THE SYNTHESIS OF NOVEL ANTICANCER DRUGS

A Thesis submitted in part fulfilment of the requirements
of the degree of Doctor of Philosophy
from the University of Glasgow

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NUMBERING

AS ORIGINAL

Dedicated to my husband, son and daughter

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ABBREVIATIONS

AA	Arachidonic acid
ADEPT	Antibody-Directed Enzyme Prodrug Therapy
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Bn	Benzyl
BOC	*t*-Butoxycarbonyl
bp	Boiling point
bs	Broad singlet(NMR spectroscopy)
CDI	1,1'-Carbonyldiimidazole
CI	Chemical ionisation
d	Doublet (NMR spectroscopy)
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	*N,N'*-Dicyclohexylurea
DHFR	Dihydrofolate reductase
DMAP	4-*N,N*-dimethylaminopyridine
DME	Dimethoxyethane
DMF	*N,N*-dimethylformamide
DNA	Deoxyribonucleic acid
dTMP	Deoxythymidine monophosphate
dUMP	Deoxyuridine monophosphate
EDCI	1-Ethyl-3-(3-(dimethylaminopropyl)carbodiimide hydrochloride
EI	Electron impact
ERK	Extracellular signal-regulated kinase
GDEPT	Gene-Directed Enzyme Prodrug Therapy
GRP	Gastrin-releasing peptide
GST	Glutathione *S*-transferase
h	Hour(s)
HETEs	Hydroxyeicosateraenoic acids
HMPA	Hexamethylphosphoramide
HPETEs	Monohydroperoxyeicosatetraenoic acids
IC_{50}	Concentration required to inhibit 50 % of cell growth
IGF-1	Insulin-like growth factor-1
IR	Infrared
JNK	c-Jun N-terminal kinase
Lawesson reagent	2,3-Bis (*p*-methoxyphenyl)-1,3-dithiadiphosphetane

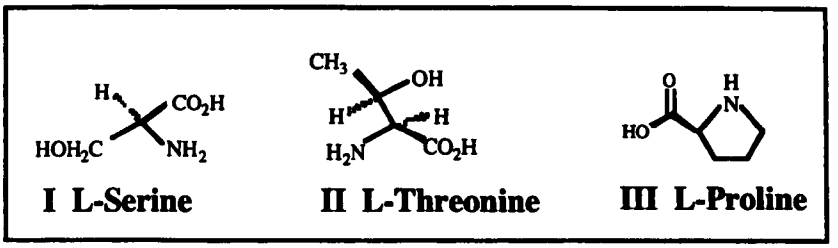
	-2,4-disulfide
lit.	Literature value
LO	Lipoxygenase
m	Multiplet (NMR spectroscopy)
m	Medium (IR spectroscopy)
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MEK	MAP/ERK kinase
MOMO	Methoxymethoxy
mp	Melting point
ms	Mass spectrometry
MTX	Methotrexate
NDGA	Nordihydroguaiaretic acid
NMR	Nuclear magnetic resonance
NSCLC	Non-small cell lung cancer
PAH	Polycyclic aromatic hydrocarbon
FAB	Fast atom bombardment
PCC	Pyridinium chlorochromate
PDGF	Platelet-derived growth factor
PPTS	Pyridinium *p*-toluenesulfonate
q	Quartet (NMR spectroscopy)
RNA	Ribonucleic acid
rt	Room temperature
s	Singlet (NMR spectroscopy)
s	Strong (IR spectroscopy)
SAPK	Stress-activated protein kinase
SCF	Stem cell factor
SCLC	Small cell lung cancer
SDS	Sodium dodecyl sulfate
ser	Serine
t	Triplet (NMR spectroscopy)
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography
TMEDA	*N,N,N',N'*-Tetramethylethylenediamine
tt	Triple triplet (NMR spectroscopy)
tyr	Tyrosine
UV	Ultraviolet
w	Weak (IR spectroscopy)

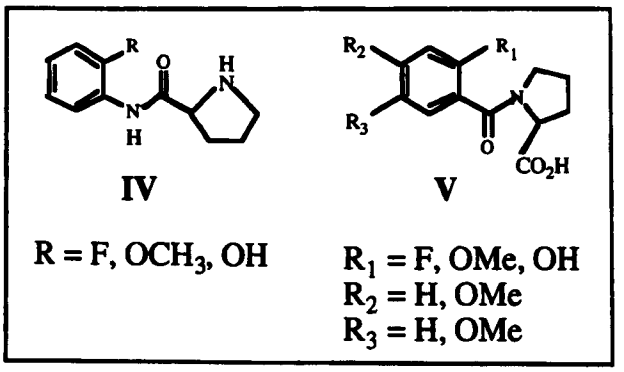
SUMMARY

Our studies on the synthesis and biological evaluation of novel anticancer drugs consist of three research areas; namely, synthesis of Mitogen Activated Protein (MAP) kinase inhibitors, Checkpoint (Chk1) inhibitors and nordihydroguaiaretic acid (NDGA) analogues.

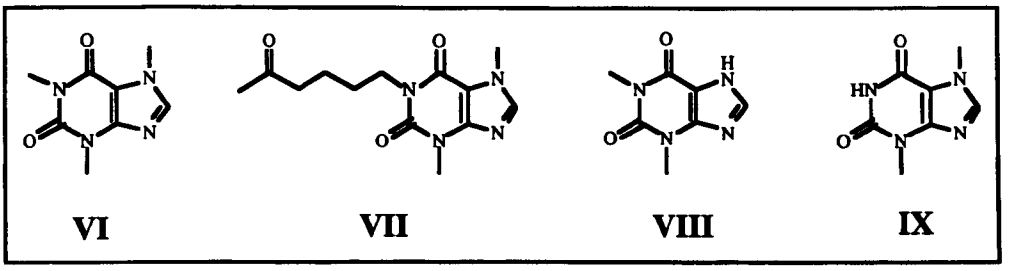
The first research area involved synthesis of MAP kinase inhibitors. MAP kinases are a family of serine **I** and threonine **II** kinases which can act together to generate a process of phosphorylation events within the cell signalling pathway leading eventually to cell division. The compounds made in this project were specifically designed to target the stress related kinases, a MAP kinase pathway which controls the expression of genes involved in cell proliferation.

The stress related kinases are known to have serine or threonine joined to a proline **III** residue. In an attempt to prepare selective inhibitors of stress related kinases, compounds of types **IV** and **V** were designed in which a conformationally restricted serine analogue is joined to L-proline *via* an amide link in one of two possible ways. Examples of these two sets of compounds were synthesised and those that were tested by Professor David Gillespie at the Beatson Institute for Cancer Research, Glasgow were shown not to be inhibitors of these kinases.

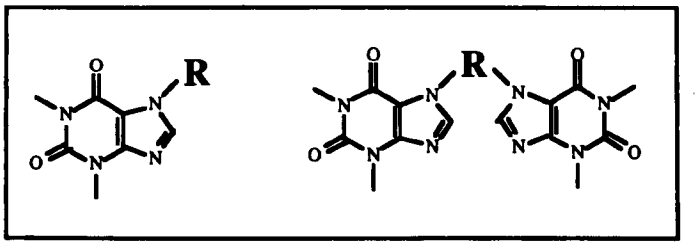




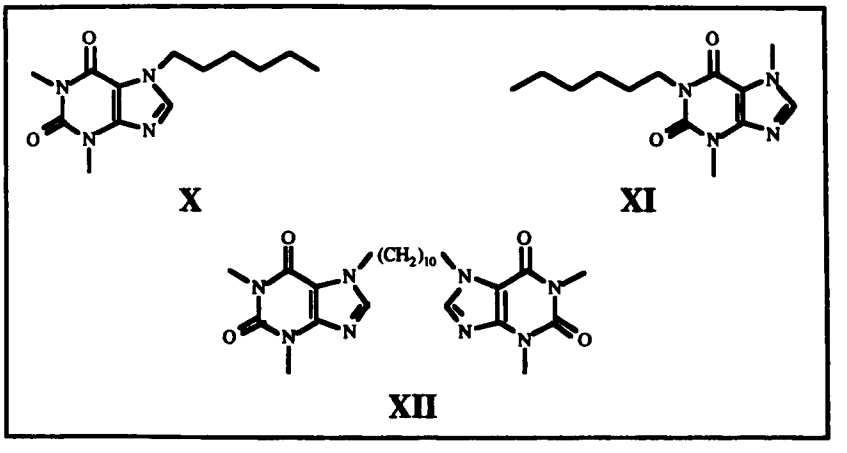
The second research area concentrated on the checkpoint signalling pathway. Components in the DNA damage checkpoint signalling pathway such as ChK1 could be potential targets for chemical intervention. Caffeine **VI** and pentoxifylline **VII** have been shown to sensitise p53-deficient tumour cells to killing by DNA damage. We envisaged that the xanthine derivatives, caffeine **VI** and pentoxifylline **VII** might also disrupt the G_2 checkpoint by preventing activation of Chk1. To test this hypothesis, a range of xanthine derivatives shown below were prepared by alkylation of theophylline **VIII** or theobromine **IX**.



VI VII VIII IX



The biological evaluation of these xanthine derivatives by Professor Gillespie revealed that three of these compounds, **X**, **XI** and **XII**, suppressed G_2/M arrest very effectively. All three active compounds possess a long aliphatic chain that provides a large degree of flexibility to the structures. The long aliphatic chains could bind to a hydrophobic pocket in the enzyme's active site that might confer selectivity on the compounds.



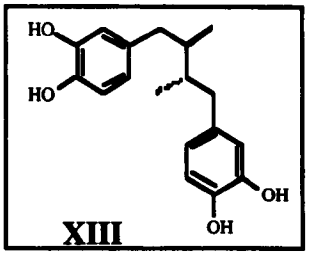
X **XI**

XII

The third area, synthesis of NDGA analogues, was the major part of the synthetic work. NDGA **XIII** is known to be a selective inhibitor of lipoxygenase and blocks small cell lung cancer growth *in vitro* and *in vivo*. In addition to its lipoxygenase activity, NDGA was demonstrated to inhibit c-kit, a tyrosine kinase that has been observed preferentially in SCLC. The main drawbacks to the use of NDGA in cancer treatment are its poor solubility and moderate potency. Therefore chemical modifications are required to provide better compounds for clinical use. Preliminary work in our group was performed by McDonald and Macleod. They synthesised a range of analogues of NDGA which were tested for their activity *in vitro* by Professor Michael Seckl at the Medical Oncology Department of Hammersmith Hospital, London. Improved potency over NDGA for new analogues with 4-6 atoms between the two

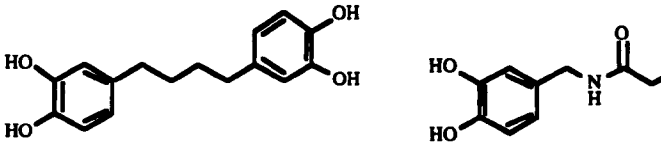
aromatic rings was observed. Furthermore introduction of an amide linkage between the two aromatic residues resulted in NDGA analogues which are more active than NDGA.

Based on these preliminary results, the structural modifications proposed for this project focused on three areas. The main programme of research was drug solubilisation of new analogues which have higher potency than NDGA for *in vivo* work. The second area of study sought to introduce position variations of the amide linkage between the two aromatic residues. The third area of work involved modification of the substituents on the two aromatic rings.



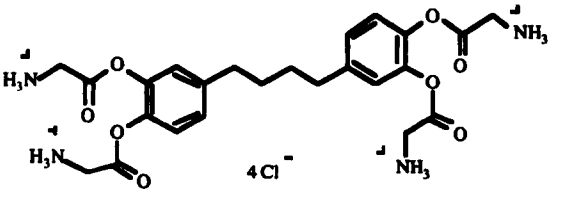
XIII

A range of NDGA analogues was successfully synthesised and evaluated for anticancer activity *in vitro*. Compounds **XIV** and **XV** were confirmed as lead compounds which are ten times more active than NDGA. Compound **XIV** was successfully transformed into a water soluble form **XVI** which is now available for *in vivo* work. In addition NDGA was converted into a water soluble form which was more potent than NDGA *in vitro*. Moreover a NDGA analogue **XVII** with no free hydroxy groups was found to be as active as NDGA, which was an unexpected discovery.



XIV

XV



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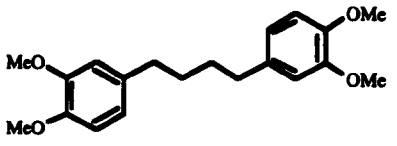


XVII

CONTENTS

CHAPTER 1

General Overview of Cancer and its Treatment

1.1 Basic Facts About Cancer

The World Health Organisation (WHO) reported that an estimated 10 million cases of cancer occurred worldwide in 1998. Furthermore WHO predicted that the situation would get worse with numbers rising to 14.7 million over the next 20 years.[1]

Cancer has afflicted our ancestors throughout the history of mankind, but it has become a major cause of mortality only in the last century.[2] Although cancer can occur at all ages, it becomes more prevalent as people grow older. Epidemiological analysis shows dramatic increases in cancer incidence with age, which accounts for the prevalence of cancer in our time. The average life expectancy has increased substantially as a result of the elimination of infectious diseases this century.[2] This has resulted in a larger fraction of older individuals in our population and consequently an increased incidence of cancer.

The goal of an ultimate conquest of cancer remains a distant hope. Cancer cells closely resemble normal cells, as cancer results from uncontrolled growth of otherwise normal cells.[2] Most of the drugs currently available for use in cancer treatment act against all rapidly proliferating cells. Thus, they kill not only cancer cells but also some normal cells, particularly those that are rapidly dividing. The effectiveness of treatment is often severely limited by the toxic side effects of the drugs. The fundamental problem in cancer treatment is to kill cancer cells selectively without adverse side effects to the patient.[2]

1.2 Cell Proliferation and Cancer

1. 2. 1 Stages of the Cell Cycle

DNA replication had been thought to be a continual process between the period of cell division, but early in the 1950s it was found this was not the case as it then became possible to carry out some radioisotopic labelling experiments using the nitrogenous base thymidine.[3] These experiments revealed a distinct gap during and after DNA formation even in rapidly proliferating cells and led to the discovery of the cell cycle. The process of mitosis is now a well-defined sequence of events that can be conveniently represented as shown in Figure 1.[3]

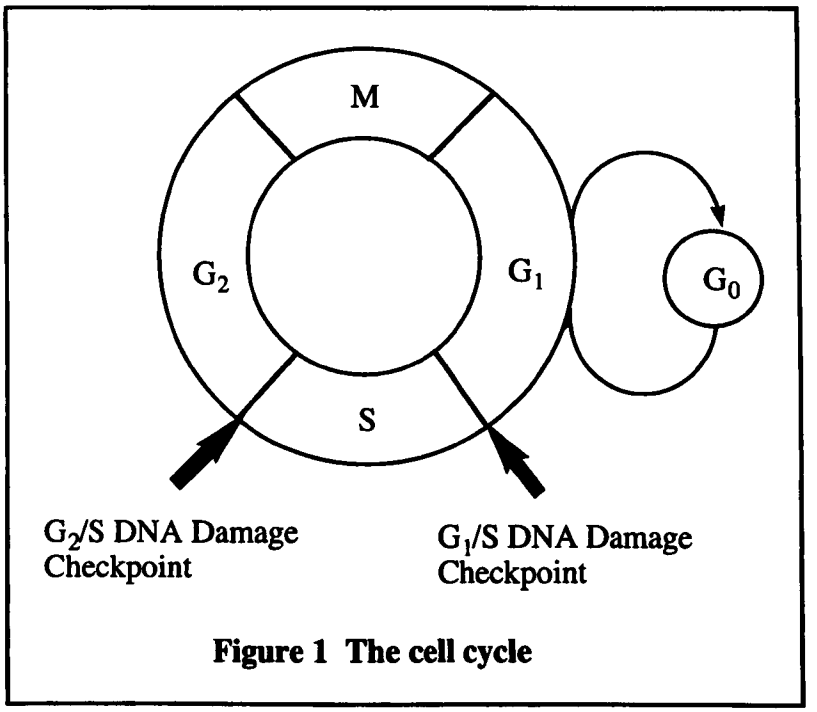


Figure 1 The cell cycle

RNA and proteins are synthesised in the G_1 (gap 1) phase and preparations are made to begin DNA synthesis. The S (synthesis) phase then follows where DNA is replicated and the DNA packing proteins, namely histones, are synthesised. The DNA is complexed with the proteins and preparations are made for cell division in the G_2 (Gap 2) phase and finally the cell nucleus and cytoplasm divide in the process of mitosis, which is the M Phase. The two daughter cells then undergo the cycle again or enter a resting phase, G_0. All cells undergo this cell cycle. Cell replication is normally controlled in response to the needs of the whole organism. However in some cases, these control mechanisms are lost resulting in excessive cell division to form a mass of cancer cells.[4]

1. 2. 2 Differences between Cancer Cells and Normal Cells[5]

Comparative studies of cell growth and behaviour have identified the characteristic differences between cancer cells and their normal counterparts.

Most tumour cells divide indefinitely regardless of the density-dependent inhibition of proliferation that is characteristic of normal cells in culture. In other words, tumour cells remain in the M-phase of the cell cycle until they die as a result of exhaustion of nutrients in the culture media. However, cancer cells stop proliferation at random points during the cell cycle rather than at the DNA checkpoints.

One type of cell normally responds to growth factors secreted by a different type of cell. By contrast, some tumour cells secrete growth factors that drive their own proliferation. In such cases, abnormal production of growth factor results in continual autostimulation of cell proliferation (autocrine growth stimulation). In other cases, tumour cells are continually

stimulated to proliferate independent of extracellular growth factors as a result of alteration of their growth factor receptors.

In addition to their reduced dependence on extracellular growth factors, cancer cells proliferate and migrate in an unregulated manner regardless of the contact with neighbouring cells or the extracellular matrix.

Cancer cells produce enzymes, called proteases, that digest other proteins. Proteases break down extracellular materials enabling the cancer cells to penetrate through surrounding normal tissues. The further expansion of a tumour requires new blood vessels to provide nutrients for tumour cell proliferation. The formation of new blood vessels is termed angiogenesis. Secretion of proteases and angiogenesis play crucial roles in cancer metastases.

Cancer cells are characterised by loss of capacity to differentiate at early stages of maturation. On the other hand, cancer cells fail to undergo programmed cell death, apoptosis, which is relevant to the invasiveness and spread (metastases) of cancers. Finally, cancer cells are the products of genetic mutations, which can lead to resistance to chemotherapeutic agents.

Taken together, these properties of cancer cells are found to contribute to the progressive growth, invasiveness, and metastasis of malignant neoplasms.

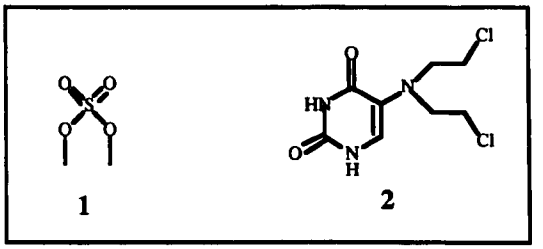
1. 3 Causes of Cancer

Tobacco smoking is undoubtedly the major cause of human cancer, accounting for about 30% of all cancer deaths. Smoking is responsible for the majority of lung cancers and has been associated with the development of many other cancers; for instance, cancer of the oral cavity, pharynx, bladder, kidney, pancreas and so on. Other common causes of human cancer include alcohol abuse, radiation, carcinogenic medicines, and occupational carcinogens, each of which accounts for only a few percent of total cancer deaths.[6] Taken together, it is estimated that up to 80% of human cancers may be attributable to environmental risk factors.

Most cancers are a result of a mutation in the sequence of DNA.[7] The generation of cancer (carcinogenesis) is closely correlated to the induction of DNA changes (mutagenesis) brought about by the following three classes of agents.

1. 3. 1 Chemical Carcinogenesis

The common feature of most chemical carcinogens is their ability to generate active electrophilic forms that can attack the nucleophilic sites on DNA. These chemical carcinogens can either undergo enzyme metabolism prior to action or can react directly like dimethyl sulfate **1** and uracil mustard **2** that are intrinsically electrophilic.[8]



1 2

The activation of the procarcinogen to the active form may proceed directly or go through a number of less reactive intermediates called proximate forms.[9] A variety of enzymes are involved in these processes often catalysing conversion of the foreign lipophilic agent into a more hydrophilic form, and this enzymatic manipulation results in the production of a carcinogenic electrophile.

An example of such a process is shown in Figure 2[9] involving the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene **3** which is one of the carcinogens present in tobacco smoke. A series of enzymatic oxidations of the procarcinogen PAH leads to the 7,8-dihydrodiol-9,10-epoxide. The most reactive electrophilic epoxides are formed near the bay region of the PAH. This epoxide can in turn react with the 2-amino group of guanine residues in DNA.

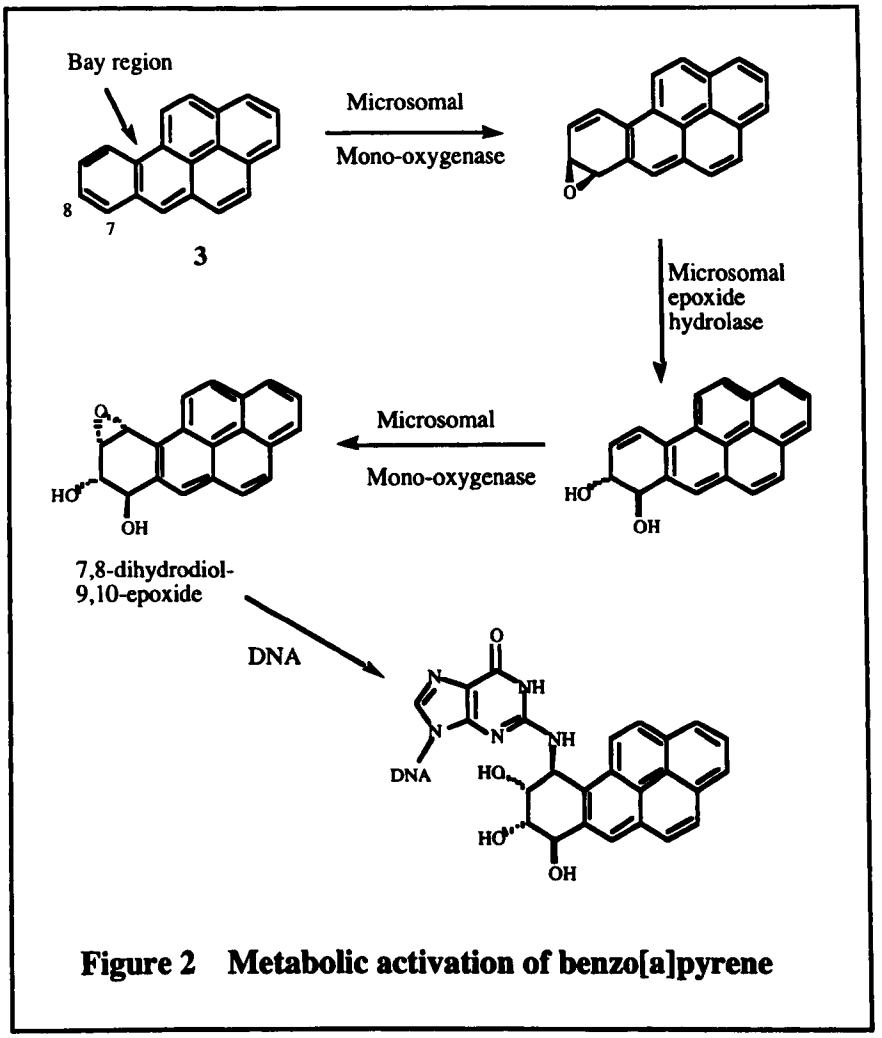


Figure 2 Metabolic activation of benzo[a]pyrene

1. 3. 2 Radiation

Ionising Radiation

Ionising radiation has a higher energy than ultraviolet (UV) light and includes X-rays and radiation produced by the decay of radioactive particles. The harmful nature of X-rays was evident shortly after their discovery in 1895 by Roentgen. Radiologists who used X-rays extensively in the early 1900s experienced a relatively high incidence of leukaemia, skin cancers and brain tumours. Subsequent animal experiments revealed the induction of sarcomas in laboratory animals after radiation exposure.[10]

The high energy associated with ionising radiation causes genetic mutations within the DNA. Absorption of dissipated energy by water is critically important in mutagenesis; ionisation ultimately leads to the generation of hydroxyl radicals, hydrogen radicals and solvated free electrons.[11] On interaction with DNA, these can lead to base deletions and alterations and attack on the sugar phosphate backbone, which can cause single, or double DNA strand breaks (Figure 3).[10]

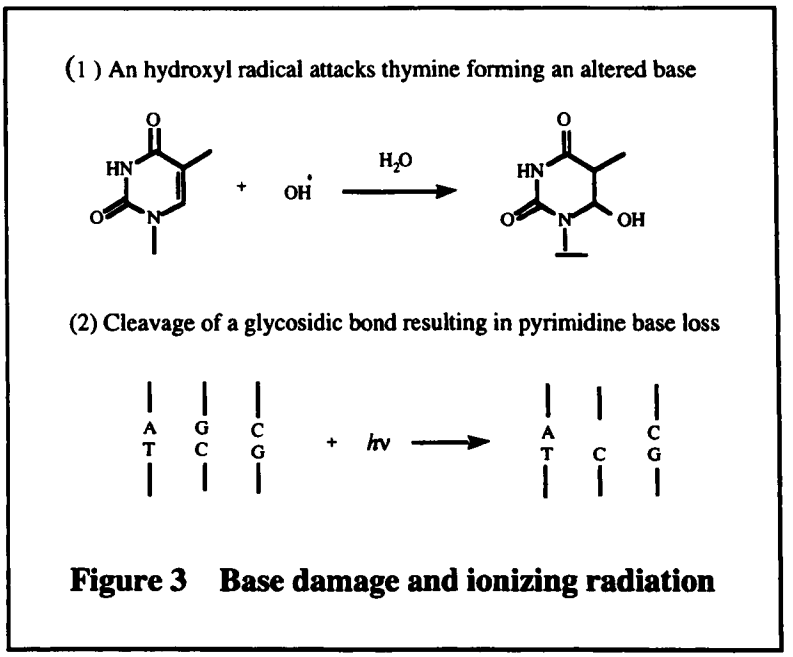


Figure 3 Base damage and ionizing radiation

Ultraviolet Radiation

Ultraviolet light is the major cause of human skin cancer. Skin cancer is very common but seldom lethal since the non-melanoma skin cancers metastasise slowly and are consequently highly curable. On the other hand melanoma is a much more serious form of skin cancer which rapidly spreads to other parts of the body.[6]

In contrast to high-energy ionising radiation, UV radiation is not thought to cause genetic mutation through widespread nucleic acid bond-breaking processes. Its activity appears to be associated with the generation of excited states in DNA bases, which lead to mutagenic photochemical reactions. For example, excitation of pyrimidine bases (thymine or cytosine) allows them to dimerise to give a chemically stable cyclobutane linkage (Figure 4).[12]

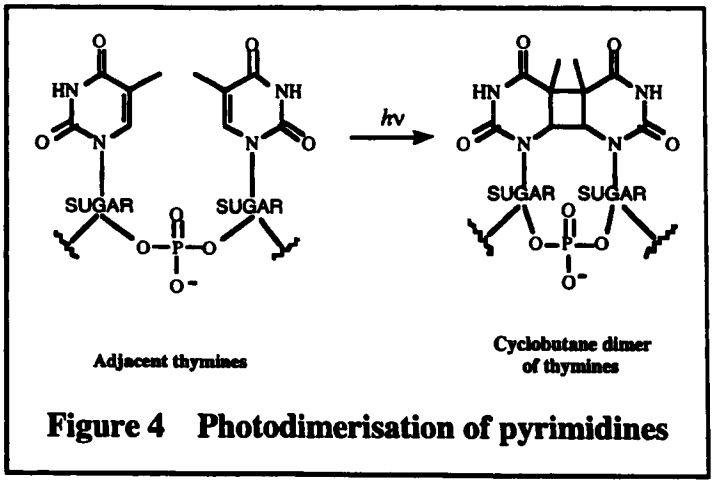


Figure 4 Photodimerisation of pyrimidines

1. 3. 3 Viruses

Several kinds of viruses are found to induce human cancers. Hepatitis B virus-induced liver cancer and papilloma virus-induced cervical cancers are thought to account for 10-20% of overall cancer incidence.[13] The study of animal tumour viruses is extremely important to understanding the mechanisms of cancer in general.

Oncogenic viruses do not kill their host cell but alter their critical genes resulting in the onset of cancer.[14] Structurally a virus consists of genetic material (DNA or RNA) encased in a protein capsid. On entry into the host cell the protein coat disappears and the viral DNA is integrated into the host's genome. After viral DNA integration, some of the viral genes are copied into mRNA molecules that in turn are translated into viral protein. Some of these can affect the activities of the host cell so that it becomes cancerous. Furthermore, these viral proteins can become incorporated into new viral particles able to leave and infect surrounding cells.[14]

1. 4 Treatment of Cancer

A variety of methods have been developed in cancer treatment, and the choice of treatment is largely dependent on the nature of the cancer and its extent of progression.[15]

1. 4. 1 Surgery

Surgery is the first treatment option for many cancers. Early stage cancers that have not yet metastasised can be completely removed and are therefore highly curable. Unfortunately, over half of cancers by the time of diagnosis have already spread and cannot be cured solely by surgery. To improve the efficiency of treatment, the combination of surgery with other therapies described below is the most common strategy adopted today.[16]

1. 4. 2 Radiation Therapy[16]

Radiation therapy is similar to surgery being primarily a treatment for localised cancers although it can be used to attack cancer cells that have already spread beyond the surgical scope.[16] Radiation is frequently used in conjugation with surgery to eliminate cancer cells that have infected surrounding tissues outwith the primary tumour site. In addition, some cancers are particularly vulnerable to radiation, making it preferential in treatment of these diseases.

A variety of types of irradiation sources are used in radiation therapy, including X-rays, and radioactive nuclei, such as cobalt-60 and radium. The basis for radiation therapy is to damage DNA, either directly or by the production of free radicals within cells.[16] This damage is

most pronounced in actively proliferating cells, which unfortunately includes some normal cells as well as cancer cells.[15]

1. 4. 3 Chemotherapy

Chemotherapy is now the most widely used form of cancer treatment. The major advantage of chemotherapy is that, unlike surgery and radiation that are localised treatments, it can target cancer cells throughout the body.[15] However chemotherapy also has its drawbacks. Most of the currently used anticancer drugs target all rapidly proliferating cells including normal cells as well as cancer cells. They can consequently produce undesired side effects in the patient. Another major problem in cancer treatment is the development of resistance, particularly multidrug resistance to anticancer drugs. In this case, exposure to one particular drug results in resistance to a variety of other agents in addition to the initial drug.[15]

In spite of these limitations, cancer chemotherapy has achieved some remarkable successes. The chemotherapeutic agents briefly surveyed below are categorised according to their mechanism of action.

1. 4. 3. 1 DNA Interactive Compounds

A large number of anticancer drugs exert their effect by damaging DNA or by interfering with DNA synthesis. Some drugs cause covalent interactions with DNA or between the base pairs of DNA that can result in crosslinks in either the minor or major grooves in an intramolecular or intermolecular fashion.[15]

1. 4. 3. 1. 1 Crosslinking Agents

Nitrogen mustards

Studies on sulfur mustard gas [$S(CH_2CH_2Cl)_2$] led to the nitrogen mustards; the first class of compounds to be investigated as anticancer agents. Mechlorethamine **4**, the first nitrogen mustard to be synthesised, was found to depress the white blood cell count effectively and was used in the treatment of certain leukaemias.[15]

Aliphatic nitrogen mustards

Nitrogen mustards are highly reactive and react readily with a wide range of nucleophiles, which results in the observed toxicities. The mechanism of action for aliphatic nitrogen mustards is illustrated in Figure 5.[15] Under physiological conditions, the nitrogen mustards first undergo an intramolecular cyclisation with anchimeric assistance of the nitrogen

lone pair of electrons to form cyclic aziridinium ions. This cation is highly strained and hence highly reactive towards attack by the nucleophilic N-7-atom of a guanine residue in the major groove of DNA. The second chloroethyl group then reacts in a similar manner with a second guanine base on the complementary DNA strand. Thus the two strands of DNA are crosslinked and as a result DNA replication is terminated.[15]

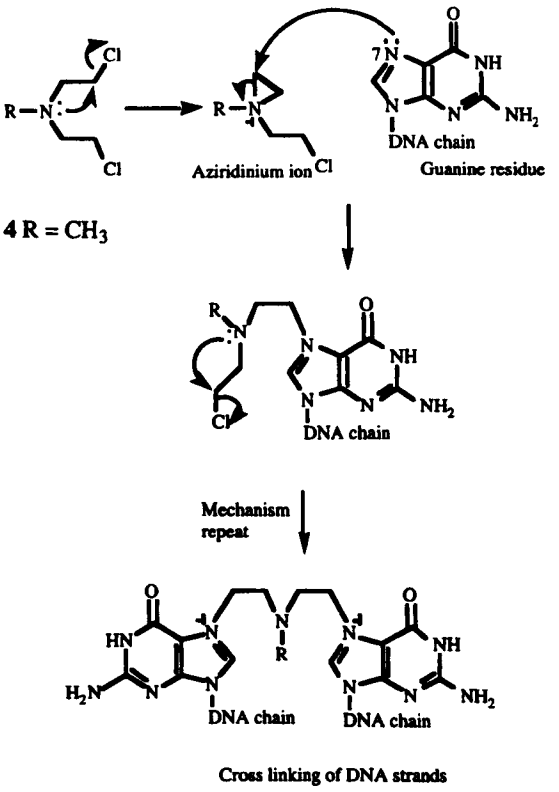
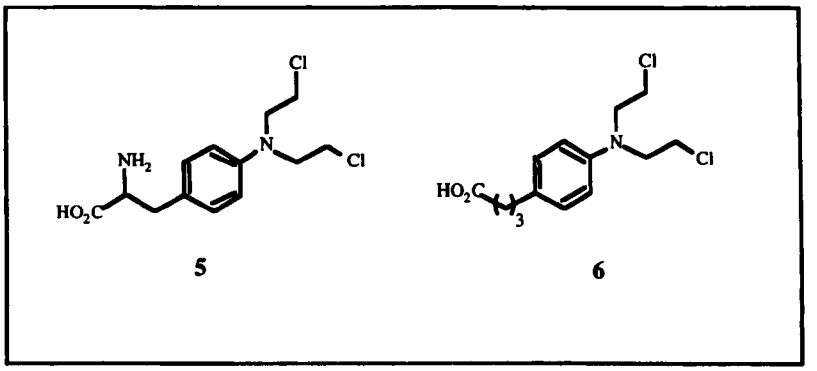


Figure 5 Mode of action of the aliphatic nitrogen mustards

Due to its severe toxicity and short time of action mechloroethamine has only very limited application and is now confined for treatment of Hodgkin's disease and other lymphomas. In order to solve these problems, aromatic nitrogen mustards were developed as milder alkylating agents.

Aromatic mustards[15]



Melphalan **5** and chlorambucil **6** are typical examples of aromatic mustards. The electron-withdrawing effects of the aromatic ring of these agents decreases the rate of alkylation considerably. This decreased activity allows time for absorption and distribution before extensive alkylation occurs and these drugs can be taken orally which is a significant advantage.

Melphalan **5** was developed in the hope that attachment of the amino acid phenylalanine to the nitrogen mustard might facilitate selective uptake by tumour cells. Although there is no evidence suggesting this to be the case, it has found clinical utility in multiple myeloma, breast and ovarian carcinoma and in the rare condition of macroglobulinaemia.

Chlorambucil **6** is probably the slowest acting and least toxic nitrogen mustard, often used in chronic lymphocytic leukaemia and carcinoma of the breast and ovary.[15]

Cyclophosphamide

Cyclophosphamide **7** was synthesised with a view to producing more selective mustards as it was found that some tumours possess high concentrations of phosphoramidases.[15] The introduction of the P=O group into the lead compound should partially delocalise the nitrogen electron pair in a similar fashion to the phenyl ring of the aromatic mustards, thus decreasing the rate of alkylation. The cyclophosphamide was designed as a prodrug that would release the active drug at the required site of action. In practice, the drug was found to undergo initial metabolism in the liver prior to acting on tumour cells.

Figure 6 illustrates the metabolism of cyclophosphamide.[17] Cyclophosphamide **7** remains inert until metabolised in the liver by hepatic cytochrome P450 oxidases. The oxidised product 4-hydroxycyclophosphamide **8** exists in equilibrium with aldophosphamide **9**. Aldophosphamide **9** is then conveyed to other tissues where it is transformed into phosphoramide mustard **13** and acrolein **12**. The metabolite phosphoramide mustard **13** is the actual cytotoxic species and the side product acrolein **12** is responsible for haemorrhagic cystitis of the bladder.[17]

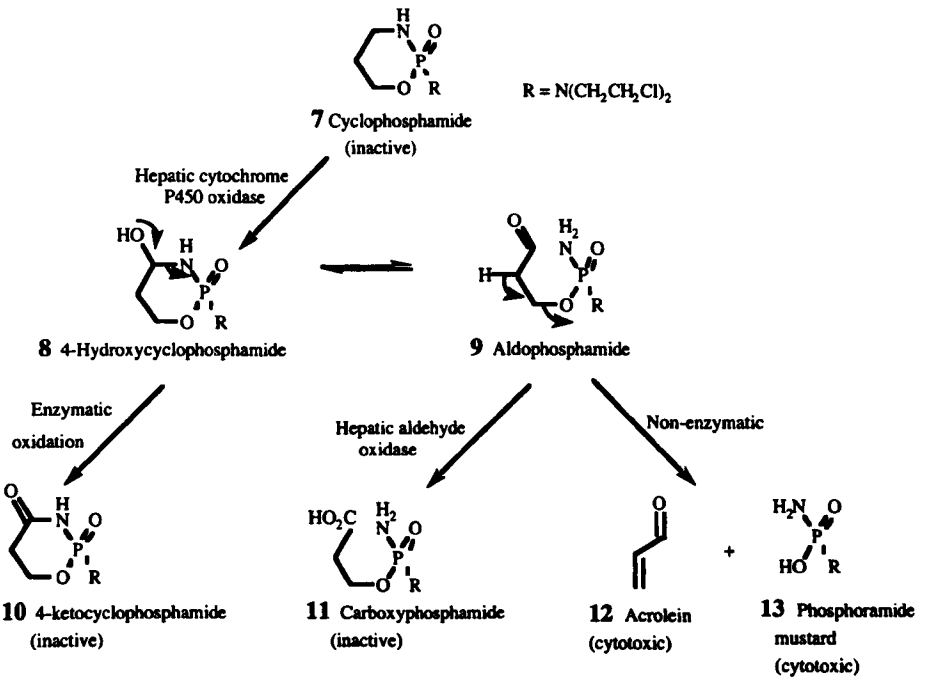
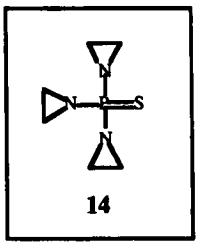


Figure 6 The metabolism of cyclophosphamide

Cyclophosphamide **7** proved to be the most successful mustard and has now been in use for over 20 years. Cyclophosphamide exhibits a broad spectrum of activity in treating malignant lymphomas and lymphoblastic leukaemia as well as carcinomas of the bronchus, breast, ovary and various sarcomas.[15]

Aziridines[15]

The aziridines closely resemble the aziridium ions formed from the nitrogen mustards. Examples of this group of agents are thiotepa **14** and mitomycin **15** (mitomycin C). Their mode of action is similar to the mustards. Aziridines have an aziridine ring already in their structure and the uncharged aziridine ring in **14** is less reactive than the fully charged aziridinium ions of the mustards. However, at physiological pH the aziridine ring in **15** is likely to be protonated due to the pKa of the aziridine nitrogen. Thus, in practice, the aziridinium ion may be the reactive species.[15]



14

Mitomycin C is a natural antitumour antibiotic isolated from *Streptomyces* species, and is considered to be the prototype bioreductive alkylating agent.[15] The three components, quinone, aziridine and carbamate moieties of the mitomycin molecule are believed to be essential for its mode of action (shown in Figure 7)[18]. It is thought that the action starts with an enzyme-catalysed one electron reduction of the quinone ring system to a semiquinone radical. This radical can rapidly disproportionate to mitomycin C and a hydroquinone, which acts as a bifunctional alkylating agent. A molecule of methanol is lost from the hydroquinone and then the aziridine ring opens to generate the alkylating agent. Research has now revealed that the main mode of DNA interaction for mitomycin involves alkylation of two guanine-N-2 residues on different DNA strands that give rise to an interstrand crosslink within the minor groove. However the most prominent feature of mitomycin is its capability for bioreductive activation prior to DNA interaction.[15] It has been revealed that the centres of some tumours are hypoxic due to a poor blood supply. Therefore the bioreductive ability of mitomycin is thought to produce selective toxicity to hypoxic tumour cells. Mitomycin has now been successfully used in the treatment of solid tumours such as those of the colon, lung and breast.[15]

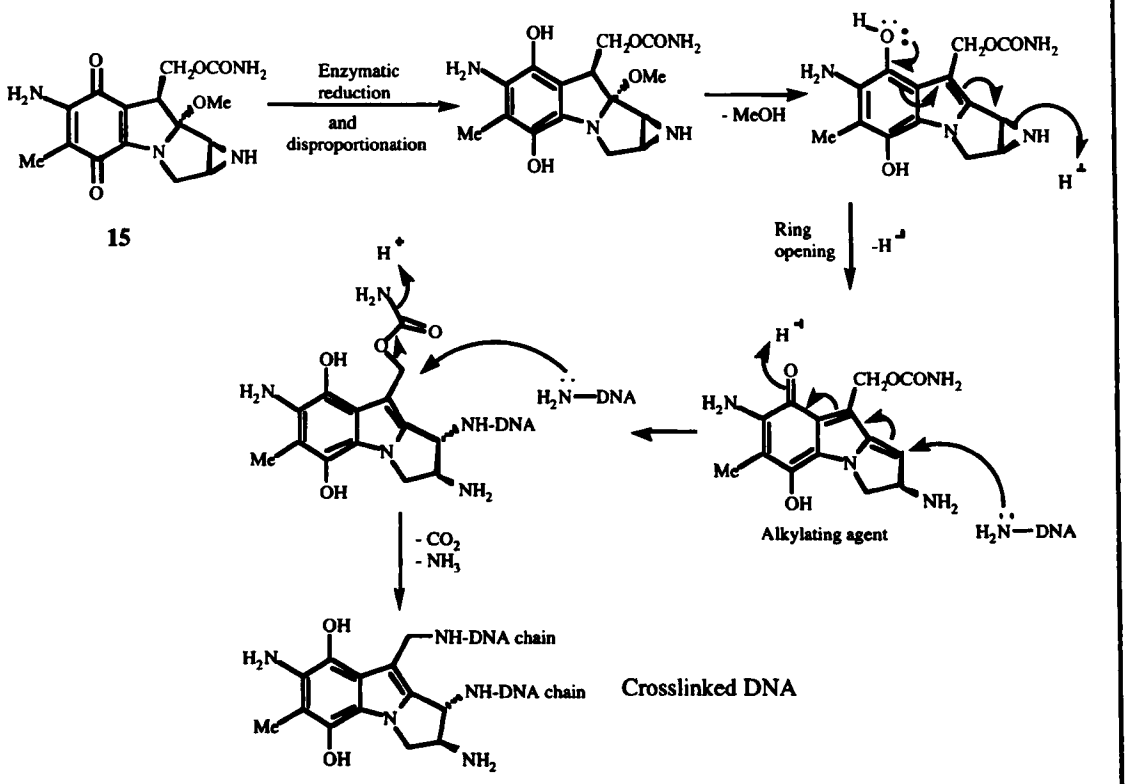
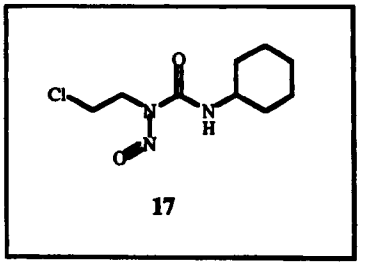


Figure 7 DNA crosslinking by mitomycin C

Nitrosoureas

Random screens performed by the USA National Cancer Institute in 1959 prompted the first work into nitrosoureas as potential chemotherapeutic agents.[19]

Two examples of clinically useful nitrosoureas are carmustine **16** [*N,N'*-bis(2-chloroethyl)-*N*-nitrosourea, BCNU] and lomustine **17** [*N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea, CCNU].



17

The mechanism of action of this group of agents has not been firmly established. The mode of action shown here (Figure 8)[20] was proposed by Reed *et al.*[21] The nitrosoureas are unstable at physiological pH and they decompose to an isocyanate and 2-chloroethyldiazene

hydroxide. It has been suggested that the latter compound spontaneously decomposes to 2-chloroethylcarbonium ion that is the major alkylating moiety. The carbonium ion is then attacked by the O-6 position of a guanine (X) in one DNA strand followed by the slow loss of chloride ion resulting in a second alkylation of base (Y) in the complementary strand. This explains the important fact that all of the nitrosoureas, even those with only one chloroethyl moiety such as lomustine can form interstrand crosslinks in DNA.

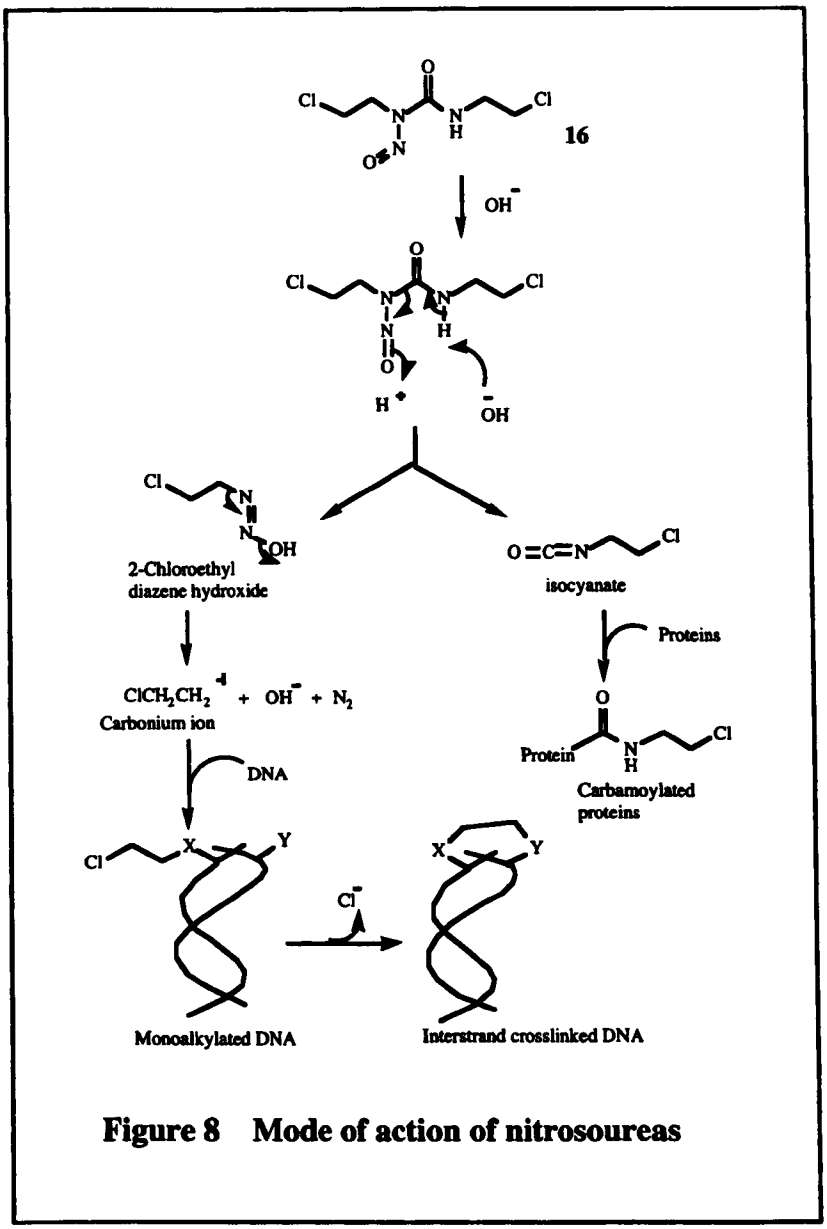
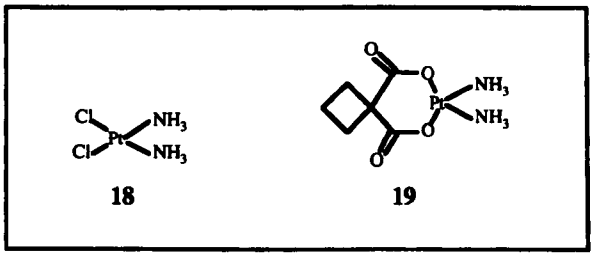


Figure 8 Mode of action of nitrosoureas

The most important feature of the nitrosoureas is their ability to cross the blood-brain barrier, which has allowed their successful application to cerebral cancers.[15] This feature is due to the high lipophilicity of these agents. BCNU and CCNU have also been used to treat malignant lymphomas and carcinomas of the breast, bronchus and colon. Unfortunately, the nitrosoureas suffer from dose-limiting adverse effects such as severe bone marrow toxicity.[15]

Platinum Compounds[22]

Cisplatin was discovered by chance as has happened with many other clinically used drugs. In 1965, Rosenberg and co-workers observed inhibition of bacterial division in an electric cell containing platinum electrodes. They discovered that it was the electrolysis products from the platinum electrode that caused the inhibition. Four years later, the same research group demonstrated the anticancer activity of several platinum complexes and *cis*-diamminedichloroplatinum (**18**, cisplatin) was identified as the most active one. Interestingly, the configurational isomer, *trans*-platin does not show any activity towards cancer cells.



18 **19**

Unlike most other alkylating agents, cisplatin forms *intrastrand* crosslinks predominately in the major groove of DNA with preferential interaction between the N-7 atoms of adjacent guanines, which results in the local denaturation of the DNA chain.

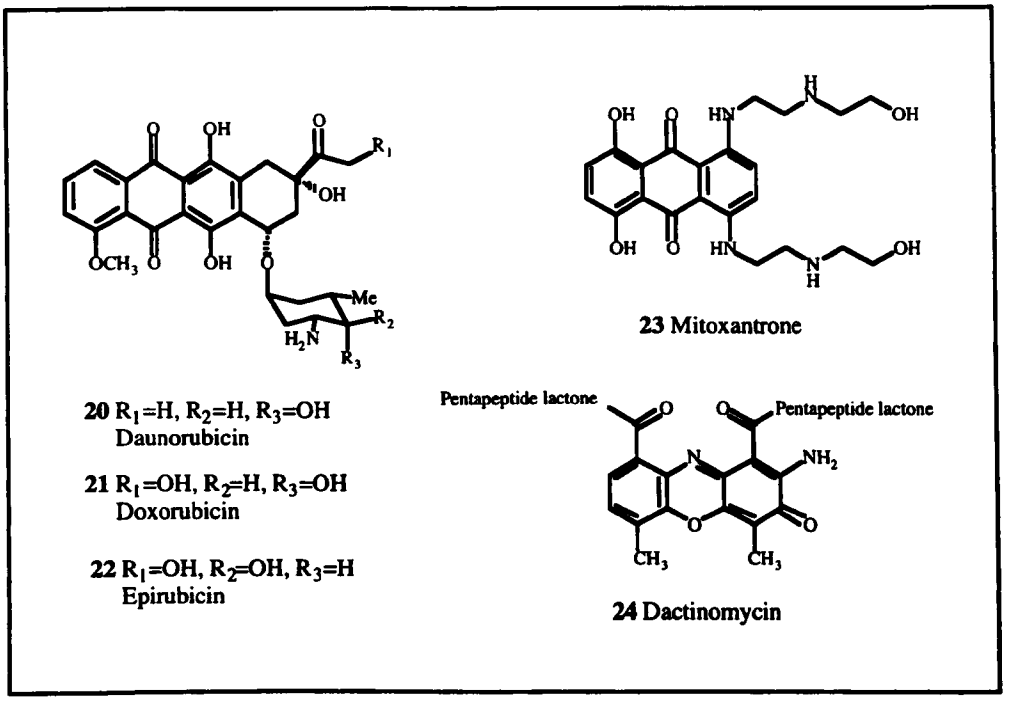
Cisplatin was introduced into clinical use in the UK in 1979 and it is claimed to be the most effective drug for the chemotherapy of testicular and ovarian cancers. 59% of patients with testicular cancer and 30% with ovarian cancer were cured in clinical trials using cisplatin in combination with vinblastine.[15] Cisplatin has also shown activity towards many other tumours including cancers of the lung, bladder, head and neck. Major side effects of cisplatin are leukopaenia, extreme nausea and renal dysfunction that are usually dose limiting. Many analogues have been prepared in the hope of reducing the side effects of cisplatin. Of these, carboplatin **19** is now in clinical use and offers lower side effects towards the kidneys and nervous system.

1. 4. 3. 1. 2 Intercalating agents

These agents have a flat shaped structure usually containing three or four fused aromatic rings. The molecular structure allows them to insert between the hydrophobic faces of the base pairs of DNA perpendicular to the axis of the helix. The insertion is held by interactions with DNA base pairs via hydrogen bonding and van der Waals forces.[15]

Most examples of this class of anticancer drug were originally discovered from natural sources in screening programmes that tested antibiotics for cytotoxic activity.[23] The

intercalating agents normally used in the clinic are the anthracycline antibiotics (**20**, **21** and **22**), mitoxantrone **23**, and dactinomycin **24**.



20 R_1=H, R_2=H, R_3=OH
Daunorubicin

21 R_1=OH, R_2=H, R_3=OH
Doxorubicin

22 R_1=OH, R_2=OH, R_3=H
Epirubicin

23 Mitoxantrone

24 Dactinomycin

Anthracyclines[15]

The anthracycline antibiotics were first isolated from a variety of strains of *Streptomyces* fungi. They are the best known family of intercalating agents, consisting of a characteristic four-ring structure that is linked to an amino sugar.

The exact mechanism for the activity of the anthracyclines has still not been fully elucidated but they are known to have several cytotoxic actions. Firstly, intercalation of the complementary strands of DNA is thought to interfere with DNA processing and transcription. The planar polycyclic moiety is inserted perpendicular to the axis of the helix and the amino sugar moiety is believed to interact with the sugar phosphate backbones of DNA to stabilise the adduct through hydrogen bonding.

In addition, the anthracyclines are known to form complexes with topoisomerase II leading to the inhibition of this group of enzymes which normally direct strand breakage, uncoiling and annealing during DNA replication (see Section 1.4.3.1.3).

Moreover, it has been observed that anthracyclines can bind to cell membranes, which could cause alteration of membrane fluidity and ion transport, and consequently change the biochemical equilibria in the cell.

Finally, the quinone moiety of the anthracyclines can be reduced by a variety of quinone reductases.[23] Generation of semiquinone and dihydroquinone can produce free radicals that cause damage to DNA and cells. As shown in Figure 9,[23] the one electron reduction of

anthracyclines provides the semiquinone, which then reacts with oxygen to generate superoxide anion $O_2^{\bullet-}$, while the two-electron reduction affords the dihydroquinone derivative, which reacts with oxygen to generate $2O_2^{\bullet-}$ or H_2O_2.[23] Superoxide anion can dismutate to peroxide which in combination with an iron chelated drug complex can produce hydroxyl radicals which are believed to be responsible for the cardiotoxicity observed with the anthracyclines.

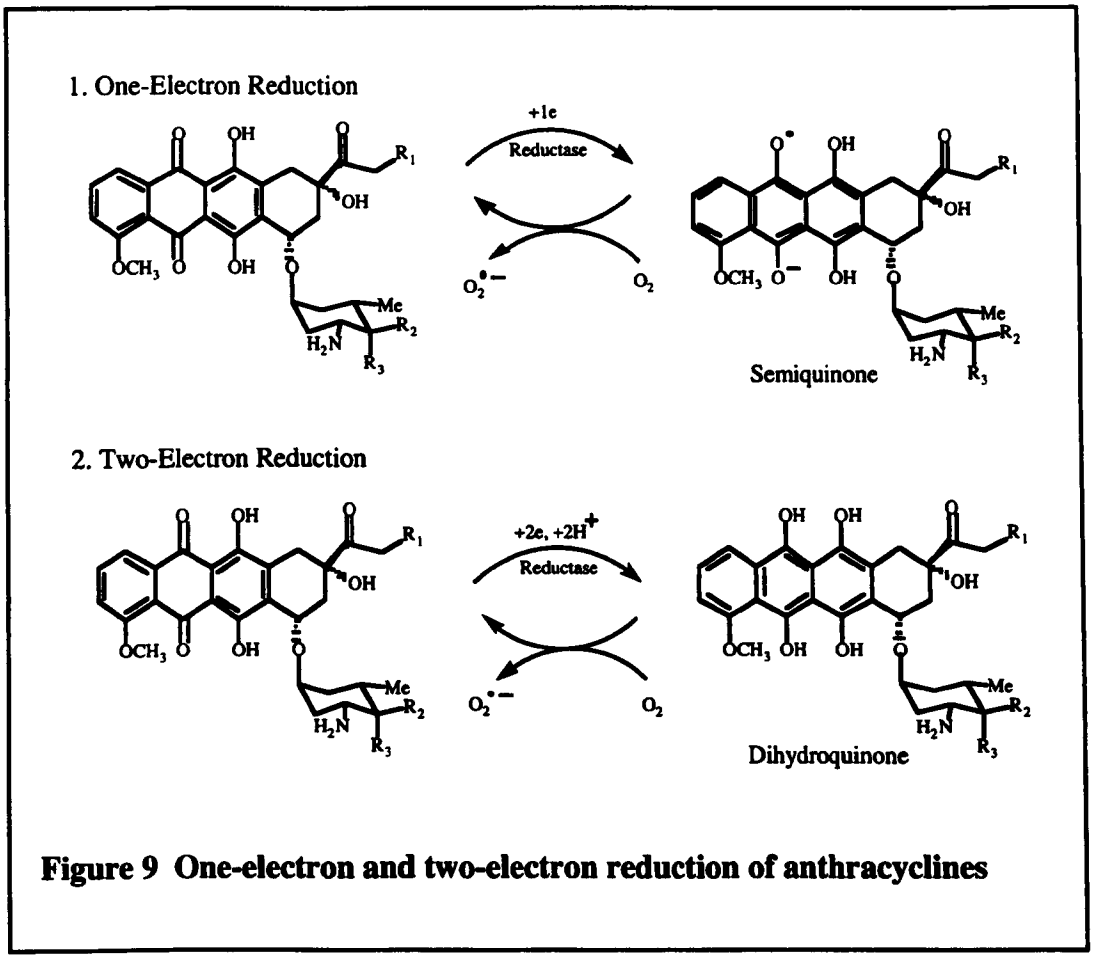


Figure 9 One-electron and two-electron reduction of anthracyclines

Doxorubicin **21** is an important anticancer drug exhibiting a wide range of activity against a variety of solid tumours including carcinoma of the breast, lung, thyroid and ovary. Daunorubicin **20** has an important role to play in the treatment of acute lymphocytic and myelocytic leukaemias. As doxorubicin and daunorubicin are valuable in clinical use, efforts have been made to develop semi-synthetic analogues including epirubicin **22**. The anthracyclines produce unique cardiotoxic reactions in both adults and children, and also cause severe bone marrow suppression, nausea and vomiting.[15]

Anthracenes[15]

These compounds were synthesised as anthracycline analogues and have three rather than four fused rings. Mitoxantrone **23** is an example of an anthracene that is used clinically to

treat childhood and adult myelogenous leukaemia, non-Hodgkin's lymphoma and breast cancer. These drugs have very low cardiotoxicity compared with the anthracyclines, as they are unable to generate free radicals.

Phenoxazines[23]

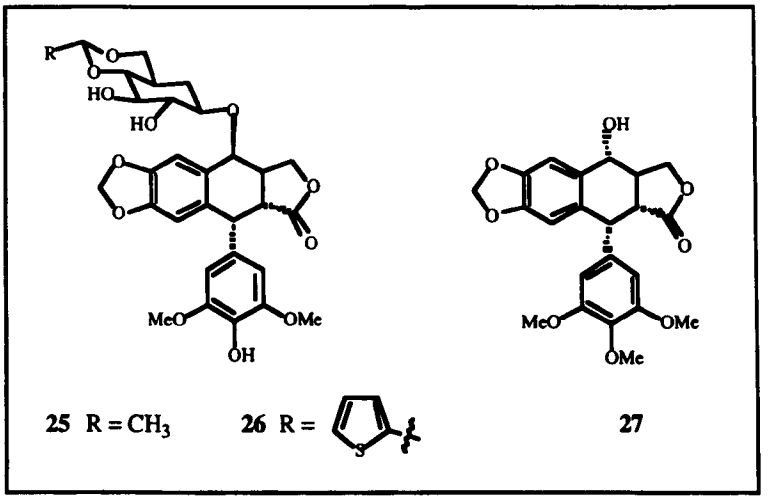
Dactinomycin **24** was isolated from *Streptomyces parvulus* in the 1940s. The compound has two identical pentapeptide lactones that are linked to a phenoxazone ring system. Much work has been done on the synthesis, biological action, and use of this drug.

At low concentrations, dactinomycin inhibits DNA-directed RNA synthesis preventing chain elongation whereas at higher concentrations DNA synthesis is also inhibited. The phenoxazone ring can interact with the N-2-amino groups when inserted preferentially between guanine-cytosine base pairs of DNA. The cyclic pentapeptide moieties interact with functional groups in the narrow groove of DNA via hydrogen bonding and hydrophobic interactions, which stabilise the DNA adduct with dactinomycin and block RNA polymerase. It is notable that dactinomycin also causes single-strand DNA breaks either through radical formation or by interaction with topoisomerase.

