4',5'-tetramethoxyflavone-8-acetic acid **199**

The method of C1f was followed using 8-allyl-2',3',4',5'-tetramethoxyflavone (4.20 g, 11 mmol) to afford 2',3',4',5'-tetramethoxyflavone-8-acetic acid (2.07 g, 47%) as colourless needles, m.p. 248–250 °C (Found: C, 61.6; H, 4.7. $C_{21}H_{20}O_8$ + 0.5 $H_2O$ requires C, 61.6; H, 5.1%); $\nu_{max}$ 1711, 1589, 1240, 1119, 1058, 867 and 760 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.01 (1 H, dd, *J* 8, 2, H-5), 7.79 (1 H, dd, *J* 8, 2, H-7), 7.50 (1 H, t, *J* 8, H-6), 7.23 (1 H, s, H-6'), 6.63 (1 H, s, H-3), 3.97 (2 H, s), 3.82 (6 H, s), 3.80 (3 H, s) and 3.79 (3 H, s) (acid proton not apparent); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.9 ($4^{ry}$), 170.5 ($4^{ry}$), 165.5 ($4^{ry}$), 163.0 ($4^{ry}$), 154.4 ($4^{ry}$), 152.9 ($4^{ry}$), 150.9 ($4^{ry}$), 136.1 (CH), 125.2 (CH), 123.7 (CH), 123.2 ($4^{ry}$), 112.4 (CH), 111.1 ($4^{ry}$), 107.5 (CH), 62.3 (2 x $OCH_3$), 56.6 ($OCH_3$), 54.1 ($OCH_3$) and 35.0 ($CH_2$); *m/z* 400 ($M^+$,

38%), 356 (12), 296 (43), 253 (20), 162 (100), 133 (17), 119 (88), 97 (32) and 77 (84).

**e.** Preparation of sodium 2',3',4',5'-tetramethoxyflavone-8-acetate **201**

The method of C2f was followed using 2',3',4',5'-tetramethoxyflavone-8-acetic acid (1.0 g, 2.5 mmol) to yield sodium 2',3',4',5'-tetramethoxyflavone-8-acetate (0.77 g, 73%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1555, 1240, 1156, 1099, 989 and 756 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.37 (2 H, m, H-5,7), 7.10 (1 H, t, *J* 8, H-6), 6.66 (1 H, s, H-3), 6.31 (1 H, s, H-6'), 3.63 (6 H, s), 3.57 (2 H, s) and 3.52 (6 H, s); $\delta_C$ ($D_2O$) 182.6 ($4^{ry}$), 180.9 ($4^{ry}$), 166.4 ($4^{ry}$), 157.1 ($4^{ry}$), 154.7 ($4^{ry}$), 152.9 ($4^{ry}$), 146.9 ($4^{ry}$), 139.4 ($4^{ry}$), 130.4 ($4^{ry}$), 129.9 (CH), 128.5 (CH), 125.9 ($4^{ry}$), 124.5 (CH), 114.0 (CH), 112.9 (CH), 111.2 ($4^{ry}$), 64.0 (2 x $OCH_3$), 63.9 ($OCH_3$), 58.9 ($OCH_3$) and 41.0 ($CH_2$).

## E. Preparation of 2-Heteroaryl Analogues of Flavone-8-acetic Acid

### 1. 2-(3-Thienyl)benzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl thiophene-3-carboxylate **204**

The method of C2b was followed using thiophene-3-carboxylic acid (20.0 g, 156 mmol) to afford methyl thiophene-3-carboxylate (20.5 g, 94%) as a colourless liquid, b.p. (oven temp.) 84 °C at 15 mmHg (lit.,[118] 90–91 °C at 17 mmHg).

**b.** Preparation of 8-allyl-2-(3-thienyl)benzopyran-4-one **202**

The method of C1e was followed using methyl thiophene-3-carboxylate (1.56 g, 11 mmol) to afford 8-allyl-2-(3-thienyl)benzopyran-4-one (2.15 g, 73%) as colourless needles, m.p. 154–155.5 °C (Found: C, 71.5; H, 4.3. $C_{16}H_{12}O_2S$ requires C, 71.6; H, 4.5%); $\nu_{max}$ 1733, 1637, 1597, 1258, 1098, 948, 823, 759 and 699 $cm^{-1}$; $\delta_H$ 8.09 (1 H, dd, *J* 8, 2, H-5), 8.00 (1 H, m, H-2'), 7.53 (1 H, dd, *J* 8, 2, H-7), 7.46 (2 H, m, H-4',5'), 7.34 (1 H, t, *J* 8, H-6), 6.71 (1 H, s, H-3), 6.15–6.01 (1 H, m), 5.20–5.13 (2 H, m) and 3.73 (2 H, d, *J* 8); $\delta_C$ 178.7 ($4^{ry}$), 159.3 ($4^{ry}$), 154.0 ($4^{ry}$), 135.8 (CH), 134.4 ($4^{ry}$), 134.2 (CH), 129.3 ($4^{ry}$), 127.5 (CH), 126.7 (CH), 125.0 (CH), 124.9 (CH), 123.93 (CH), 123.91 ($4^{ry}$), 117.0 ($CH_2$), 106.9 (CH) and 34.1 ($CH_2$); *m/z* 268 ($M^+$, 100%), 240 (5), 167 (10), 160 (15), 132 (33), 103 (14) and 77 (19).

**c.** Preparation of 2-(3-thienyl)benzopyran-4-one-8-acetic acid **206**

The method of C1f was followed using 8-allyl-2-(3-thienyl)flavone (5.37 g, 20 mmol) to afford 2-(3-thienyl)benzopyran-4-one-8-acetic acid (2.36 g, 41%) as colourless crystals, m.p. 239–242 °C (Found: C, 62.0; H, 3.3. $C_{15}H_{10}O_4S$ + 0.25 $H_2O$ requires C, 62.0; H, 3.4%); $\nu_{max}$ 1710, 1628, 1611, 1588, 1269, 1225, 1093 and 974 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$)

12.6 (1 H, br s, OH), 8.39 (1 H, t, *J* 1, H-2'), 7.97 (1 H, dd, *J* 8, 2, H-5), 7.74 (2 H, m, H-4',5'), 7.73 (1 H, dd, *J* 8, 2, H-7), 7.42 (1 H, t, *J* 8, H-6), 6.93 (1 H, s, H-3) and 4.01 (2 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.9 ($4^{ry}$), 158.7 ($4^{ry}$), 153.8 ($4^{ry}$), 135.5 (CH), 133.8 ($4^{ry}$), 128.2 (CH), 127.8 (CH), 125.4 (CH), 125.3 (CH), 124.8 (CH), 123.6 (CH), 123.3 ($4^{ry}$), 106.4 (CH) and 35.6 ($CH_2$); *m/z* 286 ($M^+$, 100%), 241 (42), 134 (78), 105 (54) and 77 (60).

**d.** Preparation of sodium 2-(3-thienyl)benzopyran-4-one-8-acetate **208**

The method of C2f was followed using 2-(3-thienyl)benzopyran-4-one-8-acetic acid (1.0 g, 3.5 mmol) to give sodium 2-(3-thienyl)benzopyran-4-one-8-acetate (0.95 g, 95%) as a white powder, m.p. >337 °C; $\nu_{max}$ 1700, 1561, 1240, 1090, 1020, 853 and 752 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.88 (1 H, d, *J* 8), 7.58 (1 H, d, *J* 8), 7.46 (1 H, d, *J* 8), 7.32–7.18 (3 H, m), 6.22 (1 H, s) and 3.56 (2 H, s); $\delta_C$ ($D_2O$) 183.2 ($4^{ry}$), 181.4 ($4^{ry}$), 164.0 ($4^{ry}$), 163.1 ($4^{ry}$), 139.0 (CH), 135.3 ($4^{ry}$), 131.5 (CH), 130.6 (CH), 129.8 ($4^{ry}$), 128.2 (CH), 127.3 (CH), 125.5 (CH), 124.6 ($4^{ry}$), 107.4 (CH) and 41.5 ($CH_2$); *m/z* (FAB, glycerol) 639 (([2M+Na]$^+$, 6%), 331 ([M+Na]$^+$, 34), 309 ($M^+$, 30), 176 (72), 155 (20), 109 (44) and 95 (100).

## 2. 2-(3-Methyl-2-thienyl)benzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl 3-methylthiophene-2-carboxylate **205**

The method of C2b was followed using 3-methylthiophene-2-carboxylic acid (20.0 g, 143 mmol) to yield methyl 3-methylthiophene-2-carboxylate (14.7 g, 67%) as a colourless liquid, b.p. 120 °C (lit.,[119] 116–117.5 °C); $\delta_H$ 7.40 (1 H, d, *J* 7, H-4), 6.92 (1 H, d, *J* 7, H-5), 3.86 (3 H, s) and 2.56 (3 H, s).

**b.** Preparation of 8-allyl-2-(3-methyl-2-thienyl)benzopyran-4-one **203**

The method of C1e was followed using methyl 3-methylthiophene-2-carboxylate (1.72 g, 11 mmol) to yield a brown liquid. This was distilled under reduced pressure (130 °C at 0.1 mmHg) and the resulting solid recrystallised from methanol to yield 8-allyl-2-(3-methyl-2-thienyl)benzopyran-4-one (1.58 g, 51%) as colourless needles, m.p. 146–147 °C (Found: C, 72.5; H, 4.7. $C_{17}H_{14}O_2S$ requires C, 72.3; H, 5.0%); $\nu_{max}$ 1631, 1578, 1206, 1090, 925, 839, 796 and 753 $cm^{-1}$; $\delta_H$ 8.10 (1 H, dd, *J* 8, 2, H-5), 7.55 (1 H, dd, *J* 8, 2, H-7), 7.48 (1 H, d, *J* 6, H-4'), 7.35 (1 H, t, *J* 8, H-6), 7.00 (1 H, d, *J* 6, H-5'), 6.62 (1 H, s, H-3), 6.18–5.95 (1 H, m), 5.20–5.10 (2 H, m), 3.70 (2 H, d, *J* 6) and 2.55 (3 H, s); $\delta_C$ 178.1 ($4^{ry}$), 159.3 ($4^{ry}$), 154.0 ($4^{ry}$), 140.1 ($4^{ry}$), 135.2 (CH), 134.0 (CH), 132.6 (CH), 129.3 ($4^{ry}$), 129.2 ($4^{ry}$), 128.5 (CH), 124.9 (CH), 123.74 ($4^{ry}$), 123.72 (CH), 117.0 ($CH_2$), 108.1 (CH), 33.7 ($CH_2$) and 16.8 ($CH_3$); *m/z* 282 ($M^+$, 100%), 267 (13), 251 (6), 224 (3), 200 (3), 161 (33), 132 (56), 121 (34), 115 (17), 103 (19), 91 (31) and 77 (42).

**c.** Preparation of 2-(3-methyl-2-thienyl)benzopyran-4-one-8-acetic acid **207**

The method of C1f was followed using 8-allyl-2-(3-methyl-2-thienyl)benzopyran-4-one (5.65 g, 20 mmol) to afford 2-(3-methyl-2-thienyl)benzopyran-4-one-8-acetic acid (2.16 g, 36%) as colourless rhomboids, m.p. 225–227 °C (Found: C, 64.0; H, 4.0. $C_{16}H_{12}O_4S$ requires C, 64.0; H, 4.0%); $\nu_{max}$ 1713, 1619, 1575, 1210, 1139, 1031, 863, 759 and 729 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 12.7–12.5 (1 H, br s, OH), 7.95 (1 H, dd, *J* 8, 2, H-5), 7.84 (1 H, d, *J* 6, H-4'), 7.75 (1 H, dd, *J* 8, 2, H-7), 7.43 (1 H, t, *J* 8, H-6), 7.15 (1 H, d, *J* 6, H-5'), 6.57 (1 H, s, H-3), 3.96 (2 H, s) and 2.55 (3 H, s); $\delta_C$ ($CD_3SOCD_3$) 176.5 ($4^{ry}$), 171.7 ($4^{ry}$), 158.8 ($4^{ry}$), 154.1 ($4^{ry}$), 140.8 ($4^{ry}$), 135.9 (CH), 133.0 (CH), 130.0 (CH), 128.2 ($4^{ry}$), 125.2 ($4^{ry}$), 125.0 (CH), 123.8 (CH), 123.1 ($4^{ry}$), 107.5 (CH),

35.1 ($CH_2$) and 16.3 ($CH_3$); *m/z* 300 ($M^+$, 100%), 255 (18), 227 (8), 179 (16), 161 (12), 133 (20), 122 (20), 106 (12) and 77 (13).

**d.** Preparation of sodium 2-(3-methyl-2-thienyl)benzopyran-4-one-8-acetate **209**

The method of C2f was followed using 2-(3-methyl-2-thienyl) benzopyran-4-one-8-acetic acid (1.0 g, 3.3 mmol) to afford sodium 2-(3-methyl-2-thienyl) benzopyran-4-one-8-acetate (0.05 g, 5%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1696, 1610, 1580, 1239, 1093, 860 and 754 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.50 (1 H, dd, *J* 8, 2, H-5), 7.35 (1 H, dd, *J* 8, 2, H-7), 7.26 (1 H, d, *J* 6, H-4'), 7.14 (1 H, t, *J* 8, H-6), 6.60 (1 H, d, *J* 6, H-5'), 6.02 (1 H, s, H-3), 3.45 (2 H, s) and 2.08 (3 H, s); $\delta_C$ ($D_2O$) 182.6 ($4^{ry}$), 181.1 ($4^{ry}$), 163.7 ($4^{ry}$), 156.6 ($4^{ry}$), 145.3 ($4^{ry}$), 139.1 (CH), 136.0 (CH), 133.4 (CH), 130.2 ($4^{ry}$), 129.7 ($4^{ry}$), 128.3 (CH), 125.6 (CH), 124.5 ($4^{ry}$), 107.6 (CH), 41.1 ($CH_2$) and 19.4 ($CH_3$); *m/z* (FAB, glycerol) 667 ($[2M+Na]^+$, 9%), 345 ($[M+Na]^+$, 56), 323 ($[M+H]^+$, 30), 301 ($[M-Na]^+$, 16), 278 (16), 256 (14), 176 (100) and 154 (57).

**3. 2-(3-Methoxy-2-thienyl)benzopyran-4-one-8-acetic Acid**

**a.** Preparation of methyl 3-methoxythiophene-2-carboxylate **210**

The method of C2a was followed by Dr R. B. Ritchie using 3-methoxythiophene-2-carboxylic acid (5.0 g, 32 mmol) to yield methyl 3-methoxythiophene-2-carboxylate (4.7 g, 86%) as colourless needles, m.p. 51–52 °C (lit.,[120] 54 °C); $\delta_H$ 7.39 (1 H, d, *J* 4, H-5), 6.83 (1 H, d, *J* 4, H-4), 3.94 (3 H, s) and 3.80 (3 H, s).

**b.** Preparation of 8-allyl-2-(3-methoxy-2-thienyl)benzopyran-4-one **211**

The method of C1e was followed by Dr R. B. Ritchie using methyl 3-thiophene-2-carboxylate (4.7 g, 27 mmol) to afford 8-allyl-2-(3-methoxy-2-thienyl)benzopyran-4-one (1.4 g, 17%) as colourless needles, m.p. 106–108 °C (Found: C, 68.0; H, 5.0. $C_{17}H_{14}O_3S$ requires C, 68.4; H, 4.7%); $\nu_{max}$ 1600, 1548, 1210, 1121, 1052, 999, 900, 831 and 718 $cm^{-1}$; $\delta_H$ 8.04 (1 H, dd, *J* 8, 2, H-5), 7.43 (2 H, m, H-7,5'), 7.28 (1 H, t, *J* 8, H-6), 7.09 (1 H, s, H-3), 6.90 (1 H, d, *J* 4, H-4'), 6.15–5.93 (1 H, m), 5.20–5.08 (2 H, m), 3.96 (3 H, s) and 3.63 (2 H, d, *J* 6); $\delta_C$ 178.4 ($4^{ry}$), 159.2 ($4^{ry}$), 158.1 ($4^{ry}$), 153.6 ($4^{ry}$), 135.4 (CH), 133.6 (CH), 129.1 ($4^{ry}$), 128.9 (CH), 124.5 (CH), 123.9 ($4^{ry}$), 123.7 (CH), 116.8 ($CH_2$), 116.3 (CH), 111.4 ($4^{ry}$), 106.6 (CH), 58.8 ($OCH_3$) and 33.8 ($CH_2$); *m/z* 298 ($M^+$, 100%), 267 (21), 255 (11), 199 (9), 161 (30), 138 (63), 123 (41), 103 (22) and 77 (32).

**c.** Preparation of 2-(3-methoxy-2-thienyl)benzopyran-4-one-8-acetic acid **212**

The method of C1f was followed using 8-allyl-2-(3-methoxy-2-thienyl)benzopyran-4-one (5.64 g, 20 mmol) to afford 2-(3-methoxy-2-thienyl)benzopyran-4-one-8-acetic acid (2.84 g, 45%) as colourless crystals, m.p. 252–254 °C (Found: C, 61.0; H, 3.6. $C_{16}H_{12}O_5S$ requires C, 60.8; H, 3.8%); $\nu_{max}$ 1700, 1579, 1320, 1118, 981, 839, 799 and 749 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 13.0–12.0 (1 H, br s, OH), 7.95 (1 H, dd, *J* 8, 2, H-5), 7.72 (1 H, dd, *J* 8, 2, H-7), 7.42 (1 H, t, *J* 8, H-6), 7.36 (2 H, m, H-4',5'), 7.12 (1 H, s, H-3), and 3.92 (5 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.1 ($4^{ry}$), 171.8 ($4^{ry}$), 161.8 ($4^{ry}$), 154.0 ($4^{ry}$), 153.2 ($4^{ry}$), 140.7 ($4^{ry}$), 135.6 (CH), 126.3 (CH), 125.4 ($4^{ry}$), 124.8 (CH), 123.6 (CH), 123.2 ($4^{ry}$), 106.5 (CH), 103.9 (CH), 60.1 ($OCH_3$) and 35.8 ($CH_2$); *m/z* 316 ($M^+$, 41%), 272 (26), 162 (87), 132 (59), 91 (12) and 77 (100).

**d.** Preparation of sodium 2-(3-methoxy-2-thienyl)benzopyran-4-one-8-acetate **213**

The method of C2f was followed using 2-(3-methoxy-2-thienyl) benzopyran-4-one-8-acetic acid (1.0 g, 3.2 mmol) to afford sodium 2-(3-methoxy-2-thienyl) benzopyran-4-one-8-acetate (0.70 g, 65%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1700, 1569, 1241, 1085, 860 and 751 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.45 (2 H, m, H-5,7), 7.18 (1 H, t, *J* 8, H-6), 6.71 (2 H, m, H-3, 4'), 6.52 (1 H, d, *J* 2, H-5'), 3.72 (3 H, s) and 3.70 (2 H, s); $\delta_C$ ($D_2O$) 182.4 ($4^{ry}$), 180.4 ($4^{ry}$), 164.6 ($4^{ry}$), 154.5 ($4^{ry}$), 154.2 ($4^{ry}$), 141.9 ($4^{ry}$), 138.5 (CH), 129.5 (CH), 128.4 (CH), 127.9 (CH), 125.1 ($4^{ry}$), 124.2 (CH), 107.0 ($4^{ry}$), 105.1 (CH), 58.4 ($OCH_3$) and 41.4 ($CH_2$); *m/z* (FAB, glycerol), 415 ($M^+$, 21%), 371 (6), 348 (5), 329 (16), 176 (100), 165 (11), 154 (46), 119 (30), 105 (42), 91 (65) and 77 (49).

## 4. 2-(2-Furyl)benzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl 2-furoate **222**

The method of C2a was followed using 2-furoic acid (20.0 g, 179 mmol) to yield a yellow liquid which was Kugelrohr distilled to afford methyl 2-furoate (14.2 g, 63%) as a colourless liquid, b.p. (oven temp.) 182-183 °C (lit.,[121] 181.3 °C); $\delta_H$ 7.59 (1 H, d, *J* 2, H-5), 7.18 (1 H, d, *J* 2, H-3), 6.51 (1 H, t, *J* 2, H-4) and 3.90 (3 H, s).

**b.** Preparation of 8-allyl-2-(2-furyl)benzopyran-4-one **220**

The method of C1e was followed using methyl 2-furoate (1.39 g, 11 mmol) to afford 8-allyl-2-(2-furyl)benzopyran-4-one (1.54 g, 56%) as yellow cubes, m.p. 164–166 °C (Found: C, 76.2; H, 4.8. $C_{16}H_{12}O_3$ requires C, 76.2; H, 4.8%); $\nu_{max}$ 1732, 1632, 1601, 1275, 1174, 1078, 988, 922, 883 and 825 $cm^{-1}$; $\delta_H$ 8.08 (1 H, dd, *J* 8, 2, H-5), 7.62 (1 H, d, *J* 8, H-5'), 7.53 (1 H, dd, *J* 8, 2, H-7), 7.32 (1 H, t, *J* 8, H-6), 7.09 (1 H, d, *J* 8, H-3'), 6.72 (1 H, s, H-3), 6.61 (1 H, m, H-4'), 6.12–5.98

(1 H, m), 5.22–5.13 (2 H, m) and 3.69 (2 H, d, *J* 8); $\delta_C$ 178.0 ($4^{ry}$), 154.8 ($4^{ry}$), 153.7 ($4^{ry}$), 146.5 ($4^{ry}$), 145.8 (CH), 135.3 (CH), 134.0 (CH), 129.2 ($4^{ry}$), 124.9 (CH), 124.2 ($4^{ry}$), 123.9 (CH), 116.9 ($CH_2$), 112.8 (CH), 112.5 (CH), 105.3 (CH) and 33.9 ($CH_2$); *m/z* 252 ($M^+$, 100%), 224 (19), 190 (10), 160 (37), 140 (28), 131 (89), 103 (40), 92 (25), 84 (53) and 77 (52).

**c.** Preparation of 2-(2-furyl)benzopyran-4-one-8-acetic acid **224**

The method of C1f was followed using 8-allyl-2-(2-furyl)benzopyran-4-one (5.1 g, 20 mmol) to afford 2-(2-furyl)benzopyran-4-one-8-acetic acid (2.3 g, 43%) as colorless needles, m.p. 244–246 °C (lit.,[91] 240–242 °C); $\nu_{max}$ 1718, 1642, 1603, 1245, 1082, 873 and 760 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.07 (1 H, d, *J* 2, H-5'), 7.93 (1 H, dd, *J* 8, 2, H-5), 7.73 (1 H, dd, *J* 8, 2, H-7), 7.43 (1 H, t, *J* 8, H-6), 7.33 (1 H, d, *J* 2, H-3'), 6.84 (1 H, d, *J* 2, H-4'), 6.62 (1 H, s, H-3) and 3.95 (2 H, s); $\delta_C$ ($CD_3SOCD_3$) 176.6 ($4^{ry}$), 172.3 ($4^{ry}$), 154.4 ($4^{ry}$), 153.8 ($4^{ry}$), 147.7 (CH), 145.6 ($4^{ry}$), 136.0 (CH), 125.7 (CH), 125.3 ($4^{ry}$), 123.9 (CH), 123.7 ($4^{ry}$), 114.2 (CH), 113.3 (CH), 104.4 (CH) and 35.6 ($CH_2$); *m/z* 270 ($M^+$, 99%), 256 (85), 225 (43), 134 (57), 133 (76), 120 (43), 106 (48), 92 (100) and 77 (48).

**d.** Preparation of sodium 2-(2-furyl)benzopyran-4-one-8-acetate **226**

The method of C2f was followed using 2-(2-furyl)benzopyran-4-one-8-acetic acid (1.0 g, 3.7 mmol) to give sodium 2-(2-furyl)-benzopyran-4-one-8-acetate (0.98 g, 91%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1702, 1578, 1290, 1237, 1088, 1014, 850 and 752 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.62 (2 H, m, H-5,4'), 7.45 (1 H, d, *J* 8, H-7), 7.24 (1 H, t, *J* 8, H-6), 7.05 (1 H, d, *J* 2, H-5'), 6.58 (1 H, d, *J* 2, H-3'), 6.26 (1 H, s, H-3) and 3.55 (2 H, s); $\delta_C$ ($D_2O$) 182.8 ($4^{ry}$), 181.6 ($4^{ry}$), 158.7 ($4^{ry}$), 156.4 ($4^{ry}$), 150.0

(CH), 147.7 ($4^{ry}$), 139.1 (CH), 129.8 ($4^{ry}$), 128.3 (CH), 125.7 (CH), 124.9 ($4^{ry}$), 118.0 (CH), 115.9 (CH), 105.7 (CH) and 41.3 ($CH_2$).

**5. 2-(3-Furyl)benzopyran-4-one-8-acetic Acid**

a. Preparation of methyl 3-furoate **223**

The method of C2a was followed using 3-furoic acid (20.0 g, 179 mmol) to afford methyl 3-furoate (15.6 g, 69%) as a colourless liquid b.p. (oven temp.) 159–160 °C (lit.,[122] 160 °C); $\delta_H$ 8.04 (1 H, d, *J* 1, H-2), 7.45 (1 H, dd, *J* 2, 1, H-4), 6.73 (1 H, d, *J* 2, H-5) and 3.87 (3 H, s).

b. Preparation of 8-allyl-2-(3-furyl)benzopyran-4-one **221**

The method of C1e was followed using methyl 3-furoate (1.39 g, 11 mmol) to afford 8-allyl-2-(3-furyl)benzopyran-4-one (2.11 g, 76%) as colourless needles, m.p. 131–132 °C (Found: C, 76.5; H, 4.7. $C_{16}H_{12}O_3$ requires C, 76.2; H, 4.8%); $\nu_{max}$ 1634, 1587, 1213, 1169, 1018 and 875 $cm^{-1}$; $\delta_H$ 8.05 (1 H, dd, *J* 8, 2, H-5), 8.02 (1 H, s, H-2'), 7.51 (1 H, d, *J* 2, H-5'), 7.49 (1 H, dd, *J* 8, 2, H-7), 7.31 (1 H, t, *J* 8, H-6), 6.72 (1 H, d, *J* 2, H-4'), 6.49 (1 H, s, H-3), 6.11–5.96 (1 H, m), 5.19–5.14 (2 H, m) and 3.65 (2 H, d, *J* 7); $\delta_C$ 178.2 ($4^{ry}$), 158.2 ($4^{ry}$), 153.9 ($4^{ry}$), 144.7 (CH), 142.8 (CH), 135.3 (CH), 134.0 (CH), 129.1 ($4^{ry}$), 124.8 (CH), 124.0 ($4^{ry}$), 124.0 (CH), 120.5 ($4^{ry}$), 116.9 ($CH_2$), 107.5 (CH), 107.0 (CH) and 34.0 ($CH_2$); *m/z* 252 ($M^+$, 100%), 224 (7), 160 (12), 145 (9), 131 (44), 115 (9), 103 (15) and 77 (18).

c. Attempted preparation of 2-(3-furyl)benzopyran-4-one-8-acetic acid **225**

The method of C1f was followed using 8-allyl-2-(3-furyl)benzopyran-4-one (2.1 g, 8.2 mmol) to afford a white solid only after evaporation to dryness. This solid dissolved in the minimum of water and was shown by $^1H$ NMR not to be the expected product.

**6. 2-(3-Chloro-2-benzothienyl)benzopyran-4-one-8-acetic acid**

**a.** Preparation of methyl 3-chlorobenzo[b]thiophene-2-carboxylate **214**

Following a procedure by Krubsack and Higa (lit.,[123]) a solution of cinnamic acid (14.8 g, 100 mmol) in thionyl chloride (25 g, 210 mmol) was heated under reflux for 2 h and then pyridine (2.0 g, 20 mmol) in thionyl chloride (5.0 g, 4.2 mmol) was added with continued heating under reflux for 12 h and the mixture allowed to cool. The elemental sulphur was filtered off and the filtrate evaporated. The resulting solid was recrystallised from hexane to give 3-chlorobenzo[b]thiophene-2-carbonylchloride **216** (3.9 g, 16%) as a yellow powder, m.p. 111–113 °C (lit.,[123] 113.5–114.5 °C)

The acid chloride was added portionwise into boiling methanol (100 ml) and the solvent removed to give methyl 3-chlorobenzo[b]thiophene-2-carboxylate (3.61 g, 95%) as a light yellow needles, m.p. 80-81 °C (Found: C, 53.0; H, 3.2. $C_{10}H_7ClO_2S$ requires C, 53.0; H, 3.1%); $\nu_{max}$ 1710, 1302, 1222, 1083, 1051, 932, 814 and 743 $cm^{-1}$; $\delta_H$ 7.93 (1 H, d, *J* 7), 7.77 (1 H, d, *J* 7), 7.47 (2 H, m) and 3.94 (3 H, s); $\delta_C$ 161.4 ($4^{ry}$), 138.5 ($4^{ry}$), 136.9 ($4^{ry}$), 128.2 (CH), 128.2 ($4^{ry}$), 127.5 ($4^{ry}$), 125.4 (CH), 123.8 (CH), 122.7 (CH) and 52.5 ($OCH_3$); *m/z* 226 ($^{35}Cl$-$M^+$, 55%), 195 (100), 167 (34), 132 (30), 123 (29) and 88 (15).

**b.** Preparation of 8-allyl-2-(3-chloro-2-benzothienyl)benzopyran-4-one **217**

The method of C1e was followed using methyl 3-chlorobenzo[b]thiophene-2-carboxylate (2.49 g, 11 mmol) to afford 8-allyl-2-(3-chloro-2-benzothienyl)benzopyran-4-one (0.85 g, 22%) as cream needles, m.p. 148–149 °C (Found: C, 68.3; H, 4.0. $C_{20}H_{13}ClO_2S$ requires C, 68.1; H, 3.7%); $\nu_{max}$ 1732, 1635, 1594, 1511, 1222, 1133, 1067, 938, 757 and 614 $cm^{-1}$; $\delta_H$ 8.05 (1 H, dd, *J* 8, 2, H-5), 7.95 (1 H, m), 7.85 (1 H, m),

7.60 (1 H, dd, *J* 8, 2, H-7), 7.58–7.50 (2 H, m), 7.38 (1 H, t, *J* 8, H-6), 7.29 (1 H, s, H-3), 6.16–6.00 (1 H, m), 5.24–5.14 (2 H, m), 3.71 (2 H, d, *J* 8); $\delta_C$ 177.9 ($4^{ry}$), 156.5 ($4^{ry}$), 153.8 ($4^{ry}$), 137.4 ($4^{ry}$), 137.3 ($4^{ry}$), 135.2 (CH), 134.2 (CH), 129.5 ($4^{ry}$), 127.7 ($4^{ry}$), 127.5 (CH), 125.6 (CH), 125.1 (CH), 123.83 ($4^{ry}$), 123.81 (CH), 123.0 (CH), 122.4 (CH), 117.1 ($CH_2$), 110.3 (CH) and 33.7 ($CH_2$) (one quaternary carbon not apparent); *m/z* 352 ($^{35}$Cl-$M^+$, 66%), 318 (38), 226 (55), 195 (100), 168 (38), 132 (48) and 77 (17).

c. Preparation of 2-(3-chloro-2-benzothienyl)benzopyran-4-one-8-acetic acid **218**

The method of C1f was followed using 8-allyl-2-(3-chloro-2-benzothienyl)benzopyran-4-one (7.04 g, 20 mmol) to afford 2-(3-chloro-2-benzothienyl)benzopyran-4-one-8-acetic acid (0.89 g, 12%) as a cream powder, m.p. 132–134 °C (Found: $M^+$, 371.0157. $C_{19}H_{11}ClO_4S$ requires *M*, 371.0145); $\nu_{max}$ 3400 (br), 1681, 1560, 1251, 1125, 1013, 879, 839 and 748 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.10 (1 H, dd, *J* 8, 2, H-5), 7.97 and 7.57 (4 H, AB pattern, *J* 7), 7.78 (1 H, dd, *J* 8, 2, H-7), 7.47 (1 H, t, *J* 8, H-6), 7.12 (1 H, s, H-3) and 3.98 (2 H, s) (acid proton not apparent); $\delta_C$ ($CD_3SOCD_3$) 176.6 ($4^{ry}$), 171.9 ($4^{ry}$), 156.2 ($4^{ry}$), 153.9 ($4^{ry}$), 139.2 ($4^{ry}$), 136.53 (CH), 136.50 ($4^{ry}$), 129.3 ($4^{ry}$), 128.4 (CH), 126.62 (CH), 126.60 ($4^{ry}$), 125.7 (CH), 124.1 ($4^{ry}$), 123.9 (CH), 123.7 ($4^{ry}$), 123.5 (CH), 122.9 (CH), 110.0 (CH) and 35.4 ($CH_2$); *m/z* (FAB, glycerol) 371 ($^{35}$Cl-$M^+$+$H^+$, 4%), 326 (9), 305 (28), 289 (10), 262 (10), 199 (18), 176 (22), 154 (100), 136 (93), 119 (41), 105 (43), 91 (90) and 77 (54).

**d.** Preparation of sodium 2-(3-chloro-2-benzothienyl)benzopyran-4-one-8-acetate **219**

The method of C2f was followed using 2-(3-chloro-2-benzothienyl)benzopyran-4-one-8-acetic acid (0.7 g, 1.9 mmol) to afford sodium 2-(3-chloro-2-benzothienyl)benzopyran-4-one-8-acetate (0.58 g, 78%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1615, 1561, 1211, 1129, 1067, 1010, 874, 840 and 743 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.43 (3 H, m), 7.15 (2 H, m), 6.89 (2 H, m), 6.78 (1 H, m) and 3.62 (2 H, s); $\delta_C$ ($D_2O$) 182.1 ($4^{ry}$), 180.9 ($4^{ry}$), 168.3 ($4^{ry}$), 159.6 ($4^{ry}$), 156.0 ($4^{ry}$), 139.5 ($4^{ry}$), 139.1 (CH), 138.9 ($4^{ry}$), 130.4 (CH), 129.6 (CH), 128.0 (CH), 125.7 (CH), 125.5 ($4^{ry}$), 125.2 (CH), 124.72 (CH), 124.70 ($4^{ry}$), 124.2 ($4^{ry}$), 109.4 (CH) and 40.7 ($CH_2$).

