Synthesis and Biological Evaluation of Tyrphostins as Anticancer Agents

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

Gerard John Montgomery

Department of Chemistry
University of Glasgow
Glasgow G12 8QQ

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Dedicated to my Dad and Mum

Whose love and support
were my inspiration

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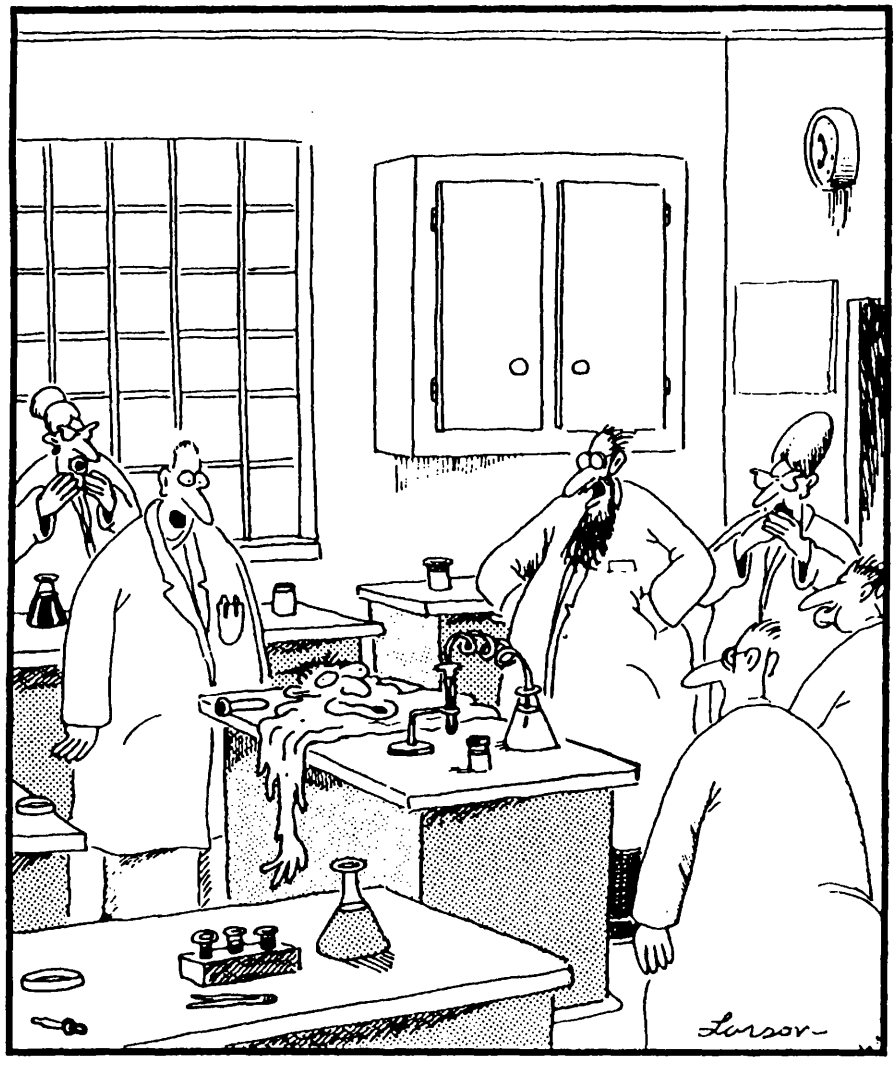
I will never drink Tequila again!

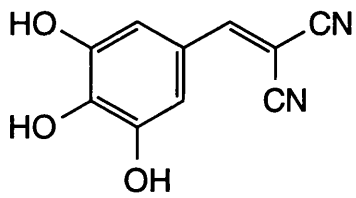


"My God! It *is* Montgomery!... Lant, see if you can figure out what the devil he was working on, and the rest of you get back to your stations."

Summary

Recent developments in molecular biology have led to a greater understanding of signalling mechanisms which are overexpressed or distorted in the cancer cell, resulting in the identification of a number of new molecular targets for cancer chemotherapy. Protein Tyrosine Kinases (PTKs) are enzymes which play an important role in the signal transduction pathways leading to mitogenesis. Growth factors, such as epidermal growth factor (EGF) are responsible for stimulating cells to synthesise DNA prior to mitosis. The EGF receptor, which is present at elevated levels in certain cancer cells, has been shown to display PTK activity.

Tyrphostins are low molecular weight molecules which are structurally similar to tyrosine. These have been shown to display PTK inhibition activity. The earliest reported tyrphostins such as **(a)** were based upon a benzylidenemalononitile nucleus, but more recently a number of tyrphostins containing a 5-membered heterocyclic nucleus **(b)** have been reported.



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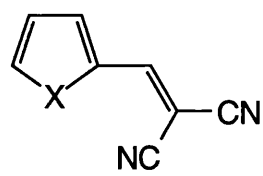
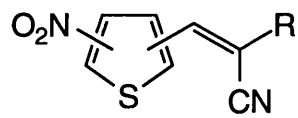


(b)

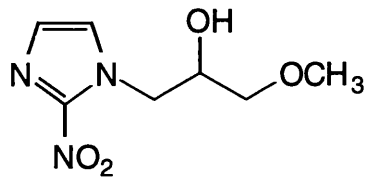
X= O, S, NH



(c)

R= CN,$C(NH_2)$=$C(CN)_2$, CO_2R' $CONH_2$, $CSNH_2$, 2-thienyl

Tyrphostins containing a nitrothiophene fragment **(c)** were prepared using standard methodology. These compounds were shown to display excellent *in vitro* growth inhibition in cells with a high EGF concentration. More detailed studies later showed that a number of these compounds were not selective for the EGF receptor, and instead displayed wide-spectrum cytotoxicity by an unknown mechanism. The crystal structure of one tyrphostin was solved, showing that the aromatic and cyano groups are *cis* across the extra-annular double bond.



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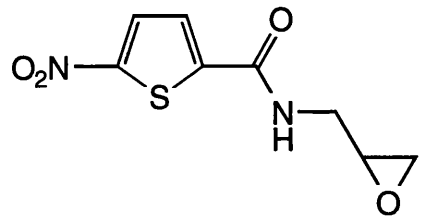
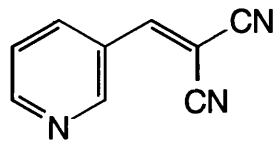


(e)

Previous studies had shown that analogues of misonidazole **(d)** which contained a nitrothiophene group **(e)** possessed the ability to become reduced to cytotoxic species in hypoxic tumour cells, which have a low oxygen saturation. The reduction potentials of a number of nitrothienyl tyrphostins were measured, and some of these fell within the parameters required for *in vivo* bioreduction.



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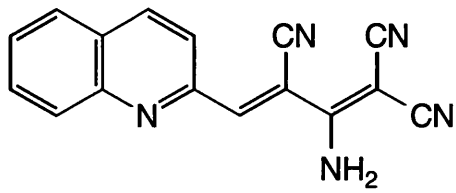

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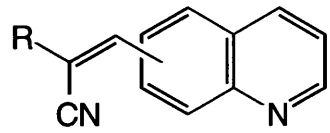
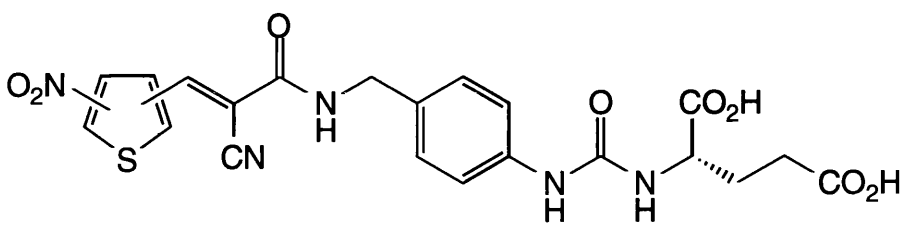


(h)

Pyridyl and quinolyl tyrphostins such as **(f)** and **(g)** have been reported to display interesting biological profiles. In particular, 2- and 4-substituted quinolines were particularly active, and this may be perhaps due at least in part to substitution at these electropositive centres. Substitution at the 3-position gave rise to a series of inactive compounds. In order to extend the study of this structure-activity relationship, a series of 6-, 7- and 8-quinolyl tyrphostins **(h)** were synthesised.

Antibody-Directed Enzyme Prodrug Therapy (ADEPT) is a sophisticated method to deliver cytotoxic agents at their preferred site of action. An antibody which recognises surface antigens on a cancer cell also possesses an enzyme which hydrolyses a prodrug molecule such as **(i)** and leads to the release of the drug molecule. A methodology to synthesise such a prodrug was investigated, but it was not possible to complete the urea linkage between L-glutamate and the benzene ring by attempting to generate an isocyanate at either of the amino groups in the starting materials.



(i)

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Abbreviations

ADEPT	Antibody-Directed Enzyme Prodrug Therapy
AEC	Antibody-Enzyme Conjugate
ATP	Adenosine Triphosphate
BMN	Benzylidenemalononitrile
br	broad
CDI	carbonyldiimidazole
CNS	Central Nervous System
CPG_2	Carboxypeptidase G_2
CV	Cyclic Voltammogram
d	doublet (NMR spectroscopy)
d	day(s)
DCM	dichloromethane
decomp.	decomposition
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EGF	Epidermal Growth Factor
FGF	Fibroblast Growth Factor
FSBA	5'-[4-(fluorosulfonyl)benzoyl]adenosine
GAP	GTPase activating protein
GI_{50}	concentration required to give 50% inhibition of growth
GTP	guanosine triphosphate
h	hour(s)
IC_{50}	concentration required to give 50% inhibition of function
ICR	Insitute of Cancer Research
lit.	literature value
m	medium (IR spectroscopy)
m	multiplet (NMR spectroscopy)
m.p.	melting point
MEM	methoxyethoxymethyl
min	minute(s)
ml	millilitre(s)
mmol	millimole(s)
mol	mole(s)

MTX	Methotrexate
n.d.	not determined
NCI	National Cancer Institute
NHE	Normal Hydrogen Electrode
PDGF	Platelet-Derived Growth Factor
PEG	Poly(ethyleneglycol)
PI	Phosphatidyl Inositol
PLC	phospholipase c_γ
poly (GAT)	poly(Glutanine-Alanine-Tyrosine)
PTK	Protein Tyrosine Kinase
q	quartet (NMR spectroscopy)
s	sharp (IR spectroscopy)
s	singlet (NMR spectroscopy)
SAR	Structure-Activity Relationship
ser	serine
SH	*src* homology
sp.act.	specific activity
t	triplet (NMR spectroscopy)
TBDMS	tertiarbutydimethylsilyl
TEG	tri(ethyleneglycol)
TGF	Transformed Growth Factor
thr	threonine
TIPS	triisopropylsilyl
tyr	tyrosine
v. br	very broad
w	weak (IR spectroscopy)

1. Introduction

> **Cancer** (kæ·nsəɪ), *sb.*
>
> *Pathol.* A malignant growth or tumour in different parts of the body, that tends to spread indefinitely and to reproduce itself, as also to return after removal; it eats away or corrodes the part in which it is situated, and generally ends in death.
>
> ***Oxford English Dictionary***

1.1 Cancer

For a very long time, cancer was defined as a 'killer' disease for which there was little or no hope of cure. In the past century, however, our knowledge of the various types of cancer has increased dramatically, and many treatments have been developed to combat these diseases. More recently, in the last ten years or so, science has begun to understand that cancer is a genetic disorder,[1, 2] and we have started to understand the molecular mechanisms underpinning the carcinogenic process.[3, 4]

Cancer is a disease of abnormal growth. Healthy cells undergo a number of structural and molecular changes which result in them escaping from the normal control mechanisms. If left untreated, they will continue to increase in number. They often form tumour masses which resemble the surrounding tissue less and less as the disease progresses. Local invasion occurs, and tumour cells may break off and enter the lymphatic system, becoming deposited at remote sites to form secondary growth or *metastases*. It is these invasive and metastatic properties which distinguishes malignant cancer cells from benign growths. Unlike bacterial infection, there is relatively little immune response by the body to cancer. When there is any, it soon becomes overwhelmed and is only weakly effective.

Whilst there have been some degrees of success in developing therapeutic agents to treat cancers, including lymphatic leukaemia of childhood, Hodgkin's disease, Burkitt's lymphoma, Wilm's tumour (a kidney cancer in children), choriocarcinoma (a malignancy of placental trophoblast), testicular cancer and certain ovarian cancers, these only account for a relatively small percentage of all malignant tumours. In other cases such as breast, lung, colon and prostate cancer (the most common types in adults), the most effective treatments remain

early diagnosis and surgery combined with radiotherapy. Most of these cancers cannot be treated effectively with the current range of relatively non-specific anticancer drugs, and whilst solid tumours show a limited response to these drugs, resistance usually develops and renders the treatment ineffective.

1.2 Cancer Epidemiology

Every 63 seconds, someone dies of cancer in the United States alone.[5] Of the 500,000 cancer-related deaths in the USA in 1992, some 76,000 were children aged between 3 and 14 years; 46,000 were women suffering from breast cancer and 43,000 were men with prostate cancer. However, it is important to note that thanks to improved methods of diagnosis and treatment, the latter two figures represent 26% and 42% of the total number of diagnoses for the same year. The five-year survival rate for all cancers has increased from 20% in the 1930s to almost 50% in the early 1990s.

1.3 Factors Leading to Cancer

It is extremely difficult to identify the various factors, be they chemical, viral, inherited or a result of exposure to radiation, which increase the likelihood of cancer.

Few would disagree with the observation that smoking is a key factor in causing 30% of *all* cancers; as well as lung cancer, smoking has also been shown to contribute to cancers of the mouth, pharynx, larynx, oesophagus, urinary bladder, pancreas and kidney.[6] Lifestyle is the next most common cause of cancer. Diet, alcohol use, reproduction and sexual behaviour and exposure to sunlight are some of the risk factors involved. Not surprisingly, exposure to chemicals, particularly in an industrial environment, has been linked to cancer.

Patients who have been infected by viruses such as hepatitis B have displayed a higher rate of liver cancer. There is little doubt that cancers may also arise from genetic predisposition. However, inherited malignancies account for only 1-2% of the total number of cancers.[7] But a high percentage of certain tumours are genetically determined. Examples include 40% of retinoblastomas, 20-40% of Wilm's tumours and almost 80% of familial multiple polyposis of the large bowel.

In certain cases specific chromosomal abnormalities have been identified. Some patients suffering from Wilm's tumour have been shown to have a deletion in band 13 of the short arm of chromosome 11.[8] Other genetic abnormalities are only triggered by environmental factors. These include xeroderma pigmentosum, which is characterised by extreme sensitivity to sunlight and incidences of skin cancer approaching 100%, and Fanconi's anaemia, which is a haematological deficiency giving rise to anaemia and haemorrhaging and an increased risk for leukaemia.

1.4 Cancer Chemotherapy

If cancer is in fact many diseases which can be caused or triggered by one or more factors, how can we as scientists develop new therapeutic agents to treat these diseases? This question has been asked for a long time, and many treatments have been tested with varying degrees of success. In 1768, the natural historian Gilbert White wrote of *"The wonderful method of curing cancer by means of toads"*.[9] It is true to say that the drugs we use today to treat cancer are far-removed from the treatments of White's days; but too few drugs are available to treat effectively all but a handful of cancers.

Why has it taken so long? The reason is, at least in part, due to the inability of cancer researchers to understand fully the complex series of molecular events which occur in normal healthy cells, and to identify the stages at which these mechanisms fail or become disrupted to give rise to cancer. But dramatic breakthroughs in molecular biology and biochemistry have led to the identification and greater understanding of key processes which are overexpressed or initiated in the abnormal cell. Within the apparent chaos of the tumour cell genome lie the specific alterations to DNA which provide the genetic basis for the initiation of the tumour. We now know that tumour suppressor genes are rendered inactive by mutation, deletion or whole chromosomal loss.[10, 11] Oncogenes, which encode for the expression or overexpression of key molecules in the cancer cell, are activated by translocation, point mutation or amplification in the chromosomes.

Ultimately, a clinically effective cancer drug should be one which can identify and kill malignant cells at a concentration which will allow survival of the patient's critical tissues (such as bone marrow and the gastrointestinal tract). This is difficult to achieve owing to the rapidity of cellular proliferation in all malignant tumour cells. Surgery, together with combinatorial treatments of radiotherapy and chemotherapy are still the most widely-used techniques.

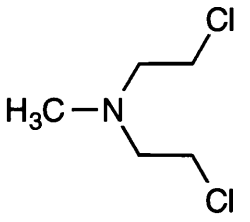
However, the low degree of specificity of these methods make them unfavourable owing to the debilitating effects which they have on the patient's health.

It is perhaps ironic that the first systemic cancer chemotherapy was originally developed as a war gas. This was nitrogen mustard, whose precursor, sulfur mustard, was used on the battlefield during World War I. In the 1930s, the nitrogen mustards were shown to have an antitumour effect in lymphosarcoma-bearing mice. These mustards entered clinical trials in 1942, but the results remained unpublished until 1946 after the war ended.[12] The following year, Farber *et al.* treated children with acute leukaemia using aminopterin or its non-methylated derivative methotrexate, which are folic acid antagonists.[13]

1.4.1 Anticancer Agents in Use Today

There are currently around 40 anticancer agents in general clinical use today. These fall into 4 categories.

1. Alkylating Agents. These are highly reactive species which form covalent links with nucleophilic sites in nucleic acids, proteins, amino acids and nucleotides. The common property of all alkylating agents is that they can undergo transformation to produce reactive, electron-deficient species. Examples of drugs in this class include nitrogen mustard **(1)**, chlorambucil **(2)**, melphalan **(3)**, and cyclophosphamide **(4)**.



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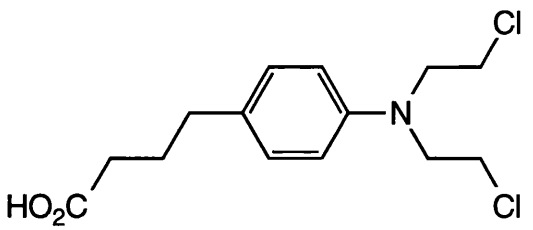

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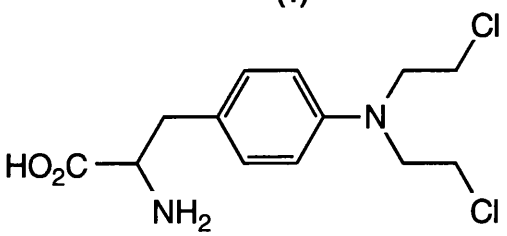

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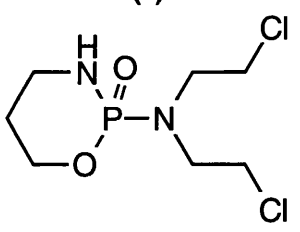
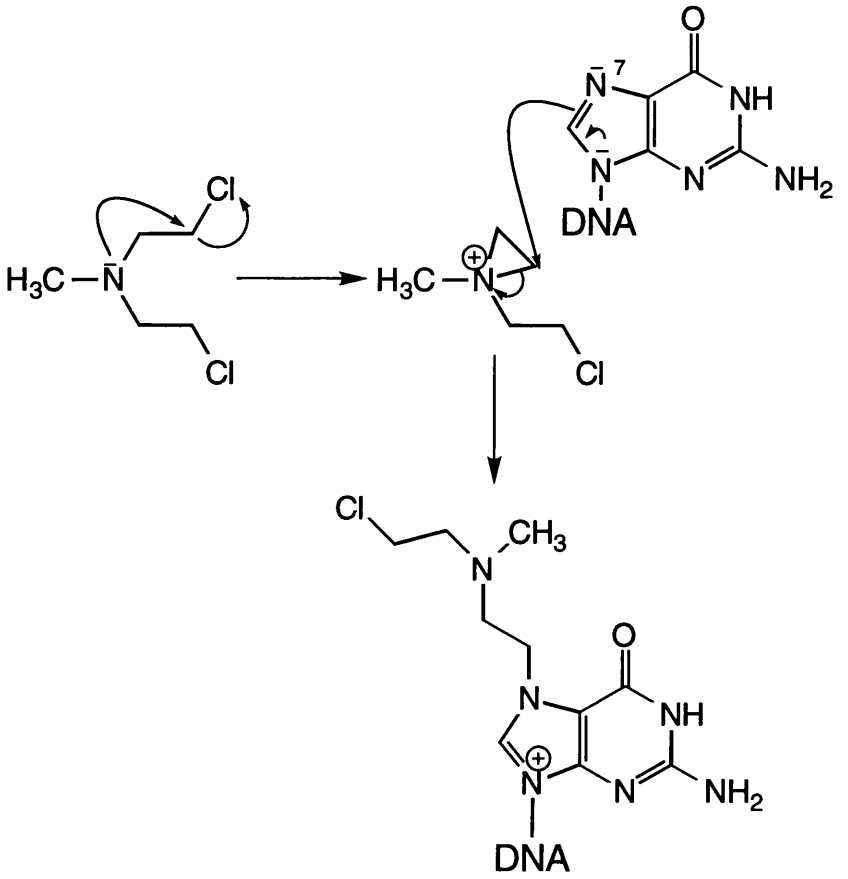


(4)

All of the alkylating agents shown are bifunctional, possessing two chloroethyl groups which form reactive aziridinium groups which are responsible for

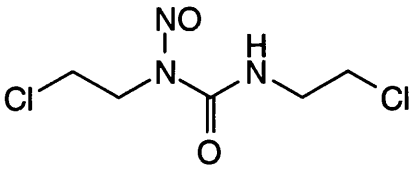
alkylation of DNA. This is normally at the N-7 position of the base guanine. **Scheme 1** shows the action of nitrogen mustard on a guanine base. Initial formation of a highly reactive positively-charged aziridine ring is followed by covalent binding with the electronegative N-7 group on guanine, resulting in alkylation. This may lead to mispairing of the alkylated guanine with thymine, or to strand break. Alternatively, the second chloroethyl side-chain may undergo a similar reaction with a guanine on the opposite DNA strand, causing cross-linking and thereby preventing DNA replication and mitosis. In this case, the result is cell death.



Scheme 1

Nitrogen mustard is still used today as part of a cocktail of drugs in the treatment of Hodgkin's disease, but its reactivity and vesicant properties make it unfavourable for treatment of most cancers. On the other hand, cyclophosphamide is devoid of these properties until it undergoes metabolic activation, and is much more widely used.

Nitrosoureas such as BCNU **(5)** and CCNU **(6)** are lipid-soluble, and have the potential to penetrate into the central nervous system for treatment of intracranial tumours. Whilst they have been successfully used in the treatment of experimental tumours in mice, their clinical application has been limited owing to the fact that they cause prolonged myelosuppression.



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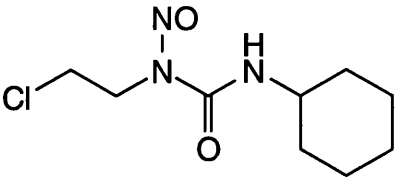
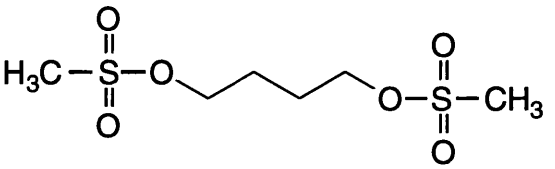


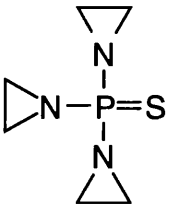
(6)

Busulfan **(7)** has been used in the treatment of myeogeneous leukaemia. This compound generates electron-deficient sulfonates which can form covalent bonds to DNA, and may cause cross-linking.



(7)

A number of compounds containing aziridine rings, such as Thio-TEPA (triethylenethiophosphamide) **(8)** have been employed as anticancer drugs. Thio-TEPA is administered topically to treat papillary carcinoma of the bladder.