Dactinomycin is employed in the treatment of paediatric solid tumours, including Wilms' tumour and Ewing's sarcoma. It has been proved effective for gestational choriocarcinoma, and is also useful in the chemotherapy of some testicular sarcomas and Kaposi's sarcoma; a tumour associated with AIDS patients.

1. 4. 3. 1. 3 Topoisomerase inhibitors[24]

Etoposide **25** and teniposide **26** are semisynthetic analogues of podophyllotoxin **27**, a microtubule inhibitor found in extracts of the American mandrake plant. It was initially expected that etoposide, too, would inhibit microtubule function. Further investigation indicated that its major activity arises from topoisomerase II inhibition.



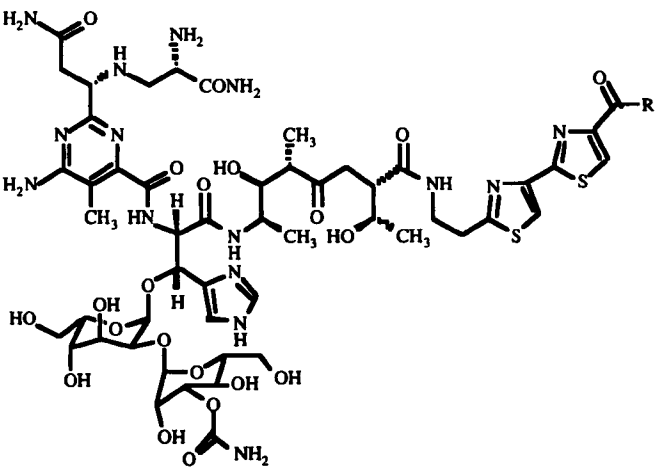
The normal function of topoisomerase II involves initial interaction with DNA forming a noncovalent complex that is noncleavable, as removal of the enzyme at this stage would leave no strand breaks. The enzyme then cuts both DNA strands, and one of the newly formed 5'-phosphate ends of the DNA attaches to a protein subunit via covalent bonding. This enzyme-DNA complex is considered cleavable, since dissociation of the complex at this point would result in a permanent double strand break. However the cleavable complex is a transient form. In the absence of enzyme inhibitors, the complex either goes back to the noncleavable complex or, after a topological change has been affected, the breaks are resealed with subsequent release of the free enzyme from the DNA. Etoposide is known to stabilise the cleavable complex, thus preventing it from converting back into the noncleavable complex or from completing topological changes. As a result of this, the drug-complex may then undergo spontaneous denaturation leading to the generation of unrepairable double-strand breaks at critical sites.

It has been observed that etoposide treatment prevents activation of the protein kinase p34. This kinase is usually activated at the end of the G_2 phase of the cell cycle and has a pivotal role in the initiation of mitosis. It is thought that the observed arrest of cells in the G_2 phase, after treatment with etoposide, may be due to interference with p34 function.[25]

Etoposide is claimed to be one of the most effective drugs for the chemotherapy of lung cancer and testicular cancer; combination therapy with cisplatin and bleomycin is now the standard treatment for the latter condition. Etoposide has found clinical use in the treatment of lymphocytic leukaemia and brain tumours in adults.

1. 4. 3. 1. 4 DNA Cleaving Agents

The bleomycins **28** are a group of metal-chelating glycopeptide antibiotics isolated from cultures of *Streptomyces verticillus*. The general structure of the bleomycins is shown below. These compounds have very complex structures and have attracted much interest among organic chemists. The first total synthesis of bleomycin was reported in 1982.[15]



28 General structure of the bleomycins

The most prominent feature of bleomycins is their ability to degrade DNA.[26] Bleomycin has been found to break single strands of DNA predominantly and thus causes DNA fragmentation, but some double-strand breakage has also been observed.[26] These agents exhibit their anticancer activity by inhibition of DNA synthesis and have much less effect on RNA and protein synthesis.[25]

Three distinct regions of bleomycin are believed to be essential to its mechanism of action.[15] First, the planar shaped bithiazole moiety (top right as drawn) is thought to act as an intercalator which is inserted perpendicular to the axis of the DNA helix. The second part (top left) consists of primary amine, pyrimidine, and imidazole nitrogens which function as ligands to chelate with ferrous iron forming a metal coordination site. The Fe(II) bleomycin complex interacts with oxygen in a redox cycle to generate superoxide and/or hydroxyl radicals leading to single- and double-strand breaks. The third region of bleomycin (bottom left) has a glycopeptide structure which has no direct antitumour activity of its own, but may contribute to the water solubility of these drugs. Bleomycin has been found to affect the G_2 phase of the cell cycle and mitosis profoundly and is also effective in G_0 phase.[15]

Bleomycin produces very little bone-marrow depression which is a big advantage compared with most anticancer drugs. It has been widely used, in particular for the treatment of testicular carcinoma, squamous cell carcinomas, and lymphomas.[23]

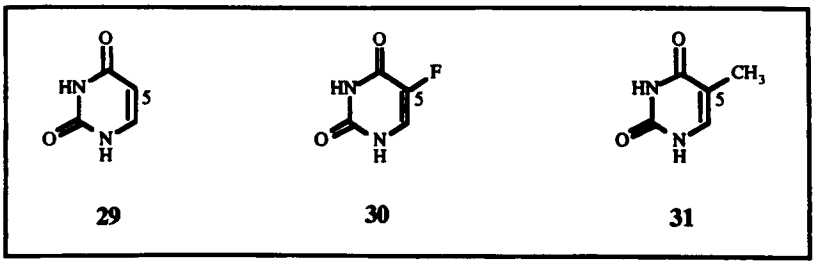
1. 4. 3. 2 Antimetabolites

An accumulation of nucleic acids and proteins is required in preparation for cell division. The antimetabolites interfere with this process by either of the following pathways.[27] Some of

the agents prevent the synthesis of DNA by poisoning the enzymes required in the formation of the deoxyribonucleoside triphosphates that are the immediate precursors for DNA synthesis, and this occurs in the S phase of the cell cycle. Alternatively, some antimetabolites are designed as structurally closed mimics of normal purines or pyrimidines that are able to compete for places in the nucleotide pathways.[27] The main antimetabolites include pyrimidine analogues, folate antagonists and purine analogues.

1. 4. 3. 2. 1 Pyrimidine analogues

A well known antimetabolite is 5-fluorouracil **30** which was designed as a close analogue of uracil **29** and thymidine **31**, the corresponding bases in RNA and DNA respectively. It is known to inhibit cell division in several ways.



The structural similarity with a nucleotide allows 5-fluorouracil to be incorporated into DNA and RNA in place of thymidine and uracil, respectively, causing mutations in DNA and RNA.

On the other hand, 5-fluorouracil exhibits its cytotoxic effect through inhibition of thymidylate synthetase, the key enzyme in the conversion of deoxyuridine monophosphate (dUMP) **32** into deoxythymidine monophosphate (dTMP) **33** (Figures 10 and 11)[28] with the assistance of a cofactor called tetrahydrofolate **34**. Inhibition of thymidylate synthetase interferes with the production of thymidine and consequently DNA synthesis and hence cell division is inhibited.

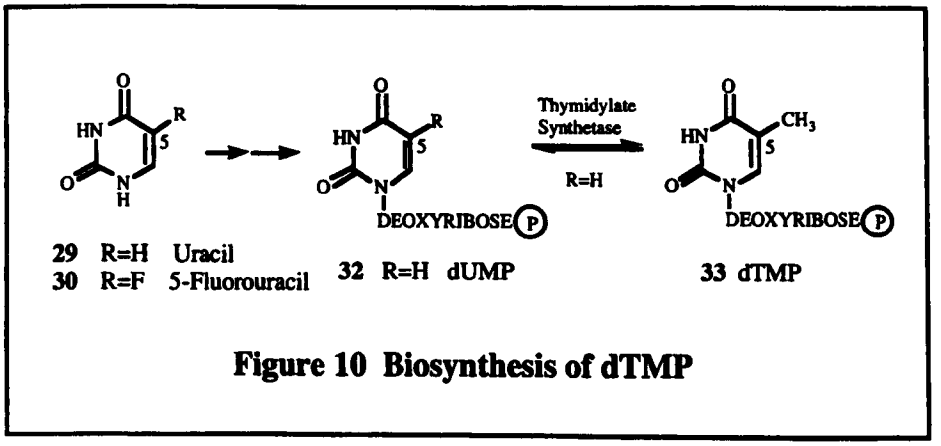


Figure 10 Biosynthesis of dTMP

In the absence of inhibitor, thymidylate synthetase catalyses the conversion of dUMP into dTMP transferring a single carbon unit from tetrahydrofolate to the 5-position on the uracil skeleton, and the thymidine formed is subsequently used in DNA synthesis.

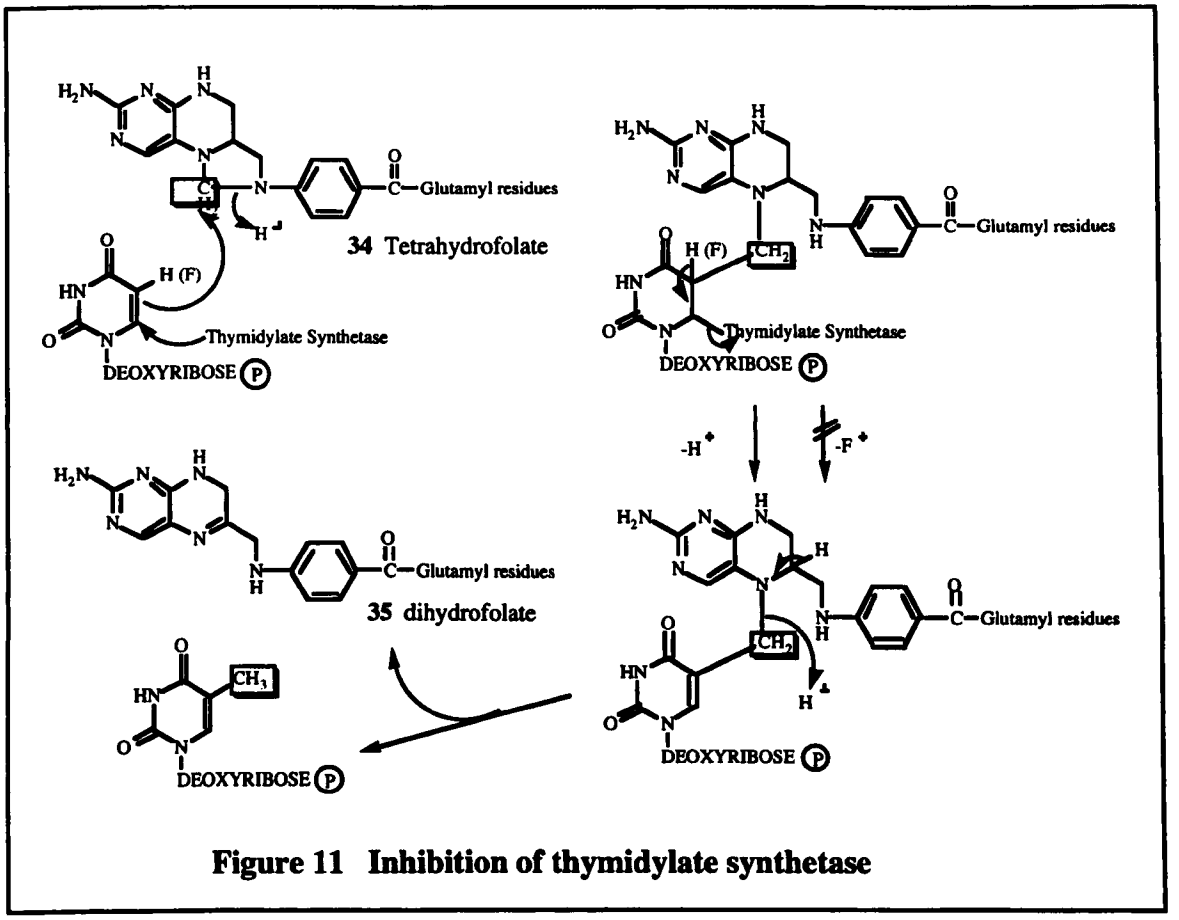


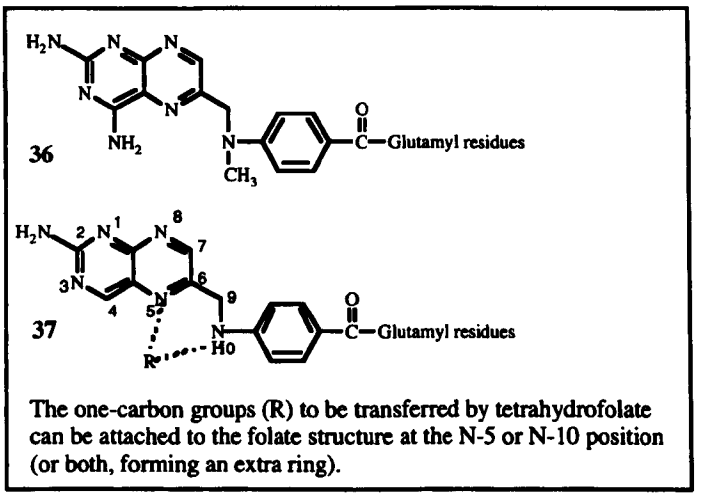
Figure 11 Inhibition of thymidylate synthetase

The mode of action of 5-fluorouracil is illustrated in Figure 11.[28] 5-Fluorouracil first combines with thymidylate synthetase and the cofactor to form a transition state complex. In this intermediate a methylene unit of the tetrahydrofolate bonds to the 5-position on the uracil skeleton. The next step, which usually involves the loss of a proton from the 5-position of uracil to release the enzyme, cannot occur as 5-fluorouracil has a fluorine atom at the 5-position instead of a hydrogen. Therefore further reaction is impossible since it would require fluorine to leave as a positive ion. Consequently 5-fluorouracil remains bound to the active site of the enzyme irreversibly. The synthesis of thymidine and hence DNA synthesis is thus terminated at this stage.[28]

5-Fluorouracil is successfully used in the treatment of skin cancer as a 5% cream, and has proved clinically useful in treating certain solid tumours such as those of the gastro-intestinal tract, breast and pancreas.

1. 4. 3. 2. 2 Folate antagonists

Methotrexate (MTX) **36** is a folate antagonist and has been widely used in cancer chemotherapy.



The one-carbon groups (R) to be transferred by tetrahydrofolate can be attached to the folate structure at the N-5 or N-10 position (or both, forming an extra ring).

The vitamin folic acid **37**, the core structure for all folates, is essential for the synthesis of purine and pyrimidine nucleotides needed for the biosynthesis of DNA. In order to act as a coenzyme, folic acid must be reduced in two successive steps by dihydrofolate reductase (DHFR) to afford 7,8-dihydrofolate **35** (FH_2) and then 5,6,7,8-tetrahydrofolate **34** (FH_4) (Figure 12).[17] It is this final reduced form that delivers single carbon units in the methylation of dUMP to form dTMP (Figures 10 and 11), and also in the *de novo* synthesis of purines.[27]

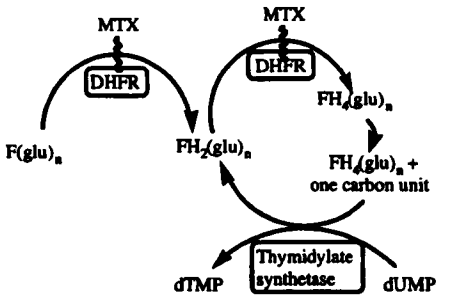


Figure 12 Action of methotrexate on thymidylate synthesis

The primary biochemical function of methotrexate is to inhibit DHFR activity. Methotrexate binds more strongly to the active site on DHFR than **37** and FH_2 due to the presence of an extra amino group that increases the basicity of the pyrimidine ring. Once this inhibition occurs, and the tetrahydrofolate is used up in dTMP synthesis, *de novo* production of uridine and thymidine nucleotide cease.[27]

Methotrexate is highly effective in the treatment of choriocarcinoma. It is also employed in the treatment of acute leukaemia and many solid tumours.

1. 4. 3. 2. 3 Purine analogues[27]

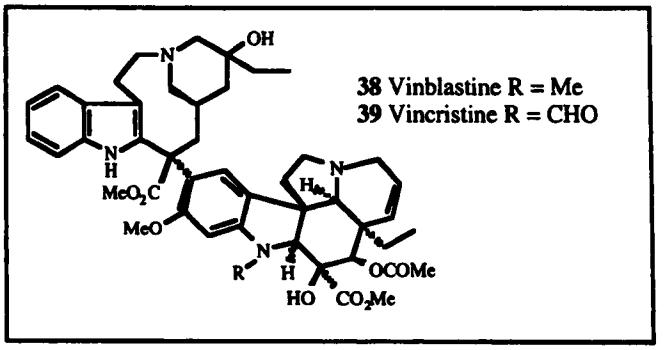
The main purine analogues are 6-mercaptopurine and 6-thioguanine. They are first subject to enzymatic conversion into their active forms that in turn inhibit several enzymatic pathways in the biosynthesis of purines. Eventually DNA synthesis ceases due to a lack of the purine bases adenine and guanine. The major problem of these drugs is their lack of selectivity, since purines are involved in many cellular processes apart from nucleic acid synthesis. They are used primarily to treat acute childhood leukaemias.

1. 4. 3. 3 Antitubulin Agents[29]

Microtubules are involved in various cellular functions including mitosis, cell movement and cell shape. The major component of microtubules is protein tubulin. Polymerisation of tubulin dimers to form microtubules is an integral part of mitosis. Microtubules exist in a very sensitive equilibrium between free tubulin dimers and assembled polymers. Antitubulin agents act by disrupting this equilibrium.

Vinca alkaloids

Two Vinca alkaloids, vinblastine **38** and vincristine **39**, which were isolated from the Madagascar periwinkle plant (*Vinca rosea*), play an important role in cancer chemotherapy. Their cytotoxic effects arise from binding to the tubulin dimers, which disrupts the equilibrium between microtubule polymerisation and depolymerisation. As a result the mitosis is blocked by metaphase cell arrest.[29]

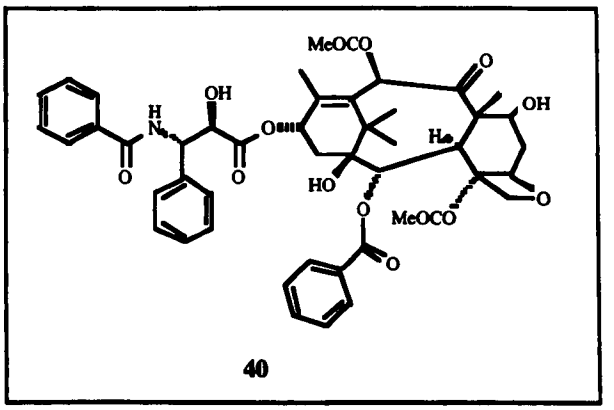


38 Vinblastine R = Me
39 Vincristine R = CHO

Vinblastine is included in several drug regimens for treating Hodgkin's disease, breast cancer and testicular carcinoma. Vincristine is commonly used in drug combinations for the treatment of acute lymphoblastic, childhood leukaemias and many other solid tumours.

Paclitaxel (Taxol)[30]

The important microtubule antagonist paclitaxel (Taxol) **40** was originally isolated from the bark of the yew *Taxus brevifolia*. In contrast to the Vinca alkaloids, Taxol disturbs the delicate tubulin-microtubule equilibrium in favour of microtubule formation. This causes stabilisation of the microtubules and hence the construction of an abnormal bundle of microtubules, and as a consequence the mitotic phase of the cell cycle is disrupted.



40

The clinical application of Taxol has been hampered by a lack of a synthetic source of this very scarce natural product. The yew tree *Taxus brevifolia* has remained as the major source of this compound until recently. Taxol is now generally obtained by semi-synthesis from 10-deacetylbaccatin III, isolated from the needles of a related plant. Taxol has attracted much interest among cancer biologists and organic synthetic chemists for its potential clinical value. Its total synthesis has been reported by the groups of Holton[31], Nicolaou[32], Danishefsky[33] and Mukaiyama.[34]

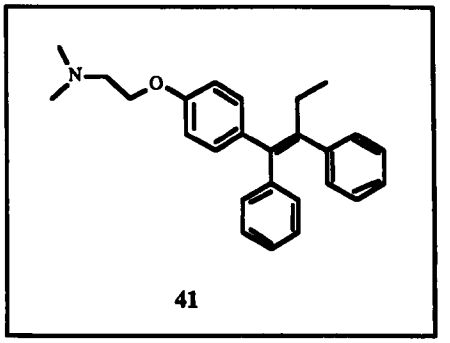
Taxol exhibits significant activity in the treatment of a variety of cancers including ovarian, breast, lung and head cancers. This promising agent is particularly useful in the treatment of the advanced metastatic forms of many cancers.

1. 4. 3. 4 Anti-hormonal agents[35]

Tumours derived from sexually differentiated tissues such as the endometrium, prostate and breast can be strongly influenced by the levels of related hormones in the body. This hormone-dependent response can be exploited chemotherapeutically to control the growth of cancer.[35] Tamoxifen **41** and leuprolide are two successful anticancer drugs that have resulted from this work.

Tamoxifen, an anti-oestrogenic agent, has pronounced activity in some cases of hormone-dependent breast cancer, and may be useful in preventing these cancers. Tamoxifen is known to exert its activity by binding to the oestrogen receptors of the tumour cells preventing

them from binding to endogenous oestrogens[35] and it therefore inhibits the growth of oestrogen-dependent tumour.



41

Prostate cancers are hormone responsive and are known to be stimulated by androgens like testosterone. Testosterone is secreted by the Leydig cells of the testes in a process controlled by gonadotrophin releasing hormone (GnRH) and leuteinising hormone (LH). Leuprolide, a clinically used decapeptide drug, works by competitive inhibition of the GnRH receptors thereby reducing androgen production.

1. 5 The Search for New Anticancer Drugs

The preceding sections have outlined the causes and treatments of cancer, with particular emphasis on the anticancer drugs and their mechanism of action. The discovery of clinically useful anticancer drugs has arisen from a variety of areas such as screening of natural products, analogue studies of active compounds and serendipity. The recent use of combinatorial methods has allowed the generation of libraries of compounds for screening purposes. Also, more emphasis has been placed on rational drug design based on key biological targets since substantial knowledge has been acquired of the fundamental biochemical differences between normal and tumour cells. In addition X-ray crystal structures are now available for a number of important enzymes and computer modelling is being used to design new compounds which should be prepared for evaluation as anticancer compounds.

There have been prodigious advances in the understanding of the basis of malignancy, in particular the role of oncogenes and tumour suppressor genes. A large number of oncogenes have now been identified and sequenced, and the technology to design and produce gene-targeted agents is rapidly advancing. Exciting developments have been made in gene therapy in which tumour suppressor genes are inserted into the DNA of cancer cells.

Recent advances in molecular biology have led to the development of new approaches towards selectivity enhancement of anticancer drugs. For example, The ADEPT[15] (Antibody-Directed Enzyme Prodrug Therapy) and GDEPT[15] (Gene-Directed Enzyme Prodrug Therapy) approaches have been investigated to enhance the selectivity of cytotoxic agents. In ADEPT,

firstly an antibody-enzyme conjugate is administered to the patient and this is delivered to the tumour site. A prodrug is then administered and this prodrug can be converted into the active cytotoxic agent only when contacted with the enzyme at the tumour site.

As these and other novel therapies emerge, it is likely that dramatic advances in the treatment of cancer will occur in this century.

CHAPTER 2

Cell Signalling and MAP Kinase Inhibitors

2. 1 Intracellular signal transduction

Intracellular signal transduction is a process by which a signal is transmitted from the cell surface to the nucleus.[36] The signalling cascade, initiated by the binding of many diverse extracellular stimuli to specific receptor molecules on the target cell surface, serves to transmit a signal through the cytoplasm to the cell nucleus, ultimately resulting in cell division or other appropriate biological response.

2. 1. 1 Protein kinases

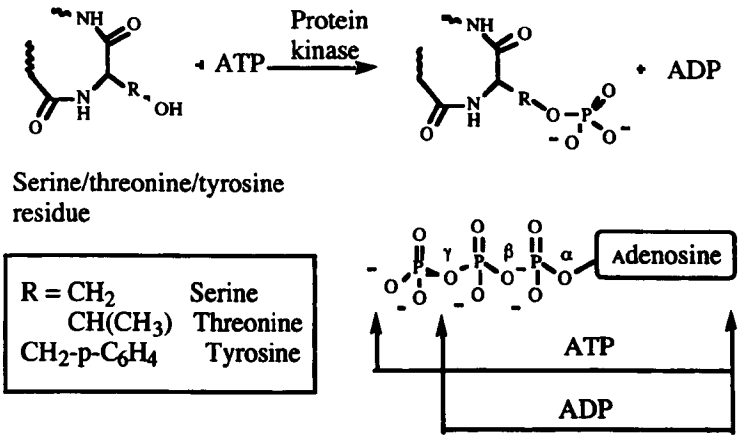
Protein kinases play a vital regulatory role within the cell signalling pathways in that they catalyse the phosphorylation of target proteins at specific sites in the pathways. Protein phosphorylation is a common process for signal transduction within cells.[37] The protein kinases are activated by phosphorylation catalysed by upstream kinases and, in turn, phosphorylate several different downstream kinases and other functional substrates. Thus a series of protein kinases can act as "molecular relay runners" to transmit signals from the cell surface to the nucleus, leading to changes in gene expression, DNA synthesis, and cell proliferation.

The protein kinases comprise two main subdivisions: the protein tyrosine kinases and the protein serine/threonine kinases.[38] The two groups of kinases are distinguished by the specific amino acid residues which they modify by phosphorylation. In the phosphorylation process the γ-phosphate of ATP is transferred to the phenolic group of tyrosine residues when catalysed by tyrosine kinases, and to the alcohol groups of serine or threonine residues when catalysed by serine/threonine kinases. As a result the phosphate monoester and ADP are formed (Figure 13).[39]

Phosphorylation of these phenolic and hydroxyl groups disrupts their hydrogen bonding and changes their polarity causing the enzyme to change its tertiary structure, and as a consequence the activity of the phosphorylated protein is frequently altered.

Multiple signalling pathways integrate into a network within individual cells to elicit various physiological responses including cell proliferation. One of the most important of these pathways is the mitogen-activated protein (MAP) kinase pathway.

Figure 13 Phosphorylation Catalysed by Protein Kinase



2. 1. 2 Mitogen-Activated Protein (MAP) Kinase Cascade[40]

MAP kinases are a family of serine/threonine kinases which can act together to generate a process of phosphorylation events within the signalling pathway. It has been revealed that the MAP kinases are divided into three main subfamilies. The extracellular signal regulated kinases (ERK1/2) comprise the first subdivision, which contains a mitogen-activated MAP kinase subfamily. The other two principal subfamilies are composed of the JNK/SAPK (stress-activated protein) and p38 kinases.

Four distinct MAP kinase pathways have been so far identified in yeast and three in mammals. As an example, the best known *ras*/ERK MAPK pathway represents signalling transduction in the phosphorylation cascade (Figure 14).[40]

2. 1. 2. 1 *Ras*/ERK MAPK pathway[41]

The ERKs are activated by MEKs (MAP/ERK kinases) *via* phosphorylation of threonine and tyrosine residues. These MAPK-activating enzymes are unusual in their ability to catalyse phosphorylation of both threonine and tyrosine residues. MEKs are in turn activated by phosphorylation of two adjacent serine/threonine residues by upstream kinases known as *raf*. *Raf* is a serine/threonine kinase oncogene which appears to require activation by association with *ras* in an undefined event. Ras, a G-protein (guanine nucleotide binding protein), which is only active in a GTP-bound state, links receptor tyrosine kinase activation to the intracellular MAP kinase pathway.

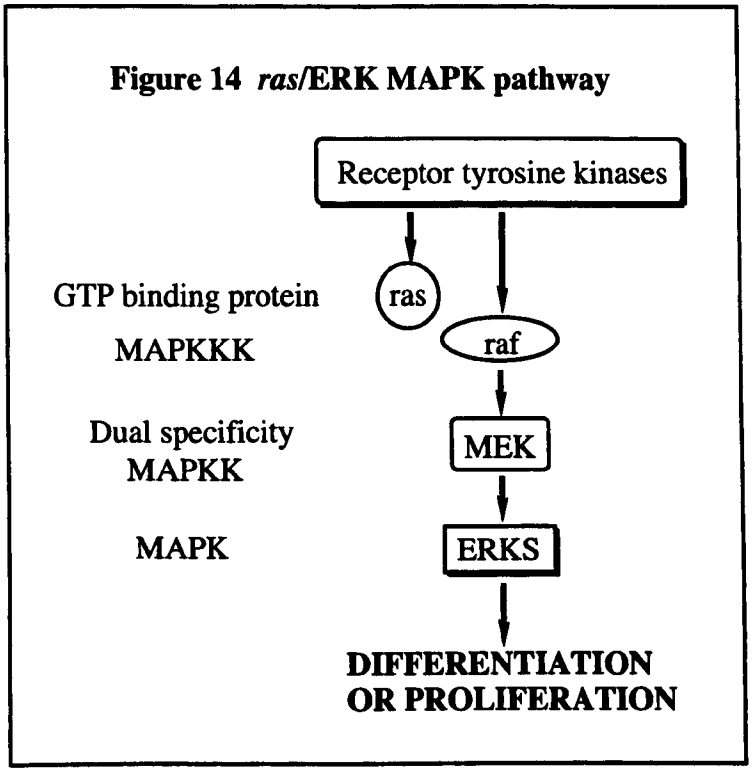


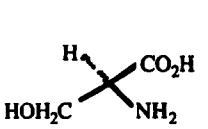
Figure 14 *ras*/ERK MAPK pathway

2. 1. 2. 2 JNK/SAPK MAPK pathway[42]

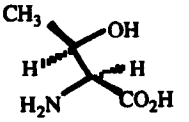
The JNK/SAPK pathway, which is activated in response to stress stimuli, represents a distinct MAPK pathway, targeting transcription factor *c-jun* which controls the expression of genes involved in cell proliferation.

JNK/SAPKs are observed to be strongly activated by cellular stress stimuli and hence the name stress-activated protein kinase (SAPK) is derived. The SAPKs have been shown to stimulate phosohorylation and activation of the transcription factor *c-jun* and they are believed to be the main *c-jun* kinases (JNK).

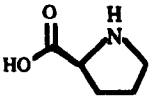
Molecular biological studies have revealed that the amino acid sequences (Ser/Thr-Pro-Glu or Ser/Thr-Pro-Asp) represent a preferred consensus sequence for phosphorylation by JNK/SAPKs, and that JNK/SAPKs often bind directly to their substrates via a distinct docking domain as exemplified by the delta domain of *c-jun*.



42 L-Serine



43 L-Threonine



44 L-Proline

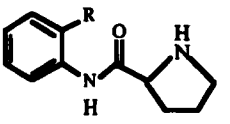
SAPKs are activated by chemicals, irritants or chemotherapeutic drugs and they are important for growth arrest, repair, apoptosis, etc.

2. 2 Model for MAP Kinase Inhibitors

MAP kinases are involved in the regulation of a variety of transcription factors necessary for the transcription of DNA. Mutations or enhanced activity by overexpression in receptor tyrosine kinase or oncogenic forms of *ras, raf* or transcription factors, *jun* in the MAP kinase pathway can lead to cancer. Consequently, targeting the MAP kinase family of enzymes may lead to new drugs for the treatment of cancer.

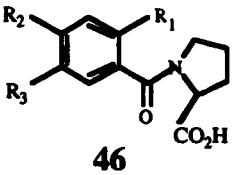
Previous work in the area of synthesis of MAP kinase inhibitors in our research group was carried out by Pearson.[43] A series of aromatic compounds with oxygen substituents modelled on serine **42** were synthesised and several of them showed inhibitory activities in the MAP kinase biological assay.

The compounds in this project were designed to target the stress related kinases specifically. As mentioned above, the stress related kinases are known to have a serine/threonine joined to a proline **44** residue. In an attempt to prepare selective inhibitors of stress related kinases, compounds **45** and **46** were designed in which a conformationally restricted serine analogue is joined to L-proline *via* an amide link in two possible ways.



45

R = F, OCH_3, OH



46

R_1 = F, OMe, OH
R_2 = H, OMe
R_3 = H, OMe

These two set of compounds were synthesised and tested for biological assay. The synthsis and biological test results are discussed in Chapter 5.

CHAPTER 3

Checkpoint Control

Cell cycle checkpoints are parts of signalling pathways which control key transitions in the life of a cell to ensure the fidelity of DNA replication and chromosome segregation.[44] In the event of DNA damage checkpoint regulatory pathways are activated resulting in cell cycle arrests at both the G_1/S and G_2/M transitions to provide time for the repair of damaged DNA, or in the case of severe damage, for activation of programmed cell death.[45]

In response to DNA damage, mammalian cells activate the p53 tumour suppressor gene, which induces transcription of $p21^{CIPI}$, a cyclin-dependent kinase inhibitor, resulting in cell cycle arrest at the G_1/S checkpoint.[46] On the other hand, G_2/M arrest is caused by inhibitory phosphorylation of $p34^{cdc2}$ protein kinase in both fission yeast and mammalian cells[47]. In the following section we will discuss the major features of the G_2/M checkpoint that regulate the onset of M-phase during the cell cycle.

3. 1 Checkpoint pathways

The pioneering work in the checkpoint control field was carried out by Hartwell and Weinert[48] who identified checkpoint mutants which were able to undergo mitosis even if DNA damage was not repaired. In 1997 three groups of scientists[44,45,49] reported their breakthrough work in the understanding of the functions of checkpoint proteins. Combining their work, an elegant model[47] (shown in Figure 15) for a checkpoint pathway emerged.

3. 1. 1 Rad3 Protein Kinase

After DNA damage, the protein kinase Rad3 is activated. Rad3 is a *rad* mutant in the fission yeast *Schizosaccharomyces pombe* as it is sensitive to DNA damage caused by radiation.[50] Sequence analysis revealed that *rad3* is related to ATM, a gene mutated in ataxia telangiectasia patients.[51] It is believed that Rad3 is required in activation of the DNA damage checkpoint in which phosphorylation of Chk1 is increased by a Rad3-dependent process.[45]

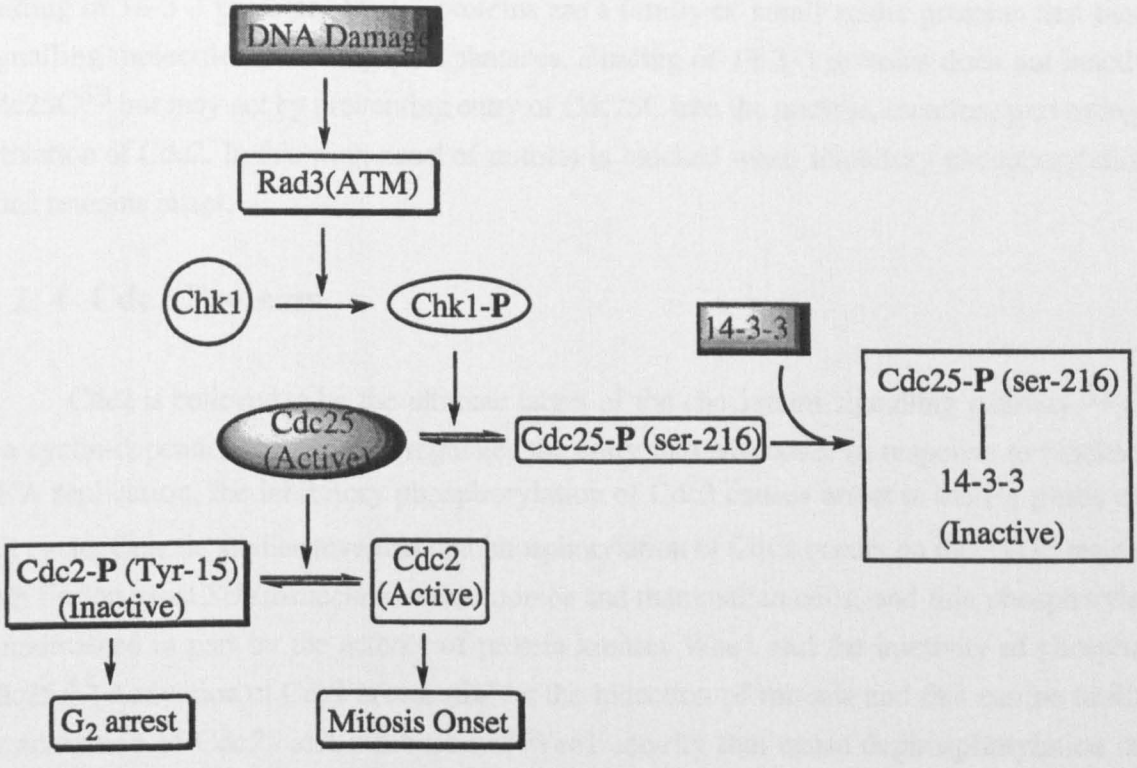


Figure 15

3. 1. 2 Chk1 Protein Kinase

In 1993 Walworth *et al.*[50] reported the identification of Chk1 protein kinase in fission yeast which links the *rad* checkpoint pathway to *cdc2*, a crucial regulator for the onset of mitosis.[52] The sequence of Chk1 was found to be 30-40% identical to the family of serine/threonine protein kinases.[44] Researchers suggested that in response to DNA damage Chk1 is activated by phosphorylation in a Rad3-dependent manner.[50] Activated Chk1 then functions upstream of Cdc25 in fission yeast and in mammalian cells.[45]

3. 1. 3 Cdc25 Protein Kinases and 14-3-3 Protein Kinases[44]

Cdc25 are dual-specificity protein phosphatases which can form complexes with Chk1.[44] There are three Cdc25 homologues including Cdc25A, Cdc25B and Cdc25C, and it is Cdc25C which regulates Cdc2 at the onset of mitosis. Research identified Cdc25 as a direct substrate of activated Chk1 which phosphorylates Cdc25C on a physiologically significant residue, Ser216. It appeared that phosphorylation of Cdc25 itself does not directly inhibit Cdc25.[69] Peng *et al.*[49] found phosphorylation of Cdc25C on Ser216 facilitates the binding of

14-3-3 protein as the Ser216 residue is embedded in a potential recognition motif for the binding of 14-3-3 proteins. 14-3-3 proteins are a family of small acidic proteins that bind to signalling molecules including phosphatases. Binding of 14-3-3 proteins does not inactivate Cdc25C[53] but may act by preventing entry of Cdc25C into the nucleus, therefore preventing the activation of Cdc2. In this way, onset of mitosis is blocked when inhibitory phosphorylation of Cdc2 remains intact.

3. 1. 4 Cdc2 Proteins

Cdc2 is believed to be the ultimate target of the checkpoint signalling pathway.[52] Cdc2 is a cyclin-dependent kinase that regulates the entry into M-phase. In response to blockade of DNA replication, the inhibitory phosphorylation of Cdc2 causes arrest in the G_2 phase of the cell cycle. Genetic studies revealed that phosphorylation of Cdc2 occurs on the Tyr15 residue in both fission yeast *Schizosaccharomyces pombe* and mammalian cells, and this phosphorylation is maintained in part by the activity of protein kinases Wee1 and the inactivity of phosphatase Cdc25.[53] Activation of Cdc2 is essential for the induction of mitosis and this can be facilitated by activation of Cdc25 and inhibition of Wee1 activity that cause dephosphorylation of the Tyr15 residue in Cdc2. The phosphotyrosine15 in Cdc2 is located in the ATP binding site, which suggests that part of the activation mechanism involves the removal of this phosphate group allowing the kinase to use ATP and hence the kinase activity appears.[52]

3. 2 Implications in Cancer Treatment

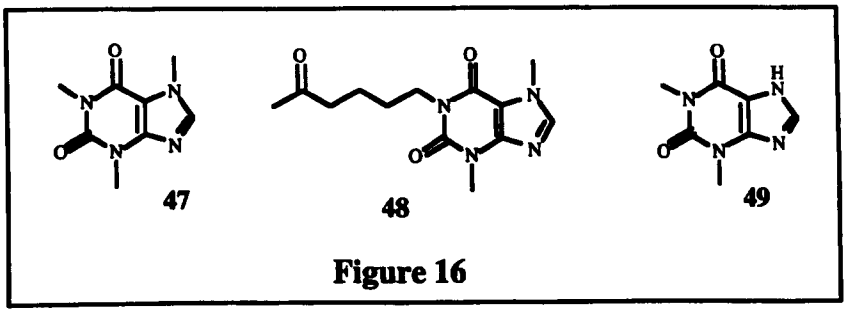
The tremendous advance in understanding of the DNA damage checkpoint signalling pathway provides a new approach to cancer treatment. The cancer cells undergo mitosis with damaged genomes when the DNA damage checkpoint control is abrogated, and this eventually leads to tumour death. Components in the checkpoint signalling pathway such as Chk1, 14-3-3 or Cdc25 could be potential targets for chemical intervention. It is of interest that the chromosomal location of Chk1 was mapped to 11q24 which is adjacent to the gene encoding ATM at 11q23, and this region has been associated with a number of cancers including lung, ovary and breast cancers.[44]

3. 3 Caffeine-mediated Activation of Cdc2[46]

The tumour suppressor gene *p53* plays a crucial role in the induction of cell cycle arrest at the G_1 checkpoint in response to DNA damage. Its inactivation is believed to be responsible for the resistance of many cancers to genotoxic anticancer agents. Inactivation of the tumour suppressor gene p53 is observed in many cancer cells. DNA damage results in preferential accumulation of p53-deficient cancer cells at the G_2/M transitions as these cells fail to arrest at

G_1. Thus abrogation of G_2 arrest via chemical intervention may render those cancer cells more sensitive to DNA damaging agents.

Caffeine **47** and pentoxifylline **48** have been demonstrated to suppress the G_2/M checkpoint and prevent activation of Chk1,[46,54] presumably by inhibiting one or more upstream steps in the DNA damage signal transduction process. Although the mechanism of action of caffeine has not yet been fully elucidated, the upstream checkpoint kinases Rad3 in yeast and ATM/ATR in vertebrate cells are likely targets for inhibition. Caffeine has been shown to sensitise p53-deficient tumour cells to killing by DNA damage. This may provide a promising therapeutic strategy for circumventing the resistance of p53-deficient cancers to DNA damaging agents.



47 **48** **49**

Figure 16

3. 4 Proposed Work

We envisage that xanthine analogues of caffeine **47** and pentoxifylline **48** may also disrupt the G_2 checkpoint by preventing activation of Chk1. To test this hypothesis, we sought to prepare a range of xanthine derivatives (as shown in Figure 17) by alkylation of theophylline **49**.

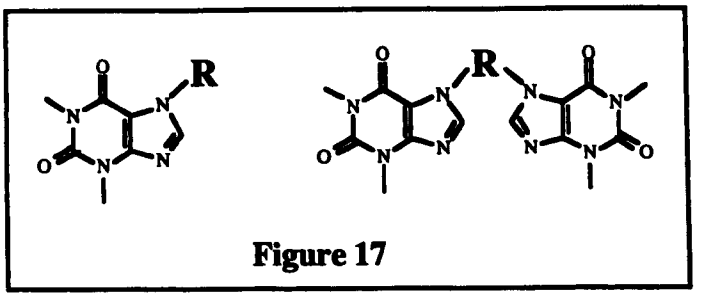


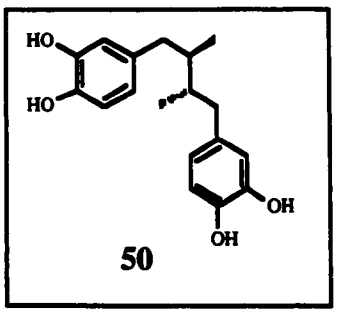
Figure 17

CHAPTER 4

NDGA and Its Mode of Action

4. 1 Introduction

Nordihydroguaiaretic acid **50**, more commonly abbreviated to NDGA, is a phenolic lignan isolated from the resinous extracts of many plants, especially *Larrea divaricata*.[55]



50

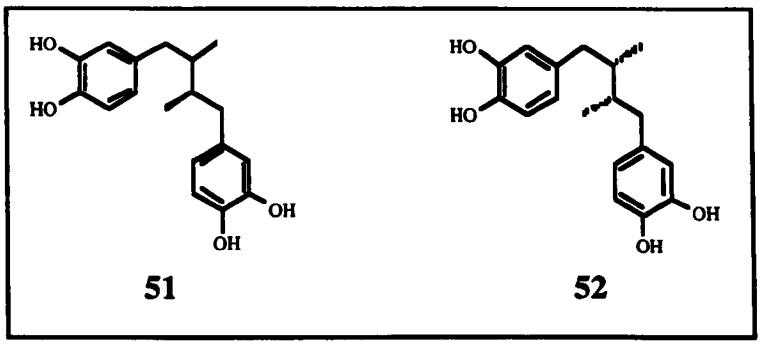
The species *Larrea divaricata*, also known as the creosote bush, is indigenous to the south western USA. The creosote bush grows in the desert and is one of the most drought tolerant plants of the region. It can survive a period of drought of up to two years and individual plants are known to live for 100-200 years.[56] It has been used in treatment of a variety of diseases by native Americans for thousands of years.[57]

The first isolation of NDGA **50** from the creosote bush was reported by Waller and Gisbold in 1945[55]. NDGA has been used as an antioxidant in food and pharmaceutical preparations. NDGA has a non-toxic nature[58] and found potential applications in cancer treatment in the 1980s. It has provoked much interest amongst cancer biologists in the past twenty years.

4. 2 Structure Determination

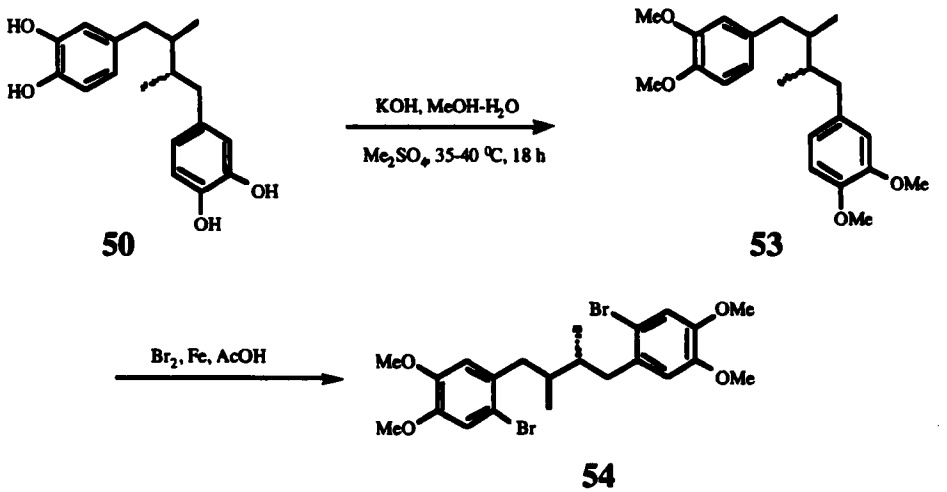
The structure of NDGA was established by synthesis. NDGA was prepared by Schroeter and his co-workers in 1918 from the dimethyl ether of guaiaretic acid by hydrogenation and subsequent demethylation.[59] The authors suggested the *meso* configuration for the tetramethyl ether of NDGA. This compound has since been synthesised by Haworth *et al.*,[60] who assigned the compound as the racemate (the two enantiomers of NDGA are shown

below). Later the work of Schrecker[61] demonstrated that the naturally occurring optically inactive NDGA was the *meso* rather than the racemic form.



Perry *et al.*[62] designed a number of experiments in order to provide unambiguous evidence for the configuration of NDGA. The commercial NDGA was methylated to give the tetramethyl ether **53** whose melting point was identical with that previously reported.[59,60,61] Bromination yielded the dibromo derivative **54** in high yield (Figure 18). A single crystal X-ray analysis of **54** confirmed that NDGA has the *meso* configuration.[62]

Figure 18 Structure Determination of NDGA



4. 3 Synthesis of NDGA[62]

Several syntheses of the natural product *meso* nordihydroguaiaretic acid have been reported in the literature. Among these synthetic pathways the approach[62] (Figure 19) reported by Perry *et al.* in 1972 is the most efficient one and of practical interest.[62] The creosote bush had remained the only commercial source of NDGA before Perry's synthesis was published.

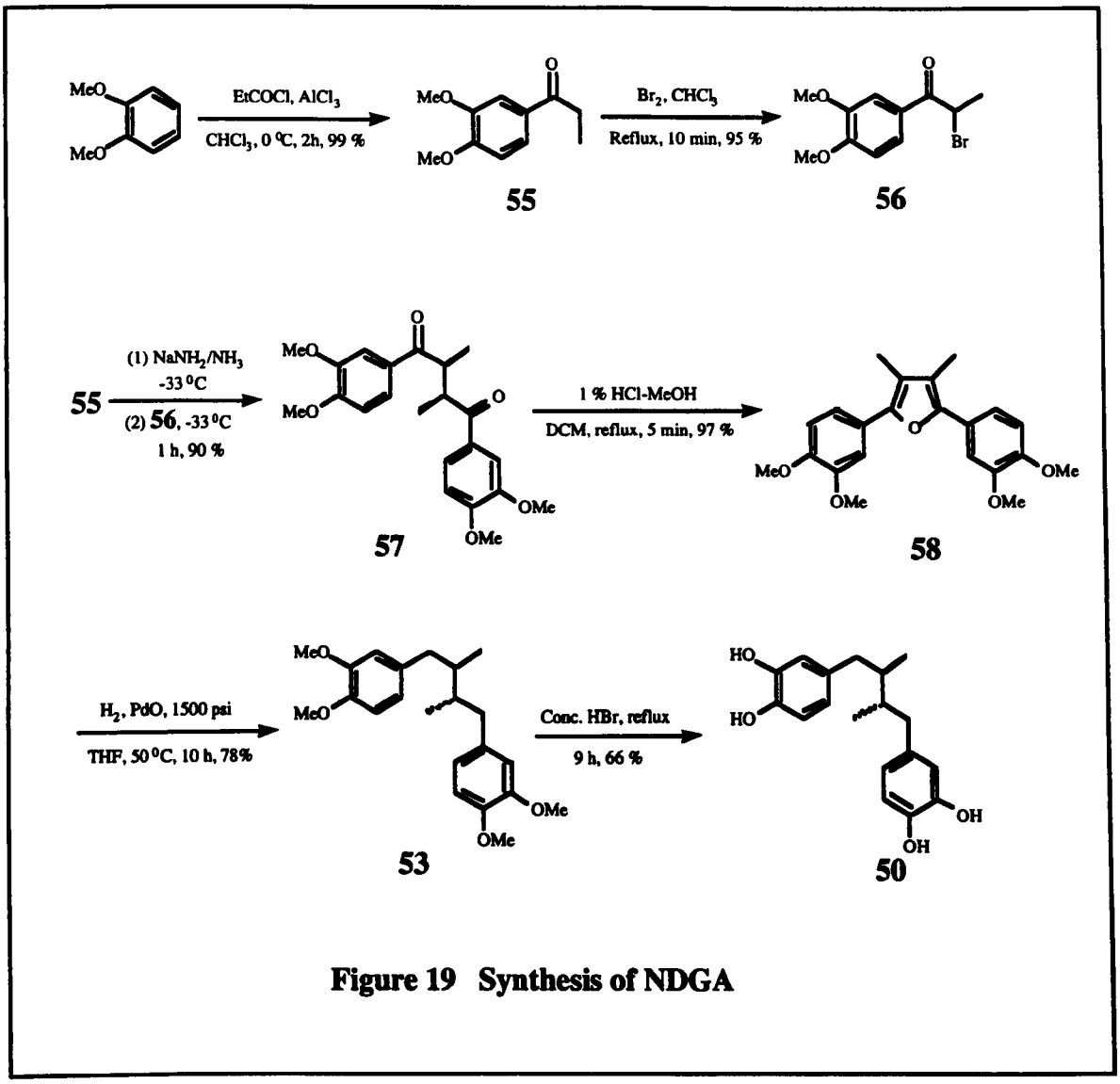
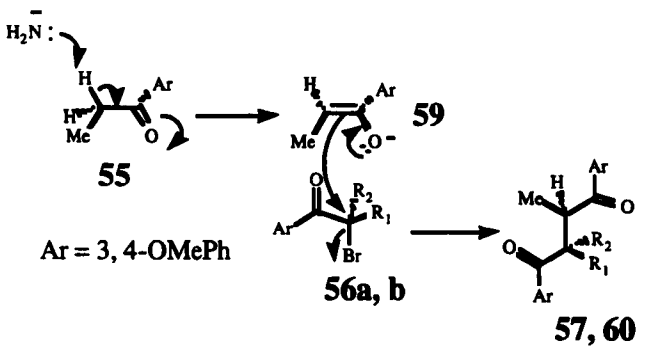


Figure 19 Synthesis of NDGA

Perry's synthesis started with a Friedel-Crafts acylation of 1,2-dimethoxybenzene with propionyl chloride which afforded the ketone **55** in quantitative yield. The ketone was transformed into the α-bromoketone **56** in a high yield of 95%. The highly stereoselective condensation of ketone **55** with bromoketone **56** in liquid ammonia at -33 ^{0}C gave the racemic diketone almost exclusively. The proposed mechanism leading to this stereoselectivity is depicted in Figure 20. The cyclodehydration was achieved by refluxing the CH_2Cl_2 solution of diketone **57** in the presence of methanolic HCl to give the furan **58** in high yield. A remarkable solvent effect was observed in the hydrogenation of furan **58**. The use of THF as a solvent and palladium oxide as a catalyst, together with high pressure and elevated temperature, gave rise to a good yield of 78% in the conversion of furan **58** into NDGA tetramethyl ether **53**. Finally, demethylation of **53** with refluxing concentrated hydrobromic acid afforded NDGA **50** as white crystals in 66% yield after three recrystallisations from 20% aqueous AcOH. The final product was reported to be identical with a purified sample of the natural product.

Figure 20



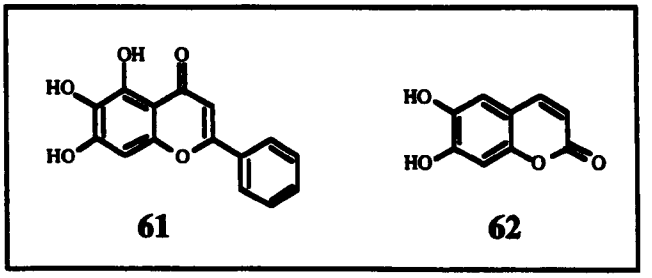
56a $R_1 = H, R_2 = CH_3$
56b $R_1 = CH_3, R_2 = H$

57 $R_1 = CH_3, R_2 = H$ (racemic)
60 $R_1 = H, R_2 = CH_3$ (meso)

The mechanism proposed for the stereoselectivity is as follows. Enolisation of ketone **55** should give predominantly the *(Z)*-enolate **59** which can react with either enantiomer **56a** or **56b** from either above or below **59**. However it would be less hindered when the aroyl group of **56** is furthest from the aroyl group of **59**, and the methyl group of **56** is towards the oxygen of **59** rather than the aryl group. Thus in the sample shown in Figure 20, the *trans*-enolate **59** would more likely react with **56b** than **56a**, and substitution of bromide with Walden inversion would lead to one enantiomer of the racemic diketone **57**. Likewise, with equal possibility, attack of **56** from the top face of **59** would give the other enantiomer of **57**, with the net result that the racemic diketone **57** is formed.

4. 4 Mode of Action of NDGA

All known lipoxygenase (LO) inhibitors have a catechol group which is believed to contribute towards their mechanism of action. Baicalein **61** and esculetin **62**, both containing the catechol group, are found to be LO inhibitors.[63]



It was observed that replacement of the two hydroxyl groups with methoxy groups reduced the activity of both baicalein and esculetin, which confirmed that the catechol group is essential for their activities. This observation prompted work into NDGA as a potential anticancer agent since it has two catechol groups.

NDGA, known as a 5-lipoxygenase inhibitor, was found to suppress the *in vitro* growth of several different human cancers such as those arising in the breast, lung, stomach, pancreas, brain and cervix.[64] Moreover, NDGA also blocks the *in vivo* growth of several of these cancers including both small cell (SCLC) and non-small cell lung cancer (NSCLC). Lung cancer is the commonest cancer killer in the developed world and identification of novel therapeutic strategies are urgently required. Thus, NDGA has become a potentially important lead compound for the treatment of this cancer.

Patients with SCLC usually die sooner than those with NSCLC. Consequently, the identification of new treatments prolonging survival are likely to be most rapidly discovered in patients with SCLC. In order to arrive at the rational design of novel anticancer agents, it is important to understand the biology of the cancer cell and of SCLC in particular.

4. 4. 1 Small Cell Lung Cancer

Small cell lung cancer (SCLC) constitutes about 20% of all lung cancers and causes significant morbidity and mortality around the world.[65] SCLC follows a highly aggressive clinical course and is a distinct clinicopathological entity among lung cancers. SCLC has nearly always metastasised at the time of diagnosis,[65] and although it is initially sensitive to chemo/radiotherapy, most patients rapidly relapse with treatment-resistant disease. Therefore, the five year survival rate for these patients is $< 5\%$.[65] consequently, the development of novel therapeutic strategies for SCLC is urgently required. The risk of developing SCLC is directly linked to the number of cigarettes smoked and it is very rare for a non-smoker to suffer from this cancer type.

The search for novel therapeutic strategies has been directed by growth factors which are implicated in the proliferation of tumour cells in autocrine/paracrine loops. The factors and signalling pathways that stimulate the proliferation of the tumour cell have been extensively studied in SCLC.[66] Multiple neuropeptide and certain polypeptide growth factors are now recognised to drive the proliferation of SCLC cells in both an autocrine and paracrine fashion.[66, 67] These growth factors bind to specific cell surface receptors and elicit intracellular signalling pathways communicating with various cellular compartments including the nucleus to orchestrate the synthesis of new proteins necessary for DNA synthesis and cell division as well as survival and migration. It has become clear that the signals used by distinct growth factors can overlap and so inhibition of such common signalling molecules may be an attractive therapeutic strategy to inhibit the actions of these different growth factors.[68] One such common

signalling pathway involves the metabolism of arachidonic acid (AA) which is involved in tumour growth and metastasis.[58,69,70] Therefore the development of small molecule inhibitors which target lipoxygenases provides a novel therapeutic approach. However, targeting a single molecule may be insufficient as cancers rapidly mutate their phenotype and so a better strategy might be to identify small molecule inhibitors which inhibit several targets in cancer cells.

The elements of growth factors and their receptors and arachidonic acid metabolism pathways are considered in the succeeding section.

4. 4. 1. 1 Growth Factors and Their Receptors

Growth factors are secreted proteins which serve as stimulators/inhibitors to regulate the proliferation of specific cell types in response to the needs of the whole organism. There are at least two types of growth factors, the neuropeptides and the larger polypeptide growth factors. Both of these are involved in SCLC biology.[66,71]

Extracellular growth factors act by binding to specific receptor on the surface of their target cells which then trigger a cascade of intracellular signal transduction. The intracellular signals are ultimately transmitted to the nucleus whereby they alter the programmes of gene expression which control cell behaviour.[72]

Growth factor receptors act as external sensors by transducing signals from outside the cell to its interior, and broadly can be divided into at least two types.[66, 71] Neuropeptide growth factors receptors are members of the seven transmembrane domain receptor family and lack intrinsic kinase activity. Instead, they couple to intracellular signals via the heterotrimeric G protein family. In contrast, the polypeptide receptors span the membrane once consisting of three parts which are extracellular, transmembrane and intracellular domains. The extracellular domain is exposed outside the cell surface to bind growth factors. The transmembrane domain passes through the plasma membrane to connect the extracellular domain with the intracellular domain which interacts with signal transducting molecules inside the cell. The binding of a growth factor to the extracellular domain results in dimerisation of two receptor molecules,[73] and the activation of the intracellular tyrosine kinase which phosphorylates the receptor (auto/transphosphorylation) and a number of second messenger signalling molecules attracted to the activated receptor, thus conveying signals from the outside to the inside of the cell.

Many growth factor receptors function as protein-tyrosine kinases, that is the intracellular domains of these receptors are enzymes that activate their target protein molecules by phosphorylation of the tyrosine residue. These receptors are therefore called protein tyrosine kinases (see Chapter 3).[72] Intracellular signal transduction is a process of phosphorylation events which ultimately induce alterations in gene expression in the nucleus and hence, cell proliferation. The SCF receptor c-kit, highly expressed in the SCLC cell line, belongs to the protein tyrosine kinase family.

In 1983 two groups of scientists,[74] who were studying oncogene and growth factors, serendipitously discovered, for the first time, that normal cell growth factors can act as oncogenes.[74] Growth factors secreted by one kind of cell normally stimulate proliferation of a different kind of cell. The basic principle is that the type of cell does not respond to the growth factor that it also produces. When a growth factor acts as an oncogene, this requirement is no longer met, and a cell begins to produce a growth factor that stimulates its own proliferation. This situation is called autocrine growth stimulation.[72]

Normal receptor protein-tyrosine kinases can be activated as oncogenes by either of two kinds of alterations in their normal function.[74] First, overexpression of the normal receptor proto-oncogene is sufficient to induce an exaggerated cellular response to growth factor binding, resulting in excess cell proliferation. Secondly, structure changes of the normal proto-oncogene encoded receptor proteins can result in cell transformation. For instance, deletions of the extracellular domains can convert these receptors into biologically active oncogenes as the binding domains regulate the catalytic activity of the tyrosine kinase domain. Thus, the oncogene protein functions constitutively, independent of growth factor binding, leading to neoplastic transformation. In other cases, point mutations at critical positions in normal receptor proteins are also responsible for activating oncogenes.