## 7. 2-(4-Pyridyl)benzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl isonicotinate **229**

The method of C2a was followed using isonicotinic acid (20.0 g, 148 mmol) to afford methyl isonicotinate (14.2 g, 65%) as a colourless liquid, b.p. (oven temp.) 104 °C at 21 mmHg (lit.,[124] 207–209 °C); $\delta_H$ 8.79 and 7.82 (4 H, AB pattern, *J* 9) and 3.95 (3 H, s).

**b.** Preparation of 8-allyl-2-(4-pyridyl)benzopyran-4-one **227**

The method of C1e was followed using methyl isonicotinate (1.51 g, 11 mmol) to afford 8-allyl-2-(4-pyridyl)benzopyran-4-one (2.60 g, 89%) as yellow needles, m.p. 146–148 °C (Found: C, 77.7; H, 4.8; N, 5.4. $C_{17}H_{13}NO_2$ requires C, 77.6; H, 5.0; N, 5.3%); $\nu_{max}$ 1664, 1598, 1549, 1209, 1158, 1014 and 992 $cm^{-1}$; $\delta_H$ 8.83 (2 H, dd, *J* 6, 2, H-3',5'), 8.09 (1 H, dd, *J* 8, 2, H-5), 7.76 (2 H, d, *J* 6, H-2',6'), 7.57 (1 H, dd, *J* 8, 2, H-7), 7.37 (1 H, t, *J* 8, H-6), 6.92 (1 H, s, H-3), 6.17–6.04 (1 H, m), 5.23–5.13 (2 H, m) and 3.69 (2 H, d, *J* 6); $\delta_C$ 178.1 ($4^{ry}$), 159.9 ($4^{ry}$), 153.9 ($4^{ry}$), 150.7 (2 x CH), 139.2 ($4^{ry}$), 134.9 (CH), 134.5 (CH), 129.4

(4ry), 125.3 (CH), 123.91 (CH), 123.90 (4ry), 119.5 (2 x CH), 117.1 ($CH_2$), 108.9 (CH) and 33.8 ($CH_2$); *m/z* 263 ($M^+$, 100%), 234 (11), 208 (6), 145 (7), 131 (21), 115 (11), 103 (11), 89 (11) and 77 (18).

**c.** Attempted preparation of 2-(4-pyridyl)benzopyran-4-one-8-acetic acid **231**

The method of C1f was followed using 8-allyl-2-(4-pyridyl)benzopyran-4-one (5.3 g, 20 mmol) to afford a black powder (0.6 g) which did not melt at the literature temperature m.p. >350 °C (lit.,[91] 275–277 °C).

## 8. 2-(2-Quinolyl)benzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl quinoline-2-carboxylate **230**

The method of C2a was followed using quinaldic acid (20.0 g, 116 mmol) to afford methyl quinoline-2-carboxylate (18.4 g, 85%) as pink needles, m.p. 83 °C (lit.,[125] 85 °C).

**b.** Preparation of 8-allyl-2-(2-quinolyl)benzopyran-4-one **228**

The method of C1e was followed using methyl quinoline-2-carboxylate (2.06 g, 11 mmol) to yield 8-allyl-2-(2-quinolyl)benzopyran-4-one (2.41 g, 70%) as yellow needles, m.p. 148–150 °C (Found: C, 80.3; H, 4.7 N, 4.4. $C_{21}H_{15}NO_2$ requires C, 80.5; H, 4.8; N, 4.5%); $\nu_{max}$ 1645, 1583, 1504, 1209, 1125, 916, 830 and 752 $cm^{-1}$; $\delta_H$ 8.30 (1 H, d, *J* 8), 8.13–8.05 (3 H, m), 7.83–7.70 (2 H, m), 7.65–7.51 (3 H, m), 7.35 (1 H, t, *J* 8, H-6), 6.21–6.05 (1 H, m), 5.20–5.13 (2 H, m) and 3.80 (2 H, d, *J* 6); $\delta_C$ 178.8 (4ry), 161.3 (4ry), 154.1 (4ry), 149.3 (4ry), 147.9 (4ry), 137.3 (CH), 135.2 (CH), 134.1 (CH), 130.4 (CH), 130.2 (CH), 129.5 (4ry), 128.7 (4ry), 128.0 (CH), 127.6 (CH), 125.1 (CH), 124.6 (4ry), 124.1 (CH), 117.5 ($CH_2$), 117.1 (CH), 109.0 (CH) and 34.1 ($CH_2$); *m/z* 313 ($M^+$, 100%), 287 (22), 284 (19), 256 (10), 154 (14), 128 (11), 103 (6) and 77 (5).

**c.** Preparation of 2-(2-quinolyl)benzopyran-4-one-8-acetic acid **232**

The method of C1f was followed using 8-allyl-2-(2-quinolyl)benzopyran-4-one (6.27 g, 20 mmol) to afford 2-(2-quinolyl)benzopyran-4-one-8-acetic acid (0.38 g, 6%) as a orange needles, m.p. 234–236 °C (Found: C, 70.0; H, 4.4; N, 3.7. $C_{20}H_{13}NO_4$ + 0.75 $H_2O$ requires C, 69.7; H, 4.2; N, 4.0%); $\nu_{max}$ 1739, 1634, 1581, 1250, 1210, 1117, 1089, 841 and 771 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 14.0–12.0 (1 H, br s, OH), 8.65 and 8.27 (2 H, AB pattern, *J* 9, H-3',4'), 8.12 (2 H, dd, *J* 8, 2), 8.01 (1 H, dd, *J* 8, 2, H-5), 7.87 (1 H, t, *J* 7), 7.81 (1 H, dd, *J* 8, 2, H-7), 7.71 (1 H, t, *J* 7), 7.49 (1 H, t, *J* 8, H-6), 7.41 (1 H, s, H-3) and 4.10 (2 H, s); $\delta_C$ ($CD_3SOCD_3$) 177.0 ($4^{ry}$), 171.4 ($4^{ry}$), 160.8 ($4^{ry}$), 154.0 ($4^{ry}$), 148.6 ($4^{ry}$), 147.1 ($4^{ry}$), 137.9 (CH), 135.8 (CH), 130.7 (CH), 129.4 (CH), 128.5 ($4^{ry}$), 128.2 (CH), 128.0 (CH), 125.7 ($4^{ry}$), 125.2 (CH), 123.71 ($4^{ry}$), 123.7 (CH), 117.6 (CH), 107.9 (CH) and 35.6 ($CH_2$); *m/z* 331 ($M^+$, 46%), 287 (100), 273 (44), 259 (13), 245 (13), 230 (17), 216 (10), 154 (50), 128 (21), 105 (16) and 77 (26).

## F. Preparation of Derivatives with Different Groups in the 2-Position

### 1. 2-Benzylbenzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl phenylacetate **233**

The method of C2b was followed using phenylacetic acid (20.0 g, 148 mmol) to yield a yellow oil which was Kugelrohr distilled to yield methyl phenylacetate (18.3 g, 84%) as a colourless liquid b.p. (oven temp.) 81 °C at 0.3 mmHg (lit.,[126] 220 °C); $\delta_H$ 7.18 (5 H, m), 3.67 (3 H, s) and 3.59 (2 H, s).

**b.** Preparation of 8-allyl-2-benzylbenzopyran-4-one **234**

The method of C1e was followed using methyl phenylacetate (1.65 g, 11 mmol) to afford 8-allyl-2-benzylbenzopyran-4-one (1.48 g, 49%) as yellow plates, m.p. 59–60 °C (Found: C, 82.6; H, 6.2. $C_{19}H_{16}O_2$ requires C, 82.6; H, 5.8%); $\nu_{max}$ 1643, 1599, 1272, 1167, 1078, 993, 891, 804, 772 and 759 $cm^{-1}$; $\delta_H$ 8.03 (1 H, dd, *J* 8, 2, H-5), 7.47 (1 H, dd, *J* 8, 2, H-7), 7.38–7.25 (6 H, m, H-6,2',3',4',5',6'), 6.13 (1 H, s, H-3), 5.97–5.83 (1 H, m), 5.06 (2 H, m), 3.93 (2 H, s) and 3.54 (2 H, d, *J* 8); $\delta_C$ 178.6 ($4^{ry}$), 167.5 ($4^{ry}$), 154.4 ($4^{ry}$), 135.3 (CH), 134.9 ($4^{ry}$), 133.8 (CH), 129.32 ($4^{ry}$), 129.30 (2 x CH), 128.9 (2 x CH), 127.4 (CH), 124.7 (CH), 123.7 (CH), 123.6 ($4^{ry}$), 116.7 ($CH_2$), 110.3 (CH), 40.8 ($CH_2$) and 33.8 ($CH_2$); *m/z* 276 ($M^+$, 100%), 185 (6), 161 (22), 132 (23), 115 (25), 91 (20) and 77 (16).

**c.** Preparation of 2-benzylbenzopyran-4-one-8-acetic acid **237**

The method of C1f was followed using 8-allyl-2-benzylbenzopyran-4-one (5.52 g, 20 mmol) to afford 2-benzylbenzopyran-4-one-8-acetic acid (0.71 g,12%) as yellow cubes, m.p. 140–142 °C (lit.,[25] 143–145 °C); $\nu_{max}$ 1716, 1650, 1603, 1590, 1228, 1169, 1132, 935, 758 and 702 $cm^{-1}$;

$\delta_H$ 12.5–11.5 (1 H, br s, OH), 7.90 (1 H, dd, *J* 8, 2, H-5), 7.67 (1 H, dd, *J* 8, 2, H-7), 7.40–7.33 (5 H, m, H-2',3',4',5',6'), 7.27 (1 H, t, *J* 8, H-6), 6.26 (1 H, s, H-3), 3.99 (2 H, s) and 3.83 (2 H, s); $\delta_C$ 176.9 ($4^{ry}$), 171.8 ($4^{ry}$), 167.8 ($4^{ry}$), 154.2 ($4^{ry}$), 135.6 ($4^{ry}$), 135.4 (CH), 129.1 (2 x CH), 128.6 (2 x CH), 127.0 (CH), 125.1 ($4^{ry}$), 124.7 (CH), 123.6 (CH), 123.0 ($4^{ry}$), 109.5 (CH), 39.5 ($CH_2$) and 34.6 ($CH_2$); *m/z* 294 ($M^+$, 100%), 277 (30), 249 (29), 179 (26), 133 (34), 115 (22), 105 (16), 91 (46) and 77 (15).

**d.** Preparation of sodium 2-benzylbenzopyran-4-one-8-acetate **239**

The method of C2f was followed using 2-benzylbenzopyran-4-one-8-acetic acid (1.0 g, 3.4 mmol) to afford sodium 2-benzylbenzopyran-4-one-8-acetate (0.95 g, 88%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1610, 1560, 1209, 1070, 1031, 980, 874, 820, 748 and 683 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.67 (1 H, d, *J* 8, H-5), 7.50–7.20 (7 H, m), 7.15 (1 H, s, H-3), 3.55 (2 H, s) and 3.53 (2 H, s); $\delta_C$ ($D_2O$) 183.9 ($4^{ry}$), 181.5 ($4^{ry}$), 161.8 ($4^{ry}$), 157.4 ($4^{ry}$), 139.9 (CH), 138.8 ($4^{ry}$), 134.7 (CH), 132.5 (2 x CH), 131.4 (2 x CH), 130.8 ($4^{ry}$), 126.4 (CH), 124.5 ($4^{ry}$), 120.2 (CH), 111.4 (CH), 47.2 ($CH_2$) and 41.4 ($CH_2$); *m/z* (FAB, glycerol) 339 ($[M+Na]^+$, 39%), 317 ($[M+H]^+$, 20), 250 (15), 199 (16), 176 (100), 167 (31), 154 (30), 133 (32), 105 (40), 91 (56) and 77 (35).

## 2. 2-Diphenylmethylbenzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl diphenylacetate **236**

The method of C2b was followed using diphenylacetic acid (20.0 g, 90 mmol) to yield methyl diphenylacetate (19.4 g, 91%) as cream plates, m.p. 58–60 °C (lit.,[127] 59–62 °C); $\delta_H$ 7.31 (10 H, m), 5.05 (1 H, s) and 3.76 (3 H, s).

**b.** Preparation of 8-allyl-2-diphenylmethylbenzopyran-4-one **235**

The method of C1e was followed using methyl diphenylacetate (2.49 g, 11 mmol) to afford 8-allyl-2-diphenylmethylbenzopyran-4-one (1.63 g, 42%) as yellow rhomboid crystals, m.p. 135–137 °C (Found: C, 85.0; H, 5.7. $C_{25}H_{20}O_2$ requires C, 85.2; H, 5.7%); $\nu_{max}$ 1647, 1598, 1164, 1074, 957, 898 and 782 $cm^{-1}$; $\delta_H$ 8.05 (1 H, dd, *J* 8, 2, H-5), 7.45 (1 H, dd, *J* 8, 2, H-7), 7.40–7.20 (11 H, m, H-6, 2 x Ph), 6.17 (1 H, s, H-3), 5.85–5.64 (1 H, m), 5.37 (1 H, s), 4.95–4.81 (2 H, m) and 3.42 (2 H, d, *J* 8); $\delta_C$ 178.6 ($4^{ry}$), 169.2 ($4^{ry}$), 154.4 ($4^{ry}$), 138.9 (2 C, $4^{ry}$), 135.1 (CH), 133.9 (CH), 129.6 ($4^{ry}$), 129.0 (4 x CH), 128.8 (4 x CH), 127.5 (2 x CH), 124.8 (CH), 123.8 ($4^{ry}$), 123.7 (CH), 116.6 ($CH_2$), 112.1 (CH), 56.1 (CH) and 33.9 ($CH_2$); *m/z* 352 ($M^+$, 100%), 274 (12), 191 (16), 161 (84), 115 (19) and 77 (14).

**c.** Preparation of 2-diphenylmethylbenzopyran-4-one-8-acetic acid **238**

The method of C1f was followed using 8-allyl-2-diphenylmethylbenzopyran-4-one (7.05 g, 20 mmol) to afford 2-diphenylmethylbenzopyran-4-one-8-acetic acid (0.67 g, 9%) as yellow plates, m.p. 171–172 °C (Found: C, 77.8; H, 4.7. $C_{24}H_{18}O_4$ requires C, 77.8; H, 4.9%); $\nu_{max}$ 3400-2800 (br), 1733, 1635, 1260, 1207, 1134, 1031, 935, 864, 817 and 702 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.12 (1 H, dd, *J* 8, 2, H-5), 7.52 (1 H, dd, *J* 8, 2, H-7), 7.36–7.19 (11 H, m, H-6, 2 x Ph), 6.32 (1 H, s, H-3), 5.38 (1 H, s) and 3.59 (2 H, s) (acid proton not apparent); $\delta_C$ ($CD_3SOCD_3$) 178.7 ($4^{ry}$), 174.8 ($4^{ry}$), 169.4 ($4^{ry}$), 154.7 ($4^{ry}$), 138.8 (2 C, $4^{ry}$), 135.3 (CH), 129.0 (4 x CH), 128.8 (4 x CH), 127.5 (2 x CH), 125.3 ($4^{ry}$), 125.0 (CH), 123.62 (CH), 123.60 ($4^{ry}$), 112.0 (CH), 56.1 (CH) and 34.9 ($CH_2$); *m/z* 370 ($M^+$, 23%), 325 (20), 283 (18), 213 (23), 184 (33), 167 (100), 133 (33), 106 (84), 89 (18) and 77 (100).

**d.** Preparation of sodium 2-diphenylmethylbenzopyran-4-one-8-acetate **240**

The method of C2e was followed using 2-diphenylmethylbenzopyran-4-one-8-acetic acid (1.0 g, 2.7 mmol) to afford sodium 2-diphenylmethylbenzopyran-4-one-8-acetate (0.84 g, 79%) as a white powder, m.p. >350 °C; $\nu_{max}$ 1610, 1031, 980, 820 and 683 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.84 (2 H, m, H-4'), 7.58 (1 H, dd, J 8, 2, H-5), 7.41–7.22 (11 H, m, H-7,2',3',5',6'), 7.07 (1 H, t, J 8, H-6), 6.25 (1 H, s, H-3), 6.18 (1 H, s) and 3.52 (2 H, s); $\delta_C$ ($D_2O$) 183.4 ($4^{ry}$), 181.4 ($4^{ry}$), 172.8 ($4^{ry}$), 157.3 ($4^{ry}$), 138.6 (CH), 137.9 ($4^{ry}$), 136.1 (CH), 132.2 (4 x CH), 131.7 (4 x CH), 130.2 (CH), 129.6 ($4^{ry}$), 127.8 (CH), 125.4 (CH), 125.0 ($4^{ry}$), 124.7 (CH), 111.5 (CH), 57.1 (CH) and 41.1 ($CH_2$).

## 3. 2-(4-Chlorostyryl)benzopyran-4-one-8-acetic Acid

**a.** Preparation of methyl 4-chlorocinnamate **245**

The method of C2b was followed using 4-chlorocinnamic acid (20.0 g, 110 mmol) to yield methyl 4-chlorocinnamate (14.9 g, 69%) as colourless needles, m.p. 75–76 °C (lit.,[128] 75–76 °C).

**b.** Preparation of 8-allyl-2-(4-chlorostyryl)benzopyran-4-one **243**

The method of C1e was followed using methyl 4-chlorocinnamate (2.16 g, 11 mmol) to afford 8-allyl-2-(4-chlorostyryl)benzopyran-4-one (0.64 g, 18%) as yellow needles, m.p. 180–182 °C; $\nu_{max}$ 1652, 1593, 1208, 1088, 908, 837, 815 and 763 $cm^{-1}$; $\delta_H$ 8.09 (1 H, dd, *J* 8, 2, H-5), 7.58–7.22 (7 H, m), 6.79 (1 H, half AB pattern, *J* 15), 6.31 (1 H, s, H-3), 6.20–6.00 (1 H, m), 5.21–5.17 (2 H, m) and 3.73 (2 H, d, *J* 8); $\delta_C$ 178.6 ($4^{ry}$), 160.9 ($4^{ry}$), 153.9 ($4^{ry}$), 135.7 ($4^{ry}$), 135.4 (CH), 135.3 (CH), 134.2 (CH), 133.5 ($4^{ry}$), 129.3 (2 x CH), 129.2 ($4^{ry}$), 128.8 (2 x CH), 124.8 (CH), 124.1 ($4^{ry}$), 124.0 (CH), 121.1 (CH), 117.0 ($CH_2$), 110.8 (CH) and

34.1 ($CH_2$); *m/z* 322 ($^{35}Cl$-$M^+$, 100%), 305 (48), 189 (27), 161 (26), 131 (58), 103 (28) and 77 (42).

c. Attempted preparation of 2-(4-chlorostyryl)benzopyran-4-one-8-acetic acid **241**

The method of C1f was followed using 8-allyl-2-(4-chlorostyryl)benzopyran-4-one (6.5 g, 20 mmol) to afford a white solid only on evaporation of solvent. This solid dissolved readily in water and was shown by $^1H$ NMR not to be the correct product.

**4. 2-(2-(2-thienyl)ethenyl)benzopyran-4-one-8-acetic acid**

a. Preparation of methyl 3-(2-thienyl)acrylate **246**

The method of C2b was followed using 3-(2-thienyl)acrylic acid (20.0 g, 130 mmol) to afford methyl 3-(2-thienyl)acrylate (15.6 g, 72%) as a colourless liquid, b.p. (oven temp.) 85 °C at 1 mmHg (lit.,[129] 135 °C at 12 mmHg).

b. Preparation of 8-allyl-2-(2-(2-thienyl)ethenyl)benzopyran-4-one **244**

The method of C1e was followed using methyl 3-(2-thienyl)acrylate (1.85 g, 11 mmol) to afford 8-allyl-2-(2-(2-thienyl)ethenyl)benzopyran-4-one (0.57 g, 18%) as yellow needles, m.p. 136–139 °C; $\nu_{max}$ 1603, 1589, 1294, 1212, 1171, 1070, 955, 859 and 695 $cm^{-1}$; $\delta_H$ 8.06 (1 H, dd, *J* 8, 2, H-5), 7.67 and 6.57 (2 H, AB pattern, *J* 16), 7.51 (1 H, dd, *J* 8, 2, H-7), 7.41–7.03 (4 H, m, H-6,3',4',5'), 6.29 (1 H, s, H-3), 6.20–6.00 (1 H, m), 5.27–5.13 (2 H, m) and 3.73 (2 H, d, *J* 8); $\delta_C$ 178.5 ($4^{ry}$), 161.1 ($4^{ry}$), 153.9 ($4^{ry}$), 140.4 ($4^{ry}$), 135.4 (CH), 134.0 (CH), 129.5 (CH), 129.12 ($4^{ry}$), 129.11 (CH), 128.2 (CH), 128.0 (CH), 124.7 (CH), 124.1 ($4^{ry}$), 123.9 (CH), 119.5 (CH), 116.9 ($CH_2$), 110.2 (CH) and 34.1 ($CH_2$); *m/z* 294 ($M^+$, 100%), 277 (57), 261 (32), 161 (30), 134 (63), 103 (17) and 77 (23).

**c.** Attempted preparation of 2-(2-(2-thienyl)ethenyl)benzopyran-4-one-8-acetic acid **242**

The method of C1f was followed using 8-allyl-2-(2-(2-thienyl)ethenyl)benzopyran-4-one (5.9 g, 20 mmol) to afford a white solid only on evaporation of the solvent. This solid was shown not to be the correct product on analysis by $^1$H NMR.

## 5. Attempted preparation of 2-phenylethynylbenzopyran-4-one-8-acetic Acid

**a.** Preparation of phenylpropiolic acid **250**

A solution of phenylacetylene **247** (20.0 g, 196 mmol) in dry THF (200 ml) was stirred vigorously under nitrogen while butyl lithium (2.5M, solution in hexanes, 12.8 ml) was added dropwise. The mixture was stirred for 2 h and then carbon dioxide was bubbled through the mixture for 2 h. The suspension that formed was added to water (200 ml) and extracted with ether (2 x 50 ml). The aqueous portion was acidified with concentrated hydrochloric acid to pH 4 and then extracted with ether (2 x 100 ml) which was dried and evaporated to give phenylpropiolic acid (12.4 g, 43%) as a yellow solid, m.p. 134–137 °C. (lit.,[130] 135–137 °C); $\delta_H$ 11.7 (1 H, br s, OH) and 8.09 (5 H, s).

**b.** Preparation of methyl phenylpropiolate **251**

The method of C2b was followed using phenylpropiolic acid (12.4 g, 85 mmol) to afford a yellow oil which was Kugelrohr distilled to yield methyl phenylpropiolate (10.5 g, 77%) as a colourless liquid, b.p. (oven temp.) 107 °C at 0.13 mmHg (lit.,[131] 132–133 °C at 16 mmHg); $\delta_H$ 3.80 (3 H, s) and 7.95 (5 H, s).

**c.** Preparation of 2-(3-allyl-2-hydroxyphenyl)-6-phenylpyran-4-one **255**

The method of C1e was followed using methyl phenylpropiolate (1.58 g, 11 mmol) to yield 2-(3-allyl-2-hydroxyphenyl)-6-phenylpyran-4-one (2.48 g, 74%) as yellow needles, m.p. 178–180 °C (Found: C, 79.3; H, 5.3. $C_{20}H_{16}O_3$ requires C, 78.9; H, 5.3%); $\nu_{max}$ 2520 (br, OH), 1652, 1585, 1568, 1287, 1217, 948, 877, 738 and 685 $cm^{-1}$; $\delta_H$ 9.3 (1 H, br s, OH), 7.85 (1 H, d, *J* 2), 7.83 (1 H, d, *J* 4), 7.71 (1 H, dd, *J* 8, 2), 7.59 (1 H, d, *J* 2), 7.53–7.47 (3 H, m), 7.29 (1 H, dd, *J* 8, 2), 6.98 (1 H, t, *J* 8), 6.83 (1 H, d, *J* 6), 6.13–6.02 (1 H, m), 5.27–5.11 (2 H, m) and 3.70 (2 H, d, *J* 6); $\delta_C$ 181.8 ($4^{ry}$), 164.1 ($4^{ry}$), 162.7 ($4^{ry}$), 154.5 ($4^{ry}$), 136.2 (CH), 133.4 (CH), 131.7 ($4^{ry}$), 131.5 (CH), 129.2 (2 x CH), 128.4 ($4^{ry}$), 126.7 (CH), 126.1 (2 x CH), 120.1 (CH), 118.9 ($4^{ry}$), 116.4 ($CH_2$), 115.1 (CH), 110.75 (CH) and 34.7 ($CH_2$); *m/z* 304 ($M^+$, 100%), 276 (83), 157 (52), 129 (44), 115 (62), 102 (43), 91 (32) and 77 (59).

**d.** Preparation of 2-(3-allyl-2-methoxyphenyl)-6-phenylpyran-4-one **252**

A suspension of sodium hydride (0.24 g, 9.8 mmol) in THF was prepared by firstly washing the hydride in light petroleum (40 ml), decanting the liquid and transferring the solid to THF (200 ml). This solution was then heated under reflux for 10 min and 2-(3-allyl-2-hydroxyphenyl)-6-phenylpyran-4-one was added portionwise over 10 min followed by dimethyl sulphate (3.7 g, 39 mmol). The mixture was allowed to cool and concentrated ammonia (40 ml) was added after stirring for 1 day, water (200 ml) was added and the mixture extracted into methylene chloride, dried and evaporated to yield a yellow oil. Column chromatography (ethyl acetate/petrol 2:1) on this crude oil yielded 2-(3-allyl-2-methoxyphenyl)-6-phenylpyran-4-one as a light yellow oil, b.p. 194 °C (Found: $M^+$, 318.1256. $C_{21}H_{18}O_3$ requires *M*, 318.1254); $\nu_{max}$ 1600, 1259, 1089, 1005, 873, 771 and 693 $cm^{-1}$; $\delta_H$ 7.88–7.81 (2 H, m), 7.63 (1 H, dd, *J* 8, 2), 7.52–7.49 (3 H, m), 7.37 (1 H, dd, *J* 8, 2),

7.20 (1 H, t, *J* 8), 7.00 (1 H, d, *J* 2), 6.83 (1 H, d, *J* 2), 6.10–5.90 (1 H, m), 5.20–5.03 (2 H, m), 3.69 (3 H, s) and 3.50 (2 H, d, *J* 7); $\delta_C$ 180.5 ($4^{ry}$), 163.7 ($4^{ry}$), 162.0 ($4^{ry}$), 156.8 ($4^{ry}$), 136.6 (CH), 134.8 ($4^{ry}$), 133.5 (CH), 131.4 (CH), 129.1 (2 x CH) 129.0 ($4^{ry}$), 127.7 (CH), 125.9 (2 x CH), 125.3 ($4^{ry}$), 124.4 (CH), 116.5 ($CH_2$), 115.6 (CH), 111.1 (CH), 61.3 ($OCH_3$) and 33.8 ($CH_2$); *m/z* 318 ($M^+$, 100%), 290 (83), 275 (9), 258 (14), 247 (41), 230 (9), 185 (13), 172 (74), 157 (39), 147 (79), 141 (40), 128 (60), 115 (67), 105 (77), 91 (42) and 77 (70).

**e.** Preparation of 2-(3-carboxymethyl-2-methoxyphenyl)-6-phenylpyran-4-one **253**

The method of C1f was followed using 2-(3-allyl-2-methoxyphenyl)-6-phenylpyran-4-one (0.25 g, 0.82 mmol) to yield 2-(3-carboxymethyl-2-methoxyphenyl)-6-phenylpyran-4-one (0.10 g, 38%) as a cream powder, m.p. 212–214 °C (Found: C, 71.3; H, 4.6. $C_{20}H_{16}O_5$ requires C, 71.4; H, 4.8%); $\nu_{max}$ 3060–2940 (br), 1720, 1621, 1546, 1196, 1004, 947, 892, 781 and 682 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.06 (2 H, m), 7.79 (1 H, d, *J* 8), 7.63 (3 H, m), 7.59 (1 H, d, *J* 8), 7.36 (1 H, t, *J* 8), 7.11 (1 H, s), 6.83 (1 H, s), 3.75 (2 H, s) and 3.69 (3 H, s) (acid proton not apparent); *m/z* 336 ($M^+$, 100%), 308 (75), 292 (13), 265 (44), 236 (9), 190 (61), 147 (96), 135 (36), 115 (41), 105 (81), 91 (24) and 77 (51).

**f.** Preparation of sodium 2-(3-methylcarboxylato-2-methoxyphenyl)-6-phenylpyran-4-one **254**

The method of C2f was followed using 2-(3-carboxymethyl-2-methoxyphenyl)-6-phenylpyran-4-one (0.10 g, 0.3 mmol) to afford sodium 2-(3-methylcarboxylato-2-methoxyphenyl)-6-phenylpyran-4-one as a cream powder (0.094 g, 89%), m.p. 267–268 °C; $\nu_{max}$ 1713, 1612, 1555, 1119, 1000, 980, 739 and 680 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.51–7.20 (5 H, m), 7.16–7.01 (3 H, m), 6.82 (1 H, s), 6.55 (1 H, s) and 3.57 (5 H, m);

$\delta_C$ ($D_2O$) 184.7 ($4^{ry}$), 182.3 ($4^{ry}$), 166.7 ($4^{ry}$), 164.6 ($4^{ry}$), 159.1 ($4^{ry}$), 138.3 (CH), 135.2 (CH), 134.6 ($4^{ry}$), 131.9 ($4^{ry}$), 131.5 (CH), 129.9 ($4^{ry}$), 128.0 (2 x CH), 127.3 (CH), 125.6 (2 x CH), 116.2 (CH), 111.6 (CH), 63.2 ($OCH_3$) and 41.4 ($CH_2$).

**6. 2,2'-bi(8-carboxymethylbenzopyran-4-one)**

**a.** Preparation of 2,2'-bi(8-allylbenzopyran-4-one) **269**

The method of C1e was followed using dimethyl oxalate **266** (Aldrich, 0.65 g, 5.5 mmol) to yield 2,2'-bi(8-allylbenzopyran-4-one) (0.87 g, 43%) as colourless needles, m.p. 200–203 °C (Found: C, 77.8; H, 4.6. $C_{24}H_{18}O_4$ requires C, 77.8; H, 4.9%); $\nu_{max}$ 1620, 1591, 1187, 1119, 913, 845, 796 and 750 $cm^{-1}$; $\delta_H$ 8.10 (2 H, dd, *J* 8, 2, H-5), 7.60 (2 H, dd, *J* 8, 2, H-7), 7.40 (2 H, t, *J* 8, H-6), 7.10 (2 H, s, H-3), 6.20–6.00 (2 H, m), 5.25–5.11 (4 H, m) and 3.72 (4 H, d, *J* 6); $\delta_C$ 177.8 (2 C, $4^{ry}$), 154.8 (2 C, $4^{ry}$), 153.8 (2 C, $4^{ry}$), 135.1 (2 x CH), 134.7 (2 x CH), 129.7 (2 C, $4^{ry}$), 125.6 (2 x CH), 124.2 (2 C, $4^{ry}$), 124.1 (2 x CH), 117.5 (2 x $CH_2$), 110.2 (2 x CH) and 33.8 (2 x $CH_2$); *m/z* 370 ($M^+$, 100%), 330 (61), 301 (8), 255 (6), 211 (12), 185 (10), 161 (15), 140 (31), 131 (32), 115 (33), 103 (29), 92 (10) and 77 (34).

**b.** Attempted preparation of 2,2'-bi(8-carboxymethylbenzopyran-4-one)

The method of C2f was followed using 2,2'-bi(8-allylbenzopyran-4-one) (7.40 g, 20 mmol), however, the compound was essentially insoluble in the reaction mixture and therefore the powder obtained was confirmed by $^1H$ NMR to be the starting material.

**7. 2,2'-(1,4-phenylene)di(8-carboxymethylbenzopyran-4-one)**

**a.** Preparation of dimethyl terephthalate **267**

The method of C2b was followed using terephthalic acid (20.0 g, 120 mmol) to yield dimethyl terephthalate (9.4 g, 40%) as a white

powder, m.p. 141–143 °C (lit.,[132] 140–142 °C); $\delta_H$ 8.09 (4 H, s) and 3.92 (6 H, s).

**b.** Preparation of 2,2'-(1,4-phenylene)di(8-allylbenzopyran-4-one) **270**

The method of C1e was followed using dimethyl terephthalate (1.07 g, 5.5 mmol) to yield 2,2'-(1,4-phenylene)di(8-allylbenzopyran-4-one) (0.98 g, 40%) as a yellow powder, m.p. 288–290 °C (Found: M+, 446.1528. $C_{30}H_{22}O_4$ requires *M*, 446.1518); $\nu_{max}$ 1563, 1345, 1195, 1109, 1055, 987, 906, 820, 784 and 740 $cm^{-1}$; $\delta_H$ 8.15–8.09 (6 H, m, H-5,2',3',5',6'), 7.60 (2 H, dd, *J* 8, 2, H-7), 7.39 (2 H, t, *J* 8, H-6), 6.93 (2 H, s, H-3), 6.18–6.01 (2 H, m), 5.24–5.16 (4 H, m) and 3.77 (4 H, m); $\delta_C$ 178.5 (2 C, $4^{ry}$), 161.5 (2 C, $4^{ry}$), 154.2 (2 C, $4^{ry}$), 135.2 (2 x CH), 134.8 (2 C, $4^{ry}$), 134.4 (2 x CH), 129.5 (2 C, $4^{ry}$), 126.8 (4 x CH), 125.3 (2 x CH), 124.1 (2 C, $4^{ry}$), 124.0 (2 x CH), 117.2 (2 x $CH_2$), 108.3 (2 x CH) and 34.0 (2 x $CH_2$); *m/z* 446 ($M^+$, 100%), 418 (5), 391 (1), 287 (5), 223 (7), 160 (9), 131 (48), 115 (16), 103 (20) and 77 (23).

