Synthesis and Biological Evaluation of Potential Anti-cancer Agents

Julie Simpson

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ASTON UNIVERSITY

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Abstract: The new technology of combinatorial chemistry has been introduced to pharmaceutical companies, improving and making more efficient the process of drug discovery. Automated combinatorial chemistry in the solution-phase has been used to prepare a large number of compounds for anti-cancer screening. A library of caffeic acid derivatives has been prepared by the Knoevenagel condensation of aldehyde and active methylene reagents. These products have been screened against two murine adenocarcinoma cell lines (MAC) which are generally refractive to standard cytotoxic agents. The target for anti-proliferative action was the 12- and 15-lipoxygenase enzymes upon which these tumour cell lines have been shown to be dependant for proliferation and metastasis. Compounds were compared to a standard lipoxygenase inhibitor and if found to be active anti-proliferative agents were tested for their general cytotoxicity and lipoxygenase inhibition.

A solid-phase bound catalyst, piperazinomethyl polystyrene, was devised and prepared for the improved generation of Knoevenagel condensation products. This piperazinomethyl polystyrene was compared to the traditional liquid catalyst, piperidine, and was found to reduce the amount of by-products formed during reaction and had the advantage of easy removal from the reaction. ^{13}C NMR has been used to determine the *E/Z* stereochemistry of Knoevenagel condensation products.

Soluble polymers have been prepared containing different building blocks pendant to the polymer backbone. Aldehyde building blocks incorporated into the polymer structure have been subjected to the Knoevenagel condensation. Cleavage of the resultant pendant molecules have proved that soluble linear polymers have the potential to generate combinatorial mixtures of known composition for biological testing.

Novel catechol derivatives have been prepared by traditional solution-phase chemistry with the intention of transferring their synthesis to a solid-phase support. Catechol derivatives prepared were found to be active inhibitors of lipoxygenase. Soluble linear supports for the preparation of these active compounds were designed and tested. The aim was to develop a support suitable for the automated synthesis of libraries of catechol derivatives for biological screening.

Keywords: **Combinatorial, Lipoxygenase, Knoevenagel, Soluble, Polymer**

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Abbreviations

AA	Arachidonic acid
AIBN	Azo*bis*isobutyrylnitrile
BBr_3	Boron tribromide
BHPP	*N*-Benzyl-*N*-hydroxy-5-phenylpentamide
BSA	Bovine serum albumin
CCl_4	Tetrachloromethane
$CDCl_3$	Deuterated chloroform
$(CD_3)_2SO$	Deuterated dimethylsulphoxide
$CHCl_3$	Chloroform
CO	Cyclo-oxygenase
DCE	Dichloroethane
DCM	Dichloromethane
DHA	Dehydroarachidonic acid
DMAP	Dimethylaminopyridine
DME	Dimethoxyethane
DMF	Dimethylformamide
DMSO	Dimethylsulphoxide
DNA	Deoxyribose nucleic acid
EDC	*N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylene diaminetetra acetic acid
EET	Epoxyeicosatetraenoic acid
EPA	Eicosapentanoic acid
Eq	Equivalent
ETYA	Eicosatraynoic acid
FAP	Focal adhesion plaque
FCS	Foetal calf serum
FLAP	Five lipoxygenase activating protein
HCl	Hydrochloric acid
HETE	Hydroxyeicosatetraenoic acid
HPETE	Hydroperoxyeicosatetraenoic acid
HTS	High throughput screening
IC_{50}	Inhibitory concentration, 50%
LA	Linoleic acid
LDL	Low density lipoprotein
MAC	Murine adenocarcinoma
MNL	Mononuclear leukocytes
MVK	Methyl vinyl ketone
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide

$Na_2S_2O_4$	Sodium dithionite
NDGA	Nordihydroguaiaretic acid
NSAID	Non-steroidal anti-inflammatory drug
Ov. m	Overlapping multiplet
PBS	Phoshate buffered saline
Pd-C	Palladium on carbon
PEG	Polyethylene glycol
PGI_2	Prostacyclin
PKC	Protein Kinase C
PLA_2	Phospholipase A_2
PUFA	Polyunsaturated fatty acid
St	Styrene
TEA	Triethylamine
TEAF	Triethyl ammonium formate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIC	Total ion current
TLC	Thin layer chromatography
p-TsOH	*para*-Toluene sulphonic acid
TXA	Thromboxane
WBC	White blood cell

Chapter 1

Drug discovery

Pharmaceutical companies are finding that the time taken from initiation of a new drug discovery program to the successful marketing of a new drug is ever increasing. During the 1960s the drug discovery process averaged 8 years and doubled in the 1990s to 16 years[1]. An increased development time leads to increases in development costs and decreases the profits that can be made from a successful drug candidate. The cost of the discovery of a single new drug has been estimated to have escalated from 100 million dollars in the 1960s to 650 million dollars in the late 1990s[1].

1.1 The process of drug discovery

First there must be identification of a biological target, and hypothesis that interaction with this target may lead to therapy in a given area. Compounds are screened against biological targets. Active compounds, 'hits' are then subjected to chemical optimisation in order to identify 'lead' compounds as potential drug candidates. Lead compounds are then subjected to extensive studies in a development phase before entry into clinical trials. Development studies include toxicology, metabolism within and excretion from the body. In the mid-1990s, for every approved drug in the US, an average of 6,200 chemical compounds were synthesised, 21 of which were put into the development phase. On average 6.5 of these were tested in humans and only 2.5 of these made it to phase III clinical trials. Up to this point the process cost an average of 350 million dollars and took an average of 12.8 years[2].

The overall research and development spend in the US pharmaceutical industry rose from 6 billion dollars in 1988 to approximately 21 billion dollars in 1998[1]. To maintain the spiralling costs of research and development pharmaceutical companies have had to re-evaluate the drug discovery process. The objective has been to make the drug discovery process faster and more efficient, introducing more drugs to the market at a greater rate.

1.2 Redesigning drug discovery

1.2.1 Screening

Pre 1990 screening programmes for drug discovery were traditionally performed manually, utilising large numbers of chemicals from natural products and 'in-house' catalogues of previously synthesised compounds. The introduction of computers led to the robotic automation of this process and has been termed 'high through-put screening' (HTS). The introduction of HTS has seen the compound screening rate increase to allow the testing of thousands of compounds in the time it would previously have taken to manually screen a few dozen.

1.2.2 Compound preparation

Traditionally, one medicinal chemist was able to synthesise between 30 and 100 compounds per year for biological screening[1]. The increased rate of compound screening has placed a greater demand on the medicinal chemists to provide compounds for the HTS programmes. To meet the increased compound demand, in the early 1990s, chemists began to synthesise large collections of compounds called ‘libraries’. These libraries of compounds were made in many cases by making all possible combinations of a series of reactants and the approach was christened ‘combinatorial chemistry’[1].

1.2.3 Combinatorial chemistry

Combinatorial chemistry is based on two main premises: The probability of finding a ‘hit’ (an active molecule) in a random screening process is proportional to the number of places one looks for it; and the simultaneous generation of numerous molecules provides numerous places to look. Combinatorial chemistry relies on producing all possible combinations of a basic set of modular components or ‘building blocks’. The number of products obtainable from a given set of building blocks increases exponentially, while the number of building blocks increases only arithmetically. A range of compounds can be produced using similar reaction conditions in the same reaction vessel, to give a mixture of compounds, or in different reaction vessels to give one compound per vessel. For example, if coupling the building block A with building block B gives the product AB, combinatorial synthesis can take a range of building blocks $A_{(1-n)}$ and react those with $B_{(1-n)}$ to make any product combination (**Figure 1.1**). The process of combinatorial chemistry may be automated, increasing the speed in which large numbers of compounds can be made aiding the drug discovery process by increasing the number of compounds synthesised in a given time, thereby generating more potential novel leads. Once these novel leads are generated combinatorial chemistry may be used to optimise the activity of initial leads (see **Section 1.2.4.2**).

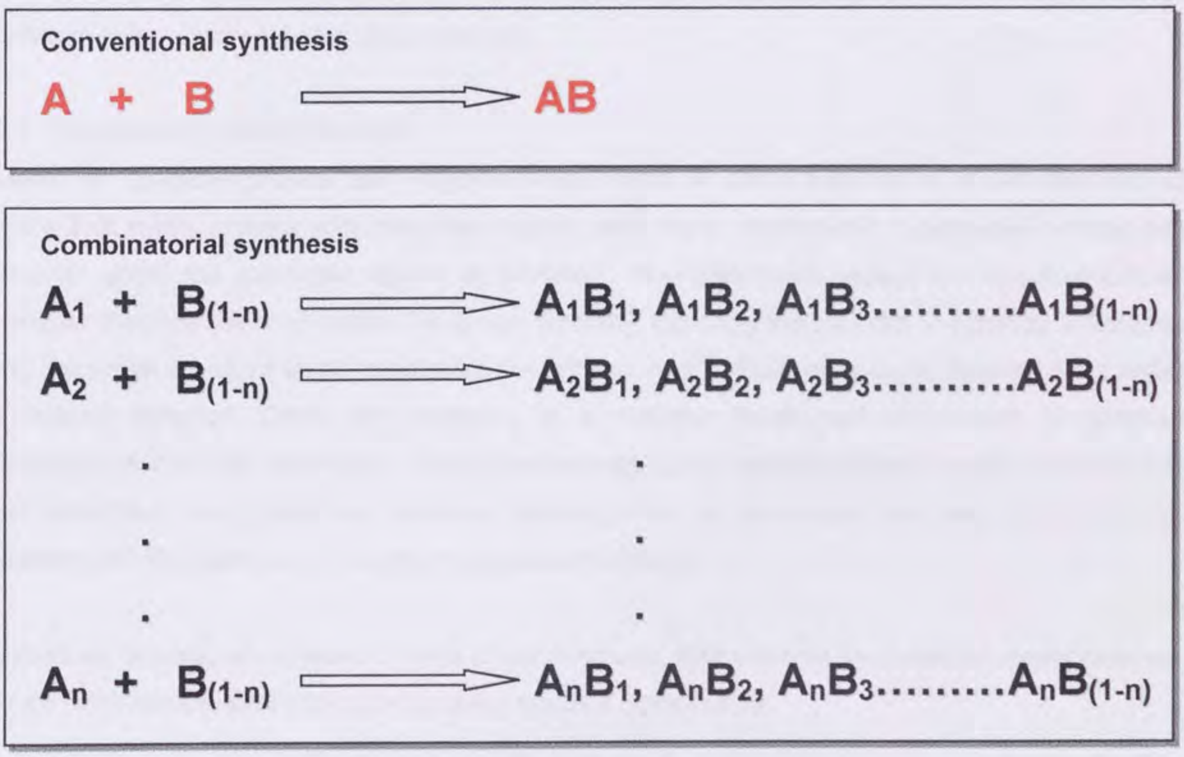


Figure 1.1 Combinatorial chemistry synthesis

Initial attempts in combinatorial chemistry focussed on the synthesis of peptide and oligonucleotide libraries, because the chemistry involved was high yielding and reproducible. Solid-phase synthesis approaches were used to prepare mixtures of peptides or oligonucleotides, and encoding strategies were developed in which molecular tags were attached to linker groups or solid-phase beads so as to identify active molecules after screening. Peptides and oligonucleotides are, however, problematic for drug development because of their poor oral bioavailability and their rapid enzymatic degradation. As the idea of combinatorial chemistry has grown, the field's original predominant focus on peptide and oligonucleotide libraries has changed towards the production of small organic molecules with molecular weights of up to 600 daltons, i.e. drug-like molecules.

1.2.4 Compound libraries

Compound libraries can be divided into two types in terms of their diversity; random primary libraries and focussed libraries. The diversity of a library can be measured by a variety of parameters, including pharmacophores, molecular volume, molecular weight, polarity, charge, solubility and membrane permeability that the compounds within the library exhibit.

1.2.4.1 Random primary libraries

Random primary libraries are prepared when there is little or no information about the target of interest, such as structure-activity relationships, receptor or enzyme structure[2,3]. Random primary libraries are designed to cover a wide chemical diversity. With no information about a target the chance of finding active compounds in a library increases with increasing diversity. An infinite diversity, or as many different molecular shapes and sizes as possible is needed, to find the maximum number of active compounds[4]. For this reason primary random libraries tend to be large and contain many different types of compounds.

1.2.4.2 Focussed/directed libraries

Focussed or directed libraries are prepared when there is some information about the kind of molecule that might interact with the given target, such as a mechanistic hypothesis or structural information about the substrate, ligand or inhibitor[5]. Any information about the structure-activity relationship changes the requirement for library diversity, directing the process to produce analogues around the active structure so as to optimise the original lead[3,6]. Such compound libraries are smaller than random libraries. Often the members of a directed library will all contain a common pharmacophore that has historically yielded compounds active against related targets. Beyond that shared pharmacophore, maximum chemical diversity may be generated in a way believed to be compatible with the retention of the pharmacophore's activity[2].

Combinatorial libraries are created by one of two methods, split and mix synthesis to create mixtures or parallel synthesis to give individual spatially discrete compounds.

1.2.4.3 Mixtures

Screening of mixtures is not a new idea, pharmaceutical companies had previously searched for hits from the natural product mixtures derived from micro-organisms and of plant extracts, which often contained many hundreds of molecules.

The split and mix technique (**Figure 1.2**) was first used in the generation of peptide libraries[2,4,7,8,9]. In split and mix combinatorial synthesis, compounds are assembled on the surface of a resin support material such as microparticles or beads. The beads are divided into a number of equal portions (x) and each of these are individually reacted with a single different reagent or building block (A, B, C etc). After completion of the reaction and washing away of the excess reagents, the individual portions are recombined, mixed and again divided into x portions. Reaction with a further set of reagents gives the complete set of possible dimers as mixtures. The process may be repeated n times to give a total of x^n products. Each bead in the library holds multiple copies of a single library member. The compounds may be tested still attached to the resin or as a mixture following cleavage from the resin[2,7,8]. Peptides do not make good drug molecules, they have low bioavailability and are susceptible to proteolysis and are extremely difficult to translate into non-peptidic drug candidates. For these reasons split and mix synthesis has been adapted using organic building blocks to produce large numbers of non-peptidic organic molecules[2].

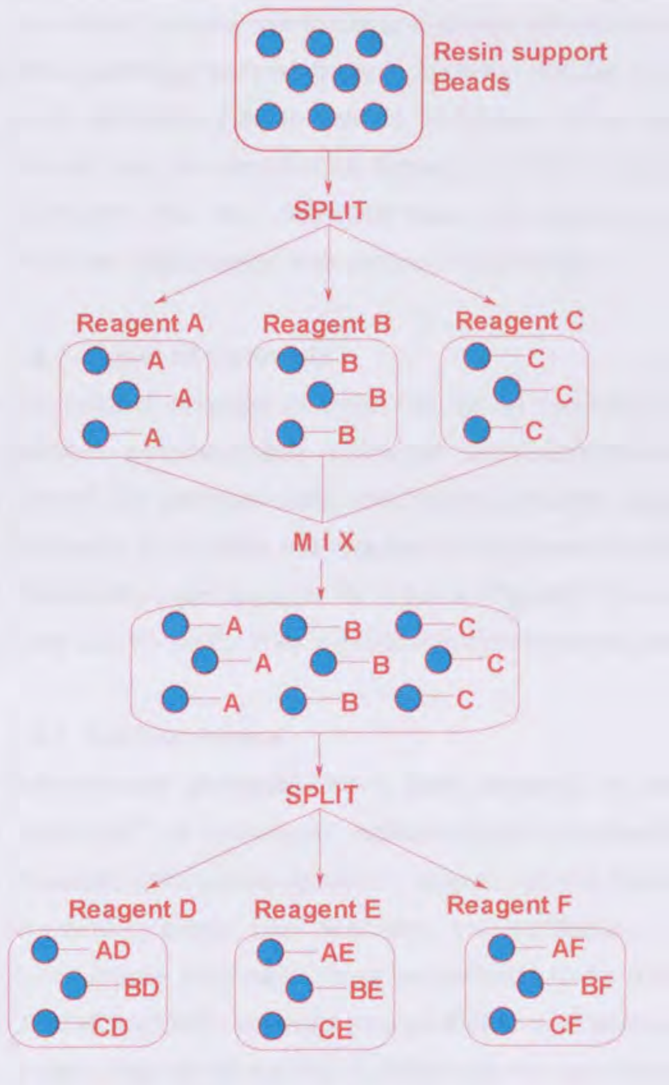


Figure 1.2 Split and Mix combinatorial chemistry

Split and mix combinatorial chemistry may also be used for solution-phase chemistry, the advantage being a guaranteed mixing during the recombination stage. The disadvantage being lack of control over reaction completion and purification at each step. Solution-phase mixtures have a large potential for the production of unwanted by-products.

The use of mixtures allows many compounds to be synthesised and tested in a given time. The synthesis, however, must deliver all the compounds in the mixture in approximately equimolar amounts. The optimal mixture complexity for biological testing and deconvolution has been determined at 20 substances per mixture. It has been found that with more complex mixtures the detection of active compounds within the mixture was no longer reliable[4].

1.2.4.4 Single compounds/Parallel synthesis

A more recent trend in combinatorial chemistry has been the move away from making complex mixtures of compounds for biological screening, followed by a process of deconvolution (identifying active compounds by iterative library re-synthesis) to find the active component of the mixture. Increasingly, libraries of compounds are being prepared individually, using discrete reaction chambers laid out in a spatially addressable format, giving one compound per vial/well. This has been termed parallel synthesis and allows individual compounds to be produced simultaneously in greater quantities with relatively high purity. Smaller numbers of compounds are usually produced by parallel synthesis than in mixture synthesis. While testing the individual compounds from parallel synthesis may be slower than testing a mixture, it is often advantageous for the analysis of active compounds and also does not have the problems of false positive or false negative results sometimes encountered with compound mixtures.

1.2.5 Types of synthesis

Combinatorial libraries as mixtures or as individual compounds, may be prepared using both traditional solution-phase synthesis and solid-phase techniques. Solution-phase chemistry is preferred for between one and three synthetic steps and where relatively small numbers of compounds (10s-100s) are required. Solid-phase synthesis is often preferred when more than two synthetic steps are required for reasons of purity. The quantities of compounds available are smaller, but the size of the libraries synthesised may be much larger than solution-phase synthesis.

1.2.5.1 Solution-phase

Solution-phase strategies have been applied to make both mixtures and spatially separate compounds[10]. A successful solution-phase approach can have significant advantages over a comparable solid-phase approach. Solution-phase chemistry can be automated and is often used for high yielding single step reactions, but purification becomes an issue for multistep syntheses. Solution-phase synthesis allows compounds to be analysed at each stage of the synthesis using traditional methods, whereas special methods of analysis are required for solid supported reactions. The vast majority of organic transformations have been performed in solution and have yet to be optimised for the solid-phase, therefore, a shorter development time is required. The number of reaction steps are reduced with solution-phase chemistry as there is no need to attach the initial starting material onto support or to cleave the final product from the support. Product purification may often be conveniently accomplished for reactions in solution by liquid-liquid or solid-liquid partitioning or the use of scavengers to remove undesired material[10,11]. Solution-phase chemistry allows for access to a larger variety of reactions and therefore, to more classes of compounds (**Section 4.4.2**). Compounds may be prepared in larger quantities than with solid-phase chemistry and may be scaled-up easily in a traditional manner, if an active compound is found.

1.2.5.2 Solid-phase

Solid-phase synthesis of peptides and oligonucleotides is well established and the majority of published work on small molecule library synthesis has been performed using solid supports[7,8]. Due to their favourable pharmacokinetic properties, the focus of combinatorial libraries is on the synthesis of small organic 'drug-like' molecules (<600 Daltons molecular weight) rather than peptides. Development of the chemistry on the solid-phase is often the rate limiting step, but an increasing number of synthetic transformations that are routine in traditional solution-phase organic chemistry are being established on various solid supports. There are advantages to solid-phase synthesis: First, simple removal of non-resin bound by-products and excess reagents by washing in the appropriate solvent makes it easier to perform multistep reactions. Second, products may be obtained in high purity avoiding time consuming and costly chromatographic purification. Solid-phase synthesis is easily automated and the varying reactivity of a range of substrates may be accommodated by the use of excess reagent to drive every reaction to completion. Solid-phase resins give 'site isolation', whereby a reactive site on a resin has in effect infinite dilution. If a molecule with two equally active functions is reacted with the resin, only one site can attach to the resin, the other will remain free to react with other molecules in solution[12].

The main disadvantages of solid-phase chemistry are the extra labour, longer development times required and the cost. There are limitations in the current range of commercially available supports and linkers as well as a limited means of monitoring reactions and identifying intermediates attached to the resin. Solid-phase routes also necessitate additional steps to link and cleave to and from the support. The amounts of products produced are often small because of the limited loading capacity of solid-phase resins. Generally, solid-phase synthesis is used to prepare very small amounts of the final product, <5mg[13] and cost factors usually prohibit the production of large quantities of product.

For most solid-phase combinatorial libraries two types of supports have been used. Polystyrene cross-linked with 1-2% divinylbenzene, and a polystyrene-polyethylene glycol co-polymer (PEG-PS). One major limitation of polystyrene resin is that the resin beads are not well solvated in protic solvents resulting in poor reaction site accessibility and diminished reaction rates[13]. PEG-PS, in contrast is well solvated in protic solvents and because the PEG is not cross-linked the reaction sites may be more accessible[8,13]. PEG-PS has the disadvantage of being more expensive, not as mechanically stable as polystyrene and of lower loading.

1.2.5.3 Synthesis on soluble polymers

Synthesis on soluble polymers combines the advantages of solid-phase synthesis with those of homogeneous solution-phase synthesis. Excess reagents may be used to drive a reaction to completion. The polymeric support is soluble in many organic solvents but may be precipitated in others, to allow simple purification by precipitation of the polymer and filtration. With synthesis on soluble polymers the general difficulties of heterogeneous solid-phase reactions, namely lower reactivity and characterisation of the polymer-bound intermediate are overcome. Reaction development may be analysed by routine methods, such as UV, IR and NMR without cleavage of the product or intermediate from the soluble polymer.

The majority of examples of synthesis using soluble polymeric materials utilise polyethylene glycol (PEG) as a polymeric support[14,15,16,17,18,19,20,21]. This polymer is soluble in many solvents yet can be precipitated as required by addition of a large amount of diethyl ether[22]. PEG-bound substrates generally appear to have reaction kinetics similar to those observed in solution chemistry therefore, the slow reaction times of solid-phase cross-linked polymers are eliminated[4,23,24,25]. An added advantage is that PEG is inexpensive when compared to commercially available insoluble solid-phase supports. There is little literature precedent for using soluble polymers other than PEG, as solid supports for small organic molecule synthesis. Oligonucleotides have been synthesised on polyvinyl alcohol and trityl functionalised linear polystyrene[15], and the synthesis of oligosaccharides has been demonstrated on polyvinyl alcohol and polyacrylamide. A soluble linear chloromethylated polystyrene has been synthesised and used in place of Merrifield resin in the synthesis of peptides and proteins to increase reaction yields and to decrease reaction times in contrast to its cross-linked counterpart, Merrifield resin. The soluble linear polymer was found to have solubility profiles amenable to organic chemistry and has been used to support prostaglandin synthesis[15,25]. The soluble chloromethylated polymer being soluble in THF, DCM, $CHCl_3$ and ethyl acetate even at low temperatures (-78°C) and insoluble in methanol and water, allowed the implementation of solvent extraction for removal of inorganic material, in conjunction with the polymer precipitation techniques already used in the PEG liquid phase approach. The solubility of the polymer also allowed analysis of intermediates in a non-destructive manner without the need for any specialised NMR techniques[26]. PEG monomethyl ether has been used as a foundation for combinatorial library assembly[27] and with further development, PEG and other soluble polymers will be of greater use to combinatorial chemists.

1.3 Current directions

1.3.1 Miniaturisation

As the applications of combinatorial chemistry proliferate, the ability to keep the equipment size and reagent quantities to a minimum is an issue of growing importance. The trend for initial library synthesis is towards smaller amounts of product being made which can be screened in miniaturised screening units. Small-scale equipment makes it possible to use less sample so decreasing reagent costs, for example there are now available 2 inch square chips that can be used to carry out chemical reactions at submicrolitre volumes in 144-well arrays[28]. The miniaturisation of combinatorial synthesis will drive forward the miniaturisation of HTS which will save on the cost of the proteins and reagents used in the assays.

1.3.2 Analysis and purification

Considerable efforts are being devoted to the chemical analysis of combinatorial libraries. Not all reactions work, so for screening it is a great advantage to be able to characterise compound libraries for purity and structure. High through-put NMR is being developed and other analytical instruments such as mass spectrometers are being customised for combinatorial applications. High performance liquid chromatography systems are in use for library purification.

1.3.3 Artificial intelligence

The development of combinatorial chemistry, automated analysis and HTS now allows the synthesis, characterisation and biological screening of thousands of compounds per month. The enormous amount of data that this process generates and the work involved in processing and interpreting the information gained presents a major challenge for information management, especially if the structural information obtained from biological screening is to be maximised. The pharmaceutical industry has applied artificial intelligence to predict which molecules within a given library are most likely to display the biological profile required in a given drug discovery programme[1]. Compound libraries, be they virtual (those not prepared physically but stored in computer memory) or actual (physically prepared) are often subjected to a diversity analysis. This determines the minimum number of compounds needed to cover all of the chemical and biological properties of the entire library, providing the diverse set of a library. Diversity analysis of actual compound libraries minimises the number of compounds put into the screening process, so decreasing screening costs. More effectively, diversity analysis of a virtual library can direct and minimises product preparation as well as decreasing screening costs. The objective is therefore changing from synthesis and screening of the maximum number of compounds to the synthesis and screening of the minimum number of compounds needed to achieve the required profile for a potential drug candidate of a given biological target. In this way artificial intelligence and automation is continually improving the effectiveness of the drug discovery process.

Chapter 2

Cancer

In Britain, four in ten of us will suffer from some form of cancer at some time in our lives[29,30]. Cancer is the cause of a quarter of all deaths in this country, around 130,000 per year[29,30]. In 1991 over 273,000 people in this country were registered with a malignant neoplasm (a potentially invasive tumour). Different cancers affect people at different ages, but the risk of getting the disease rises rapidly as we get older. More than 70 per cent of cancers in this country occur in the over 65s[29]. Almost 90,000 people die in this country every year before they reach their 65th birthday. Of these, more than 32,000 die of cancer, and 25,000 die of heart diseases, strokes and related illnesses[30].

2.1 What causes cancer?

Cancer results from cellular dysfunction and a failure of the body's normal regulatory processes to detect and eliminate these abnormal cells. The cellular dysfunction results from faults in the cell DNA which can be genetic or more often a result of damage by cancer-causing agents such as tobacco, viruses, sunlight or dietary factors. It takes more than one fault to turn a cell into a cancer cell, but as we go through our lives, we tend to accumulate these genetic faults. The fact that the body produces abnormal cells is not surprising when it is considered that in each cell of the human body there are 50,000 to 100,000 genes, each of which codes for a specific protein product, which has a unique function in the maintenance of the normal cell[31].

2.2 How do cancers develop?

Healthy cells divide and increase in number as we grow, or as the body repairs itself. When enough cells have been produced for a particular purpose this dividing mechanism switches off naturally. In cancer cells, the dividing process is uncontrolled. As a result, cancer cells continually divide and increase in number, forming a mass of abnormal cells. These cells do not function as part of the organ or tissue where they began. Normal cells are pre-programmed to die when they are too old or if they start malfunctioning. Cancer cells override this pre-programming and become immortal. The uncontrolled growth of cancer cells gives them the capacity to grow into neighbouring tissues, blood and lymphatic systems.

Cancer can affect every system in the body, originating from single cells. By the time it is detectable by X-ray it will contain 10^7 cells. By the time a tumour is palpable by a clinician it will contain 10^9 cells, be about 1cm^3 in size and will weigh about 1g[31]. For one single cancer cell to grow to 10^9 cells it only needs to double in size thirty times[31].

2.3 How do cancers spread?

A tumour that has the potential to spread is said to be malignant. Cancers spread by invading the surrounding tissue until they reach a blood or lymph vessel. Small groups of cells may then be carried via the circulation to other parts of the body, where they may settle and grow to form secondary cancers, a process called metastasis. The clinical manifestations of malignancy depend in part on the tumours innate potential for growth and also host factors that may encourage or inhibit the cell proliferative process. The resulting spectrum of malignancy extends from tumours which may stay unchanged for several years, and very rarely metastasise having no impact on life expectancy, to tumours which may kill within days of the first indication of their presence[31].

2.4 Incidence

The commonest cancer for men is lung cancer accounting for 20% of all male cancers. For women the most common cancer is breast cancer accounting for 25% of all female cancers. In both men and women large bowel cancer (cancer of the colon and rectum) is the second most frequently occurring at 12%. In men, however, there are almost as many cases of prostate cancer[29]. Survival from cancer varies greatly depending on the type of cancer and the stage of development at which it is diagnosed.

2.5 Standard chemotherapy

The major problem associated with conventional chemotherapeutic agents is the side effects experienced by the patient. The majority of anti-cancer drugs are cytotoxic, they can damage or kill cells. These cytotoxic agents are designed to be most effective in rapidly dividing cells. They lack selectivity for tumour cells and so affect the normal faster growing cells of the body, producing bone marrow suppression, hair loss, nausea and vomiting. The toxic effects to the normal tissue generally means that standard chemotherapeutic agents have a low therapeutic index, the ratio of the maximum non-toxic dose to the minimum effective dose. Targeting the differences of tumour intra- and intercellular signalling pathways, i.e. those that regulate oncogenes, proliferation, metastasis and angiogenesis, may be a way of developing future anti-cancer agents that do not cause the unacceptable damage of standard chemotherapy.

2.6 Lipoxygenases and their possible role in cancer

Dietary fat and in particular the polyunsaturated fatty acids (PUFAs) have been implicated as mediators of tumour development and growth[32]. The type of PUFA appears to be important, n-6 fatty acids, such as linoleic acid (LA), which may be synthesised from arachidonic acid (AA) *in vivo* and AA itself appear to promote tumour cell growth and metastasis. This has been demonstrated in animal models of colon, breast and pancreatic cancer[32,33]. In contrast, n-3 fatty acids [such as eicosapentanoic acid (EPA) found in fish oils] may inhibit tumour cell growth[32,33]. AA and LA enhance the growth of established murine adenocarinoma (MAC) cell lines, the fast growing MAC13, chemotherapy resistant MAC16 and the slow growing tumour MAC26 *in vitro* and *in vivo*[34]. This stimulation of tumour growth can be blocked by lipoxygenase inhibitors and is, therefore, thought to be dependant on the metabolism of AA and LA through the lipoxygenase (LO) pathway[34].

AA (5,8,11,14-eicosatetraenoic acid, a 20 carbon chain fatty acid) is an essential component of cell membrane phospholipids which is primarily liberated by the action of phospholipase A_2 (PLA_2) in response to stimuli such as growth factors, cytokines, chemokines and circulating hormones[35]. Once AA is released it may be oxidised by one of three different oxygenases to produce the eicosanoids: Cytochrome P450 epoxygenase produces epoxyeicosatetraenoic acids (EETs); Cyclo-oxygenase produces the prostanoids; prostacyclin (PGI_2), various prostaglandins and thromboxane (TXA_2); Lipoxygenase produces a variety of hydroperoxyeicosatetraenoic acids (HPETEs), hydroxyeicosatetraenoic acids (HETEs), leukotrienes, hepolixins and lipoxins.

Lipoxygenases may be found in plants and animals[36]. They constitute a family of non-haem iron containing dioxygenases that catalyse the stereospecific oxygenation of AA. The first lipoxygenase studied was isolated and crystallised from soybean[37,38,39,40,41,42]. In mammalian cells there are three major enzymes, 5-, 12 and 15-lipoxygenases, which form the corresponding 5-, 12- and 15-HPETEs. These products are unstable and are reduced either spontaneously, or by the action of peroxidases, to the corresponding stable monohydroxylated products, HETEs (**Figure 2.1**)[43].

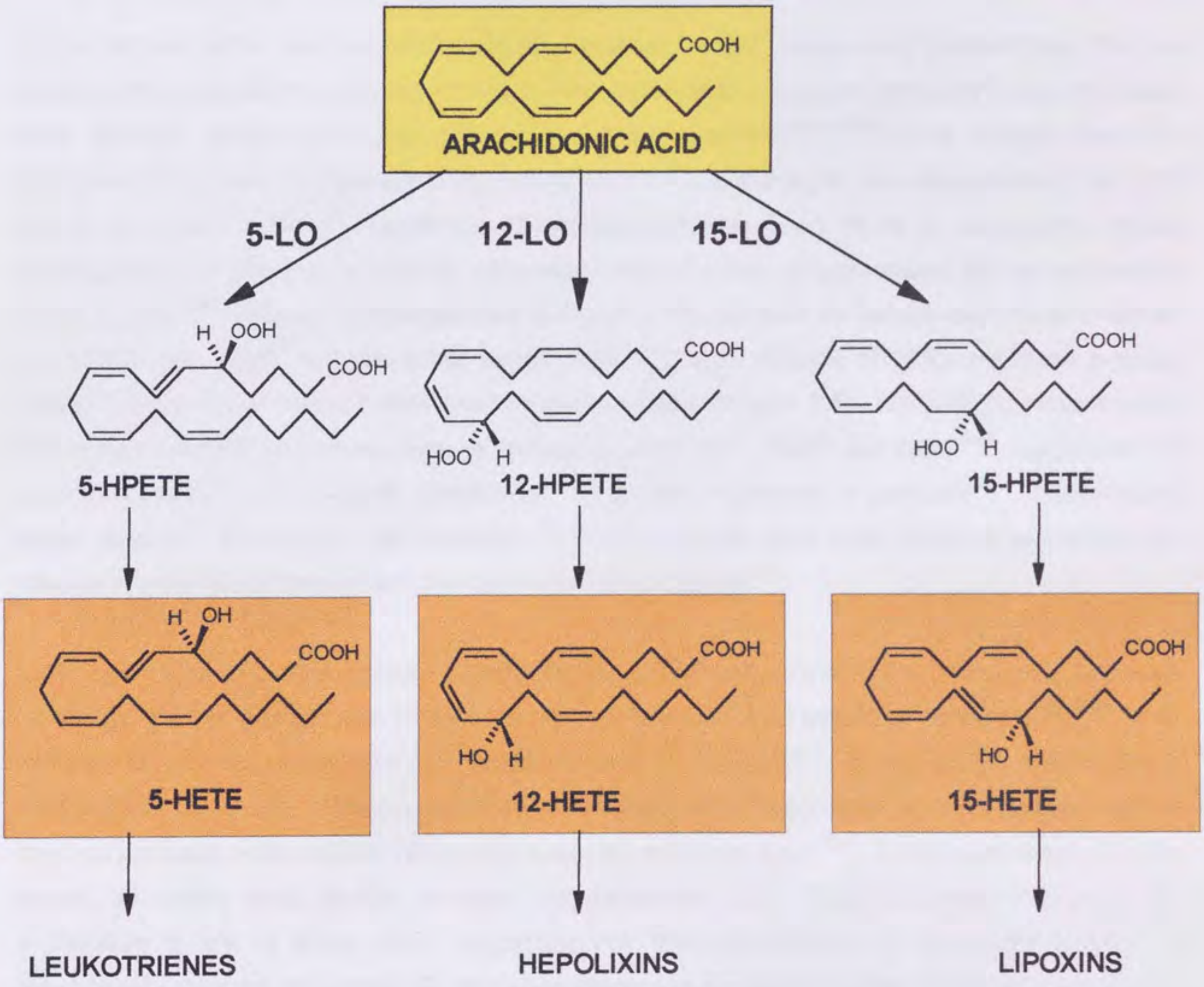


Figure 2.1 Metabolites of arachidonic acid metabolism through lipoxygenase enzymes

Of the enzyme subtypes, 5-lipoxygenase has been subject to the most thorough investigation. 5-Lipoxygenase is present in a number of different cell types including neutrophils, basophils, monocytes and macrophages and mast cells[43]. The product of 5-Lipoxygenase is 5(*S*)-HETE which is further metabolised to form the leukotrienes. These products form the so-called slow reacting substance of anaphylaxis[36,43,44,45] the components of which play a major role in inflammatory reactions and diseases such as bronchial asthma, arthritis and psoriasis[36,44,45,46].

15-Lipoxygenase dioxygenates AA to produce 15(*S*)-HETE which may be metabolised to form the lipoxins. Human 15-lipoxygenase is abundant in reticulocytes, airway, eosinophils, alveolar macrophages, monocytes and epithelial cells[46,47,48,49]. The products of 15-lipoxygenase have been implicated in a variety of responses including pulmonary inflammation, endothelial cell proliferation, arterial vasoconstriction/relaxation and psoriasis[36,38,45,48,49,50]. Unlike other lipoxygenase enzymes, 15-lipoxygenase has the ability to oxidise low density lipoproteins (LDLs) which may play a vital role in the primary stage of atheroschlerotic lesion formation[36,38,44,45,47,49,50,51,52]. Lipoxins mediate immunological responses and haemodynamic responses[37,43,44,48].

There are two isoforms of mammalian 12-lipoxygenase, leukocyte-type and platelet-type. The two isoforms are quite different, having less than 70% amino acid sequence homology[44], are expressed from different genes and have different substrate specificity[44,53,54,55]. The platelet type 12-lipoxygenase primarily oxygenates C-20 PUFAs whereas the leukocyte type oxygenates C-18, C-20 and C-22 PUFAs[37,38,45,53,55]. Leukocyte 12-lipoxygenase has been found in leukocytes, bovine tracheal cells, porcine pituitary glands, pancreas, canine cerebra, mouse spleen and rat and mouse pineal glands[37,55]. Platelet 12-lipoxygenase is found in the platelets of various animals and human and mouse skin cells[55] and metastatic tumour cells[54,56]. Both isoforms of 12-lipoxygenase produce 12(*S*)-HETE which is further metabolised to the hepolixins (**Figure 2.1**). 12(*S*)-HETE plays a major role in many cellular processes, such as platelet aggregation[37], insulin secretion[43,44], suppression of renin production[38] and leukocyte chemotaxis[37]. It is also implicated in psoriasis[37,44], inflammatory bowel disease[37] and tumour cell metastasis[44]. The hepolixins have been reported to mediate the release of intracellular calcium and the opening of ion channels[37,44].

Leukocyte 12-lipoxygenase is more closely related to 15-lipoxygenase (86% amino acid sequence homology) than to platelet type 12-lipoxygenase (66% amino acid sequence homology)[37,38,46]. Both isoforms of 12-lipoxygenase can form small amounts of 15(*S*)-HETE as well as the major product 12(*S*)-HETE. Leukocyte 12-lipoxygenase produces 15(*S*)-HETE equivalent to approximately 10% of the total products, while platelet 12-lipoxygenase only produces 1-2%[37,57]. It has been shown that the degree of amino acid identity between 5-lipoxygenase and 12-lipoxygenase/15-lipoxygenase subfamilies is low at about 40%, suggesting that their contribution to the body's function is considerably different. In contrast 12- and 15-lipoxygenase subfamilies show almost 80% amino acid identity[44]. This explains the ability of 12-lipoxygenase to produce small amounts of 15(*S*)-HETE as well as 12(*S*)-HETE.

Until recently, structural information for mammalian lipoxygenases has been derived from crystal structures of soybean lipoxygenase-1[40,41,42]. Comparisons between the mammalian and plant lipoxygenases are of limited value because they share only 25% sequence identity[58]. The crystal structure of mammalian 15-lipoxygenase with a bound inhibitor has now been determined[58]. The lipoxygenase enzymes all consist of two domains, an *N*-terminal β-barrel domain that is thought to localise the enzyme near its substrates, and a second larger catalytic domain[58]. The catalytic domain of 15-lipoxygenase consists of 18 helices creating a hydrophobic pocket for substrate binding. Two of these helices co-ordinate the non-haem catalytic ferrous ion with four histidine residues (361, 366, 541, 545) to give an octahedral geometry. The fifth protein ligand consists of the C-terminal residue, isoleucin663[44,58,59] and the sixth ligand is ascribed to a hydroxide ion[60].

The active site of mammalian 15-lipoxygenase is a boot shaped cavity. It is proposed that the methyl end of AA becomes buried deep into the hydrophobic pocket while the acid end becomes attached to the enzyme surface by the basic residue, Arginine403[58,61]. The site of AA oxygenation appears to be controlled by the volume of the hydrophobic pocket, with the 12-lipoxygenase pocket being 6%, and 5-lipoxygenase 20% larger than the 15-lipoxygenase pocket thus allowing AA to slide deeper into the pocket aligning different carbon atoms with the catalytic iron[44,58].

The iron in lipoxygenase exists in one of two oxidation states, the inactive ferrous (Fe^{2+}) or the active ferric form (Fe^{3+}). The native enzymes exist in the ferrous form and must be oxidised to the active ferric form for reaction with the substrate[40,42,60,62]. During the catalytic process, a hydrogen atom is abstracted from the substrate by the lipoxygenase ferric-hydroxide complex to generate a substrate radical and a lipoxygenase ferrous-water complex. Reaction of the substrate radical with molecular oxygen produces a peroxyl radical which reoxidises the ferrous-water complex back to the active ferric-hydroxide complex and generates the hydroperoxide product[39,42,58,59,63,60].

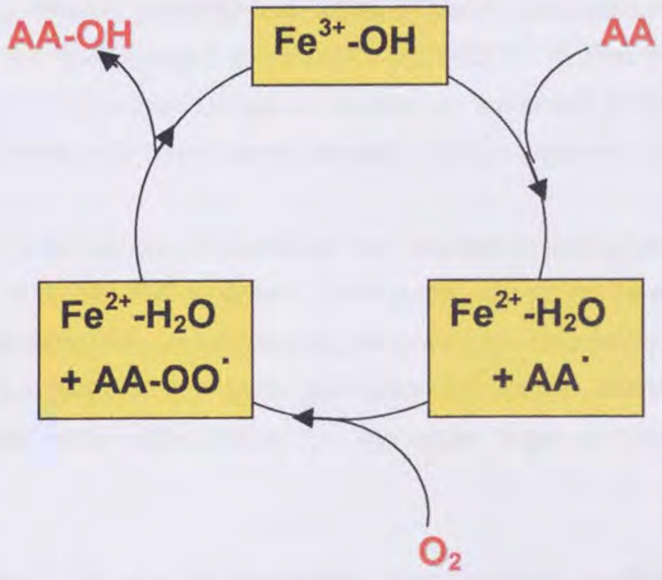


Figure 2.2 Mechanism of lipoxygenase catalysis

For most lipoxygenase reactions, there is a short lag phase before full activity because the majority of the enzyme rests in the inactive ferrous state[37,39,64]. The reaction of the substrate with the minority of ferric active sites produces the hydroperoxide product, which in turn oxidises the inactive ferrous state to the active ferric state, accounting for the lag phase[39,63]. Enzyme inactivation is a common characteristic of 5-, 15- and leukocyte 12-lipoxygenase but not of platelet 12-lipoxygenase[37,55,57]. As soon as the reaction with the substrate starts the enzyme slows down rapidly and stops in several minutes. Inactivation is thought to be caused by binding of metabolites such as leukotrienes to the enzyme[37].

Many studies have examined the relationship between the prostanoids and malignant disease and the ability of cyclo-oxygenase inhibitors, such as the non-steroidal anti-inflammatory drugs (NSAIDs) to diminish cancer growth and metastasis[65,66,67,68,69]. Studies of both cyclo-oxygenase and lipoxygenase inhibitors have suggested that prostaglandins enhance only tumour growth, while lipoxygenase metabolites enhance tumour cell proliferation and also metastasis[69,70]. It has been shown that metastatic tumour cell lines produce predominantly lipoxygenase products whereas non-metastatic cell lines produce mainly cyclo-oxygenase metabolites[69]. Of the lipoxygenase metabolites in highly metastatic cells 12(*S*)-HETE is the predominant AA metabolite and is synthesised in a greater quantity than in less metastatic cells[53,71]. It appears that a tumour cells ability to synthesise 12(*S*)-HETE is a strong correlate of their metastatic potential[71]. Inhibition of lipoxygenase pathways renders tumour cells non-invasive and non-metastatic[65].

2.6.1 Lipoxygenase and metastasis

Tumour cells exist as heterogeneous populations that differ in many phenotypic and genotypic characteristics, this means that tumour cells within a tumour may have different proliferative and metastatic abilities[53]. The differing metastatic potentials of tumour cells are expressed as cell surface differences in structure, glycoprotein, and protein content as well as phospholipid composition[69,72]. Approximately 0.001-0.0001% of the cells in a primary tumour develop malignant character and are able to metastasise[44]. The different phenotypic structure of highly metastatic cells appears in some ways to be regulated by the lipoxygenase metabolite 12(*S*)-HETE[65,73]. Poorly invasive cancer cell lines when transfected with 12-lipoxygenase genes develop an increased invasive capacity, with an enhanced capacity for migration, this effect can be blocked with lipoxygenase inhibitors[74].

The dissemination of malignant tumours (metastasis) is a cascade involving the detachment of cells from the primary tumour, invasion and digestion of the surrounding local host tissue (extracellular matrix of collagen, glycoproteins and proteoglycans), entry into the circulatory (intravasation) blood vessels or lymphatic system, dissemination within that circulatory system, arrest in a distant vascular bed by adhesion to vessel walls, extravasation into the target organ and then proliferation as a secondary colony[75].

The epidermal growth factor (EGF) receptor participates in epithelial cell proliferation and plays a role in malignant cell growth[76,77]. EGF causes a PKC dependant translocation of 12-lipoxygenase from the cytosol to the membrane and also increases transcription of the 12-lipoxygenase gene to further increase 12-lipoxgenase activity[54,76,77,78]. 12(*S*)-HETE has also been shown to upregulate oncogene expression, and down-regulate the expression of tumour suppressor genes in highly metastatic tumour cell lines[44,71,79]. 15(*S*)-HETE also upregulates DNA synthesis and proto-oncogene expression[49].

For tumour cells to metastasise they must first penetrate the extracellular matrix surrounding the developing tumour. Tumour cells interact with the host's extracellular matrix by adhesion receptors known as integrins, particularly αIIbβ3 and avβ3. These integrin receptors on the tumour cell surface can recognise and bind to a large number of extracellular matrix proteins (for example, fibronectin, fibrinogen and vitronectin)[72,80]. 12(*S*)-HETE activates protein kinase C (PKC) in tumour cells stimulating a reversible rearrangement of cytoskeletal filaments within the cell. This rearrangement of cellular structure promotes the translocation of the integrin receptors αIIbβ3 to specific areas (called focal adhesion plaques, FAPs) in the tumour cell membrane. The increased number of integrin receptors at the FAP stimulate tumour cell adhesion to the extracellular matrix[71,72,80,81]. The tumour cell begins to spread, changing its round shape to extend cylindrical pseudopods which move it forward[71,82]. The adhesion of the tumour cell integrin receptors to proteins of the extracellular matrix induces an increase of endogenous 12-lipoxygenase activity provoking the generation more of 12(*S*)-HETE[71,81].

The tumour cell has now started to move but to continue it must make a path through the extracellular matrix. The PKC-dependant cytoskeletal rearrangements invoked by 12(*S*)-HETE also triggers the release of proteolytic enzymes at the tip of the tumour cell pseudopods. A focal degradation of the extracellular matrix is produced at or adjacent to the integrin receptor-matrix attachment sites[83]. The released proteolytic enzymes, including type IV collagenase, heparinases, urokinases and cathepsins B, D and L, degrade the extracellular matrix and previous integrin-matrix adhesions allowing the tumour cells to cross tissue boundaries[65,67,71,81,82,83,84].

Autocrine motility factor, AMF, regulates tumour cell motility acting through the cell surface receptor, gp78[85]. AMF like 12(*S*)-HETE induces pseudopodial extension as well as the translocation of integrin receptors to the leading edge or pseudopod of the tumour cell. Endogenous 12(*S*)-HETE also induces up-regulation of gp78 receptors and their translocation to the leading edge of the tumour cell. In this way the 12(*S*)-HETE enhances the tumour cells response to AMF, moving the cell even further through the extracellular matrix[71,81,85].

The tumour cell will continue to move through the extracellular matrix until it reaches the endothelium of the blood or lymphatic circulation. The tumour cell must now adhere to the endothelium encountered. The endothelial cell integrin receptor, αvβ3, has the biological function of adhering endothelial cells to the subendothelial matrix but also promotes tumour cell adhesion to the endothelium and is upregulated by 12(*S*)-HETE[44,71,72]. The adhesion itself provokes an increase in 12(*S*)-HETE production in the tumour cells. The endogenous 12(*S*)-HETE produced in turn induces a transient, concentration dependant, non-destructive, localised and reversible retraction of the vessel endothelial cells[70]. 12(*S*)-HETE stimulates the endothelial cell retraction by activation of PKC. The subsequent cytoskeletal rearrangements cause retractions of cells in both large vessels and microvessels allowing the tumour cell into the circulatory system (intravasation)[69,71,84,86].

In the circulatory system tumour cells must overcome host immune cell killing. Less than 0.01% of circulating tumour cells ultimately initiate successful metastatic colonies[75]. After intravasation, tumour cells interact with platelets and induce platelet aggregation and concomitant synthesis of platelet 12(*S*)-HETE. Platelets attach themselves with preferentially tumour cell-endothelial cell junctions. These activated platelets produce 12(*S*)-HETE which signals the translocation of platelet $\alpha IIb\beta 3$ to the membrane surface which recognises circulating fibrinogen. Additional platelets aggregate at this point and more tumour cells are recruited until the structure is stable enough to travel through the circulation without damage to the encapsulated tumour cells[44]. The structure will travel through the circulation and come to rest in the microvasculature[70]. The 12(*S*)-HETE produced by the tumour cells and activated platelets at the point of arrest, enhances endothelial retraction and tumour cell adhesion to the subendothelial matrix, allowing extravasation of the tumour cell from the circulatory system[69,70,71,84,86,87]. On adherence to the subendothelial matrix the production of 12(*S*)-HETE is stimulated and the tumour cell can produce focal degradation and migrate to the target organ tissue. The tumour cell then proliferates in the target organ in response to local growth factors to form a secondary colony.

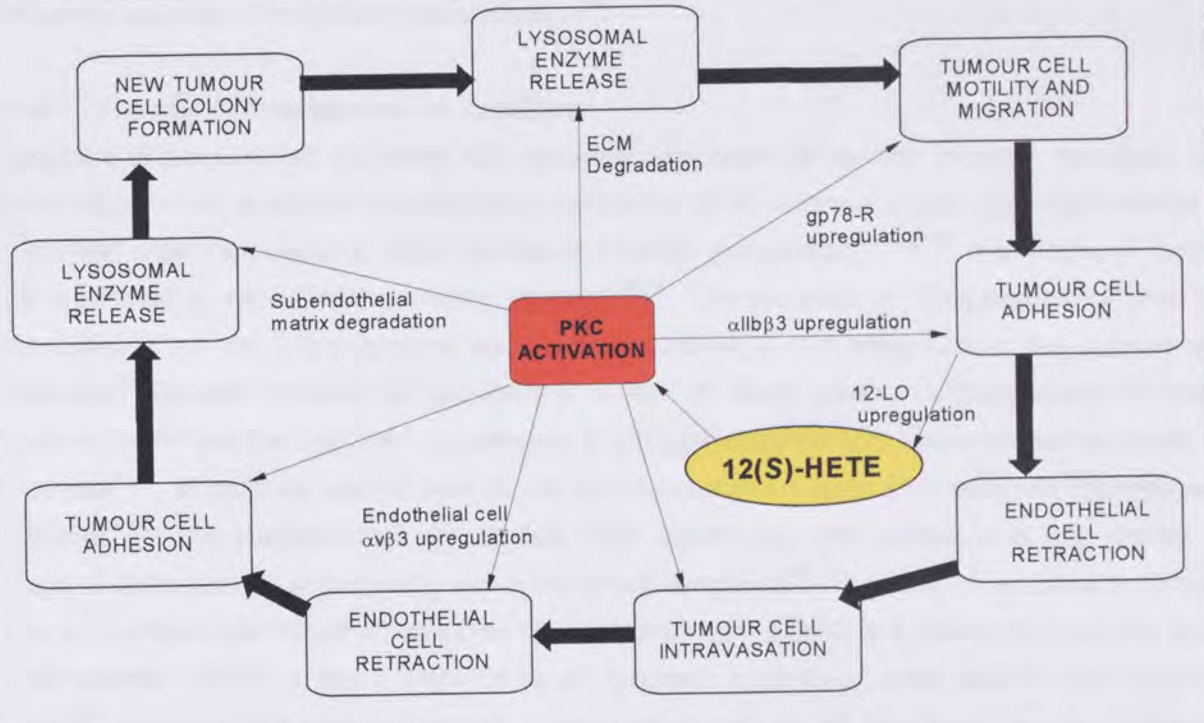


Figure 2.3 Role of 12(*S*)-HETE in tumour cell metastasis

Lipoxygenase inhibitors have been demonstrated to decrease the metastatic potential of tumour cells lines *in vitro* and decrease metastatic colony formation *in vivo*[87]. Selective inhibitors of platelet type 12-lipoxygenase, such as *N*-benzyl-*N*-hydroxy-5-phenylpentamide (BHPP, **Figure 2.6**) and also more general inhibitors of lipoxygenase, such as nordihydroguaiaretic acid (NDGA, **Figure 2.8**), BW755c (**Figure 2.4**), can dose dependently inhibit adhesion–induced tumour cell 12(*S*)-HETE biosynthesis, endothelial retraction and tumour cell adhesion to the endothelium and extracellular matrix[76,80,84,86,87].

2.6.2 The role of lipoxygenase and tumour angiogenesis

Angiogenesis is the formation of new blood vessels from pre-existing ones[88]. The growth and metastasis of solid tumours are dependent upon the ability of tumour cells to form new blood vessels. Both 15(*S*)-HETE and 12(*S*)-HETE stimulate endothelial cell DNA synthesis and cell proliferation in endothelial cells[73,89,90]. Tumour cells expressing high levels of 12-lipoxygenase are more angiogenic than those expressing low levels of 12-lipoxygenase[88]. The pro-angiogenic 15(*S*)-HETE has been shown to accumulate in tissues from disease states associated with abnormal neovascularisation[89]. Sequential steps in this process include degradation of the subendothelial matrix, endothelial cell migration and their final alignment into capillary sprouts[75,88,89,90]. The sprout subsequently expands and assumes a tubular structure, and endothelial proliferation extends these tubules and allows the formation of loops of a functioning circulatory network[75]. 12(*S*)-HETE may be involved in the angiogenic process by its ability to increase the degradation of the extracellular matrix. It also increases the surface expression of integrin $\alpha v\beta 3$ in both macro- and microvascular endothelial cells. Integrin $\alpha v\beta 3$ is predominantly associated with angiogenic blood vessels and plays an essential role in human cancer angiogenesis[88]. Inhibition of angiogenesis induces tumour cell necrosis and apoptosis because of insufficient vascularisation[88].

2.6.3 The role of lipoxygenase in apoptosis

Apoptosis or programmed cell death is a genetically encoded cell suicide program. Apoptosis has been implicated in embryonic development, maturation of the immune system and maintenance of tissue and organ homeostasis, tumorigenesis and tumour progression[56,91,92,93]. A multitude of factors are implicated in regulating/modulating apoptosis[56,91]. The products of 12-lipoxygenase and 15-lipoxygenase but not 5-lipoxygenase have been implicated in regulating tumour cell survival and apoptosis[56]. General inhibitors of lipoxygenase as well as more specific 12-lipoxygenase inhibitors (such as BHPP and baicalein) but not selective 5-lipoxygenase inhibitors cause tumour cell death by apoptosis[91,93]. In contrast, normal cells do not undergo apoptosis upon treatment with lipoxygenase inhibitors[56,92]. This suggests that tumour cells differ significantly from normal cells with respect to usage of lipoxygenase in regulating cell survival and apoptosis[56]. The ratio of bcl-2/bax in tumour cells is a critical determinant of apoptosis. The proto-oncogene bcl-2 is a protein found in the inner mitochondrial membrane, and it functions as an apoptosis suppressor, while bax is a pro-apoptotic protein[94]. Lipoxygenase inhibitors lead to a decrease in tumour cell bcl-2/bax ratio thus triggering apoptosis[93]. In the same way, 12- and 15-lipoxygenase gene expression may be down regulated by antisense oligonucleotides to trigger a decrease in the ratio of bcl-2/bax and tumour cell apoptosis[56,91,93]. Lipoxygenase inhibitors can trigger tumour cell apoptosis[93]. Lipoxygenase inhibitors such as NDGA and esculetin have been shown to suppress the proliferation of lung, breast and leukaemia tumour cell lines *in vitro* and *in vivo*[65,66,73].

2.7 Lipoxygenase inhibitors

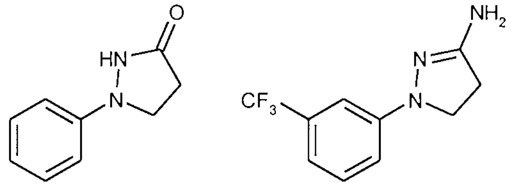
Lipoxygenase inhibitors may be classified into four main categories according to their mechanism of inhibition; antioxidants and/or free radical scavengers, iron chelators, substrate analogues and lipoxygenase activating protein inhibitors. Although quite a few lipoxygenase inhibitors are known, very few are selective towards specific lipoxygenases. A wide variety of agents have been reported as 5-lipoxygenase inhibitors and many of them are not selective 5-lipoxygenase inhibitors but often affect cyclo-oxygenase and other lipoxygenases.

2.7.1 Antioxidants

The majority of lipoxygenase inhibitors are antioxidants. Most of these compounds competitively inhibit the active centre of the enzyme and reduce the active ferric ion (Fe^{3+}) to the inactive ferrous state (Fe^{2+}). They may also interact with the fatty acid radical intermediate produced during the catalytic step (**Figure 2.2**)[62,95]. Depending upon the class of inhibitor, reversible or irreversible lipoxygenase inhibition can be achieved[44]. A problem identified with these compounds is their lack of specificity because of the ubiquitous nature of the oxidative processes in biological systems[62].

2.7.1.1 Pyrazoline derivatives

The pyrazoline derivatives, phenidone (1-phenyl-3-pyrazolidone) **1** and BW-755c (3-aminopyrazoline) **2** are examples of irreversible iron reducing agents. Phenidone, **1** inhibits both cyclo-oxygenase and platelet 12-lipoxygenase while BW-755c **2** inhibits 5-lipoxygenase and cyclo-oxygenase[96].



1 **2**

Figure 2.4 Structure of the pyrazoline lipoxygenase inhibitors, phenidone **1** and BW755c **2**

The presence of a hydrazine moiety which is easily oxidised in BW-755c **2** could be considered as responsible for its inhibitory activity[96]. It has been shown, however, that it is not the pyrazoline derivatives that are responsible for the inactivation of the lipoxygenase enzymes. It is thought that a radical species formed during the oxidation of the inhibitor binds a methionine residue in the catalytic centre inhibiting the enzyme irreversibly[44]. Compounds in this group tend to be dual cyclo-oxygenase and lipoxygenase inhibitors with relatively low specificity.

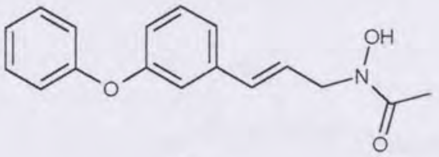
2.7.1.2 Hydroxamic acids

Hydroxamic acid containing molecules rank among the most potent inhibitors of 5-lipoxygenase. Their action on other lipoxygenases and cyclo-oxygenases when investigated suggests that generally, they have a low specificity[97,98,99,100]. Hydroxamates have the ability to chelate ferric iron (Fe^{3+}) which contributes towards their inhibitory effect on lipoxygenase enzymes. Hydroxamic acids are also able to reduce the catalytic iron of lipoxygenase to its inactive ferrous (Fe^{2+}) state[44,62,97,98,99,101].

Type A Type B

Figure 2.5 Type A and Type B hydroxamic acid lipoxygenase inhibitors

Type A Hydroxamic acids with a relatively large group attached to the carbonyl of the hydroxamate functionality and a small substituent appended to the nitrogen are extremely potent 5-lipoxygenase inhibitors *in vitro*, but lose their activity *in vivo* due to their rapid metabolism to the corresponding carboxylic acid[98,99]. Type B hydroxamic acids have the opposite substitution pattern which makes them generally resistant to this metabolism and makes them more potent orally active inhibitors[98,99]. The type B hydroxamic acid (*N*-[(*E*)-3-(3-phenoxyphenyl)prop-2-enyl]acetohydroxamic acid), BW-A4C **3** is quite selective for 5-lipoxygenase inhibition (IC_{50}=0.14μM)[62,96,102,103]. Conversion of type B hydroxamic acids to hydroxyureas has led to the further enhancements of oral bioavailability and *in vivo* potency for 5-lipoxygenase, for example, zileuton **4** and BW-B70C **5**[96]. (*N*-(1-Benzo[*b*]thien-2-ylethyl)-*N*-hydroxyurea), Zileuton **4** was the first lipoxygenase inhibitor to make it to the market. It is relatively selective for 5-lipoxygenase (5-lipoxygenase IC_{50}=0.5μM, platelet 12-lipoxygenase IC_{50}>100μM, cyclo-oxygenase IC_{50}>100μM)[104] and so is indicated for asthma therapy[44,104]. BHPP **6**, a phenylpentamamide, is a classic example of a hydroxamic acid with antioxidant qualities. *N*-hydroxylamines and phenylhydrazone derivatives are related compounds that have been shown to have similar lipoxygenase inhibitor properties as the hydroxamic acids[44].



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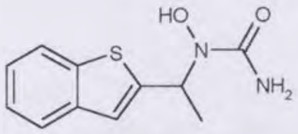

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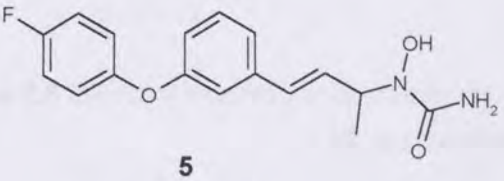

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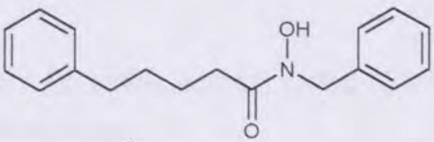

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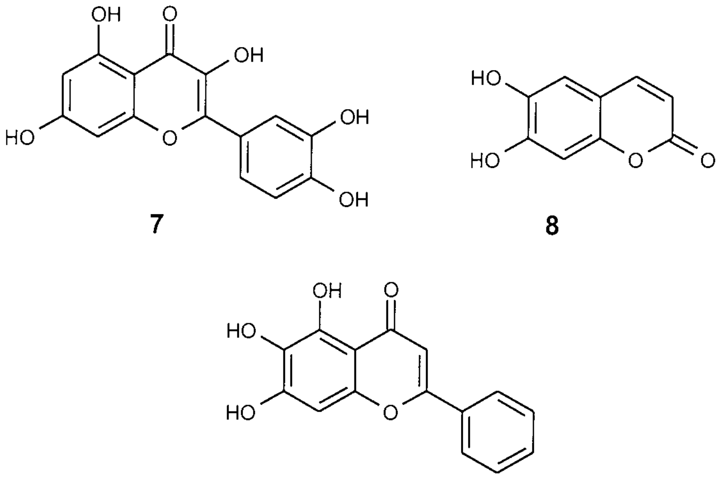
Figure 2.6 Structure of BW-A4C **3**, Zileuton **4**, BW-B70C **5**, and BHPP **6**

2.7.1.3 Phenolic compounds and related substances

Phenols are well known as reducing agents and inhibitors of lipoxygenase enzymes. Certain flavonoids, such as quercetin **7** and esculetin **8** and baicalein **9** have been identified as natural lipoxygenase inhibitors[36,96].

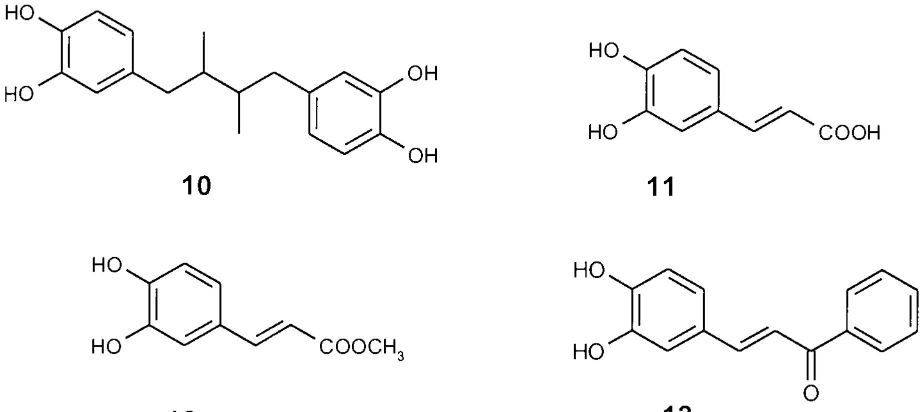


7 **8**

9

Figure 2.7 Natural phenolic lipoxygenase inhibitors, quercetin **7**, esculetin **8** and baicalein **9**

Esculetin **8** and baicalein **9** are reported to exhibit some selectivity towards 12-lipoxygenase[44,105]. A very large number of lipoxygenase inhibitors contain a catechol group. The general lipoxygenase inhibitor NDGA **10** is by far the most commonly used in pharmacological studies[36,96]. Although a potent inhibitor NDGA **10** demonstrates a very low specificity and inhibits lipoxygenase and cyclo-oxygenase families[44]. A large number of natural catechol compounds inhibit 5-lipoxygenase, such as pyrogallol, gossypol and caffeic acid **11**[96].



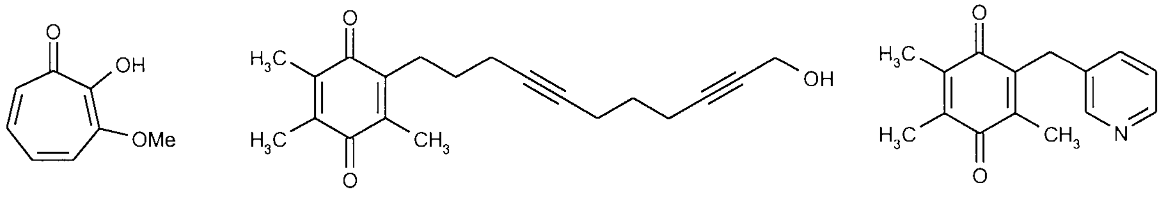
10 **11**

12 **13**

Figure 2.8 Catechol inhibitors of lipoxygenase, NDGA **10**, caffeic acid **11**, caffeic acid methyl ester **12** and 3,4-dihydroxychalcone **13**

Caffeic acid **11** and its methyl ester **12** have 5-lipoxygenase activity (IC_{50}=3.7μM and 0.48μM respectively), although they are not specific. Caffeic acid and caffeic acid methyl ester inhibit 5-lipoxygenase and platelet 12-lipoxygenase although they do not inhibit cyclo-oxygenase[105]. Various methyl ester derivatives masking the catechol moiety fail to inhibit lipoxygenase enzyme activity[105]. Hydroxychalcones have been described as more potent inhibitors of 5-lipoxygenase than the catechols NDGA **10**, quercetin **7** and caffeic acid **11**[106]. 3,4-Hydroxychalcone **13** inhibits 5-lipoxygenase with an IC_{50} of 0.004μM but is not specific to lipoxygenase, cyclo-oxygenase is also inhibited[106]. The development of caffeic acid analogues with a 3,4-dihydroxycinnamoyl and a cyano group has led to relatively selective inhibitors of 12-lipoxygenase[44,107,134]. Unique to this group of catechols is the non-competitive mechanism of inhibition shown by kinetic studies[44].

Other lipoxygenase inhibitors have been described that may be metabolised to phenols. The microbial metabolite, 3-methoxytropolone **14**, isolated from *Streptoverticillium hadanonense* was reported to have inhibitory selectivity against platelet 12-lipoxygenase (IC_{50}=1.8μM) over 5-lipoxygenase and cyclo-oxygenase[37,44,108]. Quinones are easily reduced to the potentially lipoxygenase inhibiting hydroquinones[96], examples include AA861 **15** and CV-6504 [2,3,5-trimethyl-6-(3-pyridylmethyl)-1,4-benzoquinone] **16**[62,96,109]. CV-6504 **16** is an orally active 5-lipoxygenase inhibitor (IC_{50} 0.062μM) and also a potent inhibitor of TXA_2 synthase (IC_{50}=0.4μM)[62,109].



14 **15** **16**

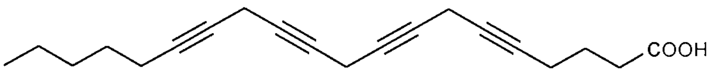
Figure 2.9 Structure of 3-methoxytropolone **14** and the quinone lipoxygenase inhibitors, AA861 **15** and CV-6504 **16**

2.7.2 Iron chelators

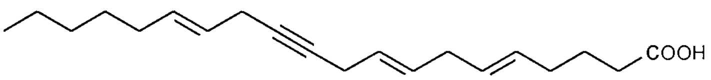
Iron chelators are substances with the ability to build complexes with the protein bound ferric ion (Fe^{3+}) of the lipoxygenase enzyme. Classic examples have already been mentioned, catechols and hydroxamic acids. Catechols have been shown to form bidentate complexes with the ferric ion in the active site of lipoxygenases[95,110]. Many of these compounds exhibit an additional reducing ability which strengthens their inhibitory effect. There are, however, some substances that are purely complex building inhibitors. Catechols with electron-withdrawing groups form stable ferric complexes, whereas those with electron-rich substituents are rather prone to reducing the iron[44].

2.7.3 Substrate analogues

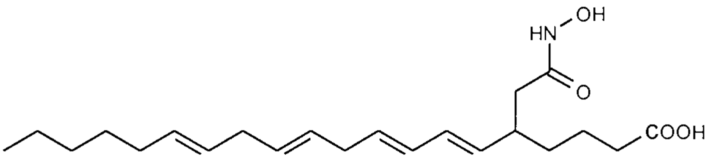
Early efforts to inhibit lipoxygenases focussed on lipoxygenase substrate analogues, with the aim of creating mechanism-based irreversible inhibitors by removing or replacing the substrate protons abstracted by the enzymes, thus mimicking fatty acids. The majority of compounds in this class contain acetylenic groups, which are responsible for the irreversible inactivation of the enzyme[111]. It is proposed that these compounds form highly reactive allene hydroperoxides, which covalently bind to an essential methionine residue in the catalytic centre, thus inhibiting the enzyme irreversibly[44]. The first acetylenic acids to be made contained triple bonds in place of all the double bonds in AA to give 5,8,11,14-eicosatraynoic acid (ETYA) **17** but these exhibited a low specificity, being inhibitory to both lipoxygenases and cyclo-oxygenases[44,111,112]. Related compounds have been developed to increase the selectivity of inhibition. Dehydroarachidonic acids (DHAs) are modified AA, in which the single acetylenic bond is positioned according to the targeted lipoxygenase subfamily, e.g. 14,15-DHA inhibits 15-lipoxygenase selectively just as 11,12-DHA **18** inhibits 12-lipoxygenase[44].



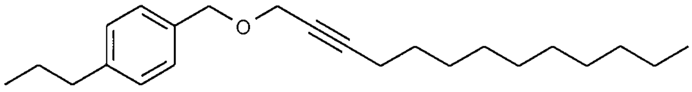
17



18



19



20

Figure 2.10 Structure of substrate analogue type lipoxygenase inhibitors, ETYA **17**, 11,12-DHA **18**, 5-hydroxamylmethyl-6,8,11,14-eicosatetraenoic acid **19**, carboxyalkylbenzylpropyl ether **20**

Eicosatetraenoic acids have been synthesised with various other functional groups at the C-5 position such as acids and amines to examine the possibility of ionic binding. Other groups such as hydroxyl and thiol groups have been put there to explore potential H-bonding interactions, all have been found as non-specific cyclo-oxygenase and lipoxygenase inhibitors[113]. As already mentioned the hydroxamic acid moiety is known to chelate iron. Eicosatetraenehydroxamates such as 5-hydroxamylmethyl-6,8,11,14-eicosatetraenoic acid **19**, have been synthesised with a hydroxamic acid moiety placed at the C-5 position of AA to give *in vitro* competitive inhibition of 5-lipoxygenase (IC_{50}=0.19μM), with no reported specificity or action on other 5-lipoxygenase substrates[113,114].

Other approaches have included methylated and cyclised analogues of AA[96,113,115]. The (carboxyalkyl)benzyl proparyl ether derivatives **20** are thought to act as acetylenic substrate analogues of AA to give a mechanism based inhibition of lipoxygenase. With these compounds a triple bond and a phenyl ring has been introduced to the molecule giving a series of substrate analogues exhibiting relative selectivity for leukocyte 12-lipoxygenase below 10μM[44,116].

2.7.4 Indirect Inhibitors

A novel target for the inhibition of 5-lipoxygenase is the transmembrane 5-lipoxygenase activating protein (FLAP) which is essential for 5-lipoxygenase activity[95]. The mechanism of co-operativity between FLAP and 5-lipoxygenase is not completely understood. Two general proposals have been offered. FLAP may act to anchor the cytosolic 5-lipoxygenase to the cell membrane following activation[46,62,96] or FLAP may be a substrate presenting protein for 5-lipoxygenase[101]. Two types of compounds have been found to inhibit FLAP, the indole containing compounds, for example MK-866 **21** and quinoline containing compounds which are second generation, more potent FLAP inhibitors. Both of these classes of compounds bind to FLAP and block the formation of the FLAP-LO complex[38,44,46,96,101].

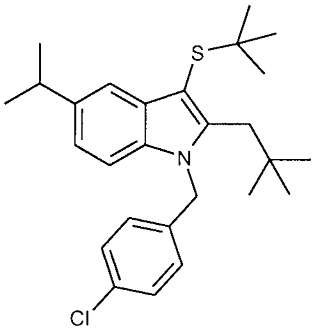


Figure 2.11 Structure of the FLAP inhibitor, MK-866 **21**

A transmembrane binding protein for 15-lipoxygenase has not been identified, although one has been found for platelet 12-lipoxygenase[117]. As yet no inhibitors of 12-lipoxygenase binding protein have been described.

Chapter 3

Library synthesis and biological testing

The products of the 12- and 15-lipoxygenase pathway play an important role in tumour cell metastasis and formation of secondary colonies. It has been shown that inhibitors of lipoxygenase can induce tumour cell apoptosis and reduce metastasis. The 5-lipoxygenase inhibitor CV-6504 **16** has been found to have a potent anti-tumour effect against the established murine adenocarcinoma cell lines, MAC13 and MAC16[119,123]. The non-specific action of the CV-6504 **16** on the 12 and 15-lipoxygenase pathways within the tumour cells was thought to bring about the anti-tumour effect. The aim of this work was to synthesise a library of compounds with the potential to inhibit the 12 and 15-lipoxygenase pathways of MAC13 and MAC16 tumour cells. The compound library prepared was based on caffeic acid analogues previously reported as active 12- and 15-lipoxygenase inhibitors. The library compounds were tested for MAC13 and MAC16 anti-proliferative effect *in vitro* and each was compared to the standard CV-6504 **16** which was used as a control for each assay. Compounds which were found to have a similar or better anti-tumour activity than CV-6504 **16** in the first assay were synthesised again as fully characterised compounds. These active compounds were screened again and IC_{50} values obtained for MAC13 and MAC16 activity. Active compounds were then subjected to a test for general toxicity to normal cells. The least toxic to normal cells with the best anti-tumour activity were tested to determine their 15-lipoxygenase inhibitory activity.

There were three main aims of this work: Firstly, was to find a compound with greater anti-proliferative activity than the 5-lipoxygenase inhibitor, CV-6504 **16** against the two cancer cell lines, MAC13 and MAC16. Secondly, to assess any compounds found with anti-proliferative activity, for their general toxicity to healthy cells. Thirdly, to determine whether the mode of action of active compounds was through the lipoxygenase pathway.

3.1 Why were MAC tumour cell lines chosen for anti-cancer screening?

Previous studies[118] have demonstrated that the established tumours of the MAC series are generally refractory to standard cytotoxic agents. Of these MAC tumour cell lines MAC13 is the most responsive to standard cytotoxic agents[73,118,119]. Growth of MAC13 and MAC16 tumours is stimulated by PUFAs suggesting that lipoxygenase or cyclo-oxygenase pathways are important in the growth and proliferation of these cell lines[119]. In addition, MAC tumour cell lines produce solid tumours which are difficult to treat with standard cytotoxic agents, because they are slower growing than the leukaemias and also have areas of hypoxia where there is no blood supply to deliver chemotherapeutic agents to the tumour cells.

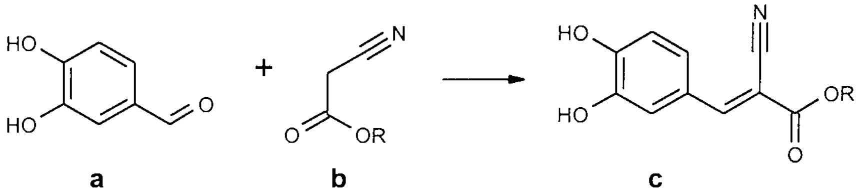
3.2 Why was CV-6504 chosen as the control compound?

The 5-lipoxygenase inhibitor, (CV-6504) **16**, has previously been tested along with various other lipoxygenase inhibitors (BW-A4C **3**, zileuton **4** and BWB-70C **5**) for its effect against MAC13 and MAC16 *in vitro* and *in vivo*[119]. BW-A4C **3** and BWB-70C **5** were found to be potent inhibitors of MAC tumour cell growth. In contrast, Zileuton **4** had lower activity towards the MAC cell lines. CV-6504 **16**, however demonstrated the most marked anti-tumour effect of all the lipoxygenase inhibitors tested[73,119]. The anti-tumour activity of the 5-lipoxygenase inhibitors was thought to have been due to the non-specific nature of their lipoxygenase inhibition. This was confirmed when the relatively 5-lipoxygenase specific zileuton showed little MAC growth inhibition and that CV-6504 **16** inhibited the production of 5-,12- and 15-HETE in MAC13 cells *in vitro*[73]. MAC16 has been found to be generally unresponsive to standard chemotherapeutic agents, however, CV-6504 **16** had a marked anti-tumour activity on both MAC16 and MAC13[118,119]. Histological studies showed that CV-6504 **16** also exerted its MAC16 anti-tumour activity in regions distant from a vascular supply and therefore generally inaccessible to standard drugs[119].

For most anti-tumour agents activity is usually only demonstrated at the maximum tolerated dose, CV-6504 **16**, however, exerted its anti-tumour activity at a dose with a high margin of safety and was found to have a therapeutic index of at least 10[73,119]. This high measure of safety together with the potent anti-tumour activity suggests that CV-6504 and other 12- and 15-lipoxygenase inhibitors may act as potential anti-tumour agents without the unacceptable side effects of traditional chemotherapeutic agents.

3.3 Why were caffeic acid derivatives chosen for initial library synthesis?

Caffeic acid has been reported to inhibit 5-lipoxygenase[134] but not 12- or 15-lipoxygenase. Previous work[134] has found that a series of caffeic acid derivatives (**c**) synthesised by the Knoevenagel condensation of 3,4-dihydroxybenzaldehyde (**a**) and cyanoacetate derivatives (**b**), exhibited potent inhibitory activity on platelet 12-lipoxygenase. These types of compounds have also been described as tyrophostins, inhibitors of protein kinase[120,121,122].



a **b** **c**

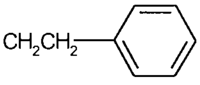
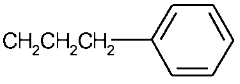
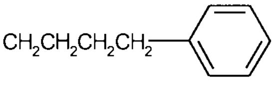
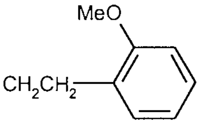
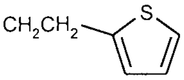
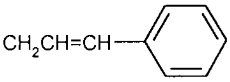
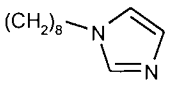
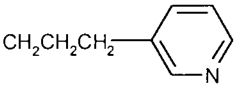
	R	IC_{50} (μM)		
		12-Lipoxygenase	15-Lipoxygenase	5-Lipoxygenase
22	CH_2CH_3	0.033	0.33	>10
23		0.051	0.43	0.16
24		0.053	1.40	0.16
25		0.47	3.59	0.22
26		0.064	1.62	0.14
27		0.013	0.50	0.09
28		0.063	3.33	1.89
29		0.18	>10	>10
30		0.47	3.93	>10

Table 3.1 Inhibitory activity of caffeic acid derivatives formed by the Knoevenagel condensation of 3,4-benzaldehyde and various cyanoacetic esters[134]

The compounds **22-30** were tested in assays against cyclo-oxygenase, 12-, 5- and 15-lipoxygenase. The screening showed that none of the compounds exhibited any activity against cyclo-oxygenase[134]. All of the compounds showed platelet 12-lipoxygenase inhibitory activity, some such as **29** and **30** were relatively selective for 12-lipoxygenase over the other two enzymes[134]. None of these compounds have been reported as anti-tumour agents. Hence, it was decided to prepare a library of compounds resembling the caffeic acid derivatives **22-30**, by performing the Knoevenagel condensation in a parallel automated solution-phase procedure with a range of aldehydes and cyanoacetic esters. This library could then be subjected to the MAC13 and MAC16 anti-cancer screen.

3.4 Initial library synthesis

An initial library of Knoevenagel products was prepared using automated parallel solution-phase synthesis (**Section 1.2.4.4**). A robotic pipetting station was used to transfer stock solutions of 13 active methylene reagents and piperidine in ethanol, and stock solutions of 48 aldehydes in ethanol to a matrix of 90 empty vials. Most of the cyanoacetic esters were chosen from commercially available compounds. Others were prepared by a transesterification reaction of methyl cyanoacetate and the appropriate alcohol. A heating block was used to heat the matrix of reactions for 2 hours at 77°C. Within each matrix of 90 vials, each separate vial contained only one active methylene reagent and only one aldehyde building block, to give one product per vial. A total of 13 active methylene reagents and 48 aldehydes were combined in a total of 624 separate reaction products (**Table 3.2** and **Table 3.3**). On cooling many of the products formed solids. Automation was used to wash the products of the Knoevenagel reaction in an attempt to remove the excess unreacted aldehyde and the piperidine catalyst. Ethanol was transferred via pipette to the product vials, allowed to stand and then removed; a process that was repeated a further two times. To increase the recovery of the more soluble products that dissolved in the ethanol, diethyl ether was used to wash the compounds three times. For products soluble in diethyl ether, the first wash was performed with diethyl ether and the second and third with petrol 40-60°.

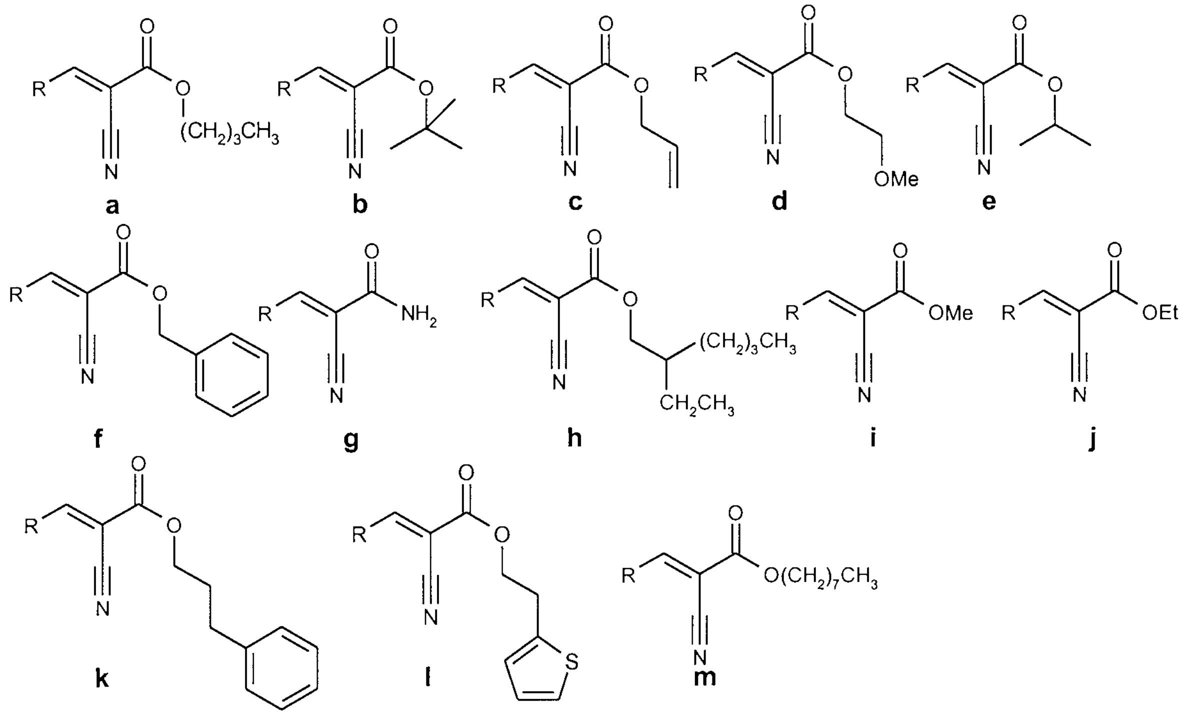


Table 3.2 General structures of the initial library products

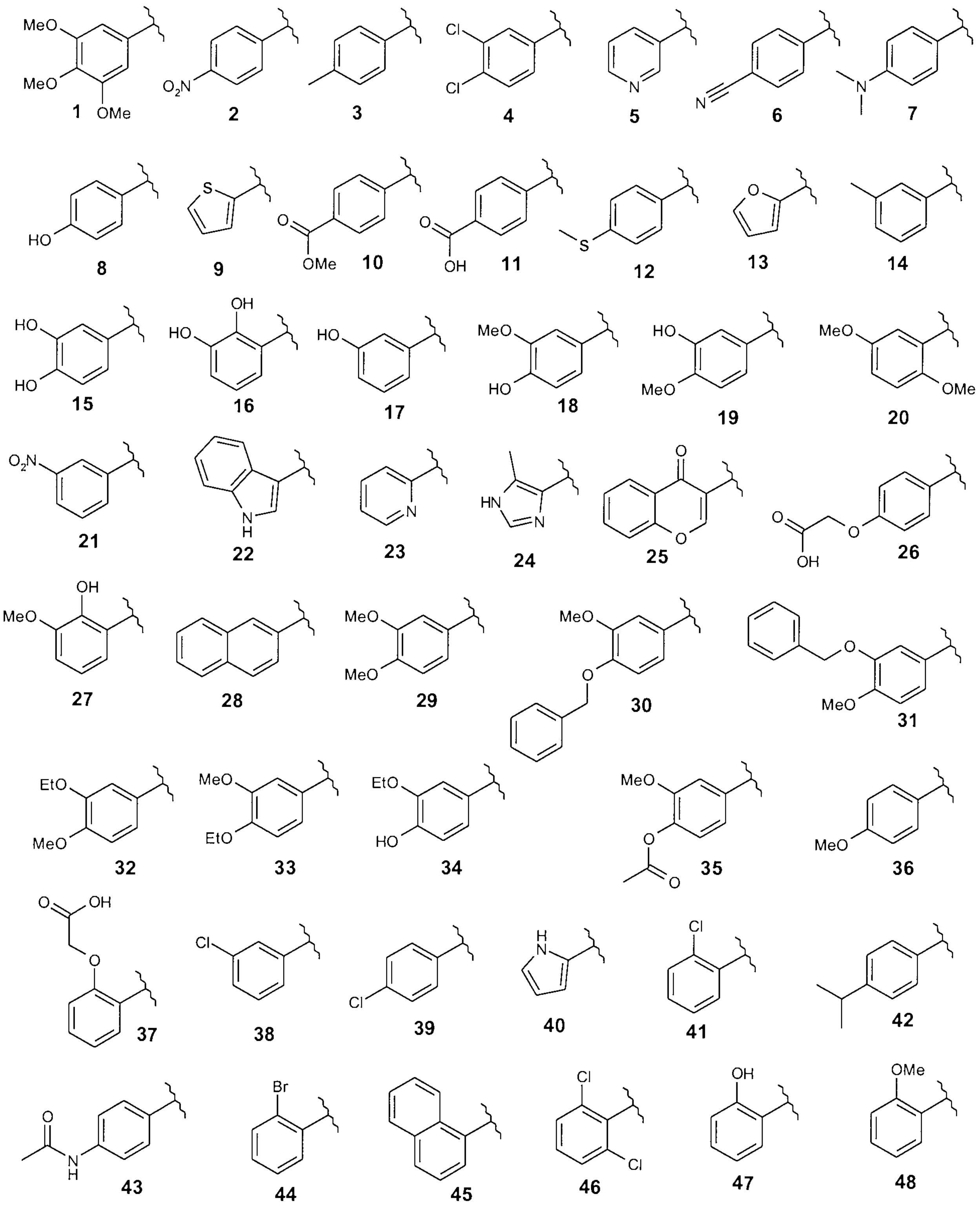


Table 3.3 Aromatic R functionalities of the initial library products

After the initial library synthesis all compounds were analysed by TLC and a range of compounds were analysed by ^1H NMR. At this stage all the products prepared were screened for inhibitory activity against the two cancer cell lines MAC13 and MAC16. Each compound was tested once at concentrations of 100, 50, 25, 10, 5 and 2.5μM, so that an indication of the IC_{50} values of any active compounds could be obtained. For each set of Knoevenagel products tested the compound CV-6504 **16** was used as a control.

3.5 Biological results

A MAC16 and MAC13 screen was set up in a 96 well plate format using resazurin dye to measure the extent of cell growth/death. The preliminary MAC13 and MAC16 96 well screens were run concomitantly with a 24 well plate screen used in the original literature, measuring total cell count. The reference compound in these experiments was the lipoxygenase inhibitor CV-6504 **16**. The two screens were found to give comparable IC_{50} values for both MAC16 and MAC13. The results obtained, however, were not the same as those expressed in the original literature, the IC_{50} values were found to be much higher (IC_{50} MAC16 5-10μM and MAC13 25-50μM) and different for the two cell lines. The original literature had found CV-6504 to have the same IC_{50} for each cell line, IC_{50} 3μM. Further tests were performed on the lipoxygenase inhibitors BW-A4C **3** and BWB-70C **5** which had also been reported in the original literature. Again the IC_{50} values obtained for these compounds were higher than originally reported[123].

Compound	MAC16 IC_{50} (μM)		MAC13 IC_{50} (μM)	
	Literature value	Our value	Literature value	Our value
CV-6504	3.0±1	10.9±0.2	3.0±1	48.0±0.5
BWB-70C	5.0±0	64.1±1.7	2.0±1	>100
BW-A4C	4.0±1	11.7±1.0	3.0±1	26.2±4.5

Table 3.4 Screening values for the lipoxygenase inhibitors, CV-6504 **16**, BW-B70C **5** and BW-A4C **3**

It was decided to proceed with the biological screen using CV-6504 as the standard whose results were to be the target and as the control for each assay. All library products were tested once in the two screens. Stock solutions of initial library products were made up on the assumption that the compound tested was the desired product.

The results of the initial biological screen suggested that it was the aromatic or heterocyclic portion of the compound (i.e. the part of the compound provided by the starting aldehyde) that conferred the anti-proliferative activity to the molecule. Only the products of certain aldehydes showed activity, see **Table 3.5**. None of the compounds tested showed a greater anti-proliferative activity than the test compound, CV-6504, for the MAC16 cell line. One compound, **g46** showed a greater anti-proliferative activity than CV-6504 for the MAC13 cell line.

Compound	IC$_{50}$ (μM)	
	MAC16	MAC13
CV-6504	10.9±0.2*	48.0±0.5*
a15	50-100	>100
b15	>100	>100
c15	>100	>100
d15	>100	>100
e15	>100	>100
f15	>100	>100
g15	50-100	>100
h15	>100	>100
i15	50-100	>100
j15	50-100	>100
k15	>100	>100
l15	50-100	>100
m15	>100	>100
a16	25-50	>100
b16	25-50	>100
c16	25-50	>100
d16	25-50	X
e16	50-100	>100
f16	25-50	>100
h16	25-50	>100
i16	10-25	25-50
j16	25-50	>100
k16	25-50	50-100
l16	25-50	X
m16	25-50	>100
a20	>100	X
d20	>100	>100
e20	>100	>100
a25	50-100	>100
b25	25-50	50-100
c25	50-100	>100
d25	50-100	50-100
e25	25-50	>100
f25	50-100	>100
g25	>100	>100
f20	>100	>100
g20	>100	>100
h20	>100	X
i20	>100	X
j20	>100	X
k20	>100	X
l20	>100	>100
m20	>100	X
h25	>100	>100
i25	50-100	>100
j25	50-100	>100
k25	50-100	50-100
l25	>100	>100
m25	50-100	50-100
a27	50-100	X
b27	50-100	X
c27	50-100	X
d27	50-100	X
e27	25-50	>100
f27	50-100	X
g27	50-100	X
h27	25-50	>100
i27	50-100	>100
j27	25-50	X
k27	25-50	50-100
l27	>100	X
m27	25-50	X
a46	>100	>100
b46	>100	>100
c46	>100	>100
d46	>100	>100
e46	>100	>100
f46	>100	>100
g46	25-50	25-50
h46	>100	X
i46	>100	>100
j46	>100	>100
k46	>100	>100
l46	>100	>100
m46	>100	>100
a47	25-50	>100
b47	>100	X
c47	25-50	>100
d47	25-50	>100
e47	25-50	>100
f47	>100	X
h47	25-50	X
i47	25-50	X
j47	>100	X

***n=96. X=Inactive**

Table 3.5 Products with some biological activity from the initial library

The caffeic acid derivatives expected to be active from the original literature[134], (the **15** series of compounds, see **Table 3.3**) showed minimal anti-proliferative activity in the MAC16 and MAC13 tumour cell lines. None of these compounds (**a-m15**) had greater activity than the standard CV-6504 **16**. Three of these compounds, also found in the original paper **j15** and **l15** were synthesised again and purified by traditional methods. When subjected to the biological screen (n=3) their activity was confirmed as being less than that of CV-6504 **16** (**Table 3.6**).

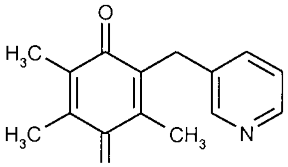
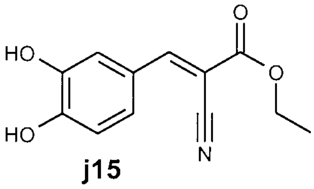
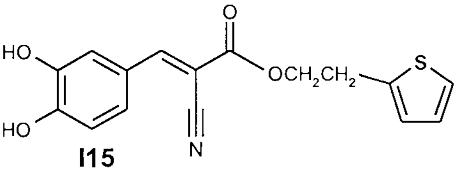
Compound	IC_{50} (µM)	
	MAC16	MAC13
 CV-6504 **16**	10.9±0.2 (n=96)	48.0±0.5 (n=96)
 **j15**	57.6±4.3 (n=3)	>100 (n=3)
 **l15**	86.4±3.0 (n=3)	>100 (n=3)

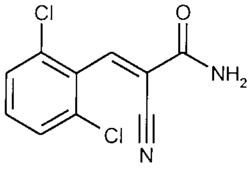
Table 3.6 Screening results of caffeic acid derivatives from original literature against MAC16 and MAC13 tumour cell lines compared with CV-6504 **16**

Compounds of the series **15, 16, 20, 25, 27, 46** and **47** (**Table 3.3**) were found to have some anti-proliferative activity. Series **15** and **20** gave minimal anti-proliferative activity, none of the compounds tested showed activity as good as or better than CV-6504 for the two tumour cell lines. It was decided to concentrate on the products which indicated that they would have an IC_{50} of less that 100µM for either cell line, for the creation of focussed libraries of compounds.

3.6 Focussed libraries

3.6.1 Compounds of series 46

One compound 2-cyano-3-(2,6-dichlorophenyl)prop-2-enamide **g46** showed good anti-proliferative activity in the primary screen (**Table 3.5**, MAC16 and MAC13 IC_{50} 25-50).