4. 4. 1. 2. Coexpression of the Stem Cell Factor and *C-kit* genes in Small Cell Lung Cancer

Growth deregulation in human SCLC results from multiple genetic defects acting in concert, rather than as independent events, to induce complete loss of growth control. Autocrine/paracrine stimulation has been a popular hypothesis used to explain the growth deregulation of SCLC.[70, 75] Three autocrine growth loops involving gastrin-releasing peptide (GRP) growth factor, insulin-like growth factor-1 (IGF-1) and stem cell factor (SCF) and their three receptors have been implicated in deregulated SCLC growth.[70, 75]

SCF, alternatively named mast cell growth factor (MGF),[75] *kit* ligand (KL), or *steel* factor, is a haemopoietic growth factor which, cooperating with other haemopoietic growth factors, regulates the proliferation and differentiation in various types of foetal and adult tissues as the ligand of the *c-kit* proto-oncogene product.[70, 76] The proto-oncogene *c-kit* encodes a transmembrane tyrosine kinase growth factor receptor which is involved in cell differentiation and proliferation in various murine tissues including the lung.[77]

SCF gene expression has been observed in a broad spectrum of human cancers including SCLC. In contrast, *c-kit* transcripts are expressed in very restricted types of cancers.[78] Co-expression of both the ligand SCF and the receptor *c-kit* has been observed preferentially in SCLC when compared with NSCLC and various other cancers, suggesting an

autocrine/paracrine mode of action *via* this autocrine growth loop in the pathogenesis of this aggressive cancer.

Furthermore, evidence exists that no *c-kit* protein has been detected in foetal and adult normal bronchial epithelial cells, indicating that *c-kit* expression in SCLC is ectopic.[78] It is notable that SCLC metastasise to the haemopoietic environment of bone marrow much more frequently than non-small cell lung cancer.[78] Strong evidence has been provided that ectopic expression of *c-kit* in SCLC would enhance the migration of SCLC cells *in vivo*. It has been demonstrated[78] that the *c-kit* protein in SCLC was autophosphorylated by the addition of exogenous SCF, resulting in the induction of positive chemotactic response of SCLC cells *in vitro*. This study has indicated that SCF can stimulate chemotaxis of the porcine aortic endothelial cells transfected with the *c-kit* expression construct, resulting in the acquisition of a higher invasive (metastatic) phenotype.

It is also of considerable interest that molecular biological analysis revealed an amino acid substitution [CTG (leucine) instead of ATG (methionine)] within the transmembrane domain of *c-kit* in an SCLC cell line. This result suggests the possibility of either a germ line mutation or polymorphism.[78]

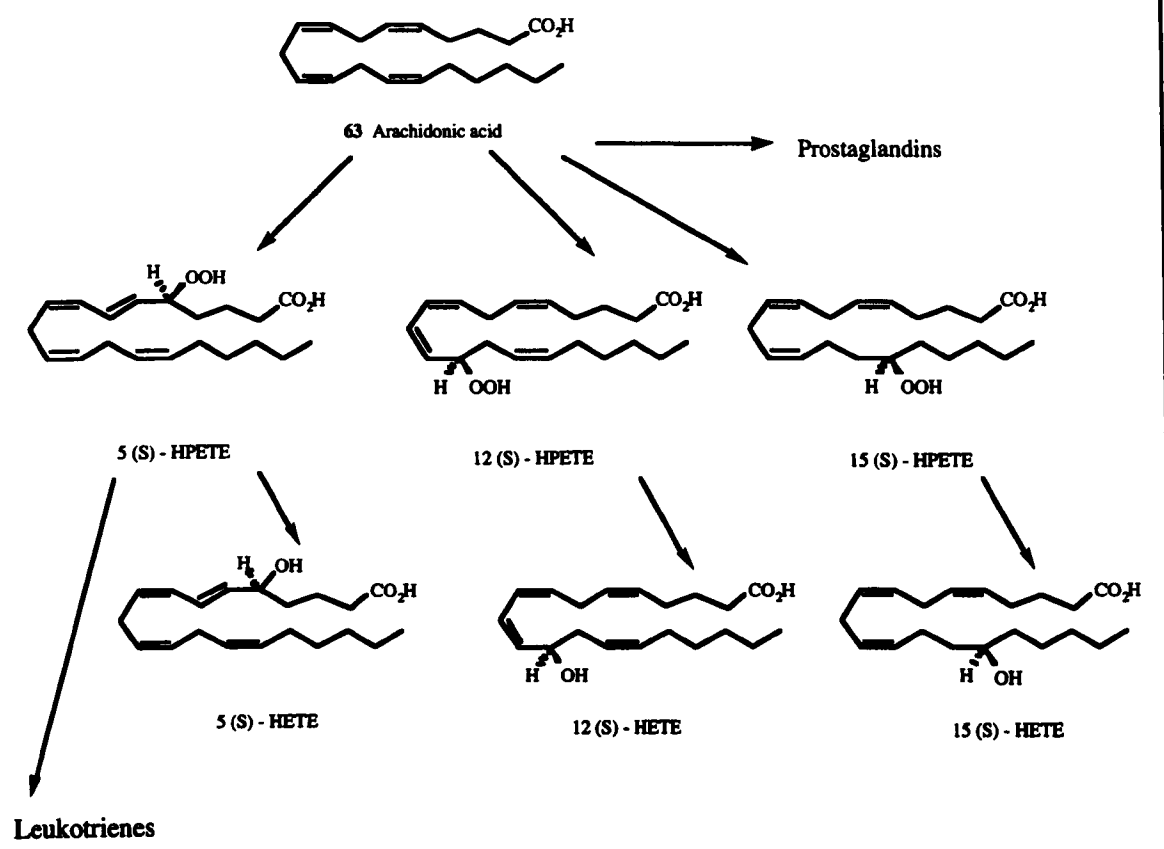
The remarkable advance achieved in the understanding of the pathogenesis of lung cancer on the molecular and biochemical basis is of great interest for the management of this highly aggressive cancer. The important autocrine growth factors, SCF and its receptor *c-kit* provide attractive targets for therapeutic intervention in SCLC.

In addition, the molecular biological study on the pathogenesis of SCLC has revealed that autocrine growth factors activate the lipoxygenase pathway of arachidonic acid (AA) metabolism in SCLC cells.[79] The eicosanoids, products of the AA metabolism, have been found to be implicated in tumour promotion, cell proliferation, metastases, and immune surveillance.[58]

4. 4. 1. 3 Arachidonic Acid Pathway[58, 70]

Arachidonic acid (5,8,11,14-eicosatetraenoic acid) **63** is a polyunsaturated fatty acid which is provided from the diet. Arachidonic acid metabolism consists of two major pathways: the cyclooxygenase and the lipoxygenase pathways (Figure 21).[58, 70]

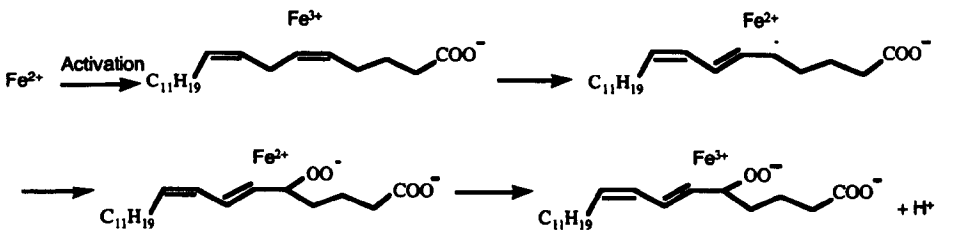
Figure 21 The Metabolism of Arachidonic Acid



Lipoxygenase catalyses the hydroperoxy substitution of AA to give allylic hydroperoxides which are designated monohydroperoxyeicosatetraenoic acids (HPETEs). There is a family of lipoxygenases (LO), namely 5-, 12- and 15-LO, that differ in the position (C-5, 12 and 15) at which they oxygenate the arachidonic acid (AA) backbone. The three hydroperoxides (5, 12 and 15-HPETE) are unstable, and they can be either reduced to the corresponding hydroxyeicosateraenoic acids (HETEs) or converted into leukotrienes (LTs).

Lipoxygenases are iron containing enzymes in which the iron exists in either the 2+ or 3+ oxidation state. To exert their oxidative activities, the Fe^{2+} state must first be oxidised to the Fe^{3+} state. In the mode of action of 5-LO (Figure 22),[8] Fe^{3+} specifically oxidises the 5,8 diene system of AA to generate a pentadienyl radical with the iron being reduced to the Fe^{2+} state. This radical then stereospecifically reacts with molecular oxygen at C-5 to generate the 5-S-hydroperoxy radical which undergoes electron transfer and protonolysis regenerating the active Fe^{3+} and 5-HPETE.

Figure 22 Mode of Action of 5-Lipoxygenase



The cyclooxygenase pathway leads to the formation of prostaglandins which contribute solely to the growth of tumours. On the other hand, the metabolites of the LO pathway have been found to play an important role in tumour initiation, promotion and metastases.[58, 70] Thus lipoxygenase enzymes present attractive targets for developing treatment and chemopreventive strategies.

4. 4. 2 Nordihydroguaiaretic acid (NDGA)

Research has suggested that NDGA exerts its antiproliferative effects in the treatment of SCLC mainly by the following two mechanisms.

Lipoxygenase inhibitor

NDGA is known to be a selective inhibitor of lipoxygenases and blocks SCLC growth *in vitro* and *in vivo*.[64] Studies of the molecular events involved in growth factor signalling suggest stimulation of or re-establishment of apoptotic growth regulation resulting from the interruption of the lipoxygenase pathway, as a possible mechanism for the antiproliferative effect of the lipoxygenase inhibitors.[80, 81] Thus, lipoxygenase inhibitors represent a new pharmacological tool to renew growth regulation in transformed cell populations.

Inhibition of *c-kit* receptor

The preliminary data obtained by Seckl *et al.*[64] are consistent with the above findings, but they noticed that NDGA is a better inhibitor of SCLC growth than other more potent lipoxygenase inhibitors. Their results also suggest that NDGA has an alternative mechanism of action in SCLC cells. It was demonstrated that NDGA inhibits *c-kit* directly and also inhibits the platelet-derived growth factor (PDGF) receptor,[64, 82] which like *c-kit* has tyrosine kinase activity. This dual mechanism of lipoxygenase and c-kit inhibition may explain its superior effectiveness as an antitumour agent.

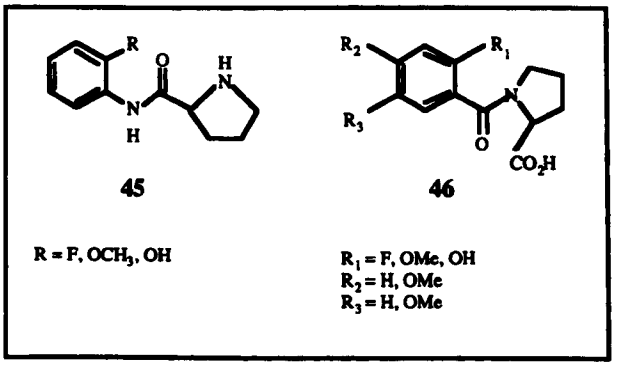
Nevertheless, NDGA itself has poor solubility and chemical modifications are required to provide a compound for optimal clinical use. In addition, the structure of NDGA is distinct from other lipoxygenase inhibitors and has not yet been exploited. Preliminary investigative work was performed by McDonald[8] and Macleod[83], who synthesised a range of analogues of NDGA which have been tested for their activity *in vitro* by Dr Michael Seckl at the Medical Oncology Department of Hammersmith Hospital, London. Their results demonstrated improved potency for new analogues with 4-6 atoms between the two aromatic rings over NDGA[8], and furthermore the structural modification work revealed that introduction of an amide linkage between the two aromatic residues results in NDGA analogues which are more active than NDGA itself.[8, 83]

Based on these preliminary results, the programmed structural modifications proposed for this project focused on three areas. The main programme of research was drug solubilisation of the candidate analogues which have better potency over NDGA. The second area of study introduced position variations of the amide linkage between the two aromatic residues. The third area of work involved modification of substitutions on the two aromatic rings.

CHAPTER 5

Synthesis of L-Proline Derivatives

5. 1 Introduction



The general biological background of the inhibition of stress related kinases was discussed in Chapter 2 together with the general structures **45** and **46** for the proposed stress related kinase inhibitors. These were modelled on serine **42** and L-proline **44**. It is a common tactic to design drugs to mimic the natural substrates in order to make a good fit at the active sites of the enzymes. As long as the drugs are in the active sites, they should block access to the natural substrates and the enzymatic reactions should slow or stop. In the designed structures the serine portion is in a conformationally restricted form and the amide link also adds rigidity to the molecules. These rigid functional groups were introduced based on the concept that rigid compounds often have enhanced biological activities and selectivities. In addition a number of fluorine compounds were synthesised with a view to introducing a fluorine atom as a bioisostere for a hydroxyl group in the hope of improving the potency and duration of action of the new compounds.

5.2 Synthesis of L-Proline Derivatives containing Fluorine and Methoxy groups

The proposed structures for the L-proline derivatives have an amide link between the conformationally restricted serine portion and the L-proline. The amide bridge can be positioned in two possible ways, therefore two sets of L-proline derivatives were synthesised.

5. 2. 1 Synthesis of *N*-Terminal L-Prolineamide Derivatives

This set of compounds was prepared in a three step procedure as shown in Figure 23. The carboxyl group of the L-proline reacts with the amino group of the aniline derivative to form the amide bond in this set of compounds. This is the key step in the synthetic route and was achieved using DCC as coupling reagent and DMAP as a base and acyl transfer reagent.

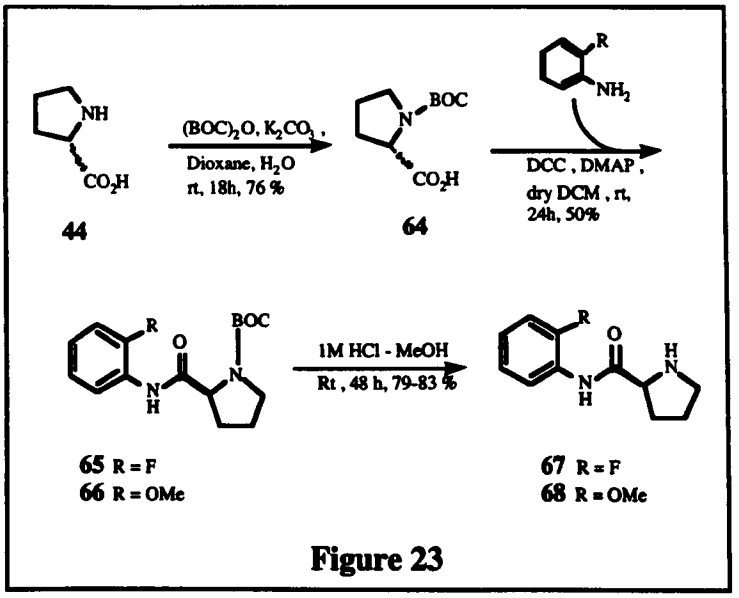


Figure 23

Our first approach to make the amide link by condensing the aniline derivatives directly with unprotected L-proline was unsuccessful. No signals expected for the products were found in the NMR spectra for the complex reaction mixtures. Therefore we decided to protect the amine group of the L-proline prior to carrying out the coupling reactions.

L-Proline was protected as BOC-proline **64** in a 75% yield by a modified procedure used by Ookawa and co-workers.[84] The product was obtained as long white crystals giving a melting point consistent with that reported in the literature.[84] The ^{1}H NMR spectrum revealed two singlets at δ 1.38 for the three methyl groups indicating the presence of rotamers, while a 4H and 2H multiplet in the aliphatic region along with two deshielded methine multiplets at δ 4.17 and 4.28 gave evidence for the proline ring. The *N*-protected L-proline was then subjected to a coupling reaction with aniline in the presence of DCC and DMAP according to the procedure of Hassner and Alexania.[85] The side product DCU of the reaction caused problems during purification. The majority of the byproduct DCU precipitated out of the reaction mixture and was filtered off, and the filtrate was stored in a freezer to encourage precipitation of the remaining DCU. After filtration of the DCU precipitate and concentration of the reaction mixture, the crude product was crystallised from alcoholic solvents. After all these attempts to remove DCU, the ^{1}H NMR spectra for the white crystalline products still had small peaks for

DCU, indicating contamination with a trace of DCU. This problem might be overcome by using a water soluble version of DCC.

Finally the deprotection of the amine group was achieved in good yields under acidic conditions.[86] In the ^1H NMR spectra compounds **65** and **66** each gave peaks from δ 6.9 to 8.2 for the aromatic ring and also gave a sharp peak for the tert-butyl group at about δ 1.4, but for compounds **67** and **68** there was no peak at δ 1.4. This indicates that the BOC group was removed. The IR spectrum of the two final products **67** and **68** revealed characteristic absorption frequencies in the 1684-1692 cm^{-1} region for the carbonyl bond and the NH stretching vibrations at about 3327-3494 cm^{-1}. The ^{13}C NMR spectra supported these findings with the carbonyl carbon signals at δ 169 and 177. The parent ions at *m/z* 208 and 220 in the mass spectrum gave further evidence for the proposed structure for compounds **67** and **68**, respectively.

5. 2. 2 Synthesis of *C*-Terminal L-Prolineamide Derivatives

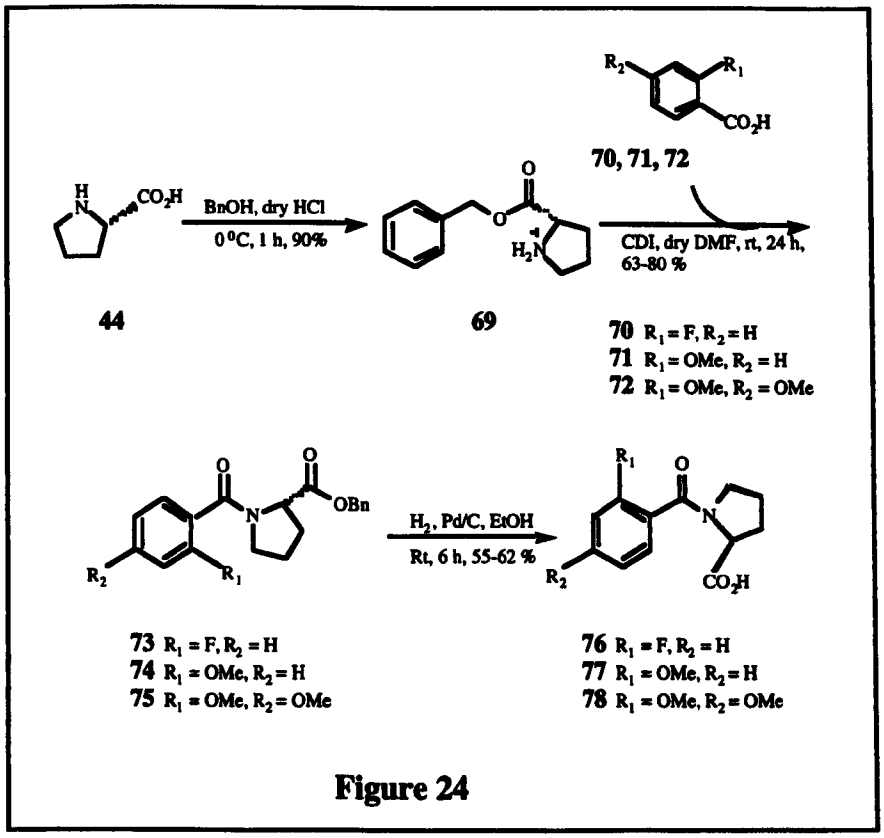


Figure 24

In order to prepare proline derivatives with the alternative amide linkage, the three step route shown in Figure 24 was used. L-Proline was protected as benzylproline **69** in high yield following the procedure of Neuman and Smith.[87] The structure of this known compound **69** was confirmed by the spectroscopic data collected and the melting point recorded was found to be in agreement with that in the literature.[87] Our initial synthetic attempts towards amide formation using DCC and DMAP proved unsuccessful. It was found that the pH value of the

sample withdrawn from the reaction mixture was about 2. This indicated that the amine group of the benzylproline would be protonated in this acidic condition, which could make the reaction difficult. In order to change the pH value of the reaction, CDI was chosen as an alternative coupling reagent and a modified version of the method by Paul and Anderson[88] was employed. The pH value of the sample taken from the reaction mixture was about 8 and the reaction was monitored by TLC (2:1 EtOAc-hexane + 5% AcOH or 5% NH_3-H_2O). After stirring under N_2 at rt for 24 hours, completion of the reaction was confirmed by TLC which showed no sign of starting material and a product spot which was stained bright orange by dragendorff reagent. Following the procedure of Challis and coworkers,[89] catalytic hydrogenation of **73-75** over palladium removed the benzyl group with ease affording the final products **76-78** as crystals with sharp melting points and analytical purity.

In the ^1H NMR spectra, compounds **73**, **74** and **75** each gave an AB system at about δ 5.1 for the benzyl methylene protons and also gave peaks at δ 4.6 and 4.3 for the methine proton on the proline ring while compounds **76**, **77** and **78** had no AB system signals at δ 5.1, which provided evidence for the removal of the benzyl groups. It is worthy of note that both the ^1H and ^{13}C NMR spectra gave two sets of signals for compounds **73** to **78**, due to the presence of rotamers. Integration of peak heights on the ^1H NMR spectra enabled the rotameric ratio for each compound to be established. The ratio for the minor:major rotamer signals were calculated as about 1:2 for the six compounds. The ^1H and ^{13}C NMR spectra of compound **77** were chosen to exemplify this NMR phenomenon exhibited by the amide series (see Appendixes 1 and 2). From the ^1H NMR spectrum of compound **77** (Appendix 1) a ratio of 1:2 for the minor:major rotamers was established. The signals on the ^1H NMR spectrum for the five-membered proline ring were evident in the region δ 1.78 to 4.67. The three methylene groups produced three pairs of multiplets at δ 1.78 and 1.90, δ 2.10 and 2.37, δ 3.31 and 3.69 respectively while the methine proton on the ring gave a pair of multiplets at δ 4.14 and 4.67. The ^{13}C NMR spectrum of **77** (Appendix 2) also provided a very clear demonstration of paired signals, a feature preserved throughout the six compound series.

Rotameric forms of amides are constantly interconverting and, in most cases, the rate of interconversion is faster than the gap between the NMR pulses. Consequently, only one set of signals in the spectrum is usually observed. In the case of amides **73** to **78**, the nitrogen atom is on the proline ring and the carbonyl group is also conjugated to the aromatic ring. The two bulky substituents decrease the rate of interconversion of the two rotamers due to the increased energy requirement for the process. As a result a set of signals for each rotamer was observed in each NMR spectrum.

The ^1H NMR spectrum of compounds **73** to **78** showed multiplets in the aromatic region for the benzene ring while compounds **74**, **77** and **75**, **78** gave a 3H singlet and a 6H singlet, respectively, at δ 3.8 for the methoxy groups. Three characteristic stretches at 1743-1757 cm^{-1} region were recorded in the IR spectrum for the carbonyl groups in compounds **73**

to **78**. The ^{13}C NMR spectrum of **73** to **78** provided evidence for the carbonyl groups through the signals at *ca* δ 168-176.

5. 3 Synthesis of L-Proline Derivatives Containing a Phenolic Moiety

In the foregoing section a fluorine atom was chosen as a bioisostere to replace the hydroxyl group on the rigid serine mimic in the L-proline derivatives. Alternatively, the hydroxy group was converted into a methyl ether in the conformationally restricted form of serine. This section will discuss the synthesis of L-proline derivatives containing rigid serine portions with a free hydroxyl group.

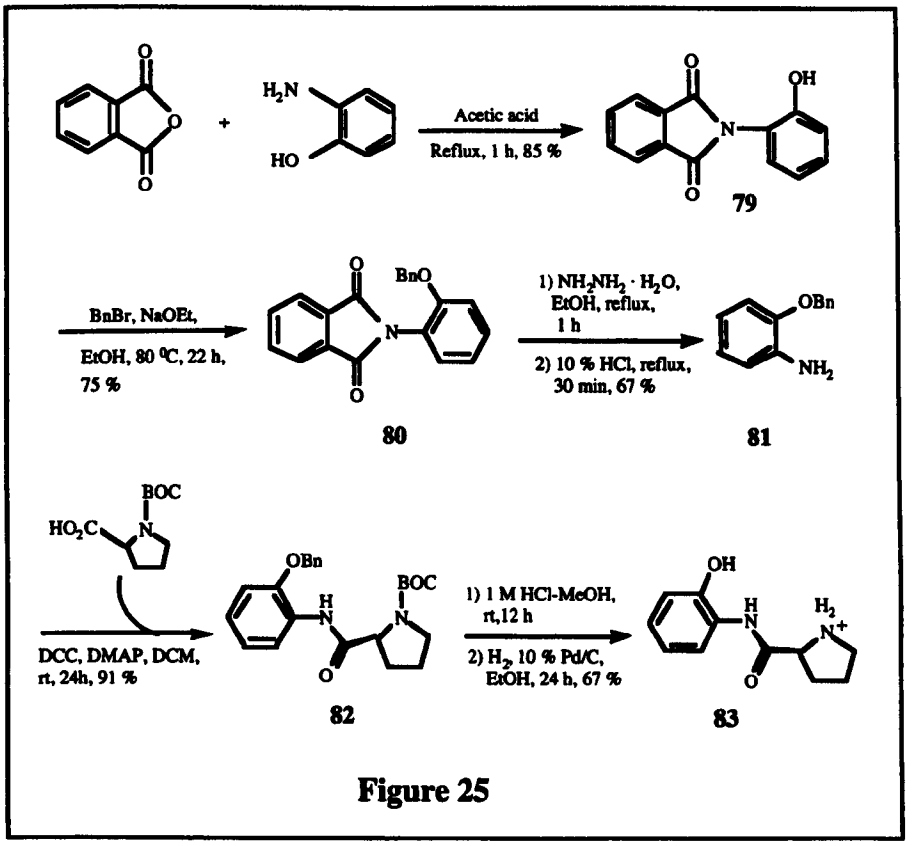


Figure 25

In the initial approach towards the target compound **83**, 2-aminophenol was coupled with BOC-proline in the presence of DCC and DMAP without protection of the hydroxy group. TLC analysis of the reaction mixture showed a streak indicating an intractable mixture of materials while the ^1H NMR spectrum of the reaction mixture gave no signals for the desired product.

This failure suggested that the free hydroxy group on 2-aminophenol should be protected as the benzyl ether **81**. Conversion of the 2-aminophenol into the corresponding

benzyl ether **81** was accomplished by a three-step procedure described by Humm and Schneider[90] (Figure 25). 2-Aminophenol was first protected as a phthalimide by heating at reflux with phthalic anhydride in acetic acid for one hour. The mixture was then diluted with water and heated to boiling. On cooling in ice, compound **79** crystallised in nearly quantitative yield. This procedure demonstrated a great simplicity in the work up and purification processes. The presence of the phthalimide moiety in compound **79** was confirmed by two characteristic multiplets at δ 7.68 and 7.85 observed in the ^{1}H NMR spectrum. Subsequent benzylation of the *N*-protected 2-aminophenol **79** using benzyl bromide and sodium in dry EtOH proceeded smoothly affording compound **80**, after crystallisation from hexane, in 75% yield. The ^{1}H NMR spectrum and the melting point of the known compound **80** were in agreement with those reported in the literature.[90] Deprotection of the amino group in compound **80** was achieved by treatment with hydrazine hydrate. After the mixture was heated at reflux for one hour, TLC analysis indicated that no starting material was left and there was a spot on the origin. On completion of the reaction the side product phthaloyl hydrazide was removed by filtration from the reaction mixture and the solution was made alkaline to yield the crude *o*-benzyloxyaniline **81** as the free base. All attempts to purify the crude aniline derivative by crystallisation were unsuccessful due to the low melting point (30-40 ^{0}C) of the compound. However trituration of the crude aniline derivative with water afforded compound **81** pure enough for synthetic work according to the ^{1}H NMR spectrum.

o-Benzyloxyaniline was in turn coupled with BOC-proline in the presence of DCC and DMAP to form the amide bond in compound **82**. TLC analysis showed, after the reaction mixture was stirred at rt for 24 hours, a homogeneous slower running spot for the product that was stained bright orange by Dragendorff reagent. ^{1}H NMR spectroscopy identified compound **82** from the multiplets at δ 6.90 for the aromatic ring, δ 5.04 for the benzyl methylene group and a singlet at δ 1.29 for the *tert*-butyl group, a 1H multiplet at δ 8.38 for the secondary amide together with the two multiplets at δ 4.21 and 4.39 for the methine proton on the proline ring. There only remained the need to unmask the NH_2 and OH from the carbamate and the benzyl ether as the free amine and the hydroxy group, respectively. As described in the above section the BOC protecting group was successfully removed under acidic conditions and the deprotection of the hydroxy group by hydrogenation was straightforward. The completion of deprotection of the BOC group was evident from TLC analysis showing no starting material and a homogeneous slower running spot stained orange by Dragendorff reagent. The crude product was used directly in the subsequent hydrogenation without further purification. The debenzylation via catalytic hydrogenation over palladium proceeded smoothly and afforded the crude product as a brown oil. Difficulties were experienced in the purification of the final compound **83** and we were unable to crystallise it. The target compound **83** was also unstable to chromatography on both silica and alumina columns, and this was evident from the ^{1}H NMR spectrum of the column fractions. In an attempt to purify the compound **83** the crude product was triturated sequentially with petroleum ether and dichloromethane and dried *in vacuo*

affording compound **83** as a brown solid in 67% yield. We also suspected that the final compound **83** tended to be oxidised by O_2 based on the observation that the brown solid compound **83** became a dark oil readily after exposure to the air. The ^1H and ^{13}C NMR spectra provided another demonstration of clearly paired signals. The successful deprotection of the amino group and the hydroxy group was evident from the ^1H NMR spectrum with the absence of the benzyl methylene signal at δ 5.04 and of the *tert*-butyl signal at δ 1.29. A broad phenolic stretch and NH stretching vibrations at *ca* 3500 cm^{-1} in the IR spectrum along with a parent ion at *m/z* 206 in the mass spectrum were also consistent with the proposed structure.

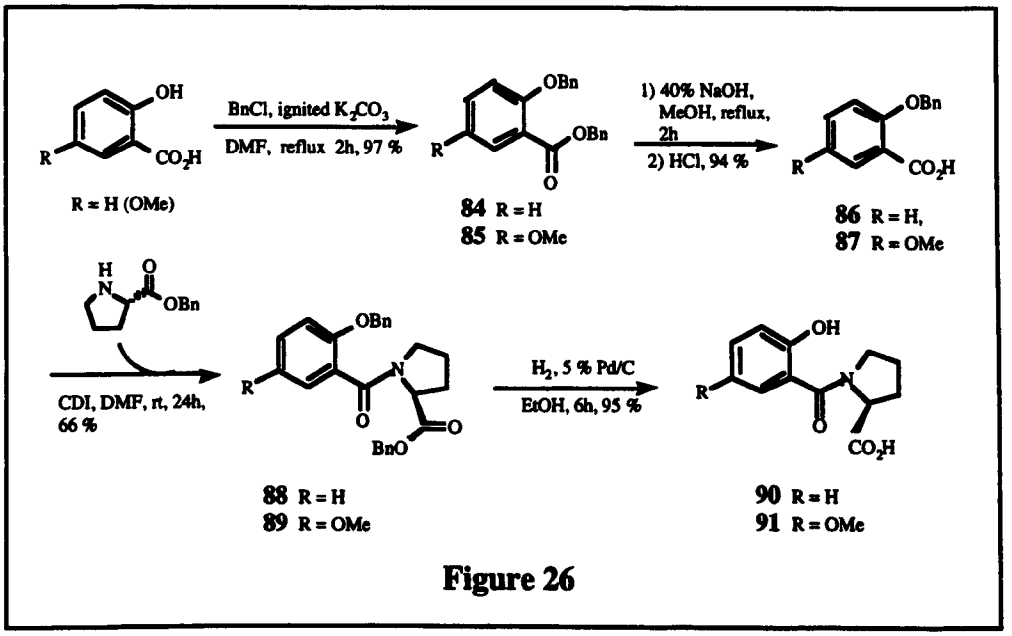


Figure 26

Attempted coupling of proline benzyl ester **69** with salicylic acid failed as had the attempt to couple BOC-proline with unprotected 2-aminophenol. Both failures indicated the coupling reaction conditions were not tolerant of free hydroxyl groups. Protection of the hydroxyl group was therefore necessary. The conversion of salicylic acid into the corresponding benzyloxybenzoic acid proceeded in two steps (Figure 26) according to the procedure of Farkas *et al.*[91] Benzylations of the *o*-hydroxybenzoic acids with benzyl chloride in the presence of ignited potassium carbonate afforded compounds **84** and **85**, after crystallisation as yellow crystals in high yield. The melting point for the known compound **84** was identical with that reported.[91] The ^1H NMR spectrum revealed two 2H singlets at δ 5.08 and 5.26 for the two benzyl methylene groups along with a 14H multiplet in the aromatic region providing further evidence for the proposed structure. In the ^1H NMR spectrum of the novel compound **85**, the two benzyl methylene groups and the methoxy group produced two 2H singlets at δ 5.01 and 5.27 and a 3H singlet at δ 3.69 respectively. The corresponding signals in the ^{13}C NMR spectrum resonated at δ 67.2, 72.2 and 56.2, respectively.

The benzyl esters were then hydrolysed in concentrated NaOH solutions followed by acidification of the carboxylate salts with 2M HCl producing the *o*-benzyloxybenzoic acids in

quantitative yields. The successful deprotections of carboxyl groups in compounds **84** and **85** were apparent through the absence of the benzyl methylene signals at δ 5.08 and 5.01 in the ^{1}H NMR spectrum while the carboxylic acid proton produced a broad singlet at *ca* δ 11.0. The melting point for the known compound **86** was in agreement with that reported in the literature,[91] while the novel compound **87** was fully characterised. Following the modified version of the procedure of Paul and Anderson as before, the *o*-benzyloxybenzoic acids **86** and **87** were coupled with benzylproline **69** using CDI in DMF at ambient temperature to form the amide bonds. TLC (1:1 EtOAc-hexane, 3% acetic acid) of the reaction mixture after stirring for 24 hours revealed no starting material and a homogeneous faster running product spot stained orange by Dragendorff reagent. The crude products were purified by column chromatography affording compounds **88** and **89** in 66-70% yields. The benzyl ester methylene groups of **88** and **89** produced ^{1}H NMR signals characteristic of two AB systems at δ 5.15 and 5.13 with *J* values of 9.6 and 12.4 Hz, respectively. Two singlets at δ 5.03 and 4.96 for the benzyl ether methylene groups in **88** and **89**, respectively, were observed in the ^{1}H NMR spectrum. The two methine protons on the proline ring of **88** and **89** gave two multiplets at δ 4.25, 4.63 and δ 4.31, 4.63, respectively, in the ^{1}H NMR spectrum. Hydrogenation over palladium carbon at rt in ethanol simultaneously removed the two benzyl groups with ease. TLC of the reaction mixture after 6 hours showed no starting material indicating the efficiency of the debenzylation. Purification of the two desired compounds proved problematic. Attempted crystallisations of the crude products were unsuccessful while chromatography on both silica and alumina resulted only in extensive decomposition evident from the ^{1}H NMR spectra for the column fractions. Trituration of the crude products with petroleum ether and concentration *in vacuo* afforded compounds **90** and **91** in 90% yield. Compounds **90** and **91** were found, like compound **83**, to be unstable in air possibly due to the oxidation of the phenolic moiety. The ^{1}H NMR spectrum showed no resonances for the benzyl methylene groups confirming the accomplishment of the debenzylation. Clearly paired signals were again observed in both the ^{1}H and ^{13}C NMR spectra for all four compounds **88** to **91**. Broad phenolic stretches at *ca.* 3500 cm^{-1} and carbonyl absorptions at *ca.* 1740-1750 cm^{-1} in the IR spectrum along with parent ions found in the mass spectrum were also in agreement with the proposed structures.

5. 4 Biological Test Results

The proline derivatives prepared were assessed for their SAP kinase inhibition by Professor David Gillespie at the Beatson Institute for Cancer Research, Glasgow.

5. 4. 1 Method of Testing[43]

The MAP kinase enzyme employed in the test was the *c-jun N*-terminal kinase, a component of the SAPK pathway. This assay involved the use of a recombinant GST-*c-jun*

protein (purified from *E. coli*) bound to Sepharose beads. This behaved as a combined affinity matrix and substrate for the SAPK kinases. Cell extracts were prepared from primary avian fibroblasts treated with anisomycin to provide the source of activated indigenous JNK to be incubated with the GST-*c-jun* beads. The assay followed standard experimental procedure and reactions were performed for each proline derivative at concentrations of 500, 50 and 5 micromolar. SDS gel electrophoresis was used to resolve the bead-bound reaction mixtures and following electroblotting, the labelling of the GST-*c-jun* from each reaction was visualised by autoradiography.

5. 4. 2 Results

At the 5, 50 and 500 μM compound concentrations none of the proline derivatives **67**, **68**, **76** to **78** containing fluorine or methoxy groups were found to exhibit inhibitory activity in the assay, which is an indication that the these compounds may be poor substrates for stress related kinases. In the light of this failure we thought the free hydroxy group on the rigid serine mimic moiety might be essential for their inhibitory activities, and therefore we sought to prepare the proline derivatives **83**, **90** and **91** containing the rigid serine portion with the free hydroxyl group. Compounds **83**, **90** and **91** have improved structure similarity with the natural substrates, which could make them more competitive for the active site of the enzyme. Unfortunately, the compounds **83**, **90** and **91** prepared have not yet been tested for biological activity.

CHAPTER 6

Synthesis of Theophylline Derivatives

As outlined in Chapter 3, recent advances in the research area of DNA damage checkpoint control raised the possibility of manipulating the checkpoint pathways. In particular the Chk1 pathway, which is implicated in a number of cancers, could be a potential target for therapeutic intervention in the development of anticancer agents.

We intend to test caffeine and theophylline as reference compounds with a range of related derivatives prepared by alkylation of theophylline **49**. The theophylline derivatives will be assessed to see if they override G2/M arrest by inhibiting Chk 1 kinase function.

6. 1 Synthesis of Alkylated Theophyllines

A series of oxoalkyldimethylxanthines has been synthesised by Mohler *et al.*[92] as solubilisers for various therapeutically useful substances. Based on this preliminary work our proposed structural modifications involved variations of the oxoalkyl moiety. A range of theophylline derivatives was synthesised by alkylation at N-7 on the xanthine ring of theophylline with bromides probably *via* an SN_2 mechanism.

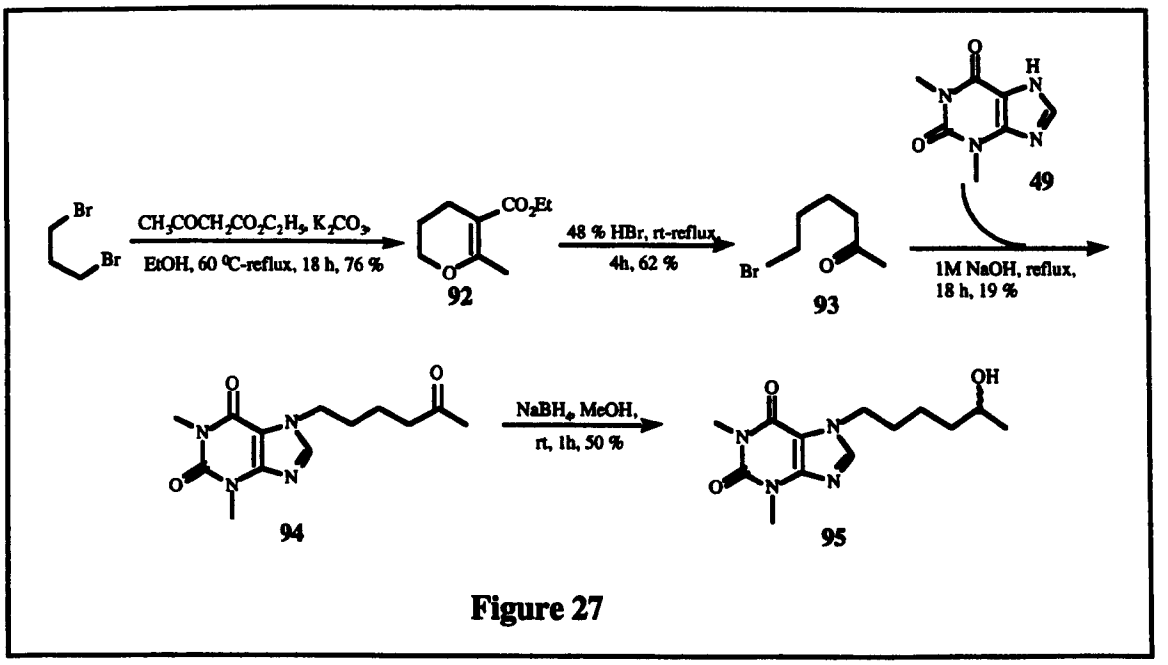


Figure 27

The preparation of the target compound **95** was achieved by a four step synthesis which is depicted in Figure 27. The known compound **94** was prepared in three steps following the

patent procedure.[92] The patent gave the melting points (or boiling points) but no spectroscopic data for each compound prepared. Reaction of 1,3-dibromopropane with ethyl acetoacetate under basic condition in ethanol proceeded uneventfully according to the TLC analysis, and the standard work-up followed by fractional distillation under reduced pressure afforded dihydropyran **92** in 76% yield as a pure clear oil. The pertinent features in the ^{1}H NMR spectrum were a 2H multiplet at δ 1.75, a 2H triplet at δ 2.23 with a *J* value of 6.4 Hz and a deshielded 2H triplet at δ 3.94 with a *J* value of 4.2 Hz for the three methylene groups on the pyran ring. The expected signals at δ 1.21 and 4.11 for the ethyl group and a 3H singlet at δ 2.15 for the exomethylene protons provided further evidence for the proposed structure. The pyran ring was opened after stirring with 48% HBr at rt for two days followed by heating at reflux for a further two hours. Following work-up and low pressure fractional distillation, bromoketone **93** was isolated as a yellow oil in 62% yield. The four methylene groups were observed as two 2H triplets and two 2H multiplets while the methyl group was seen as a 3H singlet in the ^{1}H NMR spectrum of **93**. The ^{13}C NMR spectrum revealed the expected six resonances including the carbonyl functionality at δ 208.6. The nucleophilic substitution of bromoketone **93** with theophylline was performed in 1 M NaOH and the reaction mixture was heated at reflux for 12 hours. TLC analysis showed a new spot for the product along with two spots for the starting materials indicating that the reaction was incomplete. In an attempt to force this reaction to completion, the reaction mixture was heated at reflux for two days; however no improvement in yield was observed. After workup the crude product was crystallised from isopropanol on cooling in a ice-NaCl bath to give the oxoalkyldimethylxanthine **94** in 19% yield. The pure product **94** gave a melting point in agreement with that reported[92] and was further characterised by its ^{1}H NMR spectrum. The most pertinent features of the ^{1}H NMR spectrum were two 2H multiplets at δ 1.49 and 1.82 along with two 2H triplets at δ 2.34 and 4.21 for the aliphatic chain between the 7-N and the carbonyl group. The ^{1}H NMR spectrum also produced signals characteristic of the xanthine skeleton at δ 3.33, 3.35 and 7.49. Finally $NaBH_4$ reduction of the aliphatic ketone **94**, according to the procedure of Maugras *et al.* [93] proceeded smoothly at rt for one hour and the reaction was quenched by addition of a few drops of conc. HCl. The by-product NaCl was removed by chromatography (Florisil®) and crystallisation from isopropanol afforded the racemic alcohol **95** as a white solid in 50% yield. The characteristic appearance of the aliphatic proton resonances for the alkyl alcohol moiety was observed in the δ 1.04-4.30 region of the ^{1}H NMR spectrum giving evidence for the transformation of the ketone **94** into alcohol **95**, while the OH stretching vibration at *ca* 3475 cm^{-1} was also found in the IR spectrum. The presence of the xanthine skeleton was verified from the key features in the ^{1}H NMR spectrum at δ 3.26, 3.45 and 8.27. The characteristic bands at 1600 and 1649 cm^{-1} found in the IR spectrum for the imidazole ring and the carbonyl groups gave further support for these findings. The thirteen resonances anticipated in the ^{13}C

NMR spectrum coupled with a parent ion at *m/z* 280 in the mass spectrum were also consistent with the proposed structure.

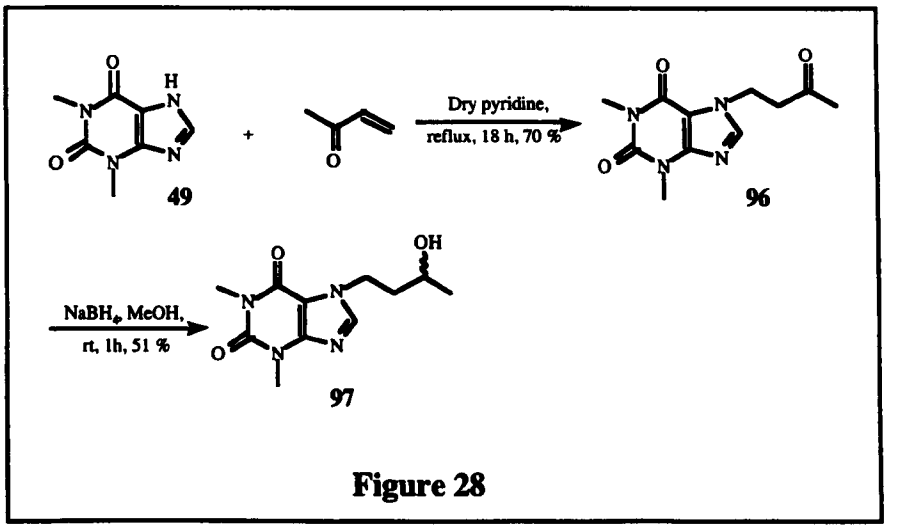


Figure 28

The synthetic route for theophylline alkyl alcohol derivative **97** is illustrated in Figure 28. The known compound **96** was prepared by a Michael addition according to a modified version of the literature method.[92] The Michael reaction carried out in dry pyridine afforded, after workup and crystallisation from isopropanol, the oxoalkyldimethylxanthine **96** as a pale yellow solid in 70% yield giving a melting point identical with that reported.[92] The identity of the compound **96** was further verified by the ^{1}H NMR spectrum with two 2H triplets and a 3H singlet in the aliphatic region for the alkyl ketone moiety together with two 3H singlets and a 1H singlet characteristic of the xanthine skeleton. Conversion of the ketone **96** into the corresponding alcohol **97** was accomplished by NaBH4 reduction as before giving the racemate of alcohol **97**. The ^{1}H NMR spectrum of the product **97** revealed a 3H doublet, a 1H multiplet and two 2H multiplets in the aliphatic region for the alkyl alcohol moiety while the IR spectrum showed characteristic OH absorption frequenceis at *ca* 3450 cm^{-1} confirming the completion of the reduction of the ketone. The xanthine skeleton produced the expected resonances at δ 3.20, 3.39 and 7.89 while the characteristic pattern for the imidazole ring and carbonyl groups were found at *ca* 1715 and 1596 cm^{-1}. The expected eleven resonances including the carbonyl resonances at δ 152.8 and 156.0 were observed in the ^{13}C NMR spectrum and a parent ion at *m/z* 252 in the mass spectrum were in agreement with the proposed structure.

In addition some more alkylated theophyllines (shown in Figure 29) were prepared *via* nuleophilic substitions.

Compound **98** was prepared in a fashion similar to that described for compound **94**. The alkylation of theophylline with hexyl bromide in 1M NaOH-EtOH afforded, after crystallisation from hexane, the theophylline derivative **98** as white crystals in 14% yield. The presence of the six carbon aliphatic chain was clearly evident from the ^{1}H NMR spectrum which contained two triplets, a multiplet and a singlet in the alkyl region and the ^{13}C NMR spectrum showed the six

resonances anticipated. The xanthine skeleton was clearly observed as three singlets at δ 3.33, 3.52 and 7.46 in the ^{1}H NMR spectrum, and the ^{13}C NMR and IR spectra of the product **98** were also in agreement with these findings. A parent ion at *m/z* 264 recorded in the mass spectrum provided further support for the proposed structure.

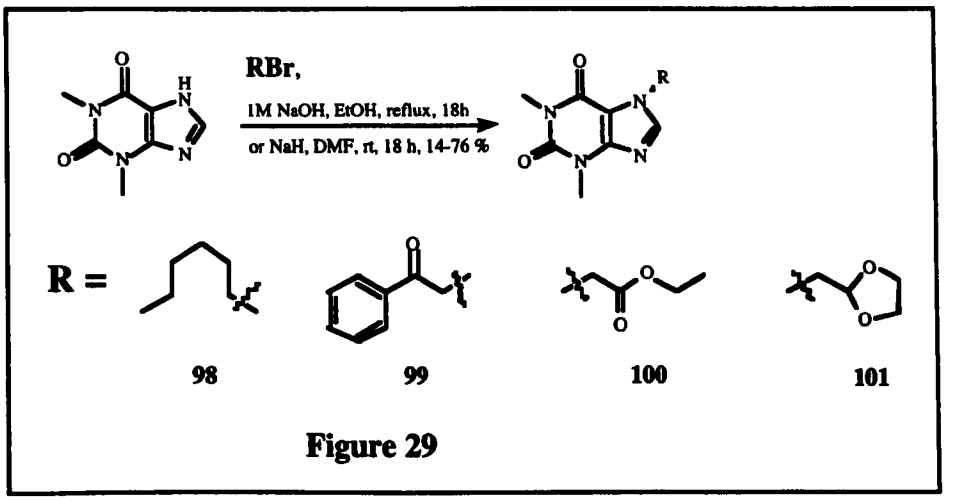


Figure 29

It is worthy of note that, despite the claimed yield of 80% for compound **94** in the literature,[92] compounds **94** and **98** were prepared in very low yields (19 and 14%, respectively) following the patent method[92] *via* SN_2 reaction performed in protic solvents, *e.g.* water/EtOH. The low yields we obtained were expected since the protic solvent mixture water/EtOH with NaOH is not appropriate for SN_2 reactions. It is well known that the SN_2 reaction is favoured by a high concentration of a strong (negatively charged) nucleophile in an aprotic polar solvent which dissolves ionic reagents chiefly through their bonding to the cation leaving the anion relatively free and highly reactive. In order to improve the conditions for the preparation of the other theophylline derivatives, the aprotic solvent DMF and stronger base NaH were employed in place of the NaOH and water/EtOH mixture.

A mixture of theophylline **49** and 2-bromoacetophenone in DMF with NaH was stirred at rt overnight and TLC analysis showed no starting material was left. The solvent was removed and the crude product was crystallised from EtOH affording compound **99** as yellow crystals in 76% yield. The melting point for the known compound **99** was in agreement with that reported[94] and the proposed structure was confirmed from its ^{1}H NMR spectrum showing a multiplet in the aromatic region for the benzene ring and a singlet at δ 5.96 for the methylene group along with the characteristic patterns resembling those found in the ^{1}H NMR spectra of compounds **94** and **95** to **98** for the xanthine skeleton.

Compound **100** was prepared by the procedure used to prepare compound **99** except for the method of workup. Dichloromethane was added to the reaction mixture to precipitate the unreacted theophylline which was filtered off. The filtrate was concentrated and the crude product was crystallised from isopropanol to give compound **100** as a grey solid in 19% yield

despite the apparent efficiency of the reaction by TLC analysis. A 3H triplet at δ 1.24, a 2H quartet at δ 4.20 and a 2H singlet at δ 5.01 in the ^{1}H NMR spectrum along with a characteristic ester resonance at δ 167 in the ^{13}C NMR spectrum confirmed that the ethyl ester was present. The same characteristic patterns for the xanthine skeleton were observed in the NMR and the IR spectra as for those recorded for the other theophylline derivatives. A parent ion at *m/z* 266 in the mass spectrum provided further evidence for the proposed structure.

Compound **101** was prepared in 55% yield following the procedure used to prepare compound **100**. The acetal moiety produced a 4H multiplet at δ 3.79, a 2H doublet at δ 4.51 and a 1H triplet at δ 5.51 in the ^{1}H NMR spectrum together with four characteristic resonances in the ^{13}C NMR spectrum. The NMR and IR spectra revealed the characteristic patterns for the xanthine skeleton. The proposed structure was also confirmed by high resolution mass spectrometry. Initially we envisaged that compound **101** should be readily converted into the corresponding aldehyde which appeared to be a good point for subsequent functional group manipulations. Unfortunately, attempts to hydrolyse the acetal to the aldehyde in 10% HCl or by treatment with PPTS were not successful. In consequence this plan was abandoned.

6. 2 Synthesis of Theophylline Dimers

A series of theophylline dimers was synthesised (Figure 30) in order to establish the optimum bridging distance between the two heterocycles. We postulated that the theophylline dimers might act as intercalating agents which could interact with both DNA strands.

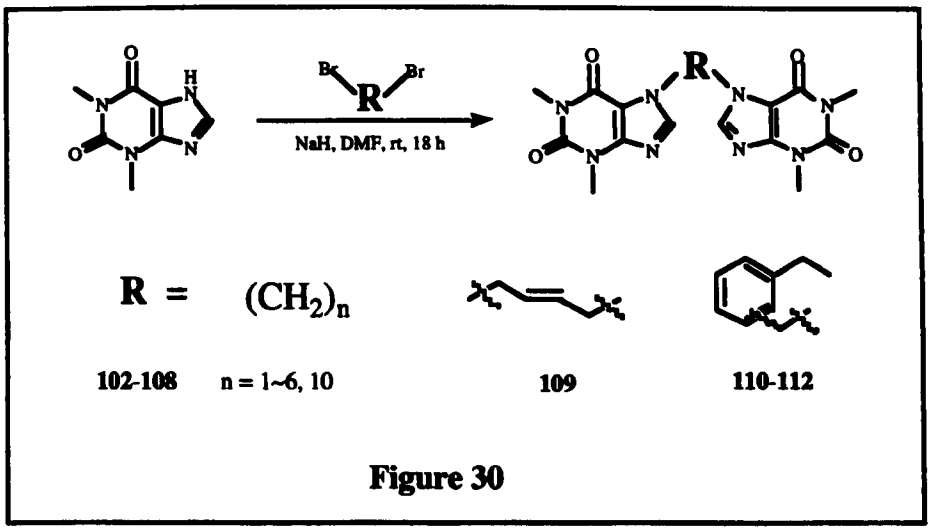


Figure 30

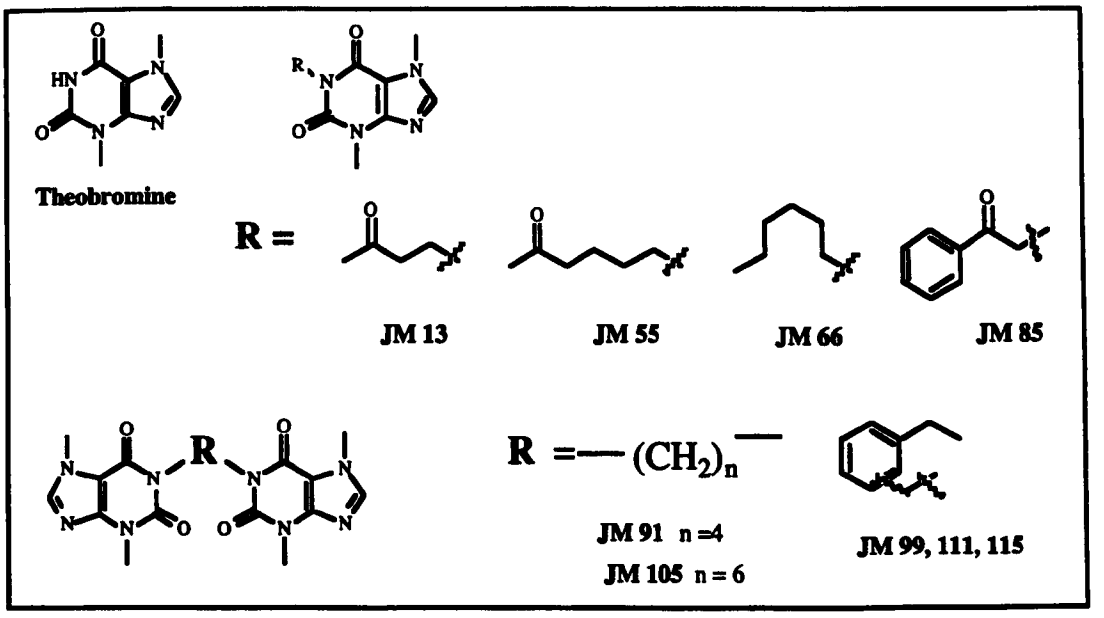
All eleven theophylline dimers **102** to **112** were prepared in the same manner following the procedure of Itahara and Imamura.[95] Alkylations of the dibromides with theophylline in a suspension of NaH in DMF were carried out at ambient temperature overnight. To encourage the completion of these reactions, the reaction mixtures were then heated at 70 °C for 3h. TLC analysis showed no starting material and a homogeneous spot for the product. Following

completion of the reaction, product precipitation was achieved by the addition of water to the cooled reaction mixture. The melting points for the seven known compounds **102** to **108** were identical to those reported in the literature.[95] No commonly used solvents were found to dissolve compounds **103** and **104**, therefore there are no NMR spectral data for them. The parent ions at *m/z* 386 and 400 in the high resolution mass spectra gave evidence for the proposed structures of compounds **103** and **104**, respectively. The ^{1}H NMR spectra of compounds **102** and **105** to **108** revealed characteristic resonances for the two xanthine skeletons with two 6H singlets at δ 3.34 and 3.49 along with a 2H singlet at δ 8.29, a feature preserved throughout the five compound series, which were consistent with literature findings[95] for these known compounds. Compounds **110** and **111** produced a 4H singlet at *ca* δ 5.5 and multiplets in the aromatic region in the ^{1}H NMR spectrum for the xylylene moiety together with distinct resonances for the two xanthine skeletons. The proposed structures were also confirmed by ^{13}C NMR, IR and accurate mass spectrometry. Compounds **109** and **112** were insoluble in commonly used solvents, and therefore NMR spectra could not be obtained. IR and accurate mass spectrometry provided evidence for the proposed structures of compounds **109** and **112**, and the purity of compound **109** was confirmed by combustion analysis.

6. 3 Biological Evaluation of Theophylline Derivatives

6. 3. 1 Water Solubility of Theophylline Derivatives

The water solubility of each of our theophylline derivatives was estimated by Miss Isabel Freer of the Tissue Culture Unit, University of Glasgow. The results are shown in Table 1. For comparison the theobromine derivatives (shown below) were prepared by Ms J. Milne in her MSci project in 1999 and their water solubility data are provided in Table 2.[96]



Theophylline Derivatives	Compound	Water Solubility
Caffeine		20 g/L
Theophylline	**49**	10 g/L
ThN(CH_2)4COMe	**94**	10 mM
ThN(CH_2)4CHOHMe	**95**	10 mM
ThN(CH_2)2COMe	**96**	100 mM
ThN(CH_2)2CHOHMe	**97**	10 mM
ThN(CH_2)5Me	**98**	1 - 10 mM
ThNCH$_2$COPh	**99**	< 0.1 mM
ThNCH$_2$CO$_2$Et	**100**	< 0.1 mM
	**101**	< 0.1 mM
ThNCH$_2$NTh	**102**	< 0.01 mM
ThN(CH_2)2NTh	**103**	< 0.01 mM
ThN(CH_2)3NTh	**104**	< 0.01 mM
ThN(CH_2)4NTh	**105**	< 0.01 mM
ThN(CH_2)5NTh	**106**	< 0.01 mM
ThN(CH_2)6NTh	**107**	< 0.01 mM
ThN(CH_2)10NTh	**108**	< 0.01 mM
ThNCH$_2$CH=CHCH$_2$NTh	**109**	< 0.01 mM
o - ThNCH$_2$C$_6$H$_4$CH$_2$NTh	**110**	< 0.01 mM
m - ThNCH$_2$C$_6$H$_4$CH$_2$NTh	**111**	< 0.01 mM
p - ThNCH$_2$C$_6$H$_4$CH$_2$NTh	**112**	< 0.01 mM

Theophylline = ThNH

Table 1

Theobromine Derivative	Compound	Water Solubility
Theobromine		0.5 g/L
$TbNCH_2CH_2COMe$	**JM 13**	100 mM
$TbN(CH_2)_4COMe$	**JM 55**	1 mM
$TbN(CH_2)_5Me$	**JM 66**	< 0.1 mM
$TbNCH_2COPh$	**JM 85**	< 0.1 mM
$TbN(CH_2)_4NTb$	**JM 91**	< 0.01 mM
$TbN(CH_2)_6NTb$	**JM 105**	< 0.01 mM
o - $TbNCH_2C_6H_4CH_2NTb$	**JM 115**	< 0.01 mM
m - $TbNCH_2C_6H_4CH_2NTb$	**JM 99**	< 0.01 mM
p - $TbNCH_2C_6H_4CH_2NTb$	**JM 111**	< 0.01 mM

Theobromine = TbNH

Table 2

A range of theophylline and theobromine derivatives was assessed for water solubility. Much to our disappointment but not to our surprise, most of the compounds except for the theophylline and theobromine keto and alcohol derivatives were found to be almost insoluble in water (< 0.1 mM).