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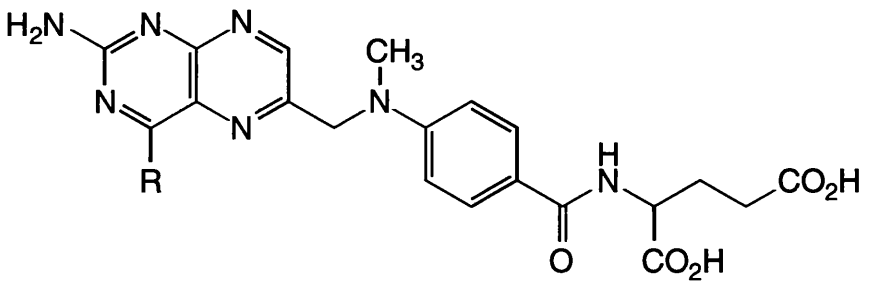
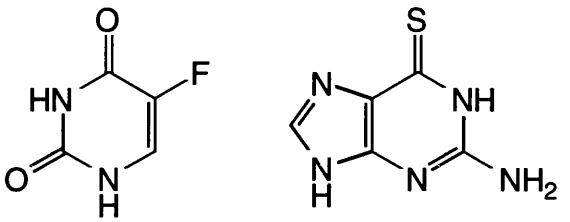
(2) Antimetabolites. These compounds interfere with nucleic acid production by one of two mechanisms. They may either inhibit production of the deoxyribonucleoside triphosphates which are the immediate precursors for DNA synthesis, or they may be sufficiently similar in structure to purines or pyrimidines to be substituted for them. A major clinical advantage of antimetabolites is that their toxicity is reversible. An example is methotrexate (MTX) **(9)**, which is a folic acid antagonist or *antifolate*. Folic acid **(10)** is required in the biosynthetic pathway to purines.



(9) R = -NH_2

(10) R = -OH

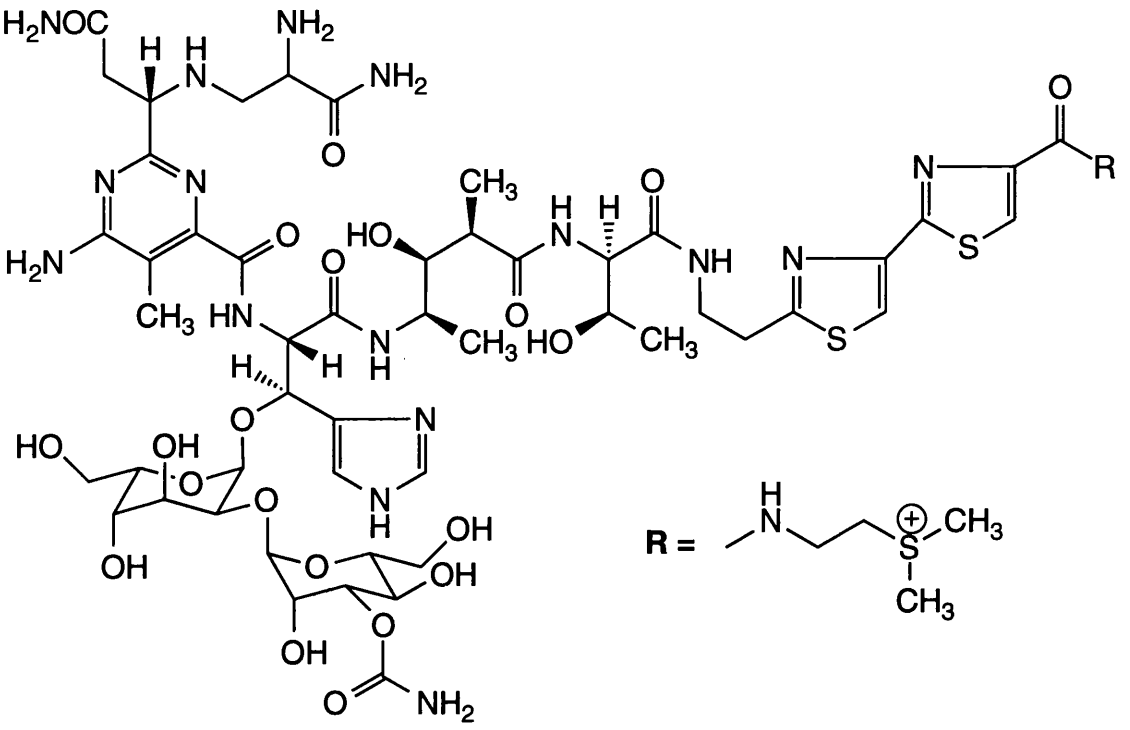
Pyrimidine antagonists include 5-fluorouracil **(11)** and 5-fluorodeoxyuridine, which prevent thymine and uracil production in DNA and RNA respectively. Mercaptopurine and 6-thioguanine **(12)** inhibit purine biosynthesis.



(11) **(12)**

(3) Natural Products. A number of anticancer agents have been isolated from natural sources. These include bleomycin, the anthracyclines (such as doxorubicin and daunorubicin), the vinca alkaloids, mitomycin C and taxol.

Oppenheimer *et al.* showed that the structure of bleomycin **(13)**, isolated from *Streptomyces verticullus*, was a complex glycopeptide with two major domains.[14] These constituted a bisthiazole ring moiety, which interacts with DNA by intercalation, and a large fragment containing primary amine, pyrimidine and imidazole nitrogens which function as ligands to form a metal co-ordination site. This domain essentially acts as a ferrous oxidase. Fe (II) bleomycin can be regenerated from Fe (III) bleomycin by an NADPH-dependent enzyme system in the nucleus, and this redox recycling of the iron-co-ordinated drug is accompanied by increased DNA cleavage preferentially at *5'*-guanine-cytosine-*3'* and *5'*-guanine-thymine-*3'* sequences.[15, 16]



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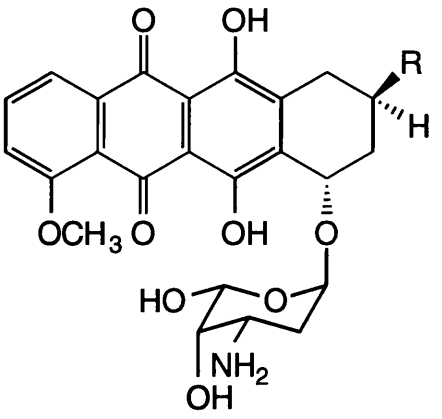
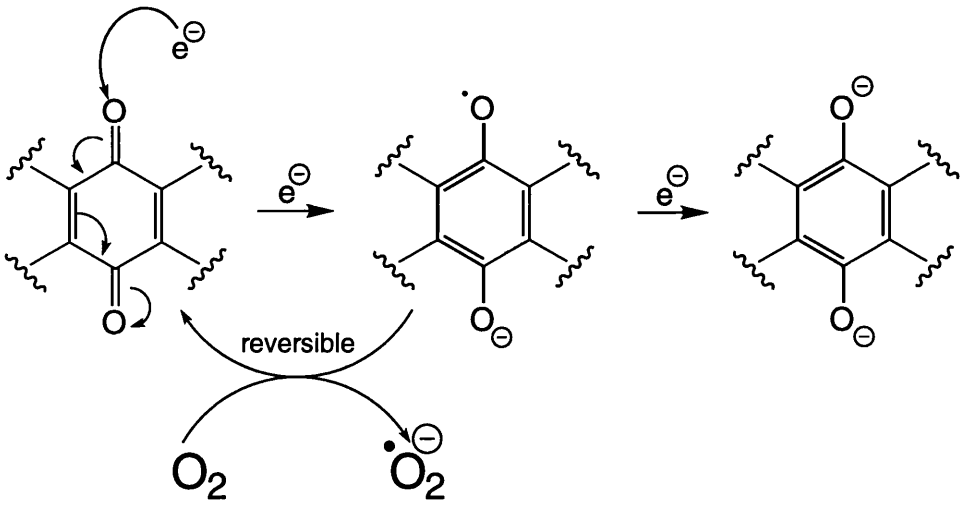
Doxorubicin **(14)** and daunorubicin **(15)** display broad spectrum antitumour activity. They have a wide range of clinical uses, and are particularly effective against solid tumours such as breast cancer (doxorubicin) and acute leukaemia (daunorubicin). Whilst these compounds display topoisomerase (TI)-2 dependent DNA intercalation (TI-2 is an enzyme which recognises and cleaves on either side of species intercalated in DNA), an additional mode of action is *via* radical generation of superoxide ($^{\cdot}O_2^-$) **(Scheme 2)**, which causes irreversible membrane damage.



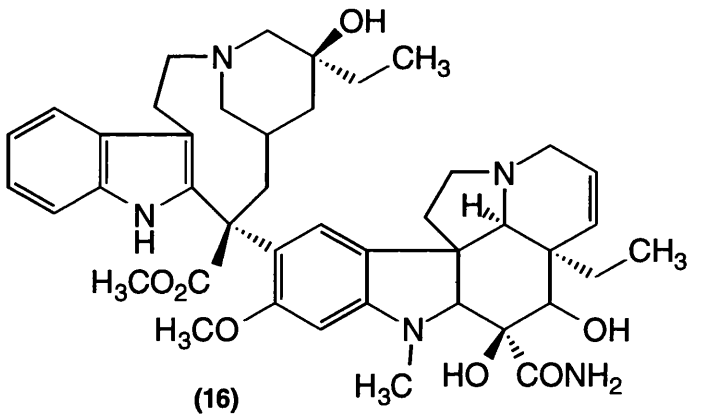
(14) R= -$COCH_2OH$

(15) R= -CH_3



Scheme 2

Microtubule inhibitors include the vinca alkaloids (e.g. vindesine, **(16)**) and taxol **(17)**. Microtubules are involved in a number of functions, including mitosis, transport of solutes, cell motility and maintenance of the cell's structural integrity. The vinca alkaloids act by binding to a site on tubulin and prevent polymerisation, which inhibits microtubule formation. Conversely, taxol enhances tubulin polymerisation. The net result of both of these mechanisms is the prevention of mitosis.



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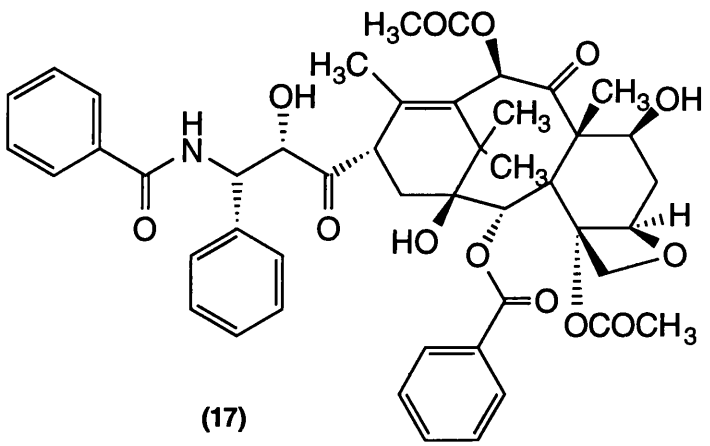

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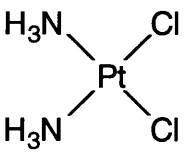
Mitomycin C **(22)**, derived from a species of *Streptomyces*, can be described as a bioreducible alkylating agent. Reductive activation of the quinone moiety by a variety of enzymes (including NADPH-cytochrome *c* reductase, xanthine reductase and quinone reductase) is followed by spontaneous elimination of the methoxy group attached to C-10. The protonated aziridine ring is then opened, and the semiquinone undergoes nucleophilic attack at C-10, most often by N-2 on guanine. Subsequent intramolecular displacement of the carbamate group yields a cross-linked drug-DNA adduct **(Scheme 3)**. Mitomycin C has been used in the treatment of cancers of the stomach, colon, pancreas, breast, lung and head and neck.

(4) Other Compounds. These include non-classical alkylating agents such as cisplatin **(18)** and carboplatin **(19)** and methylating agents such as dacarbazine **(20)** and procarbazine **(21)**. Procarbazine is metabolised to produce a methyldiazonium cation, which methylates at N-7 of guanine.



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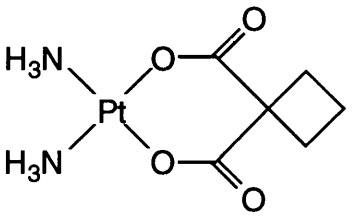

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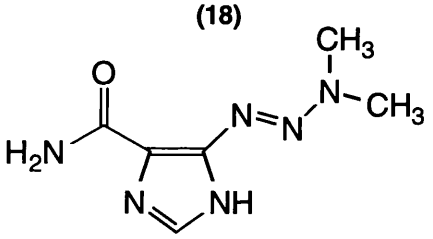

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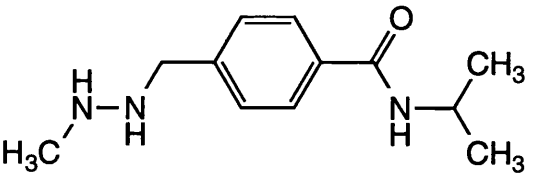
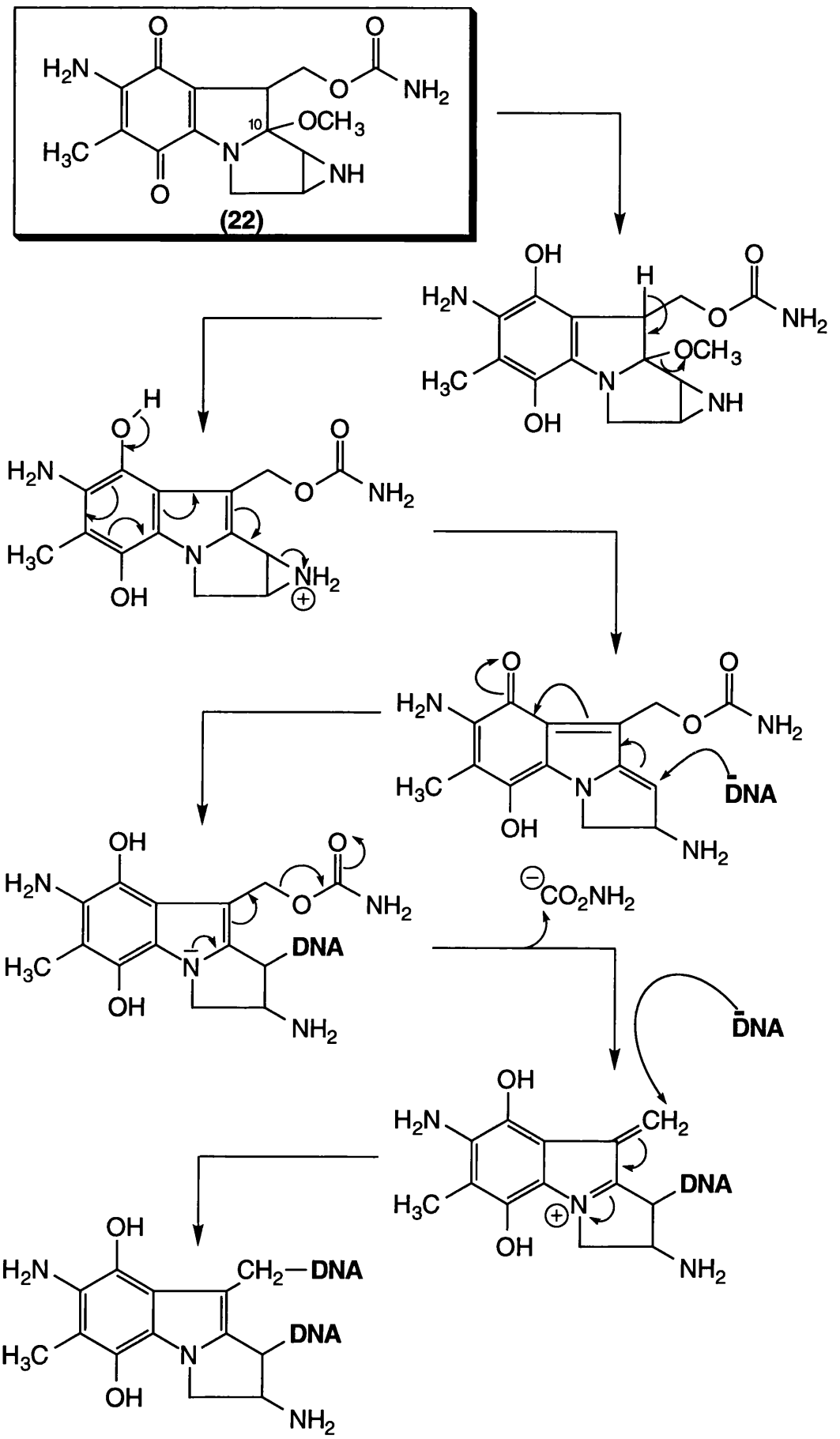


(21)

1.5 Targets for New Anticancer Agents

New developments in biochemistry and molecular biology have led to a substantial increase in our knowledge of the processes which regulate cellular proliferation and differentiation, as well as the responses of cells to external signals such as hormones and growth factors. It is on this latter group which this thesis will concentrate.



Scheme 3 (Adapted from Tomasz *et al.*[17]).

1.5.1 Growth Factors

Growth factors are small polypeptides with molecular weights in the region of 6,000 to 25,000. They are responsible for stimulating cells in the G_0 phase to enter and proceed through the cell division cycle **(Figure 1)**. The mitogenic response occurs in two distinct ways.[18, 19]

(1) Quiescent cells are acted upon by "competence" factors, which allow entry and subsequent traversion of the G_1 phase. Examples are Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), and Fibroblast Growth Factor (FGF).

(2) Cells in the G_1 phase are committed to DNA synthesis by the action of "progression" factors such as Insulin Growth Factor (IGF) and Insulin.

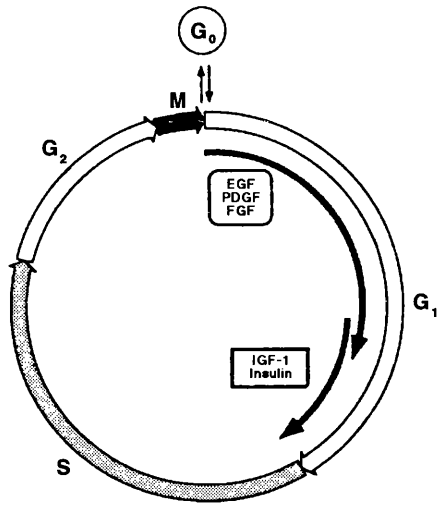


Figure 1 The Cell Division Cycle. **G_0**, quiescent phase; **$G_{1,2}$**, gap phases (normal cell function); **M**, mitosis; **S**, synthesis of DNA prior to mitosis. Adapted from Aaronson[20]

Transition through the G_1 phase requires sustained growth factor stimulation over a period of several hours; if the signal is disrupted for a short period of time, the cell reverts back to G_0. There is also a critical period in the G_1 phase where simultaneous stimulation by both the competence and progression factors is required. Beyond this point of restriction, only the progression factor is required.

In some cell types, the absence of growth factor stimulation causes the rapid onset of apoptosis (programmed cell death).

Almost all of the genetic changes involved in the evolution of the cancer cell affect molecules involved in signal transduction pathways. Many malignant cells have been shown to have uncontrolled expression or mutation of growth factors, their receptors or components of their signal transduction pathways.

In the late 1970s, Collett and Erikson demonstrated that a number of retroviral oncogene products were similar to the protein kinase encoded by the product of v-*src*.[21] Further studies of this oncogene revealed that the protein pp60^{src}, which was encoded by v-*src*, phosphorylated the tyrosine residue of target protein substrates.[22] Purification and sequencing of growth factors and their receptors revealed that the v-*erb*B product (and its equivalent rat viral oncogene, v-*neu*) was a truncated form of the EGF receptor.[23] Subsequently, protein tyrosine kinase (PTK) activity has been shown to be associated with the cytoplasmic domains of several growth factor receptors.[24]

There are now known to be 3 types of growth factor receptor which possess PTK activity.[25]

(1) Those associated with the cytoplasmic domains of the growth factor receptor (e.g. EGF and PDGF).

(2) Those located in the cytoplasm, but often attached to the surface of a membrane (e.g. the products of the *src* and *lck* oncogenes).

(3) Those located in the nucleus, at least one of which is the *abl* oncogene tyrosine kinase.

Further examples of PTKs are given in **Table 1**.

Oncogene	Associated Protein Tyrosine Kinase
src, *yes*, *fgr*, *lck*	Membrane-associated non-receptor PTKs
fps/fes	Non-receptor PTK
abl/bcr-abl	Non-receptor PTK
ros	Membrane-associated receptor-like PTK
*erb*B	Truncated EGF PTK
neu	Membrane-receptor PTK
fms	Mutated CSF-1 receptor tyrosine kinase
met	soluble truncated receptor-like PTK
trk	Mutated NGF receptor PTK
kit	Truncated stem cell receptor PTK
sea	Membrane-associated truncated receptor-like PTK
ret	Truncated receptor-like PTK

Table 1 (taken from reference 25)

1.5.2 Signalling Mechanisms of Growth Factors with Tyrosine Kinase Activity

Although our knowledge of the signal transduction mechanisms which lead to the activation of cytoplasmic and particularly membrane-bound tyrosine kinases is far from complete, we do have a reasonable understanding of the mode of action of the EGF and PDGF receptor kinases.[26-28] In both these growth factor receptors, binding of substrate to its cognate receptor results in dimerisation and *trans*-autophosphorylation in the cytoplasmic domain. The resultant phosphorylated tyrosine induces a conformational change which allows the docking of signalling proteins such as GTPase Activating Protein (GAP), Phosphatidyl inositol 3'-kinase (PI3'-kinase) and phospholipase C_γ (PLC) (all binding by their *src*-homology (SH2) domains), and also the serine/threonine (ser/thr) kinase *raf*-1. Although our understanding of this molecular cascade is incomplete, effects on MAP kinase, S6 kinase, cyclin/cdc kinase and certain transcription factors have been implicated.

Many growth signalling pathways are activated in human tumours, including PDGF which is implicated in a high fraction of sarcomas and glially deprived neoplasms, and TGFα which occurs frequently in carcinomas expressing large amounts of EGF. Members of the EGF family of receptor kinases, especially *erb*B2 and *erb*B3 are most frequently implicated in human cancers, including adenosarcomas of the breast, stomach and ovary. Of particular importance is the observation that overexpression of these receptor kinases is correlated with cell survival.[29]

1.6 Protein Tyrosine Kinases as Targets for Drug Development

Clearly, the high PTK activity found in many cancer cells presents these enzymes and their corresponding genes as promising targets for new therapeutic agents. Recombinant DNA technology has shown that inactivation of kinase activity results in abolition of cellular transformation. It follows, then, that developing drugs which act as antagonists for the growth factor receptors would have the same antiproliferative, prodifferentiating effect.

One obvious problem is that PTKs are involved in a plethora of normal cell functions. It would be essential to develop tyrosine kinase inhibitors which have a high molecular specificity for one or more oncoproteins. Further therapeutic selectivity may also arise from the excessive dependence of certain tumours on

particular signalling pathways. A number of PTKs have been identified as viable targets for inhibition by new anticancer agents.[30]

1.6.1 The EGF Tyrosine Kinase

The EGF receptor (also called HER1) is a 170 kD plasma membrane protein possessing an extracellular ligand binding domain, a single transmembrane region and an intracellular domain possessing PTK activity.[31] Fibroblasts which overexpress the EGF receptor have been shown to be tumourigenic,[32, 33] whilst expression of the related growth factor TGF_{α} in transgenic mice results in higher incidences of liver and breast cancer.[34, 35] A very high percentage of primary tumours of the brain, bladder, thyroid, lung, ovary and oesophagus express significant levels of EGF. Antibodies raised against EGF or TGF_{α} have been shown to inhibit tumour cell growth, implicating the involvement, at least in part, of EGF in the mitogenesis of these cells.[36, 37]

1.6.2 The *erb*B2 Tyrosine Kinase

Also known as HER2, the human *erb*B2 proto-oncogene is a 185 kD protein which has 44% structural affinity with the EGF receptor.[38] It is a homologue of the nitrosourea-induced neuroblastoma rat oncogene *neu*. Breast tissue is particularly susceptible to overexpression of $pp185^{erbB2}$, which induces transformation and tumourigenicity. Antibodies to $pp185^{erbB2}$ inhibit growth of a number of breast and ovarian cell lines.[39] Although structurally similar to the EGF receptor, *erb*B2 does not bind EGF.