**c.** Attempted preparation of 2,2'-(1,4-phenylene)di(8-carboxymethyl-benzopyran-4-one)

The method of C1f was followed using 2,2'-(1,4-phenylene)di(8-allylbenzopyran-4-one) (8.92 g, 20 mmol), however, the compound was essentially insoluble in the reaction medium and the powder obtained was shown to be the starting material by $^1H$ NMR.

## 8. 4',4''-Oxydi(8-carboxymethylflavone)

**a.** Preparation of dimethyl 4,4'-oxydibenzoate **268**

The method of C2b was followed using 4,4'-oxydibenzoic acid (20.0 g, 77 mmol) to afford dimethyl 4,4'-oxydibenzoate (13.3 g, 60%) as colourless needles, m.p. 154–155 °C (lit.,[133] 156–158 °C); $\delta_H$ 8.05 and 7.05 (8 H, AB pattern, *J* 8) and 3.90 (3 H, s).

**b.** Preparation of 8-allyl-4'-(4-methoxycarbonylphenoxy)flavone **271**

The method of C1e was followed using dimethyl 4,4'-oxydibenzoate (3.15 g, 11 mmol) to yield 8-allyl-4'-(4-methoxycarbonylphenoxy)flavone (0.73 g, 16%) as a yellow powder, m.p. 143–145 °C; $\nu_{max}$ (KBr) 3151, 1710, 1660, 1600, 1500, 1381, 1281, 1252, 1173, 1121, 1007, 862 and 782 $cm^{-1}$; $\delta_H$ 8.11 (1 H, dd, *J* 8, 2, H-5), 8.07 and 7.17 (4 H, AB pattern, *J* 9), 7.93 and 7.09 (4 H, AB pattern, *J* 9), 7.55 (1 H, dd, *J* 8, 2, H-7), 7.36 (1 H, t, *J* 8, H-6), 6.79 (1 H, s, H-3), 6.15–6.02 (1 H, m), 5.19–5.12 (2 H, m), 3.92 (3 H, s) and 3.75 (2 H, d, *J* 6); $\delta_C$ 178.6 ($4^{ry}$), 166.4 ($4^{ry}$), 162.3 ($4^{ry}$), 160.2 ($4^{ry}$), 159.1 ($4^{ry}$), 154.2 ($4^{ry}$), 135.3 (CH), 134.2 (CH), 131.9 (2 x CH), 129.4 ($4^{ry}$), 128.2 (2 x CH), 127.6 ($4^{ry}$), 125.9 ($4^{ry}$), 125.0 (CH), 124.0 (CH), 124.0 ($4^{ry}$), 119.6 (2 x CH), 118.6 (2 x CH), 117.0 ($CH_2$), 107.0 (CH), 52.1 ($OCH_3$) and 34.0 ($CH_2$); *m/z* 412 ($M^+$, 100%), 381 (10), 286 (8), 255 (10), 221 (11), 190 (13), 165 (11), 150 (12), 104 (10), 91 (15) and 77 (8).

**c.** Attempted preparation of 4'-(4-methoxycarbonylphenoxy)flavone-8-acetic acid

The method of C1f was followed using 8-allyl-4'-(4-methoxycarbonylphenoxy)flavone (8.24 g, 20 mmol) but this was essentially insoluble in the reaction medium and afforded a yellow powder which on analysis by $^1H$ NMR was shown to be the starting material.

**d.** Preparation of 4',4''-oxydi(8-allylflavone) **272**

The method of C1e was followed using dimethyl 4,4'-oxydibenzoate (1.57 g, 5.5 mmol) to yield 4',4''-oxydi(8-allylflavone) as a yellow powder (1.72 g, 29%), m.p. 189–191 °C (Found: C, 81.0; H, 3.7. $C_{36}H_{26}O_5$ requires C, 81.2; H, 3.8%); $\nu_{max}$ 1733, 1634, 1582, 1293, 1238, 1173, 1117, 921, 838 and 751 $cm^{-1}$; $\delta_H$ 8.12 (2 H, dd, *J* 8, 2, H-5),

7.96 and 7.21 (8 H, AB pattern, *J* 9, H-2',3',5',6'), 7.56 (2 H, dd, *J* 8, 2, H-7), 7.37 (2 H, t, *J* 8, H-6), 6.81 (2 H, s, H-3), 6.16–6.03 (2 H, m), 5.20–5.13 (4 H, m) and 3.76 (4 H, d, *J* 6); $\delta_C$ 178.6 (2 C, 4ry), 162.3 (2 C, 4ry), 159.2 (2 C, 4ry), 154.2 (2 C, 4ry), 135.3 (2 x CH), 134.2 (2 x CH), 129.4 (2 C, 4ry), 128.3 (4 x CH), 127.7 (2 C, 4ry), 125.0 (2 x CH), 124.04 (2 x CH), 124.01 (2 C, 4ry), 119.6 (4 x CH), 117.0 (2 x $CH_2$), 107.0 (2 x CH) and 34.0 (2 x $CH_2$); *m/z* 538 ($M^+$, 6%), 298 (3), 256 (5), 241 (2), 211 (3), 182 (4), 170 (7), 163 (10), 131 (11), 119 (10), 107 (36), 91 (100) and 77 (21).

e. Preparation of 4',4''-oxydi(8-carboxymethylflavone)

The method of C1f was followed using 4',4''-oxydi(8-allylflavone) (10.8 g, 20 mmol) to afford 4',4''-oxydi(8-carboxymethylflavone) (1.5 g, 13%) as a white powder, m.p. 267–269 °C (Found: $M^+-CO_2$, 530.1355. $C_{34}H_{22}O_5$ requires *M*, 530.1366); $\nu_{max}$ 3400, 1701, 1640, 1595, 1241, 1179, 834 and 785 $cm^{-1}$; $\delta_H$ ($CD_3SOCD_3$) 8.15 (2 H, dd, *J* 8, 2, H-5), 8.01 and 7.20 (8 H, m), 7.77 (4 H, d, *J* 8, H-7), 7.47 (2 H, t, *J* 8, H-6), 7.14 (2 H, s, H-3) and 4.04 (4 H, s) (acid protons not apparent); $\delta_C$ ($CD_3SOCD_3$) 177.2 (2 C, 4ry), 172.3 (2 C, 4ry), 161.9 (2 C, 4ry), 159.1 (2 C, 4ry), 154.4 (2 C, 4ry), 136.0 (2 x CH), 132.1 (4 x CH), 126.6 (2 C, 4ry), 125.4 (2 x CH), 125.2 (2 C, 4ry), 124.0 (2 x CH), 123.5 (2 C, 4ry), 119.0 (4 x CH), 106.8 (2 x CH) and 35.8 (2 x $CH_2$); *m/z* 574 ($M^+$, 10%), 530 (17), 500 (58), 486 (100), 472 (41), 458 (46), 416 (59), 402 (46), 366 (36), 338 (25), 238 (56), 218 (46), 189 (34), 165 (40), 147 (46), 120 (50) and 106 (56).

e. Preparation of sodium 4',4''-oxydi(8-carboxylatomethylflavone)

The method of C2f was followed using 4',4''-oxydi(8-carboxymethylflavone) (1.0 g, 1.7 mmol) to afford sodium 4',4''-oxydi(8-carboxylatomethylflavone) (0.95 g, 94%) as a white powder,

m.p. >350 °C; $\nu_{max}$ 1571, 1530, 1223, 1149, 1082, 998, 861, 827 and 772 $cm^{-1}$; $\delta_H$ ($D_2O$) 7.87 and 7.11 (8 H, AB pattern, *J* 9), 7.73 (2 H, d, *J* 8, H-5), 7.52 (2 H, d, *J* 8, H-7), 7.01 (2 H, t, *J* 8, H-6), 6.71 (2 H, s, H-3) and 3.70 (4 H, s); $\delta_C$ ($D_2O$) 181.9 (2 C, $4^{ry}$), 177.7 (2 C, $4^{ry}$), 161.5 (2 C, $4^{ry}$), 156.7 (2 C, $4^{ry}$), 139.1 (2 C, $4^{ry}$), 135.0 (2 x CH), 133.8 (4 x CH), 131.1 (2 x CH), 128.3 (2 x CH), 128.2 (2 C,$4^{ry}$), 121.7 (2 x CH), 121.3 (2 C,$4^{ry}$), 121.1 (4 x CH), 109.4 (2 x CH) and 41.7 (2 x $CH_2$).

## G. Preparation of Derivatives with Different Groups in the 8-Position

### 1. 8-(3-Hydroxy-2-oxopropyl) Compounds

**a.** Preparation of 8-(3-hydroxy-2-oxopropyl)flavone

To a stirred solution of 8-allylflavone (2.88 g, 11 mmol) in glacial acetic acid (100 ml), water (100 ml) and acetone (100 ml) was added potassium permanganate (2.6 g, 16.5 mmol) over a period of 1 h. On standing 1 h further a precipitate was seen which was decolourised by the addition of a saturated solution of sodium metabisulphite (approx. 50 ml). The mixture was reduced in volume and extracted with $CH_2Cl_2$ (400 ml). The extract was washed with water (2 x 100 ml), dried and evaporated. The resulting yellow solid was triturated with dry ether at 0 °C and filtered to yield 8-(3-hydroxy-2-oxopropyl)flavone (2.1 g, 66%) as a yellow powder, m.p. 200–202 °C (Found: C, 71.6; H, 4.7. $C_{18}H_{14}O_4 + 0.5\ H_2O$ requires C, 71.3; H, 5.0%); $\nu_{max}$ 3300 (br), 1723, 1641, 1591, 1490, 1054, 888, 779, 759 and 701 $cm^{-1}$; $\delta_H$ 8.16 (1 H, dd, *J* 8, 2, H-5), 7.77 (2 H, dd, *J* 6, *J* 2, H-2',6'), 7.56 (1 H, dd, *J* 8, 2, H-7), 7.52–7.39 (3 H, m, H-3',4',5'), 7.38 (1 H, t, *J* 8, H-6), 6.79 (1 H, s, H-3), 4.41 (2 H, s), 4.09 (2 H, s) and 3.30–3.00 (1 H, br s, OH,); $\delta_C$ 205.9 ($4^{ry}$), 178.3 ($4^{ry}$), 163.4 ($4^{ry}$), 154.3 ($4^{ry}$), 135.5 (CH), 131.8 (CH), 131.7 ($4^{ry}$), 129.3 (2 x CH), 129.1 ($4^{ry}$), 126.2 (2 x CH), 125.6 (CH), 125.1 (CH), 122.7 ($4^{ry}$), 107.9 (CH), 68.0 ($CH_2$) and 40.2 ($CH_2$); *m/z* 294 ($M^+$, 15%), 279 (100), 252 (5), 235 (53), 207 (6), 178 (9), 160 (3), 133 (69), 106 (21) and 77 (30).

**b.** Preparation of 2',3'-dimethoxy-8-(3-hydroxy-2-oxopropyl)flavone

The method of G1a was followed using 8-allyl-2',3'-dimethoxyflavone (3.5 g, 11 mmol) to yield 2',3'-dimethoxy-8-(3-hydroxy-2-oxopropyl)flavone (2.6 g, 66%) as a yellow powder, m.p. 155–157 °C

(Found: C, 68.1; H, 5.1. $C_{20}H_{18}O_6$ requires C, 67.8; H, 5.1%); $\nu_{max}$ 3306 (br), 1730, 1624, 1599, 1279, 1015, 881, 804 and 764 $cm^{-1}$; $\delta_H$ 8.00 (1 H, dd, *J* 8, 2, H-5), 7.70 (1 H, d, *J* 8, 2, H-7), 7.47 (1 H, t, *J* 8, H-6), 7.31 (3 H, m, H-4',5',6'), 6.84 (1 H, s, H-3), 5.50–5.30 (1 H, br s, OH), 4.25 (2 H, s), 4.18 (2 H, s), 3.90 (3 H, s) and 3.79 (3 H, s); $\delta_C$ 207.6 ($4^{ry}$), 177.1 ($4^{ry}$), 160.5 ($4^{ry}$), 154.2 ($4^{ry}$), 152.9 ($4^{ry}$), 147.2 ($4^{ry}$), 135.9 (CH), 125.4 ($4^{ry}$), 124.9 (CH), 124.6 ($4^{ry}$), 124.5 (CH), 123.4 (CH), 123.0 ($4^{ry}$), 120.2 (CH), 115.8 (CH), 111.2 (CH), 67.4 ($CH_2$), 60.4 ($OCH_3$), 55.9 ($OCH_3$) and 40.5 ($CH_2$); *m/z* 354 ($M^+$, 52%), 324 (61), 310 (31), 295 (44), 267 (28), 254 (8), 239 (68), 162 (43), 147 (84), 133 (90), 119 (22), 105 (50) and 77 (64).

**c.** Preparation of 2',5'-dimethoxy-8-(3-hydroxy-2-oxopropyl)flavone

The method of G1a was followed using 8-allyl-2,5-dimethoxyflavone (3.55 g, 11 mmol) to yield 2',5'-dimethoxy-8-(3-hydroxy-2-oxopropyl)flavone (2.22 g, 57%) as a white powder, m.p. 182–184 °C (Found: C, 68.0; H, 5.4. $C_{20}H_{18}O_6$ requires C, 67.8; H, 5.1%); $\nu_{max}$ 3440, 1720, 1630, 1581, 1500, 1243, 1037, 890, 806 and 774 $cm^{-1}$; $\delta_H$ 8.19 (1 H, dd, *J* 8, 2, H-5), 7.57 (1 H, dd, *J* 8, 2, H-7), 7.39 (1 H, t, *J* 8, H-6), 7.27 (1 H, d, *J* 2, H-6'), 7.07 (1 H, s, H-3), 7.03–6.96 (2 H, m, H-3',4'), 4.37 (2 H, s), 4.05 (2 H, s), 3.87 (3 H, s), 3.85 (3 H, s) and 3.20 (1 H, br s); $\delta_C$ 206.1 ($4^{ry}$), 178.6 ($4^{ry}$), 160.8 ($4^{ry}$), 154.4 ($4^{ry}$), 153.5 ($4^{ry}$), 152.2 ($4^{ry}$), 135.3 (CH), 125.5 (CH), 124.9 (CH), 124.1 ($4^{ry}$), 122.6 ($4^{ry}$), 121.2 ($4^{ry}$), 117.8 (CH), 114.3 (CH), 113.1 (CH), 112.8 (CH), 67.9 ($CH_2$), 56.1 ($OCH_3$), 55.9 ($OCH_3$) and 40.2 ($CH_2$); *m/z* 354 ($M^+$, 95%), 324 (14), 311 (6), 295 (63) 281 (16), 267 (31), 235 (7), 187 (10), 161 (38), 147 (39), 133 (100), 119 (200), 105 (42), 91 (10) and 77 (44).

## 2. 8-(5-Tetrazolylmethyl)flavone

a. Preparation of methyl 2-methoxy-3-methylbenzoate **274**

A solution of sodium hydroxide (17.2 g, 430 mmol) in water (100 ml) was added to a solution of 3-methylsalicylic acid **273** (21.3 g, 140 mmol) and dimethyl sulphate (160 ml, 1.68 mol) in dichloromethane (100 ml). Benzyltributylammonium chloride (4.42 g, 30 mmol) was then added. The mixture was then stirred vigorously for 12 h. The organic layer was separated and evaporated and the residue was extracted with petroleum (b.p. 40–60 °C). The extract was dried and evaporated to yield methyl 3-methyl-2-methoxybenzoate (23.4 g, 93%) as a colourless liquid, b.p. (oven temp.) 250 °C at atmospheric pressure (lit.,[134] 249.5–250.5 °C); $\delta_H$ 7.59 (1 H, dd, *J* 8, 2, H-6), 7.25 (1 H, dd, *J* 8, 2, H-4), 6.98 (1 H, t, *J* 8, H-5), 3.84 (3 H, s), 3.78 (3 H, s) and 2.25 (3 H, s).

b. Preparation of methyl 3-bromomethyl-2-methoxybenzoate **275**

A solution of 1,3-dibromo-5,5-dimethylhydantoin (2.38 g, 8.3 mmol), methyl 2-methoxy-3-methylbenzoate (3.0 g, 16.7 mmol) and azobis(isobutyronitrile) (0.13 g, 0.8 mmol) in carbon tetrachloride (50 ml) was heated under reflux for 8 h and then left to cool overnight. The colourless precipitate of 5,5-dimethylhydantoin was filtered off and the filtrate evaporated. The residue was extracted with hexane (100 ml), and the extract dried and evaporated to afford methyl 3-bromomethyl-2-methoxybenzoate (3.31 g, 77%) as a colourless liquid, b.p. 262 °C at atmospheric pressure (lit.,[135] 125 °C at 1.5 mmHg); $\delta_H$ 7.81 (1 H, dd, *J* 8, 2, H-6), 7.57 (1 H, dd, *J* 8, 2, H-4), 7.13 (1 H, t, *J* 8, H-5), 4.58 (2 H, s), 3.95 (3 H, s) and 3.91 (3 H, s).

c. Preparation of methyl 3-methoxymethyl-2-methoxybenzoate **276**

A solution of methyl 3-bromomethyl-2-methoxybenzoate (2.40 g, 9.3 mmol) in methanol (50 ml) was stirred vigorously as sodium (0.4 g,

17 mmol) was added in small pieces. The solution was then heated under reflux for 4 h and evaporated leaving a solid which was dissolved in water (20 ml). Extraction with ethyl acetate (50 ml), drying and evaporation yielded methyl 3-methoxymethyl-2-methoxybenzoate (2.03 g, 95%) as a pale brown liquid, b.p. 97 °C at 1 mmHg (lit.,[89,136] 87–95 °C at 0.06 mmHg); $\delta_H$ 7.72 (1 H, dd, *J* 7, 2), 7.54 (1 H, dd, *J* 7, 2), 7.11 (1 H, t, *J* 7), 4.50 (2 H, s), 3.87 (3 H, s), 3.81 (3 H, s) and 3.40 (3 H, s).

**d.** Attempted preparation of 8-methoxymethylflavone **277**

A suspension of sodium hydride (1.00 g, 24 mmol) in THF (200 ml) was stirred vigorously and heated under reflux for 10 min after which a solution of methyl 3-methoxymethyl-2-methoxybenzoate (5.04 g, 24 mmol) and acetophenone (2.88 g, 24 mmol) in THF (50 ml) were added dropwise. The mixture was heated under reflux for a further 4 h and allowed to cool. A solution of hydrobromic acid (20 ml) in acetic acid (40 ml) was added carefully and the mixture heated under reflux for 3 h, the solvent evaporated and the brown tar analysed by $^1$H NMR which showed that the correct material was present only in minute quantities with the main components being starting materials.

**e.** Preparation of 1-(2-methoxy-3-methylphenyl)-3-phenylpropane-1,3-dione **282**

A solution of methyl 2-methoxy-3-methylbenzoate (3.80 g, 22 mmol) and acetophenone (2.70 g, 22 mmol) in THF (30 ml) was added dropwise into a suspension of sodium hydride (0.52 g, 22 mmol) in THF (100 ml) being stirred and heated under reflux. After 24 h the mixture was poured onto crushed ice (200 g), neutralised with 10% hydrochloric acid and extracted into dichloromethane (100 ml). The extract was dried and evaporated to give 1-(2-methoxy-3-methylphenyl)-3-phenylpropane-1,3-dione (4.76 g, 79%) as a colourless oil, b.p. 163 °C; $\nu_{max}$ (Neat) 3040,

2906, 2837, 1585, 1451, 1211, 1080, 993, 762 and 682 $cm^{-1}$; $\delta_H$ 8.01 (2 H, m), 7.70 (1 H, dd, *J* 8, 2), 7.59–7.43 (3 H, m), 7.36 (1 H, dd, *J* 8, 2), 7.20–7.10 (2 H, m), 3.81 (3 H, s) and 2.37 (3 H, s); $\delta_C$ 185.9 ($4^{ry}$), 185.2 ($4^{ry}$), 134.7 (CH), 132.4 (CH), 132.2 ($4^{ry}$), 129.7 ($4^{ry}$), 128.7 (2 x CH), 127.9 (CH), 127.2 (2 x CH), 127.2 ($4^{ry}$), 124.2 (CH), 97.5 (CH), 61.4 ($OCH_3$) and 16.0 ($CH_3$); *m/z* 268 ($M^+$, 69%), 164 (77), 120 (27), 91 (100) and 77 (43).

**f.** Preparation of 8-methylflavone **283**

A solution of 1-(2-methoxy-3-methylphenyl)-3-phenylpropane-1,3-dione (2.5 g, 9.3 mmol) in hydrobromic acid (15 ml) and acetic acid (15 ml) was heated at 80 °C for 2 h then water (50 ml) was added and the mixture extracted with methylene chloride (2 x 50 ml), which was dried and evaporated to give a white solid. This was recrystallised from methanol to afford 8-methylflavone (1.6 g, 73%) as colourless thin plates, m.p. 168–169 °C (lit.,[137] 170 °C); $\delta_H$ 8.07 (1 H, dd, *J* 8, 2, H-5), 7.96 (2 H, m, H-2',6'), 7.55 (4 H, m, H-7,3',4',5'), 7.32 (1 H, t, *J* 8), 6.85 (1 H, s, H-3) and 2.62 (3 H, s).

**g.** Preparation of 8-bromomethylflavone **278**

A solution of 8-methylflavone (7.43 g, 31 mmol) was stirred in carbon tetrachloride (450 ml) was heated under reflux for 10 min. A catalytic amount of azobis(isobutyronitrile) (0.3 g, 1.8 mmol) was added followed by 1,3-dibromo-5,5-dimethylhydantoin (4.50 g, 16 mmol) and the mixture heated under reflux for 8 h after which it was allowed to cool and the precipitate that formed during the reaction filtered off and washed with carbon tetrachloride (20 ml). The solid was then recrystallised from ethanol to afford 8-bromomethylflavone (6.73 g, 69%) as colourless needles, m.p. 180–182 °C (lit.,[89] 182–184 °C); $\delta_H$ 8.24 (1 H, dd, *J* 8, 2, H-5), 8.05–8.00 (2 H, m, H-3',5'), 7.75 (1 H, dd, *J* 8, 2, H-7), 7.60–7.56

(3 H, m, H-2',4',6'), 7.40 (1 H, t, *J* 8, H-6), 6.89 (1 H, s, H-3) and 4.84 (2 H, s).

**h.** Preparation of 8-cyanomethylflavone **279**

A solution of potassium cyanide (0.85 g, 13 mmol) in ethanol (100 ml) and water (20 ml) was heated under reflux for 5 min. 8-Bromomethylflavone (3.70g, 13 mmol) was then added portionwise as a slurry in boiling ethanol (50 ml) and the mixture heated under reflux for 4 h after which it was allowed to cool overnight. The precipitate that formed was filtered off and washed with copious amounts of water then recrystallised from ethanol to afford 8-cyanomethylflavone (2.82 g, 83%) as orange plates, m.p. 178–180 °C (lit.,[89] 180–181 °C); $\delta_H$ 8.23 (1 H, dd, *J* 8, 2, H-5), 7.98–7.90 (2 H, m, H-3',5'), 7.78 (1 H, dd, *J* 8, 2, H-7), 7.59–7.54 (3 H, m, H-2',4',6'), 7.46 (1 H, t, *J* 8, H-6), 6.85 (1 H, s, H-3) and 4.09 (2 H, s); $\delta_C$ 177.7 ($4^{ry}$), 163.2 ($4^{ry}$), 153.6 ($4^{ry}$), 133.7 (CH), 132.0 (CH), 131.4 ($4^{ry}$), 129.3 (2 x CH), 126.3 (2 x CH), 125.3 (CH), 124.2 ($4^{ry}$), 119.7 ($4^{ry}$), 116.7 ($4^{ry}$), 108.0 (CH) and 18.9 ($CH_2$).

**i.** Preparation of 8-(5-tetrazolylmethyl)flavone **281**

A solution of 8-cyanomethylflavone (0.52 g, 2.0 mmol), sodium azide (0.14 g, 2.2 mmol) and ammonium chloride (0.12 g, 2.2 mmol) in dry DMF (20 ml) was heated under reflux under nitrogen for 8 h. The solvent was evaporated and the brown residue purified by chromatography with ethyl acetate/$CH_2Cl_2$ to remove impurities to yield 8-(5-tetrazolylmethyl)flavone as a beige solid (0.11 g, 16%), m.p. >350 °C. This compound was converted directly into the potassium salt.

**j.** Preparation of potassium 8-tetrazolatomethylflavone **280**

To a hot solution of potassium carbonate (0.02 g, 0.2 mmol) in water (5 ml) was added 8-tetrazolylmethylflavone (0.06 g, 0.2 mmol). The

solution was allowed to cool and the undissolved material was filtered off. This solution was then evaporated to dryness to yield potassium 8-tetrazolatomethylflavone as a cream powder (0.05 g, 73%), m.p. >350°C; $\delta_H$ 8.13 (1 H, m), 8.01 (1 H, m), 7.83 (1 H, m), 7.63–7.41 (5 H, m), 6.95 (1 H, s) and 4.39 (2 H, s).

## H. *In vitro* and *in vivo* Testing

### 1. Materials and methods

Cell lines and culture conditions

A panel of human and murine tumour cell lines were used including; MAC 15A[138] (derived from an ascitic murine adenocarcinoma of the colon), MAC 16[56] (slow growing, solid and cachectic murine adenocarcinoma of the colon), MAC 26[56] (well differentiated solid murine adenocarcinoma of the colon) WEHI-3B[139] (murine myelo-monocytic leukaemia), K562[140] (human chronic myelogenous leukaemia with erythroid characteristics), HT-29,[141] HCLO, DLD-1[142] and HCT-18 (human adenocarcinomas of the colon), HRT-18[143] (human rectal adenocarcinoma) and BM (murine bone marrow). With the exception of WEHI-3B and K562, all cell lines were routinely maintained as monolayer cultures in RPMI 1640 medium supplemented with 10% foetal calf serum, penicillin/streptomycin (50 IU $ml^{-1}$/50 $\mu g\ ml^{-1}$), sodium pyruvate (1 mM) and buffered with HEPES (25 mM). The exceptions, WEHI-3B and K562 cell lines, were maintained as suspension cultures in RPMI 1640 as above. Primary bone marrow cultures were set up as follows; Bone marrow cells were obtained from the femurs of non tumour bearing NMRI mice and collected in RPMI 1640 at 4 °C. Immediately prior to chemosensitivity testing, cells were cultured in 96 well plates containing RPMI 1640 supplemented with 20% foetal calf serum and 10% WEHI-3B conditioned medium.

### 2. *In vitro* chemosensitivity

An MTT assay[144] was used to assess chemosensitivity following the continuous (96 h) exposure of cell lines to each compound as described below. Approximately 0.5 to 1 x $10^4$ viable cells (BM cells were plated

out at 5 x $10^5$ cells per well) were plated into 96 well culture vessels containing 180 µl of RPMI 1640 medium. 20 ml of drug solution was added to each well to give a final concentration of 500 µg $ml^{-1}$ (8 wells per drug exposure were used). Following a 4 day incubation at 37 °C in an atmosphere containing 5% $CO_2$, 150 ml of old medium was replaced with 150 ml of fresh RPMI 1640 immediately prior to the addition (20 ml) of MTT solution (5 mg $ml^{-1}$). Following a further 4 hour incubation at 37 °C, 180 ml of medium was removed and discarded from each well and the formazan crystals dissolved in 150 ml of DMSO. Absorbance of the resulting solution was read at 550 nm using an ELISA spectrophotometer. All results were expressed in terms of % survival taking the control absorbance values to represent 100% survival. From the dose response curves constructed, $IC_{50}$ (the concentration required to reduce cell survival by 50%) values were estimated.

### 3. Solubility of compounds

FAA sodium salt analogues were reconstituted in saline. A range of physiologically acceptable solvents ie saline, saline +/- NaOH/HCl, DMSO or ethanol were available if any analogues proved insoluble in saline. The final concentration of solvents used was less than 0.01% and solvent controls were used throughout.

### 4. Anti-tumour activity *in vivo*

Animals and tumour system

Pure strain NMRI mice were used from the Bradford Clinical Oncology Unit inbred colony. NMRI mice were housed in cages in an air conditioned room where regular alternate 12 h cycles of light and darkness were maintained. Animals were supplied with pellet diet (CRM Labsure, Croydon, UK) and water *ad libitum*.

The development of several adenocarcinoma of the colon in NMRI mice from primary tumours induced by the prolonged administration of 1,2-dimethylhydrazine has been described elsewhere.[145] The tumour line used in this study was MAC 15A grown subcutaneously as a poorly differentiated, solid tumour in NMRI mice.

**5. Chemotherapy**

Chemotherapy began when tumours had reached a size that could be accurately measured and had an established vasculature. Anti-tumour activity was assessed by tumour weights and all tumours were of comparable size. All drugs were administered intraperitoneally (ip) at comparable doses to FAA. Drug vehicles differed depending on the analogue used with the majority administered using saline. FAA itself was administered in 20% cremophor/saline, saline + NaOH and arachis oil as positive controls.

**6. Statistical analysis**

Statistical analysis was performed using one way analysis of variance on tumour weights.[146] Where significant differences between mean tumour weights were obtained Tukeys test[146] was performed to determine whether or not treated tumour weights were significantly different from control tumour weights.

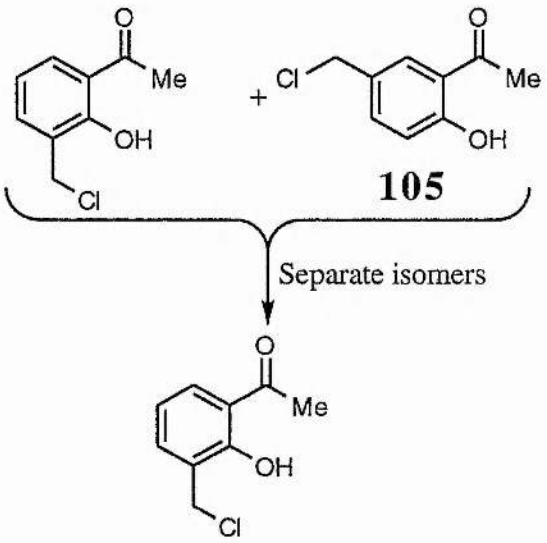
# DISCUSSION

## A. Development of a General Method for Flavone Production

Various literature methods were available for flavone production as described in the Introduction to the thesis. Factors considered in choosing the appropriate reaction scheme were cost of starting materials, ease of synthesis and overall yield.

### 1. The Basic Route

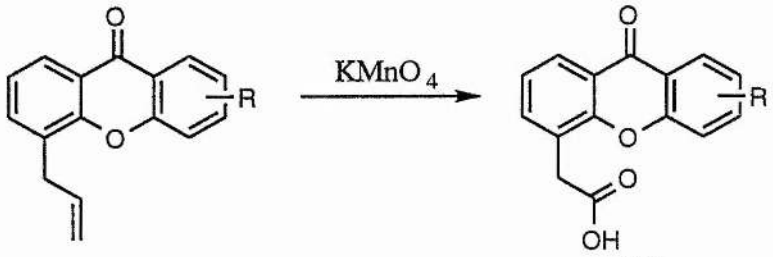
In previous work in this laboratory Sharma had found that a base induced condensation was a viable method to making flavones.[93] This method was chosen due to the wealth of information already available on the reaction. However, several problems remained and the synthesis could not be directly used to make 6-unsubstituted flavones. In his synthesis the penultimate step to flavone acetic acid formation was the acid hydrolysis of a cyanomethyl group. The cyanomethyl group originated from cyanide attack on a chloromethyl moiety, a group which was difficult to introduce into the correct position to give **96**.



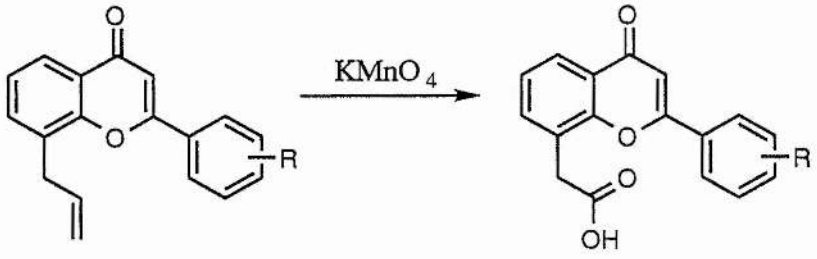

**96**

In his synthesis he had to block the site *para* to the hydroxyl with a methyl group to force the chloromethyl onto the 3-position since the mixture of

isomers formed, **96** and **105**, was impossible to separate efficiently. In our case we wanted flavones and not 6-methylflavones and therefore had to find another way of introducing the acetic acid group. This masked acetic acid had to be inert in the base condensation and subsequent acid cyclisation reactions. The patent by Denny *et al.*[147] on the production of xanthenone-4-acetic acids showed that an allyl group could be oxidised to give the target acetic acid **20**. Likewise flavone-8-acetic acid **107** could be formed by oxidative cleavage of the corresponding 8-allylflavone **106**.