6. 3. 2 Method of Biological Assay

The theophylline and theobromine derivatives prepared were assessed for their inhibition of G_2/M arrest by Professor David Gillespie at the Beatson Institute for Cancer Research, Glasgow.

DT40 B-lymphoma cells in exponential growth phase (200 ml total culture volume) were divided into 33 individual replicate 5 ml cultures. One replicate 5 ml culture was not treated with irradiation or addition of test compound apart from addition of 5 μl of vehicle (DMSO). The other 32 replicate culture were irradiated with 10 Gy γ-irradiation followed by addition of 5 μl of vehicle or test compound to 1 mM. 33 cultures were then incubated at 37 °C overnight. After fixation the harvested cells were stained with propidium iodide and analysed by flow cytometry.

6. 3. 3 Results and Discussion

The process of mitosis is a sequence of events called the cell cycle which was briefly described in Chapter 1. As shown in Figure 1, the cell cycle consists of a number of sequential

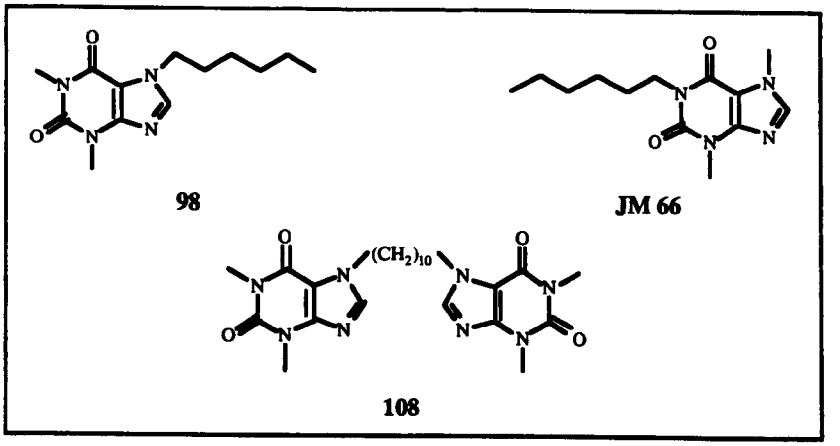
stages: G_1 phase; S (synthesis) phase; G_2 phase; and M (mitosis) phase. After mitosis, the two daughter cells undergo the cycle again or enter a resting phase, G_0. All cells undergo this cell cycle. The sequential events in the cycle for DT40 B-lymphoma cells before irradiation treatment were analysed by flow cytometry in our biological assay and they are shown in Figure 31 at the top.

The cell cycle also has two checkpoints: G_1/S and G_2/M. In the event of DNA damage checkpoint regulatory pathways are activated resulting in cell cycle arrests at both the G_1/S and G_2/M transitions to provide time for the repair of damaged DNA, or in case of severe damage, for activation of programmed cell death. DT40 B-lymphoma cells, used in the biological assay, have inactivated tumour suppressor gene p53, a critical component for induction of G_1/S arrest. DNA damage (such as irradiation) results in preferential accumulation of p53-deficient cancer cells at the G_2/M transition as these cells have an incompetent G_1/S checkpoint. The effect of the irradiation in inducing G_2/M arrest in DT40 B-lymphoma cells is shown in Figure 31 at the bottom, where it is apparent that the number of events at the G_2/M boundary has increased substantially after irradiation (DNA damage).

The preferential accumulation of these cells on the G_2/M checkpoint raised the possibility that pharmacological inhibition of G_2/M may render these tumour cells more sensitive to DNA damaging agents. The biological evaluation of the xanthine derivatives is shown in Figure 32. Most of the compounds show no effect on the G_2/M boundary events after irradiation except for three of them, **98**, **JM66** and **108**. The effects of these compounds on irradiation of cells is shown in more detail in Figure 33. These three compounds suppressed G_2/M arrest very effectively. The cancer cells undergo mitosis with damaged genomes when the DNA damage checkpoint G_2/M is inhibited, and this eventually leads to tumour death.

All three active compounds have in common a long aliphatic chain which provides a large degree of flexibility to the structures. It is worthy of note that the ten-carbon chain of compound **108** is nearly twice as long as the side chain (six carbons long) of compounds **98** and **JM66**. The long aliphatic chains could bind to a hydrophobic pocket in an enzyme's active site giving rise to the activities of compounds **98**, **JM66** and **108**. On the other hand the large alkyl chains increase the bulk of the compounds as a whole and this might assist the uptake of the compounds into cells. If this is the case, it is not clear why these three compounds are effective whereas compounds with slightly different alkyl chains such as **107** are not.

Caffeine **47** has been demonstrated to suppress the G_2/M checkpoint and to prevent activation of Chk1,[46, 54] presumably by inhibiting one or more upstream checkpoint kinases Rad3 in the DNA damage signal transduction process. Compounds **98**, **108** and **JM66** may exhibit their inhibitory activity by a similar mechanism. It is not known why caffeine **47** failed to induce G_2/M arrest in our experiments. It should be noted that the mode of action of caffeine and its derivatives has not yet been fully elucidated.

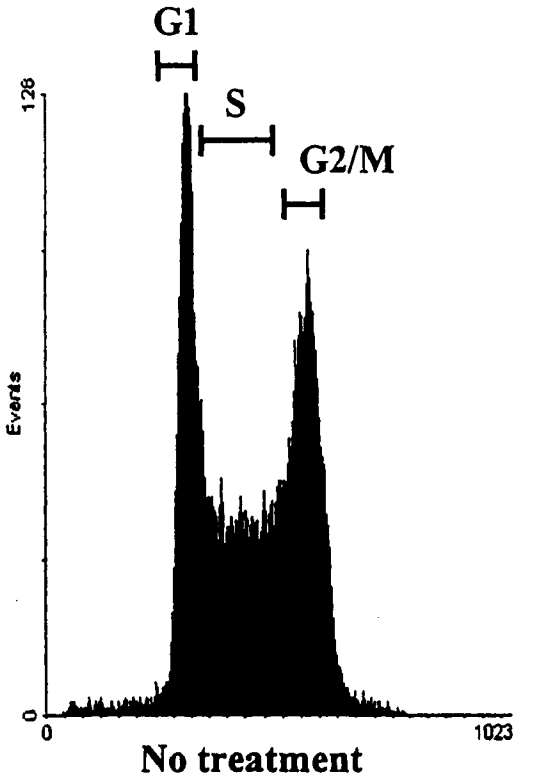


98

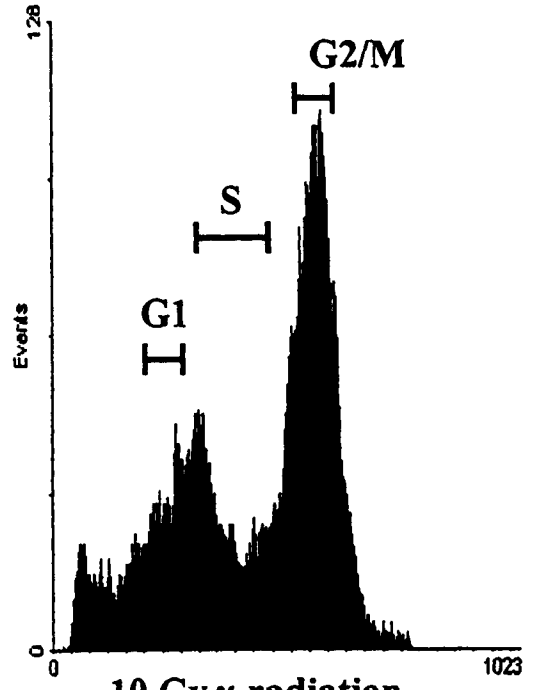
JM 66

108

Figure 31 Ionising radiation-induced G2/M arrest



No treatment



10 Gy γ-radiation
16 h

Figure 32 Compound Testing

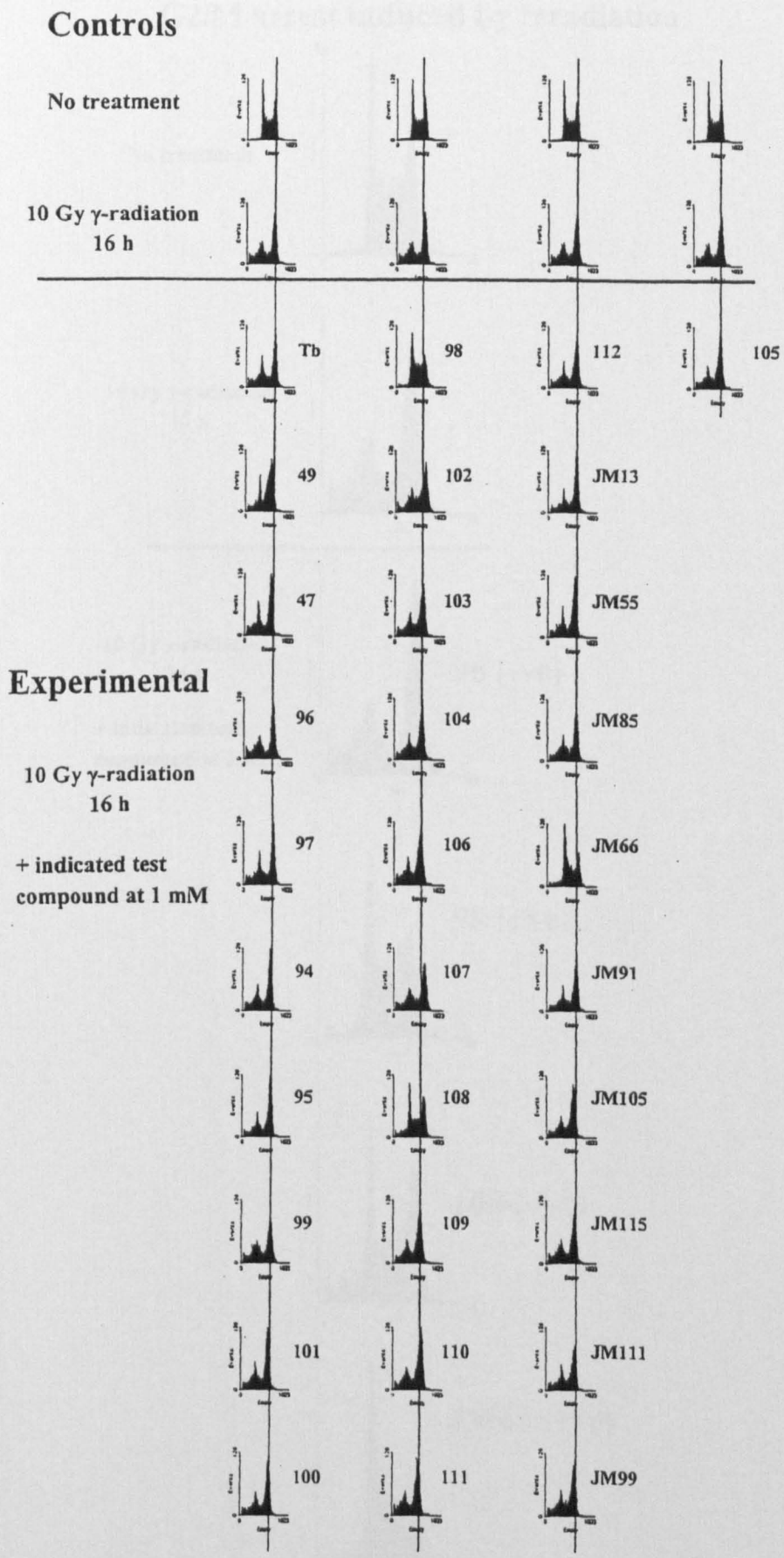
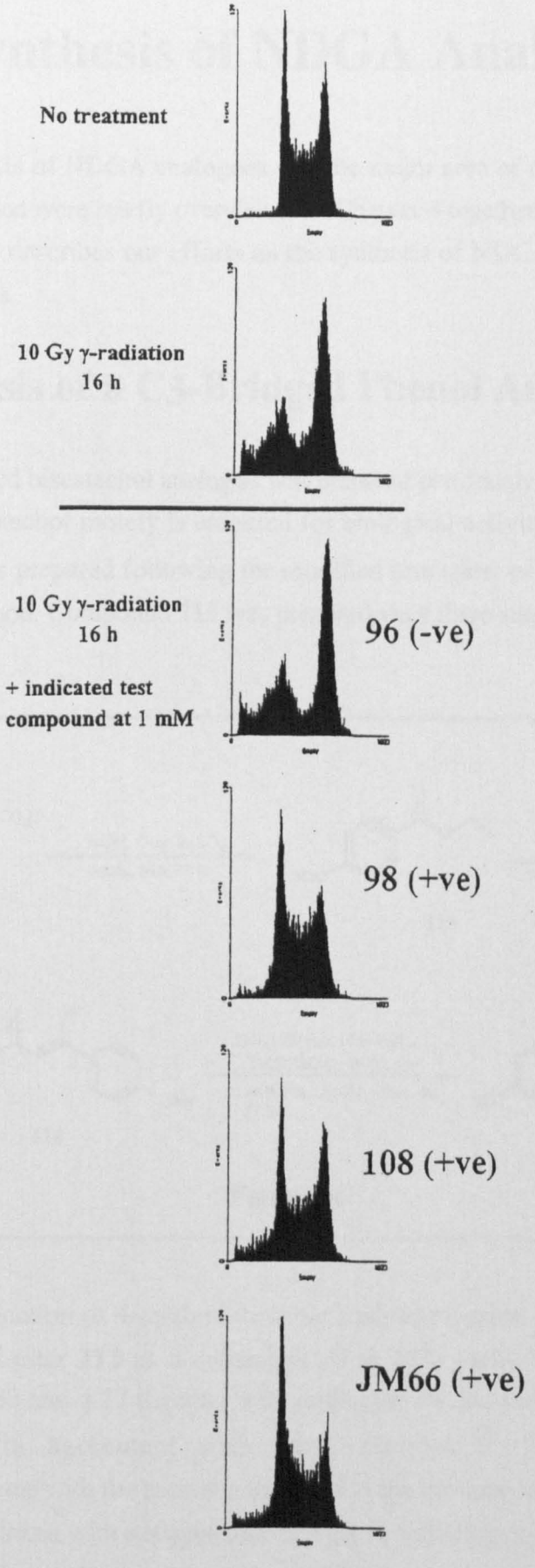


Figure 33 3 Compounds (98, 108, and JM66) inhibit G2/M arrest induced by Irradiation



CHAPTER 7

Synthesis of NDGA Analogues

The synthesis of NDGA analogues was the major area of our synthetic work. NDGA and its mode of action were briefly overviewed in Chapter 4 together with the proposed research work. This chapter describes our efforts on the synthesis of NDGA analogues along with the preliminary test data.

7. 1 Synthesis of a C3-Bridged Phenol Analogue

A C3-bridged biscatechol analogue was prepared previously by McDonald.[8] In order to determine if the catechol moiety is essential for biological activity, the C3-bridged phenolic compound **115** was prepared following the modified procedure of McDonald[8] except for the demethylation method. Compound **115** was prepared via a three step synthetic route depicted in Figure 34.

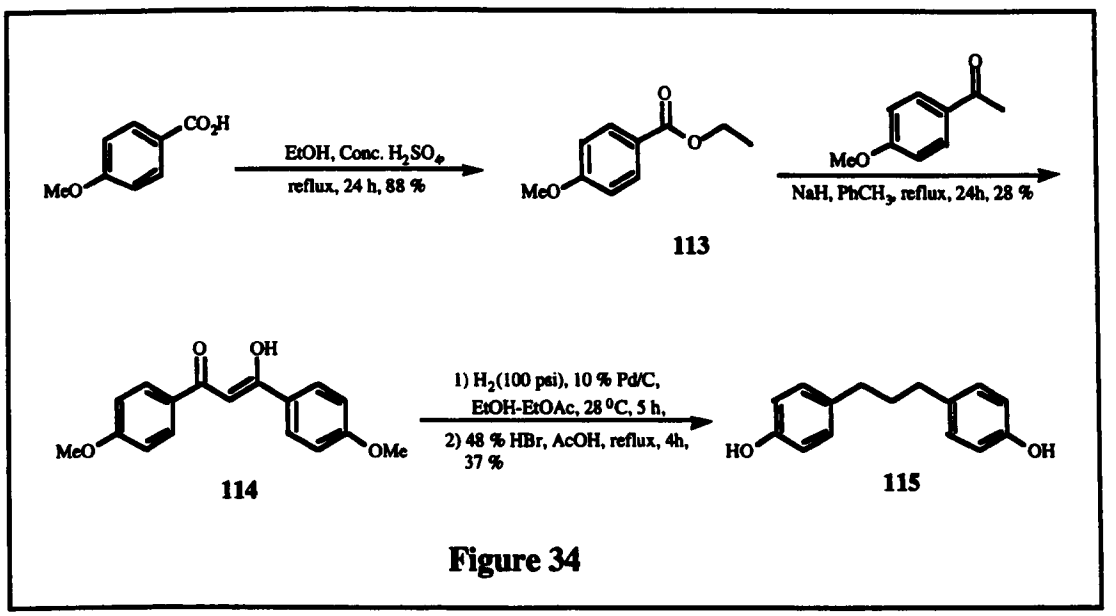


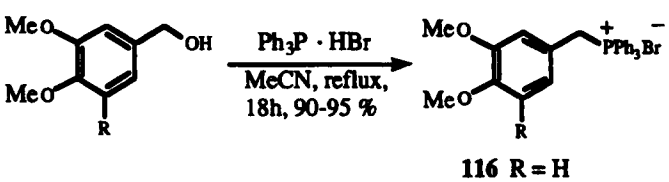
Figure 34

The esterification of 4-methoxybenzoic acid was carried out under acidic conditions affording the ethyl ester **113** as a colourless oil in 88% yield. The characteristic ethyl ester resonances at δ 1.30 and 4.27 together with multiplets for the aromatic ring in the ^{1}H NMR spectrum were in agreement with those reported.[97] The condensation of 4-methoxyacetophenone with the aromatic ester **113** is the key step in the synthetic approach. The reaction was undertaken with a suspension of NaH in refluxing toluene. 10% HCl was used to

quench the reaction and, after workup in a standard manner, the crude product was crystallised from EtOH affording the β-diketone **114** in 28% yield. Despite the low yield crystallisation was achieved in a straightforward manner to yield the pure compound **114**. The melting point and ^{1}H NMR spectrum of the known compound **114** were identical with those reported.[97] Conversion of the β-diketone **114** into the target compound **115** was achieved by a two step procedure. The β-diketone **114** was hydrogenated over Pd/C for five hours and then, without purification, the crude product was deprotected in a refluxing mixture of 48% HBr and AcOH affording the crude product **115** as a yellow oil. Deprotection with 48% HBr was carried out following the procedure of Kawasaki *et al.*[98] Trituration of the crude product **115** in petroleum ether and the following recrystallisation afforded the final product **115** as a yellow solid giving a melting point in agreement with that reported.[99] The ^{1}H NMR spectrum revealed a 2H multiplet and a 4H triplet in the aliphatic region for the three carbon alkyl bridge together with two 4H multiplets in the aromatic region for the two benzene rings. The demethylation was confirmed by the characteristic appearance of an OH proton resonance at δ 4.76.

7. 2 Synthesis of C4-Bridged Analogues

The trihydroxy substituted diarylbutane **127** is more polar than the dicatecholbutane **126**, hence is likely to be more soluble in water. Therefore we sought to examine the effect of adding one more hydroxy group to each aromatic ring on the biological activity. For this purpose the diarylbutanes **126** and **127** were synthesised via a six step approach shown in Figure 35 and 36.



116 R = H
117 R = OMe

Figure 35

The substitutions of the benzyl alcohols with triphenylphosphine hydrobromide in refluxing acetonitrile proceeded smoothly and afforded, after crystallisation from EtOH, the phosphonium salts **116** and **117** in high yields (Figure 35). The melting points of the products **116** and **117** were identical with those reported[8,100] while the ^{1}H NMR spectra revealed a 15H multiplet in the aromatic region for the three phenyl groups and a deshielded doublet at δ 5.2 for the methylene protons coupled with the adjacent P atom. The propionic acids were elaborated to the corresponding aldehydes **120** and **121** in two steps (Figure 36). Reduction of the acids to the corresponding alcohols **118** and **119** proceeded well with $LiAlH_4$ in THF at rt.

After quenching by 1M HCl-ether the products **118** and **119** were isolated in nearly quantitative yields as viscous oils satisfactory for synthetic work without further purification. The ^{1}H NMR spectra of the two alcohols **118** and **119** were in agreement with those reported.[101,102] The subsequent oxidations proceeded in a straightforward manner following the standard Swern oxidation procedure[103] to furnish the aldehydes **120** and **121** in high yields as yellow oils pure enough to use without further purification. In their ^{1}H NMR spectra each oil **120** and **121** exhibited the characteristic peak at δ 9.7 for the aldehyde proton and two 2H triplets at δ 2.7 and 2.8 together with signals for the aromatic ring and the methoxy groups, which were consistent with literature findings.[104,105]

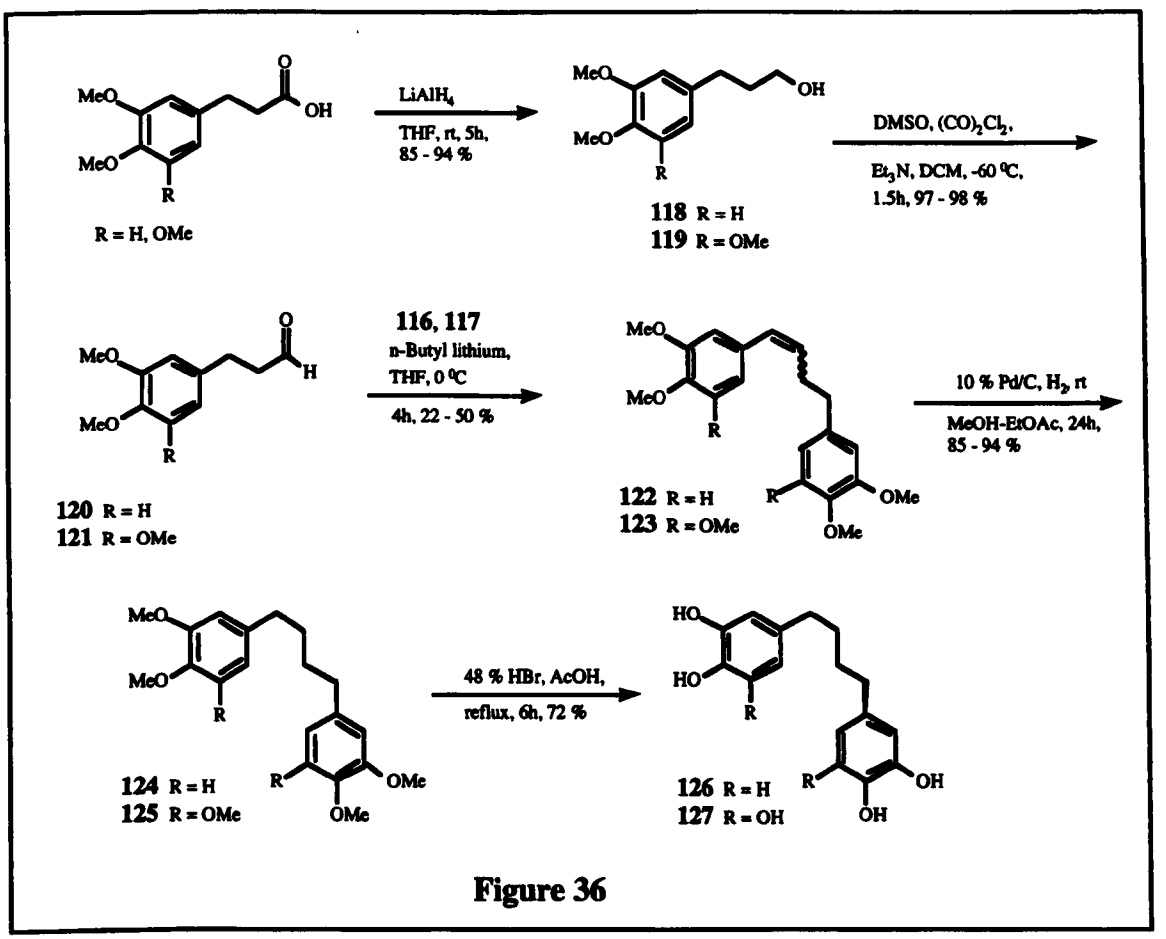


Figure 36

With the aldehydes (**120** and **121**) and the phosphonium salts (**116** and **117**) in hand, attention turned to the employment of the Wittig reaction to install the double bonds. Treatment of the phosphonium salts **116** and **117** with *n*-butyllithium in THF at 0 °C generated the phosphonium ylids which then reacted with the aldehydes **120** and **121** at the same temperature to form the double bonds. To facilitate the purification of compound **122**, the reaction mixture, after workup, was stirred with MeOH to precipitate the majority of the product as a yellow solid while the filtrate was concentrated and then subjected to chromatography to yield the rest of the product. The alkene **122** was isolated as a mixture of geometrical isomers and the purification procedure was found to affect the isomeric ratio. Integration of the characteristic peaks in the

^{1}H NMR spectrum showed *Z:E* ratios of *ca.* 1:9 and 2:1 for the alkene **122** purified from trituration in MeOH and by chromatography, respectively. The two isomers could be readily distinguished by the *J* value for the doublet of triplets for the homobenzylic methine proton with the *cis*-isomer giving *J* values of 11.6 and 6.8 Hz and the *trans*-isomer 15.8 and 6.8 Hz. Difficulties were experienced in the preparation of the trimethoxyphenylbutene **123** with a streak observed on a TLC plate and a low yield of 14-20% obtained after chromatography. The problem appeared to result from the inherent unstable nature of the anion generated from the trimethoxybenzylphosphonium salt **117** by treatment with *n*-butyllithium. The three methoxy substituents, as strong electron donors, increased the electron density of the conjugated system of the anion and hence destablised the ylid. As a consequence, this Wittig olefination gave a low yield. The melting point and the ^{1}H NMR spectrum for the known compound **122** were found to be consistent with those reported.[8] The geometrical isomers of compound **123** were observed as a pair of doublets of triplets in the ^{1}H NMR spectrum, one at δ 5.59 with *J* values of 11.6 and 6.8 Hz, the other at δ 6.11 with *J* values of 15.8 and 6.8 Hz for the homobenzylic methine proton. The six methoxy groups of compound **123** were clearly seen as a 18H multiplet at *ca* δ 3.7 in the ^{1}H NMR spectrum and there was a C-H stretching band at *ca* 2930 in the IR spectrum. A parent ion at *m/z* 388 in the high resolution mass spectrum was in agreement with the proposed structure. The mixture of alkene geometrical isomers was not separated as the subsequent hydrogenation transformed the alkenes **122** and **123** into the corresponding alkanes **124** and **125**. Catalytic hydrogenations over Pd/C were carried out with ease to produce the butanes **124** and **125** in good yields. The accomplishment of the hydrogenation was quite apparent through the absence of the characteristic signals for the double bond protons in the ^{1}H NMR spectrum. The known compound **124** provided a melting point identical with that reported[104] and produced signals in the ^{1}H NMR spectrum in agreement with data cited in the literature.[8] The symmetrical structure of the novel compound **125** was clearly evident from the NMR spectra with the ^{1}H spectrum showing a 4H multiplet and a 4H triplet in the aliphatic region together with a 4H singlet at δ 6.31, and the ^{13}C NMR spectrum gave the expected eleven resonances. The mass spectrum revealed a parent ion at the expected *m/z* 390 and a combustion analysis provided further evidence for the proposed structure for compound **125**. The demethylation of the methyl ethers **124** and **125** was carried out by treatment with refluxing 48% HBr affording the corresponding phenolic diarylbutanes **126** and **127** in good yields. The absence of peaks for the methyl ether and the characteristic appearance of the phenolic proton resonances in the ^{1}H NMR spectra confirmed that demethylation had been successful. The melting point and the ^{1}H NMR spectrum of the known compound **126** were found to be consistent with those reported in the literature.[106,8] The presence of the six hydroxy groups in compound **127** was observed in the ^{1}H NMR spectrum as a 2H broad singlet at δ 7.7 and a 4H singlet at δ 8.6 along with the broad phenolic stretches at *ca* 3500 cm^{-1} in the IR spectrum. Two 4H multiplets in the aliphatic region and a 4H singlet at δ 6.06 were also observed in the

^{1}H NMR spectrum and the ^{13}C NMR spectrum showed the expected six resonances providing support for the structure assignment of compound **127**. A parent ion at *m/z* 306 in the mass spectrum accompanied by a combustion analysis were also consistent with the proposed structure.

In conclusion, the syntheses of target compounds **126** and **127** were accomplished in five steps with overall yields of 40 and 9% for compounds **126** and **127**, respectively. The low yield (14-22%) obtained in the preparation of alkene **123** led to the low overall yield of 9% for target compound **127**. Further efforts are needed in the development of an alternative route to circumvent the difficulties in the installation of the C_4 bridge in compound **127**.

7. 3 Synthesis of Conformationally Restricted Analogues

As mentioned in Chapter 4 preliminary work in the Robins group revealed that NDGA analogues with an amide linkage between the two aromatic rings are more potent than NDGA itself. To extend this work, a series of amide (or thioamide) analogues of NDGA with both 4 and 5 atoms between the rings were synthesised.

7. 3. 1 Synthesis of Amide (or thioamide) Analogues with a 4 Atom Bridge

Given the improved potency of amide analogues of NDGA, we envisaged that replacement of the amide group by the thioamide group might result in the formation of an analogue with improved activity because of the increased lipophilicity. To test this hypothesis, the thioamide compound **132** was prepared. The thioamide **132** was prepared by thionation of the amide **130** and the synthesis is depicted in Figures 37 and 38.

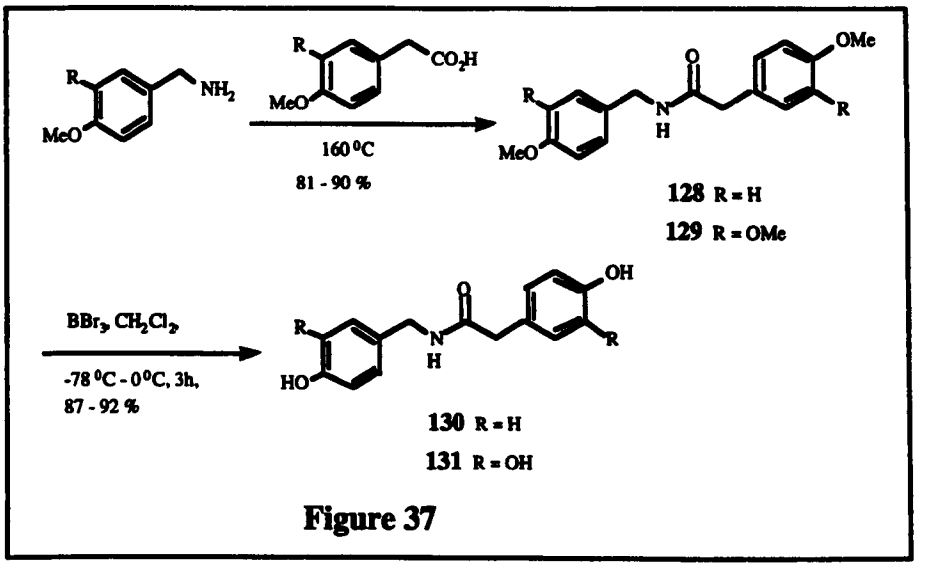


Figure 37

We investigated two methods to form the amide bond. The coupling reagent system EDCI-DMAP was employed in the procedure of McDonald[8] to install the amide bond affording the amides **128** and **129** in good yields. However, considering the stability of the starting materials and the products, we sought to simplify the reaction conditions. It was later found that the amides **128** and **129** could be readily obtained in high yields by simply heating the acids and the amines together at 160 ^{0}C, and this method aided the procedure of workup and purification. After washing sequentially with dilute acid, base and water, the amides were obtained free of contaminants, and could be used directly in the next step. The melting point and ^{1}H NMR spectrum of the known compound **129** were found to be identical with those reported[8] while the novel compound **128** was fully characterised. Demethylation of the methyl ether **129** was reported[8] to proceed in a low yield (15-20%), therefore effort was focused on improving the efficiency of this reaction. Following the reported procedure[8] 1M BBr_3-DCM solution was introduced to a DCM solution of the methyl ether **129** at -78 ^{0}C. The mixture was stirred at the same temperature for 20 min and then allowed to warm to 0 ^{0}C and stirred for a further 50 minutes. TLC analysis (EtOAc-hexane, 5% AcOH or NH_3-H_2O) revealed only a single spot sticking at the origin indicating that the starting material was used up. However, after workup, the ^{1}H NMR spectrum showed that two-thirds of the crude product was the desired compound and the rest was starting material. The carbonyl oxygen atom in the amide **129**, as an electron donor, could chelate with BBr_3 to form a boron complex which stuck at the origin. This might explain the absence of the starting material spot on the TLC plate. This indicated that the reaction did not proceed long enough to be complete. Furthermore, the reported procedure[8] of workup involved washing the crude product **131** with saturated sodium bicarbonate and this was found to cause further loss of product. TLC analysis of both the $NaHCO_3$ layer and the organic layer revealed that most of the crude product had been washed into the $NaHCO_3$ solution. This is not surprising given the considerably higher acidity of the dicatechol system of compound **131** compared with a phenolic system. In addition, it was also noticed that following an ice/water-mediated quench of the reaction, the mixture should be left stirring for 30 minutes to ensure the complete decomposition of the product-boron complex as only the free hydroxy product could be extracted into EtOAc and hence be isolated. The purification of compound **131** also proved problematic as the compound is so polar that it produced a streak on both silica and alumina TLC plates eluting with solvent systems containing 3% AcOH or NH_3-H_2O. Thus the product could be lost on the column when purified by chromatography. Attempted crystallisation was not successful as the compound was highly hygroscopic and susceptible to oxidation. It was also found that compound **131** was only soluble in water and alcoholic solvents and EtOAc. In the modified procedure of purification, the crude compound **131** was extracted from the EtOAc extracts into the $NaHCO_3$ solution. The $NaHCO_3$ extracts were then acidified and re-extracted with EtOAc to remove organic contaminants. Trituration with petroleum ether and DCM afforded the product **131** as a brown solid in 87% yield reasonably pure for synthetic work giving a melting point and ^{1}H NMR spectrum identical with those

reported.[8] BBr_3 demethylation of **128** was carried out following the procedure of McOmie *et al.*[107] The purification of the diphenol amide **130** was easier than that of dicatechol amide **131** as the diphenol amide **130** with two fewer hydroxy groups was less polar, less hygroscopic and less susceptible to oxidation. The EtOAc solution of crude product **130** could be washed with saturated $NaHCO_3$ solution without loss of the product in the aqueous layer. Crystallisation from EtOAc afforded the novel compound **130** as yellow crystals in 92% yield. Two 2H singlets in the aliphatic region and two 4H multiplets in the aromatic region were recorded in the ^1H NMR spectrum of compound **130** for the two methylene groups and the two benzene rings, respectively while the absence of the characteristic methoxy resonance indicated the completion of the demethylation. The phenolic groups were characterised by the OH stretching band at *ca* 3300 cm^{-1} in the IR spectrum. The amide bond was shown to be intact with a NH stretching band near 3300 cm^{-1} and a carbonyl absorption frequency at 1650 cm^{-1} observed in the IR spectrum together with a carbonyl resonance at δ 175 in the ^{13}C NMR spectrum. A parent ion at *m/z* 257 in the mass spectrum and a combustion analysis provided further support for the structure assignment of compound **130**.

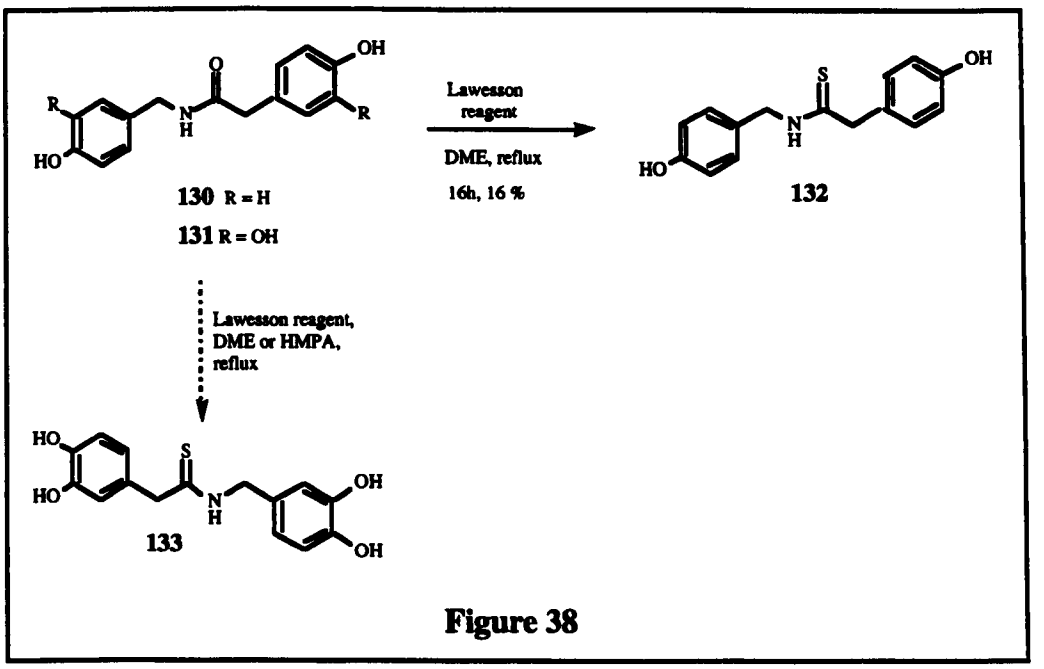


Figure 38

Lawesson and his coworkers[108] have demonstrated that the conversion of amides into thioamides could proceed smoothly in very mild conditions when Lawesson's reagent was employed. They also reported[109] that the thionation using HMPA or DME as solvent was tolerant of free hydroxy groups as HMPA and DME are known to form complexes with hydroxyaromatic compounds. Lawesson suggested that HMPA and DME act as a protecting agent for hydroxy groups in these reactions. Therefore we decided to carry out the thionation following Lawesson's procedure with some modifications. Amide **130** was treated with Lawesson's reagent in DME and the suspension was heated at reflux for 48 hours. DME was

chosen as the solvent in this reaction rather than HMPA for two reasons: DME was reported[109] to protect the phenol group more satisfactorily than HMPA in thionation; and HMPA is much more toxic than DME. TLC analysis of the reaction mixture revealed several higher running spots and a starting material spot together with a lower running streak. In an attempt to force the reaction to completion, the reaction mixture was heated at reflux for a further 24 hours; however TLC analysis showed no improvement. One of the higher running spots, which was stained light blue and relatively easy to separate, was isolated and its NMR characteristics were those expected for the thioamide **132**, but the yield was only 17%. The conversion from compound **130** into compound **132** was confirmed by a major spectroscopic difference between the thioamide and the parent amide in the ^{13}C NMR spectra, namely the carbonyl carbon in amide **130** came into resonance at δ 175 while the thiocarbonyl carbon in thioamide **132** resonated at δ 204. This shift is due to poor orbital overlap, as the π-bonding electrons in thioamide groups are not as delocalised as those of amides. This causes deshielding of the thioamide sp^2-hybridised carbon atom leading to a ^{13}C NMR chemical shift of *ca* δ 200, some 30 ppm higher than that of the corresponding amide. Two 2H singlets at δ 3.76 and δ 4.58 for the two methylene groups of compound **132** were recorded in the ^1H NMR spectrum which were 0.4 ppm higher than those for the methylene groups in the corresponding amide **130**. The IR spectrum and a parent ion at *m/z* 273 in the high resolution mass spectrum were in agreement with the proposed structure.

Thionation of compound **131** under the same conditions as those for compound **130** was unsuccessful. Compound **131** was found to be insoluble in DME even at elevated temperatures and this might be the reason for the failure. The thionation of compound **131** was then performed in HMPA in place of DME as compound **131** was soluble in HMPA; unfortunately this attempt only gave a complex mixture without a trace of the desired product as revealed by ^1H NMR spectroscopic analysis. Further effort to transform the dicatechol amide **131** into the corresponding thioamide **133** was not attempted.

Compound **135** was prepared in an attempt to ascertain the position of the amide group required for optimum activity of NDGA amide analogues (Figure 39).

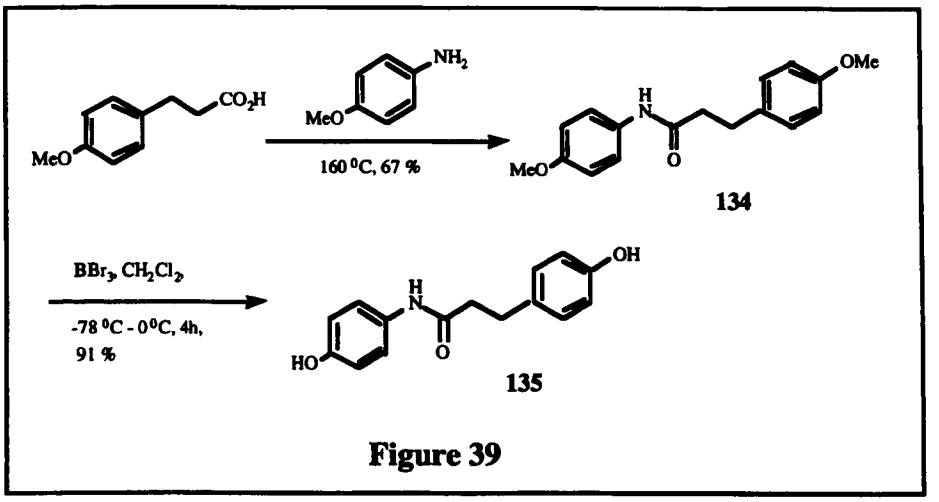


Figure 39

Compound **135** was prepared by the same route under the same reaction conditions employed in the preparation of compounds **130** and **131** except for the workup and purification after demethylation. To quench the demethylation, the reaction mixture was poured into iced water and shaken for 30 minutes and the resulting grey suspension was filtered affording compound **135** as a grey solid in 91% yield pure enough without further purification. Two 2H triplets in the aliphatic region for the two methylene groups and two singlets at δ 3.70 and 3.71 for the methoxy groups in compound **134** were observed in the ^{1}H NMR spectrum along with multiplets in the aromatic region characteristic of protons on benzene rings. The ^{13}C NMR and IR spectra together with the accurate mass spectrum were found to be consistent with the proposed structure of compound **134**. The successful deprotection of compound **134** was clearly evident from the ^{1}H NMR spectrum with the absence of methoxy resonances at *ca* δ 3.7. The ^{1}H NMR spectrum of compound **135** revealed two triplets in the aliphatic region and multiplets in the aromatic region while the ^{13}C NMR spectrum showed the expected resonances including the carbonyl functionality at δ 174. Characteristic phenolic and NH stretching frequencies at *ca* 3148 cm^{-1} were found in the IR spectrum. A parent ion at *m/z* 258 in the high resolution mass spectrum was consistent with the proposed structure.

7. 3. 2 Synthesis of Amide Analogues with a Five Atom Bridge

Compound **137** (Figure 40) was prepared by the same procedures used to prepare compound **131**.

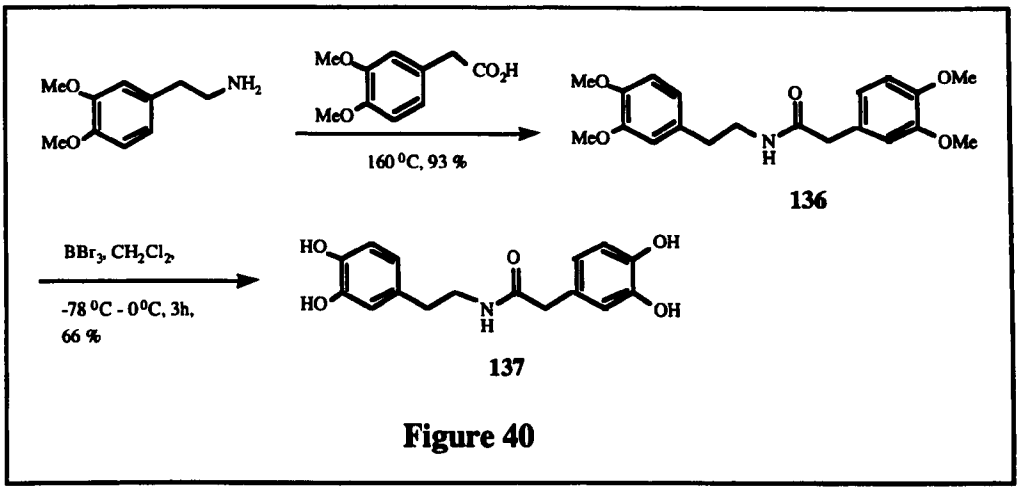


Figure 40

Difficulty was encountered in the purification of compound **137**. Like compound **131**, the dicatechol amide **137** was too polar to be purified by chromatography and it was also hygroscopic and susceptible to oxidation when exposed to air. Attempted crystallisation in a variety of solvents was unsuccessful and therefore the crude product was purified by trituration from DCM and petroleum ether affording compound **137** as a brown solid in 66% yield. The ^{1}H and ^{13}C NMR spectra of compound **137** are shown in Appendixes 3 and 4. The three methylene groups of compound **137** came into resonance as a 2H triplet and a 4H multiplet in the aliphatic region and the characteristic appearance of aromatic proton resonances was also observed in the ^{1}H NMR spectrum. The characteristic OH and NH absorption bands at *ca* 3400 cm^{-1} along with the carbonyl and aromatic absorption frequencies at 1600-1657 cm^{-1} were recorded in the IR spectrum. A parent ion at *m/z* 304 in the high resolution mass spectrum provided further support for these findings.

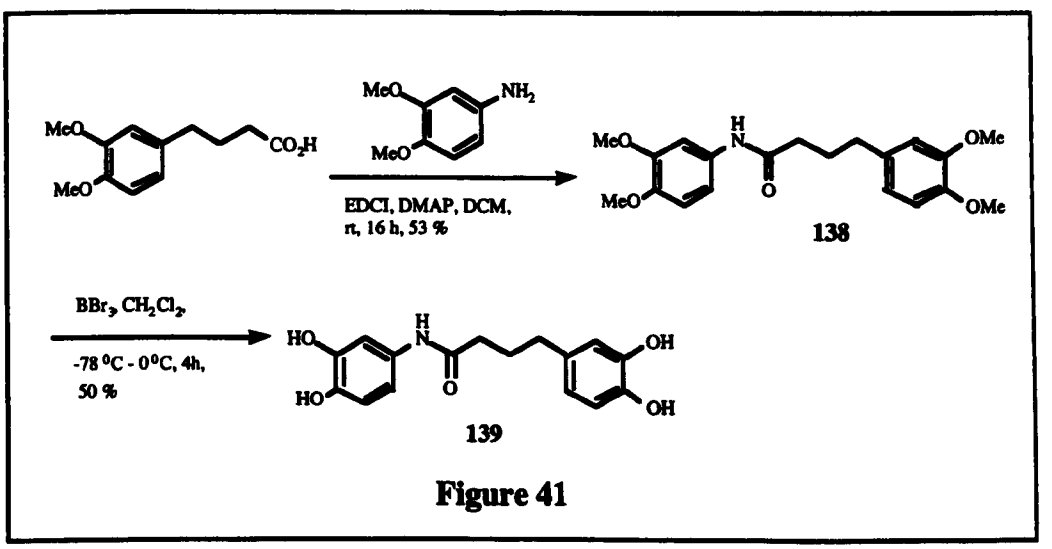


Figure 41

The intended amide coupling reaction of 3,4-dimethoxyaniline with 4-(3,4-dimethoxyphenyl)butyric acid following the procedure used in preparation of compounds **128**

and **129** proved unsuccessful. In an attempt to improve the efficiency of the reaction, EDCI was employed as the coupling reagent which has the advantage of facilitating the purification of the product as the urea by-product resulting from EDCI could be readily removed by an aqueous wash. Characteristic NH and carbonyl stretching bands observed at *ca* 3300 cm^{-1} and 1657 cm^{-1} respectively in the IR spectrum along with carbonyl resonance in the ^{13}C NMR spectrum were consistent with the expected compound from the successful amide coupling reaction. The ^{1}H NMR spectrum of compound **138** revealed a 2H triple triplet and two 2H triplets in the aliphatic region and three singlets for methoxy groups together with aromatic proton resonances. The proposed structure was further confirmed by ^{13}C NMR and accurate mass spectra. Deprotection of the methyl ether **138** was undertaken following the procedure employed in the preparation of compound **131** with the exception of the isolation. The demethylation was quenched in iced water and the reaction mixture was shaken for 30 minutes. A grey solid was observed to precipitate between the aqueous and the DCM layers. The grey solid was collected and crystallised from EtOAc-EtOH to furnish the dicatechol amide **139** as a brown solid in 50% yield. The accomplishment of demethylation in compound **138** was evident from the absence of methoxy proton resonances in the ^{1}H NMR spectrum along with the presence of the phenolic stretching band at *ca* 3400 cm^{-1} in the IR spectrum. The amide bond was proved to remain intact with characteristic absorption frequencies near 1650 cm^{-1} for the carbonyl bond and the NH stretching vibrations at *ca* 3400 cm^{-1} in the IR spectrum together with carbonyl resonance at *ca* δ 175 in the ^{13}C NMR spectrum. The NMR and the accurate mass spectra were consistent with the proposed structure.

7. 4 Synthesis of Analogues Containing Heterocycles

Isosteric interchanges are a common strategy used to determine the structure-activity relationships of a lead compound and its analogues. Among the five-membered heteroaromatic compounds containing one heteroatom, thiophene, with the least electronegative heteroatom, has the greatest aromaticity and is usually introduced into a lead structure as an isostere of a benzene ring. The preparation of a number of NDGA analogues containing thiophenes is described in this section.

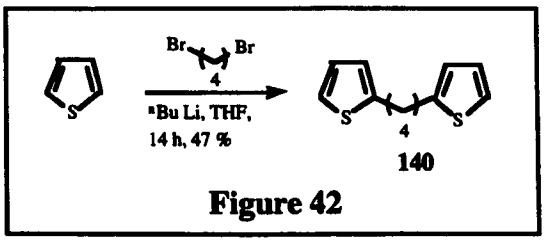


Figure 42

Koβmehl *et al.*[110] have reported the preparation of 1,4-di(2-thienyl)butane in 49% yield. We carried out the reaction following their procedure with a few modifications; prolonged reaction time and elevated temperature were employed to force the reaction to completion. The crude product was purified by chromatography affording compound **140** as a light yellow oil in 47% yield. The four methylene groups produced two 4H multiplets in the aliphatic region and the six thiophene proton resonances were recorded as three 2H multiplets in the aromatic region in the ^{1}H NMR spectrum of compound **140**.

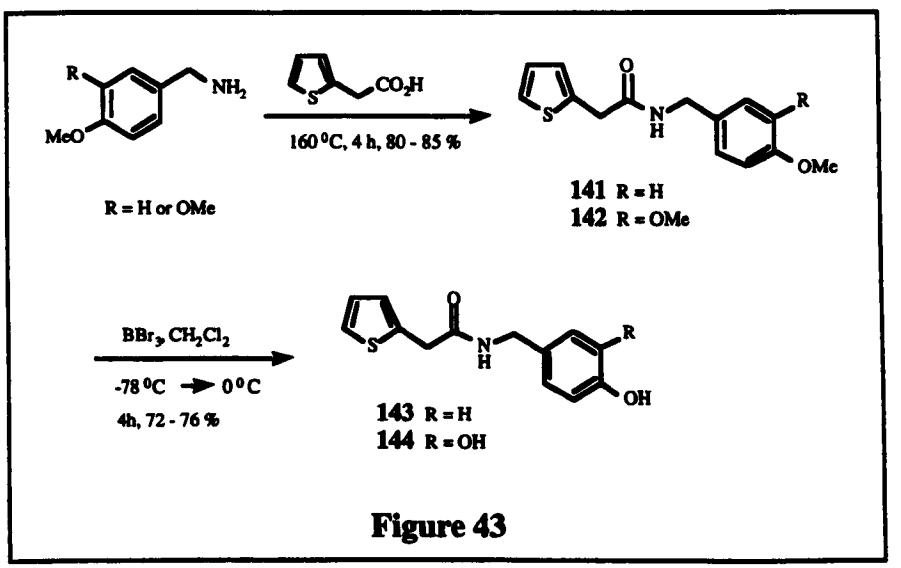


Figure 43

The preparations of compounds **141** and **142** were accomplished in a similar fashion to that described for compound **128**. Novel compounds **141** and **142** were fully characterised. The successful deprotection of the methyl ethers **141** and **142** were confirmed by the ^{1}H NMR

spectra with the absence of the methoxy proton resonance at *ca* δ 3.8 and the presence of the OH peaks at *ca* δ 8.9 together with the IR phenolic stretching band. The IR spectra of compounds **143** and **144** revealed characteristic absorption bands for NH and carbonyl functionalities indicating that the amide bonds remain intact while the carbonyl resonance at *ca* δ 170 in the ^{13}C NMR spectrum was also consistent with those findings. The accurate mass spectra of the novel compounds **143** and **144** were in agreement with the proposed structures.

7. 5 Attempted Synthesis of an Analogue Containing an Alkene with *Z* Geometry

As mentioned in the foregoing section preliminary work demonstrated that more rigid NDGA analogues are superior in biological activity by incorporating a rigid functional group such as an amide. This result prompted us to investigate an alternative tactic to introduce rigidity into the lead structure, and therefore compound **151** (Figure 46) was designed in which the amide group was replaced by a double bond. We intend to prepare an NDGA alkene analogue as a single geometrical isomer given the likely different biological activities of the *E*- and *Z*-isomers. The control of double-bond geometry in the preparation of alkenes is well documented. Schlosser's modification of the Wittig reaction provides an easy entry to *cis*-alkenes. Therefore we sought to prepare a NDGA alkene analogue with *Z*-geometry following Schlosser's method.[111]

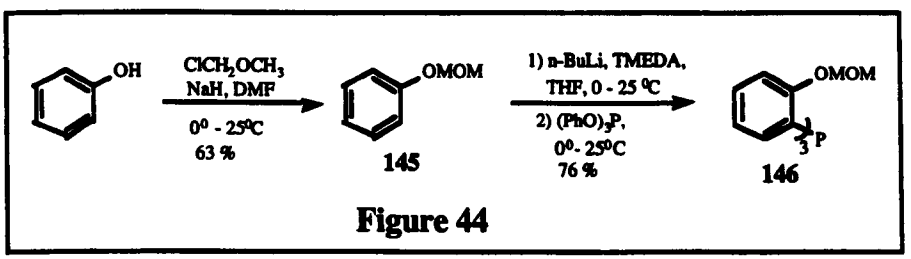


Figure 44

The phosphonium salt **150** was prepared via a five-step procedure as depicted in Figures 44 and 46. According to Schlosser's procedure the phosphine was prepared as follows. Conversion of phenol into the corresponding phenyl ether **145** was performed by treatment with sodium hydride and chloromethyl methyl ether in DMF affording **145** as a colourless oil in 63% yield. Two deshielded 3H and 2H singlets in the aliphatic region along with the aromatic proton resonances in the ^{1}H NMR spectrum confirmed the proposed structure. It was known that the introduction of the acetal function in compound **145** could facilitate the metalation of neighbouring positions required for the preparation of the phosphine **146**. Treatment of phenyl ether **145** with butyllithium at 0 ^{0}C in THF with TMEDA as cosolvent generated a 2-alkoxyphenyllithium intermediate which was then reacted with triphenyl phosphite to provide

the phosphine **146** in 76% yield. The melting point and the ^{1}H NMR spectrum for the known compound **146** were found to be consistent with those reported.[111]

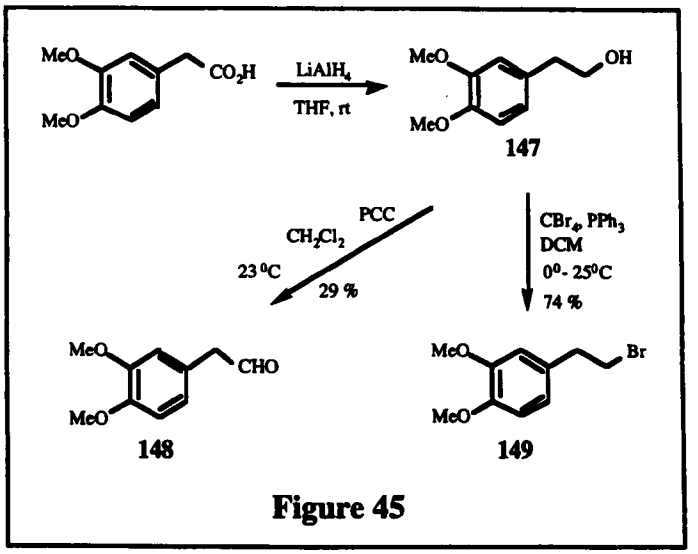


Figure 45

$LiAlH_4$ reduction of (3,4-dimethoxyphenyl)acetic acid in THF at rt produced the corresponding alcohol **147** which was then elaborated to the bromide **149** and the aldehyde **148**, respectively. Following the procedure of Kocienski *et al*[112] bromination of alcohol **147** with carbon tetrabromide and triphenylphosphine proceeded smoothly affording the bromide **149** in 74% yield while the oxidation of the alcohol **147** was surprisingly difficult. Attempted Swern oxidation of **147** gave a reaction mixture whose NMR spectra showed no traces of the desired aldehyde. Oxidation of **147** with PCC was carried out following the general procedure of Corey and Suggs.[113] The crude residue was purified by flash chromatography affording the aldehyde **148** as a colourless oil in 29% yield. The melting point for the known bromide **149** was identical with that reported[114] and the ^{1}H NMR spectrum of the two known compounds **148** and **149** were found to be in agreement with those reported.[115,114]

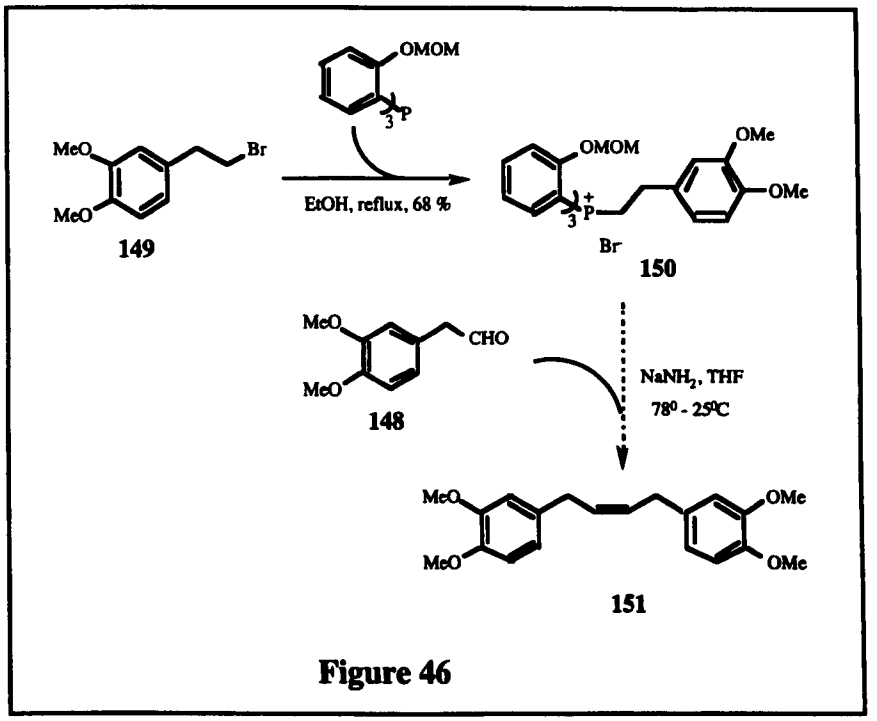


Figure 46

The substitution of the bromide **149** with the phosphine **146** in refluxing EtOH proceeded smoothly and provided the phosphonium salt **150** in 68% yield. The phosphonium salt **150** produced two multiplets in its ^1H NMR spectrum at δ 2.83 and 3.70 for the four methylene protons adjacent to the phosphorus atom which were different from those in the bromide **149** giving two triplets at δ 3.02 and 3.47. The characteristic acetal resonances appeared in the ^1H NMR spectrum as two singlets at δ 2.99 and 5.05. The proposed structure was confirmed by ^{13}C NMR and IR spectra. A parent ion at *m/z* 607 in the high resolution mass spectrum provided further support for these findings. The attempted Wittig reaction employing Schlosser's procedure was unsuccessful because the ylid failed to be generated from the phosphonium salt. The reaction using $NaNH_2$ was carried on a 1.17 mmol scale, *i.e.* 0.04 g $NaNH_2$ was needed. Practical difficulty was encountered when 0.04 g chemical was weighed in a drying box due to the pressure changing, and also the purity of the $NaNH_2$ in a tiny amount was suspicious. Lack of time prevented further work in this area.

7. 6 Biological Test Results

The compounds synthesised in this project were assessed for their inhibitory potential on small cell lung cancer (SCLC) cells *in vitro* by Prof. Michael Seckl at the Medical Oncology Department of Hammersmith Hospital, London. The preliminary test data are shown in Table 3.

7. 6. 1 Method of Biological Assay[8]

Cell Culture: SCLC cell lines H-69 were maintained in RPMI 1640 supplemented with 10% (v/v) foetal bovine serum (heat inactivated at 57 °C for 1 hour) in a humidified atmosphere of 10% CO_2/90% air at 37 ^{0}C. They were passaged every 7 days. For experimental purposes, the cells were grown in HITESA which consisted of RPMI 1640 supplemented with 10 nM hydrocortisone, 5 μg/ml insulin, 10 μg/ml transferrin, 10nM oestradiol, 30nM selenium and 0.25% bovine serum albumin.