1.6.3 The *abl* Tyrosine Kinase

This 145 kD non-receptor tyrosine kinase has been implicated in several leukaemic states since the discovery of the Philadelphia chromosome. It is understood that the *abl* proto-oncogene, which is located on chromosome nine in humans, becomes juxtaposed with the *bcr* gene from chromosome twenty to form two protein products, namely $p185^{bcr/abl}$ and $p210^{bcr/abl}$, which both possess tyrosine-specific kinase activity. The mechanisms of action of these fused proteins is poorly understood, although evidence exists to suggest that *bcr* is a ser/thr kinase which interferes with the regulation of *abl*.[40, 41]

1.6.4 The FGF Tyrosine Kinases

The Fibroblast Growth Factor (FGF) family is a group of related proteins which has been created either as distinct gene products, or as the result of splicing of a number of genes. Several ligands are known to bind to these proteins, which are involved in growth regulation, embryogenesis and angiogenesis. Genes

encoding analogues of FGF have been found in cancers of the stomach, and in Kaposi's sarcoma.[42, 43] Overexpression of FGF results in transformation of cellular phenotype, and FGF has also been shown to stimulate new blood vessel growth in tumours.[44]

1.6.5 The PDGF Tyrosine Kinase

Platelet-Derived Growth Factor (PDGF) is an extremely abundant growth factor, and is a major mitogen for cells involved in connective tissue organs including smooth muscle tissue and fibroblasts. PDGF, which consists of two polypeptide chains labelled A and B, is assembled as two homodimers (PDGF-AA or PDGF-BB) or as a heterodimer (PDGF-AB). There are two PDGF receptors; the first, a 170 kD protein labelled α, binds both the homodimers as well as the heterodimer, whilst the second, β (180 kD), binds only PDGF-BB. Cells transformed by the simian sarcoma virus secrete a protein, p28^{sis}, which is encoded by the v-*sis* viral oncogene. V-*sis* is homologous to PDGF-B, and cellular transformation by v-*sis* requires the presence of PDGF receptors.[45, 46] PDGF-B is ten times more efficient at cellular transformation than PDFG-A, and has been shown to be present in elevated amounts in the plasma or tissue of patients with breast cancer.[47-49]

1.6.6 The pp60$^{c\text{-}src}$ Tyrosine Kinase

The c-*src* proto-oncogene encodes for a 60 kD phosphoprotein which is homologous to v-*src*, a highly transforming oncogene found in the Rous sarcoma virus.[50] Whilst the precise function of *c-src* is not known, it appears to be involved in the processes of mitogenic signal transduction, cell cycle progression and cell adhesion, as well as cytoskeletal activities. Elevated c-*src* activity has been observed in tumours of the breast, colon and bladder.[51, 52, 53] In the case of the former, elevated tyrosine activity has been observed in 100% of all breast cancer cases; at least 70% of this activity is known to be caused by pp60$^{c\text{-}src}$.[54, 55] In transgenic mice carrying a disrupted c-*src* gene, no tumours were observed to develop.[56]

1.6.7 Structure of The Active Site of a Tyrosine Kinase

To date the crystal structure of only one growth factor receptor possessing a tyrosine kinase domain has been reported.[57] The Insulin receptor, refined to a resolution of 2.1 Å, was shown to possess a tyrosine residue (Tyr 1,162) in the active site. It has been hypothesised that binding of ligand moves the kinase domains of the heterotetrameric receptor closer together, resulting in transient cross-phosphorylation of Tyr 1,162 and adjacent tyrosines on the activation

loop. Tyr 1,162 is then probably displaced from the active site, and the resulting change in conformation allows ATP and exogenous substrate to bind.[58] Whilst there is a degree of structural diversity throughout the family of tyrosine kinases, all are known to contain at least one tyrosine in the activation loop at a position corresponding to Tyr 1,162 in the Insulin receptor.

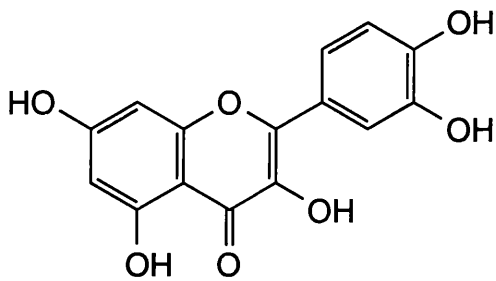
The structure of a catalytic subunit of a ser/thr kinase (which are known to have around 70% structural affinity with tyrosine kinases) has also been solved at 2.7 Å resolution.[59] However, as yet no example of a PTK inhibitor designed using these data has been reported.

Considerable progress has been made in identifying a number of potent PTK inhibitors.[25, 80] A number of these are natural products, which whilst displaying good PTK inhibition are often non-specific towards sub-classes of PTK and often show inhibition of ATP binding. It is in the development of a number of synthetic tyrosine kinase inhibitors that most progress has been made.

1.7 Tyrosine Kinase Inhibitors

1.7.1 Flavonoids

The flavone quercetin **(23)** (a natural product) is capable of growth inhibition in a number of human tumour cell lines.[60-62] Quercetin has been shown to inhibit the tyrosine phosphorylation activity of pp60$^{v\text{-}src}$ both *in vitro* and *in vivo*.[63] It has been shown, however, that quercetin is not a specific PTK inhibitor, but also inhibits other enzymes such as phospholipase A_2, phosphorylase kinase, phosphodiesterases and a number of ser/thr kinases, as well as being competitive with respect to ATP.



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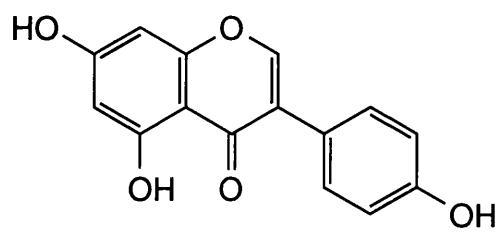


(24)

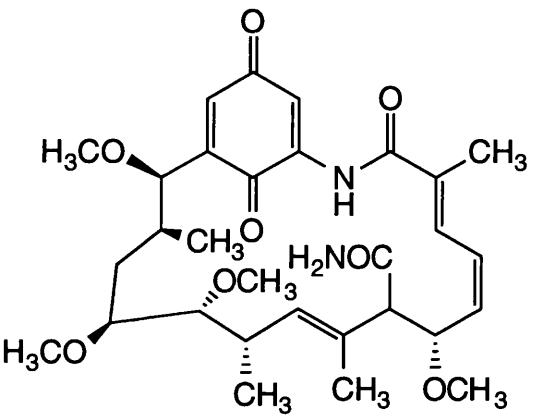
The isoflavone genistein **(24)** is a more potent EGF inhibitor than quercetin at concentrations which have no effect on ser/thr kinases and phosphorylase kinase.[64] Genistein is also a competitive inhibitor of ATP, and inhibits S6

tyrosine phosphorylation[65] and TI I and II.[66] It is unclear whether TI inhibition by this compound involves competition with the ATP binding site.

A number of flavonoid analogues have been reported,[67] although the antiproliferative and antitumour activity of these compounds have not yet been ascertained.

1.7.2 Benzoquinoid Ansamycin Antibiotics

A number of benzoquinoid ansamycin antibiotics, including herbimycin A **(25)**, geldamycin and macbecin have been reported to have moderate antitumour activity.[68, 69] Herbimycin A has also been shown to reverse the transformed morphologies of chicken and mammalian cells which have been transformed by the *yes*, *fps*, *ros*, *abl*, *erb*B and *lck* tyrosine kinase oncogenes, but not by *raf*, *ras* or *myc*.[70, 71] Consequentially, *in vitro* studies have revealed inhibition of $p60^{v\text{-}src}$, $p210^{bcr\text{-}abl}$ and EGF autophosphorylation.



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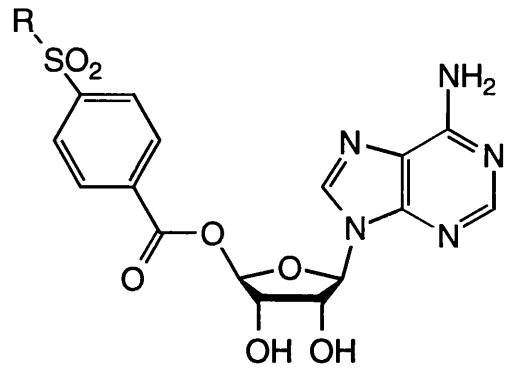
1.7.3 Peptide and Nucleotide Analogues

Analogues corresponding to Tyr419 (the residue phosphorylated by $p60^{v\text{-}src}$) have been synthesised.[72] A 21 non-phosphorylated residue was shown to inhibit both $p60^{v\text{-}src}$ and EGF autophosphorylation, but not ser/thr kinases.[73] Several other analogues have shown effective inhibition of the EGF, Insulin and $p60^{v\text{-}src}$ tyrosine kinases.[74, 75]

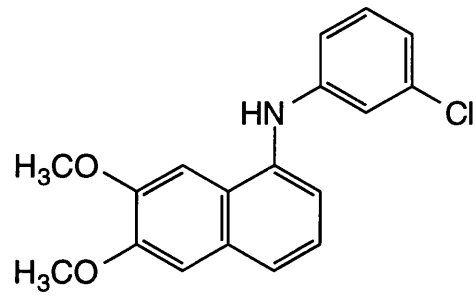


(26) R= -F
(27) R= -NHPh

Nucleotide analogues such as the ATP analogue 5'-[4-(fluorosulfonyl)benzoyl]adenosine (FSBA) **(26)** and Ciberon Blue have been reported as potent PTK inhibitors.[76, 77]

1.7.4 Anilinoquinazolines

Anilinoquinazolines such as compound **(28)** display high potency and selectivity for the EGF receptor.[78-82] Ward *et al.* showed that anilinoquinazolines are competitive with ATP, but their degree of selectivity remained high even in cells with high levels of ATP.[81] Zeneca Pharmaceuticals have synthesised a series of anilinoquinazolines which inhibit growth function at concentrations in the micromolar range (unpublished data).



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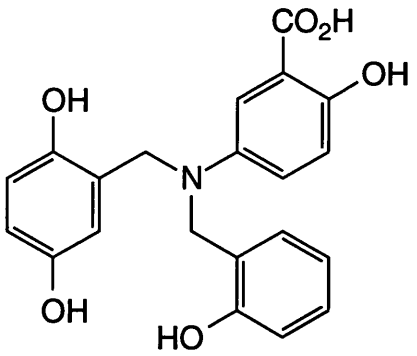
1.7.5 Multisubstrate Tyrosine Kinase Inhibitors

The majority of multisubstrate tyrosine kinase inhibitors possess a tyrosine mimic covalently linked to a triphosphate mimic. It is intended that these compounds will simultaneously inhibit both the tyrosine and ATP binding sites. The most potent compound **(27)** is an analogue of FSBA.

These compounds display limited specificity against various tyrosine kinases, and also inhibit serine-specific phosphorylation of phosphorylase b by phosphorylase kinase.[83, 84]



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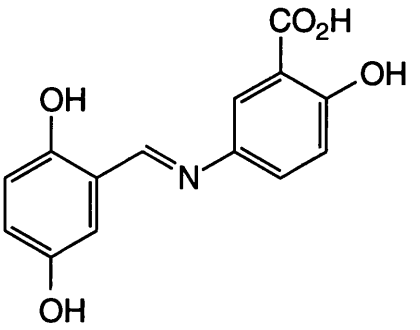

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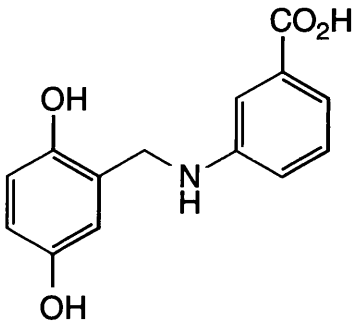
The fermentation product lavendustin A **(29)** displays bisubstrate inhibition at the EGF receptor tyrosine kinase.[85] Analogues of this compound which have had the core pharmacophore trimmed such as **(30)** show similar inhibition of EGF tyrosine kinase, and some have also been shown to distinguish between this and the p56^{lck} receptor kinase. AG814 **(31)**, a lavendustin analogue, is competitive with respect to growth factor and ATP.[86, 87]



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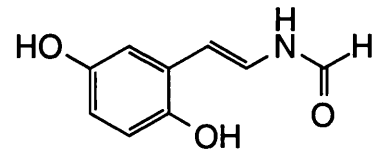
1.7.6 Erbstatin

Erbstatin **(32)** was isolated and characterised by Umezawa *et al.* from the medium of an actinomycete, and was shown to inhibit EGF receptor autophosphorylation in the membranes of A431 epidermoid carcinoma cells, which have a high concentration of the EGF receptor.[88] Although erbstatin displays potent inhibition of a number of tyrosine kinases, it has recently been shown to be competitive both with substrate and ATP.[86] Bishop and co-workers later showed that erbstatin also inhibits ser/thr kinases such as protein kinase C in the same range of concentrations.[89] Other properties include observed inhibition of DNA Topoisomerase I and II.[90] *In vivo* studies in EGF-positive mammary and oesophageal solid tumour xenografts revealed the necessity for feroxymithine (a ferric iron chelator) for antitumour activity. Feroxymithine was shown to suppress greatly the breakdown of erbstatin, which is unstable in serum.[91]



(32)

More stable analogues such as methyl 2,5-dihydroxycinnamate have been reported.[92] This compound inhibits EGF receptor autophosphorylation in intact cells, as well as in a cell-free system with potency equal to erbstatin.

1.7.7 Tyrphostins

The striking similarity in structure between erbstatin and tyrosine led to a series of compounds based on the erbstatin nucleus, which had similar potencies.[93] This class of compounds was defined as *tyrphostins*, or tyrosine phosphorylation inhibitors. Several hundred tyrphostins have been reported in the literature in the last six years.

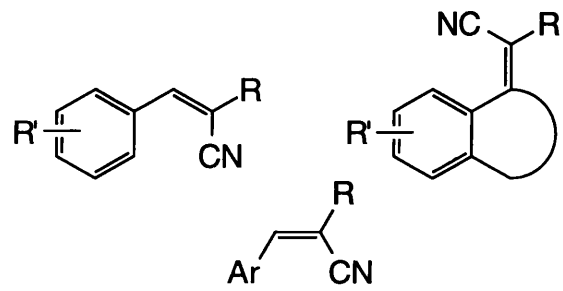
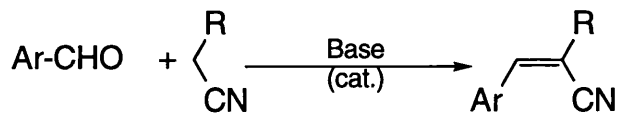


Figure 2

Tyrphostins have the general structure shown **(Figure 2)**, and are normally prepared by Knöevenagel condensation of an aromatic aldehyde with a substituted malonate derivative in the presence of a catalytic amount of base, normally piperidine or pyridine **(Scheme 4)**.[94-96] The aromatic moiety may be phenyl, heteroaromatic or fused heteroaromatic. The group labelled **R** is normally an electron-withdrawing group such a -CN, $-C(NH_2)=C(CN)_2$, $-CO_2R''$ $-CONH_2$, $-CSNH_2$ or a heteroaromatic moiety. One or both of the aromatic or heteroaromatic moieties may also be substituted with functional groups such as hydroxy, methoxy or nitro, or with a halogen atom (labelled **R'**).



Scheme 4

An extensive number of assays are available for the biological evaluation of tyrphostins. Whilst these give detailed insight into the *modus operandi* of these compounds, it makes direct comparison of drugs extremely difficult.

There are two main methods used for the primary screening of tyrosine kinase. The first involves measurement of the antiproliferative effect by *in vitro* exposure of cells expressing a high concentration of tyrosine kinase receptors, commonly for the Epidermal Growth Factor, to the compound. These include MCF-7 human breast adenocarcimona cells, HN5 human squamous carcinoma cells, A431 vulval carcinoma cells, SKOV-3 ovarian cancer cells, HT29 colon cancer cells or HER14 cells, which are a cloned form of NIH3T3 carcinoma cells transfected with the EGF receptor.

The second method gives a more accurate indication as to whether the compounds are inhibiting tyrosine kinase activity. The EGF receptor kinase activity is determined by measuring the EGF-dependant phosphorylation of a synthetic substrate, polyGAT (glutamine-alanine-tyrosine) in the presence of the drug. The system is exposed to ^{32}P-ATP, and the uptake of the labelled ATP is directly proportional to tyrosine kinase activity.

In both groups of assay systems, potency is measured in terms of IC_{50} values, that is the concentration of drug required to inhibit 50% of the function being observed. Where possible the IC_{50} values quoted in this Chapter are for drugs tested in MCF-7 cells or for activity of the drugs at the EGF receptor in a cell-free system.

Further details of the MCF-7 cell and EGF receptor kinase assays are given in Chapters 6 and 7.

Tyrphostins may be considered as falling into two broad classes.

1. 'Classical' Tyrphostins. The first series of tyrphostins were derived from the benzylidenemalononitile (BMN) nucleus. The direction of the amide bond in erbstatin was inverted and dehydrated to the cyano group in BMN. The nitrile group on BMN was reported to increase the biological activity of these compounds.[97] Similar to erbstatin, the presence of hydroxyl groups in the phenyl ring was associated with increased biological activity. In particular, compounds containing two or more hydroxyl groups **(33-35)** showed increased activities **(Figure 3)**.

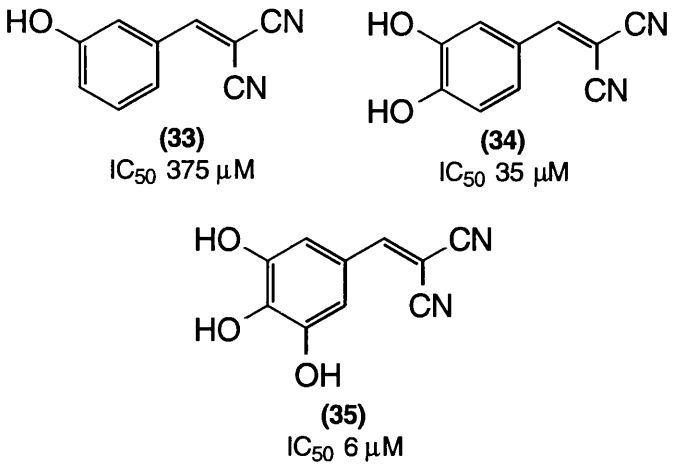


Figure 3 IC_{50} values are for inhibition of phosphorylation of a poly(GAT) substrate (see section 6.2.2 for further details)

Coplanarity of the sidearm with the aromatic ring is extremely important, having a dramatic effect on the biological activity. This was shown when Gazit *et al.* synthesised a series of conformationally constrained tyrphostins.[98] By fixing the dicyanoethylene moiety to a bicyclic structure, it was determined that optimal potency was achieved with tyrphostins having a coplanar catechol ring with the 3-hydroxy and cyano group "syn" **(Figure 4)**.

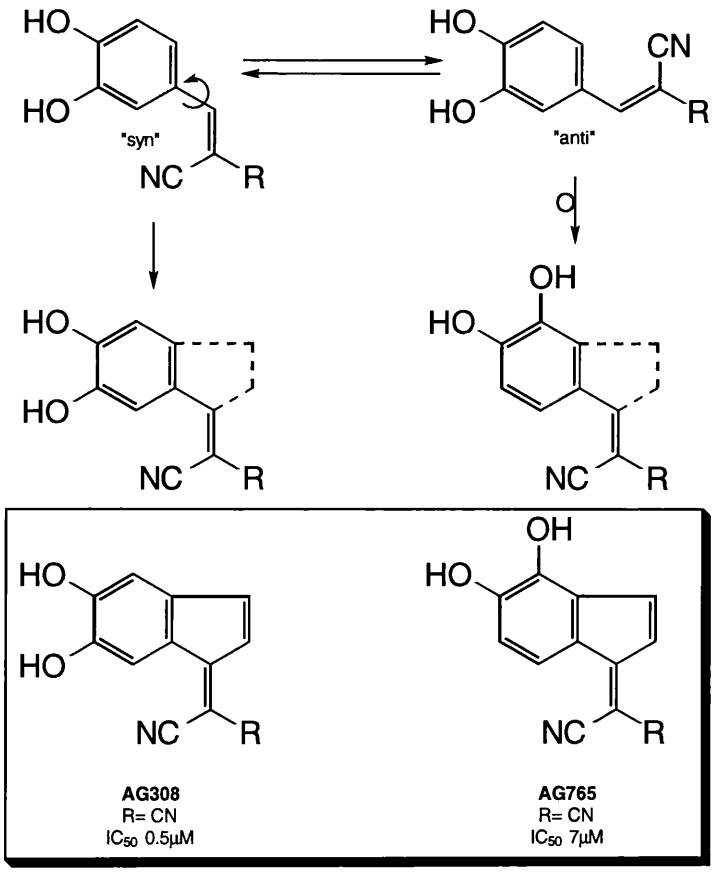
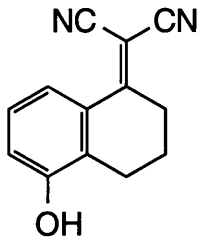


Figure 4

In contrast, when the rigidity of these tyrphostins was decreased by incorporating the dicyanoethylene moiety *exo* to a more flexible 6-membered ring **(36)**, or when the sidearm was saturated **(37)**, the tyrosine kinase inhibitory activity was lost.



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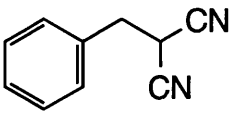

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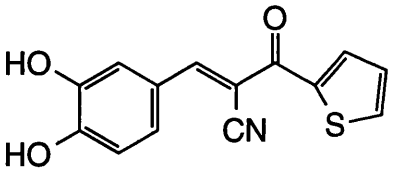


(38)

Tyrphostins containing α-keto and α-amido moieties in the sidearm generally showed good results both in biochemical and biological screens. A number of aromatic ketones were also included in the study, including **(38)** which contains a 2-thienyl moiety, and displayed IC_{50} values of 31 μM and 35 μM in the EGF receptor autophosphorylation and *erb*B2/*neu* autophosphorylation assays respectively.

Tyrphostins containing α-amido moieties also displayed good biological profiles, although no correlation between chain length and activity was apparent. This is in contrast to tyrphostins with ester moieties, where a substantial decrease in activity was observed when the ester chain was lengthened.[93]

In 1993, Gazit *et al.* reported a further series of tyrphostins, which contained a 5-*S*-aryl moiety with the general structure shown in **Figure 5**.[99] Many of these compounds inhibited autophosphorylation of the EGF receptor and *erb*B2/*neu* receptors at least one order of magnitude less than erbstatin. Moreover, these showed greater selectivity between the two receptor types, acting preferentially at *erb*B2/*neu*. The best results were observed for compounds where $\mathbf{R^1}$ = substituted phenyl or benzyl, $\mathbf{R^2}$ = $-CONH_2$ and $\mathbf{R^3}$ = $-CH_3$ or -H. This is in contrast to other BMN tyrphostins, where a marked difference in activity was observed for compounds containing hydroxy as opposed to methoxy residues on the aromatic ring.

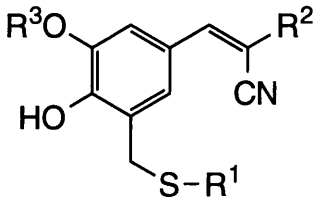
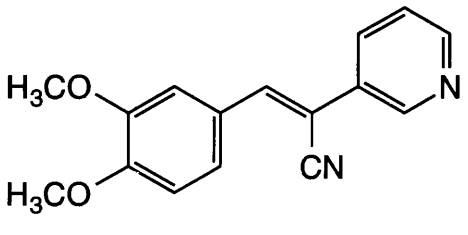


Figure 5

2. Tyrphostins Containing Heteroaromatic Moieties. A few tyrphostins containing heteroaromatic moieties in the sidearm have been reported to inhibit EGF kinase activity. RG13022 **(39)** and RG14620 **(40)** also inhibit the growth of human squamous cell carcinoma (which over-expresses the EGF receptor) when implanted in nude mice.[100]

Subsequent studies in which the BMN nucleus was replaced with indole[101, 102] or quinoline[103] gave a number of compounds which displayed similar biological profiles to the lead compounds. Several tyrphostins containing an isoquinoline ring were also prepared such as **(41)**. These may be considered as cyclic BMNs, and have been shown to inhibit the EGF receptor tyrosine kinase as opposed to p56^{lck} *in vitro*.[104, 105]



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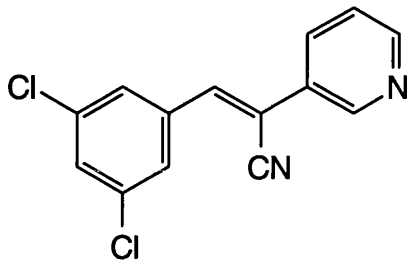

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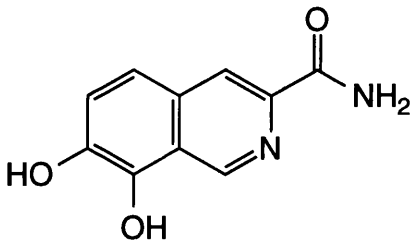
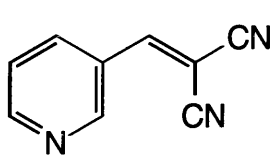
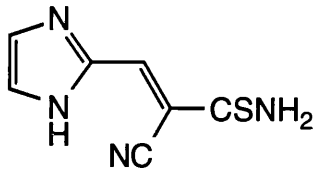


(41)

Robins and co-workers recently reported a series of tyrphostins containing pyridyl **(42)**, imidazyl **(43)** and thienyl **(44)** moieties.[106] These showed moderate activities *in vitro* when tested in MCF-7 human breast carcinoma cells (which over-express the EGF receptor). Whilst these initial results were very promising and contradicted the results reported earlier by Gazit *et al.*, further studies in a cell-free system measuring EGF-dependent phosphorylation indicated that the activity of these compounds was not due to inhibition of the EGF receptor kinase.