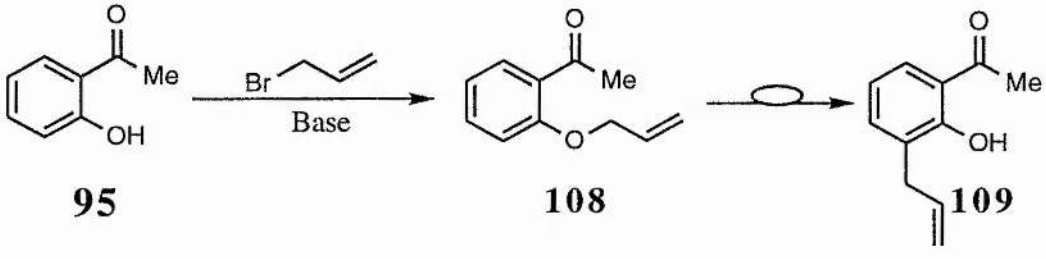



**20**




**106** **107**

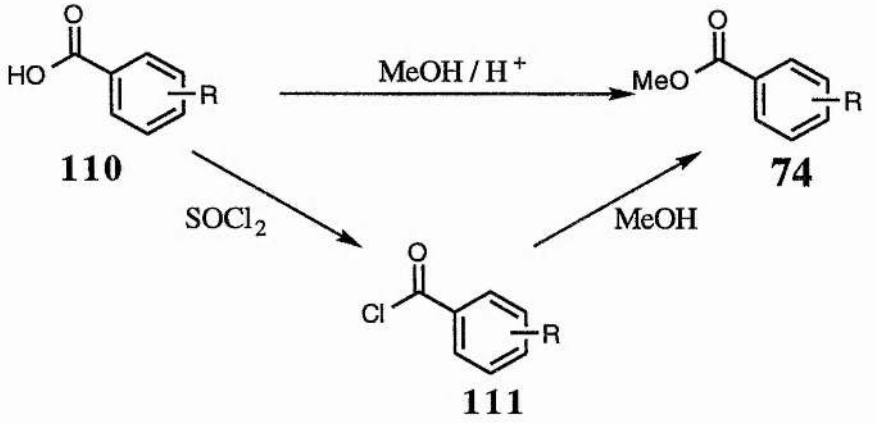
The starting materials for the condensation consisted of an aryl ester **74** and the acetophenone **109**. The problem was to place an allyl group at the 3-position on the acetophenone. This was readily solved by making use of the Claisen rearrangement.[148]




**95** **108** **109**

Using standard procedures the hydroxyacetophenone **95** was reacted with allyl bromide to give the allyl ether **108** which could be rearranged under Claisen conditions to yield the 3-allyl-2-hydroxyacetophenone **109**. This material was produced on a large scale as it would always form one of the two starting materials in the base condensation.

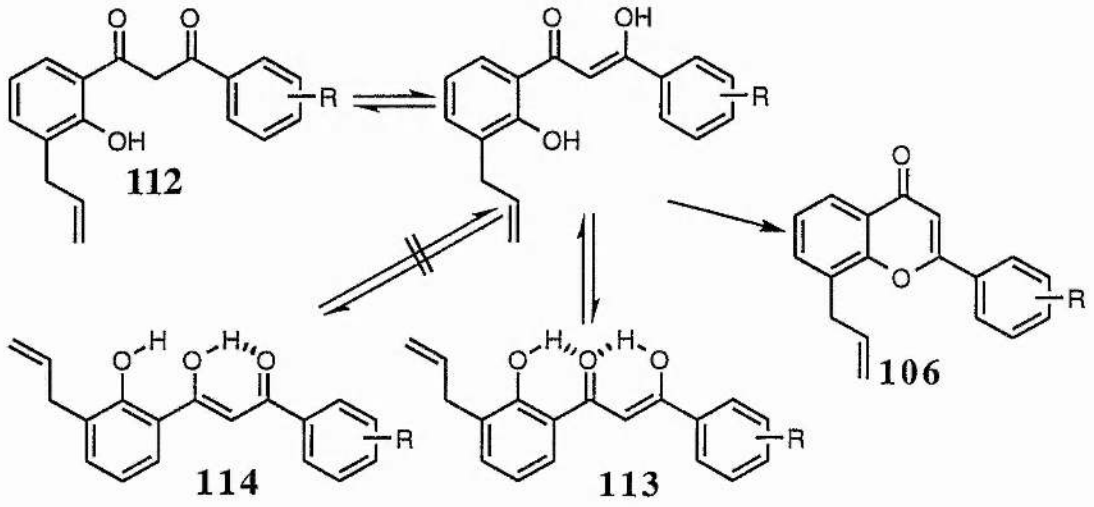
The other reactants in the condensation were the aromatic esters **74**. The esters used were commercially available or synthesised using known procedures from the corresponding carboxylic acids **110**.




Initially acidic methanol was used for the conversion of the carboxylic acids **110** into their corresponding esters **74**. This method, however, gave low yields and was therefore superceded by using thionyl chloride to firstly produce the acid chloride **111** in excellent yield, followed by reaction with methanol to synthesise the ester in very good overall yield.

The condensation of the dianion of **109** with **74** initially gives a β-diketone **112** and in the early stages of the project the β-diketones were isolated. Isolation and purification of the yellow 1-(3-allyl-2-hydroxyphenyl)-3-aryl-1,3-propanediones **112** was followed by acid catalysed cyclisation in methanol to give the allylflavone **106**. Various tautomeric forms of **112** are possible, but it was clear from the NMR spectra that the compounds existed exclusively in one form. It is most

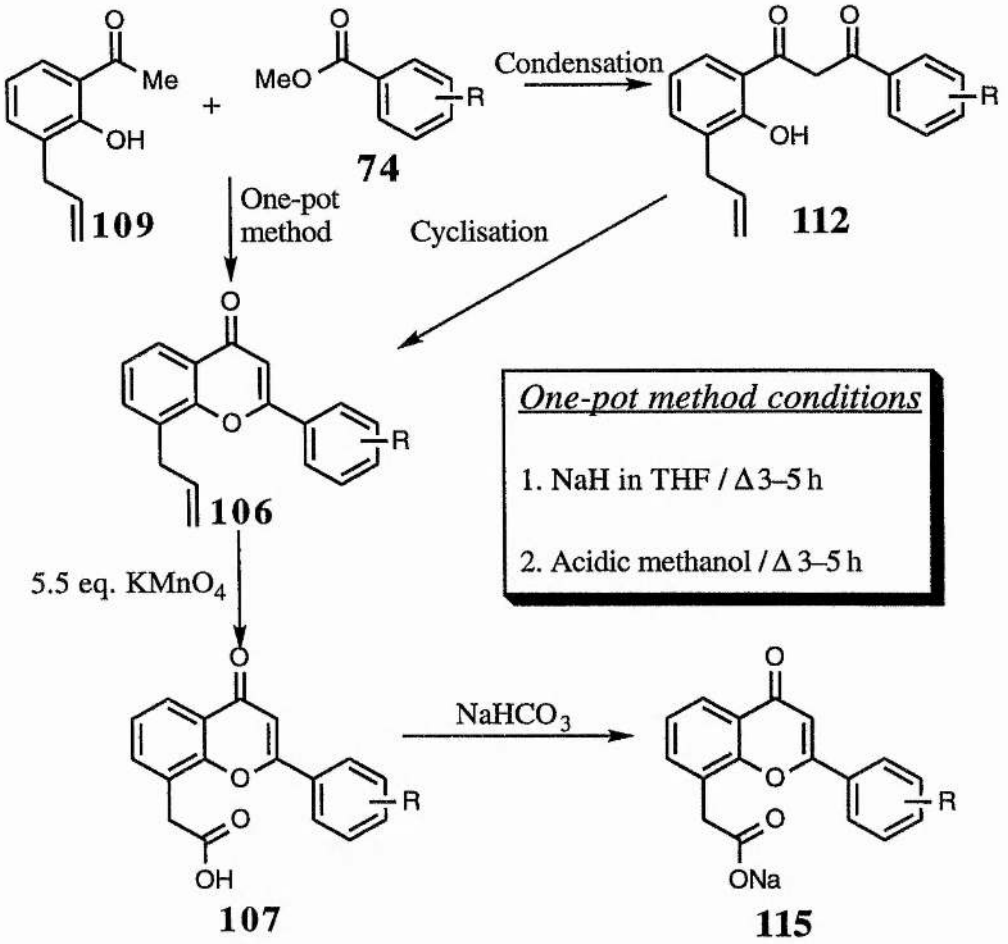
likely that this is the form **113** in which hydrogen bonding across two six-membered rings is possible and this is clearly preferred to the alternative **114** in which there is only one such interaction.




2. Optimisation of the Condensation to Allow a One-pot Synthesis

After several derivatives were prepared using this route it seemed probable that the condensation and cyclisation steps could be combined. The reasoning behind this was that, at the end of the condensation reaction, the mixture had to be acidified to slightly acidic pH. If the mixture was made more acidic and methanol was added then cyclisation could be brought about without isolation of the intermediate.

Colour changes during this one-pot synthesis were indicative of product formation. A colourless solution of the ester in THF with NaH in suspension changed to a bright orange on completion of the condensation reaction. Addition of methanol to destroy any unreacted base changed the solution to burgundy, which dissipated to an orange hue upon acidification. After heating under reflux for several hours to form the flavone itself, the colour of the solution was bright green. The many colours, although never analysed spectrally, were used in conjunction with thin layer chromatography to assess reaction progress.




All reactions were followed on silica plates (run predominantly in ether) with rf values showing relative polarities of component side groups. Workup of the green allylflavone was via evaporation of the methanol and THF followed by aqueous extraction with several base washings. These washings designed to remove any unreacted hydroxyacetophenones and β-diketone intermediates may, with hindsight, have resulted in a reduced yield of desired product due to flavone B-ring opening. Recrystallisation from ethanol with addition of charcoal yielded the pure compounds as colourless or yellow needles or cubes.

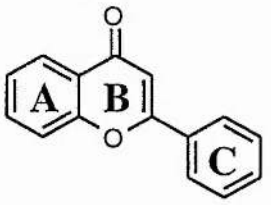
3. Oxidation

Oxidation of the allyl moieties proved more problematic than anticipated. Various oxidising agents were used such as oxone, ozone and acidic

potassium permanganate. Oxone, an effective agent for conversion of sulphides to sulphoxides and sulphones proved completely ineffective for double bond cleavage. Ozone, on the other hand, was too powerful an oxidising agent as it attacked the internal enone double bond causing breakdown of the molecule. Finally permanganate was decided upon and after optimisation of the reaction conditions this was used as a standard method. A solution of the allylflavone in acetic acid/acetone/water (2:2:1) was stirred at <5 °C while solid $KMnO_4$ (5.5 eq.) was added in portions over 6 hours. Addition of saturated sodium metabisulphite to the resulting brown suspension gave a white solid, and the mixture was then partially evaporated under reduced pressure and poured onto water. A white precipitate of FAA then precipitated which could be recrystallised from a 1:1 mixture of acetic acid / water or ethanol. The yield of this reaction was consistently low at 30 to 50 percent.

4. Sodium Salt Formation

The sodium salts of the flavone-8-acetic acids **115** were formed, initially, using sodium hydroxide in the minimum of water. The route, although successful in preparing the salts, gave brown decomposition products when the water was removed. This may be due to the instability of the B-ring of the flavone **116** to nucleophilic attack leading to breakdown products.




**116**

A new route to salt formation was sought and the literature yielded a method by Denny *et al.*.[147] In their work they took the minimum volume of water, normally 3–5 ml and heated it to about 70 °C. They then added

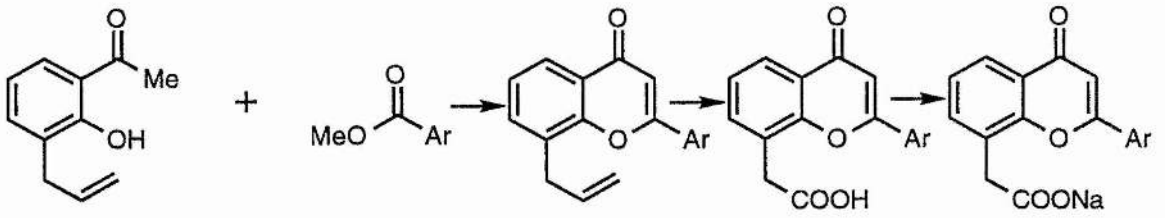
the desired amount of sodium bicarbonate and the corresponding acid and waited until the solid had disappeared. They then filtered any remaining solid material, cooled the mixture to room temperature and poured it into a large volume of A. R. acetone to yield the sodium salt as a fine white precipitate. This precipitate was filtered off and washed with more acetone and dried in the air. Although this method gave a slightly reduced yield this was more than offset by the improved purity of the product.

## B. Mono-substituted Flavone-8-acetic Acid Derivatives

### 1. Synthesis

As the previous literature on flavone-8-acetic acids contained many synthetic derivatives but few *in vitro* and *in vivo* results, it was considered prudent to firstly synthesise simple derivatives. These target flavones were mono-substituted on the C-ring and the pendant groups differed in chemical and electronic nature.

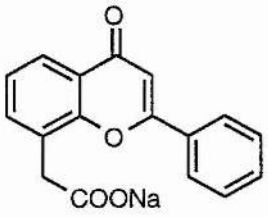
Seven aromatic methyl esters were prepared or bought including the unsubstituted parent **117**, 2-methoxybenzoate **118**, 3-methoxybenzoate **119**, 4-methoxybenzoate **120**, 4-methylbenzoate **121** and 4-chlorobenzoate **122**. The one-pot method proved robust for these examples forming **123**, **124**, **125**, **126**, **127** and **128** respectively. It was decided that the method would become the standard route for flavone synthesis due to the acceptable yields obtained. Oxidation of these compounds gave the flavone-8-acetic acids **129**, **130**, **131**, **132**, **133** and **134** in poor to reasonable yields. The sodium salts **135**, **136**, **137**, **138**, **139** and **140** were formed in good to excellent yields.



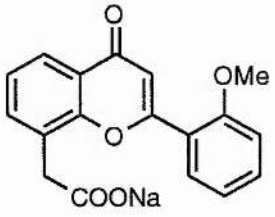

| | | | | |
|---|---|---|---|---|
| Ar = Phenyl | **117** | **123** | **129** | **135** |
| 2'-Methoxyphenyl | **118** | **124** | **130** | **136** |
| 3'-Methoxyphenyl | **119** | **125** | **131** | **137** |
| 4'-Methoxyphenyl | **120** | **126** | **132** | **138** |
| 4'-Methylphenyl | **121** | **127** | **133** | **139** |
| 4'-Chlorophenyl | **122** | **128** | **134** | **140** |
| 4'-Nitrophenyl | **141** | **142** | —— | —— |

Methyl 4-nitrobenzoate **141** was also prepared and the coupling reaction attempted to form **142**. In this case unfavourable electronic effects probably inhibited the formation of the diketone and so the flavone **142** was never formed.

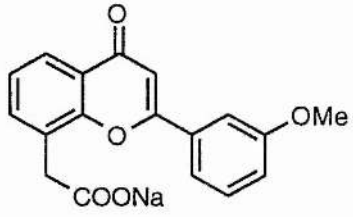
The following compounds were then ready to test –



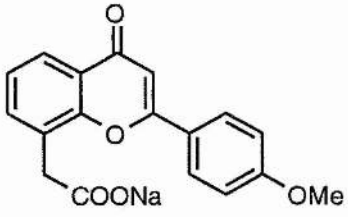

**135**



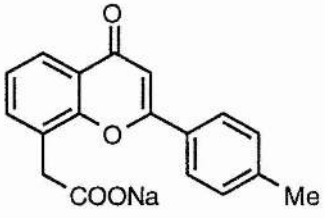

**136**



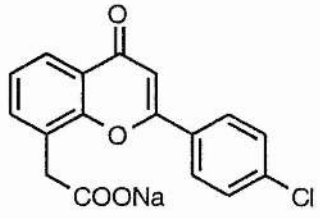

**137**




**138**



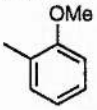
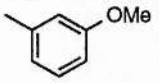
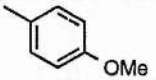
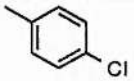

**139**




**140**

2. *In vitro* results

MAC15A tumour cells were cultured as described in the Experimental section. The results for synthesised compounds are shown in tabular form below, with the percentage survival of cells shown inside the boxes.

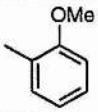
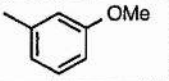
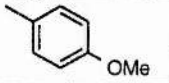
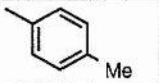
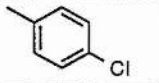
| | | % Survival of Tumour Cells | | | | | |
|---|---|---|---|---|---|---|---|
| | | Dose Rate µg/ml | | | | | |
| Compound | Ar = | 0 | 0.1 | 1 | 10 | 100 | 500 |
| **135** |  | 100 | 100 | 100 | 100 | 70 | 5 |
| **136** |  | 100 | 88 | 85 | 69 | 19 | 3.5 |
| **137** |  | 100 | 100 | 100 | 92 | 57 | 11 |
| **138** |  | 100 | 96 | 100 | 86 | 48 | 31 |
| **140** |  | 100 | 100 | 100 | 97 | 26 | 31 |

The table shows that at the low drug dose of 1 µg/ml all the cells survived except those treated with the 2-methoxy derivative. Increasing the dosage led to activity for all derivatives with the 2-methoxy derivative **136** showing greatest activity at each dosage. The 3-methoxy **137** and 4-methoxy **138** show reasonable activity at median doses with the former being most potent at the highest drug concentration. The 4-chloro derivative **140** gave very similar results to the 4-methoxy congener showing the importance of not only electronic effects but also steric effects to elicit the cytotoxic response.

At the higher doses, the pharmaceutical window conceptualised by Zaharko *et al.*[59] seems to operate as seen especially in the 4-chloro case.

3. *In vivo* results

All of the mono-substituted flavone-8-acetic acid salts were tested *in vivo* against MAC15A as described in the Experimental section. Each compound was tested at a certain dosage as dictated by the previous activity *in vitro*. The results are presented in terms of tumour growth delay in the table below.

| Ar = | Dose 1 $mgkg^{-1}$ | Growth Delay (days) | Significance | Dose 2 $mgkg^{-1}$ | Growth Delay (days) | Significance |
|---|---|---|---|---|---|---|
|  | 50 | 0 | ----- | 100 | 1 | 0.05 |
|  | 300 | 4.1 | <0.01 | 400 | 3.9 | <0.01 |
|  | 294 | 1.8 | <0.01 | ----- | ----- | ----- |
|  | 300 | 1.6 | <0.05 | 500 | 3.6 | <0.05 |
|  | 80 | 0 | ----- | ----- | ----- | ----- |
|  | 300 | 0.8 | ----- | 450 | 0 | ----- |

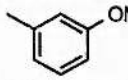
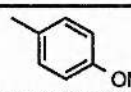
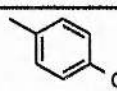
Unsubstituted FAA salt was inactive at the lowest dose but showed a significant growth delay of 1 day at double the initial dose. Statistical analysis was carried out using a Mann-Whitney test on treated cancer sizes.

The most startling result was that for the 2-methoxy derivative which elicited the largest growth delay at 300 $mgkg^{-1}$. This significant result was mirrored at the 400 $mgkg^{-1}$ concentration, however, as with the *in vitro* results a pharmaceutical window was apparent and the increased drug dose resulted in a lower growth delay. The 4-methoxy and 3-methoxy derivatives gave much smaller growth delays at 300 $mgkg^{-1}$ than their 2-methoxy counterpart, however, at the higher dose, 4-methoxy

derivative gave a comparable growth delay of 3.6 days to the 2-methoxy derivative.

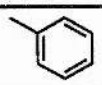
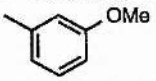
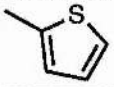
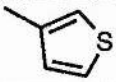
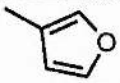
No significant growth delays were noted for the 4-methyl and 4-chloro analogues.

All of the monosubstituted sodium salt FAA derivatives had been previously synthesised by other workers.[25,89,90] The only previously reported *in vivo* results for these compounds are shown below.[26,27] The percentage tumour growth inhibition (TGI) is a measure of the effectiveness of the drug, and the other column shows the non-invasive measurement of anti-cancer drug effectiveness as a increase in lifespan (ILS) as compared to control animals. It should be noted that these results are for colon 38 (C38) and pancreatic (P) cancers, rather than the MAC15A adenocarcinoma used in our studies.

| Compound | Ar = | TGI | ILS |
|---|---|---|---|
| **135** |  | 96% (C38) | 17% (PS) |
| **137** |  | 100% (C38) | Not quoted |
| **138** |  | Not quoted | 41% (PS) |
| **140** |  | 18% (C38) | Not quoted |

Sharma synthesised many 6-methyl FAA derivatives in this laboratory.[93] These compounds were tested against various tumour cell lines *in vitro* and *in vivo* including MAC15A[138] (derived from an ascitic murine adenocarcinoma of the colon), MAC16[56] (slow growing, solid and cachectic murine adenocarcinoma of the colon), MAC26[56] (well differentiated solid murine adenocarcinoma of the colon), WEHI-3B[139] (murine myelo-monocytic leukaemia), K562[140] (human chronic myelogenous leukaemia with erythroid characteristics), HT-29[141], HCLO, DLD-1[142], HCT-18 (human adenocarcinoma of the colon), HRT-18[143]

(human rectal carcinoma) and BM (murine bone marrow). However only the results for MAC15A will be shown as direct comparison can be drawn with our results. The compounds were delivered to the tumour cells using saline.

| Compound **104** Ar = | *In vitro* $IC_{50}$ $\mu gml^{-1}$ | *In vivo* % TGI at | | |
|---|---|---|---|---|
| | | 100 $mgkg^{-1}$ | 200 $mgkg^{-1}$ | 300 $mgkg^{-1}$ |
|  | 210 | 32 | 4 | 16 |
|  | 68 | 39 | 27 | 25 |
|  | 100 | 14 | 36 | 0 |
|  | 350 | 36 | 56 | 94† |
|  | 60 | 21 | 21 | 36 |

† 4/5 deaths

It can be seen that the most effective compound *in vitro* is the heterocyclic 3-furyl derivative. It will be seen later that these encouraging results helped us decide on using certain analogues in our study.

From the activity data it was seen that the methoxy group gave very promising results. It was therefore decided to try different polymethoxy derivatives and more elaborate heterocyclic examples for testing in an attempt to increase drug potency.

## C. Polymethoxylated Flavone-8-acetic Acid Derivatives

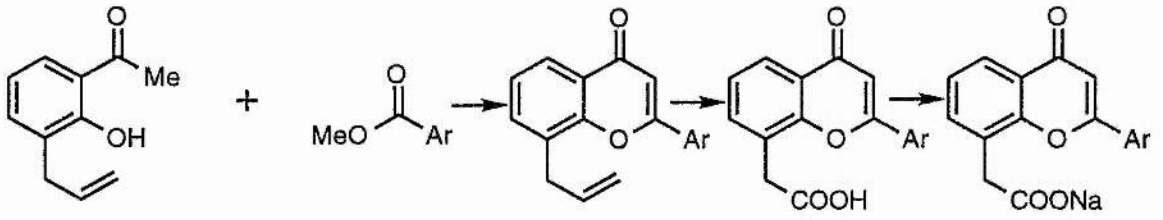
1. Synthesis

a. Dimethoxy Analogues

Dimethoxyflavone-8-acetic acid derivatives were made using the same robust method as that for the monosubstituted derivatives. Early *in vitro* results showed that the most effective compound possessed a 2-methoxy group on the C-ring and on that basis many 2-methoxy containing disubstituted analogues were synthesised. The corresponding methyl esters, 2,3-dimethoxybenzoate **143**, 2,4-dimethoxybenzoate **144**, 2,5-dimethoxybenzoate **145** and the 2,6-dimethoxybenzoate **146** proved straightforward to synthesise. All of these esters condensed and cyclised without any problems forming **147**, **148** and **149** in good to excellent yields with the exception of the 2,6-dimethoxy analogue **146** which failed to condense with the anion of the acetophenone probably due to steric hindrance. This compound **150**, complete with its loss in degrees of freedom, would have been interesting to test as the C-ring would have been twisted and locked into an almost perpendicular conformation relative to A- and B-rings.

The sequence was then continued using the normal method to give the 2-methoxy disubstituted FAA derivatives **151, 152** and **153** and their corresponding sodium salts **154**, **155** and **156**. Of these compounds only the 2,4-dimethoxy FAA **152** had been reported before.[25,89]

It was decided to make several 3-methoxy containing derivatives due to the previous testing results to complement the 2-methoxy derivatives in establishing structure-activity relationships. Methyl 3,4-dimethoxybenzoate **157**, methyl 3,5-dimethoxybenzoate **158** and methyl 3,4-methylenedioxybenzoate **159** were synthesised and coupled with 3-allyl-2-hydroxyacetophenone to form **160**, **161**, and **162** respectively.

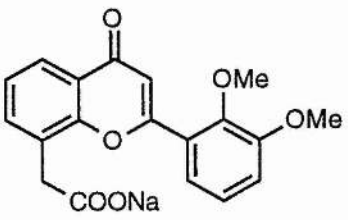



| Ar = | | | | |
|---|---|---|---|---|
| 2',3'-Dimethoxyphenyl | **143** | **147** | **151** | **154** |
| 2',4'-Dimethoxyphenyl | **144** | **148** | **152** | **155** |
| 2',5'-Dimethoxyphenyl | **145** | **149** | **153** | **156** |
| 2',6'-Dimethoxyphenyl | **146** | **150** | ——— | ——— |
| 3',4'-Dimethoxyphenyl | **157** | **160** | **163** | **166** |
| 3',5'-Dimethoxyphenyl | **158** | **161** | **164** | **167** |
| 3',4'-Methylene-dioxyphenyl | **159** | **162** | **165** | **168** |
| 4'-Chloro-2'-methoxyphenyl | **169** | **171** | **173** | **175** |
| 5'-Chloro-2'-methoxyphenyl | **170** | **172** | **174** | **176** |

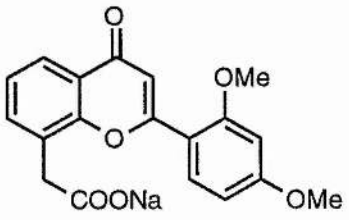
The standard reaction conditions then gave the three carboxylic acids, **163**, **164** and **165** and their salts **166**, **167** and **168**, of which only two, **163** and **164**, had previously been synthesised in patent work.[25,89] **163** had a TGI of 70% against colon 38 carcinoma while no activity results existed for the latter. The other 3,4-disubstituted FAA derivative, **165**, and its corresponding salt **168**, was designed to check if the 'locked' alkoxy function helped activity.

Two other di-substituted ester derivatives were synthesised in which one group was a chlorine and the other an 2-methoxy group. These isomeric structures, **169** and **170**, differed in the position of the chlorines, one *meta* to the methoxy and the other *para* to the methoxy group. The 4-chloro-2-methoxyphenyl allyl derivative **171** and the 5-choro-2-methoxyphenyl congener **172** were oxidised to their corresponding acetic acids **173** and **174**. The salts **175** and **176** were formed in high yields.

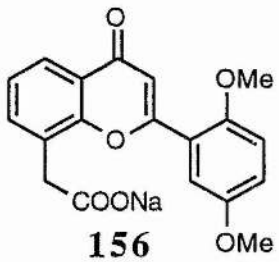
The following disubstituted derivatives were then ready to test –



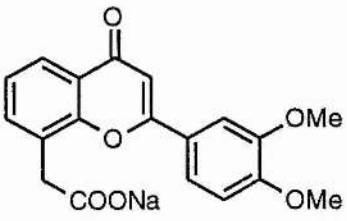

**154**



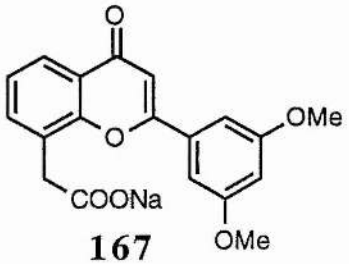

**155**



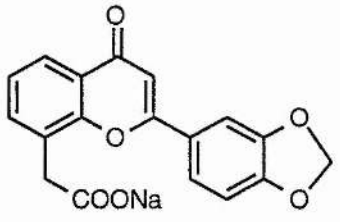

**156**



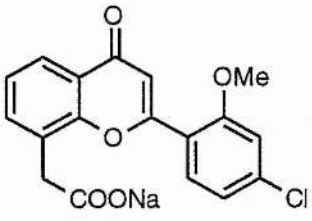

**166**



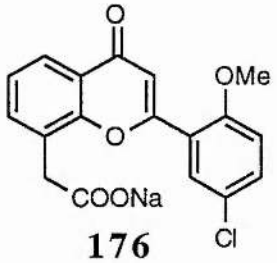

**167**




**168**



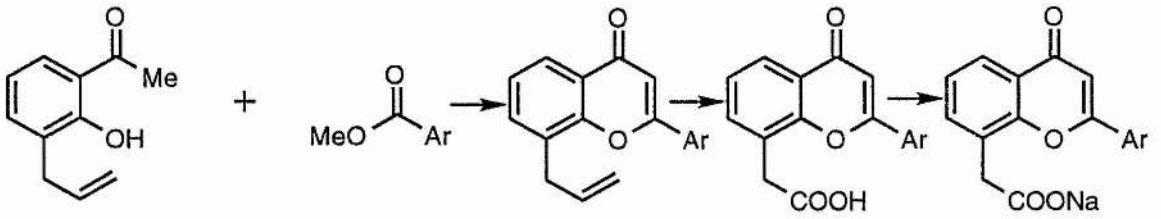

**175**




**176**

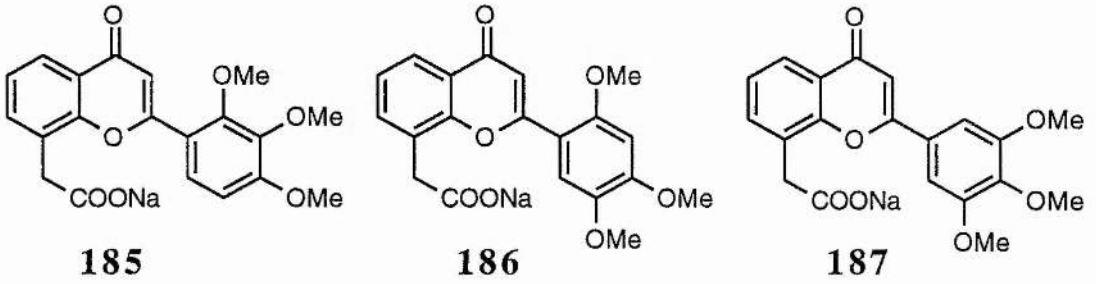
b. Trimethoxy Analogues

Trimethoxy allyl analogues **177**, **178** and **179**, as with the di-substituted derivatives, proved straightforward to make from their corresponding methyl esters **180**, **181**, and **182**. However the 8-allyl-2',4',6'-trimethoxyflavone **183** like the 2,6-dimethoxy derivative, could not be obtained since the ester **184** failed to undergo condensation presumably due to steric hindrance. The corresponding salts **185**, **186** and **187** of the acid analogues **188**, **189** and **190** were obtained readily using the normal methods.




| Ar = | | | | |
|---|---|---|---|---|
| 2',3',4'-Trimethoxyphenyl | **180** | **177** | **188** | **185** |
| 2',4',5'-Trimethoxyphenyl | **181** | **178** | **189** | **186** |
| 2',4',6'-Trimethoxyphenyl | **184** | **183** | —— | —— |
| 3',4',5'-Trimethoxyphenyl | **182** | **179** | **190** | **187** |

The following trisubstituted derivatives were ready to test –

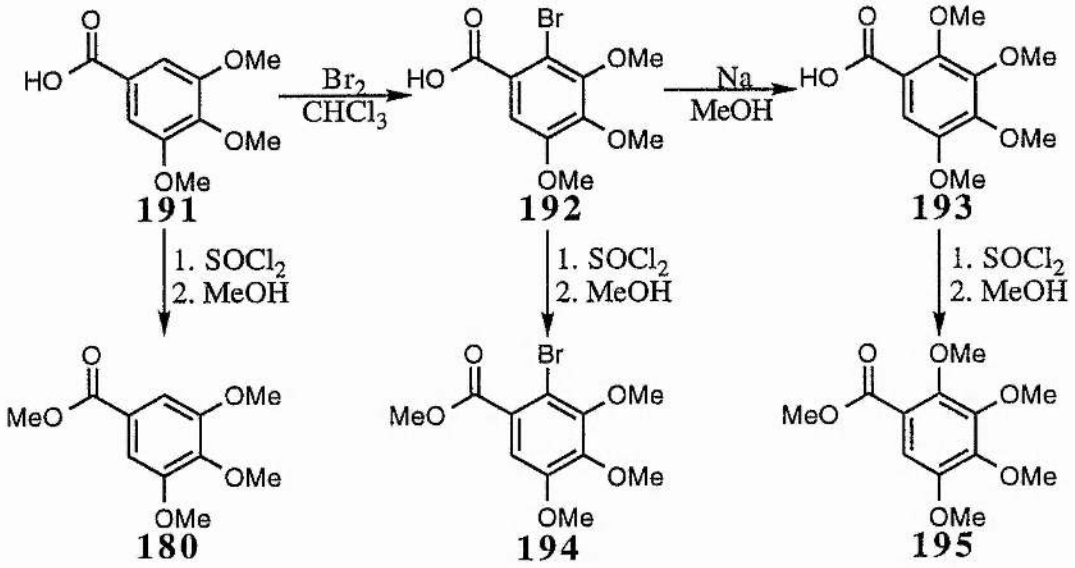



**185** **186** **187**

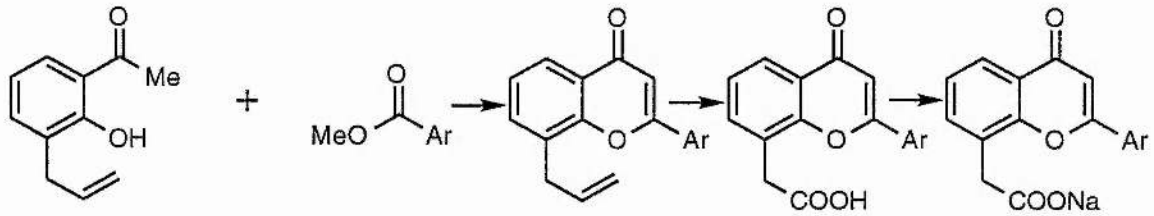
To date there have been no trisubstituted derivatives documented in the literature.

c. Tetramethoxy Analogues

The tetra-substituted derivatives were all made from 3,4,5-trimethoxybenzoic acid **191** following Mayer and Fikentscher's procedure.[115] In their procedure they took the carboxylic acid **191** and brominated the 2-position using bromine in chloroform with a catalytic quantity of water present. This 2-bromo-3,4,5-trimethoxybenzoic acid **192** was then made into the 2,3,4,5-tetramethoxybenzoic acid **193** by reaction with excess sodium methoxide in methanol in the presence of very finely divided copper bronze.