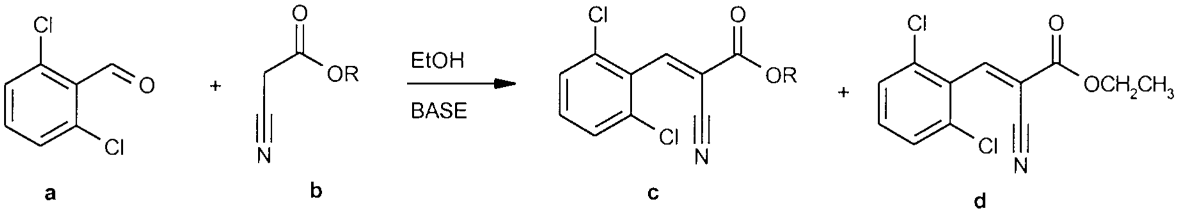


g46

Figure 3.1 Compound **g46** of the **46** compound series

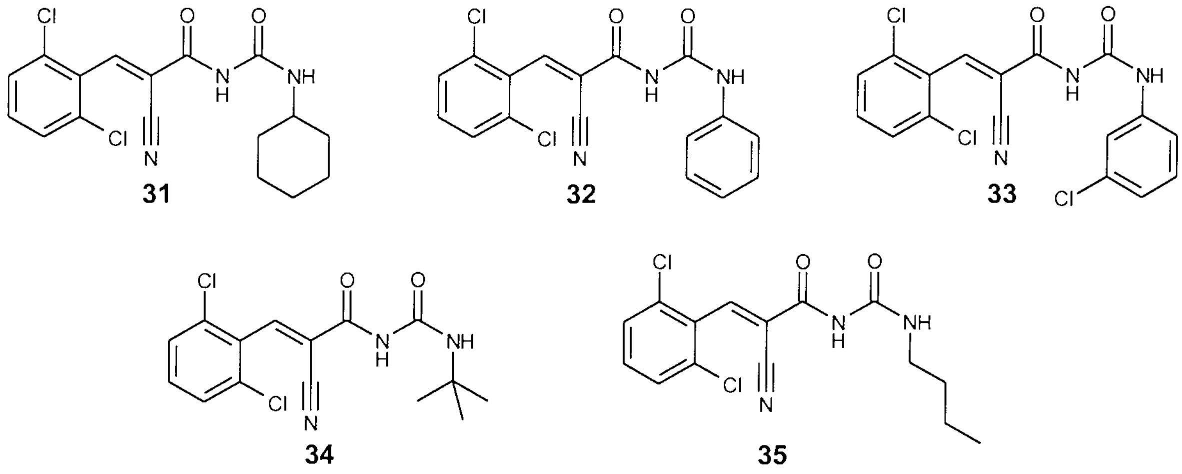
The compounds of the **46** series were subjected to complete analysis by ^1H NMR spectroscopy and mass spectrometry. This further analysis identified a problem of transesterification occurring between the ester group of the product and the solvent ethanol (**Scheme 3.1**). This meant that the solids tested were mixtures of the ethyl ester (**d**) and the desired products (**c**). After this finding it was decided to re-synthesise the **46** compound series and subject the new single compounds to the biological screen. A small focussed library of compounds was prepared by the Knoevenagel reaction of 2,6-dichlorobenzaldehyde (**a**) and a set of active methylene compounds (**b**). Toluene was used as the solvent to stop the secondary transesterification reaction occurring.

Biological screening of this focussed library confirmed the weak activity of the ester compounds against both MAC13 and MAC16 cell lines, and also confirmed the more potent activity of 2-cyano-3-(2,6-dichlorophenyl)prop-2-enamide **g46**. Biological screening of **g46** showed it to have an IC_{50} value for MAC16 of 45.9±8.3μM and MAC13 of 39.2±5.3μM (n=3).



Scheme 3.1 Knoevenagel reaction products where transesterification has occurred with ethanol

The effect of altering the amide functionality of 2-cyano-3-(2,6-dichlorophenyl)prop-2-enamide **g46** was investigated. 2-Cyano-3-(2,6-dichlorophenyl)prop-2-enamide **g46** was reacted successfully with the isocyanates, cyclohexylisocyanate to give **31**, phenylisocyanate to give **32**, 3-chlorophenylisocyanate to give **33**, *t*-butylisocyanate to give **34** and unsuccessfully with *n*-butylisocyanate **35** (**Figure 3.2**). The new analogues **31**, **32**, **33** and **34** were tested against both the MAC13 and MAC16 cell lines and all of them were found to have no anti-proliferative activity.

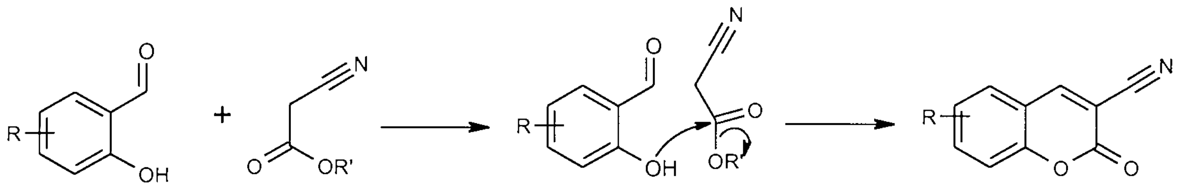


31 **32** **33** **34** **35**

Figure 3.2 Products of the reaction of the biologically active 2-cyano-3-(2,6-dichlorophenyl)prop-2-enamide **g46** with various isocyanates

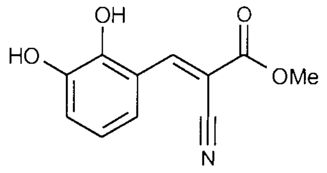
3.6.2 3-Cyanocoumarins

Products of the compound series **16**, **27** and **47** (**Table 3.3**) all gave good inhibitory activity against the MAC16 cell line and also showed some activity against the MAC13 cell line (**Table 3.5**). Further analysis of the products by mass spectrometry, however, showed that the parent ion expected was not present. ^{1}H NMR spectroscopy of these compounds showed that the products that were not clean and that the characteristic olefinic proton peak was missing from the spectra. It was clear from both the mass spectrum and the ^{1}H NMR that the starting aldehyde was no longer present. These three series of compounds have the common feature that they were all prepared from aldehydes with a hydroxyl substituent at the 2-position of the aromatic ring. Previous work[124,125,126,127,128] has reported the reaction of 2-hydroxybenzaldehydes and 2-hydroxyacetophenones with cyanoacetic esters to yield 3-cyanocoumarin products. It was anticipated that coumarins may have been formed with the **16, 27** and **47** series of compounds. It is possible that the lone pair of electrons on the 2-hydroxy group of the starting aldehyde had attacked the carbonyl carbon liberating the ester alkoxy group as an alcohol before the Knoevenagel condensation occurred which then yielded the 3-cyanocoumarin (**Scheme 3.2**).



Scheme 3.2 Possible coumarin formation after the Knoevenagel reaction of 2-hydroxybenzaldehydes and cyanoacetic esters.

It had to be determined what it was in the screened mixtures that was giving the anti-proliferative activity. The mixture that showed the greatest activity was that of **i16** (**Table 3.5**), IC_{50} for MAC16 10-25μM and MAC13 25-50μM. The clean Knoevenagel product of **i16** (**Figure 3.3**) was synthesised and purified using traditional methods.



i16

Figure 3.3 Structure of the Knoevenagel product **i16**

This clean Knoevenagel product **i16** when tested against both the MAC13 and MAC16 cell lines was shown to have only weak anti-proliferative activity with an IC_{50} value of greater than 100μM for both cell lines. This led to the suggestion that the coumarin compound was formed and was responsible for the activity of these products. Various substituted 3-cyanocoumarins have been described as anti-cancer agents with no mention of their mechanism of action[129]. Previous workers[130] have reported the coumarin, esculetin **8** (**Figure 2.7** and **3.4**) to be a potent and selective inhibitor of platelet 12-lipoxygenase (Platelet 12-lipoxygenase, IC_{50} value of 0.65μM). It was decided to put esculetin and a small selection of prepared 3-cyanocoumarins into the biological screen for anti-proliferative activity against the MAC13 and MAC16 cell lines.

The 3-cyanocoumarins prepared and biologically tested were selected as a diverse set from a virtual library of the possible 3-cyanocoumarins that could potentially be synthesised. The combinatorial chemistry software for Chem-X™ was used to build a library of the 3-cyanocoumarins that could potentially be prepared in solution. This virtual library of 3-cyanocoumarins molecules was constructed from commercially available 2-hydroxylbenzaldehydes. One way to select a small set of compounds for screening from a larger library is to find the smallest set of compounds which exhibit all the pharmacophores of the molecules within the entire library, the diverse set. The 35 molecule virtual library was then ordered for diversity and searched for the minimum number of compounds to cover the full pharmacophore diversity of the library. This diversity search gave a set of 12 coumarins (**Figure 3.4**).

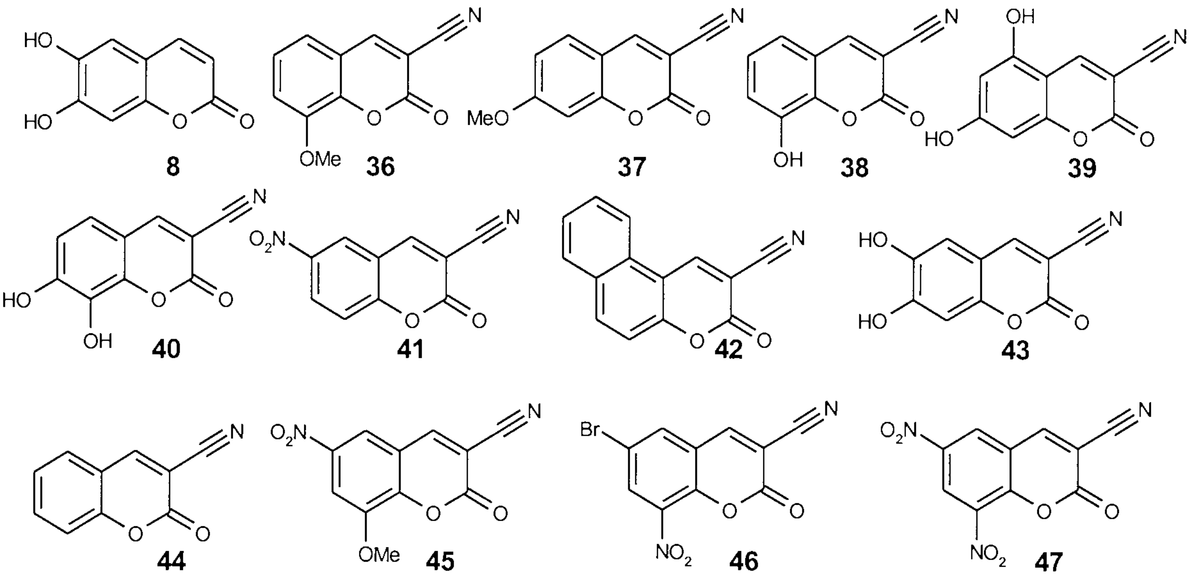


Figure 3.4 The structures of esculetin **8** and the 3-cyanocoumarin diverse set **36-47**

According to literature reports[126,127,128] the desired 3-cyanocoumarins (**36-47**) could be prepared by the reaction of ethyl cyanoacetate and the relevant 2-hydroxybenzaldehyde in the presence of catalytic piperidine, that is, the same reaction conditions as used in the initial Knoevenagel library. Previous experience with the initial Knoevenagel library products indicated that this method would give products difficult to purify. Previous workers[131] successfully prepared 3-cyanocoumarins in a one-pot procedure by the reaction, at room temperature of substituted 2-hydroxybenzaldehydes with malononitrile in heterogeneous alkaline aqueous medium (aqueous sodium hydrogen carbonate) with a subsequent acid treatment (concentrated HCl) at 90°C. This method provided the 3-cyanocoumarins as pure compounds by simple filtration[131]. All the 3-cyanocoumarins in the diverse set, except for coumarin **47** were synthesised, characterised and tested for their anti-tumour action against MAC13 and MAC16, esculetin **8** was also tested. CV-6504 **16**, was used as the control compound in each assay. Esculetin **8** gave no anti-proliferative activity in MAC13 or MAC16 tumour cell lines. The 3-cyanocoumarin **38**, showed the greatest activity against both cell lines, but was no more effective than CV-6504 **16**. The coumarins **36** and **44** did show some activity which would account for the positive results obtained in the initial biological screen, but they were still no better than the control CV-6504 **16**.

Compound	IC$_{50}$ (μM) (n=3 unless stated otherwise)	
	MAC16	MAC13
Esculetin 8	X	X
36	54.6±4.5	>100
37	>100	>100
38	43.9±0.8	47.3±1.5
39	>100	>100
40	88.2±5.5	>100
41	>100	>100

Compound	IC$_{50}$ (μM) (n=3 unless stated otherwise)	
	MAC16	MAC13
CV-6504 16	10.9±0.2 (n=96)	48.0±0.5 (n=96)
42	85.3±6.3	78.8±8.5
43	86.5±4.8	>100
44	79.0±4.5	74.8±3.8
45	>100	>100
46	>100	>100
47	-	-

X=Inactive, -=Not available for testing.

Table 3.7 Screening results of 3-cyanocoumarins compared to CV-6504 and esculetin

3.6.3 Chromenone library

The **(a-m)25** series of compounds (**Table 3.2** and **3.3**) from the initial library showed some anti-proliferative activity against MAC13 and MAC16. Some of these compounds gave activity in the same range as CV-6504 **16** for MAC13 and MAC16 (**Table 3.5**). Automated parallel synthesis was used to prepare a focussed library of Knoevenagel products using 12 commercially available 3-formylchromenones and a group of active methylene reagents (**Table 3.8** and **3.9**).

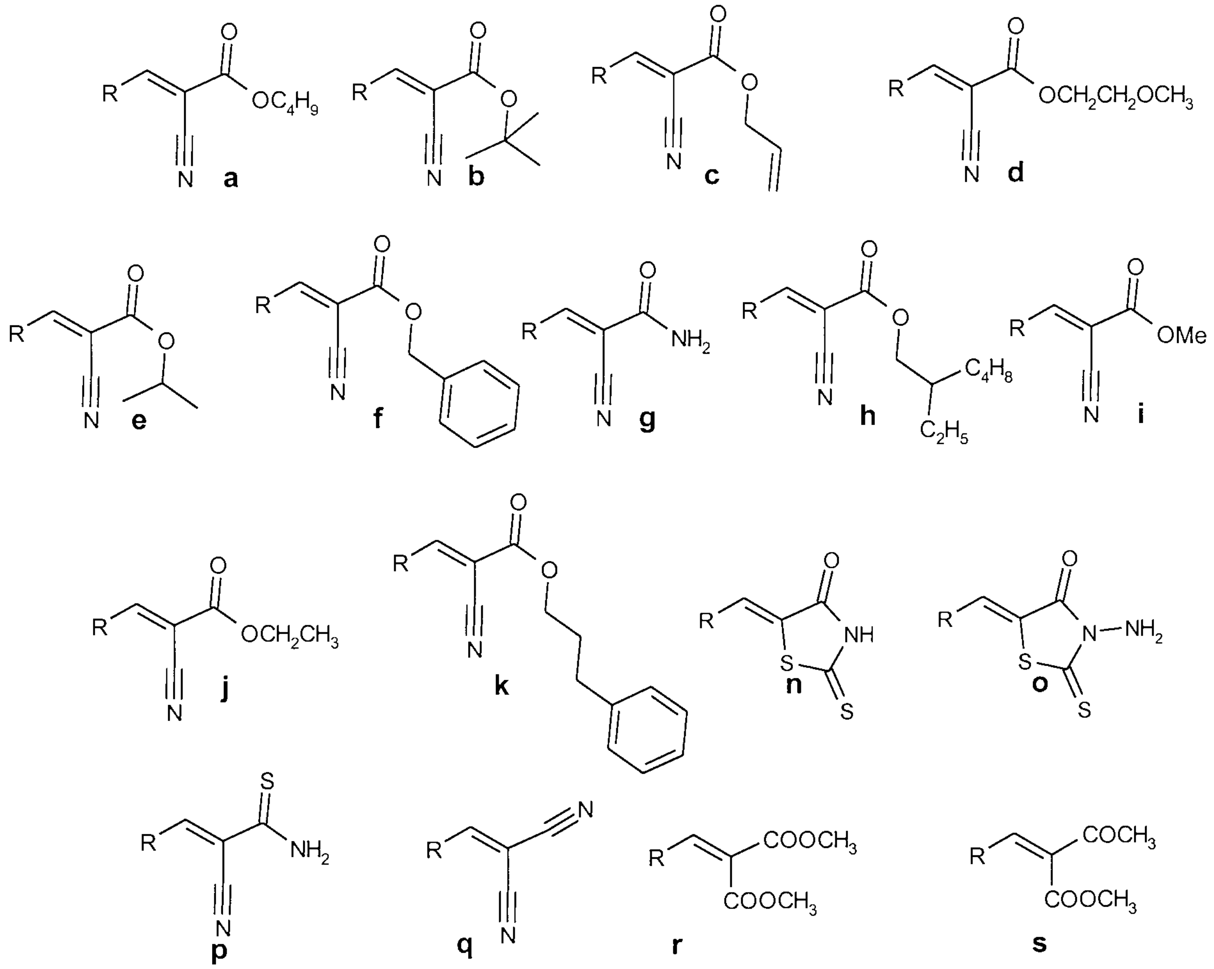


Table 3.8 General structures of the focussed chromenone library synthesis

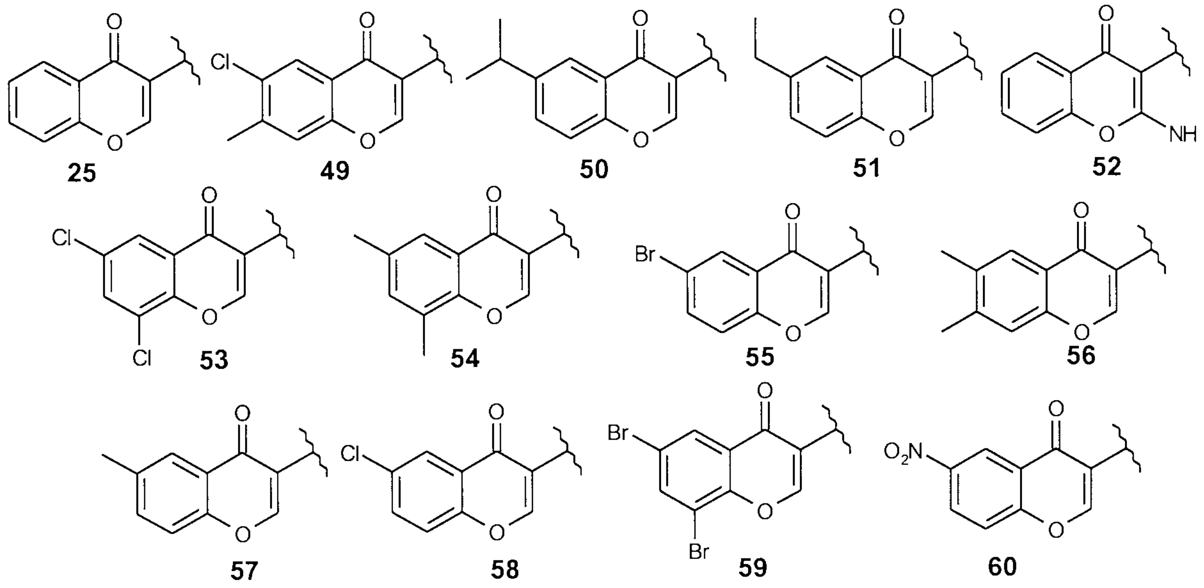


Table 3.9 Aromatic R functionalities of the focussed chromenone library

Each compound was characterised by TLC and mass spectrometry. Compounds without conclusive mass spectra were subjected to ^{1}H NMR analysis. Compounds prepared successfully in this focussed chromenone library were tested for anti-proliferative activity against both the MAC13 and MAC16 tumour cell lines. Active compounds were each tested at least three times to give IC_{50} values for each compound on each cell line. This focussed library gave rise to a large number of active compounds (**Table 3.10**). It was decided to concentrate on those compounds that gave IC_{50} values of <50μM against the MAC16 tumour cell line. The reason for concentrating on the MAC16 cell line being that it is reported to be the cell line that is the most resistant to standard chemotherapeutic agents[118].

3.7 Toxicity

Challenging freshly isolated human peripheral blood leukocytes with a drug is a useful alternative to *in vivo* challenge to assess drug toxicity[132,133]. The addition of a metabolising system, such as rat hepatic microsomes and nicotinamide adenine dinucleotide phosphate (NADPH), to the isolated blood leukocytes enables the leukocytes to be challenged with the reactive metabolites of the drugs[132,133]. Lymphocytes are attractive targets to determine cytotoxicity because they are relatively easy to obtain. Lymphocytes have a limited capacity to produce metabolites of drugs but do possess cellular defences such as epoxide hydrolase and glutathione which are able to detoxify reactive metabolites produced by the microsomal system[132,133]. Compounds and their reactive intermediates found to be toxic to leukocytes may overcome the leukocyte detoxification enzymes to cause cell death. Lymphocytes are useful because they do not proliferate and so the percentage of cell death can be determined by trypan blue exclusion.

Knowledge of the general toxicity of the selected active compounds was required. Standard chemotherapeutic agents are toxic to healthy cells as well as cancer cells leading to low tolerated doses and unacceptable side-effects. One of the reasons for using a lipoxygenase inhibitor to give anti-proliferative and anti-metastatic activity is the hope that lipoxygenase inhibitors will not cause these deleterious effects to healthy cells. It was not known at this stage how the compounds from the focussed chromenone library were acting to bring about their anti-cancer activity. The leukocyte cytotoxicity assay was performed on human leukocytes to determine the general toxicity of the chromenone anti-proliferative agents. Each active compound was incubated at a concentration of 250μM with the white blood cells of three different healthy volunteers for one hour. Percentage leukocyte death after a further incubation of 18 hours gave a crude indication of the cytotoxicity of the active compounds in relation to the control CV-6504 **16**. All the compounds had a much greater cytotoxicity than the control compound CV-6504 **16**, suggesting that the anti-proliferative activity observed with MAC13 and MAC16 cells was due to their general cytotoxicity.

In the body the majority of drugs are metabolised by the liver, in an attempt to make them more easily excreted. Often compounds are metabolised to less toxic products. The chromenone anti-proliferative agents could despite their cytotoxicity be of some use if they were to undergo metabolism to a less cytotoxic intermediate. The most active chromenones were tested for their cytotoxic action in the presence of a metabolising system of rat hepatocyte microsomes and NADPH. A control was also performed with rat hepatocyte microsomes and compound without NADPH. For all of the compounds the presence of a metabolising system did not significantly change the cytotoxicity of the compounds towards the leukocytes. These results suggest that any metabolites are just as cytotoxic as the compounds themselves. Compound toxicity could not be reduced by the presence of a metabolising system (**Table 3.10**).

Compound	IC$_{50}$ (μM) (n=3 unless stated otherwise)		% WBC* death	% WBC* death + microsomes	% WBC* death + microsomes + NADPH	% Inhibition of 15-lipoxygenase at 30μM (Panlabs)
	MAC16	MAC13				
CV-6504 16	10.9±0.2	48.0±.0.5	12.3±4.4	14.8±2.1	16.1±2.2	3
a25	32.4±3.1	52.9±0.9	31.9±6.7	29.8±6.2	35.5±6.1	2
e25	42.1±1.3	>100	33.4±2.5			
g46	45.9±8.3	39.2±5.3	24.7±7.9	20.8±8.9	20.5±10.8	
d49	41.1±1.3	51.2±3.5	28.1±8.4	24.3±9.9	18.7±7.0	
i50	28.6±2.3	>100	28.5±10.9	20.5±3.7	29.1±0.6	16
j50	37.3±1.9	>100	31.3±3.0			
b51	40.9±1.4	66.4±2.7	26.3±1.4			
e51	45.4±1.2	99.3±2.3	42.5±0.4			
b54	25.1±0.9	66.2±3.0	26.6±0.4	30.5±5.2	29.6±4.2	8
c54	47.4±1.9	85.5±3.5	26.8±2.3			
e54	40.1±0.4	66.9±0.5	55.7±2.4			
h54	32.5±1.7	62.9±0.6	32.8±2.1	26.5±6.3	30.6±6.7	8
i54	39.3±2.8	>100	22.2±5.3			
j54	47.4±1.3	>100	24.6±2.6			
b57	33.3±1.1	46.7±5.3	41.2±6.0	56.1±8.1	50.8±5.1	4
c57	34.1±2.9	72.6±1.3	27.5±6.0	27.2±0.5	24.4±5.3	11
e57	24.3±0.6	76.1±4.3	27.8±4.2	29.0±3.7	29.5±0.4	8
i57	30.5±4.3	>100	26.7±5.6	33.4±7.0	27.9±5.8	0
b58	47.2±2.8	79.9±4.3	25.3±4.0			
b94	45.8±1.8	>100	25.8±2.6			
a95	38.2±0.7	81.8±1.5	61.6±1.4			
b95	31.8±1.7	69.2±0.6	37.8±3.8	38.5±2.1	39.0±3.8	1
c95	35.9±0.2	57.8±1.5	51.4±6.2	58.2±2.0	53.5±8.4	4
d95	42.7±4.8	61.8±0.0	30.5±3.7			
i95	31.5±4.7	60.5±1.0	21.5±9.2	18.8±1.5	22.6±3.8	7

*Leukocytes may also be described as white blood cells (WBCs)

Table 3.10 Results of a general cytotoxicity screen for the anti-tumour chromenones

These results suggested that the chromenone compounds were generally cytotoxic rather than having specific toxicity to cancer cells, suggesting that their mechanism of cytotoxic action may not be through the lipoxygenase pathway. The most active chromenone compounds (**a25**, **i50**, **b54**, **h54**, **b57**, **c57**, **e57**, **i57**, **b95**, **c95** and **i95**) for MAC16 were also tested in a screening assay by Panlabs for 15-lipoxygenase activity at a concentration of 30μM (**Table 3.10**). None of these compounds had any 15-lipoxygenase inhibitory activity confirming the suggestion that their mode of anti-tumour action was one of general cytoxicity.

3.8 Conclusion

Lipoxygenase inhibitors have been shown to induce tumour cell apoptosis and are known to inhibit tumour cell growth and metastasis. It has been well documented that products of 12- and 15-lipoxygenase play an important role in tumour cell metastasis. It has been suggested that the anti-cancer effect of lipoxygenase inhibitors is effected through their inhibition of the 12- and 15-lipoxygenase enzymes found within the tumour cells.

Caffeic acid derivatives had previously been described as inhibitors of the lipoxygenase enzyme family, with a greater specificity for 12-lipoxygenase than for 5- and 15-lipoxygenase[107]. These caffeic acid derivatives were prepared by the Knoevenagel condensation of 3,4-dihydroxybenzaldehyde and various cyanoacetic esters. These literature compounds were originally tested directly for their lipoxygenase inhibitory activity and have not to date been described as anti-tumour agents. An initial caffeic acid analogue library of compounds was prepared, by automated parallel solution-phase synthesis, based on the Knoevenagel reaction of a range of aldehydes and active methylene reagents. All of the initial library products were tested against two murine tumour cell lines, MAC13 and MAC16 with the aim of finding a compound with a greater anti-tumour action than a standard lipoxygenase inhibitor CV-6504 **16**. MAC13 and MAC16 tumour cell lines were chosen because they are solid tumours which are generally difficult to treat and also because MAC16 is also generally resistant to standard chemotherapeutic agents.

Problems were identified with the initial library synthesis. Transesterification of certain products and the solvent ethanol was overcome by the use of different solvents for the preparation of a focussed 2,6-benzaldehyde library of Knoevenagel products. The formation of coumarins from Knoevenagel products with a 2-hydroxyl moiety on their aromatic ring, was identified and these compounds were found to have some anti-tumour activity. Computational analysis was used to create a virtual library of 3-cyanocoumain compounds and to find the diverse set of the library. This diverse set of coumarins was prepared and biologically screened but was found to have a lesser anti-tumour action than CV-6504.

The activity of the Knoevenagel products against MAC13 and MAC16, appeared to correlate to the aldehyde reagent used for their preparation, that is, a series of compounds could be identified as active. Interestingly, compounds described as lipoxygenase inhibitors in the original literature[107] when prepared and screened showed minimal anti-proliferative action. Compounds found to have the same range of activity as the standard CV-6504 were identified, focussed libraries of analogues were prepared and specific IC_{50} values were determined for them against both MAC13 and MAC16. From a focussed library of chromenone products a number of compounds were found to have a similar range of anti-tumour activity as the standard CV-6504 in the most resistant cell line MAC16.

Unlike standard chemotherapeutic agents lipoxygenase inhibitors are not generally cytotoxic making them an attractive alternative as potential anti-tumour agents. The active anti-tumour chromenone compounds were tested for their general cytotoxicity and also for their action against rabbit reticulocyte 15-lipoxygenase. It was found that library chromenone compounds were generally cytotoxic to healthy cells, an effect which was unchanged by the introduction of a metabolising system. The most active anti-tumour chromenone compounds were shown to have no inhibitory activity against 15-lipoxygenase. It is, therefore, suggested that these compounds, although able to exhibit an anti-tumour action were doing so through general cytotoxicity rather than through their ability to inhibit lipoxygenase enzymes within the tumour cell.

Chapter 4

Improved synthesis of Knoevenagel products using a solid-phase catalyst

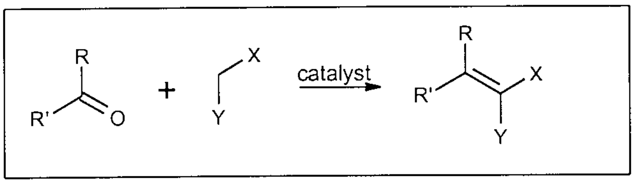
Automation of the solution-phase Knoevenagel condensation highlighted the problem of product purification when synthesising a large number of compounds in parallel. Automated purification depended on solid-solvent partitioning, which although it was efficient in removing the liquid catalyst piperidine, was not efficient in removing any insoluble by-products. Another problem that arose with this purification procedure was the low yields obtained when liquid products had been effectively 'washed away' by being dissolved in the solvent used to remove the catalyst.

An attempt was made to synthesise a catalyst bound to an insoluble polymer, that could be used in the Knoevenagel condensation. It was anticipated that the use of a polymer-bound catalyst would aid the purification procedure by easy removal by filtration and would provide improved recovery of liquid products.

This section will briefly discuss the reason for using the Knoevenagel condensation in automated synthesis (**Chapter 3**). Possible mechanistic reasons for the impurity problems encountered will be postulated. The synthesis and the use of a solid-bound catalyst will be described and compared to the use of the traditional catalyst, piperidine. Finally, the stereochemistry of the Knoevenagel products from both catalysts will be confirmed.

4.1 Why was the Knoevenagel condensation chosen for automated synthesis?

Caffeic acid analogues synthesised by the Knoevenagel condensation of 3,4-hydroxybenzaldehyde with a range of cyanoacetic esters, are known to be potent inhibitors of 12-lipoxygenase[134]. Inhibitory activity against 12-lipoxygenase suggested that these compounds may have some anti-cancer activity (**Section 3.3**). The Knoevenagel condensation, in principle is applicable to any aldehyde or ketone and any active methylene compound[136] (**Scheme 4.1**). The reaction usually proceeds in high yield making it suitable for solution-phase automated synthesis (**Section 1.2.5**).



X,Y=NO_2, CN, COAr/COAlkyl, CONHAr/CONHAlkyl,COOAlkyl, COOH, SO_2, S, Ar.

R= Ar, Alkyl R'=H, Ar, Alkyl.

Scheme 4.1 General Knoevenagel condensation equation

As previously described a range of aromatic aldehydes was heated under reflux for 2 hours in ethanol, with a set of active methylene substrates which were mainly cyanoacetic esters, in the presence of catalytic piperidine (**Chapter 3**, **Section 3.4**)[135]. Most of the products were free of impurities after three washes with organic solvents. Two disadvantages arose from these conventional synthetic conditions: First, there was a difficulty in catalyst removal from some liquid products and an unidentified catalyst-derived by-product removal from all products in which it was formed; secondly, certain products underwent a transesterification reaction with the alcohol solvent.

4.2 Why were the reaction conditions used chosen?

Many different solvents have been reported for the Knoevenagel reaction including, alcohols[136], benzene[136], DMF[137] etc. Knoevenagel catalysts include ammonia and ammonium salts, primary and secondary amines and their salts. Weak and strong basic ion exchange resins have catalysed the reaction[136]. More recently, zinc chloride ($ZnCl_2$)[138], bismuth (III) chloride[139], cadmium iodide[140] titanium tetrachloride[141], doped xonotlite[142], basic alumina (Al_2O_3) [143,144,145,146], $AlPO_4$-Al_2O_3[147], silica gel functionalised with amino groups[148], and heterogeneous clays such as montmorillonite[149] have been reported as catalysts. In condensations involving aldehydes and cyanoacetic esters, the most common catalyst reported is piperidine in a solution of alcohol[136,150], therefore, these conditions were chosen for automated synthesis.

4.3 Knoevenagel condensation by-products

4.3.1 Transesterification of Knoevenagel products

Analysis of the robotically synthesised Knoevenagel library products, by automated APCI mass spectrometry and ^{1}H NMR indicated a second problem. Caffeic acid analogues derived from certain aromatic aldehydes underwent significant amounts of transesterification with the alcoholic solvent (**Section 3.6.1**). The problem of transesterification could be overcome by the use of aprotic solvents but this led to problems with limited solubility of reactants and products.

4.3.2 Piperidine catalyst derived by-products.

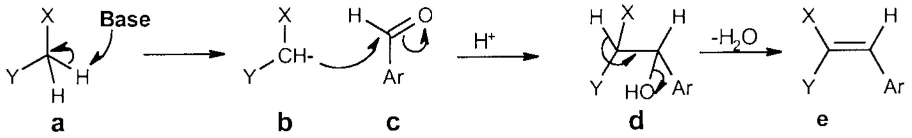
Analysis of some compounds synthesised by ^{1}H NMR spectroscopy showed that, as well as the desired product, a by-product had been formed which had not been removed in the washing process. This by-product had ^{1}H NMR resonances similar to those of piperidine but were shifted downfield in the ^{1}H NMR spectra. It was thought that the mechanism of reaction may hold some clues as to the identity of the unwanted reaction by product.

4.3.2.1 Mechanism of the Knoevenagel reaction

Two different mechanisms for the Knoevenagel condensation have been proposed, both of the mechanisms are thought to occur with the extent of one mechanism over the other thought to be dependant on reagents and catalyst used.

4.3.2.2 The Hann-Lapworth mechanism

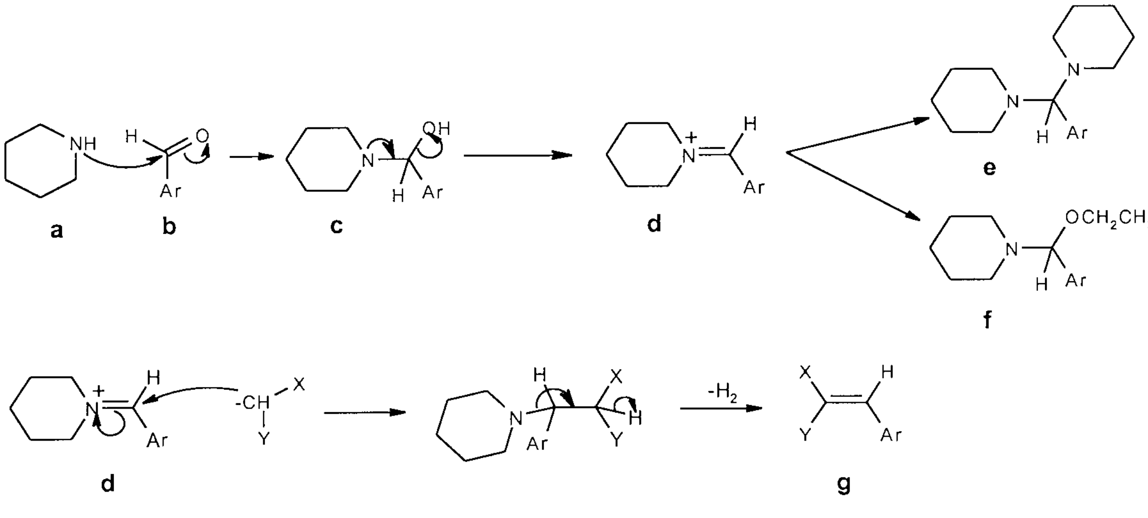
The Hann-Lapworth mechanism[136,151] (**Scheme 4.2**) suggests that the basic catalyst deprotonates the active methylene reagent (**a**) to form a carbanion (**b**) which can then attack the carbonyl function of the aldehyde (**c**). The Knoevenagel product (**e**) is obtained by dehydration of the tetrahedral intermediate (**d**). Evidence for this mechanism is dependant partly on isolation of the tetrahedral intermediate and partly on kinetic studies which show that addition of acid slows the rate of reaction. Condensations in polar media tend to favour this mechanism.



Scheme 4.2 Hann-Lapworth mechanism for the Knoevenagel condensation, X and Y are electron withdrawing moieties

4.3.2.3 The Knoevenagel mechanism[136]

The Knoevenagel mechanism (**Scheme 4.3**) was the first mechanism to be postulated. In this mechanism, the basic catalyst attacks the carbonyl function of the aldehyde (**b**) resulting with a secondary amine catalyst, for example piperidine (**a**) in the formation of a hemi-aminal (**c**) which rearranges to form the iminium ion (**d**). The iminium ion (**d**) can be further attacked by another catalyst molecule to form the aminal/*bis*-piperidine compound (**e**) or by the alcohol solvent, such as ethanol, resulting in the ether compound (**f**). It was suggested that the basic medium allows deprotonation of the active methylene compound which could then combine with the iminium ion (**d**), aminal (**e**) or ether (**f**), to give the final Knoevenagel product (**g**)[152]. Evidence of this mechanism has been gathered by isolation of the above intermediates **c**, **e** and **f** and their subsequent reaction with active methylenes to give desired Knoevenagel products (**g**)[132].



Scheme 4.3 The Knoevenagel mechanism, X and Y are electron withdrawing moieties

4.3.2.4 Attempted identification of the Knoevenagel impurity

The Hann and Lapworth mechanism (**Scheme 4.2**) gives no obvious potential impurities. The Knoevenagel mechanism (**Scheme 4.3**), however, has the potential to produce several different impurities, the hemi-aminal and the aminal and the ether compound. Examples of these impurities were synthesised so that their spectra could be compared with those of the products of the Knoevenagel reaction using the traditional catalyst, piperidine. The contaminating by-product was observed in Knoevenagel condensations involving the aldehyde 2,6-dichlorobenzaldehyde, hence, the hemi-aminal (2,6-dichlorophenyl)(piperidin-1-yl)methanol **48**, the aminal[152,153] 1-[(2,6-dichlorophenyl)(piperidin-1-yl)methyl]piperidine **49**, and the ether (2,6-dichlorophenyl)(piperidin-1-yl)methyl ethyl ether **50** (**Figure 4.1**) were prepared, but in each case the ^{1}H NMR spectra was inconsistent with the observed ^{1}H NMR of the contaminating by-product.

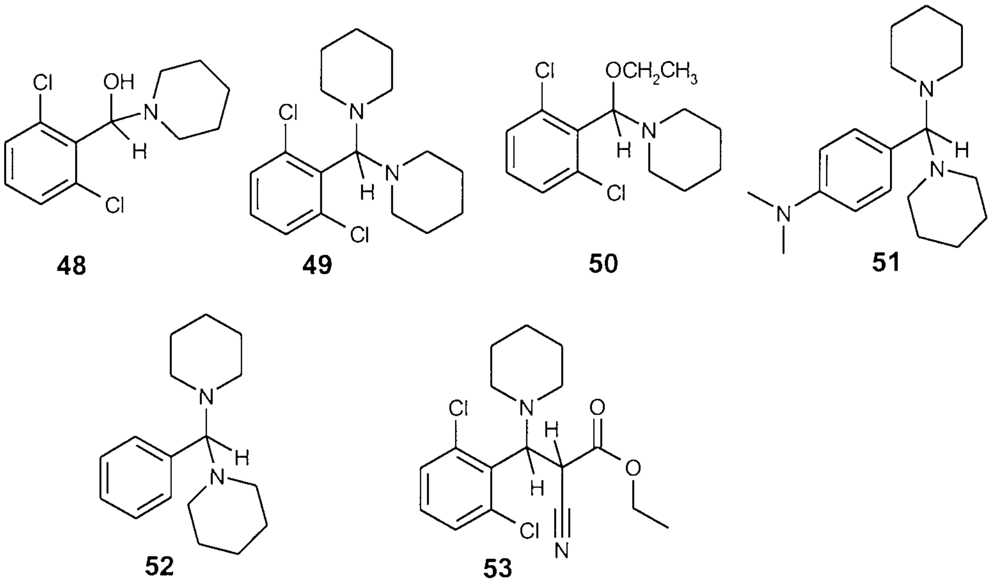


Figure 4.1 Potential Knoevenagel condensation impurities

Although it has been reported that aldehydes form aminal products[136,152,153], there appear to be exceptions, for example, 4-dimethylaminobenzaldehyde did not form the expected aminal product, *N*-[4-(dipiperidin-1-ylmethyl)phenyl]-*N*-dimethylamine **51**, perhaps due to the electron donating ability of the dimethylamino group, making the aldehyde carbonyl less reactive. The *N*-[4-(dipiperidin-1-ylmethyl)phenyl]-*N*-dimethylamine **51** was not readily synthesised in contrast, to 1-[(2,6-dichlorophenyl)(piperidin-1-yl)methyl]piperidine **49** and 1-[phenyl(piperidin-1-yl)methyl]piperidine **52** which were readily prepared. Despite the difficulty in preparing **51**, a catalyst based by-product was still detected in the Knoevenagel product, suggesting that the by-product is not the hemi-aminal, aminal or ether. Attempts, under Knoevenagel reaction conditions, to perform the Michael addition of piperidine onto the Knoevenagel product to form **53** were unsuccessful, the double bond was left intact, suggesting that this is not the by-product formed. Knoevenagel reactions performed in deoxygenated solvent under argon gave the same by-products as those performed open to the atmosphere, therefore, eliminating the possible formation of *N*-oxides of piperidine.

The unwanted by-product of the Knoevenagel reaction could be seen by ^{1}H NMR analysis, showing peaks at 3.00ppm and 1.65ppm consistent with a piperidine salt, for example piperidine hydrochloride. ^{13}C NMR of the unwanted by-product could essentially be superimposed with the piperidine salt. Piperidine itself shows peaks at 2.60ppm and 1.40ppm in ^{1}H NMR, and it could be seen that none of this was present. When products were subjected to LC-MS analysis peaks were seen that were consistent with a protonated piperidine. Efforts to conclusively identify this unwanted material were unsuccessful, but it could be concluded that it was derived from the catalyst piperidine, hence, removing the piperidine catalyst should remove the impurity.

4.4 Overcoming problems of traditional catalysts

A method was sought to catalyse the Knoevenagel condensation that did not produce the unwanted by-products of the traditional catalyst piperidine.

4.4.1 Polymer-bound catalysts/reagents

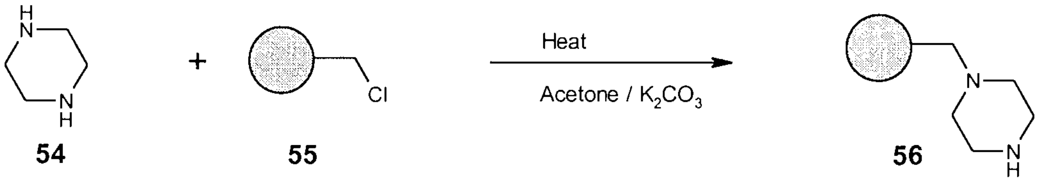
Liquid catalysts have the disadvantage of difficult removal from the final product, especially if these too are in the liquid form. Polymer-bound catalysts and reagents or ion exchange resins may overcome reaction work-up problems in organic synthesis[154]. Polymer-bound catalysts are insoluble, so allow easy work-up where products in solution may be isolated by simple filtration. A large excess may be used to increase yields or rates of reaction, due to ease of removal. Termination of reaction is achieved by removal of the insoluble catalyst with no need to quench the reaction.

4.4.2 Scavenger resins

Polymer-bound reagents, known as scavenger resins, may be used to remove unwanted components from a reaction[155,156]. Specific ligands may be polymer-bound to remove metal ions. In solution-phase synthesis, polymer-bound reagents may also be chosen to quench excess reactants and remove known impurities from the crude reaction products dependant on the reactants used in excess. Polymer-bound amines may remove electrophiles, carboxylic acids may remove nucleophiles, and isocyanates can remove amine starting materials[156]. Reactive groups on separate polymer-supports are known not to interact[156] therefore, a multiplicity of quenching reagents may be added concurrently to remove a multiplicity of impurities and excess reagents.

4.4.3 Polymer-bound knoevenagel catalyst (piperazinomethyl-polystyrene)

The principles of polymer-bound catalysts and scavenger resins can be applied to a catalyst for the Knoevenagel condensation. Binding piperazine **54** to an insoluble polymer, such as Merrifield polystyrene resin **55**, allows the piperazine to act as a secondary amine to catalyse the Knoevenagel condensation. Piperazinomethyl-polystyrene **56** was synthesised by the addition of an excess of piperazine **54** to Merrifield solid-phase resin (2% cross-linked with DVB, 200-400 mesh, 2.3 mmol Cl/g) **55** under reflux in acetone in the presence of potassium carbonate, to give a resin-bound piperidine equivalent (**Scheme 4.4**). Other polyamine resins have been made in the same manner, for example by heating excess *tris*-(2-aminoethyl)amine under reflux with commercially available Merrifield resin[156].

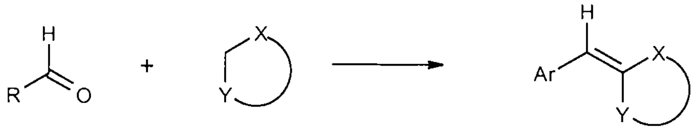


Scheme 4.4 Synthesis of a polymer-bound piperazine bound reagent (piperazinomethyl-polystyrene)

Piperazinomethyl-polystyrene **56** has previously been made by taking commercially available cross-linked polystyrene, chloromethylating it and reacting this with a monofunctional t-butoxycarbonyl (BOC) protected derivative of piperazine. Removal of BOC generates the piperazinomethyl-polystyrene. This was used as a deblocking reagent for Fmoc systems and had the added advantage of removing unwanted by-products so also acting as a scavenging reagent[156,157,158]. Piperazinomethyl-polystyrene has also been used in the development of a traceless triazine linker for the synthesis of arenes[159,160,161]. Such polymer-bound reagents may be more expensive than traditional catalysts but, the small quantity of resin and solvent employed and the reduction in work-up time make their use beneficial. Additionally, these polymer-bound reagents are easily made in large quantity.

4.4.4 Comparison of piperidine with piperazinomethyl-polystyrene

A pair of libraries of caffeic acid derivatives were synthesised in parallel using piperazinomethyl-polystyrene **56** and piperidine for direct comparison of the two catalysts (**Table 4.1** and **4.2**). Aldehydes and active methylene reagents were chosen which had previously yielded impure products contaminated by transesterification and/or piperidine derived by-products.



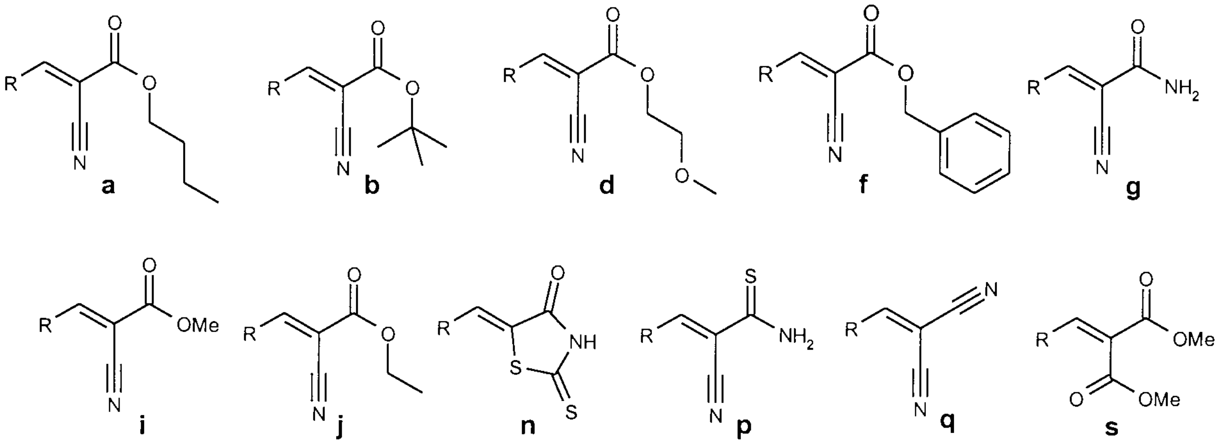


Table 4.1 Knoevenagel products prepared in the catalyst comparison libraries

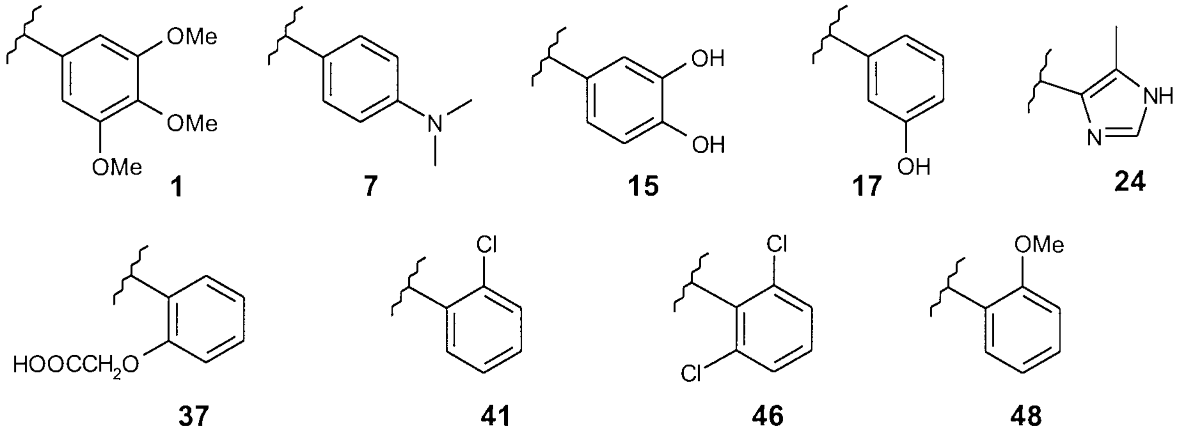


Table 4.2 R functionalities of Knoevenagel products

The solid-phase catalyst was found to eliminate the problem of the catalyst based by-product and to reduce the transesterification of the Knoevenagel product (**Table 4.3**). A major advantage of the solid-phase catalyst was its ease of removal by filtration.

Compound	SOLID CATALYST				PIPERIDINE CATALYST		
	Product	By-Product	Transesterified Product	% Yield clean product*	Product	By-Product	Transesterified Product
a17	1.00	0.00	0.00	93	1.00	0.16	0.00
a48	1.00	0.00	0.00	96	1.00	0.04	0.42
b15	1.00	0.00	0.00	88	1.00	0.25	0.00
b46	1.00	0.00	0.00	89	1.00	0.25	0.12
f1	1.00	0.00	0.00	96	1.00	0.24	1.68
g15	1.00	0.00	NA	79	1.00	0.12	NA
j15	1.00	0.00	NA	96	1.00	0.12	NA
j37	1.00	0.00	NA	79	1.00	0.39	NA
j41	1.00	0.00	NA	91	1.00	0.04	NA
n15	1.00	0.00	NA	96	1.00	0.21	NA
p15	1.00	0.00	NA	100	1.00	0.21	NA
q15	1.00	0.00	NA	87	1.00	0.09	NA
s15	1.00	0.00	0.00	65	1.00	0.24	0.00
d46	1.00	0.00	15.89		1.00	0.69	27.70
f7	1.00	0.00	0.31		0.00	0.04	1.00
f15	1.00	0.00	0.18		1.00	0.65	0.93
f17	1.00	0.00	0.39		1.00	0.65	3.55
f24	1.00	0.00	0.08		1.00	0.18	1.96
f46	1.00	0.00	3.67		1.00	0.43	4.28
i46	1.00	0.00	2.91		0.00	0.03	1.00

*Judged to be >90% pure by ^{1}H NMR spectroscopy

Table 4.3 Comparison of molar ratios of products to by-products using the solid-phase catalyst and piperidine

4.5 Stereochemistry

Stereochemical configuration of the Knoevenagel product of aromatic aldehydes appears to be independent of the catalyst employed. With piperidine, zinc chloride, doped xonotlite and $AlPO_4$-Al_2O_3 only *E* isomers have been produced[136,138,142,147]. Malonic acid condensed with aromatic aldehydes gives rise to the *trans*-cinnamic acid exclusively[1]. Ethyl cyanoacetate and cyanoacetamide condensed with aromatic aldehydes are configured with the smaller cyano group *cis* to the aromatic ring, this was found to be the same when using a polymer-bound catalyst.

Previous workers[162] starting from X-ray crystal structure established that the ^{13}C-proton coupling constant of the olefinic proton *trans* to the nitrile carbon or ester carbonyl carbon is in the range 13-

14Hz and that the corresponding *cis* coupling constant is in the range 3-8Hz. ^{1}H NMR spectroscopy confirmed that all the compounds synthesised, by both the traditional and solid-phase catalyst, were produced as single isomers. Analysis by ^{13}C NMR spectroscopy showed that the coupling constants of the compounds synthesised confirmed the stereochemistry of the Knoevenagel products as described (**Table 4.4**). All molecules had a configuration consistent with the smallest functionality *cis* to the aromatic group of the molecule which led to the majority of compounds being assigned as *E* isomers.

Compound	Proton-Coupled ^{13}C NMR data				*E/Z* configuration
	C=O		CN		
	PPM	Hz	PPM	Hz	
a17	162.1	3.3	115.7	14.0	*E*
a48	162.2	3.2	115.7	14.0	*E*
b15	161.9	6.8	116.2	13.7	*E*
b46	159.0	6.3	113.6	13.9	*E*
f1	162.6	3.3	116.2	13.8	*E*
g15	163.8	6.2	117.6	14.1	*E*
j15	163.0	3.4	116.6	13.0	*E*
j37	162.3	3.8	116.0	14.1	*E*
j41	161.3	3.5	114.9	13.8	*E*
n15	177	5.4	NA	NA	*Z*
p15	161.2	2.4	117.3	13.9	*E*
q15	NA	NA	115.5 114.5	8.4 14.2	-
s15	167.4 164.4	12.3 3.8	NA	NA	-

Table 4.4 *E/Z* configuration of Knoevenagel products

4.6 Conclusion

In condensations involving aldehydes and cyanoacetic esters the most common catalyst is an amine, usually piperidine in a solution of an alcohol[136]. Two problems were encountered in the use of piperidine as a catalyst. Products contained catalyst-derived by-products and a transesterification reaction occurred between ester group of product and the alcoholic solvent.

A polymer-bound piperazine was synthesised by a simple route and used to overcome the problem of the unwanted by-product, producing clean products, and easy removal from the reaction by filtration, in contrast to piperidine. The polymer-bound catalyst is easily synthesised. The piperazinomethyl-polystyrene appeared to act as a scavenger resin removing catalyst-based by-products. It also has the potential to remove excess aldehyde if used to drive the reaction to completion, an important consideration in automated synthesis.

The piperazinomethyl-polystyrene is especially useful in the automated synthesis of liquid Knoevenagel products where the solvent wash purification procedure tends to reduce the recovery of product obtained. Simple catalyst removal by filtration will purify the products and leave them in a high enough yield for biological testing.

Removal of the polymer-bound piperazine terminates the reaction on removal, thereby reducing the amount of transesterification occurring. Although transesterification could be avoided by the use of non-alcohol solvents, the solid catalyst gives the freedom to use alcohols when the solubility profile of substrates makes it necessary.

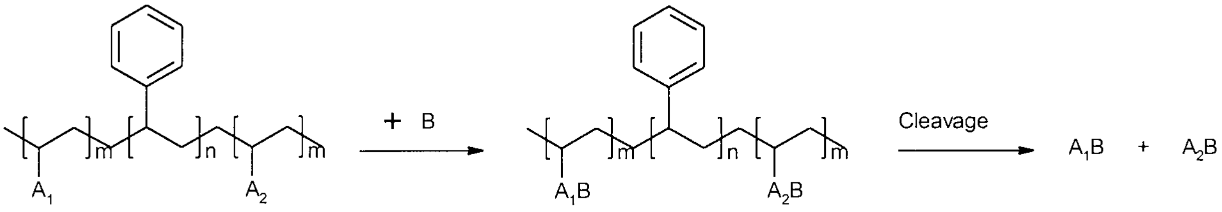
Chapter 5

Soluble polymer use in combinatorial chemistry

Combinatorial chemistry has emerged as a powerful way of accelerating the drug discovery process. The biological screening of large diverse compound libraries synthesised by combinatorial chemistry, raises the potential to find active compounds. Solid-phase synthesis has advantages over traditional solution-phase chemistry that make it attractive for the generation of combinatorial compound libraries. Most combinatorial syntheses of small organic compounds employing a solid-phase approach, use an insoluble cross-linked polymer as the carrier. The aim of this work was to use a soluble linear polymer as a support for organic synthesis and to determine the suitability of soluble linear polymers to produce mixtures of compounds. It was anticipated that a soluble polymer could be synthesised containing different building blocks pendant to its backbone. The building blocks within the polymer structure could be subjected to one or more organic reaction steps and then be cleaved from the polymer support. Cleavage would, in theory, yield a mixture of clean compounds in a known composition. The ratio of products obtained being the same as that making up the original polymer.

5.1 Theory

When mixtures are synthesised in a combinatorial fashion on solid-phase supports, each separate bead of solid-phase support has a different compound attached to it. To date the use of soluble polymers as a support for organic synthesis has after cleavage provided a single compound. A soluble polymer could be prepared to incorporate a number of different building blocks. The same reaction or a number of different reactions could then be performed on these building blocks. Upon cleavage of products from the soluble polymer a mixture of compounds would be obtained. If the components and their ratio in this mixture were predictable, then soluble polymers could be used in the combinatorial synthesis of mixtures for biological testing.

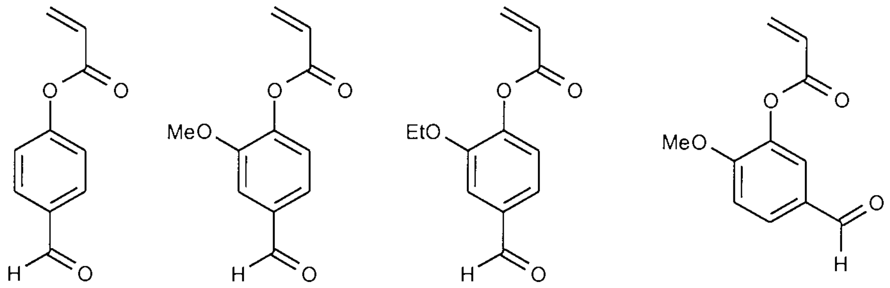


Scheme 5.1 Proposed combinatorial mixture synthesis on soluble linear polymers

5.2 Soluble co-polymers for solution-phase supported reactions

5.2.1 Synthesis of functional monomers

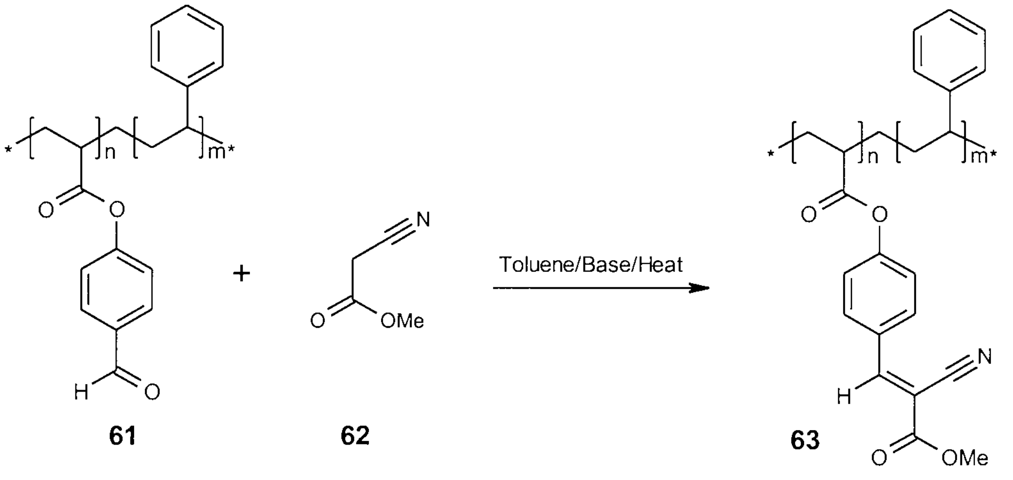
Linear polystyrene is a soluble polymer, and it was anticipated that the incorporation of functionalised aromatic rings into a polystyrene back-bone would provide a final co-polymer with a solubility profile that would allow the further organic reaction of pendant reactive moieties. To test the theory that soluble polymers could be prepared to produce mixtures of clean products a simple high yielding reaction was required. Previous experience had shown that aldehydes react readily in high yields with active methylene components, in the Knoevenagel condensation (see **Section 3.4**). Benzaldehydes, therefore, were chosen for incorporation into a polystyrene co-polymer. The functionalised monomer acrylates **57**, **58**, **59** and **60** (**Figure 5.1**) were prepared for radical polymerisation, using the radical initiator AIBN, with styrene.



57 **58** **59** **60**

Figure 5.1 Structure of acrylates used for preparation of the co-polymers for mixture synthesis

It is known that linear soluble polymers have been used for the organic synthesis of discrete small molecule libraries[15]. If the incorporation of one pendant group into polystyrene could give a discrete product, it was anticipated that incorporation of more than one pendant group into polystyrene would enable the production of mixtures of compounds of known composition as in the more traditional combinatorial chemistry[2,4,7,8,9].

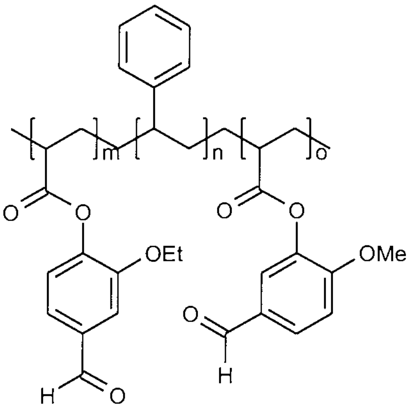


61 **62** **63**

Scheme 5.2 Knoevenagel condensation of methyl cyanoacetate and co-polymer **61**

First, a polystyrene co-polymer was synthesised incorporating **57** to give a co-polymer **61** of maximum theoretical loading of 1.69mmol/g. The synthesis was successful and ^{1}H NMR showed the broad aldehyde peak clearly visible at 9.96ppm and 2 pendant aromatic protons as a broad peak at 7.76ppm, in a ratio of 1:2 protons. Once completely dried, however, this co-polymer **61** was only sparingly soluble in $CHCl_3$, THF and toluene. In an attempt to perform the Knoevenagel condensation on the pendant aldehyde group, the co-polymer **61** was semi-dissolved in toluene and heated under reflux for 2 hours with methyl cyanoacetate **62** in an attempt to prepare **63**. By ^{1}H NMR analysis, the sparingly soluble product showed that the aldehyde peak had diminished in intensity but was still present. The insolubility of the polymer suggests that aldehyde sites not in solution could not undergo the organic transformation resulting in the incomplete reaction. Similarly, when a Knoevenagel reaction was carried out with malononitrile, the resulting polymer **65** was completely insoluble.

It was found that the addition of methoxy and ethoxy groups to the pendant acrylate groups improved the solubility profile of the final polymer. A co-polymer was, therefore synthesised containing the two pendant groups, the ethoxy compound **59** and the methoxy compound **60**, in equimolar concentrations to give a final maximum loading potential of 1.36mmol/g (**Figure 5.2**).



66

Figure 5.2 Structure of the co-polymer **66**

The methoxy and ethoxy groups could be seen at different positions in the ^{1}H NMR spectrum (**Figure 5.3**). With this co-polymer, reaction at the aldehyde function could be monitored by the disappearance of the two aldehyde peaks seen at 9.80 and 9.95ppm in the ^{1}H NMR spectrum. The polymer **66** was subjected to Knoevenagel conditions with the active methylene reagent, malononitrile. ^{1}H NMR of the resulting co-polymer **67** showed both the broad pendant aldehyde peaks, to have disappeared and a new peak corresponding to the olefinic proton to have emerged at 7.95ppm. The polymer became insoluble on drying, however, so cleavage was not considered.

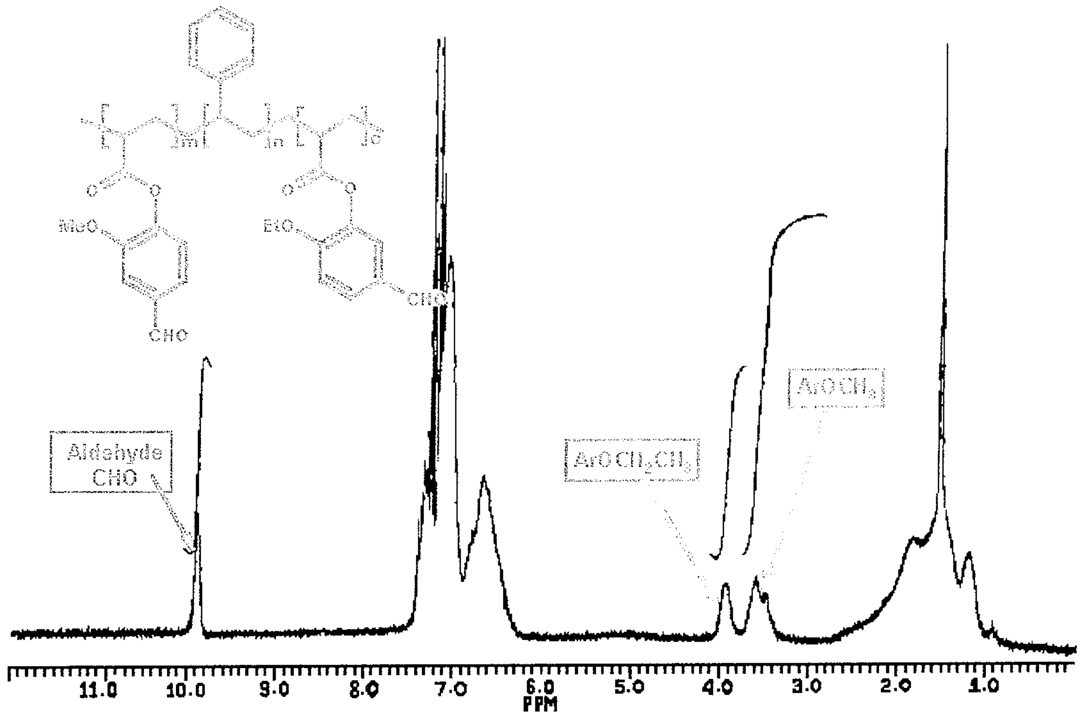


Figure 5.3 ^{1}H NMR spectrum of co-polymer **66**

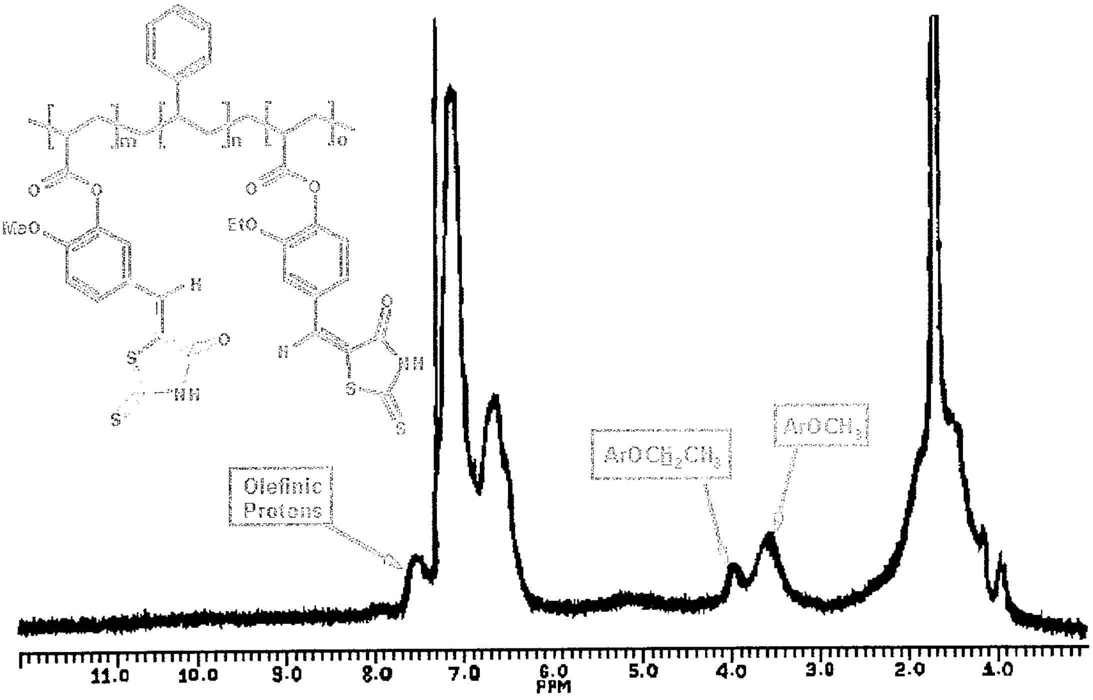
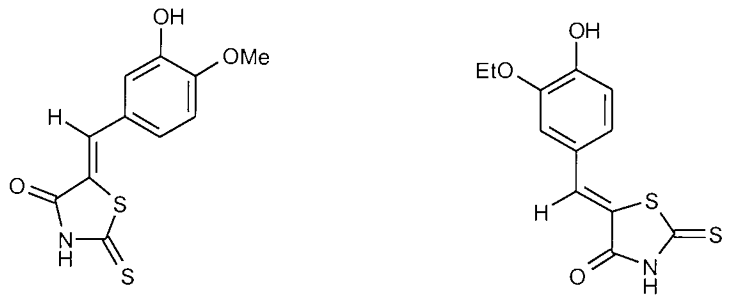


Figure 5.4 ^{1}H NMR spectrum of co-polymer **69**

Cyanoacetamide was then condensed with the pendant aldehyde functions on the co-polymer **66**, with the aim of producing a polymer **68** that could undergo a further addition at the pendant amide functionality, with for example isocyanates, thereby increasing the lipophilicity of the pendant molecules. After the initial Knoevenagel condensation, the polymer **68** was only sparingly soluble and it was found that not all the aldehyde functions had reacted. If, due to the condensation of cyanoacetamide some aldehyde moieties the polymer had became insoluble the remaining aldehyde functions would have been removed from solution and unable to react, explaining the incomplete reaction of all the aldehyde functions.

The co-polymer **66** was then reacted using Knoevenagel condensation conditions with rhodanine to give the co-polymer **69**. It was clear by ^{1}H NMR that all the pendant co-polymer aldehyde functions had reacted (**Figure 5.4**). The bright yellow polymer obtained was soluble in organic solvents and cleavage of the pendant molecules could then be attempted. The pendant molecules were, therefore, subjected to cleavage from the ester linkage using *n*-propylamine. After cleavage from the polymer, the two desired compounds were collected together (**Figure 5.5**) in a ratio of 0.89 to 1.00 (**70** to **71**). The polymer they had been cleaved from, however, was difficult to remove and could not be precipitated by pouring onto methanol. The clean-up procedure adopted was that of flash column chromatography, the desired compounds were collected together as one fraction and analysed as such.

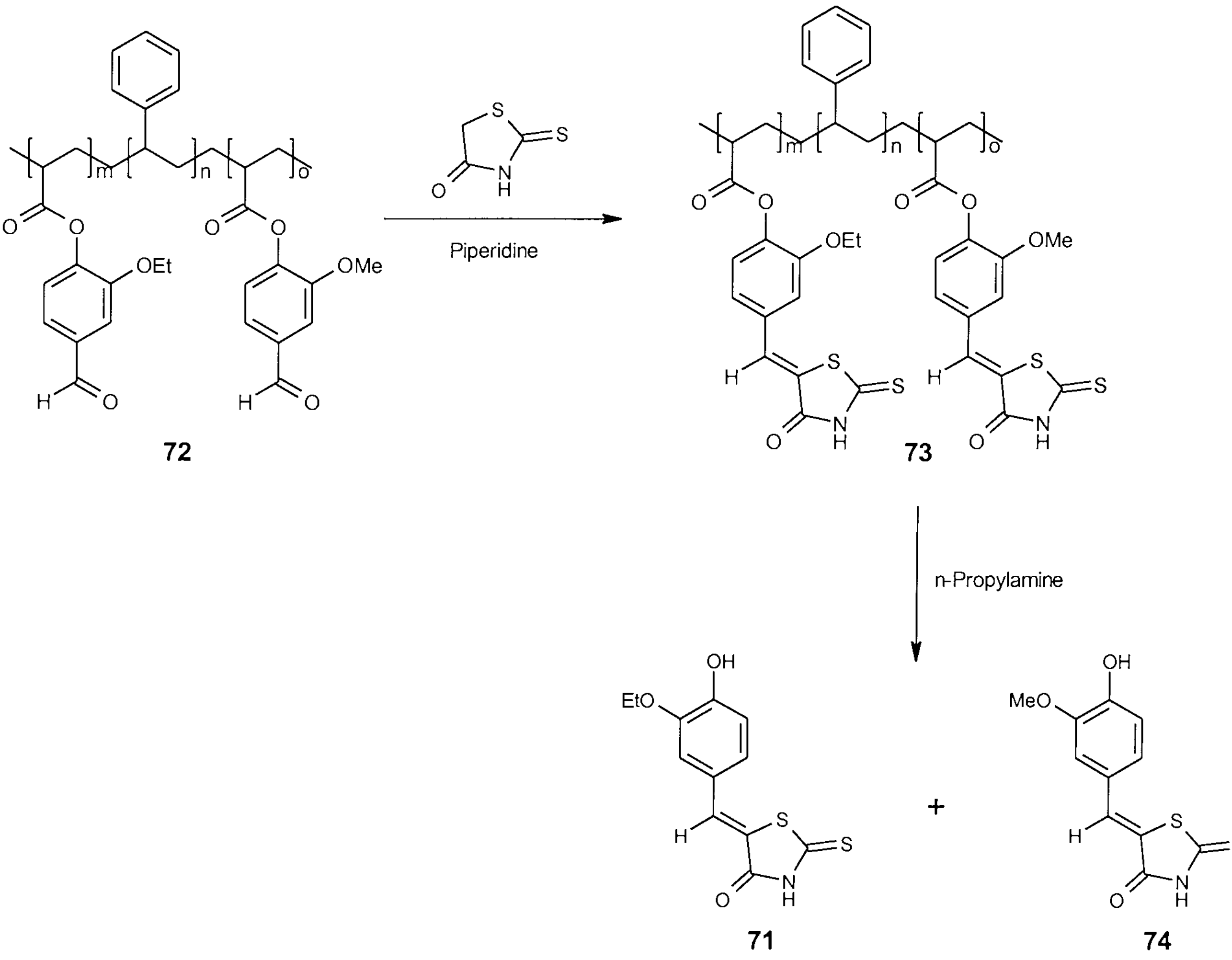


70

71

Figure 5.5 Compounds cleaved from the co-polymer as a mixture

For final proof that soluble polymers could be used to obtain defined mixtures, a similar co-polymer **72** was synthesised and reacted with rhodanine under Knoevenagel condensation conditions to give the co-polymer **73** (**Scheme 5.3**).



Scheme 5.3 Preparation and cleavage of Knoevenagel compounds from the soluble polymer **72**

After cleavage with *n*-propylamine and flash column chromatography the ratio of the products (**71** to **74**) obtained from the polymer was 1.11 to 1.00 by ^{1}H NMR, again both compounds were collected as one fraction and analysed as such.

5.3 Conclusion

It has been shown that soluble co-polymers can be synthesised and made to undergo further transformations. Cleavage from the polymer has shown that defined mixtures of known compounds may be obtained in known ratios. Varying the number and ratio of functional groups used to make the polymer will give the same number and ratio of products. This could be useful in the combinatorial synthesis of defined mixtures.

The higher the loading of a polymer, the more likely it is to take on the characteristics of its pendant molecules. This work showed the significance of the pendant group on the solubility profile of the resultant polymer. If soluble polymers are to be used to create mixtures in the future, a balance between the solubility of the pendant groups and the loading of the polymer has to be considered.

The soluble polymer approach has the advantage of non-destructive reaction monitoring. This is especially important when producing mixtures. Different building blocks have different reactivities, so it is important to drive the reaction on all the different functionalities to completion. The use of the soluble polymer enabled an excess of reagent to be used to drive the reactions to completion with easy removal of excess reagent by precipitation of the polymer onto methanol.

It can be seen from this work that the cleavage conditions used were not ideal. *n*-Propylamine was used because excess could easily be removed under vacuum and its addition in excess did not cause the polymer to precipitate. The resulting polymer, however, was soluble in methanol making it difficult to remove from the desired products. Other solvents, such as diethyl ether could be used to precipitate the polymer, but in the case studied would also precipitate the products.

Future work in this area could expand and improve upon the cleavage techniques. Cleavage conditions would have to leave the polymer in solution until the pendant groups had been removed. Aqueous sodium hydroxide, therefore, would be of little use. Benzylic amines could be used for cleavage, and may make the resulting polymer less soluble. Diamines have the potential to remove the desired product from the polymer backbone and cross-link the remaining polymer making it inherently less soluble. Benzylic amines and diamines, if used in excess for cleaving molecules from the polymer, would be collected along with the desired cleaved products. An acidic ion exchange resin would overcome this problem and could be easily removed along with the insoluble polymer by filtration.

In theory, by judicious choice of functional monomers, a soluble polymer could be synthesised containing more than one pendant functionality, for example, a ketone and an aldehyde, or an amide and an aldehyde. Each of these funtionalities could be reacted together under the same conditions, for example both a ketone and an aldehyde will undergo the Knoevenagel reaction. Alternatively, the different moieties could also be made to undergo different reactions, for example, the aldehyde could be condensed with an active methylene and cleaned up and then the amide could be reacted with an isocyanate. A polymer containing different classes of compound could, therefore, be prepared with a large diversity of pendant molecules. The more diverse the mixture they produce, the more likely they are to produce an active compound and the easier it will be to finally deconvolute final mixture to determine which if any of the compounds present is the active constituent. The concept of structurally distinct molecules synthesised on the same resin has already been used, but, for a different purpose. Solid-phase resins have been tagged in combinatorial synthesis, whereby a chemical tag encodes for a specific building block. Cleavage and analysis of the chemical tag, therefore, decodes the compound synthesised on the resin[7,8].

Chapter 6

Solution-phase preparation of catechol derivatives for lipoxygenase inhibition

The aim of this work was to synthesise novel compounds, containing a catechol moiety to inhibit lipoxygenase enzymes. The objective was to use traditional solution-phase chemistry with a view to develop a solid-phase synthesis protocol that could ultimately be automated to produce a library of compounds for biological testing.

6.1 Catechol based Inhibitors of lipoxygenase

Various compounds containing a catechol moiety have been described as inhibitors of 12-[134], 15-[163], and 5-lipoxygenase[164] enzymes. Tait et al.[163] found that the catechol (**Figure 6.1**) showed considerable rabbit reticulocyte 15-lipoxygenase inhibitory activity (n=2, $IC_{50}=0.3\mu M$). A range of analogues were also tested and found to have inhibitory activity to a lesser extent ($0.4\text{-}25\mu M$)[163]. All of these analogues were reported as inhibitors of 12- as well as 15-lipoxygenase, (12-lipoxygenase data was not published). The chemical literature yielded a structure activity relationship for the 16 catechol analogues synthesised and tested. Masking the catechol moiety as dimethylated compounds reduced their inhibitory activity by two orders of magnitude. Removal or modification of the salicylic acid hydroxyl group slightly decreased the 15-lipoxygenase inhibitory activity. Methylation of the amide nitrogen significantly reduced inhibitory activity[163].

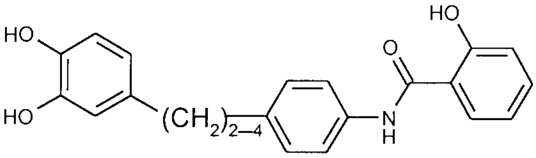


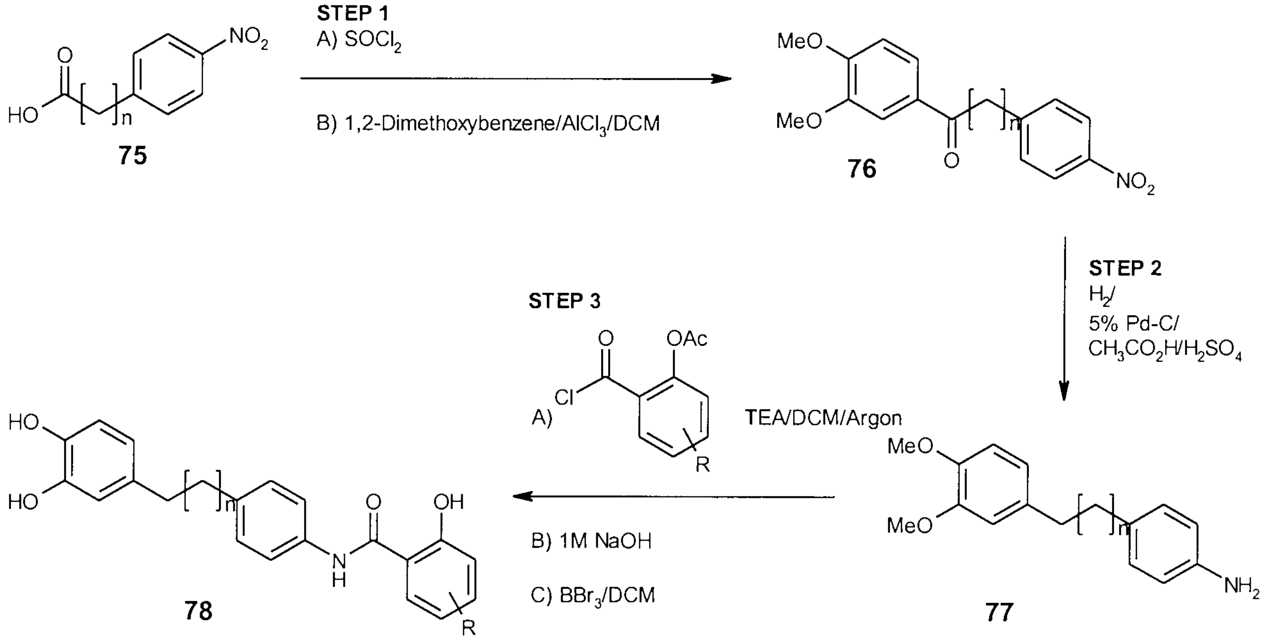
Figure 6.1 Structure of the most active catechol 15-lipoxygenase inhibitors

The length of the chain connecting the catechol and the aromatic ring was also investigated and found to have no significant effect on inhibitory activity when varied between two to four carbons in length.

6.2 Novel lipoxygenase inhibitors

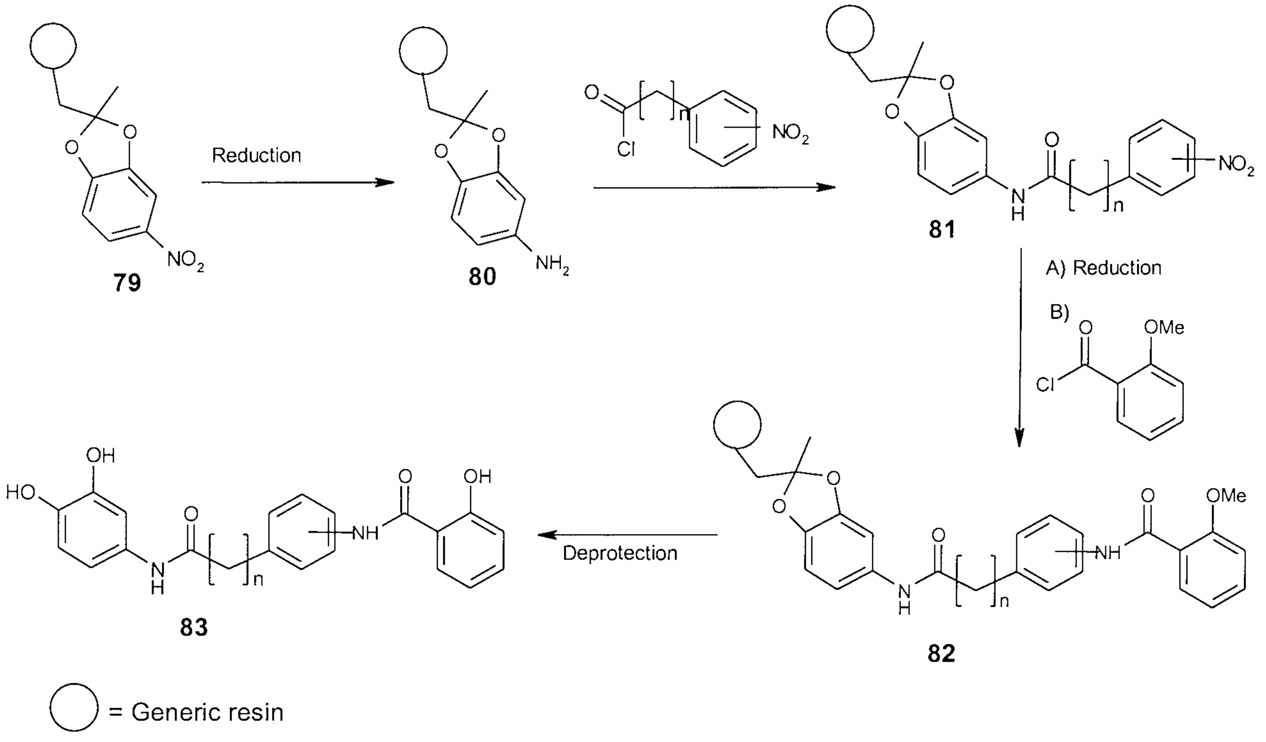
It was decided to take the literature catechol compounds known to inhibit 12- and 15-lipoxygenase (**Figure 6.1**) and change these molecules in ways in which the original literature had not reported. Preparation of the original active compounds in solution involved a four step synthesis (**Scheme 6.1**). The first step involved the conversion of the carboxylic acid **75**, using thionyl chloride, to the acid chloride which was then reacted with 1,2-dimethoxybenzene under standard Friedel-Crafts acylation conditions to give a nitroketone **76**. The nitro and the ketone functionalities were then reduced by hydrogenation over 5% Pd-C to give the amine **77**. This amine was then converted to the amide by its reaction with the appropriate acid chloride in the presence of triethylamine (TEA). Lastly, the acetyl protecting group was removed with base, and boron tribromide (BBr_3) in DCM was used to cleave the methyl ethers to give the target molecules **78**.

The objective of this work was to make similar compounds to those previously reported in solution and then transfer their synthesis to a solid support. The catechol moiety was to be retained as it was described as essential for lipoxygenase inhibitory activity[163]. It was decided that the catechol moiety could be attached to a solid-phase support by the formation of an acid-labile cyclic ketal. This would give the advantage of protecting the hydroxyl groups whilst linking them to the solid support.



Scheme 6.1 Synthesis of literature 12- and 15-lipoxygenase inhibitors[163].

If the reaction scheme from the original literature (**Scheme 6.1**) was to be developed for solid-phase synthesis using the cyclic ketal to link the catechol to the resin, the Lewis acid used to catalyse the Friedel-Crafts reaction in the first step may cause a problem. The Lewis acid may have the effect of hydrolysing the proposed ketal linkage from the solid support. It was, therefore, decided to change the nature of the carbon chain connecting the catechol and the next aromatic ring. Changing the composition of the connecting carbon chain to the catechol was not investigated in the original report[163]. If 4-nitrocatechol could be attached via the catechol moiety to a solid-phase resin to give a ketal **79** then the nitro functionality could be reduced to give an amine **80**. This amine could then be reacted with an appropriate nitro containing acid chloride to give an amide **81**. Repetition of the nitro reduction and amide formation using 2-methoxybenzoyl chloride would give **82**, which could be deprotected to give the catechol **83** (**Scheme 6.2**).



Scheme 6.2 Postulated synthesis of catechol analogues on a solid-phase resin

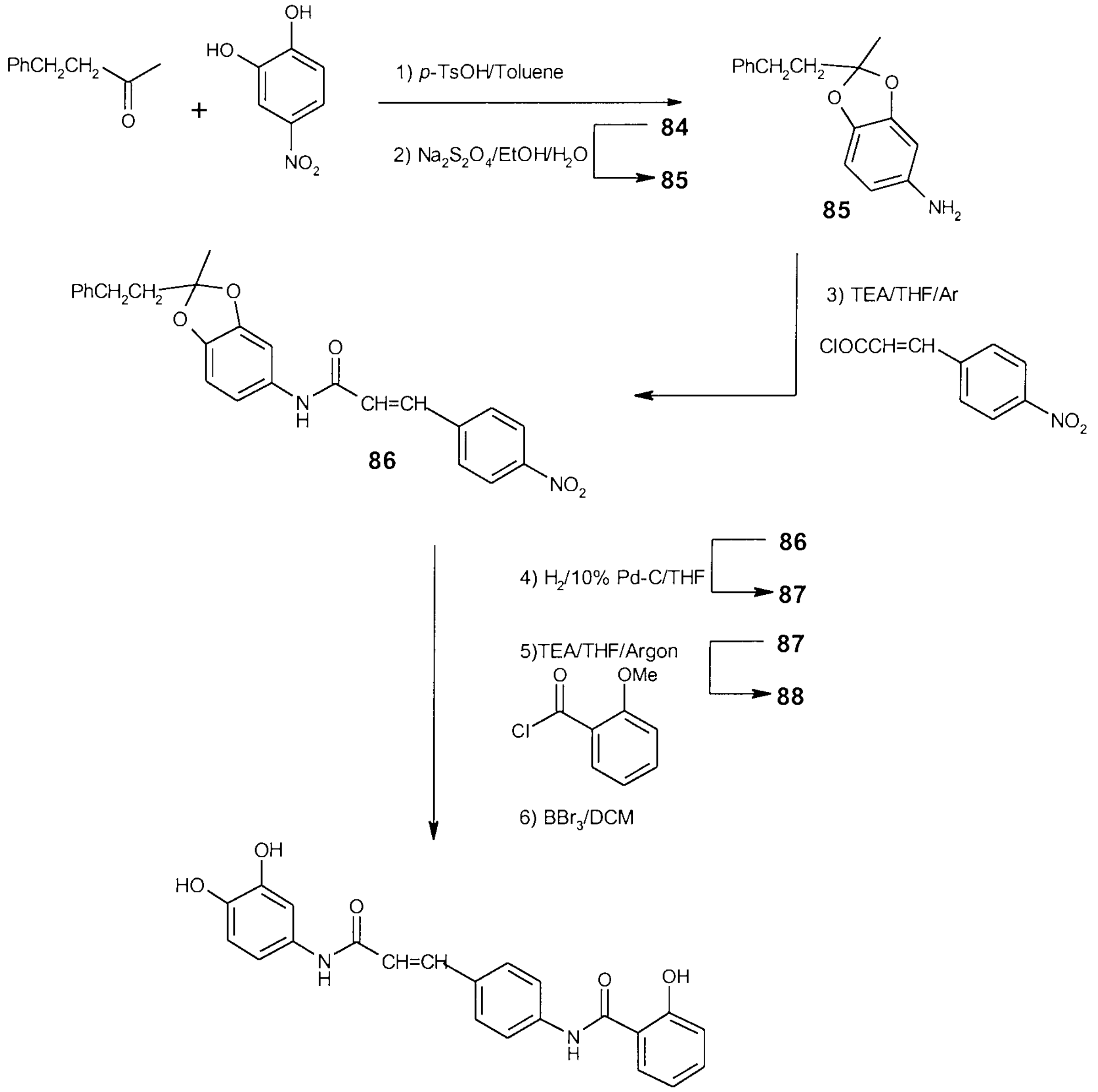
By following **Scheme 6.2** the resulting catechol compounds **83** would provide a novel set of compounds for biological screening against 12- and 15-lipoxygenase and therefore potential anti-cancer agents.

6.3 Preparing analogues in solution

Before preparing the catechol analogues **83** on a solid-phase support, the chemistry had to be tested in solution. Each step of the solution-phase synthesis will be described.

6.3.1 Step 1; Protecting the catechol moiety of 4-nitrocatechol

Catechols may be protected using a ketone and a catalytic amount of p-toluene sulphonic acid (*p*-TsOH) to form an acid labile cyclic ketal. Previous workers[165] have reported the preparation of a phenethyl ketone resin from the commercially available chloromethylated Merrifield resin. This was subsequently reacted with catechol to form a cyclic-ketal and further synthetic steps were performed to prepare a library of resin-bound dihydroxybenzopyrans, before the simple cleavage of desired products using 5M HCl[165]. It was therefore decided to prepare the desired catechol compounds on the phenethyl ketone resin prepared from commercially available chloromethylated Merrifield resin (**Chapter 7**). Before solid-phase synthesis reactions were attempted, 4-phenylbutan-2-one was used as solution-phase model for the solid-phase support (**Scheme 6.3**). The ketal **84** was synthesised from the ketone, 4-phenylbutan-2-one heated under reflux in conjunction with a Dean-Stark trap with 4-nitrocatechol in the presence of catalytic *p*-toluene sulphonic acid (*p*-TsOH) in yields ranging from 52-85%.



Scheme 6.3 Solution-phase synthesis of catechol analogues

6.3.2 Step 2; reduction of the aromatic nitro group.

When using an insoluble polymeric support resin, the chemistry used must be homogeneous. The resin has to be solvated so that the attached molecules are available for reaction. Heterogeneous catalysts commonly used for reduction of aromatic nitro groups would not access the solid supported resin due to the steric hindrance provided by the polymeric resin support. For this reason sodium dithionite ($Na_2S_2O_4$) was chosen for the hydrogenation of the aromatic nitro groups, rather than a heterogeneous catalyst. An attempt was made to reduce the nitro group of **84** in ethanol and water (1:1) heating in the presence of sodium dithionite, (3 eq) at 65°C[166]. After 24 hours there was no consumption of starting material. In a second attempt, the temperature was increased to 100°C and monitoring the reaction by TLC showed a new product being formed after 30 minutes. To ensure that the new spot observed by TLC was not due to hydrolysis of the ketal under acidic conditions, the pH of the solution was tested and found to be at pH 7. Further monitoring showed that the reaction had gone no further after 24 hours. Addition of another 3 eq of sodium dithionite and another 18 hours of heating at 100°C transformed all of the starting material into the new product **85**. A white precipitate was formed when the solution was poured onto ice, which when subjected to filtration turned to a brown oil. Extraction with ethyl acetate and analysis by TLC and ^{1}H NMR spectroscopy suggested that one new product had been formed (one spot by TLC) but had decomposed in the presence of air to give a complex mixture. The instability of this compound meant that in order to minimise its degradation, the subsequent coupling with an acid chloride would have to be performed as soon as possible or *in situ* after the reduction.

6.3.3 Step 3; acid chloride coupling

In an attempt to stop the degradation of the amine **85** it was decided to form the amine and couple it immediately with the acid chloride of choice. The nitro compound **84** was reduced with sodium dithionite, dried under argon and immediately reacted with 4-nitrocinnamoyl chloride. The coupled product **86** was obtained after column chromatography in yields ranging between 10% and 31% yield.

6.3.4 Step 4; aromatic nitro group reduction and step 5 acid chloride coupling

To prepare the final protected compound a second aromatic nitro group reduction and acid chloride coupling had to be achieved (**Scheme 6.3**). The second reduction procedure was performed as before, and the product isolated **87**, which again appeared to be unstable to the air as judged by the change in the TLC from one spot to many. This suggested that the second amine and acid chloride coupling would also need to be performed immediately after the reduction of the nitro group. This was performed and it gave the desired product **88** in a 10% yield.

6.4 Methods of hydrogenation

6.4.1 Problems associated with the sodium dithionite aromatic nitro group reduction

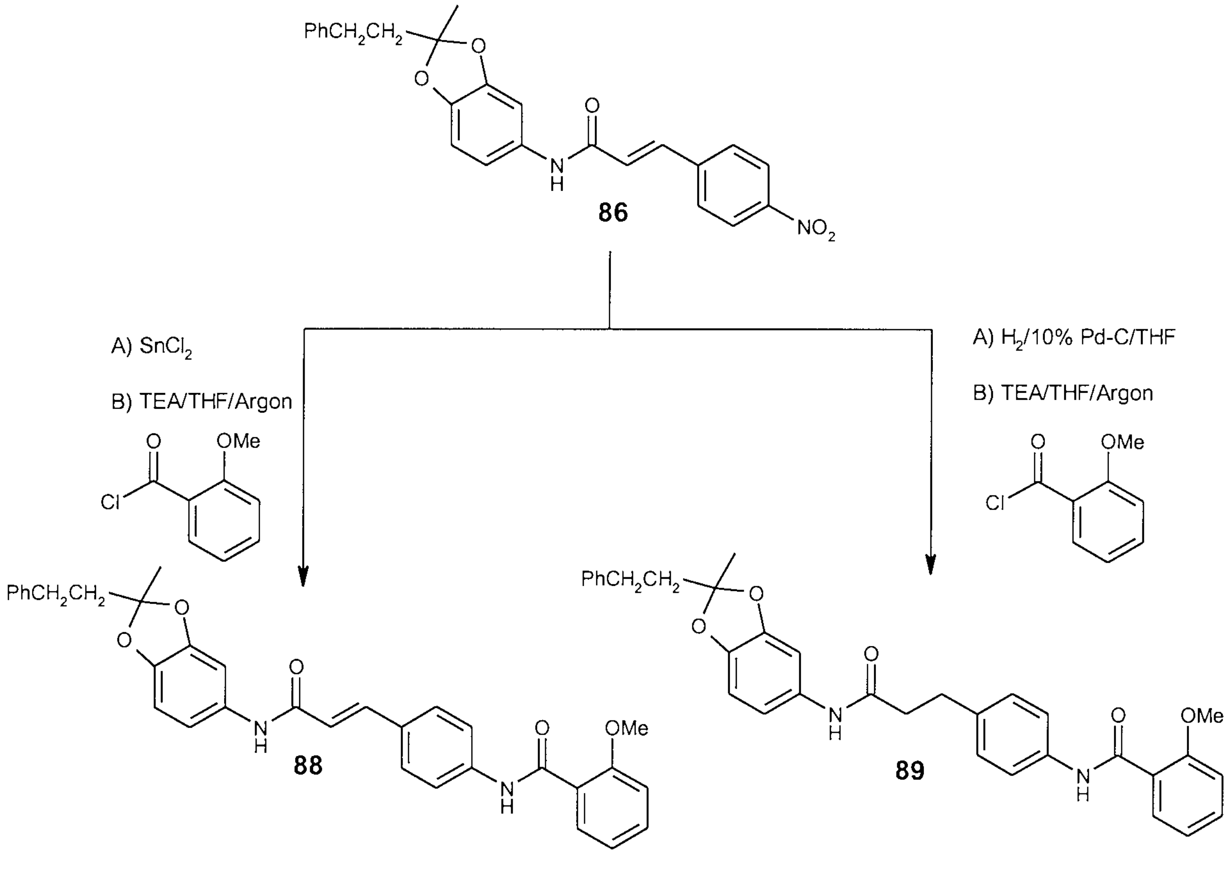
After the reduction using sodium dithionite, organic products were partitioned between diethyl ether and water under argon. A complex procedure was then employed to transfer the organic layer by cannula to a new dry vessel charged with anhydrous magnesium sulphate to dry the organic layer. A second cannula was used to remove the dry organic layer to a second dry vessel where the acid chloride coupling was to be performed. The difficult procedure may account for the low yields obtained. Separation of the organic and aqueous layers, under argon, was not easy and product may have been lost at this stage. The next stage of the procedure involved drying the organic layer under argon. The organic layer was often very wet and product may easily have been trapped in the magnesium sulphate as it solidified. Another problem may have been the poor solubility profile of the products in diethyl ether when separating the organic and aqueous layers. The problems listed should be overcome by attaching the compound to a solid-phase support.