(42)



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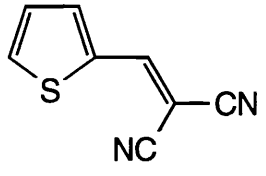
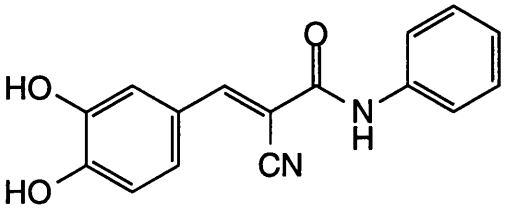


(44)

Tyrphostins were originally proposed to be competitive inhibitors with respect to substrate at the binding site, and non-competitive with respect to ATP. It was subsequently shown that a greater degree of selectivity by some tyrphostins was apparent, particularly in the cases of AG494 **(45)**, which was selective for EGF receptor, and AG825 **(46)**, an *erb*B2/*neu* -selective tyrphostin.[107]

Subsequent and more detailed studies suggested that the mode of inhibition was more complicated.[86, 108] Many tyrphostins are competitive both with substrate *and* ATP.



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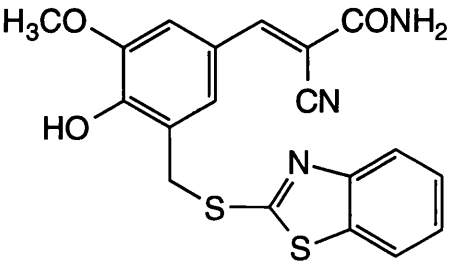


(46)

Several tyrphostins containing *S*-aryl moieties have also been shown to be potent antiproliferative agents.[86] Interestingly, tyrphostins in this series which could discriminate between *erb*B1 and the *erb*B1-2 chimera *in vitro* did not show the same effect when assayed in NIH3T3 cells which expressed either *erb*B1 or *erb*B1-2. A number of possible explanations accounting for this discrepancy included the suggestion that the agents must overcome high intracellular concentrations of ATP, which masked their differential ability. However it is more likely that alternatively, or even additionally, tyrphostins do not act *solely* at the level of *erb*B1 and *erb*B1-2 kinases, but also at a signal transduction element further downstream in the pathway. To date, the nature or location of this other binding site is not known.

It has become increasingly apparent that although many tyrphostins are more selective than genistein, lavendustin A and herbimycin A, each group inhibits several tyrosine kinases to some degree, either competitively with respect to substrate, or ATP, or both. The improvement of selectivity remains a continuing learning process. Of particular interest are tyrphostins containing a heteroaromatic moiety, since synthesis of compounds of this type would allow comparison of the potency of electron-rich and electron-deficient systems, as well as the investigation of 5-membered and fused ring systems. Drugs such as RG13022 **(39)** and RG14620 **(40)** show promising results both *in vitro* and *in vivo* and act as interesting leads for new compounds. The mode of action of the tyrphostins reported by Robins and co-workers may not yet be fully understood, but these compounds are worth further exploitation since few tyrphostins

containing heteroaromatic moieties have been reported in the literature, and they fall outwith existing patents.[104, 109-118]

There are clearly a number of potent tyrphostins currently available, but their limited selectivity and stability may prevent them from ever becoming viable anticancer agents. There have been a number of new developments in the methodology available for identifying cancer cells by their environment and expression of surface antigens, and these are discussed in Chapter 2.

2. New Targets for Cancer Chemotherapy

2.1 Tumour Hypoxia

Cancer cells are identified by their rapid proliferation and lack of differentiation. Since solid tumours grow at a rate which far exceeds that of the surrounding tissue, the intercapillary distance increases and results in areas of the tumour being deprived of essential materials such as oxygen, which is supplied through the vascular structure.[119] Hypoxic cells, therefore, exist at an oxygen concentration which is suboptimal for cell growth and metabolism, but is sufficient to maintain viability. These cells can be around 3 times more resistant to ionising radiation, since molecular oxygen is a potent radiosensitiser. When oxic cells are exposed to radiation from X-ray or γ-ray sources, only a small amount of lethal damage results as a direct interaction between DNA and the radiation source. Secondary damage occurs as a result of the action of high-energy electrons expulsed from atoms through the action of this radiation. These produce a track of short-lived ion pairs, which may react with each other and nearby molecules, producing a wide selection of free radicals and reactive species, which in turn may react with DNA or other important biological molecules. The result of this can be mutations or cell death. Reaction of molecular oxygen (the 'oxygen effect')[120] with these electron-affinic molecules can result in a significant amount of cell damage. Conversely, there is little damage by radiation to hypoxic cells.

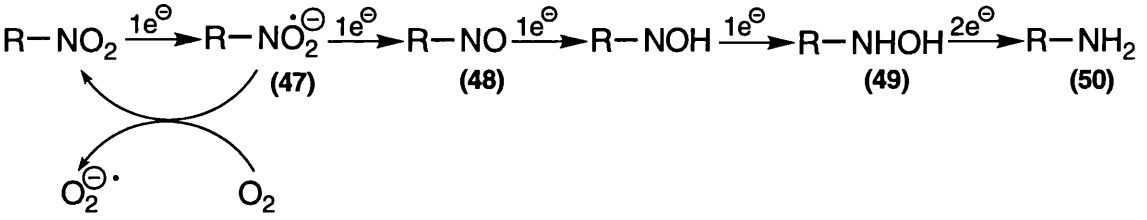
Hypoxic cells also show a reduced response to various chemotherapeutic agents, since they may be too far removed from blood vessels to allow significant concentrations of the drugs to reach the cells. The cells may also be moving much more slowly through the cell cycle, or the cycle may be totally blocked.

In order to overcome this very real problem, methods have been developed in which the tumour is re-oxygenated between treatments of radiotherapy. The

specificity of this method is extremely limited, and a number of other ways to improve oxygen delivery have been explored.[121]

It is in the development of agents which in a sense substitute for oxygen by radiosensitising hypoxic cells that the greatest progress has been made. These compounds, called *electron-affinic radiosensitisers*, take the place of oxygen by accepting an electron from the initially-formed DNA radicals. The drugs can reach the interior of solid tumours because they are less actively metabolised.

An alternative approach is in the development of compounds which display *hypoxic cytotoxicity*, which involves metabolic reduction of a functional group on the molecule to a toxic species in a hypoxic cell. An example of these are nitro compounds, which undergo a series of 1-electron and 2-electron reductions, giving rise to several toxic intermediates **(Scheme 5)**. Initial addition of an electron gives rise to a cytotoxic free radical **(47)**. This can occur *via* radical transfer from radiation-induced $CO_2^{-\bullet}$ radicals, or by enzymic reduction under hypoxic conditions. This $NO_2^{-\bullet}$ radical may be either oxidised back to the initial nitro species, in which case a toxic superoxide radical is formed, or may be further reduced to a nitroso compound **(48)**. Two subsequent 1-electron reductions give rise to a hydroxylamine **(49)**, which is reduced *via* a 2-electron mechanism to an amine **(50)**.

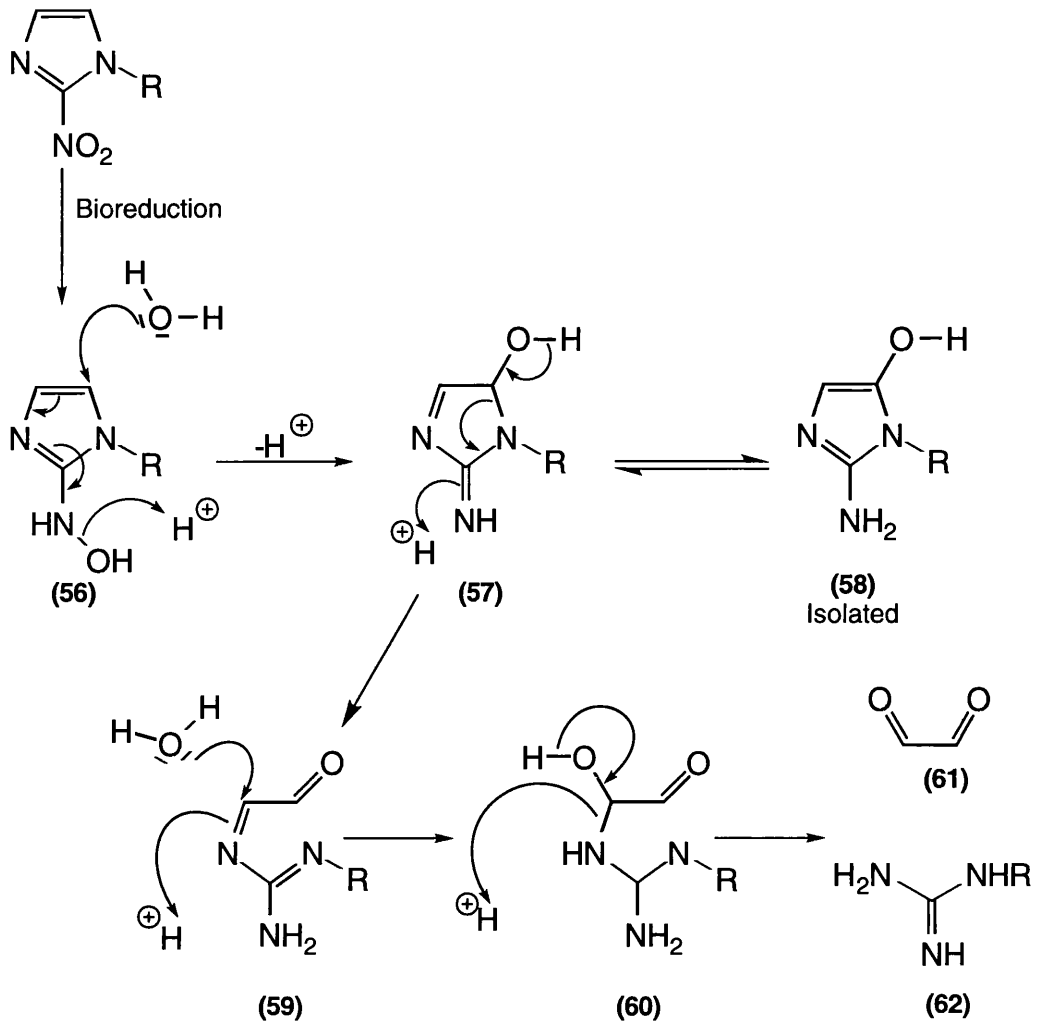


Scheme 5

A prerequisite of electron-affinic radiosensitisers is that at least their first reduction potential (E_7^1) must fall within physiological limits. This represents a value of between -200 and -500 mV *vs.* the Normal Hydrogen Electrode (NHE).

2.1.1 Nitroimidazole Radiosensitisers

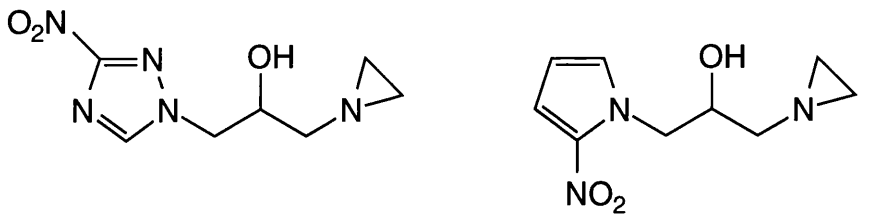
Misonidazole **(51)** has received a great deal of clinical study as an electron-affinic radiosensitiser.[122] Unfortunately due to neurotoxicity it was found to be impossible to administer this compound at a sufficient dose to achieve radiosensitisation of tumours.[123] The second generation of agents to be the subject of clinical trials include etanidazole (SR2058, **(52)**),[124, 125] pimonidazole (Ro 03-8799, **(53)**)[126, 127] and RSU1069 **(54)** [128, 129, 130] which can be considered as a 'dual-function' agent, possessing electrophilic and



Scheme 6

2.1.2 Other Nitroheterocyclic Radiosensitisers

A series of azole compounds containing either one or three nitrogen atoms based upon the nitroimidazole nucleus has been reported.[134] The triazole derivative **(63)** displayed a high degree of selectivity for hypoxic cells, whereas the pyrrole **(64)** showed no specificity. Both groups of compounds displayed similar E_7^1 values to RSU1069, whose first one-electron reduction potential is -389 mV *vs.* NHE.

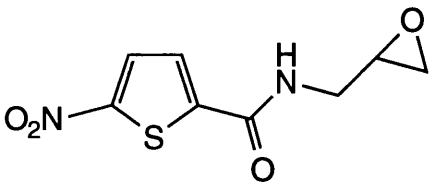


(63) **(64)**

A similar series of 2-nitrofuran-4-carboxamides and 2-nitrofuran-5-carboxamides was shown to have E_7^1 values between -210 to

-350 mV *vs.* NHE.[135] These are much better radiosensitisers than RSU1069, with their degree of sensitisation being dependent upon their reduction potential and structure.

Threadgill *et al.* later showed that a series of 2-nitrothiophene-5-carboxamides and 2-methyl-3-nitrothiophene-5-carboxamides such as **(65)** and **(66)**, had E_7^1 values between -240 and -540mV *vs.* NHE.[136] Once again, these compounds proved to be more potent radiosensitisers than predicted[137, 138] and were toxic with respect to hypoxic V79 cancer cells at concentrations equal to or one order of magnitude lower than misonidazole. However, their general toxicity under aerobic conditions implies that they are unsuitable for use as bioreducible anticancer agents.



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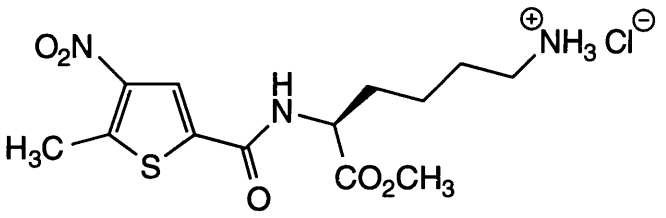
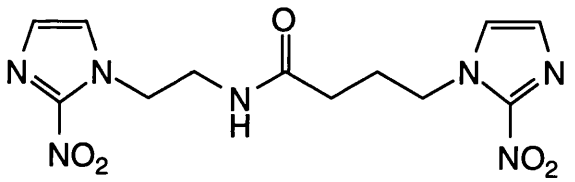


(66)

More recently, Hay *et al.* synthesised a number of bis(nitroimidazoles).[139] These compounds displayed increased solubility and hypoxia selectivity compared to a previously-published bis(imidazole) **(67)**.[140] Symmetrical bis(nitroimidazoles) such as **(68)** were up to nine times more potent than **(67)**, having much more rapid kinetics of cell kill under hypoxic conditions.



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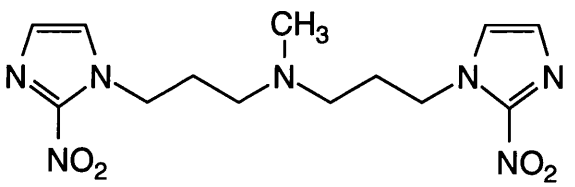


(68)

2.2 Antibody Directed Enzyme Prodrug Therapy

Since the advent of monoclonal technology, attempts have been made to attach anticancer agents to antibodies which have been raised against surface proteins (antigens) unique to cancer cells.[141, 142] Once the antibody-drug conjugate is bound to the cancer cell, the drug or toxin can be released and is taken up by the cell. The earliest methods involved attaching a nitrogen mustard such as *N*-acetylmelphalan to an antibody, but a major drawback of this strategy was that the antibody-drug conjugate failed to gain sufficient tumour access. Another disadvantage is that every molecule of the cytotoxic agent of choice would have to be bound to a separate antibody.

One other approach relies upon the ligation of a radioactive element such as indium 111 or yttrium 90 to an antibody, a process known as radioimmunology.[143] The element would only be released once the antibody was bound to the cancer cell. Whilst the limited success of this method has been reported,[144] it is severely limited by the poor attachment of the antibody conjugate to tumours. Indeed, it has been shown that only between 0.0001 to 0.01% of the administered conjugate is present per gram of tumour.[145] This could be explained by the crude method of attachment of the multidentate ligand to the antibody, a process which involved stirring an anhydride of the ligand with the antibody. The anhydride may have been attacked by the portion of the antibody which recognises the antigen, thus rendering the antibody inactive.

A third approach would be to encapsulate the cytotoxic drug into a liposome, and to attach antibodies to the liposome's external surface. Once bound to the surface of a cancer cell, the immunoliposome is engulfed within endosomes, so releasing its contents into the cancer cell **(Figure 6)**.[146] Studies have confirmed the viability of this approach, with 84% of the immunoliposomes binding to tumour cells within 2 hours.[147]

In order to overcome the problems relating to the stability and delivery of an antibody-enzyme conjugate, it has been proposed that it may be advantageous to separate the two functions.[148] Antibody-directed enzyme prodrug therapy (ADEPT)[149] separates the targeting function from the cytotoxic function by adopting a two-phase approach. An antibody-enzyme conjugate (AEC) is first administered to the patient, and after sufficient time is allowed to optimise localisation of the AEC at the tumour and clearance from the blood and normal tissue (a period of between 3-7 days), the patient is then given a prodrug.[150, 151] Upon exposure of the prodrug to the AEC, the prodrug is converted into a toxic drug close to the cell membrane, which is then taken up by the cell or by a

neighbouring cell (the 'bystander' effect) and so causes cell death (**Figure 7**). A major advantage of this approach is that the AEC may act upon several prodrug molecules, and it is free to bind to another cancer cell after acting upon the prodrug.

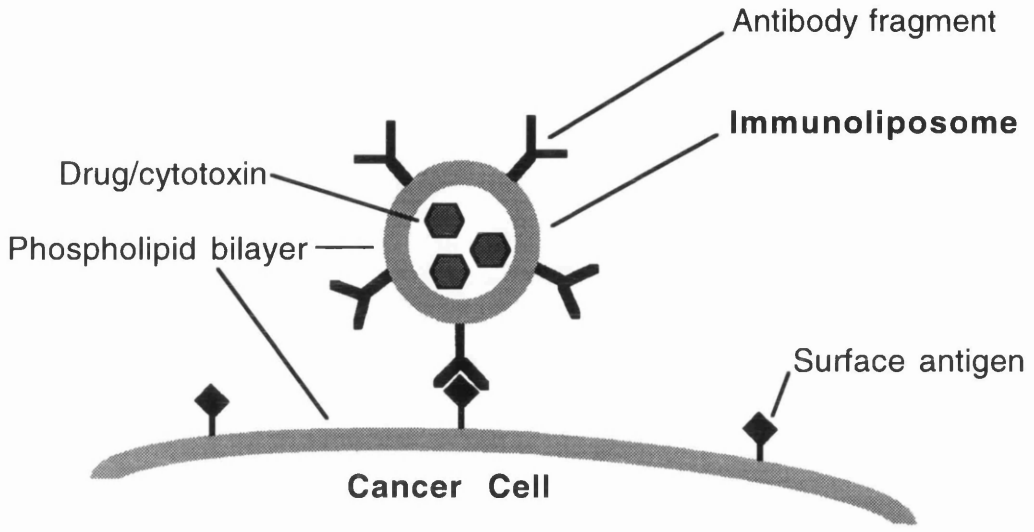


Figure 6

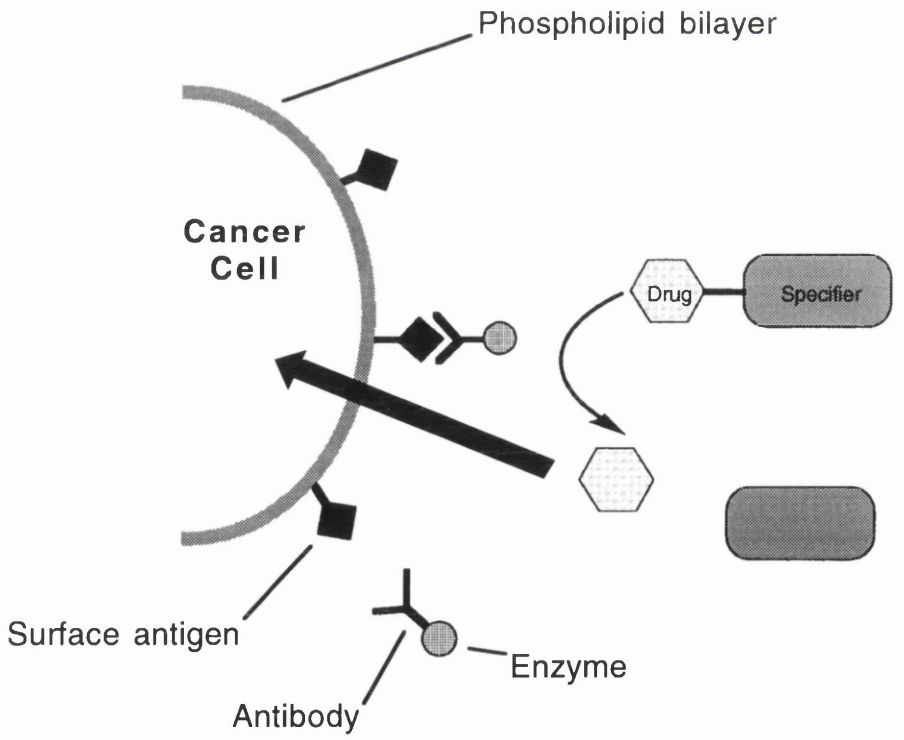


Figure 7

Whilst the nature of the antibody and antigen are outwith the scope of this thesis, it is important to review the possible target prodrugs and enzymes which may act upon them.