Liquid Culture Assay: SCLC cells, 3-5 days post passage, were washed and resuspended in HITESA. Cells were then aliquoted in 24 well Falcon plates at a density of 50,000 cells in 1 ml HITESA in the presence or absence of increasing concentrations of NDGA analogues. One and two weeks later, cell number was determined from a minimum of 3 wells per condition using a Coulter counter, after cell clumps were disaggregated by passing the cell suspension 5 times through a 19 and subsequently 21 gauge needle. These times were chosen to coincide with log phase and plateau phase growth of the cells.

7. 6. 2 Results and Discussion

The C_3-bridged bisphenol analogue **115** is surprisingly about as active as NDGA and much more potent than its counterpart C_3-bridged biscatechol analogue which had shown no inhibitory activity against the H-69 SCLC cell line.[8] However the C_4-bridged dicatechol analogue **126** is the most active compound and is ten times more active than NDGA. The C_4-bridged tetramethoxy analogue **124** is also surprisingly active as it had been thought that the free hydroxy groups were obligatory. The tetramethoxy compound **124** may exhibit its inhibitory potential *via* a different mechanism, and this needs further investigation. The C_4-bridged bis(trihydroxy) analogue **127** shows no increase in activity arising from the presence of the extra hydroxy groups. The biscatecholamide **131** is nearly equipotent to analogue **126**, therefore compound **131** is a new lead compound. Thioamide **132** and its amide counterpart **130** were both found to be inactive. The bisphenolic amide **135** is more active than **130** but it does appear that the presence of catechol groups is necessary to increase the activity. The dihydroxy analogue **144** containing thiophene is less active than NDGA whilst the monophenolic analogue **143** and the dithiophene compound **140** have no appreciable activity. This indicates that the presence of catechol groups is obligatory for good activity of amide analogues.

In summary compounds **126** and **131** have been identified as two new lead compounds which are ten times more active than NDGA when tested against the H-69 small cell lung cancer cell line. Both new lead compounds possess two catechol groups joined by a C_4 bridge or a 4-

atom amide link. Part of the above results have already been published,[116] although we still await the biological results of the amide analogues **137** and **139** with five atom bridges.

NDGA and its analogues are believed to exert their inhibition of SCLC cell growth by targeting several signalling systems like SCF and its receptor *c-kit*, lipoxygenases and the PDGF receptor. The precise relationship between the inhibitory effect of NDGA on lipoxygenases and the other two signalling systems has not been elucidated. Inhibition of several targets in the SCLC cell line gives NDGA and its analogues superior efficacy, and this approach might provide a better strategy for developing effective anticancer agents than having a single target.

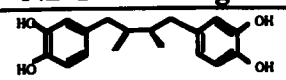
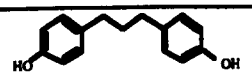
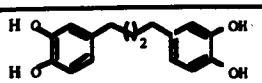
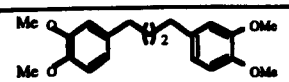
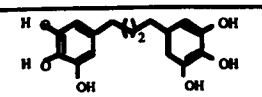
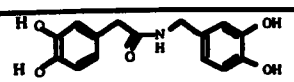
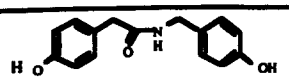
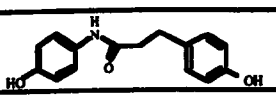
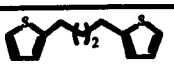
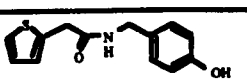
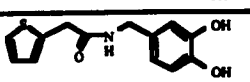
NDGA Analogue	Compound	IC50 (μmol)
	NDGA	3.5
Bisphenol derivative		
	**115**	3.5
Biscatechol derivatives		
	**126**	0.3
	**124**	3
	**127**	6.0
Amide analogues		
	**131**	0.8
	**130**	>30
	**132**	>30
	**135**	6.9
Heterocyclic analogues		
	**140**	>30
	**143**	>30
	**144**	15

Table 3

CHAPTER 8

Solubilisation of NDGA and Analogues

Preliminary work on the synthesis of NDGA analogues revealed several compounds that have improved potency over NDGA. We sought to transform those promising candidates into their water soluble forms as greater solubility is essential for *in vivo* work.

8. 1 Synthesis of a Tetraglycyl Ester

1,4-Bis-(3,4-dihydroxyphenyl)butane **126** is the most active analogue identified from the structural modification work. A water soluble tetraglycyl salt of this compound was synthesised for *in vivo* work.

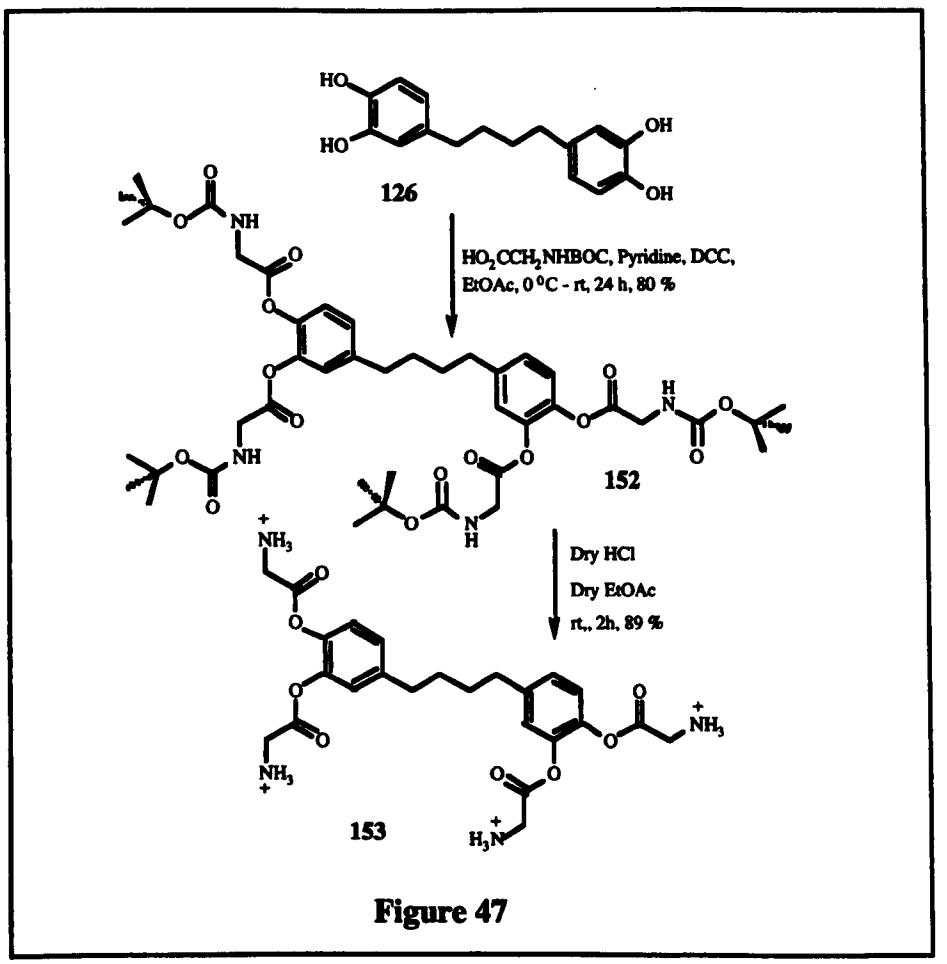


Figure 47

BOC protected glycine was esterified with the dicatecholbutane **126** utilising DCC and pyridine as coupling reagents to install the four ester bonds in compound **152** (Figure 47). The

presence of the four BOC-glycine moieties in compound **152** was evident from the NMR spectra with the ^{1}H NMR spectrum showing a 36H multiplet at δ 1.38 for the four tert-butyl groups and a deshielded 8H multiplet at δ 4.04 for the four methylene groups adjacent to the amino groups. The ^{13}C NMR spectrum contained carbonyl resonances at δ 157 and 169 for the carbamate and ester functionalities respectively which were further confirmed with characteristic absorptions at 1681 and 1783 cm^{-1}, respectively, in the IR spectrum. The successful installation of all four ester functionalities was also confirmed by an observed peak in the mass spectrum at *m/z* 925 for $(M+Na)^{+}$ run in the FAB+ mode. Deprotection of the amino groups with subsequent formation of the HCl salt was achieved by passing dry HCl through a cold solution of compound **152** in anhydrous EtOAc. After three minutes the amine salt **153** precipitated and the reaction mixture was allowed to warm to rt and stirred for a further two hours. The four ester functionalities proved inert to these reaction conditions. The ^{1}H NMR spectrum of compound **153** showed the deshielded 8H multiplet at δ 4.22 for the four glycyl methylene groups while the removal of all four tert-butyl groups was evident from the absence of the large peaks at δ 1.38 in the ^{1}H NMR spectrum. An ester absorption at 1774 cm^{-1} was observed in the IR spectrum and a $(M+H)^{+}$ peak at *m/z* 503 in the mass spectrum was consistent with the proposed structure.

8. 2 Synthesis of Amine Salts

A number of aromatic amines and analogues containing polyamines were prepared with a view to introducing solubilising ammonium groups into the structures of some of the active compounds.

8. 2. 1 Synthesis of C4-Bridged Aromatic Amine Salts

In addition to the glycyl HCl salt **153**, a water soluble form of the most potent compound **126** was prepared with solubilising ammonium groups directly on the aromatic rings.

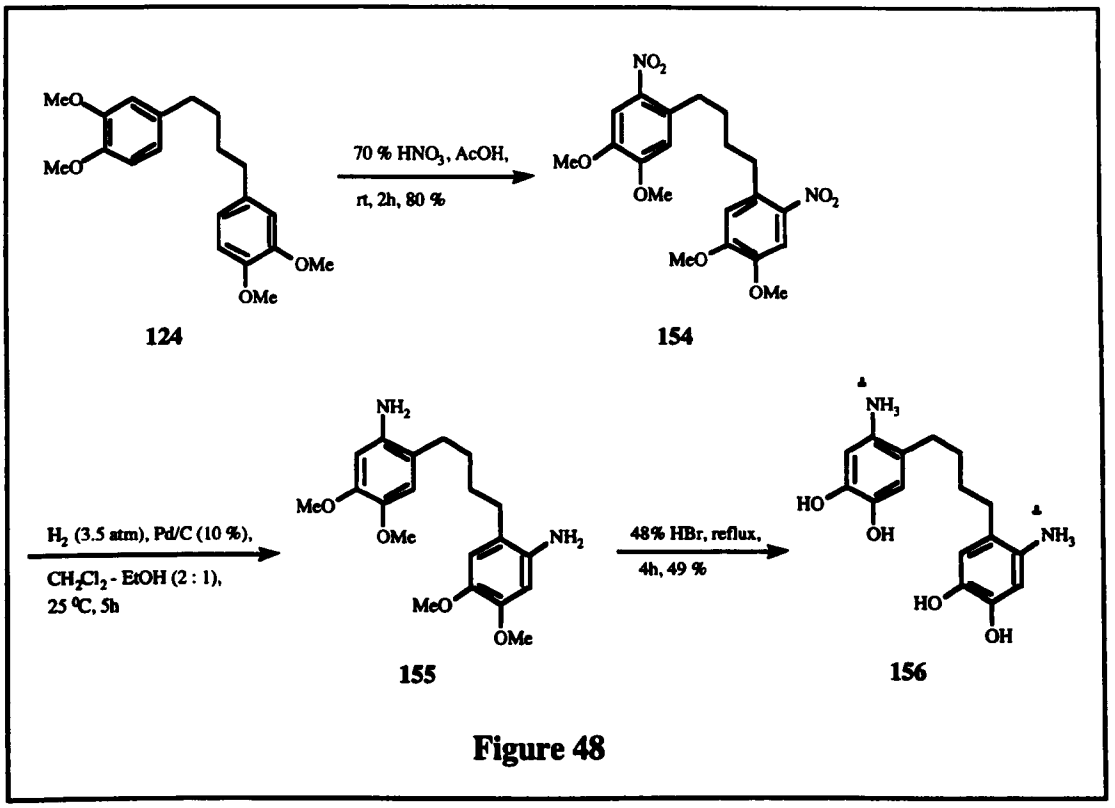


Figure 48

Nitration of compound **124** with 70% HNO_3 in glacial AcOH provided the nitrobenzene **154** in 89% yield. ^1H NMR spectroscopy distinguished the nitrobenzene **154** from compound **124** by the integral of the aromatic protons which was reduced from 6H to 4H. The regiochemistry of the addition was apparent from the ^1H NMR spectrum with only two singlets observed in the aromatic region confirming the introduction of the nitro groups in the 6-positions. This novel nitro compound **154** was further characterised by ^{13}C NMR and IR spectroscopy. A parent ion at *m/z* 420 in the mass spectrum together with the combustion analysis were consistent with the proposed structure. Catalytic hydrogenation of the nitrobenzene **154** at 3.5 bar over palladium in a mixed solvent of DCM and EtOH afforded the aromatic amine **155** as a dark green solid. The completion of the reduction of the nitro groups was evident from the ^1H NMR spectrum. A broad 4H singlet at δ 4.57 was observed for the two NH_2 groups in compound **155** and the position of the aromatic singlet for the H-5 proton shifted from δ 7.52 to δ 6.32 as a result of being *ortho* to two electron donating groups. The subsequent demethylation of the crude amine **155** with 48% HBr proceeded smoothly affording the target compound **156** in good yield. The removal of the methyl groups was apparent from the absence of the methyl singlets at *ca.* δ 3.6 in the ^1H NMR spectrum. The identity of the water soluble amine salt **156** was further confirmed by ^{13}C NMR spectrum with the expected eight resonances. The IR spectrum of the amine salt **156** and a peak recorded at *m/z* 305 in the accurate mass spectrum for $[M(-2HBr)+H]^+$ run in the FAB+mode provided further support for those findings.

8. 2. 2 Synthesis of NDGA Amine Salts

As mentioned above NDGA is poorly soluble in water and therefore it is one of our objectives to convert NDGA into a water soluble form. The synthesis of a water soluble NDGA amine salt **158** is depicted in Figure 49.

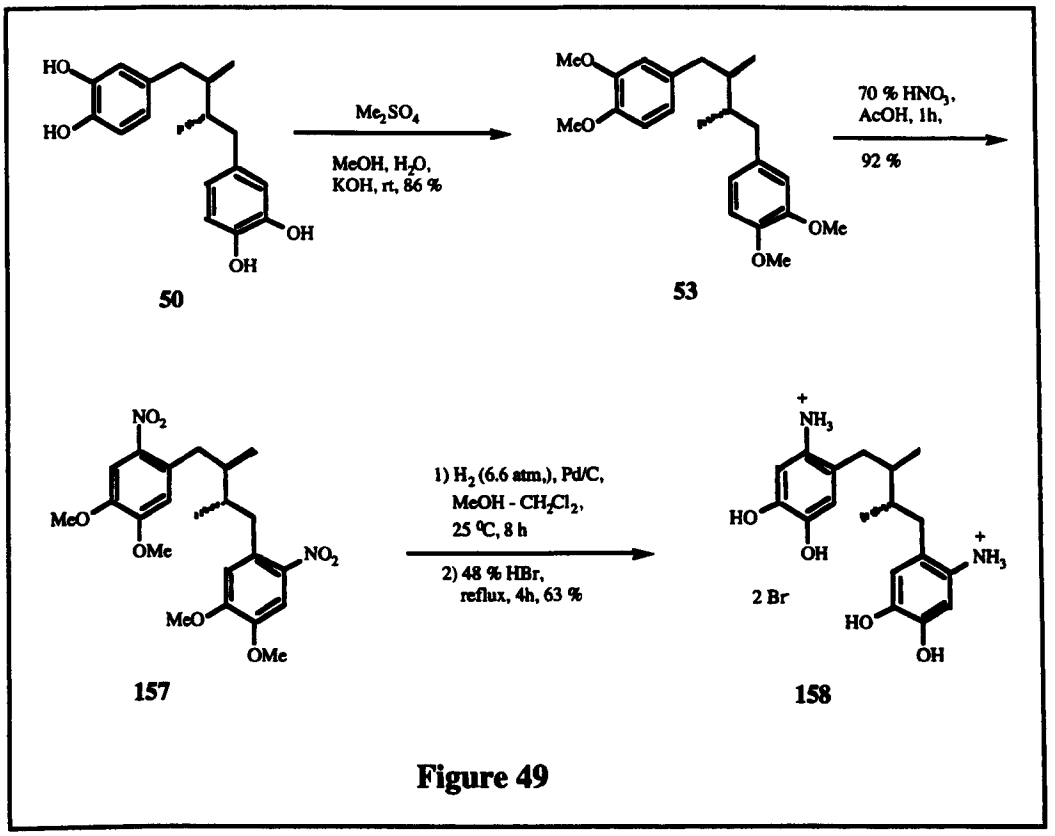


Figure 49

The preparation of the NDGA amine salt **158** was achieved by a four step synthesis following the protocol of McDonald.[8] Methylation of commercially available NDGA with dimethyl sulfate produced the tetramethyl ether **53** in good yield. The product was obtained as a yellow solid giving a melting point identical with that reported.[62] The presence of the four methoxy groups was evident from the ^1H NMR spectrum with a 12H singlet at δ 3.78. The subsequent nitration of the tetramethyl ether **53** was undertaken following the procedure employed in the preparation of the nitro compound **154** in high yield. The melting point and the ^1H NMR spectrum of compound **157** were found to be consistent with those reported.[8] Hydrogenation of the nitrobenzene **157** to the corresponding aromatic amine was carried out under 6.6 bar of hydrogen over palladium in a mixed solvent of DCM and MeOH and then, without purification, demethylation with refluxing 48% HBr provided the NDGA amine salt **158** in good yield. The nitrobenzene **157** and the NDGA amine salt **158** were identified as

known compounds and the data recorded for these two compounds were found to be consistent with the findings reported in the literature.[8]

8. 2. 3 Synthesis of C6-Bridged Aromatic Amine Salts

The C6-bridged analogue, 1,6-bis-(3,4-dihydroxyphenyl)hexane, was found to be equipotent with NDGA. Thus we set about the synthesis of an amine salt of this analogue which is shown in Figure 50.

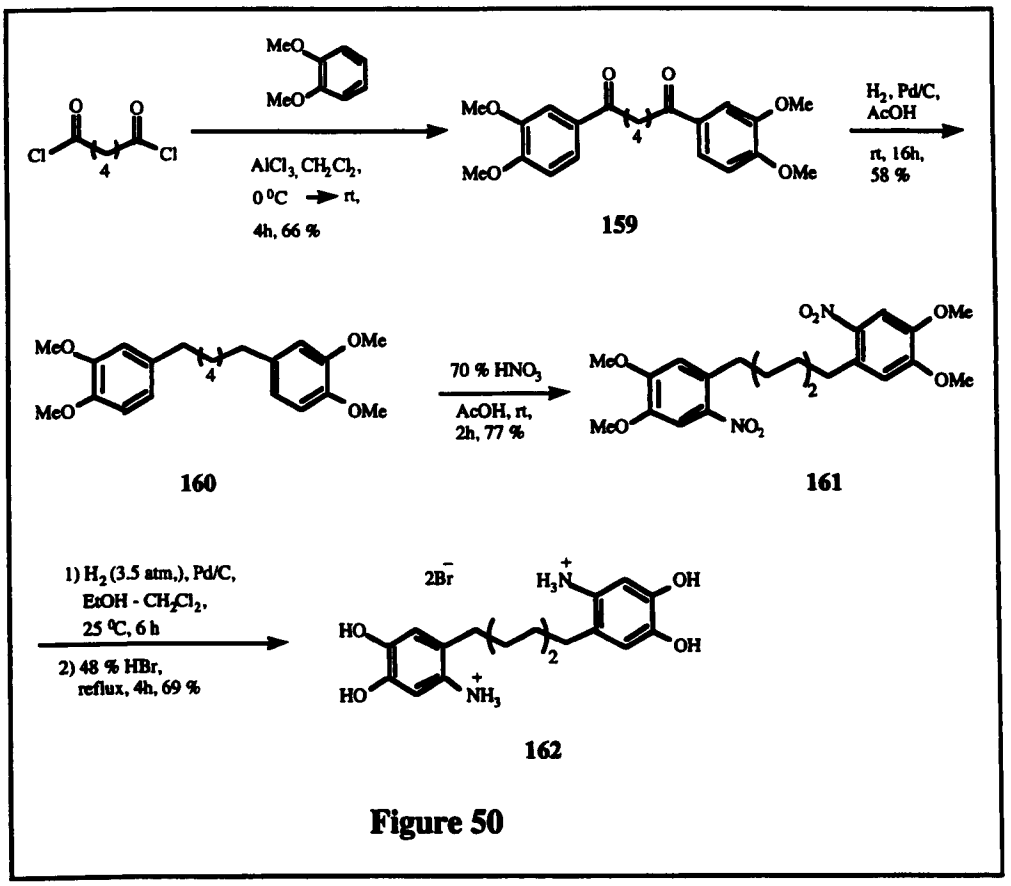


Figure 50

Friedel-Crafts acylation of veratrol with adipoyl dichloride was undertaken according to the procedure of Fliedner *et al.*[117] using anhydrous chloroform in place of tetrachloroethane. The product was obtained as pale yellow crystals in 66% yield giving a melting point identical with that reported.[117] The regiochemistry of the addition was identified by the ^{1}H NMR spectrum with two 1H doublets and a 1H doublet of doublets in the aromatic region. Diketone **159** was then subjected to hydrogenation over palladium in AcOH to furnish the diarylhexane **160** in moderate yield. The melting point and the ^{1}H NMR spectrum of this yellow crystalline product **160** were found to be consistent with the literature findings.[117] The subsequent nitration of the diarylhexane **160** followed by hydrogenation and demethylation were carried out in an analogous fashion to that used in the synthesis of C4-bridged aromatic amine **156**. The ^{1}H NMR spectrum of the novel nitro compound **161** revealed three 4H multiplets in the

aliphatic region for the six carbon bridge along with two 6H singlets for the four methoxy groups, and the aromatic protons were observed as two 2H singlets at δ 6.64 and 7.50. The ^{13}C NMR spectrum showed the expected nine resonances and the proposed structure of the nitro compound was further confirmed by its IR spectrum and combustion analysis. A parent ion at *m/z* 448 in the mass spectrum was also in agreement with these findings. The identity of the water soluble amine salt **162** was confirmed by the ^{1}H NMR spectrum with the characteristic appearance of the benzylic proton resonance at δ 2.41 and two 4H multiplets in the aliphatic region together with two singlets in the aromatic region. The expected nine peaks were recorded in the ^{13}C NMR spectrum and the IR spectrum revealed characteristic phenolic stretching bands at *ca* 3600 cm^{-1}. An $(M+H)^+$ peak at *m/z* 333 in the high resolution mass spectrum provided further support for the proposed structure.

8. 2. 4 Synthesis of an Analogue with an NH Group in the Chain

As previously mentioned, the preliminary work produced a new lead compound, 1,4-bis-(3,4-dihydrophenyl)butane **126**. We envisaged that isosteric replacement of one of the bridging methylene moieties with an ammonium group could afford a water soluble derivative as its HBr salt. The three step synthetic route developed by McDonald[8] is illustrated in Figure 51.

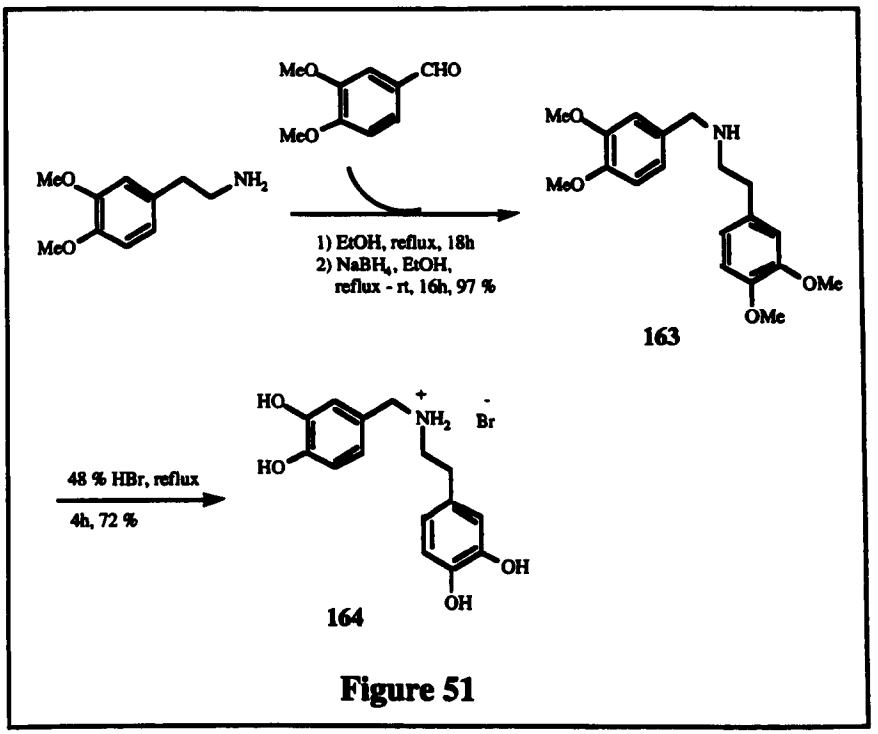


Figure 51

Condensation of 3,4-dimethoxybenzaldehyde with 2-(3,4-dimethoxyphenyl)ethylamine was performed in refluxing EtOH for 16 hours and then, without purification, the intermediate imine was subjected to a $NaBH_4$ reduction to furnish the secondary amine **163** in high yield. The product **163** was obtained as a yellow solid that was identified as a known compound and

the data recorded for this compound were found to be consistent with the findings reported in the literature.[8] Demethylation of the amine **163** with 48% HBr proceeded smoothly to provide the dicatecholamine salt **164** as tan coloured crystals giving a melting point and a ^{1}H NMR spectrum identical with those reported.[8] The proposed structure was further confirmed by the ^{13}C NMR and IR spectrum together with the high resolution mass spectrum.

8. 3 Synthesis of NDGA Analogues Containing Polyamines

Polyamines are known to be implicated in the regulation of cell growth, proliferation and differentiation through interactions with a variety of cellular macromolecules,[118] and furthermore researchers have suggested that polyamines may also play a role in the cellular process as "second messagers".[118] Interference with polyamine biosynthesis may thus provide access to anticancer treatment. We intended to introduce a polyamine component into the structure of a lead compound to produce a number of water soluble analogues of NDGA as their HBr salts.

8. 3. 1 Synthesis of an Analogue Containing Diaminobutane

Samejima *et al*[119] have reported the synthesis of a range of polyamines using KF-Celite to promote the efficiency of alkylations under mild conditions. Therefore we decided to apply their reaction conditions to the alkylation of the secondary amine **163**. A mixture of the amine **163** and one equivalent of N-(4-bromobutyl)phthalimide in acetonitrile was heated at reflux for 48 hours. The reaction proved sluggish with the TLC analysis showing only a trace of product. To drive the reaction to completion, three equivalents of 4-bromobutylphthalimide were employed and a catalytic amount of triethylamine was used. The reaction mixture was heated at reflux for four days and the starting material could hardly be seen on a TLC plate. The crude product was purified by chromatography affording the diamine as a yellow oil in only 35% yield. To improve the efficiency of this reaction, attention was turned to an alternative literature method. Ozaki *et al*[120] have reported the synthesis of several diamines via alkylations of secondary amines with *N*-(2-bromoethyl)phthalimide or *N*-(4-bromobutyl)phthalimide in the presence of K_2CO_3 with DMF as solvent. Therefore we chose to utilise this method in our synthesis of the 1,4-diaminobutane **167** which is shown in Figure 52.

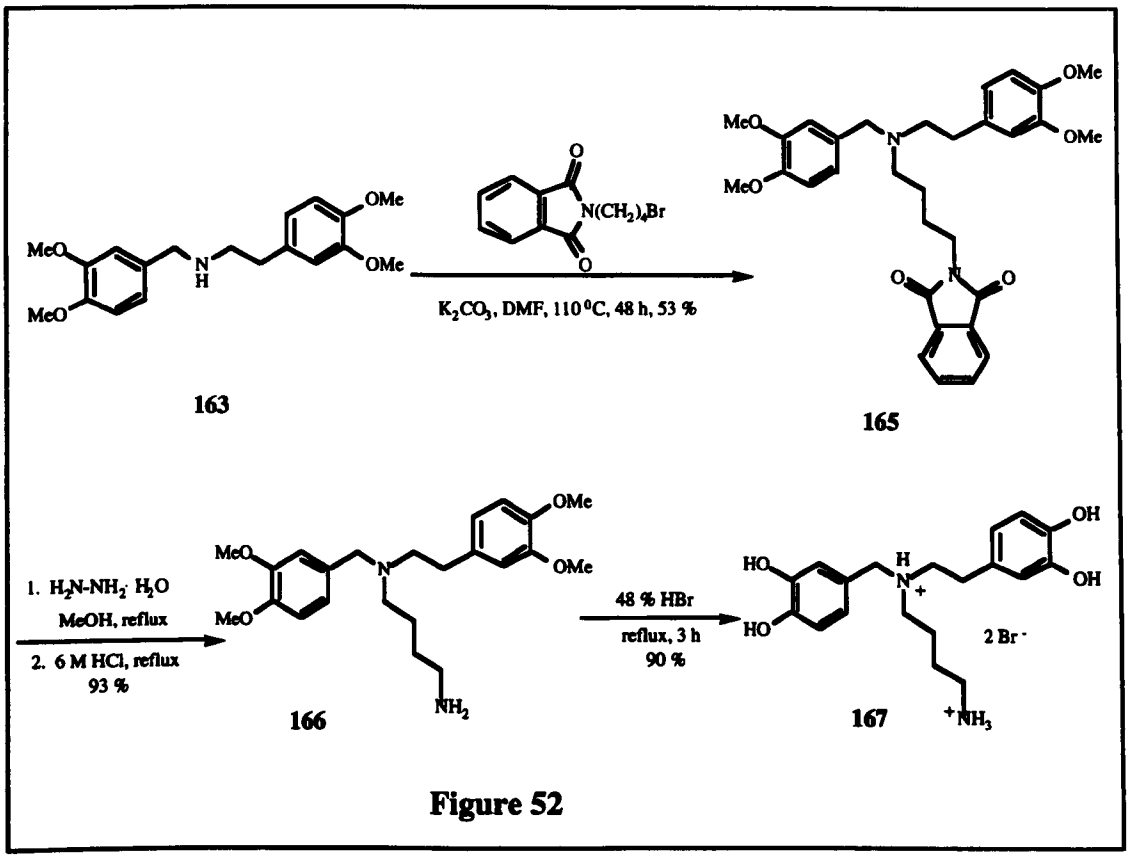


Figure 52

The method of Ozaki *et al*[120] *was* modified as follows. A mixture of the secondary amine **163**, *N*-(4-bromobutyl)phthalimide and potassium carbonate was heated at 110 ^{0}C in DMF for 48 hours. TLC analysis showed a trace of starting material and a product spot that was stained bright orange by Dragendorff reagent. The crude residue was purified by chromatography to furnish the product **165** as a light yellow oil in 53% yield. The conversion of the secondary amine **163** into the tertiary amine **165** was verified by NMR spectroscopy. The phthalimide protons provided a prominent feature in the ^{1}H NMR spectrum as two 2H multiplets at δ 7.6 and 7.7. The benzylic protons adjacent to the nitrogen atom were observed as a 2H singlet at δ 3.48 and a 4H multiplet was recorded for the two methylene groups between the protected catechol moiety and the nitrogen atom. The four carbon chain between the two nitrogen atoms was identified by two 2H multiplets and two 2H triplets in the aliphatic region and the twelve methoxy protons came to resonance at *ca.* δ 3.7. The characteristic appearance of the imide stretching band was observed at 1720-1770 cm^{-1} in the IR spectrum and the ^{13}C NMR spectrum supported these findings with the carbonyl carbon signals at *ca.* δ 167. The structural assignment of compound **165** was further confirmed by accurate mass spectrometry with a peak at *m/z* 555 for $(M+Na)^+$ run in the FAB+ mode.

The subsequent deprotection of the amino group in compound **165** with hydrazine hydrate was carried out in refluxing MeOH and, in turn, in refluxing 6M HCl according to the procedure described by Ozaki *et al.*[120] to produce the diamine **166** in high yield. The completion of the deprotection was apparent from the absence of the two characteristic

multiplets in the ^1H NMR spectrum at δ 7.63 and 7.75 for the phthalimide protons. On the other hand the diamine **166** produced a triplet at δ 2.42 for the methylene group adjacent to the primary amine group which was previously found to resonate at δ 3.58 when next to the imide group. Moreover, the primary amine protons of the diamine **166** were observed as a broad 2H singlet at δ 1.69.

Finally the demethylation of compound **166** was performed in refluxing 48% HBr to yield the NDGA analogue as a diamine HBr salt **167** in good yield. The ^1H and ^{13}C NMR spectra of compound **167** are shown in Appendixes 5 and 6. The NMR spectrum of the diamine HBr salt **167** showed the absence of the signals for the methoxy groups confirming the removal of the four methyl groups. The diamine HBr salt **167** was further characterised by its IR spectrum with phenolic and primary ammonium bands at 3609 and 2402 cm^{-1} respectively, together with an aromatic band at 1609 cm^{-1}. A $(M+H)^+$ peak at *m/z* 347 in the high resolution mass spectrum was consistent with the proposed structure.

8. 3. 2 Synthesis of an Analogue Containing Diaminopropane

The synthetic route for the preparation of the NDGA analogue **170** containing diaminopropane is illustrated in Figure 53.

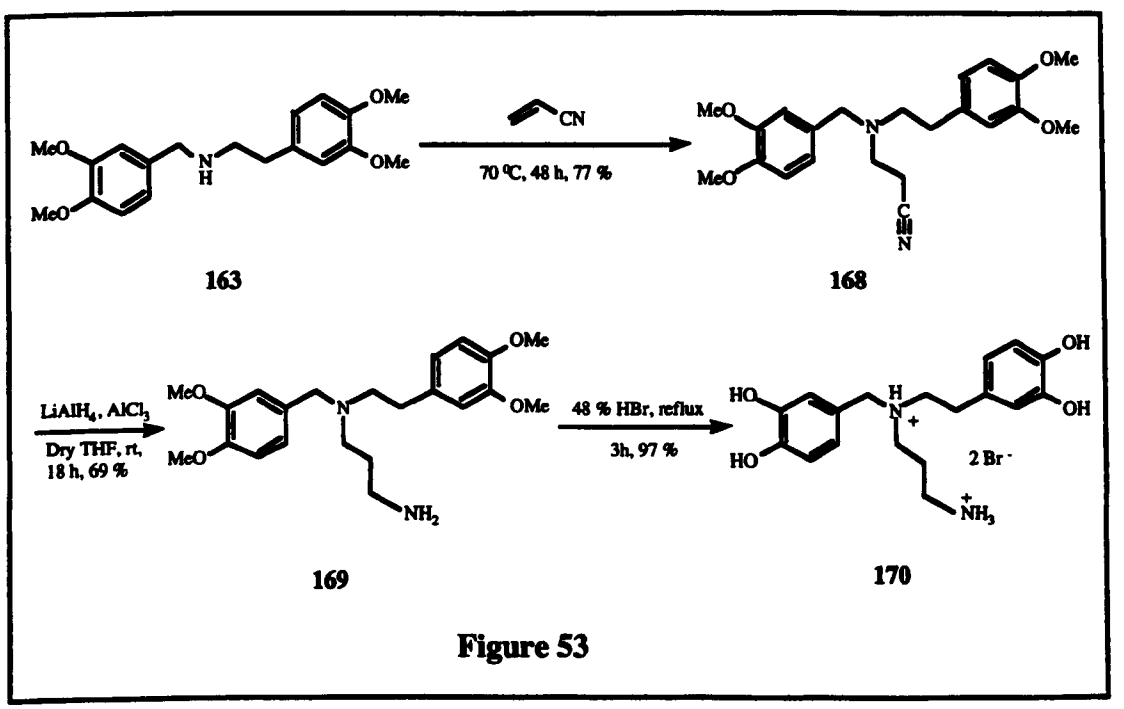


Figure 53

The synthesis started with a Michael addition of the amine **163** to acrylonitrile following a modified version of the method used by Slater.[121] A mixture of the secondary amine **163** and acrylonitrile was heated at 60-70 ^0C in a sealed tube for 48 hours and the starting material could hardly be seen on a TLC plate. The crude residue was then purified by column chromatography

to produce the nitrile **168** as a colourless oil in good yield. The identity of the nitrile **168** was verified by NMR, IR and accurate mass spectra. A 2H triplet at δ 2.30 was observed for the methylene group adjacent to the cyanide group and a 2H singlet at δ 3.56 was recorded for the benzylic protons next to the tertiary amine group while the remaining methylene protons came into resonance as a 6H multiplet at δ 2.72. The methoxy groups produced four singlets at *ca.* δ 3.8 and the characteristic aromatic proton resonances were found as a 6H multiplet at δ 6.70. The expected twenty-two resonances were recorded in the ^{13}C NMR spectrum including the cyanide resonance at δ 119. The important spectroscopic features of compound **168** in the IR spectrum were the characteristic nitrile band at 2245 cm^{-1} and an aromatic band at 1606 cm^{-1}. A parent ion at *m/z* 384 in the mass spectrum provided further support for the structural assignment.

Reduction of the nitrile **168** with a mixture of $LiAlH_4$ and $AlCl_3$ in THF according to the procedure of Slater[121] afforded the primary amine **169** as a light yellow oil in good yield. The completion of the reduction was evident with the appearance of a 2H broad singlet in the ^1H NMR spectrum at δ 1.29 characteristic of the amino group along with a 2H triplet at δ 2.46 for the methylene group adjacent to the amino group. Furthermore, the diamine **169** exhibited a 2H triple triplet at δ 1.54 for the central methylene group on the propyl moiety that was previously found to resonate at δ 2.30 when adjacent to the cyanide group. The IR spectrum provided further support for those findings with the presence of amino stretches at 3370 cm^{-1} and the absence of the nitrile band at 2245 cm^{-1}. The ^{13}C NMR spectrum revealed the expected twenty-two peaks and the proposed structure was further confirmed with a $(M+H)^+$ peak at *m/z* 389 in the high resolution mass spectrum.

The diamine **169** was finally demethylated with 48% HBr to yield the diamine salt **170** in high yield. The demethylation was apparent from the NMR spectra of compound **170** with the absence of the signals for the four methoxy groups while the IR spectrum showed the presence of the characteristic phenol stretches at 3570 cm^{-1}. The structure was confirmed by accurate mass spectrometry with an observed peak at *m/z* 333 in the FAB+ mode.

8. 3. 3 Synthesis of a Diamine Bridged Analogue

In designing NDGA diamine analogue, the diimine **171**, which had been prepared by Kliegman and Barnes,[122] was viewed as an appropriate precursor, and indeed the diamine analogue **173** was obtained therefrom by several functional group manipulations.

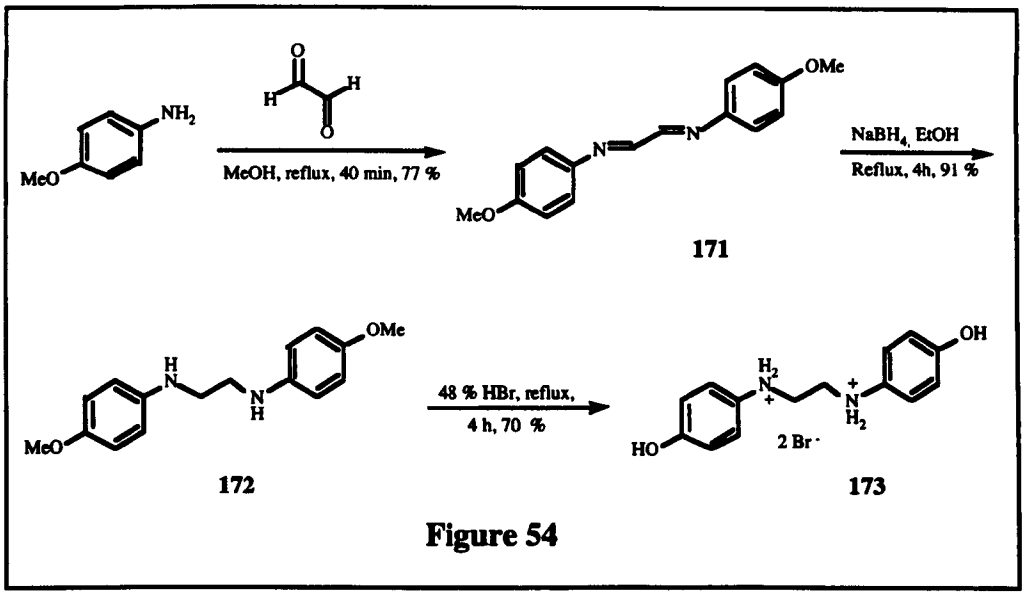


Figure 54

The condensation of glyoxal with *p*-anisidine was performed in refluxing MeOH according to the procedure of Kliegman and Barnes[122] to furnish the diimine **171** as bright yellow crystals giving a melting point and ^{1}H NMR spectrum in agreement with those previously cited in the literature.[122] Reduction of the diimine **171** with sodium borohydride in refluxing EtOH afforded the diamine **172** as a yellow solid in 91% yield. The characteristic appearance of the aromatic proton resonances for the two *para* disubstituted benzene rings was found in the ^{1}H NMR spectrum of the diamine **172** as an AA'BB' system at δ 6.55 and 6.72. Furthermore the diamine **172** provided a 6H singlet at δ 3.68 characteristic of the two methoxy groups along with another 4H singlet at δ 3.25 for the two methylene groups. The signals for the two NH groups collapsed to a 4H singlet after shaking the sample with D_2O. The expected six peaks were observed in the ^{13}C NMR spectrum, and the IR spectrum revealed a NH stretching band at 3274 cm^{-1} along with a characteristic absorption for the benzene ring at 1593 cm^{-1}. A parent ion at *m/z* 272 that was observed in the mass spectrum was consistent with the structural assignment.

The diamine **172** was then subjected to demethylation with 48% HBr to afford the analogue **173** as its HBr salt. The removal of the methyl groups was confirmed by the ^{1}H NMR spectrum with no sign of methyl ether functionality and the diamine salt **173** was further characterised by its ^{13}C NMR and IR spectra together with a combustion analysis. The structural assignment was also verified by accurate mass spectrometry with a peak at *m/z* 245 run in the FAB+ mode.

8. 3. 4 Attempted Synthesis of an Analogue containing Spermidine

Since we had an analogue containing a diamine in hand, we sought to extend the diamine backbone to incorporate a spermidine moiety in the NDGA analogue.

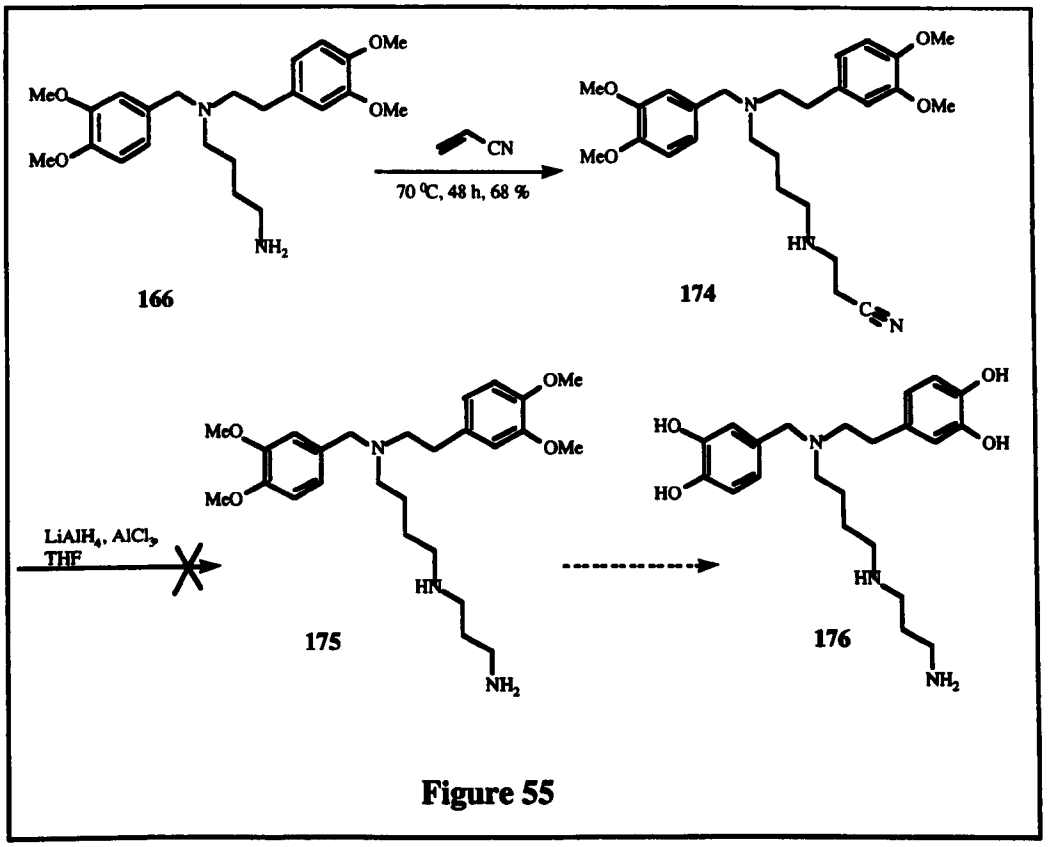


Figure 55

Michael addition of the diamine **166** to acrylonitrile according to the procedure of Slater[121] afforded the nitrile **174** as a light yellow oil in 68% yield. The extension of the diamine backbone was confirmed by the ^1H NMR spectrum with each integral of the two multiplets at δ 2.70 and δ 2.35 increased by 2H and the IR spectrum showed the characteristic nitrile stretching band. The subsequent reduction of the nitrile **174** with a mixture of $LiAlH_4$ and $AlCl_3$ led to the cleavage of the carbon-nitrogen bond of the tertiary amine moiety. Alternative ways to reduce the cyanide group to an amine group could be tried in future work. The cyanide group may be hydrogenated over Rh/Al_2O_3 or Rh/C under mild conditions to minimise cleavage of the benzylic C-N band, and reduction of the nitrile by borane hydride reagents could be considered.[123]

8.4 Biological Results and Discussion

The biological evaluation data for the NDGA analogues are shown in Table 4. All of the new compounds were shown to be much more soluble in water (> 1 mM) than NDGA as expected. The activity of the tetraglycyl tetrahydrochloride salt against SCLC cell line H-69 encouragingly matched its parent compound, the new lead compound 1,4-bis-(3,4-dihydroxyphenyl)butane **126** in *vitro*. The diamine salt **156** of the new lead compound was found to be as active as NDGA at one week but less potent after two weeks in culture. The diamine salt analogue **158** of NDGA appeared to have slightly more activity than NDGA and

the diamine salt **162** of the 1,6-bis-(3,4-dihydroxyphenyl)hexane was found to be slightly less potent than its parent compound. The C_4-bridged analogue **164** containing an amine salt in the bridge was found to be significantly less active than NDGA and adding an aminopropyl group in **170** reduced the activity further. The diamine salt **173** was also inactive. We still await the results of the other analogues containing amine salts.

In conclusion the tetraglycyl tetrahydrochloride salt of the new lead compound **126** was successfully synthesised and is now available for testing on animals. NDGA diamine salt **158** displays potency in the same region as NDGA. However the introduction of two amino groups on the aromatic rings of the new lead compound **126** produces a compound 10 times less active than its parent.

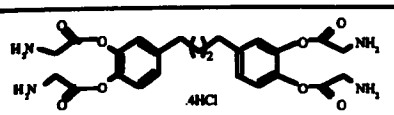
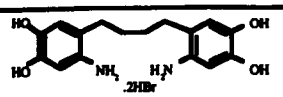
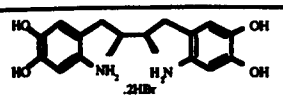
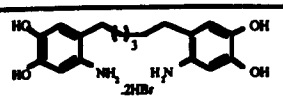
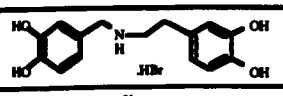
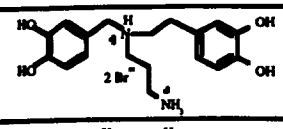
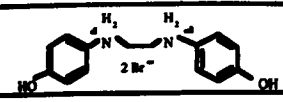
NDGA Analogue	Number	IC_{50} (μmol)
	**NDGA**	3.5
	**153**	0.3
	**156**	3.5
	**158**	2.0
	**162**	8.0
	**164**	27
	**170**	>100
	**173**	>100

Table 4

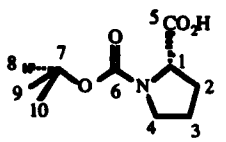
CHAPTER 9

Experimental

Reagents were purchased from Aldrich Chemical Company (Gillingham, UK) or Lancaster Synthesis (UK) and were used without further purification. Organic solvents were obtained from Rhône-Poulenc Rorer and were dried, as necessary, using the procedures described in Leonard, Lygo and Procter.[145] Melting points were determined in open capillaries using Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DPX/400 spectrometer operating at 400 MHz and 100 MHz respectively. ^{13}C NMR spectra were assigned with the aid of Distortionless Enhancement by Polarisation Transfer (DEPT)-edited spectra and ^{1}H NMR coupling constants were calculated and reported in Hz. The numbering schemes shown are used for ease of assigning the NMR spectra and do not refer to the system of nomenclature. Thin layer chromatography was performed using Merck aluminium-backed silica plates of 0.25 mm thickness and chromatograms were visualised using UV conditions at 254nm or using a variety of common stains prepared using the procedures described in Leonard, Lygo and Procter.[124] Column chromatography was carried out on silica gel (particle size 70-230 mesh). Mass spectra (MS) were recorded on AEI MS12 or MS902 spectrometers using the electron-impact ionisation (EI) mode or, if stated, chemical ionisation (CI) or fast atom bombardment (FAB) modes. Infra-red (IR) spectra were recorded on a Perkin Elmer PU 9800 FT-IR spectrometer. Combustion analysis was carried out on a Carlo-Erba 1106 elemental analyser.

Experimental to Chapter 5

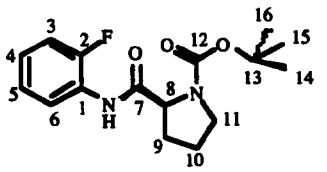
N-(t-Butoxycarbonyl)-L-proline **64**[84]



A modified version of the method by Ookawa and Soai[84] was used. To a solution of L-proline (5 g, 43.5 mmol) in dioxane (25 ml) and water (28 ml) was added

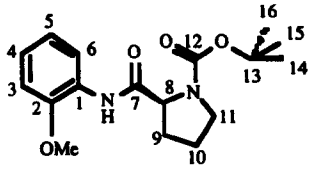
potassium carbonate (13.1 g, 95 mmol) slowly with stirring. $(BOC)_2O$ (9.9 g, 47 mmol) in dioxane/water was added dropwise, and the reaction mixture was stirred overnight. The reaction mixture was acidified with citric acid (saturated solution), and the product extracted with DCM (3 x 50 ml). The organic extracts were washed with brine (40 ml), dried over $MgSO_4$, and concentrated *in vacuo* to give a white solid. Recrystallisation from ethyl acetate/hexane yielded compound **64** as white needles (7.1 g, 33 mmol, 76%) mp 129-131 ^0C (lit.,[84] 136-137 ^0C). δ_H (400 MHz, $CDCl_3$) 1.35 and 1.41 (9H, two s, H-8 to 10), 1.82 to 2.25 (4H, m, H-2 and 3), 3.26 to 3.52 (2H, m, H-4), 4.23 (1H, m, H-1), 9.6 (1H, bs, CO_2H).

N-*(t-Butoxycarbonyl)*-N'-*(2-fluorophenyl)-L-prolineamide* **65**



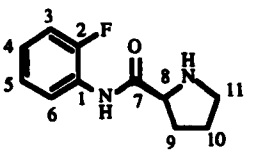
A modified version of the literature method[85] was used. To a solution of *N*-(t-butoxycarbonyl)-L-proline (2 g, 9.3 mmol) in DCM (40 ml) was added DCC (2.42 g, 11.2 mmol) then DMAP (181.8 mg, 1.5 mmol) with stirring. 1 h later 2-fluoroaniline (1.2 ml, 1.41 g, 12.6 mmol) was added. The reaction mixture was stirred for 24 h under N_2 at room temperature. The DCU precipitate was filtered off, and the filtrate stored in a freezer overnight to encourage precipitation of the remaining DCU. The DCU was filtered, acetic acid was added dropwise to the filtrate, and the filtrate washed with brine, dried (anhydrous $MgSO_4$) and the solvent evaporated *in vacuo* to give a pale yellow solid. Recrystallisation from EtOH yielded compound **65** as white needles (2.4 g, 7.8 mmol, 84%), mp 150-152 ^0C. ν_{max} (KBr disc)/cm^{-1} 3265s (NH), 3062m (Ar-H), 1699-1620s (CO), 1598s (Ar), 1367s (C-F); δ_H (400 MHz, $CDCl_3$) 1.42 (9H, s, H-14 to 16), 1.89 (2H, m, H-10), 2.14 and 2.45 (2H, two bs, H-9), 3.38 (2H, m, H-11), 4.42 (1H, m, H-8), 6.97 to 8.25 (4H, m, H-3 to 6); δ_C (100 MHz, $CDCl_3$) 24.0 (C-10), 27.2 (C-14 to 16), 26.0 (C-9), 46.1 (C-11), 59.8 (C-8), 80.0 (C-13), 113.7 (C-6), 113.8 (C-3, J_{CF} 20 Hz), 120.6 and 123.3 (C-4 and 5), 126.0 (C-1, J_{CF} 23 Hz), 151.5 (C-2, J_{CF} 243 Hz), 169.0 (CO); *m/z* (EI) 308.1537 ($M^{+\bullet}$). $C_{16}H_{21}O_3N_2F$ requires 308.1538; *m/z* (%) 308 (10), 235 (12), 207 (5), 114 (95), 70 (100), 57 (55).

N-*(t-Butoxycarbonyl)*-N'-*(2-methoxyphenyl)-L-prolineamide* **66**



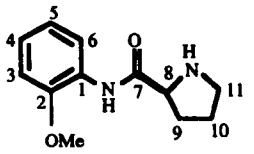
Compound **66** was prepared from **64** (1.92 g, 8.95 mmol) and *o*-anisidine (1.50 g, 8.95 mmol) in the presence of DCC (2.33 g, 11.28 mmol) and DMAP (0.18 g, 1.41 mmol) by the modified method used to prepare compound **65**. Recrystallisation from isopropanol/hexane yielded the title compound **66** (1.11 g, 3.50 mmol, 40%). mp 213-220 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3327m (CONH), 1774s and 1691s (CONH), 1601s (Ar); δ_H (400 MHz, d4-MeOH) 1.44 (9H, two m, H-14 to 16), 1.84 to 2.33 (4H, m, H-9 and 10), 3.50 (3H, m, NH and H-11), 3.89 (3H, s, OCH_3), 4.40 (1H, bs, H-8), 6.94 to 8.09 (4H, m, H-3 to 6); δ_C (100 Mz, d4-MeOH) 26.5 and 27.2 (C-10), 28.9 (C-14 to 16), 32.9 and 35.2 (C-9), 48.4 (C-11), 49.6 (OCH_3), 56.4 and 56.6 (C-8), 82.1 (C-13), 111.9 and 112.2 (C-3 and 6), 121.9 and 126.6 (C-4 and 5), 124.0 (C-1), 140.0 (C-2), 176.3 (CO); *m/z* (EI) 320.1736 ($M^{+\bullet}$). $C_{17}H_{24}O_4N_2$ requires 320.1736; *m/z* (%) 320 (30), 247 (13), 224 (8). 170 (22), 114 (92), 70 (100), 57 (50).

N'-*(2-Fluorophenyl)-L-prolineamide* **67**



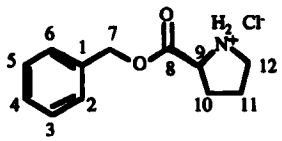
A modified version of the method by Stahl and co-workers[86] was used. Compound **65** (0.5 g, 1.62 mmol) was dissolved in 1M HCl-MeOH (9 ml). The reaction mixture was stirred at room temperature for 48 h. The solvent was removed *in vacuo* to yield a pale yellow solid which was recrystallised from absolute ethanol giving the compound **67** as white needles (280 mg, 1.35 mmol, 83%), mp 223-229 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3327s (NH), 1684s (CONH), 1608s (Ar), 1105 and 1384 (C-F); δ_H (400 MHz, d4-MeOH) 2.03 and 2.48 (4H, two m, H-9 and 10), 3.33 (2H, m, H-11), 4.44 (1H, m, H-8), 7.08 (3H, m, H-3 to 5), 7.82 (1H, m, H-6); δ_C (100 MHz, d4-MeOH) 25.5 and 26.5 (C-10), 31.7 and 35.1 (C-9), 47.9 (C-11), 62.0 (C-8), 117.1 (C-3, J_{CF} 19.6 Hz), 125.9 (C-6, J_{CF} 4 Hz), 126.0 (C-4, J_{CF} 10 Hz) 126.7 (C-1, J_{CF} 14 Hz), 128.2 (C-5, J_{CF} 7 Hz), 156.2 (C-2, J_{CF} 240 Hz), 169.0 (CO); *m/z* (EI) 208.1015 ($M^{+\bullet}$). $C_{11}H_{13}ON_2F$ requires 208.1018; *m/z* (%) 208 (5), 111 (10), 83 (5), 70 (100), 68 (5).

N-*(2-Methoxyphenyl)-L-prolineamide* **68**



Compound **68** was prepared from **66** (0.4 g, 1.2 mmol) by the method used to prepare **67**. Purification was by column chromatography eluting with hexane-ethyl acetate-conc. NH_3 (2:1:0.3). The fractions R_f 0.2 were combined and the solvent evaporated *in vacuo* to yield **68** as a grey solid (0.2 g, 0.91 mmol, 79%), mp 218 ^{0}C (from acetonitrile). ν_{max} (KBr disc)/cm^{-1} 3494s (NH), 1692s (CO), 1600s (Ar); δ_H (400 MHz, d4-MeOH) 2.05 and 2.42 (4H, two m, H-9 and 10), 3.26 (2H, m, H-11), 4.29 (1H, m, H-8), 6.94 to 7.14 (3H, m, H-3 to 5), 8.05 (1H, dd, J 1.56 Hz, J 8 Hz, H-6); δ_C (100 MHz, d4-MeOH) 26.4 (C-10), 32.0 (C-9), 48.1 (C-11), 56.7 (OCH_3), 62.3 (C-8), 112.2 (C-6), 121.9 (C-3), 123.1 (C-4), 126.8 (C-5), 128.0 (C-1), 151.7 (C-2), 176.8 (CO); *m/z* (EI) 220.1209 ($M^{+\bullet}$) $C_{12}H_{16}O_2N_2$ requires 220.1206; *m/z* (%) 220 (10), 123 (18), 108 (8), 70 (100), 68 (5).

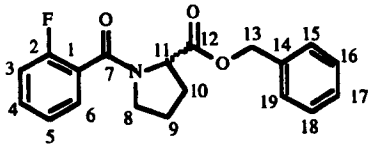
L-Proline Benzyl Ester Hydrochloride **69**[87]



The method of Neuman and Smith[87] was used. L-Proline (6 g, 52.2 mmol) was suspended in benzyl alcohol (84 ml) and dry HCl was passed through for 1 h at 0 ^{0}C. The mixture was then heated at 85 ^{0}C for 1 h under reduced pressure to remove water and HCl. Ether (840 ml) was added to the cooled residue (0 ^{0}C) to give a white precipitate, which was stored at 0 ^{0}C overnight. The solvent was removed and the solid was dried *in vacuo* to give compound **69** as a white powder (9.7 g, 47 mmol, 90%) which was satisfactory for synthetic work, mp 147-148 ^{0}C (lit.,[87] 148-148.5 ^{0}C).

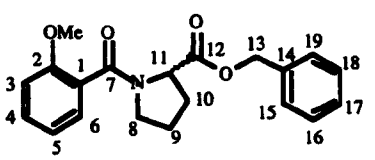
New data: δ_H (400 MHz, d4-MeOH) 2.00 and 2.32 (4H, two m, H-10 and 11), 3.25 (2H, m, H-12), 4.39 (1H, dd, J 6.4 Hz, J 8.8 Hz, H-9), 5.18 (2H, AB system, J 6.8 Hz, H-7), 7.26 (5H, m, H-2 to 6); δ_C (100 MHz, d4-MeOH) 24.9 and 25.1 (C-11), 29.7 and 30.2 (C-10), 47.5 (C-12), 61.1 (C-9), 69.7 (C-7), 130.1 to 130.2 (C-2 to 6), 136.8 (C-1), 170.4 (C-8).