At this stage 4-nitrocatechol was attached to the phenethyl ketone derivative of Merrifield resin and subsequent attempts were made to reduce the aromatic nitro group attached to the resin using sodium dithionite. The use of Merrifield resin proved to be impractical due to problems encountered with difficulty in analysis (see **Chapter 7**) and alternative soluble linear polymers were considered as supports for organic synthesis. The use of soluble linear polymeric supports would enable the use of heterogeneous catalytic hydrogenation.

6.4.2 Heterogeneous catalytic hydrogenation.

Reactions on Merrifield solid-phase resin proved difficult to monitor and gave disappointing results (see **Chapter 7**). It was anticipated that the type of chemistry required would be more easily carried out on a soluble polymer, for two main reasons. First, reaction progress on a soluble linear polymer can be monitored by ^{1}H NMR. Secondly, the pendant groups on a soluble polymer should in theory be amenable to the heterogeneous catalysts commonly used for the reduction of aromatic nitro groups. Considering that the next step, after the nitro group reduction, in the synthesis of these molecules required anhydrous conditions, it was proposed that heterogeneous catalytic hydrogenation would give better yields because water would not be used as a solvent during reduction. Heterogeneous catalytic hydrogenation with 10% Pd-C with immediate acid chloride coupling was used to prepare *N*-{4[(*E*)-3-(2-methyl-2-phenyl-1,3-benzodioxol-5-ylamino)-3-oxopro-1-enyl]phenyl}-2-methoxybenzamide **86** from **84** in better yields (73% and 92%) compared to the homogeneous reduction with immediate acid chloride coupling (10-31%).

6.4.3 Selective reduction of aromatic nitro groups without reduction of double bonds

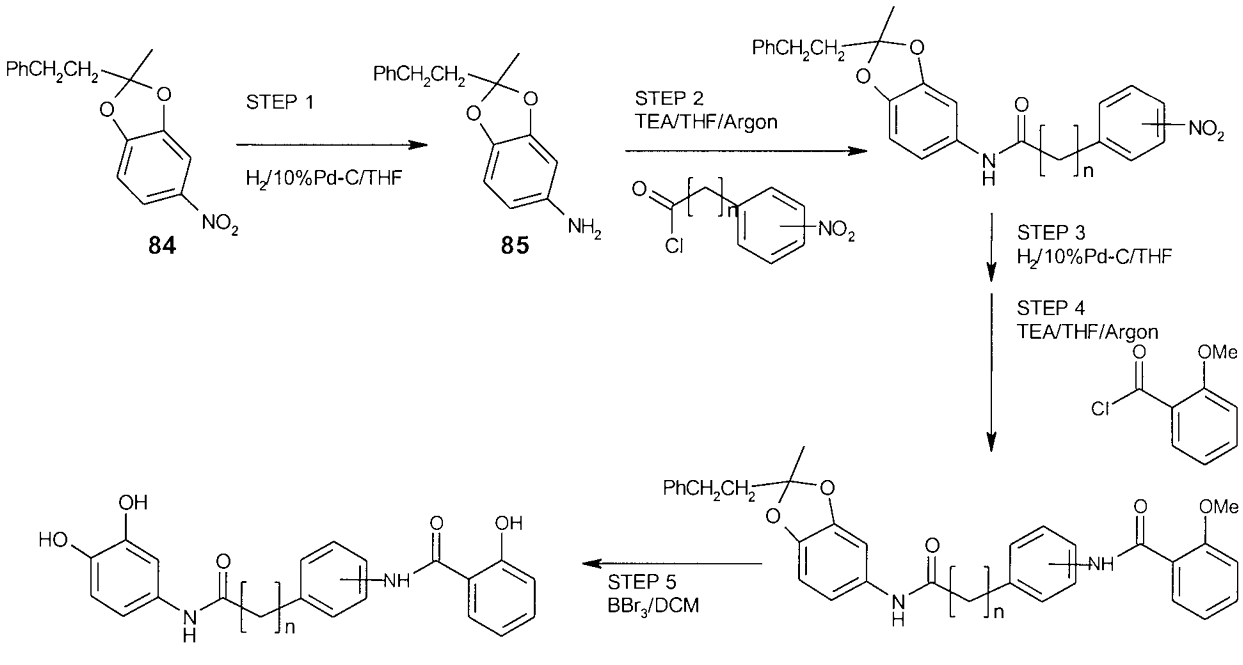


Scheme 6.4 Methods of aromatic nitro group reduction to reduce double bonds or leave them intact

The compound **86** was subjected to heterogeneous catalytic hydrogenation with 10% Pd-C and hydrogen and coupled to give the single bonded compound **89**, in 43% Yield. Both the double bond in the connecting chain and the aromatic nitro group had been reduced. It was decided to develop a protocol for the selective reduction of aromatic nitro groups that could be used in automated library synthesis. Reduction of the aromatic nitro group, while leaving the double bond of **86** intact, was achieved by the use of tin (II) chloride dihydrate, to give the desired compound **88** in 11% yield. The low yield may have been due to the difficulty in partitioning the tin (II) chloride dihydrate between the organic and aqueous layers, a problem that would not be encountered using a soluble polymeric support. With a soluble polymeric support the polymer could be precipitated onto methanol to remove the unwanted tin (II) chloride.

6.5 Preparing a small library of catechol analogues

Once initial experiments had proven that the reduction of the aromatic nitro group was feasible along with the subsequent acid chloride coupling to give desired products in reasonable yield, a small test library of ketal-protected catechol compounds was prepared for cleavage and subsequent biological screening. It was decided to introduce diversity into the central portion of the molecule, which meant varying the central acid chloride in step 2 (**Scheme 6.5**), while retaining the catechol and salicyl moiety.

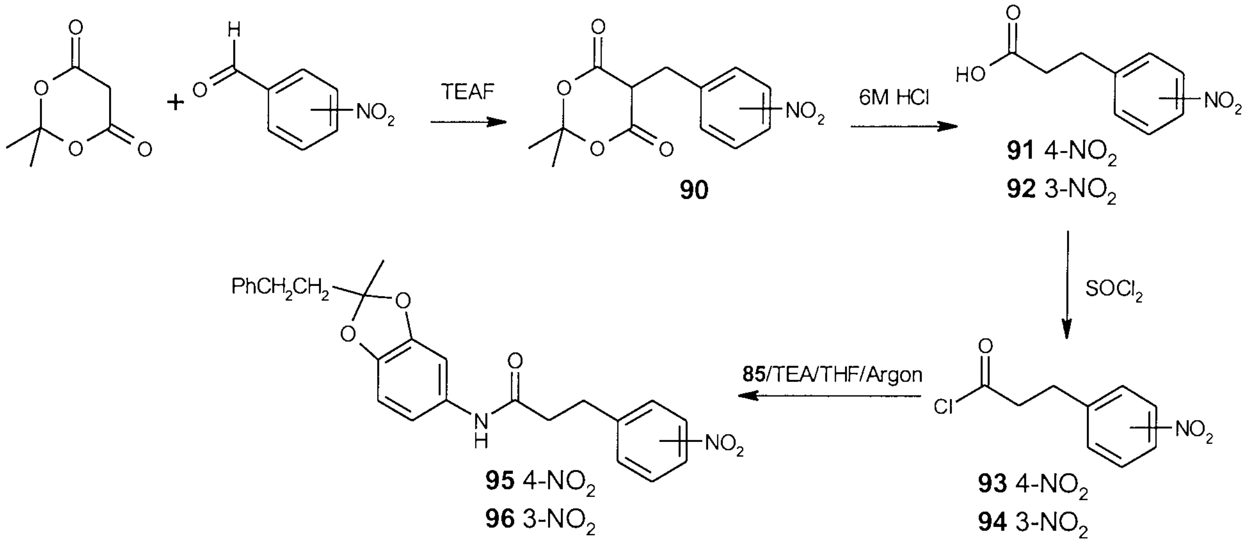


Scheme 6.5 Total synthesis of the catechol analogues with potential lipoxygenase inhibitory activity

Three commercially available nitrophenyl containing acid chlorides, 4-nitrocinnamoyl chloride (already discussed above), 4-nitrobenzoyl chloride and 5-nitro-2-furoyl chloride were chosen. Two acid chlorides, 3-(4-nitrophenyl)propanoyl chloride **91** and 3-(3-nitrophenyl)propanoyl chloride **92** were also prepared so that biological activity could be compared with that of the original molecules.

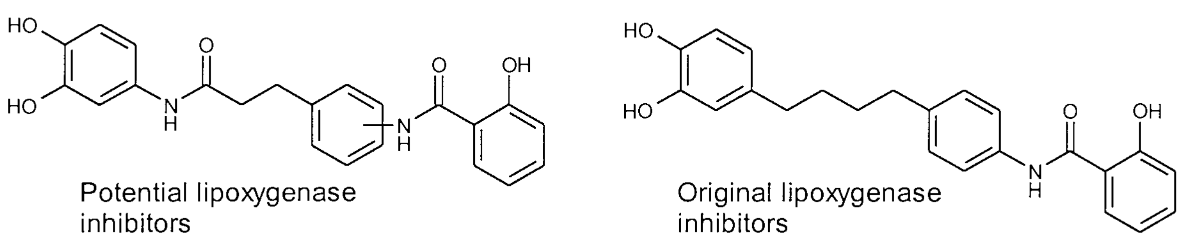
6.5.1 Preparation of acid chloride building blocks

Nitrophenyl aldehydes were condensed with Meldrum's acid in the presence of formic acid and triethylamine (TEAF, triethyl ammonium formate) to form 1,3-dioxane-4,6-diones **90**. Hydrolysis of these products with 6M HCl gave 3-(4-nitrophenyl)propanoic acid **91** and 3-(3-nitrophenyl)propanoic acid **92** in yields of 78% and 68%, respectively[167]. The arylpropanoic acids were transformed to the acid chlorides by reaction with thionyl chloride **93** and **94** (**Scheme 6.6**). The resulting acid chlorides were not isolated but reacted immediately with 2-methyl-2-phenethyl-1,3-benzodioxol-5-amine, **85** (**Scheme 6.5**) to give the compounds **95** and **96**.



Scheme 6.6 Synthesis of acid chloride building blocks and their coupling with 2-methyl-2-phenethyl-1,3-benzodioxol-5-amine **85** to give compounds **95** and **96**

These two acid chlorides **93** and **94** were prepared to provide a four-atom amide connecting chain in the target catechol molecules. In contrast, the original active compounds[163] contained a four-carbon alkyl connecting chain. In addition the effect of different substitution around the central aromatic ring was investigated (**Figure 6.2**).


Potential lipoxygenase inhibitors

Original lipoxygenase inhibitors

Figure 6.2 Structures of original and potential lipoxygenase inhibitors

6.5.2 Protected intermediate products

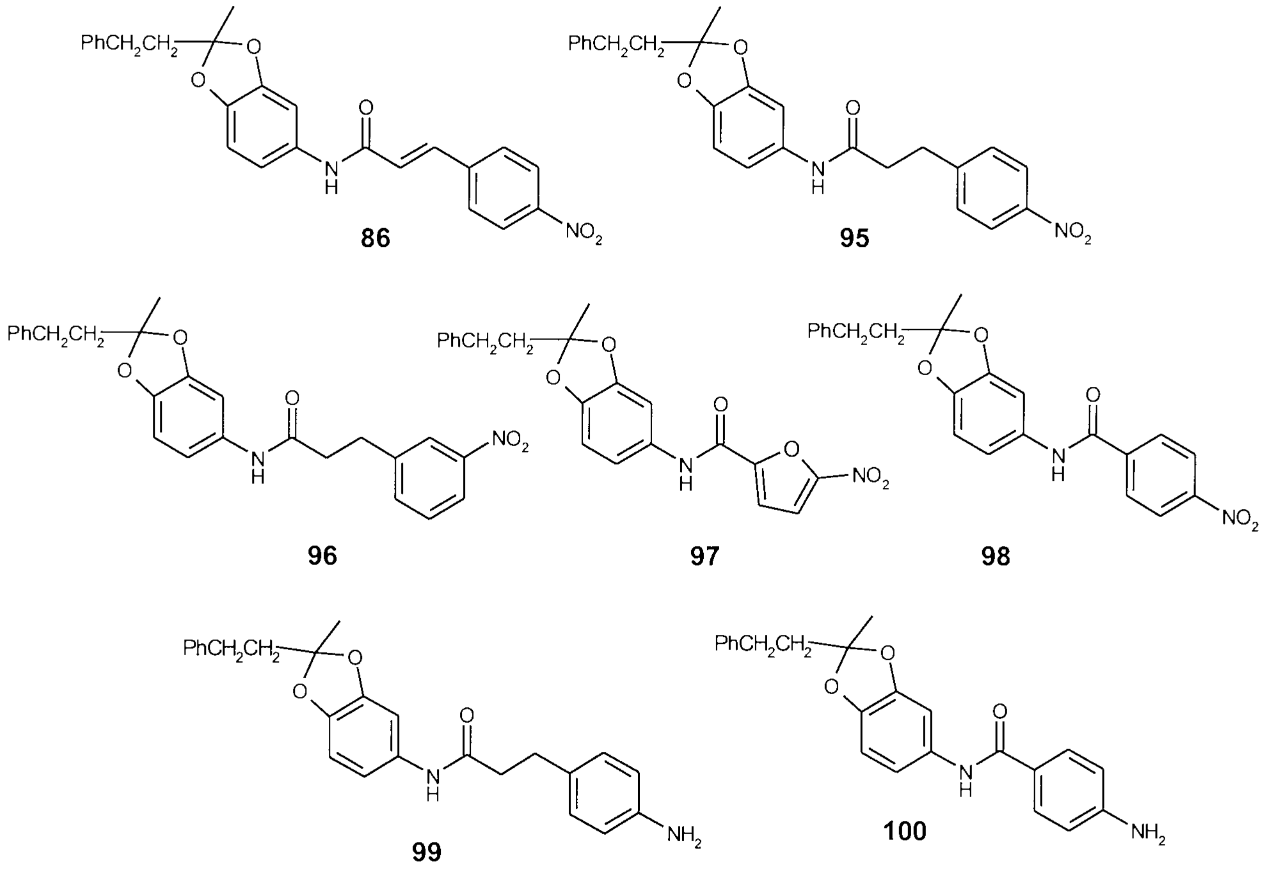
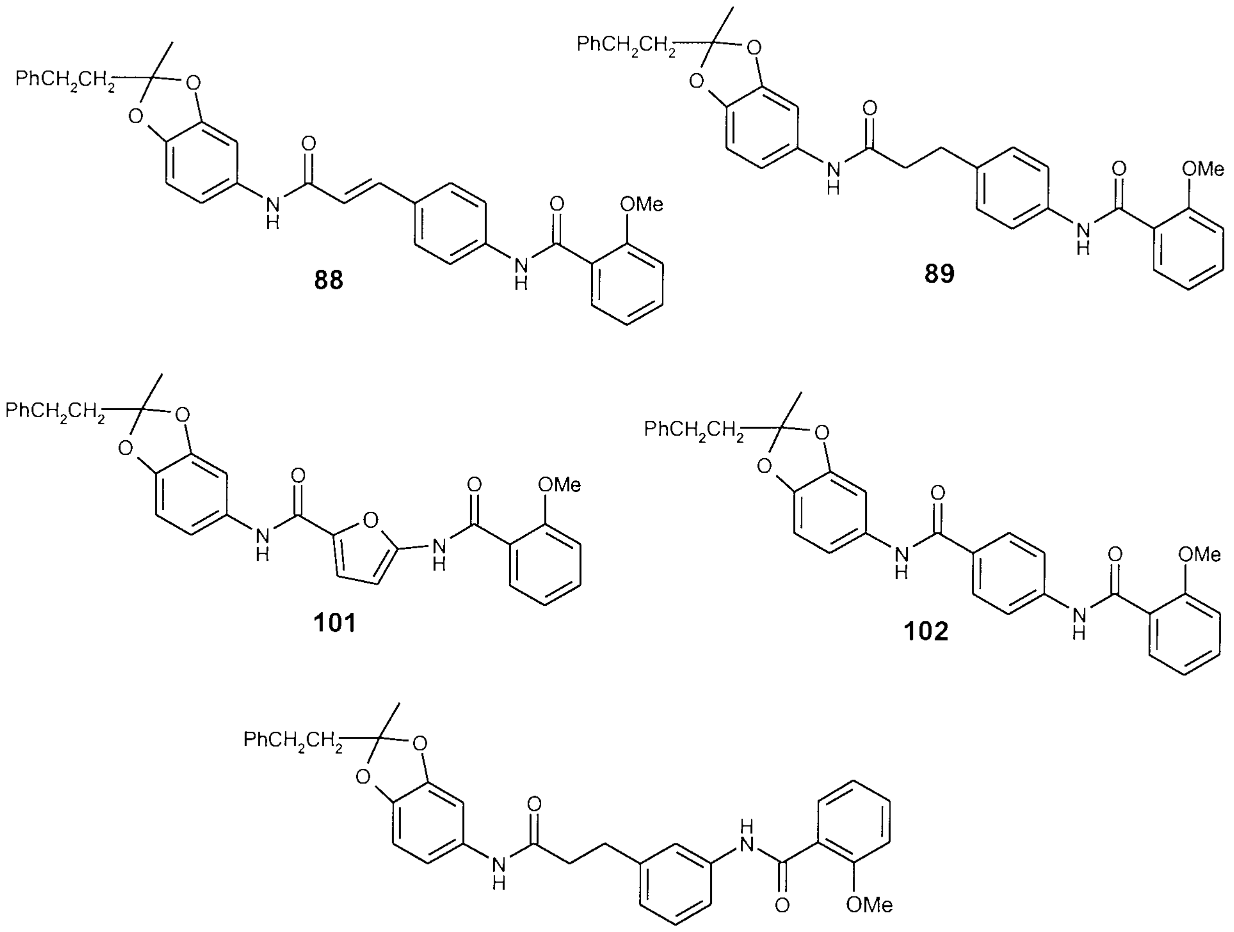


Figure 6.3 Structures of the protected intermediate products

Five nitro ketal compounds **86**, **95**, **96**, **97** and **98** (**Figure 6.2**) were prepared using sequential aromatic nitro group reduction and acid chloride coupling. These five compounds were subjected to hydrogenation conditions after which only two the amines **99** and **100** were isolated before coupling to acid chlorides to form the protected compounds **88**, **89**, **99**, **101** and **102** (**Figure 6.3**). Problems were only encountered with compounds that contained other groups liable to reduction as well as the aromatic nitrogen. Heterogeneous catalytic hydrogenation is non-selective, an example of this was seen with compound **86** where both the double bond and the nitro group were reduced. This problem was overcome by the use of tin (II) chloride dihydrate to reduce the aromatic nitro group and leave the double bond intact (**Scheme 6.4**). The same problem occurred with the use of 5-nitro-2-furoyl chloride in the second step (**Scheme 6.5**). The first reduction-coupling procedure gave the desired product **97** in 61% yield. The second reduction procedure resulted in a large number of products, and the desired compound **101** could only be isolated in a 4% and 5% yield. It was speculated that, after the hydrogenation of **97** with 10% Pd-C, more than one reduced compound was formed, due perhaps to reduction of one or both of the furan ring double bonds, as well as the nitro group. Future automated synthesis would have to take into account the problems seen with furan rings and reduction. The use of tin (II) chloride dihydrate would theoretically overcome the reduction of the furan double bonds.



88

89

101

102

103

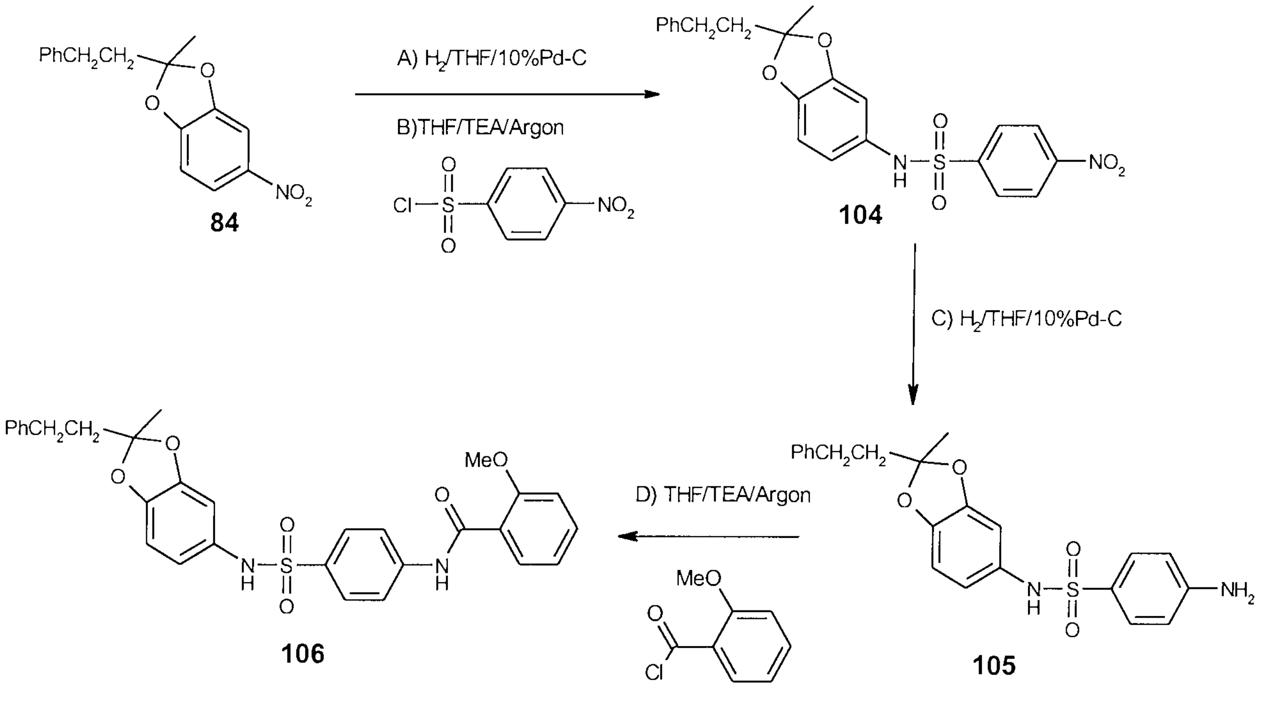
Figure 6.4 Structures of the protected compounds

6.6 Increasing library diversity

Amines as well as coupling to acid chlorides, may also be coupled with sulphonyl chlorides, isocyanates, isothiocyanates and aldehydes. Introducing these new reagents/building blocks at steps 2 and 4 (see **Scheme 6.5**) after the reduction of the aromatic nitro groups would increase the diversity of the final compounds available for biological testing. For the purposes of preparing an initial solution-phase library, it was decided to vary only the central part of the molecule by coupling the amine **85** with a sulphonyl chloride, an isocyanate and an isothiocyanate. It was also decided to investigate variation in the terminal connecting chain by reacting the amine formed in step 5 (**Scheme 6.5**) with an aldehyde. This introduction of different types of building blocks would give an idea as to the sorts of compound libraries that could be made, when the process had been optimised for solid-phase chemistry and automation.

6.6.1 Sulphonamides

2-Methyl-5-nitro-2-phenethyl-1,3-benzodioxole **84** was reduced by catalytic heterogeneous hydrogenation to give the amine **85**, this was immediately coupling with 4-nitrobenzene sulphonyl chloride to give **104** in 35% yield (**Scheme 6.7**). The subsequent attempts to reduce of the aromatic nitro group of **104** and further acid chloride coupling, proved problematic. TLC showed that many compounds were formed, and although mass spectrometry indicated that the desired compound **106** was present it could not be isolated as a pure product in significant yield.



Scheme 6.7 Attempted preparation of sulphonamide compounds

Due to previous problems of stability of reduced products, initial attempts to reduce and couple the aromatic nitro group of **104** were performed without isolation of the reduced intermediate. When reactions were unsuccessful, it was decided to isolate the amine to determine the effectiveness of the reduction procedure. The reduced compound **105** was isolated in 99% yield, suggesting that the highly electron withdrawing sulphonyl group deactivated the amine making it stable to air but imparted a decreased reactivity towards acid chloride coupling to form **106**. This suggested that in future automated synthesis, after the first reduction the next coupling step should not be carried out with a sulphonyl chloride, because of the problems of further reduction and acid chloride coupling. To create a diverse library of compounds, however, a range of sulphonyl chlorides could be used in the final amine coupling step 5 (**Scheme 6.5**).

6.6.2 Ureas and thioureas

Coupling reactions of amines are not only possible with acid chlorides, but may be performed with isocyanates and also isothiocyanates to form ureas and thioureas, respectively. Trial coupling reactions were carried out with the commercially available 4-nitrophenyl isocyanate and 4-nitrophenyl isothiocyanate with the amine **84** to yield the isocyanate derivative **107** (17%) and the isothiocyanate derivative **108** (20%). These compounds were then reduced using heterogeneous catalytic hydrogenation and the resulting amines coupled to the acid chloride, 2-methoxybenzoyl chloride to give the protected compounds, **109** (96%) and **110** (87%).

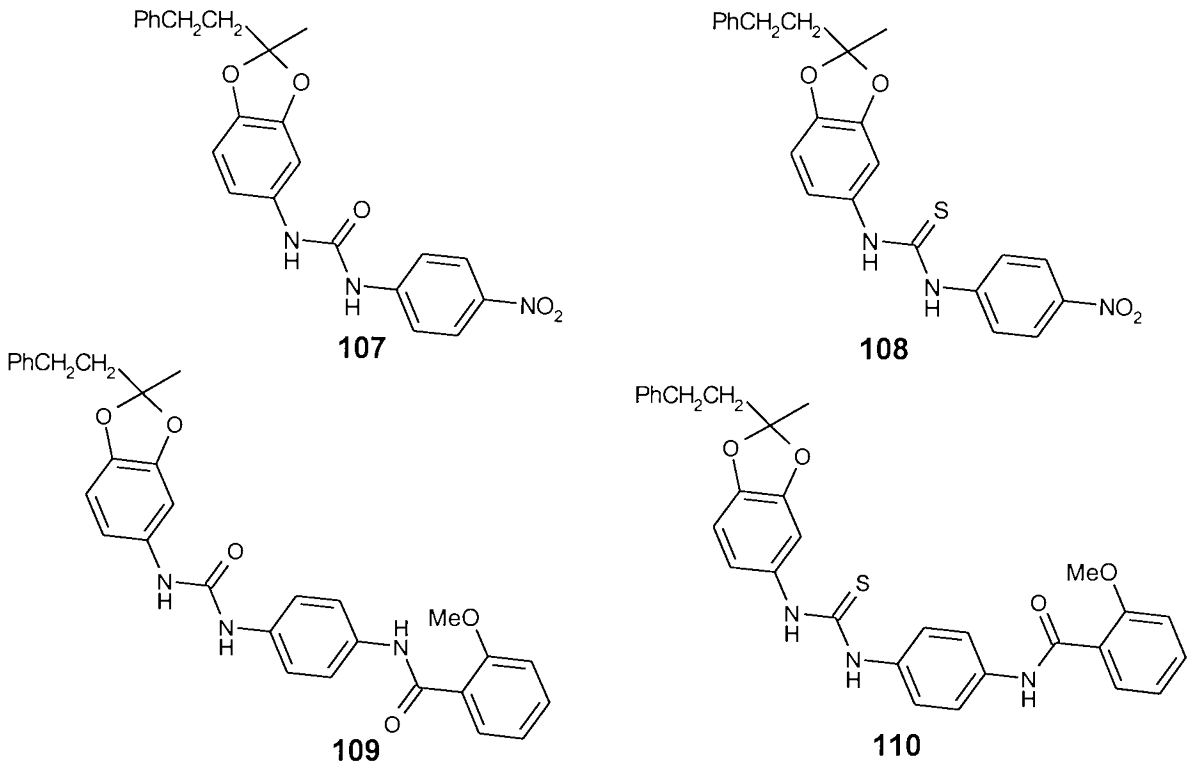
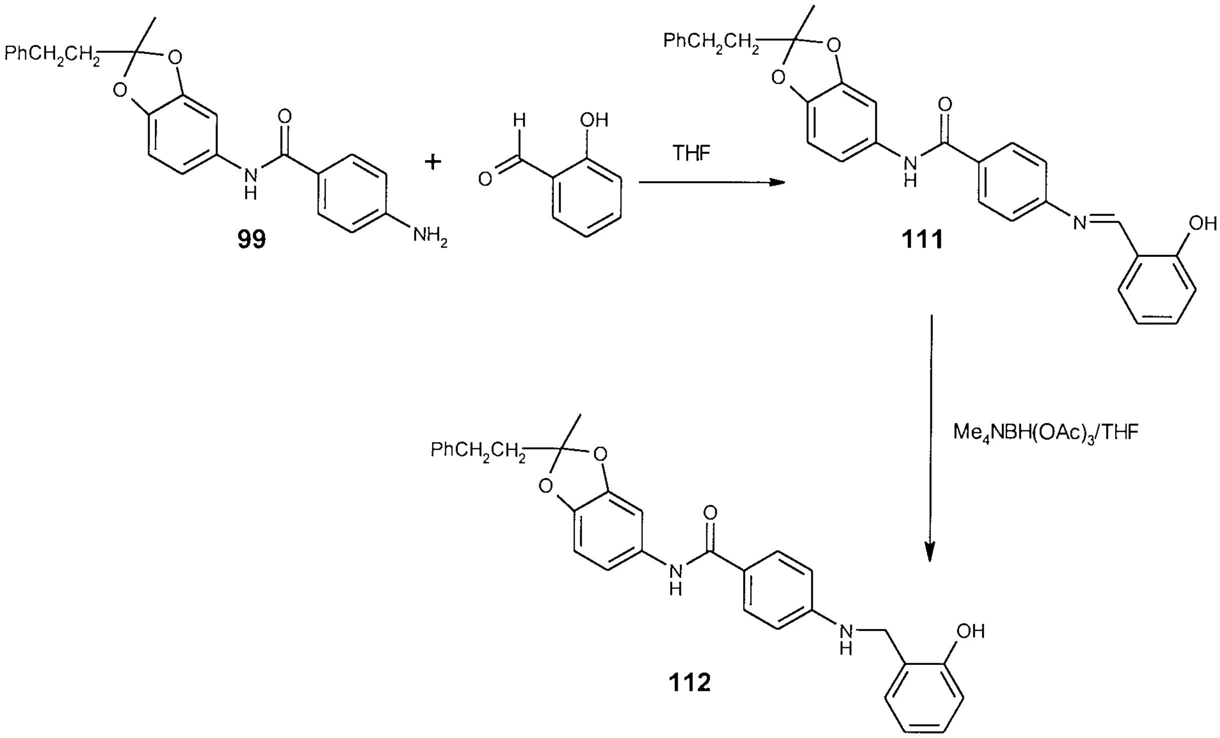


Figure 6.5 Structures of the protected ureas and thioureas prepared

6.6.3 Secondary amines

Aldehydes may be coupled with amines to form imines which may then be reduced to give secondary amines. Salicylaldehyde was coupled with the amine **99** to give the imine **111** in 79% yield.



Scheme 6.8 Reductive amination

The imine **111** was reduced to the corresponding amine, by heating with tetramethylammonium triacetoxyborohydride [$Me_4NBH(OAc)_3$] to give the amine **112** in 63% yield. Reductive aminations are commonly carried out using cyanoborohydride or sodium borohydride[167,168]. Tetramethylammonium triacetoxyborohydride was chosen as the reagent for the reductive amination because of previous success in automated library synthesis[169], the lack of formation of toxic by-products and the ease of use and ease of removal. This entire procedure may be carried out in 'one pot' without the isolation of the imine intermediate. Compounds of this nature on biological testing would investigate the effect of changing the terminal amide to an amine function.

6.7 Deprotection of ketal-protected catechols analogues and their ether protected hydroxy groups

The ketal protecting group is acid-labile. Previous workers using Merrifield resin and catechol functions attached to the resin by a ketal linkage, had used 5M HCl to cleave the products from the resin[165]. Other solid-phase ketal cleavages have employed 90-95% TFA and water[170]. Hydrolysis of the ketal protecting group was tested after the first acid chloride coupling reaction with compounds **86** and **98**. An initial concentration of 10% TFA was increased to 30% TFA in dioxane to cleave the ketal group from **86** to give the catechol **113** in 35% yield. Use of 1M HCl under reflux resulted in a more efficient deprotection of **98** to give the catechol **114** in 71% yield. The deprotection of analogues using HCl or TFA would enable the preparation of analogues where the terminal aromatic group contains a methoxy substituent. It seems likely, however, that for biological activity this would need to be deprotected to give the free hydroxyl group[163].

Previous workers[163] preparing the catechol compounds (**Scheme 6.1**) prepared the final products by demethylation of the methoxy groups using one equivalent of BBr_3 per methoxy group. Aromatic methyl ethers and acetal groups are known to be cleaved in inert solvent by BBr_3[171]. In the light of these observations it was decided to deprotect the methoxy group and try to cleave the cyclic ketal using three equivalents of BBr_3 in DCM. The protected compounds **88**, **89**, **102**, **103**, **109**, and **110** were all subjected to at least three equivalents of BBr_3 in DCM and heated under reflux. The resulting compounds **115**, **116**, **117**, **118**, **119**, and **120** were all fully deprotected, and were collected in yields ranging from 29% to 90%. Deprotection of the catechol group of the secondary amine compound **112** was achieved using 2.5M HCl, to give the catechol analogue **121** in 18% yield. The ability to cleave the secondary amine analogue suggested that a library of these compounds could be prepared using a polymer support and automated synthesis.

6.8 Biological testing

The catechol analogues prepared were synthesised in an attempt to explore the feasibility of performing the coupling reactions involved on a polymeric support with a view to making large libraries of compounds on a polymeric support using automated synthesis. The final seven compounds prepared and the control CV-6504 were subjected to an initial biological screen (Panlabs pharmacology services, Taiwan) to show inhibition of rabbit reticulocyte 15-lipoxygenase at a preliminary concentration of 30μM. All the compounds at this concentration gave an inhibition of ≥99%; in contrast the control compound CV-6504 gave negligible inhibitory activity. The active compounds were then subjected to a five point IC_{50} determination (n=2) on rabbit reticulocyte 15-lipoxygenase (**Table 6.1**).

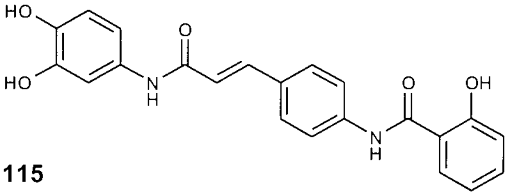
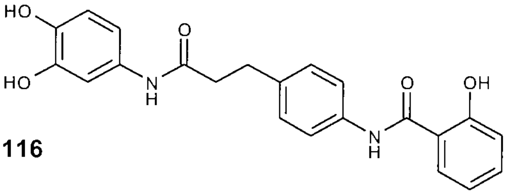
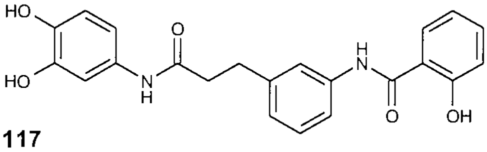
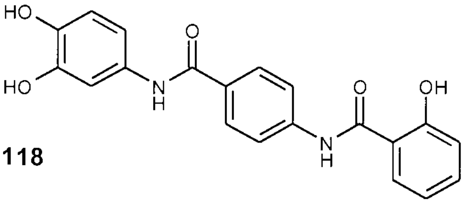
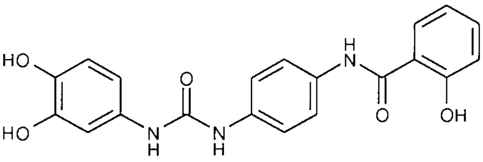
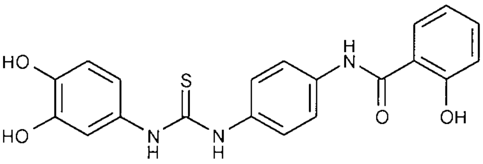
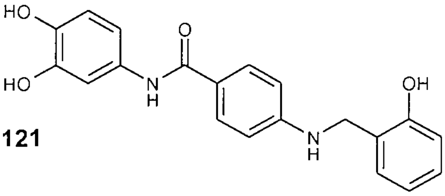
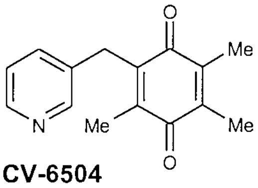
COMPOUND	% Inhibition of Rabbit Reticulocyte 15-LO at 30μM	IC$_{50}$ for Rabbit reticulocyte 15-LO (μM)
 **115**	102%	1.47
 **116**	99%	1.45
 **117**	102%	1.67
 **118**	100%	1.27
 **119**	104%	1.51
 **120**	103%	1.95
 **121**	103%	1.01
 **CV-6504**	3%	-

Table 6.1 Inhibitory activity of catechol analogues towards 15-lipoxygenase

6.9 Conclusion

This piece of work showed that novel compounds containing a catechol moiety may be prepared by traditional chemistry, in solution, to give potent inhibitors of 15-lipoxygenase. This project showed that it is feasible to protect the catechol moiety with a ketal functionality and carry out aromatic nitro group reductions and subsequent amine coupling reactions with a variety of functions in solution, with conditions that may be applied to a polymeric support.

The validity of making such compounds was confirmed by the good 15-lipoxygenase inhibitory activity shown by the seven test compounds prepared. The preliminary biological results for inhibition of 15-lipoxygenase suggest that the central aromatic core of the molecule and the linking groups between this and the catechol moiety and the salicyl moiety are not of great importance to the biological activity. These results justify the preparation of analogues of these compounds for biological testing. This may best be done using automated synthesis on a polymeric support. The next section will describe the search for a suitable polymeric support and test reactions performed on these supports.

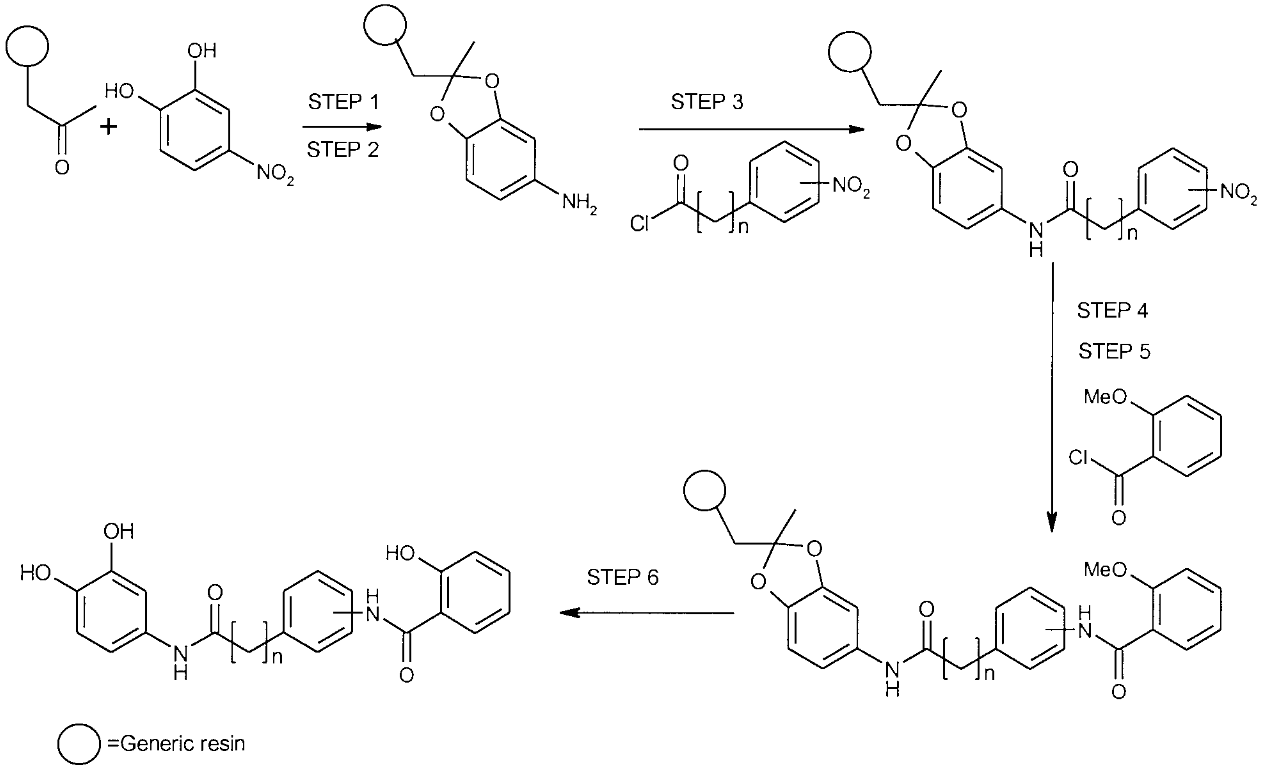
Chapter 7

Transfer of solution-phase synthesis to polymer-supported synthesis

The aim of this work was to find a suitable support for the preparation of catechol derivatives previously made in the solution-phase (**Chapter 6**). The objective was to transfer the solution-phase synthesis to a polymeric support in a way that would be amenable to the automated synthesis of small molecule libraries of catechol derivatives for biological testing.

7.1 Merrifield resin

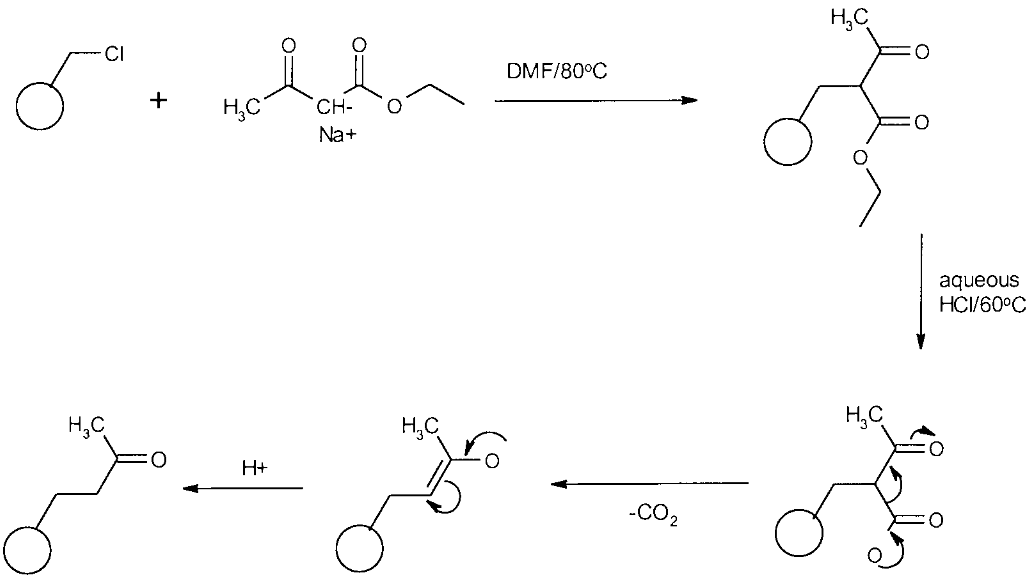
Initial literature investigations led to the concept of transforming Merrifield chloromethylated resin to the phenethyl ketone resin, and then attaching the catechol via ketal formation and applying the reduction/coupling regime employed for the solution-phase synthesis (**Chapter 6**).



Scheme 7.1 Postulated synthesis of catechol derivatives on a solid-phase resin

7.1.1 Conversion of Merrifield chloromethylated resin to the phenethyl ketone resin

Merrifield resin was chosen initially for the attachment of a catechol group to a solid support. This is a cross-linked polystyrene resin containing pendant chloromethyl groups[172]. Work has been reported where the chloromethyl group of Merrifield resin has been derivatised to give a pendant ketone[173,174]. Thus Merrifield resin was reacted with excess sodium ethyl acetylacetate to produce the β-keto ester and then hydrolysed with HCl to yield the Merrifield phenylethyl ketone resin **122** (**Scheme 7.2**).



122

Scheme 7.2 Preparation of Merrifield phenethyl ketone resin

The transformation of the chloromethyl function of the Merrifield resin to the ketone function was confirmed by the appearance of a broad strong carbonyl absorbance at 1716cm^{-1} by IR spectroscopy.

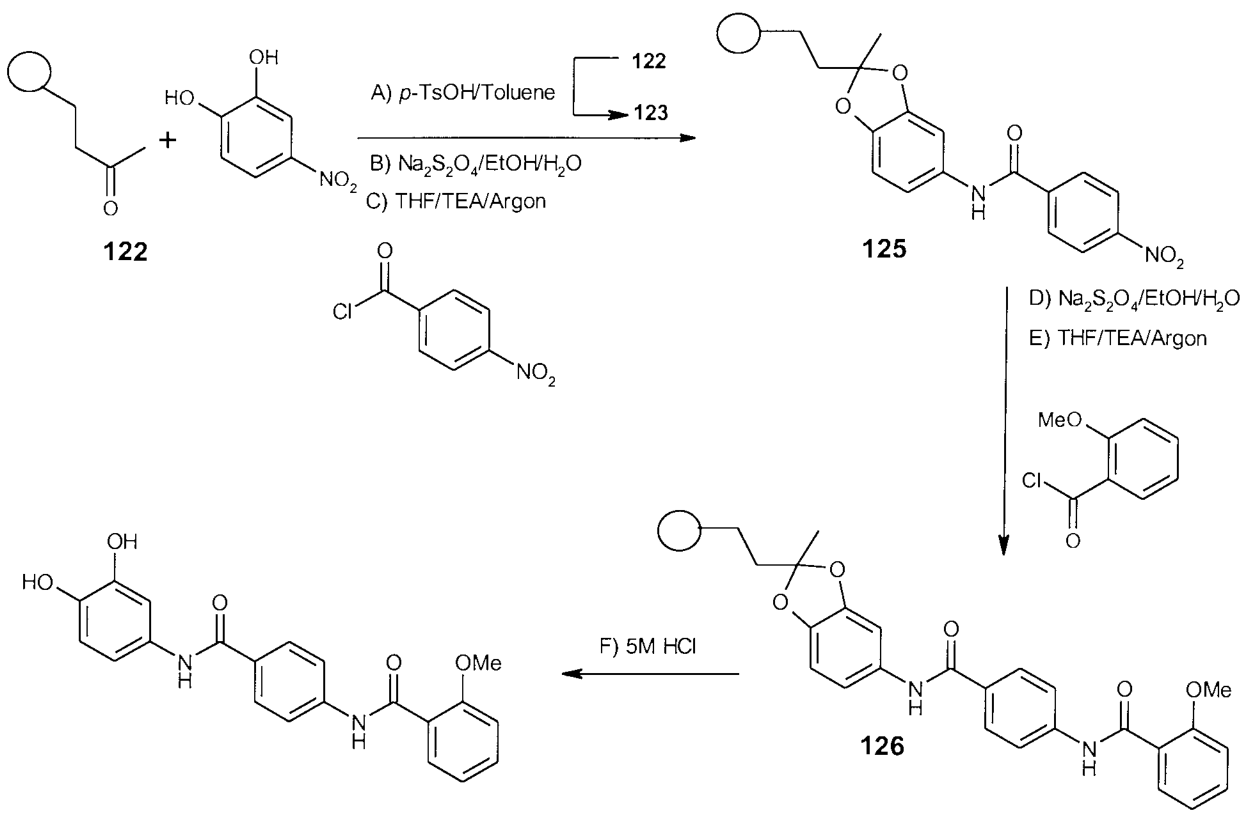
7.1.2 Protection of 4-nitrocatechol with Merrifield phenethyl ketone resin (Step 1, Scheme 7.1)

With the successful formation of the phenethyl ketone resin **122**, attachment of the 4-nitrocatechol group was attempted using the same procedure as was used for the solution-phase preparation of catechol analogues (see **Chapter 6**). The phenethyl ketone resin **122** was heated under reflux in toluene with excess 4-nitrocatechol and a catalytic amount of p-toluene sulphonic acid. The reaction was monitored by IR spectroscopy for the disappearance of the ketone absorbance at 1716cm^{-1}. IR spectroscopy of the products **123** showed a reduction in the intensity of the ketone absorbance after 24 hours. After 4 days, however, the ketone absorbance was still present and could not be decreased in intensity any further. It was decided that the ketal formation should be performed at a higher temperature. By heating the reaction under reflux in the solvent xylene, an attempt was made to try and react all the ketone sites within the resin with the excess 4-nitrocatechol. The same result was obtained for xylene as for toluene. It was concluded that despite the excess catechol and the higher temperatures used, some ketone sites within the matrix of the cross-linked resin were not available for ketal formation. When the IR spectrum of the Merrifield phenethyl ketone resin **122** was later compared with the IR spectrum of soluble poly(MVK-*co*-St) (see **Section 7.2.1**) the ketone absorbance was found to be much broader for the solid resin, suggesting that there were ketone functionalities in more than one environment within the solid Merrifield phenethyl ketone resin **122**.

7.1.3 Amine formation and acid chloride coupling on Merrifield phenethyl ketone resin (step 2 and 3, Scheme 7.1)

An attempt was made to reduce the aromatic nitro group of the resin bound catechol **123** using sodium dithionite in the homogeneous reduction method used for the initial solution-phase reductions (**Chapter 6**). The original solution-phase reduction reactions using sodium dithionite gave poor yields which were thought to be due to the difficulty in phase separation encountered during the work up procedure. The problem of difficult phase separation should not be encountered with solid-phase resin synthesis because of the ease of resin recovery by filtration. Nitro-group reduction was attempted and the subsequent acid chloride coupling reactions, with 4-nitrocinnamoyl chloride to give **124** and with 4-nitrobenzoyl chloride to give **125** were performed without isolation of the intermediate amine product.

Both of the products **124** and **125** gave similar IR spectra which implied that the reduction and coupling steps had not occurred or that the differences in the compounds were not significant enough to be seen by IR spectroscopy. Absorbances representing the resin-bound compounds were difficult to identify, because the resin itself gave a complex spectrum which may have been obscuring absorbances associated with the attached moieties. For **124** and **125** IR spectrum absorbances could be still be identified for the unreacted ketone at 1716cm^{-1}, and new absorbances were also observed in the regions of 3460 and 1750cm^{-1}, which could have been interpreted as absorbances representing a new amide functionality, suggesting that the coupling had occurred. Although there was some evidence that the reduction and coupling steps had occurred, the IR spectroscopy was not conclusive, therefore, an attempt to determine the success of the reduction and coupling reactions was made by cleavage of the pendant molecules from the solid support.



Scheme 7.3 Attempted synthesis of catechol analogues on Merrifield phenethyl ketone resin

7.1.4 Cleavage of resin-bound products

Cleavage of the resin-bound compounds of **124** was attempted using the conditions reported in the original literature, namely, 5M HCl in dioxane, heated at 80°C for 8 hours[173,174]. Analysis of the resulting cleaved solid showed no assignable peaks by ^{1}H NMR spectroscopy or mass spectrometry. It is conceivable that the reduction and coupling procedures did not work. If this were the case, however, then the starting material 4-nitrocatechol should have been recovered, but was not. Assuming that the reduction and coupling did occur, it is possible that the compound intended for isolation was unstable to the air (as found in the solution-phase preparation, **Chapter 6**). It is also feasible that the strongly acidic conditions used to hydrolyse the ketal function, also hydrolysed the amide bonds of the desired products.

Further reduction of **125** in homogeneous solution and its subsequent coupling with 2-methoxybenzoyl chloride to give **126** was attempted (**Scheme 7.3**). Analysis of **126** proved even more difficult than in previous steps. The IR spectrum of the product looked the same as that of its starting material **125**. This result could be interpreted in two ways; one, the reactions did not occur, or two, the newly added function gave no new IR absorbances and masked the disappearance of the aromatic nitro group absorbance in the IR spectrum. Cleavage of the product from the resin was attempted because it was the only way of interpreting the success of the previous reactions. As before, 5M HCl in dioxane at 80°C was used, in an attempt to hydrolyse only the ketal bonds, the reaction time was reduced from 8 to 4 hours. Analysis of the resulting brown solid by ^{1}H NMR showed many peaks in the aromatic region but no identifiable product peaks by mass spectrometry. No purification or further reactions were attempted using the solid-phase phenethyl ketone resin.

7.1.5 Problems encountered with Merrifield resin

The lack of traditional analytical methods that could be used to interpret intermediates of resin-bound compounds made the success of the synthesis difficult to judge. Problems may have been encountered with the reduction step. Polar solvents were required to dissolve the sodium dithionite for the reduction the aromatic nitro groups. The polar solvents used may, however, not have allowed the resin to swell[13], so reduction would not have occurred at unsolvated sites within the resin. The instability of amine intermediates made it hard to determine whether or not the reduction had occurred. The acid chloride coupling step with the aromatic amine is dependant on anhydrous conditions suggesting a second problem. The reduction step involves water which may not be completely removed before the acid chloride coupling, resulting in the formation of the carboxylic acid before the acid chloride can couple to the amine.

Lastly, the manufacturers of the Merrifield resin could not guarantee the stability of the resin itself at high temperatures. Verbal communication with the manufacturer suggested that the resin would withstand 150°C for 30 minutes, but they had no other stability information and did not recommend long periods of heating. In the original patent, catechol was reacted with the phenethyl ketone resin, at 90°C for 30 hours. The catechol function of 4-nitrocatechol is less reactive than that of catechol itself because of the electron withdrawing effect of the nitro group and therefore, needs a longer reaction time. The literature suggests that polystyrene can withstand temperatures of 220°C[175] before breaking down, but the sustained heating at 115°C for up to 96 hours required for the attachment of the 4-nitrocatechol may have degraded the resin in the first step. If this were the case it would explain why the resin was black after the first ketal formation step and also after the cleavage step. IR spectra of the resin after cleavage showed only small differences from the IR spectra of the resin before cleavage, making it difficult to know whether the product was cleaved from the resin at all. There were too many unknown factors to make further synthesis on solid-phase resin a feasible project with which to continue.

7.2 Soluble polymers with pendant ketone functionalities

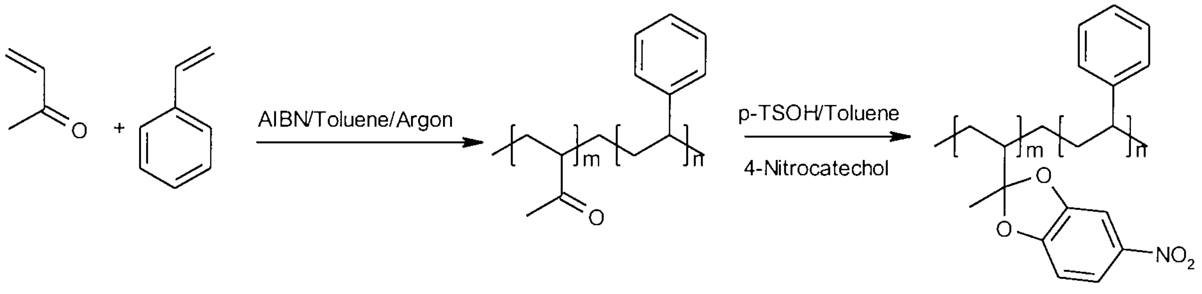
The main problem encountered with the insoluble Merrifield resin was the lack of analysis that could be performed using traditional methods on the intermediate products. A second problem encountered was the difficulty of transferring solution-phase chemical synthetic protocols to a solid-phase support. The use of a soluble polymer should overcome both of these problems. A soluble polymer may be analysed by ^{1}H NMR. Pendant groups of soluble polymers have a reaction profile similar to that of the functional groups in solution. In the light of theses two advantages a soluble polymer containing a pendant ketone function was sort.

7.2.1 MVK-*co*-ST soluble linear polymer

MVK is known to successfully co-polymerise with styrene[175]. Radical polymerisation was used to prepare a co-polymer from the monomers, styrene (St) and methylvinylketone (MVK). The poly(MVK-

co-St) was prepared with a range of maximum potential loadings based on 100% incorporation of the starting monomers into the final polymer. The higher the loading of a soluble co-polymer the more likely that the polymer will take on pendant molecule characteristics, hence a range of co-polymers of different loading potentials were prepared to determine the best one to use as a support for compound synthesis. Poly(MVK-*co*-St) co-polymers **127**, **128** and **129** were prepared with maximum potential loadings of 1.97, 3.60 and 5.75 mmol/g, respectively. The maximum potential loading of the polymer was taken to be the number of mmoles of the functionalised monomer (e.g. MVK) in 1g of the co-polymer. This was calculated from the amounts of each monomer added in the initial polymerisation reaction.

7.2.1.1 Ketal formation with poly(MVK-*co*-St)



127-129

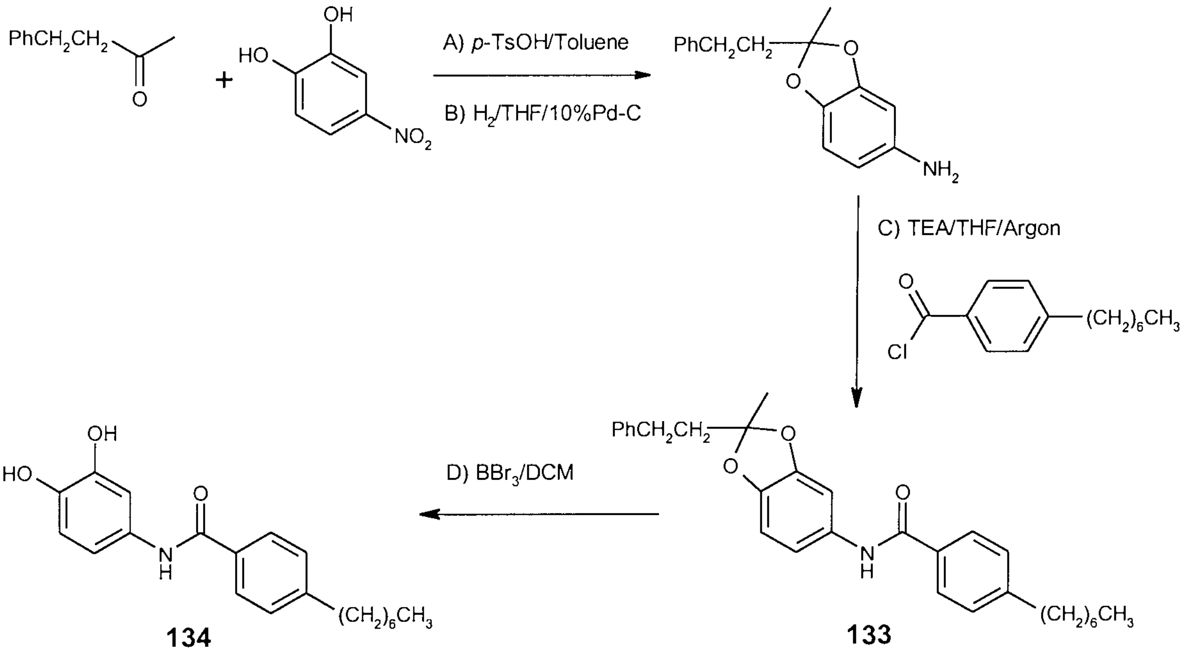
130-132

Scheme 7.4 Poly(MVK-*co*-St) formation and Ketal formation with 4-nitrocatechol

The formation of the ketal from 4-nitrocatechol with the pendant ketone functionalities of poly(MVK-*co*-St) was attempted in the same way as for the solution-phase synthesis (see **Chapter 6**). An attempt was made with the (MVK-co-St) polymers for each different maximum potential loading **127**, **128** and **129** to give **130**, **131** and **132**. It was found with the polymers of higher loading (3.50 mmol/g **128** and 5.75mmol/g **129**) that the 4-nitrocatechol conferred its solubility properties to the polymer. Once dry, the higher loading polymers (**131** and **132**) could not be re-dissolved in the organic solvents tried. The first attempt to form the ketal with the lower loading co-polymer (**127**, 1.97mmol/g), was performed over 24 hours. The resulting polymer **130** had only partially reacted. The IR spectrum showed a reduction in the intensity of the ketone absorbance, but it had not completely disappeared. The ^{1}H NMR did, however, show the appearance of a new broad resonance at 7.75ppm, downfield from the aromatic backbone of the polymerised styrene at 6.20-7.23ppm suggesting ketal formation. When the ketal formation was monitored by IR spectroscopy it was found that a longer reaction time of 48 hours, was needed for complete disappearance of the ketone absorbance of the polymer. When complete, the excess catechol was removed by precipitation of the polymer **130** onto methanol. The 4-nitrocatechol being soluble in methanol could be washed away and the polymer was collected as a precipitate. It was found that some unreacted catechol could become entrained in the solid polymer during the precipitation process, it was therefore necessary to re-dissolve and re-precipitate the polymer a further three times to completely rid the polymer of the excess reagent. This technique was, therefore, employed for any subsequent reactions on this and other soluble polymers.

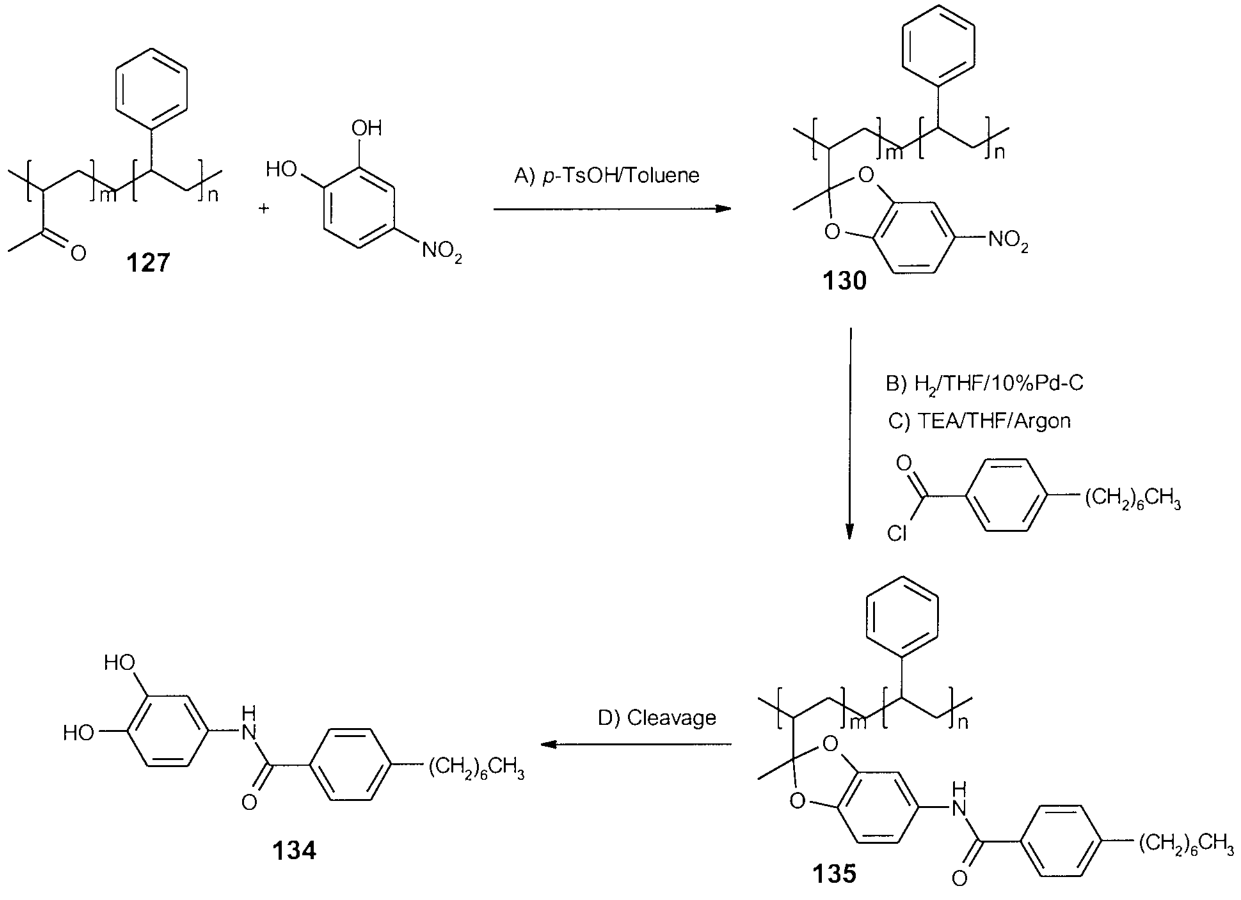
7.2.1.2 Model compound, *N*-(3,4-dihydroxyphenyl)-4-heptylbenzamide

To illustrate that pendant molecules could be prepared on, and cleaved successfully from, the poly(MVK-*co*-St) it was decided to prepare a lipophilic test compound **134** on the polymeric support. Firstly, traditional solution-phase chemistry was used to prepare the test compound **134** (**Scheme 7.3**). This process provided a standard for the test compound **134** and confirmed that the chemistry worked in solution.



Scheme 7.5 Solution-phase synthesis of the model compound *N*-(3,4-dihydroxyphenyl)-4-heptylbenzamide **134**

Once made in solution the same reaction scheme was applied to the poly(MVK-*co*-St) **127** (**Scheme 7.6**). The 4-nitrocatechol was attached to the co-polymer through ketal formation to give **130**. The aromatic nitro group was then reduced by heterogeneous catalytic hydrogenation and the resulting amine was immediately coupled to 4-*n*-heptylbenzoyl chloride to give the co-polymer **135**. IR spectroscopy could not confirm the reduction and coupling reaction, but the ^{1}H NMR spectrum showed new peaks representing the pendant alkyl group and also the newly formed amide proton at 8.15ppm.



Scheme 7.6 Synthesis of the model compound using poly(MVK-co-St) as a soluble support

Cleavage of the pendant test compound **134** was attempted with 17% TFA and 34% TFA, but this method did not yield the desired compound as judged by TLC and ^{1}H NMR and comparison with the authentic material obtained by solution-phase synthesis (**Chapter 7**). Although the TLC was the same as that of the test compound, the ^{1}H NMR and the mass spectrum indicated that it was not **134**. A second method of ketal hydrolysis for the test co-polymer **135** was attempted using a two phase approach with aqueous HCl and DCM (1:1). The idea was to maximise the HCl concentration without precipitating the polymer, by keeping the polymer dissolved in the DCM while forming an emulsion by vigorous stirring. This method left the pendant groups on the polymer intact and so was not pursued. Cleavage was then tried using 3M HCl in dioxane. The test compound was collected successfully, to give a minimum polymer loading of 0.17mmol/g. Analysis by ^{1}H NMR of the remaining polymer precipitate was consistent with incomplete cleavage.

The pendant compounds on the polymer appeared to be cleaved with BBr_3 at 2eq per mole of pendant molecule attached to the polymer, at room temperature for 16 hours. The ^{1}H NMR of the co-polymer after the cleavage procedure showed no peaks that could be associated with any pendant molecules. The cleaved residue collected, however, contained five isolatable components that could not be identified by ^{1}H NMR spectroscopy or mass spectrometry. Heating the polymer under reflux for 2 hours with the same amount of BBr_3, as before, also appeared to cleave the pendant groups from the polymer. The ^{1}H NMR spectrum of the final polymer showed no pendant compound protons.

Purification of the residue remaining after cleavage yielded the test compound **134**, to give a minimum loading 0.18mmol/g. It was considered that the test compound had given enough evidence that catechol compounds of this nature could be prepared on and cleaved from the poly(MVK-*co*-St).

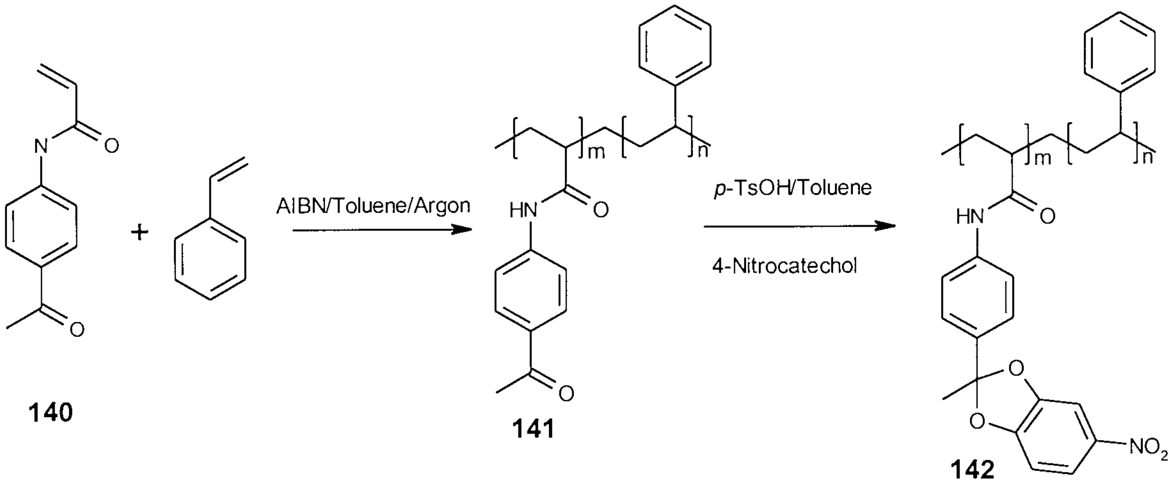
Hydrogenation of the pendant aromatic nitro groups of the co-polymer **130** and immediate coupling of the reduced co-polymer with the acid chloride, 4-nitrocinnamoyl chloride was performed. It was decided to cleave the bound product from the co-polymer in order to establish the extent of reaction on the pendant groups of the polymer. The aim was to cleave any pendant molecules from the polymer **136** using 5M HCl. Since the polymer was insoluble in water, it was dissolved in dioxane and treated with concentrated HCl until just below the point of precipitation of the polymer. It was found that a maximum concentration of 2M HCl could be obtained before the polymer precipitated. Heating the acidic mixture under reflux for 8 hours and then removing the polymer by precipitation and filtration, did not yield the desired product but a complex mixture of compounds. ^{1}H NMR analysis of the polymer indicated that cleavage had not occurred implying that the conditions were not strong enough to hydrolyse the ketal group attached to the polymer. Similarly, the use of 10% TFA at 80^{o}C for 18 hours, also failed to hydrolyse the ketal group and the co-polymer with its intact pendant groups could still be seen by ^{1}H NMR analysis after the attempted cleavage.

Considering that the pendant groups could be observed by ^{1}H NMR, it was decided to continue with the second aromatic nitro group reduction and coupling procedure should be performed with 2-methoxybenzoyl chloride to give **137**. Then cleavage of pendant groups from the co-polymer could be attempted again. The success of the second reduction and acid chloride coupling procedure was confirmed by the appearance of a new broad peak in the ^{1}H NMR spectrum at 9.85ppm representing the NH proton of the newly formed amide. The same cleavage conditions were employed as were used for the previous solution-phase synthesis (**Chapter 6**). A solution of 1M boron tribromide (BBr_3) in DCM was used with 3 equivalents per mole of pendant molecule on the co-polymer. A precipitate of the desired product formed immediately in the solution-phase preparation. This effect was not observed with the co-polymer, even after heating under reflux. When the polymer was removed by methanol-induced precipitation and filtration, analysis by ^{1}H NMR spectroscopy showed the resulting polymer to have no pendant functions. The desired product however, could not be isolated. Possibly the poor solubility profile of the desired product hindered its isolation and purification.

Another attempt was, therefore, made to synthesise one of the catechol analogues supported by this polymer **130**. 4-Nitrocatechol was attached in the usual manner, the aromatic nitro group was reduced as before, and immediately coupled to 4-nitrobenzoyl chloride to give **138**. The reduction and coupling procedure was confirmed by ^{1}H NMR analysis. The second reduction step was then carried out and the second acid chloride coupling was performed with 2-methoxybenzyl chloride and again confirmed by ^{1}H NMR to give **139**. Cleavage of the resultant polymer bound molecules was then attempted by heating the polymer under reflux for 2 hours with BBr_3, 3 equivalents per mole of pendant compound as done before for the test molecule **134**. After the cleavage step, the resulting polymer showed none of the previously present pendant groups, suggesting that the ketal had been hydrolysed, but as before the desired compound could not be isolated. The difficulty in isolating the product was again, put down to its poor solubility. It was envisaged that any attempt to purify this and other compounds like it by flash column chromatography would lead to its precipitation on the column from the eluting phase and therefore, its loss.

7.2.2 n-(4-Acetylphenyl) acrylamide-*co*-St soluble linear polymer

Poly(MVK-*co*-St) **127** although promising as a support for the test molecule **134** did not prove to be of use for the more insoluble target catechol compounds. It was hypothesised that moving the pendant molecule further away from the polymer backbone would make it easier to cleave from the supporting polymer. New polymers, were therefore sought with pendant ketone functionalities further away from the polymer backbone. The monomer *N*-(4-acetylphenyl) acrylamide **140** was made in 43% yield by the reaction of acryloyl chloride with *p*-aminoacetophenone. This monomer was co-polymerised with styrene, to give a co-polymer of maximum potential loading of 0.73mmol/g. Confirmation of the co-polymerisation could be seen in both the ^{1}H NMR and IR spectrum. In the ^{1}H NMR spectrum a broad peak at 7.85ppm, could be seen, representing the amide proton of the polymerised acrylamide and a broad peak at 2.65ppm could be seen for the methyl group of the polymerised ketone function. IR spectroscopy showed amide absorbances and a ketone absorbance in the expected positions.

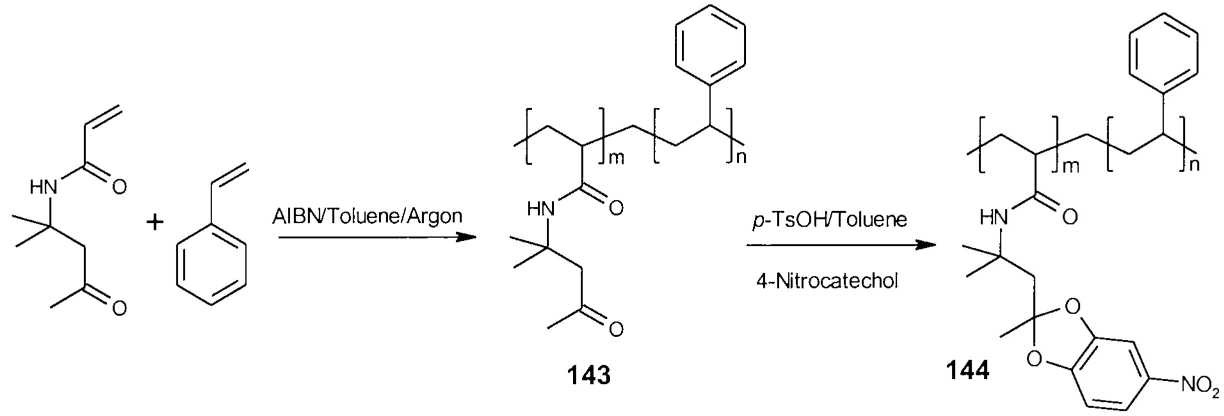


Scheme 7.7 Synthesis of *N*-(4-Acetylphenyl) acrylamide-*co*-St and ketal formation with 4-nitrocatechol

Formation of a ketal from the ketone functionality of the co-polymer **141** and 4-nitrocatechol was attempted in the usual manner, by heating under reflux in toluene with *p*-TsOH (**Scheme 7.7**). Analysis of the product **142** by IR spectroscopy showed that the ketone function had not reacted completely, although its abundance had been decreased. The ^{1}H NMR spectrum of the product showed new peaks at 7.60-7.70ppm, in the aromatic region, suggesting attachment of the catechol to polymer support. This product **142** was reduced in the same manner as for the solution-phase chemistry and acid chloride coupling was attempted with 4-nitrocinnamoyl chloride. No further work was performed on this polymer because of the lack of solubility encountered and other polymers were prepared.

7.2.3 Diacetone acrylamide–*co*-St soluble linear polymer

Diacetone acrylamide was commercially available and it was thought that the co-polymerisation of this with styrene might overcome the solubility problems encountered with the other polymers. A co-polymer of diacetone acrylamide and styrene **143** was prepared to give a maximum potential loading of 1.00mmol/g. Evidence for the successful co-polymerisation could not be seen in the ^{1}H NMR spectrum, however, IR spectroscopy showed amide peaks and a ketone peak could be seen at 1710cm^{-1}.



Scheme 7.8 Diacetone acrylamide–*co*-St soluble linear polymer

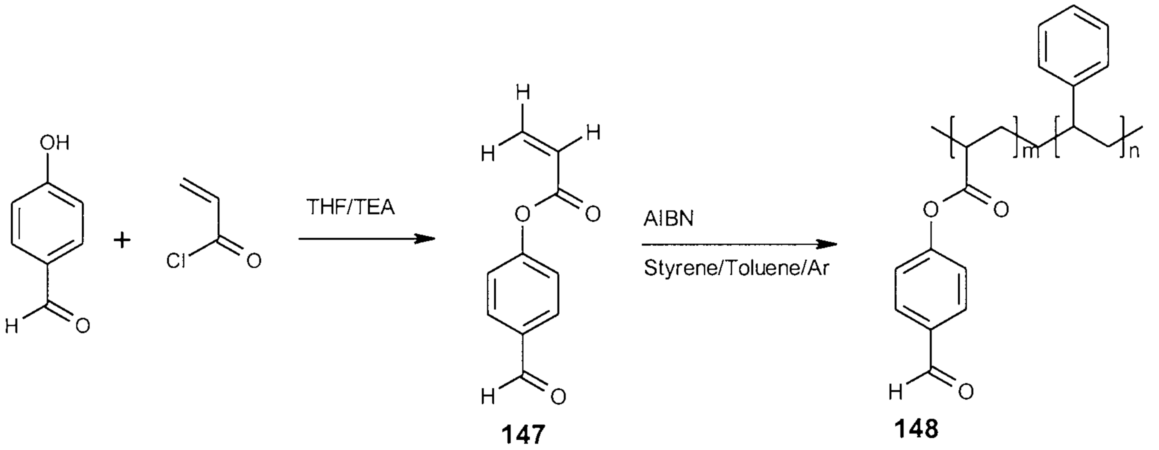
7.2.3.1 Further reactions on poly(Diacetone acrylamide–*co*-St)

Ketal formation with the pendant ketone functionalities and 4-nitrocatechol was performed in the usual manner on the co-polymer **143**. ^{1}H NMR analysis of the resulting polymer **144** showed a new peak at 7.68ppm, indicating the presence of new aromatic protons after the ketal formation. The IR spectrum showed that the ketone absorbance had completely disappeared when compared to the spectrum of the starting polymer, suggesting complete transformation of the ketone to the ketal. Heterogeneous catalytic hydrogenation of the aromatic nitro group was performed followed by the immediate coupling with 4-nitrobenzoyl chloride. The resulting co-polymer **145** showed new peaks in the aromatic region of the ^{1}H NMR suggesting that the hydrogenation and coupling steps had been successful. The second hydrogenation step and coupling with 2-methoxybenzoyl chloride, were performed and the final co-polymer **146** again gave new peaks in the aromatic region of the ^{1}H NMR spectrum. The IR spectrum, however, showed no notable change, but this was expected because no new functional groups were being introduced into the molecule. Analysis by ^{1}H NMR showed that after each stage of the reaction process peaks representing the pendant molecules in the polymer were becoming smaller with respect to the polymer back-bone. This suggested that the solubility of the polymer was being decreased by the pendant molecules formed on the polymer and that insoluble particles were being removed during work up procedures. Cleavage of the pendant molecules from the polymer backbone with BBr_3 gave a mixture of compounds with no assignable peaks when analysed by ^{1}H NMR or mass spectrometry.

7.3 Soluble polymers with pendant aldehyde functionalities

It appeared from previous results that hydrogenation and coupling reactions could be performed on a soluble polymeric support. The problems seemed to be the cleavage and retrieval of the desired compound from the polymeric support at the end of the synthesis and also the solubility of the final polymers. It was hypothesised that the polymer back-bone was stabilising the ketal formed, making it difficult to hydrolyse for the subsequent recovery of the desired catechol compound. Another way of protecting a catechol function is by formation of an acetal moiety. An acetal group may be formed in the same way as a ketal, by using an aldehyde instead of a ketone. Acetals are less stable than ketals and are, therefore, more easily hydrolysed. It was proposed that a soluble polymer with pendant aldehyde moieties could be used to support the preparation of catechol containing compounds and yield the desired compounds through easier cleavage from the support.

The monomer **147** was prepared in 93% yield by the coupling of 4-hydroxybenzaldehyde and acryloyl chloride. It was then co-polymerised with styrene in 78% yield, **148** (**Scheme 7.9**). Verification of the co-polymerisation was given by ^{1}H NMR and IR spectroscopy. A large broad aldehyde proton peak was visible in the ^{1}H NMR spectrum at 9.96ppm and one pair of protons of the para-disubstituted aromatic ring could be seen at 7.76ppm. Both of these peaks gave integrals in the ratio of one and two pendant protons respectively, providing a means of calibrating the products of future reactions on the supporting polymer. The visible aldehyde proton would also provide a useful means of monitoring the formation of the acetal, by its disappearance, in the next step of acetal formation.

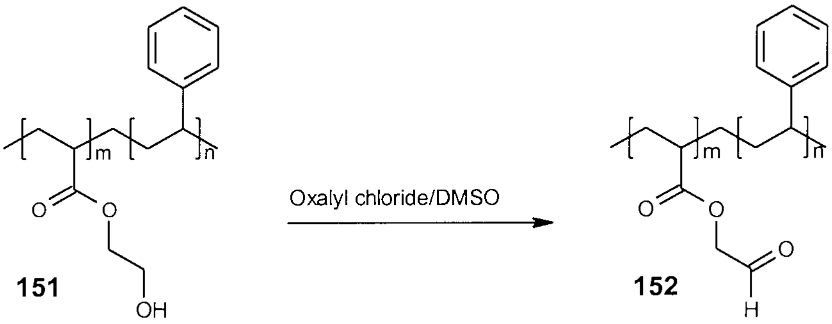


Scheme 7.9 Synthesis of 4-formylphenyl acrylate-co-styrene polymer

An attempt was made to form the acetal on the soluble polymer **148**, using 4-nitrocatechol. After heating under reflux for 18 hours the co-polymer **149** had precipitated out of solution and when collected and dried proved only sparingly soluble in a range of organic solvents. The acetal formation was confirmed by the diminished aldehyde peak at 1705cm^{-1} in the IR spectrum. The poor solubility of the resulting polymer, however, meant that it could not be reacted further. The maximum loading potential of the polymer **148** was 1.7mmol/g, it is possible that a lower loading polymer would not have become insoluble on conversion to the acetal. A lower loading polymer was not prepared, because it was considered that using a lower loading polymer would not provide a large enough quantity of final product to make the process feasible. A second aldehyde co-polymer of the commercially available vinyl benzaldehyde and styrene with a maximum loading potential of 1.8mmol/g was prepared **150**. The co-polymer once prepared and dried had a very poor solubility profile, no further reactions were therefore performed with this polymer as a soluble support.

7.3.1 Oxidation of soluble polymer pendant hydroxyl groups to form pendant aldehyde moieties

The idea of forming a soluble polymer with pendant aldehyde group was further investigated by the preparation of poly(2-hydroxyethylmethacrylate-*co*-St) **151**. The co-polymer **151** was prepared by radical co-polymerisation of commercially available 2-hydroxyethylmethacrylate and styrene in 74% yield, to give a maximum loading potential of 1.8mmol/g.



Scheme 7.10 Proposed oxidation of pendant polymer hydroxyl groups to aldehyde functionalities

Confirmation of the co-polymerisation could be seen in the ^{1}H NMR spectrum where two broad peaks could be seen for the pendant CH_2 groups. It was proposed that the pendant alcohol function could be oxidised to form an aldehyde function **152**, which could then be used to protect a catechol in the form of an acetal. Previous workers have effectively oxidised a cross-linked polystyrene-bound alcohol[176], with oxalyl chloride and DMSO. This method has also previously been used oxidise long chain alcohols[177,178]. Two attempts to oxidise the alcohol function of the soluble polymer **151** were made using this method. Both of the resulting polymers, on analysis, showed only one CH_2 group peak in contrast to the starting 2 peaks by ^{1}H NMR. Analysis by ^{1}H NMR, however, did not show an aldehyde proton peak. The IR spectrum was inconclusive, because any new aldehyde absorbance may have been masked by the ester absorbance. The IR spectrum also showed peaks that could represent hydroxyl functionalities. The products were both collected as a viscous gel, which suggests that the product had cross-linked in some way. If the aldehyde function formed had reacted with the polymer or the starting terminal hydroxyl function had transesterified with the linking ester moiety, this would explain the lack of an aldehyde peak and subsequent cross-linking. Another explanation for the lack of an aldehyde peak could be that the oxidation did not stop at the aldehyde and a carboxylic acid was formed, and this would explain the appearance of only one pendant CH_2 group in the ^{1}H NMR spectrum. The reaction could not be quantified and so was left at this point. This starting polymer **151**, however, does have the potential to be used as a polymeric support with its terminal hydroxyl group acting as the linkage.

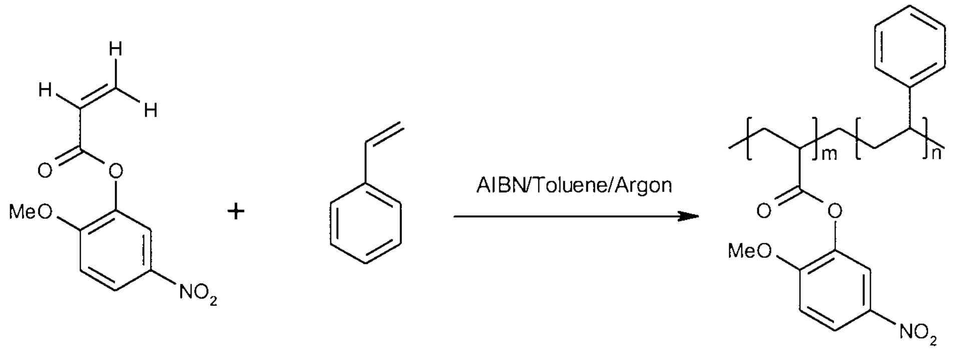
7.3.2 Polyethylene glycol

The soluble polymer polyethylene glycol (PEG) has been used as a soluble support in organic synthesis (see **Chapter 2**). PEG has been used to solubilise drug molecules[15]. Using PEG as a support would offer the advantages of; one increasing the solubility of the attached/pendant molecules and two; aiding analysis of reaction intermediates by ^1H NMR due to the lack of aromatic protons so providing a greater 'window' in the ^1H NMR spectrum.

It was anticipated that if the terminal hydroxyl function of PEG could be oxidised to the aldehyde, the product would provide a good support for the catechol analogues. PEG 5000 monomethyl ether was chosen because the methoxy group is visible in the ^1H NMR spectrum providing an opportunity to calibrate the conversion of the PEG hydroxyl group to the aldehyde. Attempts made to oxidise PEG using DMSO activated by phosphorus pentoxide only resulted in conversion to the aldehyde of 7-10%. When DMSO was activated with oxalyl chloride in large excess, the conversion was increased to 32%. For the PEG to be a successful support, it was considered that 100% conversion to the aldehyde would be necessary to allow for loading to be calculated and to minimise the unwanted reaction between pendant hydroxyl groups and added reagents, such as acid chlorides. No further attempts were made to oxidise the PEG momomethyl ether.

7.4 Incorporating the catechol function into a monomer for polymerisation

The next option available was to prepare a co-polymer containing the catechol moiety within the polymer backbone. The concept of incorporating the first building block in the synthesis into the polymer back-bone could be applied to the commercially available 2-methoxy-5-nitrophenol.



153 **154**

Scheme 7.11 Incorporating the first building block of synthesis into a soluble linear polymer

The acrylate **153** was formed in 90% yield and co-polymerised with styrene to give the co-polymer **154** in 77% yield. The maximum potential loading of the prepared polymer was 1.6mmol/g. The success of the co-polymerisation was supported by analysis of the ^1H NMR spectrum which showed peaks distinct from the polystyrene backbone representing the aromatic protons and the methoxy protons of the pendant group (**Figure 7.1**). The aromatic methoxy group increased the probability of the polymer staying soluble, would protect the potential hydroxyl group and could be removed with BBr_3 if necessary. BBr_3 being the final cleavage step in the solution-phase synthesis of the catechol analogues desired, made the co-polymer **154** ideal for the catechol derivative synthesis.

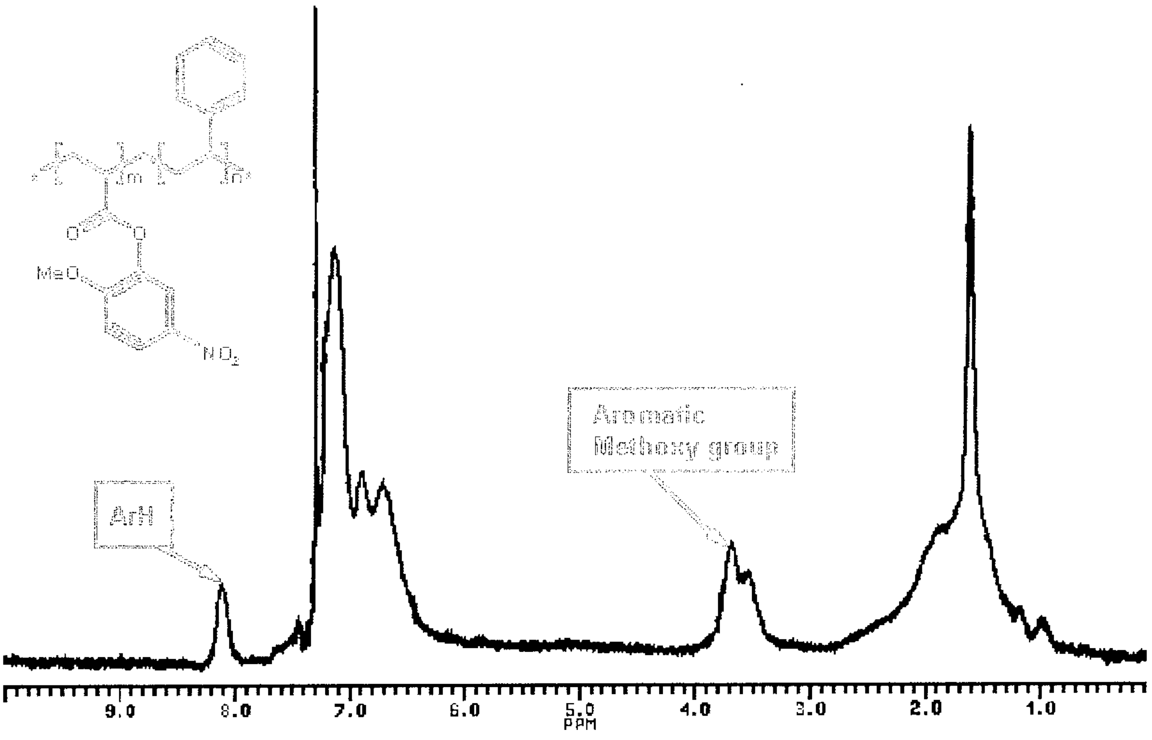
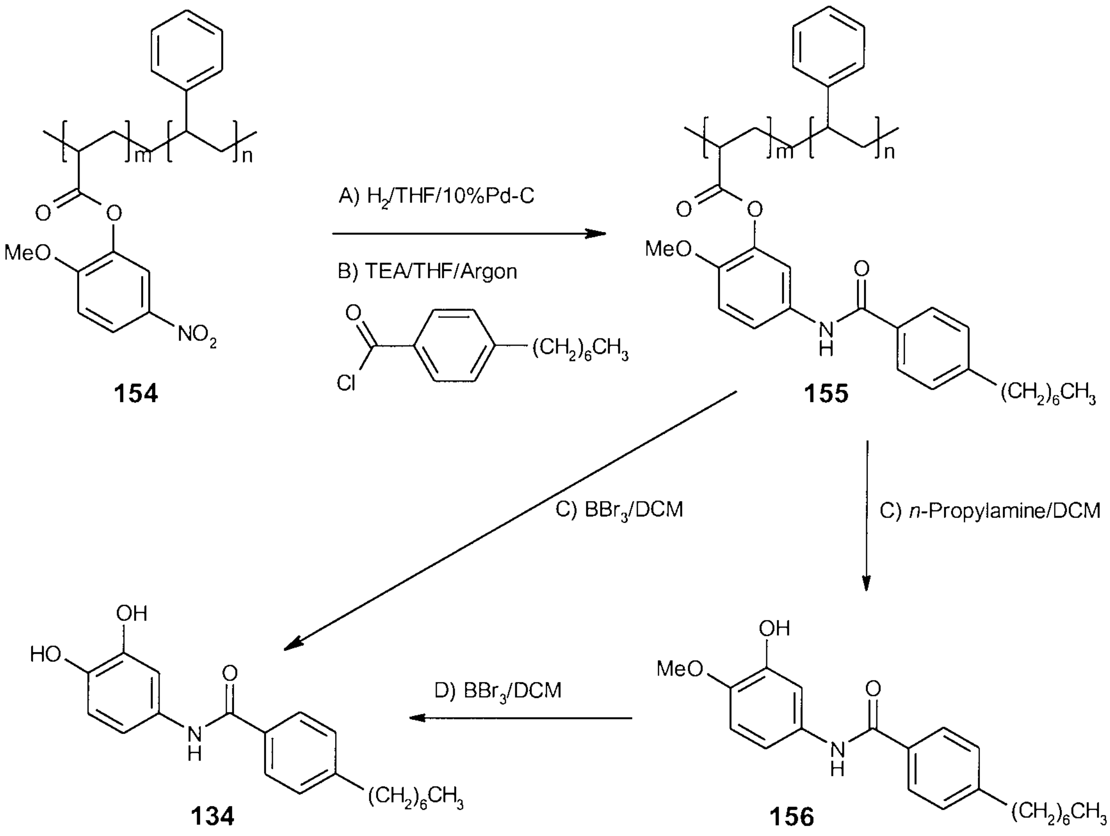


Figure 7.1 ^{1}H NMR spectrum of poly(2-methoxy-5-nitrophenyl acrylate-*co*-St) **154**

It was decided, as with poly(MVK-*co*-St) **127** to perform a test heterogeneous catalytic hydrogenation and coupling using the acid chloride, 4-*n*-heptylbenzoyl chloride. The intention of these test reactions was to prepare a lipophilic and therefore, more soluble pendant molecule than the final catechol analogues eventually required. Preparing a more lipophilic pendant group would keep the polymer soluble and the cleaved compound would be more easily collected after cleavage from the polymer. In contrast, the desired catechol analogues tend to have poor solubility profiles and are therefore, difficult to separate from a precipitated polymer.

Heterogeneous catalytic hydrogenation was firstly performed on the co-polymer **154**, in the usual manner with 10% Pd-C (10% by weight of polymer) at atmospheric pressure for 18 hours. As before, the acid chloride, 4-*n*-heptylbenzoyl chloride, was added immediately after the reduction procedure in case the amine formed was susceptible to oxidation in the air. Analysis of the product collected **155** by ^{1}H NMR and IR spectroscopy suggested that no nitro group reduction had occurred. Increasing the pressure of the hydrogenated mixture to 80psi for 18 hours had no effect as judged by the ^{1}H NMR and IR spectra of the product. Further proof that no reduction had occurred was seen when cleavage of the pendant molecules was attempted with *n*-propylamine. The main component collected was the starting material 2-methoxy-5-nitrocatechol, the amount collected gave a minimum loading of 1.08mmol/g, which was a good recovery when it is considered that the maximum loading was 1.56mmol/g, giving a percentage recovery of 69%. A second problem highlighted during this cleavage procedure was that after the cleavage procedure the resulting polymer could not be precipitated and had to be removed by flash column chromatography. Although flash column chromatography is not an ideal way of purifying the final product, it was considered better to undertake one flash column at the end of the synthesis, rather than one at each step of the synthesis. The amount of heterogeneous catalyst used for the hydrogenation was increased to 50% of the weight of the polymer, and the hydrogenation time was increased to 72 hours before the addition of the acid chloride. Analysis of the final product **155** indicated that the hydrogenation and coupling had occurred. Pendant CH_2 groups could be seen in the ^1H NMR at 2.65ppm and amide peaks were observed using IR spectroscopy at 3390 and $1680cm^{-1}$.