2.2.1 Nature of the Enzyme

It would be desirable for the enzyme of choice to be non-mammalian, since this will ensure that lysis of the prodrug does not occur as a result of the action of the host's enzymes. A disadvantage of this approach, however, is that this increases the likelihood of an immune response by the host. The enzyme must also be easily conjugated to the antibody, either by chemical means, or perhaps through the development of genetically engineered antibodies possessing enzymatic domains.[152]

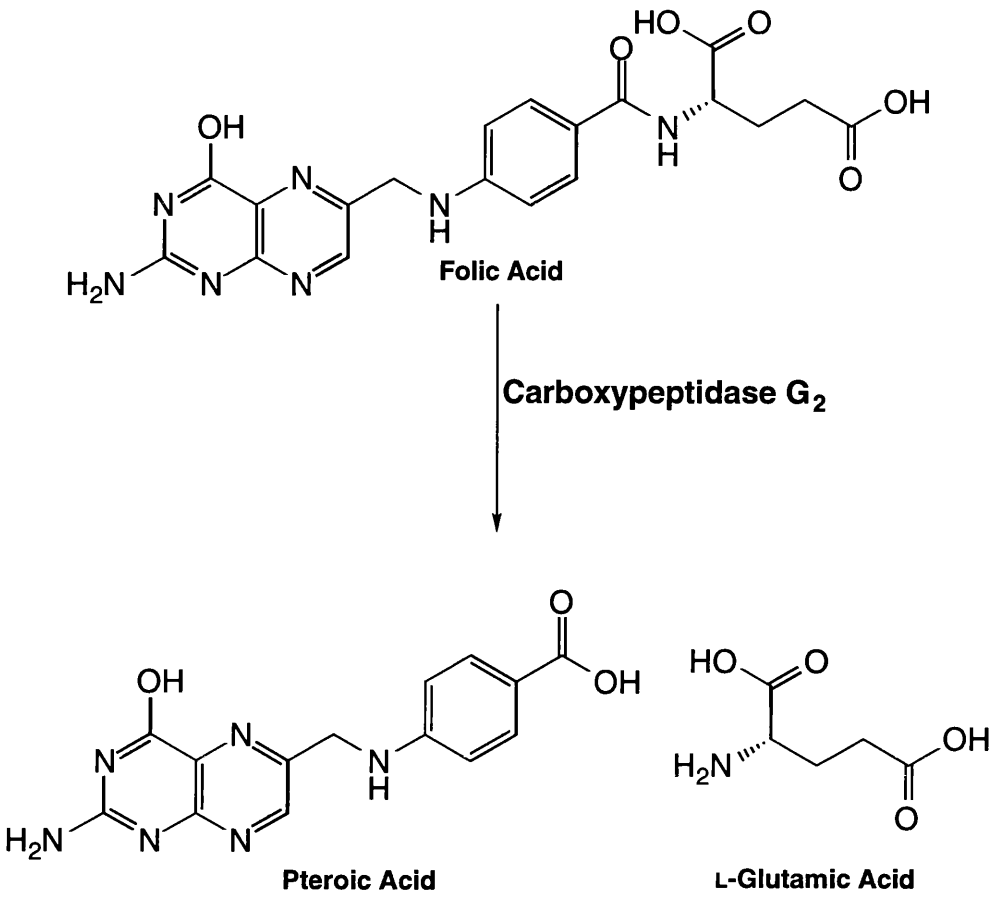
2.2.2 Nature of the Prodrug

Ideally, the prodrug should be a compound possessing high water solubility, low toxicity and a relatively long half-life, and should only be converted into the active form by the action of the AEC. The active drug by comparison would be a small, lipid-soluble molecule which could easily traverse the cell membrane, such as a mustard. Its half-life would be of the order of seconds rather than minutes (but long enough to allow the 'bystander' effect to occur), helping to minimise any toxic effects with respect to healthy tissue surrounding the tumour.

2.2.3 Enzymes and Prodrugs used in ADEPT

There are several enzymes which have been identified as possible targets for action upon ADEPT prodrugs.

(1) Carboxypeptidase G_2. This zinc metalloenzyme, which was isolated from a *Pseudomonas* strain[153] catalyses the hydrolytic cleavage of reduced and non-reduced folates to pteroates and L-glutamates (**Scheme 7**). The enzyme has been linked to a monoclonal antibody raised to human chorionic gonadotrophin.[154, 155] A number of prodrugs which are acted upon by an AEC containing carboxypeptidase G_2 (CPG_2) have been reported.[156-158] Many of these are based on a *para*-*N*-bis(dischloroethyl)aminobenzoyl glutamic acid skeleton which incorporates a nitrogen mustard moiety (**Figure 8**).



Scheme 7

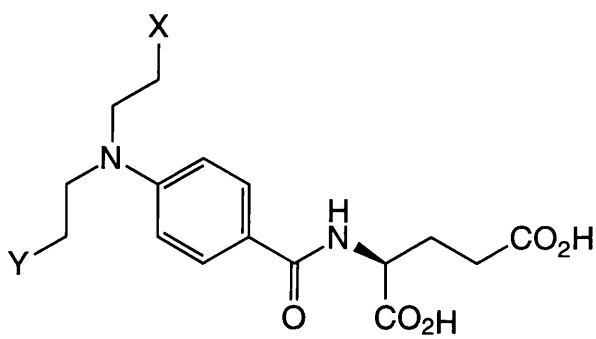
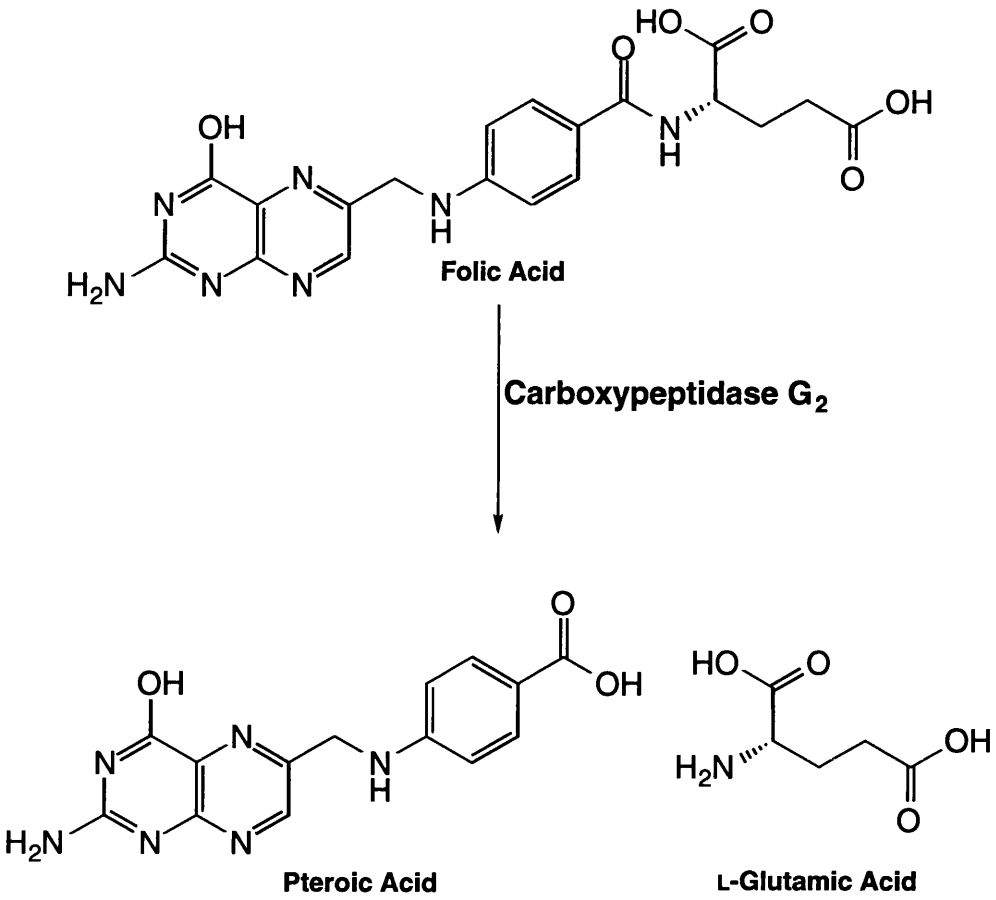


Figure 8: X, Y, -Cl or $-SO_3CH_2CH_3$

These prodrugs fill the criterion of having a longer half-life compared with their equivalent active drugs. By exchanging one of the chloroethyl moieties for a mesyloxyethyl group, which reacts more quickly with DNA, the authors were able to increase the biological activity of the free drug by a factor of 70. In addition, it was shown that incorporating a 2-fluoro substituent in the aromatic ring had a deactivating effect on the alkylating moieties, whereas a 3-substituted fluorine activated the alkylation of DNA, although generally the non-fluorinated drugs showed a stronger binding affinity for CPG_2.[158] This clearly indicates the requirement for non-substituted benzenoid prodrugs by this enzyme.



Scheme 7

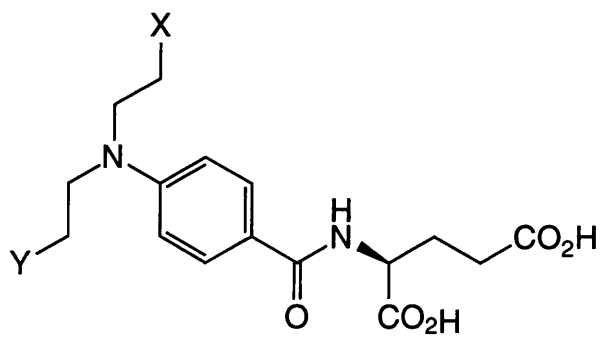


Figure 8: X, Y, -Cl or $-SO_3CH_3$

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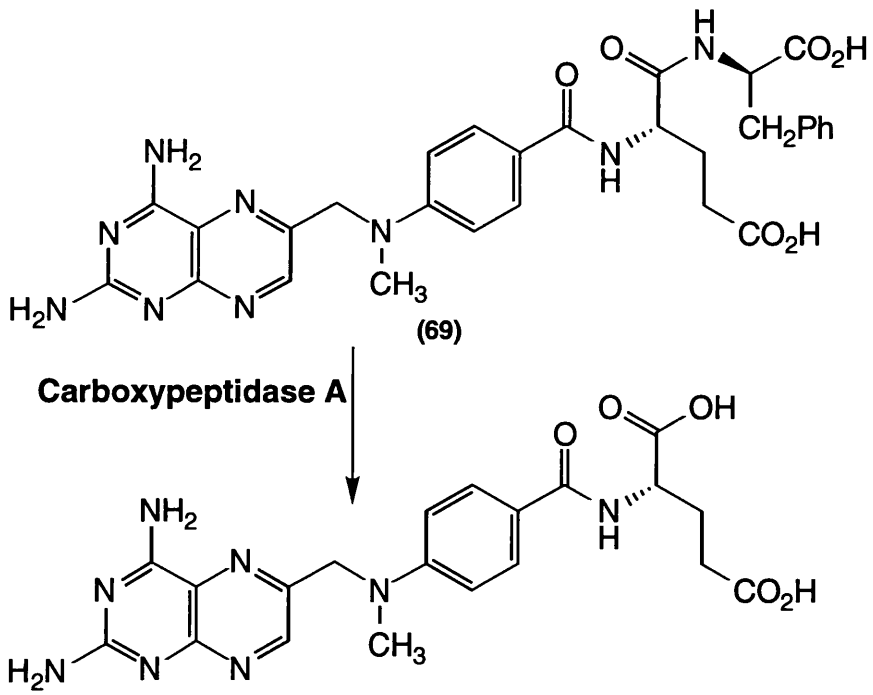
In vitro experiments showed that the prodrugs of this type exert minimal cytotoxicity both in normal and cancerous cells.[156] It is anticipated that this will be reflected *in vivo*, although clinical trials are incomplete.[159]

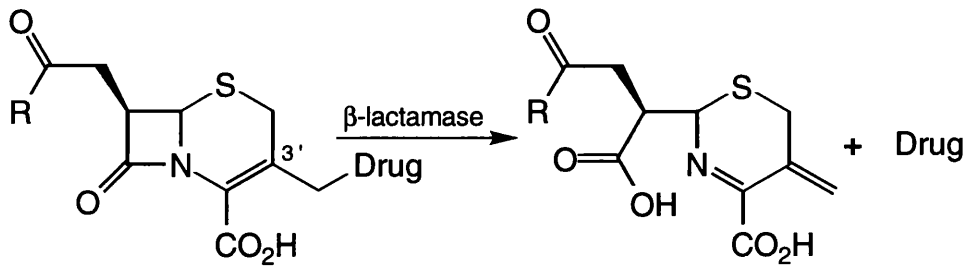
(2) Carboxypeptidase A. This enzyme has been used in the breakdown of a methotrexate-phenylalanine conjugate **(69) (Scheme 8)** by an AEC.[160] By breaking the amide bond between the MTX subunit and the peptide, the AEC generates free MTX **(9)**. *In vitro* studies have shown this conjugate to display similar cytotoxicity with respect to cancer cells as MTX; *in vivo* data have not yet been reported.

However, since MTX is an antimetabolite which is dependent upon the cell being in the S-phase of the cell cycle for its action, the question remains as to the feasibility of using this drug and its analogues in ADEPT. It may be more desirable to choose cytotoxic agents whose activities are independent of the cell cycle.



Scheme 8

(3) β Lactamases This class of enzymes not only hydrolyses penicillins, but also cephalosporins *via* a mechanism which involves expulsion of a 3'-leaving group **(Scheme 9)**. The enzyme is also tolerant of a wide variety of substituents at this position,[161] making it a good candidate for ADEPT.

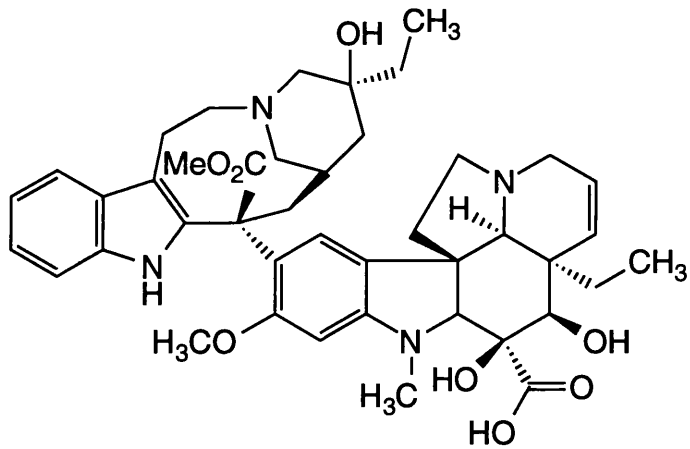


Scheme 9

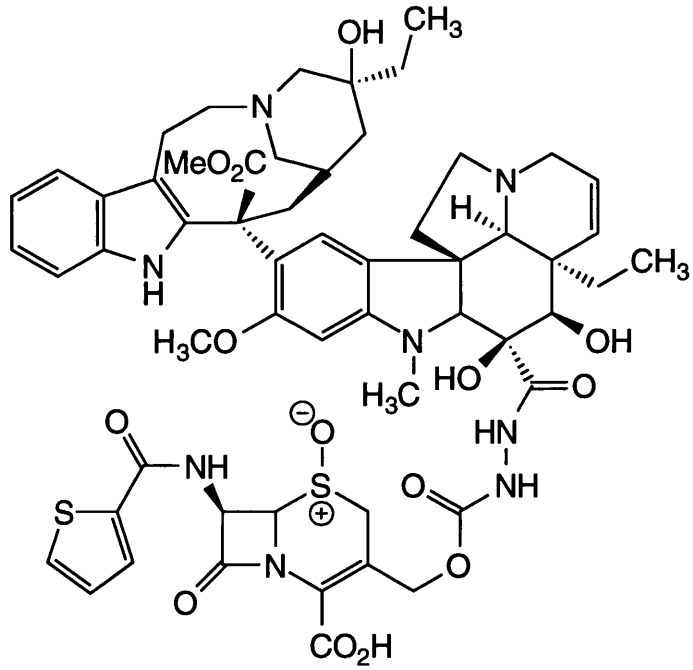
A number of prodrugs of this type containing nitrogen mustards attached *via* a carbamate linkage have been reported.[162] In addition, MTX has been conjugated to the cepham sulfoxide nucleus *via* nucleophilic displacement of the cepham alkyl iodide by the terminal carboxyl group of MTX.[163] The vinblastine analogue, DAVLB **(70)** has been similarly attached to the cepham moiety.[164]

Whilst the MTX prodrug and free drug showed similar toxicities, the DAVLB conjugate **(71)** was five times less active compared to the free drug, and also induced tumour regression in mice xenografts which had been previously exposed to the β-lactamase AEC.[165] This conjugate has also been shown to be a good substrate for the AEC.[166]

(4) β-Glucuronides. These enzymes are present at low concentrations in the blood, but due to their high polarity they have poor cell membrane permeability.

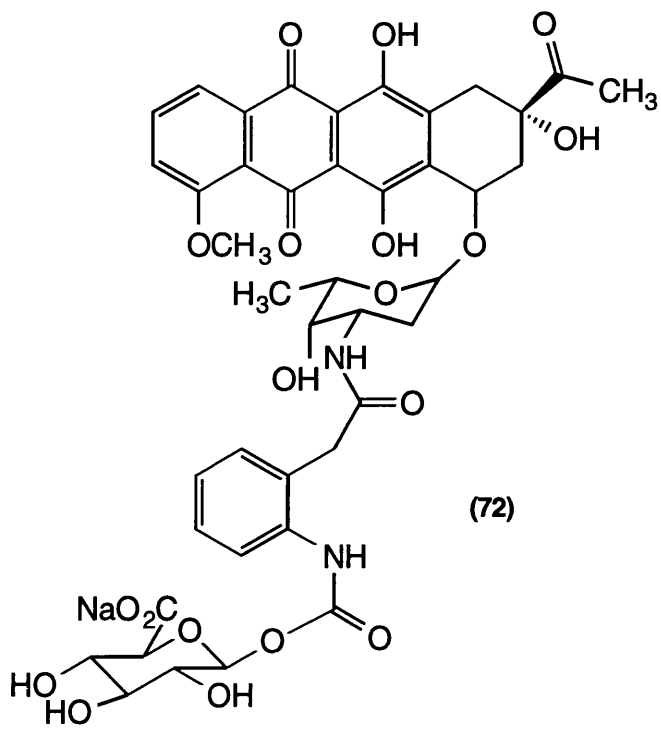


(70)



(71)

A series of prodrugs which are acted upon by a β-glucuronide has been recently reported, an example of which is **(72)**.[167] Of particular interest is the fact that these drugs contain a spacer unit. The reasons for this are two-fold. Firstly, the spacer provides added stability to the prodrug, and it secondly also separates the 'specifier' fragment, i.e. the β-glucuronide, from the drug fragment, in this case daunomycin. This helps to reduce the steric crowding around the specifier, and should, in theory, increase the binding affinity of the prodrug with the AEC.



(72)

2.2.4 Possible New Targets for ADEPT

Carboxypeptidase G_2 is, at present, perhaps the most promising enzyme for new ADEPT chemotherapy treatment. Prodrugs possessing nitrogen mustard moieties have been shown to be less toxic compared to the corresponding free drug, and studies have confirmed the hydrolysis of these compounds by the AEC *in vitro*. Traditionally, the approach has been to attach a 'hit and run' drug, such as a mustard or one which may induce apoptosis, since these do not rely on an extensive exposure time to exert a toxic effect. However, the relative non-specificity of these types of drug remains an area of concern. It would be perhaps more desirable to design a series of prodrugs which would release an extremely specific anticancer agent, such as one which would interfere with a key signalling mechanism in the cancer cell, but would also be sufficiently toxic to display an effect within a relatively short timescale. It is for this reason that tyrosine kinase inhibitors, and tyrphostins in particular, are interesting targets for new ADEPT drugs.

2.3 Conclusions

Cancer, unlike many other diseases, is not peculiar to race, or climate. It is a disease which is universally widespread. The first generation of anticancer agents such as the nitrogen mustards made an enormous impact in reducing the numbers of deaths from this affliction. These drugs, and the current second-generation compounds including the antimetabolites, anthracyclines and microtubule inhibitors are much more specific and potent substances, and their discovery and synthesis remain one of the most important contributions to medical science in the twentieth century.

The almost exponential increase in our understanding of molecular biology in the past twenty years has led to the development of a third generation of cancer treatments. By appreciating how cellular signalling mechanisms function, we have been able to identify new targets such as growth factor receptors which can be acted upon to inhibit the function of abnormal cells. By preventing the phosphorylation of signal transduction proteins, it may be possible to halt completely the proliferation of these cells.

The problem of the specificity of new agents remains. In particular, it is apparent that many current treatments display suboptimal potency at hypoxic centres in solid tumours. The development of electron-affinic radiosensitisers which owe their activity, at least in part, to their ability to become bioreduced

under anaerobic conditions may help to overcome this dilemma. By synthesising new prodrugs for ADEPT, it may be possible to achieve new chemotherapeutic treatments which are so subtle, yet so precise in their targeting, that the enormous side-effects of so many of the current drug treatments may become a thing of the past.

There is clearly an enormous amount of scope for developing new anticancer agents, which will present many new challenges for the medicinal chemist.

2.4 Outline of the Following Chapters

This thesis will concentrate on the development of a number of new compounds based on those discussed in this and the preceding Chapter.

In Chapter 3, a series of tyrphostins containing a thienyl nucleus will be discussed. These were chosen to extend the earlier work of Robins and co-workers,[106] which showed that compounds of this type containing a 5-membered heterocycle performed well *in vitro*. The thiophene ring also contained a nitro group, as it was considered necessary to investigate the biological potential of tyrphostins which might be active under hypoxic conditions. Threadgill and co-workers had already shown that a number of nitrothiophenes have first 1-electron reduction potentials which fall within physiological parameters, and are effective anticancer agents.[136] It was also of interest to investigate the effect of an electron-withdrawing group, attached to the thiophene ring at various positions, on the biological activity of these compounds. The planarity of the sidearm with the aromatic ring was confirmed when the crystal structure of one nitrothienyl tyrphostin was solved.

Chapter 4 considers the synthesis of tyrphostins containing a 6-,7- or 8-substituted quinolyl moiety. Previous work carried out in this department (unpublished) showed that 2 and 4-substituted quinolyl tyrphostins displayed promising *in vitro* activity, whereas 3-substituted quinolines did not. The Chapter concludes with the synthesis of two 8-nitro-2-quinolyl tyrphostins.

In Chapter 5, the identification of a target prodrug containing a tyrphostin for use in ADEPT is discussed. This tripartate molecule would be acted upon by an AEC incorporating the CPG_2 enzyme. Of particular note are the methods used in the unsuccessful attempts to link the L-glutamic acid specifier to the spacer unit *via* a urea linkage.

Chapter 6 details some of the methods used in the biological and biochemical evaluation of the compounds synthesised in Chapters 4 and 5, and draws attention to data which suggest that some of these compounds may not be tyrosine kinase inhibitors, but exhibit their cytotoxicity by causing DNA strand breaks. The Chapter contains electrochemical data for the nitrothiophene and nitroquinoline tyrphostins synthesised, which confirm that some of these compounds possess the requisite reduction potentials to allow bioreduction under hypoxic conditions, although no clear pattern for the nature of these reductions was observed.

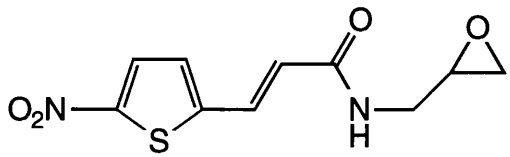
Finally, Chapter 7 gives details of the experimental procedures used in the synthesis of the compounds discussed in Chapters 3 to 5.

3. Synthesis of Nitrothienyl Tyrphostins

3.1 Introduction

Tyrphostins containing a thienyl moiety have been made, and one of those prepared displayed considerable *in vitro* tyrosine kinase inhibition.[106] Whilst a significant number of tyrphostins based on the BMN nucleus have been reported in the literature,[93, 97, 98, 99] only a few containing 5-membered heterocycles have been synthesised.

Nitrothiophenes generally undergo nucleophilic attack at the 2- or 5-position depending on the position of the nitro moiety. The deactivating effect of the nitro group generates electropositive centres at ring positions either *'ortho'* or *'para'* to it.

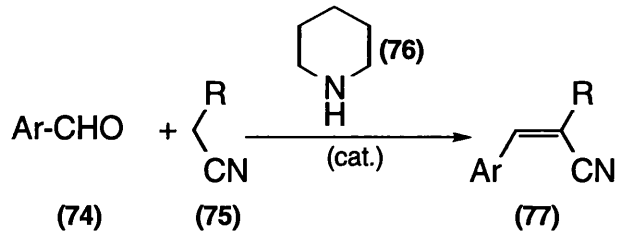


(73)

Threadgill and co-workers have shown that the substitution pattern had little effect on the ability of nitrothiophene radiosensitisers to display potent *in vitro* activity in hypoxic cells.[136] However, substantial differences in the first 1-electron reduction potentials were reported. In 2-substituted 4-nitrothiophenes, this value was around -510 mV *vs.* NHE, whereas in compounds based upon the 5-nitro-2-thiophene nucleus a much more positive value of between -240 and -275 mV was recorded, the lowest values corresponding to compounds in which the sidearm contained a group such as an amide (e.g. **(73)**). These findings have clear implications for the synthesis of other anticancer agents which may be reduced under hypoxic conditions.

3.2 General Route to Tyrphostins- The Knöevenagel Condensation

The majority of tyrphostins which have been reported in the literature were prepared by Knöevenagel Condensation of an aromatic aldehyde **(74)** with a substituted malonate derivative **(75)** in the presence of a catalytic amount of base **(Scheme 10)**. Whilst the nature of the base can vary, it is generally a secondary amine such as piperidine **(76)** or a tertiary amine such as pyridine. Two mechanisms have been proposed, depending upon the nature of the base.