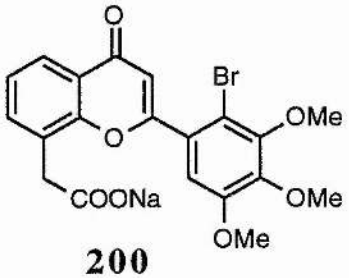



Both of these carboxylic acids were esterified forming **194** and **195** respectively and used to produce the corresponding allylflavones **196** and **197**. The flavone-8-acetic acids **198** and **199** and their salts **200** and **201** were then formed with little difficulty.

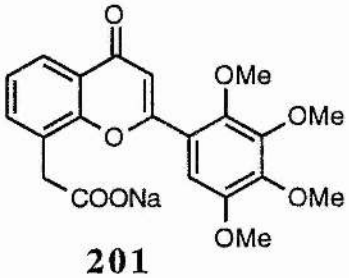



| Ar = | | | | |
|---|---|---|---|---|
| 2'-Bromo-3',4',5'-trimethoxyphenyl | **194** | **196** | **198** | **200** |
| 2',3',4',5'-Tetramethoxyphenyl | **195** | **197** | **199** | **201** |

The following tetrasubstituted derivatives were ready to test –



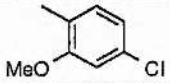
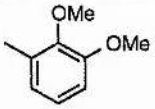
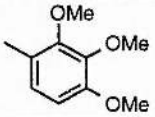
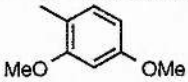
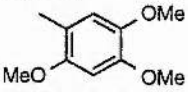
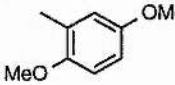
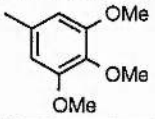
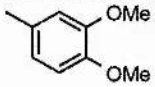
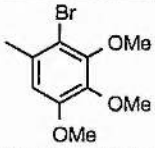
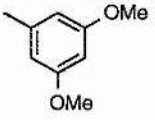
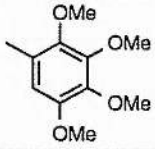
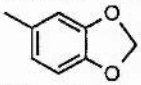

**200**




**201**

2. *In vitro* Testing

The *in vitro* activity against MAC15A was determined as described in the Experimental section and the results are presented in terms of $IC_{50}$ values in $mgkg^{-1}$.

| Cmpd No. | Ar = | $IC_{50}$ values $mgkg^{-1}$ | Cmpd No. | Ar = | $IC_{50}$ values $mgkg^{-1}$ |
|---|---|---|---|---|---|
| **135** |  | 6.8±1.5 | **175** |  | 3.5±2.2 |
| **154** |  | 325±74 | **185** |  | 16±7 |
| **155** |  | 17±3.5 | **186** |  | 123±10 |
| **156** |  | 8.8±2.4 | **187** |  | 41±9 |
| **166** |  | >500 | **200** |  | 19±1.2 |
| **167** |  | 65±10 | **201** |  | 30±7.2 |
| **168** |  | 17±4 | | | |

It can be seen from these results that the 2-methoxysubstituted derivatives, in the main, show the best activity. Analysis of these results is indicative of an electronic factor which may be influencing anti-tumour activity. If

the tetramethoxy derivative is compared to any of the methoxy derivatives it is plain that there can be no additional steric factors operating in these di or trisubstituted analogues which are not present in the tetrasubstituted derivative.

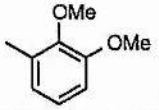
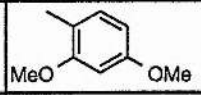
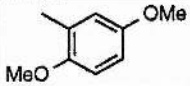
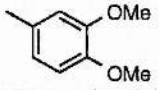
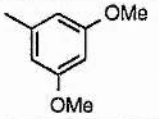
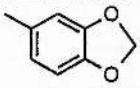
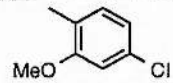
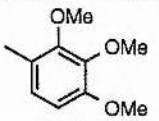
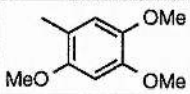
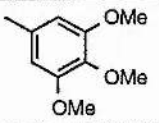
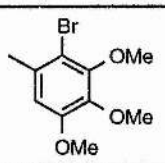
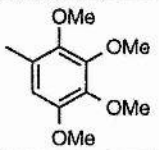
If compounds with equivalent steric bulk i.e. the 4-chloro-2-methoxy and the 2,4-dimethoxy derivatives are compared then there has to be a combination of electronic and steric factors affecting activity.

All of the 2-methoxy containing disubstituted compounds show significant activity *in vitro*. The 2,4-dimethoxy **155** and 2,5-dimethoxy **156** compounds showed twenty fold or greater activity than the 2,3-dimethoxy derivative **154**. The 3-methoxy containing disubstituted derivatives showed good to reasonable activity except for the 3,4-dimethoxy compound **166** which proved effectively inactive. It is interesting to note that the close relative of this compound, 3,4-methylenedioxy **168**, was very active. Once again this activity must be due to an electronic effect, however it could be argued that this derivative has fewer degrees of freedom with regards to space and hence fits more snugly into the active site. However, the trisubstituted derivatives and tetrasubstituted derivatives tend to disprove this argument.

The trisubstituted compounds especially the 2,3,4-trimethoxy **185** and the 3,4,5-trimethoxy **187** derivatives showed significant activity in the MAC15 screen. Both of the tetrasubstituted derivatives gave good results in the *in vitro* screen with the halo derivative slightly more active than its congener.

3. *In vivo* testing

Using the *in vitro* results as the basis for the dosage regime *in vivo*, the polymethoxylated derivatives were tested against subcutaneous MAC15A tumours. The results are presented in terms of tumour growth delay.

| Cmpd No | Ar = | Dose 1 $mgkg^{-1}$ | Growth Delay (days) | Significance | Dose 2 $mgkg^{-1}$ | Growth Delay (days) |
|---|---|---|---|---|---|---|
| **135** |  | 50 | 0 | | 100 | 1.0 |
| **154** |  | 500 | 1.7 | ----- | ----- | ----- |
| **155** |  | 450 | 2.8 | <0.05 | 550 | 3.2 |
| **156** |  | 500 | 0 | ----- | ----- | ----- |
| **166** |  | 324 | 1.9 | <0.01 | 400 | 1.1 |
| **167** |  | 400 | 1.4 | ----- | ----- | ----- |
| **168** |  | 37 | 0 | ----- | 50 | 0 |
| **175** |  | 39 | 0.8 | ----- | 50 | 0 |
| **185** |  | 450 | 0.5 | ----- | ----- | ----- |
| **186** |  | 500 | 0 | ----- | ----- | ----- |
| **187** |  | 500 | 1.7 | <0.05 | ----- | ----- |
| **200** |  | 500 | 3.1 | <0.01 | ----- | ----- |
| **201** |  | 500 | 1.2 | ----- | ----- | ----- |

At equivalent doses the dimethoxy derivatives showed variable results. The 2,4-dimethoxy derivative, with the best *in vitro* result, stopped the cancer growing for about 3 days with the 2,3-dimethoxy derivative inhibiting growth for about 2 days. It is interesting to note that even at such a high dosage the 2,5-dimethoxy derivative is inactive *in vivo*. Whether this effect is due to under/overshooting the pharmaceutical window requires further study. The very good *in vitro* result perhaps indicates that the concentration of the drug used here was too high to elicit a response.

The 3,4-dimethoxy derivative shows a contrasting result to that obtained *in vitro*. A median dose of this drug caused a respectable growth delay of about 2 days whereas at the higher dose of 600 $mgkg^{-1}$ no growth delay was observed. Even more startling was the discovery that the close congener, 3,4-methylenedioxy **168**, which was very active *in vitro*, showed no activity, albeit at low dosage. This effect may be due to undershooting the pharmaceutical window.

Of the three trisubstituted derivatives only the 3,4,5-trimethoxy derivative **187** showed any statistically significant growth delay. These results at least showed correlation between the *in vitro* and *in vivo* tests with the 2,4,5-trimethoxy derivative **186**, which was least active *in vitro*, also proving inactive *in vivo* at high dose.

The halo-substituted tetrasubstituted derivative **200** gave significantly the best growth delay of 3.1 days at high dose whereas its tetramethoxy congener **201** gave a growth delay of 1.2 days at the same dose.

## D. Heterocyclic Flavone-8-acetic Acid Derivatives

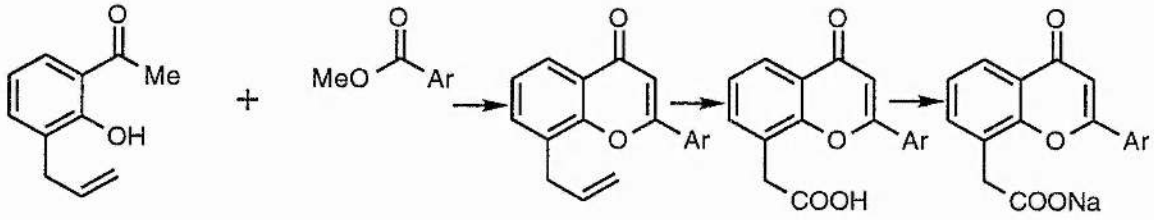
Since it appeared that the presence of one or more electron-donating methoxy groups on the 2-aryl substituent was generally beneficial for

activity, it was decided to synthesise compounds with the 2-aryl substituent replaced by a range of π-excessive heteroaromatic groups and for comparison some π-deficient heterocyclic groups.

1. Synthesis

a. Sulphur containing heterocyclic derivatives

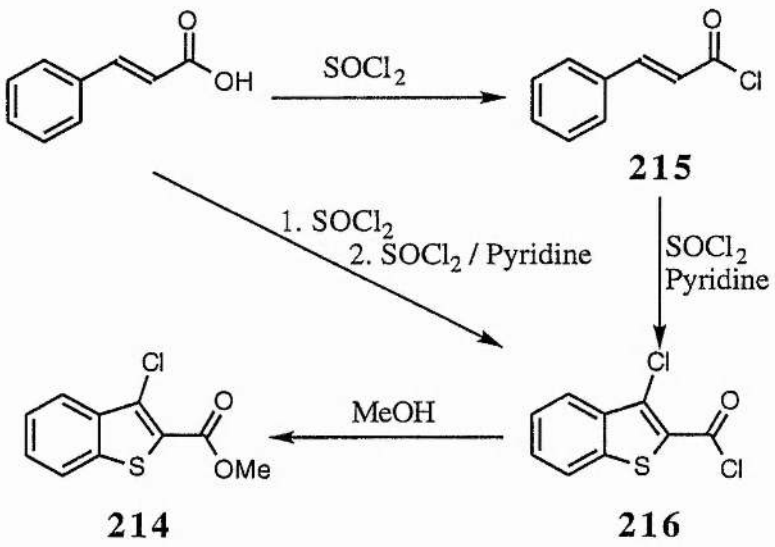
Sulphur, oxygen, and nitrogen heterocyclic carboxylic acid esters were all made. The allyl derivatives including 3-thienyl **202** and 3-methyl-2-thienyl **203** proved straightforward to synthesise from their corresponding esters **204** and **205**. These allyl derivatives were oxidised under normal conditions to form the corresponding carboxylic acids **206** and **207** and converted to their sodium salts **208** and **209**. The 8-allyl-(3-methoxythienyl)flavone **210** was synthesised by Dr. Robert Ritchie in this laboratory from the methyl ester **211** of 3-methoxythiophene-2-carboxylic acid (gifted by Synthetic Chemicals Ltd.). This derivative was then oxidised by the author to the carboxylic acid **212** which was converted to its sodium salt **213**.




| Ar = | | | | |
|---|---|---|---|---|
| 3-Thienyl | **204** | **202** | **206** | **208** |
| 3-Methyl-2-thienyl | **205** | **203** | **207** | **209** |
| 3-Methoxy-2-thienyl | **211** | **210** | **212** | **213** |
| 3-Chloro-2-benzo[b]thienyl | **214** | **217** | **218** | **219** |

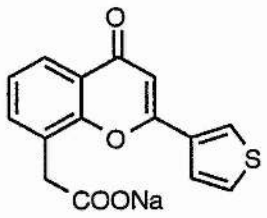
The most exotic sulphur-containing methyl ester **214** was prepared using the procedure of Krubsack and Higa.[123] The action of thionyl chloride on cinnamic acid produced firstly the acid chloride **215**. Addition of

pyridine and more thionyl chloride to the reaction mixture gave the fused chlorobenzothiophene acid chloride **216** and elemental sulphur after heating for 12 hours under reflux. After filtration of the solid sulphur and evaporation of all volatile components in the mixture a white solid, which was the acid chloride **216**, was obtained. This compound was added slowly into boiling methanol to form the methyl ester **214** and then the methanol was removed. This ester itself was a new compound, only the ethyl ester being isolated before by Krubsack and Higa.[123]

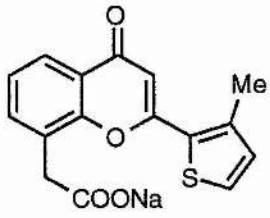



The methyl ester was then coupled to 3-allyl-2-hydroxyacetophenone to form the allyl compound **217** in reasonable yield. The low yielding oxidation of the allyl compound to the acid **218** proved straightforward and the salt **219** was formed without too much difficulty.

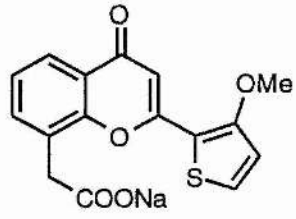
The following sulphur-containing derivatives were ready to test –



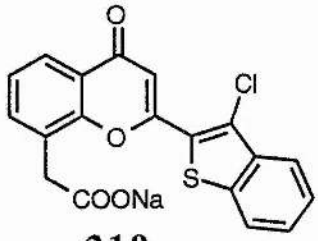

**208**




**209**



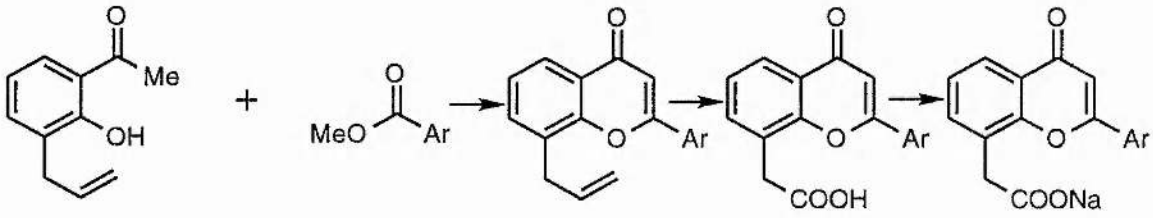

**213**




**219**

b. Oxygen containing heterocyclic derivatives

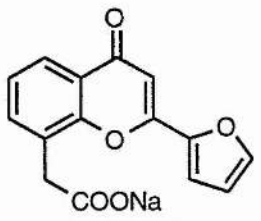
Two allyl derivatives **220** and **221** were synthesised without problem from the corresponding esters **222** and **223**. Of the two oxygen containing heterocyclic allyl derivatives made only the 2-furyl derivative oxidised properly to give the desired product. These acids **224** and **225** were designed to be complimentary to their sulphur analogues. Isolation of the acid **224** was surprising due to the inherent instability of the oxygen heterocycle under acidic oxidising conditions.




| Ar = | | | | |
|---|---|---|---|---|
| 2-Furyl | **222** | **220** | **224** | **226** |
| 3-Furyl | **223** | **221** | **225** | ——— |

The 3-furyl allyl precursor **221** was apparently too sensitive for the oxidising conditions and no solid material was obtained. The sodium salt **226** of the 2-furyl acid was synthesised easily and in excellent yield.

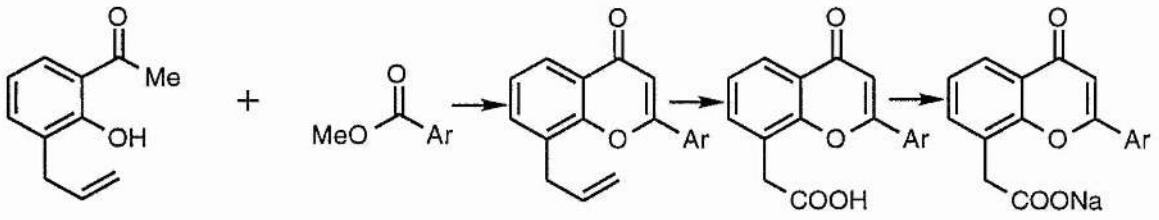
Only one oxygen-containing derivative was ready to test –




**226**

c. Nitrogen containing heterocyclic derivatives

The nitrogen heterocyclic allyl derivatives, **227** and **228**, likewise showed no difficulty in preparation from their corresponding methyl esters **229** and **230** and 3-allyl-2-hydroxyacetophenone under basic conditions.



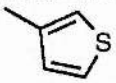
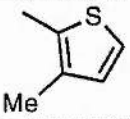
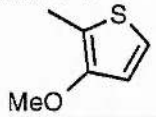
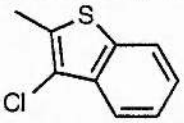
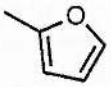

| Ar = | | | | |
|---|---|---|---|---|
| 4-Pyridyl | **229** | **227** | **231** | ——— |
| 2-Quinolyl | **230** | **228** | **232** | ——— |

The oxidation of **227** to **231** gave the same problem as the 3-furyl derivative in that no precipitate was found on normal workup. This was possibly due not only to the breakup of the benzopyranone fragment but also to the oxidation of the nitrogen to the N-oxide. The quinoline derivative **232** also gave the same problem in that no solid was obtained at the end of the oxidation reaction. This was unfortunate as these nitrogen containing derivatives would have been useful in obtaining quantitative structure activity relationships.

No nitrogen-containing derivatives were ready to test.

2. *In vitro* Testing

The heterocyclic derivatives were evaluated against MAC15A *in vitro* and the results are shown below.

| Cmpd No | Ar = | $IC_{50}$ value $mgkg^{-1}$ |
|---|---|---|
| **135** |  | 6.8±1.5 |
| **208** |  | 129±40 |
| **209** |  | 6.4±1.3 |
| **213** |  | 19±2.6 |
| **219** |  | 4.8±3.0 |
| **226** |  | 26±10 |

These *in vitro* results showed that heterocyclic analogues had comparable activity to their carbocyclic counterparts. All the heterocyclic analogues gave promising activity results with the 3-methyl-2-thienyl **209** and the 3-chloro-2-benzo[b]thienyl derivatives **219** giving results among the best of all the compounds tested.

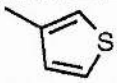
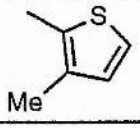
Other *in vitro* results

Only one heterocyclic derivative, synthesised by Dr. Ritchie, had been tested. This 2-thienyl derivative had an $IC_{50}$ value of 30±9.6 which was almost four-fold more active than the 3-thienyl derivative. This value

compares well with the 2-furyl derivative shown above with the 2-furyl analogue being slightly more active.

3. *In vivo* activity

Only two of the heterocyclic derivatives were tested against MAC15A *in vivo*. Both of these compounds were significantly active with the 3-methyl-2-thienyl derivative **209** giving the best activity recorded for the smallest amount of drug. This compound would possibly have been inactive at much higher drug dose whereas the 3-thienyl derivative **208** would probably be less active at lower doses.

| Cmpd No | Ar = | Dose 1 $mgkg^{-1}$ | Growth Delay (days) | Significance | Dose 2 $mgkg^{-1}$ | Growth Delay (days) | Significance |
|---|---|---|---|---|---|---|---|
| **135** |  | 50 | 0 | ----- | 100 | 1.0 | 0.05 |
| **208** |  | 272 | 2.3 | <0.01 | 350 | 2.9 | <0.01 |
| **209** |  | 80 | 4.1 | <0.01 | | | |

The above results are startling as they show probably the most active derivative synthesised to date. It is difficult to imagine why this is the case except for the fact that it perhaps fits into the active site more snugly than the other sulphur derivative. This activity is mirrored by its remarkable activity *in vitro* followed closely by its 3-methoxy congener.

## E. Extended Analogues of Flavone-8-acetic Acid

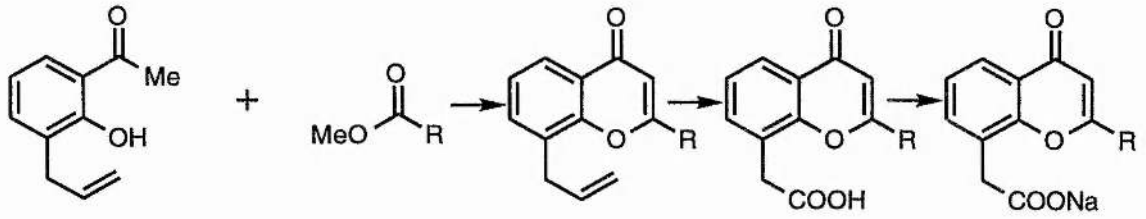
It seemed clear that for activity both the carboxylic acid group of FAA and the 2-aryl substituent are essential. In order to further examine the

structure-activity relationships for this class of compounds, it was decided to vary the distance between these groups.

1. Synthesis

a. Alkyl or alkenyl extension

Phenylacetic acid was made into the acid chloride and esterified to produce **233** which was then condensed with the acetophenone to form **234** in reasonable yield. The methylene extension of the phenyl ring disrupted the conjugation of the system giving an allyl molecule which has a lower melting point than usual.

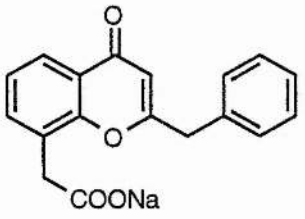



| R = | | | | |
|---|---|---|---|---|
| Benzyl | **233** | **234** | **237** | **239** |
| Diphenylmethyl | **236** | **235** | **238** | **240** |
| 4-Chlorostyryl | **243** | **241** | **245** | — |
| 2-(2-Thienyl)-ethenyl | **244** | **242** | **246** | — |

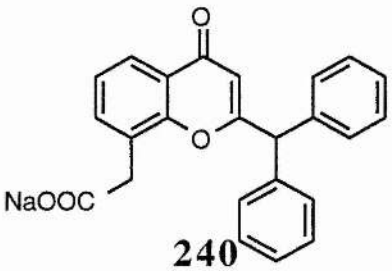
The other extended derivative, 8-allyl-2-diphenylmethylbenzopyran-4-one **235**, was also straightforward to make from methyl diphenylacetate **236**. Both of the corresponding carboxylic acids, **237** and **238** readily formed by the usual method, differed from the normal flavone-8-acetic acids in that they were soluble in dichloromethane. This increase in solubility was probably due to an increased flexibility and hence decreased planarity of the structure. Formation of the sodium salts, **239** and **240**, was therefore problematic as both the carboxylic acids and the sodium salts were soluble in the acetone/water mixture used to isolate the pure sodium salt but the compounds were obtained in good yield.

The double-bond extended allyl compounds, **241** and **242**, proved straightforward to synthesise from their esters, **243** and **244**, albeit with very poor yields. Unfortunately these compounds did not give rise to the expected acids **245** and **246** since the double-bonds of the 2-substituent were oxidatively cleaved under the conditions used.

The following compounds were ready to test–

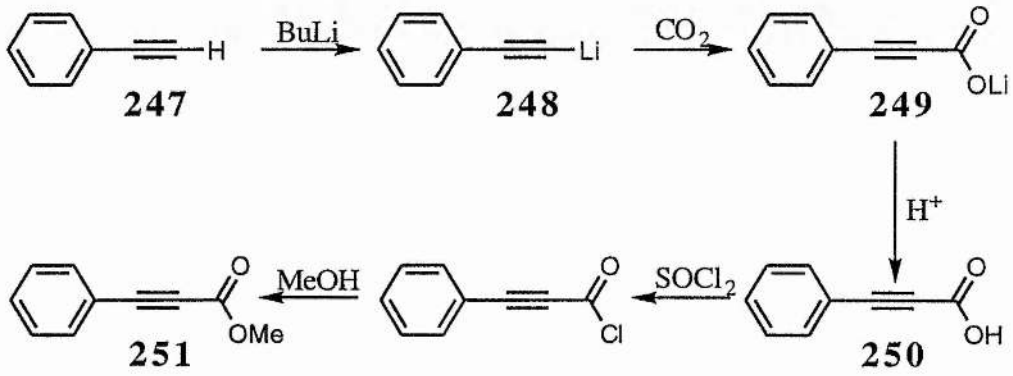



**239**




**240**

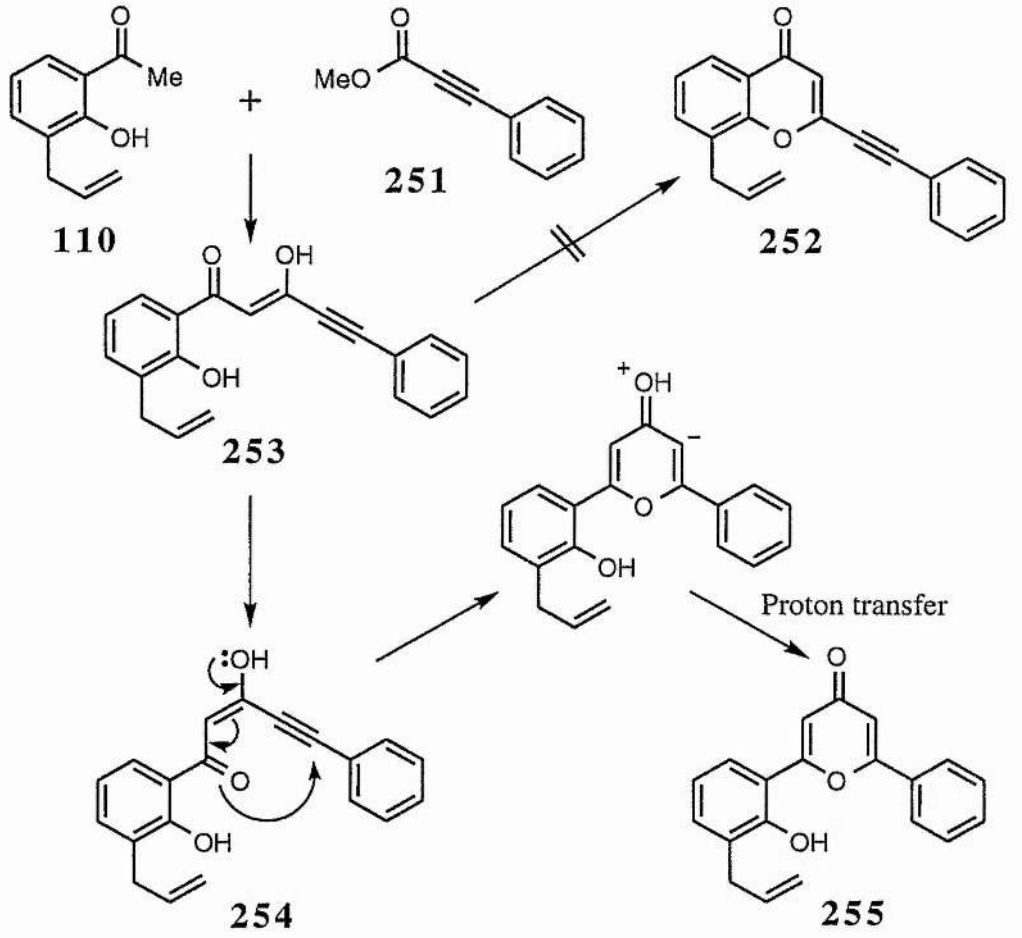
The most interesting of the extended derivatives was obtained serendipitously in an attempt to prepare the analogue of FAA with a 2-phenylethynyl group. This would have had an unsaturated spacer group which could have withstood the oxidative conditions needed to generate the acid from the allyl group. The required acetylenic ester was prepared starting from phenylacetylene **247**. This alkyne was lithiated to give **248** and carbonylated using gaseous carbon dioxide to afford **249**.




**247** **248** **249** **250** **251**

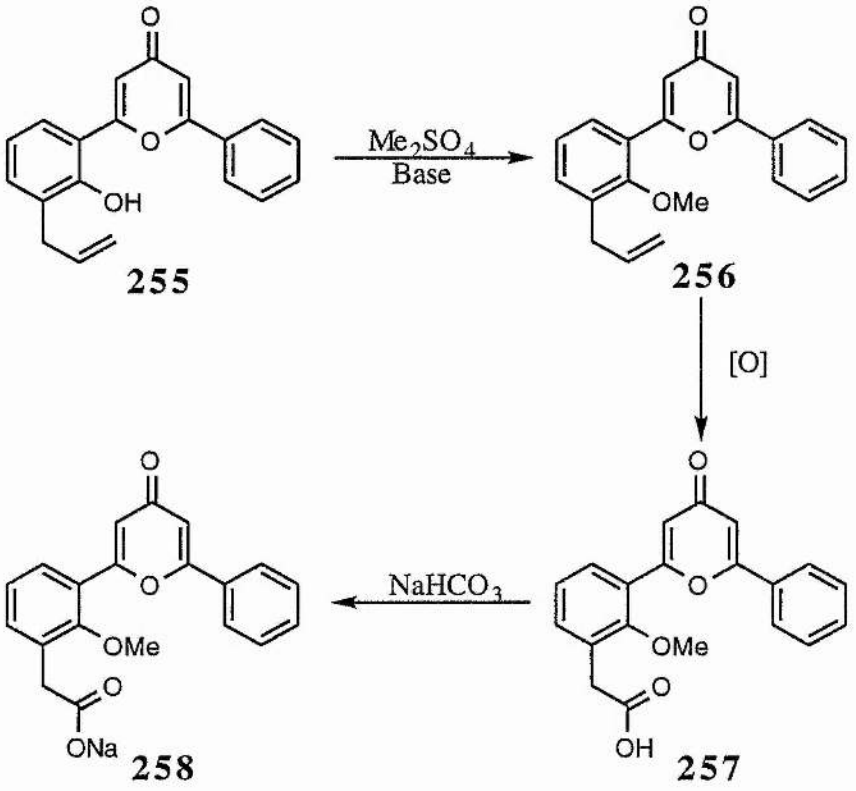
Acidification of the lithium salt gave **250** and subsequent esterification by reaction with thionyl chloride and then methanol gave **251**. The normal route for flavone formation did not, however, lead to the benzopyranone **252**. Instead the quite different compound **255** was obtained.

It seems from experiments carried out on the system later that the β-diketone adduct **253** does form as normal. However, attempted cyclisation of this results in an alternative mode of reaction as shown in **254** to give an extended pyranone system **255** formed by Michael addition.

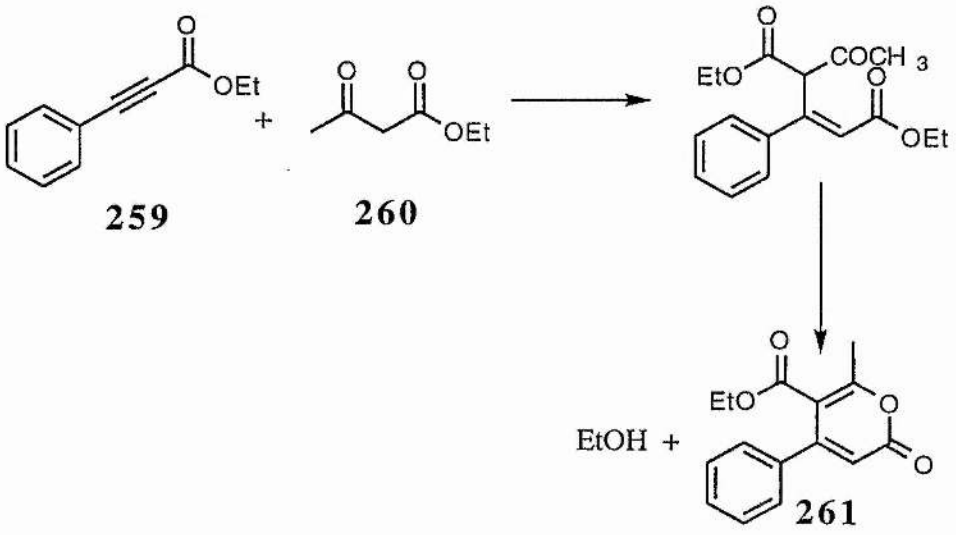



This 'stretched flavone' **255** was deemed important enough to continue along the reaction pathway to the acid. Firstly the free hydroxyl group had to be methylated to stop unfavourable side reactions under the

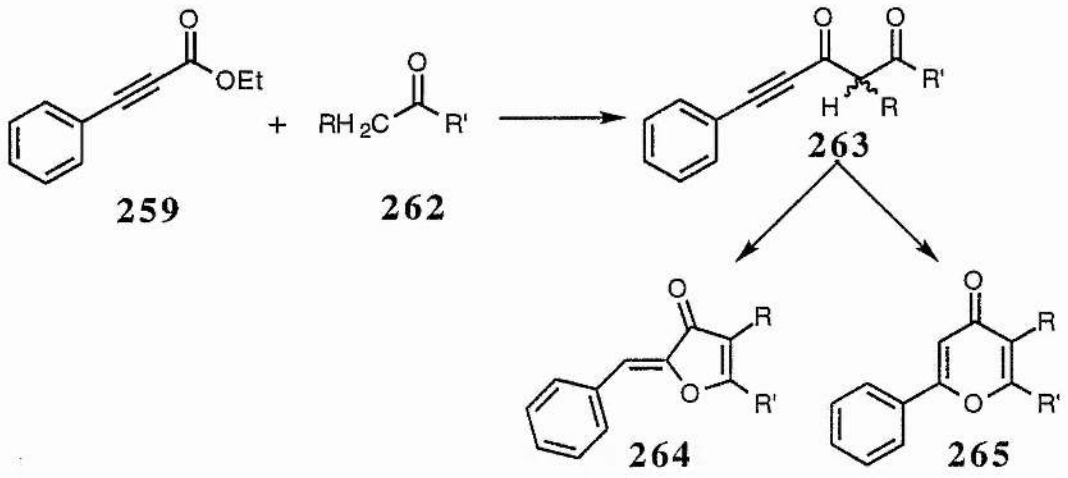
oxidising conditions. The resulting compound **256** was a liquid at room temperature, however after undergoing permanganate oxidation a white solid was formed. This carboxylic acid **257** was converted into its sodium salt **258** using the standard method.