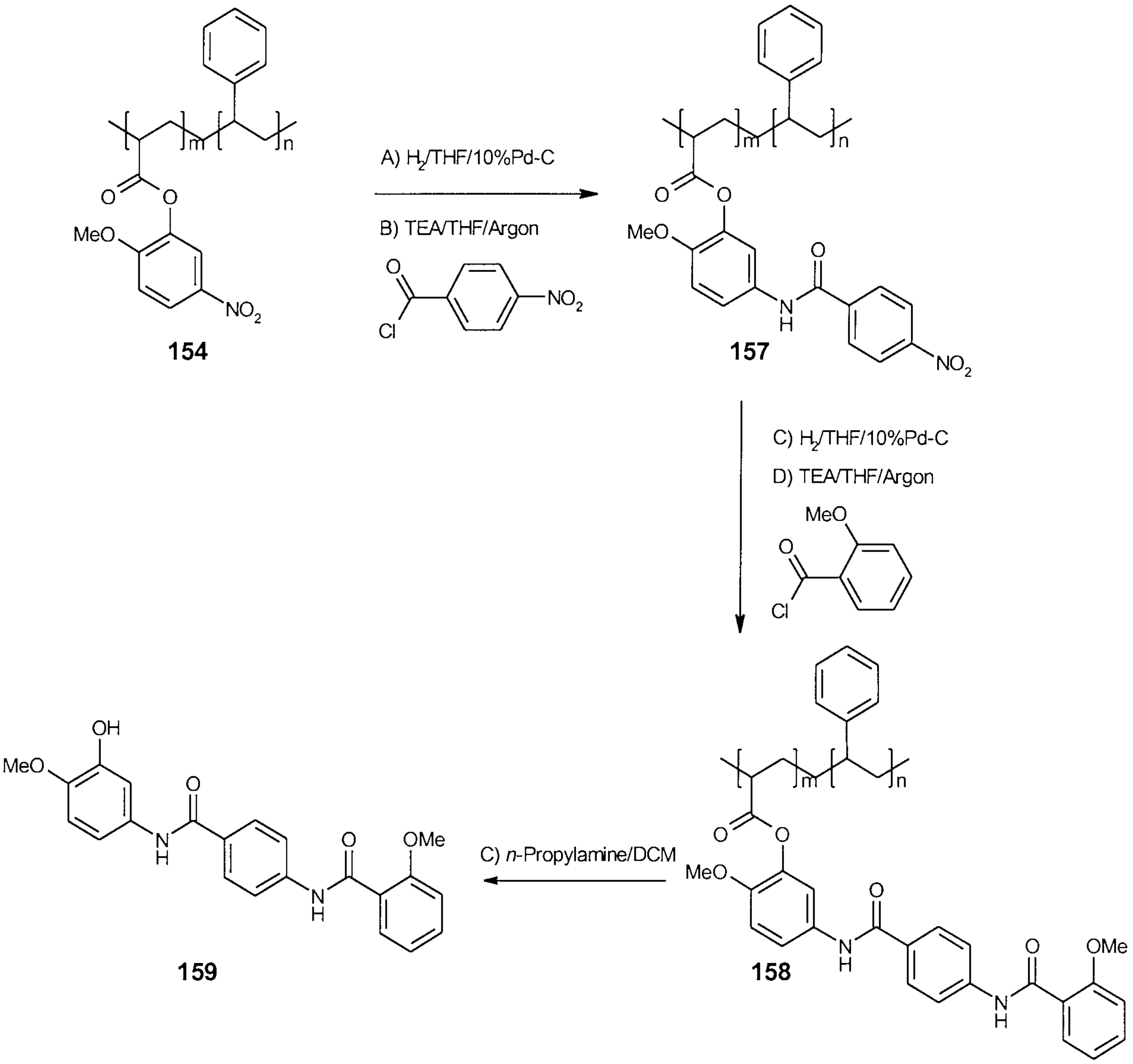
Cleavage of the pendant molecules from the polymer was performed by aminolysis of the ester linkage using a reactive amine. The amine used was *n*-propylamine, chosen because it could be easily removed under vacuum and so could be used in a large excess. The protected test compound **156** was cleaved from the co-polymer **155**, with *n*-propylamine and gave a minimum loading in the range 0.48-0.53mmol/g. Once cleaved, BBr_3 was used to demethylate the aromatic methoxy group of **156** to give the catechol **134** in 52% yield.



Scheme 7.12 Formation, cleavage and deprotection of model compound **134** from co-polymer **154**

If this method was employed to synthesise large libraries of catechol derivatives, it would be preferable if the demethylation step and cleavage step from the polymer were combined. A attempt was, therefore, made to cleave and demethylate the test compound in one step with BBr_3. The catechol derivative **134** was successfully obtained giving a minimum polymer loading of 0.31mmol/g.

The fact that the hydrogenation and acid chloride coupling on polymeric support was proven to be successful with a lipophilic test compound meant that the concept needed to be used to prepare an example of the desired catechol analogues.



Scheme 7.13 Formation and cleavage of the protected catechol compound **159** from co-polymer **154**

4-Nitrobenzoyl chloride was coupled to the reduced co-polymer of **154**. Confirmation of the coupling could be seen by a new broad peak at 7.85ppm in the aromatic region of the ^1H NMR (**Figure 7.2**). The second reduction and coupling with 2-methoxybenzoyl chloride was performed and confirmed, again, by new peaks seen in the ^1H NMR. New peaks were observed in the aromatic region and also an amide proton peak was seen at 9.55ppm (**Figure 7.3**). The protected product **159** was purified after cleavage with *n*-propylamine by flash column chromatography. The polymer gave a minimum loading of 0.15mmol/g. An attempt to obtain the deprotected product **115** was made by trying to cleave the pendant compound from the polymer with BBr_3. The desired catechol analogue could not be isolated, perhaps due to the poor solubility of the product and therefore, the difficulty in separating it from the resulting polymer.

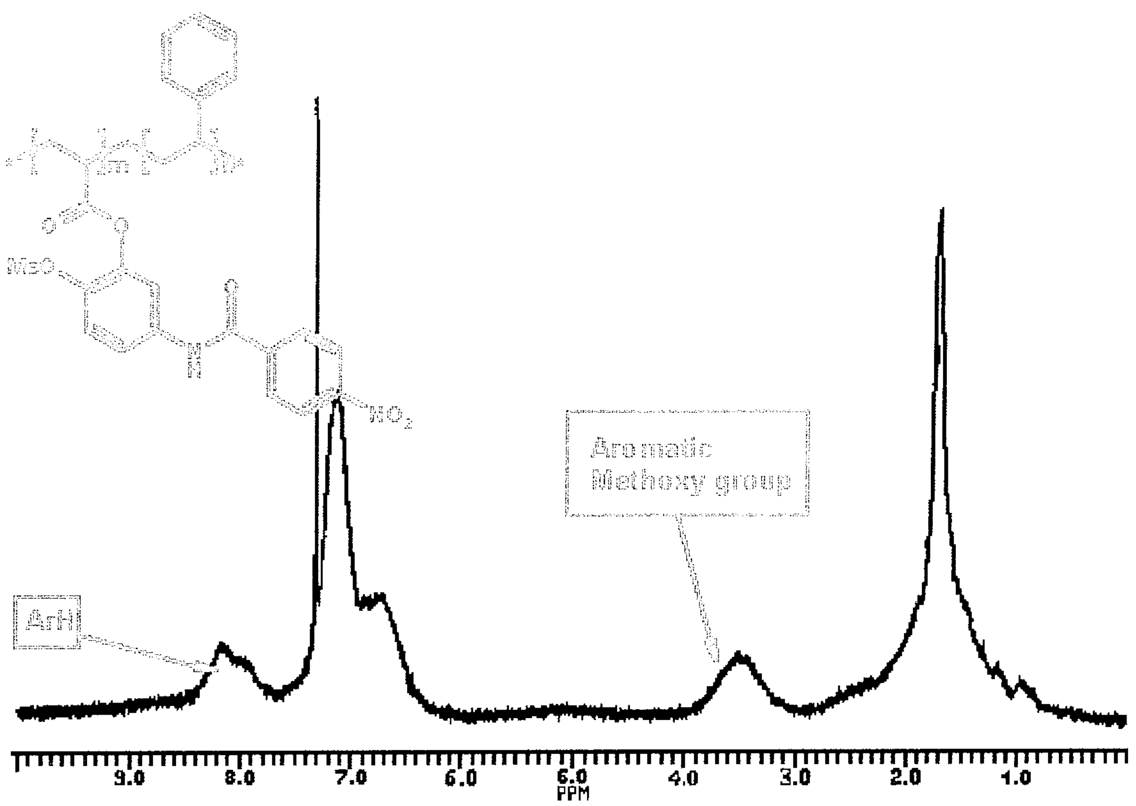


Figure 7.2 ^{1}H NMR spectrum of co-polymer **157** after reduction and acid chloride coupling

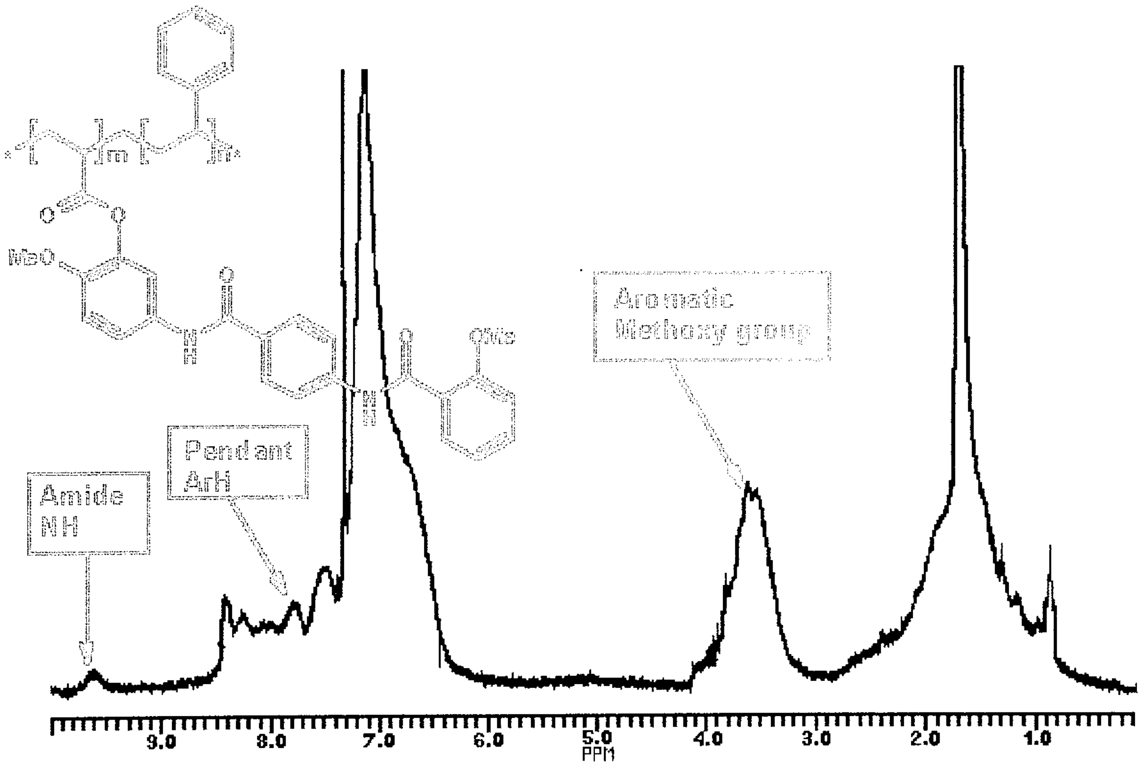
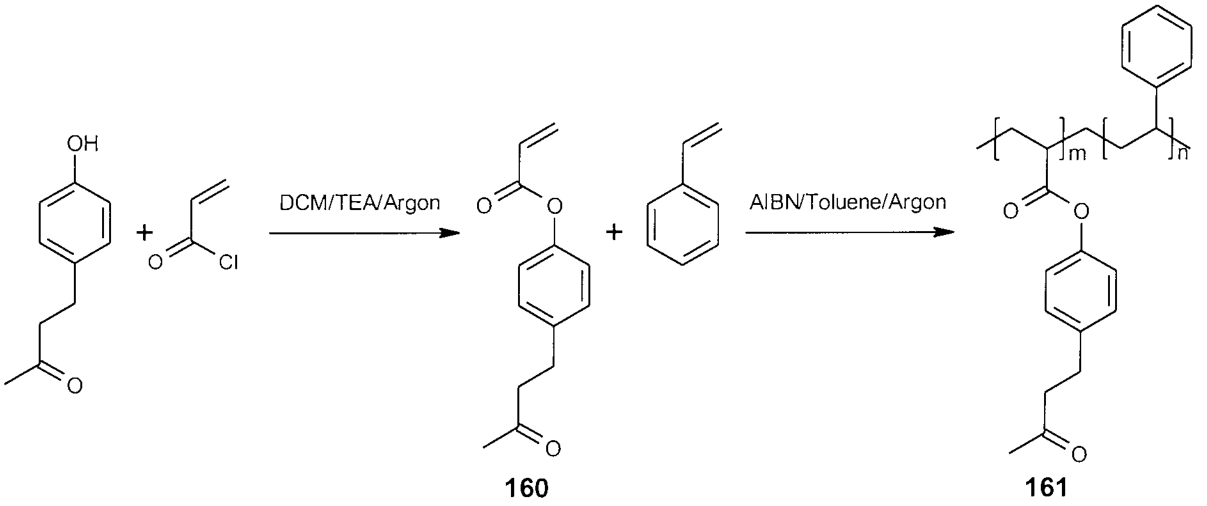


Figure 7.3 ^{1}H NMR spectrum of co-polymer **158** after the second reduction and acid chloride coupling reactions

7.5 Poly[4-(3-oxobutyl)phenyl acrylate-*co*-St]



Scheme 7.14 Preparation of poly[4-(3-oxobutyl)phenyl acrylate-*co*-St] **160**

The final polymer prepared was synthesised by the co-polymerisation of the acrylate of 4-(-4-hydroxyphenyl)-2-butanone **160** and styrene to give the co-polymer **161**. The IR spectrum for this polymer had a strong ketone absorbance at 1712cm^{-1}, which would be useful for monitoring the ketal formation with 4-nitrocatechol. Analysis of this polymer by ^{1}H NMR spectroscopy showed the pendant methyl protons of the ketone function at 2.15ppm, and also the pendant alkyl protons at 2.75 and 2.88ppm. The advantage of preparing the catechol analogues supported by this polymer would be that they may be cleaved by *n*-propylamine, from the polymer at the carbonyl group, leaving them in a protected form. The protected catechol analogues would be much easier to work with, to isolate and purify, due to their greater lipophilicity. This would provide the opportunity to screen the protected analogues for biological activity. The deprotection of these analogues should be exactly the same as for the solution-phase scheme (see **Chapter 6**).

7.6 Conclusion

Merrifield cross-linked resin was found to be unsuitable to support the preparation of the desired biologically active catechol derivatives previously made in solution (see **Chapter 6**). The main problem with the Merrifield resin was found to be the lack of traditional techniques available to analyse the intermediates of the reaction schemes. Greater analytical data can be gathered from soluble polymers, so in this work, linear polymer supports were prepared containing pendant ketone and aldehyde functionalities with the intent of attaching, and therefore protecting, 4-nitrocatechol by way of an ketal/acetal function. The aromatic nitro group could then be reduced and coupled to the acid chloride of choice as done in the solution-phase scheme (**Chapter 6**).

Analysis of reaction products proved easier with the soluble polymers prepared and some success was achieved in preparing and cleaving the test compound **134** from poly(MVK-*co*-St) polymer **127** and also from the catechol incorporated co-polymer, **154**. With both these polymers, difficulties were encountered in isolating the final catechol products from the cleaved polymer residue. This was thought to be due to the difficulty in handling the final catechol analogues. This demonstrated that the soluble linear polymers appear to be suitable to support the preparation of lipophilic compounds but are not suitable for the insoluble catechol derivative target compounds. Finally, a co-polymer **161** was devised that would overcome this problem, providing the catechol derivative in a protected and hence more lipophilic form. The time was not available to test this polymer as a soluble linear support.

Chapter 8

Experimental

8.1 General methods

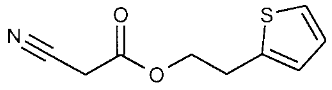
Mass spectrometric analyses were carried at Aston University in APCI$^+$ or APCI$^-$ mode on a Hewlett-Packard HP 5989B quadruple mass spectrometer using a HP 59987A APCI-electrospray LC/MS interface. NMR spectra were recorded on a Bruker AC-250 spectrometer. Infrared spectra were recorded on a Mattson Galaxy 3020 FT-IR spectrophotometer. Flash chromatography was performed using Sorbsil C60 silica gel. TLC was performed using plastic-backed Kiesel 60 silica gel plates containing a fluorescent indicator and visualised under UV (254nm). Aqueous potassium permanganate solution was used where appropriate to develop TLC plates. Rabbit reticulocyte 15-lipoxygenase assays were carried out by MDS Panlabs Pharmacology services (Taiwan) according to their standard protocol. Elemental analyses were performed by Butterworth Laboratories (Middlesex).

8.2 Gifts

Lipoxygenase inhibitors BW-A4C **3**, BW-B70C **5** and CV-6504 **16** were kind gifts of Dr. Helen Hussey, Pharmaceutical Sciences Research Institute, Aston University.

8.3 Initial automated parallel synthesis library

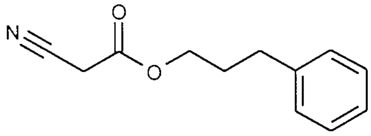
Preparation of 2-(2-thienyl)ethyl 2-cyanoacetate



Method A: Titanium (IV) ethoxide (4.39mmol, 1g) was added to a mixture of methyl cyanoacetate (19mmol, 1.881g) and 2-(2-thienyl)ethanol (38mmol, 4.864g). The mixture was heated at 80°C for 16 hours. The reaction mixture was allowed to cool to room temperature and then mixed with 1M HCl (45ml). The organic components were extracted into diethyl ether (3x100ml). The organic layer was separated, combined and washed with a saturated solution of sodium hydrogen carbonate (3x100ml). The organic layer was separated, dried with anhydrous magnesium sulphate and concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with ethyl acetate-petrol 40:60 (1:3). The title compound was collected as a clear yellow liquid (2.12g, 57%). TLC [ethyl acetate-petrol 40:60, (1:3)] R_f 0.31. ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 3.15 (t, 2H, *J*=6.6Hz, $COOCH_2C\underline{H}_2$), 4.03 (s, 2H, $CNCH_2COO$), 4.32 (t, 2H, *J*=6.5Hz, $COOCH_2$), 6.96-6.99 (ov. m, 2H, ArH-3/5), 7.37 (dd, 1H, *J*=4.5, 4.5Hz, ArH-4) ppm; Mass spectrum (APCI$^+$): *m/z* 196M$^+$, 111[M-$CNCH_2COO$]$^+$.

Method B: Dowex 50xB-100 (2.4g) was added to a mixture of methyl cyanoacetate (30mmol, 2.973g) and 2-(2-thienyl)ethanol (60mmol, 7.68g). The reaction mixture was heated at 80°C for 72 hours. The ion exchange resin was removed by filtration and the title compound was separated by distillation using Kugelrohr apparatus at 65°C. The title compound was collected as a clear yellow liquid (4.943g, 84%). TLC, ^{1}H NMR and mass spectrum as above.

Preparation of 3-phenylpropyl-2-cyanoacetate

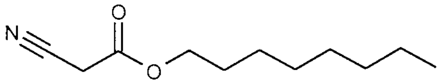


Method A: EDC (6mmol, 1.782g) and DMAP (0.6mmol, 74mg) were added to a mixture of cyanoacetic acid (510mg, 6mmol) and 3-phenylpropan-1-ol (6.0mmol, 817mg) in anhydrous DMF (15ml) at 0°C. The mixture was allowed to warm to room temperature and was stirred for 48 hours. The mixture was concentrated under vacuum. The resulting residue was dissolved in ethyl acetate (100ml) and washed with brine (3x100ml). The organic layer was dried with anhydrous magnesium sulphate and concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with petrol 40:60-diethyl ether (2:1). The title compound was collected as a golden liquid (339mg, 32%). TLC [petrol 40:60-diethyl ether (2:1)] R_f 0.26. ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.90 (quintet, 2H, J=6.4Hz, $COOCH_2C\underline{H}_2$), 2.65 (t, 2H, J=7.3Hz, $COOCH_2CH_2C\underline{H}_2C_6H_5$), 4.01 (s, 2H, $CNCH_2COO$), 4.11 (t, 2H, J=6.5Hz, $COOCH_2$), 7.16-7.32 (ov. m, 5H, C_6H_5) ppm; Mass spectrum (APCI$^+$): m/z 204M$^+$, 119[M-$CNCH_2COO$]$^+$.

Method A was repeated as above on a 17mmol scale, the title compound was collected (2.975g, 79%). TLC, ^1H NMR and mass spectrum as above.

Method B: Dowex 50xB-100 (2.4g) was added to a mixture of methyl cyanoacetate (30mmol, 2.973g) and 3-phenylpropanol (60mmol, 8.172mg). The reaction mixture was heated at 80°C for 72 hours. The ion exchange resin was removed by filtration and the title compound was separated by distillation using Kugelrohr apparatus at 95°C. The title compound was collected as a clear yellow liquid (5.284g, 87%). TLC [DCM] R_f 0.51. ^1H NMR and mass spectrum as above.

Preparation of octyl-2-cyanoacetate



Titanium (IV) ethoxide (4.39mmol, 1g) was added to a mixture of methyl cyanoacetate (20mmol, 1.98g) and octan-1-ol (40mmol, 5.2g). The mixture was heated at 80°C for 16 hours. The reaction mixture was allowed to cool to room temperature and then mixed with 1M HCl (45ml). The organic components were extracted into diethyl ether (3x100ml). The organic layer was separated, combined and washed with a saturated solution of sodium hydrogen carbonate (3x100ml). The organic layer was separated, dried with anhydrous magnesium sulphate and concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with ethyl acetate-petrol 40:60 (1:3). The title compound was collected as a clear yellow liquid (2.47g, 63%). TLC [ethyl acetate-petrol 40:60 (1:3)] R_f 0.36; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.86 (t, 3H, J=6.6Hz, CH_3), 1.15-1.26 (ov. m, 10H, $COOCH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 1.57 (quintet, 2H, J=6.7Hz, $COOCH_2C\underline{H}_2$), 4.00 (s, 2H, $CNCH_2COO$), 4.10 (t, 2H, J=6.5Hz, $COOC\underline{H}_2CH_2$) ppm; Mass spectrum (APCI$^+$): m/z 198M$^+$, 114[M-$CNCH_2COO$]$^+$.

Initial automated parallel solution-phase library synthesis

Automation was used to dispense 1.5ml of solutions of various aldehydes in ethanol (0.45mmol/1.5ml) into separate vials. To each vial was added 1.5ml of a single active methylene reagent solution in ethanol (0.4mmol/1.5ml) containing piperidine (0.04mmol/1.5ml). The reaction vials were heated at 75°C for 2 hours. The remaining residues were subjected to automated ethanol, diethyl ether or petrol 40:60 washes (3x1.5ml) depending upon solubility. The resulting solids were then dried under vacuum.

8.4 Anti-cancer biological screening

Cell origins and storage

The cell lines MAC13 and MAC16 were originally derived from colon tumours induced by 1,2-dimethylhydrazine (DMH) in NMRI mice and were provided by Prof. J.A. Double and Dr M.C. Bibby (University of Bradford, UK). Cells were stored in RPMI 1640 culture media with a supplement of 20% foetal calf serum (FCS) and 10% DMSO at $2x10^6$ cells/ml and kept under liquid nitrogen. Once cells had been taken out of storage, they were kept at passage numbers of less than 45 to minimise any differentiation that may occur from continuous culture.

Preparation of cell lines for use

MAC13 cells have a doubling time of 18 hours and grow as a monolayer. The cells were cultured in the phenol-red free growth media RPMI 1640 , supplemented with 10% FCS, 1% glutamine and 1% penicillin and streptomycin. Cells were washed in phosphate buffered saline (PBS) and were removed from the flask surface with trypsin (0.025% EDTA) diluted in PBS. The cells were resuspended in RPMI 1640 medium at $0.5x10^4$ cells/ml and were subcultured every 3 or 4 days.

MAC16 cells have a doubling time of 32 hours and grow in suspension. The cells were cultured in phenol-red free growth media RPMI 1640 medium supplemented with 10% FCS, 1% glutamine and 1% penicillin and streptomycin. Any cells adhering to the flask surface were removed with a firm tap. Cells were then resuspended in RPMI 1640 medium at $2x10^4$ cells/ml and were subcultured every 3 or 4 days. Cell number was determined by means of a ZM electronic particle coulter counter.

MAC13 and MAC16 total cell count

MAC16 and MAC13 cells were maintained in RPMI 1640 medium supplemented with 10% FCS at 37°C under an atmosphere of 5% carbon dioxide in air. MAC13 cells were seeded at $1x10^4$ cell/ml. MAC16 cells were seeded at $2x10^4$ cells/ml. Both cell lines were seeded in 24 well plates and incubated for 2 hours before the test compound addition. Compounds were dissolved in DMSO to make a 20mM stock solution, further diluted in medium and added to wells in 10µL aliquots to give final compound concentrations ranging from 100 to 1µM. Each concentration had three replicates and a control with three replicates. Cell counts were made 72 hours after drug addition using a ZM electronic particle coulter counter.

MAC13 and MAC16 viability

Both cell lines were seeded in 96 well plates at a concentration of 2×10^4 cells/ml in 200μL aliquots incubated at 37°C for 2 hours. Compounds were dissolved in DMSO and serially diluted in medium, 10μL of the final dilutions was added to each of 6 wells, to give final concentrations of 100, 50, 25, 10, 5 and 2.5μM. Resazurin dye was added after a further 72 hours incubation, and read on a plate reader after 6 hours. With each set of compounds tested the control compound CV-6504 was tested simultaneously for each cell line. Each library compound was tested once to give a general range of cytotoxic activity/growth inhibition. Active compounds were tested on both MAC13 and MAC16 cell lines on three separate occasions to obtain an IC_{50} value.

Chemical analysis and screening results from initial library products

Compound structures **a-m** and **1-48** can be found in **Tables 3.2** and **3.3**, pages 45 and 46. Inactivity against MAC16 and MAC13 is denoted by X. Where compounds could not be identified as the desired products, the recovery, TLC, mass spectra and ^{1}H NMR spectra have been recorded with X. Where ^{1}H NMR spectra were not obtained no data have been entered into the table.

*a, b and c represent different solvent systems; a (diethyl ether), b (petrol 40:60-diethyl ether (1:1)) and c (ethyl acetate).

Compound	Recovery	TLC*	Mass spectrum APCI$^+$: *m/z*	^{1}H NMR (250.1MHz, $(CD_3)_2SO$) δ_H (ppm)	MAC16 IC_{50} (μM)	MAC13 IC_{50} (μM)
a1	80mg, 63%	0.30 b	320M$^+$, 264[M-$CH_2CH_2CH_2CH_3$]$^+$, 246[M-$OCH_2CH_2CH_2CH_3$]$^+$		X	X
b1	75mg, 59%	0.36 b	320M$^+$, 264[M-$C(CH_3)_3$]$^+$, 246[M-$OC(CH_3)_3$]$^+$		X	X
c1	73mg, 60%	0.29 b	304M$^+$, 246[M-$CH_2CH{=}CH_2$]$^+$		X	X
d1	53mg, 41%	0.22 b	322M$^+$, 264[M-$CH_2CH_2OCH_3$]$^+$, 246 [M-$OCH_2CH_2OCH_3$]$^+$,		X	X
e1	85mg, 70%	0.24 b	306M$^+$, 264[M-$CH(CH_3)_2$]$^+$, 246[M-$OCH(CH_3)_2$]$^+$		X	X
f1	98mg, 69%	0.26 b	354M$^+$, 264[M-$CH_2C_6H_5$]$^+$, 246[M-$OCH_2C_6H_5$]$^+$		X	X
g1	98mg, 94%	0.03 b	263M$^+$		X	X
h1	106mg , 71%	0.31 b	264[M-$CH_2CH(C_2H_5)C_4H_9$]$^+$, 246[M-$OCH_2CH(C_2H_5)$ C_4H_9]$^+$		X	X
i1	75mg, 68%	0.18 b	278M$^+$, 264[M-CH_3]$^+$, 246[M-OCH_3]$^+$		X	X
j1	68mg, 59%	0.78 a	292M$^+$, 264[M-CH_2CH_3]$^+$, 246[M-OCH_2CH_3]$^+$	1.31 (t, 3H, $COOCH_2C\underline{H}_3$), 3.83 (s, 9H, 3xOCH_3), 4.32 (q, 2H, *J*=7.1Hz, $COOC\underline{H}_2CH_3$), 7.51 (s, 2H, ArH-2/6), 8.33 (s, 1H, CH=)	X	X
k1	122mg , 80%	0.38 b	382M$^+$		X	X
l1	55mg, 37%	0.16 b	374M$^+$, 264[M-$CH_2CH_2C_4H_3S$]$^+$, 246[M-$0CH_2CH_2C_4H_3S$]$^+$		X	X
m1	86mg, 57%	0.29 b	376M$^+$, 264[M-$(CH_2)_7CH_3$]$^+$, 246[M-$O(CH_2)_7CH_3$]$^+$		X	X
a2	60mg, 55%	0.33 b	275M$^+$, 219[M-$(CH_2)_3CH_3$]$^+$, 201[M-$O(CH_2)_3CH_3$]$^+$		X	X
b2	71mg, 65%	0.34 b	275M$^+$, 291[M-$C(CH_3)_3$]$^+$, 201[M-$OC(CH_3)_3$]$^+$		X	X

c2	56mg, 54%	0.30 b	$259M^+$, $219[M-CH_2CH=CH_2]^+$, $201[M-OCH_2CH=CH_2]^+$		X	X
d2	87mg, 79%	0.26 b	$291[M-CH_2CH_2OCH_3]^+$, $202[M-OCH_2CH_2OCH_3]^+$		X	X
e2	75mg, 72%	0.29 b	$261M^+$, $219[M-CH(CH_3)_2]^+$		X	X
f2	99mg, 80%	0.27 b	$309M^+$, $219[M-CH(CH_3)_2]^+$, $91[CH_2C_6H_5]^+$		X	X
g2	81mg, 93%	0.02 b	$218M^+$, 166	7.97 (br d, 2H, NH_2), 8.13 (d, 2H, J=8.8Hz, 2xArH), 8.30 (s, 1H, CH=), 8.39 (d, 2H, J=8.8Hz, 2xArH)	X	X
h2	82mg, 62%	0.40 b	$331M^+$, $219[M-CH_2CH(C_2H_5)C_4H_9]^+$		X	X
i2	54mg, 58%	0.30 b	$233M^+$		X	X
j2	63mg, 64%	0.32 b	$247M^+$, $219[M-CH_2CH_3]^+$		X	X
k2	76mg, 57%	0.35 b	$219[M-(CH_2)_3C_6H_5]^+$, $201[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$,		X	X
l2	66mg, 50%	0.35 b	$329M^+$, $219[M-CH_2CH_2C_4H_3S]^+$		X	X
m2	59mg, 45%	0.38 b74	$331M^+$		X	X
a3	45mg, 46%	0.50 b	$244M^+$, $188[M-(CH_2)_3CH_3]^+$, 170 $[M-O(CH_2)_3CH_3]^+$		X	X
b3	63mg, 65%	0.54 b	$244M^+$, 188 $[M-C(CH_3)_3]^+$, $170[M-OC(CH_3)_3]^+$		X	X
c3	73mg, 80%	0.48 b	$228M^+$, $188[M-CH_2CH=CH_2]^+$, $170[M-OCH_2CH=CH_2]^+$		X	X
d3	67mg, 68%	0.45 b	$246M^+$, $188[M-CH_2CH_2OCH_3]^+$, $170[M-OCH_2CH_2OCH_3]^+$		X	X
e3	73mg, 80%	0.50 b	$230M^+$, $188[M-CH(CH_3)_2]^+$, $170[M-OCH(CH_3)_2]^+$		X	X
f3	65mg, 59%	0.52 b	$278M^+$, $188[M-CH_2C_6H_5]^+$, 91 $[CH_2C_6H_5]^+$		X	X
g3	60mg, 81%	0.10 b	$187M^+$, $170[M-NH_2]^+$		X	X
h3	78mg, 65%	0.52 b	$188[M-CH_2CH(C_2H_5)\ C_4H_9]^+$, $170[M-CH_2CH(C_2H_5)\ C_4H_9]^+$,		X	X
i3	37mg, 46%	0.44 b	$202M^+$, $188[M-CH_3]^+$, $170[M-OCH_3]^+$		X	X
j3	58mg, 67%	0.48 b	$216M^+$, $188[M-CH_2CH_3]^+$, $170[M-OCH_2CH_3]^+$		X	X
k3	72mg, 59%	0.56 b	$306M^+$, $91[CH_2C_6H_5]^+$		X	X
l3	61mg, 51%	0.43 b	$298M^+$, $188[M-CH_2CH_2C_4H_3S]^+$, $170[M-0CH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m3	84mg, 70%	0.50 b	$300M^+$, $188[M-(CH_2)_7CH_3]^+$, $170[M-O(CH_2)_7CH_3]^+$		X	X
a4	66mg, 56%	0.45 b	$299M^+$, $242[M-(CH_2)_3CH_3]^+$, $224[M-O(CH_2)_3CH_3]^+$		X	X
b4	62mg, 52%	0.58 b	$299M^+$, $242[M-C(CH_3)_3]^+$, $224[M-OC(CH_3)_3]^+$,		X	X
c4	67mg, 59%	0.46 b	$283M^+$, $242[M-CH_2CH=CH_2]^+$, $224[M-OCH_2CH=CH_2]^+$		X	X
d4	78mg, 65%	0.47 b	$301M^+$, $242[M-CH_2CH_2OCH_3]^+$, $224[M-OCH_2CH_2OCH_3]^+$		X	X
e4	84mg, 74%	0.49 b	$285M^+$, $242[M-CH(CH_3)_2]^+$, $224[M-OCH(CH_3)_2]^+$,	1.30 (s, 3H, $CH(CH_3)C\underline{H}_3$), 1.33 (s, 3H, $CH(C\underline{H}_3)CH_3$), 5.11 (septet, 1H, J=6.6Hz, $CH(CH_3)_2$), 7.89 (d, 1H, J=8.5Hz, ArH-5), 8.05 (dd, 1H, J=8.5, 1.7Hz, ArH-6), 8.28 (d, 1H, J=1.6Hz, ArH-2), 8.40 (s, 1H, CH=)	X	X
f4	108mg, 81%	0.46 b	$332M^+$, $91[CH_2C_6H_5]^+$		X	X
g4	76mg, 79%	0.02 b	$242M^+$		X	X
h4	96mg,	0.54	$354M^+$, 242[M-		X	X

	68%	b	$CH_2CH(C_2H_5)C_4H_9]^+$, 224$[M-OCH_2CH(C_2H_5)C_4H_9]^+$			
i4	69mg, 67%	0.43 b	256M+, 242$[M-CH_3]^+$		X	X
j4	61mg, 56%	0.44 b	270M+, 242$[M-CH_2CH_3]^+$		X	X
k4	106mg, 74%	0.57 b	361M^+		X	X
l4	83mg, 59%	0.41 b	353M^+, 111$[CH_2CH_2C_4H_3S]^+$		X	X
m4	78mg, 55%	0.45 b	355M^+, 242$[M-(CH_2)_7CH_3]^+$		X	X
a5	80mg, 87%	0.17 b	231M^+		X	X
b5	48mg, 52%	0.11 b	231M^+, 175$[M-C(CH_3)_3]^+$		X	X
c5	58mg, 69%	0.09 b	215M^+		X	X
d5	61mg, 66%	0.09 b	233M^+		X	X
e5	67mg, 78%	0.09 b	217M^+, 175$[M-CH(CH_3)_2]^+$		X	X
f5	69mg, 65%	0.10 b	265M^+		X	X
g5	61mg, 88%	0.01 b	174M^+		X	X
h5	69mg, 60%	0.10 b	287M^+, 175$[M-CH_2CH(C_2H_5)C_4H_9]^+$		X	X
i5	66mg, 88%	0.04 b	189M^+		X	X
j5	44mg, 54%	0.48 a	203M^+	1.32 (t, 3H, J=7.1Hz, CH_3), 4.35 (q, 2H, J=7.1Hz, CH_2), 7.64 (dd, 1H, J=8.2, 4.8Hz, ArH-6), 8.46-8.49 (ov. m, 2H, CH=, ArH-5), 8.77 (dd, 1H, J=4.8, 1.4Hz, ArH-4), 9.10 (d, 1H, J=2.2Hz, ArH-2)	X	X
k5	75mg, 64%	0.09 b	293M^+		X	X
l5	73mg, 64%	0.16 b	285M^+		X	X
m5	67mg, 59%	0.13 b	287M^+	0.83-0.88 (m, 3H, CH_3), 1.24-1.39 (ov. m, 10H, 5xCH_2), 1.55-1.79 (m, 2H, $COOCH_2C\underline{H}_2$), 4.30 (t, 2H, J=7.1Hz, $COOC\underline{H}_2$), 7.64 (dd, 1H, J=8.2, 4.8Hz, ArH-5), 8.46-8.49 (ov. m, 2H, CH=, ArH-6), 8.77 (dd, 1H, J=4.7, 1.5Hz, ArH-4), 9.10 (d, 1H, J=2.0Hz, ArH-2)	X	X
a6	87mg, 86%	0.35 b	255M^+, 199$[M-(CH_2)CH_3]^+$, 181$[M-O(CH_2)_3CH_3]^+$		X	X
b6	58mg, 57%	0.40 b	255M^+, 199$[M-C(CH_3)_3]^+$		X	X
c6	62mg, 61%	0.38 b	239M^+, 199$[M-CH_2CH=CH_2]^+$, 181$[M-OCH_2CH=CH_2]^+$		X	X
d6	68mg, 66%	0.30 b	257M^+, 199$[M-CH_2CH_2OCH_3]^+$, 181$[M-OCH_2CH_2OCH_3]^+$		X	X
e6	56mg, 58%	0.32 b	241M^+, 199$[M-CH(CH_3)_2]^+$, 181$[M-OCH(CH_3)_2]^+$		X	X
f6	70mg, 61%	0.38 b	289M^+, 91$[CH_2C_6H_5]^+$		X	X
g6	68mg, 86%	0.01 b	198M^+		X	X
h6	69mg, 56%	0.33 b	311M^+, 199$[M-CH_2CH(C_2H_5)C_4H_9]^+$		X	X
i6	75mg, 88%	0.23 b	213M^+		X	X
j6	48mg, 53%	0.24 b	227M^+, 199$[M-CH_2CH_3]^+$, 181$[M-OCH_2CH_3]^+$		X	X
k6	88mg, 70%	0.38 b	NO TIC	2.03 (quintet, 2H, J=7.4Hz, $COOCH_2C\underline{H}_2$) 2.73 (t, 2H, J=7.6Hz, $COOCH_2CH_2C\underline{H}_2$), 4.29 (t, 2H, J=6.4Hz, $COOC\underline{H}_2$), 7.33-7.18 (m, 5H, $CH_2C_6\underline{H}_5$), 8.08 (d, 2H, J=8.4Hz, $C_6\underline{H}_4CN$), 8.19 (d, 2H, J=8.4Hz, $C_6\underline{H}_4CN$), 8.47 (s, 1H, $C\underline{H}$=)	X	X
l6	38mg,	0.26	199$[M-CH_2CH_2C_4H_3S]^+$,	3.28 (t, 2H, J=6.3Hz, $COOCH_2C\underline{H}_2$), 4.48 (t,	X	X

	31%	b	$111[CH_2CH_2C_4H_3S]^+$	2H, *J*=6.3Hz, $COOC\underline{H}_2$), 6.87 (dd, 1H, *J*=3.5, 0.8Hz, Ar'H), 6.93 (dd, 1H, *J*=5.1, 3.4Hz, Ar'H), 7.30 (dd, 1H, *J*=5.1, 1.2Hz, Ar'H), 8.08 (d, 2H, *J*=8.5Hz, 2xArH), 8.18 (d, 2H, *J*=8.5Hz, 2xAr'H), 8.51 (s, 1H, CH=)		
m6	69mg, 56%	0.28 b	$311M^+$, $199[M-(CH_2)_7CH_3]^+$		X	X
a7	68mg, 63%	0.24 b	$273M^+$, $217[M-(CH_2)_3CH_3]^+$, $199[M-O(CH_2)_3CH_3]^+$		X	X
b7	74mg, 68%	0.31 b	$273M^+$, $217[M-C(CH_3)_3]^+$, $199[M-OC(CH_3)_3]^+$		X	X
c7	68mg, 66%	0.25 b	$257M^+$, $217[M-CH_2CH=CH_2]^+$, $199[M-OCH_2CH=CH_2]^+$		X	X
d7	71mg, 65%	0.21 b	$275M^+$, $217[M-CH_2CH_2OCH_3]^+$, $199[M-OCH_2CH_2OCH_3]^+$		X	X
e7	63mg, 61%	0.28 b	$259M^+$, $217[M-CH(CH_3)_2]^+$		X	X
f7	94mg, 77%	0.28 b	$307M^+$, $217[M-CH_2C_6H_5]^+$, $199[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$.	3.09 (s, 6H, $N(CH_3)_2$), 5.29 (s, 2H, $COOC\underline{H}_2C_6H_5$), 7.41 (dd, 4H, *J*=283.4, 9.1Hz ArH-2/3/5/6), 7.38 (ov. m, 5H, $COOCH_2C_6\underline{H}_5$), 8.14 (s, 1H, CH=C)	X	X
g7	74mg, 86%	0.01 b	$216M^+$			
h7	77mg, 59%	0.24 b	$329M^+$, $217[M-CH_2CH(C_2H_5)C_4H_9]^+$		X	X
i7	57mg, 62%	0.20 b	$231M^+$		X	X
j7	58mg, 59%	0.24 b	$245M^+$, $202[M-N(CH_3)_2]^+$, $199[M-OCH_2CH_3]^+$.	1.28 (t, 3H, $COOCH_2CH_3$), 3.09 (s, 6H, $N(CH_3)_2$), 4.25 (q, 2H, CH_2), 7.4 (dd, 4H, *J*=280.9, 9.1Hz, ArH-2/3/5/6), 8.11 (s, 1H, CH=C)	X	X
k7	101mg, 76%	0.34 b	$335M^+$, $217[M-(CH_2)_3C_6H_5]^+$, $199[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$,		X	X
l7	48mg, 37%	0.24 b	$327M^+$, $217[M-CH_2CH_2C_4H_3S]^+$		X	X
m7	71mg, 54%	0.28 b	$329M^+$		X	X
a8	66mg, 67%	0.23 b	$246M^+$, $190[M-(CH_2)_3CH_3]^+$, $172[M-O(CH_2)_3CH_3]^+$		X	X
b8	53mg, 54%	0.22 b	$246M^+$, $190[M-C(CH_3)_3]^+$, $172[M-O(CH_3)_3]^+$		X	X
c8	57mg, 62%	0.19 b	$230M^+$, $190[M-CH_2CH=CH_2]^+$, $172[M-OCH_2CH=CH_2]^+$	4.77 (d, 2H, *J*=5.4Hz, $CH_2CH=C\underline{H}_2$) 5.43-5.26 (m, 2H, $C\underline{H}_2CH=CH_2$), 6.96-5.70 (m, 1H, $CH_2C\underline{H}=CH_2$), 7.99 (d, 2H, *J*=8.8Hz, Ar-H), 8.24 (d, 2H, *J*=8.8Hz, Ar-H), 8.26 (s,1H, CH=),10.75 (br s, 1H, Ar-OH)	X	X
d8	79mg, 80%	0.18 b	$248M^+$, $190[M-CH_2CH_2OCH_3]^+$, $172[M-OCH_2CH_2OCH_3]^+$		X	X
e8	34mg, 37%	0.17 b	$232M^+$, $190[M-CH(CH_3)_2]^+$, $172[M-OCH(CH_3)_2]^+$		X	X
f8	86mg, 77%	0.16 b	$280M^+$, $190[M-CH_2CH_3]^+$, $172[OCH_2CH_3]^+$, $91[CH_2C_6H_5]^+$		X	X
g8	53mg, 70%	0.01 b	$189M^+$		X	X
h8	84mg, 70%	0.18 b	$190[M-CH_2CH(C_2H_5)C_4H_9]^+$, $172[M-OCH_2CH(C_2H_5)C_4H_9]^+$	0.89-0.95 (ov. m, 6H, $2xCH_3$), 1.24-1.44 (ov. m, 8H, $4xCH_2$), 1.57-1.68 (m, 1H, CH), 4.24 (d, 2H, *J*=6.8Hz, $COOCH_2$), 6.90 (d, 2H, *J*=8.8Hz, 2xArH), 7.98 (d, 2H, *J*=8.8Hz, 2xArH), 8.19 (s, 1H, CH=)	X	X
i8	67mg, 83%	0.12 b	$204M^+$, $190[M-CH_3]^+$, $172[M-OCH_3]^+$		X	X
j8	41mg, 47%	0.17 b	$218M^+$, $190[M-CH_2CH_3]^+$		X	X
k8	95mg, 77%	0.25 b	$308M^+$, $190[M-(CH_2)_3C_6H_5]^+$, $172[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l8	44mg, 37%	0.12 b	$300M^+$, $190[M-CH_2CH_2C_4H_3S]^+$, $172[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X

m8	70mg, 58%	0.26 b	$302M^+$, $180[M-(CH_2)_7CH_3]^+$, $162[M-O(CH_2)_7CH_3]^+$		X	X
a9	49mg, 52%	0.37 b	$236M^+$, $180[M-(CH_2)_3CH_3]^+$, $162[M-O(CH_2)_3CH_3]^+$		X	X
b9	49mg, 52%	0.44 b	$236M^+$, $180[M-C(CH_3)_3]^+$,		X	X
c9	51mg, 58%	0.34 b	$220M^+$, $180[M-CH_2CH=CH_2]^+$, $162[M-OCH_2CH=CH_2]^+$		X	X
d9	63mg, 66%	0.32 b	$238M^+$, $180[M-CH_2CH_2OCH_3]^+$, $162[M-OCH_2CH_2OCH_3]^+$		X	X
e9	75mg, 85%	0.37 b	$222M^+$, $162[M-OCH(CH_3)_2]^+$		X	X
f9	71mg, 66%	0.36 b	$270M^+$, $162[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g9	58mg, 81%	0.02 b	$179M^+$		X	X
h9	67mg, 58%	0.42 b	$292M^+$		X	X
i9	35mg, 45%	0.36 b	$194M^+$, $162[M-OCH_3]^+$		X	X
j9	53mg, 64%	0.57 a	$240[M+32]^+$, $208M^+$, $162[M-OCH_2CH_3]^+$	1.29 (t, 3H, J=7.0Hz, CH_3), 4.30 (q, 2H, J=7.1Hz, CH_2), 7.36 (dd, 1H, J=5.6, 4.9Hz, ArH), 8.06 (d, 1H, J=3.7Hz, ArH), 8.21 (dd, 1H, J=2.0, 0.9Hz, ArH)	X	X
k9	69mg, 58%	0.48 b	$298M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l9	31mg, 27%	0.31 b	$290M^+$, $162[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m9	63mg, 54%	0.40 b	$292M^+$, $280[M-(CH_2)_7CH_3]^+$, $162[M-O(CH_2)_7CH_3]^+$		X	X
a10	88mg, 77%	0.38 b	$288M^+$, $232[M-(CH_2)_3CH_3]^+$, $200[M-O(CH_2)_3CH_3]^+$		X	X
b10	71mg, 62%	0.42 b	$288M^+$, $232[M-C(CH_3)_3]^+$, $200[M-C(CH_3)_3-OCH_3]^+$		X	X
c10	91mg, 84%	0.35 b	$272M^+$, $232[M-CH_2CH=CH_2]^+$, $200[M-OCH_2CH=CH_2]$+		X	X
d10	64mg, 55%	0.34 b	$290M^+$, $232[M-CH_2CH_2OCH_3]^+$, $200[M-OCH_2CH_2OCH_3]^+$		X	X
e10	99mg, 91%	0.39 b	$274M^+$, $232[M-CH(CH_3)_2]^+$, $200[M-CH(CH_3)_2-OCH_3]^+$	1.31 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 3.89 (s, 3H, $COOCH_3$), 5.11 (septet, 1H, J=6.2Hz, $C\underline{H}(CH_3)_2$), 8.11 (d, 2H, J=8.9Hz, 2xArH), 8.15 (d, 2H, J=8.9Hz, 2xArH), 8.46 (s, 1H, CH)	X	X
f10	91mg, 71%	0.34 b	$322M^+$, $232[M-CH_2C_6H_5]^+$, $200[M-CH_2C_6H_5-OCH_3]^+$, $91[CH_2C_6H_5]^+$		X	X
g10	74mg, 80%	0.02 b	$231M^+$		X	X
h10	97mg, 71%	0.40 b	$232[M-CH_2CH(C_2H_5)C_4H_9]^+$, $200[M-CH_2CH(C_2H_5)C_4H_9-OCH_3]^+$		X	X
i10	57mg, 58%	0.26 b	$246M^+$, $232[M-CH_3]^+$, $200[M-CH_3-OCH_3]^+$		X	X
j10	57mg, 55%	0.32 b	$260M^+$, $232[M-CH_2CH_3]^+$, $200[M-CH_2CH_3-OCH_3]^+$		X	X
k10	95mg, 68%	0.35 b	$350M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l10	70mg, 51%	0.27 b	$342M^+$, $232[M-CH_2CH_2C_4H_3S]^+$, $200[M-OCH_2CH_2C_4H_3S-OCH_3]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m10	74mg, 54%	0.49 b	$344M^+$, $232[M-(CH_2)_7CH_3]^+$, $200[M-(CH_2)_7CH_3-OCH_3]^+$		X	X
a11	75mg, 69%	0.11 b	$274M^+$, $218[M-CH_2CH_2CH_2CH_3]^+$, $200[M-OCH_2CH_2CH_2CH_3]^+$		X	X
b11	82mg, 75%	0.13 b	$274M^+$, $218[M-C(CH_3)_3]^+$		X	X
c11	71mg, 69%	0.14 b	$258M^+$, $218[M-CH_2CH=CH_2]^+$, 200[M-		X	X

			$OCH_2CH=CH_2]^+$			
d11	88mg, 80%	0.10 b	$276M^+$, $218[M-CH_2CH_2OCH_3]^+$, $200[M-OCH_2CH_2OCH_3]^+$		X	X
e11	79mg, 76%	0.12 b	$260M^+$, $218[M-CH(CH_3)_2]^+$, $200[M-OCH(CH_3)_2]^+$		X	X
f11	102mg, 83%	0.19 b	X	X	X	X
g11	65mg, 75%	0.01 b	$217M^+$		X	X
h11	115mg, 87%	0.15 b	$218[M-CH_2CH(C_2H_5)C_4H_9]^+$, $200[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i11	63mg, 68%	0.02 b	$232M^+$, $218[M-CH_3]^+$		X	X
j11	60mg, 61%	0.20 b	$246M^+$, $218[M-CH_2CH_3]^+$, $200[M-CH_2CH_3]^+$		X	X
k11	65mg, 49%	0.18 b	$336M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l11	72mg, 55%	0.15 b	$328M^+$, $218[M-CH_2CH_2C_4H_3S]^+$, $200[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m11	79mg, 60%	0.48 b	$330M^+$, $218[M-(CH_2)_7CH_3]^+$, $200[M-O(CH_2)_7CH_3-OCH_3]^+$		X	X
a12	74mg, 67%		$276M^+$, $220[M-(CH_2)_3CH_3]^+$, $202[M-O(CH_2)_3CH_3]^+$		X	X
b12	68mg, 62%	0.49 b	$276M^+$, $220[M-C(CH_3)_3]^+$, $202[M-OC(CH_3)_3]^+$		X	X
c12	X	X	X	X	X	X
d12	X	X	X	X	X	X
e12	103mg, 98%	0.37 b	$262M^+$, $220[M-CH(CH_3)_2]^+$, $202[M-OCH(CH_3)_2]^+$		X	X
f12	91mg, 74%	0.46 b	$310M^+$, $220[M-CH_2C_6H_5]^+$, $202[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g12	X	X	X	X	X	X
h12	84mg, 63%	0.60 b	$332M^+$, $220[M-CH_2CH(C_2H_5)C_4H_9]^+$, $202[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i12	58mg, 62%	0.21 b	$234M^+$, $220[M-CH_3]^+$, $202[M-OCH_3]^+$		X	X
j12	47mg, 48%	0.35 b	$248M^+$, $220[M-CH_2CH_3]^+$, $202[M-OCH_2CH_3]^+$		X	X
k12	103mg, 76%	0.48 b	$338M^+$, $220[M-(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l12	75mg, 57%	0.33 b	$330M^+$, $220[M-CH_2CH_2C_4H_3S]^+$, $202[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m12	75mg, 57%	0.51 b	$332M^+$, $220[M-(CH_2)_7CH_3]^+$, $202[M-O(CH_2)_7CH_3]^+$		X	X
a13	57mg, 65%	0.39 b	$220M^+$, $164[M-(CH_2)_3CH_3]^+$, $146[M-O(CH_2)_3CH_3]^+$		X	X
b13	54mg, 62%	0.42 b	$220M^+$, $164[M-C(CH_3)_3]^+$		X	X
c13	X	X	X	X	X	X
d13	X	X	X	X	X	X
e13	75mg, 91%	0.02 b	$206M^+$, $164[M-CH(CH_3)_2]^+$, $146[M-OCH(CH_3)_2]^+$		X	X
f13	68mg, 67%	0.20 b	X	X	X	X
g13	X	X	X	X	X	X
h13	60mg, 55%	0.42 b	$276M^+$, $164[M-CH_2CH(C_2H_5)C_4H_9]^+$, $146[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i13	31mg, 44%	0.21 b	$178M^+$, $164[M-CH_3]^+$, $146[M-OCH_3]^+$		X	X
j13	57mg, 75%	0.62 a	$164[M-CH_2CH_3]^+$	1.29 (t, 3H, J=7.1Hz, CH_3), 4.29 (q, 2H, J=7.1Hz, CH_2), .87 (dd, 1H, J=3.6, 1.7Hz, ArH), 7.53 (d, 1H, J=3.6Hz, ArH), 8.14 (s, 1H, CH=), 8.21 (d, 1H, J=1.7Hz, ArH)	X	X
k13	48mg, 43%	0.45 b	$282M^+$, $164[M-(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l13	60mg, 55%	0.31 b	$274M^+$, $164[M-CH_2CH_2C_4H_3S]^+$, $146[M-$	2.92 (t, 2H, J=6.8Hz, $COOCH_2C\underline{H}_2$), 3.60 (t, 2H, J=6.8Hz, $C\underline{H}_2CH_2$), 6.88-6.95 (ov. m,	X	X

			$OCH_2CH_2C_4H_3S]^+$	.4H, 4xArH), 7.30 (dd, 1H, *J*=4.0, 1.1Hz, ArH), 7.53 (d, 1H, *J*=3.6Hz, ArH), 8.15 (s,1H, $C\underline{H}$=)		
m13	62mg, 56%	0.49 b	$276M^+$, $164[M-(CH_2)_7CH_3]^+$, $146[M-O(CH_2)_7CH_3]^+$		X	X
a14	66mg, 68%	0.50 b	$244M^+$, $188[M-(CH_2)_3CH_3]^+$, $170[M-O(CH_2)_3CH_3]^+$		X	X
b14	54mg, 56%	0.53 b	$244M^+$, $188[M-C(CH_3)_3]^+$, $170[M-OC(CH_3)_3]^+$		<100	X
c14	X	X	X	X	X	X
d14	X	X	X	X	X	X
e14	72mg, 77%	0.51 b	$230M^+$, $188[M-CH(CH_3)_2]^+$, $170[M-OCH(CH_3)_2]^+$,		X	X
f14	86mg, 78%	0.48 b	$278M^+$, $188[M-CH_2C_6H_5]^+$, $170[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g14	51mg, 69%	0.03 b	$187M^+$		X	X
h14	70mg, 59%	0.54 b	$300M^+$, $188[M-CH_2CH(C_2H_5)C_4H_9]^+$, $170[M-OCH_2CH(C_2H_5)\ C_4H_9]^+$		X	X
i14	40mg, 50%	0.29 b	$202M^+$, $188[M-CH_3]^+$, $170[M-OCH_3]^+$		X	X
j14	34mg, 40%	0.48 b	$216M^+$, $188[M-CH_2CH_3]^+$, $170[M-OCH_2CH_3]^+$		X	X
k14	77mg, 63%	0.61 b	$306M^+$, $188[M-(CH_2)_3C_6H_5]^+$, $170[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l14	64mg, 54%	0.44 b	$298M^+$, $188[M-CH_2CH_2C_4H_3S]^+$, $170[M-OCH_2CH_2C_4H_3S]^+$		X	X
m14	67mg, 56%	0.56 b	$300M^+$, $188[M-(CH_2)_7CH_3]^+$, $170[M-(CH_2)_7CH_3]^+$		X	X
a15	59mg, 57%	0.19 b	$262M^+$, $206[M-(CH_2)_3CH_3]^+$, $188[M-O(CH_2)_3CH_3]^+$	0.93 (t, 3H, *J*=7.3Hz, CH_3), 1.40 (sextet, 2H, *J*=7.4Hz, $CH_2CH_2C\underline{H}_2CH_3$), 1.66 (quintet, 2H, *J*=7.0Hz, $COOCH_2CH_2C\underline{H}_2CH_3$), 4.25 (t, 2H, *J*=6.8Hz, $COOC\underline{H}_2$), 6.85 (d, 1H, *J*=8.3Hz, ArH-5), 7.38 (dd, 1H, *J*=8.**4**, 2.2Hz, ArH-6), 7.63 (d, 1H, *J*=2.2Hz, ArH-2), 8.08 (s, 1H, CH=), 9.80 (br s, 2H, 2xOH)	50-100	>100
b15	62mg, 59%	0.16 b	$206[M-C(CH_3)_3]^+$, $188[M-OC(CH_3)_3]^+$	1.51 (s, 3H, $(CH_3)_3$), 6.86 (d, 1H, *J*=8.3Hz, ArH-5), 7.35 (dd, 1H, *J*=8.**4**, 2.2Hz, ArH-6), 7.60 (d, 1H, *J*=2.2Hz, ArH-2), 8.01 (s, 1H, CH=), 9.80 (br s, 2H, 2xOH)	>100	>100
c15	70mg, 71%	0.12 b	$246M^+$, $206[M-CH_2CH=CH_2]^+$, $188[M-OCH_2CH=CH_2]^+$		>100	>100
d15	69mg, 66%	0.10 b	$264M^+$, $206[M-CH_2CH_2OCH_3]^+$, $188[M-OCH_2CH_2OCH_3]^+$		>100	>100
e15	56mg, 57%	0.13 b	$248M^+$, $206[M-CH(CH_3)_3]^+$, $188[M-OCH(CH_3)_2]^+$		>100	>100
f15	83mg, 70%	0.15 b	$206[M-CH_2C_6H_5]^+$, $188[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$	4.49 (s, 2H, $COOC\underline{H}_2C_6H_5$), 6.82 (d, 1H, *J*=8.3Hz, ArH-5), 7.23-7.38 (ov. m, 6H, ArH-6, 5xAr'H), 7.63 (d, 1H, *J*=2.3Hz, ArH-2), 8.07 (s, 1H, CH=), 9.80 (br s, 2H, 2xOH)	>100	>100
g15	70mg, 86%	0.01 b	$205M^+$, $188[M-NH_2]^+$	6.88 (d, 1H, *J*=8.3Hz, ArH-5), 7.28 (dd, 1H, *J*=8.4, 2.1Hz, ArH-6), 6.53 (d, 1H, *J*=2.1Hz, ArH-2), 7.64 (br d, 2H, NH_2), 7.94 (s, 1H, CH=), 9.81 (br s, 2H, 2xOH)	>100	>100
h15	80mg, 63%	0.14 b	$318M^+$, $206[M-CH_2CH(C_2H_5)C_4H_9]^+$, $188[M-OCH_2CH(C_2H_5)C_4H_9]^+$		>100	>100
i15	81mg, 92%	0.03 b	$220M^+$, $206[M-CH_3]^+$, $188[M-OCH_3]^+$	3.82 (s, 3H, $COOC\underline{H}_3$), 6.90 (d, 1H, *J*=8.3Hz, ArH-5), 7.41 (dd, 1H, *J*=8.4, 2.1Hz, ArH-6), 7.65 (d, 1H, *J*=2.2Hz, ArH-2), 8.14 (s, 1H, CH=), 9.95 (br s, 2H, 2xOH)	50-100	>100
j15	40mg, 43%	0.55 a	$234M^+$, $206[M-CH_2CH_3]^+$, $188[M-OCH_2CH_3]^+$	1.28 (t, 3H, *J*=7.1Hz, $COOCH_2C\underline{H}_3$), 4.27 (q, 2H, *J*=7.1Hz, $COOC\underline{H}_2CH_3$), 6.82 (d, 1H, *J*=8.3Hz, ArH-5), 7.37 (dd, 1H, *J*=8.4, 2.2Hz, ArH-6), 7.63 (d, 1H, *J*=2.2Hz, ArH-2), 8.07 (s, 1H, CH=), 9.80 (br s, 2H, 2xOH)	50-100	>100
k15	97mg, 75%	0.41 b	$324M^+$	1.92 (quintet, 2H, *J*=7.1Hz, $COOCH_2C\underline{H}_2$), 2.65 (t, 2H, *J*=7.7Hz, $C\underline{H}_2C_6H_5$), 4.11 (t, 2H, *J*=6.5Hz, $COOC\underline{H}_2$), 6.91 (d, 1H, *J*=8.0Hz, ArH-5), 7.19-7.32 (ov. m, 5H, $CH_2C_6\underline{H}_5$),	>100	>100

				9.52 (s, 1H, ArH), 9.70 (s, 1H, ArH), 10.08 (s, 1H, CH=)		
l15	69mg, 55%	0.04 b	$316M^+$, $206[M-CH_2CH_2C_4H_3S]^+$, $188[M-OCH_2CH_2C_4H_3S]^+$	2.92 (t, 2H, *J*=6.8Hz, $OCH_2C\underline{H}_2$), 3.59 (t, 2H, *J*=6.8Hz, $OC\underline{H}_2$), 6.82-6.85 (ov. m, 2H, 2xArH), 6.92 (dd, 1H, *J*=8.4, 3.4Hz, ArH), 7.29 (d, 1H, *J*=5.2Hz, ArH-5), 7.37 (dd, 1H, *J*=8.4, 2.3Hz, ArH-6), 7.63 (d, 1H, *J*=2.2Hz, ArH-2), 8.08 (s, 1H, CH=)	50-100	>100
m15	73mg, 58%	0.12 bg	$318M^+$, $206[M-(CH_2)_7CH_3]^+$, $188[M-O(CH_2)_7CH_3]^+$	0.83-0.88 (m, 3H, CH_3), 1.24-1.39 (ov. m, 10H, $5xCH_2$), 1.55-1.79 (m, 2H, $COOCH_2C\underline{H}_2CH_2$), 4.30 (t, 2H, *J*=7.1Hz, $COOC\underline{H}_2$), 6.83 (d, 1H, *J*=8.4Hz, ArH-5), 7.38 (dd, 1H, *J*=8.4, 1.8Hz, ArH-6), 7.62 (d, 1H, *J*=1.8Hz, ArH-2), 8.07 (s, 1H, CH=), 9.60 (br s, 2H, 2xOH)	>100	>100
a16	X	X	390, 273, 235, 220, 207, 189, 163	X	25-50	>100
b16	X	X	390, 189, 188, 163	X	25-50	>100
c16	X	X	390, 274, 235, 220, 189, 163	X	25-50	>100
d16	X	X	390, 274, 235, 220, 189, 163	X	25-50	X
e16	X	X	390, 234, 189, 188	X	50-100	>100
f16	X	X	274, 255, 235, $189[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$	X	25-50	>100
g16	X	X	X	X	X	X
h16	X	X	390, 375, 273, 255, 256, 235, 229, 220, 207, 189, 163	X	25-50	>100
i16	X	X	390, 234, 189, 188, 220	X	10-25	25-50
j16	X	X	390, 274, 255, 235, 220, 189, 163	X	25-50	>100
k16	X	X	390, 338, 325, 255, 207, 189, 163, 119, 91	X	25-50	50-100
l16	X	X	274, $189[M-OCH_2CH_2C_4H_3S]^+$, 188, $111[CH_2CH_2C_4H_3S]^+$	X	25-50	X
m16	X	X	390, 273, 255, 256, 235, 220, 207, 189, 163		25-50	>100
a17	62mg, 63%	0.35 b	$246M^+$, $190[M-CH_2CH_2CH_2CH_3]^+$, $172[M-OCH_2CH_2CH_2CH_3]^+$		X	X
b17	56mg, 57%	0.28 b	$246M^+$, $190[M-C(CH_3)_3]^+$, $172[M-O(CH_3)_3]^+$		>100	X
c17	64mg, 70%	0.29 b	$230M^+$, $190[M-CH_2CH=CH_2]^+$, $172\ [M-OCH_2CH=CH_2]^+$		X	X
d17	61mg, 62%	0.26 b	$248M^+$, $190[M-CH_2CH_2OCH_3]^+$, $172[M-OCH_2CH_2OCH_3]^+$		X	X
e17	56mg, 61%	0.28 b	$190[M-CH(CH_3)_2]^+$, $172[M-OCH(CH_3)_2]^+$	1.30 (s, 3H, $CH(C\underline{H}_3)$) 1.32 (s, 3H, $CH(C\underline{H}_3)$), 5.23 (septet, 1H, *J*=6.2Hz, $C\underline{H}(CH_3)_2$), 7.04 (d, 1H, *J*=7.9Hz, Ar-$\underline{H}$5), 7.39-7.49 (ov. m, 3H, Ar-$\underline{H}$1,2,3), 8.28 (s, 1H, C$\underline{H}$), 10.1 (br s, 1H, O$\underline{H}$)	X	X
f17	79mg, 71%	0.28 b	$280M^+$, $190[M-CH_2C_6H_5]^+$, $172[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$	5.35 (s, 2H, CH_2), 7.04 (d, 1H, *J*=9.1Hz, ArH-2), 7.30-7.50 (ov. m, 8H, ArH), 8.34 (s, 1H, CH=C), 10.05 (br s, 1H, OH)	X	X
g17	61mg, 81%	0.01 b	$189M^+$		X	X
h17	75mg, 62%	0.32 b	$302M^+$, $190[M-CH_2CH(C_2H_5)C_4H_9]^+$, $172[M-OCH_2CH(C_2H_5)C_4H_9]^+$	0.90-0.96 (ov. m, 6H, $2xCH_3$), 1.28-1.42 (ov. m, 8H, $4xCH_2$), 1.64-1.67 (m, 1H, CH), 4.29 (d, 2H, *J*=6.8Hz, $COOCH_2$), 7.04 (d, 1H, *J*=7.3Hz, ArH), 7.38-7.49 (ov. m, 3H. 3xArH), 8.28 (s, 1H, CH=), 9.89 (br s, 1H, OH)	>100	X
i17	49mg, 60%	0.13 b	$204M^+$, $190[M-CH_3]^+$, $172[M-OCH_3]^+$		X	X
j17	38mg, 44%	0.24 b	$218M^+$, $190[M-CH_2CH_3]^+$, $172[M-OCH_2CH_3]^+$		X	X
k17	83mg, 68%	0.30 b	$308M^+$, $204[M-O(CH_2)_3C_6H_5+32]^+$, $190[M-(CH_2)_3C_6H_5]^+$, $172[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l17	64mg,	0.21	$300M^+$, $190[M-$		X	X

	54%	b	$CH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$			
m17	68mg, 56%	0.37 b	$302M^+$, $190[M-(CH_2)_7CH_3]^+$, $172[M-(CH_2)_7CH_3]^+$		X	X
a18	69mg, 63%	0.23 b	$276M^+$, $220[M-(CH_2)_3CH_3]^+$, $202[M-O(CH_2)_3CH_3]^+$		X	X
b18	69mg, 63%	0.20 b	$276M^+$, $220[M-C(CH_3)_3]^+$, $202[M-OC(CH_3)_3]^+$		X	X
c18	60mg, 58%	0.18 b	$260M^+$, $220[M-CH_2CH=CH_2]^+$, $202[M-CH_2CH=CH_2]+$		X	X
d18	62mg, 56%	0.17 b	$278M^+$, $220[M-CH_2CH_2OCH_3]^+$, $202[M-OCH_2CH_2OCH_3]^+$		X	X
e18	47mg, 45%	0.18 b	$262M^+$, $220[M-C(CH_3)_2]^+$, $202[M-OCH(CH_3)_2]^+$		X	X
f18	92mg, 74%	0.24 b	$310M^+$, $220[M-CH_2C_6H_5]^+$, $202[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g18	72mg, 83%	0.01 b	$219M^+$		X	X
h18	84mg, 63%	0.20 b	$332M^+$, $220[M-CH_2CH(C_2H_5)C_4H_9]^+$, $202[M-OCH_2CH(C_2H_5)\ C_4H_9]^+$		X	X
i18	57mg, 61%	0.09 b	$234M^+$, $220[M-CH_3]^+$, $202[M-OCH_3]^+$		X	X
j18	69mg, 70%	0.68 a	$248M^+$, $220[M-CH_2CH_3]^+$, $202[M-OCH_2CH_3]^+$	1.29 (t, 3H, J=7.1Hz, $CH_2C\underline{H}_3$), 3.81 (s, 3H, OCH_3), 4.29 (q, 2H, J=7.1Hz, CH_2), 6.95 (d, 1H, J=8.3Hz, ArH-5), 7.61 (dd, 1H, J=8.4, 2.1Hz, ArH-6), 7.77 (d, 1H, J=2.0Hz, ArH-2), 8.24 (s, 1H, CH=)	>100	X
k18	99mg, 73%	0.32 b	$220[M-(CH_2)_3C_6H_5]^+$, $202[M-O(CH_2)_3C_6H_5]^+$	1.99 (quintet, 2H, J=7.4Hz, $COOCH_2C\underline{H}_2$) 2.71 (t, 2H, J=7.6Hz, $C\underline{H}_2C_6H_5$), 3.82 (s, 3H, OCH_3), 4.23 (t, 2H, J=6.5Hz $COOC\underline{H}_2$), 6.96 (d, 1H, J=8.3Hz), 7.18-7.33 (ov. m, 5H, $CH_2C_6\underline{H}_5$), 7.65 (dd, 1H, J=8.4Hz, 2.6Hz), 8.22 (s, 1H, CH=), 7.77 (s, 1H, ArH-5), 10.58 (br s, 1H, OH)	X	X
l18	72mg, 55%	0.12 b	$330M^+$, $220[M-CH_2CH_2C_4H_3S]^+$, $202[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m18	76mg, 57%	0.24 b	$332M^+$, $220[M-(CH_2)_7CH_3]^+$, $202[M-O(CH_2)_7CH_3]^+$		X	X
a19	69mg, 63%	0.15 b	$276M^+$, $220[M-(CH_2)_3CH_3]^+$, $202[M-O(CH_2)_3CH_3]^+$		X	X
b19	68mg, 62%	0.16 b	$234[M-OC(CH_3)_3-32]^+$, $220[M-C(CH_3)_3]^+$, $202[M-OC(CH_3)_3]^+$		X	X
c19	39mg, 38%	0.15 b	$260M^+$, $234[M-OCH_2CH=CH_2+32]^+$, $220[M-CH_2CH=CH_2]^+$, $202[M-OCH_2CH=CH_2]^+$		X	X
d19	93mg, 84%	0.14 b	$278M^+$, $220[M-CH_2CH_2OCH_3]^+$, $202[M-OCH_2CH_2OCH_3]^+$		X	X
e19	90mg, 86%	0.12 b	$262M^+$, $220[M-CH(CH_3)_2]^+$, $202[M-OCH(CH_3)_2]^+$	1.29 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 5.06 (septet, 1H, J=6.2Hz, $CH(CH_3)_2$), 7.13 (d, 1H, J=8.5Hz, ArH-5), 7.52 (dd, 1H, J=8.5, 1.5Hz, ArH-6), 7.64 (d, 1H, J=1.5Hz, ArH-2), 9.45 (br s, 1H, OH)	>100	X
f19	90mg, 73%	0.11 b	$310M^+$, $220[M-CH_2C_6H_5]^+$, $202[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g19	68mg, 78%	0.01 b	$219M^+$		X	X
h19	89mg, 67%	0.14 b	$332M^+$, $220[M-CH_2CH(C_2H_5)C_4H_9]^+$, $202[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i19	59mg, 63%	0.04 b	$234M^+$, $220[M-CH_3]^+$, $202[M-OCH_3]^+$		X	X
j19	70mg, 71%	0.62 a	$248M^+$, $220[M-CH_2CH_3]^+$, $202[M-OCH_2CH_3]^+$	1.29 (t, 3H, J=7.1Hz, $COOCH_2C\underline{H}_3$), 3.88 (s, 3H, OCH_3), 4.28 (q, 2H, J=7.1Hz, $COOC\underline{H}_2CH_3$), 7.12 (d, 1H, J=8.5Hz, ArH-5), 7.52 (dd, 1H, J=6.3, 2.2Hz, ArH-6), 7.65 (d, 1H, J=2.1Hz, ArH-2), 8.20 (s, 1H, CH=), 9.68	>100	X

				(br s, 1H, OH)		
k19	101mg, 75%	0.35 b	$338M^+$, $220[M-(CH_2)_3C_6H_5]^+$, $202[M-O(CH_2)_3C_6H_5]^+$		X	X
l19	70mg, 53%	0.08 b	330M+, $220[M-CH_2CH_2C_4H_3S]^+$, $202[M-CH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m19	74mg, 56%	0.17 b	$332M^+$, $220[M-(CH_2)_7CH_3]^+$, $202[M-O(CH_2)_7CH_3]^+$		X	X
a20	91mg, 79%	0.38 b	$290M^+$, $234[M-(CH_2)_3CH_3]^+$, $216[M-O(CH_2)_3CH_3]^+$		>100	X
b20	72mg, 62%	0.36 b	$290M^+$, $234[M-C(CH_3)_3]^+$, $216[M-OC(CH_3)_3]^+$		X	X
c20	73mg, 67%	0.32 b	$274M^+$, $216[M-OCH_2CH=CH_2]^+$		X	X
d20	83mg, 71%	0.33 b	$292M^+$		>100	>100
e20	107mg, 97%	0.33 b	$276M^+$, $234[M-CH(CH_3)_2]^+$, $216[M-OCH(CH_3)_2]^+$		>100	>100
f20	74mg, 57%	0.30 b	$324M^+$, $216[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		>100	>100
g20	80mg, 86%	0.01 b	$233M^+$		>100	>100
h20	94mg, 68%	0.36 b	$346M^+$, $234[M-CH_2CH(C_2H_5)C_4H_9]^+$, $216[M-OCH_2CH(C_2H_5)C_4H_9]^+$		>100	X
i20	63mg, 64%	0.19 b	$248M^+$, $216[M-OCH_3]^+$		>100	X
j20	56mg, 54%	0.20 b	$262M^+$, $216[M-OCH_2CH_3]^+$		>100	X
k20	108mg, 80%	0.37 b	$352M^+$, $234[M-(CH_2)_3C_6H_5]^+$, $216[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		>100	X
l20	73mg, 53%	0.23 b	$344M^+$, $216[M-OCH_2CH_2C_4H_3S]^+$		>100	>100
m20	73mg, 53%	0.39 b	$346M^+$, $234[M-(CH_2)_7CH_3]^+$, $216[M-O(CH_2)_7CH_3]^+$		>100	X
a21	68mg, 62%	0.25 b	$275M^+$, $219[M-(CH_2)_3CH_3]^+$, $201[M-O(CH_2)_3CH_3]^+$		X	X
b21	63mg, 57%	0.33 b	$275M^+$		X	X
c21	X	X	X	X	X	X
d21	X	X	X	X	X	X
e21	97mg, 93%	0.28 b	$261M^+$, $219[M-CH(CH_3)_2]^+$, $201[M-CH(CH_3)_2]^+$	1.32 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 5.13 (septet, 1H, J=6.2Hz, $C\underline{H}(CH_3)_2$), 7.89 (dd, 1H, J=8.0Hz, ArH-5), 8.61 (d, 1H, J=5.0Hz, ArH-4), 8.91 (s, 1H, CH=)	>100	>100
f21	78mg, 63%	0.24 b	$309M^+$, $219[M-CH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g21	67mg, 77%	0.01 b	$218M^+$, $201[M-NH_2]^+$	7.86 (dd, 1H, J=8.1, 8.1Hz, ArH-5), 7.93 (br d, 2H, NH_2), 8.30-8.33 (ov. m, 2H, ArH-2/6), 8.40 (dd, 1H, J=8.4, 1.8Hz, ArH-4), 8.77 (s, 1H, CH=)	X	X
h21	76mg, 58%	0.36 b	$331M^+$, $219[M-CH_2CH(C_2H_5)C_4H_9]^+$,		>100	X
i21	59mg, 64%	0.14 b	$233M^+$, $219[M-CH_3]^+$		X	X
j21	62mg, 63%	0.19 b	$247M^+$, $219[M-CH_2CH_3]^+$		X	X
k21	92mg, 68%	0.30 b	$337M^+$, $119[CH_2CH_2CH_2C_6H_5]^+$		X	X
l21	70mg, 53%	0.19 b	$329M^+$, $219[M-CH_2CH_2C_4H_3S]^+$, $201[M-CH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m21	74mg, 56%	0.44 b	$331M^+$, $219[M-(CH_2)_7CH_3]^+$		X	X
a22	48mg, 45%	0.18 b	$269M^+$, $213[M-(CH_2)_3CH_3]^+$, $195[M-O(CH_2)_3CH_3]^+$	0.94 (t, 3H, J=6.7Hz, $COOCH_2CH_2CH_2C\underline{H}_3$), 1.31 (sextet, 2H, J=6.9Hz, $COOCH_2CH_2C\underline{H}_2$), 1.68 (quintet, 2H, J=6.9Hz, $COOCH_2C\underline{H}_2$), 4.28 (t, 2H, J=6.9Hz, $COOC\underline{H}_2$), 7.23-7.35 (ov. m, 2H, ArH-4/5), 7.55 (dd, 1H, J=6.8, 1.7Hz, ArH-3), 7.98 (dd, 1H, J=6.4, 1.8Hz, ArH-6), 8.57 (s, 1H, ArH-2), 8.58 (s, 1H, CH=), 12.51 (br s,	>100	X

				1H, NH)		
b22	70mg, 65%	0.20 b	269M$^+$, 227[M-OC(CH$_3$)$_3$+32]$^+$, 213[M-C(CH$_3$)$_3$]$^+$, 195[M-OC(CH$_3$)$_3$]$^+$		>100	X
c22	79mg, 78%	0.20 b	253M$^+$, 213[M-CH$_2$CH=CH$_2$]$^+$, 195[M-OCH$_2$CH=CH$_2$]$^+$		>100	X
d22	87mg, 81%	0.17 b	271M$^+$, 213[M-CH$_2$CH$_2$OCH$_3$]$^+$, 195[M-OCH$_2$CH$_2$OCH$_3$]$^+$	3.40 (s, 3H, OCH$_3$), 3.62 (t, 2H, *J*=4.7Hz, $\mathrm{C\underline{H}_2OCH_3}$), 4.40 (t, 2H, *J*=4.6Hz, $\mathrm{C\underline{H}_2CH_2OCH_3}$), 7.26-7.33 (ov. m, 2H, ArH-4/5), 7.58 (dd, 1H, *J*=6.8, 1.7Hz, ArH-3), 7.98 (dd, 1H, *J*=6.4, 1.8Hz, ArH-6), 8.57 (s, 1H, ArH-2), 8.58 (s, 1H, CH=), 12.51 (br s, 1H, NH)	>100	>100
e22	90mg, 88%	0.12 b	255M$^+$, 213[M-CH(CH$_3$)$_2$]$^+$, 195[M-OCH(CH$_3$)$_2$]$^+$		>100	>100
f22	72mg, 60%	0.14 b	X	X	>100	X
g22	X	X	X	X	X	X
h22	80mg, 62%	0.18 b	325M$^+$, 213[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$, 195[M-OCH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		>100	>100
i22	70mg, 77%	0.07 b	227M$^+$, 213[M-CH$_3$]$^+$, 195[M-OCH$_3$]$^+$		X	X
j22	78mg, 81%	0.67 a	241M$^+$, 213[M-CH$_2$CH$_3$]$^+$, 195[M-OCH$_2$CH$_3$]$^+$, 91[CH$_2$C$_6$H$_5$]$^+$	1.31 (t, 3H, *J*=7.1Hz, CH$_3$), 4.29 (q, 2H, *J*=7.1Hz, CH$_2$), 7.26-7.31 (ov. m, 2H, ArH-4/5), 7.58 (dd, 1H, *J*=6.8, 1.7Hz, ArH-3), 7.98 (dd, 1H, *J*=6.3, 1.5Hz, ArH-6), 8.57 (s, 1H, ArH-2), 8.59 (s, 1H, CH=), 12.46 (br s, 1H, NH)	X	X
k22	X	X	X	X	X	X
l22	74mg, 53%	0.08 b	323M$^+$, 213[M-CH$_2$CH$_2$C$_4$H$_3$S]$^+$, 195[M-OCH$_2$CH$_2$C$_4$H$_3$S]$^+$, 111[CH$_2$CH$_2$C$_4$H$_3$S]$^+$		50-100	>100
m22	45mg, 35%	0.18 b	325M$^+$, 213[M-(CH$_2$)$_7$CH$_3$]$^+$, 195[M-O(CH$_2$)$_7$CH$_3$]$^+$		50-100	X
a23	X	X	X	X	X	X
b23	X	X	X	X	X	X
c23	X	X	X	X	X	X
d23	X	X	X	X	X	X
e23	X	X	X	X	X	X
f23	X	X	X	X	X	X
g23	X	X	X	X	X	X
h23	X	X	X	X	X	X
i23	X	X	X	X	X	X
j23	X	X	X	X	X	X
k23	X	X	X	X	X	X
l23	X	X	X	X	X	X
m23	X	X	X	X	X	X
a24	75mg, 80%	0.36 c	234M$^+$		X	X
b24	56mg, 60%	0.01 b	234M$^+$, 178[M-C(CH$_3$)$_3$]$^+$		>100	X
c24	85mg, 98%	0.29 c	218M$^+$		X	X
d24	86mg, 91%	0.31 c	236M$^+$		X	X
e24	80mg, 91%	0.01 b	220M$^+$, 178[M-CH$_2$CH$_2$OCH$_3$]$^+$, 160[M-OCH$_2$CH$_2$OCH$_3$]$^+$		X	X
f24	77mg, 72%	0.05 b	268M$^+$, 160[M-OCH$_2$C$_6$H$_5$]$^+$, 134 [M-OCH$_2$CH$_3$, -CN]$^+$, 91 [CH$_2$C$_6$H$_5$]$^+$.	2.40 (s, 3H, CH$_3$), 5.30 (s, 2H, $\mathrm{C\underline{H}_2C_6H_5}$), 7.33-7.45 (ov. m, 5H, $\mathrm{CH_2C_6\underline{H}_5}$), 7.88 (s, 1H, $\mathrm{N{=}C\underline{H}NH}$), 8.08 (s, 1H, $\mathrm{C\underline{H}{=}}$), 12.80 (br s, 1H, NH)	X	X
g24	X	X	X	X	X	X
h24	68mg, 59%	0.05 b	290M$^+$, 178[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$, 160[M-OCH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		X	X
i24	62mg, 81%	0.19 b	192M$^+$, 178[M-CH$_3$]$^+$, 160[M-OCH$_3$]$^+$		X	X
j24	21mg, 26%	0.79 a	206M$^+$	1.28 (t, 3H, *J*=7.1Hz, $\mathrm{COOCH_2C\underline{H}_3}$), 2.40 (s, 3H, CH$_3$), 4.25 (q, 2H, *J*=7.1Hz, $\mathrm{COOC\underline{H}_2CH_3}$), 7.86 (s, 1H, N=CH), 8.03 (s, 1H, $\mathrm{C\underline{H}{=}}$), 12.21 (br s, 1H, NH)	X	X

k24	110mg, 93%	0.05 b	296M$^+$		>100	X
l24	67mg, 58%	0.04 b	288M$^+$, 178[M-$CH_2CH_2C_4H_3S$]$^+$, 160[M-$CH_2CH_2C_4H_3S$]$^+$		X	X
m24	68mg, 59%	0.05 b	290M$^+$		>100	>100
a25	101mg, 85%	0.36 b	298M$^+$, 330[M+32]$^+$	0.93 (t, 3H, *J*=7.4Hz, CH_3), 1.41 (sextet, 2H, *J*=7.5Hz, $COOCH_2CH_2C\underline{H}_2$), 1.68 (quintet, 2H, *J*=7.3Hz, $COOCH_2C\underline{H}_2$), 4.30 (t, 2H, *J*=6.5Hz, $COOC\underline{H}_2$), 7.61 (t trying to split to a ddd, 1H, *J*=7.2Hz, ArH-7), 7.79 (d, 1H, *J*=8.4Hz, ArH-8), 7.92 (ddd, 1H, *J*=7.1, 7.0, 1.6Hz, ArH-6), 8.14 (dd, 1H, *J*=7.9, 1.6Hz, ArH-5), 8.33 (s, 1H, ArH-2), 9.21 (s, 1H, CH=)	50-100	>100
b25	97mg, 82%	0.18 b	298M$^+$		25-50	50-100
c25	71mg, 63%	0.30 b	314[M+32]$^+$	4.82 (d, 2H, *J*=5.4Hz, $COOCH_2$), 5.32 (dd, 1H, *J*=11.8, 1.6Hz, $CH=C\underline{H}$), 5.43 (dd, 1H, *J*=17.2, 1.6Hz, $CH=C\underline{H}$), 5.97-6.10 (m, 1H, $CH_2C\underline{H}=CH_2$), 7.61 (ddd, 1H, *J*=7.9, 7.1, 0.9Hz, ArH-7), 7.79 (d, 1H, *J*=8.3Hz, ArH-8), 7.92 (ddd, 1H, *J*=8.5, 6.9, 1.6Hz, ArH-6), 8.15 (dd, 1H, *J*=6.4, 1.6Hz, ArH-5), 8.36 (s, 1H, ArH-2), 9.22 (s, 1H, CH=)	50-100	>100
d25	118mg, 98%	0.27 b	332[M+32]$^+$, 300M$^+$	3.38 (s, 3H, OCH_3), 3.64 (t, 2H, *J*=4.7Hz, $C\underline{H}_2OCH_3$), 4.42 (t, 2H, *J*=4.6Hz, $C\underline{H}_2CH_2OCH_3$), 7.61 (ddd, 1H, *J*=7.9, 7.1, 0.9Hz, ArH-7), 7.79 (d, trying to split to a dd, 1H, *J*=8.5Hz, ArH-8), 7.92 (ddd, 1H, *J*=8.5, 8.3, 1.2Hz, ArH-6), 8.16 (dd, 1H, *J*=7.9, 0.9Hz, ArH-5), 8.34 (d, 1H, *J*=0.9Hz, ArH-2), 9.22 (s, 1H, CH=)	50-100	50-100
e25	82mg, 72%	0.22 b	316[M+32]$^+$, 284M$^+$		25-50	>100
f25	77mg, 58%	0.24 b	364[M+32]$^+$, 332M$^+$, 91[$CH_2C_6H_5$]$^+$		50-100	>100
g25	71mg, 74%	0.01 b	241M$^+$		>100	>100
h25	110mg, 78%	0.24 b	354M$^+$		>100	>100
i25	90mg, 88%	0.23 b	256M$^+$, 288[M+32]$^+$	3.84 (s, 3H, OCH_3), 7.39-7.46 (ov. m, 2H, ArH-7/8), 7.77 (ddd, 1H, *J*=8.6, 7.1, 1.6Hz, ArH-6), 7.92 (dd, 1H, *J*=7.7, 1.6Hz, ArH-5), 8.79 (s, 1H, CH=)	50-100	>100
j25	82mg, 76%	0.72 a	302[M+32]$^+$, 270M$^+$,	1.31 (t, 3H, *J*=7.1Hz, CH_3), 4.33 (q, 2H, *J*=7.1Hz, $C\underline{H}_2CH_3$), 7.61 (ddd, 1H, *J*=7.9, 7.0, 0.9Hz, ArH-7), 7.79 (d, trying to split to a dd, 1H, *J*=8.4Hz, ArH-8), 7.91 (ddd, 1H, *J*=8.4, 7.0, 1.2Hz, ArH-6), 8.16 (dd, 1H, *J*=7.9, 0.9Hz, ArH-5), 8.34 (d, 1H, *J*=0.9Hz, ArH-2), 9.21 (s, 1H, CH=)	50-100	>100
k25	81mg, 56%	0.29 b	392[M+32]$^+$, 360M$^+$		50-100	50-100
l25	58mg, 53%	0.20 b	352M$^+$, 111[$CH_2CH_2C_4H_3S$]$^+$		>100	>100
m25	58mg, 41%	0.36 b	354M$^+$		50-100	50-100
a26	X	X	X	X	X	X
b26	X	X	X	X	X	X
c26	X	X	X	X	X	X
d26	63mg, 52%		306M$^+$, 230[M-$OCH_2CH_2OCH_3$]$^+$	3.30 (s, 1H, OCH_3), 3.63 (t, 2H, *J*=4.5Hz, CH_2OCH_3), 4.39 (t, 2H, *J*=4.5Hz, $COOCH_2CH_2OCH_3$), 4.84 (s, 2H, $OC\underline{H}_2COOH$), 7.15 (d, 2H, *J*=8.9Hz, 2xArH), 8.09 (d, 2H, *J*=8.9Hz, 2xArH), 8.33 (s, 1H, CH=) 13.00 (br s, 1H, COOH)	X	X
e26	X	X	X	X	X	X
f26	55mg, 41%		338M$^+$, 91[$CH_2C_6H_5$]$^+$	4.72 (s, 2H, $OC\underline{H}_2COOH$), 5.33 (s, 2H, $COOC\underline{H}_2$), 7.10 (d, 2H, *J*=8.9Hz, 2xArH), 7.36-7.83 (ov. m, 5H, $CH_2C_6\underline{H}_5$), 8.07 (d, 2H, *J*=8.9Hz, 2xArH), 8.35 (s, 1H, CH=) 13.95 (br s, 1H, COOH)	X	X
g26	X	X	X		X	X
h26	X	X	X		X	X

i26	X	X	X		X	X
j26	X	X	X		X	X
k26	X	X	X		X	X
l26	X	X	X		X	X
m26	X	X	X		X	X
a27	X	X	288, 269, 249, 234, 202$[M-O(CH_2)_3CH_3]^+$		50-100	X
b27	89mg, 90%	0.18 b	220$[M-(CH_3)_3]^+$, 202$[M-O(CH_3)_3]^+$, 177	1.53 (s, 9H, $(CH_3)_3$), 3.91 (s, 3H, OCH_3), 7.33-7.45 (ov. m, 3H, ArH-4/5/6), 8.63 (s, 1H, CH=), 9.90 (br s, 1H, OH)	50-100	X
c27	X	X	288, 249, 234, 202$[M-OCH_2CH=CH_2]^+$		50-100	X
d27	X	X	288, 269, 249, 234, 202$[M-OCH_2CH=CH_2]^+$		50-100	X
e27	X	X	288, 249, 234, 202$[M-CH(CH_3)_2]^+$	Coumarin 3.93 (s, 3H, OCH_3), 7.34-7.49 (ov. m, 3H, ArH-5/6/7), 8.95 (s, 1H, ArH-4)	25-50	>100
f27	87mg, 77%	0.10 b	288, 269, 234, 202$[M-OCH_2C_6H_5]^+$, 91$[CH_2C_6H_5]^+$		50-100	X
g27	X	X	X		50-100	X
h27	X	X	288, 269, 234, 202$[M-OCH_2CH(C_2H_5)C_4H_9]^+$		25-50	>100
i27	44mg, 47%	0.05 b	288, 269, 249, 234M^+, 202$[M-OCH_3]^+$		50-100	>100
j27	52mg, 53%	0.07 b	288, 249, 234, 202$[M-OCH_2CH_3]^+$		25-50	X
k27	111mg, 82%	0.32 b	338M^+, 202$[M-O(CH_2)_3C_6H_5]^+$, 119$[(CH_2)_3C_6H_5]^+$		25-50	50-100
l27	72mg, 55%	0.13 b	288, 269, 202$[M-OCH_2CH_2C_4H_3S]^+$, 111$[CH_2CH_2C_4H_3S]^+$		>100	X
m27	66mg, 50%	0.23 b	332M^+, 288, 269, 234, 220$[M-(CH_2)_7CH_3]^+$, 202$[M-O(CH_2)_7CH_3]^+$		25-50	X
a28	87mg, 78%	0.45 b	280M^+, 224$[M-(CH_2)_3CH_3]^+$, 206$[M-O(CH_2)_3CH_3]^+$		50-100	X
b28	80mg, 72%	0.56 b	280M^+, 224$[M-C(CH_3)_3]^+$, 206$[M-OC(CH_3)_3]^+$		X	X
c28	79mg, 75%	0.44 b	264M^+, 224$[M-CH_2CH=CH_2]^+$, 206$[M-OCH_2CH=CH_2]^+$		X	X
d28	65%, 58%	0.42 b	282M^+, 224$[M-CH_2CH_2OCH_3]^+$, 206$[M-OCH_2CH_2OCH_3]^+$		X	X
e28	80mg, 75%	0.44 b	266M^+, 224$[M-CH(CH_3)_2]^+$, 206$[M-OCH(CH_3)_2]^+$		X	X
f28	110mg, 88%	0.46 b	314M^+, 224$[M-CH_2C_6H_5]^+$, 206$[M-CH_2C_6H_5]^+$, 91$[CH_2C_6H_5]^+$		X	X
g28	X	X	X	X	X	X
h28	93mg, 69%	0.48 b	224$[M-CH_2CH(C_2H_5)C_4H_9]^+$, 206$[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i28	61mg, 64%	0.43 b	238M^+, 224$[M-CH_3]^+$, 206$[M-OCH_3]^+$		X	X
j28	64mg, 64%	0.45 b	252M^+, 224$[M-CH_2CH_3]^+$, 206$[M-OCH_2CH_3]^+$		X	X
k28	97mg, 71%	0.43 b	342M^+, 224$[M-(CH_2)_3C_6H_5]^+$, 206$[M-O(CH_2)_3C_6H_5]^+$, 119$[(CH_2)_3C_6H_5]^+$		X	X
l28	68mg, 51%	0.37 b	334M^+, 224$[M-CH_2CH_2C_4H_3S]^+$, 206$[M-OCH_2CH_2C_4H_3S]^+$, 111$[CH_2CH_2C_4H_3S]^+$		X	X
m28	71mg, 53%	0.57 b	336M^+, 224$[M-(CH_2)_7CH_3]^+$, 206$[M-(CH_2)_7CH_3]^+$		X	X
a29	97mg, 84%	0.26 b	290M^+, 234$[M-(CH_2)_3CH_3]^+$		X	X
b29	72mg, 82%	0.22 b	234$[M-C(CH_3)_3]^+$, 216$[M-OC(CH_3)_3]^+$	1.52 (s, 9H, $C(CH_3)_3$), 3.80 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.18 (d, 1H, J=8.3Hz, ArH-5), 7.69-7.73 (ov. m, 2H, ArH-2/6), 8.21 (s, 1H, CH=)	X	X
c29	80mg, 71%	0.21 b	274M^+, 234$[M-CH_2CH=CH_2]^+$, 216[M-		X	X