Scheme 10

It is generally accepted that the extra-annular double bond in each tyrphostin has the aromatic and nitrile moieties *cis* (as drawn above) although there is little supporting evidence in the literature. The crystal structure of one thienyl tyrphostin **(100)** was solved (see Section 3.9), and this geometry **(77)** was observed for this compound.

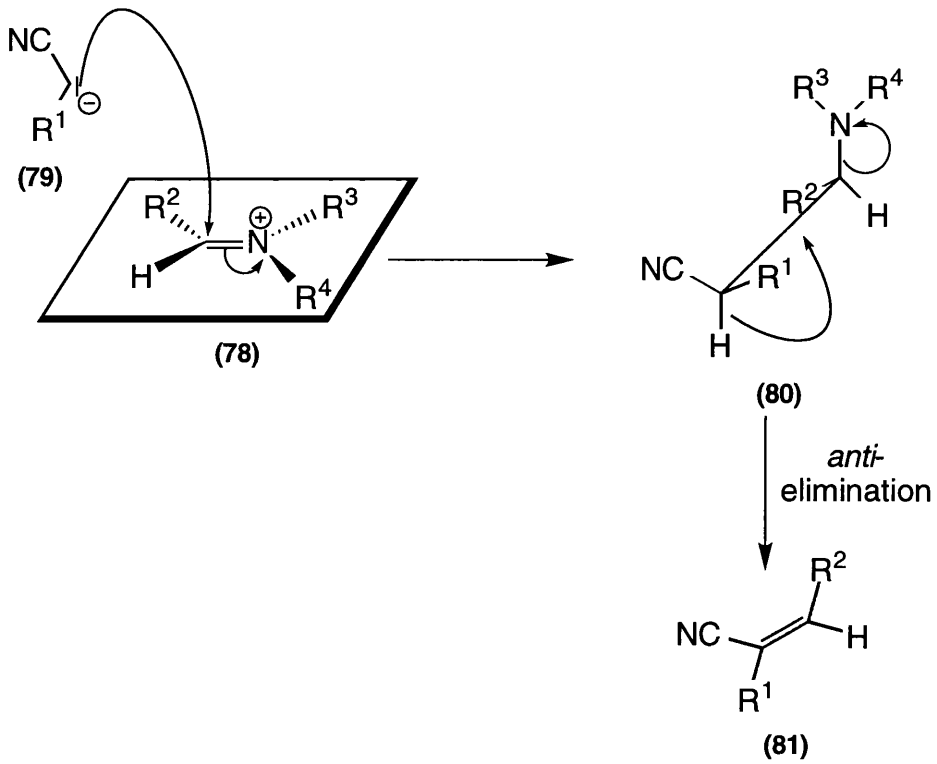
3.2.1 'Classical' Mechanism

Condensation of the aldehyde with a primary or secondary amine gives rise to an iminium salt **(78)**. Nucleophilic attack by a preformed malonate carbanion **(79)** gives rise to an intermediate amine **(80)**, which undergoes *anti*-elimination of HNR_2 to give the Knöevenagel product **(81)** and results in regeneration of the base **(Scheme 11)**.

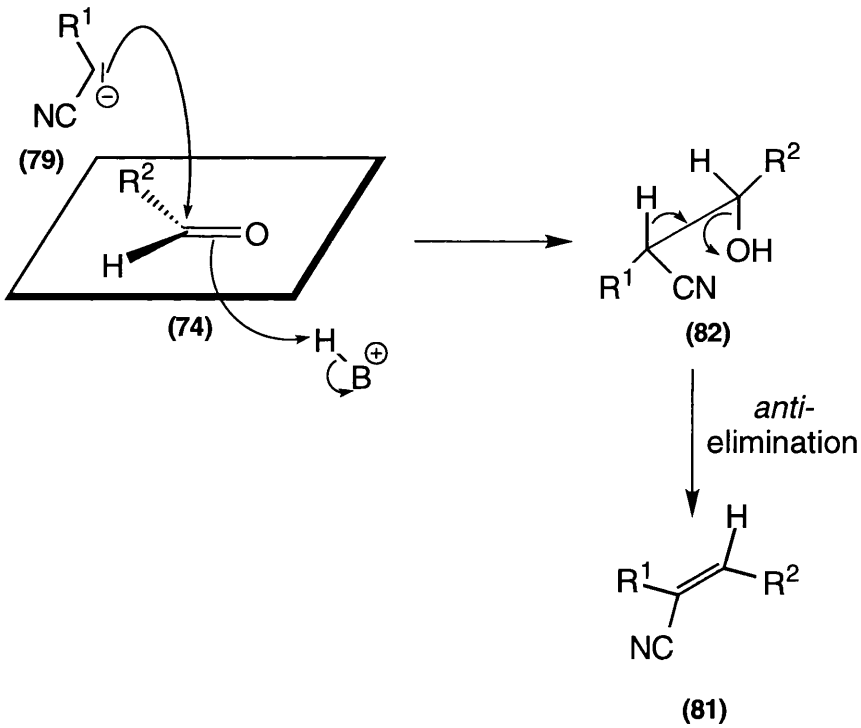
3.2.2 Hann-Lapworth Mechanism

Deprotonation of the malonate derivative by the base gives rise to a carbanion **(79)** which attacks at the electrophilic carbonyl carbon of the aldehyde **(74)**. The β-hydroxycarbonyl compound **(82)** normally spontaneously dehydrates to give the Knöevenagel product **(81)** **(Scheme 12)**. This mechanism hold true when tertiary amines such as pyridines are used, although it is possible to isolate a β-hydroxycarbonyl species when other bases are used.

Many other bases, including the ammonium salts of primary, secondary or tertiary amines, Lewis acids such as $TiCl_4$ and K_2CO_3, or potassium fluoride have been employed as catalysts for this reaction. It is widely held that the reaction follows the Hann-Lapworth mechanism when these catalysts are used.



Scheme 11



Scheme 12

3.3 Synthetic Targets

There are six possible sites for the nitro and aldehyde groups around the thiophene ring **(Figure 9)**. 5-Nitro-2- and 2-nitro-4-thiophenecarbaldehyde **(85** and **84)** are commercially available, whilst 2-nitro-3- and 4-nitro-2-thiophenecarbaldehyde **(83** and **87)** can be easily prepared from readily available starting materials. Extensive literature searches were unable to identify feasible routes to the two other nitrothiophenecarbaldehydes.

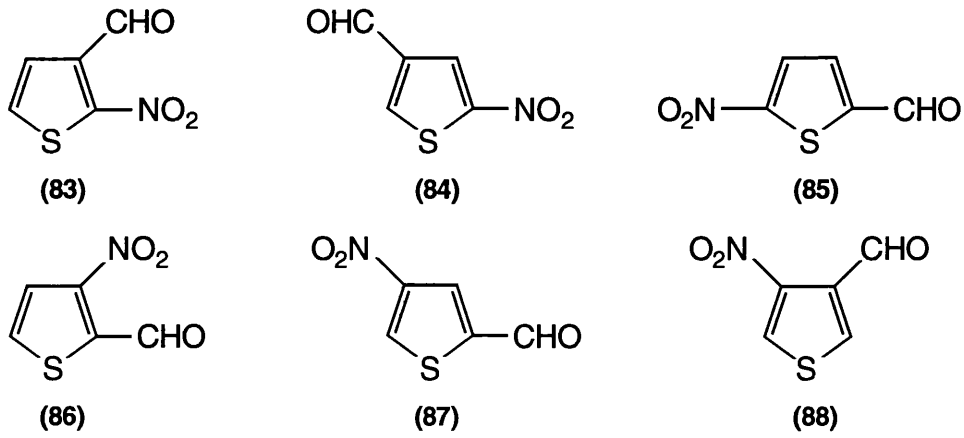


Figure 9

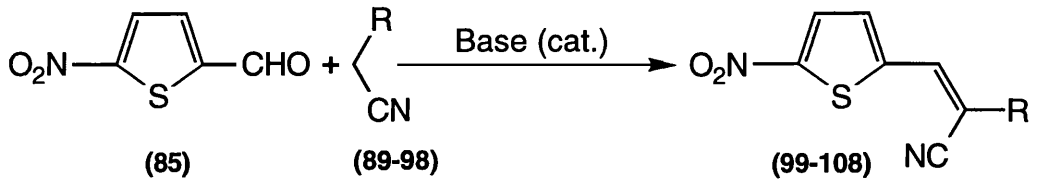
Our initial targets were tyrphostins in which the nitro group and sidearm were *'ortho'* or *'para'*. 2, 5-Substituted nitrothienyl compounds had already been shown to possess 1-electron reductions of around -255 mV *vs.* NHE,[136] and so a series of 5-nitro-2-thienyl tyrphostins was prepared. Compounds containing a 3-nitro-2-thiophene moiety were then prepared.

For comparison, a second group of tyrphostins in which the nitro group and sidearm were *meta* was then prepared. Compounds based upon the 2-nitro-4- and 4-nitro-2-thiophene nucleus are discussed in Sections 3.6 and 3.7.

A general procedure was employed in the synthesis of the tyrphostins reported in this thesis.[93] The aromatic aldehyde was dissolved in a minimal amount of solvent, and to this was added the malonate derivative, either neat in the case of the methyl, ethyl, ^{n}butyl or ^{t}butyl ester derivatives, or as a solution in the same solvent for all the other malonate derivatives. This mixture was stirred for several minutes at room temperature until the product precipitated. If no reaction occurred after about one hour, piperidine or some other base was added until the product formed. Further details are given in Sections 7.2 and 7.3.

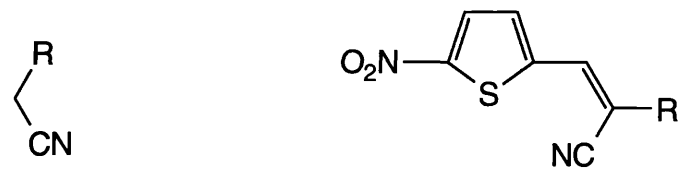
3.4 2-(5-Nitrothienyl) Tyrphostins

A procedure involving reaction of 5-nitro-2-thiophene carbaldehyde **(85)** with a series of malonate derivatives **(89** to **98)** in the presence of a catalytic amount of base gave rise to ten tyrphostins **(Scheme 13** and **Table 2)**. Most of these compounds were isolated as powders which could not be crystallised due to their insolubility in an extensive range of solvent systems. The compounds were characterised by NMR, IR and mass spectrometry, gave acceptable microanalytical data and were submitted for biological evaluation.



(85) **(89-98)** **(99-108)**

Scheme 13



R	No.	Base	No.	Yield (%)
CN	**(89)**	piperidine	**(99)**	38
$C(NH_2)=C(CN)_2$	**(90)**	piperidine	**(100)**	40
CO_2Me	**(91)**	dilute piperidine	**(101)**	94
CO_2Et	**(92)**	piperidine	**(102)**	49
$CO_2{}^nBu$	**(93)**	dilute piperidine	**(103)**	65
$CO_2{}^tBu$	**(94)**	piperidine	**(104)**	61
$CO_2(TEG)OMe$†	**(95)**	piperidine	**(105)**	89
$CONH_2$	**(96)**	piperidine	**(106)**	15
$CSNH_2$	**(97)**	diethylamine	**(107)**	44
2-thienyl	**(98)**	piperidine	**(108)**	15

† Discussed in Section 3.8.3

Table 2

The relatively low yield of compound **(99)** (38 %) may be explained in part by the fact that a spot with R_F 0.71 was observed by TLC, corresponding to an acetal which had formed as a result of the reaction of the starting aldehyde with the solvent (propan-2-ol). The reaction was also carried out at 0 °C after previous attempts at room temperature resulted in polymerisation. The ^1H NMR spectrum consisted of three signals of equal intensity. The most deshielded signal, a broad singlet at δ 8.79 was assigned to the olefinic proton on the sidearm, whilst signals consisting of an AB system at δ 8.23 and 7.89 with a coupling of 4.4 Hz were from the aromatic protons. The more downfield signal of these two corresponds to the proton α to the nitro group. These data were

consistent with previously published values.[168] The ^{13}C NMR spectrum showed two extremely deshielded signals in the aromatic region at δ 155.9 and 139.2, corresponding to the carbons bearing the nitro group and the sidearm respectively. The aromatic C-H carbons appeared at δ 152.5 and 137.9, the more deshielded signal corresponding to the carbon in the 4-position on the thiophene ring. A signal at δ 129.6 for the olefinic C-H and signals at δ 114.9 and 112.9 for the two nitriles were also observed. In the IR spectrum, characteristic signals were observed at 2240 cm^{-1} (nitrile), 1590 cm^{-1} (extended conjugation to the aromatic system) and 3050 and 815 cm^{-1} (aromatic-H). The mass spectrum showed signals at *m/z* 205 and 159, for the molecular ion and for loss of the nitro group. Accurate mass spectrometry and microanalytic data were consistent with the proposed product.

Compound **(100)** was crystallised from 1:1 acetone/chloroform, giving crystals of sufficient size and purity to be submitted for X-ray crystallography (see Section 3.8). The ^{1}H NMR spectrum of **(100)** contained by a broad singlet at δ 9.17, corresponding to the $-NH_2$ protons. The aromatic protons consisted of an AB system at δ 8.23 and δ 7.88, with a coupling of 4.4 Hz. The aromatic signal at δ 7.88 was further split by the olefinic proton at δ 8.41, with a long range coupling of 0.4 Hz. The ^{13}C NMR spectrum included signals at δ 115.5, 115.2 and 114.4 corresponding to three nitrile groups, which were observed as a weak signal at 2222 cm^{-1} in the IR spectrum. Signals at *m/z* 271 and 153 in the mass spectrum were assigned to the molecular ion and loss of the $NC\text{-}C(NH_2)=C(CN)_2$ fragment of the sidearm from the parent molecule.

The tyrphostins containing methyl **(101)**,[168] ethyl **(102)**, *n*butyl **(103)** and *t*butyl **(104)** ester groups in the sidearm were prepared by reaction of the aromatic aldehyde with the corresponding alkyl cyanoacetate **(91-94)**. All products gave good microanalytical and accurate mass spectrometric data. The ^{1}H NMR spectra showed characteristic deshielded signals for the olefinic proton around δ 8.60 and the aromatic protons around δ 8.20-8.00, with signals upfield corresponding to the methyl, ethyl, *n*butyl and *t*butyl protons. The carbonyl carbon was observed as a singlet at around δ 160 in the ^{13}C NMR spectra, with four signals in the aromatic region between δ 155-137. The olefinic carbons came around δ 130 (CH) and at about δ 104. IR spectra included weak signals between 2220-2120 cm^{-1} for the nitrile group, and sharp signals around 1715 cm^{-1} corresponding to an αβ-unsaturated carbonyl. Signals for the molecular ion and loss of the nitro and ester moieties were observed in the mass spectra of these compounds.

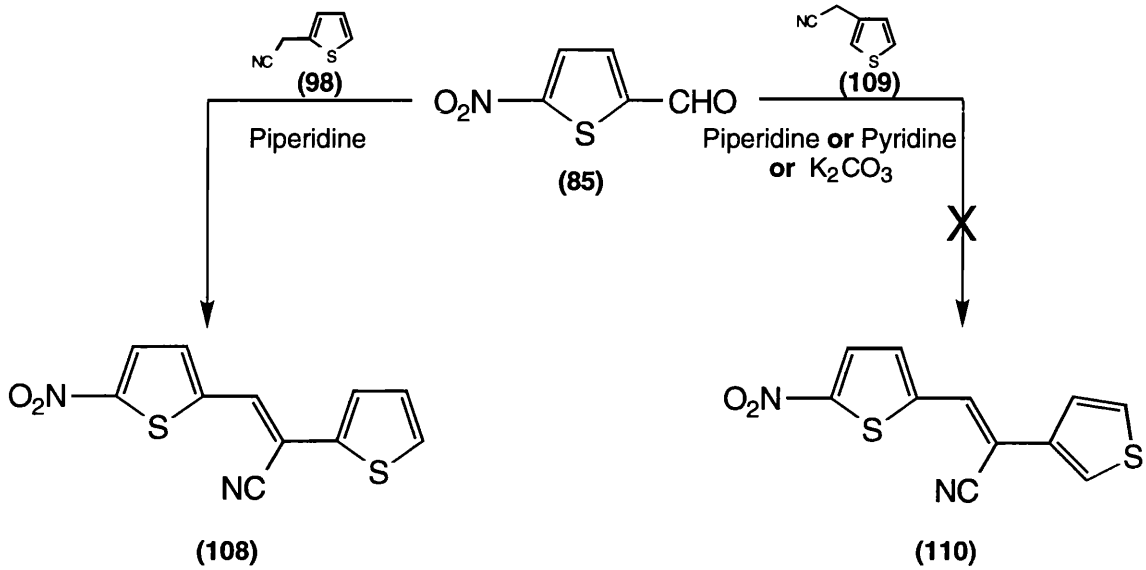
The IR spectrum of 2-cyano-3[5-(2-nitrothienyl)]propenamide **(106)** contained distinguishing signals at 3350 and 3250 cm^{-1}, corresponding to the amide N-H stretch, a weak signal at 2200 cm^{-1} for the nitrile and a sharp signal at 1690 cm^{-1} for an alkene in conjugation to an aromatic species. The most distinctive signals in the ^1H NMR spectrum were two broad singlets at δ 8.03 and δ 7.96. These very deshielded signals were lost after D_2O exchange, and were assigned to the $-NH_2$ protons. The reason for the observation of these two separate line-broadened signals for these chemically equivalent protons arises from the delocalisation of the nitrogen lone pair into the π system, which slows down rotation around the C-N bond. When the sample was heated in the NMR probe, the singlets broadened further and eventually coalesced. The ^{13}C NMR spectrum displayed a signal at δ 161.5 corresponding to the amide carbonyl carbon, and other signals which were consistent with previously published data.[169] Signals for the molecular ion and for loss of the nitro and amide groups from this ion were observed in the mass spectrum.

Compound **(107)** was prepared from 2-cyanothioacetamide **(97)** and 5-nitro-2-thiophenecarbaldehyde using diethylamine as the base since polymerisation of starting materials was observed when piperidine was used. The product was a dark brown solid which was very insoluble and could not be recrystallised. However, microanalysis and accurate mass spectrometry confirmed the purity and the presence of a molecular ion consistent with the molecular formula. Two broad singlets in the ^1H NMR spectrum at δ 7.98 and δ 7.95 correspond to the thioamide protons, whilst a quaternary carbon at δ 162.4 was assigned to the thiocarbonyl carbon in the ^{13}C NMR spectrum. The IR spectrum contained relevant signals for an aromatic system (3145 cm^{-1}), a nitrile group (2190 cm^{-1}), a $C-NO_2$ stretch (1470 cm^{-1}) and a broad peak at 1460 cm^{-1} which was assigned to the thioamide N-H stretch.

Gazit *et al.* had previously reported a benzylidene tyrphostin which contained a 2-substituted thiophene on the sidearm.[93] This had been prepared by the reaction of 2-thiopheneacetonitrile with the appropriate aromatic aldehyde using Knöevenagel methodology. In an attempt to develop this work further to include tyrphostins based on a nitrothienyl nucleus, 2-cyano-3-[5-(2-nitrothienyl)]-1-(2-thienyl)propene **(108)** was synthesised **(Scheme 14)**. 5-Nitro-2-thiophenecarbaldehyde **(85)** was stirred in ethanol in the presence of 2-thiopheneacetonitrile **(98)** and piperidine for eight days; however the yield was very low at only 15 %. When the reaction time was extended, no significant increase in yield was observed, and when the base was changed to pyridine, diethylamine or potassium carbonate, a black tar formed. The ^1H NMR spectrum of the product was dominated by a complex multiplet in the aromatic

region between δ 8.30-7.45 with a relative intensity of five, with a singlet further downfield at δ 8.43 with a relative intensity of one for the olefinic proton. Signals in the ^{13}C NMR spectrum were assigned to the thiophene rings, and the nitrile group came as a singlet at δ 116.4. The IR spectrum showed a very sharp signal at 1615 cm^{-1}, corresponding to a C=C bond in conjugation with an aromatic system, and the mass spectrum showed signals for the molecular ion accompanied by signals at *m/z* 216 and 179 for loss of the nitro and thiophene moieties respectively.

5-Nitro-2-thiophenecarbaldehyde **(85)** was treated with 3-thiopheneacetonitrile **(109)** in the presence of a number of bases **(Scheme 14)**. In each case however, a mixture of up to seven products was observed by TLC and could not be separated by column chromatography.

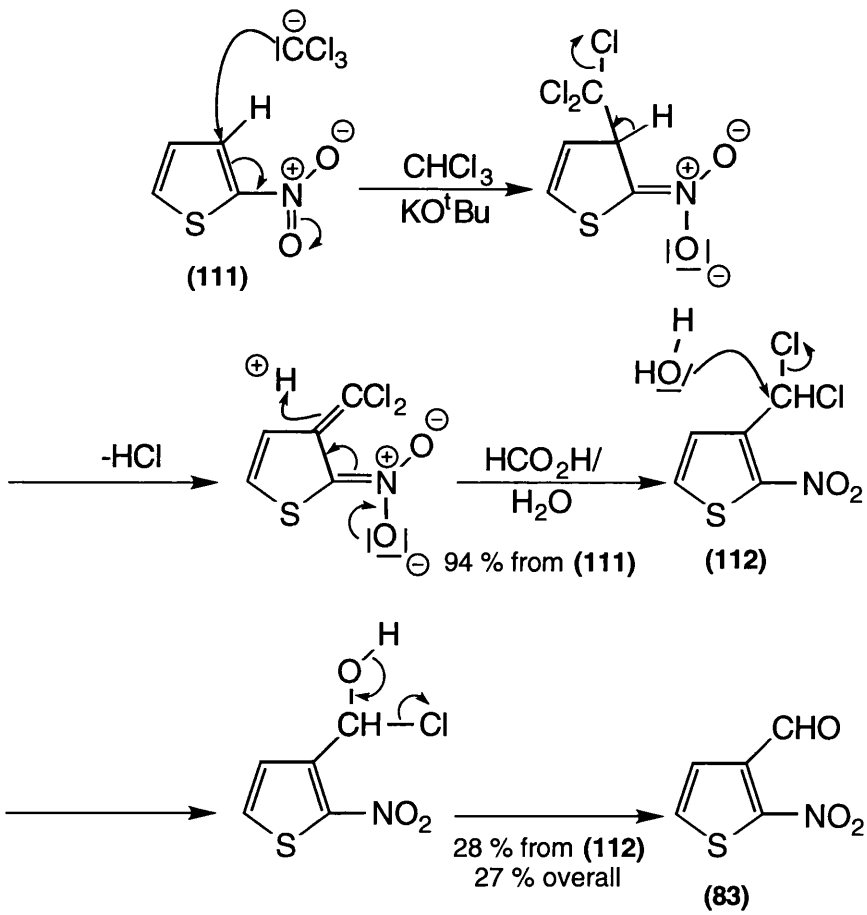


Scheme 14

3.5 3-(2-Nitrothienyl) Tyrphostins

We then turned our attention to the synthesis of a series of nitrothienyl tyrphostins in which the sidearm and nitro group were *'ortho'* to each other. Mąkosza and Owczarozyk had previously reported[170] a method involving the nucleophilic substitution of hydrogen by a dichloromethyl carbanion and the subsequent hydrolysis of the dichloromethyl group to a formyl moiety at the α position in a nitroaromatic system. We treated a solution of 2-nitrothiophene **(111)** and chloroform in THF with a slight excess of potassium ^{t}butoxide. The base generates a trichloromethyl carbanion, which attacks exclusively at the α-position **(Scheme 15)**. Subsequent β-elimination of HCl and rapid quenching

of the reaction by a 1:1 mixture of formic acid and methanol results in restoration of aromaticity and formation of 3-dichloromethyl-2-nitrothiophene **(112)**. Upon neutralisation of excess acid using sodium bicarbonate, the product was extracted into diethyl ether and the solvent was removed under reduced pressure to give a very viscous black tar. This was easily purified by column chromatography over silica gel to give the product. The ^{1}H NMR spectrum of **(112)** showed two doublets at δ 8.04 and 7.70 with a coupling of 5.7 Hz corresponding to the protons in the 5- and 4-positions on the thiophene ring, with a singlet at δ 7.75 for the dichloromethyl group. The ^{13}C NMR spectrum contained five signals, consisting of quaternary carbons at δ 140.9 and 125.0 for the ring carbons attached to the nitro and dichloromethyl moieties respectively, and CH groups at δ 130.0 and δ 128.9 for the aromatic carbons in the 5- and 4-positions. A CH group at δ 63.9 corresponded to the dichloromethyl group.