This use for the Michael reaction was discovered by Ruhemann *et al.*[149] in 1899 and is widely applicable.



They discovered that esters of acetylenic acids **259** reacted with β-keto esters **260** or β-diketones to form 2-pyrone **261**. From their previous work on the acetylenic esters **259** and substituted ketones **262** they expected these acetylenic β-diketones **263** to condense forming five **264** or six membered rings **265**.

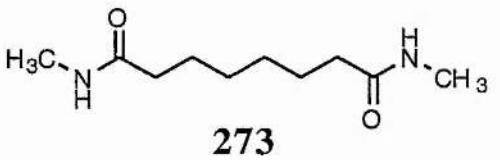



It is possible that the identical process happens for the alkene derivatives, hence explaining the much reduced yields. However, only the correct products were retrieved after recrystallisation.

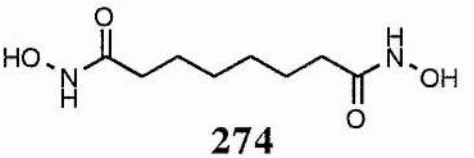
c. Dimeric Derivatives

The last series of 2-substituted derivatives stemmed from the idea of increased activity via two active groups on the same molecule. This was based on work carried out by Breslow *et al.*[150] wherein they discovered that bisacetamides were more active as anti-cancer agents than monoacetamides by more than a factor of two. Polar solvents were shown[151] to cause differentiation in murine erythroleukaemia cell lines thereby nullifying the cancer. This innovative form of anti-cancer treatment was unfortunately not viable due to the low *in vivo* concentration of the polar compound. Rapid deacetylation of the compound and rapid renal clearance led to short *in vivo* exposures.

Extensive structure-activity relationships showed that the two groups, separated by a six carbon spacer unit, were binding at two separate receptor sites on the target cell. Further studies showed that if the acetamide groups were replaced by hydroxamic acids then even greater activity was observed. The most effective compound that induced cell differentiation was suberic bishydroxamic acid **274** which was >100 times more effective than its acetamide counterpart **273**.

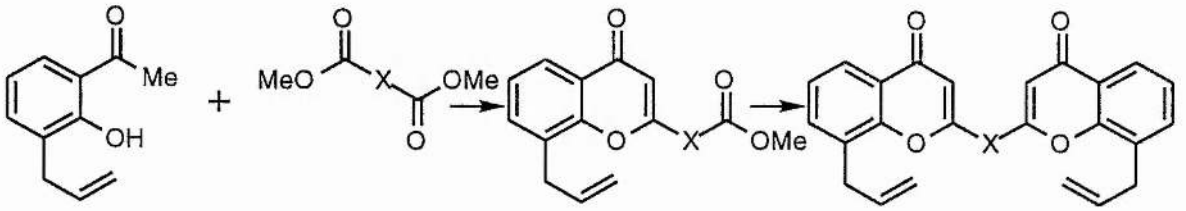


**273**

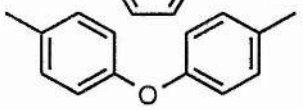


**274**

As the cell receptor for FAA was unknown it was considered prudent to try out Breslow's hypothesis for several representative compounds. This was brought about by using a series of diesters with which the acetophenone anions would add twice.



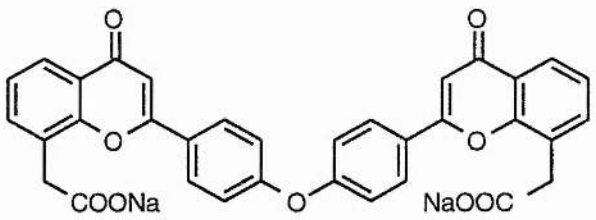
X =

| | | | |
|---|---|---|---|
| — | **266** | Not Isolated | **269** |
|  | **267** | Not Isolated | **270** |
|  | **268** | **271** | **272** |

Preparation of the dimethyl esters **266**, **267** and **268** proved straightforward from their corresponding carboxylic acids. The solid diesters required sonication at the start of the condensation reaction to help the crystals to dissolve in the medium.

The first diester, dimethyl oxalate, was condensed with the two equivalents of acetophenone **110** using four equivalents of base. The resulting compound **269** however was not really a flavone dimer but a benzopyranone dimer. This compound **269**, as with the rest of the allyl dimers **270**, **271** and **272** dissolved only sparingly in the medium dictated by the oxidation. Unfortunately this meant that only the diphenyl ether linked compound was isolated in very low yield from the oxidation reaction. The sodium salt **275** was formed from the carboxylic acid. Interestingly, compound **271** was formed by using only half the amount of starting acetophenone.

The following dimeric compound was ready to test–



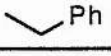
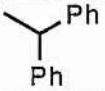
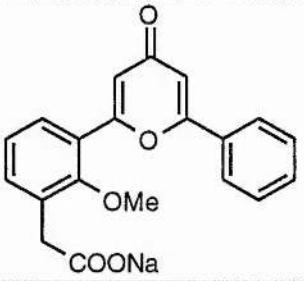

**275**

2. *In vitro* testing

Four extended derivatives were tested on MAC15A cell cultures. Two of these compounds were similar in that they both possessed an alkyl extension. As discussed earlier they also dissolved in less polar solvents due to greater degrees of freedom.

It is perhaps not surprising that the phenylmethyl derivative was extremely active in comparison with the parent FAA whereas the diphenylmethyl derivative was quite inactive when the theoretical active site is envisaged. The benzyl group of **239** can occupy the site intended for the phenyl group of FAA with the whole molecule planar, but with

**240** it is impossible for both phenyl groups to be in the plane and the significant out-of-plane bulk completely removes the activity.

| Cmpd No | R = | $IC_{50}$ value $mgkg^{-1}$ |
|---|---|---|
| **135** |  | 6.8±1.5 |
| **239** |  | 3.7±1.3 |
| **240** |  | >500 |
| **258** |  | 75±18 |
| **275** | Ether dimer | 37±7 |

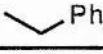
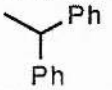
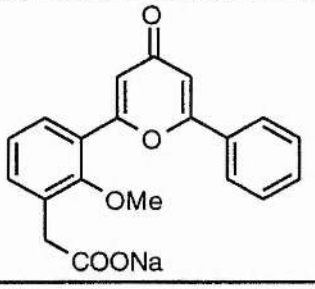
It was hoped that the derivative **258** originating from phenylacetylene would prove active in the *in vitro* test. If this was so, it would show that the distance between the C-4 carbonyl and the acetic acid grouping could be increased. The results were indeed startling as the compound was active and comparable to the majority of derivatives synthesised.

Only one dimeric compound **275** was submitted for testing. This diphenylether dimer was expected to be at least as active as a 4-methoxy derivative. The extra bulk of the molecule would hopefully be able to avoid unfavourable interaction with the active site due to the flexibility of the oxygen linkage. Unfortunately, there was so little of the drug available due to the inherent solubility problems that only the *in vitro* testing could be carried out. It is hoped that the next worker on the project will be able to synthesise a greater amount of compound and

submit it for the *in vivo* test. It is difficult to deduce, at this early stage, whether the effect that Breslow saw is operating on the flavone system. Certainly good activity was shown but many more derivatives would be required to be tested in order to have a more informed opinion.

3. *In vivo* activity

Only three extended derivatives were tested on the murine tumours *in vivo*. The outstanding $IC_{50}$ value for the phenylmethyl derivative prompted the testers to try a very low dosage in the first instance. When this showed no cytotoxicity they increased the dosage of the drug a further two times whereupon it should have caused tumour regression. The diphenylmethyl derivative was inactive under the testing conditions. The results are as follows.

| Cmpd No | Structure | Dose 1 mgkg$^{-1}$ | Growth Delay (days) | Dose 2 mgkg$^{-1}$ | Growth Delay (days) |
|---|---|---|---|---|---|
| **135** |  | 50 | 0 | 100 | 1.0 |
| **239** |  | 34 | 0 | 60 | 0 |
| **240** |  | 500 | 0 | | |
| **258** |  | 500 | 1.7 | | |

The probable cause for the inactivity of both **239** and **240** is metabolic removal of the drug before is could reach the tumour site or the failure to

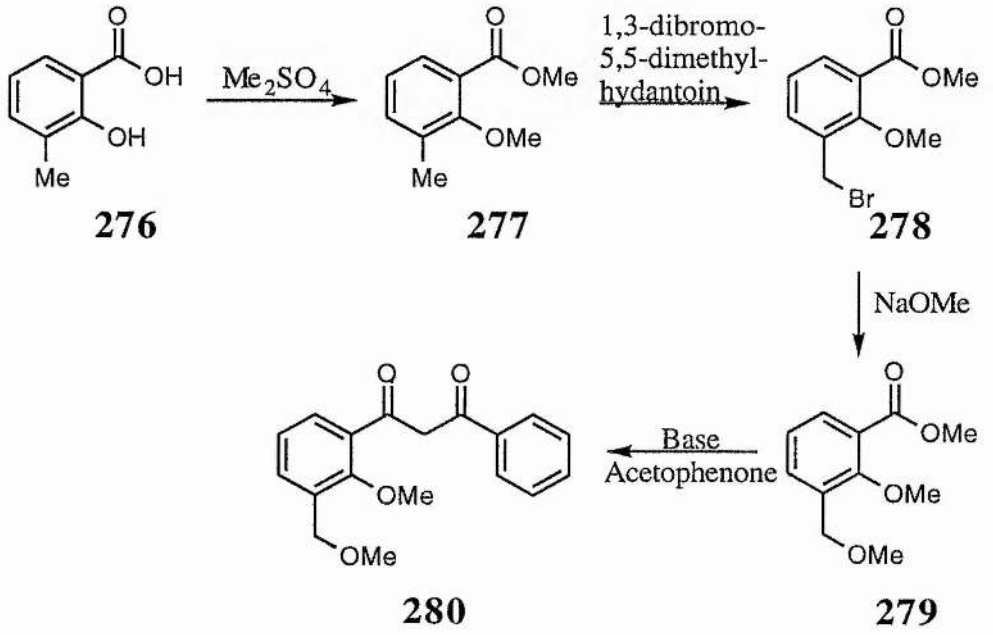
reach the pharmaceutical window. The benzylic CH's are clearly vulnerable to biological oxidation.

The significant activity of the extended analogue, derived from phenylacetylene, **258** shown *in vitro* was borne out *in vivo* where there was also significant activity albeit at high drug dosage. This result was encouraging and will perhaps lead, in the future, to many other optimised similar structures. This results shows that it is indeed possible to increase the distance between the C-4 carbonyl and the acetic acid group and retain activity. It would also be possible to construct a desmethoxy derivative by using an acetophenone instead of a hydroxyacetophenone.

## F. Derivatives with Different Groups in the 8-Position

1. Synthesis

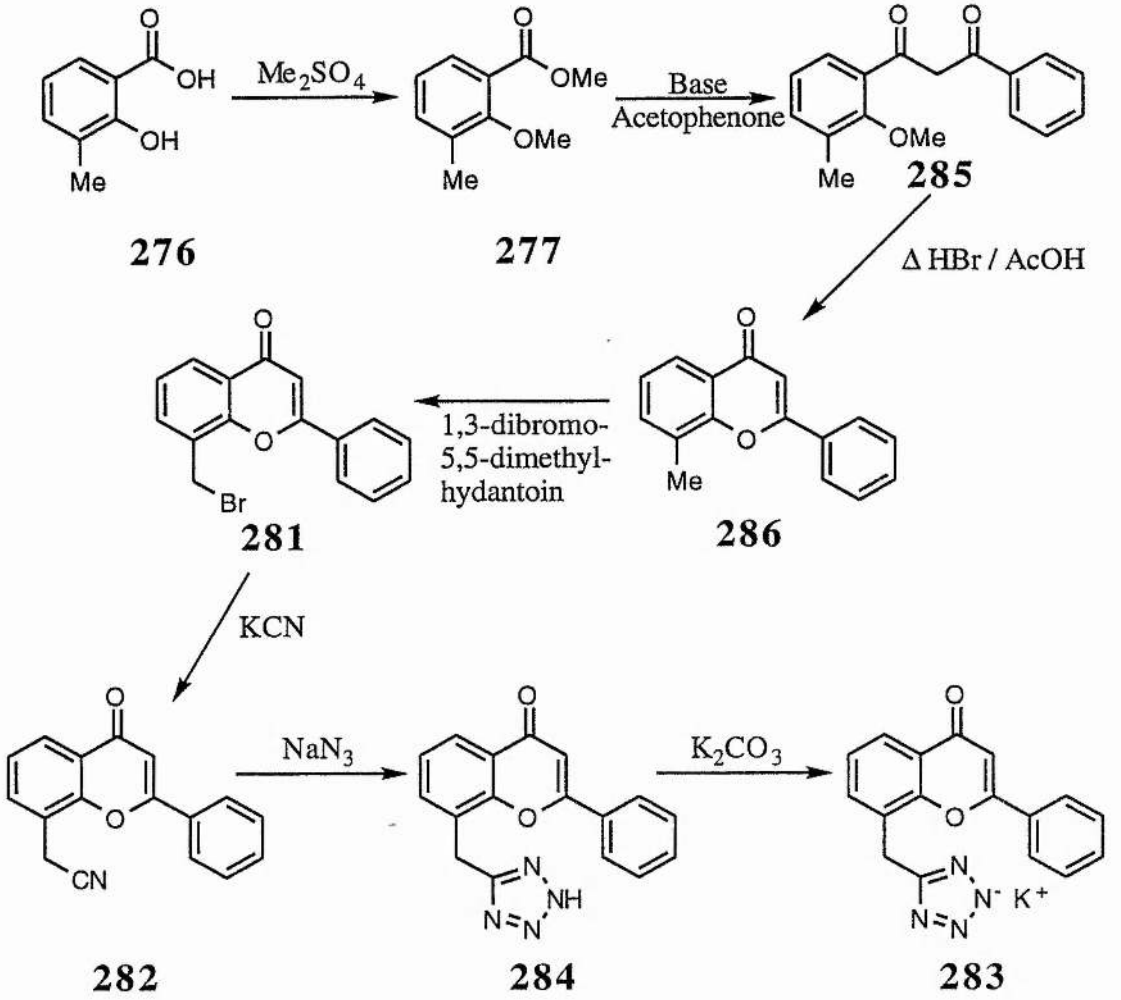
The penultimate change to the flavone moiety involved changing the 8-position's acetic acid group into the isosteric tetrazole. This has been successfully applied in a number of pharmaceutical compounds.[152] The route chosen was developed from previous work[25,89,90,93] starting from 3-methylsalicylic acid **276**.



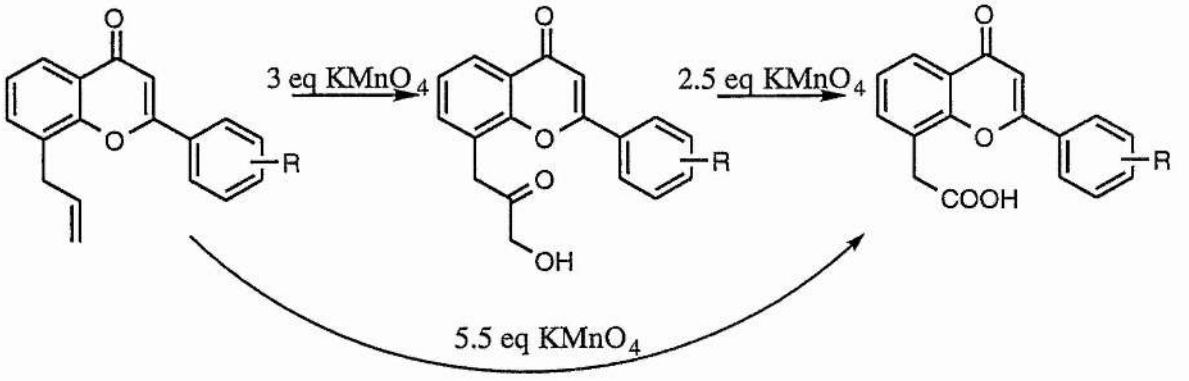

**276** **277** **278** **279** **280**

This was methylated twice to give **277** with some difficulty and the methyl group brominated to give **278**. This bromo-compound was converted into the corresponding methoxy derivative **279**. It was this ester that was condensed with acetophenone under basic conditions. This procedure, although identical in conditions to previous condensations, is the exact reverse in terms of the starting materials, in that the A-ring is formed from the ester and the C-ring formed from the acetophenone. The β-diketone **280** formed after the condensation was cyclised using acidic conditions of hydrobromic acid in acetic acid to form the bromomethylflavone **281**. Unfortunately, due to the difficulty in separating the cyclised product from the starting materials this route was abandoned in favour of the synthesis below.

Once again 3-methylsalicylic acid was used and methylated twice to give **277**. The condensation with acetophenone gave β-diketone **285** and subsequent formation of flavone **286** was brought about by warming the diketone to 80 °C in a hydrobromic acid and acetic acid mixture. 8-Methylflavone **286** had been synthesised previously in the literature.[137] For the conversion to **281** 1,3-dibromo-5,5-dimethylhydantoin was used as the brominating agent instead of N-bromosuccinimide since it was found to be a more active and selective agent for this process. Potassium cyanide was used to displace the bromine forming **282**. The cyano compound was reacted with sodium azide by heating under reflux in dry DMF.[152] The crude product was treated directly with potassium carbonate and the potassium salt **283** that was formed was separated from unreacted cyanide starting material using column chromatography. The acidic silica of the column also converted the potassium salt back to the free tetrazole **284**. Addition of potassium carbonate to the free tetrazole followed by complete removal of water then gave the purified potassium tetrazole salt **283**.

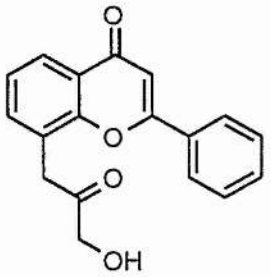



The last group of compounds synthesised had a β-hydroxyketone moiety on the 8-position instead of the acetic acid group. These compounds were formed, by using fewer equivalents of permanganate in the oxidation reaction. Only 3 molar equivalents were used instead of 5.5 equivalents which led to the isolation of these 8-(3-hydroxy-2-oxopropyl) compounds at the end of the reaction. Three derivatives were synthesised, the unsubstituted 2-phenyl- **287**, the 2,3-dimethoxyphenyl- **288** and the 2,5-dimethoxyphenyl derivative **289**.

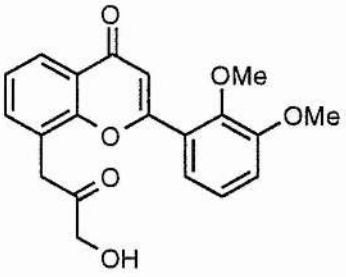



The following derivatives were ready to test -

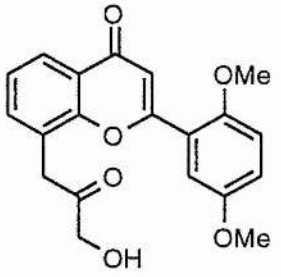
**283**



**287**



**288**



**289**

2. *In vitro* testing

The tetrazole compound **283** was evaluated against MAC15A *in vitro*. Unfortunately, however, it proved to be completely inactive. It is unclear why this compound failed to show activity unless it failed to reach the pharmaceutical window.

| Cmpd No | Structure | $IC_{50}$ value mgkg$^{-1}$ |
|---|---|---|
| **283** |  | >500 |

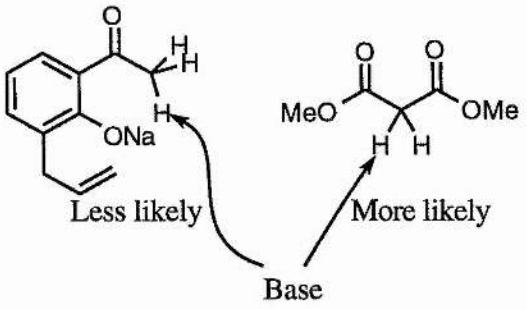
Similarly disappointing results were seen for the three β-hydroxyketone flavone compounds which proved insoluble in the testing medium and therefore were not tested against the MAC15A cell line. A 8-(3-hydroxy-2-oxopropyl)-3',4',5'-trimethoxyflavone synthesised by Ritchie in this laboratory elicited a growth delay of 0.2 days when administered at 100 mgkg$^{-1}$ to murine hosts. This indeed bodes well for other compounds of this type should they be synthesised in the future.

3. *In vivo* testing

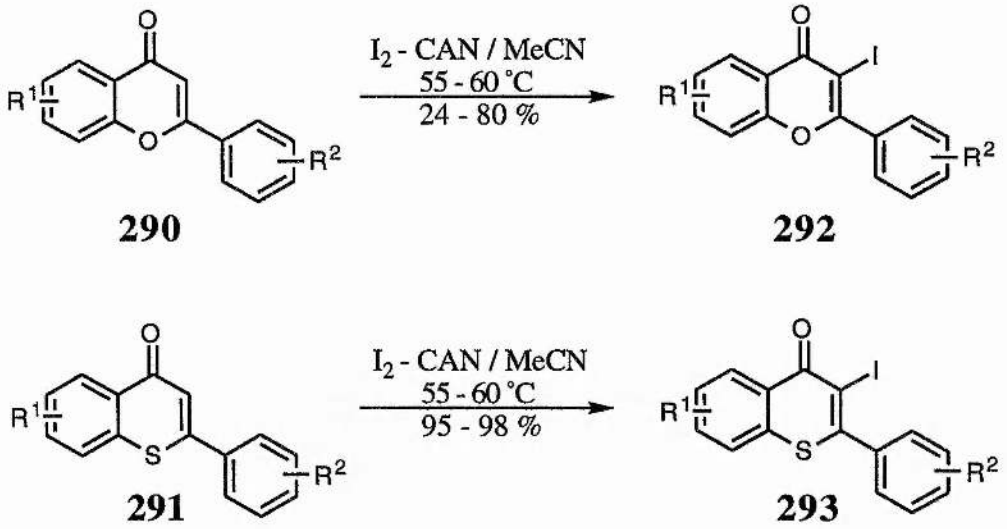
Due to insufficient quantity of drug no *in vivo* testing was carried out on the tetrazole derivative.

4. Other Syntheses Attempted

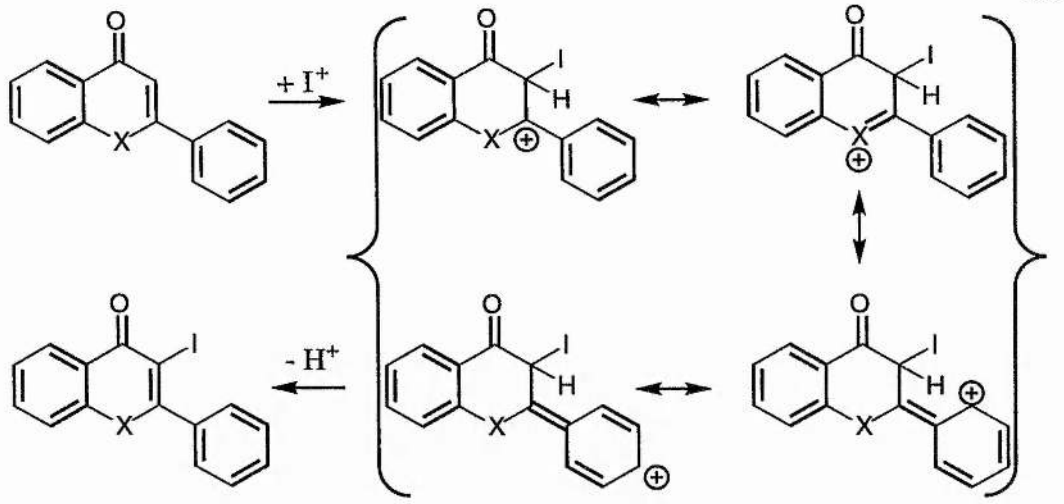
It seemed clear that spacer groups could indeed be useful for increasing activity, as seen in the work of Breslow *et al.*.[150] However methylene spacer groups could not be used as the acidic hydrogens that they contain would react unfavourably under the basic conditions of the one-pot reaction. This reaction was tried once however to confirm this hypothesis and the consequent analysis showed failure of condensation.




From the abnormal NMR properties (see later) of the 8-allyl-2′-methoxyflavone and its corresponding acid and salt it was decided to synthesise the 3-substituted derivative and observe the effect on the 2'-position. A synthesis was attempted which aimed to functionalise the 3-position and comes from the work of Li *et al.*[153] This preparation takes substituted flavones **290**, and thioflavones **291** under mild conditions with $I_2$ and ceric ammonium nitrate (CAN) in acetonitrile and results in iodination at the 3-position with varying degrees of success to give **292** and **293**.




CAN is used to form $I^+$ in essence and it is this species which brings about an electrophilic substitution at the 3-position. A series of canonical forms are possible which stabilise the intermediate in this process as shown.




It has been reported however that various side reactions can occur e.g. a double iodination at the 3 and 8 positions.[153] The yields of this reaction vary tremendously with the change of the pyranone oxygen for sulphur. It has been proposed that the sulphur lone pair is much more basic and can therefore stabilise the intermediate cation.

Unfortunately the synthetic procedure failed to produce the required 3-iodinated compounds for both parent allyl and acetic acid compounds **123** and **129**.

## G. Correlation between Structure and Activity

### 1. Computational Analysis

It can be seen from the preceeding results that one of the most effective anti-cancer agents is the 2′-OMe derivative. Exactly why this is so effective is unknown as the protein binding site is unresolved but it has been noted in the NMR and molecular modelling that something strange is happening.

Computational studies were initiated on various derivatives of the flavone-8-acetic acid system to firstly check on the overall optimised

geometry and secondly to perhaps correlate electron density at various positions on the molecule with *in vitro* and *in vivo* activity. These calculations were carried out on a Sun workstation running SunOS4 using the program MOPAC5 developed for computational analysis. All derivatives studied were entered into the program in the form of cartesian coordinates and were subjected to restricted Hartree Fock calculations using the AM1 Hamiltonian.[154] The program was also run under the PRECISE qualifier which increased selection criteria for the energy gradient by 100 fold. The cartesian coordinates section of the output file was entered into Chem3D, a molecular viewing package, and the graphic file displayed as can seen below. Electron density at various positions were also noted for later analysis. It should be noted, however, that there are many energy minima on the potential surface varying only by a few kcal and because of this the calculations were checked by having different starting conformations.

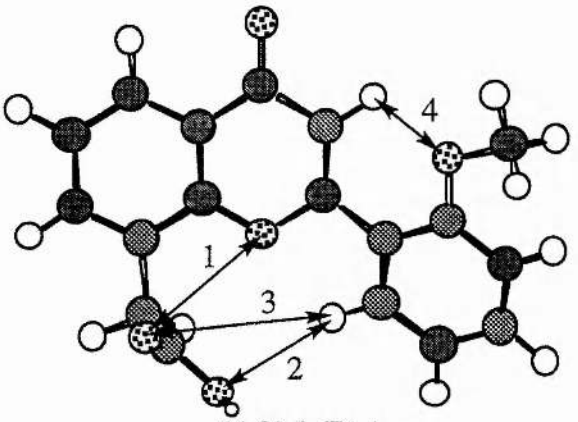
A general feature of the results for FAA, confirmed later by X-ray structure determination, was that the flavone moiety, as expected, was almost planar. However the acetic acid group was invariably orientated almost perpendicular to the flavone. Calculations on the sodium salt of FAA however showed a different effect.

The model of the sodium salt analogue below shows that the carboxymethyl group has swung around until it is close to the hydrogen on C-6′. A similar interaction can be seen occurring between the oxygen of the 2'-methoxy and the hydrogen on the 3-position. This would infer some kind of interaction if not intramolecular hydrogen bonding.

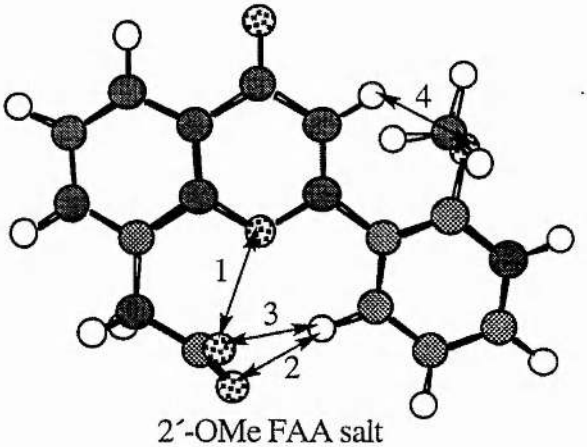
This type of bonding is rare but has also been postulated by Baguley, Ching, Denny *et al.*[70] in the xanthenone-4-acetic acid system. Although these results are only high level calculations, the experimental facts seem to hold with the theory of some interactions at C-3 hydrogen and the C-6′ hydrogen and hence their respective carbons. A good

review[155] of these C–H...O hydrogen bonds explains that although the effect may be small in energy terms such contacts do exist and help such molecules to pack more effectively in the crystal lattice.

Computational Results for 2′-OMe Flavoneacetic Acid and its Salt




2′-OMe FAA

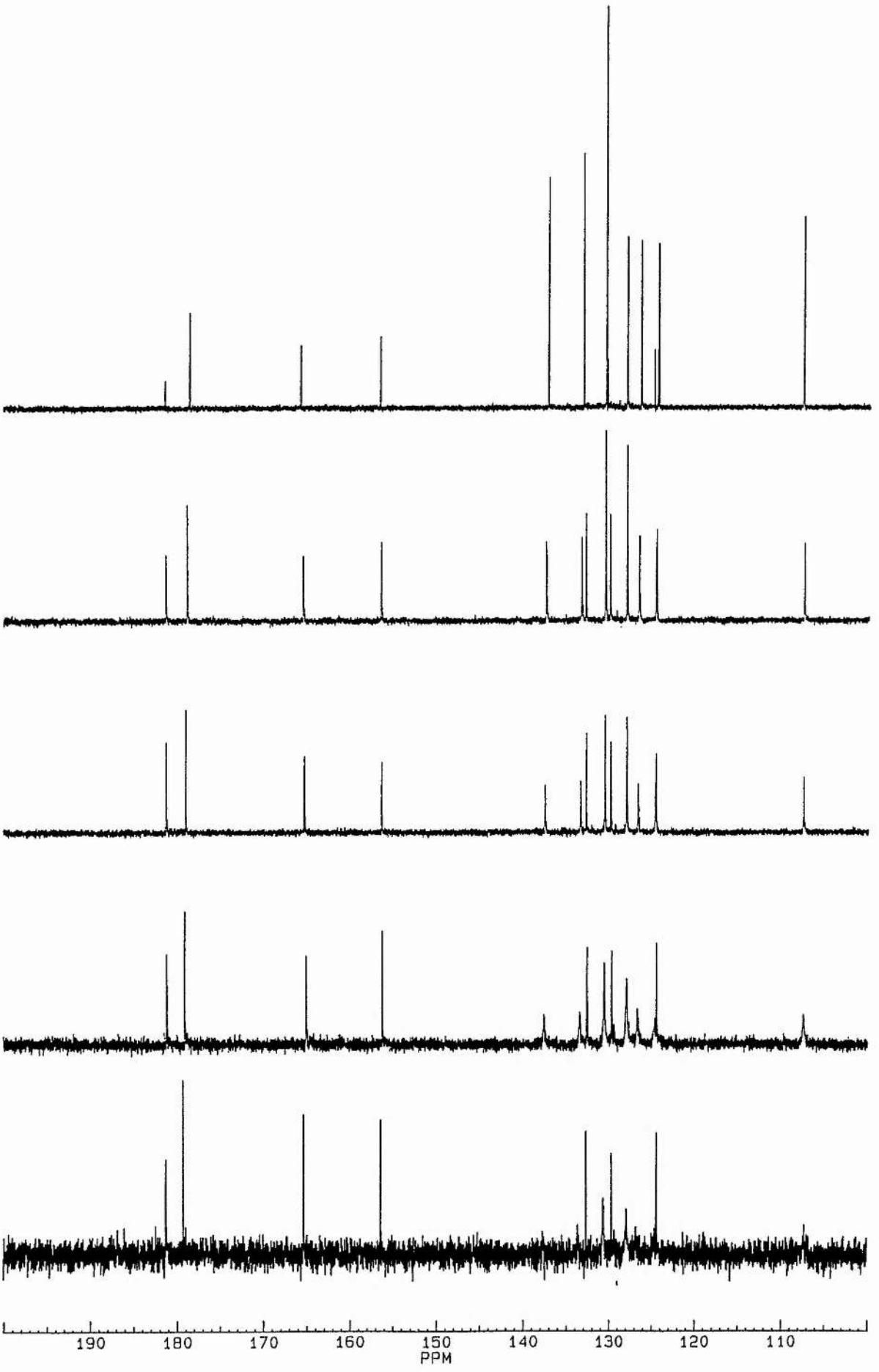



2′-OMe FAA salt

| | Interatomic Distance / Å | | | | Dihedral Angle / ° | |
|---|---|---|---|---|---|---|
| Analogue | 1 | 2 | 3 | 4 | C7-C8-$CH_2$-CO | C3-C2-C1′-C6′ |
| 2′-OMe FAA | 3.84 | 3.40 | 5.03 | **2.27** | -157.2 | -145.3 |
| 2′-OMe FAA salt | 2.89 | **2.06** | 2.86 | **2.20** | -110.0 | -162.7 |

2. Low Temperature NMR Study

A low temperature $^{1}H$ and $^{13}C$ NMR study was carried out on the sodium salt of flavoneacetic acid **135** to ascertain the barrier to rotation of the 2-phenyl ring. The salt **135** was dissolved in $d_4$-methanol and then, starting at 30°C, cooled to –100°C in a series of five steps. Small changes in shift positions occurred as the probe cooled which were more dramatic at low temperatures. Results from this study are inconclusive as the sample froze before the coalescence temperature was reached. A stacked plot of the $^{13}C$ NMR spectra at each temperature follows.