			$OCH_2CH=CH_2]^+$			
d29	91mg, 78%	0.22 b	$292M^+$, $234[M-CH_2CH_2OCH_3]^+$, $216[M-OCH_2CH_2OCH_3]^+$		X	X
e29	79mg, 72%	0.19 b	$276M^+$, $234[M-CH(CH_3)_2]^+$, $216[M-OCH(CH_3)_2]^+$		X	X
f29	106mg, 83%	0.16 b	$324M^+$, $234[M-CH_2C_6H_5]^+$, $216[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g29	61mg, 66%	0.11 b	$233M^+$		X	X
h29	91mg, 66%	0.16 b	$346M^+$, $234[M-CH_2CH(C_2H_5)C_4H_9]^+$, $216[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i29	60mg, 61%	0.15 b	$248M^+$, $234[M-CH_3]^+$, $216[M-OCH_3]^+$	3.74 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, $COOCH_3$), 7.19 (d, 1H, J=8.2Hz, ArH-5), 7.73-7.77 (ov. m, 2H, ArH-2/6), 8.31 (s, 1H, CH=)	X	X
j29	43mg, 41%	0.54 a	$262M^+$, $234[M-CH_2CH_3]^+$, $216[M-OCH_2CH_3]^+$	1.30 (t, 3H, J=7.1Hz, $COOCH_2C\underline{H}_3$), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.30 (q, 2H, J=7.1Hz, $COOC\underline{H}_2CH_3$), 7.18 (d, 1H, J=8.3Hz, ArH-5), 7.75 (dd, 1H, J=9.9, 2.1Hz, ArH-6), 7.77 (d, 1H, J=2.0Hz, ArH-2), 8.30 (s, 1H, CH=)	X	X
k29	93mg, 66%	0.20 b	$352M^+$, $234[M-(CH_2)_3C_6H_5]^+$, $216[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l29	75mg, 55%	0.13 b	$344M^+$, $234[M-CH_2CH_2C_4H_3S]^+$, $216[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$	2.92 (t, 2H, J=6.8Hz, $OCH_2C\underline{H}_2$), 3.61 (t, 2H, J=6.4Hz, $OC\underline{H}_2$), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.87-6.93 (ov. m, 2H, 2xArH), 7.19 (d, 1H, J=8.5Hz, ArH), 7.28 (d, 1H, J=4.7Hz, ArH), 7.73-7.77 (ov. m, 2H, 2xArH), 8.31 (s, 1H, CH=)	X	X
m29	76mg, 55%	0.28 b	$346M^+$, $234[M-(CH_2)_7CH_3]^+$, $216[M-(CH_2)_7CH_3]^+$	0.85-0.87 (m, 3H, CH_3), 1.05-1.33 (ov. m, 10H, $5xCH_2$), 1.65-1.72 (m, 2H, $COOCH_2C\underline{H}_2$), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.32 (t, 2H, J=7.1Hz, $COOC\underline{H}_2$), 7.20 (d, 1H, J=8.3Hz, ArH-5), 7.73-7.77 (ov. m, 2H, ArH-2/6), 8.31 (s, 1H, CH=)	X	X
a30	124mg, 85%	0.28 b	$366M^+$, $91[CH_2C_6H_5]^+$		X	X
b30	103mg, 71%	0.33 b	$366M^+$, $91[CH_2C_6H_5]^+$		X	X
c30	133mg, 95%	0.34 b	$350M^+$, $91[CH_2C_6H_5]^+$		X	X
d30	139mg, 95%	0.32 b	X		X	X
e30	107mg, 76%	0.31 b	$352M^+$, $91[CH_2C_6H_5]^+$		X	X
f30	93mg, 58%	0.34 b	$400M^+$, $91[CH_2C_6H_5]^+$		X	X
g30	93mg, 75%	0.01 b	$309M^+$, $91[M-CH_2C_6H_5]^+$		X	X
h30	120mg, 71%	0.35 b	$422M^+$, $91[CH_2C_6H_5]^+$		X	X
i30	116mg, 90%	0.25 b	$324M^+$, $91[M-CH_2C_6H_5]^+$		X	X
j30	132mg, 98%	0.67 a	$338M^+$, $91[CH_2C_6H_5]^+$	1.30 (t, 3H, J=7.1Hz, $CH_2C\underline{H}_3$). 3.81 (s, 3H, $OC\underline{H}_3$), 7.28 (d, 1H, J=7.1Hz, $C\underline{H}_2CH_3$), 7.48-7.35 (ov. m, 5H, C_6H_5), 7.73 (dd, 1H, J=8.5,2.2Hz, Ar-H1 of $C_6H_3(OCH_3)(OC_6H_5)$, 7.80 (d, 1H, J=1.9Hz, ArH-5 of $C_6H_3(OCH_3)(OC_6H_5)$, 8.31 (s, 1H, CH=)	X	X
k30	109mg, 64%	0.36 b	$428M^+$, $310[M-(CH_2)_3C_6H_5]^+$, $292[M-O(CH_2)_3C_6H_5]^+$, $119[M-(CH_2)_3C_6H_5]^+$		X	X
l30	90mg, 54%	0.22 b	$420M^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m30	91mg, 54%	0.46 b	$422M^+$, $91[CH_2C_6H_5]^+$		X	X

a31	122mg, 84%	0.28 b	366M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$	0.93 (t, 3H, *J*=7.3Hz, COO(CH$_2$)$_3$C$\underline{H}_3$), 1.28-1.45 (sextet, 2H, *J*=7.3Hz, COOCH$_2$CH$_2$C$\underline{H}_2$), 1.67 (quintet, 2H, *J*=7.0Hz, COOCH$_2$C$\underline{H}_2$), 3.89 (s, 3H, ArOCH$_3$), 4.28 (t, 2H, *J*=7.8Hz, COOC$\underline{H}_2$), 5.13 (s, 2H, OC$\underline{H}_2$C$_6$H$_5$), 7.22 (d, 1H, *J*=8.6Hz, ArH-5), 7.35-7.48 (ov. m, 5H, OCH$_2$C$_6\underline{H}_5$), 7.79 (dd, 1H, *J*=8.5, 2.0Hz, ArH-6), 7.86 (d, 1H, *J*=2.0Hz, ArH-2), 8.29 (s, 1H, CH=)	X	X
b31	96mg, 66%	0.29 b	366M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
c31	115mg, 82%	0.28 b	350M$^+$, 91[M-CH$_2$C$_6$H$_5$]$^+$		>100	X
d31	111mg, 76%	0.26 b	368M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
e31	58mg, 41%	0.24 b	352M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
f31	122mg, 76%	0.32 b	400M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
g31	X	X	X	X	X	X
h31	118mg, 70%	0.35 b	422M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
i31	91mg, 70%	0.20 b	324M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
j31	130mg, 96%	0.63 a	338M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$	1.30 (t, 3H, *J*=7.1Hz, COOCH$_2$CH$_3$), 3.89 (s, 3H, ArOCH$_3$), 4.30 (q, 2H, *J*=7.1Hz, COOC$\underline{H}_2$CH$_3$), 5.12 (s, 2H, OC$\underline{H}_2$C$_6$H$_5$), 7.21 (d, 1H, *J*=8.6Hz, ArH-5), 7.34-7.59 (ov. m, 5H, OCH$_2$C$_6\underline{H}_5$), 7.76 (dd, 1H, *J*=8.6, 1.9Hz, ArH-6), 7.85 (d, 1H, *J*=2.0Hz, ArH-2), 8.28 (s, 1H, CH=)	X	X
k31	103mg, 60%	0.35 b	428M$^+$, 292[M-O(CH$_2$)$_3$C$_6$H$_5$]$^+$, 119[M-(CH$_2$)$_3$C$_6$H$_5$]$^+$		X	X
l31	97mg, 58%	0.17 b	420M$^+$, 111[CH$_2$CH$_2$C$_4$H$_3$S]$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
m31	82mg, 49%	0.33 b	422M$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
a32	79mg, 65%	0.33 b	304M$^+$, 248[M-(CH$_2$)$_3$CH$_3$]$^+$, 230[M-O(CH$_2$)$_3$CH$_3$]$^+$		X	X
b32	76mg, 63%	0.29 b	304M$^+$, 248[M-C(CH$_3$)$_3$]$^+$, 230[M-C(CH$_3$)$_3$]$^+$		X	X
c32	63mg, 55%	0.27 b	288M$^+$, 248[M-CH$_2$CH=CH$_2$]$^+$, 230[M-CH$_2$CH=CH$_2$]$^+$		X	X
d32	21mg, 17%	0.26 b	306M$^+$, 248[M-CH$_2$CH$_2$OCH$_3$]$^+$, 230[M-OCH$_2$CH$_2$OCH$_3$]$^+$		X	X
e32	79mg, 68%	0.23 b	290M$^+$, 248[M-CH(CH$_3$)$_2$]$^+$, 230[M-OCH(CH$_3$)$_2$]$^+$		X	X
f32	128mg, 95%	0.22 b	338M$^+$, 248[M-CH$_2$C$_6$H$_5$]$^+$, 230[M-OCH$_2$C$_6$H$_5$]$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
g32	66mg, 67%	0.01 b	247M$^+$		X	X
h32	88mg, 61%	0.27 b	360M$^+$, 248[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$, 230[M-OCH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		X	X

i32	72mg, 60%	0.20 b	$262M^+$, $248[M-CH_3]^+$, $230[M-OCH_3]^+$		X	X
j32	93mg, 85%	0.57 a	$276M^+$, $248[M-CH_2CH_3]^+$, $230[M-OCH_2CH_3]^+$	1.30 (t, 3H, *J*=7.1Hz, $ArOCH_2C\underline{H}_3$), 1.36 (q, 3H, *J*=6.9Hz, $COOCH_2C\underline{H}_3$), 3.88 (s, 3H, OCH_3), 4.06 (q, 2H, *J*=6.9Hz, $ArOC\underline{H}_2CH_3$), 4.29 (q, 2H, *J*=7.1Hz, $COOC\underline{H}_2CH_3$), 7.18 (d, 1H, *J*=8.5Hz, ArH-5), 7.70 (dd, 1H, *J*=8.5, 2.0Hz, ArH-6), 7.76 (d, 1H, *J*=2.0Hz, ArH-2), 8.29 (s, 1H, CH=)	X	X
k32	91mg, 62%	0.29 b	$366M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l32	75mg, 53%	0.17 b	$358M^+$, $248[M-CH_2CH_2C_4H_3S]^+$, $230[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m32	82mg, 57%	0.33 b	$360M^+$, $248[M-(CH_2)_7CH_3]^+$, $230[M-O(CH_2)_7CH_3]^+$		X	X
a33	85mg, 70%	0.38 b	$304M^+$, $248[M-(CH_2)_3CH_3]^+$, $230[M-O(CH_2)_3CH_3]^+$	0.92 (t, 3H, *J*=7.3Hz, $COOCH_2CH_2CH_2C\underline{H}_3$) 1.27-1.47 (ov. m, 8H, $COOCH_2CH_2C\underline{H}_2CH_3$, $COCH_2C\underline{H}_3$), 1.66 (quintet, 2H, *J*=7Hz, $COOCH_2C\underline{H}_2CH_2CH_3$), 4.14 (t, 2H, *J*=7Hz, $COC\underline{H}_2CH_2CH_2CH_3$), 3.8 (s, 3H, $OC\underline{H}_3$), 4.28 (q, 2H, *J*=7.0 Hz, $COC\underline{H}_2CH_3$), 7.18 (d,1H,*J*=8Hz, Ar-H2), 7.78 (d,1H, *J*=8.0, 2.6Hz, Ar-H1), 8.30 (s,1H, C$\underline{H}$=), 9.20 (br s, 1H, OH)	X	X
b33	81mg, 67%	0.30 b	$304M^+$, $248[M-C(CH_3)_3]^+$, $230[M-OC(CH_3)_3]^+$, $204[M-C(CH_3)_3-CH_3-CH_2CH_3]^+$		X	X
c33	101mg, 88%	0.27 b	$288M^+$, $248[M-CH_2CH=CH_2]^+$, $230[M-OCH_2CH=CH_2]^+$		X	X
d33	104mg, 85%	0.30 b	$306M^+$, $248[M-CH_2CH_2OCH_3]^+$, $230[M-OCH_2CH_2OCH_3]^+$		X	X
e33	105mg, 91%	0.25 b	$290M^+$, $248[M-CH(CH_2)_2]^+$, $230[M-OCH(CH_2)_2]^+$		X	X
f33	96mg, 71%	0.27 b	$338M^+$, $248[M-CH_2C_6H_5]^+$, $230[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g33	70mg, 71%	0.01 b	$247M^+$		X	X
h33	95mg, 66%	0.31 b	$360M^+$, $248[M-CH_2CH(C_2H_5)C_4H_9]^+$, $230[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i33	68mg, 65%	0.23 b	$262M^+$, $248[M-CH_3]^+$, $230[M-OCH_3]+$		X	X
j33	89mg, 81%	0.61 a	$276M^+$, $248[M-CH_2CH_3]^+$, $230[M-OCH_2CH_3]^+$	1.27-1.39 (ov. m, 6H, OCH_2CH_3, $COOCH_2CH_3$), 3.81 (s, 3H, OCH_3), 4.15 (q, 2H, *J*=6.9Hz, $OC\underline{H}_2CH_3$), 4.30 (q, 2H, *J*=7.1Hz, $COOC\underline{H}_2CH_3$), 7.17 (d, 1H, *J*=8.5Hz, ArH-6), 7.70-7.77 (ov. m, 2H, ArH-2/4), 8.29 (s, 1H, CH=), 9.21 (br s, 1H, OH)	X	X
k33	106mg, 73%	0.26 b	$366M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l33	77mg, 54%	0.20 b	$358M^+$, $248[M-CH_2CH_2C_4H_3S]^+$, $230[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m33	81mg, 56%	0.39 b	$360M^+$, $248[M-(CH_2)_7CH_3]^+$, $230[M-O(CH_2)_7CH_3]^+$		X	X
a34	68mg,	0.30	290^+, $234[M-(CH_2)_3CH_3]^+$		X	X

	59%	b				
b34	81mg, 70%	0.29 b	$290M^{+}$, $234[M\text{-}C(CH_3)_3]^{+}$, $216[M\text{-}OC(CH_3)_3]^{+}$		X	X
c34	81mg, 74%	0.27 b	$274M^{+}$, $234[M\text{-}CH_2CH{=}CH_2]^{+}$, $216[M\text{-}OCH_2CH{=}CH_2]^{+}$		X	X
d34	88mg, 76%	0.24 b	$292M^{+}$, $234[M\text{-}CH_2CH_2OCH_3]^{+}$, $216[M\text{-}OCH_2CH_2OCH_3]^{+}$		X	X
e34	48mg, 45%	0.26 b	$276M^{+}$, $234[M\text{-}CH(CH_3)_2]^{+}$		X	X
f34	101mg , 78%	0.24 b	$324M^{+}$, $234[M\text{-}CH_2C_6H_5]^{+}$, $216[M\text{-}OCH_2C_6H_5]^{+}$		X	X
g34	68mg, 73%	0.01 b	$233M^{+}$		X	X
h34	93mg, 67%	0.27 b	$346M^{+}$, $234[M\text{-}CH_2CH(C_2H_5)C_4H_9]^{+}$		X	X
i34	82mg, 83%	0.20 b	$248M^{+}$, $234[M\text{-}CH_3]^{+}$, $216[M\text{-}OCH_3]^{+}$		X	X
j34	41mg, 39%	0.63 a	$262M^{+}$, $234[M\text{-}CH_2CH_3]^{+}$, $216[M\text{-}OCH_2CH_3]^{+}$	1.29 (t, 3H, J=7.1Hz, $ArOC\underline{H}_2CH_3$), 1.36 (t, 3H, J=7.0Hz, $COOCH_2C\underline{H}_3$), 4.07 (q, 2H, J=6.9Hz, $ArOC\underline{H}_2CH_3$), 4.28 (q, 2H, J=2.9Hz, $COOC\underline{H}_2CH_3$), 6.96 (d, 1H, J=8.4Hz, ArH-5), 7.60 (dd, 1H, J=8.4, 2.1Hz, ArH-6), 7.75 (d, 1H, J=2.0Hz, ArH-2), 8.22 (s, 1H, CH=), 9.18 (br s, 1H, OH)	X	X
k34	99mg, 71%	0.30 b	$352M^{+}$, $119[(CH_2)_3C_6H_5]^{+}$		X	X
l34	73mg, 53%	0.17 b	$344M^{+}$, $234[M\text{-}CH_2CH_2C_4H_3S]^{+}$, $216[M\text{-}OCH_2CH_2C_4H_3S]^{+}$, $111[CH_2CH_2C_4H_3S]^{+}$		X	X
m34	77mg, 56%	0.31 b	$346M^{+}$, $234[M\text{-}(CH_2)_7CH_3]^{+}$, $216[M\text{-}O(CH_2)_7CH_3]^{+}$		>100	X
a35	54mg, 43%	0.35 b	$318M^{+}$, $220[M\text{-}(CH_2)_3CH_3\text{-}COCH_3]^{+}$, $202[M\text{-}O(CH_2)_3CH_3\text{-}COCH_3]^{+}$		X	X
b35	76mg, 60%	0.24 b	$318M^{+}$, $202[M\text{-}OC(CH_3)_3\text{-}COCH_3]^{+}$		>100	X
c35	82mg, 68%	0.29 b	$302M^{+}$, $220[M\text{-}CH_2CH{=}CH_2\text{-}COCH_3]^{+}$		X	X
d35	77mg, 60%	0.24 b	$320M^{+}$, $220[M\text{-}CH_2CH_2OCH_3\text{-}COCH_3]^{+}$		X	X
e35	79mg, 65%	0.23 b	$304M^{+}$, $262[M\text{-}CH(CH_3)_2]^{+}$, $220[M\text{-}\ CH(CH_3)_2\text{-}COCH_3]^{+}$, $202[M\text{-}OCH(CH_3)_2\text{-}COCH_3]^{+}$		X	X
f35	102mg , 73%	0.24 b	$352M^{+}$, $220[M\text{-}CH_2C_6H_5\text{-}COCH_3]^{+}$, $202[M\text{-}OCH_2C_6H_5\text{-}COCH_3]^{+}$, $91[CH_2C_6H_5]^{+}$		X	X
g35	X	X	X		X	X
h35	114mg , 76%	0.29 b	$374M^{+}$, $220[M\text{-}CH_2CH(C_2H_5)C_4H_9\text{-}COCH_3]^{+}$, $202[M\text{-}OCH_2CH(C_2H_5)C_4H_9\text{-}COCH_3]^{+}$		X	X
i35	74mg, 67%	0.20 b	$276M^{+}$, $220[M\text{-}CH_3\text{-}COCH_3]^{+}$,		X	X
j35	52mg, 45%	0.50 a	$290M^{+}$, $220[M\text{-}CH_2CH_3\text{-}COCH_3]^{+}$		X	X
k35	X	X	X		X	X
l35	80mg, 54%	0.15 b	$372M^{+}$, $220[M\text{-}CH_2CH_2C_4H_3S\text{-}COCH_3]^{+}$, $202[M\text{-}OCH_2CH_2C_4H_3S\text{-}COCH_3]^{+}$, $111[CH_2CH_2C_4H_3S]^{+}$		X	X
m35	83mg, 56%	0.24 b	$374M^{+}$		X	X
a36	76mg, 73%	0.40 b	$260M^{+}$, $218[M\text{-}(CH_2)_3CH_3{+}32]^{+}$, $204[M\text{-}(CH_2)_3CH_3]^{+}$, $186[M\text{-}O(CH_2)_3CH_3]^{+}$		X	X
b36	62mg, 60%	0.24 b	$260M^{+}$, $218[M\text{-}OC(CH_3)_3{+}32]^{+}$, $204[M\text{-}C(CH_3)_3]^{+}$, $186[M\text{-}OC(CH_3)_3]^{+}$		X	X
c36	68mg, 70%	0.37 b	$244M^{+}$, $204[M\text{-}CH_2CH{=}CH_2]^{+}$, 186[M-		X	X

			OCH$_2$CH=CH$_2$]$^+$			
d36	67mg, 64%	0.33 b	262M$^+$, 204[M-CH$_2$CH$_2$OCH$_3$]$^+$, 186[M-OCH$_2$CH$_2$OCH$_3$]$^+$		X	X
e36	53mg, 54%	0.37 b	246M$^+$, 204[M-CH(CH$_3$)$_2$]$^+$, 186[M-OCH(CH$_3$)$_2$]$^+$		X	X
f36	77mg, 66%	0.31 b	294M$^+$, 204[M-CH$_2$C$_6$H$_5$]$^+$, 186[M-OCH$_2$C$_6$H$_5$]$^+$		X	X
g36	74%	0.04 b	203M$^+$, 186[M-NH$_2$]$^+$	3.86 (s, 3H, CH$_3$), 7.14 (d, 2H, J=8.8Hz, 2xArH), 7.72 (br d, 2H, NH$_2$), 7.96 (d, 2H, J=8.8Hz, 2xArH), 8.11 (s, 1H, CH=)	X	X
h36	80mg, 63%	0.37 b	316M$^+$, 204[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$, 186[M-OCH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		X	X
i36	48mg, 55%	0.24 b	218M$^+$, 204[M-CH$_3$]$^+$, 186[M-OCH$_3$]$^+$		X	X
j36	41mg, 44%	0.28 b	232M$^+$, 204[M-CH$_2$CH$_3$]$^+$, 186[M-OCH$_2$CH$_3$]$^+$		X	X
k36	98mg, 76%	0.33 b	322M$^+$, 204[M-(CH$_2$)$_3$C$_6$H$_5$]$^+$, 186[M-O(CH$_2$)$_3$C$_6$H$_5$]$^+$, 119[(CH$_2$)$_3$C$_6$H$_5$]$^+$		X	X
l36	71mg, 57%	0.26 b	346[M+32]$^+$, 204[M-CH$_2$CH$_2$C$_4$H$_3$S]$^+$, 186[M-OCH$_2$CH$_2$C$_4$H$_3$S]$^+$, 111[CH$_2$CH$_2$C$_4$H$_3$S]$^+$		X	X
m36	75mg, 60%	0.44 b	316M$^+$, 204[M-(CH$_2$)$_7$CH$_3$]$^+$, 186[M-O(CH$_2$)$_7$CH$_3$]$^+$		X	X
a37	X	X	X	X	X	X
b37	X	X	X	X	X	X
c37	X	X	X	X	X	X
d37	X	X	X	X	X	X
e37	X	X	X	X	X	X
f37	X	X	X	X	X	X
g37	X	X	X	X	X	X
h37	X	X	X	X	X	X
i37	X	X	276M$^+$, 230[M-OCH$_2$CH$_3$]$^+$	X	X	X
j37	X	X	X	X	X	X
k37	X	X	X	X	X	X
l37	X	X	X	X	X	X
m37	X	X	X	X	X	X
a38	60mg, 57%	0.40 b	264M$^+$, 208[M-(CH$_2$)$_3$CH$_3$]$^+$, 190[M-O(CH$_2$)$_3$CH$_3$]$^+$		X	X
b38	61mg, 58%	0.35 b	264M$^+$, 208[M-C(CH$_3$)$_3$]$^+$, 190[M-OC(CH$_3$)$_3$]$^+$		X	X
c38	64mg, 65%	0.38 b	248M$^+$, 208[M-CH$_2$CH=CH$_2$]$^+$, 190[M-OCH$_2$CH=CH$_2$]$^+$		X	X
d38	72mg, 68%	0.34 b	266M$^+$, 208[M-CH$_2$CH$_2$OCH$_3$]$^+$, 190[M-OCH$_2$CH$_2$OCH$_3$]$^+$		X	X
e38	84mg, 84%	0.54 b	250M$^+$, 208[M-CH(CH$_3$)$_2$]$^+$, 190[M-OCH(CH$_3$)$_2$]$^+$		>100	X
f38	83mg, 70%	0.44 b	298M$^+$, 208[M-CH$_2$C$_6$H$_5$]$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
g38	X	X	X	X	X	X
h38	82mg, 64%	0.54 b	320M$^+$, 208[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$, 190[M-OCH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		>100	X
i38	51mg, 58%	0.35 b	222M$^+$, 208[M-CH$_3$]$^+$		X	X
j38	59mg, 53%	0.37 b	236M$^+$, 208-210[M-CH$_2$CH$_3$]$^+$		X	X
k38	93mg, 72%	0.47 b	326M$^+$, 119[(CH$_2$)$_3$C$_6$H$_5$]$^+$		X	X
l38	69mg, 54%	0.39 b	318M$^+$, 208[M-CH$_2$CH$_2$C$_4$H$_3$S]$^+$, 111[CH$_2$CH$_2$C$_4$H$_3$S]$^+$		X	X
m38	71mg, 56%	0.59 b	320M$^+$, 208[M-(CH$_2$)$_7$CH$_3$]$^+$		X	X
a39	74mg, 70%	0.34 b	264M$^+$, 208[M-(CH$_2$)$_3$CH$_3$]$^+$, 190[M-O(CH$_2$)$_3$CH$_3$]$^+$		X	X
b39	59mg, 56%	0.36 b	264M$^+$, 208[M-C(CH$_3$)$_3$]$^+$		X	X
c39	65mg,	0.34	248M$^+$, 208[M-		X	X

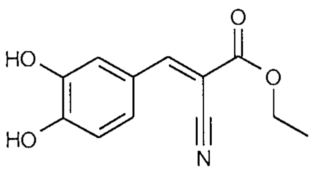
	66%	b	CH$_2$CH=CH$_2$]$^+$, 190[M-OCH$_2$CH=CH$_2$]$^+$			
d39	68mg, 64%	0.31 b	266M$^+$, 208[M-CH(CH$_3$)$_2$]$^+$, 190[M-OCH(CH$_3$)$_2$]$^+$		>100	X
e39	97mg, 97%	0.54 b	208[M-CH(CH$_3$)$_2$]$^+$		>100	X
f39	91mg, 77%	0.49 b	298M$^+$, 208[M-CH$_2$C$_6$H$_5$]$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	>100
g39	X	X	X	X	X	X
h39	57mg, 45%	0.58 b	320M$^+$, 208[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$, 190[M-OCH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		>100	X
i39	78mg, 88%	0.40 b	222-223M$^+$, 208[M-CH$_3$]$^+$		X	X
j39	50mg, 53%	0.43 b	236M$^+$, 208[M-CH$_2$CH$_3$]$^+$		X	X
k39	93mg, 72%	0.58 b	326M$^+$, 119[(CH$_2$)$_3$C$_6$H$_5$]$^+$		X	X
l39	72mg, 57%	0.46 b	318M$^+$, 111[CH$_2$CH$_2$C$_4$H$_3$S]$^+$		X	X
m39	76mg, 60%	0.61 b	321M$^+$, 208[M-(CH$_2$)$_7$CH$_3$]$^+$		X	X
a40	50mg, 57%	0.24 b	219M$^+$, 163[M-(CH$_2$)$_3$CH$_3$]$^+$, 146[M-O(CH$_2$)$_3$CH$_3$]$^+$		X	X
b40	60mg, 69%	0.31 b	219M$^+$, 163[M-C(CH$_3$)$_3$]$^+$, 145[M-OC(CH$_3$)$_3$]$^+$		X	X
c40	65mg, 80%	0.23 b	203M$^+$, 163[M-CH$_2$CH=CH$_2$]$^+$, 145[M-OCH$_2$CH=CH$_2$]$^+$		X	X
d40	54mg, 61%	0.23 b	221M$^+$, 163[M-CH$_2$CH$_2$OCH$_3$]$^+$, 146[M-OCH$_2$CH$_2$OCH$_3$]$^+$		X	X
e40	47%, 58%	0.16 b	205M$^+$, 163[M-CH(CH$_3$)$_2$]$^+$, 146[M-OCH(CH$_3$)$_2$]$^+$		>100	X
f40	83mg, 82%	0.21 b	253M$^+$, 163[M-CH$_2$C$_6$H$_5$]$^+$, 145[M-OCH$_2$C$_6$H$_5$]$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
g40	51mg, 79%	0.01 b	NO TIC	6.40 (s, trying to split to a d, 1H, ArH), 7.28-7.29 (ov. m, 2H, 2xArH), 7.47 (br s, 2H, NH$_2$), 8.01 (s, 1H, CH=), 11.82 (br s, 1H, NH)	X	X
h40	61mg, 56%	0.23 b	275M$^+$, 163[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$, 145[M-OCH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		>100	X
i40	60mg, 85%	0.20 b	177M$^+$, 163[M-CH$_3$]$^+$, 145[M-OCH$_3$]$^+$		>100	X
j40	29mg, 38%	0.56 a	191M$^+$, 163[M-CH$_2$CH$_3$]$^+$, 146[M-OCH$_2$CH$_3$]$^+$		>100	X
k40	76mg, 68%	0.32 b	281M$^+$, 163[M-(CH$_2$)$_3$C$_6$H$_5$]$^+$, 145[M-O(CH$_2$)$_3$C$_6$H$_5$]$^+$, 119[(CH$_2$)$_3$C$_6$H$_5$]$^+$	1.91 (quintet, 2H, J=6.7Hz, COOCH$_2$C$\underline{H}_2$), 2.65 (t, 2H, J=7.7Hz, C$\underline{H}_2$C$_6$H$_5$), 4.11 (t, 2H, J=6.5Hz, COOCH$_2$), 6.26-6.29 (m, 1H, ArH), 6.98-7.01 (m, 1H, ArH), 7.16-7.32 (ov. m, 6H, 6xArH), 8.13 (s, 1H, CH=), 12.11 (br s, 1H, NH)	X	X
l40	57mg, 52%	0.14 b	163[M-CH$_2$CH$_2$C$_4$H$_3$S]$^+$, 145[M-CH$_2$CH$_2$C$_4$H$_3$S]$^+$, 111[CH$_2$CH$_2$C$_4$H$_3$S]$^+$		>100	X
m40	65mg, 59%	0.34 b	275M$^+$, 163[M-(CH$_2$)$_7$CH$_3$]$^+$, 145[M-O(CH$_2$)$_7$CH$_3$]$^+$		>100	X
a41	97mg, 92%	0.48 b	264M$^+$, 208[M-(CH$_2$)$_3$CH$_3$]$^+$, 190[M-O(CH$_2$)$_3$CH$_3$]$^+$		X	X
b41	73mg, 69%	0.51 b	264M$^+$, 208[M-C(CH$_3$)$_3$]$^+$, 190[M-OC(CH$_3$)$_3$]$^+$	1.53 (s, 9H, C(C$\underline{H}_3$)$_3$), 7.53-7.71 (ov. m, 3H, ArH-4/5/6), 8.09 (dd, 1H, J=6.1, 1.4 Hz, ArH-3), 8.47 (s,1H, CH=)	X	X
c41	88mg, 89%	0.43 b	248M$^+$, 208[M-CH$_2$CH=CH$_2$]$^+$, 190[M-OCH$_2$CH=CH$_2$]$^+$		X	X
d41	92mg, 87%	0.41 b	266M+, 208[M-CH$_2$CH$_2$OCH$_3$]+, 190[M-OCH$_2$CH$_2$OCH$_3$]+		X	X
e41	56mg, 56%	0.61 b	250M$^+$, 208[M-CH(CH$_3$)$_2$]$^+$, 141[M-OCH(CH$_3$)$_2$]$^+$		X	X
f41	98mg, 82%	0.46 b	299M$^+$, 208[M-CH$_2$C$_6$H$_5$]$^+$, 91[CH$_2$C$_6$H$_5$]$^+$		X	X
g41	63mg,	0.02	207M$^+$, 190[M-NH$_2$]$^+$	7.51-7.62 (ov. m, 2H, ArH-5/6), 7.65 (ddd,	X	X

	76%	b		1H, *J*=8.3, 7.3, 1.7Hz, ArH-4), 7.97 (br d, 2H, NH_2), 7.99 (dd, 1H, *J*=7.1, 2.2Hz, ArH-3), 8.36 (s, 1H, CH=)		
h41	88mg, 69%	0.64 b	$320M^+$, $208[M-CH_2CH(C_2H_5)C_4H_9]^+$, $190[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i41	54mg, 61%	0.52 b	$222M^+$, $208[M-CH_3]^+$, $190[M-OCH_3]^+$		X	X
j41	60mg, 64%	0.53 b	$237M^+$, $208[M-CH_2CH_3]^+$		>100	X
k41	94mg, 72%	0.39 b	$326M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l41	76mg, 60%	0.19 b	$318M^+$, $208[M-CH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m41	77mg, 60%	0.48 b	$321M^+$, $208[M-(CH_2)_7CH_3]^+$, $190[M-O(CH_2)_7CH_3]^+$		X	X
a42	74mg, 68%	0.45 b	$272[M]^+$		X	X
b42	68mg, 63%	0.50 b	$272M^+$, $216[M-C(CH_3)_3]^+$, $198[M-OC(CH_3)_3]^+$		X	X
c42	71mg, 70%	0.45 b	$256M^+$, $216[M-CH_2CH=CH_2]^+$, $198[M-OCH_2CH=CH_2]^+$		X	X
d42	69mg, 63%	0.40 b	$274M^+$, $216[M-CH_2CH_2OCH_3]^+$, $198[M-OCH_2CH_2OCH_3]^+$		X	X
e42	88mg, 86%	0.57 b	$258M^+$, $216[M-CH(CH_3)_2]^+$, $198[M-OCH(CH_3)_2]^+$		X	X
f42	72mg, 59%	0.48 b	$306M^+$, $216[M-CH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		X	X
g42	61mg, 71%	0.02 b	$215M^+$		X	X
h42	56mg, 43%	0.60 b	$328M^+$, $216[M-CH_2CH(C_2H_5)C_4H_9]^+$, $198[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i42	67mg, 73%	0.44 b	$230M^+$, $216[M-CH_3]^+$, $198[M-OCH_3]^+$		X	X
j42	63mg, 65%	0.45 b	$244M^+$, $216[M-CH_2CH_3]^+$, $198[M-OCH_2CH_3]^+$		X	X
k42	84mg, 63%	0.49 b	$334M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l42	72mg, 55%	0.44 b	$326M^+$, $216[M-CH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m42	75mg, 57%	0.48 b	$328M^+$, $216[M-(CH_2)_7CH_3]^+$, $198[M-O(CH_2)_7CH_3]^+$		X	X
a43	92mg, 80%	0.57 c	$287M^+$, $245[M-(CH_2)_3CH_3+32]^+$, $231[M-(CH_2)_3CH_3]^+$, $213[M-O(CH_2)_3CH_3]^+$	0.92 (t, 3H, *J*=7.3Hz, $CH_2CH_2CH_2C\underline{H}_3$) 1.36 (ov. m, 4H, $CH_2C\underline{H}_2C\underline{H}_2CH_3$), 2.10 (s, 3H, $C\underline{H}_3CO$), 4.25 (t, 2H, *J*=7.0Hz, $COOC\underline{H}_2$) 7.77 (d, 2H, *J*=8.8Hz, 2xArH), 8.04 (d, 2H, *J*=8.8Hz, 2xArH), 8.26 (s, 1H, CH=), 10.45 (s, 1H, N$\underline{H}$)	>100	X
b43	79mg, 73%	0.56 c	$287M^+$, $231[M-C(CH_3)_3]^+$, $213[M-OC(CH_3)_3]^+$		X	X
c43	102mg, 94%	0.56 c	$271M^+$, $245[M-OCH_2CH=CH_2+32]^+$, $231[M-CH_2CH=CH_2]^+$, $213[M-OCH_2CH=CH_2]^+$	2.10 (s, 3H, $COCH_3$), 4.79 (d, 2H, *J*=5.3Hz, $COOCH_2$), 5.30 (dd, 1H, *J*=10.5, 1.5Hz, $CH=C\underline{H}$), 5.42 (dd, 1H, *J*=17.3, 1.5Hz, $CH=C\underline{H}$), 5.96-6.10 (m, 1H, $CH_2C\underline{H}=CH_2$), 7.78 (d, 2H, *J*=8.6Hz, 2xArH), 8.05 (d, 2H, *J*=8.6Hz, 2xArH), 8.29 (s, 1H, CH=), 10.43 (br s, 1H, NH)	>100	X
d43	105mg, 91%	0.02 b	$289M^+$, $231[M-CH_2CH_2OCH_3]^+$, $213[M-OCH_2CH_2OCH_3]^+$		X	X
e43	96mg, 88%	0.01 b	$273M^+$, $231[M-C(CH_3)_2]^+$, $213[M-OC(CH_3)_2]^+$	1.29 (s, 3H, $CH(C\underline{H}_3)CH_3$), 1.32 (s, 3H, $CH(CH_3)C\underline{H}_3$), 2.10 (s, 3H, $COCH_3$), 5.09 (septet, 1H, *J*=6.2Hz, $C\underline{H}(CH_3)_2$), 7.77 (d, 2H, *J*=8.8Hz, 2xArH), 8.03 (d, 2H, *J*=8.8Hz, 2xArH), 8.25 (s, 1H, CH=), 10.42 (br s, 1H, NH)	>100	X
f43	X	X	X	X	X	X
g43	X	X	X	X	X	X
h43	81mg, 59%	0.03 b	$343M^+$, $231[M-CH_2CH(C_2H_5)C_4H_9]^+$, 213[M-		>100	X

			$OCH_2CH(C_2H_5)C_4H_9]^+$			
i43	68mg, 70%	0.53 c	$245M^+$, $231[M-CH_3]^+$, $213[M-OCH_3]^+$		X	X
j43	72mg, 64%	0.53 c	$259M^+$, $231[M-CH_2CH_3]^+$, $213[M-OCH_2CH_3]^+$		X	X
k43	86mg, 58%	0.56 c	$349M^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l43	X	X	X	X	X	X
m43	82mg, 56%	0.56 c	$343M^+$, $231[M-(CH_2)_7CH_3]^+$, $213[M-O(CH_2)_7CH_3]^+$		X	X
a44	X	X	X	X	X	X
b44	66mg, 54%	0.52 c	$307\text{-}309M^+$, $251\text{-}253[M-C(CH_3)_3]^+$, $234\text{-}236[M-OC(CH_3)_3]^+$		X	X
c44	X	X	X	X	X	X
d44	X	X	X	X	X	X
e44	37mg, 31%	0.51 b	$293\text{-}295M^+$, $251\text{-}253[M-CH(CH_3)_2]^+$, $234\text{-}236[M-OCH(CH_3)_2]^+$		X	X
f44	X	X	X	X	X	X
g44	X	X	X	X	X	X
h44	99mg, 68%	0.60 b	$280\text{-}282M^+$ethyl ester, $252\text{-}254[M-CH_2CH_3]^+$, $234\text{-}236[M-OCH_2CH_3]^+$		X	X
i44	X	X	X	X	X	X
j44	X	X	X	X	X	X
k44	X	X	X	X	X	X
l44	X	X	X	X	X	X
m44	X	X	X	X	X	X
a45	72mg, 64%	0.48 b	$280M^+$, $224[M-(CH_2)_3CH_3]^+$, $206[M-O(CH_2)_3CH_3]^+$		X	X
b45	95mg, 85%	0.49 b	$280M^+$, $224[M-C(CH_3)_3]^+$, $206[M-OC(CH_3)_3]^+$		>100	>100
c45	X	X	X		X	X
d45	X	X	X		X	X
e45	86mg, 81%	0.46 b	$266M^+$, $224[M-CH(CH_3)_2]^+$, $206[M-OCH(CH_3)_2]^+$		X	X
f45	105mg, 84%	0.54 b	$314M^+$, $224[M-OCH_2C_6H_5]^+$, $91[CH_2C_6H_5]^+$		>100	X
g45	X	X	X	X	X	X
h45	82mg, 61%	0.58 b	$336M^+$, $238[M-OCH_2CH(C_2H_5)C_4H_9+32]^+$, $224[M-CH_2CH(C_2H_5)C_4H_9]^+$, $206[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i45	60mg, 63%	0.35 b	$238M^+$, $224[M-CH_3]^+$, $206[M-OCH_3]^+$		X	X
j45	65mg, 65%	0.38 b	$252M^+$, $224[M-CH_2CH_3]^+$, $206[M-OCH_2CH_3]^+$		X	X
k45	102mg, 75%	0.53 b	$342M^+$, $206[M-O(CH_2)_3C_6H_5]^+$		X	X
l45	69mg, 52%	0.37 b	$334M^+$, $206[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m45	69mg, 51%	0.55 b	$336M^+$, $224[M-(CH_2)_7CH_3]^+$, $206[M-O(CH_2)_7CH_3]^+$		>100	X
a46	X	X	X		>100	>100
b46	96mg, 81%	0.53 b	$299M^+$, $224[M-C(CH_3)_3]^+$		>100	>100
c46	X	X	X	X	>100	>100
d46	X	X	X	X	>100	>100
e46	X	X	X	X	>100	>100
f46	107mg, 81%	0.48 b	$332M^+$, $271M^+$ (ethyl ester), $91[CH_2C_6H_5]^+$		>100	>100
g46	X	X	X		25-50	25-50
h46	90mg, 64%	0.51 b	$354M^+$, $242[M-CH_2CH(C_2H_5)C_4H_9]^+$, $224[M-OCH_2CH(C_2H_5)C_4H_9]^+$, $113[CH_2CH(C_2H_5)C_4H_9]^+$	0.86-0.89 (ov. m, 6H, $2xCH_3$), 1.30-1.34 (ov. m, 8H, $4xCH_2$), 1.65-1.67 (m, 1H, CH), 4.25 (d, 2H, J=7.0Hz, $COOCH_2$), 7.53-7.69 (ov. m, 3H, 3xArH), 8.49 (s, 1H, CH=) plus ethyl ester	>100	X
i46	67mg, 63%		X (ethyl ester)		>100	>100
j46	72mg, 67%	0.49 b	$302\text{-}304[M+32]^+$, $270\text{-}272M^+$, $242[M-CH_2CH_3]^+$, $224[M-$	1.32 (t, 3H, J=7.1Hz, CH_3), 4.37 (q, 2H, J=7.1Hz, CH_2), 7.53-7.69 (ov. m, 3H,	>100	>100

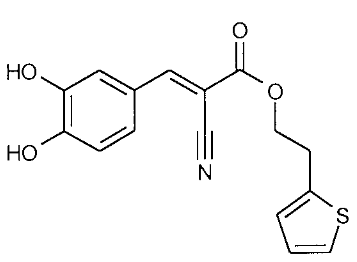
			$OC_2H_5]^+$,	3xArH), 8.50 (s, 1H, CH=)		
k46	X	X	X (ethyl ester)		>100	>100
l46	72mg, 51%	0.37 b	352-354M^+, 270-272M^+ (ethyl ester), $242[M-CH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		>100	>100
m46	75mg, 53%	0.55 b	$354M^+$, $242[M-(CH_2)_7CH_3]^+$		>100	>100
a47	X	X	343, 258, 219, 204, $173[M-OCH_2CH_2CH_2CH_3]^+$		25-50	>100
b47	X	X	$173[M-C(CH_3)_3]^+$, 147		>100	X
c47	X	X	343, 258, 219, $173[M-OCH_2CH=CH_2]^+$		25-50	>100
d47	X	X	343, 258, 239, 219, 204, $173[M-CH_2CH_2OCH_3]^+$		25-50	>100
e47	X	X	343, 258, 219, $173[M-OCH(CH_3)_2]^+$		25-50	>100
f47	X	X	343, 258, 219, $91[CH_2C_6H_5]^+$		>100	X
g47	X	X	X		X	X
h47	X	X	358, 239, 213, $173[M-OCH_2CH(C_2H_5)C_4H_9]^+$, $172M^+$ (coumarin)		25-50	X
i47	X	X	343, 258, 239, 219, $173[M-OCH_3]^+$		25-50	X
j47	X	X	258, 239, 219, 204, $173[M-OCH_2CH_3]^+$		>100	X
k47	X	X	X	X	X	X
l47	X	X	X	X	X	X
m47	X	X	X	X	X	X
a48	59mg, 57%	0.38 b	$260M^+$, $204[M-(CH_2)_3CH_3]^+$, $186[M-O(CH_2)_3CH_3]^+$		X	X
b48	69mg, 67%	0.37 b	$260M^+$, $204[M-C(CH_3)_3]^+$, $186[M-OC(CH_3)_3]^+$		X	X
c48	71mg, 73%	0.37 b	$244M^+$, $204[M-CH_2CH=CH_2]^+$, $186[M-OCH_2CH=CH_2]^+$		X	X
d48	67mg, 64%	0.35 b	$262M^+$, $204[M-CH_2CH_2OCH_3]^+$, $186[M-OCH_2CH_2OCH_3]^+$		X	X
e48	76mg, 78%	0.38 b	$246M^+$, $204[M-CH(CH_3)_2]^+$, $186[M-OCH(CH_3)_2]^+$		X	X
f48	88mg, 75%	0.40 b	$294M^+$, $204[M-CH_2C_6H_5]^+$, $186[M-OCH_2C_6H_5]^+$		X	X
g48	61mg, 75%	0.01 b	$202M^+$,		X	X
h48	96mg, 76%	0.41 b	$316M^+$, $204[M-CH_2CH(C_2H_5)C_4H_9]^+$, $186[M-OCH_2CH(C_2H_5)C_4H_9]^+$		X	X
i48	56mg, 65%	0.37 b	$218M^+$, $204[M-CH_3]^+$, $186[M-OCH_3]^+$		X	X
j48	67mg, 73%	0.37 b	$232M^+$, $204[M-CH_2CH_3]^+$, $186[M-OCH_2CH_3]^+$		X	X
k48	101mg , 79%	0.40 b	$322M^+$, $204[M-(CH_2)_3C_6H_5]^+$, $186[M-O(CH_2)_3C_6H_5]^+$, $119[(CH_2)_3C_6H_5]^+$		X	X
l48	89mg, 71%	0.38 b	$346[M+32]^+$, $204[M-CH_2CH_2C_4H_3S]^+$, $186[M-OCH_2CH_2C_4H_3S]^+$, $111[CH_2CH_2C_4H_3S]^+$		X	X
m48	90mg, 71%	0.39 b	$316M^+$, $204[M-(CH_2)_7CH_3]^+$, $186[M-O(CH_2)_7CH_3]^+$		X	X

Preparation of ethyl (*E*)-2-cyano-3-(3,4-dihydroxyphenyl)prop-2-enoate j15



Piperidine (0.2mmol, 17mg) was added to a solution of 3,4-dihydroxybenzaldehyde (2.0mmol, 276mg) and ethyl-2-cyanoacetate (2.0mmol, 226mg) in ethanol (10ml). The reaction was heated under reflux for 2 hours. The reaction mixture was concentrated under vacuum. The resultant residue was subjected to flash column chromatography, eluted with petrol 40:60-diethyl ether (1:1). The title compound was collected as a yellow solid (316mg, 68%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.20; ^{1}H NMR (399.95MHz, $(CD_3)_2SO$): δ_H 1.29 (t, 3H, *J*=7.1Hz, CH_3), 4.28 (q, 2H, *J*=7.1Hz, CH_2), 6.90 (d, 1H, *J*=8.3Hz, ArH-5), 7.40 (dd, 1H, *J*=8.4, 2.3Hz, ArH-6), 7.65 (d, 1H, *J*=2.2Hz, ArH-2), 8.07 (s, 1H, CH=), 9.84 (br s, 1H, OH), 10.30 (br s, 1H, OH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 14.28 (CH_3), 62.11 (CH_2), 96.69 (q), 116.20 (q), 116.60 (CH), 116.61 (CH), 123.13 (q), 127.26 (CH), 146.06 (q), 152.35 (q), 155.18 (CH), 162.96 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 234M$^+$, 206[M-CH_2CH_3]$^+$; IR (KBr disc): υ_{max} 3367 (OH), 2985, 2921 (CH_2/CH_3), 2223 (CN), 1697 (COO), 1583, 1533 (ArH/CH_2), 1467, 1448 (CH_2/CH_3), 1375 (CH_3), 1344, 1300, 1271, 1213, 1122, 1099 (OH/CO), 1002 (CO), 867 (2 adjacent ArH), 804 (isolated ArH) cm^{-1}. Calculated for $C_{12}H_{11}NO_4$: C, 61.80%; H, 4.75%; N, 6.01%. Found: C, 61.61%; H, 4.63%; N, 5.63%.

Preparation of 2-(2-thienyl)ethyl-(*E*)-2-cyano-3-(3,4-dihydroxyphenyl)prop-2-enoate I15



Piperidine (0.14mmol, 12mg) was added to a solution of 3,4-dihydroxybenzaldehyde (1.43mmol, 197mg) and 2-(2-thienyl)ethyl 2-cyanoacetate (1.43mmol, 279mg) in ethanol (10ml). The reaction was heated under reflux for 2 hours. The reaction mixture was concentrated under vacuum. The resultant residue was subjected to flash column chromatography, eluted with petrol 40:60-diethyl ether (1:1). The title compound was collected as a yellow solid (362mg, 80%). TLC [petrol 40-60-diethyl ether (1:1)] R_f 0.18; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 3.23 (t, 2H, *J*=6.4Hz, $OCH_2C\underline{H}_2$), 4.42 (t, 2H, *J*=6.4Hz, OCH_2), 6.90 (d, 1H, *J*=8.3Hz, ArH-5), 6.97-6.99 (ov. m, 2H, 2xAr'H), 7.36-7.41 (ov. m, 2H, ArH-6, Ar'H), 7.64 (d, 1H, *J*=2.1Hz, ArH-2), 8.12 (s, 1H, CH=), 10.15 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 28.69 (CH_2), 66.21 (CH_2), 96.45 (q), 115.76 (q), 116.23 (CH), 116.48 (CH), 116.66 (q), 123.09 (CH), 124.83 (CH), 127.22 (CH), 139.77 (q), 146.07 (q), 152.43 (q), 155.38 (CH), 162.86 (COO) ppm; Mass spectrum (APCI$^+$): *m/z* 316M$^+$, 206[M-$CH_2CH_2C_4H_3S$]$^+$, 188[M-$OCH_2CH_2C_4H_3S$]$^+$; IR (KBr disc): υ_{max} 3178 (OH), 2921, 2854 (CH_2), 2227 (CN), 1724 (COO), 1585, 1513 (CH/ArH), 1380 (OH), 1249 (CO), 1171, 1101 (CO/CS), 812 (2 adjacent ArH) cm^{-1}. Calculated for $C_{16}H_{13}NO_4S$: C, 60.94%; H, 4.16%; N, 4.44%. Found: C, 61.58%; H, 4.60%; N, 5.84%.

8.5 Focussed compound libraries

Preparation of the 46 series focussed library

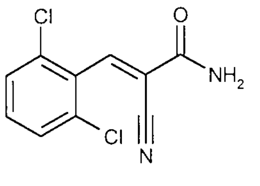
Automation was used to dispense 1.5ml of a solution of 2,6-dichlorobenzaldehyde in toluene (0.66mmol/1.5ml, 116mg/1.5ml) into separate vials. To each vial was added 1.5ml of a single active methylene reagent solution in toluene (0.6mmol/1.5ml) containing piperidine (0.06mmol/1.5ml). The reaction vials were heated at 75°C for 48 hours. The remaining residues were subjected to an 3x1.5ml automated diethyl ether washes. The resulting solids were then dried under vacuum.

Compound structures (**a-s**)**46** can be found in **Tables 3.3** and **3.8**, pages 46 and 54. Inactivity against MAC16 and MAC13 is denoted by X. Where compounds could not be identified as the desired products, the recovery, TLC, mass spectra and ^{1}H NMR spectra have been recorded with X. Where ^{1}H NMR spectra were not obtained no data have been entered into the table.

*a, b and c represent different solvent systems; a (diethyl ether), b (petrol 40:60-diethyl ether (1:1)) and c (ethyl acetate).

Compound	Recovery	TLC*	Mass spectrum APCI$^+$	^{1}H NMR (250.1MHz, $(CD_3)_2SO$) δ_H ppm	MAC16 IC$_{50}$ (μM)	MAC13 IC$_{50}$ (μM)
a46	88mg, 49%	0.55 b	298-300M$^+$, 242$[M-(CH_2)_3CH_3]^+$, 224$[M-O(CH_2)_3CH_3]^+$	0.94 (t, 3H, *J*=7.3Hz, CH_3), 1.43 (sextet, 2H, *J*=7.4Hz, $COOCH_2CH_2C\underline{H}_2CH_3$), 1.70 (quintet, 2H, *J*=7.0Hz, $COOCH_2C\underline{H}_2CH_2CH_3$), 4.34 (t, 2H, *J*=6.5Hz, $COOC\underline{H}_2CH_2$), 7.54-7.70 (ov. m, 3H, 3xArH), 8.50 (s, 1H, CH=)	>100	>100
b46	21mg, 12%	0.55 b	299M$^+$, 242-244$[M-C(CH_3)_3]^+$	1.55 (s, 9H, $C(CH_3)_3$), 7.52-7.68 (ov. m, 3H, 3xArH), 8.40 (s, 1H, CH=)	>100	>100
c46	126mg, 75%	0.52 b	282M$^+$, 256$[M-CH_2CH{=}CH_2]^+$, 224$[M-OCH_2CH{=}CH_2]^+$	4.86 (d, 1H, *J*=5.5Hz, $C\underline{H}_2CH{=}CH_2$), 5.33 (dd, 1H, *J*=10.4, 1.0Hz, $CH_2CH{=}C\underline{H}$), 7.44 (dd, 1H, *J*=17.2, 1.0Hz, $CH_2CH{=}C\underline{H}$), 5.98-6.09 (m, 1H, $CH_2C\underline{H}{=}CH_2$), 7.53-7.68 (ov. m, 3H, 3xArH), 8.53 (s, 1H, CH=)	>100	>100
d46	156mg, 87%	0.49 b	256$[M-OCH_2CH_2OCH_3+32]^+$, 224$[M-OCH_2CH_2OCH_3]^+$,	3.32 (s, 3H, OCH_3), 3.63 (m, 2H, $COOCH_2C\underline{H}_2OCH_3$), 4.43-4.50 (m, 2H, $COOC\underline{H}_2$), 7.53-7.68 (ov. m, 3H, 3xArH), 8.50 (s, 1H, CH=)	>100	>100
e46	121mg, 71%	0.49 b	242$[M-CH(CH_3)_2]^+$, 22$[M-OCH(CH_3)_2]^+$	1.57-1.66 (ov. m, 6H, $CH(C\underline{H}_3)_2$), 3.02 (septet, 1H, *J*=5.1Hz, $C\underline{H}(CH_3)_2$), 7.40-7.59 (ov. m, 3H, ArH-2/3/4), 7.73 (s, 1H, CH=)	>100	>100
f46	119mg, 60%	0.48 b	224$[M-OCH_2C_6H_5+32]^+$, 224$[M-OCH_2C_6H_5]^+$	5.39 (s, 2H, $COOC\underline{H}_2C_6H_5$), 7.38-7.53 (ov. m, 5H, 5xAr'H), 7.55-7.68 (ov. m, 3H, 3xArH), 8.54 (s, 1H, CH=)	>100	>100
g46	114mg, 79%	0.16 b	241M$^+$	7.50-7.69 (ov. m, 3H, ArH-3/4/5), 8.03 (br s, 1H, NH), 8.21 (s, 2H, NH and CH=)	45.9 ±2.2	39.2 ±4.0
h46	145mg, 68%	0.57 b	354M$^+$, 242$[M-CH_2CH(C_2H_5)C_4H_9]^+$, 224 $[M-OCH_2CH(C_2H_5)C_4H_9]^+$	0.87-0.92 (ov. m, 6H, $2xCH_3$), 1.30-1.43 (ov. m, 8H, $4xCH_2$), 1.65-1.70 (m, 1H, CH), 4.25 (d, 2H, *J*=5.7Hz, $COOCH_2$), 7.53-7.70 (ov. m, 3H, 3xArH), 8.49 (s, 1H, CH=)	>100	>100
i46	69mg, 68%	0.47 b	256 M$^+$, 224$[M-OCH_3]^+$	3.91 (s, 3H, OCH_3), 7.68-7.55 (ov. m, 3H, ArH-3/4/5), 8.48 (s, 1H, CH=C)		
i46	128mg, 79%	0.47 b	258-256M$^+$, 224$[M-OCH_3]^+$	3.90 (s, 3H, OCH_3), 7.53-7.69 (ov. m, 3H, 3xArH), 8.51 (s, 1H, CH=)	>100	>100
j46	126mg, 66%	0.49 b	302[M+32]$^+$, 242$[M-CH_2CH_3]^+$, 224$[M-OCH_2CH_3]^+$	1.32 (t, 3H, *J*=7.1Hz, CH_3), 4.37 (q, 2H, *J*=7.1Hz, $COOC\underline{H}_2$), 7.53-7.69 (ov. m, 3H, 3xArH), 8.49 (s, 1H, CH=)	>100	>100
n46	70mg, 40%	0.46 b	290-292M$^+$	7.47 (ov. m, 4H, ArH, CH=), 14.00 (br s, 1H, NH)	77.5 ±1.3	X
o46	x	X	305-307M$^+$, 493-495	X	>100	90.5 ±3.7
p46	142mg, 92%	0.21 b	257-258M$^+$	7.50-7.67 (ov. m, 3H, 3xArH), 8.08 (s, 1H, CH=), 9.86 (br s, 1H, NH), 10.37 (br s, 1H, NH)	>100	>100
q46	26mg, 19%	0.47 b	255[M+32]$^+$		>100	>100
r46	X	X	257	X		
s46	X	X	X	X		

Preparation of 2-cyano-3-(2,6-dichlorophenyl)pro-2-enamide g46



Piperidine (1.5mmol, 128mg) was added to a solution of 2,6-dichlorobenzaldehyde (15mmol, 1.26g) and cyanoacetamide (15mmol, 2.625g) in ethanol (50ml). The reaction was heated under reflux for 4 hours. The title compound formed as a white solid on cooling and was removed by filtration (2.486g, 69%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.17; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 7.50-7.69 (ov. m, 3H, ArH-3/4/5), 8.03 (br s, 1H, NH), 8.21 (s, 2H, NH and CH=) ppm; Mass spectrum (APCI$^+$): *m/z* 241-243M$^+$.

Preparation of *N*-[(*E*)-2-cyano-3-(2,6-dichlorophenyl)prop-2-enoyl]-*N'*-cyclohexylurea 31

Cyclohexylisocyanate (0.55mmol, 69mg) was added to a solution of 2-cyano-3-(2,6-dichlorophenyl)pro-2-enamide, **g46** (0.5mmol, 121mg) in anhydrous toluene (10ml) under an atmosphere of argon. After 18 hours the mixture was concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 40:60 (1:1). The title compound was collected as a white solid (60mg, 33%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.21; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.26-1.65 (ov. m, 8H, 4xCH_2), 1.81-1.85 (ov. m, 2H, 2xCH), 3.36-3.41 (m, 1H, CH), 7.53-7.69 (ov. m, 3H, 3xArH), 8.14 (br d, 1H, CONHCON$\underline{H}$), 8.47 (s, 1H, CH=), 11.10 (br s, 1H, NH) ppm; Mass spectrum (EI): *m/z* 365M, 322, 284$[M-C_6H_{10}]^+$, 241$[M-CONHC_6H_{10}]^+$, 231, 207, 205, 161, 124, 98, 82, 56.

Preparation of *N*-[(*E*)-2-cyano-3-(2,6-dichlorophenyl)prop-2-enoyl]-*N'*-phenylurea 32

Phenylisocyanate (0.5mmol, 60mg) was added to a solution of 2-cyano-3-(2,6-dichlorophenyl)pro-2-enamide, **g46** (0.5mmol, 121mg) in anhydrous toluene (10ml) under an atmosphere of argon. After 18 hours the mixture was concentrated under vacuum. The title compound was crystallised from ethyl acetate and collected as fine white crystals (52mg, 29%). TLC [diethyl ether-petrol 40-60 (1:1)] R_f 0.16; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$) δ_H 7.10-7.16 (t, trying to split further, 1H, *J*=7.4Hz, Ar'H-4), 7.33-7.40 (m, t trying to split further , 2H, *J*=7.6Hz, Ar'H-3/5), 7.54-7.70 (ov. m, 5H, 3xArH, Ar'H-2/6), 8.52 (s, 1H, CH=), 10.17 (s, 1H, NH), 11.31 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$) *m/z* 360M$^+$, 137$[M-C_6H_3(Cl)_2CH=C(CN)CO]^+$.

Preparation of *N*-(3-chlorophenyl)-*N'*-[(*E*)-2-cyano-3-(2,6-dichlorophenyl)prop-2-enoyl]urea 33

3-chlorophenylisocyanate (2.2mmol, 337mg, 267μl) was added to a solution of 2-cyano-3-(2,6-dichlorophenyl)pro-2-enamide, **g46** (2.0mmol, 482mg) in anhydrous toluene (50ml) under an atmosphere of argon. After 18 hours the mixture was concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 40:60 (1:1). The title compound was collected as a yellow solid (336mg, 43%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.38; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 7.18 (dd, 1H, *J*=7.4, 1.5Hz, ArH), 7.35-7.79 (ov. m, 5H, 5xArH), 8.51 (s, 1H, CH=), 10.29 (br s, 1H, NH), 11.40 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 394-396M$^+$, 241-243$[M-CONHC_6H_4Cl]^+$, 171$[M-C_6H_3(Cl)_2CH=C(CN)CO]^+$.

Attempted preparation of *N*-(*t*-butyl)-*N'*-[(*E*)-2-cyano-3-(2,6-dichlorophenyl)prop-2-enoyl]urea 34

Method A: *t*-Butyl isocyanate (2.2mmol, 218mg, 251μl) was added to a solution of 2-cyano-3-(2,6-dichlorophenyl)pro-2-enamide, **g46** (2.0mmol, 482mg) in anhydrous toluene (50ml) under an atmosphere of argon. After 18 hours the mixture was concentrated under vacuum. The remaining solid was analysed and found to be the starting amide **g46**.

Method B: *t*-Butyl isocyanate (2.2mmol, 218mg, 251μl) was added to a solution of (*E*)-2-cyano-3-(2,6-dichlorophenyl)pro-2-enamide, **g46** (2.0mmol, 482mg) in anhydrous toluene (50ml) under an atmosphere of argon. The mixture was heated under reflux. After 18 hours the mixture was concentrated under vacuum. The remaining solid was analysed and found to be the starting amide **g46**.

Preparation of *N*-butyl-*N'*-[(*E*)-2-cyano-3-(2,6-dichlorophenyl)prop-2-enoyl]urea 35

n-Butylisocyanate (2.2mmol, 218mg, 248μl) was added to a solution of (*E*)-2-cyano-3-(2,6-dichlorophenyl)pro-2-enamide, **g46** (2.0mmol, 482mg) in anhydrous toluene (50ml) under an atmosphere of argon. The mixture was heated under reflux. After 18 hours the mixture was concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 40:60 (1:1). The title compound was collected as a white solid (392mg, 64%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.17; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.90 (t, 3H, *J*=7.2Hz, CH_3), 1.32 (sextet, 2H, *J*=7.4Hz, $CH_2CH_2C\underline{H}_2CH_3$), 1.48 (quintet, 2H, *J*=7.2Hz, $CH_2C\underline{H}_2CH_2CH_3$), 3.22 (q, 2H, *J*=6.5Hz, $C\underline{H}_2CH_2CH_2CH_3$), 7.52-7.67 (ov. m, 3H, ArH-3/4/5), 8.18 (br s, 1H, NH), 8.47 (s, 1H, CH=), 11.03 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 340-342[M+32]$^+$, 241-243[M-CONHC$_4$H$_9$+32]$^+$, 117[M-C$_6$H$_3$(Cl)$_2$CH=C(CN)CO]$^+$.

8.6 Preparation of the focussed coumarin library

Preparation of methyl (*E*)-2-cyano-3-(2,3-dihydroxyphenyl)prop-2-enoate i16

Piperidine (0.14mmol, 12mg) was added to a solution of 2,3-dihydroxybenzaldehyde and methyl cyanoacetate in ethanol (10ml). The reaction mixture was stirred for 1 hour at room temperature. The title compound started to precipitate out of solution after 30 minutes. The title compound was collected as a yellow solid (173mg, 56%). TLC [diethyl ether] R_f 0.17. ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 3.84 (s, 3H, OCH_3), 7.03-7.12 (ov. m, 3H, 3xArH), 8.29 (s, 1H, CH=), 9.32 (br s, 1H, OH), 10.1 (br s, 1H, OH) ppm; Mass spectrum (APCI$^+$): *m/z* 220M$^+$.

Preparation of 3-cyano-8-methoxycoumarin 36

Piperidine (0.4mmol, 34mg) was added to a solution of 2-hydroxy-3-methoxybenzaldehyde (4.0mmol, 608mg) and ethyl cyanoacetate (4.0mmol, 452mg) in ethanol (25ml). The reaction mixture was heated under reflux for 2 hours. The title compound was formed as an orange solid upon cooling of the reaction mixture (393mg, 49%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.08. ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 3.93 (s, 3H, OCH_3), 7.33-7.44 (ov. m, 2H, ArH-6/7), 7.50 (dd, 1H, *J*=7.8, 1.8Hz, ArH-5), 8.95 (s, 1H, ArH-4) ppm; Mass spectrum (APCI$^+$): *m/z* 234[M+32]$^+$, 202M$^+$.

Preparation of 3-cyano-7-methoxycoumarin 37

A mixture of 2-hydroxy-4-methoxybenzaldehyde (2.5mmol, 380mg) and malononitrile (2.5mmol, 165mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 12.5ml) for 60 hours. On addition of concentrated HCl (313μl) the original grey changed to a yellow solution. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a yellow precipitate formed which was removed by filtration and washed with water (3x50ml). The resulting solid was dried under vacuum to give title compound as a pale yellow solid (334mg, 66%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.17; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 3.91 (s, 3H, CH_3), 7.07

(dd, 1H, *J*=8.7, 2.4Hz, ArH-6), 7.13 (d, 1H, *J*=2.4Hz, ArH-8), 7.74 (d, 1H, *J*=8.7Hz, ArH-5), 8.85 (s, 1H, ArH-4) ppm. Mass spectrum (APCI$^+$): *m/z* 202M$^+$.

Preparation of 3-cyano-8-hydroxycoumarin 38

A mixture of 2,3-dihydroxybenzaldehyde (5mmol, 690mg) and malononitrile (5mmol, 330mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 25ml) for 60 hours. On addition of concentrated HCl (626μl) the original brown solution did not change colour. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a brown precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give title compound as a pale brown solid (753mg, 81%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.16; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 7.20-7.28 (ov. m, 3H, ArH-5/6/7), 8.91 (s, 1H, ArH-4), 10.55 (br s, 1H, OH) ppm; Mass spectrum (APCI$^+$): *m/z* 188M$^+$.

Preparation of 3-cyano-5,7-dinitrocoumarin 39

Method A: A mixture of 2,4,6-trihydroxybenzaldehyde (2.5mmol, 385mg) and malononitrile (2.5mmol, 165mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 12.5ml) for 60 hours. On addition of concentrated HCl (313μl) the original orange solution changed to a brown solution. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a dark brown precipitate formed which was removed by filtration and washed with water (3x50ml). The product could not be identified by ^{1}H NMR analysis.

Method B: A mixture of 2,4,6-trihydroxybenzaldehyde (0.5mmol, 77mg) and malononitrile (0.5mmol, 33mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 2.5ml) for 60 hours. On addition of concentrated HCl (63μl) the original brown solution did not change colour. The mixture was then heated at 90°C for 1 hour. The mixture was cooled in an ice bath and a brown precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give the title compound as a brown solid (65mg, 64%). TLC [ethyl acetate] R_f 0.19; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.82 (s, 1H, ArH), 7.07 (s, 1H, ArH), 8.74 (s, 1H, ArH-4), 9.88 (br s, 1H, OH), 10.80 (br s, 1H, OH) ppm; Mass spectrum (APCI$^+$): *m/z* 204M$^+$.

Preparation of 3-cyano-7,8-dihydroxycoumarin 40

A mixture of 2,3,4-trihydroxybenzaldehyde (2.5mmol, 385mg) and malononitrile (2.5mmol, 165mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 12.5ml) for 60 hours. On addition of concentrated HCl (313μl) the original brown solution did not change colour. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a brown precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give the title compound as a pale brown solid (340mg, 67%). TLC [diethyl ether] R_f 0.06; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.91 (d, 1H, *J*=8.5Hz, ArH), 7.17 (d, 1H, *J*=8.5Hz, ArH), 8.77 (s, 1H, ArH-4), 9.77 (br s, 1H, OH), 10.98 (br s, 1H, OH) ppm; Mass spectrum (APCI$^+$): *m/z* 236[M+32]$^+$, 204M$^+$.

Preparation of 3-cyano-6-nitrocoumarin 41

A mixture of 2-hydroxy-5-nitrobenzaldehyde (2.5mmol, 493mg) and malononitrile (2.5mmol, 165mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 12.5ml) for 60 hours. On addition of concentrated HCl (313μl) the original yellow solution changed colour to form a pale yellow solution. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a yellow precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give the title compound as a yellow solid (451mg, 84%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.17; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 7.75 (d, 1H, *J*=9.2Hz, ArH-8), 8.57 (dd, 1H, *J*=9.2, 2.8Hz, ArH-7), 8.78 (d, 1H, *J*=2.8Hz, ArH-5), 9.07 (s, 1H, ArH-4) ppm; Mass spectrum (APCI$^+$):*m/z* 249[M+32]$^+$,217M$^+$.

Preparation of 3-oxo-3*H*-benzo[*f*]chromene-2-carbonitrile 42

A mixture of 2-hydroxy-1-napthaldehyde (5mmol, 860mg) and malononitrile (5mmol, 330mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 25ml) for 60 hours. On addition of concentrated HCl (625μl) the original orange solution changed to a yellow solution. The mixture was then heated at 90°C for 2 hours. The mixture was allowed to cool and a brown precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give the title compound as a pale brown solid (931mg, 79%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.22; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 7.65-7.72 (ov. m, 2H, ArH-5/7), 7.82 (t, trying to split to a ddd, 1H, *J*=7.2Hz, ArH-6), 8.12 (d, 1H, *J*=7.9Hz, ArH), 8.40 (d, 1H, *J*=9.11Hz, ArH), 8.66 (d, 1H, *J*=8.47Hz, ArH), 9.82 (s, 1H, ArH-4) ppm; Mass spectrum (APCI$^+$): *m/z* 254[M+32]$^+$, 222M$^+$.

Preparation of 3-cyano-6,7-dihydroxycoumarin 43

A mixture of 2,4,5-trihydroxybenzaldehyde (1mmol, 154mg) and malononitrile (1mmol, 66mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 5ml) for 60 hours. On addition of concentrated HCl (126μl) the original brown solution did not change colour. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a brown precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give title compound as a pale brown solid (203mg, 42%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.10; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.82 (s, 1H, ArH), 7.07 (s, 1H, ArH), 8.73 (s, 1H, ArH-4), 9.88 (br s, 1H, OH), 11.10 (br s, 1H, OH) ppm; Mass spectrum (APCI+): *m/z* 236[M+32]$^+$, 204M$^+$.

Preparation of 3-cyanocoumarin 44

A mixture of 2-hydroxybenzaldehyde (5mmol, 610mg) and malononitrile (5mmol, 330mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 25ml) for 60 hours. On addition of concentrated HCl (626μl) the original brown solution did not change colour. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a brown precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give the title compound as a pale brown solid (698mg, 82%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.22; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 7.18-7.29 (ov. m, 4H, ArH-5/6/7/8), 8.91 (s, 1H, ArH-4) ppm; Mass spectrum (APCI$^+$): *m/z* 172M$^+$.

Preparation of 3-cyano-6-nitro-8-methoxycoumarin 45

A mixture of 2-hydroxy-3-methoxy-5-nitrobenzaldehyde (2.5mmol, 493mg) and malononitrile (2.5mmol, 165mg) was shaken vigorously with a solution of aqueous sodium hydrogen carbonate (0.05M, 12.5ml) for 60 hours. On addition of concentrated HCl (313μl) the original orange solution changed colour to form a cream solution. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a cream precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give the title compound as a cream solid (479mg, 78%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.15; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 4.07 (s, 3H, OCH_3), 8.14 (d, 1H, *J*=2.5Hz, ArH-7), 8.36 (d, 1H, *J*=2.5Hz, ArH-5), 9.03 (s, 1H, ArH-4) ppm; Mass spectrum (APCI$^+$): *m/z* 279[M+32]$^+$, 247M$^+$.

Preparation of 3-cyano-6-bromo-8-nitrocoumarin 46

A mixture of 5-bromo-3-nitrosalicylbenzaldehyde (1mmol, 246mg) and malononitrile (1mmol, 66mg) was shaken vigorously with a solution of aqueous sodium hydrogen carbonate (0.05M, 5ml) for 60 hours. On addition of concentrated HCl (126μl) the original orange solution changed colour to form a pale orange solution. The mixture was then heated at 90°C for 2 hours. The mixture was cooled in an ice bath and a orange precipitate formed which was removed by filtration and washed with water (3x50ml). The solid was dried under vacuum to give the title compound as an orange solid (209mg, 71%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.12; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 8.77 (d, 1H, *J*=2.7Hz, ArH-7), 8.86 (d, 1H, *J*=2.6Hz, ArH-5), 9.03 (s, 1H, ArH-4) ppm; Mass spectrum (APCI$^+$):*m/z* 327-330[M+32]$^+$, 296-298M$^+$.

Attempted synthesis of 3-cyano-6,8-dinitrocoumarin 47

Method A: A mixture of 3,5-dinitro-2-hydroxybenzaldehyde (0.5mmol, 106mg) and malononitrile (0.5mmol, 33mg) was shaken vigorously with a solution of aqueous sodium hydrogen carbonate (0.05M, 2.5ml) for 60 hours. On addition of concentrated HCl (63μl) a yellow/orange precipitate formed from the original orange solution. The mixture was then heated at 90°C for 2 hours. The mixture was allowed to cool and the yellow/orange precipitate was removed by filtration and washed with water (3x50ml). The product could not be identified by ^{1}H NMR analysis.

Method B: 3,5-dinitro-2-hydroxybenzaldehyde (0.5mmol, 106mg) and malononitrile (0.5mmol, 33mg) was shaken vigorously with a solution of sodium hydrogen carbonate (0.05M, 2.5ml) for 48 hours. On addition of concentrated HCl (63μl) a yellow/orange precipitate formed from the original orange solution. The yellow/orange precipitate was removed by filtration and washed with water (3x50ml). The product could not be identified by ^{1}H NMR analysis.

8.7 Preparation of the Knoevenagel chromenone focussed library

Automation was used to dispense 1.5ml of a solution of a set of chromenone aldehydes in ethanol (0.15mmol/1.5ml) into separate vials. To each vial was added 1.5ml of a single active methylene reagent solution in ethanol (0.15mmol/1.5ml) containing piperidine (0.015mmol/1.5ml). The reaction vials were heated at 75°C for 4 hours. The remaining residues were subjected to automated ethanol washes (3x1.5ml). The resulting solids were then dried under vacuum.

Compound structures (**a-s**)**25-60** can be found in **Tables 3.8** and **3.9**, page 54. Inactivity against MAC16 and MAC13 is denoted by X. Where compounds could not be identified as the desired product, the recovery, TLC, mass spectra and ^{1}H NMR spectra have been recorded with X and – shows that they were not tested against MAC16 and MAC13. Where ^{1}H NMR spectra were not obtained no data have been entered into the table.

*a, b and c represent different solvent systems; a (diethyl ether), b (petrol 40:60-diethyl ether (1:1)) and c (ethyl acetate).

Compound	Recovery	TLC*	Mass spectrum APCI$^+$	^{1}H NMR (250.1MHz, $(CD_3)_2SO$) δ_H ppm	MAC16 IC_{50} (μM)	MAC13 IC_{50} (μM)
a25	32mg, 72%	0.33 b	330[M+32]$^+$, 298M$^+$, 242[M-$(CH_2)_3CH_3$]$^+$, 224[M-O$(CH_2)_3CH_3$]$^+$		65.8±1.7	>100
b25	32mg, 72%	0.34 b	330[M+32]$^+$, 274[M-$C(CH_3)_3$+32]$^+$, 242[m-$C(CH_3)_3$]$^+$	1.53 (s, 9H, $C(CH_3)_3$), 7.60 (ddd, 1H, *J*=8.1, 7.3, 0.8Hz, ArH-7), 7.79 (d, trying to split to a dd, 1H, *J*=8.5Hz, ArH-8), 7.91 (ddd, 1H, *J*=8.1, 6.9, 1.6Hz, ArH-6), 8.15 (dd, 1H, *J*=8.1, 1.6, ArH-5), 8.26 (s, 1H, ArH-2), 9.17 (s, 1H, CH=)	32.4±3.1	52.9±0.9
c25	29mg, 69%	0.29 b	282M$^+$, 314[M+32]$^+$	4.82 (d, 1H, *J*=5.5Hz, $C\underline{H}_2CH=CH_2$), 5.32 (dd, 1H, *J*=10.4, 1.0Hz, $CH_2CH=C\underline{H}$), 5.43 (dd, 1H, *J*=17.2, 1.0Hz, $CH_2CH=C\underline{H}$), 5.86-6.50 (m, 1H, $CH_2C\underline{H}=CH_2$), 7.64 (ddd, 1H, *J*=8.4, 7.0, 1.7Hz, ArH-7), 7.80 (d, trying to split to a dd, 1H, *J*=8.5Hz, ArH-8), 7.92 (ddd, 1H, *J*=8.5, 6.9, 1.7Hz, ArH-6), 8.16 (dd, 1H, *J*=8.0, 1.7, ArH-5), 8.36 (s, 1H, ArH-2), 9.22 (s, 1H, CH=)	54.1±0.2	>100
d25	33mg, 74%	0.33 b	332[M+32]$^+$, 300M$^+$, 224[M-$OCH_2CH_2OCH_3$]$^+$		82.7±2.9	53.4±2.8
e25	19mg, 45%	0.28 b	316[M+32]$^+$, 274[M-$CH(CH_3)_2$+32]$^+$, 242[M-$CH(CH_3)_2$]$^+$		42.1±1.3	>100
f25	13mg, 26%	0.30 b	364[M+32]$^+$		56.4±1.2	57.3±1.6
g25	31mg, 86%	0.01 b	241M$^+$		>100	>100
h25	20mg, 38%	0.43 b	386 [M+32]$^+$, 354M$^+$, 242 [M-$CH_2CH(C_2H_5)C_4H_9$]$^+$		>100	>100
i25	29mg, 76%	0.25 b	288[M+32]$^+$, 256M$^+$, 224[M-OCH_3]$^+$		83.3±6.0	>100
j25	28mg, 68%	0.29 b	302[M+32]$^+$, 270M$^+$, 224[M-OCH_2CH_3]$^+$		>100	>100
k25	41mg, 76%	0.29 b	360M$^+$, 392[M+32]$^+$		95.1±4.1	65.9±0.6
n25	42mg, 97%	0.09 b	290M$^+$	7.52 (s, 1H, ArH-2), 7.57 (t, trying to split to a ddd, 1H, *J*=7.2, ArH-7), 7.75 (d trying to split to a dd, 1H, *J*=8.5 Hz, ArH-7), 8.89 (t, trying to	63.3±0.8	52.7±3.1

				split to a ddd, 1H, J=8.4Hz, ArH-6), 8.14 (d, trying to split to a dd, 1H, J=7.9Hz, ArH-5), 8.98 (s, 1H, CH=), 13.62 (br s, 1H, NH)		
q25	X	X	X	X	-	-
r25	31mg, 72%	0.16 b	289M^{+}, 257[M-32]$^{+}$		X	X
s25	28mg, 69%	0.19 b	273M^{+}, [M-OCH$_3$]$^{+}$	1.31 (s, 3H, CH$_3$), 1.33 (s, 3H, CH$_3$), 5.12 (m, 1H, CH(CH$_3$)$_2$), 7.60 (ddd, 1H, J=8.3, 7.9, 1.1Hz, ArH-7), 7.79 (d, trying to split to a dd, 1H, J=8.7Hz, ArH-8), 7.92 (ddd, 1H, J=8.7, 7.0, 1.7Hz, ArH-6), 8.15 (dd, 1H, J=7.9, 1.2Hz, ArH-5), 8.32 (s, 1H, ArH-2), 9.20 (s, 1H, CH=)	79.9±8.5	X
a49	48mg, 93%	0.39 b	378[M+32]$^{+}$, 346M^{+}		>100	>100
b49	28mg, 54%	0.42 b	378 [M+32]$^{+}$, 322[M-C(CH$_3$)$_3$+32]$^{+}$		69.8±0.8	76.8±1.9
c49	41mg, 87%	0.34 b	362[M+32]$^{+}$, 330M^{+}		>100	>100
d49	36mg, 69%	0.32 b	380[M+32]$^{+}$, 348M^{+}		41.1±1.3	51.3±3.5
e49	38mg, 77%	0.37 b	364[M+32]$^{+}$		>100	>100
f49	39mg, 69%	0.34 b	412[M+32]$^{+}$, 380M^{+}		>100	>100
g49	29mg, 67%	0.01 b	289M^{+}		X	X
h49	31mg, 52%	0.53 b	434[M+32]$^{+}$, 402M^{+}, 290[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^{+}$		>100	>100
i49	30mg, 66%	0.48 b	336[M+32]$^{+}$, 304M^{+}		>100	>100
j49	37mg, 78%	0.49 b	350[M+32]$^{+}$, 318M^{+}		>100	>100
k49	59mg, 97%	0.54 b	440[M+32]$^{+}$, 408M^{+}		>100	>100
n49	36mg, 71%	0.07 b	338M^{+}		61.8±3.9	X
q49	X	X	X	X	-	-
r49	X	X	X	X	-	-
s49	X	X	X	X	-	-
a50	38mg, 75%	0.54 b	372[M+32]$^{+}$, 340M^{+}		64.6±1.7	>100
b50	21mg, 41%	0.49 b	372[M+32]$^{+}$, 316[M-C(CH$_3$)$_3$+32]$^{+}$		53.4±0.7	51.4±0.2
c50	33mg, 71%	0.50 b	356[M+32]$^{+}$, 324M^{+}		86.3±1.2	75.6±2.4
d50	46mg, 90%	0.26 b	374[M+32]$^{+}$, 342M^{+}		73.8±2.7	>100
e50	29mg, 59%	0.46 b	358[M+32]$^{+}$, 326M^{+}, 316[M-CH(CH$_3$)$_2$+32]$^{+}$, 284[M-CH(CH$_3$)$_2$]$^{+}$		56.1±1.6	90.8±2.7
f50	21mg, 38%	0.28 b	374M^{+}, 406[M+32]$^{+}$		49.0±1.5	57.7±3.4
g50	42mg, 99%	0.01 b	283M^{+}		>100	>100
h50	23mg, 39%	0.63 b	428[M+32]$^{+}$, 396M^{+}, 284[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^{+}$		95.6±3.7	60.3±0.7
i50	26mg, 58%	0.29 b	330[M+32]$^{+}$, 298M^{+}		28.6±2.3	>100
j50	26mg, 56%	0.33 b	344[M+32]$^{+}$, 312M^{+}		37.3±1.9	>100
k50	16mg, 27%	0.50 b	434[M+32]$^{+}$, 402M^{+}		49.1±1.4	92.7±2.5
n50	30mg, 60%	0.13 b	332M^{+}		>100	>100
q50	X	X	X	X	-	-
r50	X	X	X	X	-	-
s50	X	X	X	X	-	-
a51	27mg, 55%	0.46 b	358[M+32]$^{+}$, 326M^{+}, 270[M-(CH$_2$)$_3$CH$_3$]$^{+}$		62.6±2.6	>100
b51	29mg, 59%	0.48 b	358[M+32]$^{+}$, 326M^{+}, 270[M-C(CH$_3$)$_3$]$^{+}$		40.9±1.4	66.4±2.7

c51	31mg, 67%	0.40 b	342[M+32]$^+$, 310M$^+$		71.3±1.0	73.8±2.7
d51	32mg, 65%	0.33 b	360[M+32]$^+$, 328M$^+$, 252[M-$OCH_2CH_2OCH_3$]$^+$		51.2±2.7	78.6±2.9
e51	37mg, 79%	0.44 b	344 [M+32]$^+$, 312M$^+$, 302[M-$CH(CH_3)_2$+32]$^+$, 270[M- $CH(CH_3)_2$]$^+$		45.4±1.2	99.3±2.3
f51	22mg, 41%	0.39 b	392[M+32]$^+$, 360M$^+$		48.1±3.7	63.9±2.0
g51	18mg, 45%	0.01 b	269M$^+$		>100	>100
h51	11mg, 19%	0.43 b	414[M+32]$^+$, 382M$^+$, 270[M-$CH_2CH(C_2H_5)C_4H_9$]$^+$		50.7±0.5	70.1±1.1
i51	34mg, 80%	0.33 b	316[M+32]$^+$, 284M$^+$		56.3±2.3	95.6±0.5
j51	29mg, 65%	0.38 b	330[M+32]$^+$, 298M$^+$		60.9±0.4	>100
k51	38mg, 65%	0.35 b	388M$^+$, 420[M+32]$^+$		53.8±1.6	89.2±2.0
n51	34mg, 72%	0.10 b	318M$^+$	0.87-0.93 (ov. m, 6H, $2XCH_3$), 1.20-1.44 (ov. m, 11H, $4xCH_2$, CH_3), 1.65 (septet, 1H, *J*=5.8Hz, $COOCH_2C\underline{H}$), 2.76 (q, 2H, *J*=7.6Hz, $ArC\underline{H_2}CH_3$), 4.22 (d, 2H, *J*=5.5Hz, $COOC\underline{H_2}$), 7.71 (d, 1H, *J*=8.6Hz, ArH-8), 7.77 (dd, 1H, *J*=8.6, 2.0Hz, ArH-7), 7.95 (d, 1H, *J*=2.0Hz, ArH-5), 8.34 (s, 1H, ArH-2), 9.20 (d, 1H, *J*=0.8Hz, CH=)	>100	>100
q51	X	X	X	X	-	-
r51	X	0.33 b/0.19b	317M$^+$, 203M$^+$ (aldehyde)	X	-	-
s51	20mg, 44%	0.33 b	301M$^+$, 269[M-OCH_3]$^+$		75.6±3.0	>100
a52	X	X	X190M$^+$ (aldehyde)	X	-	-
b52	X	X	X190M$^+$ (aldehyde)	X	-	-
c52	X	X	X190M$^+$ (aldehyde)	X	-	-
d52	X	X	X190M$^+$ (aldehyde)	X	-	-
e52	X	X	X190M$^+$ (aldehyde)	X	-	-
f52	X	X	X190M$^+$ (aldehyde)	X	-	-
g52	X	X	X190M$^+$ (aldehyde)	X	-	-
h52	X	X	X190M$^+$ (aldehyde)	X	-	-
i52	X	X	X190M$^+$ (aldehyde)	X	-	-
j52	X	X	X190M$^+$ (aldehyde)	X	-	-
k52	X	X	X190M$^+$ (aldehyde)	X	-	-
n52	X	X	X190M$^+$ (aldehyde)	X	-	-
q52	X	X	X190M$^+$ (aldehyde)	X	-	-
r52	X	X	X190M$^+$ (aldehyde)	X	-	-
s52	X	X	X190M$^+$ (aldehyde)	X	-	-
a53	X	X	X	X	-	-
b53	X	X	X	X	-	-
c53	X	X	X	X	-	-
d53	X	X	X	X	-	-
e53	X	X	X	X	-	-
f53	X	X	X	X	-	-
g53	23mg, 50%	0.01 b	309-313 M$^+$		>100	>100
h53	X	X	X	X	-	-
i53	X	X	X	X	-	-
j53	X	X	X	X	-	-
k53	X	X	X	X	-	-
n53	31mg, 58%	0.12 b	No TIC	7.39 (s, 1H, ArH-2), 8.04 (d, 1H, *J*=2.5Hz, ArH-7), 8.28 (d, 1H, *J*=2.5Hz, ArH-5), 9.06 (s, 1H, CH=), 13.65 (br s, 1H, NH)	65.0±1.0	93.4±2.5
q53	X	X	X	X	-	-
r53	X	X	X	X	-	-
s53	X	X	X	X	-	-
a54	21mg, 43%		358[M+32]$^+$, 326M$^+$		48.7±1.6	75.2±1.8
b54	33mg, 68%	0.48 b	358[M+32]$^+$, 302[M-$C(CH_3)_3$+32]$^+$, 270[M-$C(CH_3)_3$]$^+$, 225[M-$OC(CH_3)_3$]$^+$		25.1±0.9	66.2±3.0

c54	38mg, 86%	0.45 b	342[M+32]$^+$, 310M$^+$		47.4±1.9	85.5±3.5
d54	33mg, 67%	0.43 b	360[M+32]$^+$, 328M$^+$	2.41 (s, 3H, $ArOCH_3$), 2.46 (s, 3H, $ArOCH_3$), 3.31 (s, 3H, $CH_2CH_2OC\underline{H}_3$), 3.63 (m, 2H, $COOCH_2C\underline{H}_2$), 4.42 (m, 2H, $COOC\underline{H}_2$), 7.61 (s, trying to split to a d, 1H, ArH-7), 7.77 (s, trying to split to a d, 1H, ArH-5), 8.35 (s, 1H, ArH-2), 9.23 (s, 1H, CH=)	>100	>100
e54	45mg, 96%	0.45 b	344[M+32]$^+$, 312M$^+$, 302[M-$CH(CH_3)_2$+32]$^+$, 270[M- $CH(CH_3)_2$]$^+$		40.1±0.4	66.9±0.5
f54	37mg, 69%	0.39 b	392[M+32]$^+$, 360M$^+$		69.2±1.5	77.0±2.3
g54	31mg, 77%	0.01 b	301[M+32]$^+$, 284, 269M$^+$, 252	NMR shows the presence of the desired compound, no starting materials, but also another unidentified compound	-	-
h54	48mg, 84%	0.53 b	414 [M+32]$^+$, 382M$^+$, 270 [M-$CH_2CH(C_2H_5)C_4H_9$]$^+$		32.5±1.7	62.9±0.6
i54	41mg, 97%	0.31 b	316[M+32]$^+$, 284M$^+$		39.4±2.8	>100
j54	38mg, 85%	0.33 b	330[M+32]$^+$, 298M$^+$		47.4±1.3	>100
k54	38mg, 66%	0.54 b	420[M+32]$^+$, 388M$^+$		48.8±2.0	87.7±2.7
n54	44mg, 93%	0.08 b	318M$^+$	2.40 (s, 3H, $ArCH_3$), 2.44 (s, 3H, $ArCH_3$), 7.40 (s, 1H, ArH), 7.57 (s, 1H, ArH), 7.75 (s, 1H, ArH-2), 8.95 (s, 1H, CH=), 13.6 (br s, 1H, NH)	>100	>100
q54	X	X	X	X	-	-
r54	X	X	X	X	-	-
s54	X	X	X	X	-	-
a55	12mg, 21%	0.42 b	408-410[M+32]$^+$, 376-378M$^+$, 320-322[M-$(CH_2)_3CH_3$]$^+$		51.8±1.5	>100
b55	24mg, 43%	0.45 b	408-410[M+32]$^+$, 352[M-$C(CH_3)_3$+32]$^+$		45.8±1.8	70.3±0.3
c55	32mg, 62%	0.38 b	392-394[M+32]$^+$, 360-362M$^+$	4.82 (d, 1H, J=5.3Hz, $C\underline{H}_2CH=CH_2$), 5.32 (d, trying to split to a dd, J=10.4Hz, $CH_2CH=C\underline{H}$), 5.42 (d, trying to split to a dd, J=17.2Hz, $CH_2CH=C\underline{H}$), 5.96-6.08 (m, 1H, $CH_2C\underline{H}=CH_2$), 7.80 (d, 1H, J=8.9Hz, ArH-8), 8.09 (dd, 1H, J=9.0, 2.6Hz, ArH-7), 8.21 (d, 1H, J=2.4Hz, ArH-5), 8.32 (s, 1H, ArH-2), 9.22 (s, 1H, CH=)	77.8±2.7	>100
d55	X	X	X	X	-	-
e55	29mg, 53%	0.41 b	396-394[M+32]$^+$, 354-352[M-$CH(CH_3)_2$+32]$^+$, 321-320[M-$CH(CH_3)_2$]$^+$		59.6±1.0	>100
f55	34mg, 55%	0.31 b	442-446[M+32]$^+$		62.2±1.1	72.6±1.1
g55	34mg, 71%	0.01 b	320-321M$^+$		>100	>100
h55	X	X	X	X	-	-
i55	47mg, 94%	0.31 b	366-368[M+32]$^+$, 334-336M$^+$	3.88(s, 1H, OCH_3), 7.79 (d, 1H, J=8.9Hz, ArH-8), 8.09 (d, trying to split to a dd, 1H, J=8.9Hz, ArH-7), 8.21 (s, trying to split to a d, 1H, ArH-5), 8.30 (s, 1H, ArH-2), 9.21 (s, 1H, CH=)	>100	>100
j55	49mg, 94%	0.38 b	380-382[M+32]$^+$, 348-350M$^+$		>100	>100
k55	33mg, 50%	0.38 b	438-440M$^+$, 470-472[M+32]$^+$		49.7±2.2	98.3±3.1
n55	20mg, 36%	0.04 b	No TIC	7.40 (s, 1H, ArH-2), 7.76 (d, 1H, J=9.0Hz, ArH-8), 8.05 (dd, 1H, J=9.0, 2.4Hz, ArH-7), 8.2 (d, 1H, J=2.4Hz, ArH-5), 8.99 (s, 1H, CH=), 13.65 (br s, 1H, NH)	73.9±5.6	>100
q55	X	X	X	X	-	-
r55	X	X	X	X	-	-
s55	X	X	X	X	-	-

a56	34mg, 70%	0.39 b	358[M+32]$^+$, 326M$^+$		38.2±0.7	81.8±1.5
b56	32mg, 66%	0.44 b	358[M+32]$^+$, 302[M-C(CH$_3$)$_3$+32]$^{+\cdot}$ 270[M-C(CH$_3$)$_3$]$^+$		31.8±1.7	69.2±0.6
c56	21mg, 45%	0.37 b	342[M+32]$^+$		33.9±0.2	57.8±1.5
d56	29mg, 59%	0.13 b	360[M+32]$^+$, 328M$^+$, 252[M-OCH$_2$CH$_2$OCH$_3$]$^+$		42.7±4.8	61.8±0.1
e56	42mg, 90%	0.41 b	344[M+32]$^+$, 312M$^+$, 302[M-CH(CH$_3$)$_2$+32]$^{+\cdot}$ 270[M-CH(CH$_3$)$_2$]$^+$		66.6±2.0	>100
f56	40mg, 74%	0.35 b	No TIC	2.36 (s, 3H, ArCH$_3$), 2.40 (s, 3H, ArCH$_3$), 5.36 (s, 2H, CH$_2$), 7.38-7.48 (ov. m, 5H, 5xAr'H), 7.60 (s, 1H, ArH), 7.89 (s, 1H, ArH), 8.37 (d, 1H, *J*=0.8Hz, ArH-2), 9.17 (d, 1H, *J*=0.7Hz, CH=)	>100	>100
g56	30mg, 75%	0.01 b	30[M+32]$^+$, 269M$^+$		>100	>100
h56	13mg, 23%	0.51 b	414[M+32]$^+$, 382M$^+$, 270[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		69.1±1.6	73.4±1.8
i56	34mg, 80%	0.31 b	316[M+32]$^+$, 284M$^+$		31.5±4.7	60.5±1.1
j56	32mg, 76%	0.37 b	298M$^+$, 330[M+32]$^+$		57.3±0.8	97.3±4.1
k56	21mg, 36%	0.42 b	420[M+32]$^+$, 388M$^+$		86.6±2.2	67.0±1.0
n56	40mg, 84%	0.04 b	318M$^+$		58.4±0.9	>100
q56	X	X	X	X	-	-
r56	19mg, 40%	0.31 b	317M$^+$, 285[M-OCH$_3$]$^+$	X	-	-
s56	22mg, 49%	0.19 b	419, 399, 367, 269	X	-	-
a57	37mg, 79%	0.39 b	344[M+32]$^+$, 312M$^+$, 256[M-(CH$_2$)$_3$CH$_3$]$^+$		92.3±2.0	53.9±1.7
b57	32mg, 69%	0.43 b	344[M+32]$^+$, 288[M-C(CH$_3$)$_3$+32]$^+$, 256[M-C(CH$_3$)$_3$]$^+$, 212[M-OC(CH$_3$)$_3$]$^+$		33.3±1.1	46.7±5.3
c57	31mg, 70%	0.33 b	328[M+32]$^+$, 296M$^+$		34.1±2.9	72.6±1.3
d57	26mg, 55%	0.14 b	346[M+32]$^+$, 313M$^+$, 270[M-OCH$_2$CH$_2$OCH$_3$]$^+$, 238[M-OCH$_2$CH$_2$OCH$_3$]$^+$		79.0±2.0	56.7±2.6
e57	40mg, 90%	0.39 b	330[M+32]$^+$, 298[M-C(CH$_3$)$_2$]$^{+\cdot}$ 256[M-CH(CH$_3$)$_2$]$^+$		24.3±0.6	76.1±4.3
f57	21mg, 41%	0.37 b	378[M+32]$^+$		53.5±4.4	57.2±3.3
g57	33mg, 87%	0.01 b	255M$^+$		X	X
h57	16mg, 29%	0.41 b	400[M+32]$^+$, 367M$^+$, 256[M-CH$_2$CH(C$_2$H$_5$)C$_4$H$_9$]$^+$		62.2±5.3	75.3±4.2
i57	33mg, 82%	0.31 b	302[M+32]$^+$, 270M$^+$, 238[M-OCH$_3$]$^+$		30.5±4.3	>100
j57	35mg, 82%	0.35 b	316[M+32]$^+$, 284M$^+$, 238[M-OCH$_2$CH$_3$]$^+$		65.2±3.1	>100
k57	40mg, 71%	0.41 b	406[M+32]+, 373M$^+$		58.7±2.3	81.0±3.4
n57	40mg, 88%	0.10 b	304 M$^+$		86.81±2.31	>100
q57	X	X	X	X	-	-
r57	X	X	X, 271[M-OCH$_3$]$^+$, 189 (aldehyde)	X	-	-
s57	28mg, 65%	0.29 b	287M$^+$, 255[M-OCH$_3$]$^+$		X	X
a58	27mg, 54%	0.37 b	364[M+32]$^+$, 332M$^+$, 276[M-(CH$_2$)$_3$CH$_3$]$^+$		59.3±1.4	>100
b58	31mg, 62%	0.43 b	364[M+32]$^+$, 308[M-C(CH$_3$)$_3$+32]$^+$, 276[M-C(CH$_3$)$_3$]$^+$, 226[M-OC(CH$_3$)$_3$]$^+$	1.53 (s, 1H, C(CH$_3$)$_3$), 7.86 (d, 1H, *J*=8.8Hz, ArH-8), 7.98 (dd, 1H, *J*=9.3, 1.9Hz, ArH-7), 8.08 (d, 1H, *J*=1.9Hz, ArH-5), 8.22 (s, 1H, ArH-	47.2±2.8	79.9±4.3

				2), 9.18 (s, 1H, CH=)		
c58	35mg, 74%	0.34 b	348[M+32]$^+$, 316M$^+$		86.1±3.2	>100
d58	34mg, 68%	0.12 b	366[M+32]$^+$, 334M$^+$, 258[M-OCH$_2$CH$_2$OCH$_3$]$^+$		>100	>100
e58	39mg, 82%	0.36 b	350[M+32]$^+$, 318M$^+$, 308[M-CH(CH$_3$)$_2$+32]$^{+\cdot}$ 276[M-CH(CH$_3$)$_2$]$^+$		53.1±1.9	>100
f58	22mg, 40%	0.17 b	398[M+32]$^+$		58.9±3.0	73.0±2.4
g58	34mg, 83%	0.01 b	275M$^+$		>100	>100
h58	X	X	X	X	-	-
i58	24mg, 55%	0.24 b	322[M+32]$^+$, 290M$^+$, 258[M-OCH$_3$]$^+$		56.0±0.6	>100
j58	29mg, 64%	0.30 b	336[M+32]$^+$, 304M$^+$, 258[M-OCH$_2$CH$_3$]$^+$		78.7±3.4	>100
k58	36mg, 61%	0.32 b	426[M+32]$^+$, 393M$^+$		78.7±3.4	84.3±0.2
n58	39mg, 80%	0.06 b	324M$^+$		62.4±4.4	>100
q58	X	X	X	X	-	-
r58	X	X	X	X	-	-
s58	X	X	X	X	-	-
a59	X	X	X	X	-	-
b59	X	X	X	X	-	-
c59	X	X	X	X	-	-
d59	X	X	X	X	-	-
e59	X	X	X	X	58.1±0.8	>100
f59	X	X	X	X	-	-
g59	28mg, 47%	0.01 b	397-399M$^+$		>100	>100
h59	X	X	X	X	-	-
i59	X	X	X	X	88.6±3.6	>100
j59	X	X	X	X	>100	>100
k59	X	X	X	X	78.7±3.4	84.3±0.2
n59	52mg, 78%	0.13 b	No TIC	7.38 (s, 1H, ArH-2), 8.18 (d, 1H, *J*=2.3Hz, ArH-5), 8.47 (d, 1H, *J*=2.3Hz, ArH-7), 9.06 (s, 1H, CH=), 13.68 (br s, 1H, NH)	54.6±1.5	>100
q59	X	X	X	X	-	-
r59	X	X	X	X	-	-
s59	X	X	X	X	-	-
a60	32mg, 62%	0.46 b	375[M+32]$^+$, 287[M-(CH$_2$)$_3$CH$_3$]$^+$		X	X
b60	X	X	X	X	-	-
c60	X	X	X	X	-	-
d60	38mg, 74%	0.14 b	X	X	-	-
e60	X	X	X	X	-	-
f60	X	X	X	X	-	-
g60	X	X	X	X	-	-
h60	X	X	X	X	-	-
i60	X	X	X	X	-	-
j60	X	X	X	X	-	-
k60	35mg, 58%	0.46 b	437[M+32]$^+$, 405M$^+$, 287[M-(CH$_2$)$_3$C$_6$H$_5$]$^+$		X	X
n60	X	X	X	X	-	-
q60	X	X	X	X	-	-
r60	29mg, 58%	0.32 b	334M$^+$, 302[M-OCH$_3$]$^+$	3.74 (s, 3H, OCH$_3$), 3.79 (s, 3H, OCH$_3$), 7.55 (s, 1H, ArH-2), 8.00 (d, 1H, *J*=9.1Hz, ArH-8), 8.63 (dd, 1H, *J*=9.2, 2.8Hz, ArH-7), 8.75 (d, 1H, *J*=2.7Hz, ArH-5), 8.90 (s, 1H, CH=)	>100	>100
s60	33mg, 69%	0.14 b	318M$^+$, 286[M-OCH$_3$]$^+$		X	X

Preparation of *t*-butyl-(*E*)-2-cyano-3-(4-oxo-4*H*-chromen-3-yl)prop-2-enoate b25

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formylchromone (2.0mmol, 348mg) and *t*-butyl cyanoacetate (2.0mmol, 282mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give fine cream crystals (349mg, 59%). TLC [petrol 40:60-diethyl ether] R_f 0.44; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.54 (s, 9H, $C(C\underline{H}_3)_3$), 7.60 (ddd, 1H, *J*=7.1, 6.4, 0.8Hz, ArH-7), 7.79 (d, 1H, *J*=8.1Hz, ArH-8), 7.91 (ddd, 1H, *J*=8.5, 7.0, 1.6Hz, ArH-6), 8.15 (dd, 1H, *J*=8.0, 1.6Hz, ArH-5), 8.26 (d, 1H, *J*=0.7Hz, ArH-2), 9.17 (s, 1H, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 27.71 ($C(\underline{C}H_3)_3$), 83.99 ($\underline{C}(CH_3)_3$), 106.10 (q), 115.05 (CN), 117.31 (q), 119.06 (CH), 123.12 (q), 125.79 (CH), 127.00 (CH), 135.57 (CH), 145.81 (CH), 155.85 (q), 159.77 (CH), 160.21 (q), 174.00 (COO)ppm; Mass spectrum (APCI$^+$): *m/z* 298M$^+$; IR (KBr disc): υ_{max} 3085 (ArH/CH=), 2981, 2938 (CH_2/CH_3), 2225 (CN), 1710 (COO), 1654 (aromatic ketone), 1620, 1560 (CH/ArH), 1463 (CH_2/CH_3), 1396, 1361 (CH_3), 1294, 1255, 1228, 1147, 1090 (CO), 842, 756, 692 (4 adjacent ArH) cm^{-1}.