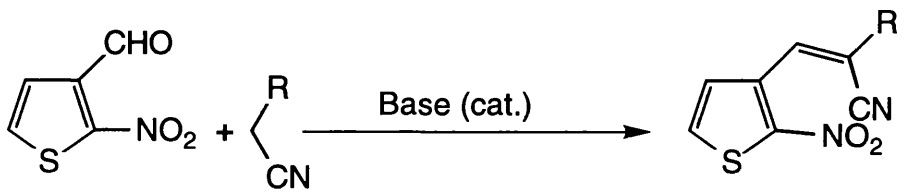


Scheme 15

The next step involved heating **(112)** with aqueous formic acid to reflux for 22 hours. TLC showed the absence of the spot at R_F 0.75 for the starting material which was replaced by a new spot at R_F 0.58. The reaction mixture was allowed to cool to room temperature and the excess acid was neutralised using aqueous sodium bicarbonate solution, and extraction into diethyl ether

using aqueous sodium bicarbonate solution, and extraction into diethyl ether was followed by purification using flash column chromatography over silica to give 2-nitro-3-thiophenecarbaldehyde **(83)** in moderate overall yield. The IR spectrum contained strong signals at 3096 and 3080 cm^{-1} for the aromatic C-H stretch, a sharp signal at 1694 cm^{-1} for an aromatic aldehyde and a signal at 1534 cm^{-1} corresponding to a C-NO_2 stretch. The ^1H NMR spectrum showed a very deshielded aldehydic proton at δ 10.61, and two aromatic doublets at δ 7.95 and 7.78 with a coupling of 5.4 Hz, assigned to the protons on the 5- and 4-positions on the thiophene ring. These values were consistent with those which were reported in the original paper.[170] The ^{13}C NMR spectrum had a CH group at δ 183.9 (-CHO), a quaternary carbon at δ 133.0 for the aromatic carbon attached to the nitro group, another signal at δ 126.0 for the aromatic carbon attached to the aldehyde, and two CH groups at δ 131.0 and 126.7 for the 5- and 4-carbons on the ring. The mass spectrum possessed a minor peak for the molecular ion and a significantly larger signal at *m/z* 111 corresponding to loss of the nitro group from the parent compound.

Compound **(83)** was then treated with a series of malonate derivatives in the usual manner to yield a series of tyrphostins **(Scheme 16** and **Table 3)**.



(83) **(89-97)** **(113-121)**

Scheme 16

R	No.	Base	No.	Yield (%)
CN	**(89)**	-	**(113)**	77
$C(NH_2)=C(CN)_2$	**(90)**	dilute piperidine	**(114)**	75
CO_2Me	**(91)**	piperidine	**(115)**	77
CO_2Et	**(92)**	dilute piperidine	**(116)**	45
$CO_2{}^nBu$	**(93)**	piperidine	**(117)**	33
$CO_2{}^tBu$	**(94)**	piperidine	**(118)**	80
CO_2(TEG)OMe†	**(95)**	dilute piperidine	**(119)**	62
$CONH_2$	**(96)**	piperidine	**(120)**	31
$CSNH_2$	**(97)**	-	**(121)**	85

† Discussed in Section 3.8.3

Table 3

The first tyrphostin to be synthesised containing this nucleus was **(113)**, which was produced from the reaction of 2-nitro-3-thiophenecarbaldehyde **(83)** with malononitrile (89) without the addition of base. The product was isolated as a brown precipitate which was filtered and washed to give the product in 77 % yield.

In the ^{1}H NMR spectrum a singlet at δ 8.72 was assigned to the olefinic proton, and doublets with a coupling of 5.7 Hz at δ 8.22 and 7.76 corresponding to the protons in the 5- and 4-positions on the thiophene ring were observed. The ^{13}C NMR spectrum contained a CH group at δ 152.2 for the olefinic *C*H=C, with the corresponding CH=*C* coming at δ 88.3. In the aromatic region, signals at δ 134.3, 131.6, 131.5 and 125.7 were assigned to the carbons in the 5-, 2-, 3- and 4-positions. The nitrile groups were observed as two quaternary carbons at δ 113.3 and 112.2. IR spectroscopy of the product showed the presence of an aromatic system, with signals at 3038 cm^{-1} (Ar-H), 1580 cm^{-1} (aromatic C=C), and weak signals at 870 and 834 cm^{-1} for two *ortho*-substituted Ar-H stretches. Other signals at 2230 and 1522 cm^{-1} confirmed the presence of a nitrile group and nitro moiety. In the mass spectrum, the molecular ion was observed at *m/z* 205, and a signal at *m/z* 159 corresponded to loss of the nitro group from the parent compound.

The tyrphostin formed from the reaction of 2-nitro-3-thiophenecarbaldehyde **(83)** with malononitrile dimer **(90)**, compound **(114)**, was prepared in the usual manner in the presence of a catalytic amount of piperidine. In the ^{1}H NMR spectrum, a broad singlet at δ 9.27 was seen for the $-NH_2$ protons. The olefinic proton came as a singlet at δ 8.40, and the aromatic protons were doublets at δ 8.23 and 7.78 with a coupling of 5.6 Hz. The ^{13}C NMR spectrum included signals at δ 164.0 (aromatic C-NO_2), and three quaternary carbons for the nitrile groups at δ 115.1, 114.4 and 114.0. In the IR spectrum, a sharp signal at 3052 cm^{-1} and a weak signal at 820 cm^{-1} were assigned to an aromatic C-H stretch, and a sharp signal at 2726 cm^{-1} corresponded to an N-H stretch. Medium bands at 2223 and 2209 cm^{-1} were assigned to nitrile groups, and a sharp signal at 1647 cm^{-1} corresponded to a C=C bond conjugated to an aromatic system. In the mass spectrum, a small signal at *m/z* 270 was observed for the molecular ion, with other signals at *m/z* 245 and 225 corresponding to loss of the cyano and nitro groups from the parent molecule respectively.

Tyrphostins which contained methyl **(115)**, ethyl **(116)**, ^{n}butyl **(117)** and ^{t}butyl **(118)** ester groups in the sidearm were then prepared, using piperidine as the base. Again, the low yield (33 %) of the ^{n}butyl ester may be attributed to the fact

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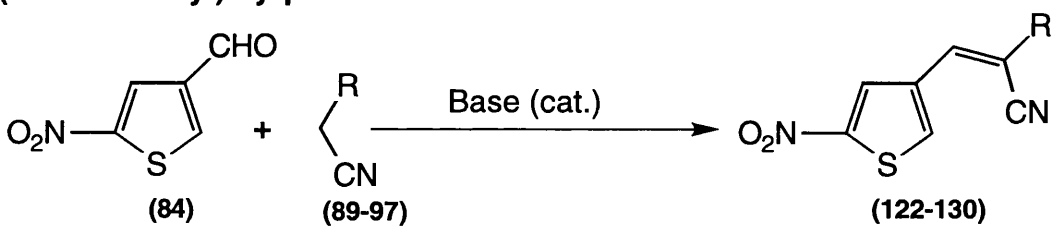
Tyrphostins which contained methyl **(115)**, ethyl **(116)**, ^{n}butyl **(117)** and ^{t}butyl **(118)** ester groups in the sidearm were then prepared, using piperidine as the base. Again, the low yield (33 %) of the ^{n}butyl ester may be attributed to the fact

that ethanol was used as the solvent for this reaction, perhaps resulting in ester exchange with ^{n}butyl cyanoacetate. This was not observed in the case of the ^{t}butyl ester derivative, where propan-2-ol was used as the solvent. In the ^{1}H NMR spectra of these products, signals for the olefinic proton were observed as singlets between δ 9.00-8.72. The aromatic protons came as doublets with couplings of 5.6-5.7 Hz at δ 8.82-7.83. Characteristic signals for the methyl, ethyl, ^{n}butyl and ^{t}butyl moieties were seen further upfield. In the ^{13}C NMR spectra, the ester carbonyls were observed at about δ 162. Two quaternary carbons and two CH groups were observed in the aromatic region between δ 145-126, and olefinic C-H groups were observed at about δ 127. The nitrile groups came at *ca.* δ 115. In the IR spectra, sharp absorptions at around 1720 cm^{-1} were assigned to an αβ-unsaturated carbonyl stretch. Weak signals at *ca.* 2220-2250 cm^{-1} and signals of medium intensity at 1530-1540 cm^{-1} corresponded to a nitrile group and a C-NO_2 stretch respectively. Broad signals of weak to medium intensity for an aromatic C-H stretch came at about 3120 cm^{-1}. The mass spectra of the products contained signals corresponding to the molecular ions, accompanied by other signals for loss of the nitro moiety. All the mass spectra contained a signal at *m/z* 179 for loss of CO_2R from the parent compound.

2-Cyano-3-[3-(2-nitrothienyl)]propenamide **(120)** was then prepared in the usual manner over an extended time of 50 hours until TLC showed the presence of a spot at R_F 0.56 and the absence of unreacted aldehyde. After filtration, the product was isolated as a brown precipitate which could not be crystallised but was analytically pure for biological evaluation. In the ^{1}H NMR spectrum, a singlet at δ 8.20 was assigned to the olefinic proton, with doublets at δ 8.19 and 7.79 for the aromatic protons, with a coupling of 5.6 Hz. The NH_2 protons came as a broad singlet at δ 8.08. In the ^{13}C NMR spectrum, a quaternary carbon at δ 161.3 for the carbonyl carbon was accompanied by two CH groups and two quaternary carbons in the aromatic region at δ 141.9, 139.5, 133.8 and 131.7 were assigned to the ring carbons in the 5-, 2-, 4- and 3-positions. The olefinic carbons came as a CH group and a quaternary carbon at δ 127.7 and 112.3. The nitrile group came at δ 115.3. The IR spectrum contained characteristic signals at 3077, 1495 and 824 cm^{-1} for aromatic C-H and C=C stretches, with the lower signal corresponding to two *ortho*-substituted aromatic C-H stretches. Amide N-H stretches were observed as sharp absorptions at 3455 and 3380 cm^{-1}, accompanied by a weak amide N-H stretch at 1516 cm^{-1}, and a weak absorption at 2200 cm^{-1} for the nitrile group. In the mass spectrum a signal at *m/z* 223 for the molecular ion was accompanied by signals at *m/z* 179 and 177 for loss if the -$CONH_2$ and nitro moieties from the parent molecule.

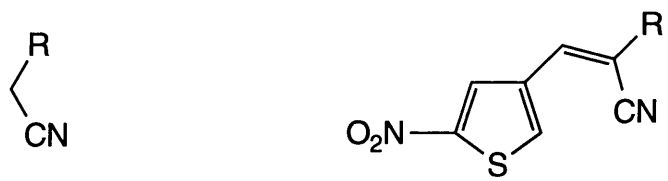
Finally, 2-cyano-3-[2-nitrothienyl)]propenethioamide **(121)** was prepared from 2-cyanothioacetamide and the appropriate nitrothiophenecarbaldehyde without the need for base. In the ^1H NMR spectrum, two broad singlets at δ 9.84 and 9.55 were assigned to the $-NH_2$ protons, with the olefinic proton coming at δ 7.73. The aromatic protons in the 5- and 4-positions were seen as doublets with a coupling of 5.6 Hz at δ 8.05 and δ 7.62 respectively. The ^{13}C NMR spectrum showed a characteristically deshielded quaternary signal at δ 168.2 for the thiocarbonyl carbon, and the aromatic carbons were seen as two quaternary signals and two CH groups between δ 143.1 and 134.2. A CH group at δ 128.4 corresponded to the olefinic carbon β to the thiocarbonyl, with the α olefinic carbon at δ 111.8. The nitrile group came at δ 116.1. In the IR spectrum, a sharp signal at 3096 cm^{-1} and a weak signal at 818 cm^{-1} were assigned to aromatic C-H stretches; the nitrile group showed a medium absorption at 2188 cm^{-1}. A sharp absorption at 1625 cm^{-1} resulted from a C=C conjugated to an aromatic system. A thioamide N-H stretch came as a sharp signal at 1459 cm^{-1}, and a sharp signal corresponding to a $C-NO_2$ stretch was observed at 1507 cm^{-1}. In the mass spectrum, the molecular ion was observed at *m/z* 239.

3.6 4-(2-Nitrothienyl) Tyrphostins



(84) **(89-97)** **(122-130)**

Scheme 17



R	No.	Base	No.	Yield (%)
CN	**(89)**	-	**(122)**	63
$C(NH_2)=C(CN)_2$	**(90)**	piperidine	**(123)**	75
CO_2Me	**(91)**	dilute piperidine	**(124)**	86
CO_2Et	**(92)**	dilute piperidine	**(125)**	95
$CO_2{}^nBu$	**(93)**	dilute piperidine	**(126)**	57
$CO_2{}^tBu$	**(94)**	piperidine	**(127)**	62
$CO_2(TEG)OMe$†	**(95)**	piperidine	**(128)**	11
$CONH_2$	**(96)**	-	**(129)**	69
$CSNH_2$	**(97)**	-	**(130)**	62

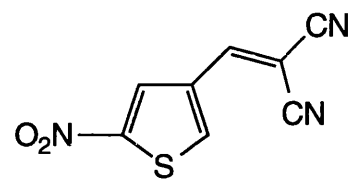
† Discussed in Section 3.8.3

Table 4

These compounds were prepared in the usual manner outlined in Section 3.2. A total of nine compounds were made **(Scheme 17** and **Table 4)**.

The ^{1}H NMR spectrum of compound **(122)** (detail shown in **Figure 10**) showed three deshielded doublets of doublets. The furthest downfield signal at δ 8.76-8.74 corresponds to the olefinic proton on the sidearm, and signals at δ 8.48-8.44 were assigned to the aromatic protons in the 3- and 5-positions on the thiophene ring. The olefinic methine carbon was observed in the ^{13}C NMR spectrum at δ 153.4 **(Figure 11)**. Aromatic singlets at δ 152.8 and 132.0 were accompanied by CH groups at δ 143.5 and 127.5. The nitrile carbons came at δ 113.6 and 112.9, with the quaternary olefinic carbon at δ 82.0. This compound has been reported as an agent which could be used in the treatment of diabetic complications such as retinopathy, nephropathy and neuropathy,[116, 118] although no analytical data were given.

In the IR spectrum, signals were observed for an Ar-H stretch at 3103 cm^{-1}, a nitrile group (2233 cm^{-1}), a sharp signal at 1597 cm^{-1} for a diene conjugated to an aromatic system and a sharp signal at 819 cm^{-1} for an out-of-plane bending by an isolated aromatic C-H. The signal for the molecular ion at *m/z* 205 was accompanied by one for loss of the nitro group from the parent compound at *m/z* 159.



(122)

Figure 10

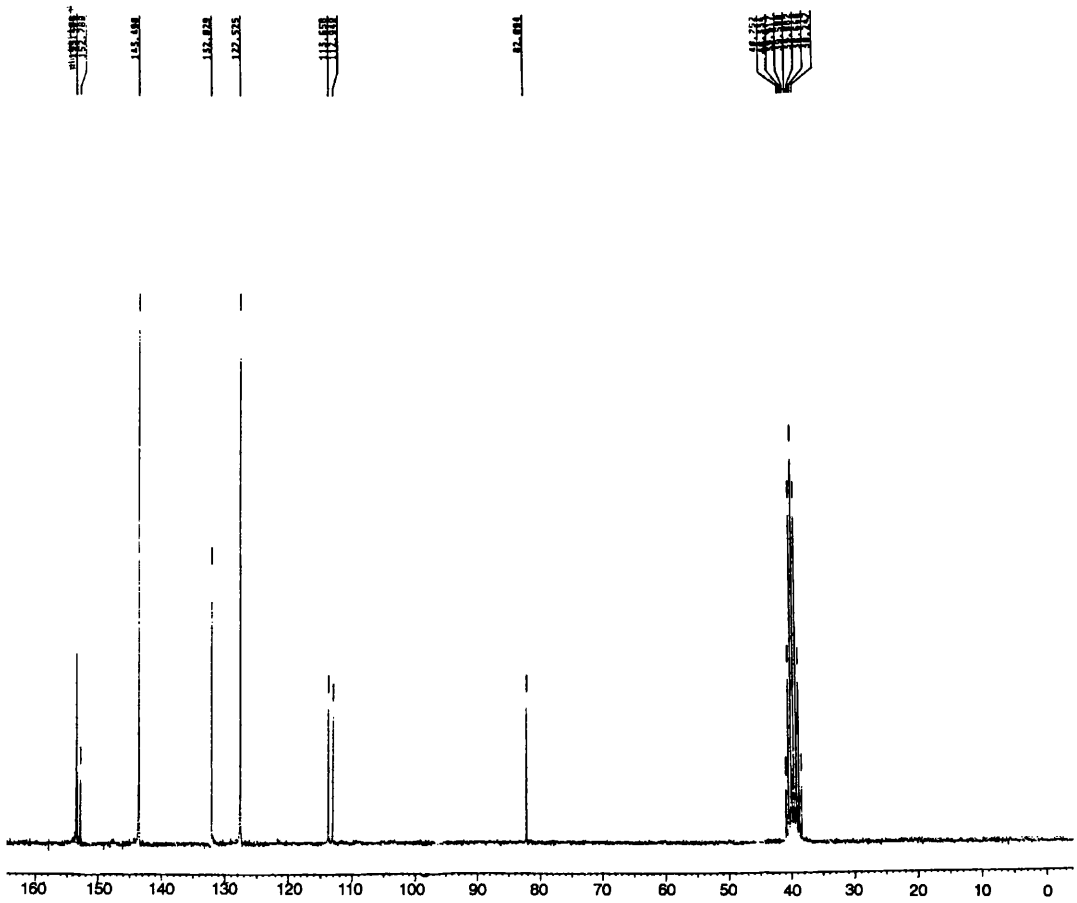


Figure 11

Compound **(123)** was isolated in 75 % yield as large brown crystals after an extended reaction time of eight days. The ^{1}H NMR spectrum displayed a broad signal at δ 9.10 for the $-NH_2$ protons, whilst the aromatic protons were seen as doublets with a coupling of 2.3 Hz at δ 8.70 and 8.58. The olefinic proton came as a singlet at δ 8.15. Quaternary carbons at δ 132.4, 102.6 and 50.1 were assigned to the quaternary olefinic carbons in the ^{13}C NMR spectrum, and three signals corresponding to the nitrile groups were observed at δ 115.4, 114.7 and 114.6. A signal at 1636 cm^{-1} in the IR spectrum showed the presence of extended conjugation to an aromatic system, and the molecular ion was observed at *m/z* 271 in the mass spectrum.

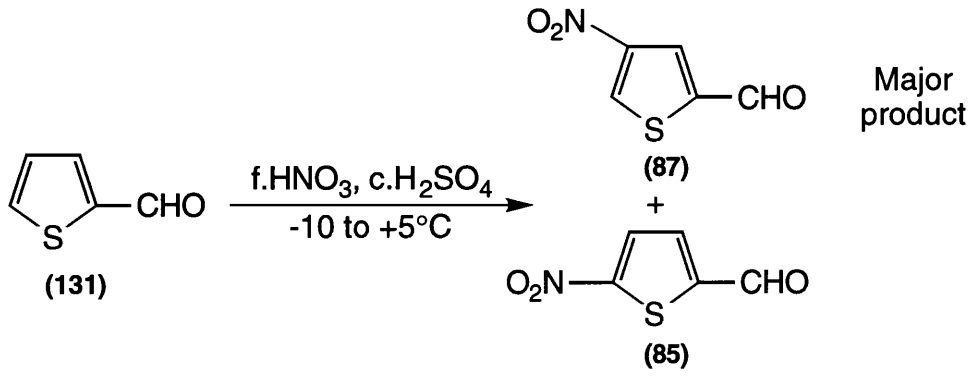
Syntheses of the tyrphostins which contained either methyl **(124)**, ethyl **(125)**, ^{n}butyl **(126)** or ^{t}butyl **(127)** ester groups then followed. Whilst the methyl and ethyl esters were isolated in >85 % yields, the reactions to produce the two butyl esters only gave yields of around 60 %, which may be a result of transesterification of the starting materials (the cyanoacetates) with the solvents used for these reactions (propan-2-ol and ethanol). This was incidental since purity of the products (confirmed by microanalysis) and not efficiency of their synthesis was considered important. The ^{1}H and ^{13}C NMR spectra showed characteristic upfield signals corresponding to the alkyl portions of the esters

accompanied by signals in the aromatic region. In all cases no signal was observed for the aldehydic group. In the IR spectra, signals corresponding to αβ-unsaturated carbonyls, extended conjugation to an aromatic system and isolated aryl-H stretches were observed at about 1720, 1610 and 860-820 cm^{-1}. The molecular ions were observed in the mass spectra, and the methyl and ethyl derivatives possessed a signal at *m/z* 179 corresponding to the loss of CO_2R.

2-Cyano-3-[4-(2-nitrothienyl)]propenamide **(129)** was quickly prepared and isolated as a brown crystalline solid when the aldehyde was treated with 2-cyanoacetamide **(97)** without the addition of base. The ^1H NMR spectrum showed two broad signals at δ 7.86 and 7.80 for the $-NH_2$ protons, which were further upfield from the aromatic protons (doublets at δ 8.63 and 8.50, ^4J 1.7 Hz) and the olefinic proton (a singlet at δ 8.13). The ^{13}C NMR spectrum contained a deshielded signal at δ 162.0 (C=O), a CH group at δ 128.0 and a signal at δ 106.9 (olefinic carbons). In the IR spectrum, three weak signals at 3422, 3335 and 3289 cm^{-1} were assigned to a primary amide N-H stretch, with another N-H stretch at 2849 cm^{-1}. An amide carbonyl stretch was seen as a sharp peak at 1597 cm^{-1}. Signals at *m/z* 223 and 179 were assigned to the molecular ion and for loss of $-CONH_2$ from the parent compound.

The equivalent thioamide derivative **(130)** was also prepared without the use of a catalyst in acceptable yield. The signals in the ^1H NMR spectrum for the $-NH_2$ protons were further downfield this time at δ 8.69 and 8.61, whilst the aromatic protons with a coupling of 1.9 Hz were observed as doublets at δ 8.17 and 8.10. A singlet at δ 8.11 was assigned to the olefinic proton. In the ^{13}C NMR spectrum, a signal for the thiocarbonyl carbon was seen at δ 156.8, and the olefinic carbons were seen at δ 128.2 (CH) and δ 112.8, with the nitrile carbon coming at δ 116.2. The mass spectrum showed the molecular ion at *m/z* 239, and a smaller signal at *m/z* 82 was assigned to the thiophene ring having lost the nitro group and sidearm.