190
180
170
160
150
140
130
120
110
PPM

The top spectrum was recorded at 303 K and shows the region from 100 to 200 ppm. The overall trend of the spectra shows the quaternaries increasing in intensity with the majority of the CH's decreasing. The reason for this is unclear. Another facet of the study is the movement of the peaks. Although the difference between each spectrum is slight, the general trend to lower temperatures shows the hidden quaternaries becoming visible. At the lowest temperature the C-3 almost merges with the background noise as do some other CH's.

Unfortunately although there is some indication of a slowing down of the rotations at 213 K, the coalescence temperature is clearly even lower and so not accessible.

3. Comparison between activity and trends in $^{1}H$ and $^{13}C$ chemical shifts

Analysis of $^{1}H$ and $^{13}C$ chemical shifts of the 2, 3 and 4 positions provides a good method for quickly identifying flavones. When experimental data is tabulated it becomes clear that, within experimental error of 0.05ppm, certain trends emerge. Such tables for all compounds, both allylflavones and flavoneacetic acids, synthesised during the project follow.

Table 1: $^1$H NMR data for 2-aryl-8-allylbenzopyranones

| Cmpd | H-3 | H-5 | H-6 | H-7 | H-2' | H-3' | H-4' | H-5' | H-6' | $CH_2$ | CH range | $=CH_2$ | Substituent Shifts |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 123 | 6.83 | 8.10 | 7.32 | 7.53 | 7.91 | 7.53 | 7.53 | 7.53 | 7.91 | 3.75 | 6.17–6.00 | 5.17 | |
| 124 | 7.16 | 8.10 | 7.33 | 7.52 | OMe | 7.88 | 7.09 | 7.44 | 7.03 | 3.70 | 6.13–6.00 | 5.13 | 3.93 (OMe) |
| 125 | 6.77 | 8.07 | 7.32 | 7.52 | 7.39 | OMe | 7.05 | 7.45 | 7.38 | 3.71 | 6.14–6.00 | 5.14 | 3.85 (OMe) |
| 126 | 6.83 | 8.12 | 7.35 | 7.53 | 7.93 | 7.04 | OMe | 7.04 | 7.93 | 3.76 | 6.16–6.02 | 5.16 | 3.89 (OMe) |
| 127 | 6.74 | 8.06 | 7.30 | 7.50 | 7.74 | 7.29 | Me | 7.29 | 7.74 | 3.70 | 6.12–5.98 | 5.14 | 2.37 (Me) |
| 128 | 6.77 | 8.07 | 7.33 | 7.52 | 7.82 | 7.47 | Cl | 7.47 | 7.82 | 3.72 | 6.13–5.98 | 5.13 | |
| 147 | 7.04 | 8.10 | 7.32 | 7.50 | OMe | OMe | 7.04 | 7.17 | 7.37 | 3.69 | 6.10–6.00 | 5.11 | 3.90 (OMe), 3.85 (OMe) |
| 148 | 7.15 | 8.10 | 7.30 | 7.50 | OMe | 6.53 | OMe | 6.63 | 7.88 | 3.70 | 6.15–6.01 | 5.13 | 3.93 (OMe), 3.88 (OMe) |
| 149 | 7.20 | 8.11 | 7.34 | 7.53 | OMe | ~7.0 | ~7.0 | OMe | 7.46 | 3.72 | 6.16–6.02 | 5.12 | 3.90 (OMe), 3.84 (OMe) |
| 160 | 6.77 | 8.10 | 7.32 | 7.55 | 7.39 | OMe | OMe | 6.99 | 7.55 | 3.75 | 6.19–6.00 | 5.13 | 3.97 (OMe), 3.96 (OMe) |
| 161 | 6.83 | 8.05 | 7.38 | 7.50 | 7.07 | OMe | 6.65 | OMe | 7.07 | 3.71 | 6.20–6.00 | 5.15 | 3.89 (2xOMe) |
| 162 | 6.69 | 8.10 | 7.34 | 7.53 | 7.34 | —$OCH_2O$— | | 6.92 | 7.49 | 3.73 | 6.18–6.00 | 5.17 | 6.10 ($OCH_2O$) |
| 171 | 7.13 | 8.09 | 7.33 | 7.52 | OMe | 7.02 | Cl | 7.10 | 7.82 | 3.68 | 6.12–5.98 | 5.14 | 3.94 (OMe) |
| 172 | 7.12 | 8.08 | 7.33 | 7.53 | OMe | 6.95 | 7.38 | Cl | 7.87 | 3.70 | 6.11–5.98 | 5.20 | 3.93 (OMe) |
| 177 | 7.12 | 8.12 | 7.38 | 7.53 | OMe | OMe | OMe | 6.83 | 7.61 | 3.71 | 6.10–6.00 | 5.17 | 3.97 (3xOMe) |
| 178 | 7.19 | 8.08 | 7.30 | 7.49 | OMe | 6.58 | OMe | OMe | 7.45 | 3.70 | 6.17–6.04 | 5.10 | 3.96 (2xOMe), 3.91 (OMe) |
| 179 | 6.76 | 8.09 | 7.34 | 7.53 | 7.13 | OMe | OMe | OMe | 7.13 | 3.73 | 6.19–6.01 | 5.12 | 3.94 (2xOMe), 3.92 (OMe) |
| 196 | 6.91 | 8.10 | 7.35 | 7.52 | Br | OMe | OMe | OMe | 6.55 | 3.67 | 6.12–5.92 | 5.08 | 4.00 (2xOMe), 3.91 (OMe) |
| 197 | 6.90 | 8.10 | 7.34 | 7.53 | OMe | OMe | OMe | OMe | 6.56 | 3.65 | 6.10–5.97 | 5.07 | 3.95 (3xOMe), 3.88 (OMe) |
| 202 | 6.71 | 8.09 | 7.34 | 7.53 | 8.00 | — | 7.46 | 7.46 | — | 3.73 | 6.15–6.01 | 5.17 | |
| 203 | 6.62 | 8.10 | 7.35 | 7.55 | — | Me | 7.48 | 7.00 | — | 3.70 | 6.18–5.95 | 5.15 | 2.55 (Me) |
| 206 | 7.09 | 8.04 | 7.28 | 7.43 | — | OMe | 6.90 | 7.43 | — | 3.63 | 6.15–5.93 | 5.14 | 3.96 (OMe) |
| 220 | 6.72 | 8.08 | 7.32 | 7.53 | — | 7.09 | 6.61 | 7.62 | — | 3.69 | 6.12–5.98 | 5.18 | |
| 221 | 6.49 | 8.05 | 7.31 | 7.49 | 8.02 | — | 6.72 | 7.51 | — | 3.65 | 6.11–5.92 | 5.16 | |
| 217 | 7.29 | 8.05 | 7.38 | 7.60 | — | Cl | — | — | — | 3.71 | 6.16–6.00 | 5.19 | |
| 227 | 6.92 | 8.09 | 7.37 | 7.57 | 7.76 | 8.83 | — | 8.83 | 7.76 | 3.69 | 6.17–6.04 | 5.18 | |
| 228 | ~7.60 | 8.13 | 7.35 | 7.55 | — | ~8.10 | ~8.10 | — | — | 3.80 | 6.21–6.05 | 5.16 | |
| 234 | 6.13 | 8.03 | 7.30 | 7.47 | 7.31 | 7.31 | 7.31 | 7.31 | 7.31 | 3.54 | 5.97–5.83 | 5.06 | 3.93 ($CH_2$) |
| 235 | 6.17 | 8.05 | ~7.30 | 7.45 | ~7.30 | ~7.30 | ~7.30 | ~7.30 | ~7.30 | 3.42 | 5.85–5.64 | 4.89 | 5.37 (CH) |
| 243 | 6.31 | 8.09 | 7.33 | 7.54 | 7.42 | 7.50 | 7.50 | 7.50 | 7.42 | 3.73 | 6.20–6.00 | 5.19 | 6.79 (CH) |
| 244 | 6.29 | 8.06 | 7.35 | 7.51 | — | 7.38 | 7.07 | 7.31 | — | 3.73 | 6.20–6.00 | 5.21 | 7.67 (CH), 6.57 (CH) |
| 269 | 7.10 | 8.10 | 7.40 | 7.60 | — | — | — | — | — | 3.72 | 6.20–6.00 | 5.17 | |
| 270 | 6.93 | 8.15 | 7.39 | 7.60 | 8.09 | 8.09 | — | 8.09 | 8.09 | 3.77 | 6.18–6.01 | 5.20 | |
| 271 | 6.79 | 8.11 | 7.36 | 7.55 | 7.93 | 7.09 | — | 7.09 | 7.93 | 3.75 | 6.15–6.02 | 5.15 | 3.92 (OMe) |
| 272 | 6.81 | 8.12 | 7.37 | 7.56 | 7.96 | 7.21 | — | 7.21 | 7.96 | 3.76 | 6.13–6.03 | 5.17 | |

Table 2: $^1$H NMR data for 2–aryl–8–carboxymethylbenzopyranones

| Cmpd | H-3 | H-5 | H-6 | H-7 | H-2' | H-3' | H-4' | H-5' | H-6' | $CH_2$ | Substituent Shifts |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **129** | 7.03 | 8.00 | 7.45 | 7.78 | 8.10 | 7.61 | 7.61 | 7.61 | 8.10 | 4.03 | |
| **130** | 6.94 | 7.98 | 7.43 | 7.73 | OMe | 7.23 | 7.57 | 7.14 | 7.89 | 3.95 | 3.94 (OMe) |
| **131** | 7.03 | 7.98 | 7.46 | 7.75 | 7.58 | OMe | 7.15 | 7.46 | 7.64 | 4.01 | 3.88 (OMe) |
| **132** | 6.90 | 7.96 | 7.42 | 7.72 | 8.03 | 7.12 | OMe | 7.12 | 8.03 | 3.99 | 3.86 (OMe) |
| **133** | 6.99 | 7.95 | 7.43 | 7.74 | 7.95 | 7.34 | Me | 7.34 | 7.95 | 4.01 | 2.38 (Me) |
| **134** | 7.04 | 7.96 | 7.45 | 7.75 | 8.09 | 7.63 | Cl | 7.63 | 8.09 | 4.00 | |
| **151** | 6.87 | 8.00 | 7.46 | 7.76 | OMe | OMe | 7.28 | 7.43 | 7.28 | 3.96 | 3.90 (OMe), 3.83 (OMe) |
| **152** | 6.98 | 7.97 | 7.42 | 7.72 | OMe | 6.79 | OMe | 6.76 | 7.91 | 3.98 | 3.96 (OMe), 3.90 (OMe) |
| **153** | 7.01 | 7.96 | 7.43 | 7.74 | OMe | 7.16 | 7.16 | OMe | 7.46 | 3.91 | 3.89 (OMe), 3.82 (OMe) |
| **163** | 7.06 | 8.01 | 7.42 | 7.74 | 7.61 | OMe | OMe | 7.11 | 7.70 | 4.02 | 3.93 (OMe), 3.90 (OMe) |
| **164** | 7.09 | 7.97 | 7.45 | 7.74 | 7.22 | OMe | 6.70 | OMe | 7.22 | 3.99 | 3.85 (2 OMe) |
| **165** | 6.98 | 7.95 | 7.42 | 7.72 | 7.61 | —$OCH_2O$— | | 7.11 | 7.76 | 4.00 | 6.17 ($OCH_2O$) |
| **173** | 6.93 | 7.92 | 7.43 | 7.73 | OMe | 7.33 | Cl | 7.87 | 7.18 | 3.95 | 3.96 (OMe) |
| **174** | 6.98 | 7.96 | 7.44 | 7.74 | OMe | 7.29 | 7.60 | Cl | 7.91 | 3.98 | 3.95 (OMe) |
| **188** | 6.98 | 7.97 | 7.44 | 7.73 | OMe | OMe | OMe | 7.01 | 7.65 | 3.97 | 3.92 (OMe), 3.89 (OMe), 3.83 (OMe) |
| **189** | 7.00 | 7.94 | 7.40 | 7.70 | OMe | 6.84 | OMe | OMe | 7.48 | 3.95 | 3.98 (OMe), 3.91 (OMe), 3.84 (OMe) |
| **190** | 7.12 | 7.95 | 7.42 | 7.73 | 7.35 | OMe | OMe | OMe | 7.35 | 4.01 | 3.92 (2xOMe), 3.79 (OMe) |
| **198** | 6.69 | 8.04 | 7.51 | 7.79 | Br | OMe | OMe | OMe | 7.25 | 3.91 | 3.88 (OMe), 3.87 (OMe), 3.85 (OMe) |
| **199** | 6.63 | 8.01 | 7.50 | 7.79 | OMe | OMe | OMe | OMe | 7.23 | 3.97 | 3.82 (2xOMe), 3.80 (OMe), 3.79 (OMe) |
| **206** | 6.93 | 7.97 | 7.42 | 7.73 | 8.39 | — | 7.74 | 7.74 | — | 4.01 | |
| **207** | 6.57 | 7.95 | 7.43 | 7.75 | — | Me | 7.84 | 7.15 | — | 3.96 | 2.55 (Me) |
| **212** | 7.12 | 7.95 | 7.42 | 7.72 | — | OMe | 7.36 | 7.36 | — | 3.92 | 3.92 (OMe) |
| **224** | 6.62 | 7.93 | 7.43 | 7.73 | — | 7.33 | 6.84 | 8.07 | — | 3.95 | |
| **232** | 7.41 | 8.01 | 7.49 | 7.81 | — | 8.65 | 8.27 | — | — | 4.10 | |
| **237** | 6.26 | 7.90 | 7.27 | 7.67 | ~7.37 | ~7.37 | ~7.37 | ~7.37 | ~7.37 | 3.99 | 3.83 ($CH_2$) |
| **238** | 6.32 | 8.12 | 7.36 | 7.52 | 7.20 | 7.25 | 7.25 | 7.25 | 7.20 | 3.59 | 5.38 (CH) |

The preceding tables show a summary of proton shifts for both 8-allyl and 8-acetic acid substitution and adequately show comparison between the many C-ring derivatives synthesised. As would be expected, substitution on the C-ring by various groups, most notably methoxy, gives distinctive results for the C-ring protons. However other positions are affected by these substitution patterns. Taking into account experimental error, brought about by sample dilution, temperature, machine variability and errors in precise referencing, the results for the different positions are remarkably consistent.

Certain trends can be seen from the analysis of the H-3 shift. Firstly, and most importantly, the shift is increased if a methoxy group is present on the 2'-position. This effect must not only be due to an electronic component but also a through-space interaction between the steric bulk of the methoxy group and the hydrogen on the 3-position. Moreover it must be the electronegative oxygen hydrogen-bonding to this hydrogen to produce such a dramatic shift effect in comparison with the parent allyl derivative.

Increasing the number of methoxy's on the C-ring changes the H-3 shift markedly with 2'-methoxy containing compounds again having higher shifts than the parent allyl compound. The 2',5'-dimethoxy derivative has a higher 3-position shift compared to the 2',4'-dimethoxy which is in turn higher than the 3-position shift for the 2',3'-dimethoxy derivative.

Heterocyclic derivatives all have lower 3-position shifts than the parent allylflavone with the exception of the 3-methoxy-2-thienyl compound which has comparable H-3 shift with the 2'-methoxy derivative. The highest shift for the 3-position came from the 3-chlorobenzo-2-thiophene derivative attributable to interaction of the chlorine atom and the hydrogen. The extended derivatives show a quite marked decrease in 3-position shift due to an interruption of conjugation.

Analysis of the 3-position shifts for the flavone acetic acids shows that the above trend for methoxy substitution is not as marked and is generally reversed. Perhaps the less marked results are possibly due to changing the solvent to dimethyl sulphoxide, a more polar solvent than deuterochloroform. This would reduce the capacity for hydrogen bonding and hence would lessen the effect seen on the 3-position. The majority of shifts for the 3-position centre around 7 parts per million with only the heterocyclic and extended derivatives giving lower shift values. Only four results are notable. Both of the tetrasubstituted derivatives give remarkably low shifts for the 3-position in comparison with the parent flavone acetic acid. Higher than normal H-3 values come from the 3-methoxy-2-thienyl and the 3-chloro-2-benzothienyl. Precisely why these final examples have high shifts is unknown.

The following tables show the $^{13}C$ NMR results for all 8-allyl and 8-acetic acid derivatives successfully synthesised.

Table 3: $^{13}C$ NMR data for 2-aryl-8-allylbenzopyranones

| Cmpd | C-2 | C-3 | C-4 | C-5 | C-6 | C-7 | C-8 | C-9 | C-10 | $CH_2$ | CH | $=CH_2$ | Signals for Ar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 123 | 163.0 | 107.4 | 178.7 | 125.0 | 123.9 | 135.3 | 129.5 | 154.2 | 124.0 | 34.0 | 134.1 | 117.0 | 132.1 (C-1'), 126.3 (C-2', 6'), 129.1 (C-3', 5'), 131.6 (C-4') |
| 124 | 160.6 | 111.8 | 179.2 | 124.6 | 123.7 | 135.5 | 129.4 | 154.4 | 123.7 | 34.0 | 133.8 | 116.8 | 120.9 (C-1'), 158.0 (C-2'), 112.4 (C-3'), 132.4 (C-4'), 120.8 (C-5'),129.2 (C-6') |
| 125 | 162.7 | 107.4 | 178.6 | 124.9 | 123.8 | 135.2 | 129.5 | 154.1 | 123.9 | 33.9 | 134.1 | 117.0 | 133.2 (C-1'), 111.6 (C-2'), 160.0 (C-3'), 117.1 (C-4'), 130.1 (C-5'), 118.5 (C-6') |
| 126 | 162.5 | 105.8 | 178.6 | 124.9 | 123.9 | 135.3 | 129.4 | 154.2 | 124.1 | 34.0 | 134.1 | 117.0 | 123.7 (C-1'), 128.1 (C-2', 6'), 114.6 (C-3', 5'), 163.4 (C-4') |
| 127 | 163.0 | 106.6 | 178.6 | 124.8 | 123.8 | 135.3 | 129.4 | 154.1 | 123.9 | 34.0 | 133.9 | 117.0 | 129.6 (C-1'), 129.8 (C-2', 6'), 126.1 (C-3', 5'), 142.2 (C-4') |
| 128 | 161.8 | 107.4 | 178.5 | 125.1 | 123.9 | 135.1 | 129.4 | 154.1 | 123.8 | 34.0 | 134.3 | 117.1 | 130.3 (C-1'), 129.4 (C-2', 6'), 127.4 (C-3', 5'), 137.8 (C-4') |
| 147 | 160.9 | 112.2 | 179.0 | 124.8 | 123.8 | 135.4 | 129.5 | 154.4 | 123.8 | 33.8 | 133.9 | 117.0 | 126.4 (C-1'), 153.4 (C-2'), 148.1 (C-3'), 114.9 (C-4'), 124.3 (C-5'), 120.6 (C-6') |
| 148 | 160.5 | 111.1 | 179.2 | 124.4 | 123.7 | 135.5 | 129.2 | 154.3 | 123.7 | 34.0 | 133.6 | 116.8 | 113.7 (C-1'), 163.2 (C-2'), 98.9 (C-3'), 159.7 (C-4'), 105.3 (C-5'), 130.3 (C-6') |
| 149 | 160.1 | 112.6 | 179.2 | 124.7 | 123.8 | 135.4 | 129.3 | 154.4 | 123.8 | 34.0 | 134.0 | 116.8 | 121.3 (C-1'), 153.4 (C-2'), 113.0 (C-3'), 117.7 (C-4'), 152.4 (C-5'), 114.1 (C-6') |
| 160 | 163.0 | 106.2 | 178.7 | 124.9 | 124.0 | 135.3 | 129.2 | 154.2 | 124.0 | 34.1 | 134.1 | 116.9 | 124.5 (C-1'), 108.8 (C-2'), 152.1 (C-3'), 149.3 (C-4'), 111.3 (C-5'), 119.9 (C-6') |
| 161 | 162.6 | 107.5 | 178.5 | 124.9 | 123.8 | 135.2 | 129.4 | 154.1 | 123.9 | 34.0 | 134.1 | 117.0 | 133.7 (C-1'), 104.3 (C-2', 6'), 161.1 (C-3', 5'), 103.4 (C-4') |
| 162 | 162.6 | 106.4 | 178.5 | 124.9 | 123.9 | 135.3 | 129.3 | 154.1 | 123.9 | 34.0 | 134.0 | 117.0 | 125.9 (C-1'), 106.2 (C-2'), 150.6 (C-3'), 148.5 (C-4'), 108.8 (C-5'), 121.3 (C-6') |
| 171 | 159.5 | 112.4 | 179.0 | 124.7 | 123.8 | 135.4 | 129.3 | 154.3 | 123.7 | 34.0 | 134.0 | 116.9 | 119.5 (C-1'), 158.5 (C-2'), 112.5 (C-3'), 138.2 (C-4'), 121.1 (C-5'), 130.0 (C-6') |
| 172 | 158.8 | 112.8 | 179.0 | 124.8 | 123.8 | 135.4 | 129.4 | 154.4 | 123.8 | 34.2 | 134.2 | 117.0 | 122.1 (C-1'), 156.6 (C-2'), 113.1 (C-3'), 131.8 (C-4'), 126.0 (C-5'), 128.8 (C-6') |
| 177 | 160.6 | 111.0 | 179.1 | 124.6 | 123.8 | 135.5 | 129.3 | 154.1 | 123.8 | 33.9 | 133.8 | 116.9 | 119.0 (C-1'), 153.2 (C-2'), 142.6 (C-3'), 156.2 (C-4'), 107.4 (C-5'), 124.0 (C-6') |
| 178 | 160.2 | 111.2 | 179.0 | 124.6 | 123.8 | 135.4 | 128.9 | 154.2 | 123.7 | 34.1 | 134.0 | 116.7 | 111.8 (C-1'), 154.0 (C-2'), 97.1 (C-3'), 143.2 (C-4'), 152.4 (C-5'), 111.5 (C-6') |
| 179 | 162.7 | 107.1 | 178.6 | 125.0 | 124.0 | 135.3 | 129.1 | 154.2 | 123.9 | 34.2 | 134.3 | 116.8 | 127.1 (C-1'), 103.5 (C-2',6'), 153.5 (C-3',5'), 141.0 (C-4') |
| 196 | 163.7 | 112.6 | 178.4 | 125.0 | 123.8 | 135.5 | 129.4 | 154.4 | 123.9 | 33.6 | 134.2 | 116.8 | 129.7 (C-1'), 109.1 (C-2'), 152.9 (C-3'), 145.1 (C-4'), 151.6 (C-5'), 109.9 (C-6') |
| 197 | 163.7 | 112.6 | 178.4 | 125.1 | 123.9 | 135.5 | 129.4 | 154.5 | 123.9 | 33.6 | 134.2 | 116.8 | 129.8 (C-1'), 153.0 (C-2'), 145.2 (C-3'), 151.7 (C-4'), 109.1 (C-5'), 109.9 (C-6') |
| 202 | 159.3 | 106.9 | 178.7 | 124.9 | 123.9 | 135.3 | 129.3 | 154.0 | 123.9 | 34.1 | 134.2 | 117.0 | 127.5 (C-2'), 134.4 (C-3'), 125.0 (C-4'), 126.7 (C-5') |
| 203 | 159.3 | 108.1 | 178.1 | 124.9 | 123.7 | 135.2 | 129.3 | 154.0 | 123.7 | 33.7 | 134.0 | 117.0 | 140.1 (C-2'), 129.2 (C-3'), 132.6 (C-4'), 128.5 (C-5') |
| 206 | 159.2 | 106.6 | 178.4 | 124.5 | 123.7 | 135.4 | 129.1 | 153.6 | 123.9 | 33.8 | 133.6 | 116.8 | 111.4 (C-2'), 158.1 (C-3'), 116.3 (C-4'), 128.9 (C-5') |
| 220 | 154.8 | 105.3 | 178.0 | 124.9 | 123.9 | 135.3 | 129.2 | 153.7 | 124.2 | 33.9 | 134.0 | 116.9 | 146.5 (C-2'), 112.8 (C-3'), 112.5 (C-4'), 145.8 (C-5') |
| 221 | 158.2 | 107.0 | 178.2 | 124.8 | 124.0 | 135.3 | 129.1 | 153.9 | 124.0 | 34.0 | 134.0 | 116.9 | 144.7 (C-2'), 120.5 (C-3'), 107.5 (C-4'), 142.8 (C-5') |
| 217 | 156.5 | 110.3 | 177.9 | 125.1 | 123.8 | 135.2 | 129.5 | 153.8 | 123.8 | 33.7 | 134.2 | 117.1 | 137.4 (C-2'), 127.7 (C-3'), 137.3 (C-4'), 129.5 (C-5') |
| 227 | 159.9 | 108.9 | 178.1 | 125.3 | 123.9 | 134.9 | 129.4 | 153.9 | 123.9 | 33.8 | 134.5 | 117.1 | 139.2 (C-1'), 150.7 (C-2', 6'), 119.5 (C-3', 5') |
| 228 | 161.3 | 109.0 | 178.8 | 125.1 | 124.1 | 135.2 | 129.5 | 154.1 | 124.6 | 34.1 | 134.1 | 117.1 | 147.9 (C-2'), 117.5 (C-3'), 137.3 (C-4'), 128.7 (C-5'), 127.6 (C-6'), 130.2 (C-7'), 130.4 (C-8'), 149.3 (C-9'), 128.0 (C-10') |
| 234 | 167.5 | 110.3 | 178.6 | 124.7 | 123.7 | 135.3 | 129.3 | 154.4 | 123.6 | 33.8 | 133.8 | 116.7 | 40.8 ($CH_2$), 134.9 (C-1'), 128.9 (C-2',6'), 129.3 (C-3', 5'), 127.4 (C-4') |
| 235 | 169.2 | 112.1 | 178.6 | 124.8 | 123.7 | 135.1 | 129.6 | 154.4 | 123.8 | 33.9 | 133.9 | 116.6 | 56.1 (CH), 138.9 (C-1'), 128.8 (C-2', 6'), 129.0 (C-3',5'), 127.5 (C-4') |
| 243 | 160.9 | 110.8 | 178.6 | 124.8 | 124.0 | 135.3 | 129.2 | 153.9 | 124.1 | 34.1 | 134.2 | 117.0 | 135.4 (=CH), 121.1 (=CH), 135.7 (C-1'), 129.3 (C-2', 6'), 128.8 (C-3', 5'), 133.5 (C-4') |
| 244 | 161.1 | 110.2 | 178.5 | 124.7 | 123.9 | 135.4 | 129.1 | 153.9 | 124.1 | 34.1 | 134.0 | 116.9 | 128.0 (=CH), 119.5 (=CH), 140.4 (C-2'), 129.9 (C-3'), 128.2 (C-4'), 129.5 (C-5') |
| 269 | 154.8 | 110.2 | 177.8 | 125.6 | 124.1 | 135.1 | 129.7 | 153.8 | 124.2 | 33.8 | 134.7 | 117.5 | |
| 270 | 161.5 | 108.3 | 178.5 | 125.3 | 124.0 | 135.2 | 129.5 | 154.2 | 124.1 | 34.0 | 134.4 | 117.2 | 134.8 (C-1'), 126.8 (C-2',3',4',5') |
| 271 | 162.3 | 107.0 | 178.6 | 125.0 | 124.0 | 135.3 | 129.4 | 154.2 | 124.0 | 34.0 | 134.2 | 117.0 | 127.6 (C-1'), 128.2 (C-2',6'), 119.6 (C-3',5'), 159.1 (C-4') |
| 272 | 162.3 | 107.0 | 178.6 | 125.0 | 124.0 | 135.3 | 129.4 | 154.2 | 124.0 | 34.0 | 134.2 | 117.0 | 127.7 (C-1'), 128.3 (C-2',6'), 119.6 (C-3',5'), 159.2 (C-4'), 129.8 (C-5'), 109.9 (C-6') |

Table 4: $^{13}C$ NMR data for 2-aryl-8-carboxymethylbenzopyranones

| Cmpd | C-2 | C-3 | C-4 | C-5 | C-6 | C-7 | C-8 | C-9 | C-10 | $CH_2$ | $CO_2H$ | Signals for Ar |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **129** | 162.3 | 106.9 | 177.1 | 124.9 | 123.6 | 135.6 | 125.6 | 154.0 | 123.3 | 35.5 | 171.6 | 131.3 (C-1'), 126.3 (C-2', 6'), 129.1 (C-3', 5'), 131.7 (C-4') |
| **130** | 160.2 | 111.4 | 177.1 | 124.7 | 123.5 | 135.5 | 125.4 | 154.3 | 123.1 | 35.2 | 171.6 | 120.0 (C-1'), 157.7 (C-2'), 112.6 (C-3'), 132.8 (C-4'), 120.7 (C-5'), 129.0 (C-6') |
| **131** | 162.1 | 107.1 | 177.2 | 125.0 | 123.7 | 135.7 | 125.5 | 154.1 | 123.3 | 35.6 | 171.7 | 132.6 (C-1'), 111.0 (C-2'), 159.8 (C-3'), 118.0 (C-4'), 130.3 (C-5'), 118.6 (C-6') |
| **132** | 162.3 | 105.4 | 176.9 | 124.7 | 123.6 | 135.4 | 125.4 | 153.9 | 123.4 | 35.5 | 171.7 | 123.3(C-1'), 128.1 (C-2', 6'), 114.6 (C-3', 5'), 162.2 (C-4') |
| **133** | 162.3 | 106.1 | 177.0 | 124.8 | 123.6 | 135.6 | 125.5 | 153.9 | 123.2 | 35.5 | 172.0 | 128.4 (C-1'), 126.2 (C-2',6'), 129.6 (C-3',5'), 142.0 (C-4') |
| **134** | 161.0 | 107.2 | 177.0 | 125.0 | 123.7 | 135.6 | 125.5 | 153.9 | 123.3 | 35.5 | 171.7 | 130.1 (C-1'), 129.1 (C-2', 6'), 128.0 (C-3', 5'), 136.6 (C-4') |
| **151** | 160.6 | 111.4 | 177.2 | 124.9 | 123.6 | 135.7 | 125.5 | 154.4 | 123.2 | 35.2 | 171.7 | 125.5 (C-1'), 153.1 (C-2'), 147.5 (C-3'), 116.1 (C-4'), 124.5 (C-5'), 120.4 (C-6') |
| **152** | 160.2 | 110.0 | 177.2 | 124.6 | 123.6 | 135.4 | 125.4 | 154.2 | 123.1 | 35.5 | 172.0 | 112.4 (C-1'), 163.3 (C-2'), 99.1 (C-3'), 159.6 (C-4'), 106.2 (C-5'), 130.2 (C-6') |
| **153** | 159.6 | 111.5 | 177.2 | 124.7 | 123.8 | 135.6 | 125.5 | 154.2 | 123.1 | 35.5 | 171.6 | 120.2 (C-1'), 153.2 (C-2'), 113.1 (C-3'), 118.9 (C-4'), 152.0 (C-5'), 114.1 (C-6') |
| **163** | 162.3 | 105.6 | 176.9 | 124.7 | 123.6 | 135.4 | 125.4 | 153.9 | 123.3 | 35.6 | 171.9 | 123.2 (C-1'), 111.9 (C-2'), 148.9 (C-3'), 151.8 (C-4'), 108.9 (C-5'), 119.9 (C-6') |
| **164** | 161.8 | 107.2 | 177.1 | 124.9 | 123.6 | 135.7 | 125.5 | 154.0 | 123.3 | 35.6 | 171.6 | 133.2 (C-1'), 104.1 (C-2', 6'), 160.9 (C-3', 5'), 104.2 (C-4') |
| **165** | 162.0 | 105.8 | 176.9 | 124.8 | 123.5 | 135.5 | 125.4 | 154.0 | 123.1 | 35.5 | 171.9 | 102.0 ($CH_2$),125.0 (C-1'), 106.1 (C-2'), 150.3 (C-3'), 149.1 (C-4'), 109.6 (C-5'), 121.5 (C-6') |
| **173** | 159.1 | 111.4 | 177.0 | 124.8 | 123.5 | 135.6 | 125.4 | 154.1 | 122.9 | 35.2 | 171.8 | 118.8 (C-1'), 158.3 (C-2'), 113.0 (C-3'), 137.3 (C-4'), 130.2 (C-5'), 120.7 (C-6') |
| **174** | 158.6 | 111.9 | 177.1 | 124.9 | 123.5 | 135.6 | 125.5 | 154.2 | 123.0 | 35.3 | 171.6 | 121.4 (C-1'), 156.5 (C-2'), 114.5 (C-3'), 132.2 (C-4'), 124.9 (C-5'), 128.3 (C-6') |
| **188** | 160.1 | 110.0 | 177.1 | 124.8 | 123.6 | 135.6 | 125.4 | 154.2 | 123.1 | 35.3 | 172.0 | 117.7 (C-1'), 156.2 (C-2'), 142.2 (C-3'), 152.4 (C-4'), 109.2 (C-5'), 123.9 (C-6') |
| **189** | 159.8 | 110.0 | 177.1 | 124.5 | 123.6 | 135.3 | 125.2 | 154.1 | 123.5 | 35.5 | 171.6 | 110.4 (C-1'), 153.7 (C-2'), 98.4 (C-3'), 143.0 (C-4'), 152.7 (C-5'), 111.3 (C-6') |
| **190** | 161.8 | 106.5 | 177.1 | 124.9 | 123.6 | 135.6 | 125.5 | 154.0 | 123.2 | 35.8 | 171.8 | 104.3 (C-1'), 103.9 (C-2',6'), 153.3 (C-3'5'), 140.7 (C-4') |
| **198** | 163.1 | 112.4 | 177.1 | 125.8 | 125.5 | 136.3 | 124.0 | 154.6 | 123.4 | 35.2 | 172.0 | 128.7 (C-1'), 108.1 (C-2'), 153.1 (C-3'), 144.7 (C-4'), 151.1 (C-5'), 111.0 (C-6') |
| **199** | 163.0 | 112.4 | 177.1 | 125.2 | 123.7 | 136.1 | 125.5 | 154.4 | 123.2 | 35.0 | 171.9 | 111.1 (C-1'), 170.5 (C-2'), 150.9 (C-3'), 165.5 (C-4'), 152.9 (C-5'), 107.5 (C-6') |
| **206** | 158.7 | 106.4 | 177.1 | 124.8 | 123.6 | 135.5 | 125.4 | 153.8 | 123.3 | 35.6 | 171.9 | 125.3 (C-2'), 133.8 (C-3'), 127.8 (C-4'), 128.2 (C-5') |
| **207** | 158.8 | 107.5 | 176.5 | 125.0 | 123.8 | 135.9 | 125.2 | 154.1 | 123.1 | 35.1 | 171.7 | 140.8 (C-2'), 128.2 (C-3'), 133.0 (C-4'), 130.0 (C-5') |
| **212** | 161.8 | 106.5 | 177.1 | 124.8 | 123.6 | 135.6 | 125.4 | 154.0 | 123.2 | 35.8 | 171.8 | 140.7 (C-2'), 153.2 (C-3'), 103.9 (C-4'), 126.3 (C-5') |
| **224** | 153.8 | 104.4 | 176.6 | 125.3 | 123.9 | 136.0 | 125.3 | 154.4 | 123.7 | 35.6 | 172.3 | 145.6 (C-2'), 114.2 (C-3'), 113.3 (C-4'), 147.7 (C-5') |
| **232** | 160.8 | 107.9 | 177.0 | 125.2 | 123.7 | 135.8 | 125.7 | 154.0 | 123.7 | 35.6 | 171.4 | 147.1 (C-2'), 117.6 (C-3'), 137.9 (C-4'), 128.2 (C-5'), 128.0 (C-6'), 129.4 (C-7'), 130.7 (C-8'), 148.6 (C-9'), 128.5 (C-10') |
| **237** | 167.8 | 109.5 | 176.9 | 124.7 | 123.6 | 135.4 | 125.1 | 154.2 | 123.0 | 34.6 | 171.5 | 39.5 ($CH_2$), 135.6 (C-1'), 128.6 (C-2',6'), 129.1 (C-3',5'), 127.0 (C-4') |
| **238** | 169.4 | 112.0 | 178.7 | 125.0 | 123.6 | 135.5 | 125.3 | 154.7 | 123.6 | 34.9 | 174.8 | 56.1 (CH), 138.8 (C-1'), 128.8 (C-2',6'), 129.0 (C-3',5'), 127.5 (C-4') |

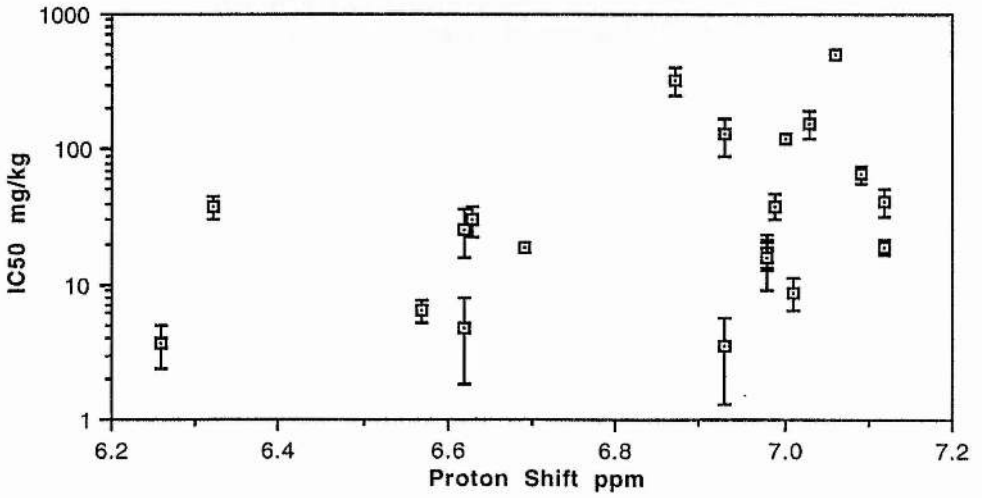
At first glance the entries in both tables seem similar for each derivative. On closer inspection certain facts are prominent. Positions 2 and 3 are the only positions to show significant variability on the A- and B-rings. The rest of the positions show remarkable similarity with each other varying on average by only 0.2 parts per million. This means that it is possible to identify the flavone produced by examination of the C-2 and C-3 positions.