Preparation of *t*-butyl-(*E*)-2-cyano-3-(6,8-dimethyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate b54

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formyl-6,8-dimethylchromone (2.0mmol, 404mg) and *t*-butyl cyanoacetate (2.0mmol, 282mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give yellow crystals (385mg, 59%). TLC [petrol 40:60-diethyl ether] R_f 0.58; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.53 (s, 9H, $C(C\underline{H}_3)_3$), 2.41 (s, 3H, $ArC\underline{H}_3$-8), 2.46 (s, 3H, $ArC\underline{H}_3$-6), 7.60 (s, 1H, ArH-7), 7.75 (s, 1H, ArH-5), 8.26 (d, 1H, *J*=0.8Hz, ArH-2), 9.17 (d, 1H, *J*=0.8Hz, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 15.10 ($ArCH_3$-8), 20.63 ($ArCH_3$-6), 27.71 ($C(\underline{C}H_3)_3$), 83.83 ($\underline{C}(CH_3)_3$), 105.67 (q), 115.31 (CN), 117.02 (q), 122.65 (q), 122.71 (CH), 127.96 (q), 136.12 (q), 137.29 (CH), 145.95 (CH), 152.61 (q), 159.25 (CH), 160.53 (q), 174.25 (COO) ppm; Mass spectrum (APCI$^+$): *m/z* 326M$^+$; IR (KBr disc): υ_{max} 3082 (ArH/CH=), 2972 (CH_2/CH_3), 2929 (CN), 1729 (COO), 1658 (aromatic ketone), 1606, 1571 (CH/ArH), 1471 (CH_2/CH_3), 1378, 1346 (CH_3), 1272, 1236, 1156 (CO) cm^{-1}.

Preparation of *t*-butyl-(*E*)-2-cyano-3-(6,7-dimethyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate b56

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formyl-6,7-dimethylchromone (2.0mmol, 404mg) and *t*-butyl cyanoacetate (2.0mmol, 282mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give cream crystals (444mg, 68%). TLC [petrol 40-60-diethyl ether] R_f 0.56; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.53 (s, 9H, $C(C\underline{H}_3)_3$), 2.36 (s, 3H, $ArC\underline{H}_3$-7), 2.40 (s, 3H, $ArC\underline{H}_3$-6), 7.58 (s, 1H, ArH-8), 7.87 (s, 1H, ArH-5), 8.26 (d, 1H, *J*=0.8Hz, ArH-2), 9.10 (d, 1H, *J*=0.7Hz, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 19.13 ($ArCH_3$), 20.12 ($ArCH_3$), 27.71 ($C(\underline{C}H_3)_3$), 83.80 ($\underline{C}(CH_3)_3$), 105.98 (q), 115.89 (CN), 117.23 (q), 118.84 (CH), 121.21 (q), 125.18 (CH), 136.26 (q), 146.10 (CH), 146.35 (q), 154.34 (q), 159.36 (CH), 173.47 (COO), ppm; Mass spectrum (APCI$^+$): *m/z* 326M$^+$; IR (KBr disc): υ_{max} 3044 (ArH/CH=), 2985 (CH_2/CH_3), 2227 (CN), 1704 (COO), 1654 (aromatic ketone), 1623, 1585, 1552 (CH/ArH), 1463, 1425 (CH_2/CH_3), 1369 (CH_3), 1272, 1093 (CO), 877, 840 (isolated ArH) cm^{-1}.

Preparation of *t*-butyl-(*E*)-2-cyano-3-(6-methyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate b57

Piperidine (0.1mmol, 9mg) was added to a solution of 3-formyl-6-methylchromone (1.0mmol, 188mg) and *t*-butyl cyanoacetate (1.0mmol, 141mg) in ethanol (10ml). The reaction mixture was heated under reflux for 2 hours and then concentrated under vacuum. The title compound was filtered off and recrystallised from ethanol three times to give white crystals (113mg, 36%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.54; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.53 (s, 9H, $C(CH_3)_3$), 2.45 (s, 3H, $ArCH_3$), 7.67 (d, 1H, *J*=8.5Hz, ArH-8), 7.73 (dd, 1H, *J*=8.6Hz, 2.0Hz, ArH-7), 7.92 (d, 1H, *J*=0.6Hz, ArH-5), 8.26 (d, 1H, *J*=0.7Hz, ArH-2), 9.13 (d, 1H, *J*=0.7Hz, CH=C) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 20.66 (CH_3), 27.71 ($C(\underline{C}H_3)_3$), 83.83 ($\underline{C}(CH_3)_3$), 105.76 (q), 115.26 (CN), 117.24 (CH), 118.84 (q), 122.86 (CH), 125.02 (q), 136.54 (q), 136.81 (CH), 145.89 (CH), 153.92 (q), 159.60 (CH), 160.42 (q), 173.96 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 312M$^+$, 256[M-C(CH$_3$)$_3$]$^+$; IR (KBr disc): υ_{max} 3081, 3050, 3004 (ArH/CH=), 2933 (CH_2/CH_3), 2217 (CN), 1710 (COO), 1654 (aromatic ketone), 1622, 1592, 1570 (CH, ArH), 1481 (CH_2/CH_3), 1385, 1380, 1351 (CH_3), 1290, 1244, 1149, 1083 (CO), 819 (2 adjacent ArH), 793, 766 (isolated ArH) cm^{-1}.

Preparation of allyl-(*E*)-2-cyano-3-(6,7-dimethyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate c56

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formyl-6,7-dimethylchromone (2.0mmol, 404mg) and allyl cyanoacetate (2.0mmol, 250mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give white crystals (343mg, 49%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.55; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 2.34 (s, 3H, $ArC\underline{H}_3$-7), 2.38 (s, 3H, $ArC\underline{H}_3$-6), 4.79 (d, 2H, *J*=5.5Hz, CH_2), 5.29 (dd, 1H, *J*=10.6, 1.5Hz, CH=C$\underline{H}$), 5.41 (dd, 1H, *J*=17.2, 1.5Hz, C$\underline{H}$=CH), 6.00 (dd, 1H, *J*=17.2, 10.6Hz, $CH_2C\underline{H}=CH_2$), 7.55 (s, 1H, ArH-8), 7.86 (s, 1H, ArH-5), 8.33 (d, 1H, *J*=0.7Hz, ArH-2), 9.12 (d, 1H, *J*=1.1Hz, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 18.54 ($ArCH_3$-7), 19.29 ($ArCH_3$-6), 30.28 (CH), 66.81 (CH_2), 101.02 (CH_2), 104.95 (q), 116.21 (q), 117.83 (q), 118.91 (q), 122.37 (q), 125.38 (CH), 131.98 (CH), 137.64 (q), 145.52 (q), 146.97 (CH), 154.41 (q), 159.68 (CH), 174.22 (COO) ppm; Mass spectrum (APCI$^+$): *m/z* 342[M+32]$^+$, 270[M-CH$_2$CH=CH$_2$]$^+$, 252[M-OCH$_2$CH=CH$_2$]$^+$; IR (KBr disc): υ_{max} 3064 (ArH/CH=), 2977, 2919 (CH_2/CH_3), 2217 (CN), 1737 (COO), 1652 (aromatic ketone), 1625, 1552 (CH/ArH), 1459 (CH_2/CH_3), 1377 (CH_3), 1261, 1242, 1160 (CO) cm^{-1}.

Preparation of allyl (*E*)-2-cyano-3-(6-methyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate c57

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formyl-6-methylchromone (2.0mmol, 376mg) and allyl cyanoacetate (2.0mmol, 250mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give fine yellow crystals (250mg, 42%). TLC [petrol 40:60-diethyl ether] R_f 0.53; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 2.46 (s, 3H, $ArC\underline{H}_3$-6), 4.82 (d, 2H, *J*=5.4Hz, $C\underline{H}_2CH=CH_2$), 5.32 (dd, 1H, *J*=10.7, 1.6Hz, CH=C$\underline{H}$), 5.42 (dd, 1H, *J*=17.2, 1.6Hz, CH=C$\underline{H}$), 6.02 (dd, 1H, *J*=17.2, 10.7Hz, C$\underline{H}$=CH), 7.69 (d, 1H, *J*=8.6Hz, ArH-8), 7.74 (dd, 1H, *J*=8.6, 1.9Hz, ArH-7), 7.93 (s, 1H, ArH-5), 8.34 (d, 1H, *J*=0.7Hz, ArH-2), 9.16 (d, 1H, *J*=0.7Hz, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 20.98 ($ArCH_3$), 67.11 (CH_2), 104.21 (q), 115.41 (q), 117.50 (q), 119.19 (CH_2), 119.20 (CH), 123.18 (q), 125.39 (CH), 132.22 (CH), 136.91 (q), 137.22 (CH), 147.10 (CH), 154.23 (q), 160.43 (q), 161.60 (q), 174.23 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 296M$^+$, 256[M-CH$_2$CH=CH$_2$]$^+$, 238[M-OCH$_2$CH=CH$_2$]$^+$; IR (KBr disc): υ_{max} 3079, 3035 (ArH/CH=), 2929 (CH_2/CH_3), 2217 (CN), 1732 (COO), 1654 (aromatic ketone), 1594, 1568 (CH/ArH), 1485 (CH_2/CH_3), 1378 (CH_3), 1272, 1243, 1199, 1147 (CO), 943 (isolated ArH), 839 (2 adjacent ArH) cm^{-1}.

Preparation of isopropyl (*E*)-2-cyano-3-(6-methyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate e57

Piperidine (0.3mmol, 25mg) was added to a solution of 3-formyl-6-methylchromone (3.0mmol, 564mg) and isopropyl cyanoacetate (3.0mmol, 381mg) in ethanol (30ml). The reaction mixture was heated under reflux for 2 hours. The title compound forms as a solid upon cooling. The title compound was filtered off and recrystallised from ethanol three times to give a white solid (286mg, 32%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.51; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.31 (s, 3H, CHC$\underline{H}_3$), 1.34 (s, 3H, CHC$\underline{H}_3$), 5.12 (septet, 1H, *J*=6.3Hz, C$\underline{H}$(CH_3)$_2$), 7.68 (d, 1H, *J*=8.6Hz, ArH-8), 7.73 (dd, 1H, *J*=8.6, 2.0Hz, ArH-7), 7.94 (s, 1H, ArH-5), 8.32 (s, 1H, ArH-2), 9.17 (s, 1H, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 20.66 (CH_3), 21.61 (CH_3), 70.79 ($\underline{C}H(CH_3)_2$), 104.92 (q), 115.00 (CN), 117.20 (q), 118.87 (CH), 122.55 (q), 125.05 (CH), 136.58 (CH), 136.87 (q), 146.40 (CH), 153.88 (q), 159.89 (CH), 174.05 (COO) ppm; Mass spectrum (APCI$^+$): *m/z* 298M$^+$; IR (KBr disc): υ_{max} 3082, 3048 (ArH/CH=), 2985 (CH_2/CH_3), 2221 (CN), 1708 (COO), 1660 (aromatic ketone), 1616, 1594, 1558 (CH/ArH), 1479 (CH_2/CH_3), 1378, 1350 (CH_3), 1321, 1272, 1238, 1103, 1081 (CO) cm^{-1}.

Preparation of 2-ethylhexyl-(*E*)-2-cyano-3-(6,8-dimethyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate h54

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formyl-6,8-dimethylchromone (2.0mmol, 404mg) and 2-ethylhexyl cyanoacetate (2.0mmol, 394mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The reaction mixture was concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with petrol 40:60-diethyl ether (1:1). The title compound was collected as a yellow solid (289mg, 38%). TLC [petrol 40:60-diethyl ether (1:1).] R_f 0.59; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.86-0.93 (ov. m, 6H, 2xCH_3), 1.32-1.44 (ov. m, 8H, 4xCH_2), 1.62-1.70 (m, 1H, CH), 2.41 (s, 3H, ArC$\underline{H}_3$-8), 2.46 (s, 3H, ArC$\underline{H}_3$-6), 4.21 (d, 2H, *J*=7.0Hz, $COOCH_2$), 7.60 (s, 1H, ArH-7), 7.76 (s, 1H, ArH-5), 8.34 (d, 1H, *J*=0.8Hz, ArH-2), 9.21 (d, 1H, *J*=0.8Hz, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 11.38 (CH_3), 14.42 (CH_3), 15.42 (CH_3), 20.95 (CH_3), 22.92 (CH_2), 23.75 (CH_2), 28.83 (CH_2), 30.26 (CH_2), 68.81 (CH_2), 104.23 (q), 104.23 (q), 115.44 (q), 117.25 (q), 123.01 (CH), 123.18 (q), 128.29 (q), 136.50 (q), 136.50 (q), 137.65 (CH), 146.79 (CH), 152.74 (q), 159.94 (CH), 161.96 (q), 174.47 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 270$[M-CH_2CH(C_2H_5)C_4H_9]^+$, 252$[M-OCH_2CH(C_2H_5)C_4H_9]^+$; IR (KBr disc): υ_{max} 3082 (ArH/CH=), 2972 (CH_2/CH_3), 2929 (CN), 1729 (COO), 1658 (aromatic ketone), 1606, 1571 (CH/ArH), 1471 (CH_2/CH_3), 1378, 1346 (CH_3), 1272, 1236, 1156 (CO) cm^{-1}.

Preparation of methyl (*E*)-2-cyano-3-(6-isopropyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate i50

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formyl-6-isopropylchromone (2.0mmol, 432mg) and methyl cyanoacetate (2.0mmol, 198mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give fine yellow crystals (352mg, 59%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.48; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.24 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 3.10 (septet, 1H, *J*=6.9Hz, C<u>H</u>(CH_3)$_2$), 3.88 (s, 3H, OCH_3), 7.72 (d, 1H, *J*=8.7Hz, ArH-8), 7.84 (dd, 1H, *J*=8.7, 2.3Hz, ArH-7), 7.96 (d, 1H, *J*=2.2Hz, ArH-5), 8.34 (d, 1H, *J*=1.1Hz, ArH-2), 9.19 (d, 1H, *J*=0.7Hz, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 23.86 (CH_3), 33.16 (CH_3), 53.72 (CH_3), 104.00 (CH), 115.05 (q), 117.31 (q), 119.09 (CH), 122.31 (q), 123.20 (CH), 134.29 (CH), 146.61 (CH), 147.45 (q), 153.87 (q), 160.07 (CH), 161.62 (q), 162.14 (q), 173.88 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 298M$^+$; IR (KBr disc): υ_{max} 3051 (ArH/CH=), 2962 (CH_2/CH_3), 2219 (CN), 1722 (COO), 1653 (aromatic ketone), 1653, 1614, 1560 (CH/ArH), 1487, 1427 (CH_2/CH_3), 1360, 1326 (CH_3), 1275, 1246, 1080 (CO), 939 (isolated ArH), 852 (2 adjacent ArH) cm^{-1}.

Preparation of methyl-(*E*)-2-cyano-3-(6,7-dimethyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate i56

Piperidine (0.2mmol, 17mg) was added to a solution of 3-formyl-6,7-dimethylchromone (2.0mmol, 404mg) and methyl cyanoacetate (2.0mmol, 198mg) in ethanol (20ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give a cream solid (364mg, 64%). TLC [petrol 40:60-diethyl ether (1:1)] R_f 0.39; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 2.35 (s, 3H, ArCH_3-7), 2.39 (s, 3H, ArCH_3-6), 3.87 (s, 3H, OCH_3), 7.57 (s, 1H, ArH-8), 7.87 (s, 1H, ArH-5), 8.33 (s, 1H, ArH-2), 9.13 (s, 1H, CH=) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 105.99 (q), 114.97 (q), 117.22 (q), 119.31 (CH), 121.11 (q), 125.24 (CH), 137.11 (q), 147.10 (CH), 146.46 (q), 154.34 (q), 159.21 (CH), 173.97 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 284M$^+$; IR (KBr disc): υ_{max} 3064, 3037 (ArH/CH=), 2954 (CH_2/CH_3), 2219 (CN), 1735 (COO), 1648 (aromatic ketone), 1619, 1550 (CH/ArH), 1466, 1446, 1420 (CH_2/CH_3), 1373, 1352 (CH_3), 1259, 1240, 1159 (CO) cm^{-1}.

Preparation of methyl-(*E*)-2-cyano-3-(6-methyl-4-oxo-4*H*-chromen-3-yl)prop-2-enoate i57

Piperidine (0.1mmol, 9mg) was added to a solution of 3-formyl-6-methylchromone (1.0mmol, 188mg) and methyl cyanoacetate (1.0mmol, 99mg) in ethanol (10ml). The reaction mixture was heated under reflux for 2 hours. The title compound was filtered off and recrystallised from ethanol three times to give cream crystals (142mg, 53%). TLC [petrol 40:60-diethyl ether] R_f 0.38; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 2.46 (s, 3H, ArCH_3), 3.88 (s, 3H, OCH_3), 7.68 (d, 1H, *J*=8.6Hz, ArH-8), 7.74 (dd, 1H, *J*=8.5, 2.0Hz, ArH-7), 7.94 (s, 1H, ArH-5), 8.35 (d, 1H, *J*=0.7Hz, ArH-2), 9.18 (s, 1H, CH=)ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 20.65 (ArCH_3), 53.72 (OCH_3), 103.98 (q), 115.21 (q), 117.15 (q), 118.87 (CH), 122.95 (q), 125.07 (CH), 136.59 (q), 136.61 (CH), 146.59 (CH), 154.23 (q), 160.02 (CH), 162.05 (q), 153.86 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 270M$^+$; IR (KBr disc): υ_{max} 3056 (ArH/CH=), 2964 (CH_2/CH_3), 2225 (CN), 1718 (COO), 1660 (aromatic ketone), 1615, 1599, 1564 (CH/ArH), 1485, 1430 (CH_2/CH_3), 1380, 1351 (CH_3), 1277, 1238, 1181, 1095 (CO), 823 (isolated ArH/2 adjacent ArH) cm^{-1}.

8.8 Toxicology

Direct toxicity

Human mononuclear leukocytes (MNL) were collected from the heparinised blood of healthy volunteers. MNLs were washed with phosphate buffered saline (PBS) and suspended in HEPEs buffer at a concentration of 1×10^6 cells/ml. MNLs were incubated with test compounds at 250μM for 1 hour, cells were recovered and resuspended in bovine serum albumin (BSA) 5mg/ml in HEPEs and incubated for a further 18 hours. Percentage cell death was determined using a trypan blue exclusion assay. At least three experiments were performed for each compound, using blood from three different healthy volunteers.

Indirect toxicity

Microsomes were prepared from rat liver homogenate. The protein content was determined using the Bio-Rad protein assay reagents package (Bio-Rad laboratories). MNLs were isolated as before and incubated with the test compounds, 1mM NADPH and 2mg of rat liver microsomes for 1 hour. Cells were recovered and resuspended in BSA 5mg/ml in HEPEs and incubated for 18 hours. Percentage cell death was determined with a trypan blue exclusion assay. At least three experiments were performed for each compound, using blood from three different healthy volunteers.

8.9 Solid-phase catalyst

Synthesis of (2,6-dichlorophenyl)(piperidin-1-yl)methanol 48

A mixture of 2,6-dichlorobenzaldehyde (2mmol, 350mg) and piperidine (2mmol, 170mg) was heated under reflux for 16 hours in cyclohexane (20ml). The cyclohexane was removed under vacuum to yield the title compound as a deep red/purple oil (252mg, 49%). TLC [petrol 60:80-ethyl acetate (2:1)] R_f 0.50; ^{1}H NMR [250.1MHz, $(CD_3)_2SO$]: δ_H 1.32-1.50 (ov. m, 6H, $3xCH_2$), 2.31-2.47 (ov. m, 4H, $2xCH_2$), 4.67 (s, 1H, CH), 7.22-7.49 (ov. m, 3H, ArH-3/4/5) ppm; Mass spectrum (APCI$^+$): *m/z* 242[M-OH]$^+$, 174[M-NC_5H_{10}]$^+$.

Synthesis of 1-[(2,6-dichlorophenyl)(piperidin-1-yl)methyl]piperidine 49

A mixture of 2,6-dichlorobenzaldehyde (2mmol, 350mg) and piperidine (4mmol, 340mg) was heated under reflux in cyclohexane (10ml) for 2 hours in the presence of 4A$^\circ$ molecular sieves. The molecular sieves were removed by filtration and the cyclohexane was removed under vacuum to yield the title compound as a white solid (398mg, 61%). TLC [petrol 60:80-ethyl acetate (2:1)] R_f 0.51; ^{1}H NMR [250.1MHz, $(CD_3)_2SO$]: δ_H 1.32-1.47 (ov. m, 12H, $6xCH_2$), 2.35-2.40 (ov. m, 4H, $2xCH_2$), 2.66-3.00 (ov. m, 4H, $2xCH_2$), 4.66 (s, 1H, CH), 7.24-7.44 (ov. m, 3H, ArH-3/4/5) ppm; Mass spectrum (APCI$^+$): *m/z* 327M$^+$, 242[M-NC_5H_{10}]$^+$.

Preparation of 1-(2,6-Dichlorophenyl)-1-piperidinyl-methyl ether 50

A mixture of 2,6-dichlorobenzaldehyde (2mmol, 350mg) and piperidine (2mmol, 170mg) was dissolved in ethanol (30ml) and heated under reflux for 2 hours. The ethanol was removed under vacuum to give the title compound as a red oil (257mg, 45%). TLC [petrol 60:80-ethyl acetate (2:1)]

R_f 0.49; ^{1}H NMR [250.1MHz, $(CD_3)_2SO$]: δ_H 1.11 (t, 3H, *J*=7.0Hz, CH_3) 1.38-1.45 (ov. m, 6H, piperidine 3xCH_2), 2.60-2.76 (ov. m, 4H, piperidine 2xCH_2), 3.21-3.29 (ov. m, 2H, CH_2 prochiral centre), 5.26 (s, 1H, CH), 7.33-7.52 (ov. m, 3H, ArH) ppm; Mass spectrum (APCI$^+$): *m/z* 242[M-OC_2H_5]$^+$.

Attempted synthesis of 1-[(dimethylamino)(piperidin-1-yl)methyl]piperidine 51

4-Dimethylaminobenzaldehyde (1mmol) was dissolved hot cyclohexane (20ml). Piperidine (2mmol, 170mg) was added and the mixture was heated under reflux for 16 hours in the presence of 4A° molecular sieves. The molecular sieves were removed by filtration and the cyclohexane and excess piperidine were removed under vacuum to yield a pale yellow solid which was shown by ^{1}H NMR analysis to be the starting aldehyde.

Preparation of di-(1-piperidinyl)-phenylmethane 52

Benzaldehyde (5mmol, 530mg) and piperidine (10mmol, 850mg) were dissolved in cyclohexane (10ml) and heated under reflux for 2 hours, in the presence of 4A° molecular sieves. The molecular sieves were removed by filtration and the cyclohexane was removed under vacuum. The title compound was washed with cyclohexane and dried under vacuum to give a white solid (847mg, 66%). TLC [petrol 60:80-ethyl acetate (2:1)] R_f 0.49; ^{1}H NMR [250.1MHz, $(CD_3)_2SO$]: δ_H 7.25-7.36 (ov. m, 3H, 3xArH), 7.15-7.18 (ov. m, 2H, 2xArH), 3.59 (s, 1H, CH), 2.15-2.38 (ov. m, 8H, 4xCH_2), 1.29-1.47 (ov. m, 12H, 6xCH_2) ppm; Mass spectrum (APCI$^+$): *m/z* 259M$^+$.

Attempted synthesis of ethyl 2-cyano-3-(2,6-dichlorophenyl)-3-piperidin-1-ylpropanoate 53

A mixture of ethyl-(*E*)-2-cyano-3-(2,6-dichlorophenyl) prop-2-enoate (2.01mmol, 545mg) and piperidine (5mmol, 425mg) was dissolved in cyclohexane (100ml) and heated under reflux in conjunction with a Dean-Stark trap for 16 hours. A white solid formed in solution which was removed by filtration and washed with cyclohexane (3x50ml) and diethyl ether (3x50ml). The unidentified white solid was dried under vacuum. TLC [ethyl acetate] R_f 0.01; ^{1}H NMR [250.1MHz, $(CD_3)_2SO$]: δ_H 1.54-1.64 (ov. m, 6H, CH_2-3/4/5), 2.98-3.36 (ov. m, 4H, piperidine CH_2-2/6), 7.40-7.47 (ov. m, 1H, ArH), 7.56-7.59 (ov. m, 2H, 2xArH), 7.73 (s, 1H, CH=C) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_c 22.01 (piperidine CH_2-4), 22.51 (piperidine CH_2-3/5), 43.78 (piperidine CH_2-2/6), 100.22 (CH=$\underline{C}$), 117.17 (CN), 123.65 (ArC-1), 128.66 (ArCH-3/5), 131.31 ($\underline{C}$H=C), 132.64 (ArCCl), 133.19 (ArCCl or COO), 143.34 (ArCH-4) ppm; Mass spectrum (APCI$^+$): *m/z* 86[NC_5H_{10}]$^+$.

Preparation of piperazinomethyl-polystyrene 56

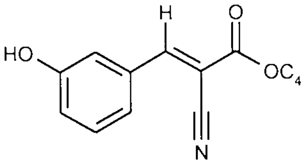
Merrifield resin [5g, (2% cross-linked with DVB, 200-400 mesh, 2.3 mmol Cl/g)] was swollen in acetone (150ml). Piperazine (10 eq, 9.9g) was then dissolved in the acetone and potassium carbonate, K_2CO_3 (20eq, 31.74g) added. The mixture was heated under reflux for 72 hours. The resin was collected by filtration and washed with THF (3x150ml), acetone (3x150ml) and then dried under vacuum. Elemental analysis found C, 86.02%; H, 7.87%; N, 4.20% indicating a loading of 1.5mmol piperazine/g.

Solution-phase synthesis of Knoevenagel Condensation products using piperazinomethyl-polystyrene catalyst (method A) or piperidine (method B)

Method A: Aldehyde (1mmol) and active methylene (1mmol) were dissolved in ethanol (10ml). Piperazinomethyl polystyrene (50mg) was added and the reaction mixture was heated under reflux for 2 hours. Piperazinomethyl-polystyrene was removed by filtration and excess ethanol was removed under vacuum.

Method B: Aldehyde (1mmol) and active methylene (1mmol) were dissolved in ethanol (10ml). Piperidine (1 drop) was added and the reaction mixture was heated under reflux for 2 hours. Excess ethanol was removed under vacuum.

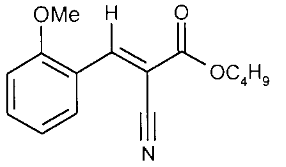
Preparation of *n*-butyl-(*E*)-2-cyano-(3-hydroxyphenyl)prop-2-enoate a17



Using 3-hydroxybenzaldehyde and *n*-butyl cyanoacetate **method A** gave the title compound as a pale yellow solid (244mg, 93%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.31; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.93 (t, 3H, *J*=7.4Hz, $OCH_2CH_2CH_2C\underline{H}_3$), 1.40 (sextet, 2H, *J*=7.5Hz, $OCH_2CH_2C\underline{H}_2CH_3$), 1.67 (quintet, 2H, *J*=6.6Hz, $OCH_2C\underline{H}_2CH_2CH_3$), 4.27 (t, 2H, *J*=6.5Hz, $OC\underline{H}_2CH_2CH_2CH_3$), 7.04 (dd, 1H, *J*=7.9, 3.9Hz, ArH-6), 7.49-7.35 (ov. m, 3H, ArH), 8.28 (s, 1H, C$\underline{H}$=C), 10.05 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 13.76 (CH_3) 18.77 (CH_2), 30.24 (CH_2), 66.12 (CH_2), 102.36 (CH=$\underline{C}$), 115.72 (CN), 116.59 ($\underline{C}$H=C), 121.03 (ArCH), 122.72 (ArCH), 130.59 (ArCH-5), 132.69 (ArC-1), 155.43 (ArCH), 158.03 (ArCOH), 162.12 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 13.89 (q, *J*=62.9Hz, CH_3), 18.41 (t, *J*=62.9Hz, CH_2), 30.24 (t, *J*=61.5Hz, CH_2), 66.09 (t, *J*=149.6Hz, CH_2), 102.36 (s, CH=$\underline{C}$), 115.78 (d, *J*=14.0Hz, CN), 116.60 (dd, *J*=161.5, 5.7Hz, $\underline{C}$H=C), 121.03 (d, trying to split, *J*=161.5Hz, ArCH), 122.72 (ddd, *J*=164.4, 15.7, 5.7Hz, ArCH), 130.64 (d, *J*=161.9Hz, ArCH-5), 132.73 (d, *J*=8.0Hz, ArC-1), 155.48 (ddd, *J*=160.9, 5.7, 4.3Hz, ArCH), 158.05 (d, *J*=8.6Hz, ArCOH), 162.11 (d, *J*=3.29Hz, COO) ppm; Mass spectrum (APCI$^+$): *m/z* 246M$^+$, 190$[M-C_4H_9]^+$, 172$[M-OC_4H_9]^+$, 144$[M-COOC_4H_9]^+$; IR (KBr disc): υ_{max} 3376 (OH), 3035 (C=CH), 2956, 2875 (CH_2/CH_3), 2229 (CN), 1726 (COO), 1606, 1575, 1488 (Ar), 1473 (CH_2/CH_3), 1396 (CH_3), 1259 (OH), 1103 (C-OH), 836 (CH=C), 778 (3 adjacent Ar-H) cm^{-1}.

Using 3-hydroxybenzaldehyde and ethyl cyanoacetate **method B** gave a mixture of the title compound and piperidine-derived by-product (251mg) in a molar ratio of (1:0.16).

Preparation of *n*-butyl-(*E*)-2-cyano-(2-methoxyphenyl)prop-2-enoate a48

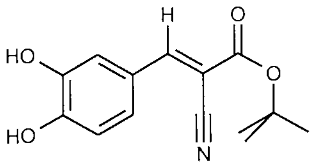


Using 2-methoxybenzaldehyde and *n*-butyl cyanoacetate **method A** gave the title compound as a pale yellow clear liquid (249mg, 96%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.40; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.92 (t, 3H, *J*=7.3Hz, $OCH_2CH_2CH_2C\underline{H}_3$), 1.38 (sextet, 2H, *J*=7.3Hz, $OCH_2CH_2C\underline{H}_2CH_3$), 1.67 (quintet, 2H, *J*=5.6Hz, $OCH_2C\underline{H}_2CH_2CH_3$), 3.90 (s, 3H, OCH_3), 4.26 (t, 2H, *J*=6.5Hz, $OC\underline{H}_2CH_2CH_2CH_3$), 7.13-7.23 (ov. m, 2H, ArH-5/6), 7.63 (ddd, 1H, *J*=8.2, 8.1, 1.6Hz, ArH-4), 8.11 (dd, 1H, *J*=6.5,1.3Hz, ArH-3), 8.58 (s, 1H, CH=C) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 13.65 (CH_3), 18.76 (CH_2), 30.20 (CH_2), 56.19

(OCH$_3$), 66.01 (CH$_2$), 102.03 (CH=$\underline{C}$), 112.24 ($\underline{C}$H=C), 115.72 (CN), 119.90 (ArC-1), 120.92 (ArCH), 128.61 (ArCH-6), 135.68 (ArCH), 148.93 (ArCH), 159.06 (Ar$\underline{C}$OCH$_3$), 162.17 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, (CD$_3$)$_2$SO): δ_C 21.49 (q, trying to split again, *J*=120.0Hz, CH$_3$), 25.60 (t, trying to split again, *J*=120.0Hz, CH$_2$), 56.21 (q, *J*=145.7Hz, OCH$_3$), 66.07 (t, trying to split, *J*=146.1Hz, CH$_2$), 102.00 (s, CH=$\underline{C}$), 112.26 (dd, *J*=161.9, 7.8Hz, $\underline{C}$H=C), 115.77 (d, *J*=14.0Hz, CN), 119.89 (t, trying to be dd, *J*=6.8Hz, ArC-1), 120.94 (dd, *J*=164.2, 7.5Hz, ArCH), 128.62 (ddd, *J*=160.4, 9.5, 6.8Hz, ArCH-6), 135.72 (dd, *J*=160.7, 8.6Hz, ArCH), 148.91 (dd, *J*=161.66, 3.6Hz, ArCH), 159.08 (complex m, Ar$\underline{C}$OCH$_3$), 162.20 (quintet, *J*=3.2Hz, COO) ppm; Mass spectrum (APCI$^+$): *m/z* 260M$^+$, 204[M-C$_4$H$_9$]$^+$, 186[M-0C$_4$H$_9$]$^+$; IR (Thin layer): υ_{max} 2957 (CH$_3$/CH$_2$), 2850 (O-CH$_3$), 2219 (CN), 1724 (COO), 1600 (Ar), 757 (4 adjacent ArH) cm^{-1}.

Using 2-methoxybenzaldehyde and *n*-butyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterified product (355mg) in a molar ratio of (1:0.04:0.42).

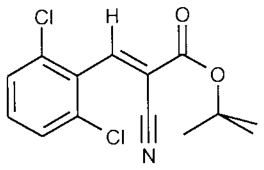
Preparation of *t*-butyl-(*E*)-2-cyano-(3,4-dihydroxyphenyl)prop-2-enoate b15



Using 3,4-dihydroxybenzaldehyde and *t*-butyl cyanoacetate **method A** gave the title compound as a yellow solid (230mg, 88%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.16; ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO): δ_H 1.51 (s, 9H, C(CH$_3$)$_3$), 6.90 (d, 1H, *J*=8.34Hz, ArH-4), 7.37 (dd, 1H, *J*=6.23, 2.18Hz, ArH-5), 7.62 (d, 1H, *J*=2.08Hz, ArH-1), 8.04 (s, 1H, C$\underline{H}$=C), 9.98 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, (CD$_3$)$_2$SO): δ_C 27.84 (OC($\underline{C}$H$_3$)$_3$) 82.78 (O$\underline{C}$(CH$_3$)$_3$), 98.25 (CH=$\underline{C}$), 116.19 (ArCH-5), 116.62 ($\underline{C}$H=C), 116.77 (CN), 123.17 (ArC-1), 126.92 (ArCH), 146.03 (ArCOH-3), 152.08 (ArCOH-4), 154.58 (ArCH), 161.91 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, (CD$_3$)$_2$SO): δ_C 27.8 (dddd, *J*=123.0, 120.0, 8.1, 4.1Hz, OC($\underline{C}$H$_3$)$_3$), 98.2 (s, CH=$\underline{C}$), 82.8 (m, O$\underline{C}$(CH$_3$)$_3$), 116.23 (d, *J*=152.8Hz, ArCH-5), 116.60 (dd, *J*=151.7, 4.7Hz, $\underline{C}$H=C), 116.84 (d, *J*=13.7Hz, CN), 123.20 (d, *J*=8.9Hz, ArC-1), 127.04 (dd, *J*=162.0, 12.8 Hz, ArCH), 146.10 (dd, *J*=10.3, 5.4Hz, ArCOH-3), 152.16 (ddd, *J*=10.0, 7.1, 2.3Hz, ArCOH-4), 155.91 (dd, *J*=157.9, 9.0Hz, ArCH), 161.95 (d, *J*=6.75Hz, COO) ppm; Mass spectrum (APCI$^+$): *m/z* 188[M-OC(CH$_3$)$_3$]$^+$, 160[M-COOC(CH$_3$)$_3$]$^+$; IR (KBr disc): υ_{max} 3234 (OH), 2987 (CH$_3$), 2229 (CN), 1712 (COO), 1585, 1511 (Ar), 1367 (C(CH$_3$)$_3$),1292, 1251 (OH), 1093 (OH), 840 (CH=C), 807 (2 adjacent Ar-H) cm^{-1}.

Using 3,4-dihydroxybenzaldehyde and *t*-butyl cyanoacetate **method B** gave the title compound and piperidine-derived by-products (249mg) in the molar ratio (1:0.25).

Preparation of *t*-butyl-(*E*)-2-cyano-(2,6-dichlorophenyl)prop-2-enoate b46

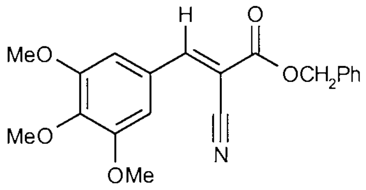


Using 2,6-dichlorobenzaldehyde and *t*-butyl cyanoacetate **method A** gave the title compound as a white solid (264mg, 89%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.51; ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO): δ_H 1.55 (s, 9H, OC(CH$_3$)$_3$), 7.55-7.68 (ov. m, 3H, ArH), 8.40 (s, 1H, C$\underline{H}$=C) ppm; ^{13}C NMR (62.9MHz, (CD$_3$)$_2$SO): δ_C 27.61(OC($\underline{C}$H$_3$)$_3$), 64.64 (O$\underline{C}$(CH$_3$)$_3$), 113.63 (CN), 114.73 (CH=$\underline{C}$), 128.91 (ArCH-3/5), 130.51 (ArC-1), 132.67(ArCCl-2/6), 132.97 ($\underline{C}$H=C), 151.83 (ArCH-4), 159.04 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, (CD$_3$)$_2$SO): δ_C

27.61 (quartet, trying to split further, *J*=127.2Hz, $\mathrm{OC(\underline{C}H_3)_3}$). 84.64 (sextet/m, *J*=3.8Hz, $\mathrm{O\underline{C}(CH_3)_3}$), 113.63 (d, *J*=13.9Hz, CN), 114.73 (s, CH=$\underline{\mathrm{C}}$), 128.91 (dd, *J*=170.9, 7.6Hz, ArCH-3/5), 130.51 (d, *J*=5.3Hz, ArC-1), 132.67 (d, trying to split further, *J*=11.6Hz, ArCCl-2/6), 132.97 (d, *J*=168.6Hz, $\underline{\mathrm{C}}$H=C), 151.83 (d, *J*=172.1Hz, ArCH-4), 159.04 (d, *J*=6.30Hz, COO) ppm; Mass spectrum (APCI$^+$): *m/z* 298M$^+$, 242$\mathrm{[M\text{-}C(CH_3)_3]^+}$, 224$\mathrm{[M\text{-}OC(CH_3)_3]^+}$, 158$\mathrm{[CH{=}C(CN)COOC(CH_3)_3]^+}$, 57$\mathrm{[C(CH_3)_3]^+}$; IR (KBr disc): υ_{max} 3054 (CH=C), 2975 ($\mathrm{CH_3}$) 2235 (CN), 1721 (COO), 1631 (C=C-Ar), 1560 (Ar), 1431 ($\mathrm{CH_3}$), 1368 ($\mathrm{C(CH_3)_3}$), 833 (CH=C), 773 (3 adjacent Ar-H and / or C-Cl) cm^{-1}.

Using 2,6-dichlorobenzaldehyde and *t*-butyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterification product (293mg) in a molar ratio of (1:0.25:0.12).

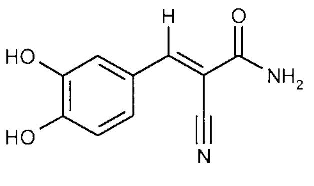
Preparation of benzyl-(*E*)-2-cyano-(3,4,5-trimethoxyphenyl) prop-2-enoate f1



Using 3,4,5-trimethoxybenzaldehyde and benzyl cyanoacetate **method A** gave the title compound as a pale yellow solid (340mg, 96%). TLC [diethyl ether-petrol 40:60 (1:1)] R$_f$ 0.22; ^{1}H NMR (250.1MHz, $\mathrm{(CD_3)_2SO}$): δ_H 3.79 (s, 3H, $\mathrm{OCH_3}$-4) 3.83 (s, 6H, 2x$\mathrm{OCH_3}$), 5.35 (s, 2H, $\mathrm{C\underline{H}_2C_6H_5}$), 7.37-7.45 (ov. m, 5H, ArH), 7.52 (s, 2H, ArH), 8.38 (s, 1H, C$\underline{\mathrm{H}}$=C) ppm; ^{13}C NMR (62.9MHz, $\mathrm{(CD_3)_2SO}$): δ_C 56.22 ($\mathrm{OCH_3}$-3/5) 60.54 ($\mathrm{OCH_3}$-4), 67.62 ($\mathrm{CH_2}$), 109.10 ($\underline{\mathrm{C}}$H=C), 116.22 (CN), 126.63 (CH=$\underline{\mathrm{C}}$), 128.28 (ArCH), 128.56 (2xArCH), 128.77 (Ar'CH), 135.58 ($\mathrm{ArOCH_3}$-4), 142.32 ($\mathrm{ArOCH_3}$-3/5), 153.04 (Ar'C-1), 155.50 (ArCH-2/6), 162.57 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $\mathrm{(CD_3)_2SO}$): δ_C 56.23 (q, *J*=145.1Hz, $\mathrm{OCH_3}$-3/5), 67.93 ($\mathrm{CH_2}$), 60.58 (q, *J*=145.3Hz, $\mathrm{OCH_3}$-4), 109.08 (ddd, *J*=162.2, 6.1, 4.6Hz, $\underline{\mathrm{C}}$H=C), 126.68 (s, CH=$\underline{\mathrm{C}}$), 116.28 (d, *J*=13.8Hz, CN), 128.39 (ddd, *J*=153.9, 5.8, 4.1Hz, ArCH), 128.78 (dd, *J*=159.7, 2.5Hz, 2xArCH), 128.89 (d, *J*=161.1Hz, Ar'CH), 128.89 (d, *J*=161.1Hz, Ar'CH), 135.58 (complex m, $\mathrm{ArOCH_3}$-4), 142.32 (ddd, *J*=5.7,4.8, 2.9Hz, $\mathrm{ArOCH_3}$-3/5), 153.07 (complex m, Ar'C-1), 155.56 (ddd, *J*=161.1, 6.4, 5.0Hz, ArCH-2/6), 162.30 (d, *J*=3.3Hz, $\underline{\mathrm{C}}$OO) ppm; Mass spectrum (APCI$^+$): *m/z* 354M$^+$, 246$\mathrm{[M\text{-}OCH_2C_6H_5]^+}$, 91$\mathrm{[CH_2C_6H_5]^+}$; IR (KBr disc): υ_{max} 3001 (C=CH), 2967, 2939 ($\mathrm{CH_2/CH_3}$), 2825 ($\mathrm{OCH_3}$), 2215 (CN), 1720 (COO), 1602, 1577, 1502 (Ar), 1239 ($\mathrm{CO\text{-}CH_3}$), 831 (C=CH), 760, 703 (5 adjacent Ar-H) cm^{-1}.

Using 3,4,5-trimethoxybenzaldehyde and benzyl cyanoacetate **method B** gave the title compound, piperidine-derived by-products and transesterified product (346mg) with molar ratio (1:0.24:1.68).

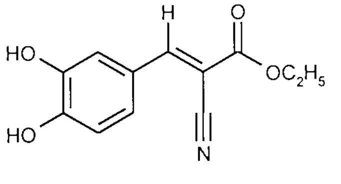
Preparation of (*E*)-2-cyano-3-(3,4-dichlorophenyl) prop-2-enamide g15



Using 3,4-dihydroxybenzaldehyde and cyanoacetamide **method A** gave the title compound as an orange/brown solid (161mg, 79%). TLC [diethyl ether] R$_f$ 0.18; ^{1}H NMR (250.1MHz, $\mathrm{(CD_3)_2SO}$): δ_H 6.88 (d, 1H, *J*=8.3Hz, ArH-5), 7.27 (dd, 1H, *J*=6.3, 2.1Hz, ArH-6), 7.53 (d, 1H, *J*=2.1Hz, ArH-2), 7.68 (br d, 2H, $\mathrm{NH_2}$), 7.94 (s, 1H, C$\underline{\mathrm{H}}$=C), 9.69 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, $\mathrm{(CD_3)_2SO}$): δ_C 101.26 (CH=$\underline{\mathrm{C}}$), 116.24 (ArCH-5), 116.39 (ArCH-2), 117.61 (CN), 123.58 (ArC-1), 125.53 (ArCH), 145.99 (ArCOH-3), 151.05 ($\underline{\mathrm{C}}$H=C), 163.81 ($\mathrm{CONH_2}$) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $\mathrm{(CD_3)_2SO}$): δ_C 101.27 (d, *J*=6.5Hz, CH=$\underline{\mathrm{C}}$), 116.24 (d, *J*=174.0Hz, ArCH-5), 116.39 (dd, *J*=160.3, 5.4Hz, ArCH-2), 117.61 (d, *J*=14.13Hz, CN), 123.58 (d, *J*=8.9Hz, ArC-1), 125.53 (ddd, *J*=160.7, 5.9, 3.3Hz, ArCH-6), 146.02 (dd, *J*=6.7, 3.3Hz, ArCOH-3), 151.09 (ddd, *J*=9.5, 6.9, 5.2,Hz, ArCOH-4), 151.1 (ddd, *J*=156.9, 5.5, 5.2Hz, $\underline{C}H=C$), 163.8 (d, *J*=6.2Hz, $CONH_2$) ppm; Mass spectrum ($APCI^+$): *m/z* 205M^+, 160$[M-CONH_2]^+$; IR data (KBr disc): υ_{max} 1656 (C=O of amide), 1630 (NH), 1594, 1527 (Ar), 816 (2 adjacent Ar-H) cm^{-1}.

Using 3,4-dihydroxybenzaldehyde and cyanoacetamide **method B** gave a mixture of the title compound and piperidine-derived by-products (173mg) in a molar ratio (1:0.12).

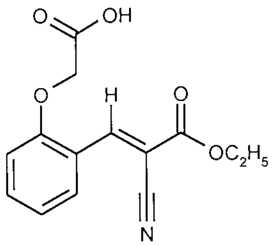
Preparation of ethyl-(*E*)-2-cyano-(3,4-dihydroxyphenyl) propan-2-enoate j15



Using 2,4-benzaldehyde and ethyl cyanoacetate **method A** gave the title compound as a yellow solid (224mg, 96%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.13; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 2.50 (t, 3H, *J*=1.8Hz, $COOCH_2C\underline{H}_3$), 4.25 (q, 2H, *J*=7.1Hz, $COOC\underline{H}_2CH_3$), 7.37 (dd, 1H, *J*=8.5, 2.2Hz, ArH-6), 7.63 (d, 1H, *J*=2.2Hz, ArH-2), 8.08 (s, 1H, $C\underline{H}=C$), 10.10 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 14.28 ($OCH_2\underline{C}H_3$) 62.11 ($O\underline{C}H_2CH_3$),96.70 ($CH=\underline{C}$), 116.21 (ArCH-5), 116.59 (ArCH-2), 116.61 (CN), 123.14 (ArC-1), 127.28 (ArCH-6), 146.08 (ArCOH-3), 152.39 (ArCOH-4), 155.17 ($\underline{C}H=C$), 162.98 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 14.33 (qd, *J*=127.0Hz, $OCH_2\underline{C}H_3$) 62.16 (td, *J*=151.1, 4.4Hz, $O\underline{C}H_2CH_3$), 96.69 (s, $CH=\underline{C}$), 116.25 (d, *J*=160.1Hz, ArCH-5), 116.59 (ddd, *J*=158.4, 8.3, 6.2Hz, ArCH-2), 116.61 (d, *J*=13.67Hz, CN), 123.18 (d, *J*=8.9Hz, ArC-1), 127.34 (ddd, *J*=162.1, 7.5, 6.4Hz, ArCH-6), 146.15 (dd, *J*=7.1, 3.9Hz, ArCOH-3), 152.45 (ddd, *J*=9.4, 7.1, 1.7Hz, ArCOH-4), 155.24 (ddd, *J*=158.0, 6.1 3.9Hz, $\underline{C}H=C$), 163.03 (quintet, *J*=3.3Hz, COO) ppm; Mass spectrum ($APCI^+$): *m/z* 234M^+, 206$[M-CN]^+$, 188$[M-OC_2H_5]^+$, 160$[M-COOC_2H_5]^+$; IR (KBr disc): υ_{max} 3203 (OH), 2989 (CH_2/CH_3), 2229 (CN), 1725 (COO), 1585, 1511 (Ar), 1364 (CH_3), 1251 (OH), 1166, 1091 (C-O), 807 (2 adjacent Ar-H) cm^{-1}.

Using 2,4-benzaldehyde and ethyl cyanoacetate **method B** gave a mixture of the title compound and piperidine-derived by-products (249mg) in a molar ratio of (1:0.12).

Preparation of 2-{2-[(*E*)-2-cyano-3-ethoxy-3-oxoprop-1-enyl]phenoxy}acetic acid j37

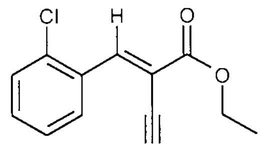


Using 2-(2-formylphenoxy)acetic acid and ethyl cyanoacetate **method A** gave the title compound as a yellow oil (249mg, 79%). TLC [diethyl ether] R_f 0.15; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.32 (t, 3H, *J*=6.40Hz, $COOCH_2C\underline{H}_3$), 4.32 (2H, *J*=7.10Hz, $COOC\underline{H}_2CH_3$), 4.89 (s, 2H, $C\underline{H}_2COOH$), 7.12-7.20 (ov. m, 2H, ArH-3/4), 7.64-7.58 (ov. m, 1H, ArH-4), 8.14 (d, 1H, *J*=7.75Hz, ArH-1), 8.69 (s, 1H, CH=C) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 14.23 ($COOCH_2\underline{C}H_3$), 62.63 ($COO\underline{C}H_2CH_3$), 65.45 ($O\underline{C}H_2COOH$), 102.63 ($CH=\underline{C}$), 113.44 ($\underline{C}H=C$), 115.92 (CN), 120.43 ($Ar\underline{C}OCH_2COOH$), 121.65 (ArCH), 128.80 (ArCH-6), 135.54 (ArCH), 149.40 (ArCH), 157.80 (ArC-2), 163.69 ($\underline{C}OOCH_2CH_3$), 169.98 (COOH) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 14.23 (qd, *J*=127.1, 2.5Hz, $COOCH_2\underline{C}H_3$), 62.85 (td, *J*=144.8, 4.4Hz, $COO\underline{C}H_2CH_3$), 65.63 (t, *J*=147.9Hz, $O\underline{C}H_2COOH$), 113.59 (dd, *J*=161.8, 7.8Hz, $\underline{C}H=C$), 116.16 (d, *J*=14.1Hz, CN), 120.43 (d,

J=6.4Hz, ArCH-1), 121.65 (dd, J=156.5, 7.4Hz, ArCH), 129.0 (ddd, J=160.5, 9.2, 6.6Hz, ArCH-6), 135.76 (dd, J=161.2, 7.5Hz, ArCH), 149.56 (dd, J=162.4, 3.6Hz, ArCH), 157.98 (complex m, ArC-2), 162.46 (quintet, J=3.3Hz, $\underline{C}OOCH_2CH_3$), 170.31 (quintet, J=4.4Hz, COOH) ppm; Mass spectrum (APCI$^+$): m/z 276M$^+$, 230[M-OC_2H_5]$^+$, 172[M-CH_2COOH-OC_2H_5]$^+$; IR (KBr disc): υ_{max} 3052 (CH=C), 3000 (COOH), 2982-2910 (CH_2), 2221 (CN), 1722 ($COOC_2H_5$/COOH), 1590 (Ar), 1457-1490 (CH_2/CH_3), 1390 (CH_3), 763 (4 adjacent Ar-H) cm^{-1}.

Using 2-(2-formylphenoxy)acetic acid and ethyl cyanoacetate **method B** gave the title compound and piperidine-derived by-products (259mg) in a molar ratio of (1:0.39).

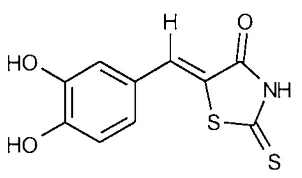
Preparation of ethyl-(*E*)-2-cyano-(2-chlorophenyl)propan-2-enoate j41



Using 2-chlorobenzaldehyde and ethyl cyanoacetate **method A** gave the title compound as a pale yellow solid (231mg, 91%). TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0.53; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.31 (t, 3H, J=7.1Hz, $CH_2C\underline{H}_3$), 4.35 (q, 2H, J=7.1Hz, $C\underline{H}_2CH_3$), 7.57-7.73 (ov. m, 3H, ArH-3/4/5), 8.12 (dd, 1H, J=5.9, 1.7Hz, ArH-6), 8.54 (s, 1H, C$\underline{H}$=C).ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 14.12 (CH_3), 62.93 ($O\underline{C}H_2CH_3$), 106.88 (CH=$\underline{C}$), 114.85 (CN), 128.14 ($\underline{C}$H=C), 129.76 (ArC-1), 129.97 (ArCH-2), 130.47 (ArCH), 134.42 (ArCH), 134.97 (ArCCl), 150.82 (ArCH), 161.33 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 14.15 (q, trying to split again, J=127.2Hz, $OCH_2\underline{C}H_3$), 62.97 (td, J=149.4, 4.4Hz, $O\underline{C}H_2CH_3$), 106.89 (s, CH=$\underline{C}$), 114.91 (d, J=13.8Hz, CN), 128.19 (dd, J=166.1, 7.3Hz, $\underline{C}$H=C), 129.80 (dd, J=8.8, 5.0Hz, ArC-1), 130.02 (d, trying to split further, J=164.2Hz, ArCH-2), 130.52 (dd, J=168.7, 7.6Hz, ArCH), 134.48 (dd, J=165.9, 8.4Hz, ArCH), 135.00 (complex m, ArCCl), 150.86 (dd, J=164.3, 3.7Hz, ArCH), 161.37 (quintet, J=3.5Hz, COO) ppm; Mass spectrum (APCI$^+$): m/z 208[M-C_2H_5]$^+$, 190[M-OC_2H_5]$^+$; IR (KBr disc): υ_{max} 3077, 2979 (CH_2/CH_3), 3039 (C=CH), 2233 (CN), 1720 (COO), 1619 (C=C-Ar), 1428 (CH_2/CH_3), 923 (C=CH), 800 (C-Cl) cm^{-1}.

Using 2-chlorobenzaldehyde and ethyl cyanoacetate **method B** gave the title compound and piperidine-derived by-products (245mg) in a molar ratio of (1:0.04).

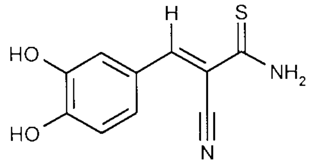
Preparation of 5-[(*Z*)-(3,4-dihydroxyphenyl)methylidene]-2-thioxothiazolidin-4-dione n15



Using 3,4-dihydroxybenzaldehyde and rhodanine **method A** gave the title compound as an orange solid (242mg, 96%). TLC [diethyl ether] R_f 0.54; ^{1}H NMR [250.1MHz, $(CD_3)_2SO$]: δ_H 6.89 (d, J=15.0Hz, ArH-5), 6.89 (d, J=1.6Hz, ArH-2), 6.99 (dd, J=2.5, 2.1Hz, ArH-6), 7.46 (s, 1H, CH=C), 9.94 (br. s, 1H, OH), 10.07 (br. s, 1H, OH), 13.42 (br. s, 1H, NH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 115.77 (Ar$\underline{C}$H-2), 116.86 (ArCH-4), 121.06 (CH=$\underline{C}$), 124.62 (ArCH-6), 133.01 ($\underline{C}$H=C), 146.30 (Ar-COH), 149.44 (Ar-COH), 169.89 (ArC-1), 176.98 (C=O), 195.88 (C=S) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 115.82 (d, J=160.2Hz, ArCH-2) 116.75 (d, J=160.1Hz, ArCH-4), 124.91 (dd, J=160.1, 7.6Hz, ArCH-6), 133.13 (ddd, J=158.5, 4.8, 3.8Hz, $\underline{C}$H=C), 146.27 (complex m, ArC-1), 149.50 (d, J=6.2Hz, Ar-COH), 169.90 (d, J=6.2Hz, Ar-COH), 177.08 (t, J=5.4Hz, C=O), 195.92 (s, C=S), 121.04 (s, CH=$\underline{C}$) ppm; Mass spectrum (APCI$^+$): *m/z* 254M$^+$; IR (KBr disc): υ_{max} 3440 (NH), 3205 (OH), 1681, 1580 (CONH), 1378 (OH), 1183 (C=S), 1114 (CO), 810 (CH=C) cm^{-1}.

Using 3,4-dihydroxybenzaldehyde and rhodanine **method B** gave a mixture of the title compound and piperidine-derived by-products (244g), of molar ratio (1:0.21).

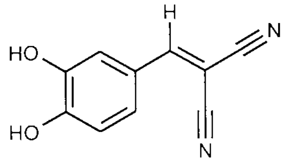
Preparation of (*E*)-2-cyano-3-(3,4-dichlorophenyl)prop-2-enthioamide p15



Using 3,4-dihydroxybenzaldehyde and cyanothioacetamide **method A** gave the title compound as an orange/brown solid (220mg, 100%). TLC [diethyl ether] R$_f$ 0.20; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.88 (d, 1H, *J*=8.3Hz, ArH-5), 7.30 (dd, 1H, *J*=8.5, 2.1Hz, ArH-6), 7.55 (d, 1H, *J*=2.1Hz, ArH-2), 7.94 (s, 1H, C$\underline{H}$=C), 9.39 (br s, 1H, NH), 9.91 (br s, 1H, NH), 10.25 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 107.48 (CH=$\underline{C}$), 116.33 (ArCH-5), 116.63 (ArCH-2), 117.33 (CN), 123.31 (ArC-1), 125.97 (ArCH-6), 146.00 (ArCOH-4), 148.27 ($\underline{C}$H=C), 151.15 (ArCOH-3), 161.19 ($CSNH_2$) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 107.50 (d, *J*=9.5Hz, CH=$\underline{C}$), 116.37 (d, *J*=159.7Hz, ArCH-5), 116.63 (ddd, *J*=157.9, 7.5, 6.6Hz, ArCH-2), 117.41 (d, *J*=13.9Hz, CN), 123.34 (d, *J*=9.0Hz, ArC-1), 126.07 (ddd, *J*=160.9, 7.1, 5.9Hz, ArCH-6), 146.07 (complex m, ArCOH-4), 148.28 (ddd, *J*=156.6, 4.8, 3.8Hz, $\underline{C}$H=C), 151.25 (dd, *J*=10.5, 6.7Hz, ArCOH-3), 161.20 (d, *J*=2.4Hz, $CSNH_2$) ppm; Mass spectrum (APCI$^+$): *m/z* 221M$^+$, 204[M-NH$_2$]$^+$; IR (KBr disc): υ_{max} 3374 (NH), 3182 (OH), 2212 (CN), 1635, 1521 (Ar), 1569 (C=SN), 1446 (OH), 1272 (amide), 1118 (C-O), 881 (2 adjacent Ar-H), 818 (C=CH) cm^{-1}.

Using 3,4-dihydroxybenzaldehyde and cyanothioacetamide **method B** gave a mixture of the title compound and piperidine-derived by-product (225mg) in the molar ratio (1:0.21).

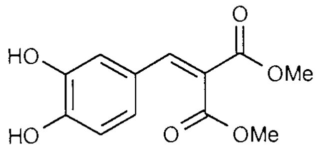
Preparation of 2-(3,4-dihydroxybenzylidene) malononitrile q15



Using 3,4-dihydroxybenzaldehyde and malononitrile **method A** gave the title compound as a beige crystalline solid (172mg, 87%). TLC [diethyl ether-petrol 40:60 (1:1)] R$_f$ 0.09; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.92 (d, 1H, *J*=8.4Hz, ArH-5) 7.33 (dd, 1H, *J*=8.5, 2.2Hz, ArH-6), 7.53 (d, 1H, *J*=2.3Hz, ArH-2), 8.20 (s, 1H, CH=C), 10.21 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 74.65 (CH=$\underline{C}$), 114.49 (CN_{trans}), 115.55 (CN_{cis}), 116.24 (ArCH-6), 116.39 (ArCH-5), 123.46 (ArC-1), 127.25 (ArCH-2), 146.25 (ArCOH-3), 153.56 (ArCOH-4), 160.84 ($\underline{C}$H=C) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 74.65 (s, CH=$\underline{C}$), 114.49 (d, *J*=14.2Hz, CN_{trans}), 116.23 (ddd, *J*=160.1,7.6, 6.2Hz, ArCH-6), 115.55 (d, *J*=8.4Hz, CN_{cis}), 116.39 (d, *J*=160.6Hz, ArCH-5), 123.46 (d, *J*=9.0Hz, ArCH-1), 127.25 (ddd, *J*=161.9, 8.3, 5.8Hz, ArCH-2), 146.29 (t, *J*=10.2Hz, ArCOH-3), 153.61 (complex m, ArCOH-4), 160.84 (ddd, *J*=162.0, 5.7, 4.5Hz, $\underline{C}$H=C) ppm; Mass spectrum (APCI$^+$): *m/z* 187M$^+$; IR data (KBr disc): υ_{max} 3460 (CH=C), 3286 (OH), 2235 (CN), 2233 (CN), 1615 (C=C-Ar), 1600, 1573, 1529 (Ar), 1403 (OH), 850 (2 adjacent Ar-H), 800 (CH=C) cm^{-1}.

Using 3,4-dihydroxybenzaldehyde and malononitrile **method B** gave a mixture of the title compound and piperidine-derived by-products (193mg) in molar ratio (1:0.09).

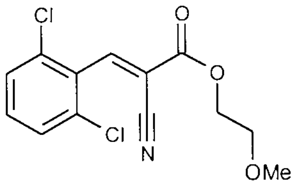
Preparation dimethyl-1,2-(3,4-dihydroxybenzylidene)malonate s15



Using 3,4-dihydroxybenzaldehyde dimethylmalonate **method A** gave the title compound as a beige solid (164mg, 65%).TLC [diethyl ether-petrol 40:60 (1:1)] R_f 0. 07; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 3.74 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.78 (d, 1H, *J*=8.8Hz, ArH-5), 7.24 (dd, 1H, *J*=3.6, 1.9Hz, ArH-6), 7.28 (d, 1H, *J*=2.0Hz, ArH-2), 7.52 (s, 1H, C$\underline{H}$=C), 9.75 (br s, 2H, 2xOH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 52.63 (CH_3), 52.92 (CH_3), 115.80 (ArCH-6), 116.18 (ArCH-5), 120.92 (CH=$\underline{C}$), 123.47 (ArC-1), 124.90 (ArCH-6), 142.65 ($\underline{C}$H=C), 146.35 (ArCOH-3), 149.84 (ArCOH-4), 164.67 (COO), 167.40 (COO) ppm; ^{13}C NMR Proton-Coupled (62.9MHz, $(CD_3)_2SO$): δ_C 52.69 (q, *J*=147.8Hz, CH_3) 53.00 (q, *J*=147.9Hz, CH_3), 115.82 (d, *J*=158.1Hz, ArCH-6), 116.21 (d, *J*=159.7Hz, ArCH-5), 120.90 (s, CH=$\underline{C}$), 123.48 (d, *J*=8.5Hz, ArC-1), 124.99 (dd, *J*=159.6, 6.5Hz, ArCH-6), 142.70 (ddd, *J*=156.8, 5.7, 4.8Hz, CH=C), 146.48 (d, *J*=6.7Hz, ArCOH-3), 149.90 (ddd, *J*=9.3, 8.6, 1.4Hz, ArCOH-4), 164.71 (quintet, *J*=3.8Hz, COO), 167.47 (quintet, *J*=12.3Hz, COO) ppm; Mass spectrum ($APCI^+$): *m/z* 253M$^+$, 221[M-OCH_3]$^+$, 189[M-2x(OCH_3)]$^+$, 161[M-OCH_3-$COOCH_3$]$^+$; IR (KBr disc): υ_{max} 3326 (OH), 1722 (COO), 1710 (COO), 1656 (C=C-Ar), 1594, 1526 (Ar), 1290 (OH), 1162 (C-OH), 815 (2 adjacent Ar-H) cm^{-1}.

Using 3,4-dihydroxybenzaldehyde dimethyl malonate **method B** gave a mixture of the title compound and by-products (181mg) of molar ratio (1:0.24).

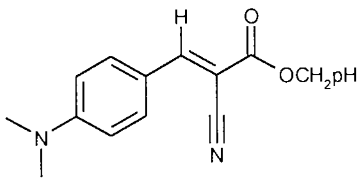
Attempted preparation of 2-methoxyethyl (*E*)-2-cyano-3-(2,6-dichlorophenyl)prop-2-enoate d46



Using 2,6-dichlorobenzaldehyde and 2-methoxyethyl 2-cyanoacetate **method A** gave a mixture of the title compound and transesterified product (286mg) in the molar ratio (1:15.89).

Using 2,6-dichlorobenzaldehyde and 2-methoxyethyl 2-cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterified product (301mg) in a molar ratio of (1:0.69:27.70).

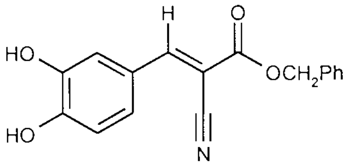
Attempted of preparation of benzyl-(*E*)-2-cyano-(4-dimethylaminophenyl) prop-2-enoate f7



Using 4-dimethylaminobenzaldehyde and benzyl cyanoacetate **method A** gave a mixture of the title compound and transesterified by-products (264mg) of molar ratio (1:0.31).

Using 4-dimethylaminobenzaldehyde and benzyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-products and transesterified by-products (311mg) of molar ratio (0:0.04:1).

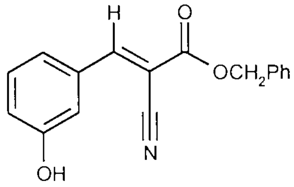
Attempted preparation of benzyl-(*E*)-2-cyano-(3,4-dihydroxyphenyl) prop-2-enoate f15



Using 3,4-dihydroxybenzaldehyde and benzyl cyanoacetate **method A** gave a mixture of the title compound and transesterified product (248mg) in the molar ratio (1:0.18).

Using 3,4-dihydroxybenzaldehyde and benzyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterified product (337mg) in a molar ratio of (1:0.65:0.93).

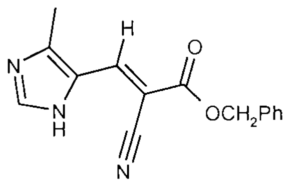
Attempted preparation of benzyl-(*E*)-2-cyano-(3-hydroxyphenyl) prop-2-enoate f17



Using 3-hydroxybenzaldehyde and benzyl cyanoacetate **method A** gave a mixture of the title compound and transesterified product (264mg) in the molar ratio (1:0.39).

Using 3-hydroxybenzaldehyde and benzyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterified product (284mg) in a molar ratio of (1:0.65:3.55).

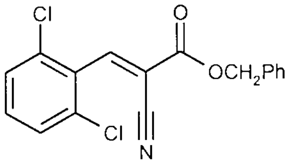
Attempted preparation of benzyl (*E*)-2-cyano-3-(5-methyl-1*H*-imidazol-4-yl)prop-2-enoate f24



Using 4-methyl-1*H*-imidazole-5-carbaldehyde and benzyl cyanoacetate **method A** gave a mixture of the title compound and transesterified product (220mg) in the molar ratio (1:0.08).

Using 4-methyl-1*H*-imidazole-5-carbaldehyde and benzyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterified product (242mg) in a molar ratio of (1:0.18:1.96).

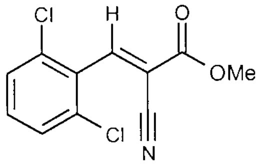
Attempted preparation of benzyl (*E*)-2-cyano-3-(2,6-dichlorophenyl)prop-2-enoate f46



Using 2,6-dichlorobenzaldehyde and benzyl cyanoacetate **method A** gave a mixture of the title compound and transesterified product (164mg) in the molar ratio (1:3.67).

Using 2,6-dichlorobenzaldehyde and benzyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterified product (172mg) in a molar ratio of (1:0.43:4.28).

Attempted preparation of methyl-(*E*)-2-cyano-(2,6-dichlorophenyl)prop-2-enoate i46



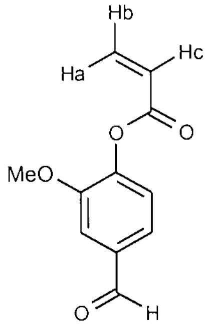
Using 2,6-dichlorobenzaldehyde and methyl cyanoacetate **method A** gave a mixture of the title compound and transesterified product (243mg) in the molar ratio (1:2.91).

Using 2,6-dichlorobenzaldehyde and methyl cyanoacetate **method B** gave a mixture of the title compound, piperidine-derived by-product and transesterified product (248mg) in a molar ratio of (1:0.03:1).

8.10 Solution-phase polymer chemistry experimental

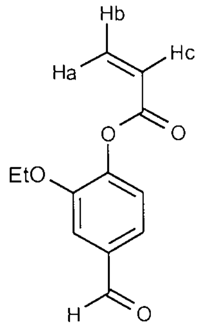
Unless otherwise stated the maximum loading of the polymers is based on the ratio of monomers used to prepare the initial polymer.

Preparation of 4-formyl-2-methoxyphenyl acrylate 58



3-Methoxy-4-hydroxybenzaldehyde (70mmol, 10.64g) was dissolved in anhydrous THF (80ml) under argon and TEA (12ml) added. A solution of acryloyl chloride (75mmol, 6.788g) in anhydrous THF (10ml) was added to aldehyde mixture slowly at 0°C. A white precipitate formed directly and the reaction was stirred at 0°C for 5 hours with constant monitoring by TLC. The white precipitate of triethylamine hydrochloride was removed by filtration. The remaining mixture was poured onto cold methanol and stirred for 2 hours at 0°C. THF and methanol were removed under at ambient temperature. The crude mixture was subjected to flash column chromatography eluted with diethyl ether-petrol 60:80 (2:1) to give the title compound as a white solid (7.07g, 41%), stored below 5°C. TLC [petrol 60:80-diethyl ether (2:1)] R_f 0.28; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 3.91 (s, 3H, OCH_3), 6.08 (dd, 1H, J=10.4, 1.3Hz, Hb), 6.37 (dd, 1H, J=17.3, 10.4Hz, Hc), 6.66 (dd, 1H, J=17.3, 1.3Hz, Ha), 7.28 (d, 1H, J=8.2Hz, ArH-5), 7.49 (d, 1H, J=1.8Hz, ArH-2), 7.50 (dd, 1H, J=8.4, 1.8Hz, ArH-6), 9.97 (s, 1H, CHO) ppm; Mass spectrum (APCI)$^-$: m/z 151[M-CH_2=CHCO]$^-$; Mass spectrum (APCI)$^+$: m/z 207M$^+$, 153[M-CH_2=CHCO]$^+$.

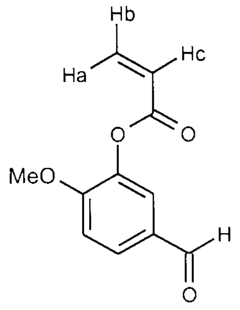
Preparation of 2-ethoxy-4-formylphenyl acrylate 59



3-Ethoxy-4-hydroxybenzaldehyde (20mmol, 5.05g) was dissolved in anhydrous THF (20ml) under argon and TEA (5ml) was added. A solution of acryloyl chloride (22mmol, 1.991g) in anhydrous THF (10ml) was added slowly at 0°C. A white precipitate formed directly and the reaction was stirred at 0°C for 5 hours with constant monitoring by TLC. The white precipitate of triethylamine hydrochloride was removed by filtration. The remaining mixture was poured onto cold methanol (600ml) and stirred for 2 hours at 0°C. THF and methanol were removed under vacuum at ambient temperature to give the title compound as a white solid (4.348g, 99%), which was stored below 5°C. TLC [petrol 60:80-diethyl ether (2:1)] R_f 0.30; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.42 (t, 3H, J=7.0Hz, CH_3), 4.16 (q, 2H, J=7.0Hz, CH_2), 6.08 (dd, 1H, J=10.4, 1.3Hz, Hb), 6.39 (dd, 1H, J=17.2, 10.4Hz, Hc), 6.67 (dd, 1H, J=17.2, 1.3Hz, Ha), 7.29 (dd, 1H, J=4.2, 3.3Hz, ArH-6), 7.49-7.52 (ov. m, 2H, ArH-2/5), 9.97 (s, 1H, CHO) ppm. Mass spectrum (APCI)$^-$: m/z 165[M-CH_2=CHCO]$^-$; Mass spectrum (APCI$^+$): m/z 221M$^+$, 167[M-CH_2=CHCO]$^+$.

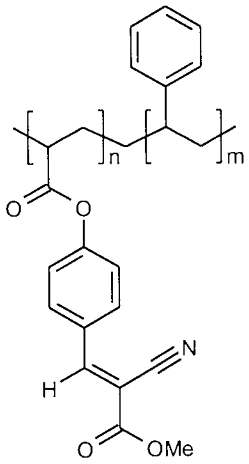
Preparation of **59** was repeated as above on a 68mmol scale. The final compound was subjected to flash column chromatography, eluted with petrol 60:80-diethyl ether (1:1). The title compound was collected as a white solid (10.53g, 62%). ^{1}H NMR and mass spectrum (APCI$^+$) were the same as for the previous batch.

Preparation of 5-formyl-2-methoxyphenyl acrylate 60



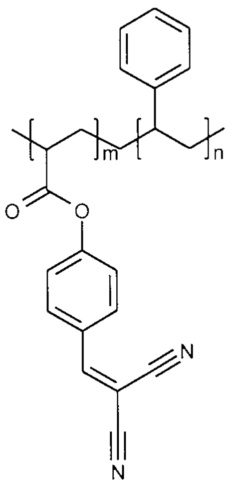
4-Methoxy-3-hydroxybenzaldehyde (20mmol, 5.05g) was dissolved in anhydrous THF (20ml) under argon and TEA (5ml) was added. A solution of acryloyl chloride (22mmol, 1.991g) in anhydrous THF (10ml) was added slowly at 0°C. A white precipitate formed directly and the reaction was stirred at 0°C for 5 hours with constant monitoring by TLC. The white precipitate of triethylamine hydrochloride was removed by filtration. The remaining mixture was poured onto cold methanol and stirred for 2 hours at 0°C. THF and methanol were removed under vacuum without heating to give the title compound as a pale yellow solid (3.978g, 97%), which was stored below 5°C. TLC [petrol 60:80-diethyl ether (2:1)] R_f 0.30; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 3.95 (s, 3H, OCH_3), 6.89 (dd, 1H, *J*=10.4, 1.3Hz, Hb), 6.39 (dd, 1H, *J*=17.3, 10.4Hz, Hc), 6.67 (dd, 1H, *J*=17.3, 1.3Hz, Ha), 7.13 (d, 1H, *J*=8.5Hz, ArH-5), 7.66 (d, 1H, *J*=2.0Hz, ArH-2), 7.82 (dd, 1H, *J*=8.4, 2.0Hz, ArH-6), 9.91 (s, 1H, CHO) ppm; Mass spectrum (APCI)$^-$: m/z 151[M-CH_2=CHCO]$^-$; Mass spectrum (APCI)$^+$: *m/z* 207M$^+$, 153[M-CH_2=CHCO]$^+$.

Preparation of co-polymer 63



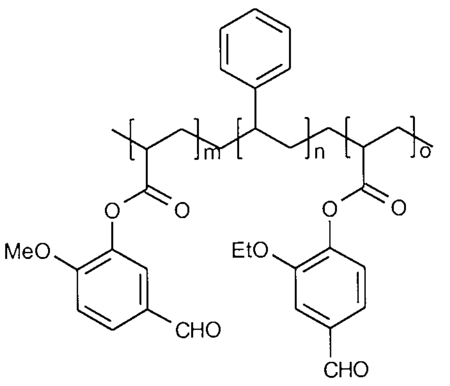
The co-polymer **61** (5g, maximum loading 1.69mmol/g) was partially dissolved in toluene (60ml). Methyl cyanoacetate (10mmol, 990mg) and piperidine (2 drops) were added and the reaction was heated under reflux for two hours. The soluble polymer was poured onto methanol (600ml) and the precipitate was collected along with the undissolved polymer precipitate by filtration. The polymer was dried under vacuum to give a pale yellow solid (4.954g). ^{1}H NMR analysis showed the pendant aldehyde functionality to be incompletely reacted and the resulting polymer was found to be only sparingly soluble in DCM, $CHCl_3$, THF and toluene.

Preparation of co-polymer 65



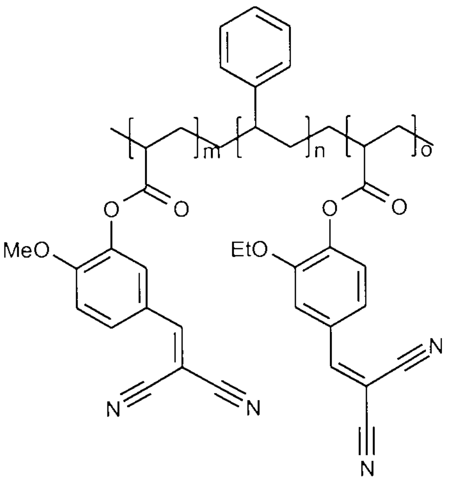
The co-polymer **61** (5.5g, maximum potential loading 1.69mmol/g) was partially dissolved in toluene (60ml). Malononitrile (15mmol, 990mg) and piperidine (2 drops) were added and the reaction was heated under reflux for two hours. The soluble polymer was poured onto methanol (600ml) and the resulting precipitate was collected with the undissolved polymer precipitate by filtration. The polymer was dried under vacuum to give a beige solid (5.304g). The resulting polymer was completely insoluble in DCM, $CHCl_3$, THF and toluene. Due to its solubility the polymer could not be analysed by ^1H NMR.

Preparation of the co-polymer of 5-formyl-2-methoxyphenyl acrylate, 2-ethoxy-4-formylphenyl acrylate and styrene 66



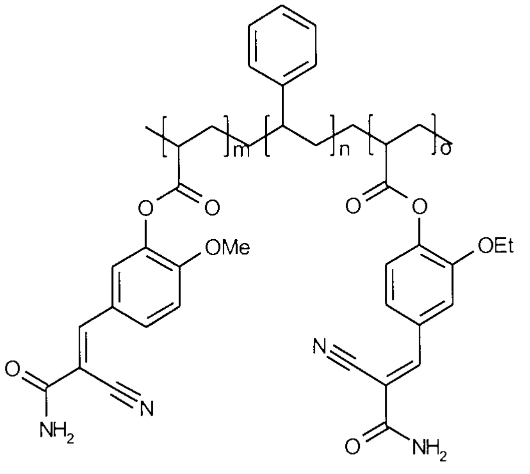
Styrene (150mmol, 15.60g) was added to a solution of **59** (15mmol, 3.3g) and **60** (15mmol, 3.09g) in toluene (100ml). The reaction vessel was covered in foil and AIBN (2% by weight, 506mg) was added. Air was removed by alternate application of vacuum and argon. The mixture was then heated at 60°C for 5 days. The polymer was poured onto methanol (600ml) and the precipitate collected by filtration. The title polymer was dried under vacuum to give a pale yellow solid (19.151g, 87%). ^1H NMR (250.1MHz, $CDCl_3$): δ_H 0.95-2.10 (br m, polymer back-bone CH_2), 3.55 (br d, polymer OCH_3), 3.95 (br s, polymer CH_2), 6.4-7.40 (br m, polymer backbone ArH), 7.75 (br s, polymer ArH), 9.8 (br s, polymer CHO), 9.95 (br s, polymer CHO) ppm; IR (KBr disc): υ_{max} 3080, 3024 (ArH), 2930 (CH_2/CH_3), 2860 (CH/OCH_3), 1760 (COOAr), 1690 (CHO), 1603 (ArH), 1498 (ArH), 1450 (CH_2), 1390 (OCH_3), 1280, 1100, 1020 (CO), 760, 700 (5 adjacent ArH) cm^{-1}.

Preparation of co-polymer 67



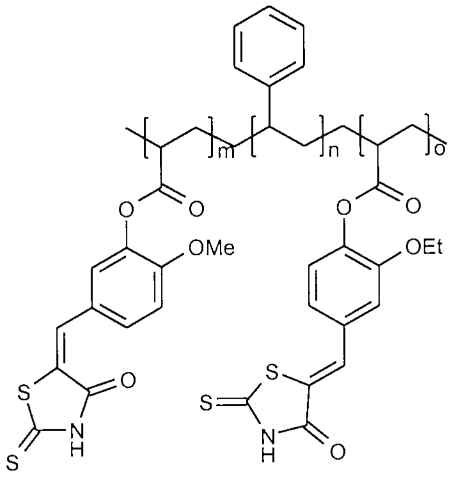
Piperidine (2 drops) and malononitrile (20.4mmol, 1.346g) were added to a solution of polymer **66** (10g, maximum potential polymer loading 1.36mmol/g) in toluene (120ml). The reaction was heated under reflux for 2 hours. The polymer was poured onto methanol (500ml) and the resulting polymer precipitate was collected by filtration, dissolved and precipitated a further three times. The resulting polymer was dried under vacuum to give the title polymer as a beige solid (8.983g, 84% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.95-2.10 (br m, polymer backbone CH_2), 3.55 (br s, polymer OCH_3), 3.95 (br s, polymer $C\underline{H}_2CH_3$), 6.40-7.20 (br m, polymer backbone ArH), 7.55 (br s, polymer ArH pendant), 7.7 (br s, polymer ArH pendant), 7.95 (br s, polymer pendant CH=) ppm.

Preparation of co-polymer 68



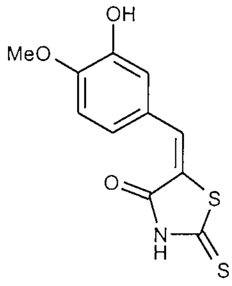
Piperidine (2 drops) and cyanoacetamide (10mmol, 840mg) was added to a solution of the polymer **66** (5g, maximum potential loading 1.34mmol/g) in toluene (100ml). The mixture was heated under reflux for two hours. During the heating the polymer became insoluble forming a beige solid. The reaction mixture was cooled, the solid was collected by filtration and then washed with methanol (3x100ml). The dry polymer was only sparingly soluble in DCM, $CHCl_3$, THF and toluene. ^{1}H NMR analysis showed that there were still aldehyde functionalities left unreacted on the resultant co-polymer.

Preparation of co-polymer 69

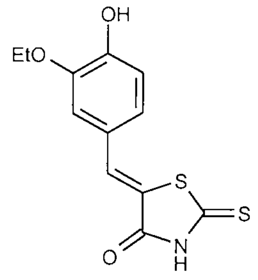


Piperidine (2 drops) and rhodanine (8mmol, 1.064g) were added to a solution of polymer **66** (3.6g, maximum potential loading 1.36mmol/g) in toluene (100ml). The reaction mixture was heated under reflux for three hours and then allowed to cool. The resulting polymer was poured onto vigorously stirred methanol (400ml). The resulting yellow precipitate was collected by filtration, dissolved and precipitated a further three times and then dried under vacuum. The title polymer was collected as a bright yellow solid (3.750g, 90% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, CDCl$_3$): δ_H 0.90-2.10 (br m, polymer CH$_2$), 3.60 (br s, pendant OCH$_3$), 3.95 (br s, pendant OC$\underline{H}_2$CH$_3$), 6.40-7.30 (br m, polymer and pendant ArH), 7.50 (br s, pendant ArH) ppm; IR (KBr disc): υ_{max} 3200 (CONH/CSNH), 3060, 3025 (ArH), 2921 (CH$_2$/CH$_3$), 2865 (CH/OCH$_3$), 1761 (COOAr), 1726 (CONH), 1598 (ArH), 1504 (ArH), 1454 (CH$_2$/CH$_3$), 1267 (CO), 1213 (CO/CS), 1118 (CO), 1070 (CO/S-CS-N), 760, 700 (5 adjacent ArH) cm^{-1}.

Preparation of the mixture 5-[(3-hydroxy-4-methoxyphenyl)methylidene]-2-thioxothiazolidine-4-one 70 and 5-[(4-hydroxy-3-ethoxyphenyl)methylidene]-2-thioxothiazolidine-4-one 71



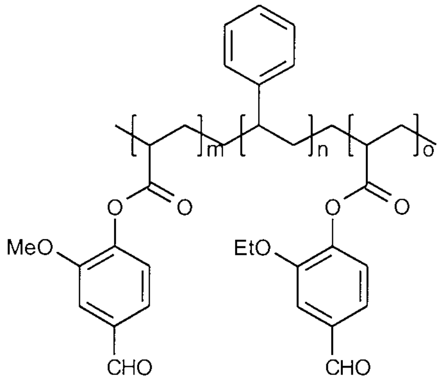
70



71

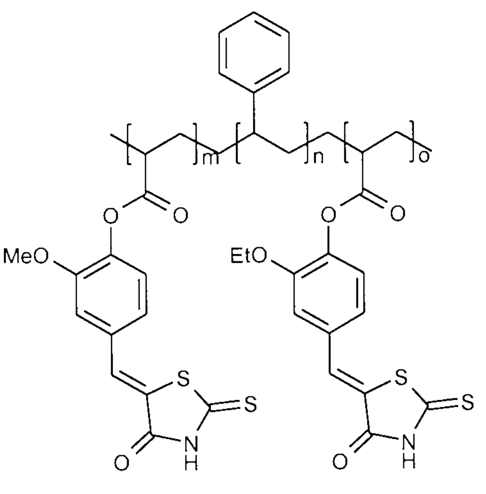
n-Propylamine (4ml) was added to a solution of polymer **69** (3.2g, maximum potential polymer loading 1.36mmol/g) in DCM (50ml). The reaction mixture was stirred for 16 hours and then concentrated under vacuum. The resultant residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (1:1). The title compounds were collected together to give a yellow solid [260mg, ratio of **70** to **71** from ^{1}H NMR (0.89:1), minimum loading 0.30mmol/g, 22% based on the maximum potential polymer loading]. TLC [diethyl ether-petrol 60:80 (1:1)] R$_f$ **70**-0.13, **71**-0.21; ^{1}H NMR (250.1MHz, CDCl$_3$) δ_H 1.54 (t, 3H, *J*=1.0Hz, **71**-OCH$_2$C$\underline{H}_3$), 4.00 (s, 3H, **70**-OCH$_3$), 4.23 (q, 2H, *J*=6.8Hz, **71**-OC$\underline{H}_2$CH$_3$), 6.15 (br s, 2H, **70** and **71**-NH), 6.96 (d, 1H, *J*=1.9Hz, **71**-ArH-3), 7.00 (d, 1H, *J*=9.6Hz, **70**-ArH-6), 7.06-7.13 (ov. m, 4H, **70** and **71**-ArH), 7.60 (s, 1H, **70**-CH=), 7.62 (s, 1H, **71**-CH=), 9.28 (br s, 2H, OH) ppm; Mass spectrum (APCI)$^+$: *m/z* 307M**70**$^+$, 321M**71**$^+$.

Preparation of co-polymer 72



Styrene (200mmol, 20.8g) was added to a solution of **59** (25mmol, 5.5g) and **58** (25mmol, 5.15g) in toluene (100ml). The reaction vessel was covered in foil and AIBN (2% by weight, 629mg) was added. Air was removed by the alternate application of vacuum and argon, and the mixture was heated at 60°C for 72 hours. The polymer was poured onto methanol (600ml) and the resulting precipitate was collected by filtration. The title polymer was dried under vacuum to give a white solid (27.334g, 87%, maximum potential loading 1.59mmol/g). ^{1}H NMR (250.1MHz, CDCl$_3$): δ_H 0.95-2.10 (br m, polymer back-bone CH$_2$), 3.55 (br d, polymer OCH$_3$), 3.95 (br s, polymer CH$_2$), 6.40-7.40 (br m, polymer backbone ArH), 7.75 (br s, polymer ArH), 9.95 (br s, polymer CHO) ppm; IR (KBr disc): υ_{max} 3080, 3024 (ArH), 2925 (CH$_2$/CH$_3$), 2835 (CH/OCH$_3$), 1756 (COOAr), 1695 (CHO), 1603 (ArH), 1498 (ArH), 1450 (CH$_2$), 1390 (OCH$_3$), 1273 1200, 1112, 1020 (CO), 755, 696 (5 adjacent ArH) cm^{-1}.

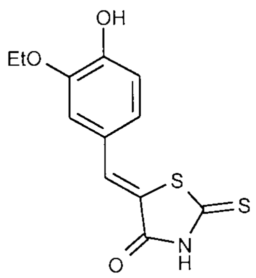
Preparation of co-polymer 73



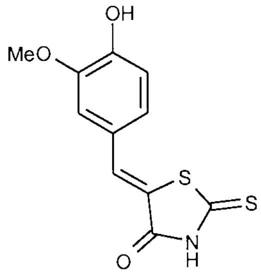
Method A: Piperidine (6 drops) and rhodanine (11.925mmol, 1.586g) were added to a solution of polymer **72** (5g, maximum potential polymer loading 1.59mmol/g) in toluene (100 ml). The reaction was heated under reflux for 2 hours. The polymer was poured onto methanol (500ml) and the resulting polymer precipitate was collected by filtration, and then re-dissolved and precipitated a further three times. The resulting polymer was dried under vacuum to give the title polymer as a yellow solid (4.667g, 75% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.95-2.10 (br m, polymer backbone CH_2), 3.60 (br d, polymer OCH_3), 3.95 (br s, polymer CH_2), 6.50-7.40 (br m, polymer backbone ArH), 7.85 (br s, polymer ArH) ppm; IR (KBr disc): υ_{max} 3200 (CONH/CSNH), 3080, 3028 (ArH), 2921 (CH_2/CH_3), 2865 (CH/OCH_3), 1761 (COOAr), 1726 (CONH), 1604, 1504 (ArH), 1454 (CH_2/CH_3), 1267 (CO), 1213 (CO/CS), 1118 (CO), 1070 (CO/S-CS-N), 765, 702 (5 adjacent ArH) cm^{-1}.

Preparation of **73** was repeated using co-polymer **72** on a 6g scale. The title polymer was collected as a bright yellow solid (6.749g, 95% based on the maximum potential polymer loading). ^{1}H NMR was the same as the previous batch.

Preparation of the mixture 5-[(3-ethoxy-4-hydroxyphenyl)methylidene]-2-thioxothiazolidine-4-one 71 and 5-[(3-methoxy-4-hydroxyphenyl)methylidene]-2-thioxothiazolidine-4-one 74



71

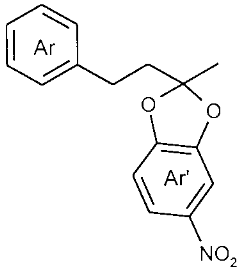


74

n-Propylamine (1ml) was added to a solution of the polymer **73** (4g, maximum potential polymer loading 1.59mmol/g) in DCM (30ml). After 18 hours the solution was concentrated under vacuum and subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (1:1) The two desired fractions were collected together to give a yellow solid 357mg, ratio of **74** to **71** from ^{1}H NMR analysis (1.11:1), minimum loading 0.33mmol/g, 21% yield based on the maximum potential polymer loading]. TLC [diethyl ether-petrol 60:80 (1:1)] R_f **74**-0.16, **71**-0.25; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.54 (t, 3H, *J*=1.0Hz, **71**-$OCH_2C\underline{H}_3$), 3.39 (s, 3H, **74**-OCH_3), 4.23 (q, 2H, *J*=6.8Hz, **71**-$OC\underline{H}_2CH_3$), 6.09 (br s, 1H, **74**-NH), 6.15 (br s, 1H, **71**-NH), 6.94-6.98(ov. m, 2H, **74**-ArH-3, **71**-ArH-3), 7.00-7.12 (ov. m, 4H, **74** and **71** ArH), 7.61 (s, 1H, **74**-CH=), 7.62 (s, 1H, **71**-CH=), 9.53 (br s, 2H, OH) ppm. Mass spectrum (APCI$^+$): *m/z* 307M**74**$^+$, 321M**71**$^+$.

8.11 Catechol derivatives

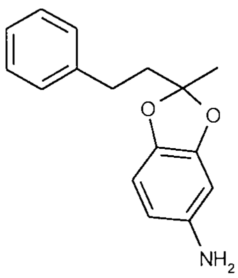
Preparation of 2-methyl-5-nitro-2-phenethyl-1,3-benzodioxole 84



4-Phenylbutan-2-one (155mmol, 22.94g) and *p*-TsOH (7.5mmol, 1.425g) were added to a solution of 4-nitrocatechol (150mmol, 23.25g) in toluene (400ml). The mixture was heated under reflux in conjunction with in conjunction with a Dean-Stark trap for 72 hours. The reaction was monitored by TLC and after the disappearance of the starting catechol, the reaction was allowed to cool to room temperature and the toluene was removed under vacuum. The remaining brown residue was dissolved in DCM (500ml) and washed with 0.5M sodium hydroxide (3x500ml) to remove any remaining catechol and *p*-TsOH. The organic layer was collected and dried with anhydrous magnesium sulphate. The DCM was removed under vacuum and a yellow solid was precipitated from toluene. The title compound was collected by filtration and washed with toluene to give a yellow solid (29.000g, 68%). TLC [petrol 60:80-diethyl ether] R_f 0.42; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.73 (s, 3H, CH_3), 2.28-2.35 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 2.69-2.75 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 7.08 (d, 1H, *J*=8.7Hz, Ar'H-6), 7.15-7.30 (ov. m, 5H, ArH), 7.70 (d, 1H, *J*=2.4Hz, Ar'H-2), 7.87 (dd, 1H, *J*=8.7, 2.4Hz, Ar'H-5) ppm; Mass spectrum (APCI$^+$): m/z 286M$^+$; IR (KBr disc): υ_{max} 2937 (CH_2/CH_3), 1604 (Ar), 1515 ($C-NO_2$), 1450 (CH), 1382 (CH_3), 1334 ($C-NO_2$), 742, 708 (5 adjacent ArH), 865 (isolated ArH), 819 (2 adjacent ArH) cm^{-1}.