N-*(2-Fluorobenzoyl)-L-proline Benzyl Ester* **73**



A modified version of the method by Paul and Anderson[88] was used. 2-Fluorobenzoic acid (0.6 g, 4.28 mmol) and compound **69** (1.76 g, 8.56 mmol) were dissolved in DMF (21 ml). Carbonyldiimidazole (1.043 g, 6.42 mmol) was added and the solution was stirred for 24 h under N_2 at room temperature. The reaction mixture was dissolved in water (210 ml), and extracted with ether (3 x 70 ml). The organic extracts were washed with saturated Na_2CO_3 solution (100 ml), dried (anhydrous Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by coloumn chromatography, eluting with hexane-ethyl acetate (1:1). The fractions R_f 0.3 were combined and the solvent was evaporated *in vacuo* to yield compound **73** as a yellow oil (0.9 g, 2.7 mmol, 63%). ν_{max} (neat)/cm^{-1} 1745s (CO), 1610m (Ar), 1171s (C-F); δ_H (400 MHz, $CDCl_3$) 1.79 and 2.28 (4H, two m, H-9 and 10), 3.35 and 3.43 (2H, two m, H-8), 4.24 and 4.66 (1H, two m, H-11), 5.16 (2H, AB system, J 12 Hz, H-13), 7.09 (9H, m, H-3 to 6 and H-15 to 19), δ_C (100 MHz, $CDCl_3$) 21.0 and 23.1 (C-9), 29.8 and 31.6 (C-10), 46.9 and 48.6 (C-8), 59.3 and 60.7 (C-11), 65.6 and 67.3 (C-13), 116.2 (C-3, J_{CF} 21), 124.9 (C-5, J_{CF} 3.6 Hz), 125.2 (C-1, J_{CF} 18 Hz), 127.9, 128.5, 128.6, 128.9 and 129.4 (C-15 to 19), 129.5 (C-6, J_{CF} 6 Hz), 131.8 (C-4, J_{CF} 10 Hz), 135.4 and 136.1 (C-14), 158.9 (C-2, J_{CF} 247 Hz), 165.9 (C-7), 172.1 (C-12); m/z (EI) 327.1271 ($M^{+\bullet}$) $C_{19}H_{18}O_3NF$ requires 327.1271; *m/z* (%) 327 (4), 230 (20), 192 (45), 160 (10), 123 (100), 98 (67), 91 (74), 70 (44).

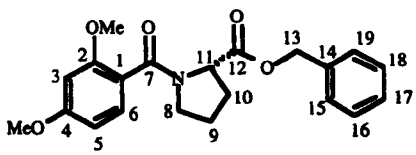
N-*(2-Methoxybenzoyl)-L-proline Benzyl Ester* **74**



Compound **74** was prepared from **69** (0.5 g, 3.29 mmol) and **71** (1.69 g, 8.23 mmol) in the presence of CDI (0.8 g, 4.94 mmol) by the method used to prepare compound **73**. Purification was by column chromatography eluting with hexane-ethyl acetate (1: 2). The fractions R_f 0.3 were combined and concentrated *in vacuo* to yield **74** as a viscous oil (0.90 g, 80%); ν_{max} (neat)/cm^{-1} 1744s and 1636s (CO), 1601s (Ar); δ_H (400 MHz, $CDCl_3$) 1.80 to 2.17 (4H, three m, H-9 and 10), 3.25 and 3.33 (2H, two m, H-8), 3.71 (3H, s, OCH_3), 4.18 and 4.65 (1H, two m, H-11), 5.14 (2H, AB

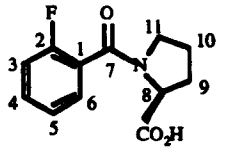
system, J 13.2 Hz, H-13), 6.86, 7.07 and 7.26 (9H, three m, H-3 to 6 and H-15 to 19); δ_C (100 MHz, $CDCl_3$) 23.2 and 25.0 (C-10), 30.0 and 31.6 (C-9), 46.6 and 48.4 (C-11), 55.9 and 56.0 (OCH_3), 59.0 and 60.7 (C-8), 67.11 and 67.18 (C-13), 111.4 and 111.5 (C-3), 121.2 (C-5), 126.6 and 126.8 (C-1), 128.4 to 128.9 (C-6, 15 to 19), 131.0 (C-4), 135.6 and 136.2 (C-14), 155.3 and 155.8 (C-2), 168.4 and 168.6 (7-CO), 172.4 (12-CO); *m/z* (EI) 339.1472 ($M^{+\bullet}$). $C_{20}H_{21}O_4N$ requires 339.1474; *m/z* (%) 339 (5), 279 (5), 204 (35), 149 (22), 135 (100), 91 (13), 86 (54), 77(18), 49(15).

N-*(2,4-Dimethoxybenzoyl)-L-proline Benzyl Ester* **75**



Compound **75** was prepared from **69** (0.6 g, 3.3 mmol) and **72** (1.36 g, 6.6 mmol) in the presence of CDI (0.8 g, 4.94 mmol) by the method used to prepare **73**. Purification was by column chromatography eluting with hexane-ethyl acetate (1:2). The fractions R_f 0.4 were combined and concentrated *in vacuo* to yield **75** as a viscous oil (0.79 g, 64%); ν_{max} (neat)/cm^{-1} 1751s (ester), 1687s and 1638s (CON and Ar); δ_H (400 MHz, $CDCl_3$) 1.80, 1.91 and 2.18 (4H, three m, H-9 and 10), 3.28 and 3.36 (2H, two m, H-11), 3.68 and 3.72 (6H, two s, OCH_3), 4.21 and 4.62 (1H, two m, H-8), 5.13 (2H, AB system J 13.6 Hz, H-13), 6.34 and 7.21 (8H, each m, ArH); δ_C (100 MHz, $CDCl_3$), 23.2 and 25.1 (C-10), 29.9 and 31.6 (C-9), 46.6 and 48.5 (C-11), 55.7 and 56.0 (OCH_3), 59.1 and 60.6 (C-8), 67.0 (C-13), 98.9 and 99.0 (C-3), 105.1 (C-5), 119.5 and 119.7 (C-1), 128.4 to 128.9 (C-15 to 19), 129.7 and 129.9 (C-6), 135.6 and 136.3 (C-14), 157.3 (C-2), 162.2 (C-4), 168.3 (7-CO), 172.5 (12-CO); *m/z* (EI) 369.1573 ($M^{+\bullet}$). $C_{21}H_{23}O_5N$ requires 369.1570; *m/z* (%) 369 (9), 232 (4), 165 (100), 122 (5), 91 (21), 77 (3).

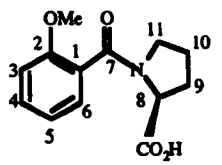
N-*(2-Fluorobenzoyl)-L-proline* **76**



A modified version of the method by Challis and co-workers[89] was used. A solution of compound **73** (0.9 g, 2.7 mmol) in absolute EtOH (20 ml) containing 5% Pd-C (45 mg) was stirred under a hydrogen balloon at rt for 6 h. The catalyst was

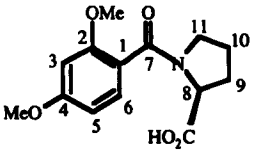
removed by filtration, and the filtrate concentrated under vacuum to give a yellow solid, which was recrystallised from ethyl acetate to give **76** (0.35 g, 1.48 mmol, 55%), mp 165-166 ^{0}C; ν_{max} (KBr disc)/cm^{-1} 2485s (N^+H), 1757s and 1621s (CO and Ar), 1033s and 1346s (C-F); δ_H (400 MHz, d4-MeOH) 2.06 and 2.40 (4H, two m, H-9 and 10), 3.47 and 3.77 (2H, two m, H-11), 4.28 and 4.61 (1H, two m, H-8), 7.32 and 7.48 (4H, each m, Ar-H); δ_C (100 MHz, d4-MeOH) 24.1 and 26.0 (C-10), 31.1 and 32.6 (C-9), 48.2 and 50.2 (C-11), 60.8 and 62.5 (C-8), 117.5 (C-3, J_{CF} 21 Hz), 126.2 (C-1, J_{CF} 15 Hz), 126.3 (C-5, J_{CF} 3.5 Hz), 130.3 (C-6, J_{CF} 7 Hz), 133.6 (C-4, J_{CF} 9 Hz), 160.1 (C-2, J_{CF} 246 Hz), 167.9 (CO), 175.5 (CO_2H); *m/z* (EI) 237.0802 ($M^{+\bullet}$). $C_{12}H_{12}O_3NF$ requires 237.0803; *m/z* (%) 237 (5), 193 (80), 192 (75), 123(100), 95 (65), 75 (22).

N-*(2-Methoxybenzoyl)-L-proline* **77**



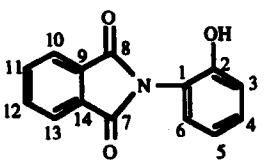
Compound **77** was prepared from **74** (0.76 g, 2.2 mmol) in absolute ethanol (20 ml) containing 5% Pd-C (45 mg) by the method used to prepare **76**. Purification by chromatography eluting with EtOAc-hexane (2:1) containing acetic acid (5%), and recrystallisation from EtOAc provided **77** as light yellow crystals (0.34 g, 62%). mp 135 ^{0}C. (Found: C, 62.71; H, 6.11; N, 5.51; $C_{13}H_{15}NO_4$ requires C, 62.65; H, 6.02; N, 5.62%); ν_{max} (KBr disc)/cm^{-1} 2975s (CO_2-H), 2496s (N^+H), 1748s (CO), 1589s (Ar); δ_H (400 MHz, $CDCl_3$) 1.80 and 1.93 (2H, two m, H-10), 2.12 and 2.33 (2H, two m, H-9), 3.29 (2H, m, H-11), 3.78 (3H, s, OCH_3), 4.10 and 4.69 (1H, two m, H-8), 6.79 to 7.34 (4H, m, ArH); δ_C (100 MHz, $CDCl_3$) 23.0 and 24.9 (C-10), 28.6 and 32.0 (C-9), 46.5 and 49.1 (C-11), 56.1 (OCH_3), 60.1 and 61.0 (C-8), 111.2 and 111.6 (C-3), 121.2 and 121.3 (C-5), 125.7 (C-1), 128.4 and 128.9 (C-6), 131.1 and 131.6 (C-4), 155.6 (C-2), 168.9 (7-CO), 170.5 (CO_2H); *m/z* (CI) 250.1077 ($M^{+\bullet}$). $C_{13}H_{15}O_4N$ requires 250.1075; *m/z* (%) 249 (2), 205 (25), 135 (100), 92 (10), 77 (18).

N-*(2,4-Dimethoxybenzoyl)-L-proline* **78**



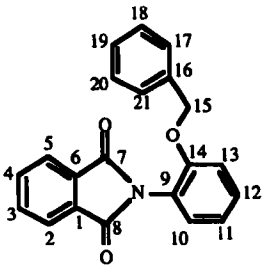
Compound **78** was prepared from **75** (0.79 g, 2.1 mmol) in absolute ethanol (20 ml) containing 5% Pd-C (40 mg) by the method used to prepare **76**. Purification by chromatography eluting with EtOAc-hexane (2:1) containing acetic acid (5%), and recrystallisation from EtOAc provided **78** as light yellow crystals (0.34 g, 57%), mp 134-135 ^{0}C. (Found: C, 59.89; H, 6.06; N, 4.94; $C_{14}H_{17}NO_5$ requires C, 60.21; H, 6.09; N, 5.02%); ν_{max} (KBr disc)/cm^{-1} 2959s (CO_2-H), 2511s (N^+H), 1743s (CO), 1591s (Ar); δ_H (400 HMz, $CDCl_3$) 1.93 and 2.10 (2H, two m, H-10), 2.29 and 2.39 (2H, two m, H-9), 3.40 and 3.69 (2H, two m, H-11), 3.80 (6H, s, OCH_3), 4.24 and 4.55 (1H, two m, H-8), 6.33 to 7.20 (3H, m, Ar-H), 8.06 (1H, bs, CO_2H); δ_C (100 Hz, $CDCl_3$) 24.9 (C-10), 28.4 (C-9), 49.3 (C-11), 55.9 and 56.1 (OCH_3), 60.4 (C-8), 99.1 (C-3), 105.3 (C-5), 118.1 (C-1), 129.8 (C-6), 157.2 (C-2), 162.8 (C-4), 171.1 and 173.2 (CO or CO_2H); *m/z* (EI) 279.1109 ($M^{+\bullet}$). $C_{14}H_{17}O_5N$ requires 279.1112; *m/z* (%) 279 (3), 235 (20), 193 (10), 165 (100), 123 (35), 95 (8), 63 (8), 63 (7).

N-*2-Hydroxyphenylphthalimide* **79** [90]



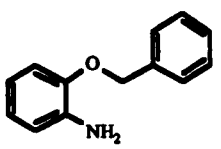
2-Aminophenol (5.45 g, 50 mmol) and phthalic anhydride (14.8 g, 100 mmol) were added to acetic acid (125 ml), and the mixture was heated at reflux for 1 h, then diluted with water (125 ml) and heated to boiling. On cooling in ice, **79** (10.1655 g, 42.5 mmol, 85%) crystallised, mp 218-219 ^{0}C (lit.,[90] 223-224 ^{0}C); δ_H (400 HMz, d_6-DMSO) 6.92 (1H, m, H-3), 7.01 (1H, m, H-5), 7.30 (2H, m, H-4 and 6), 7.91 (2H, m, H-11 and 12), 7.96 (2H, m, H-10 and 13), 9.87 (1H, s, OH).

N-*(2-Benzyloxyphenyl)phthalimide* **80** [90]



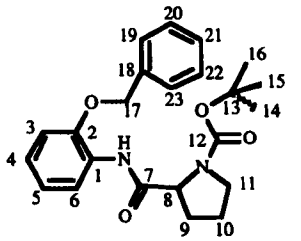
Compound **79** (5 g, 21 mmol) was added to a solution of Na (0.483 g, 21 mmol) in 99% EtOH (54 ml). Under rapid cooling, benzyl bromide (5.39 g, 31.5 mmol) was added dropwise and the mixture was heated for 22 h at 80 ^{0}C. Water (130 ml) was added to the reaction mixture while it was cooled in ice. Compound **80** was extracted with DCM (100 ml x 3). The organic extracts were washed with 10% NaOH (150 ml) and water (150 ml), dried with Na_2SO_4 and concentrated *in vacuo* to give a yellow solid which was recrystallised from hexane to yield **80** (5.1148 g, 15.5 mmol, 75%), mp 143-144 ^{0}C (lit.,[90] 147-148 ^{0}C); δ_H (400 HMz, $CDCl_3$) 5.02 (2H, s, H-15), 7.00, 7.18 and 7.29 (9H, three m, H-17 to 21 and H-10 to 13), 7.67 (2H, m, H-3 and 4), 7.85 (2H, m, H-2 and 5).

2-Benzyloxyaniline **81** [90]



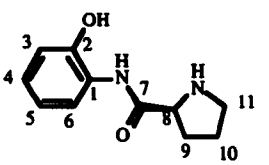
Hydrazine hydrate (0.6 ml) was added to a solution of **80** (4.0 g, 12 mmol) suspended in EtOH (50 ml) and heated to reflux for 1 h. After cooling, ether (150 ml) was added and the precipitate was filtered off. The filtrate was concentrated and the residue was heated in 10% HCl (40 ml). After cooling an excess of conc. NaOH was added. The oily precipitate was dissolved in ether, washed with water, dried (anhydrous Na_2SO_4) and concentrated *in vacuo* to yield compound **81** as a brown solid (1.588 g, 8 mmol, 67%), mp 30 ^{0}C (lit.,[90] 37-39 ^{0}C); δ_H (400 HMz, $CDCl_3$) 3.74 (2H, bs, NH_2), 4.99 (2H, s, H-7), 6.64 and 6.75 (each 2H, each m, H-3 to 6), 7.27 (5H, m, H-9 to 13).

N-*(t-Butoxycarbonyl)*-N'-*(2-benzyloxyphenyl)-L-prolineamide* **82**



Compound **82** was prepared from **64** (0.75 g, 3.5 mmol) and **81** (0.5 g, 2.5 mmol) in the presence of DCC (0.72 g, 3.5 mmol) and DMAP (0.05 g, 0.4 mmol) by the method used to prepare **65**. Purification was by column chromatography eluting with hexane-ethyl acetate (1:1). The fractions R_f 0.4 were combined and concentrated *in vacuo* to yield **82** as a yellow solid (0.9 g, 2.3 mmol, 91%), mp 111-113 ^{0}C. (Found: C, 69.70; H, 7.01; N, 7.31; $C_{23}H_{28}O_4N_2$ requires C, 69.69; H, 7.07; N, 7.07%); ν_{max} (KBr disc)/cm^{-1} 3316m (NH), 1698s (CO), 1597s (Ar); δ_H (400 MHz, $CDCl_3$) 1.29 (9H, s, H-14 to 16), 1.45 to 2.14 (4H, m, H-9 and 10), 3.29 and 3.39 (2H, two m, H-11), 4.21 and 4.39 (1H, two m, H-8), 5.04 (2H, m, H-17), 6.90 to 8.38 (9H, m, ArH); δ_C (100 MHz, $CDCl_3$) 25.0 (C-10), 28.7 (C-14 to 16), 32.0 (C-9), 47.0 (C-11), 81.0 (C-13), 111.8, 120.5, 121.8, 124.1, 127.6, 128.6, 129.1 and 147.0 (C-1 to 6 and C-18 to 23); *m/z* (EI) 396.2050 ($M^{+\bullet}$). $C_{23}H_{28}O_4N_2$ requires 396.2051; *m/z* (%) 396 (35), 340 (13), 226 (28), 170 (30), 114 (100), 70 (98), 57 (53).

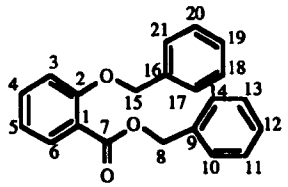
N-*(2-Hydroxyphenyl) L-prolinamide* **83**



Compound **82** (0.72 g, 1.8 mmol) was dissolved in 1 M HCl-MeOH (10 ml). The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated *in vacuo* to yield a brown oil. The brown oil was then dissolved in absolute EtOH (20 ml) and Pd-C (0.5 g) was added. The reaction mixture was stirred under a hydrogen balloon for 24 h at rt. The catalyst was removed by filtration, and the filtrate concentrated under vacuum to give a dark brown oil, which was washed with DCM. The DCM was separated and the remaining brown oil was concentrated *in vacuo* to give **83** as a dark brown gum (0.2454 g, 1.2 mmol, 67 %); ν_{max} (neat)/cm^{-1} 3532s (NH or OH), 1742s (CO), 1610s (Ar); δ_H (400 MHz, d4-MeOH) 2.01, 2.34 and 2.48 (4H, three m, H-9 and 10), 3.33 (2H, m, H-11), 4.37 and 4.50 (1H, two m, 8-H), 6.60 to

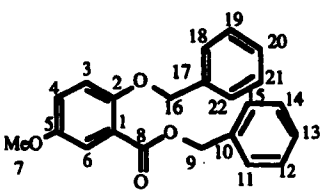
7.69 (4H, m, 3 to 6-H); δ_C (100 MHz, d4-MeOH) 25.0 and 25.5 (C-10), 31.8 and 32.3 (C-9), 47.9 and 48.0 (C-11), 61.7 and 62.2 (C-8), 116.8 and 117.6 (C-6), 120.9 and 121.4 (C-3), 124.6, 125.3, 127.6 and 131.7 (C-4 and 5), 126.5 (C-1), 150.3 (C-2), 169 (CO); *m/z* (EI) 206.1056 ($M^{+\bullet}$). $C_{11}H_{14}O_2N_2$ requires 206.1057; *m/z* (%) 206 (10), 199 (5), 122 (4), 109 (28), 80 (14), 70 (100), 62 (13), 55 (8).

Benzyl 2-benzyloxybenzoate **84**[91]



Salicylic acid (6 g, 43.4 mmol), ignited K_2CO_3 (27 g, 195.3 mmol), benzyl chloride (17.4 g, 137.5 mmol) and DMF (50 ml) were heated at reflux with stirring for 2.5 h, then the mixture was diluted with water and stored at room temperature. Compound **84** (13.5 g, 42.0 mmol, 97%) crystallised, mp 48 ^{0}C (lit.,[91] 52-54 ^{0}C). (Found: C, 79.19; H, 5.55; $C_{21}H_{18}O_3$ requires C, 79.24; H, 5.66 %).
New data: δ_H (400 MHz, $CDCl_3$) 5.08 and 5.26 (each 2H, each s, H-8 and 15), 6.90 (2H, m, H-3 and 5), 7.29 (11H, m, H-4, 10 to 14 and 17 to 21), 7.77 (1H, m, H-6); δ_C (100 MHz, $CDCl_3$) 67.0 and 71.0 (C-15 and 8), 114.1 (C-3), 120.9 (C-5), 127.5, 128.2, 128.4, 128.6, 128.9, 132.3 and 133.9 (C-4, 6, 10 to 14 and 17 to 21), 121.1 (C-1), 136.2 and 137.0 (C-9 and 16), 158.6 (C-2), 166.7 (C-7).

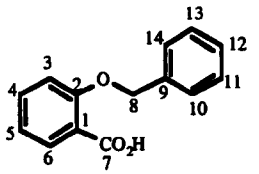
Benzyl 2-benzyloxy-5-methoxybenzoate **85**



Compound **85** was prepared from 2-hydroxy-5-methoxybenzoic acid (2.0 g, 12.1 mmol) and benzyl chloride (4.84 g, 38.6 mmol) in the presence of ignited K_2CO_3 (7.5 g) by the method used to prepare **84**. Recrystallisation from DMF/water provided **85** as a yellow solid (2.9 g, 70%), mp 43-44 ^{0}C. (Found: C, 75.82; H, 5.76; $C_{22}H_{20}O_4$ requires C, 75.86; H, 5.75%); ν_{max} (KBr disc)/cm^{-1} 1691s (CO), 1606m (Ar); δ_H (400 MHz, $CDCl_3$) 3.69 (3H, s, H-7), 5.01 and 5.27 (each 2H, each s, H-9 and 16), 6.90 (2H, m, H-3 and 4), 7.23 to 7.48 (11H, m, H-6, 11 to 15 and 18 to 22), δ_C (100 MHz, $CDCl_3$) 56.2 (C-7), 67.2 and 72.2 (C-9 and 16), 116.6 and 119.8 (C-3

and 6), 127.6, 128.2, 128.5, 128.6 and 128.9 (C-4, 11 to 15 and 18 to 22), 121.9 (C-1), 136.4 and 137.3 (C-10 and 17), 152.8 and 153.8 (C-2 and 5), 166.5 (C-8); *m/z* (EI) 348.1363 ($M^{+\bullet}$). $C_{22}H_{20}O_4$ requires 348.1364; *m/z* (%) 348 (10), 257 (6), 181 (18), 151 (17), 91 (100), 82 (23), 65 (7).

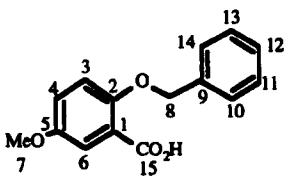
2-Benzyloxybenzoic acid **86**[91]



Compound **84** (1.2 g, 3.7 mmol) was hydrolysed by heating it at reflux in a mixture of MeOH (31 ml) and 40 % NaOH (6 ml) for 2 h. Dilution with water and acidification with HCl precipitated **86** as pale yellow crystals (0.7585 g, 3.6 mmol, 94 %), mp 64-66 ^{0}C (lit.,[91] 76-78 ^{0}C). (Found: C, 72.9; H, 4.84; $C_{13}H_{10}O_3$ requires C, 72.89; H, 4.67 %).

New data: δ_H (400 HMz, d_6-DMSO) 5.2 (2H, s, H-8), 7.01 (1H, m, H-3), 7.20 (1H, m, H-5), 7.30, 7.40 and 7.50 (6H, three m, H-4,and 10 to 14),7.62 (1H, m, H-6); δ_C (100 MHz, d_6-DMSO) 69.9 (C-8), 114.2 (C-3), 120.7 (C-5), 127.4, 127.9, 128.7, 130.9 and 133.2 (C-4, 6 and 10 to 14), 157.2 (C-2), 167.7 (C-7), 122.3 (C-1), 137.3 (C-9).

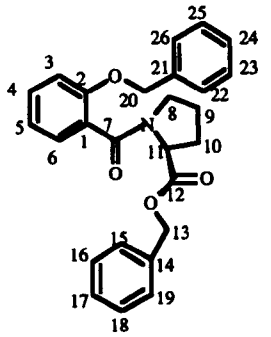
2-Benzyloxy-5-methoxybenzoic acid **87**



Compound **87** was prepared from **85** (2.8 g, 8 mmol) and 40% NaOH (11.8 ml) in MeOH (65 ml) by the method used to prepare **86**. Purification by chromatography eluting with EtOAc-hexane (1:1) containing acetic acid (2%) afforded **87** as a yellow solid (1.84 g, 90 %), mp 81-84 ^{0}C. (Found: C, 70.03; H, 5.45; $C_{15}H_{14}O_4$ requires C, 69.77; H, 5.42%); ν_{max} (KBr disc)/cm^{-1} 3234m (CO_2H), 1727s (CO), 1619m (Ar); δ_H (400 MHz, $CDCl_3$) 3.74 (3H, s, H-7), 5.17 (2H, s, H-8), 7.01 (2H, m, H-3, 4), 7.33 (5H, m, H-10 to 14), 7.61 (1H, m, H-6); δ_C (100 MHz, $CDCl_3$) 56.2 (C-7), 73.3 (C-8), 115.3 and 116.7 (C-3 and 6), 122.4 (C-4), 128.4, 129.0, 129.5 and 129.6(C-10 to 14), 119.0 (C-1), 134.8 (C-9), 151.9 154.9 (C-2 and

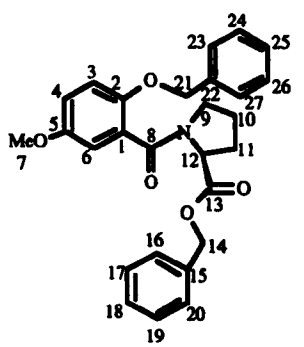
5), 165.5 (C-15); *m/z* (EI) 258.0893 ($M^{+\bullet}$). $C_{15}H_{14}O_4$ requires 258.0894; *m/z* (%) 258 (15), 150 (5), 91 (100), 82 (9), 65 (7).

N-*(2-Benzyloxybenzoyl)-L-proline benzyl ester* **88**



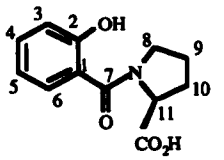
Compound **88** was prepared from **86** (0.6 g, 2.80 mmol) and **69** (0.87 g, 4.2 mmol) in the presence of CDI (0.68 g, 4.2 mmol) by the method used to prepare **73**. Purification was by column chromatography eluting with hexane-ethyl acetate (1:1). The fractions R_f 0.3 were combined and concentrated *in vacuo* to yield **88** as a viscous oil (0.77 g, 66%). (Found: C, 75.08; H, 6.16; N, 3.35; $C_{26}H_{25}NO_4$ requires C, 75.18; H, 6.02; N, 3.37 %); ν_{max} (neat)/cm^{-1} 1751s and 1624s (CO), 1600s (Ar); δ_H (400 MHz, $CDCl_3$) 1.66 to 2.19 (4H, m, H-9 and 10), 3.30 and 3.71 (2H, two m, H-8), 4.25 and 4.63 (1H, two m, H-11), 5.03 (2H, s, H-20), 5.15 (2H, AB system, J 9.6 Hz, H-13), 6.78 to 7.44 (14H, m, ArH); δ_C (100 MHz, $CDCl_3$) 23.3 and 25.0 (C-9), 29.9 and 31.5 (C-10), 46.6 and 48.4 (C-8), 59.1 and 60.5 (C-11), 66.1 and 67.1 (C-13), 70.8 (C-20), 113.2 and 113.5 (C-3), 121.7 (C-5), 127.3, 128.2, 128.3, 128.5, 128.8, 128.9, 129.0 and 130.9 (C-4, 6, 22 to 26 and 15 to 19), 127.4 (C-1), 136.3 and 137.2 (C-14 and 21), 154.8 (C-2), 168.3 (C-7), 172.4 (C-12); *m/z* (EI) 415.1785 ($M^{+\bullet}$). $C_{26}H_{25}O_4N$ requires 415.1786; m/z (%) 415 (14), 324 (13), 280 (13), 211 (80), 188(7), 120 (12), 91 (100), 70 (11), 65(7).

N-*(2-Benzyloxy-5-methoxybenzoyl)-L-proline benzyl ester* **89**



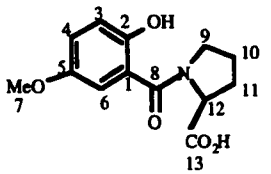
Compound **89** was prepared from **87** (0.9 g, 3.7 mmol) and **69** (1.06 g, 5.2 mmol) in the presence of CDI (0.84 g, 5.2 mmol) by the method used to prepare **73**. Purification by column chromatography eluting with hexane-ethyl acetate (1:1) afforded **89** as a viscous oil (1.19 g, 73%). (Found: C, 72.71; H, 6.20; N, 3.14; $C_{27}H_{27}O_5N$ requires C, 72.81; H, 6.06; N, 3.14%); ν_{max} (neat)/cm^{-1} 3064w (Ar-H), 1744s (CO), 1648m (Ar); δ_H (400 MHz, $CDCl_3$) 1.66 to 2.19 (4H, m, H-10 and 11), 3.33 and 3.71 (2H, each m, H-9), 3.59 and 3.66 (3H, each s, H-7), 4.31 and 4.63 (1H, each m, H-12), 4.94 and 4.96 (2H, each s, H-21), 5.13 (2H, AB system, J 12.4 Hz, H-14), 6.71 to 7.32 (13H, m, ArH); δ_C (100 MHz, $CDCl_3$) 23.3 and 25.0 (C-10), 29.9 and 31.5 (C-11), 46.7 and 48.4 (C-9), 56.0 and 56.1 (C-7), 59.1 and 31.5 (C-11), 46.7 and 48.4 (C-9), 56.0 and 56.1 (C-7), 59.1 and 60.5 (C-12), 67.0 and 67.1 (C-14), 71.9 and 72.1 (C-21), 113.4, 113.8, 115.9, 116.8 and 116.9 (C-3, 4 and 6), 127.5, 128.2, 128.3, 128.5, 128.6, 128.7, 128.8, 128.9 and 129.0 (C-1, 16 to 20 and 23 to 27), 136.2, 137.3 and 137.5 (C-15 and 22), 148.8 and 154.5 (C-2 and 5), 167.9 (C-8), 172.3 (C-13); *m/z* (EI) 445.1888 ($M^{+\bullet}$). $C_{27}H_{27}O_5N$ requires 445.1887; *m/z* (%) 445 (14), 354 (5), 241 (28), 204 (12), 150 (22), 91 (100), 44 (14).

N-*(2-Hydroxybenzoyl)-L-proline* **90**



Compound **90** was prepared from **88** (1.34 g, 3.23 mmol) in absolute ethanol (15 ml) containing 5% Pd-C (600 mg) by the method used to prepare **76**. Trituration with petroleum ether afforded **90** as a white solid (0.78 g, 92%), mp 31-33 ^{0}C. ν_{max} (neat)/cm^{-1} 3489s (OH), 1747s (CO),1594m (Ar); δ_H (400 MHz, $CDCl_3$) 1.82, 1.98 and 2.26 (4H, three m, H-9 and 10), 3.67 (2H, m, H-8), 4.15 and 4.60 (1H, two m, H-11), 6.74 to 7.44 (4H, m, H-3 to 6), 7.83 (1H, bs, OH or CO_2H); δ_C (100 MHz, $CDCl_3$) 24.9 and 26.3 (C-9), 31.1 and 32.5 (C-10), 48.8 and 50.4 (C-8), 58.7 and 60.9 (C-11), 117.0 and 117.7 (C-3), 120.8 (C-5), 124.8 (C-1), 129.5 and 130.1 (C-6), 132.0 and 132.9 (C-4), 156.4 (C-2), 170.9 (CO); *m/z* (EI) 235.0847 ($M^{+\bullet}$). $C_{12}H_{13}O_4N$ requires 235.0849; *m/z* (%) 235 (33), 217 (10), 190 (28), 121 (100), 93 (17), 70 (100), 65 (25), 41 (5).

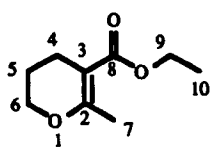
N-(2-Hydroxy-5-methoxybenzoyl)-L-proline **91**



Compound **91** was prepared from **89** (0.95 g, 2.14 mmol) in absolute ethanol (15 ml) containing 5% Pd-C (680 mg) by the method used to prepare **76**. Trituration with petroleum ether afforded **91** as a grey solid (0.54 g, 94 %), mp 32 ^{0}C; ν_{max} (KBr disc)/cm^{-1} 3438s (OH), 1743s (CO), 1583s (Ar); δ_H (400 MHz, d4-MeOH) 1.89 and 2.24 (4H, two m, H-10 and 11), 3.47 (2H, m, H-9), 3.60 and 3.64 (3H, two s, H-7), 4.33 and 4.46 (1H, two m, H-12), 6.62 to 6.78 (3H, m, ArH); δ_C (100 MHz, d4-MeOH) 24.0 and 26.2 (C-10), 31.1 and 32.5 (C-11), 48.9 and 50.0 (C-9), 56.6 (C-7), 60.9 (C-12), 113.8, 114.0, 118.8 and 118.9 (C-1, 3, 4 and 6), 148.0, 149.0 and 154.5 (C-2 and 5), 176.5 (CO); *m/z* (EI) 265.0949 ($M^{+\bullet}$). $C_{13}H_{15}O_5N$ requires 265.0948; *m/z* (%) 265 (21), 235 (7), 219 (28), 150 (100), 121 (23), 107 (12), 70 (83).

Experimental to Chapter 6

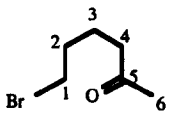
2-Methyl-3-ethoxycarbonyl-5,6-dihydropyran **92**[92]



A mixture of potassium carbonate (11.2 g), 99% ethanol (14 ml), 1,3-dibromopropane (8.08 g, 40 mmol) and ethyl acetoacetate (5.2 g, 40 mmol) was heated with stirring to 60 ^{0}C, then heated at reflux overnight. The reaction mixture was concentrated *in vacuo* and the residue was mixed with water (60 ml). The resulting oily layer was separated, and the aqueous phase was extracted with ethyl acetate (3 x 50 ml) and the ethyl acetate layer was combined with the yellow oil. After drying with sodium sulfate the ethyl acetate was evaporated and the residue was fractionally distilled to give **92** as a colourless oil bp 65-70 ^{0}C (>1 mmHg, vacuum pump), (5.15 g, 30.3 mmol, 76 %).

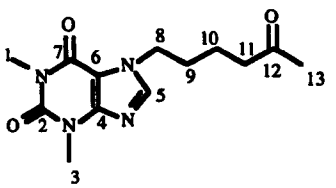
New data: δ_H (400 MHz, $CDCl_3$) 1.21 (3H, t, J 7.2 Hz, H-10), 1.75 (2H, m, H-5), 2.15 (3H, s, H-7), 2.23 (2H, t, J 6.4 Hz, H-4), 3.94 (2H, t, J 4.2 Hz, H-6), 4.11 (2H, q, J 7.2 Hz, H-9).

6-Bromohexan-2-one **93**[92]



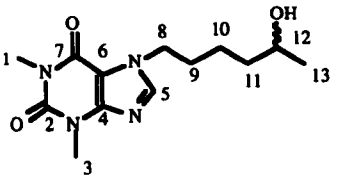
48% Hydrobromic acid (9.5 ml) was slowly added at room temperature to **92** (5.1 g, 30 mmol). After standing for 2 h at room temperature the mixture was heated to reflux for 2 h, then diluted with an equal volumn of iced water. The mixture was extracted with chloroform (20 ml x 3), and the organic extracts were washed with saturated sodium bicarbonate solution and dried with sodium sulfate. The chloroform was evaporated *in vacuo*, and the residue was fractionally distilled to yield **93** as a yellow oil, bp 60-70 ^{0}C (> 1 mmHg, vacuum pump), (3.34 g, 18 mmol, 62 %).
New data: δ_H (400 MHz, $CDCl_3$) 1.65 and 1.79 (each 2H, each m, H-2 or 3), 2.08 (3H, s, H-6), 2.40 (2H, t, J 7.2 Hz, H-4), 3.34 (2H, t, J 6.4 Hz, H-1); δ_C (100 MHz, $CDCl_3$) 22.6 (C-3), 30.2 (C-6), 32.4 (C-2), 33.6 (C-4), 42.9 (C-1), 208.6 (CO).

7-(5'-Oxohexyl)-1,3-dimethylxanthine **94**[92]



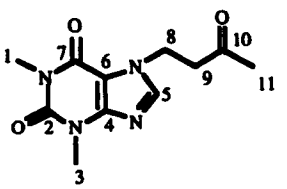
A solution of 1-bromohexan-5-one (3.4 g, 18.9 mmol) in ethanol (30 ml) was gradually mixed at the boil with theophylline (5.13 g, 28.5 mmol) in 1 M NaOH (40 ml). After heating at reflux overnight the alcohol was evaporated, and the residual aqueous phase was cooled and made alkaline and extracted with chloroform (3 x 60 ml). The chloroform was evaporated *in vacuo* and the residue recrystallised from isopropanol to yield **94** (1.0 g, 3.6 mmol, 19 %). mp 70-72 ^{0}C (lit.,[92] 75-76 ^{0}C).
New data: δ_H (400 MHz, $CDCl_3$) 1.49 and 1.82 (each 2H, each tt, each J 7.2 Hz, H-9 or 10), 2.07 (3H, s, H-13), 2.43 (2H, t, J 7.2 Hz, H-11), 3.33 and 3.51 (each 3H, each s, H-1 or 3), 4.21 (2H, t, J 7.2 Hz, H-8), 7.49 (1H, s, H-5).

7-(5'-Hydroxyhexyl)-1,3-dimethylxanthine **95**



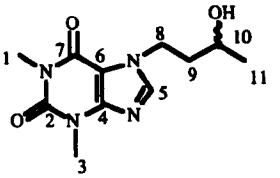
A modified version of the literature method[93] was used. Compound **94** (0.60 g, 2.16 mmol) was dissoved in MeOH (20 ml). While cooling in ice, $NaBH_4$ (0.24 g, 6.48 mmol) was added to the solution portionwise. The reaction mixture was stirred at rt for 1 h, then conc. HCl (0.4 ml) was added slowly to quench the reaction. The solvent was evaporated *in vacuo* to give a white solid. By-product NaCl was removed by chromatography (Florisil®) eluting with EtOAc-MeOH (10:1). The fractions R_f 0.36 were combined and solvent evaporated to yield compound **95** as a white solid (0.3 g, 1.08 mmol, 50 %) mp 89-90 ^{0}C (isopropanol). (Found: C, 55.86; H, 7.22; N, 20.10; $C_{13}H_{22}O_3N_4$ requires C, 55.71; H, 7.14; N, 20.0%); ν_{max} (KBr disc)/cm^{-1} 3475s (OH), 1710s, 1649s and 1600w (imidazole ring and CO); δ_H (400 MHz, d4-MeOH) 1.04 (3H, d, J 6.0 Hz, H-13), 1.30 (4H, m, H-9 and 10), 1.81 (2H, m, H-11), 3.26 and 3.45 (each 3H, each s, H-1 and 3), 3.62 (1H, m, H-12), 4.30 (2H, t, J 6.8 Hz, H-8), 8.27 (1H, s, H-5); δ_C (100 MHz, d4-MeOH) 23.9 (C-13), 28.9 and 31.1 (C-1 and 3), 32.0 (C-9 or 10), 39.7 (C-11), 49.2 (C-8), 68.6 (C-12), 108.4 (C-4), 142.2 (C-5), 148.1 (C-6), 153.1 and 156.4 (C-2 and 7); *m/z* (EI) 280.1532 ($M^{+\bullet}$). $C_{13}H_{20}O_3N_4$ requires 280.1528; *m/z* (%) 280 (67), 236 (18), 207 (23), 180 (100), 149(19), 82 (77), 78 (26), 44 (58).

7-(3'-Oxobutyl)-1,3-dimethylxanthine **96**[92]



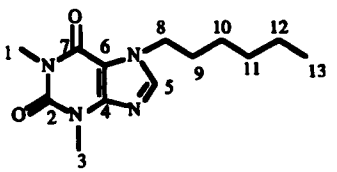
Theophylline (0.9 g, 4.99 mmol), methylvinyl ketone (0.4 g, 4.99 mmol) and dry pyridine (7.5 ml) were heated at reflux overnight. The solvent was removed *in vacuo* and the residue was recrystallized from isopropanol. Compound **96** was a pale yellow solid (0.8411 g, 3.4 mmol, 70%), mp 142 ^{0}C (lit.,[92] 144-146 ^{0}C).
New data: δ_H (400 MHz, $CDCl_3$) 2.07 (3H, s, H-11), 3.03 (2H, t, J 6Hz, H-9), 3.33 and 3.52 (each 3H, each s, H-1 and 3), 4.43 (2H, t, J 6Hz, H-8), 7.62 (1H, s, H-5).

7-(3'-Hydroxybutyl)-1,3-dimethylxanthine **97**



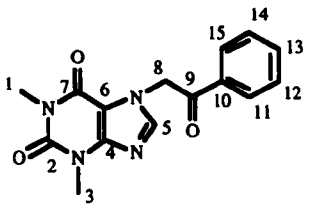
Compound **97** was prepared from **96** (0.37 g, 1.48 mmol) and $NaBH_4$(0.14 g, 3.7 mmol) by the method used to prepare **95**. Purification by chromatography eluting with EtOAc-MeOH (10:1) afforded **97** as a white solid (0.19 g, 0.75 mmol, 51%), mp 119-120 ^{0}C. (Found: C, 52.30; H, 6.39; N, 22.22; $C_{11}H_{16}O_3N_4$ requires C, 52.38; H, 6.35; N, 22.22%); ν_{max} (KBr disc)/cm^{-1} 3450s (OH), 1715s and 1596m (imidazole ring and CO); δ_H (400 MHz, D_2O) 1.12 (3H, d, J 6.4 Hz, H-11), 1.89 (2H, m, H-9), 3.20 and 3.39 (each 3H, each s, H-1 and 3), 3.70 (1H, m, H-10), 4.27 (2H, m, H-8), 7.89 (1H, s, H-5); δ_C (100 MHz, D_2O) 22.4 (C-11), 28.3 and 30.2 (C-1 and 3), 38.9 (C-9), 44.5 (C-8), 65.1 (C-10), 107.3 (C-4), 143.3 (C-5), 148.9 (C-6), 152.8 and 156.0 (C-2 and 7); *m/z* (EI) 252.1222 ($M^{+\bullet}$). $C_{11}H_{16}O_3N_4$ requires 252.1222; *m/z* (%) 252 (87), 207 (36), 194 (100), 180 (52), 149 (16), 123 (16), 95 (28), 68 (13), 44 (33).

7-Hexyl-1,3-dimethylxanthine **98**



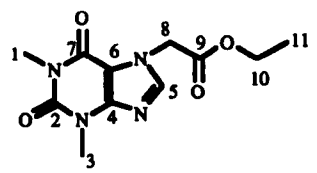
Compound **98** was prepared from 1-bromohexane (1.65 g, 10 mmol) and theophylline(1.80 g, 10 mmol) together with 1M NaOH (15 ml) by the method used to prepare **94**. Recrystallisation from hexane afforded **98** as white crystals (0.35 g, 14%), mp 50 ^{0}C. (Found: C, 59.10; H, 7.57; N, 21.20; $C_{13}H_{20}O_2N_4$ requires C, 59.09; H, 7.57; N, 21.21%); ν_{max} (KBr disc)/cm^{-1} 1715m, 1646m and 1597m (imidazole ring and CO); δ_H (400 MHz, $CDCl_3$) 0.80 (3H, t, J 6.8 Hz, H-13), 1.24 (6H, s, H-10 to 12), 1.79 (2H, tt, J 7.2 Hz, H-9), 3.33 (3H, s, H-3), 3.52 (3H, s, H-1), 4.21 (2H, t, J 7.2 Hz, H-8), 7.46 (1H, s, H-5); δ_C (100 MHz, $CDCl_3$) 12.9 (C-13), 21.4 (C-12), 25.0 (C-11), 26.9 (C-3), 28.7 (C-1), 29.8 (C-10), 30.1 (C-9), 46.3 (C-8), 105.9 (C-4), 139.7 (C-5), 147.9 (C-6), 150.7 and 154.1 (CO); *m/z* (EI) 264.1587 ($M^{+\bullet}$). $C_{13}H_{20}O_2N_4$ requires 264.1588; *m/z* (%) 264 (100), 235 (10), 221 (14), 207 (22), 180 (80), 123 (12), 95 (16), 55 (8).

7-Benzoylmethyl-1,3-dimethylxanthine **99**[94]



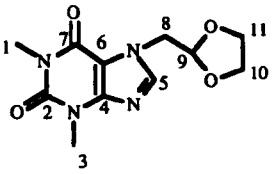
Compound **99** (1.143 g, 3.8 mmol, 76%) was obtained from theophylline (0.9 g, 5 mmol) and 2-bromoacetophenone (1 g, 5 mmol) in the presence of NaH (60% suspension, 0.3 g, 7.5 mmol) in DMF (50 ml) with stirring overnight at room temperature. DMF was evaporated off, and the residual solid was collected and recrystallised from EtOH, mp 184 ^{0}C (lit.,[94] mp 190-191 ^{0}C).
New data: δ_H (400 HMz, d$_6$-DMSO) 3.16 and 3.47 (each 3 H, each s, H-1 or 3), 5.96 (2H, s, H-8), 7.60 to 7.77 (6 H, m, H-5 and ArH); δ_C (100 HMz, d$_6$-DMSO) 27.7 and 29.8 (C-1 and 3), 52.9 (C-8), 106.9 (C-4), 128.4, 129.4 and 134.6 (C-11 to 15), 134.4 (C-10), 143.7 (C-5), 148.3 (C-6), 151.4 and 154.8 (C-2 and 7), 192.8 (C-9).

7-Ethoxycarbonylmethyl-1,3-dimethylxanthine **100**



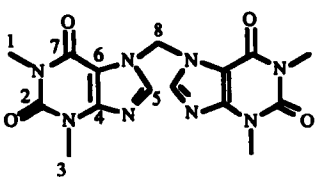
Compound **100** was prepared from theophylline (0.7 g, 3.9 mmol) and ethyl bromoacetate (0.65 g, 3.9 mmol) in the presence of NaH (60 % suspension, 0.21 g, 5.0 mmol) by the modified method used to prepare **99**. DCM was added to the reaction mixture to precipitate the unreacted theophylline which was filtered off, and the filtrate was concentrated *in vacuo* to give a dark oil. Recrystallisation from isopropanol give **100** (0.19 g, 0.72 mmol, 19%) as a grey solid, mp 136-139 ^{0}C. (Found: C, 49.45; H, 5.38; N, 21.01; $C_{11}H_{14}O_4N_4$ requires C, 49.62; H, 5.26; N, 21.05%); ν_{max} (KBr disc)/cm^{-1} 1760m, 1636m (imidazole ring and CO); δ_H (400 MHz, $CDCl_3$) 1.24 (3H, t, J 7.2 Hz, H-11), 3.31 and 3.53 (each 3H, each s, H-1 and 3), 4.20 (2H, q, J 7.2 Hz, H-10), 5.01 (2H, s, H-8), 7.53 (1H, s, H-5); δ_C (100 MHz, $CDCl_3$) 14.4 (C-11), 28.2 and 30.1 (C-1 and 3), 47.7 (C-10), 62.7 (C-8), 107.5 (C-4), 142.2 (C-5), 148.9 (C-6), 152.0 and 155.6 (C-2 and 7), 167.4 (C-9); *m/z* (EI) 266.1016 ($M^{+\bullet}$). $C_{11}H_{14}O_4N_4$ requires 266.1017; *m/z* (%) 266 (100), 252 (18), 220 (59), 193 (58), 152 (13), 109 (36), 81 (24), 67 (18).

7-(1,3-Dioxolylmethyl)-1,3-dimethylxanthine **101**



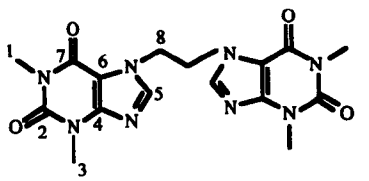
Compound **101** was prepared from theophylline (2.0 g, 11 mmol) and 2-bromomethyl-1,3-dioxolane (1.86 g, 11 mmol) in the presence of NaH (60% suspension, 0.57 g) by the modified method used to prepare **100**. Recrystallisation from EtOH provided **101** as pale yellow needles (1.61 g, 6 mmol, 55 %), mp 137-138 ^{0}C. (Found: C, 49.80; H, 5.29; N, 21.04; $C_{11}H_{14}O_4N_4$ requires C, 49.62; H, 5.26; N, 21.05%); ν_{max} (KBr disc)/cm^{-1} 1760m, 1636m (imidazole ring and CO); δ_H (400 MHz, $CDCl_3$) 3.34 and 3.52 (each 3H, each s, H-1 and 3), 3.79 (4H, m, H-10 and 11), 4.51 (2H, d, J 3.2 Hz, H-8), 5.15 (1H, t, J 3.2 Hz, H-9), 7.58 (1H, s, H-5); δ_C (100 MHz, $CDCl_3$) 28.3 and 30.1 (C-1 and 3), 48.3 (C-10 and 11), 65.8 (C-8), 101.2 (C-9), 107.7 (C-4), 142.6 (C-5), 148.7 (C-6), 152.0 and 155.7 (C-2 and 7); *m/z* (EI) 266.1014 ($M^{+\bullet}$). $C_{11}H_{14}O_4N_4$ requires 266.1013; *m/z* (%) 266 (10), 193 (6), 180 (5), 149 (2), 109 (5), 73 (100).

7,7'-Methylenebis(theophylline) **102**[95]



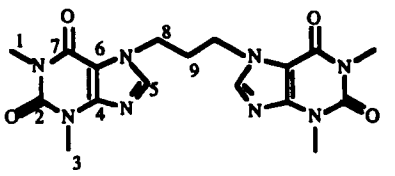
Sodium hydride (60% suspension, 0.17 g, 4.2 mmol) and dibromomethane (0.24 g, 1.4 mmol) were added to a solution of theophylline (0.5 g, 2.8 mmol) in DMF (20 ml). The mixture was stirred at room temperature overnight then heated at 70 ^{0}C for 3 h. The resulting mixture was poured into water to give **102** as a white solid (0.36 g, 0.97 mmol, 69 %), mp 327-328 ^{0}C (lit.,[95] mp > 300 ^{0}C). δ_H (400 MHz, $CDCl_3$) 3.34 and 3.49 (each 6H, each s, H-1 and 3), 6.66 (2H, s, H-8), 8.29 (2H, s, H-5).

7,7'-(1,2-Ethanediyl)bis(theophylline) **103**[95]



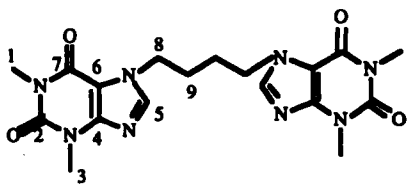
Compound **103** (0.41 g, 1.07 mmol, 77%) was prepared from theophylline (0.5 g, 2.8 mmol) and 1,2-dibromoethane (0.26 g, 1.4 mmol) in the presence of NaH (60 % suspension, 0.17 g, 4.2 mmol) by the method used to prepare **102**, mp 335 ^{0}C (lit.,[95] mp > 300 ^{0}C); *m/z* (EI) 386.1453 ($M^{+\bullet}$). $C_{16}H_{18}O_4N_8$ requires 386.1455; *m/z* (%) 386 (48), 278 (21), 221 (11), 206 (100), 180 (46), 122 (20), 95 (17), 67 (8).

7,7'-(1,3-Propanediyl)bis(theophylline) **104**[95]



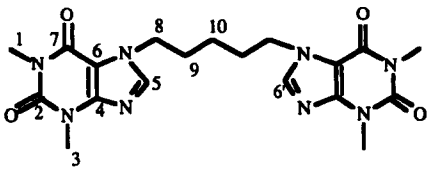
Compound **104** (0.30 g, 0.75 mmol, 54%) was prepared from theophylline (0.5 g, 2.8 mmol) and 1,3-dibromopropane (0.28 g, 1.4 mmol) in the presence of NaH (60% suspension, 0.17 g, 4.2 mmol) by the modified method used to prepare **102**, mp 268-270 ^{0}C (lit.,[95] mp 283-285 ^{0}C); *m/z* (EI) 400.1608 ($M^{+\bullet}$). $C_{17}H_{20}O_4N_8$ requires 400.1608; *m/z* (%) 400 (63), 386 (7), 207 (100), 194 (65), 180 (15), 109 (17), 82 (35), 67 (12).

7,7'-(1,4-Butanediyl)bis(theophylline) **105**[95]



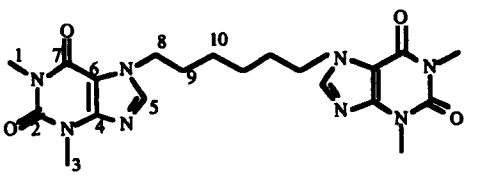
Compound **105** (0.28 g, 0.70 mmol, 55%) was prepared from theophylline (0.45 g, 2.5 mmol) and 1,4-dibromobutane (0.27 g, 1.25 mmol) in the presence of NaH (60% suspension, 0.15 g, 3.8 mmol) by the method used to prepare **102**, mp 280 ^{0}C (lit.,[95] mp 297-299 ^{0}C); δ_H (400 MHz, $CDCl_3$) 1.86 (4H, tt, J 6.4 Hz, H-9), 3.33 and 3.52 (each 6H, each s, H-1 and 3), 4.26 (4H, t, J 6.4 Hz, H-8), 7.51 (2H, s, H-5).

7,7'-(1,5-Pentanediyl)bis(theophylline) **106**[95]



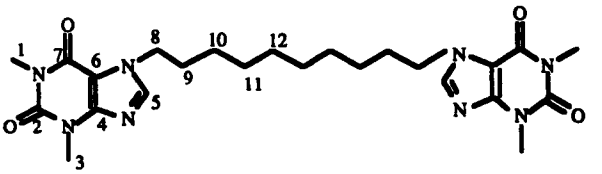
Compound **106** (0.13 g, 0.31 mmol, 22%) was prepared from theophylline (0.5 g, 2.8 mmol) and 1,5-dibromopentane (0.32 g, 1.4 mmol) in the presence of NaH (60% suspension, 0.17 g, 4.2 mmol) by the method used to prepare compound **102**, mp 221-223 ^{0}C (lit.,[95] mp 230-232 ^{0}C); δ_H (400 MHz, $CDCl_3$) 1.30 (2H, tt, J 7.6 Hz, H-10), 1.88 (4H, tt, J 7.6 Hz, H-9), 3.33 and 3.52 (each 6H, each s, H-1 and 3), 4.21 (4H, t, J 7.2 Hz, H-8), 7.49 (2H, s, H-5).

7,7'-(1,6-Hexanediyl)bis(theophylline) **107**[95]



Compound **107** (0.43 g, 0.97 mmol, 69%) was prepared from theophylline (0.5 g, 2.8 mmol) and 1,6-dibromohexane (0.34 g, 1.4 mmol) in the presence of NaH (60% suspension, 0.17 g, 4.2 mmol) by the method used to prepare compound **102**, mp 232-235 ^{0}C (lit.,[95] mp 243-245 ^{0}C); δ_H (400 MHz, $CDCl_3$) 1.30 (4H, m, H-10), 1.82 (4H, m, H-9), 3.33 and 3.51 (each 6H, each s, H-1 and 3), 4.19 (4H, t, J 7.2 Hz, H-8), 7.46 (2H, s, H-5).

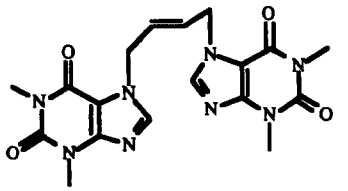
7,7'-(1,10-Decanediyl)bis(theophylline) **108**[95]



Compound **108** (0.47 g, 0.95 mmol, 68%) was prepared from theophylline (0.5 g, 2.8 mmol) and 1,10-dibromodecane (0.42 g, 1.4 mmol) in the presence of NaH (60% suspension, 0.17 g, 4.2 mmol) by the method used to prepare compound **102**, mp 161-163 ^{0}C (lit.,[95] mp 169-171 ^{0}C); δ_H (400 MHz, $CDCl_3$) 1.20 (12H, m, H-10 to

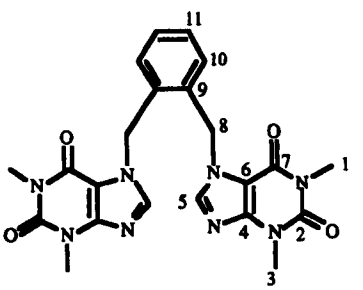
12), 1.80 (4H, m, H-9), 3.33 and 3.51 (each 6H, each s, H-1 and 3), 4.20 (4H, t, J 7.2 Hz, H-8), 7.46 (2H, s, H-5).

7,7'-(1,4-but-2-enediyl)bis(theophylline) **109**



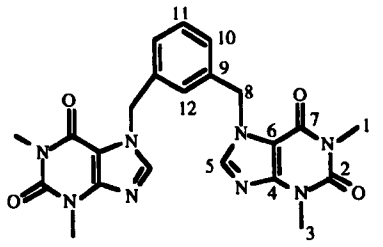
Compound **109** (0.23 g, 0.57 mmol, 41%) was prepared from theophylline (0.5 g, 2.8 mmol) and 1,4-dibromo-2-butene (0.30 g, 1.4 mmol) in the presence of NaH (60% suspension, 0.17 g, 4.2 mmol) by the method used to prepare **102**, mp 292-293 ^{0}C. (Found: C, 52.21; H, 4.89; N, 27.04; $C_{18}H_{20}O_4N_8$ requires C, 52.39; H, 4.85; N, 27.17 %); ν_{max} (KBr disc)/cm^{-1} 3117m (Ar-H), 1708s, 1646s and 1602m (imidazole ring and CO); *m/z* (EI) 412.1609 ($M^{+\bullet}$). $C_{18}H_{20}O_4N_8$ requires 412.1611; *m/z* (%) 412 (33), 232 (100), 180 (13), 147 (33), 121 (21), 94 (16), 80 (8), 67 (7).

7,7'-o-Xylylenebis(theophylline) **110**



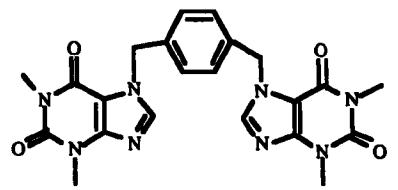
Compound **110** (0.44 g, 0.94 mmol, 75%) was prepared from theophylline (0.45 g, 2.5 mmol) and α,α-dibromo-o-xylene (0.33 g, 1.25 mmol) in the presence of NaH (60% suspension, 0.15 g, 3.8 mmol) by the method used to prepare **102**, mp 298 ^{0}C. (Found: C, 56.99; H, 4.71; N, 23.91; $C_{22}H_{22}O_4N_8$ requires C, 57.14; H, 4.76;N, 24.24%); ν_{max} (KBr disc)/cm^{-1} 3112m (Ar-H), 1719s and 1602m (imidazole ring, Ar and CO); δ_H (400 MHz, $CDCl_3$) 3.29 and 3.51 (each 6H, each s, H-1 and 3), 5.59 (4H, s, H-8), 7.04 and 7.28 (each 2H, each m, ArH), 7.46 (2H, s, H-5); δ_C (100 MHz, $CDCl_3$) 28.3 and 30.2 (C-1 and 3), 47.5 (C-8), 107.3 (C-4), 129.3 and 129.8 (C-10 and 11), 133.6 (C-9), 141.6 (C-5), 149.3 (C-6), 151.9 and 155.6 (C-7); *m/z* (EI) 462.1762 ($M^{+\bullet}$). $C_{22}H_{22}O_4N_8$ requires 462.1760; *m/z* (%) 462 (70), 282 (100), 281 (30), 198 (35), 130 (33), 104 (22), 78 (7).

7,7'-m-Xylylenebis(theophylline) **111**



Compound **111** (0.50 g, 1.08 mmol, 87%) was prepared from theophylline (0.45 g, 2.5 mmol) and α,α'-dibromo-m-xylene (0.33 g, 1.25 mmol) in the presence of NaH (60% suspension, 0.15 g, 3.8 mmol) by the method used to prepare **102**, mp 265 ^{0}C; ν_{max} (KBr disc)/cm^{-1} 3110m (Ar-H), 1731s (imidazole ring, Ar and CO); δ_H (400 MHz, $CDCl_3$) 3.33 and 3.50 (each 6H, each s, H-1 and 3), 5.40 (4H, s, H-8), 7.19 to 7.31 (4H, m, Ar-H), 7.51 (2H, s, H-5); δ_C (100 MHz, $CDCl_3$) 28.3 and 30.1 (C-1 and 3), 50.3 (C-8), 107.2 (C-4), 128.0, 128.4 and 130.2 (C-10, 11 and 12), 136.8 and 138.5 (C-9), 141.1 (C-5), 149.3 (C-6), 151.9 and 155.6 (C-2 and 7); *m/z* (EI) 462.1765 ($M^{+\bullet}$). $C_{22}H_{22}O_4N_8$ requires 462.1766; *m/z* (%) 462 (90), 282 (100), 198 (18), 171 (8), 130 (9), 104 (27), 78 (9).

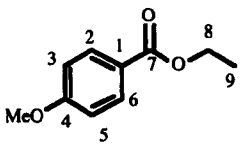
7,7'-p-Xylylenebis(theophylline) **112**



Compound **112** (0.48 g, 1.04 mmol, 84%) was prepared from theophylline (0.45 g, 2.5 mmol) and α,α'-dibromo-p-xylene (0.33 g, 1.25 mmol) in the presence of NaH (60% suspension, 0.15 g, 3.8 mmol) by the method used to prepare **102**, mp > 340 ^{0}C; ν_{max} (KBr disc)/cm^{-1} 3104m (Ar-H), 1727s and 1597m (imidazole ring, Ar and CO); *m/z* (EI) 462.1767 ($M^{+\bullet}$). $C_{22}H_{22}O_4N_8$ requires 462.1770; *m/z* (%) 462 (15), 282 (100), 231 (3), 185 (4), 104 (38), 78 (5).