Similar effects are seen in the C-2 and C-3 position shifts as with those seen in the proton NMR. However both acids and allyl compounds show the same trends. Substitution of a 2-methoxy group on the phenyl ring gives a characteristic increase in the C-3 to over 110 parts per million. Coupled with this effect is the lowering of the C-2 shift compared to the parent compound.

Other groups have also looked into the NMR of substituted flavones.[156,157] Their findings coincide with ours in that 2'-methoxy or 2'-halogen substitution on the C-ring leads to anomalous shifts for the 3-position.

Correlation was sought between activity and $^1H$ NMR shift for H-3 of substituted FAA's (table 2). A graph of this correlation follows.

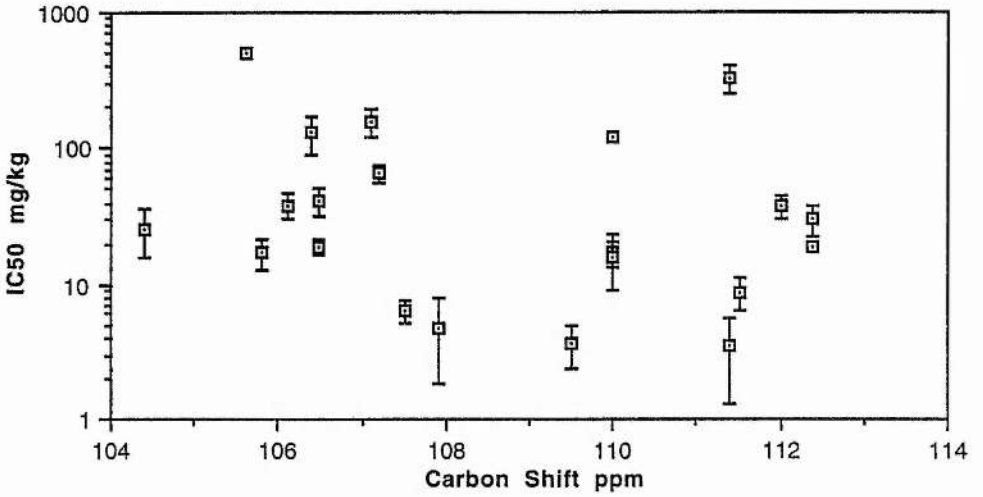
Scatter Graph of Proton Shift vs $IC_{50}$



It can be seen that the most active compounds occur at a lower chemical shift. The two lowest shifts are attributable to the benzylic derivatives and therefore should really be treated separately. However there is no definite relationship that can be seen.

Correlation was sought between activity and $^{13}C$ NMR shift for C-3. A graph of this correlation follows.

Scatter Graph of Carbon Shift vs $IC_{50}$

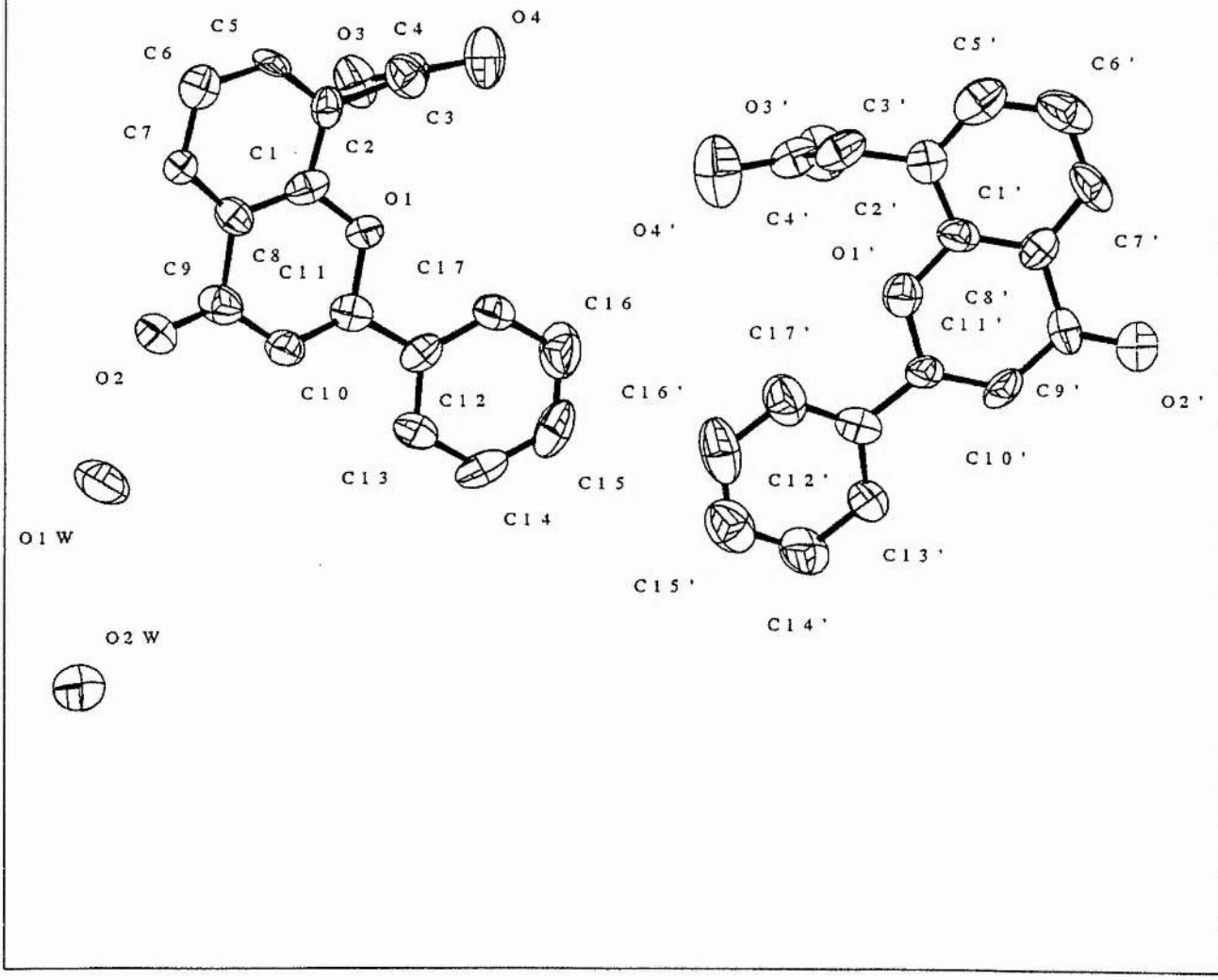



The scatter graph was used to hopefully find areas where active compounds clustered. However there seems to be no obvious correlation between proton or carbon shift as active compounds occur over the range of shifts. There is a slight tendency for the more active compounds to have higher carbon chemical shifts for C-3.

4. X-ray Structure Determination

Crystals of the parent acid were grown from glacial acetic acid and water (2:1) and sent for structure determination at the SERC X-ray Crystallography Unit, Cardiff. This is the first reported structure determination for any flavone-8-acetic acid.

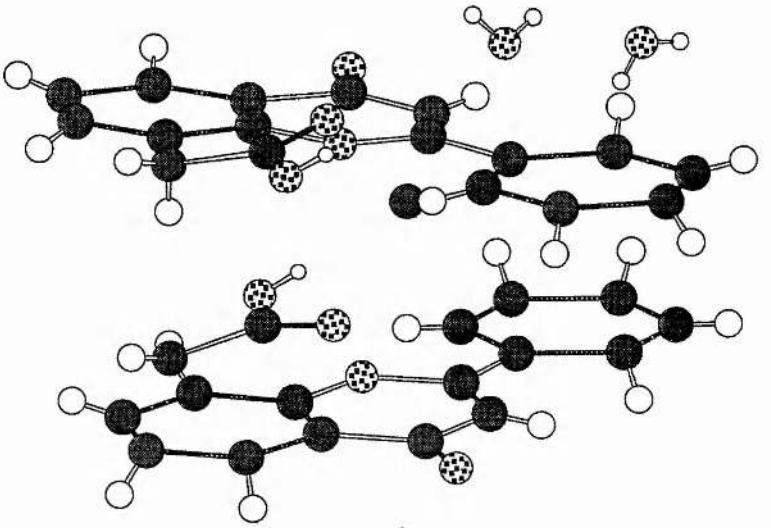
The crystal system was monoclinic and the space group was determined as P2(1). Cell volume was determined as 144.0 Å with a calculated density of 1.321 $Mgm^{-3}$. Unit cell dimensions of a=5.214 Å alpha=90 deg. b=4.242 Å beta=93.84 deg. and c=19.490 Å gamma=90 deg. The picture below is a representation of the system as sent from Cardiff. Selected bond lengths and bond angles for only one of the two independent flavone molecules are also shown in the following tables.



| Atoms | Length Å | Atoms | Length Å | Atoms | Length Å |
|---|---|---|---|---|---|
| O(1) – C(1) | 1.36(2) | C(2) – C(3) | 1.49(2) | C(11) – C(12) | 1.47(2) |
| O(1) – C(11) | 1.37(2) | C(3) – C(4) | 1.49(2) | C(12) – C(13) | 1.39 |
| O(2) – C(9) | 1.26(2) | C(5) – C(6) | 1.38(2) | C(12) – C(17) | 1.39 |
| O(3) – C(4) | 1.21(2) | C(6) – C(7) | 1.40(2) | C(13) – C(14) | 1.39 |
| O(4) – C(4) | 1.30(2) | C(7) – C(8) | 1.39(2) | C(14) – C(15) | 1.39 |
| C(1) – C(8) | 1.39(2) | C(8) – C(9) | 1.50(3) | C(15) – C(16) | 1.39 |
| C(1) – C(2) | 1.41(2) | C(9) – C(10) | 1.42(3) | C(16) – C(17) | 1.39 |
| C(2) – C(5) | 1.34(2) | C(10) – C(11) | 1.32(2) | | |

| Atoms | Angle ° | Atoms | Angle ° |
|---|---|---|---|
| C(1) – O(1) – C(11) | 118.4(13) | C(1) – C(8) – C(9) | 116(2) |
| O(1) – C(1) – C(8) | 124(2) | O(2) – C(9) – C(10) | 125(2) |
| O(1) – C(1) – C(2) | 114(2) | O(2) – C(9) – C(8) | 118(2) |
| C(8) – C(1) – C(2) | 122(2) | C(10) – C(9) – C(80 | 117(2) |
| C(5) – C(2) – C(1) | 114.8(14) | C(11) – C(10) – C(9) | 121(2) |
| C(5) – C(2) – C(3) | 123(2) | C(10) – C(11) – O(1) | 123.3(14) |
| C(1) – C(2) – C(3) | 122(2) | C(10) – C(11) – C(12) | 125(2) |
| C(2) – C(3) – C(4) | 114.7(13) | O(1) – C(11) – C(12) | 111.3(12) |
| O(3) – C(4) – O(4) | 120(2) | C(13) – C(12) – C(17) | 120.0 |
| O(3) – C(4) – C(3) | 123(2) | C(13) – C(12) – C(11) | 119.6(9) |
| O(4) – C(4) – C(3) | 116.6(13) | C(17) – C(12) – C(11) | 120.4(9) |
| C(2) – C(5) – C(6) | 127(2) | C(12) – C(13) – C(14) | 120.0 |
| C(5) – C(6) – C(7) | 117(2) | C(15) – C(14) – C(13) | 120.0 |
| C(8) – C(7) – C(6) | 120(2) | C(14) – C(15) – C(16) | 120.0 |
| C(7) – C(8) – C(1) | 120(2) | C(15) – C(16) – C(17) | 120.0 |
| C(7) – C(8) – C(9) | 124.8(14) | C(16) – C(17) – C(12) | 120.0 |

The results were interesting as not only were there two molecules of FAA in the unit cell but also two accompanying molecules of water were present. The full numerical data can be seen in Appendix 1 and the graphical representations can be seen below.



Several important observations can be made from the crystal structure. Firstly the two molecules of FAA are almost flat. This is, of course, expected for the pyranone fragment but the C-ring is, from previous calculations, shown to be slightly deviant from the benzopyranone plane. The molecules are stacked through the C-rings, implying π-stacking. However the rest of the molecule is too bulky to continue this stacking and hence it is formed into a helical structure. The C-4 carbonyls of the separate molecules of FAA point in approximately opposite directions, accounting for the spiral arrangement. The most interesting point about the structure is the manner in which the acetic acid groups are arranged. Not only do they lie, as calculated, with the carboxylic acids pointing in towards the body of each molecule but they also adopt the same angle of deviation from the plane. Also very interesting is that the carbonyl oxygen of the carboxylic acid points towards the ether oxygen of the pyranone. This, along with the corresponding distances, shows the

possibility of interaction. The two waters of recrystallisation do not seem to interact with the FAA molecules in the unit cell, however, at least one of them is close enough to hydrogen bond to the carbonyl oxygen of the pyranone. These water molecules hydrogen bond to FAA molecules in adjacent unit cells.

Accurate bond lengths and angles can now be used in the calculations where before the only accurate model was pyranone. Although inferences cannot be drawn between solid state structure and computational conformation, it is interesting to note that the calculations carried out so far have coincided directly with the X-ray structure.

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# Appendix 1

Table 5. Crystal data and structure refinement

| | |
|---|---|
| Empirical formula | $C_{17} H_{14} O_5$ |
| Formula weight | 287.26 |
| Temperature | 293(2) K |
| Wavelength | 0.71069 Å |
| Crystal system | Monoclinic |
| Space group | P2(1) |
| Unit cell dimensions | a=5.214 Å alpha=90 deg. |
| | b=4.242 Å beta=93.84 deg. |
| | c=19.490 Å gamma=90 deg. |
| Volume | 144.0 $Å^3$ |
| Z | 4 |
| Density (calculated) | 1.321 $Mgm^{-3}$ |
| Absorption coefficient | 0.097 $mm^{-1}$ |
| F (000) | 596 |
| θ range for data collection | 1.77 to 24.99 deg. |
| Index ranges | $-5 \leq h \leq 4$, $-8 \leq k \leq 15$, $-22 \leq l \leq 21$ |
| Reflections collected | 5016 |
| Data / restraints / parameters | 2042 / 1 / 374 |
| Goodness-of-fit on F2 | 1.091 |
| Final R indices [I>2sigma(I)] | $R^1 = 0.1530$, $wR^2 = 0.3760$ |
| R indices (all data) | $R^1 = 0.1800$, $wR^2 = 0.4290$ |
| Absolute structure parameter | -2(6) |
| Largest diff. peak and hole | 0.823 and -0.354 $e.A^{-3}$ |

Table 6. Atomic coordinates (x $10^4$) and equivalent isotropic displacement parameters ($A^2$ x $10^3$) for flavone-8-acetic acid.

U(eq) is defined as one third of the trace of the orthogonalised Uij tensor.

| | X | Y | Z | U(eq) |
|---|---|---|---|---|
| O(1) | 1783(21) | 8082(8) | 5400(5) | 43(3) |
| O(2) | 1796(24) | 7227(10) | 3407(6) | 64(4) |
| O(3) | 2090(24) | 10349(10) | 5743(6) | 62(4) |
| O(4) | 1141(25) | 10106(11) | 6800(6) | 74(4) |
| C(1) | 60(33) | 8441(12) | 4917(9) | 44(4) |
| C(2) | -1653(31) | 9100(12) | 5180(7) | 38(4) |
| C(3) | -1521(32) | 9372(14) | 5918(8) | 51(5) |
| C(4) | 720(30) | 9974(13) | 6141(8) | 41(4) |
| C(5) | -3464(32) | 9425(13) | 4718(8) | 50(5) |
| C(6) | -3747(34) | 9210(13) | 4029(9) | 51(5) |
| C(7) | -1999(32) | 8569(13) | 3782(8) | 43(4) |
| C(8) | -86(32) | 8187(12) | 4227(8) | 43(5) |
| C(9) | 1838(34) | 7469(14) | 4028(10) | 52(5) |
| C(10) | 3485(30) | 7089(13) | 4566(8) | 43(4) |
| C(11) | 3482(29) | 7414(11) | 5200(8) | 39(4) |
| C(12) | 5111(20) | 7062(8) | 5792(5) | 40(4) |
| C(13) | 7192(22) | 6485(8) | 5680(5) | 44(4) |
| C(14) | 8756(21) | 6158(9) | 6235(7) | 64(6) |
| C(15) | 8239(26) | 6409(11) | 6901(6) | 77(7) |
| C(16) | 6157(28) | 6986(12) | 7013(4) | 83(7) |
| C(17) | 4594(22) | 7313(9) | 6458(6) | 56(5) |
| O(1') | 1485(22) | 5662(9) | 9702(5) | 50(3) |
| O(2') | 3207(29) | 4762(11) | 11651(7) | 79(5) |

| | | | | |
|---|---|---|---|---|
| O(3') | 1486(26) | 7906(12) | 9378(7) | 73(4) |
| O(4') | -140(34) | 7504(13) | 8346(8) | 97(6) |
| C(1') | 160(34) | 6024(13) | 10238(10) | 50(5) |
| C(2') | -1806(30) | 6659(12) | 10018(9) | 41(4) |
| C(3') | -2226(33) | 6943(14) | 9291(10) | 58(5) |
| C(4') | -93(33) | 7507(13) | 9031(10) | 50(5) |
| C(5') | -3245(39) | 7033(14) | 10518(11) | 65(6) |
| C(6') | -2839(42) | 6755(16) | 11221(12) | 72(6) |
| C(7') | -883(41) | 6100(16) | 11391(9) | 66(6) |
| C(8') | 608(31) | 5728(12) | 10909(8) | 44(4) |
| C(9') | 2633(33) | 5017(14) | 11060(9) | 50(5) |
| C(10') | 3930(36) | 4682(12) | 10484(8) | 51(5) |
| C(11') | 3423(30) | 5002(11) | 9830(8) | 38(4) |
| C(12') | 4539(21) | 4689(8) | 9211(5) | 42(4) |
| C(13') | 6678(20) | 4104(9) | 9241(5) | 45(4) |
| C(14') | 7706(22) | 3806(9) | 8638(7) | 72(6) |
| C(15') | 6595(30) | 4093(11) | 8005(5) | 83(7) |
| C(16') | 4456(29) | 4678(11) | 7974(4) | 84(8) |
| C(17') | 3428(22) | 4976(9) | 8577(6) | 74(6) |
| O(1W) | 4816(29) | 6004(13) | 2772(7) | 89(5) |
| O(2W) | 7126(34) | 3670(12) | 2186(8) | 93(5) |

Table 7. Bond lengths [Å] for flavone-8-acetic acid

| | | | |
|---|---|---|---|
| O(1)–C(1) | 1.36(2) | O(1')–C(1') | 1.39(2) |
| O(1)–C(11) | 1.37(2) | O(1')–C(11') | 1.39(2) |
| O(2)–C(9) | 1.26(2) | O(2')–C(9') | 1.23(2) |
| O(3)–C(4) | 1.21(2) | O(3')–C(4') | 1.18(2) |
| O(4)–C(4) | 1.30(2) | O(4')–C(4') | 1.33(2) |
| C(1)–C(8) | 1.39(2) | C(1')–C(8') | 1.38(2) |
| C(1)–C(2) | 1.41(2) | C(1')–C(2') | 1.41(2) |
| C(2)–C(5) | 1.34(2) | C(2')–C(5') | 1.38(2) |
| C(2)–C(3) | 1.49(2) | C(2')–C(3') | 1.48(2) |
| C(3)–C(4) | 1.49(2) | C(3')–C(4') | 1.49(2) |
| C(5)–C(6) | 1.38(2) | C(5')–C(6') | 1.43(2) |
| C(6)–C(7) | 1.40(2) | C(6')–C(7') | 1.41(2) |
| C(7)–C(8) | 1.39(2) | C(7')–C(8') | 1.37(2) |
| C(8)–C(9) | 1.50(3) | C(8')–C(9') | 1.48(3) |
| C(9)–C(10) | 1.42(3) | C(9')–C(10') | 1.43(3) |
| C(10)–C(11) | 1.32(2) | C(10')–C(11') | 1.36(2) |
| C(11)–C(12) | 1.47(2) | C(11')–C(12') | 1.45(2) |
| C(12)–C(13) | 1.39 | C(12')–C(13') | 1.39 |
| C(12)–C(17) | 1.39 | C(12')–C(17') | 1.39 |
| C(13)–C(14) | 1.39 | C(13')–C(14') | 1.39 |
| C(14)–C(15) | 1.39 | C(14')–C(15') | 1.39 |
| C(15)–C(16) | 1.39 | C(15')–C(16') | 1.39 |
| C(16)–C(17) | 1.39 | C(16')–C(17') | 1.39 |

Table 8. Bond angles [deg] for flavone-8-acetic acid

| | | | |
|---|---|---|---|
| C(1)-O(1)-C(11) | 118.4(13) | C(1')-O(1')-C(11') | 120.6(13) |
| O(1)-C(1)-C(8) | 124(2) | O(1')-C(1')-C(8') | 123(2) |
| O(1)-C(1)-C(2) | 114(2) | O(1')-C(1')-C(2') | 113(2) |
| C(8)-C(1)-C(2) | 122(2) | C(8')-C(1')-C(2') | 124(2) |
| C(5)-C(2)-C(1) | 114.8(14) | C(5')-C(2')-C(1') | 117(2) |
| C(5)-C(2)-C(3) | 123(2) | C(5')-C(2')-C(3') | 121(2) |
| C(1)-C(2)-C(3) | 122(2) | C(1')-C(2')-C(3') | 122(2) |
| C(2)-C(3)-C(4) | 114.7(13) | C(2')-C(3')-C(4') | 114.1(14) |
| O(3)-C(4)-O(4) | 120(2) | O(3')-C(4')-O(4') | 123(2) |
| O(3)-C(4)-C(3) | 123(2) | O(3')-C(4')-C(3') | 125(2) |
| O(4)-C(4)-C(3) | 116.6(13) | O(4')-C(4')-C(3') | 112(2) |
| C(2)-C(5)-C(6) | 127(2) | C(2')-C(5')-C(6') | 121(2) |
| C(5)-C(6)-C(7) | 117(2) | C(5')-C(6')-C(7') | 118(2) |
| C(8)-C(7)-C(6) | 120(2) | C(8')-C(7')-C(6') | 122(2) |
| C(7)-C(8)-C(1) | 120(2) | C(7')-C(8')-C(1') | 118(2) |
| C(7)-C(8)-C(9) | 124.8(14) | C(7')-C(8')-C(9') | 124(2) |
| C(1)-C(8)-C(9) | 116(2) | C(1')-C(8')-C(9') | 118(2) |
| O(2)-C(9)-C(10) | 125(2) | O(2')-C(9')-C(10') | 123(2) |
| O(2)-C(9)-C(8) | 118(2) | O(2')-C(9')-C(8') | 121(2) |
| C(10)-C(9)-C(8) | 117(2) | C(10')-C(9')-C(8') | 116(2) |
| C(11)-C(10)-C(9) | 121(2) | C(11')-C(10')-C(9') | 123(2) |
| C(10)-C(11)-O(1) | 123.3(14) | C(10')-C(11')-O(1') | 119.1(14) |
| C(10)-C(11)-C(12) | 125(2) | C(10')-C(11')-C(12') | 128(2) |
| O(1)-C(11)-C(12) | 111.3(12) | O(1')-C(11')-C(12') | 112.8(13) |
| C(13)-C(12)-C(17) | 120.0 | C(13')-C(12')-C(17') | 120.0 |
| C(13)-C(12)-C(11) | 119.6(9) | C(13')-C(12')-C(11') | 121.1(10) |
| C(17)-C(12)-C(11) | 120.4(9) | C(17')-C(12')-C(11') | 118.9(10) |

| | | | |
|---|---|---|---|
| C(12)-C(13)-C(14) | 120.0 | C(12')-C(13')-C(14') | 120.0 |
| C(15)-C(14)-C(13) | 120.0 | C(15')-C(14')-C(13') | 120.0 |
| C(14)-C(15)-C(16) | 120.0 | C(14')-C(15')-C(16') | 120.0 |
| C(15)-C(16)-C(17) | 120.0 | C(15')-C(16')-C(17') | 120.0 |
| C(16)-C(17)-C(12) | 120.0 | C(16')-C(17')-C(12') | 120.0 |

Table 9. Anisotropic displacement parameters ($A^2 \times 10^3$) for flavone-8-acetic acid.

The anisotropic displacement factor exponent takes the form:–

$-2\pi^2 [h^2 a^{*2} U11 + ... + 2 h k a^* b^* U12]$

| | U11 | U22 | U33 | U23 | U13 | U12 |
|---|---|---|---|---|---|---|
| O(1) | 47(7) | 35(7) | 47(7) | 6(5) | 5(6) | 12(6) |
| O(2) | 81(9) | 65(9) | 44(8) | -10(7) | 4(6) | 22(8) |
| O(3) | 66(9) | 72(10) | 48(7) | -9(7) | 6(7) | -16(8) |
| O(4) | 75(9) | 94(11) | 54(9) | 19(8) | 2(6) | -34(8) |
| C(1) | 36(9) | 31(10) | 65(12) | 9(9) | -1(9) | -8(8) |
| C(2) | 48(10) | 37(10) | 30(9) | 10(8) | 0(8) | -9(9) |
| C(3) | 44(10) | 59(12) | 50(10) | 2(9) | 9(8) | -12(10) |
| C(4) | 35(9) | 55(11) | 36(10) | 12(9) | 15(8) | 11(9) |
| C(5) | 55(11) | 51(12) | 47(11) | 4(10) | 37(9) | 24(10) |
| C(6) | 49(11) | 51(12) | 53(12) | 2(9) | -4(8) | -11(10) |
| C(7) | 49(11) | 45(11) | 34(9) | 1(8) | 0(8) | 16(9) |
| C(8) | 48(11) | 45(11) | 35(10) | -12(9) | -3(8) | -3(9) |
| C(9) | 51(11) | 52(12) | 53(12) | -20(10) | -8(9) | 3(9) |
| C(10) | 45(10) | 42(11) | 44(11) | -2(9) | 9(8) | 5(8) |
| C(11) | 34(9) | 33(10) | 51(11) | -4(9) | 1(8) | -11(8) |
| C(12) | 40(9) | 24(9) | 54(11) | 5(8) | -14(8) | -8(8) |
| C(13) | 42(10) | 36(10) | 54(11) | -12(9) | 4(8) | -1(9) |
| C(14) | 51(11) | 53(13) | 85(16) | 15(11) | -17(11) | 15(10) |
| C(15) | 76(15) | 89(18) | 59(14) | 31(13) | -38(13) | -16(14) |
| C(16) | 70(15) | 124(22) | 52(13) | 13(13) | -16(11) | 0(15) |
| C(17) | 49(11) | 72(14) | 49(11) | -2(10) | 7(8) | 28(11) |
| O(1') | 54(7) | 46(7) | 49(7) | 6(6) | 1(5) | -5(6) |

| | | | | | | |
|---|---|---|---|---|---|---|
| O(2') | 102(10) | 89(11) | 47(9) | 12(7) | 5(7) | 21(9) |
| O(3') | 52(9) | 90(11) | 76(9) | -7(9) | -7(7) | -14(9) |
| O(4') | 117(12) | 104(13) | 69(10) | -3(9) | -6(8) | -61(11) |
| C(1') | 51(11) | 34(10) | 66(13) | 2(10) | 23(9) | 9(9) |
| C(2') | 36(10) | 35(10) | 51(11) | 0(9) | 1(8) | -18(9) |
| C(3') | 46(11) | 43(12) | 83(15) | 4(11) | -13(10) | 6(9) |
| C(4') | 31(10) | 47(11) | 68(14) | 0(10) | -23(9) | -1(9) |
| C(5') | 65(13) | 46(12) | 80(15) | 9(12) | -19(11) | -6(11) |
| C(6') | 73(15) | 54(14) | 95(18) | -16(12) | 41(13) | -11(12) |
| C(7') | 83(14) | 70(15) | 49(11) | -9(11) | 47(11) | -15(14) |
| C(8') | 46(10) | 40(10) | 45(11) | 11(9) | -2(8) | -6(9) |
| C(9') | 51(11) | 63(12) | 36(10) | 1(10) | 12(8) | -4(10) |
| C(10') | 71(12) | 28(10) | 53(12) | 12(9) | -13(9) | 1(9) |
| C(11') | 42(10) | 21(8) | 51(10) | 5(8) | 10(8) | -4(8) |
| C(12') | 28(9) | 40(10) | 60(12) | -9(9) | 9(8) | -6(8) |
| C(13') | 35(9) | 55(12) | 46(10) | 0(9) | 11(8) | -9(9) |
| C(14') | 56(13) | 80(16) | 82(15) | 10(13) | 36(11) | -3(11) |
| C(15') | 96(18) | 80(18) | 75(17) | -5(13) | 28(14) | -25(15) |
| C(16') | 108(18) | 103(20) | 42(12) | -14(12) | 0(12) | -47(16) |
| C(17') | 95(16) | 75(15) | 50(12) | -10(11) | -3(12) | -8(14) |
| O(1W) | 80(9) | 117(14) | 68(9) | -31(9) | 0(7) | 31(10) |
| O(2W) | 123(13) | 80(10) | 76(10) | 3(9) | 5(9) | 33(10) |