The preparation of 2-methyl-5-nitro-2-phenethyl-1,3-benzodioxole was repeated a further six times in a range of scales 10-50mmol, giving yields in the range of 52 to 85%. TLC, ^{1}H NMR and mass spectra were as for the previous batch.

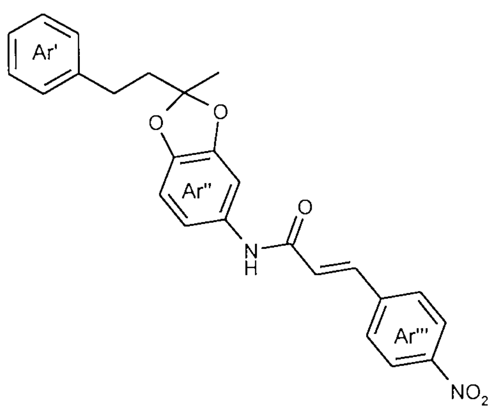
Attempted preparation of 2-methyl-2-phenethyl-1,3-benzodioxol-5-amine 85



Method A: A solution of sodium dithionite (15.6mmol, 3eq, 2.714g) in water (100ml) was added to a solution of **84** (5.2mmol, 1.496g) in ethanol (100ml). The reaction mixture was heated at 65°C and the reaction was monitored by TLC [petrol 60:80-diethyl ether]. There was no change in the TLC after 18 hours.

Method B: A solution of sodium dithionite (15mmol, 2.610g) in water (100ml) was added to a solution of **84** (5.0mmol, 1.425g) in ethanol (100ml). The reaction mixture was heated under reflux and the reaction was monitored by TLC. After 30 minutes a new spot started to appear at a lower R_f value from that of the starting material. The pH of the solution was tested and found to be at pH 7. After 24 hours, TLC showed that there was still starting material present together with the new spot. The reaction was allowed to cool and ethanol (50ml) was added to the mixture and then a further solution of sodium dithionite (15mmol, 2.610g) in water (50ml) was added. The mixture was heated under reflux for a further 18 hours. When only the new spot was visible by TLC, the yellow mixture was concentrated under vacuum to approximately half of its original volume. The remaining mixture was poured on to ice (100g). A white precipitate formed, which turned into a brown oil when filtration was attempted. The reaction mixture was partitioned between water and ethyl acetate and the organic layer was collected, dried with anhydrous magnesium sulphate and concentrated under vacuum to give a brown residue. Analysis by TLC showed many spots and suggested that the title compound was unstable and had been degraded by air. TLC of new spot before decomposition [diethyl ether-petrol 40:60 (1:1)] R_f 0.29.

Preparation of (*E*)-*N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-3-(4-nitrophenyl)prop-2-enamide 86



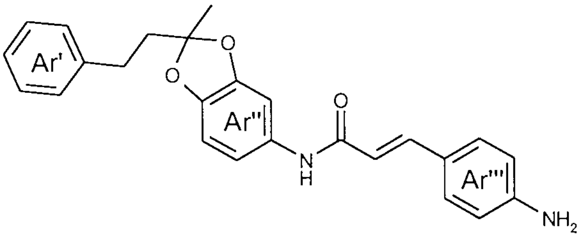
Method A: A solution of sodium dithionite (35mmol, 6.090g) in water (100ml) was added to a solution of **84** (3.5mmol, 997.5mg) in ethanol (100ml). The reaction was heated under reflux for 24 hours and the solvents were removed under vacuum. The resulting solid was dissolved in water and extracted into diethyl ether (3x100ml) under an argon atmosphere. The organic layer was removed, under argon, by a cannula to a new dry vessel charged with anhydrous magnesium sulphate. The dried organic layer was then removed, via cannula, under argon, to another new dry vessel charged with TEA (1ml). A solution of 4-nitrocinnamoyl chloride (4.2mmol, 886mg) in anhydrous THF (10ml) was slowly added to the reduced mixture and stirred for 16 hours. The reaction mixture was poured onto ice (100g), and the organic compounds were extracted into diethyl ether (3x100ml). The organic layer was combined and washed with 0.5M NaOH (3x200ml). The organic layer was then separated and dried with anhydrous magnesium sulphate. The crude brown residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 40:60 (1:2). The title compound was dried under vacuum and collected as a bright orange solid (238mg, 16%). TLC [diethyl ether-petrol 40:60 (1:2)] R_f 0.22. ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.64 (s, 3H, CH_3), 2.26 (m, 2H, $C_6H_5C\underline{H_2}CH_2$), 2.69 (m, 2H, $C_6H_5CH_2C\underline{H_2}$), 6.83 (d, 1H, *J*=8.4Hz, Ar"H-5), 6.96 (d, 2H, *J*=15.7Hz, CH=), 7.05 (dd, 1H, *J*=8.1, 1.2Hz, Ar"H-6), 7.18-7.28 (ov. m, 5H, 5xAr'H), 7.36 (d, 1H, *J*=1.8Hz, Ar"H-2), 7.67 (d, 1H, *J*=15.7Hz, CH=C), 7.88 (d, 2H, *J*=8.9Hz, 2xAr'"H), 8.29 (d, 2H, *J*=8.9Hz, 2xAr'"H), 10.24 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 431[M+32]$^+$; IR (KBr disc): υ_{max} 3323, 3246 (CONH), 3060 (CONH/ArH), 2931 (CH_2/CH_3), 1660 (CONH), 1600 (ArH), 1533 (CONH), 1498 (ArH), 1460 (CH_2/CH_3), 1215 (Ar-O-C), 977 (CH=CH), 829 (2 adjacent ArH), 702 (5 adjacent ArH) cm^{-1}.

The preparation of (*E*)-*N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-3-(4-nitrophenyl)prop-2-enamide was repeated a further three times in a range of scales 5 to 10mmol, giving yields in the range of 10 to 31%. TLC, ^{1}H NMR and mass spectra were as for the previous batch.

Method B: 10% Pd-C (185mg) was added to a solution of **84** (10mmol, 1.85g) in THF (100ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously for 16 hours. The hydrogen was then replaced with argon and TEA (3ml) was added. 4-Nitro-*trans*-cinnamoyl chloride (12mmol, 2.544g) was dissolved in anhydrous DMF (10ml) and slowly added to the reduced mixture and allowed to stir for 16 hours. The solids, 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite, and the THF and DMF were removed under vacuum. The resulting orange/brown residue was dissolved in ethyl acetate (300ml) and washed with water (3x150ml). The organic layer was dried with anhydrous magnesium sulphate. The title compound was recrystallised from ethyl acetate as a bright orange solid (3.130g, 73%). TLC [ethyl acetate] R_f 0.60; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.64 (s, 3H, CH_3), 2.26 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 2.69 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 6.83 (d, 1H, *J*=8.4Hz, Ar"H-5), 6.97 (d, 1H, *J*=15.7Hz, CH=), 7.23 (dd, 1H, *J*=8.3, 1.7Hz, Ar"H-6), 7.15-7.30 (ov. m, 5H, 5xAr'H), 7.36 (d, 1H, *J*=1.8Hz, Ar"H-2), 7.67 (d, 1H, *J*=15.7Hz, CH=C), 7.88 (d, 2H, *J*=8.9Hz, 2xAr'"H), 8.29 (d, 2H, *J*=8.9Hz, 2xAr'"H), 10.24 (br s, 1H, NH) ppm; ^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 1.68 (s, 3H, CH_3), 2.22-2.29 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 2.76-2.83 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 6.62 (d, 1H, *J*=15.5Hz, CH=C), 6.85 (dd, 1H, *J*=8.6, 1.7Hz, Ar"H-6), 6.72 (d, 1H, *J*=8.4Hz, Ar"H-5), 7.21-7.35 (ov. m, 7H, 5xArH, Ar"H-2, NH), 7.68 (d, 2H, *J*=8.7Hz, 2xAr"H), 7.79 (d, 1H, *J*=15.5Hz, CH=C), 8.26 (d, 2H, *J*=8.7Hz, 2xAr'"H) ppm; Mass spectrum (APCI$^+$): *m/z* 431[M+32]$^+$; IR (KBr disc): υ_{max} 3398 (CONH), 3068 (CONH/ArH), 2927 (CH_2/CH_3), 2848 (CH), 1666 (CONH), 1631 (C=C), 1606 (Ar), 1498 (NO_2), 1515 (CONH), 1460 (CH_2/CH_3), 1346 (Ar-O-C), 989 (CH=CH), 844 (2 adjacent ArH), 711 (5 adjacent ArH) cm^{-1}.

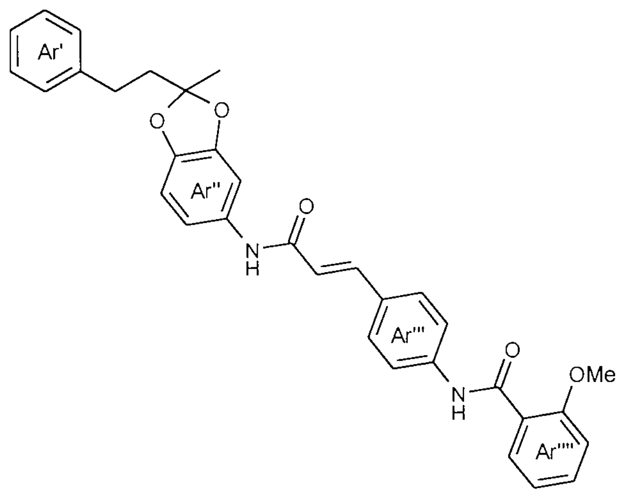
Preparation of (*E*)-3-(4-aminophenyl)-*N*-(2-methyl-2-phenyl-1,3-benzodioxol-5-yl)prop-2-enamide was repeated again on a 20mmol scale to give a 92% yield.

Preparation of (*E*)-3-(4-aminophenyl)-*N*-(2-mehyl-2-phenyl-1,3-benzodioxol-5-yl)prop-2-enamide 87



A solution of sodium dithionite (5mmol, 870mg) in water (40ml) to a solution of **86** (0.5mmol, 230mg) in ethanol (40ml). The reaction mixture was heated under reflux and was monitored by TLC. After 48 hours when all the starting material had been transformed the solvents were removed under vacuum. The resulting solid was dissolved in water (200ml) and extracted into DCM (3x100ml). The organic layer was collected then dried with anhydrous magnesium sulphate and concentrated under vacuum. The title compound was collected as an orange solid and dried under vacuum (80mg). TLC [diethyl ether] R_f 0.44; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.63 (s, 3H, CH_3), 2.12-2.30 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 2.66-2.75 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 5.66 (br s, 2H, NH_2), 6.41 (d, 1H, *J*=15.8Hz, CH=), 6.57 (d, 2H, *J*=8.5Hz, 2xAr'"H), 6.78 (d, 1H, *J*=8.3Hz, Ar"H-5), 6.98 (dd, 1H, *J*=8.3, 1.7Hz, Ar"H-6), 7.18-7.28 (ov. m, 5H, 5xAr'H), 7.35 (d, 1H, *J*=1.8Hz, Ar"H-2), 7.37 (d, 1H, *J*=15.7Hz, CH=C), 7.68 (d, 2H, *J*=8.5Hz, 2xAr"H), 9.85 (br s, 1H, NH) ppm. After 24 hours exposure to the air, the orange solid has turned to a brown solid and the TLC had changed from one to 6 spots, suggesting that the compound decomposed in air.

Attempted preparation of *N*-{4[(*E*)-3-(2-methyl-2-phenyl-1,3-benzodioxol-5-ylamino)-3-oxopro-1-enyl]phenyl}-2-methoxybenzamide 88

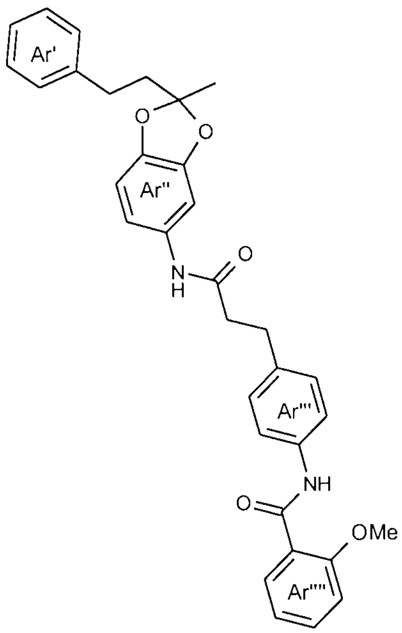


Method A: A solution of sodium dithionite (11mmol, 1.914g) in water (50ml) was added to a solution of **86** (1.10mmol, 469mg) in ethanol (50ml). The reaction mixture was heated under reflux and was monitored by TLC. After 48 hours the starting material was no longer present and the ethanol and water was removed under vacuum. The remaining organic component was dissolved in diethyl ether and the inorganic components were dissolved in water, under argon. A cannula was used to transfer the diethyl ether layer to a dry flask charged with anhydrous magnesium sulphate under argon. The resultant dry diethyl ether layer was transferred by cannula, under argon, to a final dry flask charged with TEA (1ml). A solution of 2-methoxybenzoyl chloride (2mmol, 341mg) in anhydrous DMF (10ml) was added slowly to the reduced mixture and allowed to stir for 18 hours. The mixture was concentrated under vacuum and re-dissolved in ethyl acetate (100ml). The mixture was poured directly onto ice (100g) and extracted into ethyl acetate (3x100ml). The organic layer was collected, dried with anhydrous magnesium sulphate and then concentrated under vacuum. The crude residue collected was analysed by TLC and ^1H NMR showed that the main component present was 2-methoxybenzoic acid. Isolation of the desired title compound was not attempted.

Method B: **86** was reduced as above using **86** (5.26mmol, 2.262g), sodium dithionite (52.6mmol, 9.152g). The crude reduced product was partitioned between water and diethyl ether under argon. The organic layer was transferred via a cannula to a clean dry vessel charged with anhydrous Magnesium sulphate, under argon. The dried organic layer was then transferred via cannula to another dry vessel charged with TEA (2ml). A solution of 2-methoxybenzoyl chloride (6mmol, 1.024g) in anhydrous THF (10ml) was added slowly to the reduced mixture and stirred for 16 hours. The crude mixture was poured onto ice (100g) and extracted into diethyl ether (3x200ml). The organic layer was dried with anhydrous magnesium sulphate and then concentrated under vacuum. The crude residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 40:60 (1:1) to give the title compound as a yellow solid (268mg, 10%). TLC [diethyl ether] R_f 0.33; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.64 (s, 3H, CH_3), 2.19-2.26 (m, 2H, $C_6H_5CH_2C\underline{H_2}$), 2.69-2.76 (m, 2H, $C_6H_5C\underline{H_2}CH_2$), 3.89 (s, 3H, OCH_3) 6.71 (d, 1H, *J*=15.7Hz, CH=C), 6.81 (d, 1H, *J*=8.4Hz, Ar”H-5), 7.03 (d, 2H, *J*=8.7Hz, 2xAr’”H), 7.09 (dd, 1H, 8.3, 1.1Hz, Ar”H-6), 7.18-7.31 (ov. m, 5H, 5xAr’H), 7.37 (d, 1H, *J*=2.0Hz, Ar”H-2), 7.49-7.65 (ov. m, 5H, CH=/4xAr””H), 7.82 (d, 2H, *J*=8.7Hz, 2xAr’”H), 10.08 (Br s, 1H, NH), 10.33 (Br s, 1H, NH) ppm; Mass spectrum ($APCI^+$): *m/z* $535M^+$; IR (KBr disc): υ_{max} 3309, 3239 (CONH), 3060 (CONH/ArH), 2931 (CH_2/CH_3), 1658 (CONH), 1599 (ArH), 1533 (CONH), 1498 (ArH), 1460 (CH_2/CH_3), 1215 (Ar-O-C), 977 (CH=CH), 829 (2 adjacent ArH), 754, 702 (5 adjacent ArH) cm^{-1}.

Method C: **86** (8mmol, 3.44g) was added to a 1M solution of tin (II) chloride dihydrate in DMF (100ml) and heated at 60°C until starting material disappeared by TLC. After 18 hours 4A$^{\circ}$ molecular sieves and TEA (5ml) were added to the reduced mixture. 2-Methoxybenzoyl chloride (10mmol, 1.706g) was mixed with anhydrous DMF (10ml) and added slowly to the reduced mixture and allowed to stir for 18 hours. The molecular sieves were collected by filtration and DMF was removed under vacuum. The remaining white solid was dissolved in water and partitioned between ethyl acetate and water. The organic layer was collected, dried with anhydrous magnesium sulphate and removed under vacuum. The crude orange/brown residue obtained was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (5:1). The title compound was collected as a cream solid (486mg, 11%). TLC [diethyl ether-petrol 60:80 (5:1)] R_f 0.19; TLC, ^{1}H NMR and mass spectrum were the same as for the previous batch.

Preparation of *N*-{4[(*E*)-3-(2-methyl-2-phenyl-1,3-benzodioxol-5-ylamino)-3-oxopropan]phenyl}-2-methoxybenzamide 89



Method A: 10% Pd-C was added (52mg) to a solution of **86** (1.20mmol, 516mg) in THF (150ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously at 50Psi in a hydrogenator. After 16 hours the hydrogen was replaced with argon and TEA (1ml) was added. 2-methoxybenzoyl chloride (2mmol, 340mg) was mixed with anhydrous DMF (10ml) and added slowly to the reduced mixture and stirred for a further 16 hours. The solids 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the mixture was concentrated under vacuum. The resulting pale brown oil was subjected to flash column chromatography, eluted with diethyl ether. The title compound was collected as a white solid (275mg, 43%). TLC [diethyl ether] R_f 0.33; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.64 (s, 3H, CH_3), 2.20-2.26 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 2.63 (t, 2H, *J*=7.5Hz, $NHCOCH_2C\underline{H}_2$), 2.74-2.81 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 3.03 (t, 2H, *J*=7.3Hz, $NHCOC\underline{H}_2CH_2$), 4.06 (s, 3H, OCH_3), 6.63 (d, 1H, *J*=8.3Hz, Ar''H-5), 6.70 (dd, 1H, *J*=8.3, 2.0Hz, Ar''H-6), 7.02-7.30 (ov. m, 11H, 5xAr'H, Ar''H-2, 2xAr'''H, 2xAr''''H, NH), 7.51 (ddd, 1H, *J*=8.8, 7.4, 1.9Hz, Ar'''H), 7.60 (d, 2H, *J*=8.4Hz, 2xAr'''H), 8.28 (dd, 1H, *J*=7.8, 1.8Hz, ArH), 9.80 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): m/z 537M$^+$; IR data (KBr disc): υ_{max} 3242 (CONH), 3166 (CONH), 2933 (CH_2/CH_3), 2854 (CH), 1652 (CONH), 1610 (Ar), 1535 (CONH), 1498 (Ar), 1448 (CH), 1387 (CH_3), 1196 (Ar-O-C), 1026 (Ar-O-C), 813 (2 adjacent ArH), 732 (4 adjacent ArH), 696 (5 adjacent ArH) cm^{-1}.

Method B: 10% Pd-C was added (108mg) to a solution of **84** (2.5mmol, 1.080mg) in THF (50ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously. After 16 hours the hydrogen was replaced with argon and TEA (1ml) was added. 2-methoxybenzoyl chloride (4.5mmol, 768mg) was mixed with anhydrous THF (10ml) and added slowly to the reduced mixture and stirred for a further 16 hours. The solids 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the mixture was concentrated under vacuum. The resulting pale brown oil was subjected to flash column chromatography, eluted with diethyl ether. The title compound was collected as a white solid (275mg, 43%). TLC [diethyl ether] R_f 0.33; ^{1}H NMR and mass spectrum as for the previous batch.

Preparation of 2,2-dimethyl-5-(3-nitrobenzyl)-1,3-dioxane 90

3-Nitrobenzaldehyde (50mmol, 7.55g) was dissolved in a solution of TEAF (formic acid, 9.583g and TEA, 50.5g). Meldrum's acid (50mmol, 7.20g) was added and the reaction was stirred at 60°C for 96 hours, until the emission of carbon dioxide stopped. The cloudy orange solution was poured onto ice (100g) and acidified to pH 2 using 6M HCl. The pale yellow precipitate formed was removed by filtration, dissolved in acetone (400ml) and heated at 50°C with activated charcoal (5g) for 16 hours. The activated charcoal was removed by filtration through celite. The title compound was recrystallised from cold acetone to give a white solid (9.329g, 67%). TLC [diethyl ether] R_f 0.51; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.72 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 3.60 (d, 2H, *J*=5.1Hz, CH_2), 3.84 (t, 1H, *J*=5.1Hz, CH), 7.51 (dd, 1H, *J*=8.1, 7.9Hz, ArH-5), 7.76 (dd, 1H, *J*=7.7, 1.0Hz, ArH-6), 8.14 (ddd, 1H, *J*=8.2, 2.2, 0.8Hz, ArH-4), 8.26 (dd, 1H, *J*=2.8, 1.7Hz, ArH-2) ppm.

Preparation of 3-(4-nitrophenyl) propanoic acid 91

4-Nitrobenzaldehyde (50mmol, 7.55g) was dissolved in a solution of TEAF (formic acid, 9.583g and TEA, 50.5g). Meldrum's acid (50mmol, 7.20g) was added and the reaction was stirred at 60°C for 72 hours, until the emission of carbon dioxide stopped. The deep red solution was poured onto ice (100g) and acidified to pH 2 using 6M HCl. The brown solid formed was removed by filtration and heated under reflux in concentrated HCl (150ml) for 48 hours. The title compound was recrystallised from acetone as a beige solid (7.653g, 78%). TLC [diethyl ether] R_f 0.49; ^{1}H NMR (250.1MHz, $(CD_2)_3SO$): δ_H 2.61(t, 2H, *J*=7.5Hz, $C\underline{H}_2CH_2COOH$), 2.95 (t, 2H, *J*=7.5Hz, $CH_2C\underline{H}_2COOH$), 7.53 (d, 2H, *J*=8.7Hz, ArH), 8.15 (d, 2H, *J*=8.7Hz, ArH), 12.25 (br s, 1H, COOH) ppm; Mass spectrum (APCI$^+$): *m/z* 196M$^+$; IR (KBr disc): υ_{max} 3077 (COOH), 2924 (CH_2), 2630 (CH), 1703 (COOH), 1601 (Ar), 1512 (NO_2), 1440 (CH_2), 1342 (NO_2), 1225 (CO), 860 (2 adjacent Ar) cm^{-1}.

Preparation of 3-(3-nitrophenyl)propanoic acid 92

2,2-Dimethyl-5-(3-nitrobenzyl)-1,3-dioxan-1,3-dioxone **90** (33.44mmol, 9.329g) was dissolved in concentrated HCl (150ml) and heated under reflux for 48 hours. A white solid formed on cooling which was removed by filtration and washed with water. The white solid was recrystallised from diethyl ether to give the title compound (4.425g, 68%). TLC [diethyl ether] R_f 0.51; ^{1}H NMR

(250.1MHz, $CDCl_3$): δ_H 2.78 (t, 2H, *J*=7.5Hz, CH_2), 3.10 (t, 2H, *J*=7.5Hz, CH_2), 7.50 (dd, 1H, *J*=8.5, 8.1Hz, ArH-5), 7.59 (dd, 1H, *J*=7.7, 1.0, ArH-6), 8.08-8.14 (ov. m, 2H, ArH-2/4), 13.02 (s, 1H, COOH) ppm; Mass spectrum ($APCI^+$): *m/z* 196M^+; IR (KBr disc): υ_{max} 3118 (COOH), 2931 (CH_2), 2684 (CH), 1706 (COOH), 1527 (NO_2), 1429 (CH_2), 1350 (NO_2), 1246, 1223, 1207 (C-O), 902, 738 (meta-disubstituted ArH) cm^{-1}.

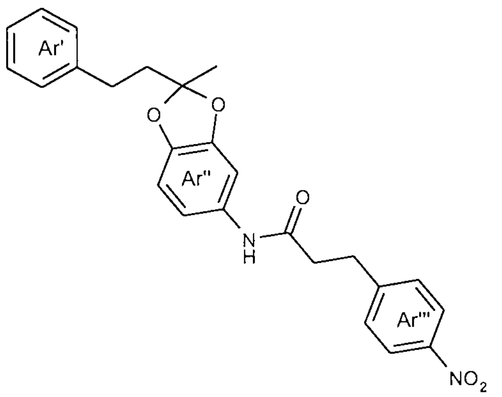
Preparation of 3-(4-nitrophenyl) propanoyl chloride 93

Thionyl chloride (30ml) was slowly added to a solution of **91** (25mmol, 4.875g) in $CHCl_3$ (30ml). The mixture was heated under reflux for 18 hours. The mixture was allowed to cool to room temperature and was then concentrated under vacuum. The remaining residue was analysed only by TLC and reacted immediately with the amine **85**. The title compound was collected as a crude brown residue. TLC [diethyl ether] R_f 0.45.

Preparation of 3-(3-nitrophenyl)propanoyl chloride 94

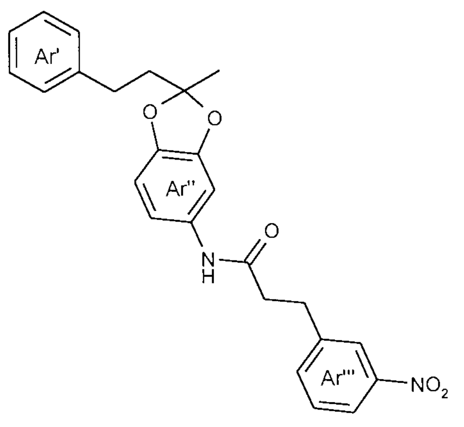
Thionyl chloride (30ml) was added slowly to a solution of **92** (20mmol, 3.900g) in $CHCl_3$ (30ml). The mixture was heated under reflux for 18 hours. The mixture was allowed to cool to room temperature and was then concentrated under vacuum. The remaining residue was analysed only by TLC and reacted immediately with the amine **85**. The title compound was collected as a crude pale brown residue. TLC [DCM-ethyl acetate (4:1)] R_f 0.38.

Preparation of *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-3-(4-nitrophenyl)propanamide 95



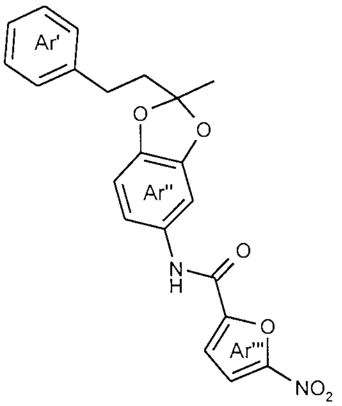
10% Pd-C (423mg) was added to a solution of **84** (15mmol, 4.275g) in THF (40ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction was shaken vigorously for 16 hours. The hydrogen was then replaced with argon and TEA (5ml) was added. Crude 3-(4-nitrophenyl)propanoyl chloride **93** (15mmol, 3.203g) was dissolved in anhydrous DMF (15ml) and added slowly to the reduced mixture and stirred for 16 hours. The mixture was concentrated under vacuum and the remaining orange/brown residue was subjected to flash column chromatography, eluted with ethyl acetate-hexane (1:1). The title compound was collected as an orange solid (49.312g, 76%). TLC [ethyl acetate-hexane (1:1)] R_f 0.45; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 2.16-2.23 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 2.61-2.72 (ov. m, 4H, $C_6H_5C\underline{H}_2CH_2$, $NHCOCH_2C\underline{H}_2$), 3.03 (t, 2H, *J*=7.4 Hz, $NHCOC\underline{H}_2CH_2$), 6.75 (d, 1H, *J*=8.3Hz, Ar'H-5), 6.87 (dd, 1H, *J*=8.4, 2.0Hz, Ar'H-6), 7.17-7.30 (ov. m, 6H, 5xAr'H, Ar"H-2), 7.53 (d, 2H, *J*=8.6Hz, 2xAr"H), 8.15 (d, 2H, *J*=8.6Hz, 2xAr"H), 9.83 (s, 1H, NH) ppm; Mass spectrum ($APCI^+$): *m/z* 433M^+; IR (KBr disc): υ_{max} 3380 , 3300 (CONH), 3068 (ArH), 2933(CH_2/CH_3), 2860 (CH), 1654 (CONH), 1606 (Ar), 1515 (CONH), 1492 (C-NO_2), 1448 (CH_2), 1342 (C-NO_2), 1232 (ArOC), 1107 (ArOC), 848 (2 adjacent ArH), 748, 696 (5 adjacent ArH) cm^{-1}.

Preparation of *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-3-(3-nitrophenyl)propanamide 96



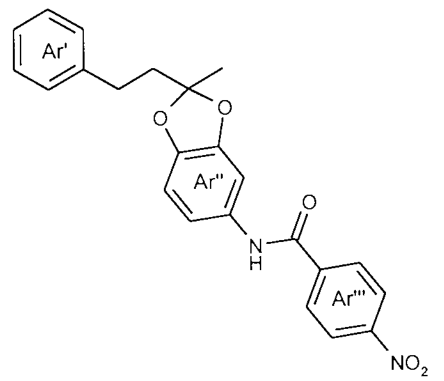
10% Pd-C (277mg) was added to a solution of **84** (15mmol, 2.775g) in THF (150ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously at 40Psi in a hydrogenator. After 18 hours the hydrogen was replaced with argon and TEA (5ml) was added. Crude 3-(3-nitrophenyl) propanoyl chloride (20mmol, 4.270g) was dissolved in anhydrous DMF (10ml) and added slowly to the reduced mixture and allowed to react for another 16 hours. The catalyst 10% Pd-C was removed by filtration through celite and the mixture was concentrated under vacuum. The resulting brown residue was dissolved in ethyl acetate and partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate. The organic layer was dried with anhydrous magnesium sulphate The title compound was precipitated in diethyl ether and collected as a white solid (2.863g, 44%). TLC [ethyl acetate-hexane (1:1)] R_f 0.43; ^1H NMR (250.1MHz, $CDCl_3$): δ_H 1.65 (s, 3H, CH_3), 2.20-2.27 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 2.69 (t, 2H, *J*=7.5Hz, $NHCOCH_2C\underline{H}_2$), 2.74-2.81 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 3.17 (t, 2H, *J*=7.5Hz, $NHCOC\underline{H}_2CH_2$), 6.67 (d, 1H, *J*=1.8Hz, Ar"H-2), 7.02-7.31 (ov. m, 8H, 5xAr'H, Ar"H-5/6, NH), 7.47 (dd, 1H, *J*=8.1, 7.9Hz, Ar'"H-5), 7.61 (d, trying to split, 1H, Ar'"H-2), 8.07-8.12 (m, 2H, Ar'"H-4/6) ppm; Mass spectrum ($APCI^+$): *m/z* 433M^+; IR (KBr disc): υ_{max} 3238 (CONH), 3049 (ArH), 2937 (CH_2/CH_3), 2866 (CH), 1653 (CONH), 1523 (CONH), 1498 ($C-NO_2$), 1444 (CH_2/CH_3), 1383 (CH_3), 1350 ($C-NO_2$), 1226 (Ar-O-C), 806 (3 adjacent ArH), 850 (2 adjacent ArH), 734, 702 (5 adjacent ArH) cm^{-1}.

Preparation of *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-5-nitro-2-furamide 97



10% Pd-C (278mg) was added to a solution of **84** (15mmol, 2.775g) in THF (150ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously for 16 hours. The hydrogen was replaced with argon and TEA (5ml) was added. 5-nitro-2-furoyl chloride (20mmol, 3.51g) was dissolved in anhydrous DMF (10ml) and added slowly to the reduced mixture. The reaction was then stirred for another 16 hours. The solids, 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the THF and DMF were removed under vacuum. The resultant brown residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (1:1). The title compound was collected as a bright yellow solid (3.579g, 61%). TLC [diethyl ether-petrol 60:80 (1:1)] R_f 0.11; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.65 (s, 3H, CH_3), 2.20-2.27 (m, 2H, CH_2), 2.69-2.76 (m, 2H, CH_2), 6.87 (d, 1H, *J*=8.3Hz, Ar"H-5), 7.09-7.31 (ov. m, 7H, 5xAr'H, Ar"H-2/6), 7.59 (d, 1H, *J*=4.0Hz, Ar'"H), 7.82 (d, 1H, *J*=4.0Hz, Ar'"H), 10.51 (s, 1H, NH) ppm; Mass spectrum ($APCI^+$): *m/z* 395M^+; IR (KBr disc): υ_{max} 3348 (CONH), 3023 (ArH), 2929 (CH_2/CH_3), 1660 (CONH), 1596 (Ar), 1534 (CONH), 1492 ($C-NO_2$), 1440 (CH_2/CH_3), 1376 (CH_3), 1318 ($C-NO_2$) cm^{-1}.

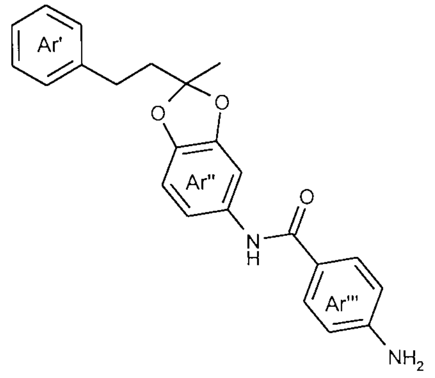
Preparation of *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-4-nitrobenzamide 98



Method A: 10% Pd-C (354mg) was added to a solution of **84** (19.14mmol, 3.54g) in THF (150ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction was shaken vigorously for 48 hours in a hydrogenator at 30Psi. The hydrogen was replaced with argon and TEA (4ml) was added. 4-Nitrobenzoyl chloride (24.14mmol, 4.48g) was dissolved in anhydrous DMF (10ml), added slowly to the reduced mixture and the reaction was allowed to stir for 16 hours. The solids, 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite, and the THF and DMF were removed under vacuum. The crude orange/brown residue was dissolved in ethyl acetate (200ml) and was then washed with water (3x100ml). The organic layer was collected and dried with anhydrous magnesium sulphate. The title compound was precipitated from ethyl acetate and hexane as a bright yellow solid (4.653g, 60%). TLC [diethyl ether-petrol 60:80 (1:1)] R_f 0.10; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.65 (s, 3H, CH_3), 2.20-2.30 (m, 2H, CH_2), 2.68-2.78 (m, 2H, CH_2), 6.86 (d, 1H, *J*=8.3Hz, Ar"H-5), 7.15-7.31 (ov. m, 6H, 5xAr'H, Ar"H-6), 7.37 (d, 1H, *J*=1.9Hz, Ar"H-2), 8.15 (d, 2H, *J*=8.3Hz, 2xAr'"H), 8.37 (d, 2H, *J*=8.3Hz, 2xAr"H), 10.49 (s, 1H, NH) ppm; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.69 (s, 3H, CH_3), 2.27 (m, 2H, CH_2), 2.78 (m, 2H, CH_2), 6.74 (d, 1H, *J*=8.2Hz, Ar"H-5), 6.88 (dd, 1H, *J*=8.3, 1.7Hz, Ar"H-6), 7.18-7.32 (ov. m, 5H, 5xAr'H), 7.77 (br s, 1H, NH), 8.02 (d, 2H, *J*=8.7Hz, 2xAr'"H), 8.34 (d, 2H, *J*=8.7Hz, 2xAr'"H) ppm; Mass spectrum (APCI$^+$): *m/z* 405M$^+$; IR (KBr disc): υ_{max} 3373 (CONH), 3077, 3070, 3050 (ArH/CONH), 2939 (CH_2), 2860 (CH), 1650 (CONH), 1606 (Ar), 1529 (CONH), 1500 (NO_2), 1456 (CH_2), 1350 ($C-NO_2$), 1275, 1243, 1209 (ArOC), 862 (isolated ArH), 810 (2 adjacent ArH), 725, 700 (5 adjacent ArH) ppm.

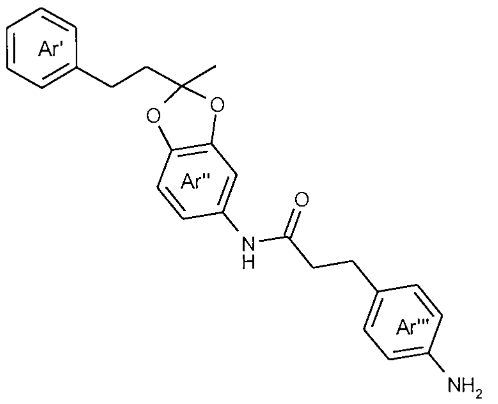
The preparation of **98** was repeated on a 15mmol scale. The crude residue was subjected to flash column chromatography, eluted with diethyl ether to give a yellow solid (5.793g, 96%). TLC [diethyl ether] R_f 0.63; ^{1}H NMR and mass spectrum as for the previous batch.

Preparation of 4-amino-*N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)benzamide 99



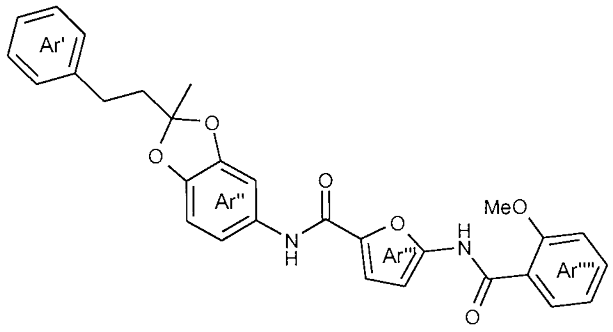
10% Pd-C (10% by weight, 263mg) was added to a solution of **84** (6.5mmol, 2.63g) in THF (50ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously for 18 hours. The hydrogen was replaced with argon and the catalyst, 10% Pd-C was removed by filtration through celite. The THF was removed under vacuum and the crude residue was subjected to flash column chromatography, eluted with ethyl acetate-petrol 40:60 (2:5). The title compound was collected as a white crystalline solid (2.070g, 85%). TLC [ethyl acetate-petrol 40:60 (2:5)] R_f 0.14; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.67 (s, 3H, CH_3), 2.22-2.29 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 4.30 (br s, 2H, NH_2), 2.79-2.83 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 6.67-6.72 (ov. m, 3H, Ar"H-5, 2xAr'"H), 6.83 (dd, 1H, J=8.3, 2.1Hz, Ar"H-6), 7.16-7.31 (ov. m, 6H, 5xAr'H, Ar"H-2), 7.62 (br s, 1H, NH), 7.69 (d, 2H, J=8.6Hz, 2xAr'"H) ppm; Mass spectrum (APCI$^+$): *m/z* 375M$^+$; IR (KBr disc): υ_{max} 3469 (NH_2), 3356 (CONH), 3210 (NH_2), 2933 (CH_2/CH_3), 1444 (CH), 1379 (CH_3),1623 (CONH), 1606 (Ar), 1540 (Ar), 1492 (CONH), 1272, 1246, 1184 (Ar-O-C), 839 (2 adjacent ArH), 696 (5 adjacent ArH) cm^{-1}.

Preparation of 4-amino-*N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-3-(3-nitrophenyl)propanamide 100



10% Pd-C was added (340mg) to a solution of **84** (7.83mmol, 3.382g) in THF (30ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously. After 16 hours the 10% Pd-C was removed by filtration through celite and the THF was removed under vacuum. The resulting residue was subjected to flash column chromatography, eluted with ethyl acetate-hexane (1:1) to give the title compound as a pale yellow solid (2.844g, 90%). TLC [ethyl acetate-hexane (1:1)] R_f 0.18; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.61 (s, 3H, CH_3), 2.16-2.23 (m, 2H, $CH_2C\underline{H}_2C_6H_5$), 2.42-2.45 (m, 2H, $COCH_2C\underline{H}_2$), 2.66-3.31 (ov. m, 4H, $COC\underline{H}_2/C\underline{H}_2CH_2C_6H_5$), 4.85 (Br s, 2H, NH_2), 4.90 (d, 2H, J=8.3Hz, 2xAr'"H), 6.75 (d, 1H, J=8.4Hz, Ar"H-5), 6.90-6.85 (ov. m, 3H, Ar"H-6, 2xAr'"H), 7.17-7.30 (ov. m, 6H, Ar"H-2, 5xAr'H), 9.74 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 403M$^+$; IR data (KBr disc): υ_{max} 3280 (CONH, NH_2), 3058, 3023 (ArH), 2927(CH_2/CH_3), 2864 (NH_2), 1658, 1637 (CONH), 1550, 1519 (CONH), 1498 (ArH), 1452 (CH_2/CH_3), 1377 (CH_3), 1228, 1160, (ArOC), 1124 (CO), 852, 820 (2 adjacent ArH), 752, 698 (5 adjacent ArH) cm^{-1}.

Preparation of *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-5-[(2-methoxybenzoyl)amino]-2-furamide 101

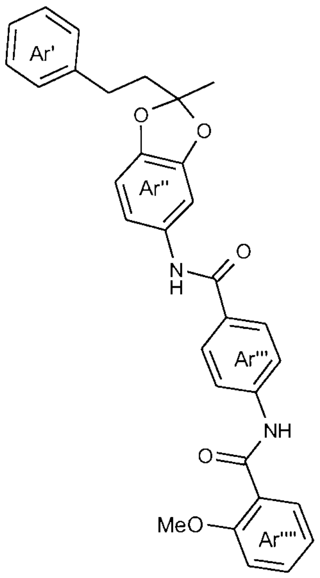


10% Pd-C (197mg) was added to a solution of **97** (5.0mmol, 1.970g) in THF (100ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously. After 18 hours the hydrogen was replaced with argon and

TEA (2ml) was added. A solution of 2-methoxybenzoyl chloride (8mmol, 1.36mg) in DMF (10ml) was added slowly to the reduced mixture and allowed to stir for 16 hours. The solids 10% Pd-C and triethylamine hydrochloride was removed by filtration through celite. The THF and DMF were removed under vacuum. The crude residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (1:1). The title compound was collected as a yellow oil (64mg, 4%). TLC [diethyl ether-petrol 60:80 (1:1)] R_f 0.14; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.67 (s, 3H, CH_3), 2.23-2.30 (m, 2H, CH_2), 2.78-2.85 (m, 2H, CH_2), 4.05 (s, 3H, OCH_3), 6.73 (d, 1H, *J*=8.3Hz, Ar”H-5), 6.92 (dd, 1H, *J*=8.3, 2.1Hz, Ar”H-6), 7.03 (d, 1H, *J*=8.2Hz, Ar”H-2), 7.10-7.32 (ov. m, 9H, 5xAr’H, Ar’”H, 2xAr””H, NH), 7.36 (d, 1H, *J*=4.1Hz, Ar’”H), 7.50 (ddd, 1H, *J*=9.0, 7.4, 1.9Hz, Ar””H), 8.29 (dd, 1H, *J*=7.8, 1.7, Ar””H), 9.69 (br s, 1H, NH) ppm; Mass spectrum ($APCI^+$): *m/z* 390$[M\text{-}C_6H_4OCH_3]^+$.

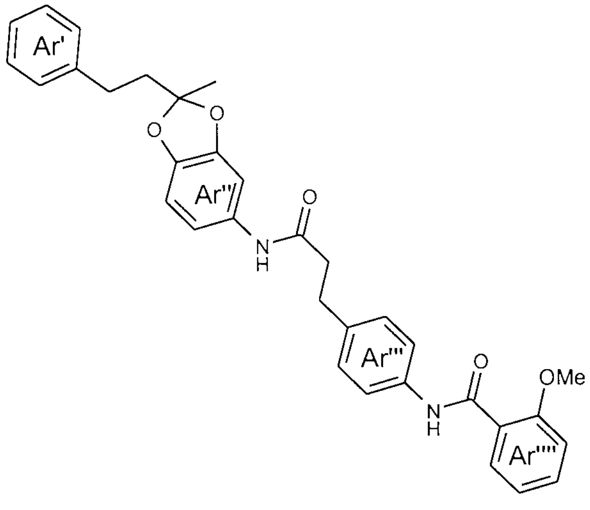
Preparation of **101** was repeated on a 3.5mmol scale to give a yield of 5%. TLC, ^{1}H NMR and mass spectrum the same as the previous batch.

Preparation of *N*-(3-{[(2-methyl-2-phenethyl-1,3-benzodioxol-5-ylamino)-3-oxopropyl]phenyl}-2-methoxybenzamide 102



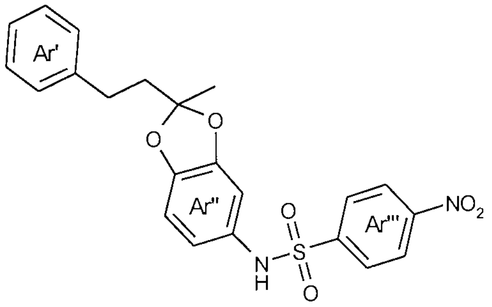
10% Pd-C (162mg) was added to a solution of **98** (4mmol, 1.616g) in THF (50ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture shaken vigorously for 16 hours. The hydrogen was replaced with argon and TEA (1ml) was added. 4-Nitrobenzoyl chloride (5mmol, 853mg) was dissolved in anhydrous DMF (10ml) and added slowly to the reduced mixture which was then stirred for 16 hours. The solids, 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the THF and DMF were removed under vacuum. The title compound was recrystallised from ethyl acetate as a white solid (1.467g, 72%). TLC [diethyl ether] R_f 0.38; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.65 (s, 3H, CH_3), 2.20-2.27 (m, 2H, $CH_2C\underline{H}_2C_6H_5$), 2.70-2.76 (m, 2H, $C\underline{H}_2CH_2C_6H_5$), 3.90 (s, 3H, OCH_3), 6.83 (d, 1H, *J*=8.4Hz, Ar”H-5), 7.05-7.31 (ov. m, 10H, 5xAr’H, Ar”H-6, 2xAr’”H, 2xAr””H), 7.37 (d, 1H, *J*=2.0Hz, Ar”H-2), 7.53 (ddd, 1H, *J*=9.7, 8.9, 1.6Hz, Ar””H), 7.63 (d, 1H, *J*=8.4Hz, 2xAr”H), 7.90 (dd, 1H, *J*=8.8. 1.9Hz, Ar””H), 10.04 (s, 1H, NH), 10.40 (s, 1H, NH) ppm; Mass spectrum ($APCI^+$): *m/z* 509M^+, 491$[M\text{-}CH_3]^+$; IR (KBr disc): υ_{max} 3352 (CONH), 3025 (ArH), 2943 (CH_2/CH_3), 2854 (OCH_3), 1652 (CONH), 1594 (Ar), 1529 (CONH), 1448 (CH), 1374 (CH_3) cm^{-1}.

Preparation of *N*-(4-{[(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)amino]carbonyl}phenyl)-2-methoxybenzamide 103



10% Pd-C (216mg) was added to a solution of **84** (5mmol, 2.160g) in THF (100ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously for 48 hours. The hydrogen was replaced with argon and TEA (5ml) was added. 2-methoxybenzoyl chloride (10mmol, 1.706g) was mixed with anhydrous DMF (10ml), and added slowly to the reduced mixture and left to stir for 16 hours. The solids, 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the THF and DMF were removed under vacuum. The resulting yellow/brown residue was subjected to flash column chromatography, eluted with petrol 60:80-diethyl ether (1:4). The title compound was collected as a pale yellow solid (1.989g, 74%). TLC [petrol 60:80-diethyl ether (1:4)] R_f 0.16; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.69 (s, 3H, CH_3), 2.18-2.25 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 2.64 (t, 2H, $NHCOCH_2C\underline{H}_2$), 2.73-2.80 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 3.05 (t, 2H, $NHCOC\underline{H}_2CH_2$), 4.05 (s, 3H, OCH_3), 6.62 (d, 1H, *J*=8.3Hz, Ar"H-5), 6.68 (dd, 1H, *J*=8.4, 2.0Hz, Ar"H-6), 6.99-7.30 (ov. m, 11H, 5xArH, Ar"H-2, 4xArH, NH), 7.42-7.53 (ov. m, 2H, 2xArH), 7.67 (s, 1H, ArH), 8.28 (dd, 1H, *J*=7.8, 1.8Hz, Ar""H), 9.83 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 537M$^+$; IR data (KBr disc): υ_{max} 3346 (CONH), 3023 (ArH), 2929 (CH_2/CH_3), 2838 (CH), 1668 (CONH), 1603 (ArH), 1552 (CONH), 1492 (ArH), 1234 (Ar-O-C), 1016 (Ar-O-C), 848 (isolated ArH/2 adjacent ArH), 749 (4 adjacent ArH), 693 (5 adjacent ArH) cm^{-1}.

Preparation of *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-4-nitrosulphonamide 104

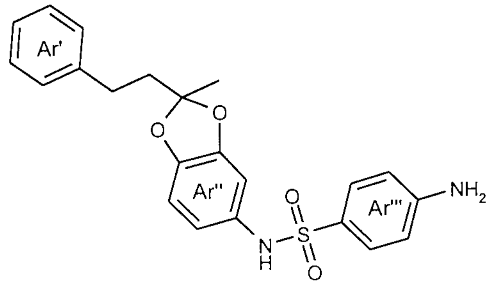


Method A: 10% Pd-C (370mg) was added to a solution of **84** (20mmol, 3.7g) in THF (50ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction was then shaken vigorously. After 18 hours TEA (5ml) was added. A solution of 4-nitrobenzene sulphonyl chloride (22mmol, 4.875g) in anhydrous DMF (10ml) was added slowly to the reduced mixture and allowed to react for 48 hours. The solids 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the THF and DMF were removed under vacuum. The brown residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (2:1). The title compound was collected as a clear brown oil (3.111g, 35%). TLC [ethyl acetate] R_f 0.56; ^1H NMR (399.95MHz, $CDCl_3$): δ_H 1.64 (s, 3H, CH_3), 2.18-2.24 (m, 2H, CH_2), 2.70-2.76 (m, 2H, CH_2), 6.43 (dd, 1H, *J*=8.2, 2.2Hz, Ar'H-6), 6.58 (d, 1H, *J*=8.2Hz, Ar'H-5), 6.63 (d, 1H, *J*=2.1Hz, ArH'-2), 7.14-7.31 (ov. m, 6H, 5xAr'H, NH), 7.95 (d, 2H, *J*=8.9 Hz, 2xAr'''H), 8.28 (d, 2H, *J*=8.9Hz, 2xAr'''H) ppm; Mass spectrum ($APCI^+$): *m/z* 441M^+, 377, 255$[M\text{-}SO_2C_6H_4NO_2]^+$. Method A was repeated on a 10mmol scale to give a 25% yield. ^1H NMR, mass spectrum and TLC the same as for the previous batch.

Method C: A solution of sodium dithionite (50mmol, 8.7g) in water (50ml) was added to a solution of **84** (5mmol, 925mg) in ethanol (50ml). The reaction mixture was heated under reflux for 18 hours. The reaction mixture was cooled and a second solution of sodium dithionite (50mmol, 8.7g) in water (50ml) was added, and the mixture was heated under reflux for a further 18 hours. The ethanol and water was removed under vacuum. Organic compounds were dissolved in ether under argon and water and added to dissolve the inorganic components of the remaining mixture. A cannula was used to siphon the ether layer off into a dry flask charged with anhydrous Magnesium sulphate under argon. The ether layer was then transferred by cannula to a fresh dry flask charged with TEA (2ml) under argon. A solution of 4-nitrobenzene sulphonyl chloride (10mmol, 2.21g) in anhydrous DMF was added slowly to the reduced mixture and allowed to react for 16 hours. The white solid, triethylamine hydrochloride was removed by filtration and the THF and DMF were removed under vacuum. The brown residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (2:1). The title compound was collected as a clear brown oil (82mg, 4%). TLC [diethyl ether-petrol 60:80 (2:1)] R_f 0.56.

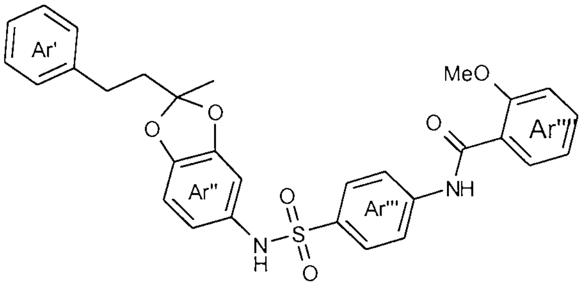
Method C was repeated a further two times in a 5 to 10mmol scale to give yields in the range of 15 to 16%. ^1H NMR, mass spectrum and TLC the same as for the previous batch.

Preparation of 4-amino-*N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-4-nitrosulphonamide 105



10% Pd-C (300mg) was added to a solution of **84** (6.8mmol, 3.00g) in THF (25ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously for 16 hours. The catalyst, 10% Pd-C was removed by filtration through celite. The THF was removed under vacuum to give a brown residue which was subjected to flash column chromatography, eluted with diethyl ether-petrol 40:60 (5:1). The title compound was isolated as a pale yellow solid (2.766g, 99%). TLC [diethyl ether-petrol 40:60 (5:1)] R_f 0.21; ^1H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.58 (s, 3H, CH_3), 1.66-1.98 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 2.62-2.69 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 5.98 (s, 2H, NH_2), 6.44 (dd, 1H, *J*=8.3, 2.0Hz, Ar''H-6), 6.53-6.58 (ov. m, 3H, Ar''H-2, 2xAr'''H), 6.70 (d, 1H, *J*=8.3Hz, Ar''H-5), 7.13-7.28 (ov. m, 5H, 5xAr'H), 7.35 (d, 2H, *J*=8.6Hz, 2xAr'''H), 9.54 (s, 1H, NH) ppm; Mass spectrum ($APCI^+$): *m/z* 411M^+, 255$[M\text{-}SO_2C_6H_4NH_2]^+$.

Attempted preparation of *N*-(4-{[(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)amino]sulphonyl}phenyl)-2-methoxybenzamide 106

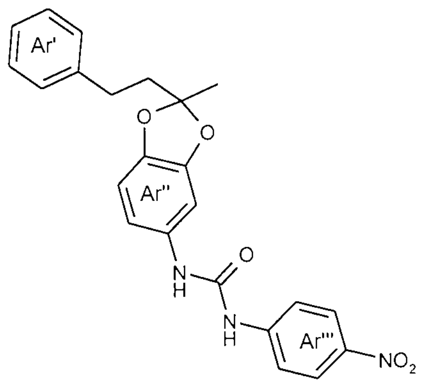


Method A: **84** (0.91mmol, 400mg) was dissolved in ethanol (30ml) and added to a solution of sodium dithionite, (18.18mmol, 3.16g) in water (30ml). The reaction mixture was heated under reflux for 36 hours. The ethanol and water were removed under vacuum and the residue was re-dissolved and partitioned between water and ethyl acetate. The organic layer was collected, dried with magnesium sulphate and concentrated under vacuum. The resulting brown residue was re-dissolved in anhydrous THF and TEA (1ml) was added. A solution of 2-methoxybenzoyl chloride (1.5mmol, 255mg) in anhydrous DMF (10ml) was added to the reduced mixture and left to stir for 18 hours. The white precipitate (triethylamine hydrochloride) was removed by filtration and the THF and DMF were removed under vacuum. The brown residue was subjected to flash column chromatography, eluted with diethyl ether. The title compound could not be isolated.

Method B: 10% Pd-C (112mg) was added to a solution of **102** (2.50mmol, 1.114g) in THF (150ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously at 40Psi in a hydrogenator. The hydrogen was replaced with argon after 18 hours. A solution of 2-methoxybenzoyl chloride (3mmol, 510mg) in anhydrous DMF (10ml) was added slowly to the reduced mixture and allowed to react for 48 hours. The catalyst 10% Pd-C was removed by filtration through celite, and the THF and DMF were removed under vacuum. Analysis of the crude brown oil showed 6 separate components by TLC. ^{1}H NMR indicated the amine to be present along with the 2-methoxybenzoyl chloride.

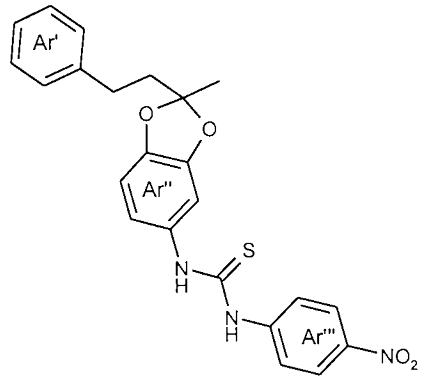
Method C: 10% Pd-C (112mg) was added to **102** (15.3mmol, 6.73g) in THF (150ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously for 18 hours, after which the hydrogen was replaced with argon. A solution of 2-methoxybenzoyl chloride (20mmol, 3.412g) in anhydrous DMF (10ml) was added slowly to the reduced mixture and allowed to react for 48 hours. The catalyst 10% Pd-C was removed by filtration through celite, and the THF and DMF were removed under vacuum. Analysis of the crude brown oil showed 6 separate components by TLC. ^{1}H NMR indicated the amine to be present along with the 2-methoxybenzoyl chloride. The mass spectrum (APCI$^+$) did not show a molecular ion at 544, but showed peaks at 527, 389, 255[$C_6H_5CH_2CH_2C(CH_3)OOC_6H_3NH$]$^+$, 180, 135[$COC_6H_4(OCH_3)$]$^+$. An attempt was not made to isolate the title compound.

Preparation of *N*-(2-methyl-2-phenthyl-1,3-benzodioxol-5-yl)-*N'*-(4-nitrophenyl)urea 107



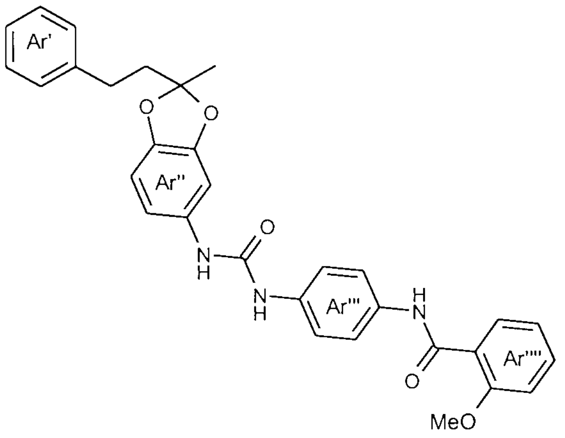
10% Pd-C (370mg) was added to a solution of **84** (20mmol, 3.70g) in THF (150ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously at 40Psi in a hydrogenator. After 16 hours. the hydrogen was replaced with argon. 4-Nitrophenyl isocyanate (22mmol, 3.608g) was dissolved in anhydrous DMF (10ml) and added dropwise to the reduced mixture and allowed to react for another 16 hours. The catalyst 10% Pd-C was removed by filtration through celite and the THF and DMF were removed under vacuum. The resulting brown residue was subjected to flash column chromatography, eluted with petrol 60:80-diethyl ether (1:2). The title compound was collected as an orange solid (1.440g, 17%). TLC [DCM] R_f 0.23; ^1H NMR (250.1MHz, $CDCl_3$): δ_H 1.62 (s, 3H, CH_3), 2.17-2.24 (m, 2H, $C_6H_5CH_2C\underline{H_2}$), 2.71-2.77 (m, 2H, $C_6H_5C\underline{H_2}CH_2$), 6.63-6.70 (ov. m, 2H, Ar"H-5/6), 6.82 (d, 1H, *J*=1.5Hz, Ar"H-2), 7.14-7.31 (ov. m, 6H, 5xAr'H, NH), 7.46 (d, 2H, *J*=9.2Hz, 2xAr'"H), 7.71 (s, 1H, NH), 8.01 (d, 2H, *J*=9.2Hz, 2xAr'"H) ppm; Mass spectrum (APCI$^+$): *m/z* 420M$^+$; IR (KBr disc): υ_{max} 2935 (CH_2/CH_3), 1668 (N-CO-N), 1611 (Ar), 1557 (C-NO_2), 1333 (C-NO_2), 1214 (C-O-C) cm^{-1}.

Preparation of *N*-(2-methyl-2-phenthyl-1,3-benzodioxol-5-yl)-*N'*-(4-nitrophenyl)thiourea 108



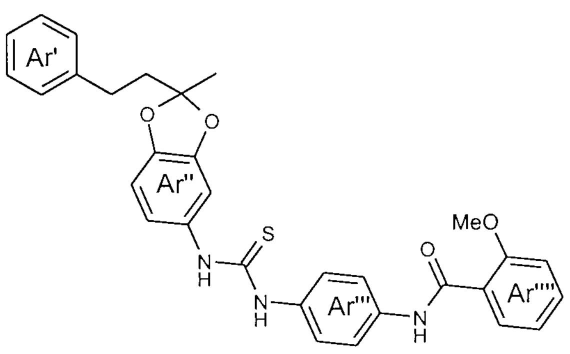
10% Pd-C (740mg) was added to a solution of **84** (40mmol, 7.40g) in THF (150ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously at 40Psi in a hydrogenator. The hydrogen was replaced with argon after 16 hours. 4-Nitrophenyl isothiocyanate (44mmol, 7.92g) was dissolved in anhydrous DMF (10ml) and added dropwise to the reduced mixture and allowed to react for 16 hours. The catalyst 10% Pd-C was removed by filtration through celite, and the THF and DMF were removed under vacuum. The remaining orange/brown residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (1:2). The title compound was collected as an orange solid (3.412g, 20%). TLC [diethyl ether-petrol 60:80 (1:2)] R_f 0.21; ^1H NMR (250.1MHz, $CDCl_3$): δ_H 1.71 (s, 3H, CH_3), 2.25-2.31 (m, 2H, $C_6H_5CH_2C\underline{H_2}$), 2.76-2.83 (m, 2H, $C_6H_5C\underline{H_2}CH_2$), 6.73-6.88 (ov. m, 3H, Ar"H-2/5/6), 7.17-7.32 (ov. m, 5H, 5xAr'H), 7.51 (d, 2H, *J*=9.1Hz, 2xAr'"H), 8.19 (d, 2H, *J*=9.1Hz, 2xAr"H), 8.39 (br s, 1H, NH), 8.41 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 436M$^+$; IR (KBr disc): υ_{max} 3330 (CSN), 2906 (CH_2/CH_3), 1594 (NHSNH), 1547 (CSN), 1495 (C-NO_2), 1400 (CH_2/CH_3), 1330 (C-NO_2), 1243 (N-CS-N) cm^{-1}.

Preparation of *N*-(4-{[(2-methyl-2-phenthyl-1,3-benzodioxol-5-ylamino)carbonyl]amino}phenyl)-2-methoxybenzamide 109



10% Pd-C (10% by weight, 105mg) was added to a solution of **107** (2.50mmol, 1.05g) in THF (50ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously and followed by TLC until starting material disappeared. After 72 hours the hydrogen was replaced with argon and TEA (1ml) was added. 2-methoxybenzoyl chloride (4.5mmol, 768mg) was mixed with anhydrous DMF (10ml) and added slowly to the reduced mixture and stirred for 18 hours. The solids, 10% Pd-C and triethylamine were removed by filtration through celite. and the THF and DMF were removed under vacuum. The resulting brown residue was subjected to flash column chromatography, eluted with ethyl acetate-petrol 40:60 (1:1). The title compound was collected as a pale yellow solid (1.36g, 96%). TLC [ethyl acetate-petrol 40:60 (1:1)] R_f 0.23; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.61 (s, 3H, CH_3), 2.18-2.23 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 2.75-2.88 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 4.04 (s, 3H, OCH_3), 6.54-6.62 (ov. m, 2H, Ar”H-5/6), 6.99-7.30 (ov. m, 10H, 5xArH, Ar”H-2, 2xAr’”H, 2xAr””H), 7.41 (d, 2H, *J*=8.8Hz, 2xAr’”H), 7.52 (ddd, 1H, *J*=9.7, 7.9, 1.8Hz, Ar””H), 7.69 (br s, 1H, NH), 7.59 (br s, 1H, NH), 8.24 (dd, 1H, *J*=7.8, 1.8Hz, Ar””H), 9.77 (Br s, 1H, NH) ppm; Mass spectrum (APCI)$^+$: *m/z* 524M$^+$; IR (KBr disc): υ_{max} 3340 (CONH), 3070, 3029 (ArH), 2985, 2937 (CH_2/CH_3), 2843 (CH), 1666 (CONH), 1655 (NCON), 1600 (Ar), 1654 (CONH), 1492 (Ar), 1198 (Ar-O-C), 1016 (Ar-O-Ar), 833 (2 adjacent ArH), 750, 702 (5 adjacent ArH) cm^{-1}.

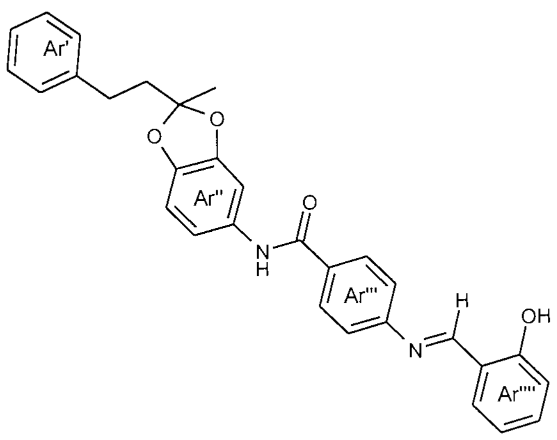
Preparation of *N*-(4-{[(2-methyl-2-phenthyl-1,3-benzodioxol-5-ylamino)sulphonyl]amino}phenyl)-2-methoxybenzamide 110



Method A: 10% Pd-C (131mg) was added to a solution of **108** (3.00mmol, 1.305g) in THF (50ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously and followed by TLC until starting material disappeared. After 72 hours the hydrogen was replaced with argon and TEA (2ml) was added. 2-methoxybenzoyl chloride (4.5mmol, 768mg) was mixed with anhydrous DMF (10ml) and added slowly to the reduced mixture and stirred for 18 hours. The solids, 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the THF and DMF were removed under vacuum. The resulting brown residue was subjected to flash column chromatography, eluted with ethyl acetate-petrol 40:60 (1:1). The title compound was collected as a pale yellow solid (1.401g, 87%). TLC [ethyl acetate-petrol 40:60 (1:1)] R_f 0.23; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.58 (s, 3H, CH_3), 2.13-2.20 (m, 2H, $CH_2C\underline{H}_2C_6H_5$), 2.67-2.73 (m, 2H, $C\underline{H}_2CH_2C_6H_5$), 3.94 (s, 3H, OCH_3), 6.52-6.78 (ov. m, 3H, Ar”H-5/6), 6.93-7.31 (ov. m, 10H, 5xAr’H, Ar”H-2, 2xAr’”H, 2xAr””H), 7.41 (ddd, 1H, *J*=9.3, 8.4, 1.8Hz, Ar””H), 7.57 (d, 2H, *J*=8.8Hz, 2xAr’”H), 8.11 (dd, 1H, *J*=7.8, 1.7Hz, Ar””H), 8.30 (br s, 1H, NH), 8.45 (br s, 1H, NH), 9.82 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 540M$^+$; IR (KBr disc): υ_{max} 3342, 3245 (CONH/CSNH), 2929 (CH_2/CH_3), 1664 (CONH), 1598 (Ar), 1511 (CONH), 1490 (CSNH), 1313 (N-CS-N), 1284 (C=S), 1228 (Ar-O-C), 1014 (Ar-O-C), 833 (2 adjacent ArH), 750 (4/5 adjacent ArH), 702 (5 adjacent ArH) cm^{-1}.

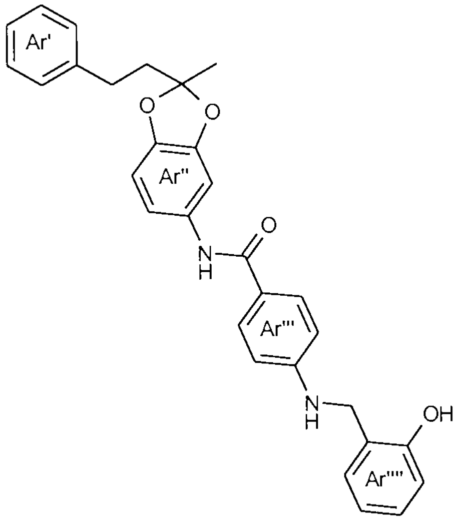
Method B: Tin (II) chloride dihydrate (21mmol, 5.368g) and 4A$^{\circ}$ molecular sieves were added to a solution of **108** (3mmol, 1.305g) in anhydrous DMF (50ml). The reaction mixture was monitored by TLC and allowed to stir under an atmosphere of argon. After 18 hours TEA (2ml) was added and then a solution of 2-methoxybenzoyl chloride (5mmol, 853mg) in anhydrous DMF (10ml) was added slowly to the reduced mixture. After 16 hours ethyl acetate was added and the white precipitate formed was removed by filtration. The ethyl acetate was removed under vacuum to give a clear yellow residue, which was partitioned between DCM and water. The organic layer was collected and dried with anhydrous Magnesium sulphate. The resulting yellow residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (1:1). The title compound was collected as a pale yellow solid (251mg, 16%). TLC [ethyl acetate] R_f 0.57.

Preparation of *N*-(2-methyl-2phenethyl-1,3-benzodioxol-5-yl)-4-{[(*E*)-(2-hydroxyphenyl)methylidene]amino}benzamide 111



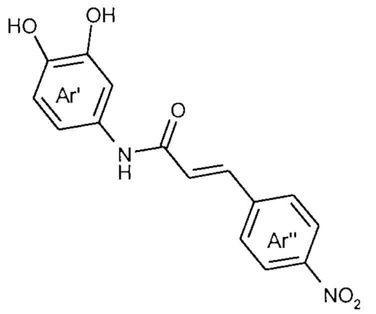
2-Hydroxybenzaldehyde (2.2mmol, 269mg) was added to a solution of **98** (2mmol, 748mg) in dichloroethane (10ml) and the mixture was stirred for 18 hours. The title compound formed as a yellow precipitate which was removed by filtration, washed with dichloroethane, and dried under vacuum (754mg, 79%). TLC [diethyl ether] R_f 0.53; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 1.65 (s, 3H, CH_3), 2.23-2.27 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 2.69-2.73 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 6.84 (d, 1H, *J*=8.4Hz, Ar”H-5), 6.99 (d, 2H, *J*=8.5Hz, 2xAr’”H), 7.15-7.31 (ov. m, 8H, 5xAr’H, Ar”H-6, 2xAr””H), 7.38 (d, 1H, *J*=2.0Hz, Ar”H-2), 7.45 (ddd, 1H, *J*=8.4, 7.7, 1.3Hz, Ar””H), 7.54 (d, 2H, *J*=8.5Hz, 2xAr’”H), 7.71 (d, 1H, *J*=6.5Hz, Ar’H-5), 8.04 (dd, 1H, *J*=8.5, 1.1Hz, Ar””H), 9.03 (s, 1H, N=CH), 10.17 (s, 1H, NH), 12.85 (br s, 1H, OH) ppm; Mass spectrum (APCI$^+$): m/z 479M$^+$; IR (KBr disc): υ_{max} 3367 (N=CH/CONH), 2923 (CH_2/CH_3), 1647 (CONH), 1600 (ArH), 1566 (ArH), 1526 (ArH), 1494 (CONH), 1243 (Ar-O-C), 848 (2 adjacent ArH), 804, 750 (5 adjacent ArH) cm^{-1}.

Preparation of *N*-(2-methyl-2phenethyl-1,3-benzodioxol-5-yl)-4-[(2-hydroxybenzyl)amino]benzamide 112



2-Hydroxybenzaldehyde (3.00mmol, 366mg) and 4A$^{\circ}$ molecular sieves were added to a solution of **98** (2.37mmol, 888mg) in THF (20ml). The reaction was heated at 70°C and monitored by TLC until complete (18 hours). The reaction was cooled, and then tetramethylammonium triacetoxyborohydride (5.22mmol, 1.372g) was added and the reaction was heated for 72 hours. A yellow solid which formed on cooling was removed by filtration and washed with THF. The yellow solid was dissolved in and partitioned between $CHCl_3$ (150ml) and 1% NaOH (150ml). The organic layer was then washed with brine (3x100ml) and dried with anhydrous magnesium sulphate. The $CHCl_3$ was removed under vacuum to give a yellow solid which was subjected to flash column chromatography, eluted with ethyl acetate-petrol 40:60 (3:2). The title compound was recovered as a yellow oil (719mg, 63%). TLC [ethyl acetate-petrol 40:60 (3:2)] R_f 0.18; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.63 (s, 3H, CH_3), 2.18-2.24 (m, 2H, $C_6H_5CH_2C\underline{H}_2$), 2.50-2.75 (m, 2H, $C_6H_5C\underline{H}_2CH_2$), 4.25 (d, 2H, *J*=5.8Hz, $NHC\underline{H}_2C_6H_4OH$), 6.61 (d, 2H, *J*=8.8Hz, 2xAr'''H), 6.70-6.84 (ov. m, 4H, Ar''H-2/5/6, Ar''''H), 7.03-7.34 (ov. m, 8H, 5xAr'H, 3xAr''''H), 7.69 (d, 2H, *J*=8.8Hz, 2xAr''H), 9.60 (s, 1H, NH), 9.64 (s, 1H, NH), 12.74 (br s, 1H, OH) ppm; Mass spectrum (APCI$^+$): m/z 481M$^+$, 375 [M-$CH_2C_6H_4OH$]$^+$; IR (KBr disc): υ_{max} 3403 (CONH/NH), 3350 (OH/NH), 3060 (CONH), 2932 (CH_2/CH_3), 2910 (CH_2/CH_3), 2867 (N-CH_2), 1722 (CONH), 1604 (CONH), 1504 (Ar), 1458 (CH_2/CH_3), 1371 (CH_3), 1352 (Ar-O-C), 1242 (OH), 1134 (Ar-O-C), 845 (2 adjacent ArH), 760, 698 (5 adjacent ArH) cm^{-1}.

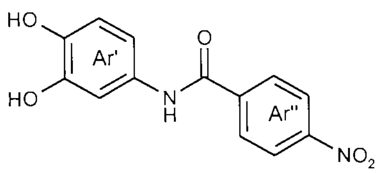
Preparation of (*E*)-*N*-(3,4-dihydroxyphenyl)-3-(4-nitrophenyl)prop-2-enamide 113



TFA (1ml) was added to a solution of **84** (1.0mmol, 430mg) in dioxane (9ml). The mixture was heated at 80°C and continually monitored by TLC. After one hour there was no change in the TLC, and more TFA (1ml) was added. Again the reaction was monitored by TLC and after 2 hours a new product could be seen along with the starting material. TFA (1ml) was added and the reaction was stirred at 100°C for 16 hours. The reaction mixture was then cooled and concentrated under vacuum. The resulting residue was subjected to flash column chromatography, eluted with diethyl ether-petrol 60:80 (1:1). The title compound was collected as a beige/brown solid (105mg, 35%). TLC [diethyl ether-petrol

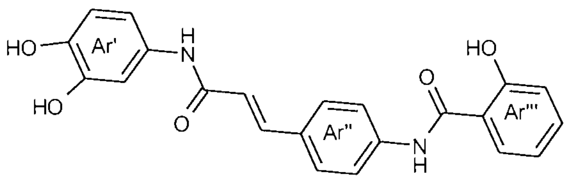
60:80 (1:1)] R_f 0.31; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.73 (d, 1H, *J*=16.0Hz, CH=), 6.89-7.03 (ov. m, 2H, Ar'H-5/6), 7.19 (br s, 1H, NH), 7.28 (d, 1H, *J*=1.9Hz, Ar'H-2), 7.65 (d, 1H, *J*=16.0Hz, CH=), 7.94 (d, 2H, *J*=8.7Hz, 2xAr"H), 8.21 (d, 2H, *J*=8.7Hz, 2xAr"H), 10.12 (s, 2H, 2xOH) ppm; Mass spectrum (APCI$^+$): *m/z* 301M$^+$.

Preparation of *N*-(3,4-dihydroxyphenyl)-4-nitrobenzamide 114



84 (15mmol, 6.094g) was dissolved in THF (36ml). Concentrated HCl (4ml) and water (500μl) were added and the reaction mixture was heated under reflux for 16 hours. The THF was removed under vacuum. The title compound formed as an orange precipitate from diethyl ether. The precipitate was collected by filtration and washed with diethyl ether. The orange solid collected (2.919g, 71%) appeared to be air sensitive and was stored under argon. TLC [diethyl ether] R_f 0.39; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.70 (d, 1H, *J*=8.5Hz, Ar'H-5), 6.99 (dd, 1H, *J*=8.8, 2.2Hz, Ar'H-6), 7.30 (d, 1H, *J*=1.8Hz, Ar'H-2), 8.14 (d, 2H, *J*=8.7Hz, 2xAr"H), 8.35 (d, 2H, *J*=8.7Hz, 2xAr"H), 8.79 (s, 1H, OH), 9.07 (s, 1H, OH), 10.27 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 275M$^+$, 229[M-NO$_2$]$^+$, 150[M-C_6H_3(OH)NH]$^+$; IR (KBr disc): υ_{max} 3419 (CONH), 3245 (OH), 1648 (CONH), 1604 (Ar), 1531 (CONH/NO$_2$), 1468 (Ar), 1354 (NO$_2$), 1166 (OH), 814 (2 adjacent ArH) cm^{-1}.

Preparation of *N*-{4[(*E*)-3(3,4-dihydroxyanilino)-3-oxoprop-1-enyl]phenyl}-2-hydroxybenzamide 115

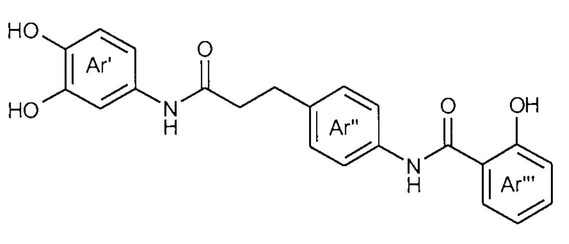


Method A: A 1M solution of BBr_3 in DCM (600μl) was added to a solution of **88** (0.094mmol, 50mg) in DCM (5ml) at –78°C. The mixture was allowed to warm to room temperature. After 16 hours the mixture was concentrated under vacuum and the residue dissolved in ethyl acetate and then partitioned between the ethyl acetate and water. The organic layer was collected and dried with anhydrous sodium sulphate and then removed under vacuum. The solid collected was shown by ^{1}H NMR to be the starting material.

Method B: A 1M solution of BBr_3 in DCM (5ml) was added to a solution of **88** (0.09mmol, 48mg) in DCM (5ml) at –78°C. The mixture was allowed to warm to room temperature and then heated under reflux for 2 hours. A yellow precipitate formed during the heating. The yellow precipitate was collected by filtration and was then dissolved in ethyl acetate (100ml) and washed with water (2x100ml). The organic layer was collected and dried with anhydrous sodium sulphate and then removed under vacuum. The title compound was collected as an orange solid (14mg, 40%). TLC [diethyl ether] R_f 0.39; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.62 (d, 1H, *J*=15.7Hz, CH=), 6.89-7.01 (ov. m, 4H, Ar'H-5/6, 2xAr"H), 7.27 (d, 1H, *J*=2.0Hz, Ar'H-2), 7.41-7.48 (ov. m, 2H, ArH), 7.58-7.75 (ov. m, 5H, ArH), 10.52 (s, 1H, NH), 10.58 (s, 1H, NH), 11.56 (br d, 2H, 2xOH), 12.75 (br s, 1H, OH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 108.03 (CH), 110.64 (CH), 117.42 (CH), 118.15 (CH), 119.40 (CH), 121.07 (2xCH), 121.76 (CH), 128.50 (CH), 129.50 (CHx2), 130.82 (q), 133.94 (CH), 138.93 (CH), 139.70 (q), 141.67 (q), 145.20 (q), 158.52 (q), 158.31 (q), 163.18 (q), 166.63 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 391M$^+$, 271[M-COC$_6$H$_5$OH]$^+$, 266[NHC$_6$H$_5$(OH)$_2$]$^+$, 158[NHCOCH=CHC$_6$H$_4$NH]$^+$; IR data (KBr disc): υ_{max} 3209 (OH/CONH), 1635 (CONH), 1589 (CONH), 1518 (CONH), 1488 (CONH), 1290 (OH), 1255 (OH), 1216 (CO), 821 (2 adjacent ArH), 757 (4 adjacent ArH) cm^{-1}.

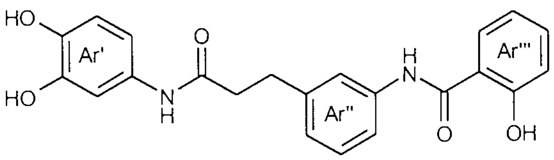
Method C: A 1Molar solution of BBr$_3$ in DCM (1.0ml) was added to a solution of 88 (0.28mmol, 149mg) in DCM (10ml). The reaction was heated under reflux for 2 hours and an orange solid formed, which was removed by filtration and washed with DCM and water. The product was dried under vacuum to give the title compound as a rust coloured solid (98mg, 90%). TLC, ^{1}H NMR and mass spectrum as for the previous batch.

Preparation of *N*-{4-[3-(3,4-dihydroxyanilino)-3-oxopropyl]phenyl}-2-hydroxybenzamide 116



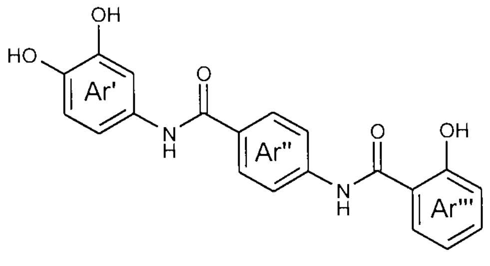
A 1M solution in DCM of BBr$_3$ (0.96ml) was added to a solution of **89** (0.32mmol, 171mg) in DCM (5ml). A white precipitate formed immediately, and the mixture was heated under reflux for 1 hour. The reaction was allowed to cool and water (50ml) was added. The white precipitate which formed was removed by filtration, washed with DCM and water, and dried under vacuum to give the title compound (112mg, 29%). TLC [ethyl acetate] R$_f$ 0.51; ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO): δ_H 2.99-2.80 (ov. m, 4H, $C\underline{H}_2C\underline{H}_2$), 6.48-6.62 (m, 2H, ArH), 6.73 (dd, 1H, *J*=7.3, 1.1Hz, Ar'H-5), 6.97 (d, 2H, *J*=8.0Hz, 2xAr"H), 7.13 (d, 1H, *J*=1.1Hz, Ar'H-2), 7.23 (d, 2H, *J*=8.0Hz, 2xAr"H), 7.43 (ddd, 1H, *J*=4.3, 3.8, 0.5Hz, Ar'"H), 7.60 (dd, 1H, *J*=8.3, 1.1Hz, Ar'"H), 7.94 (dd, 1H, *J*=7.3, 0.5Hz, ArH), 9.57 (br s, 1H, NH), 10.35 (br s, 1H, NH), 12.46 (br s, 2H, 2xOH), 12.55 (br s, 1H, OH) ppm; Mass spectrum (APCI$^+$): *m/z* 393M$^+$, 282[M-C$_6$H$_5$(OH)$_2$]$^+$; IR (KBr disc): υ_{max} 3311 (OH/CONH), 3068 (CONH), 2927 (CH$_2$), 1647 (CONH), 1612 (CONH), 1552 (CONH), 1486 (CONH), 1448 (CH$_2$), 1367 (OH), 1286 (OH), 1247 (OH), 1130 (C-OH), 861 (2 adjacent ArH), 752 (4 adjacent ArH) cm^{-1}; ^{13}C NMR (62.9MHz, (CD$_3$)$_2$SO): δ_C 30.09 (CH$_2$), 38.19 (CH$_2$), 108.10 (CH), 110.20 (CH), 115.20 (CH), 117.44 (CH), 119.20 (CH), 120.58 (CH), 121.29 (CH), 128.71 (CH), 128.89 (CH), 129.12 (CH), 131.52 (q), 133.88 (CH), 136.18 (q), 136.67 (q), 137.50 (q), 141.40 (q), 145.06 (q), 158.84 (q), 166.78 ($NH\underline{C}OC_6H_4OH$), 169.50 ($CH_2CH_2\underline{C}ONHC_6H_3(OH)_2$) ppm; Mass spectrum (APCI$^+$): *m/z* 393M$^+$, 282[M-C$_6$H$_3$(OH)$_2$]$^+$; IR (KBr disc): υ_{max} 3311 (OH/CONH), 3072 (CONH), 2931 (CH$_2$/CH$_3$), 1622 (CONH), 1610 (ArH), 1515 (CONH), 1450 (CH) cm^{-1}.

Preparation of *N*-{3-[3-(3,4-dihydroxyanilino)-3-oxopropyl]phenyl}-2-hydroxybenzamide 117



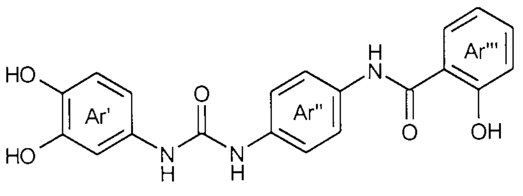
A 1M solution in DCM of BBr$_3$ (3ml) was added to a solution of the **102** (1.0mmol, 536mg) in DCM (10ml). A white precipitate formed immediately. The mixture was heated under reflux for 1 hour, allowed to cool and water (50ml) was added. The precipitate was removed by filtration, washed with DCM and water and dried under vacuum to give the title compound as a white/grey solid (392mg, 72%). TLC [ethyl acetate] R_f 0.55; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 3.95 (m, 4H, CH_2CH_2), 6.61 (d, 1H, *J*=8.4Hz, Ar'H-5), 6.75 (dd, 1H, *J*=8.3, 1.2Hz, Ar'H-6), 6.97-7.00 (ov. m, 3H, 3xArH), 7.25-7.30 (ov. m, 2H, 2xArH), 7.41-7.66 (ov. m, 3H, 3xArH), 7.95 (d, 1H, *J*=8.3Hz, ArH), 9.55 (br s, 1H, NH), 10.36 (br s, 1H, NH), 11.82 (br s, 2H, 2xOH), 12.50 (br s, 1H, OH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 31.10 (CH_2), 36.00 (CH_2), 110.80 (q), 115.28 (q), 115.60 (q), 117.45 (2xCH), 119.28 (3xCH), 121.12 (CH), 124.39 (CH), 129.28 (3xCH), 133.89 (CH), 138.5 (2xq), 140.50 (q), 145.00 (q), 158.63 (q), 166.80 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 393M$^+$, 282[M-$C_6H_3(OH)_2$]$^+$; IR (KBr disc): υ_{max} 3311(OH/CONH), 3068 (CONH), 2927 (CH_2), 1647, 1612, 1552, 1486 (CONH), 1448 (CH_2), 1367, 1286, 1247, 1225 (C-OH), 861 (2 adjacent ArH), 752 (4 adjacent ArH) cm^{-1}.

Preparation of *N*-{4-[(3,4-dihydroxyanilino)carbonyl]phenyl}-2-hydroxybenzamide 118



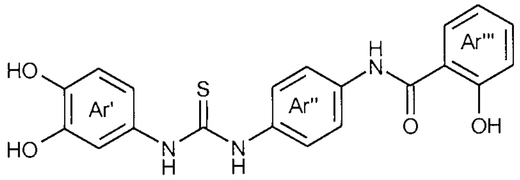
A 1M solution in DCM of BBr$_3$ (3ml) was added a solution of **103** (1mmol, 508mg) in DCM (10ml). and a yellow precipitate formed immediately. The reaction mixture was heated under reflux for 1 hour. The reaction was allowed to cool and water (50ml) was added to mixture. The precipitate was removed by filtration and washed with DCM and water to give a yellow solid (250mg, 69%). TLC [ethyl acetate] R_f 0.46; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.65-6.95 (ov. m, 6H, ArH), 7.26-7.30 (ov. m, 2H, ArH), 7.83-7.94 (ov. m, 6H, ArH), 9.70 (br s, 1H, NH), 9.80 (br s, 1H, NH), 11.3 (br d, 2H, 2xOH), 12.85 (br s, 1H, OH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 102.75 (CH), 106.36 (CH), 109.14 (CH), 111.73 (CH), 115.093 (CH), 117.55 (CH), 118.36 (CH), 120.21 (CH), 128.34 (CH), 129.45 (CH), 133.59 (CH), 141.21 (q), 141.98 (q), 144.97 (q), 148.50 (q), 151.21 (q), 159.55 (q), 164.15 ($NH\underline{C}OC_6H_4OH$), 166.61 ($CH_2CH_2\underline{C}ONHC_6H_3(OH)_2$) ppm; Mass spectrum (APCI$^+$): *m/z* 365M$^+$; IR (KBr disc): υ_{max} 3312 (OH/CONH), 3062 (CONH), 1630 (CONH), 1594 (Ar), 1525, 1510 (CONH), 1452 (CH), 1330, 1292 (OH), 1237, 1188, 1103 (CO), 846 (2 adjacent ArH), 751 (4 adjacent ArH) ppm.

Preparation of *N*-(4-{[(3,4-dihydroxyanilino)carbonyl]amino}phenyl)-2-hydroxybenzamide 119



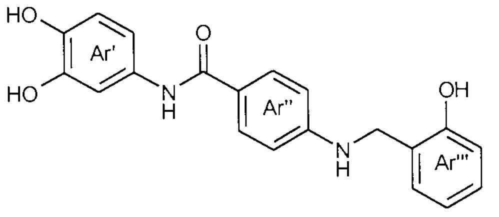
A 1M solution of BBr_3 in DCM (3ml) was added to a solution of the **109** (1mmol, 523mg) in DCM (10ml). Immediately, a yellow/beige precipitate formed. The reaction mixture was heated under reflux for 1 hour. The reaction was allowed to cool and water (50ml) was added. The precipitate was removed by filtration, washed with DCM and water and dried under vacuum to give the title compound as a yellow/beige solid (298mg, 79%). TLC [ethyl acetate] R_f 0.50; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.09-7.96 (ov. m, 11H, ArH), 8.26 (br s, 2H, NH), 8.50 (br s, 1H, NH), 10.31 (br s, 2H, 2xOH), 11.99 (br s, 1H, OH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 107.55 (CH), 109.76 (CH), 115.70 (CH), 117.21 (CH), 117.50 (CH), 118.38 (2xCH), 119.16 (CH), 122.13 (CH), 128.95 (CH), 131.84 (q), 132.06 (q), 133.89 (CH), 136.76 (q), 140.62 (q), 145.36 (q), 152.86 (q), 159.12 (q), 166.81 ($NH\underline{C}OC_6H_4OH$) ppm; Mass spectrum (APCI$^+$): *m/z* 380M$^+$; IR (KBr disc): υ_{max} 3282 (OH/CONH), 1638 (NHCONH), 1569 (CONH), 1510 (CONH), 1436 (OH), 1408 (OH), 1309 (OH), 1228 (C-O), 831 (2 adjacent ArH), 748 (4 adjacent ArH) cm^{-1}.

Preparation of *N*-(4-{[(3,4-dihydroxyanilino)sulphonyl]amino}phenyl)-2-hydroxybenzamide 120



A 1M solution of BBr_3 in DCM (1.35ml) was added to a solution of **110** (0.45mmol, 245mg) in DCM (10ml) and a beige/cream precipitate forms immediately. The reaction mixture was heated under reflux for 1 hour. Water (50ml) was added to the mixture after cooling. The precipitate removed by filtration, washed with DCM and water and dried under vacuum to give the title compound as a beige/cream solid (80mg, 45%). TLC [ethyl acetate] R_f 0.80; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.67-6.75 (m, 2H, Ar'H-5/6), 6.96-6.86 (m, 4H, ArH), 7.45 (m, 2H, ArH), 7.61 (m, 2H, ArH), 7.95 (m, 1H, ArH), 8.8 (br s, 1H, NH), 9.40 (br s, 1H, NH), 9.45 (br s, 1H, NH), 10.37 (br s, 2H, 2xOH), 11.85 (br s, 1H, OH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 112.95 (CH), 115.39 (CH), 115.81 (CH), 117.47 (CH), 119.26 (CH), 120.35 (CH), 121.20 (CH), 124.65 (CH), 129.15 (CH), 130.76 (CH), 133.92 (CH), 134.67 (q), 136.09 (q), 143.25 (q), 145.20 (q), 158.82 (q), 166.77 (q), 179.70 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 396M$^+$, 362[M-$(OH)_2$ or S]$^+$; IR (KBr disc): υ_{max} 3212 (OH/CONH), 1614 (CONH), 1510 (CONH), 1450 (Ar), 1197 (C=S) cm^{-1}.

Preparation of *N*-(3,4-dihydroxyphenyl)-4-[(2-hydroxybenzyl)amino]benzamide 121



Concentrated HCl (5ml) and water (500μl) were added to a solution of **112** (1.50mmol, 719mg) in THF (15ml). The mixture was heated under reflux for 16 hours. The THF and HCl were removed under vacuum to give a yellow solid. The yellow solid was dissolved in a solution of sodium hydrogen carbonate and extracted into ethyl acetate. The organic layer was dried with sodium sulphate and removed under vacuum to give a brown residue. The title compound was precipitated from diethyl ether as a grey solid (96mg, 18%). TLC [ethyl acetate] R_f 0.10; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 4.25 (d, 2H, *J*=5.8Hz, CH_2), 6.58-6.84 (ov. m, 5H, 2xAr"H, 3xAr'''H), 6.90 (dd, 1H, *J*=8.6, 2.3Hz, Ar'H-6), 7.05 (ddd, 1H, *J*=7.9, 7.6, 1.2Hz, Ar'''H), 7.15 (d, 1H, *J*=7.5Hz, Ar'H-5), 7.24 (d, 1H, *J*=2.4Hz, Ar'H-2), 7.68 (d, 2H, *J*=8.6Hz, 2xAr"H), 8.53 (br s, 1H, NH), 8.85 (br s, 1H, NH), 9.42 (br s, 2H, 2xOH), 9.55 (br s, 1H, OH) ppm; ^{13}C NMR (62.9MHz, $(CD_3)_2SO$): δ_C 39.32, (CH_2), 107.53 (CH), 109.36 (CH), 110.03 (CH), 113.35 (CH), 113.49 (CH), 117.27 (CH), 120.01 (CH), 123.59 (q), 126.13 (q), 126.64 (CH), 127.44 (CH), 130.13 (CH), 139.60 (q), 143.16 (q), 149.82 (q), 153.45 (q), 163.24 (q) ppm; Mass spectrum (APCI$^+$): *m/z* 351M$^+$, 245[M-$CH_2C_6H_4(OH)$]$^+$, 226[M-$NHC_6H_3(OH)_2$]$^+$, 120[COC_6H_4NH]$^+$; IR (KBr disc): υ_{max} 3477 (NH), 3376 (NHCO), 3240 (OH), 1606 (CONH), 1533 (CONH), 1458 (CH_2), 1369 (OH), 1109 (OH) cm^{-1}.

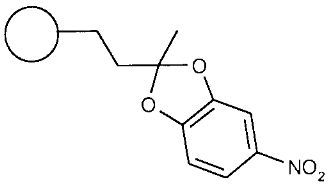
Preparation of Merrifield β-keto ester and hydrolysis to the Merrifield phenethyl ketone 122

Merrifield resin (5g, 2.3mmol Cl/g) was swollen in DMF (150ml) was added sodium ethyl acetoacetate (115 mmol, 10eq, 17.48g). The mixture was heated at 80°C with gentle stirring. After 18 hours the resin was removed by filtration and washed with DMF (2x100ml) and THF (2x100ml). The resin was dried under vacuum to give the title resin as a cream solid (4.98g). IR (KBr disc): υ_{max} 2925 (CH_2/CH_3), 2860 (CH), 1702, 1645 (COOC), 1581 (ArH), 1441 (CH_2/CH_3/OCOCH), 1259, 1022 (CO), 760, 690 (5 adjacent ArH) cm^{-1}.

The β-keto ester (4.98g) was swollen in dioxane (100ml) and 4M HCl was added slowly. The reaction mixture was stirred gently at 80°C for 18 hours. The resin was then collected by filtration and washed with dioxane and dried under vacuum to give a pale yellow solid (4.95g). IR (KBr disc): υ_{max} 3060, 3015 (Ar), 2917 (CH_2/CH_3), 2840 (CH), 1716 (CH_3CO), 1598 (ArH), 1495, 1452 (CH_2), 1370 (CH_3CO), 1255, 1190, 1150, 1114, 1030 (CO), 755, 690 (5 adjacent ArH) cm^{-1}.

Merrifield phenethyl ketone was prepared a further two times, on a 5g scale where 4.994g of resin was collected and on a 10g scale where 9.541g of resin was collected.

Preparation of Merrifield 2-methyl-5-nitro-2-phenethyl-1,3-benzodioxole resin 123

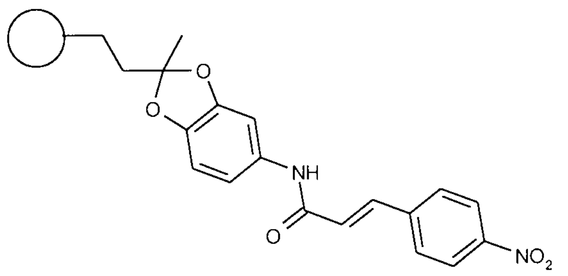


Method A: The phenethyl ketone resin **122** (4.95g) was swollen in toluene (100ml). 4-Nitrocatechol (20mmol, 3.10g) and *p*-TsOH (100mg) were added, and the mixture was heated under reflux in conjunction with a Dean-Stark trap, with gentle stirring. The reaction was monitored by the disappearance of the ketone peak at 1716cm^{-1} in the IR spectrum. After 96 hours the resin was removed by filtration and washed with toluene (100ml), dioxane (3x100ml), and CH_2Cl_2 (2x100ml). The title resin was collected as a dark brown resin (4.67g). The ketone peak was monitored by IR spectrum and ketone peak could not be completely reduced in size. IR (KBr disc): υ_{max} 3062, 3025 (Ar), 2925 (CH_2/CH_3), 2865 (CH), 1706 (residual ketone), 1602 (ArH), 1510 (NO_2), 1495, 1450 (CH_2), 1330 (NO_2), 1182, 1120, 1020, 1010 (CO), 760, 699 (5 adjacent ArH) cm^{-1}.

Method A was repeated on a 5g scale and the title resin was collected as a dark brown resin (5.189g). IR spectrum as for previous batch.