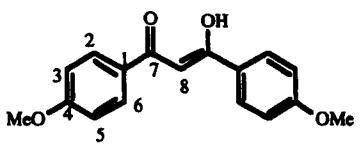
Experimental to Chapter 7

Ethyl 4-methoxybenzoate **113**[97]



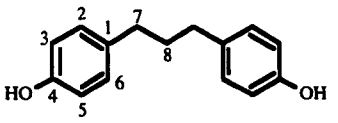
To a stirring suspension of *p*-anisic acid (3.0 g, 19.7 mmol) in EtOH (18 ml) was added concentrated H_2SO_4 (0.4 ml) dropwise and the mixture was heated at reflux for 24 h. The EtOH was evaporated and the residue was taken into EtOAc (50 ml). The EtOAc solution was washed with 1 M NaOH (2 x 25 ml), water (25 ml) and brine (10 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave **113** as a colourless oil (3.11 g, 17.3 mmol. 88%) pure enough for synthetic work without further purification. δ_H (400 MHz, $CDCl_3$) 1.30 (3H, t, J 7.2 Hz, H-9), 3.78 (3H, s, OCH_3), 4.27 (2H, q, J 7.2 Hz, H-8), 6.84 (2H, m, H-3 and 5). 7.92 (2H, m, H-2 and 6); δ_C (100 MHz, $CDCl_3$) 13.4 (C-9), 54.4 (OMe), 59.6 (C-8), 112.5 (C-3 and 5), 121.9 (C-1), 130.5 (C-2 and 6), 162.2 (C-4), 165.4 (CO).

1, 3-Bis-(4-methoxyphenyl)propane-1, 3-dione **114**[97]



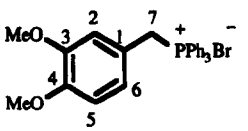
A solution of 4-methoxyacetophenone (1.17 g, 7.57 mmol) in dry toluene (10 ml) was added dropwise to a stirred suspension of NaH (0.43 g, 60% dispersion, 7.57 mmol) in dry toluene (50 ml) at rt. After 2 min a solution of ethyl ester **113** (1.50 g, 8.33 mmol) in dry toluene (5 ml) was introduced dropwise at rt, and the mixture was heated at reflux for 24 h. After cooling to rt, the reaction was quenched by the cautious addition of 10% HCl (40 ml) and the mixture was extracted with EtOAc (2 x 50 ml). The combined organic layers were washed with water (60 ml) and brine (50 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a yellow solid which was recrystallised from EtOH affording **114** as bright yellow crystals (0.60 g, 2.11 mmol, 28 %). mp 106-108 ^{0}C (lit.,[97] 108 ^{0}C); δ_H (400 MHz, $CDCl_3$) 3.80 (6H, s, OCH_3), 6.65 (1H, s, H-8), 6.88 (4H, m, H-3 and 5), 7.91 (4H, m, H-2 and 6); δ_C (100 MHz, $CDCl_3$) 54.4 (OCH_3), 90.5 (C-8), 112.9 (C-3 and 5), 127.2 (C-1), 128.1 (C-2 and 6), 161.9 (C-4), 183.6 (CO).

1, 3-Bis-(4-hydroxyphenyl)propane **115**[99]



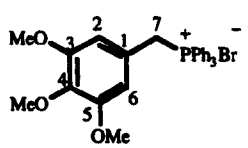
A solution of compound **114** (0.34 g, 1.19 mmol) in EtOH-EtOAc (4:1) (60 ml) containing Pd/C (10%, 1.0 g) was shaked under 7 atm of hydrogen at 28 ^{0}C for 5 h. The mixture was filtered through Celite® and concentrated in vacuo to give a coloureless oil (0.25 g) which solidified to white crystals on standing. The crude methyl ether was taken up in glacial AcOH (2.5 ml). 48% HBr (5 ml) was added to the AcOH solution and the mixture was heated at reflux for 4 h. the solvent was evaporated and the residue was taken up in EtOAC (10 ml). The EtOAC solution was washed with saturated $NaHCO_3$ (3 x 5 ml), water (5 ml) and brine (5 ml) before drying over $MgSO_4$. Concentration *in vacuo* to give a yellow oil which was then washed with petroleum ether affording crude **115** as a yellow solid. Recrystallisation from EtOAc yielded compound **115** as a yellow solid (0.10 g, 0.44 mmol, 37%), mp 105-106 ^{0}C (lit.,[99] 108-109 ^{0}C); δ_H (400 MHz, $CDCl_3$) 1.79 (2H, m, H-8), 2.48 (4H, t, J 7.8 Hz, H-7), 4.76 (2H, bs, OH), 6.68 (4H, m, H-3 and 5). 6.96 (4H, m, H-2 and 6); δ_C (100 MHz, $CDCl_3$) 33.8 (C-8), 34.8 (C-7), 115.5 (C-3 and 5), 129.9 (C-2 and 6). 135.0 (C-1), 153.8 (C-4).

3,4-Dimethoxybenzyl triphenylphosphonium bromide **116**[8]



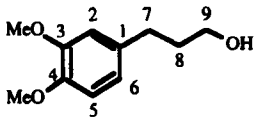
A mixture of triphenylphosphine hydrobromide (4.08 g, 11.9 mmol) and 3,4-dimethoxybenzyl alcohol (2.0 g, 11.9 mmol) in dry acetonitrile (120 ml) was heated at reflux overnight. The mixture was concentrated *in vacuo* to give a pale yellow solid which was recrystallised from ethanol affording compound **116** as white crystals (5.3 g, 95%), m.p. 235 ^{0}C (lit.,[8] 235-237 ^{0}C). δ_H (400 MHz, $CDCl_3$) 3.45 and 3.72 (each 3H, each s, OCH_3), 5.22 (2H, d, J_{H-P} 13.8 Hz, CH_2), 6.52-6.75 (3H, m, H-2,5 and 6), 7.53-7.71 (15H, m, PPh_3).

3,4,5-Trimethoxybenzyl triphenylphosphonium bromide **117**[100]



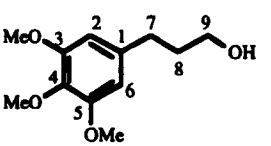
A mixture of triphenylphosphine hydrobromide (3.5 g, 10.1 mmol) and 3,4,5-trimethoxybenzyl alcohol (2.0 g, 10.1 mmol) in dry acetonitrile (140 ml) was heated at reflux overnight. Concentration *in vacuo* gave a pale yellow solid which was recrystallised from ethanol affording **117** as white crystals (4.8 g, 9.18 mmol, 90%), mp 232 ^{0}C (lit.,[100] 234-236 ^{0}C); δ_H(400 MHz, $CDCl_3$) 3.43 (6H, s, OCH_3), 3.69 (3H, s, OCH_3), 5.31 (2H, d, J_{H-P} 14.0 Hz, CH_2), 6.42 (2H, d, J 2.8 Hz, ArH), 7.51-7.71 (15H, m, PPh_3).

3-(3,4-Dimethoxyphenyl)propan-1-ol **118**[101]



A solution of 3-(3,4-dimethoxyphenyl)propionic acid (8.7 g, 42.8 mmol) in dry THF (20 ml) was added dropwise to a stirring slurry of $LiAlH_4$ (2.44 g, 64.2 mmol) and THF (100 ml) at 0 ^{0}C. After stirring at rt for 5 h the reaction was quenched by the cautious addition of moist ether followed by 1M HCl till the layers were separated. The aqueous layer was extracted with EtOAc (2 x 100 ml) and the combined organic layers were washed with brine (50 ml) before drying over $MgSO_4$. Concentration *in vacuo* afforded compound **118** as a viscous oil which was satisfactory for synthetic work without further purification (7.7 g, 94%), bp 159 ^{0}C > 1 mmHg (lit.,[8] 150-152 ^{0}C > 1 mmHg, vacuum pump); δ_H (400 MHz, $CDCl_3$) 1.52 (1H, bs, OH), 1.80 (2H, m, H-8), 2.58 (2H, t, J 7.9Hz, H-7), 3.60 (2H, t, J 6.4 Hz, H-9), 3.78 and 3.79 (each 3H, each s, OCH_3), 6.65-6.73 (3H, m, ArH).

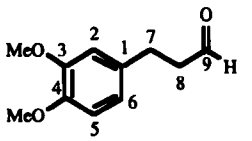
3-(3,4,5-Trimethoxyphenyl)propan-1-ol **119**[102]



A solution of 3-(3,4,5-trimethoxyphenyl)propionic acid (3.0 g, 12.5 mmol) in dry THF (10 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (0.71 g, 18.8 mmol) in THF (40 ml) at 0 ^{0}C. After stirring at rt for 5 h the reaction was quenched by the cautious addition of water and ether followed by 1M HCl till the layers were separated. The aqueous layer was extracted with EtOAc (2 x 80 ml) and the combined organic layers were washed with brine (50 ml) before drying over $MgSO_4$. Concentration *in vacuo* afforded **119** as a colourless oil which was satisfactory for synthetic work without further purification (2.4 g, 10.62 mmol, 85%). bp 148-158 ^{0}C (> 1 mmHg, vacuum pump) [lit.,[102] b.p. 120-130^{0}C (1 mmHg)]; δ_H(400 MHz,

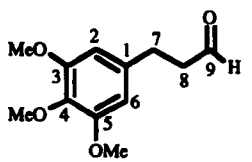
$CDCl_3$) 1.75 (1H, bs, OH), 1.81 (2H, m, H-8), 2.58 (2H, t, J 8.0 Hz, H-7), 3.61 (2H, t, J 6.4 Hz, H-9), 3.74 (3H, s, OCH_3), 3.77 (6H, s, OCH_3), 6.35 (2H, s, ArH).

3-(3,4-Dimethoxyphenyl)propionaldehyde **120**[104]



A solution of DMSO (6.7 g, 6.1 ml, 85.6 mmol) in dry DCM (25 ml) was added dropwise to a stirred solution of oxalyl chloride (5.0 g, 3.4 ml, 39.2 mmol) in dry DCM (180 ml) at -60 ^{0}C. After 2 min a solution of alcohol **118** (7.0 g, 35.7 mmol) in dry DCM (50 ml) was introduced dropwise at the same temperature and stirring was continued for 30 min. Et_3N (18.1 g, 24.9 ml, 178.9 mmol) was added dropwise to the white cloudy solution and the mixture was allowed to warm to rt over 1h. Water (250 ml) was added and the resultant two layers were separated and the aqueous layer was further extracted with DCM (150 ml). The combined organic layers were washed with 10% HCl (100 ml) and brine (150 ml) before drying over $MgSO_4$. Concentration *in vacuo* afforded compound **120** as a light yellow oil (6.7 g, 97%) pure enough to be used without further purification. δ_H(400 MHz, $CDCl_3$) 2.68 and 2.83 (each 2H, each t, J 7.2 Hz, H-7 and 8), 3.77 and 3.79 (each 3H, each s, CH_3O), 6.64-6.73 (3H, m, ArH), 9.74 (1H, s, CHO).

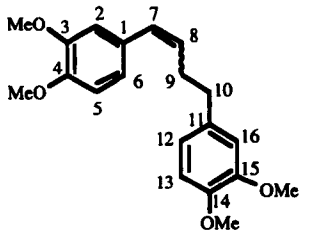
3-(3,4,5-Trimethoxyphenyl)propionaldehyde **121**[105]



A solution of DMSO (0.92 g, 0.84 ml, 11.8 mmol) in dry DCM (2 ml) was added dropwise to a stirred solution of oxalyl chloride (0.69 g, 0.47 ml, 5.4 mmol) in dry DCM (35 ml) at -60 ^{0}C. After 2 min a solution of alcohol **119** (1.1 g, 4.9 mmol) in dry DCM (5 ml) was introduced dropwise at the same temperature and stirring was continued for 30 min. Et_3N (2.49 g, 3.4 ml, 24.5 mmol) was added dropwise to the white cloudy solution and the mixture was allowed to warm to rt over 1 h. Water (30 ml) was added and the resultant two layers were separated and the aqueous layer was further extracted with DCM (30 ml). The combined organic layers were washed with 10% HCl (10 ml) and brine (15 ml) before drying over $MgSO_4$. Concentration *in vacuo* afforded **121** as a yellow oil (1.08 g, 98%) pure enough to be

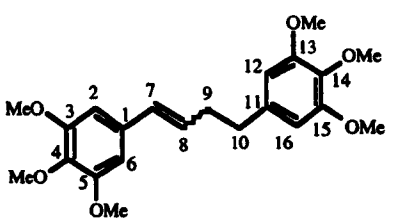
used without further purification. δ_H(400 MHz, $CDCl_3$) 2.71 and 2.83 (each 2H, each m, H-7 and 8), 3.72 (3H, s, OCH_3), 3.77 (6H, s, OCH_3), 6.34 (2H, s, ArH), 9.73 (1H, s, CHO).

1,4-Bis-(3,4-dimethoxyphenyl)but-1-ene **122**[8]



To a stirred suspension of phosphonium salt **116** (14.9 g, 30.2 mmol) in dry THF (250 ml) was added *n*-butyl lithium (15.8 ml, 2.1 M in hexanes, 33.2 mmol) dropwise at 0 ^{0}C. After stirring the dark red solution for a further 15 min aldehyde **120** (5.5 g, 28.5 mmol) in THF (40 ml) was introduced dropwise at the same temperature. The resultant fawn coloured suspension was stirred at 0 ^{0}C for 30 min then allowed to warm to rt and stirred for a further 3 h before pouring into iced water (500 ml). The mixture was extracted into ethyl acetate (3 x 250 ml) and the combined organic layers were washed with brine (200 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a dark yellow gum which became a mixture of crystals and sticky oil after leaving at rt overnight. The mixture was stirred with MeOH (150 ml) at rt for 2h. The yellow suspension was filtered and washed thoroughly with MeOH affording **122** as a yellow solid (4.7 g, 50%, E:Z = 9). Concentration of the filtrate *in vacuo* gave a yellow oil which was purified by silica gel column chromatography affording more of **122** (2.1 g, 22%, E:Z = 2). mp 60 ^{0}C (lit.,[8] 58 ^{0}C). δ_H(400 MHz, $CDCl_3$) (E)-isomer: 2.42 (2H, m, H-9), 2.66 (2H, m, H-10), 3.80 (12H, m, CH_3O), 6.05 (1H, dt, J 15.8 Hz and 6.8 Hz, H-8), 6.29 (1H, m, H-7), 6.64-6.82 (6H, m, ArH); (Z)-isomer: 2.58 (2H, m, H-9), 2.66 (2H, m, H-10), 3.80 (12H, m, CH_3O), 5.55 (1H, dt, J 11.6 Hz and 6.8 Hz, H-8), 6.29 (1H, m, H-7), 6.64-6.82 (6H, m, ArH).

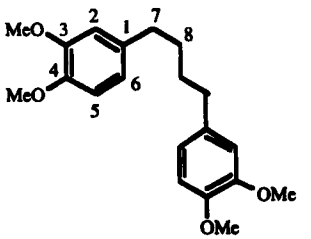
1,4-Bis-(3,4,5-trimethoxyphenyl)but-1-ene **123**



n-Butyl lithium (3.5 ml, 1.6 M in hexanes, 5.6 mmol) was added dropwise to a stirred suspension of phosphonium salt **117** (2.69 g, 5.1 mmol) in dry THF (30 ml) at 0 ^{0}C. After stirring the dark red solution for a further 15 min, aldehyde **121** (1.1 g, 4.9 mmol) in THF (6

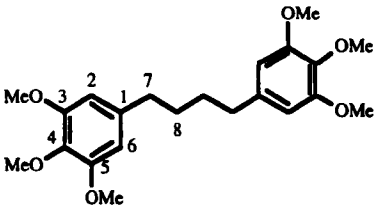
ml) was introduced dropwise at the same temperature. The resultant yellow suspension was stirred at 0 ^{0}C for 30 min then allowed to warm to rt and stirred for a further 3 h before pouring into iced water (50 ml). The mixture was extracted into ethyl acetate (3 x 40 ml) and the combined organic layers were washed with brine (20 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a dark yellow gum which became a mixture of crystals and sticky oil after leaving at rt overnight. The mixture was purified by column chromatography to give **123** as a mixture of geometrical isomers (0.61 g, 1.57 mmol, 32%, Z:E = 1); mp 98 ^{0}C; ν_{max} (neat)/cm^{-1} 2937s (OCH_3), 1588s (C=C); δ_H(400 MHz, $CDCl_3$) (E)-isomer: 2.44 (2H, m, H-9), 2.65 (2H, m, H-10), 3.75 (18H, m, OCH_3), 6.11 (1H, dt, J 15.8 Hz and 6.8 Hz, H-8), 6.28 (1H, m, H-7), 6.33-6.49 (4H, m, ArH); (Z)-isomer: 2.61 (2H, m, H-9), 2.65 (2H, m, H-10), 3.75 (18H, m, OCH_3), 5.59 (1H, dt, J 11.6 Hz and 6.8 Hz, H-8), 6.28 (1H, m, H-7), 6.33-6.49 (4H, m, ArH); δ_C(100 MHz, $CDCl_3$) 29.9, 33.8 and 35.3 (C-9 and 10), 55.1 (OCH_3), 102.0, 102.4, 104.3, 104.4, 104.9, 105.5, 128.3, 128.6 and 129.4 (CH), 129.4, 130.4, 132.4, 135.1, 136.5, 150.5, 151.8, 152.1, 152.3 and 152.4 (C); *m/z* (EI) 388.1884 (M^+). $C_{22}H_{28}O_6$ requires M 388.1882; *m/z* (%) 388 (45), 360 (12), 345 (8), 207 (86), 181 (100), 176 (34), 149(7), 106 (5).

1,4-Bis-(3,4-dimethoxyphenyl)butane **124**[104]



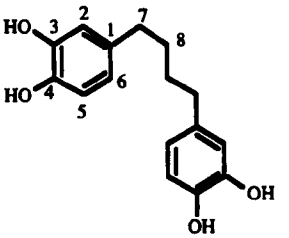
Palladium on charcoal (10%, 1.0 g) was added to a stirred solution of **122** in EtOAc-MeOH (1: 5) (370 ml) and the mixture was stirred under a hydrogen balloon at rt for 24 h. The mixture was filtered through Celite®. Concentration *in vacuo* gave a white solid which was recrystallised from aqueous MeOH affording **124** as white crystals (3.9 g, 92%), mp 89 ^{0}C (lit.,[104] 90-91 ^{0}C); δ_H(400 MHz, $CDCl_3$) 1.60 (4H, m, H-8), 2.51 (4H, t, J 6.8 Hz, H-7), 3.77 and 3.78 (each 6H, each s, OCH_3), 6.62-6.72 (6H, m, ArH).

1,4-Bis-(3,4,5-trimethoxyphenyl)butane **125**



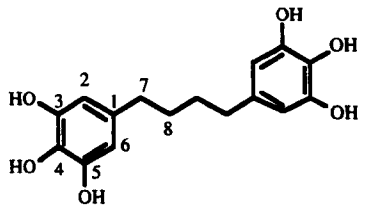
Pd/C (10 %, 0.2 g) was added to a stirred solution of **123** (0.41 g, 1.06 mmol) in EtOAc-MeOH (1:4) (40 ml) and the mixture was stirred under a hydrogen balloon at rt for 24 h. The mixture was filtered through Celite®. Concentration *in vacuo* gave a white solid which was recrystallised from aqueous MeOH affording **125** as white crystals (0.36 g, 86 %), mp 96 ^{0}C. (Found: C, 67.79; H, 7.79; $C_{22}H_{30}O_6$ requires C, 67.69; H, 7.6 %); ν_{max} (KBr disc)/cm^{-1} 2932s (OCH_3), 1587s (Ar); δ_H (400 MHz, $CDCl_3$) 1.60 (4H, m, H-8), 2.52 (4H, t, J 6.8 Hz, H-7), 3.75-3.79 (18H, m, OCH_3), 6.31 (4H, s, ArH); δ_C(100 MHz, $CDCl_3$) 31.5 (C-8), 36.6 (C-7), 56.4 and 61.2 (OCH_3), 105.7 (C-2 and 6), 136.4 and 138.7 (C-1 and 4), 153.5 (C-3 and 5); *m/z* (EI) 390.2044 (M^+). $C_{22}H_{30}O_6$ requires M 390.2046; *m/z* (%) 390 (23), 362 (25), 282 (12), 208 (4), 181 (100), 151 (10), 83 (17).

1,4-Bis-(3,4-dihydroxyphenyl)butane **126**[106]



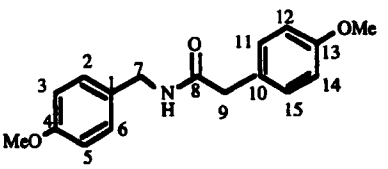
A mixture of compound **124** (1.8 g, 5.5 mmol), 48% HBr (30 ml) and glacial AcOH (12 ml) was heated at reflux under N_2 for 6 h. After cooling to rt the mixture was poured into water (300 ml) and extracted with EtOAc (2 x 200 ml). The organic layers were decolourised by activated carbon and washed with brine (2 x 60 ml), dried over $MgSO_4$ and concentrated *in vacuo* to give a light brown solid. Recrystallisation from ether-hexane afford **126** as brown crystals (1.1 g, 72%), mp 139 ^{0}C (lit.,[106] 136-138 ^{0}C). δ_H(400 MHz, d_6-DMSO) 1.47 (4H, bs, H-8), 2.40 (4H, t, J 6.2 Hz, H-7), 6.38-6.62 (6H, m, ArH), 8.60 (4H, bs, OH).

1,4-Bis-(3,4,5-trihydroxyphenyl)butane **127**



A mixture of **125** (0.13 g, 0.33 mmol), 48% HBr (3.5 ml) and glacial AcOH (1.4 ml) was heated at reflux under N_2 for 6 h. After cooling to rt the mixture was poured into water (30 ml) and extracted with EtOAc (2 x 20 ml). The organic layers were decolourised by activated carbon and washed with brine (10 ml), dried over $MgSO_4$ and concentrated *in vacuo* to give a light brown solid. Recrystallisation from ether-hexane afforded **127** as yellow crystals (0.073 g, 72%), mp 191-192 ^{0}C. (Found: C, 62.50; H, 6.23; $C_{16}H_{18}O_6$ requires C, 62.34; H, 6.49%). ν_{max} (KBr disc)/cm^{-1} 3455s (OH), 1611s (Ar); δ_H(400 MHz, d_6-DMSO) 1.36 (4H, m, H-8), 2.32 (4H, m, H-7), 6.06 (4H, s, H-2 and 6), 7.70 (2H, bs, OH), 8.57 (4H, bs, OH); δ_C(100 MHz, d_6-DMSO) 31.1 (C-8), 35.0 (C-7), 107.3 (C-2 and 6), 131.0 and 132.7 (C-1 and 4), 146.2 (C-3 and 5); *m/z* (EI) 306.1100 (M^+). $C_{16}H_{18}O_6$ requires M 306.1096; *m/z* (%) 306 (65), 246 (5), 178 (14), 139 (100), 122 (5), 65 (7), 53 (10).

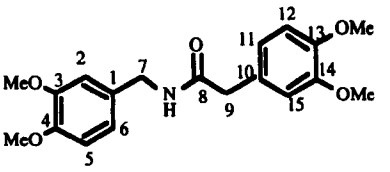
N-(4-Methoxybenzyl)-4-methoxyphenylacetamide **128**



A mixture of 4-methoxyphenylacetic acid (0.8 g, 4.81 mmol) and 4-methoxybenzylamine (0.59 g, 0.56 ml, 4.33 mmol) was heated with stirring under nitrogen at 160 ^{0}C for 4 h. The brown solid formed dissolved in CH_2Cl_2 and the organic solution was washed successively with saturated $NaHCO_3$ (10 ml), 5% citric acid (10 ml), water (10 ml) and brine (10 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave **128** as a yellow solid which was recrystallised from ethyl acetate providing **128** as yellow crystals (1.0 g, 81%), mp 136-138 ^{0}C. (Found: C, 71.61; H, 6.73; N, 4.88; $C_{17}H_{19}NO_3$ requires C, 71.58; H, 6.67; N, 4.91 %). ν_{max} (KBr disc)/cm^{-1} 3284s (CONH), 2834m (OCH_3), 1644s (CO), 1609s (Ar). δ_H (400 MHz, $CDCl_3$) 3.47 (2H, s, H-9), 3.70 and 3.71 (each 3H, each s, OCH_3), 4.25 (2H, d, J 5.6 Hz, H-7), 5.60 (1H, bs, NH), 6.78 (4H, m, H-3, 5, 12 and 14), 7.06 (4H, m, H-2, 6, 11 and 15); δ_C (100 MHz, $CDCl_3$) 41.9 and 42.0 (C-7 and 9), 54.2 (OCH_3), 113.0 and 113.4 (C-12, 14, 3 and 5), 125.7 and 129.2 (C-1 and 15), 127.9 and 129.5 (C-2, 6, 11 and 15), 157.8 and

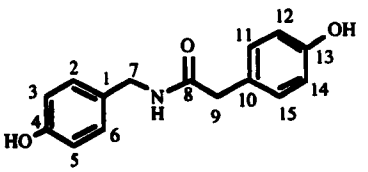
157.9 (C-4 and 13), 170.2 (CO). *m/z* (EI) 285.1363 (M^+). $C_{17}H_{19}O_3N$ requires M 285.1361; *m/z* (%) 285 (20), 227 (3), 177 (4), 121 (100), 91 (5), 78 (8), 77 (7).

N-(3,4-Dimethoxybenzyl)-3,4-dimethoxyphenylacetamide **129**[8]



Compound **129** was prepared from (3,4-dimethoxyphenyl)acetic acid (0.8 g, 4.07 mmol) and veratrylamine (0.61 g, 0.55 ml, 3.67 mmol) by the method used to prepare **128**. Recystallisation from ethyl acetate afforded **129** as a yellow solid (1.14 g, 90%), mp 130-132 ^{0}C (lit.,[8] 127-129 ^{0}C); δ_H (400 MHz, $CDCl_3$) 3.49 (2H, s, H-9), 4.27 (2H, d, J 5.6 Hz, H-7), 3.78 (9H, s, OCH_3), 3.79 (3H, s, OCH_3), 4.27 (2H, d, J 5.4 Hz, H-7), 5.63 (1H, bs, NH), 6.64-6.76 (6H, m, ArH).

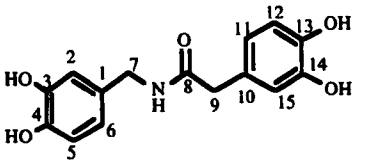
N-(4-Hydroxybenzyl)-4-hydroxyphenylacetamide **130**



The procedure of McOmie *et al.*[107] was modified as follows. To a stirred solution of **128** (2.0 g, 7.0 mmol) in dry CH_2Cl_2 (250 ml) was added boron tribromide (28.1 ml, 28.07 mmol, 1M in DCM) dropwise at -78 ^{0}C. This was stirred for 1 h then allowed to reach 0 ^{0}C and stirred for 2.5 h before pouring into iced water (50 ml). The mixture was stirred for 1 h and the two layers were separated. The aqueous layer was extracted with ethyl acetate (3 x 150 ml), and the initial organic layer was concentrated *in vacuo* to give a yellow solid which was combined with the ethyl acetate extracts. The ethyl acetate solution was washed with saturated $NaHCO_3$ (2 x 150 ml), water (150 ml) and brine (150 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a yellow solid which was recrystallised from ethyl acetate affording **130** as yellow crystals (1.66 g, 92%), mp 171-173 ^{0}C. (Found: C, 70.12; H, 5.77; N, 5.29; $C_{15}H_{15}NO_3$ requires C, 70.04; H, 5.84; N, 5.45 %). ν_{max} (KBr disc)/cm^{-1} 3349m and 3239m (OH and CONH), 1648s (CO), 1611s and 1598s (Ar). δ_H (400 MHz, d4-MeOH) 3.30 (2H, s, H-9), 4.13 (2H, s, H-7), 6.62 (4H, m, H-3, 5, 12 and 14), 6.96 (4H, m, H-2, 6, 11, and 15); δ_C (100 MHz, d4-MeOH) 43.5 and 44.2 (C-7 and 9), 116.6 and 116.7 (C-3, 5, 12 and 14), 128.1 and 131.0 (C-1 and 10), 130.4 and 131.5 (C-2, 6, 11 and 15), 157.8 and 158.1 (C-4 and

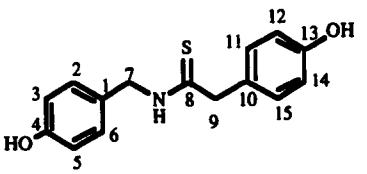
13), 174.8 (CO). *m/z* (EI) 257.1051 (M^+). $C_{15}H_{15}O_3N$ requires M 257.1050; *m/z* (%) 257 (27), 199 (2), 163 (3), 121 (7), 107 (100), 77 (12), 51 (2).

N-(3,4-Dihydroxybenzyl)-3,4-dihydroxyphenylacetamide **131**[8]



To a stirred solution of **129** (0.4 g, 1.16 mmol) in dry CH_2Cl_2 (10 ml) was added BBr_3 (8.1 ml, 8.1 mmol, 1 M in CH_2Cl_2) dropwise at -78 ^{0}C. This was stirred for 1 h then allowed to reach 0 ^{0}C and stirred for 2.5 h before pouring into iced water (30 ml). The mixture was stirred for 1 h and the two layers were separated. The aqueous layer was extracted with EtOAc (3 x 30 ml). The combined EtOAc extracts were extracted into saturated $NaHCO_3$ (40 ml) which was then acidified by 10 % HCl, and re-extracted with EtOAc (3 x 40 ml). The EtOAc solution was washed with brine (30 ml), dried over $MgSO_4$ and concentrated to give **131** as a dark brown solid (0.29 g, 1.0 mmol, 87%), mp 177-179 ^{0}C (EtOAc-EtOH) (lit.,[8] 179-182 ^{0}C); δ_H (400 MHz, d_6-DMSO) 3.23 (2H, s, H-9), 4.11 (2H, d, J 5.6 Hz, H-7), 6.42-6.73 (6H, m, ArH), 8.25 (1H, t, J 5.6 Hz, NH), 8.8 (4H, bs, OH).

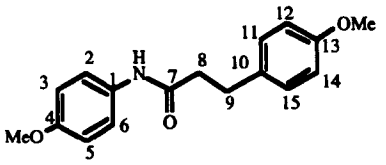
N-(4-Hydroxybenzyl)-4-hydroxyphenylthioethanamide **132**



To a stirred solution of **130** (0.12 g, 0.467 mmol) in DME (5 ml) was added Lawesson's reagent (0.25 g, 0.63 mmol) portionwise. The resultant suspension was heated at reflux overnight. After cooling to rt, the reaction mixture was poured into water (25 ml) and extracted with EtOAc (3 x 25 ml). The combined organic extracts were washed successively with saturated $NaHCO_3$ (3 x 25 ml), water (25 ml) and brine (25 ml) before drying over $MgSO_4$. The solvent was evaporated and the residue was purified by chromatography to give **132** as a yellow solid (0.02 g, 0.072 mmol, 16%), mp 134-137 ^{0}C (EtOAc). ν_{max} (KBr disc)/cm^{-1} 3379s (OH and CSNH), 1608s (Ar), 1511m (CSN). δ_H (400 MHz, d_4-MeOH) 3.76 (2H, s, H-9), 4.58 (2H, s, H-7), 6.62 (4H, m, H-3, 5, 12 and 14), 7.04 (4H, m, H-2, 6, 11 and 15); δ_C (100 MHz, d_4-MeOH) 50.8 (C-9), 52.7 (C-7), 116.6 and 116.7 (C-3, 5, 12 and 14), 129.3 and 129.6 (C-1 and 10), 130.9 and 131.3 (C-2, 6, 11 and 15), 157.9 and 158.4 (C-4 and 13), 204.5 (CS).

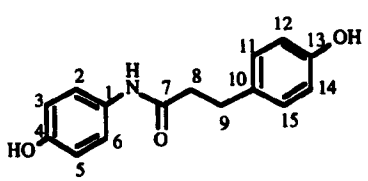
m/z (EI) 273.0824 (M^+). $C_{15}H_{15}O_2NS$ requires M 273.0824; *m/z* (%) 273 (30), 257 (3), 199 (4), 167 (9), 133 (10), 107 (100), 77 (18), 44 (10).

N-*(4-Methoxyphenyl)-4-methoxyphenylpropanamide* **134**



A mixture of 3-(4-methoxyphenyl)propionic acid (1.0 g, 5.58 mmol) and *p*-anisidine (0.69 g, 5.58 mmol) was heated with stirring under nitrogen at 160 ^{0}C for 4 h. The dark solid formed dissolved in CH_2Cl_2 and the organic solution was washed successively with saturated $NaHCO_3$ (10 ml), 5% citric acid (10 ml), water (10 ml) and brine (10 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave **134** as a dark purple solid which was pure enough for use without further purification (1.06 g, 67 %), mp 154-155 ^{0}C (EtOAc). (Found: C, 71.36; H, 6.74; N, 4.96; $C_{17}H_{19}NO_3$ requires C, 71.58; H, 6.67; N, 4.91 %). ν_{max} (KBr disc)/cm^{-1} 3309m (CONH), 2953m (OCH_3), 1656s (CO), 1601 (Ar). δ_H (400 MHz, $CDCl_3$) 2.52 (2H, t, J 7.4 Hz, H-8), 2.91 (2H, t, J 7.4 Hz, H-9), 3.70 and 3.71 (6H, each s, OCH_3), 6.75 (4H, m, H-3, 5, 12 and 14), 7.06 (2H, d, J 8.6 Hz, H-11 and 15), 7.25 (2H, m, H-2 and 6); δ_C (100 MHz, $CDCl_3$) 29.8 (C-8), 38.6 (C-9), 54.3 and 54.4 (OCH_3), 113.0, 113.1 and 120.9 (C-3, 5, 12, 14, 2 and 6), 128.3 (C-11 and 15), 129.8 and 131.7 (C-1 and 10), 155.4 and 157.1 (C-4 and 13), 169.3 (CO); *m/z* (EI) 285.1363 (M^+). $C_{17}H_{19}O_3N$ requires M 285.1361; *m/z* (%) 285 (60), 227 (15), 123 (100), 121 (38), 108 (25), 83 (12), 77 (6).

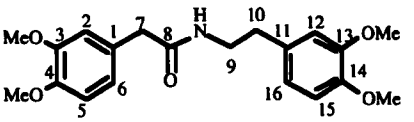
N-*(4-Hydroxyphenyl)-4-hydroxyphenylpropanamide* **135**



BBr_3 (8.42 ml, 8.42 mmol, 1M in DCM) was added dropwise to a stirred solution of **134** (0.64 g, 2.24 mmol) in DCM (16 ml) at -78 ^{0}C. The brown suspension was stirred for 1 h and then allowed to reach 0 ^{0}C and stirred for 2.5 h. The reaction mixture was poured into iced water (30 ml) and shaken for 20 min. The resulting suspension was filtered affording **135** as a grey solid (0.53 g, 91%) pure enough to use without further purification, mp 190 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3148s (OH and CONH), 1654s (CO), 1601s (Ar). δ_H (400 MHz, d4-MeOH) 2.45 (2H, t, J 7.4 Hz, H-8), 2.78 (2H, t, J 7.4 Hz, H-9), 6.60 (4H, m, H-3, 5, 12 and 14), 6.94 (2H, m, H-11 and 15), 7.14 (2H, m, H-2 and 6); δ_C (100 MHz, d4-MeOH) 32.6 (C-8), 40.5

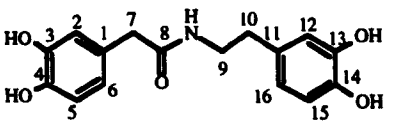
(C-9), 116.5, 116.6 and 124.0 (C-2, 6, 3, 5, 12 and 14), 130.8 (C-11 and 15), 131.9 and 133.4 (C-1 and 10), 155.8 and 157.2 (C-4 and 13), 174.1 (CO); Found:$[M+H]^+$ (FAB) 258.1133. $C_{15}H_{16}O_3N$ requires 258.1136.

N-*[2-(3,4-Dimethoxyphenyl)ethyl]-3,4-dimethoxyphenylacetamide* **136**



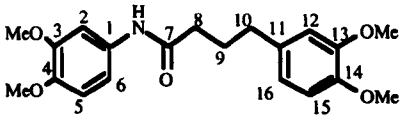
Compound **136** (1.70 g, 93%) was prepared from (3,4-dimethoxyphenyl)acetic acid (1.0 g, 5.10 mmol) and 3,4-dimethoxyphenethylamine (0.92 g, 0.86 ml, 5.10 mmol) by the method used to prepare **128**, mp 102-103 ^{0}C. (Found: C, 66.63; H, 7.05; N, 3.92; $C_{20}H_{25}NO_5$ requires C, 66.85; H, 6.96; N, 3.90%). ν_{max} (KBr disc)/cm^{-1} 3323m (CONH), 2837m and 2915m (OCH_3), 1649s (CO), 1607s (Ar). δ_H (400 MHz, $CDCl_3$) 2.61 (2H, t, J 6.8 Hz, H-10), 3.37 (2H, m, H-9), 3.40 (2H, s, H-7), 3.75 (6H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 5.38 (1H, bs, NH), 6.44-6.73 (6H, m, ArH); δ_C (100 MHz, $CDCl_3$) 35.4 (C-10), 41.1 (C-9), 43.8 (C-7), 56.1, 56.2 and 56.3 (OCH_3), 111.5, 111.8, 112.1 and 112.8 (C-2, 5, 12 and 15), 120.9 and 121.9 (C-6 and 16), 127.6 and 131.4 (C-1 and 11), 148.0, 148.7, 149.4 and 149.6 (C-3, 4, 13 and 14), 171.6 (CO). *m/z* (EI) 359.1729 (M^+). $C_{20}H_{25}O_5N$ requires M 359.1725; *m/z* (%) 359 (18). 195 (5), 164 (100), 151 (38), 107 (5).

N-*[2-(3,4-dihydroxyphenyl)ethyl]-3,4-dihydroxyphenylacetamide* **137**



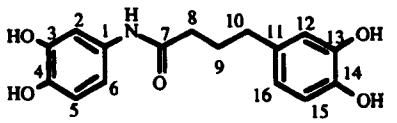
Compound **137** (0.56 g, 66 %) was prepared from **136** (1.0 g, 2.79 mmol) and BBr_3 (19.5 ml, 19.5 mmol, 1M in DCM solution) by the method used to prepare **131**, mp 38 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3418s (OH and CONH), 1600s (Ar). δ_H (400 MHz, d4-MeOH) 2.49 (2H, t, J 7.2 Hz, H-10). 3.22 (4H, m, H-7 and 9), 6.54 (6H, m, ArH); δ_C (100 MHz, d4-MeOH) 36.2 (C-10), 42.9 (C-9), 43.8 (C-7), 116.8, 116.9, 117.2 and 117.7 (C-2, 5, 12 and 15), 121.6 and 122.0 (C-6 and 16), 128.6 and 132.3 (C-1 and 11), 145.2, 145.8, 146.6 and 146.8 (C-3,4, 13 and 14), 175.1 and 175.2 (CO); Found:$[M+H]^+$ (FAB) 304.1188. $C_{16}H_{18}O_5N$ requires 304.1191.

N-*(3,4-dimethoxyphenyl)-3,4-dimethoxyphenylbutanamide* **138**



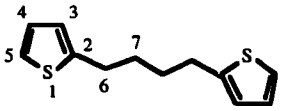
A solution of 4-(3,4-dimethoxyphenyl)butyric acid (1.22 g, 5.35 mmol) and EDCI (1.03 g, 5.35 mmol) in dry DCM (50 ml) was stirred at rt for 10 min before DMAP (72.7 mg, 0.59 mmol) was added. After stirring the mixture for a futher 15 min, 3,4-dimethoxyaniline (0.85 g, 5.6 mmol) was added and the brown mixture was stirred at rt for 24 h. The reaction mixture was washed successively with saturated $NaHCO_3$ (3 x 4 ml), 5% citric acid (3 x 40 ml), water (40 ml) and brine (20 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a brown solid which was recrystallised from MeOH affording the compound **138** as grey fluffy crystals (1.02 g, 2.84 mmol, 53 %), mp 107-109 ^{0}C (EtOAc). (Found: C, 66.74; H, 7.06; N, 3.95; $C_{20}H_{25}NO_5$ requires C, 66.85; H, 6.96; N, 3.90%). ν_{max} (KBr disc)/cm^{-1} 3299m (CONH), 2937m (OCH_3), 1657s (CO), 1603s (Ar). δ_H (400 MHz, $CDCl_3$) 1.97 (2H, tt, J 7.4 Hz, H-9), 2.26 (2H, t, J 7.4 Hz, H-8), 2.59 (2H, t, J 7.4 Hz, H-10), 3.77, 3.78 and 3.79 (12H, each s, OCH_3), 6.65-7.30 (6H, m, ArH); δ_C (100 MHz, $CDCl_3$) 26.0 (C-9), 33.7 (C-8), 35.6 (C-10), 54.8, 54.9 and 55.1 (OCH_3), 103.8, 110.2, 110.3, 110.6, 110.8 and 119.3 (C-2, 5, 6, 12, 15 and 16), 130.6 and 132.9 (C-1 and 11), 144.7, 146.3, 147.9 and 148.0 (C-3, 4, 13 and 14), 169.9 (CO). *m/z* (EI) 359.1729 (M^+). $C_{20}H_{25}O_5N$ requires 359.1725; *m/z* (%) 360 (20), 359 (80), 195 (100), 153 (40), 151 (35), 110 (8), 83 (14).

N-*(3,4-dihydroxyphenyl)-3,4-dihydroxyphenylbutanamide* **139**



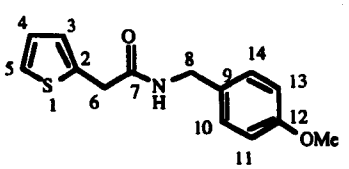
To a stirred solution of **138** (0.6 g, 1.67 mmol) in dry CH_2Cl_2 (15 ml) was added BBr_3 (11.7 ml, 11.70 mmol, 1 M in CH_2Cl_2) dropwise at -78 ^{0}C. The reaulting brown mixture was stirred for 1 h and then allowed to warm to 0 ^{0}C and stirred for 2.5 h before pouring into iced water (30 ml). After shaking the mixture for 20 min, a grey solid precipitated between the aqueous and the DCM layers. The solid was filtered off and recrystallised from EtOAc-EtOH to give **139** as a brown solid (0.25 g, 50%), mp 56 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3387s (OH and CONH), 1646s (CO and Ar). δ_H (400 MHz, d4-MeOH) 1.90 (2H, m, H-9), 2.34 (2H, t, J 7.6 Hz, H-8), 2.53 (2H, t, J 7.6 Hz, H-10), 6.47-7.17 (6H, m, ArH); δ_C (100 MHz, d4-MeOH) 29.4 (C-9), 36.1 (C-8), 37.5 (C-10), 110.6, 114.0, 116.5, 116.8, 117.0 and 121.2 (C-2, 5, 6, 12, 15 and 16), 132.2 and 134.9 (C-1 and 11), 143.9, 144.8, 146.5 and 146.6 (C-3, 4, 13 and 14), 174.9 (CO). Found:$[M+H]^+$ (FAB) 304.1191 $C_{16}H_{18}O_5N$ requires 304.1197.

1,4-Di(2-thienyl)butane **140**[110]



The procedure of Koβmehl *et al.*[110] was modified as follows. Thiophene (0.5 g, 6 mmol) was dissolved in dry THF (7 ml) under nitrogen. After the addition of n-butyl lithium (2.4 ml, 2.5 M in hexane, 6 mmol) the resulting mixture was heated at reflux for 2 h. After cooling to -60 ^{0}C a solution of 1,4-dibromobutane (0.28 ml, 2.4 mmol) was added dropwise to the reaction mixture which was stirred for 2 h then allowed to warm to rt and stirred for a further 5 h. Finally the mixture was heated at reflux for 5 h to complete the reaction before pouring into iced water (15 ml). The mixture was neutralised with 10% HCl and extracted with diethyl ether (3 x 15 ml). The combined organic layers were washed with water (20 ml) and brine (20 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a black oil which was purified by silica-gel column chromatography, eluting with hexane. The fractions R_f 0.3 were combined and concentrated *in vacuo* to yield **140** as a light yellow oil (0.25 g, 1.12 mmol, 47%). δ_H (400 MHz, $CDCl_3$) 1.69 (4H, m, H-7), 2.79 (4H, m, H-6), 6.70 (2H, m, H-3), 6.83 (2H, m, H-4), 7.02 (2H, m, H-5).

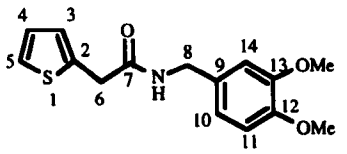
N-(4-Methoxybenzyl)thiophene-2-acetamide **141**



A mixture of thiophene-2-acetic acid (0.8 g, 5.63 mmol) and 4-methoxybenzylamine (0.69 g, 0.66 ml, 5.07 mmol) was heated with stirring under nitrogen at 160 ^{0}C for 4 h. The brown solid formed was dissolved in CH_2Cl_2 and the organic solution was washed successively with saturated $NaHCO_3$ (10 ml), 5% citric acid (10 ml), water (10 ml) and brine (10 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave **141** as a brown solid which was pure enough for use without further purification (1.28 g, 4.4 mmol, 79 %), mp 95-97 ^{0}C. (Found: C, 64.47; H, 5.81; N, 5.30; $C_{14}H_{15}O_2NS$ requires C, 64.37; H, 5.75; N, 5.36%); ν_{max} (KBr disc)/cm^{-1} 3256s (CONH), 2841m (OCH_3), 1658s (CO), 1611s (Ar). δ_H(400 MHz, $CDCl_3$) 3.71 (3H, s, OCH_3), 3.73 (2H, s, H-6), 4.28 (2H, d, J 5.6 Hz, H-8), 5.82 (1H, bs, NH), 6.74-7.19 (7H, m, aromatic H); δ_C(100 MHz, $CDCl_3$) 36.5 (C-6), 42.1 (C-8), 54.3 (OCH_3), 113.0 (C-11 and 13), 124.6, 125.3 and 125.4 (C-3 to 5), 127.9 (C-10 and14), 129.0 and 135.1 (C-2 and 9), 157.9

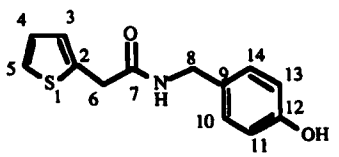
(C-12), 168.6 (CO); *m/z* (EI) 261.0823 (M^+). $C_{14}H_{15}O_2NS$ requires 261.0823; *m/z* (%) 261 (24), 203 (3), 177 (12), 121 (100), 97 (12), 82 (9), 78 (6).

N-(3,4-Dimethoxybenzyl)thiophene-2-acetamide **142**



Compound **142** was prepared from thiophene-2-acetic acid (0.8 g, 5.63 mmol) and veratrylamine (0.85 g, 0.76 ml, 5.07 mmol) by the method used to prepare **128**. Recrystallisation from ethyl acetate afforded **142** as a yellow solid, mp 91-92 ^{0}C. (Found: C, 61.73; H, 5.82; N, 4.77; $C_{15}H_{17}O_3NS$ requires C, 61.86; H, 5.84; N, 4.81 %); ν_{max} (KBr disc)/cm^{-1} 3294s (CONH), 2832m (OCH_3), 1656s (CO), 1593s (Ar). δ_H(400 MHz, $CDCl_3$) 3.75 and 3.76 (6H, two s, OCH_3), 3.78 (2H, s, H-6), 4.29 (2H, d, J 5.6 Hz, H-8), 5.86 (1H, bs, NH), 6.66-7.17 (6H, m, aromatic H); δ_C (100 MHz, $CDCl_3$) 36.6 (C-6), 42.4 (C-8), 54.8 and 54.9 (OCH_3), 109.7 and 110.1 (C-11 and 14), 118.7 (C-10), 124.6, 126.3 and 126.4 (C-3 to 5), 129.5 (C-2), 135.1, 147.4 and 148.1 (C-9, 12 and 13), 168.6 (CO); *m/z* (EI) 291.0926 (M^+). $C_{15}H_{17}O_3NS$ requires 291.0923; *m/z* (%) 291 (50), 193 (5), 176 (7), 151 (100), 97 (14), 65 (3).

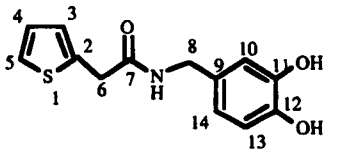
N-(4-Hydroxybenzyl)thiophene-2-acetamide **143**



The procedure of McOmie *et al.*[107] was modified as follows. To a stirred solution of **141** (0.5 g, 1.9 mmol) in dry CH_2Cl_2 (18 ml) was added boron tribromide (3.8 ml, 3.8 mmol, 1M in DCM) dropwise at -78 ^{0}C. This was stirred for 1 h then allowed to reach 0 ^{0}C and stirred for 2.5 h before pouring into iced water (50 ml). The mixture was stirred for 1 h and the two layers were separated. The aqueous layer was extracted with ethyl acetate (3 x 30 ml), and the organic layer was concentrated *in vacuo* to give a yellow solid which was combined with the ethyl acetate extracts. The ethyl acetate solution was washed with saturated $NaHCO_3$ (2 x 50 ml), water (50 ml) and brine (50 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a brown solid which was recrystallised from ethyl acetate affording **143** as colourless crystals (0.34 g, 72%), mp 115-117 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3395m and 3200s (OH and CONH), 1652s (CO). δ_H(400 MHz, d_6-DMSO) 3.67 (2H, s, H-6), 4.15 (2H, d, J 5.6 Hz, H-8), 6.68-

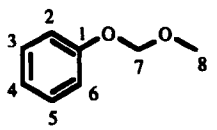
7.36 (7H, m, aromatic H), 8.45 and 9.29 (2H, m and bs, NH and OH); δ_C (100 MHz, d_6-DMSO) 36.9 (C-6), 42.2 (C-8), 115.4 (C-11 and 13), 125.1, 126.3 and 126.9 (C-3 to 5), 129.0 (C-10 and 14), 129.7 and 138.1 (C-2 and 9), 156.6 (C-12), 169.2 (CO); *m/z* (EI) 247.0664 (M^+). $C_{13}H_{13}O_2NS$ requires 247.0661; *m/z* (%) 247 (17), 163 (15), 120 (4), 107 (100), 97 (22), 77 (9), 49 (4).

N-(3,4-Dihydroxybenzyl)thiophene-2-acetamide **144**



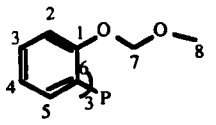
Compound **144** was prepared from **142** (0.35 g, 1.2 mmol) and BBr_3 (4.8 ml, 4.8 mmol, 1M solution in DCM) by the method used to prepare **130**. Recystallisation from ethyl acetate-hexane afforded **144** as a yellow solid (0.24 g, 76%), mp 105 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3481s and 3170s (OH and CONH), 1652s (CO), 1600s (Ar). δ_H(400 MHz, d_6-DMSO) 3.67 (2H, s, H-6), 4.09 (2H, d, J 5.6 Hz, H-8), 6.46-7.36 (6H, m, H-10, 13, 14 and 3 to 5), 8.40-8.85 (3H, m, NH and OH); δ_C (100 MHz, d_6-DMSO) 36.8 (C-6), 42.4 (C-8), 115.4 and 115.6 (C-10 and 13), 118.7 (C-14), 125.1, 126.3 and 126.8 (C-3 to 5), 130.3 (C-2), 138.1 (C-9), 144.6 and 145.5 (C-11 and 12) 169.1 (CO); *m/z* (EI) 263.0613 (M^+). $C_{13}H_{13}O_3NS$ requires 263.0610; *m/z* (%) 263 (30), 179 (15), 123 (100), 97 (27), 77 (9), 51 (4).

Methoxymethyl phenyl ether **145**[111]



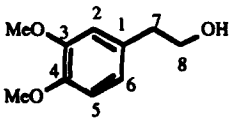
The method of Schlosser and his coworkers[111] was modified as follows. A solution of phenol (5.16 g, 54.8 mmol) in dry DMF (10 ml) was added into a slush of NaH (60% dispersion, 2.43 g, 60.9 mmol) in dry DMF (30 ml). After stirring for 30 min chloromethyl methyl ether (3.2 ml, 3.42 g, 42.5 mmol) was added dropwise at 0 ^{0}C. After stirring for 3 h at rt, the resultant grey suspension was poured into water (150 ml), which was extracted with hexane (3 x 100 ml). The combined organic extracts were washed with 1 M NaOH (100 ml), water (2 x 100 ml) and brine (100 ml) before drying over Na_2SO_4. Concentration *in vacuo* gave a colourless oil which was distilled under reduced pressure to yield **145** as a colourless oil (3.69 g, 26.74 mmol, 63%), bp 74-75 ^{0}C (> 1 mmHg,vacuum pump) (lit.,[111] bp 63-65^{0}C at 8 mmHg). δ_H (400 MHz, $CDCl_3$) 3.41 (3H, s , H-8), 5.10 (2H, s, H-7), 6.96 (3H, m, H-2, 4 and 6), 7.21 (2H, m, H-3 and 5).

Tris (2-methoxymethyleneoxyphenyl)phosphine **146**[111]



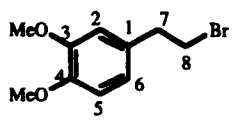
To an ice-cooled solution of **145** (4.0 g, 28.99 mmol) and *N,N,N',N'*-tetramethylethylenediamine (6.74 g, 8.7 ml, 57.98 mmol) in dry THF (55 ml) was added *n*-butyl lithium (24 ml, 2.5 M in hexanes, 60.87 mmol) dropwise. After stirring the dark red solution for 2 h at 25 ^{0}C, triphenyl phosphite (6.60 g, 5.6 ml, 21.26 mmol) was added dropwise at 0 ^{0}C. The resultant yellow solution was stirred at 25 ^{0}C for 16 h. The solvent was evaporated and the residue was purified by silica gel column chromatography to give a white solid which was recrystallised from EtOAc to yield **146** as white crystals (3.24 g, 7.32 mmol, 76%), mp 125-126 ^{0}C (lit.,[111] 128-129 ^{0}C). δ_H (400 MHz, $CDCl_3$) 3.16 (9H, s, H-8), 5.03 (6H, s, H-7), 6.73 (3H, m, H-2), 6.83 (3H, m, H-4), 7.04 and 7.22 (each 3H, each m, H-3 or 5).

2-(3,4-Dimethoxyphenyl)ethanol **147**[115]



Compound **147** (2.91 g, 75%) was prepared from (3,4-dimethoxyphenyl)acetic acid (4.0 g, 20.39 mmol) and $LiAlH_4$ (1.55 g, 40.78 mmol) by the method used to prepare **118**, mp 43-44 ^{0}C (lit.,[115] mp 44-45 ^{0}C). δ_H (400 MHz, $CDCl_3$) 1.41 (1H, bs, OH), 2.75 (2H, t, J 6.4 Hz, H-7), 3.77 (2H, t, J 6.4 Hz, H-8), 3.79 and 3.81 (6H, two s, OCH_3), 6.68-6.77 (3H, m, ArH).

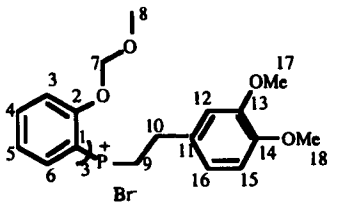
2-(3,4-Dimethoxyphenyl)ethyl bromide **149**[114]



To a stirred solution of alcohol **147** (1.50 g, 8.24 mmol) and CBr_4 (3.42 g, 10.30 mmol) in CH_2Cl_2 (25 ml) was added Ph_3P (3.25 g, 12.36 mmol) portionwise with ice-bath cooling. After addition was complete, the mixture was stirred for 40 min, whereupon the solvent was removed *in vacuo*. Ether (50 ml) was added and the white precipitate was filtered off. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography to give a pale yellow solid (1.49 g, 6.07 mmol, 74%), mp 50-51 ^{0}C (EtOH) (lit.,[114] 47-50 ^{0}C). δ_H

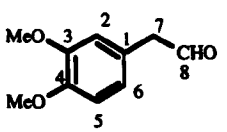
(400 MHz, $CDCl_3$) 3.02 (2H, t, J 7.6 Hz, H-7), 3.47 (2H, t, J 7.6 Hz, H-8), 3.79 and 3.81 (each 3 H, each s, OCH_3), 6.66-6.76 (3H, m, ArH).

2-(3,4-Dimethoxyphenyl)ethyltris(o-methoxymethyleneoxyphenyl)phosphonium bromide **150**



A mixture of compound **149** (1.00 g, 4.08 mmol) and **146** (1.0 g, 2.26 mmol) in EtOH (30 ml, distilled over Na_2CO_3) was heated at reflux for 8 h. The solvent was evaporated and the residue was washed with ether (3 x 100 ml). The white precipitate was recrystallised from EtOAc to yield **150** (1.05 g, 1.53 mmol, 68%), mp 140-142 ^{0}C. δ_H (400 MHz, $CDCl_3$) 2.83 (2H, m, H-10), 2.99 (9H, s, H-8), 3.70 (2H, m, H-9), 3.73 and 3.80 (each 3H, each s, H-17 or 18), 5.05 (6H, s, H-7), 6.37-6.81 (3H, m, H-12, 15 and 16), 7.19-7.67 (12H, m, H-3 to 6); δ_C (100 MHz, $CDCl_3$) 23.7 (C-10), 28.3 (C-9), 55.7 (C-8), 54.9 and 55.5 (C-17 and 18), 93.8 (C-7), 105.1 (C-1, J_{CP} 90 Hz), 110.1, 111.1 and 114.0 (C-12, 15 and 16), 118.9, 121.7, 121.8, 134.2 and 135.9 (C-3 to 6), 131.1(C-2, J_{CP} 10 Hz), 146.8, 148.1 and 158.4 (C-11, 13 and 14). Found: $[M+H]^+$ (FAB), 607.2458. $C_{34}H_{40}O_8P$ requires 607.2455.

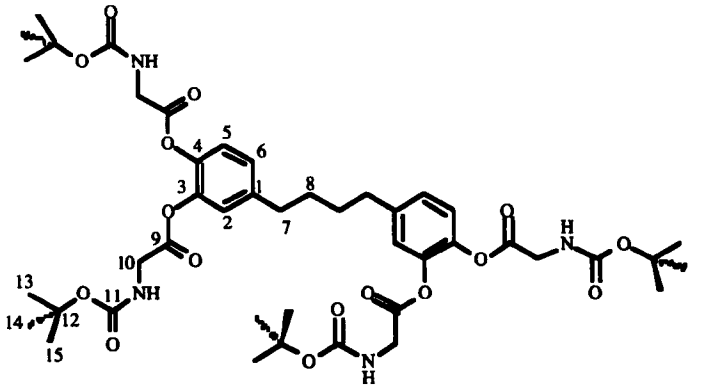
3,4-Dimethoxyphenylacetaldehyde **148**[115]



To a stirred suspension of pyridinium chlorochromate (1.61 g, 7.47 mmol) in CH_2Cl_2 (10 ml) was added **147** (0.8 g, 4.4 mmol) in CH_2Cl_2 (10 ml). After being stirred at 23 ^{0}C for 2 h, the reaction mixture was poured into dry ether (150 ml), and the precipitate was filtered off. The filtrate was concentrated *in vacuo* and the residue was purified by chromatography to give **148** as a colourless oil (0.23 g, 1.24 mmol, 29 %). δ_H (400 MHz, $CDCl_3$) 3.55 (2H, s, H-7), 3.81 (6H, s, OCH_3), 6.32-6.80 (3H, m, H-2, 5 and 6).9.62 (1H, s, H-8).

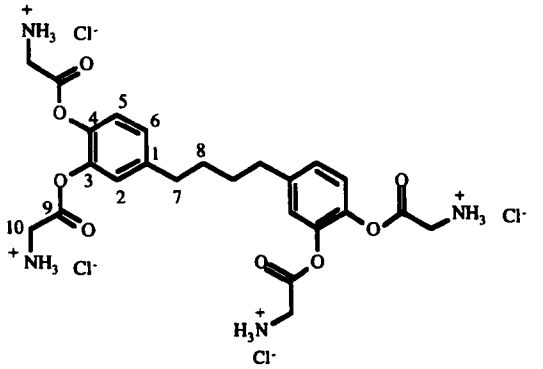
Experimental to Chapter 8

N-Boc protected glycine ester **152**



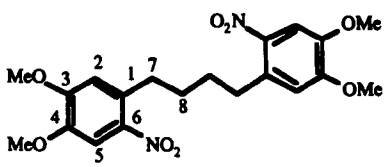
Pyridine (1.2 g, 1.2 ml, 14.6 mmol) was added dropwise at 0 ^{0}C to a stirred solution of **126** (1.0 g, 3.3 mmol) and *N-(tert*-butoxycarbonyl)glycine (2.6 g, 14.6 mmol) in dry EtOAc (80 ml). After the addition was complete the mixture was stirred for a further 5 min before DCC (3.0 g, 14.6 mmol) was added and the mixture was allowed to warm to rt. After stirring for 24 h the precipitated DCU was removed through Celite®, and the filtrate was stored in a freezer for 12 h to precipitate further DCU which was filtered off through Celite®. The solution was washed successively with 5% citric acid (2 x 60 ml), 5% $NaHCO_3$ (80 ml), water (100 ml) and brine (80 ml) before drying over $MgSO_4$. The organic solution was concentrated affording the glycine ester **152** as white crystals (2.4 g, 80%), mp 101-104 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 1681s (carbamate), 1783s (ester); δ_H(400 MHz, $CDCl_3$) 1.38 and 1.39 (36H, two s, H-13 to 15), 1.48 (4H, m, H-8), 2.47 (4H, m, H-7), 4.04 (8H, m, H-10), 6.45-7.02 (6H, m, ArH); δ_C(100 MHz, $CDCl_3$) 28.7 (C-13 to 15), 31.0 (C-8), 35.3 (C-7), 42.7 (C-10), 80.6 (C-12), 123.2, 123.3 and 127.2 (C-2, 5 and 6), 138.5, 140.0 and 141.9 (C-1, 3 and 4), 156.6 (C-11), 168.5 (C-9). Found:$[M+Na]^+$ (FAB), 925.4058. $C_{44}H_{62}N_4O_{16}Na$ requires 925.4057.