Method B: The resin **122** (5.00g) was swollen in xylene (100ml). 4-Nitrocatechol (20mmol, 3.10g) and *p*-TsOH (100mg) were added to the resin mixture and heated under reflux with gentle stirring in conjunction with a Dean-Stark trap. The reaction was monitored by the disappearance of the IR spectrum ketone peak. After 96 hours the resin was removed by filtration and washed with toluene (100ml), dioxane (3x100ml), and DCM (2x100ml). The title resin was collected as a black resin (4.97g). Despite the higher boiling point of xylene the IR spectrum showed that the ketone peak still could not be completely reduced in size. IR (KBr disc): υ_{max} 3062, 3025 (Ar), 2925 (CH_2/CH_3), 2865 (CH), 1706 (residual ketone), 1602 (ArH), 1510 (NO_2), 1495, 1450 (CH_2), 1330 (NO_2), 1182, 1120, 1020, 1010 (CO), 760, 699 (5 adjacent ArH) cm^{-1}.

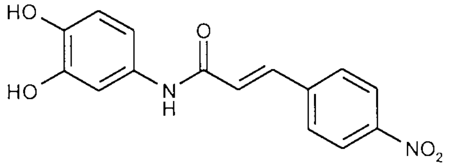
Attempted preparation of Merrifield (*E*)-*N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-3-(4-nitrophenyl)prop-2-enamide resin 124



Sodium dithionite (57.5mmol, 10.005g) in water (100ml) was added to a solution of the resin **123** (4.67g) in ethanol (100ml). The reaction mixture was heated under reflux for 7 days. The mixture was concentrated under vacuum and the washed with diethyl ether (3x100ml) and THF (3x100ml) under argon. The resin was then soaked in anhydrous THF for one hour, and then washed with more anhydrous THF in an argon atmosphere. The resin was then mixed with fresh anhydrous THF (35ml) and TEA (5ml) under an atmosphere of argon. A solution of 4-nitrocinnamoyl chloride (15mmol, 3.17g) in anhydrous THF (10ml) was added slowly to the reduced resin mixture and allowed to stir gently for 18 hours. The resulting brown/black resin was collected by filtration, washed with THF (3x100ml) and dried under vacuum (4.123g). IR (KBr disc): υ_{max} 3463 (CONH), 3064, 3021 (Ar), 2920 (CH_2/CH_3), 1748 (CONH), 1714 (CH_3CO-), 1598 (ArH), 1520 (NO_2/CONH), 1500, 1454 (CH_2), 1346 (NO_2), 1163 (ArOC), 1060, 1011 (CO), 760, 699 (5 adjacent ArH) cm^{-1}.

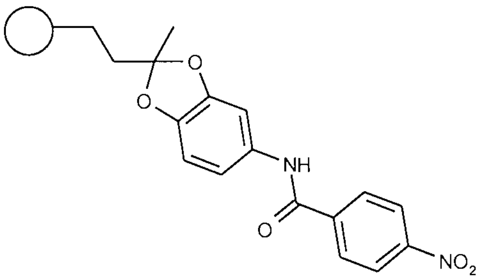
Method B: Sodium dithionite (57.5mmol, 10.005g) in water (100ml) was added to a solution of the resin **123** (5.00g) in ethanol (100ml). The reaction mixture was heated under reflux for 3 days. The reaction mixture was cooled and another batch of sodium dithionite (57.5mmol, 10.005g) in water (100ml) was added. The reaction mixture was then heated under reflux for a further 3 days. The mixture was concentrated under vacuum and the residue washed in an atmosphere of argon, with ethanol (3x100ml), dioxane (3x100ml) and THF (3x100ml). The resin was then soaked in anhydrous THF (100ml) for one hour and was then washed with more anhydrous THF (100ml) in an argon atmosphere. The resin was then mixed with fresh anhydrous THF (35ml) and TEA (5ml) in an atmosphere of argon. A solution of 4-nitrocinnamoyl chloride (15mmol, 3.17g) in anhydrous THF (10ml) was added slowly to the reduced resin and allowed to stir gently for 18 hours. The resulting brown/black resin was collected by filtration, washed with THF (3x100ml) and dried under vacuum (4.836g). IR spectrum as for previous batch.

Attempted cleavage of (*E*)-*N*-(3,4-dihydroxyphenyl)-3-(4-nitrophenyl)prop-2-enamide from Merrifield phenethyl ketone resin



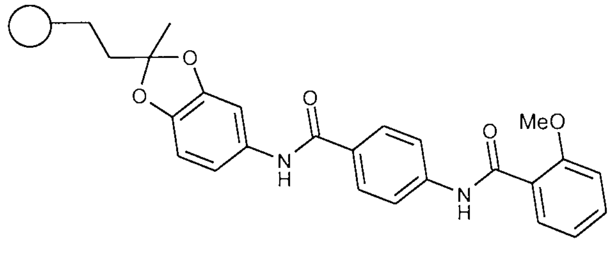
Concentrated HCl (45ml) was added slowly to a gently stirred mixture of the resin **124** (3g) in dioxane (40ml) and water (5ml). The mixture was heated at 80°C for 8 hours. The HCl, dioxane and water were removed under vacuum. The remaining brown solid was analysed by TLC and showed >8 spots and gave no identifiable peaks by ^{1}H NMR or mass spectrometry.

Attempted preparation of Merrifield *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-4-nitrobenzamide 125



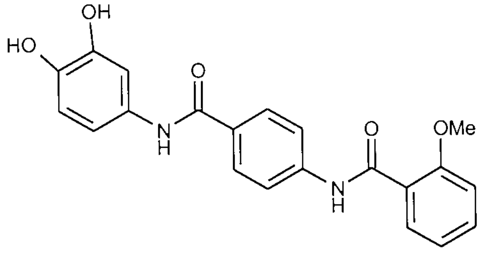
Sodium dithionite (57.5mmol, 10.005g) in 50ml water was added to a solution of the resin **123** (5.00g) in ethanol (50ml). The reaction mixture was heated under reflux for 18 hours. The reaction mixture was allowed to cool and ethanol (50ml) was added then sodium dithionite (57.5mmol, 10.005g) in water (50ml) was added and the mixture was heated under reflux for a further 18 hours. The resin was removed by filtration under argon and washed with ethanol (100ml), water (100ml) and THF (2x100ml). The resin was suspended in anhydrous THF with 4A° molecular sieves (1g) and soaked in anhydrous THF for one hour. The resin was then washed with more anhydrous THF and was then resuspended in fresh anhydrous THF (35ml) and TEA (5ml) under an atmosphere of argon. A solution of 4-nitrobenzoyl chloride (15mmol, 2.784g) in anhydrous DMF was added slowly to the reduced resin and allowed to stir gently for 18 hours. The resin was collected by filtration and washed with DMF (100ml), dioxane (100ml), and DCM (2x100ml). The resin was dried under vacuum to give a dark brown/black solid (4.381g). IR (KBr disc): υ_{max} 3460 (CONH), 3077, 3025 (Ar), 2922 (CH_2/CH_3), 2852 (CH), 1746 (CONH), 1714 (CH_3CO-, residual ketone), 1600 (ArH), 1523 (NO_2/CONH), 1493, 1445 (CH_2), 1346 (NO_2), 1255, 1178 (ArOC), 1064, 1014 (CO), 754, 690 (5 adjacent ArH) cm^{-1}.

Attempted preparation of Merrifield *N*-(4-{[(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)amino]carbonyl}phenyl)-2-methoxybenzamide resin 126



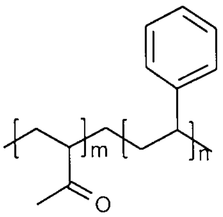
Sodium dithionite (57.5mmol, 10.005g) in 50ml water was added to a solution of the resin **125** (4.381g) in ethanol (50ml). The reaction mixture was heated under reflux for 18 hours. The reaction mixture was allowed to cool and ethanol (50ml) was added then sodium dithionite (57.5mmol, 10.005g) in water was added and the mixture was heated under reflux for a further 18 hours. The resin was removed by filtration under argon and washed ethanol (100ml), water (100ml) and THF (2x100ml). The resin was suspended in anhydrous THF with 4A° molecular sieves (1g) and soaked in anhydrous THF for one hour. The resin was then washed with more anhydrous THF and was then resuspended in fresh anhydrous THF (35ml) and TEA (5ml) under an atmosphere of argon. A solution of 2-methoxybenzoyl chloride (12mmol, 2.047g) in anhydrous DMF was added slowly to the reduced resin and allowed to stir gently for 18 hours. The resin was collected by filtration and washed with DMF (100ml), dioxane (100ml), and DCM (2x100ml). The resin was dried under vacuum to give a dark brown/black solid (4.126g). IR (KBr disc): υ_{max} 3430 (CONH), 3084, 3026 (Ar), 2917 (CH_2/CH_3), 2848 (CH), 1738 (CONH), 1714 (residual ketone), 1603 (Ar), 1529 (residual NO_2), 1493, 1452 (CH_2), 1338 (NO_2/OCH_3), 1261, 1222 (ArOC), 1176, 1064, 1018 (CO), 756, 694 (5 adjacent ArH) cm^{-1}.

Attempted cleavage of *N*-{4-[(3,4-dihydroxyanilino)carbonyl]phenyl}-2-hydroxybenzamide from Merrifield phenethyl ketone resin



Concentrated HCl (85ml) was added slowly to a mixture of the resin **126** (4g) in dioxane (80ml), and water (5ml). The reaction mixture was heated at 80°C for 4 hours. The reaction was cooled and DMF (30ml) was added to dissolve any insoluble cleaved product. The Merrifield resin was removed by filtration and the remaining organic solvent was removed under vacuum. The DMF had reacted with HCl to give the white solid dimethylamine hydrochloride. The solid was dissolved in water and a fine brown powder was left which was removed by filtration, the ^{1}H NMR spectrum of this product showed no peaks at all. The water layer was extracted with ethyl acetate, which was then dried with anhydrous sodium sulphate, and removed under vacuum to give a brown solid. The remaining brown solid was analysed by TLC and showed >6 spots and gave no identifiable peaks in ^{1}H NMR or by mass spectrometry.

Preparation of the poly(MVK-*co*-St), maximum potential loading 1.97mmol/g 127



AIBN (580mg) was added to a solution of styrene (480.769mmol, 50g) and MVK (114.286mmol, 8g) in toluene (100ml). The reaction vessel was covered with foil and sealed. Air was removed from the reaction vessel by the alternate application of vacuum and argon. The reaction mixture was heated at 60°C for 48 hours. The resulting polymer was poured onto vigorously stirred Methanol (600ml). The title polymer was collected by filtration as a white gelatinous solid and dried under vacuum to give a white solid (36.074g, 62%). Maximum potential polymer loading 1.97mmol/g. ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.90-2.39 (br m, polymer back-bone CH_2), 6.40-7.25 (br m, polymer back-bone ArH) ppm; IR (KBr disc): υ_{max} 3055, 3025 (Ar), 2925 (CH_2/CH_3), 2850 (CH), 1712 ($COCH_3$), 1602 (ArH), 1495 (CH_2), 1450 (CH_2), 1350 ($COCH_3$), 1150, 1020 (CO), 760, 700 (5 adjacent ArH) cm^{-1}.

Preparation of poly(MVK-*co*-St) **127** was repeated on the same scale to give a 68% yield.^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.95-2.10 (br m, polymer CH_2), 6.35-7.42 (br m, polymer ArH from styrene) ppm; IR (KBr disc): υ_{max} 3090, 3080, 3028, 2925 (CH_2/CH_3), 2860 (CH), 1706 ($CH\text{-}COCH_3$), 1598 (Ar), 1490 (CH), 1454 (CH), 1350 ($COCH_3$), 1159 (CO), 1025 (CO), 762, 696 (5 adjacent ArH) cm^{-1}.

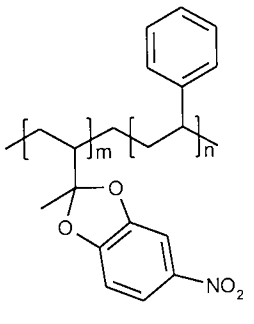
Preparation of the poly(MVK-*co*-St), maximum potential loading 3.60mmol/g 128

AIBN (556mg) was added to a solution of MVK (200mmol, 14g) and styrene (400mmol, 41.6g) in toluene (100ml). The reaction vessel was covered with foil and sealed. Air was removed from the reaction vessel by repeated application of vacuum and argon. The reaction mixture was heated at 60°C for 24 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml). The title polymer was collected by filtration as a white gelatinous solid and dried under vacuum to give a white solid (26.64g, 48%). Maximum potential polymer loading 3.60mmol/g. ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.95-2.10 (br m, polymer back-bone CH_2), 6.45-7.20 (br m, polymer back-bone ArH) ppm. IR (KBr disc): υ_{max} 3060, 3023 (ArH), 2921 (CH_2/CH_3), 2860 (CH), 1710 ($C{=}O\text{-}CH_3$), 1598 (ArH), 1490, 1450 (CH_2), 1350 ($C{=}O\text{-}CH_3$), 1161, 1026 (CO), 762, 696 (5 adjacent ArH) cm^{-1}.

Preparation of the poly(MVK-*co*-St), maximum potential loading 5.75mmol/g 129

AIBN (696mg) was added to a solution of MVK (400mmol, 28g) and styrene (400mmol, 41.6g) in toluene (100ml). The reaction vessel was covered with foil and sealed. Air was removed from the reaction vessel by the alternate application of vacuum and argon. The reaction mixture was heated at 60°C for 24 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml). The title polymer was collected by filtration as a white gelatinous solid and dried under vacuum to give a white solid (33.8g, 49%). Maximum potential polymer loading 5.75mmol/g. ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.90-2.39 (br m, polymer back-bone CH_2), 6.40-7.25 (br m, polymer back-bone ArH) ppm; IR (KBr disc): υ_{max} 3060, 3023 (ArH), 2923 (CH_2/CH_3), 2855 (CH), 1706 ($C{=}O\text{-}CH_3$), 1598 (Ar), 1490, 1454 (CH_2), 1356 ($C{=}O\text{-}CH_3$), 1157, 1026 (CO), 766, 698 (5 adjacent ArH) cm^{-1}.

Ketal formation with 4-nitrocatechol and poly(MVK-*co*-St) 127to give 130



Method A: 4-Nitrocatechol (13mmol, 2.015g) and *p*-TsOH (100mg) were added to a solution of the polymer **127** (10g) in toluene (100ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap for 24 hours. The resulting polymer was precipitated onto vigorously stirred methanol (600ml). The resulting polymer precipitate was collected by filtration as a pale brown precipitate and dried under vacuum. Analysis of the solid polymer by IR spectroscopy showed that there was still a peak at 1710cm^{-1}, suggesting that the reaction did not go to completion.

Method B: 4-Nitrocatechol (26.85mmol, 4.162g) and *p*-TsOH (100mg) were added to a solution of the polymer **127** (10g) in toluene (100ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap for 48 hours. The reaction was monitored by the disappearance of the carbonyl peak at 1706cm^{-1} in the IR spectrum. When the carbonyl peak had completely disappeared from the IR spectrum the polymer was precipitated onto vigorously stirred methanol (600ml). The title polymer was collected by filtration as a black/grey precipitate and dried under vacuum (9.550g, 84% based on maximum polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.70-2.35 (br m, polymerised CH_2), 6.20-7.23 (br m, polymerised ArH of styrene), 7.60-7.90 (br s, polymerised ArH-2) ppm; IR (KBr disc): υ_{max} 3055, 3025 (Ar), 2925 (CH_2/CH_3), 2850 (CH), 1598 (ArH), 1530 (NO_2), 1495 (CH_2), 1450 (CH_2), 1340 (NO_2), 1270, 1020 (CO), 760, 700 (5 adjacent ArH) cm^{-1}.

Method B was repeated on a 22mmol scale to give a 70% yield based on the maximum polymer loading. IR (KBr disc): υ_{max} 3055, 3025 (Ar), 2925 (CH_2/CH_3), 2850 (CH), 1598 (ArH), 1530 (NO_2), 1495 (CH_2), 1450 (CH_2), 1340 (NO_2), 1270 (ArOC), 1020 (CO), 760, 702 (5 adjacent ArH) cm^{-1}.

Method C: 4-Nitrocatechol (29mmol, 4.5g) and *p*-TsOH (100mg) was added to a solution of the polymer 127 (13g, maximum potential polymer loading 1.970mmol/g) in toluene (100ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap and monitored by IR spectroscopy, for 86 hours. The polymer was poured onto methanol (600ml). The polymer precipitate was collected by filtration and re-dissolved and precipitated again a further three times. The title polymer was dried under vacuum as a brown solid (12.2g, 74% based on maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.90-2.30 (br m polymer back-bone CH_2), 6.10-7.40 (br m, polymer back-bone ArH), 7.75 (br s, pendant ArH-6) ppm; IR (KBr disc): υ_{max} 3090, 3040, 2935 (CH_2/CH_3), 2895 (CH), 1706 (unreacted ketone), 1598 (Ar), 1520 (NO_2), 1488, 1450 (CH_2), 1345, 1280, 1225 (CO), 765, 702 (5 adjacent ArH) cm^{-1}.

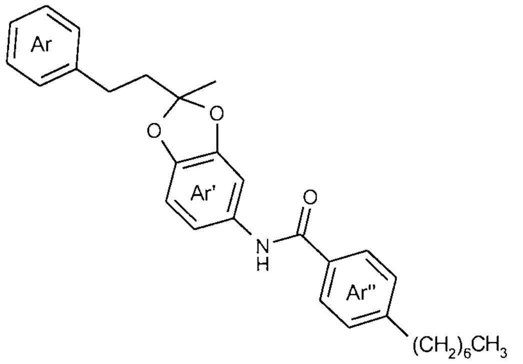
Attempted ketal formation with the higher loading poly(MVK-*co*-St) 3.6mmol/g 131

4-Nitrocatechol (20mmol, 3.1g) and *p*-TsOH (100mg) were added to a solution of the polymer **128** (5g) in toluene (100ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap for 72 hours. The reaction was monitored by disappearance of IR carbonyl peak at 1706cm^{-1}. When complete the polymer was precipitated onto methanol (600ml). The title polymer was collected by filtration as a black/grey precipitate and dried under vacuum (4.62g, 62% based on maximum polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.70-2.35 (br m, polymerised CH_2), 6.20-7.23 (br m, polymerised ArH of styrene), 7.60-7.90 (br s, polymerised ArH-2) ppm; IR (KBr disc): υ_{max} 3055, 3025 (Ar), 2925 (CH_2/CH_3), 2850 (CH), 1598 (ArH), 1530 (NO_2), 1495 (CH_2), 1450 (CH_2), 1340 (NO_2), 1270, 1020 (CO), 760, 700 (5 adjacent ArH) cm^{-1}. The IR spectrum shows a large reduction in the ketone peak, but the more the ketone peak is reduced the less soluble the polymer becomes. The final polymer is only sparingly soluble in the organic solvents, $CHCl_3$, DCM, THF, and toluene.

Attempted ketal formation with the higher loading poly(MVK-*co*-St), 5.75mmol/g 132

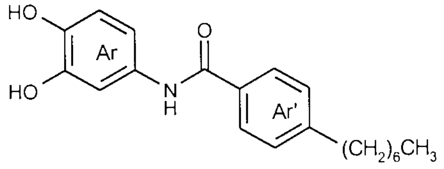
4-Nitrocatechol (20.00mmol, 3.1g) and *p*-TsOH (100mg) were added to a solution of the polymer **129** (5g) in toluene (100ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap for 72 hours. The reaction was monitored by disappearance of IR carbonyl peak at 1706cm^{-1}. When complete the polymer was precipitated onto methanol (600ml). The title polymer was collected by filtration as a black/grey precipitate and dried under vacuum (3.56g, 40% based on maximum polymer loading). ^{1}H NMR analysis was not performed because of the lack of product solubility; IR (KBr disc): υ_{max} 3067, 3022 (Ar), 2924 (CH_2/CH_3), 2860 (CH), 1600 (ArH), 1536 (NO_2), 1497 (CH_2) 1336 (NO_2), 1279, 1023 (CO), 760, 700 (5 adjacent ArH) cm^{-1}. The IR spectrum shows a large reduction in the ketone peak, but the more the ketone peak is reduced the less soluble the polymer becomes. The final polymer is not soluble in the organic solvents, $CHCl_3$, DCM, THF, and toluene.

Preparation of *N*-(2-methyl-2-phenethyl-1,3-benzodioxol-5-yl)-4-heptylbenzamide 133



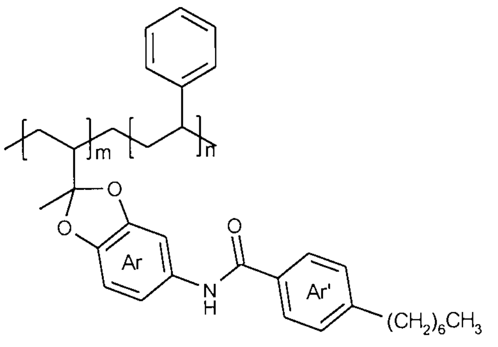
10% Pd-C (117mg) was added to a solution of **84** (4.1mmol, 1.167g) in THF (30ml). Air was removed by alternate application of vacuum and argon. Argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was shaken vigorously for 16 hours. The hydrogen was replaced with argon and TEA (1ml) was added. A solution of 4-*n*-heptylbenzoyl chloride (4.5mmol, 1.075g) in anhydrous THF (10ml) was added slowly to the reduced mixture and stirred for 16 hours. The solids, 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite and the THF was removed under vacuum. The resulting residue was subjected to flash chromatography, eluting with petrol 60:80-diethyl ether (3:1) to give the title compound as a pale yellow solid (1.757g, 94%). TLC [petrol 60:80-diethyl ether (3:1)] R_f 0.26; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.91 (t, 3H, *J*=6.8Hz, Ar"$CH_2(CH_2)_5C\underline{H}_3$), 1.31-1.34 (m, 10H, Ar"$CH_2(C\underline{H}_2)_5CH_3$), 1.70 (s, 3H, CH_3), 2.25-2.32 (m, 2H, $CH_2C\underline{H}_2C_6H_4$), 2.69 (t, 2H, *J*=7.7Hz, Ar"$C\underline{H}_2$), 2.80-2.86 (m, 2H, $C\underline{H}_2CH_2C_6H_4$), 6.74 (d, 1H, *J*=8.3Hz, Ar'H-6), 6.88 (dd, 1H, *J*=8.3, 2.1Hz, Ar'H-5), 7.21-7.34 (ov. m, 8H, ArH), 7.74 (br s, 1H, NH), 7.79 (d, 2H, *J*=8.2Hz, 2xAr"H) ppm; ^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 0.85 (t, 3H, *J*=6.6Hz, Ar"$C_6H_{12}C\underline{H}_3$), 1.27-1.25 (m, 6H, Ar"$CH_2CH_2CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_3$), 1.64-1.58 (m, 4H, Ar"$CH_2C\underline{H}_2C\underline{H}_2CH_2CH_2CH_2CH_3$), 2.26-2.19 (m, 2H, $CH_2C\underline{H}_2C_6H_4$), 2.64 (t, 2H, *J*=7.6, $COC_6H_4C\underline{H}_2$), 2.75-2.69 (m, 2H, $C\underline{H}_2CH_2C_6H_4$), 3.35 (s, 3H, CH_3), 6.82 (d, 1H, *J*=8.4Hz, ArH), 7.37-7.12 (ov. m, 10H, 10xArH), 7.84 (d, 2H, *J*=8.2Hz, 2xAr'H), 10.04 (s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 458M$^+$; IR (KBr disc): υ_{max} 3025 (Ar-H), 3330 (NHC=O), 2927 (CH_2/CH_3), 2856 (CH_2/CH_3), 1647 (NHC=O), 1607 (Ar), 1526 (NHC=O), 1504 (Ar), 1435 (CH_2/CH_3), 1377 (CH_2/CH_3), 1245 (CO) cm^{-1}.

Preparation of *N*-(3,4-dihydroxyphenyl)-4-heptylbenzamide 134



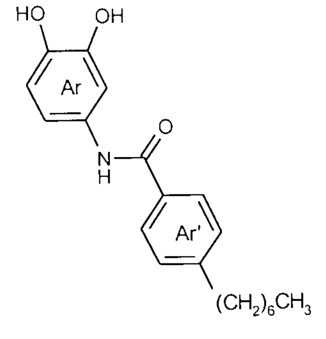
A 1M solution in DCM of BBr$_3$ (4.5ml) was added to a solution of **133** (3.8mmol, 1.73g) in DCM (10ml) at 0°C. The reaction mixture was allowed to warm to room temperature over two hours. The DCM was removed under vacuum. The residue was re-dissolved in ethyl acetate, washed with aqueous sodium hydrogen carbonate and dried with anhydrous sodium sulphate. The title compound was precipitated from diethyl ether and petrol 60:80 as a beige solid (474mg, 97%). TLC [ethyl acetate-petrol 60:80] R$_f$ 0.26; ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO): δ_H 0.85 (t, 3H, *J*=6.6Hz, CH$_3$), 1.22-1.28 (ov. m, 8H, CH$_2$), 1.58 (m, 2H, Ar'CH$_2$C$\underline{\text{H}}_2$), 2.63 (t, 2H, *J*=7.7Hz, Ar'C$\underline{\text{H}}_2$), 6.66 (d, 1H, *J*=8.5Hz, ArH-5), 6.96 (dd, 1H, *J*=8.2, 1.9Hz, ArH-6), 7.28-7.32 (ov. m, 3H, ArH-2, 2xAr'H), 7.82 (d, 2H, *J*=8.1Hz, 2xAr'H), 9.84 (br s, 1H, NH) ppm; ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO/D$_2$O): δ_H 0.76 (t, 3H, *J*=6.7Hz, CH$_3$), 1.15-1.18 (ov. m, 8H, CH$_2$), 1.51 (m, 2H, Ar'CH$_2$C$\underline{\text{H}}_2$), 2.57 (t, 2H, *J*=7.7Hz, Ar'C$\underline{\text{H}}_2$), 6.69 (d, 1H, *J*=8.6Hz, ArH-5), 6.85 (dd, 1H, *J*=8.2, 1.9Hz, ArH-6), 7.13 (d, 1H, *J*=1.8Hz, ArH-2), 7.26 (d, 2H, *J*=8.2Hz, 2xAr'H), 7.72 (d, 2H, *J*=8.2Hz, 2xAr'H), 9.84 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 328M$^+$; IR (KBr disc): υ_{max} 3432-3231 (OH/CONH), 2965, 2924 (CH$_2$/CH$_3$), 2848 (CH), 1634 (CONH), 1608 (Ar), 1550 (CONH), 1450 (CH$_2$/CH$_3$), 1245, 1195, 1120 (CO), 848, 820 (2 adjacent ArH) cm^{-1}.

Reduction and coupling of the co-polymer 130 with 4-*n*-heptylbenzoyl chloride to give 135



10% Pd-C (200mg) was added to a solution of the co-polymer **130** (2g) in THF (50ml). Air was replaced by the alternate application of vacuum and argon. The argon was then replaced by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously for 72 hours. The hydrogen was replaced with argon and TEA (1ml) was added. A solution of 4-*n*-heptylbenzoyl chloride (6mmol, 1.432g) in THF anhydrous (10ml) was added slowly to the reduced polymer mixture and allowed to stir for 18 hours. The reaction mixture was filtered by filtration through celite and the resulting polymer was poured onto vigorously stirred methanol (600ml). The precipitated polymer was collected by filtration, and then re-dissolved and precipitated a further three times. The title polymer was dried under vacuum to give a grey solid (1.82g, 65% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, CDCl$_3$): δ_H 0.80-0.98 (br m, polymer pendant CH$_2$), 0.98-1.90 (br m polymer back-bone CH$_2$), 2.65 (br s, polymer pendant CH$_3$/CH$_2$), 6.50-7.40 (br m, polymer back-bone ArH), 7.72 (br s, pendant ArH), 8.15 (br s, pendant ArH or NH) ppm; IR (KBr disc): υ_{max} 3060, 3023 (Ar), 2921 (CH$_2$/CH$_3$), 2855 (CH), 1604 (ArH), 1521 (CONH), 1488, 1450 (CH$_2$), 1336, 1267, 1085 (CO), 760, 702 (5 adjacent ArH) cm^{-1}.

Cleavage of *N*-(3,4-dihydroxyphenyl)-4-heptylbenzamide 134 from poly(MVK-*co*-St) 130



Method A: TFA (4ml) and water (500μl) was added to a solution of the co- polymer **135** (1g) in DCM (20ml). The reaction mixture was stirred and monitored by TLC. After 3 hours no cleavage appeared to have occurred and more TFA (4ml) was added. The reaction was allowed to stir for a further 16 hours. The remaining polymer was poured onto methanol. The polymer precipitate was removed by filtration and the remaining organic solution was concentrated under vacuum to gave a crude brown solid, 7 components by TLC. The brown solid was subjected to preparative TLC eluted with ethyl acetate-petrol 60:80 (1:1). The band at R_f 0.38 [ethyl acetate-petrol 60:80 (1:1)] was collected as a pale brown solid. Although the TLC showed to same R_f as the title compound the ^{1}H NMR and mass spectrum was completely different. Mass spectrum (APCI$^+$): *m/z* 288, 276, 235.

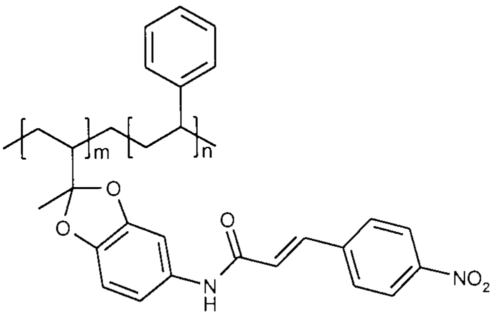
Method B: Concentrated HCl (50ml) was added to a solution of **135** (2g) in DCM (50ml). The reaction mixture was heated under reflux for two hours. The organic constituents were extracted into $CHCl_3$. The polymer was poured onto methanol and the polymer precipitate was removed by filtration. The remaining organic solution was concentrated under vacuum. A crude pale brown solid was obtained (358mg) of 2 main components by TLC. The residue was subjected to preparative TLC, eluted with ethyl acetate-petrol 60:80 (1:3). The two components were collected neither of which could be identified as the desired product, by ^{1}H NMR the products appeared to be the methyl ester of the acid chloride and the carboxylic acid. Suggesting that no cleavage occurred. The cleaved polymer appeared still to have the pendant groups previously present.

Method C: The co-polymer **135** (1g) was dissolved in dioxane (40ml). Concentrated HCl (17ml) was added to the solution with the aim of adding the maximum amount of concentrated HCl before causing the polymer to precipitate. The mixture was stirred for 18 hours. The polymer was poured onto methanol and the polymer precipitate was removed by filtration. The remaining organic solution was concentrated under vacuum and the resulting residue was subjected to preparative TLC [petrol 60:80-ethyl acetate (1:1] R_f 0.19. The title compound was collected as an orange/brown solid (55mg). ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.85 (t, 3H, *J*=6.6Hz, CH_3), 1.17-1.27 (ov. m, 8H, 4xCH_2), 1.58 (m, 2H, Ar'$CH_2C\underline{H}_2$), 2.63 (t, 2H, *J*=7.5Hz, Ar'$C\underline{H}_2$), 6.66 (d, 1H, *J*=8.5Hz, ArH-5), 6.95 (dd, 1H, *J*=8.7, 2.6Hz, ArH-6), 7.28-7.32 (ov. m, 3H, ArH-2, 2xAr'H), 7.82 (d, 2H, *J*=8.1Hz, 2xAr'H), 8.71 (s, 1H, OH), 8.99 (s, 1H, OH), 9.85 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 328M$^+$. The ^{1}H NMR of the polymer after cleavage still showed small peaks of the pendant molecule. ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.95 (br s, pendant CH_2), 1.20-2.10 (br m, polymer CH_2), 6.35-7.20 (br m, polymer ArH), 7.70 (br s, pendant ArH) ppm.

Method D: BBr_3 (12ml, 1M in DCM) was added to a solution of the co-polymer **135** (2g) in DCM (50ml) at 0°C. The reaction mixture was monitored by TLC and allowed to warm to room temperature and stirred for 16 hours. The polymer mixture was poured slowly onto a solution of aqueous sodium hydrogen carbonate. The organic layer was collected and the aqueous layer washed with DCM and then ethyl acetate. The organic layer was dried with sodium sulphate and poured onto methanol (150ml) to precipitate the polymer. The polymer precipitate was removed by filtration and the remaining organic solution was concentrated under vacuum. A crude brown solid was obtained (303mg) of 6 components by TLC. The residue was subjected to flash column chromatography eluted with ethyl acetate-petrol 60:80 (1:1). Five separate components were collected none of which could be identified as the desired product. The cleaved polymer appeared no longer to have the pendant groups previously present. Polymer after cleavage procedure ^{1}H NMR (250.1MHz, CDCl$_3$): δ_H 0.85-3.00 (br m, polymer backbone CH$_2$, including larger peaks at 1.0ppm, pendant CH$_3$ and at 1.3ppm, pendant CH$_2$, and at 2.85ppm, pendant CH$_2$), 6.40-7.60 (br m, polymer backbone ArH, no pendant ArH identifiable) ppm.

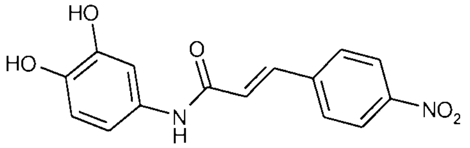
Method E: BBr$_3$ (12ml, 1M in DCM) was added to a solution of **135** (1.82g) in DCM (80ml). The reaction mixture was monitored by TLC and heated under reflux for 2 hours. The polymer mixture was poured slowly onto vigorously stirred methanol (300ml). The polymer precipitate was removed by filtration and the remaining organic solution was concentrated under vacuum. A crude brown/orange oil was obtained (274mg) of 5 components by TLC. The residue was subjected to flash column chromatography eluted with diethyl ether-petrol 40:60 (1:1). The cleaved polymer appeared no longer to have the pendant groups previously present. Polymer after cleave ^{1}H NMR (250.1MHz, CDCl$_3$): δ_H 0.85-3.00 (br m, polymer backbone CH$_2$, including larger peaks at 1.0ppm, pendant CH$_3$ and at 1.3ppm, pendant CH$_2$, and at 2.85ppm, pendant CH$_2$), 6.40-7.60 (br m, polymer backbone ArH, no pendant ArH identifiable) ppm. Band 2 was collected as a beige solid (108mg) to give a minimum loading of 0.18mmol/g. TLC [diethyl ether-petrol 40:60 (1:1)] R$_f$ 0.18; ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO): δ_H 0.85 (t, 3H, *J*=6.6Hz, CH$_3$), 1.21-1.29 (ov. m, 8H, CH$_2$), 1.62 (m, 2H, Ar'CH$_2$C$\underline{H}_2$), 2.64 (t, 2H, *J*=7.5Hz, Ar'CH$_2$), 6.41 (d, 1H, *J*=8.5Hz, ArH-5), 7.12 (s, 1H, *J*=8.1, 1.9Hz, ArH-6), 7.33-7.37 (ov. m, 3H, 2xAr'H), 7.88 (d, 2H, *J*=8.1Hz, 2xAr'H), 9.48 (br s, 1H, NH), 12.0 (br s, 2H, OH) ppm; Mass spectrum (APCI$^+$): *m/z* 360[M+32]$^+$.

Reduction and coupling of the co-polymer 130 with 4-nitrocinnamoyl chloride to give 136



10% Pd-C (100mg) was added to a solution of the co-polymer **127** (10.00g, maximum potential polymer loading 1.97mmol/g) in THF (100ml). Air was replaced by the alternate application of vacuum and argon. Argon was then replaced by the alternate application of vacuum and hydrogen. The reaction mixture was then vigorously shaken for 72 hours. The hydrogen was replaced by argon and TEA (5ml) was added. A solution of 4-nitrocinnamoyl chloride (25mmol, 5.3g) in anhydrous THF (20ml) was added slowly to the reduced mixture and allowed to stir for 16 hours. The mixture was filtered by filtration through celite and the remaining organic mixture was poured onto vigorously stirred methanol (600ml). The resulting polymer precipitate was collected by filtration and dissolved and precipitated again a further three times. The title polymer was collected and dried under vacuum to give a grey solid (9.503g, 71% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.89-1.80 (br m polymer back-bone CH_2), 6.10-7.30 (br m, polymer back-bone ArH), 7.75 (br s, pendant ArH-6), 8.25 (br s, pendant ArH) ppm; IR (KBr disc): υ_{max} 3395 (CONH), 3060, 3026 (ArH), 2924 (CH_3/CH_2), 2848 (OCH_3/CH), 1601 (Ar), 1522 (CONH), 1495, 1451 (CH_2), 1381, 1345, 1250, 1181, 1117 (CO), 825 (2 adjacent aromatic ArH), 757, 700 (5 adjacent ArH) cm^{-1}.

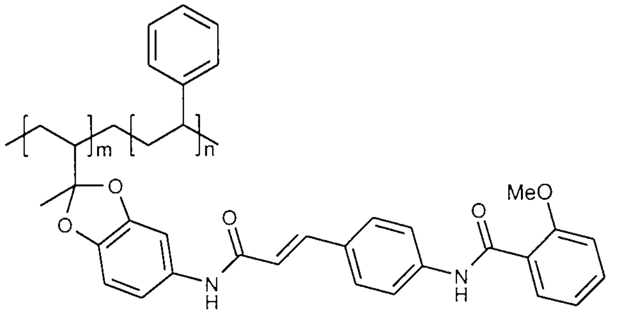
Attempted cleavage of *N*-(3,4-dihydroxyphenyl)-4-nitrobenzamide from poly(MVK-co-St)



Method A: Water (500μl) and concentrated HCl (10ml) was added slowly to a solution of the polymer **133** (2g, maximum potential polymer loading 1.97mmol/g) in dioxane (40ml) until the polymer started to precipitate. The reaction mixture was heated under reflux for 8 hours. The reaction mixture was poured onto vigorously stirred methanol (400ml). The resulting polymer precipitate was removed by filtration and the remaining organic solution was concentrated under vacuum to give a crude brown residue (231mg). The residue was subjected to preparative TLC, eluted with diethyl ether-petrol 60:80 (1:1). Three separate bands were collected, none of which could be identified as the desired product. The polymer solid was dried under vacuum and subjected to ^{1}H NMR analysis, which indicated that the pendant groups were still attached to the polymer. ^{1}H NMR was the same as **136.**

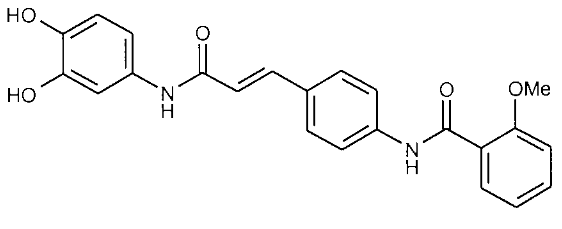
Method B: TFA (2ml) and water (2ml) was slowly added to a solution of the co-polymer **136** (1g) in dioxane (18ml). The mixture was stirred for 18 hours at 80°C. The polymer was poured onto vigorously stirred methanol (300ml). The resulting polymer precipitate was collected by filtration and the remaining organic solution was concentrated under vacuum. The residue was subjected to preparative TLC eluted with diethyl ether-petrol 60:80 (1:1). Three separate bands were collected, none of which could be identified as the desired product. The polymer solid was dried under vacuum and subjected to ^{1}H NMR analysis, which indicated that the pendant groups were still attached to the polymer. ^{1}H NMR was the same as **136**.

Reduction and coupling of the co-polymer 136 with 2-methoxybenzoyl chloride to give 137



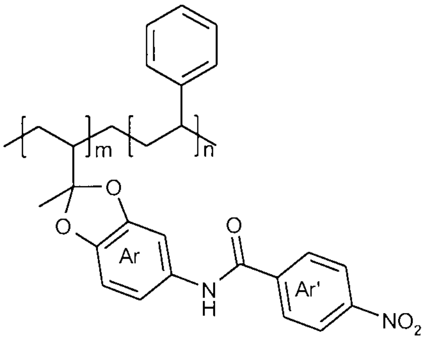
10% Pd-C (200mg) was added to a solution of the co-polymer **136** (2g) in THF (40ml). The reaction mixture was purged with argon and then hydrogen and then shaken vigorously in a hydrogenator at 40Psi. After 18 hours the hydrogen was removed and TEA (1ml) was added. A solution of 2-methoxybenzoyl chloride (8mmol, 1.368g) in anhydrous THF (10ml) was added to the reduced mixture and stirred for 16 hours. The mixture was filtered by filtration through celite and the remaining organic solution was poured onto vigorously stirred methanol (300ml). The polymer precipitate was collected by filtration. It was then re-dissolved and precipitated a further three times. The title compound was collected and dried under vacuum to give a grey/brown solid (1.782g, 74% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.60-2.15 (br m polymer back-bone CH_2), 3.60-3.98 (br m, pendant CH_2/CH_3), 6.00-7.26 (br m, polymer back-bone ArH), 7.75 (br s, pendant ArH-6), 8.25 (br d, pendant ArH), 9.85 (br s, pendant NH) ppm.

Cleavage from poly(MVK-*co*-St) with BBr_3



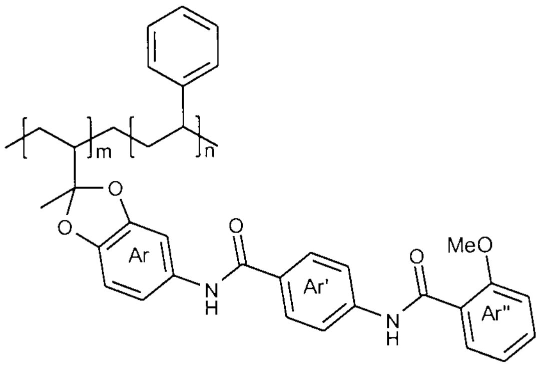
BBr_3 (3ml, 1M solution in DCM) was added to a solution of the polymer **137** (1.5g) in DCM (30ml). The reaction mixture was heated under reflux for 2 hours. The polymer was poured onto vigorously stirred methanol (200ml). The resulting polymer precipitate was removed by filtration and the remaining organic solution was concentrated under vacuum to give a crude brown solid (156mg). The polymer collected after the cleavage showed no pendant peaks. ^{1}H NMR and Mass spectroscopy gave no assignable peaks.

Reduction and coupling of the co-polymer 130 with 4-nitrobenzoyl chloride to give 138



10% Pd-C (500mg) was added to a solution of the polymer **130** (5g) in THF (40ml). Air was removed by the alternate application of vacuum and argon. The argon was then replaced by the alternate application of vacuum and hydrogen. The reaction mixture then vigorously shaken for 18 hours. The hydrogen was then replaced with argon and TEA (2ml) added. A solution of 4-nitrobenzoyl chloride in anhydrous THF (10ml) was added slowly to the reduced polymer mixture and allowed to react for 16 hours. The title polymer was precipitated onto methanol three times to give a brown precipitate (4.978g, 79% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.80-2.50 (br m, polymerised CH_2), 6.00-7.50 (br m, polymerised ArH of styrene), 7.60-7.80 (br s, polymerised ArH-2), 8.10-8.40 (br s, polymerised Ar'H) ppm; IR (KBr disc): υ_{max} 3455 (CONH), 3417, 3063 (CH_2/CH_3), 3033 (ArH), 2932 (CH_2/CH_3), 2867 (CH), 1706 (remaining reduced ketone peak), 1602 (ArH), 1525 (CONH/NO_2), 1494 (CH), 1454 (CH), 1338, 1261 (Ar-O-C), 760, 696 (5 adjacent ArH) cm^{-1}.

Reduction and coupling of the co-polymer 138 with 2-methoxybenzoyl chloride to give 139

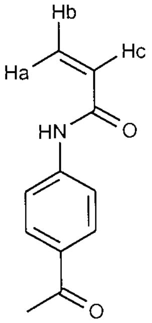


10% Pd-C (400mg) was added to a solution of the co-polymer **138** (3.9g) in THF (50ml). The reaction mixture was purged with argon and then hydrogen and vigorously shaken for 48 hours. The hydrogen was replaced with argon and TEA (2ml) was added. A solution of 2-methoxybenzoyl chloride (10.47mmol, 1.787mg) in anhydrous THF (10ml) was slowly added to the reduced mixture and stirred for 18 hours. Solids in the reaction mixture were removed by filtration through celite, and brown polymer precipitated onto vigorously stirred methanol (300ml). The precipitate was collected and then re-dissolved and precipitated a further three times. The title polymer was collected by filtration as a brown precipitate (2.814g, 58% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.90-2.70 (br m, polymer CH_2), 3.05 (br s, polymer CH_3), 3.65 (br s, polymer OCH_3), 5.90-7.30 (br m, polymer backbone ArH), 7.70-8.25 (br m, pendant ArHx3), 10.05 (br s, pendant NH) ppm; IR (KBr disc): υ_{max} 3450 (CONH), 3070, 3028 (ArH), 2925 (CH_2/CH_3), 2850 (CH), 1706 (unreacted ketone), 1598 (Ar), 1523 (CONH), 1494, 1449 (CH), 1336, 1267 (Ar-O-C), 760, 702 (5 adjacent ArH) cm^{-1}.

Cleavage of *N*-{4-[(3,4-dihydroxyanilino)carbonyl]phenyl}-2-hydroxybenzamide from poly(MVK-co-St)

BBr_3 (8ml, 1M solution in DCM) was added to a solution of the polymer **139** (3.5g) in DCM (50ml). The reaction mixture was heated under reflux for 2 hours. The polymer was poured onto vigorously stirred methanol (300ml). The resulting polymer precipitate was removed by filtration and the remaining organic solution was concentrated under vacuum. The polymer collected after the cleavage showed no pendant peaks.

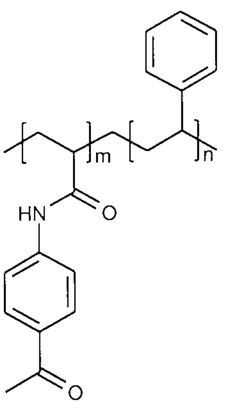
Preparation of *n*-(4-Acetylphenyl)acrylamide 140



IRA-68 (27g) was added to a solution of *p*-aminoacetophenone (50mmol, 6.75g) in anhydrous $CHCl_3$ (150ml). Air was replaced by the alternate application of vacuum and air. A solution of acryloyl chloride (55mmol, 5.0g) in anhydrous $CHCl_3$ (10ml) was added slowly at 0°C and the reaction mixture was stirred for 18 hours at 0°C. A white precipitate formed which was removed with the IRA-68 by filtration. The remaining organic solution was poured onto ice water. The organic layer collected and was dried with anhydrous magnesium sulphate and stirred with IRA-68 (5g) to remove any residual carboxylic acid. The title compound was collected and dried under vacuum as a pale yellow solid (4.030g, 43%). TLC [diethyl ether] R_f 0.31; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 2.61 (s, 3H, CH_3), 5.85 (dd, 1H, *J*=9.9, 1.4Hz, Hb), 6.35 (dd, 1H, *J*=16.8, 9.9Hz, Hc), 6.52

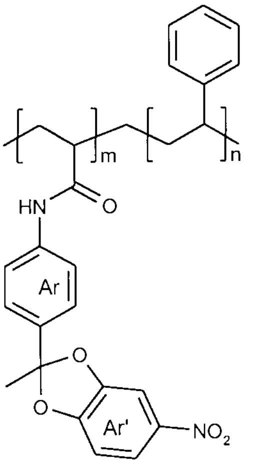
(dd, 1H, *J*=16.8, 1.5Hz, Ha), 7.76 (d, 2H, *J*=8.7Hz, 2xArH), 7.90 (br s, 1H, NH), 7.98 (d, 2H, *J*=8.7Hz, 2xArH) ppm; Mass spectrum (APCI$^+$): *m/z* 190M$^+$.

Preparation of poly[*n*-(4-acetylphenyl)acrylamide-*co*-St] 141



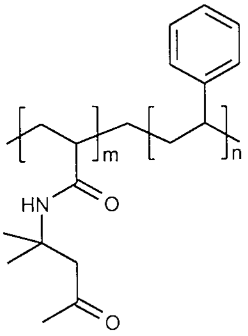
AIBN (290mg) was added to a solution of styrene (240.38mmol, 25g) and **140** (21.12mmol, 4g) in toluene (100ml). The reaction vessel covered with foil and sealed. Air was removed by the alternate application of vacuum and argon. The reaction mixture was stirred at 60°C for 72 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml) to give a cream precipitate. The title polymer was collected by filtration and dried under vacuum to give a cream solid (19.485g, 67%) with a maximum loading potential 0.73mmol/g; ^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 0.70-2.00 (br m, polymer CH_2), 2.65 (br s, polymer CH_3), 6.00-7.20 (br m, polymer ArH from styrene), 7.85 (br s, polymer NH) ppm; IR (KBr disc): υ_{max} 3400, 3315 (CONH), 3050, 3028, 2924 (CH_2/CH_3), 2838 (CH), 1682 (Ar-$COCH_3$), 1602 (Ar), 1511, 1490, 1454 (CONH), 1355 (CH_3), 1271 (C-O), 845 (2 adjacent ArH), 760, 700 (5 adjacent ArH) cm^{-1}.

Ketal formation with 4-nitrocatechol and poly(4-acetylphenyl)acrylamide-*co*-St] to give 142



4-nitrocatechol (6.38mmol, 988mg) and *p*-TsOH (200mg) were added to a solution of the co-polymer **141** (5g) in toluene (100ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap for 48 hours. The resulting polymer was poured onto vigorously stirred methanol (300ml). The polymer precipitate was collected by filtration and re-dissolved and precipitated a further three times. The title polymer was collected by filtration and dried under vacuum as a cream precipitate (4.086g, 73% based on maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 0.89-2.00 (br m, polymer CH_2), 2.55 (br s, polymer CH_3), 6.40-7.25 (br m, polymer ArH from styrene), 7.60-7.70 (br m, polymer Ar'H-2/5/6), 7.85 (br s, polymer NH) ppm; IR (KBr disc): υ_{max} 3410 (CONH), 3007 (CH_2/CH_3), 1596 (Ar), 1547 (NO_2), 1512,1490 (CONH), 1448 (NO_2), 1350 (CH_3), 1290 (C-O), 1202, 1150 (Ar-O-C), 1045, 1025 (Ar-O-C), 748, 699 (5 adjacent ArH) cm^{-1}.

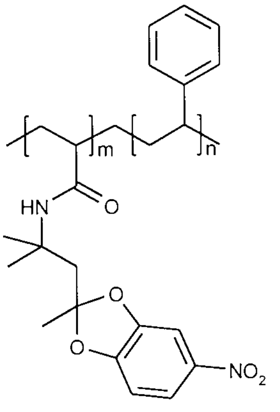
Preparation of poly(diacetone acrylamide-*co*-St) 143



AIBN (585mg) was added to a solution of diacetone acrylamide (50mmol, 8.450g) and styrene (485.4mmol, 50g) in toluene (150ml). The reaction vessel was covered in foil and sealed. Air was removed by the alternate application of vacuum and argon. The reaction mixture was then heated at 60°C for 48 hours. The resulting polymer was poured slowly onto vigorously stirred methanol (600ml). The title polymer was formed as a white precipitate, which was collected by filtration, and dried under vacuum (28.427g, 49%). Maximum potential polymer loading

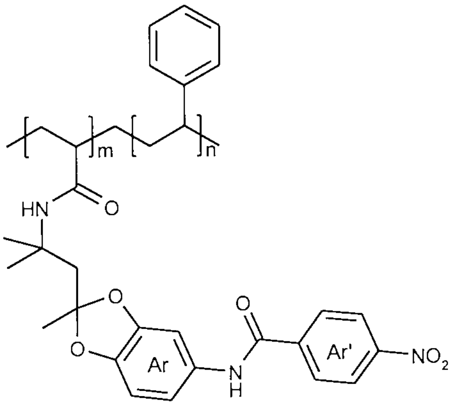
1.00mmol/g. ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.90-2.10 (br m, polymerised CH_2), 6.15-7.20 (br m, polymerised ArH from styrene) ppm; IR (KBr disc); υ_{max} 3410 (CONH), 3400 (CONH), 3060, 3050, 3300 (Ar), 2910 (CH_2/CH_3), 2860 (CH), 1710 ($CH_2C{=}OCH_3$), 1672 (CONH), 1598 (Ar), 1490, 1445 (CONH), 1360 (CH_3), 696, 752 (5 adjacent ArH) cm^{-1}.

Ketal formation with 4-nitrocatechol and poly(diacetone acrylamide-*co*-St) to give 144



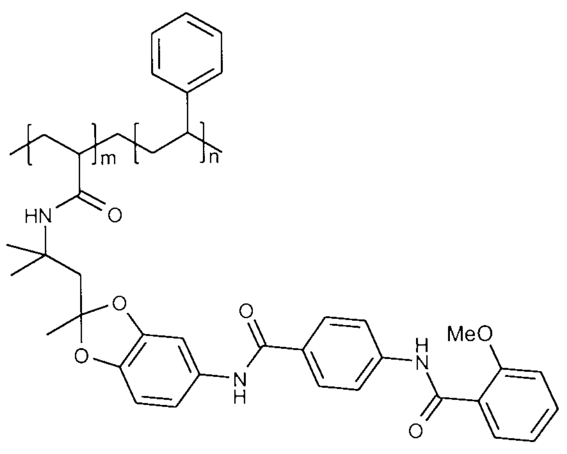
4-Nitrocatechol (14mmol, 2.170g) and *p*-TsOH (100mg) were added to a solution of the co-polymer **143** (10g) in toluene (100ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap for 48 hours. The reaction was monitored by the disappearance of the carbonyl peak at 1710cm^{-1} in the IR spectrum. The resulting polymer was poured onto vigorously stirred methanol (600ml). The resulting precipitate was collected and then re-dissolved and precipitated a further three times. The title polymer was collected by filtration as a cream precipitate and dried under vacuum (9.392g, 83% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 0.90-2.20 (br m, polymerised CH_2), 6.15-7.20 (br m, polymerised ArH from styrene), 7.38 (br s, polymerised ArH-2) ppm; IR (KBr disc): υ_{max} 3490, 3390 (CONH), 3050, 3010 (Ar), 2910 (CH_2/CH_3), 2860 (CH), 1652 (CONH), 1603 (Ar), 1521 (NO_2), 1490,1445 (CONH), 1340 (CH_3), 1280, 1170 (Ar-O-C), 745, 696 (5 adjacent ArH) cm^{-1}.

Reduction and coupling of the co-polymer 144 with 4-nitrobenzoyl chloride to give 145



10% Pd-C (800mg) was added to a solution of the co-polymer **144** (8g) in THF (50ml). Air was removed by alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously for 48 hours. The hydrogen was replaced in the manner described above with argon and TEA (1ml) then added. A solution of 4-nitrobenzoyl chloride (12mmol, 2.227g) in anhydrous DMF (10ml) and added slowly to the reduced mixture and allowed to react for 16 hours. The resulting polymer was precipitated onto vigorously stirred methanol (300ml) The polymer precipitate that formed was collected by filtration and then re-dissolved and precipitated a further three times. The title polymer was collected by filtration and dried under vacuum to give a cream precipitate (7.321g, 73% based on max. potential polymer loading).^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 0.80-2.20 (br m, polymerised CH_2), 6.30-7.30 (br m, polymerised ArH from styrene), 7.70 (br s, polymerised ArH), 8.00-8.40 (br s, polymerised Ar') ppm; IR (KBr disc): υ_{max} 3490, 3390 (CONH), 3024 (Ar), 2925 (CH_2/CH_3), 2848 (CH), 1683 (CONH), 1598 (Ar), 1550 (NO_2), 1490,1450 (CONH), 1340 (CH_3), 1280, 1170 (Ar-O-C), 745, 696 (5 adjacent ArH) cm^{-1}.

Reduction and coupling of the co-polymer 145 with 4-nitrobenzoyl chloride to give 146

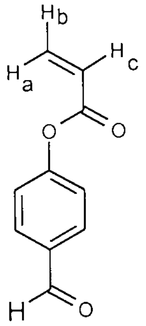


10% Pd-C (500mg) was added to a solution of the polymer **145** (5g) in THF (50ml). Air was replaced by the alternate application of vacuum and argon. The argon was then replaced by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously for 72 hours. The hydrogen was replaced with argon, in the same manner as described above, and TEA (2ml) was then added. A solution of 2-methoxybenzoyl chloride (10mmol, 1.706g) in anhydrous DMF (10ml) was added slowly to the reduced mixture and allowed to stir. After 18 hours, any solids in the mixture were removed by filtration through celite. The resulting polymer was poured onto vigorously stirred methanol (400ml). The polymer precipitate that formed was collected by filtration and then re-dissolved and precipitated a further three times. The remaining polymer was then dried under vacuum. The IR spectrum was the same as that of the previous step, and the ^{1}H NMR showed only one new, very small broad peak at 8.15ppm.

Attempted cleavage of *N*-{4-[(3,4-dihydroxyanilino)carbonyl]phenyl}-2-hydroxybenzamide from poly(diacetone acrylamide-co-St)

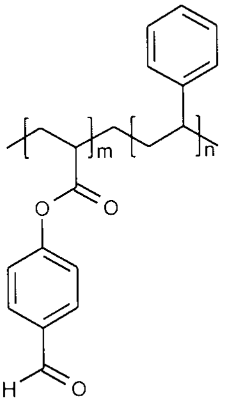
BBr_3 (12ml of a 1M solution in DCM) was added to a solution of the polymer **146** (3.974g, maximum potential polymer loading 1mmol/g) in $CHCl_3$ (50ml). The reaction mixture was heated under reflux for two hours. The polymer was poured onto methanol (300ml). The polymer precipitate was removed by filtration and the remaining organic solution concentrated under vacuum, to give a brown powder. The mass spectrum of the crude solid showed no peaks at the required molecular weight, and ^{1}H NMR showed no peaks in the required regions.

Preparation of 4-formylphenyl acrylate 147



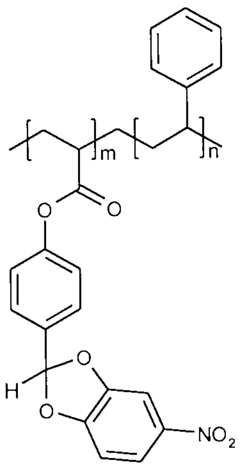
4-Hydroxybenzaldehyde (91mmol, 11.102g) dissolved in anhydrous THF (40ml) under argon and TEA (15ml) added. A solution of acryloyl chloride (92mmol, 8.326g) in anhydrous THF (10ml) was slowly added to aldehyde mixture at 0°C. A white precipitate formed directly and the reaction was stirred at 0°C for 5 hours with constant monitoring by TLC. The white precipitate of triethylamine hydrochloride was removed by filtration. The remaining mixture was poured onto cold methanol (100ml) and stirred for 2 hours at 0°C. THF and methanol were removed under vacuum at ambient temperature, to give the title compound as a beige solid, stored below 5°C (14.872g, 93%). TLC [petrol 60:80-diethyl ether (2:1)] R_f 0.28; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 6.21 (dd, 1H, *J*=10.0, 1.6Hz, Hb), 6.44 (dd, 1H, *J*=17.2, 10.0Hz, Hc), 6.59 (dd, 1H, *J*=17.2, 1.6Hz, Ha), 7.45 (d, 2H, *J*=8.5, 2xArH), 7.99 (d, 2H, *J*=8.5Hz, 2xArH), 10.01 (s, 1H, CHO) ppm; Mass spectrum (APCI$^+$): *m/z* 177M$^+$, 123[M-COCH=CH$_2$]$^+$.

Preparation of poly(4-formylphenyl acrylate-*co*-St) 148



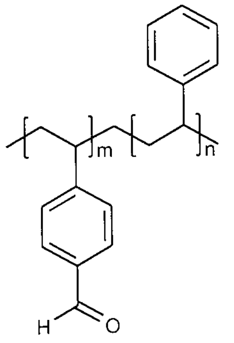
AIBN (500mg) was added to a solution of **147** (84.43mmol, 14.86g) and styrene (337.88mmol, 35.14g) in toluene (100ml). The reaction vessel was covered in foil and sealed. Air was removed by the alternate application of vacuum and argon. The reaction mixture was then heated at 60°C for 48 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml). The title polymer was collected by filtration as a cream precipitate (39.060g, 78%) with a maximum potential loading of 1.69mmol/g. ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.00-2.20 (br m, polymer CH_2), 6.20-7.29 (br m, polymer ArH of styrene), 7.76 (br s, polymer pendant 2xArH), 9.96 (br s, polymer CHO) ppm; IR (KBr disc): υ_{max} 3079, 3060, 3028 (Ar), 2924 (CH_2/CH_3), 2850 (CH), 2730 (CH_2), 1759 (COOAr), 1705 (CHO), 1596 (ArH), 1490, 1454 (CH_2), 1387 (COO), 1297, 1209, 1150, 1116, 1010 (CO), 858 (2 adjacent ArH), 758, 696 (5 adjacent ArH) cm^{-1}.

Attempted acetal formation with 4-nitrocatechol and poly(4-formylphenyl acrylate-*co*-St) to give 149



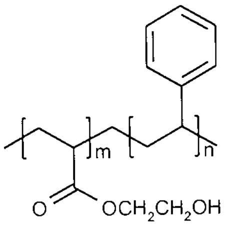
4-Nitrocatechol (20mmol, 3.100g) and *p*-TsOH (100mg) were added to a solution of polymer **148** (10g) in toluene (250ml). The reaction mixture was heated under reflux in conjunction with a Dean-Stark trap. After 18 hours an insoluble cream precipitate was formed in solution. The precipitate was collected by filtration and dried under vacuum. The solid polymer was only sparingly soluble in the organic solvents, THF, DCM, $CHCl_3$, CCl_4, benzene, DME, dioxane and toluene. ^{1}H NMR analysis could not be performed because the product was insoluble in CH_3Cl. IR (KBr disc): υ_{max} 3062, 3023 (Ar), 2921 (CH_2/CH_3), 2850 (CH), 1759 (COOAr), 1598 (ArH), 1527 (NO_2), 1490, 1450 (CH_2), 1340 (NO_2), 1209, 1120 (CO), 762, 702 (5 adjacent ArH) cm^{-1}.

Preparation of poly(vinyl benzaldehyde-*co*-St) 150



AIBN (290mg) was added to a solution of vinyl benzaldehyde (75.76mmol, 10g) and styrene (303.03mmol, 31.515g) in toluene (100ml). The reaction vessel covered with foil and sealed. Air was removed by the alternate application of vacuum and argon. The reaction mixture was then stirred at 60°C for 48 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml) to give a pale yellow precipitate. The title polymer was collected by filtration and dried under vacuum (23.524g, 42%). Maximum potential polymer loading 1.83mmol/g. ^{1}H NMR analysis could not be performed because the product was insoluble in CH_3Cl. IR (KBr disc): υ_{max} 3080, 3050, 3022 (ArH), 2919 (CH_2), 2850 (CH_2), 2725 (CH), 1699 (CHO), 1600 (ArH), 1490, 1448 (CH_2), 1350, 1209, 1180, 1103, 1055 (CO), 823 (2 adjacent ArH), 760, 700 (5 adjacent ArH) cm^{-1}.

Preparation of the poly(2-hydroxyethylmethacrylate-*co*-St) 151



AIBN (546mg) was added to a solution of 2-hydroxyethylmethacrylate (100mmol, 13g) and styrene (400mmol, 41.6g) in toluene (100ml). The reaction vessel was covered with foil and sealed. Air was removed by the alternate application of vacuum and argon. The reaction mixture was then heated at 60°C for 72 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml). The resulting precipitate was collected by filtration and dried under vacuum to give the title polymer as a white solid (40.605g, 74%) of maximum potential loading 1.83mmol/g. ^1H NMR (250.1MHz, $CDCl_3$): δ_H 0.30-1.00 (br m, pendant CH_2), 1.10-2.40 (br m, polymer back-bone CH_2), 3.00-4.20 (br m, pendant CH_2), 6.40-7.45 (br m, polymer back-bone ArH) ppm; IR (KBr disc): υ_{max} 3465 (OH), 3090, 3060 (CH_2), 3028 (Ar), 2919 (CH_2), 1726 (COO), 1598 (ArH), 1500 (ArH), 1450 (CH_2), 1386 (OH), 1180 (COH), 756, 704 (5 adjacent ArH) cm^{-1}.

Attempted oxidation of poly(2-hydroxyethylmethacrylate-co-St) to give 152

Method A: Anhydrous DMSO (4.58mmol, 3.59g, 3.26ml) was added to a mixture of oxalyl chloride (2.33mmol, 2.95g, 2.03ml) in anhydrous $CHCl_3$ (40ml) at –78°C. After 5 minutes, a solution of **151** (1g) in anhydrous $CHCl_3$ (40ml), was added at –78°C and left to stir at 30 minutes. TEA (9.93mmol, 10.03g, 13.82ml) was added and the reaction mixture was allowed to warm to room temperature. The polymer solidified on addition of the TEA. The polymer re-dissolved in $CHCl_3$ and was partitioned between $CHCl_3$ and water. The organic layer was collected and dried with anhydrous sodium sulphate. The resulting polymer was poured onto methanol and the resultant precipitate collected by filtration and dried under vacuum to give a white polymer (856mg). ^1H NMR (250.1MHz, $CDCl_3$): δ_H 0.60-1.85 (br m, polymer back-bone CH_2), 3.38 (br s, polymer pendant CH_2), 6.40-7.15 (br m, polymer back-bone ArH) ppm. The pendant CH_2 groups appear to have reduced to form a broad singlet, however, there is no sign of an aldehyde peak.

Method B: Anhydrous DMSO (11.45mmol, 8.975g, 8.15ml) was added to a mixture of oxalyl chloride (5.83mmol, 7.375g, 5.08ml) in anhydrous DCM (160ml) at –78°C. The reaction fizzed on addition of DMSO. After 5 minutes, a solution of 151 (2.5g) in anhydrous DCM (40ml), was added at –78°C and left to stir at 30 minutes. TEA (24.825mmol, 25.075g, 34.55ml) was then slowly added, resulting in the liberation of $(CH_3)S$, and the reaction mixture was allowed to warm to room temperature. Another 20ml of anhydrous DCM was added and the organic layer was washed with 10% aqueous HCl. The organic layer was collected and dried with anhydrous sodium sulphate. The resulting polymer was poured onto vigorously stirred methanol (500ml) and the resultant precipitate was collected by filtration and dried under vacuum to give a white gel (1.842g). The gel-like quality suggested a cross-linked polymer. ^1H NMR (250.1MHz, $CDCl_3$): δ_H 0.60-1.85 (br m, polymer back-bone CH_2), 3.60 (br s, polymer pendant CH_2), 6.45-7.15 (br m, polymer back-bone ArH) ppm. The pendant CH_2 groups appear to have reduced to form a broad singlet, however, there is no sign of an aldehyde peak. IR (KBr disc): υ_{max} 3448 (OH), 3080, 3070 (CH_2), 3023 (Ar), 2925 (CH_2), 2850 (CH), 1735 (COO/CHO), 1598 (ArH), 1490 (ArH), 1450 (CH_2), 1390 (OH), 1255, 1190, 1120, 1020 (COH), 760, 696 (5 adjacent ArH) cm^{-1}.

Attempted oxidation of PEG-monomethyl ether

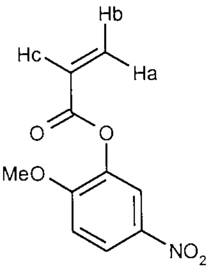
Method A: DMSO (8mmol, 643mg) and phosphorous pentoxide (8mmol, 1.17g) were added sequentially to a solution of PEG-monomethyl ether of average molecular weight 5000 (approximately 4mmol, 20g) in anhydrous DCM (50ml) at 0°C. The mixture was allowed to warm to room temperature. After 1 hour the mixture was cooled to 0°C and TEA (14.4mmol, 2.02ml) was added slowly. The reaction was allowed to stir for 30 minutes and was then quenched with 1M HCl and washed with saturated aqueous solution of sodium chloride. The organic layer was collected and dried with anhydrous sodium sulphate. The PEG solution was then poured onto vigorously stirred diethyl ether (500ml). The resulting white precipitate formed was collected by filtration and dried under vacuum (16.388g). ^1H NMR (250.1MHz, $CDCl_3$): δ_H 3.39 (m, 3H, OCH_3), 3.50-3.82 (br m, overlapping polymer OCH_2CH_2O), 3.94 (m, 2H, CH_2), 9.49 (s, 1H, CHO) ppm. ^1H NMR analysis showed 10% conversion of the alcohol to the aldehyde.

Method B: DMSO (8mmol, 643mg) and phosphorous pentoxide (8mmol, 1.17g) were added sequentially to a solution of PEG-monomethyl ether of average molecular weight 5000 (approximately 4mmol, 20g) in anhydrous DCM (50ml) at 0°C. The mixture was allowed to warm to room temperature. After 16 hours the mixture was cooled to 0°C and TEA (14.4mmol, 2.02ml) was added slowly. The reaction was allowed to stir for 7 hours and was then quenched with 1M HCl and washed with saturated aqueous solution of sodium chloride. The organic layer was collected and dried with anhydrous sodium sulphate. The PEG solution was then poured onto vigorously stirred diethyl ether (500ml). The resulting white precipitate formed was collected by filtration and dried under vacuum (16.388g). ^1H NMR (250.1MHz, $CDCl_3$): δ_H 3.39 (m, 3H, OCH_3), 3.50-3.82 (br m, overlapping polymer OCH_2CH_2O), 3.94 (m, 2H, CH_2), 9.49 (s, 1H, CHO) ppm. ^1H NMR analysis showed 7% conversion of the alcohol to the aldehyde.

Method C: DMSO (8mmol, 643mg) was added to a solution of PEG-monomethyl ether average molecular weight 5000 (approximately 4mmol, 20g) in anhydrous DCM (40ml). The mixture was cooled to 0°C and phosphorous pentoxide (8mmol, 1.17g) was added. The reaction was allowed to warm to room temperature and TEA (14.4mmol, 2.02ml) was added after 30 minutes. The reaction was quenched with 1M HCl (100ml) after 18 hours. The mixture was then washed with saturated aqueous sodium chloride. The organic layer was collected and dried with anhydrous sodium sulphate. The PEG solution was then poured onto vigorously stirred diethyl ether (500ml). The resulting white precipitate formed was collected by filtration and dried under vacuum (16.388g). ^1H NMR (250.1MHz, $CDCl_3$): δ_H 3.39 (m, 3H, OCH_3), 3.50-3.82 (br m, overlapping polymer OCH_2CH_2O), 3.94 (m, 2H, CH_2), 9.49 (s, 1H, CHO) ppm. ^{1}H NMR analysis showed 8% conversion of the alcohol to the aldehyde.

Method D: DMSO (35.2mmol, 2.746mg) was added to a solution of PEG-monomethyl ether average molecular weight 5000 (approximately 4mmol, 20g) in anhydrous DCM (100ml). The mixture was cooled to 0°C and oxalyl chloride (17.6mmol, 2.235g) was added. The reaction was allowed to warm to room temperature and TEA (60mmol, 8.35ml) was added after 30 minutes. The reaction was quenched with 1M HCl (100ml) after 18 hours. The mixture was then washed with saturated aqueous sodium chloride. The organic layer was collected and dried with anhydrous sodium sulphate. The PEG solution was then poured onto vigorously stirred diethyl ether (500ml). The resulting white precipitate formed was collected by filtration and dried under vacuum (16.388g). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 3.39 (m, 3H, OCH_3), 3.50-3.82 (br m, overlapping polymer OCH_2CH_2O), 3.94 (m, 2H, CH_2), 9.49 (s, 1H, CHO) ppm. ^{1}H NMR analysis showed 32% conversion of the alcohol to the aldehyde.

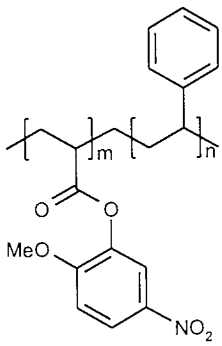
Preparation of 2-methoxy-5-nitrophenyl acrylate 153



2-Methoxy-5-nitrophenol (100mmol, 16.9g) was dissolved in anhydrous THF (30ml) under argon and TEA (14ml) added. A solution of acryloyl chloride (102mmol, 9.231g) in anhydrous THF (20ml) was slowly added at 0°C. A white precipitate formed directly. The reaction mixture was stirred at 0°C for 16 hours with constant monitoring by TLC. The white precipitate of triethylamine hydrochloride was removed by filtration. The remaining mixture was poured onto cold methanol and stirred for 2 hours at 0°C. THF and methanol were removed under vacuum at ambient temperature, to give the title compound as a yellow solid, stored below 5°C (20.00g, 90%). TLC [petrol 60:80-ethyl acetate (1:1)] R_f 0.49; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 3.96 (s, 3H, OCH_3), 6.11 (dd, 1H, *J*=10.4, 1.3Hz, Hb), 6.37 (dd, 1H, *J*=17.2, 10.4Hz, Hc), 6.67 (dd, 1H, *J*=17.2, 1.3Hz, Ha), 7.07 (d, 1H, *J*=9.1Hz, ArH-3), 8.04 (d, 1H, *J*=2.7Hz, ArH-6), 8.20 (dd, 1H, *J*=9.1, 2.8Hz, ArH-4) ppm; Mass spectrum (APCI$^+$): *m/z* 266M$^+$, 170[M-COCH=CH_2]$^+$.

Preparation of **152** was repeated on a 100mmol scale to give an 88% yield. TLC, ^{1}H NMR and mass spectrum as for previous batch.

Preparation of poly(2-methoxy-5-nitrophenyl acrylate-*co*-St) 154

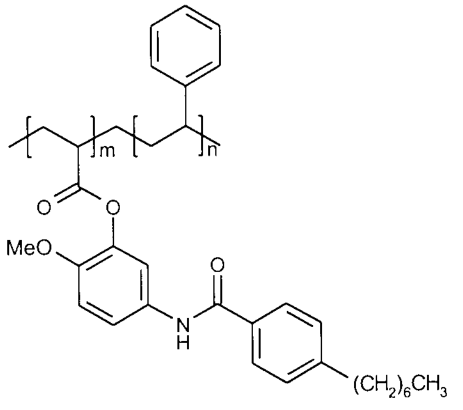


AIBN (680mg) was added to a solution of **153** (53mmol, 12g) and styrene (212mmol, 22g) in toluene (100ml). The reaction vessel was covered with foil and sealed. Air was removed from the reaction vessel, by the alternate application of vacuum and argon. The reaction mixture was heated at 60°C for 48 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml) to give a pale yellow precipitate. The title polymer was collected by filtration and dried under vacuum (26.091g, 77%); Maximum polymer loading 1.54mmol/g; ^{1}H NMR (250.1MHz,

$CDCl_3$): δ_H 0.90-2.30 (br m, polymer back-bone CH_2), 3.45-3.65 (br d, polymer OCH_3), 6.55-7.50 (br m, polymer back-bone ArH), 8.10 (br s, pendant ArH-4/6) ppm; IR (KBr disc): υ_{max} 3023 (ArH), 2921 (CH_2/CH_3), 2860 (CH/OCH_3), 1760 (COO), 1598 (Ar), 1521 (CNO_2), 1500 (Ar), 1450 (CH_2), 1342 (C-NO_2), 1278, 1202, 1140, 1020 (CO), 815 (2 adjacent ArH), 760, 698 (5 adjacent ArH) cm^{-1}.

Preparation of poly(2-methoxy-5-nitrophenyl acrylate-*co*-St) **154** was repeated on a the same scale as above to give a 75% yield. ^1H NMR and IR as for the above batch.

Reduction and coupling of the nitro groups of poly(2-methoxy-5-nitrophenyl acrylate-*co*-St) with 4-*n*-heptylbenzoyl chloride to give 155



Method A: 10% Pd-C (500mg) was added to a solution of the co-polymer **154** (5g) in THF (40ml). Air was replaced by the alternate application of vacuum and argon. The argon was then replaced by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously. After 18 hours the hydrogen was replaced with argon and TEA (2ml) was added. A solution of 4-*n*-heptylbenzoyl chloride (8.0mmol, 1.912g) in anhydrous THF (10ml) was added slowly to the reduced mixture and left stirring for 18 hours. The mixture was filtered by filtration through celite and the polymer was poured onto vigorously stirred methanol (300ml). The precipitate formed was collected by filtration and was then re-dissolved and precipitated a further three times. The resulting pale yellow polymer precipitate was dried under vacuum (1.987g). There was no difference in the IR spectrum of the final product and the starting polymer.

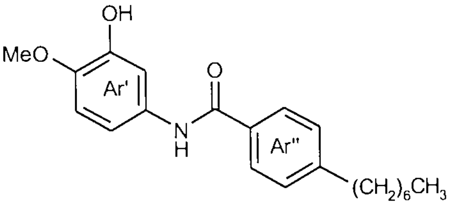
Method B: 10% Pd-C (500mg) was added to a solution of the co-polymer **153** (5g, maximum potential loading 1.54mmol/g) in THF (100ml). Air was replaced by the alternate application of vacuum and argon. The argon was then replaced by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously in a hydrogenator at 80Psi. After 18 hours the pressure was reduced and the hydrogen was replaced with argon and TEA (1ml) was added. A solution of 4-*n*-heptylbenzoyl chloride (7.0mmol, 1.673g) in anhydrous THF (10ml) was added slowly to the reduced mixture and left stirring for 18 hours. The mixture was filtered by filtration through celite and the resulting polymer was poured onto methanol (300ml). The precipitate formed was collected by filtration and was re-dissolved and precipitated a further three times. The resulting pale yellow polymer precipitate was dried under vacuum (4.42g). There was no difference in the IR of the final product and the starting polymer.

Method C: 10% Pd-C (1.5g) was added to a solution of the polymer **154** (3g) in THF (40ml). Air was removed by the alternate application of vacuum and argon. The argon was then replaced by the alternate application vacuum and hydrogen. The reaction mixture was then shaken vigorously. After 72 hours the hydrogen was replaced with argon and TEA (1ml) was added. A solution of 4-*n*-

heptylbenzoyl chloride (7.0mmol, 1.673g) in anhydrous THF (10ml) was added slowly to the reduced mixture and left stirring for 18 hours. The mixture was filtered by filtration through celite and the polymer was poured onto methanol (300ml). The precipitate formed was collected by filtration and was dissolved and precipitated a further three times to give the title polymer as a cream solid (2.395g, 63% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 0.90 (br s, pendant CH_3), 1.25-1.40 (br m, pendant CH_2), 1.55-2.00 (br m, polymer backbone CH_2), 2.65 (br m, pendant CH_2), 3.60 (br d, pendant OCH_3), 6.65-7.30 (br m, polymer backbone ArH), 7.45-8.35 (br m, pendant ArH) ppm; IR (KBr disc): υ_{max} 3023 (ArH), 3390 (CONH), 2921 (CH_2/CH_3), 2860 (CH/OCH_3), 1760 (COO), 1680 (CONH), 1598 (Ar), 1521 (CNO_2), 1500 (Ar), 1450 (CH_2), 1170, 1140, 1099 (CO), 755, 698 (5 adjacent ArH) cm^{-1}.

Method C was repeated on a 3g scale to give an 81% yield based on the maximum potential polymer loading and on a 6g scale to give a 64% yield based on the maximum potential polymer loading. ^{1}H NMR (250.1MHz, $CDCl_3$) δ_H 0.88 (br m, pendant CH_3), 1.11-1.39 (br m, pendant CH_2x5), 1.50-1.95 (br m, polymer backbone CH_2), 2.6 (br s, pendant $ArCH_2$), 3.55 (br s, pendant OCH_3), 6.55-7.40 (br m, polymer backbone ArH), 7.71 (br m, pendant ArH), 8.11 (br s, pendant NH) ppm.

Attempted cleavage of 4-heptyl-*N*-(4-hydroxy-3-methoxyphenyl)benzamide 156 from co-polymer 155



n-Propylamine (4ml) was added to a solution of **155** (2g, max potential polymer loading 1.54mmol/g) in DCM (16ml) and the reaction was stirred for 18 hours. Seven components were visible by TLC. The mixture was poured onto methanol (300ml) in an attempt to precipitate the polymer. A precipitate was not formed and the mixture was concentrated under vacuum. The brown residue was subjected to column chromatography eluted with ethyl acetate-petrol 40:60 (1:1). The major component collected was the solid beige starting material 2-methoxy-5-nitrophenol (365mg, 1.08mmol/g). TLC [ethyl acetate-petrol 40:60 (1:1)] R_f 0.10; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 4.04 (s, 3H, OCH_3), 5.87 (br s, 1H, OH), 6.94 (d, 1H, *J*=8.8Hz, ArH-3), 7.29-7.90 (ov. m, 2H, ArH-4/6) ppm.

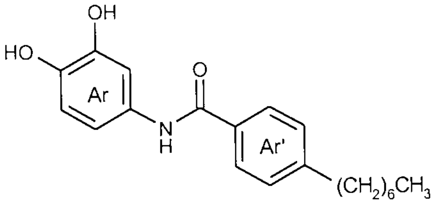
Cleavage of 4-heptyl-*N*-(4-hydroxy-3-methoxyphenyl)benzamide 156 from co-polymer 155

n-Propylamine (4ml) was added to a solution of the polymer **155** (2.053g, maximum potential polymer loading 1.54mmol/g) in DCM (16ml). The reaction mixture was stirred for 18 hours was then poured onto vigorously stirred methanol (300ml) in an attempt to precipitate the polymer. A precipitate was not formed and the mixture was concentrated under vacuum. The resulting brown residue was subjected to flash column chromatography eluted with ethyl acetate-petrol 40:60 (1:1). The title compound was collected as a pale pink solid (371mg, minimum polymer loading 0.53mmol/g). TLC [DCM] R_f 0.12; ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.91 (t, 3H, *J*=7.0Hz, CH_3), 1.02-1.32 (ov. m, 10H, $(CH_2)_5$), 2.69 (t, 2H, *J*=7.5Hz, Ar''CH_2), 3.92 (s, 3H, OCH_3), 5.72 (br s, 1H, OH), 6.87 (d, 1H, *J*=9.0Hz, ArH-5), 7.21-7.33 (ov. m, 4H, ArH-2/6, Ar'H), 7.67 (br s, 1H, NH), 7.79 (d, 2H, *J*=8.2Hz, 2xAr''H) ppm; ^{1}H NMR (250.1MHz, $(CD_3)_2SO$): δ_H 0.85 (t, 3H, *J*=6.9Hz, CH_3), 1.21-1.47 (ov.

m, 8H, (CH$_2$)$_4$), 1.55-1.68 (m, 2H, CH$_2$), 2.63 (t, 2H, *J*=8.1Hz, Ar"CH$_2$), 3.73 (s, 3H, CH$_3$), 6.86 (d, 1H, *J*=8.8Hz, Ar'H-5), 7.11 (dd, 1H, *J*=8.3, 2.6Hz, Ar'H-6), 7.29-7.33 (ov. m, 3H, Ar'H-2, Ar"H), 7.83 (d, 2H, *J*=8.1Hz, Ar"H), 9.05 (br s, 1H, OH/NH), 9.93 (br s, 1H, OH/NH) ppm; Mass spectrum (APCI$^+$): *m/z* 342M$^+$; IR (KBr disc): υ_{max} 3482 (OH), 3319, 3278 (CONH), 2925 (CH$_2$/CH$_3$), 2854 (CH/OCH$_3$), 1641 (CONH), 1612 (Ar), 1517 (CONH), 1432 (CH$_2$), 1360 (CH$_3$), 1283 (OH), 1230, 1190, 1110, 1010 (CO), 840 (meta-disubstituted), 792 (2 adjacent ArH) cm^{-1}.

This was repeated with 2.06g of polymer to give the title compound was collected as a pale pink solid (337mg, minimum polymer loading 0.48mmol/g). TLC, ^{1}H NMR, IR and mass spectrum as above.

Preparation of *N*-(3,4-dihydroxyphenyl)-4-heptylbenzamide by demethylation of 4-heptyl-*N*-(4-hydroxy-3-methoxyphenyl)benzamide 134

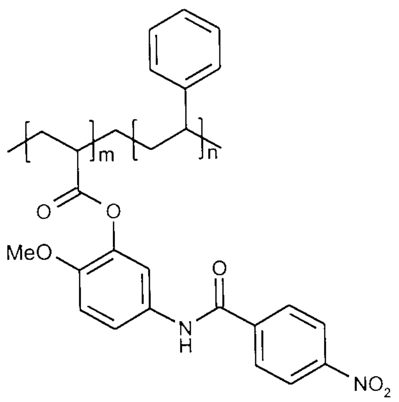


BBr$_3$ (700μl, 1M solution in DCM) was added to a solution of **155** (0.35mmol, 120mg) in DCM (10ml). A yellow precipitate formed after 2 hours, the precipitate was too fine to be removed by filtration. DCM (100ml) was added to the reaction mixture which was then washed with aqueous sodium hydrogen carbonate. The organic layer was collected and dried with anhydrous sodium sulphate and removed under vacuum. The brown residue was subjected to flash column chromatography, eluted with ethyl acetate-petrol 60:80 (1:1). The title compound was collected as a beige solid (60mg, 52%). TLC [ethyl acetate-petrol 60:80 (1:1)] R$_f$ 0.26. ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO): δ_H 0.85 (t, 3H, *J*=6.6Hz, CH$_3$), 1.22-1.28 (ov. m, 8H, CH$_2$), 1.58 (m, 2H, Ar'CH$_2$C$\underline{H}_2$), 2.63 (t, 2H, *J*=7.7Hz, Ar'C$\underline{H}_2$), 6.66 (d, 1H, *J*=8.5Hz, ArH-5), 6.96 (dd, 1H, *J*=8.2, 1.9Hz, ArH-6), 7.28-7.32 (ov. m, 3H, ArH-2, 2xAr'H), 7.82 (d, 2H, *J*=8.1Hz, 2xAr'H), 8.61 (s, 1H, OH), 9.02 (s, 1H, OH), 9.84 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 328M$^+$.

Cleavage of *N*-(3,4-dihydroxyphenyl)-4-heptylbenzamide 134 from co-polymer 155

BBr$_3$ (4.62ml, 1M solution in DCM) was added to a solution of **154** (2g, maximum potential polymer loading 1.54mmol/g) in DCM (30ml). The solution was stirred at room temperature for 18 hours and concentrated under vacuum. The crude brown residue was subjected to flash column chromatography, eluted with ethyl acetate-petrol 60:80 (1:1). The title compound was collected as a beige solid (205mg, maximum potential polymer loading 1.54mmol/g, minimum polymer loading 0.31mmol/g, 20%). TLC [ethyl acetate-petrol 60:80 (1:1)] R$_f$ 0.23. ^{1}H NMR (250.1MHz, (CD$_3$)$_2$SO): δ_H 0.85 (t, 3H, *J*=6.6Hz, CH$_3$), 1.17-1.27 (ov. m, 8H, CH$_2$), 1.58 (m, 2H, Ar'CH$_2$C$\underline{H}_2$), 2.63 (t, 2H, *J*=7.5Hz, Ar'C$\underline{H}_2$), 6.66 (d, 1H, *J*=8.5Hz, ArH-5), 6.95 (dd, 1H, *J*=8.7, 2.6Hz, ArH-6), 7.28-7.32 (ov. m, 3H, ArH-2, 2xAr'H), 7.82 (d, 2H, *J*=8.1Hz, 2xAr'H), 8.71 (s, 1H, OH), 8.99 (s, 1H, OH), 9.85 (br s, 1H, NH) ppm; Mass spectrum (APCI$^+$): *m/z* 328M$^+$.

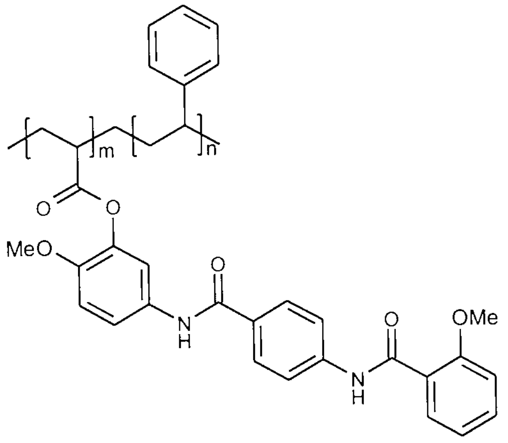
Reduction and coupling of poly(2-methoxy-5-nitrophenyl acrylate-co-St) with 4-nitrobenzoyl chloride to give 157



10% Pd-C (2.5g) was added to a solution of **154** (5g) in THF (100ml). Air was removed by the alternate application of vacuum and argon. The argon was then removed by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously. After 18 hours the hydrogen was replaced with argon and TEA (1.5ml) was added. A solution of 4-nitrobenzoyl chloride (8mmol, 1.488g) in anhydrous THF (10ml) was added slowly to the reduced polymer mixture and allowed to stir for 16 hours. The reaction mixture was filtered by filtration through celite and the resulting polymer was poured onto methanol (500ml). The polymer precipitate was collected by filtration and then re-dissolved and precipitated a further three times. The title polymer was collected as a yellow solid (5.029g, 85% based on the maximum potential polymer loading). ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 1.20-1.90 (br m, polymer backbone CH_2), 3.50 (br s, pendant OCH_3), 6.50-7.40 (br m, polymer backbone ArH), 7.85-8.25 (br m, pendant ArH) ppm; IR (KBr disc): υ_{max} 3400 (CONH), 2070, 3040 (ArH), 2923 (CH_2/CH_3), 2855 (CH/OCH_3), 1759 (COOAr), 1674 (CONH), 1604 (Ar), 1527 ($C\text{-}NO_2$/CONH), 1450 (CH_2), 1348 ($CO/C\text{-}NO_2/OCH_3$), 1278, 1126, 1028 (CO), 755, 698 (5 adjacent ArH) cm^{-1}.

Preparation of **157** was repeated with 4g of co-polymer **154** to give the title polymer was collected as a yellow solid (2.759g, 55% based on the maximum potential polymer loading). ^{1}H NMR and IR spectra were the same as for the previous batch.

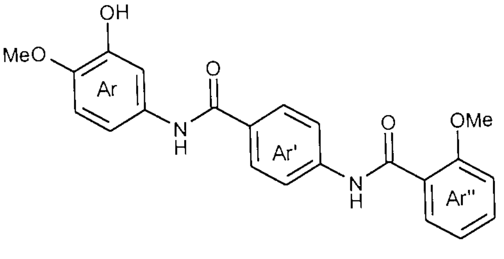
Reduction and coupling of the co-polymer 157 with 2-methoxybenzoyl chloride to give 158



10% Pd-C (1.25g) was added to a solution of co-polymer **157** (2.5g) in THF (100ml). Air was replaced by the alternate application of vacuum and argon. The argon was then replaced by the alternate application of vacuum and hydrogen. The reaction mixture was then shaken vigorously. After 18 hours the hydrogen was replaced with argon and TEA (1ml) was added. A solution of 2-methoxybenzoyl chloride (4mmol, 683mg) in anhydrous THF (10ml) was added slowly to the reduced mixture and stirred for 16 hours. The solids 10% Pd-C and triethylamine hydrochloride were removed by filtration through celite. The resulting polymer was poured onto methanol (400ml). The polymer precipitate was collected by filtration, and then re-dissolved and precipitated a further three times. The title polymer was dried under vacuum to give a white solid (2.213g, 76% based on maximum potential polymer loading). ^{1}H NMR (250.1MHz,

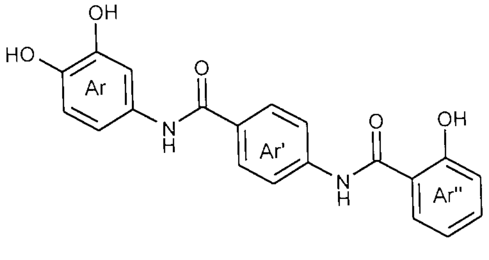
$CDCl_3$): δ_H 0.80-2.10 (br m, polymer back-bone CH_2), 3.48-3.83 (br d, 2xOCH_3), 6.50-7.30 (br m, polymer back-bone ArH), 7.48 (br s, pendant ArH), 7.79-8.25 (br m, pendant ArH), 8.40 (br s, pendant ArH), 9.60 (br s, pendant NH) ppm.

Cleavage of *N*-{4-[(4-hydroxy-3-methoxyanilino)carbonyl]phenyl}-2-methoxybenzamide 159 from co-polymer 158



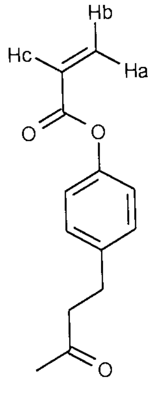
n-Propylamine (4ml) was added to a solution of **158** (2g) in DCM (25ml) and stirred for 18 hours. The mixture was concentrated under vacuum and the residue subjected to flash column chromatography, eluted with diethyl ether. The title compound was collected as a yellow solid (117mg, minimum loading 0.15mmol/g). TLC [diethyl ether] R_f 0.29; ^1H NMR (250.1MHz, $CDCl_3$): δ_H 3.99 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.93 (d, 1H, *J*=8.6Hz, ArH), 7.00 (dd, 1H, *J*=8.3, 0.8Hz, ArH), 7.10 (ddd, 1H, *J*=8.7, 7.7, 1.0Hz, ArH), 7.46 (ddd, 1H, *J*=8.3, 7.3, 1.9Hz, ArH), 7.83-7.98 (ov. m, 5H, ArH, NH/OH), 8.23 (dd, 1H, *J*=7.8, 1.9Hz, ArH), 8.29 (d, 2H, *J*=8.9Hz, Ar'H), 9.97 (s, 1H, NH/OH), 10.01 (s, 1H, NH/OH) ppm; Mass spectrum (APCI)$^-$: 391[M]$^-$, 287[M-$C_6H_4OCH_3$]$^-$.

Attempted cleavage of *N*-{4-[(3,4-dihydroxyanilino)carbonyl]phenyl}-2-hydroxybenzamide from co-polymer 158



BBr_3 (10ml, 1M solution in DCM) was added to a solution of the co-polymer **158** (2g) in DCM (30ml). After 16 hours the mixture was concentrated under vacuum and the resulting brown solid was adsorbed onto silica and subjected to flash column chromatography, eluted with diethyl ether. The chromatography failed to give the desired product.

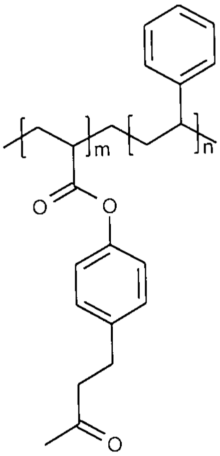
Preparation of 4-(3-oxobutyl)phenyl acrylate 160



4-(4-Hydroxyphenyl)-2-butanone (70mmol, 11.494g) was dissolved in anhydrous DCM (80ml) under argon and TEA (12ml) added. A solution of acryloyl chloride (75mmol, 6.788g) in anhydrous DCM (10ml) was added to aldehyde mixture slowly at 0°C. A white precipitate formed directly and the reaction was stirred at 0°C for 16 hours with constant monitoring by TLC. The white precipitate of triethylamine hydrochloride was removed by filtration. The remaining mixture was poured onto iced water and stirred for 2 hours at 0°C. The organic layer was collected stirred with wet IRA-67 (15g) for 30 minutes. IRA-67 was removed by filtration and the organic layer was dried with anhydrous magnesium sulphate and concentrated under vacuum to give the title compound as a clear honey coloured oil, stored below 5°C (14.291g, 94%). TLC [petrol 60:80-diethyl ether (1:1)] R_f 0.31; ^1H NMR (250.1MHz,

$CDCl_3$): δ_H 2.18 (s, 3H, CH_3), 2.75-2.82 (m, 2H, $C_6H_4C\underline{H}_2$), 2.88-2.96 (m, 2H, $C\underline{H}_2COCH_3$), 6.04 (dd, 1H, *J*=10.4, 1.4Hz, Hb), 6.34 (dd, 1H, *J*=17.2, 10.4Hz, Hc), 6.63 (dd, 1H, *J*=17.2, 1.4Hz, Ha), 7.06 (d, 2H, *J*=8.6Hz, 2xArH), 7.23 (d, 2H, *J*=8.6Hz, 2xArH) ppm; Mass Spectrum (APCI)$^-$: *m/z* 163[M-CH_2=CHCO]$^-$; Mass Spectrum (APCI)$^+$: *m/z* 219M$^+$, 161[M-CH_2=CHCO]$^+$.

Preparation of poly[4-(3-oxobutyl)phenyl acrylate-*co*-St] 161



AIBN (824mg) was added to a solution of **160** (65mmol, 14.170g) and styrene (260mmol, 27.04g) in toluene (100ml). The reaction vessel was covered with foil and sealed. Air was removed by the alternate application of vacuum and argon. The reaction mixture was then heated at 60°C for 48 hours. The resulting polymer was poured onto vigorously stirred methanol (600ml). The resulting precipitate was collected by filtration and dried under vacuum to give the title polymer as a cream solid (27.897g, 68%), maximum loading potential 1.58mmol/g. ^{1}H NMR (250.1MHz, $CDCl_3$): δ_H 0.90-2.00 (br m, polymer backbone CH_2), 2.15 (br s, polymer CH_3), 2.75 (br s, CH_2), 2.88 (br s, CH_2), 6.21-7.33 (br m, polymer ArH) ppm; IR (KBr disc): υ_{max} 3100, 3080, 3040 (ArH), 3020 ($COCH_3$), 2919, 2860 (CH_2/CH_3), 1749 (COOAr), 1712 (CH_2COCH_3), 1598, 1510, 1505 (Ar), 1454 (CH_2/CH_3), 1367 ($CH_3/COCH_3$/OCOCH), 1195, 1169, 1126 (CO), 756, 696 (5 adjacent ArH) cm^{-1}.

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