Glycine ester tetrahydrochloride salt **153**



Dry HCl was bubbled into a solution of **152** (250 mg, 0.28 mmol) under nitrogen in dry EtOAc (4 ml) at 0 ^{0}C for 3 min. A white solid precipitated from the reaction mixture which was allowed to warm to rt and stirred for a further 2h. The mixture was filtered and the solid was washed thoroughly with EtOAc affording **153** as a white solid (0.12 g, 88%), mp 192-195 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 1774s (ester), 1594s (Ar); δ_H(400 MHz, D_2O) 1.52 (4H, m, H-8), 2.52 (4H, m, H-7), 4.22 (8H, m, H-10), 6.44-7.22 (6H, m, ArH); δ_C(100 MHz, D_2O) 30.1 (C-8), 34.5 (C-7), 40.5 (C-10), 117.6, 122.7, and 128.5 (C-2, 5 and 6), 138.5, 140.4 and 144.2 (C-1, 3 and 4), 167.2 (CO). Found: $[M+H]^+$ (FAB) 503.2146. $C_{24}H_{31}N_4O_8$ requires 503.2150.

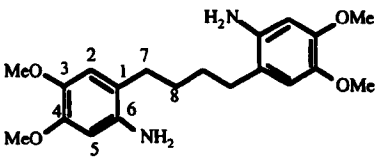
1,4-Bis-(4,5-dimethoxy-2-nitrophenyl)butane **154**



Concentrated nitric acid (70%) (0.30 ml, d 1.42, 6.85 mmol) was added dropwise at rt to a stirred solution of **124** (300 mg, 0.91 mmol) in glacial AcOH (4.3 ml). The resultant yellow suspension was stirred for 2 h. After water (27 ml) was added to the reaction mixture, the suspension was extracted with $CHCl_3$ (3 x 25 ml) and the combined organic layers were washed with saturated Na_2CO_3 (40 ml), water (40 ml) and brine (40 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a solid which was satisfactory for synthetic work without further purification (0.343 g, 0.81 mmol, 89 %), mp 170 ^{0}C. (Found: C, 56.92; H, 5.79; N, 6.49; $C_{20}H_{24}O_8N_2$ requires C, 57.14; H, 5.71; N, 6.67 %); ν_{max} (KBr disc)/cm^{-1} 1616m (Ar), 1327m and 1525s (NO_2); δ_H (400 MHz, $CDCl_3$) 1.68 (4H, tt, J 7.0 Hz, H-8), 2.91 (4H, t, J 7.0 Hz, H-7), 3.86 and 3.90 (12H, two s, OCH_3), 6.64 (2H, s, H-2), 7.52 (2H, s, H-5); δ_C(100 MHz, $CDCl_3$) 31.0 (C-8), 33.9 (C-7), 56.7 and 56.8 (OCH_3), 108.6 (C-2), 113.6 (C-5), 133.3, 141.4, 147.5 and 153.4 (C-1, 3, 4 and 6); *m/z* (EI) 420.1534 (M^+). $C_{20}H_{24}O_8N_2$ requires

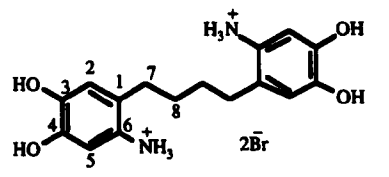
420.1535; *m/z* (%) 420 (55), 375 (20), 325 (10), 206 (65), 180 (100), 151 (50), 136(14), 77 (12).

1,4-Bis-(2-amino-4,5-dimethoxyphenyl)butane **155**



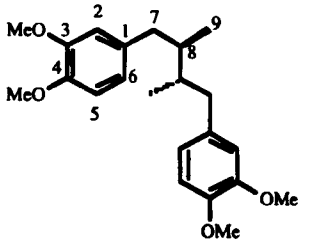
A solution of **154** (0.26 g, 0.62 mmol) in CH_2Cl_2-EtOH (2:1) (25 ml) containing 10% Pd-C (0.1 g) was stirred under 3.5 atm of hydrogen at 25 ^{0}C for 5 h. The mixture was filtered through Celite.® Concentration *in vacuo* gave a black solid (0.13 g, 0.36 mmol, 58 %), mp 149-150 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 1613 m (Ar), 3383 m (NH_2); δ_H(400 MHz, d_6-DMSO) 1.52 (4H, m, H-8), 2.38 (4H, m, H-7), 3.60 and 3.65 (12H, two s, OCH_3), 4.57 (4H, bs, NH_2), 6.32 (2H, s, H-5), 6.52 (2H, s, H-2); δ_C(100 MHz, d_6-DMSO) 29.1 (C-8), 30.2 (C-7), 55.7 and 57.0 (OCH_3), 101.9 (C-5), 115.5 (C-2), 118.8 (C-1), 138.2, 141.4 and 148.0 (C-3, 4 and 6); *m/z* (EI) 360.2047 (M^+). $C_{20}H_{28}O_4N_2$ requires 360.2044; *m/z* (%) 360 (50), 330 (8), 206 (10), 166 (100), 122 (15), 82 (9), 57 (5).

1,4-Bis-(2-amino-4,5-dihydroxyphenyl)butane dihydrobromide **156**



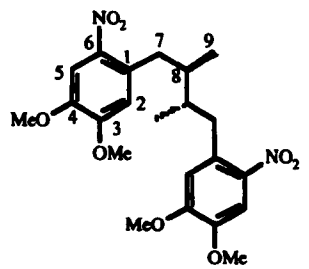
A mixture of **155** (0.2 g, 0.55 mmol) and 48% HBr (7 ml) was heated at reflux under N_2 for 4 h. After cooling to rt the precipitated brown solid was collected on suction to yield **156** as a brown solid (0.21 g, 0.47 mmol, 84%), mp > 330 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3485s (OH), 1635s (Ar); δ_H(400 MHz, D_2O) 1.52 (4H, m, H-8), 2.48 (4H, m, H-7), 6.76 and 6.77 (4H, two s, ArH); δ_C(100 MHz, D_2O) 29.2 and 29.3 (C-7 and 8), 111.5 (C-5), 117.7 (C-2), 120.1 (C-1), 128.5, 143.1 and 144.9 (C-3, 4 and 6); Found: [M (-2HBr) + H]$^+$ (FAB), 305.1497. $C_{16}H_{21}O_4N_2$ requires 305.1493.

(meso)-1,4-Bis-(3,4-dimethoxyphenyl)-2,3-dimethylbutane **53**[8]



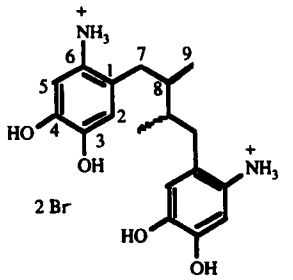
The procedure of Perry *et al.* was modified as follows.[62] A solution of KOH (0.21 g, 3.75 mmol) in MeOH (0.65 ml) and water (1 ml) was added dropwise to a stirred solution of NDGA (0.25 g, 0.83 mmol) in MeOH (2 ml). To the resultant brown solution was added dimethyl sulfate (0.84 g, 0.63 ml, 6.66 mmol) dropwise and the mixture was stirred for a further 23 h ensuring the mixture was maintained at pH 8-9 by periodic addition of more KOH solution. Ammonia solution (35%, 5 ml) was added to the tan coloured suspension and the mixture was stirred for a further 30 min. The resultant mixture was extracted with EtOAc (30 ml) and the organic extracts were washed with water (10 ml) and brine (10 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a yellow coloured solid (0.25 g, 0.71 mmol, 86 %), mp 99-100 ^{0}C (MeOH-H_2O) (lit.,[8] 100-102 ^{0}C). δ_H (400 MHz, $CDCl_3$) 0.75 (6H, d, J 6.6 Hz, H-9), 1.70 (2H, m, H-8), 2.23 (2H, dd, J 13.4 Hz and 9.2 Hz, H-7), 2.68 (2H, dd, J 13.4 Hz and 4.9 Hz, H-7), 3.78 (12H, two s, OCH_3), 6.58-6.72 (6H, m, ArH).

(meso)-1,4-Bis-(3,4-dimethoxy-6-nitrophenyl)-2,3-dimethylbutane **157**[8]



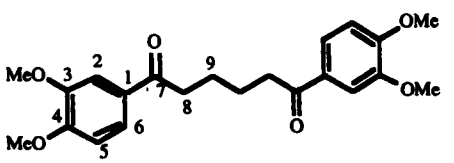
Compound **157** was prepared from **53** (1.0 g, 2.79 mmol) and 68% HNO_3 (0.67 ml, 10.32 mmol) in glacial acetic acid (8 ml) by the method used to prepare **154**. Recrystallisation from MeOH afforded **157** as yellow crystals (1.15 g, 92 %), mp 149-150 ^{0}C (lit.,[8] 150-151 ^{0}C). δ_H (400 MHz, $CDCl_3$) 0.80 (6H, d, J 6.8 Hz, H-9), 1.80 (2H, m, H-8), 2.60 (2H, dd, J 13.0 Hz and 9.5 Hz, H-7), 3.29 (2H, dd, J 13.0 Hz and 4.1 Hz, H-7), 3.86 and 3.91 (12 H, two s, OCH_3), 6.68 (2H, s, H-2), 7.54 (2H, s, H-5).

(meso)-1,4-Bis-(3,4-dihydroxy-6-aminophenyl)-2,3-dimethylbutane dihydrobromide **158**[8]



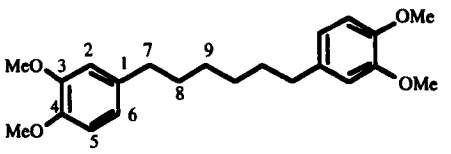
A solution of **157** (1.10 g, 2.46 mmol) in DCM-EtOH (1:1) (40 ml) containing 10% Pd/C (0.5 g) was stirred under 6 atm of hydrogen at 25 ^{0}C for 7.5 h. The mixture was filtered through Celite®. Concentration *in vacuo* gave a semi-solid (0.8 g, 84%) which was dissolved in 48% HBr (9 ml). The mixture was heated at reflux for 3 h and the resultant brown precipitate was collected to give **158** as a brown solid (0.72 g, 75%), mp > 250 ^{0}C (lit.,[8] mp>250 ^{0}C). δ_H (400 MHz, D_2O) 0.74 (6H, d, J 6.6 Hz, H-9), 1.75 (2H, m, H-8), 2.31 (2H, dd, J 14.6 Hz and 10.1Hz, H-7), 2.66 (2H, dd, J 14.6 Hz and 4.5 Hz, H-7), 6.75 and 6.80 (4H, two s, ArH).

1,6-Bis-(3,4-dimethoxyphenyl)hexane-1,6-dione **159**[117]



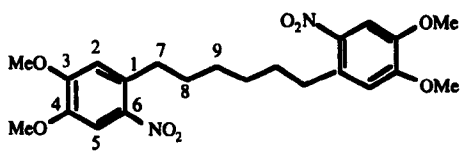
Powdered $AlCl_3$ (4.05 g, 30.37 mmol) was added in portions to a stirred solution of veratrole (3.5 g, 25.33 mmol) in dry CH_2Cl_2 (50 ml) under nitrogen at 0 ^{0}C. After the addition was complete, adipoyl dichloride (2.78 g, 2.2 ml, 15.2 mmol) was added dropwise and the mixture was stirred at 0 ^{0}C for a further 4 h. The yellow suspension was poured into a flask containing iced water (180 ml) and concentrated HCl (7 ml) and the mixture was stirred for 1 h with more CH_2Cl_2 added to ensure two homogenous layers. The layers were separated and the aqueous layer was further extacted with CH_2Cl_2 (2 x 30 ml). The combined organic layers were washed with saturated $NaHCO_3$ (2 x 50 ml), water (50 ml) and brine (60 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a yellow solid which was recrystallised from ethyl acetate affording compound **159** as pale yellow crystals (3.2 g, 8.3 mmol, 66%), mp 139-140 ^{0}C (lit.,[117] 149-150 ^{0}C). δ_H(400 MHz, $CDCl_3$) 1.4 (4H, m, H-9), 2.91 (4H, m, H-8), 3.86 (6H, s, OCH_3), 3.87 (6H, s, OCH_3) 6.84 (2H, m, H-5), 7.45 (2H, d, J 2 Hz, H-2), 7.52 (2H, m, H-6).

1,6-Bis-(3,4-dimethoxyphenyl)hexane **160**[117]



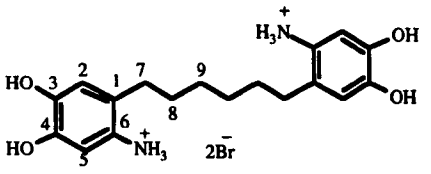
Pd/C (10%, 1 g) was added to a solution of **159** in glacial AcOH (75 ml) and the mixture was stirred under a hydrogen balloon overnight. The mixture was filtered through Celite®. Concentration *in vacuo* gave a dark brown oil which was taken into CH_2Cl_2 (20 ml). The CH_2Cl_2 solution was washed with 10% NaOH (15 ml), water (10 ml) and brine (10 ml) before drying over $MgSO_4$. The solvent was evaporated *in vacuo* to yield a brown solid which was recrystallised from MeOH affording compound **160** as pale yellow crystals (0.81 g, 2.3 mmol, 58 %), mp 72-73 ^{0}C (lit.,[117] 78-79 ^{0}C). δ_H(400 MHz, $CDCl_3$)1.28 (4H, m, H-9), 1.53 (4H, m, H-8), 2.48 (4H, m, H-7), 3.78 (6H, s, OCH_3), 3.79 (6H, s, OCH_3), 6.39 (4H, m, H-2 and 5), 6.72 (2H, m, H-6).

1,6-Bis-(4,5-dimethoxy-2-nitrophenyl)hexane **161**



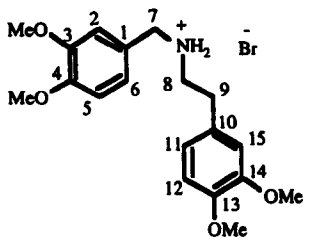
Compound **161** (0.45 g, 77 %) was prepared from **160** (0.47 g, 1.31 mmol) and concentrated nitric acid (70 %, 0.47 ml, 7.3 mmol) in glacial acetic acid (7 ml) by the method used to prepare **154**, mp 148-149 ^{0}C. (Found: C, 58.71; H, 6.35; N, 6.06; $C_{22}H_{28}N_2O_8$ requires C, 58.93; H, 6.25; N, 6.25 %); ν_{max} (KBr disc)/cm^{-1} 2932m and 2853s (OCH_3), 1614s (Ar), 1584s and 1525s (NO_2); δ_H(400 MHz, $CDCl_3$) 1.40 (4H, m, H-9), 1.58 (4H, m, H-8), 2.84 (4H, m, H-7), 3.85 and 3.90 (each 6H, each s, OCH_3), 6.64 (2H, s, H-2), 7.50 (2H, s, H-5); δ_C(100 MHz, $CDCl_3$) 28.2 (C-9), 29.5 (C-8), 32.8 (C-7), 55.2 and 55.3 (OCH_3), 107.2 (C-5), 112.2 (C-2), 132.3, 140.0, 146.0 and 152.0 (C-1, 3, 4 and 6); *m/z* (EI) 448.1842 (M^+). $C_{22}H_{28}O_8N_2$ requires 448.1839; *m/z* (%) 448 (80), 403 (44), 369 (8), 190 (35), 180 (100), 151 (84), 107 (13), 77 (11).

1,6-Bis-(2-amino-4,5-dihydroxyphenyl)hexane dihydrobromide **162**



A solution of **161** (0.4 g, 0.89 mmol) in EtOH-DCM (3:2) (40 ml) containing 10% Pd/C (0.15 g) was stirred under 3 atm of hydrogen at 25 ^{0}C for 5 h. The mixture was filtered through Celite®. Concentration *in vacuo* provided a dark green solid (0.34 g, 97%). δ_H(400 MHz, d_6-DMSO) 1.34 (4H, m, H-9), 1.47 (4H, m, H-8), 2.35 (4H, m, H-7), 3.61 and 3.65 (each 6H, each s, OCH_3), 4.66 (4H, br, NH_2), 6.33 (2H, s, H-5), 6.53 (2H, s, H-2). The dark green solid (0.21 g, 0.54 mmol) was dissolved in 48% HBr (7 ml) and the mixture was heated at reflux for 3 h and the resultant brown precipitate was collected to give **162** as a brown solid (0.18 g, 71%). mp 320 ^{0}C. ν_{max} (KBr disc)/cm^{-1} 3597s (OH), 1641s (Ar); δ_H(400 MHz, D_2O) 1.23 (4H, m, H-9), 1.43 (4H, m, H-8), 2.41 (4H, t, J 7.6 Hz, H-7), 6.73 and 6.74 (4H, two s, H-2 and 5); δ_C(100 MHz, D_2O) 28.6 (C-9), 29.4 (C-8), 29.7 (C-7), 111.5 (C-5), 117.7 (C-2), 120.1 (C-1), 128.9, 143.0 and 144.9 (C-3, 4 and 6). Found: $[M+H]^+$ (FAB) 333.1811. $C_{18}H_{25}O_4N_2$ requires 333.1808.

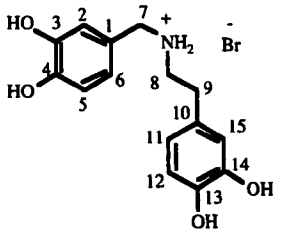
N-(3,4-Dimethoxybenzyl)-N-[2-(3,4-dimethoxyphenyl)ethyl]ammonium bromide **163**[8]



2-(3,4-Dimethoxyphenyl)ethylamine (0.57 g, 0.53 ml, 3.16 mmol) was added to a solution of 3,4-dimethoxybenzaldehyde (0.50 g, 3.01 mmol) in dry EtOH (15 ml) and the mixture was heated at reflux under N_2 for 18 h. After cooling to rt the mixture was concentrated *in vacuo* to give the intermediate imine as a brown oil. The crude imine was taken up in fresh EtOH (20 ml) and treated portionwise with $NaBH_4$ (0.57 g, 15.04 mmol). The mixture was heated at reflux for 1 h, allowed to cool and stirred overnight at rt. The resultant mixture was poured into water (60 ml), extracted with $CHCl_3$ (2 x 20 ml) and the combined organic extracts were washed with water (20 ml) and brine (20 ml) before drying over $MgSO_4$. Concentration *in vacuo* gave a white solid which was recrystallised from 48% HBr to give compound **163** as a yellow solid (1.2 g, 97%), mp 185-186 ^{0}C (lit.,[8] 187 ^{0}C). δ_H (400 MHz, d_6-DMSO) 2.91 (2H,

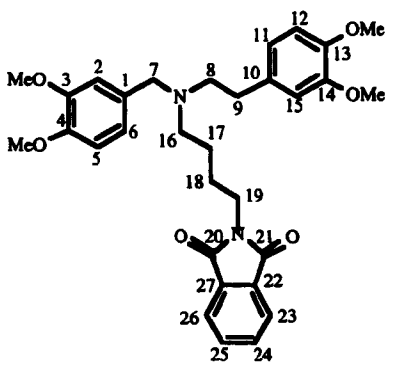
t, J 9.1 Hz, H-9), 3.11 (2H, m, H-8), 3.72, 3.74, 3.76 and 3.78 (each 3H, each s, OCH_3), 4.11 (2H, t, J 5.6 Hz, H-7), 6.74-7.26 (6H, m, ArH), 8.34 (2H, bs, NH_2).

N-(3,4-Dihydroxybenzyl)-N-[2-(3,4-dihydroxyphenyl)ethyl]ammonium bromide **164** [8]



Compound **164** (0.16 g, 72 %) was prepared from **163** (0.25 g, 0.61 mmol) and 48% HBr (10 ml) by the method used to prepare **115**. δ_H (400 MHz, d4-MeOH) 2.75 (2H, m, H-9), 3.06 (2H, m, H-8), 3.95 (2H, s, H-7), 6.47-6.96 (6H, m, ArH).
New data. mp 199-201 ^{0}C; δ_C (100 MHz, d4-MeOH) 33.0 (C-9), 50.0 (C-8), 52.6(C-7), 117.1, 118.5, 121.4 and 123.3 (C-2, 5, 6, 11, 12 and 15), 123.9 and 129.5 (C-1 and 10), 145.9, 147.3 and 148.2 (C-3, 4, 13 and 14).

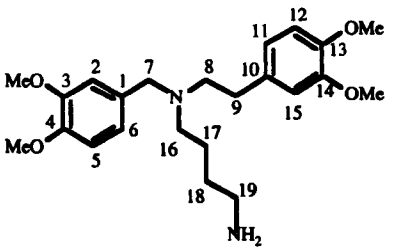
N-*{4-[*N'-*(3, 4-Dimethoxybenzyl)]*-N'-*[2-(3, 4-dimethoxyphenyl)ethyl]butyl}*
phthalimide **165**



The method of Ozaki and his coworkers[120] was modified as follows. A mixture of **163** (2.0 g, 6.04 mmol), *N*-(4-bromobutyl)phthalimide (2.05 g, 7.25 mmol) and K_2CO_3 (2.09 g, 15.1 mmol) in DMF (35 ml) was stirred at 110 ^{0}C for 48 h. The resulting mixture was diluted with water (200 ml) and extracted with $CHCl_3$ (2 x 150 ml). The combined $CHCl_3$ extracts were washed with saturated $NaHCO_3$ (2 x 200 ml), water (2 x 200ml) and brine (100 ml) before drying over Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography (EtOAc-petroleum ether 1:2 with 10% Et_3N) to give compound **165** as a light yellow oil (1.71 g, 3.2 mmol, 53%). ν_{max}(neat)/cm^{-1} 2935s (OCH_3), 1719s and 1770s (imide), 1607s (Ar); δ_H (400 MHz, $CDCl_3$) 1.48 (2H, m, H-17), 1.62 (2H, m, H-18),

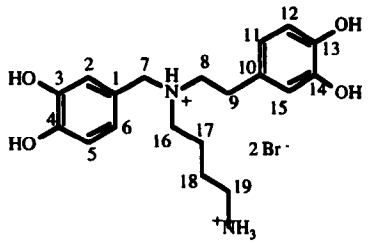
2.45 (2H, t, J 7.0 Hz, H-16), 2.59 (4H, m, H-8 and 9), 3.48 (2H, s, H-7), 3.58 (2H, t, J 7.2 Hz, H-19), 3.74, 3.75, 3.76 and 3.77 (12H, each s, OCH_3), 6.69 (6H, m, H-2, 5, 6, 11, 12 and 15), 7.63 (2H, m, H-24 and 25), 7.75 (2H, m, H-23 and 26); δ_C (100 MHz, $CDCl_3$) 23.5 (C-17), 25.5 (C-18), 32.0 (C-9), 36.9 (C-19), 52.1 and 54.5 (C-8 and 16), 57.3 (C-7), 54.8 and 54.9 (OCH_3), 109.7, 110.1, 110.8 and 111.1 (C-2, 5, 12 and 15), 119.5 and 119.7 (C-6 and 11), 122.1 (C-23 and 26), 131.4 and 132.3 (C-1 and 10), 131.1 (C-22 and 27), 146.2, 146.8, 146.8, 147.7 and 147.8 (C-3, 4, 13 and 14), 167.4 (CO). Found: $[M+Na]^+$ (FAB), 555. 2475. $C_{31}H_{36}O_6N_2Na$ requires 555.2479.

N-*(3,4-Dimethoxybenzyl)*-N-*[2-(3,4-dimethoxyphenyl)ethyl]-1,4-butanediamine* **166**



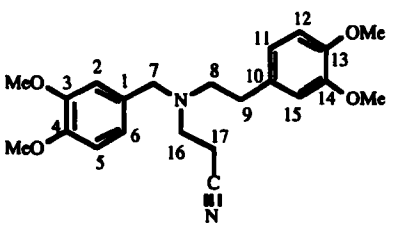
A solution of **165** (1.36 g, 2.56 mmol) and hydrazine hydrate (0.14 g, 0.14 ml, 2.82 mmol) in MeOH (7 ml) was heated at reflux for 3.5 h and concentrated *in vacuo*. The residue was taken into 6 M HCl (11 ml) and the reaction mixture was heated at reflux for 2 h. The precipitate was filtered off and the filtrate was treated with 5% NaOH (75 ml). The resulting milky mixture was extracted with $CHCl_3$ (2 x 120 ml) and the combined extracts were washed with water (2 x 120 ml), brine (120 ml) and dried over Na_2SO_4. Concentration *in vacuo* yielded **166** as a brown oil (0. 96 g, 2.39 mmol, 93 %) pure enough without further purification. ν_{max}(neat)/cm^{-1} 3367s (NH_2), 2935s (OCH_3); δ_H (400 MHz, $CDCl_3$) 1.34 and 1.44 (each 2H, each m, H-17 and 18), 1.69 (2H, bs, NH_2), 2.42 (2H, t, J 7.0 Hz, H-19), 2.78 (2H, t, J 7.0 Hz, H-16), 2.63 (4H, m, H-8 and 9), 3.50 (2H, s, H-7), 3.77, 3.78, 3.79 and 3.80 (12H, each s, OCH_3), 6.69 (6H, m, ArH); δ_C (100 MHz, $CDCl_3$) 23.5 (C-17), 30.6 (C-18), 32.1 (C-9), 41.2 (C-19), 52.6 and 54.5 (C-8 and 16), 54.8 and 54.9 (OCH_3), 57.3 (C-7), 109.7, 110.1, 110.8 and 111.1 (C-2, 5, 12 and 15), 119.6 and 119.7 (C-6 and 11), 131.5 and 132.4 (C-1 and 10), 146.2, 146.8, 147.7 and 147.8 (C-3, 4, 13 and 14). Found: $[M+H]^+$ (FAB), 403.2596. $C_{23}H_{35}O_4N_2$ requires 403.2595.

N-*(3,4-Dihydroxybenzyl)*-N-*[2-(3,4-dihydroxyphenyl)ethyl]*-*1,4-diaminobutane dihydrobromide* **167**



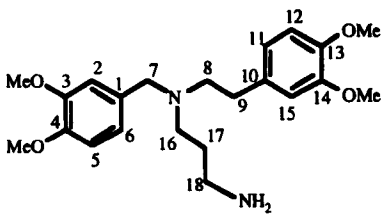
A mixture of **166** (0.31 g, 0.77 mmol) and 48% HBr (3 ml) was heated at reflux under N_2 for 3 h. The resulting dark brown solution was concentrated *in vacuo* to give **167** as a brown solid (0.35 g, 0.69 mmol, 90%), mp 156-157 ^{0}C. ν_{max}(KBr disc)/cm^{-1} 3609s (OH), 2402s (NH^+), 1609s (Ar and NH_3^+); δ_H (400 MHz, D_2O) 1.62 (4H, m, H-17 and 18), 2.73 (2H, m, H-9), 2.86 (2H, t, J 7.6 Hz, H-19), 3.02 (2H, m, H-16), 3.13 (2H, m, H-8), 4.06 (2H, dd, J 16 and 2.4 Hz, H-7), 6.68 (6H, m, ArH); δ_C (100 MHz, D_2O) 20.9 (C-18), 24.3 (C-17), 29.3 (C-9), 39.1 (C-19), 52.5 (C-16), 53.4 (C-8), 57.6 (C-7), 116.7, 116.8, 118.7, 121.4 and 124.2 (C-2, 5, 6, 11, 12 and 15), 128.9 (C-1 and 10), 143.4, 144.6, 144.8 and146.1 (C-3, 4, 13 and 14). Found: $[M+H]^+$ (FAB) 347.1975. $C_{19}H_{27}O_4N_2$ requires 347.1979.

N-*(2-Cyanoethyl)*-N-*(3,4-dimethoxybenzyl)*-N-*[2-(3,4-dimethoxyphenyl)ethyl]amine* **168**



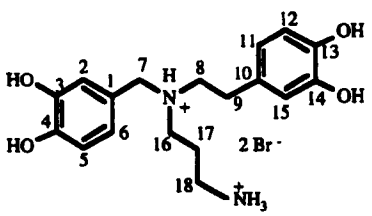
A mixture of **163** (0.50 g, 1.51 mmol) and acrylonitrile (0.08 g, 0.10 ml, 1.51 mmol) was heated at 60-70 ^{0}C in a sealed tube for 48 h. The remaining acrylonitrile was distilled off and the residue was purified by silica gel chromatography (EtOAc-petroleum ether 1:1.5 with 10% Et_3N) to yield **168** as a colourless oil (0.44 g, 1.16 mmol, 77%). ν_{max}(neat)/cm^{-1} 2936s (OCH_3), 2245s (CN), 1606s (Ar); δ_H (400 MHz, $CDCl_3$) 2.30 (2H, t, J 6.8 Hz, H-17), 2.72 (6H, m, H-8, 9 and 16), 3.56 (2H, s, H-7), 3.78, 3.79, 3.80 and 3.81 (12H, each s, OCH_3), 6.70 (6H, m, ArH); δ_C (100 MHz, $CDCl_3$) 17.0 (C-17), 33.8 (C-9), 49.6 (C-16), 56.1 (C-8), 56.2 and 56.3 (OCH_3), 58.7 (C-7), 111.2, 111.6, 112.0 and 112.5 (C-2, 5, 12 and 15), 119.4 (CN), 120.9 and 121.0 (C-6 and 11), 131.8 and 133.0 (C-1 and 10), 147.8, 148.6, 149.2 and 149.5 (C-3, 4, 13 and 14). *m/z* (EI) 384.2050 (M^+). $C_{22}H_{28}O_4N_2$ requires 384.2050; *m/z* (%) 384 (3), 233 (13), 151 (100), 107 (4), 106 (3).

N-(3,4-Dimethoxybenzyl)-N-[2-(3,4-dimethoxyphenyl)ethyl]-1,3-propanediamine **169**



To a stirred suspension of $LiAlH_4$ (0.05 g, 1.33 mmol) in dry THF (6 ml) was added $AlCl_3$ (0.18 g, 1.37 mmol) portionwise and the resulting mixture was stirred for 10 min. A solution of **168** (0.24 g, 0.62 mmol) in THF (2 ml) was added dropwise and the mixture was stirred at rt for 18 h. The mixture was cooled with an ice bath and excess $LiAlH_4$ was decomposed *via* careful addition of water (0.06 ml), 15% NaOH (0.16 ml) and water (0.06 ml). The resulting solid was filtered off and the filtrate was concentrated *in vacuo* to give a light yellow oil which was taken into $CHCl_3$ (5 ml). The $CHCl_3$ solution was washed with 15% NaOH (1.5 ml) and the aqueous layer was re-extracted with $CHCl_3$ (2 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **169** as a light yellow oil (0.167 g, 0.43 mmol, 69%) pure enough without further purification. ν_{max}(neat)/cm^{-1} 2935s (OCH_3), 3370s (NH_2), 1590s (Ar); δ_H (400 MHz, $CDCl_3$) 1.29 (2H, bs, NH_2), 1.54 (2H, tt, J 6.8 H, H-17), 2.46 (2H, t, J 6.8 Hz, H-18), 2.65 (6H, m, H-8, 9 and 16), 3.49 (2H, s, H-7), 3.77, 3.78 and 3.79(12H, each s, OCH_3), 6.70 (6H, m, ArH); δ_C (100 MHz, $CDCl_3$) 29.9 (C-17), 32.0 (C-9), 39.5 (C-18), 50.3 (C-16), 54.5 (C-8), 54.8 and 54.9 (OCH_3), 57.4 (C-7), 109.7, 110.2,110.8 and 111.1 (C-2, 5, 12 and 15), 119.6 and 119.7 (C-6 and 11), 131.5 and 132.3 (C-1 and 10), 147.2, 147.9, 147.7 and 147.8 (C-3, 4, 13 and 14). Found: $[M+H]^+$ (FAB) 389.2443. $C_{22}H_{33}O_4N_2$ requires 389.2445.

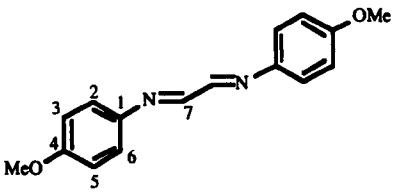
N-(3,4-Dihydroxybenzyl)-N-[2-(3,5-dihydroxyphenyl)ethyl]-1,3-diaminopropane dihydrobromide **170**



A mixture of **169** (0.10 g, 0.27 mmol) and 48% HBr (2 ml) was heated at reflux under N_2 for 3 h. Concentration *in vacuo* yielded **170** as a grey solid (0.1271 g, 0.257 g, 97%). mp 179-180 ^{0}C. ν_{max}(KBr disc)/cm^{-1} 3570s (OH), 1606s (Ar); δ_H (400 MHz, D_2O) 1.99 (2H, m, H-17), 2.75 (2H, m, H-9), 2.88 (2H, t, J 7.8 Hz, H-18), 3.14 (4H, m, H-8 and 16), 4.13 (2H, s, H-7), 6.72 (6H, m, ArH); δ_C (100 MHz, D_2O) 22.0 (C-17), 29.2 (C-9), 36.9 (C-18), 50.1 (C-

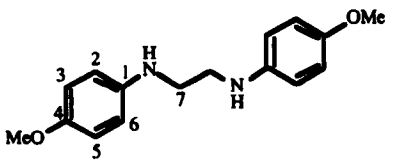
16), 53.5 (C-8), 57.7 (C-7), 116.8, 116.9, 118.8, 121.5 and 124.3 (C-2, 5, 6, 11, 12 and 15), 128.8 (C-1 and 10), 143.5, 144.6, 144.8 and 146.2 (C-3, 4, 13 and 14). Found: [Free base+H^+] (FAB) 333.1813. $C_{18}H_{25}O_4N_2$ requires 333.1812.

N, N'-*Bis(p-anisyl)ethylenediimine* **171**[122]



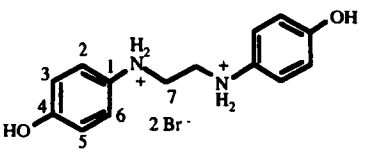
Glyoxal (0.7 ml, 0.88 g, 40% aqueous solution, 6.1 mmol) was added dropwise to a hot solution of *p*-anisidine (1.5 g, 12.18 mmol) in MeOH (10 ml). The resulting yellow suspension was heated at reflux for a further 40 min. The precipitate was collected, washed well with cold MeOH, ether and dried to yield **171** as yellow needles (1.26 g, 4.68 mmol, 77%) pure enough without further purification, mp 153 ^{0}C (lit.,[122] mp 153-154 ^{0}C). δ_H (400 MHz, $CDCl_3$) 3.77 (6H, s, OCH_3), 6.87 (4H, q, J 8.9 Hz and 3.3 Hz, AA'BB' system, H-3 and 5), 7.25 (4H, q, J 8.9 Hz and 3.3 Hz, AA'BB' system, H-2 and 6), 8.34 (2H, s, H-7); δ_C (100 MHz, $CDCl_3$) 54.5 (OCH_3), 113.6 (C-3 and 5), 122.0 (C-2 and 6), 141.9(C-1), 156.6 (C-7), 158.7 (C-4).

N, N'-*Bis(p-anisyl)-1,2-diaminoethane* **172**

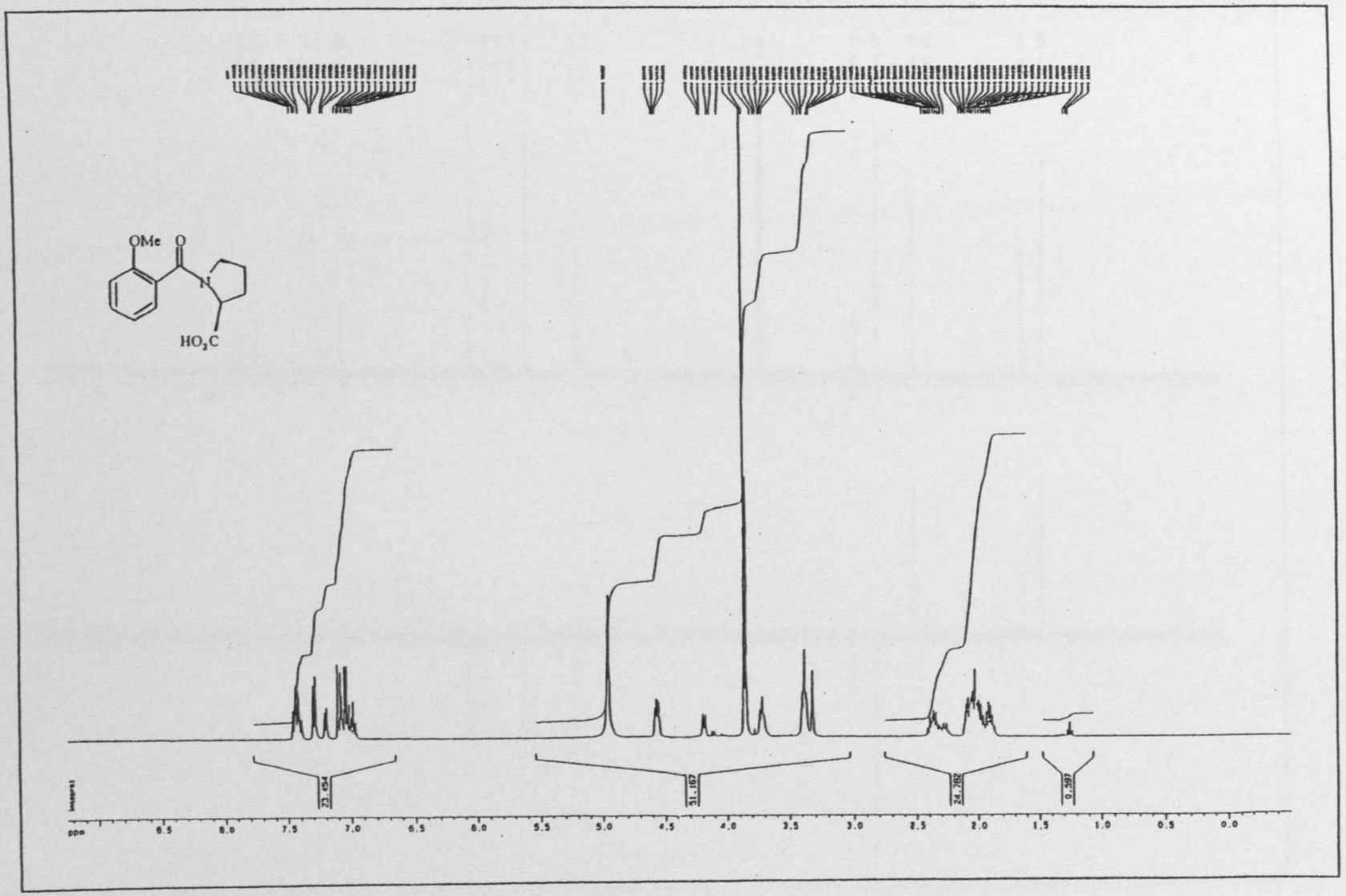


To a solution of **171** (0.3 g, 1.12 mmol) in dry EtOH (25 ml) was added $NaBH_4$ (0.42 g, 11.2 mmol) portionwise. The mixture was heated at reflux for 4 h, allowed to cool and stirred overnight at rt. The resulting mixture was poured into water (15 ml) and the EtOH was evaporated. The remaining aqueous solution was extracted with $CHCl_3$ (2 x 15 ml). The combined $CHCl_3$ extracts were washed with brine (10 ml), dried over Na_2SO_4 and concentrated *in vacuo* to give **172** as a yellow solid (0.28 g, 1.02 mmol, 91%) pure enough for synthetic work without further purification, mp 132-133 ^{0}C. ν_{max}(KBr disc)/cm^{-1} 2849s (OCH_3), 3274s (NH), 1593 (Ar); δ_H (400 MHz, D_2O + $CDCl_3$) 3.25 (4H, s, H-7), 3.68 (6H, s, OCH_3), 6.55 (4H, q, J 8.9 Hz and 3.6 Hz, AB system, H-3 and 5), 6.72 (4H, q, J 8.9 Hz and 3.6 Hz, AB system, H-2 and 6); δ_C (100 MHz, $CDCl_3$) 43.4 (C-7), 54.8 (OCH_3), 113.6 and 113.9 (C-2, 3, 5 and 6), 141.0 (C-1), 151.5(C-4). *m/z* (EI) 272.1528 (M^+). $C_{16}H_{20}O_2N_2$ requires 272.1531; *m/z* (%) 272 (30), 136 (100), 122 (14), 121 (8), 108 (6), 93 (4), 77 (4).

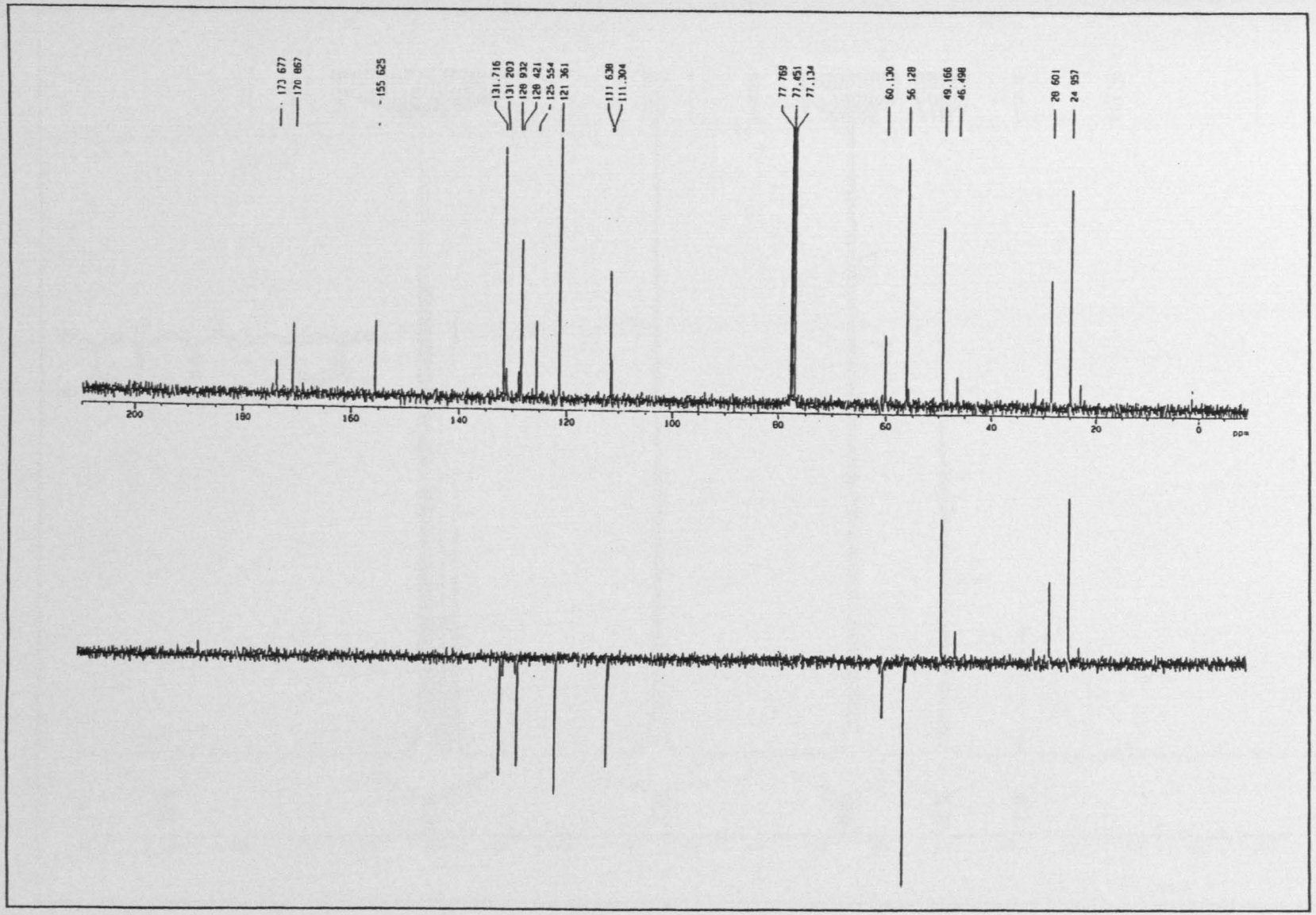
N, N'-*Bis(p-hydroxyphenyl)-1, 2-diaminoethane dihydrobromide* **173**



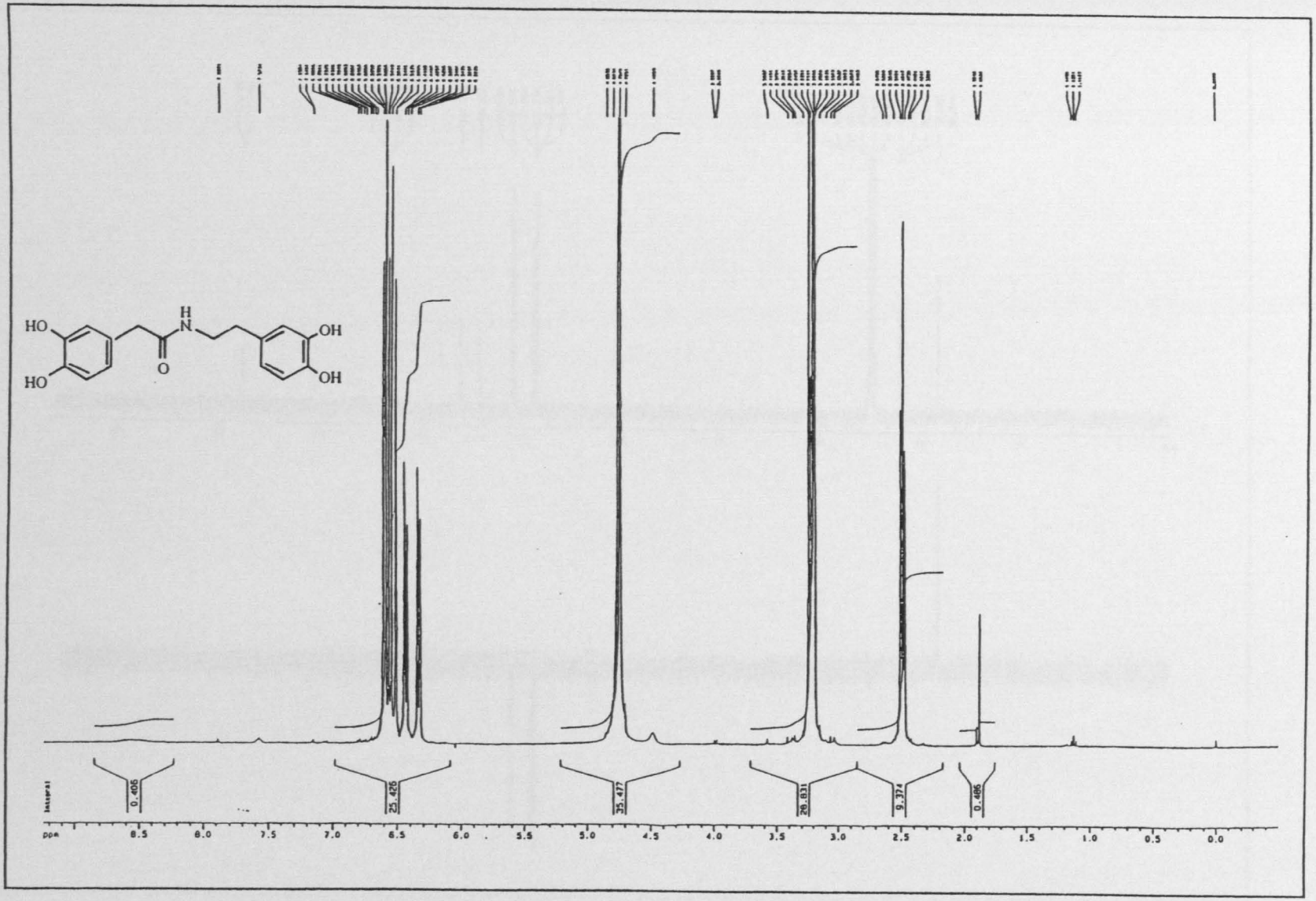
A solution of **172** (0.21 g, 0.77 mol) in 48% HBr (*20 ml) was heated at reflux for 4 h.* Concentration *in vacuo* afforded **173** as a brown solid (0.22 g, 0.54 mmol, 70%), mp 175-176 ^{0}C. (Found: C, 41.51; H, 4.37; N, 6.88; $C_{14}H_{18}N_2O_2Br_2$ requires C, 41.38; H, 4.43; N, 6.90%); ν_{max}(KBr disc)/cm^{-1} 2849s (OCH_3), 3274s (NH), 1593 (Ar); δ_H (400 MHz, D_2O) 3.65 (4H, s, H-7), 6.89 (4H, m, H-3 and 5), 7.15(4H, m, H-2 and 6); δ_C (100 MHz, D_2O) 45.7 (C-7), 117.3 (C-3 and 5), 123.6 (C-2 and 6), 156.6 (C-4), 128.0 (C-1). Found: $[M+H]^+$ (FAB) 245.1287. $C_{14}H_{17}N_2O_2$ requires 245.1284.



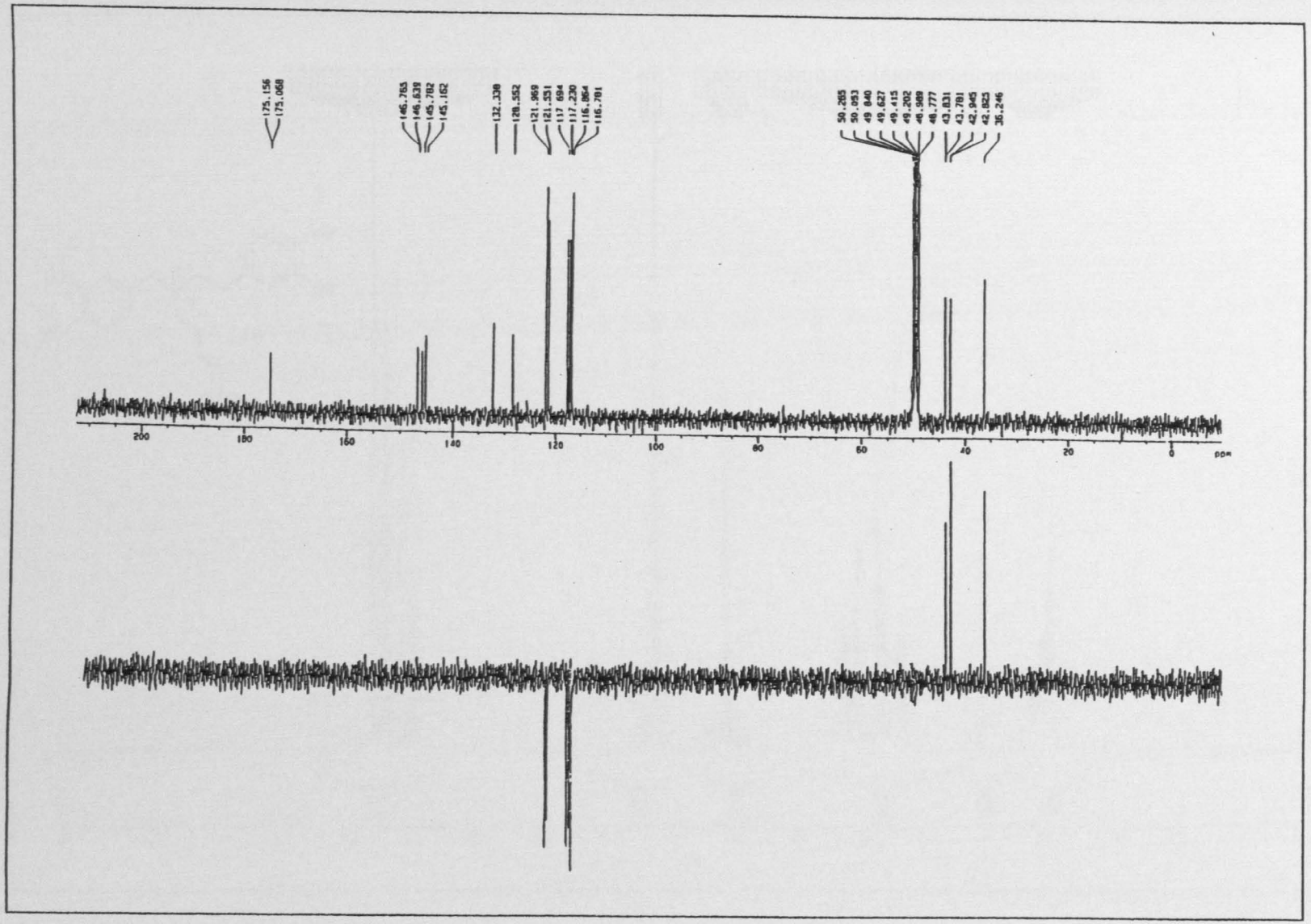
Appendix 1 ^{1}H NMR spectrum for compound **77**



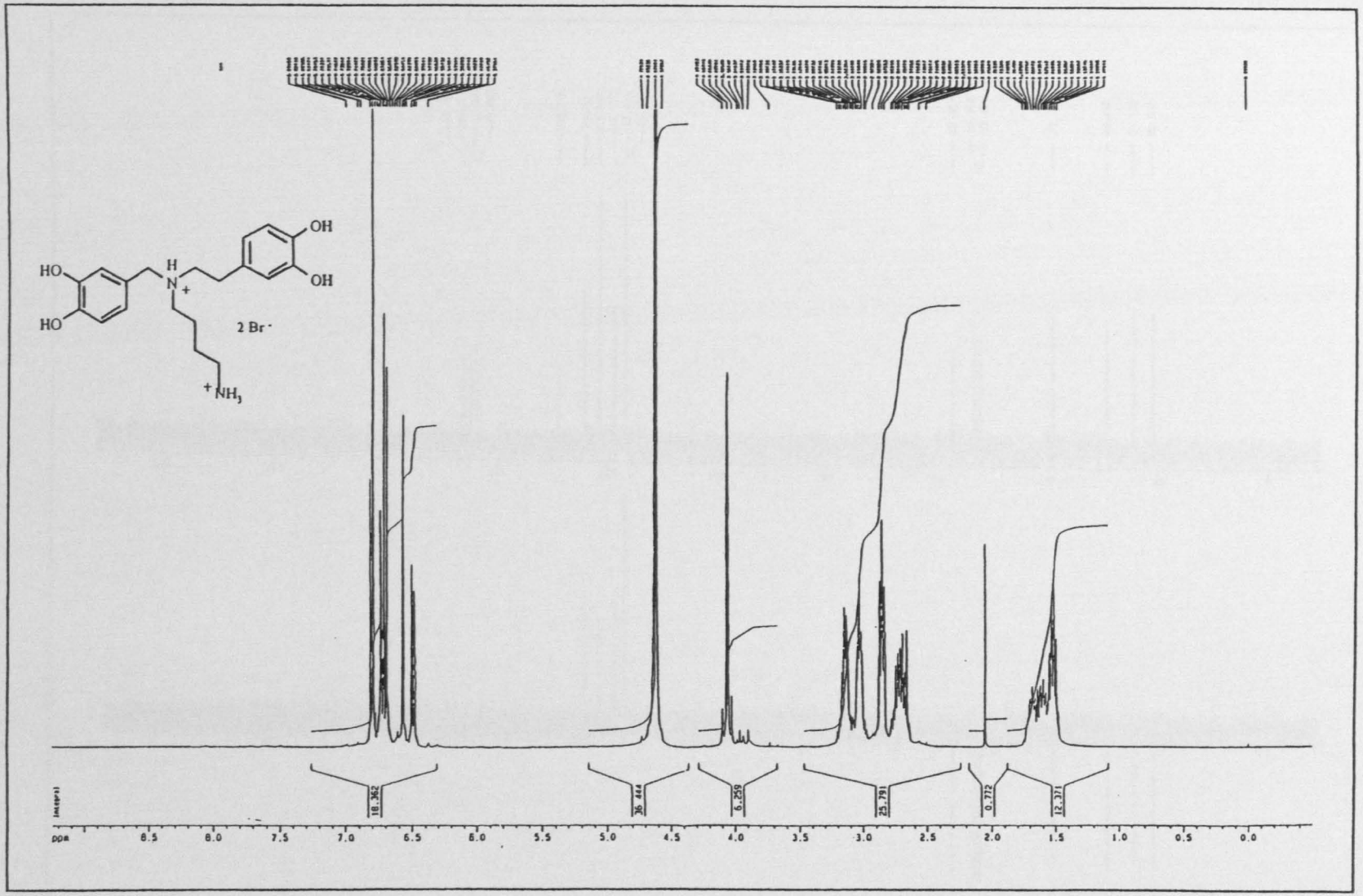
Appendix 2 ^{13}C NMR spectrum for compound 77



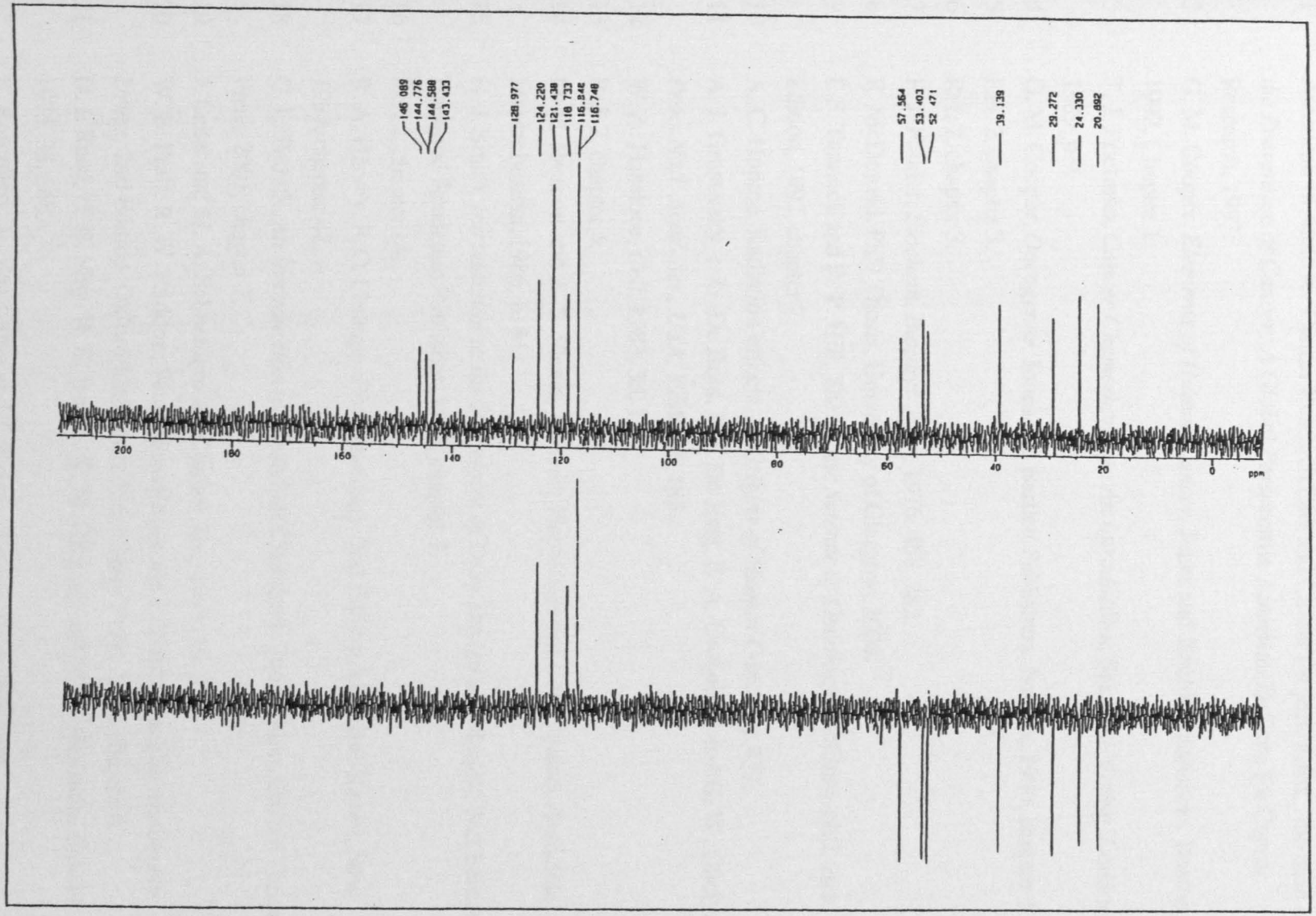
Appendix 3 ^{1}H NMR spectrum for compound **137**



Appendix 4 ^{13}C NMR spectrum for compound 137



Appendix 5 ^{1}H NMR spectrum for compound **167**



Appendix 6 ^{13}C NMR spectrum for compound 167

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