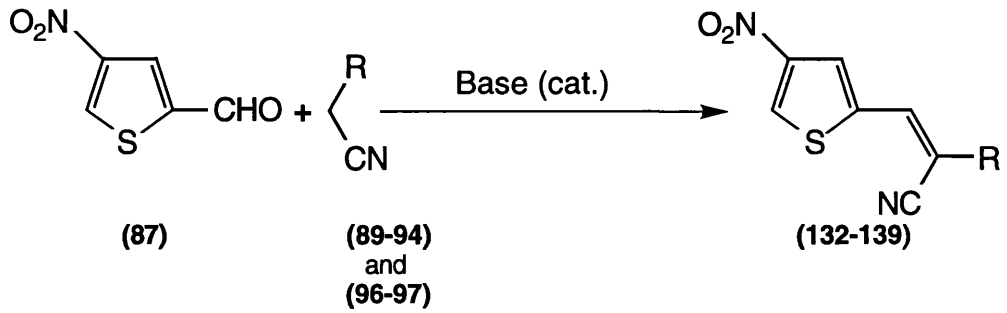
3.7 2-(4-Nitrothienyl) Tyrphostins



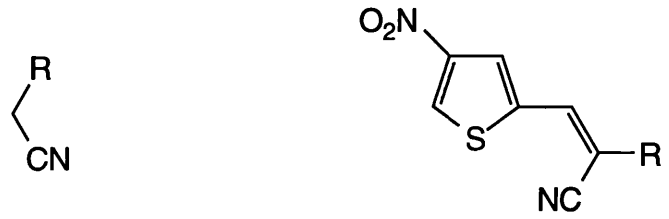
Scheme 18

4-Nitro-2-thiophenecarbaldehyde **(87)** was prepared from 2-thiophenecarbaldehyde **(131)** using standard nitration conditions **(Scheme 18)**.[171] Other approaches, such as the use of acetic acid with HNO_3 to generate the nitronium ion were not pursued owing to the acceptable yield that this approach gave. The reaction required extremely careful addition of a nitrating solution consisting of fuming nitric acid and concentrated sulfuric acid to a pre-cooled solution of 2-thiophenecarbaldeyde in concentrated sulfuric acid. After several attempts, it was established that the best method for this addition required the use of a syringe pump to ensure that the reaction temperature did not exceed 5 °C. The addition took around two hours to complete, and gave a black, turbid solution. The excess acid was then neutralised by careful addition of aqueous sodium bicarbonate solution, and the mixture was poured onto a slurry of ice and water and was slowly warmed to room temperature. Extraction of the products into ethyl acetate and removal of the solvent *in vacuo* gave a brown oil which was purified by column chromatography and recrystallisation from ethyl acetate gave the major product, 4-nitro-2-thiophenecarbaldehyde, as large orange crystals in 80 % yield. The ^1H NMR spectrum of the product showed a characteristic aldehydic proton at δ 9.26, accompanied by a singlet at δ 8.65 for the proton in the 5-position and a broader singlet between δ 8.29-8.12 for the proton on ring position 3. This broadening may be as a result of a long range coupling with the aldehydic proton. The IR spectrum contained sharp absorptions at 1508 and 1366 cm^{-1}, corresponding to asymmetric and symmetric stretches of the nitro N-O bonds. A sharp signal at 1734 cm^{-1} was assigned to the aldehydic carbonyl stretch. In the mass spectrum the molecular ion was seen as the largest signal in the spectrum at *m/z* 157, and was accompanied by a signal at *m/z* 111 corresponding to loss of the nitro group from the parent molecule.

Subsequent Knöevenagel condensation of compound **(87)** with a series of malonate derivatives in the usual manner **(Scheme 19** and **Table 5)** generated the final series of nitrothienyl tyrphostins.



Scheme 19



R	No.	Base	No.	Yield (%)
CN	**(89)**	dilute piperidine	**(132)**	70
$C(NH_2)=C(CN)_2$	**(90)**	dilute piperidine	**(133)**	55
CO_2Me	**(91)**	dilute piperidine	**(134)**	86
CO_2Et	**(92)**	dilute piperidine	**(135)**	40
$CO_2{}^nBu$	**(93)**	dilute piperidine	**(136)**	30
$CO_2{}^tBu$	**(94)**	dilute piperidine	**(137)**	40
$CONH_2$	**(96)**	dilute piperidine	**(138)**	15
$CSNH_2$	**(97)**	dilute piperidine	**(139)**	10

Table 5

Preparation of 2-cyano-3-[2-(4-nitrothienyl)]propenonitrile **(132)** followed the normal procedure using piperidine as the base. In the ^1H NMR spectrum, the olefinic proton came as a singlet at δ 8.72, with the aromatic protons in the 5- and 3-positions as doublets with a coupling of 5.7 Hz at δ 8.22 and 7.76. The ^{13}C NMR spectrum contained signals at δ 151.9 (CH) and δ 117.3 for the olefinic carbons, whilst the quaternary carbon bearing the nitro group came at δ 149.2 and the ring carbon bearing the sidearm was at δ 135.8. The two tertiary ring carbons came at δ 136.1 and 132.1. The nitrile groups were observed at δ 112.6 and 113.0. In the IR spectrum a sharp signal for an aromatic C-H stretch was observed at 3038 cm^{-1}, with weak signals at 870 and 834 cm^{-1} from two isolated aromatic C-H stretches. A signal corresponding to a nitrile group came at 2230 cm^{-1}, and a C-NO_2 stretch gave rise to a sharp absorption at 1522 cm^{-1}. A sharp absorption at 1636 cm^{-1} was assigned to a

C=C conjugated to an aromatic system. The mass spectrum contained a signal at *m/z* 205 for the molecular ion, and loss of a nitro group from this ion was signified by a peak at *m/z* 159.

The next compound to be prepared was **(133)**, from 4-nitro-2-thiophenecarbaldehyde **(87)** and 2-aminopropene-1,1,3-tricarbonitrile **(90)** using a catalytic amount of piperidine. In the ^{1}H NMR spectrum of the product, two broad singlets corresponding to the $-NH_2$ protons was observed at the characteristically downfield values of δ 9.19 and 9.17. The olefinic proton was a singlet at δ 9.18. In the ^{13}C NMR spectrum, the quaternary olefinic carbons came at δ 164.3, 101.2 and 50.2. Three nitrile signals were observed at δ 115.4, 114.6 and 114.5. The IR spectrum contained distinctive peaks at 3052, 1607 and 820 cm^{-1} for aromatic C-H and C=C stretches, and a sharp signal at 1647 cm^{-1} corresponded to a C=C conjugated to an aromatic system. A sharp absorption at 2726 cm^{-1} was assigned to an N-H stretch. The molecular ion was seen in the mass spectrum, and a signal at *m/z* 225 corresponded to loss of the nitro moiety from the parent molecule.

Reaction of 4-nitro-2-thiophenecarbaldehyde **(87)** with compounds **(91** to **94)** gave the corresponding products **(134** to **137)**. Yields for these reactions were low at around 30-40 %, although microanalysis of the products confirmed their purity. In the ^{1}H NMR spectra, the olefinic protons came as characteristic singlets at around δ 8.7, whilst the aromatic protons often showed a long range coupling of between 0.8-1.7 Hz. The ^{13}C NMR spectra all possessed a quaternary carbon at around δ 162, and a nitrile signal at δ 115. All of the NMR spectra showed signals for the alkyl fragments of the ester chain. In the IR spectra, signals corresponding to aromatic C-H stretches, nitrile moieties, αβ-unsaturated carbonyls and $C-NO_2$ stretches were observed. Mass spectra all contained signals corresponding to the molecular ion, and a signal at *m/z* 179 for loss of $-CO_2R$ from the parent molecule.

2-Cyano-3-[2-(4-nitrothienyl)]propenamide **(138)** was prepared from 2-cyanoacetamide and 4-nitro-2-thiophenecarbaldehyde **(87)** in the presence of piperidine. A brown precipitate formed after only five minutes, and despite attempts to react all of the starting materials by heating to reflux temperature, only 15 % of the product was obtained, which was easily separated from the starting materials by filtration. The ^{1}H NMR spectrum of the product contained a very broad singlet centred at δ 7.92 for the $-NH_2$ protons, and the aromatic protons were also observed as broad singlets at δ 9.16 and 8.41. In the ^{13}C NMR spectrum, a signal at δ 161.7 resulted from an amide carbonyl, and the olefinic carbons came at δ 142.6 (CH) and 105.6. The IR spectrum had two

absorptions of medium intensity at 3423 and 3181 cm^{-1} for primary amide N-H stretches, and a sharp amide C=O stretch at 1690 cm^{-1}. Aromatic C=C vibrations gave rise to signals at 1599 and 1542 cm^{-1}. In the mass spectrum, a signal at *m/z* 223 corresponding to the molecular ion was accompanied by another signal at *m/z* 179 for loss of the amide from the parent molecule.

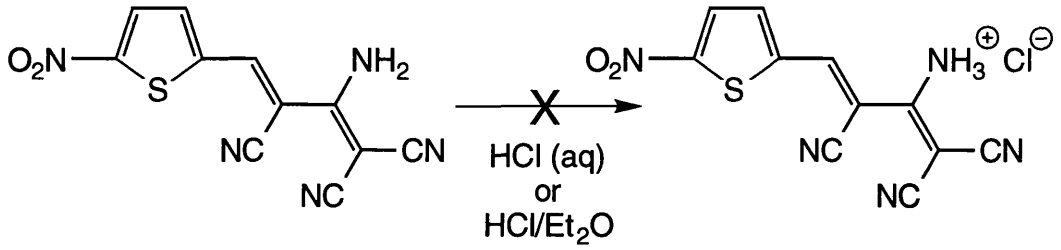
The final tyrphostin in this series to be prepared was compound **(139)**. This was prepared in the usual manner, using one equivalent of 2-cyanothioacetamide in the presence of piperidine. As in the case of the amide derivative, the yield for this reaction was particularly low at only 10 %. The NH_2 protons were observed in the ^1H NMR spectrum as two broad singlets at δ 10.22 and 9.66, and the olefinic proton came at δ 8.36. In the ^{13}C NMR spectrum, signals were observed at δ 191.0 for the thiocarbonyl carbon, at δ 147.9 (CH) and 111.3 for the olefinic carbons and at δ 116.1 for the nitrile. In the IR spectrum, signals at 3277 and 3170 cm^{-1} resulted from a thioamide N-H stretch, and a thiocarbonyl stretch came as a sharp absorption at 1628 cm^{-1}. Aromatic C=C vibrations gave rise to absorptions at 1596 and 1541 cm^{-1}. The mass spectrum showed a peak at *m/z* 239 for the molecular ion.

3.8 Attempts to Overcome the Problems of Solubility

The compounds discussed thus far were extremely insoluble in water and common organic solvents, and only displayed an acceptable level of solubility when DMSO was used as the solvent. Dimethylsulfoxide is itself toxic and may therefore mask any inherent biological activity. We therefore considered it necessary to investigate a number of methods which could be employed to increase the solubility of these compounds.

3.8.1 HCl Salt of 2-cyano-3[5-(2-nitrothienyl)]propenoic acid (100)

A procedure involving the stirring of an acetone solution of 2-cyano-3[5-(2-nitrothienyl)]propenoic acid **(100)** with 1.0 M hydrochloric acid was carried out at room temperature **(Scheme 20)**, but only starting material was observed by TLC after three hours. The same result was observed when the procedure was repeated using dry ethereal HCl and a suspension of **(100)** in dry diethyl ether. The failure of these routes can be accounted for by the reduced basicity of the lone pair on the amino nitrogen, whose electrons have been delocalised into the molecule's extended π system.



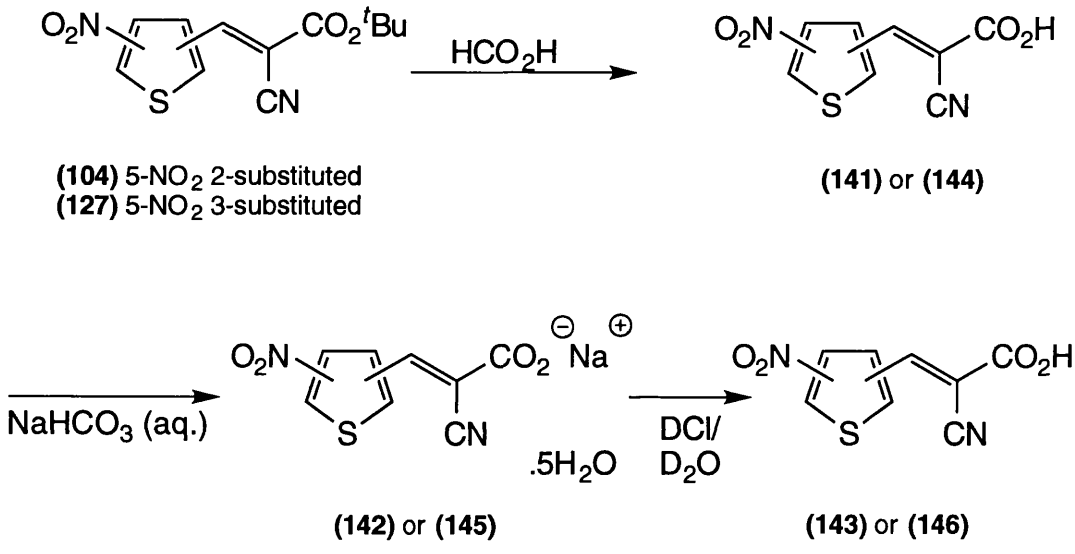
(100) **(140)**

Scheme 20

3.8.2 Sodium Salts of Acid Derivatives

The procedure outlined in **Scheme 21** was employed, and involved reaction of ^{t}butyl 2-cyano-3-[2-(5-nitrothienyl)]-propenoate **(104)** with formic acid to hydrolyse the ester. The product **(141)** was identified from its ^{1}H NMR spectrum, which showed a very broad signal for the acid proton along the baseline between δ 7.8-7.0, and a sharp signal in the IR spectrum at 1694 cm^{-1} for an $\alpha\beta$-unsaturated carboxylic acid C=O stretch.

Subsequent treatment of **(141)** with one molar equivalent of aqueous sodium bicarbonate solution yielded a yellow solid which was freeze-dried for 24 hours to give the pentahydrated sodium salt **(142)** (determined by microanalysis) in 44 % overall yield. No signal for the acid proton in the ^{1}H NMR spectrum was observed, and the IR spectrum did not posses a peak which could be assigned to the unsaturated acid. The pentahydrate was completely soluble in water, and was converted back into the deuterated acid **(143)** by stirring in a mixture of DCl and D_2O.



(104) 5-NO_2 2-substituted
(127) 5-NO_2 3-substituted

(141) or **(144)**

(142) or **(145)** **(143)** or **(146)**

Scheme 21

The above procedure was repeated using ^{t}butyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate **(127)** to give compound **(144)** in quantitative yield.

In the ^{1}H NMR spectrum of this compound, a broad signal between δ 8.0-7.0 was assigned to the acidic proton, and a signal at δ 163.1 in the ^{13}C NMR spectrum corresponded to the carbonyl carbon in the acid, compared to δ 160.3 for the ^{t}butyl ester. No signals for the ^{t}butyl moiety were observed in these spectra. A significant absorption in the IR spectrum at 3098 cm^{-1} was assigned to an O-H stretch, and was accompanied by a signal for an αβ-unsaturated acid C=O stretch at 1750 cm^{-1}. A fairly strong signal in the mass spectrum at *m/z* 224 corresponded to the molecular ion.

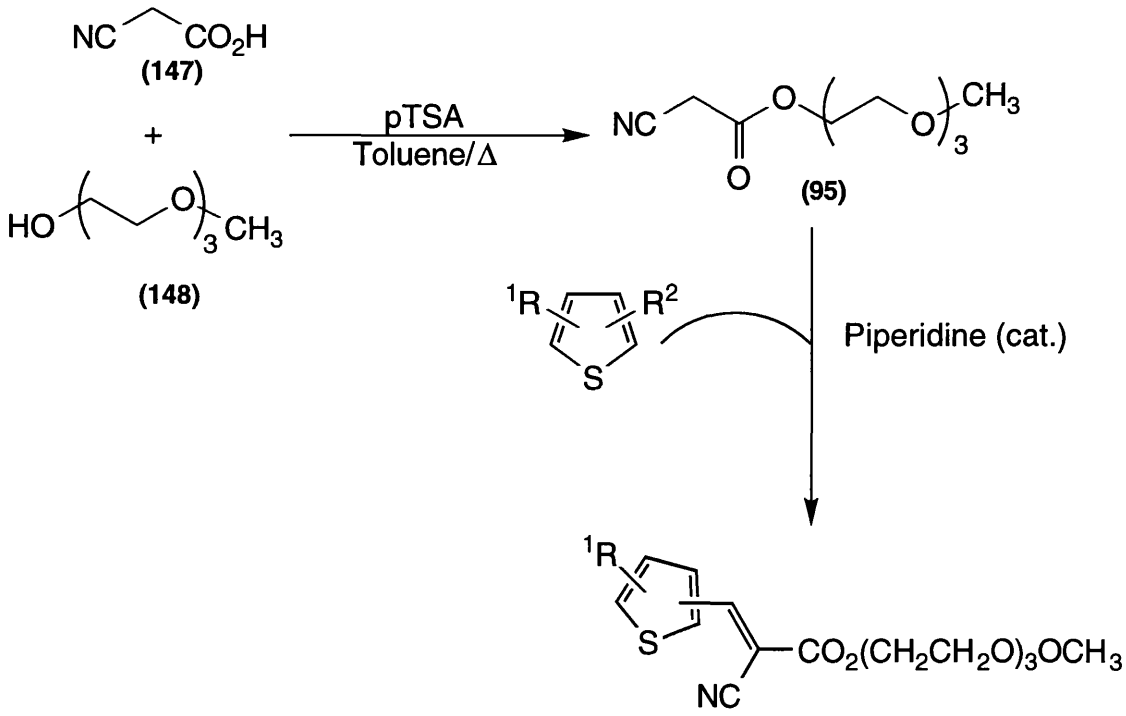
Compound **(144)** was then stirred with a slight excess of aqueous sodium bicarbonate solution until fully dissolved, and after freeze-drying, the sodium salt of **(144)** was isolated as the dihydrate **(145)**. No signal for the acidic proton was observed in the ^{1}H NMR spectrum of the product, nor was the absorption at ~3100 cm^{-1} in the IR spectrum. A signal at *m/z* 223 corresponded to the anion of this salt. The salt was converted back into the deutero acid in the manner outlined in **Scheme 21** to give **(146)** in quantitative yield.

3.8.3 Tri(ethyleneglycol) Ester Derivatives

Currently, a great deal of interest has been shown in the attachment of drugs to tri- and poly(ethyleneglycol) units (TEG and PEG) as a means of increasing the duration of activity through slow release of the compound, and to increase drug solubility. Ethyleneglycols are know to be non-toxic, non-antigenic and biocompatible, are soluble in water and organic solvents and have solubilising properties when attached to other organic compounds.[172]

A variety of drugs, such as procaine[173] and atropine[174] have been attached to TEG and PEG. The trimer/polymer-drug conjugates showed longer durations of activity due to slow release. Insulin has been attached to PEG to give a product which retained its biological activity.[175]

Attachment of a drug to ethyleneglycols is carried out *via* the terminal hydroxyl group of TEG or PEG. Monomethyl ethers of TEG or PEG are often employed to prevent bifunctionalisation of the trimer/polymer.



Aldehyde	R^1	R^2	Product
(85)	5-NO_2	2-CHO	**(105)**
(83)	2-NO_2	3-CHO	**(119)**
(84)	2-NO_2	4-CHO	**(128)**

Scheme 22 and **Table 6**

Our synthetic route, outlined in **Scheme 22**, involved the preparation of an ethyleneglycol malonate ester such as compound **(95)** and its subsequent reaction with nitrothiophenecarbaldehydes **(83-85)** to produce compounds **(105)** **(119)** and **(128)**. Treatment of 2-cyanoacetic acid **(147)** with a slight excess of tri(ethyleneglycol) monomethyl ether **(148)** using Dean-Stark conditions for 24 hours gave a yellow solution which was concentrated to a light brown oil. TLC showed the presence of two very broad spots, the lower of which (R_F 0.15) corresponding to unreacted **(148)**. These were easily separated by positive pressure column chromatography, giving the product as an amber oil in 64 % yield. The ^1H NMR spectrum of this oil was dominated by a complex multiplet at δ 3.42-3.29, corresponding to six sets of methylene protons. Signals at δ 4.01 (multiplet) and δ 3.03 (singlet) were also observed, and were assigned to the methylene protons α to the nitrile group and to the terminal methyl group, respectively. The ^{13}C NMR spectrum consisted of a downfield signal at δ 162.9 (corresponding to the carbonyl carbon), a quaternary carbon at δ 113.0 for the nitrile carbon, seven signals between δ 70.6-57.4 for the methylene carbons and the methyl carbon at δ 23.2. Signals for the nitrile group, a carbonyl stretch and a C-O-C stretch were seen in the IR spectrum, whilst the mass spectrum showed a very small peak for the molecular ion and larger signals

corresponding to loss of fragments of the tri(ethyleneglycol) portion of the molecule. Microanalytical and accurate mass data were consistent with the structure shown.

Compound **(95)** was then reacted with 5-nitro-2-thiophenecarbaldehyde in the presence of piperidine to give **(105)** as an orange solid in 89 % yield after a short reaction time of ten minutes. In the ^{1}H NMR spectrum **(Figure 12)**, three signals of equal intensity were observed in the aromatic region. These consisted of a singlet at δ 8.29 corresponding to the olefinic proton, and two AB systems with a coupling of 4.4 Hz at δ 7.97 and δ 7.76 for the ring protons β and α to the sidearm. A large multiplet at δ 3.86-3.39 with an intensity of 12 for the methylene protons was accompanied by a singlet at δ 3.37 with an intensity of three for the terminal methyl group. In the ^{13}C NMR spectrum **(Figure 13)**, a very deshielded signal at δ 161.2 was assigned to the carbonyl carbon. Signals at δ 156.0 and 145.2 were assigned to the ring carbons bearing the nitro group and the sidearm respectively, whilst two CH signals corresponding to the aromatic carbons in the 3- and 4- positions came at δ 128.2 and 135.0. The olefinic methine carbon was a signal at δ 145.2, and the quaternary olefinic carbon was at δ 104.8. The nitrile came as a singlet at δ 114.2. The six methylene carbons on the sidearm were observed between δ 71.9-66.2, and the terminal methyl group came at δ 59.0. The IR spectrum of **(105)** contained a signal of medium intensity at 2892 cm^{-1} corresponding to an ether C-H stretch. A weak absorption at 2222 cm^{-1} was assigned to a nitrile moiety, and a C-NO_2 stretch came as an absorption of medium intensity at 1504 cm^{-1}. Signals at 1725 cm^{-1}, and at 1338 and 1271 cm^{-1} could be assigned to an αβ-unsaturated carbonyl and ester C-O stretch, respectively. In the mass spectrum, a signal at *m/z* 355 corresponding to loss of a methyl group from the molecular ion was accompanied by other signals at *m/z* 252 and 235 for loss of fragments of the ester chain from the parent molecule.

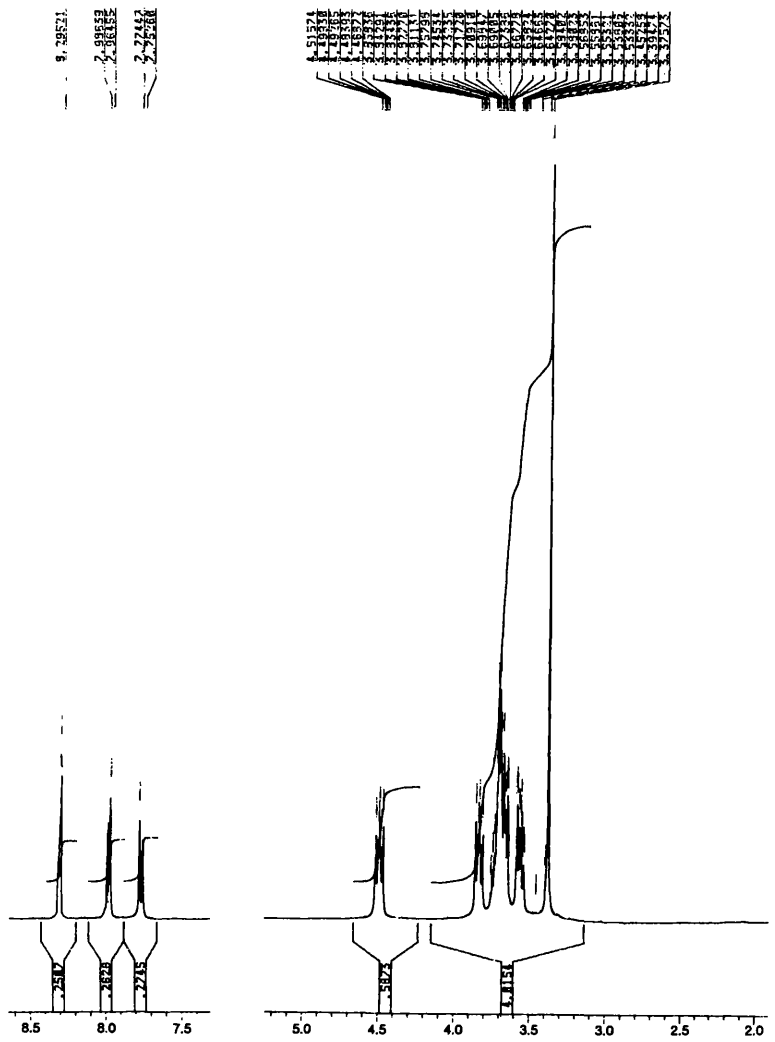


Figure 12

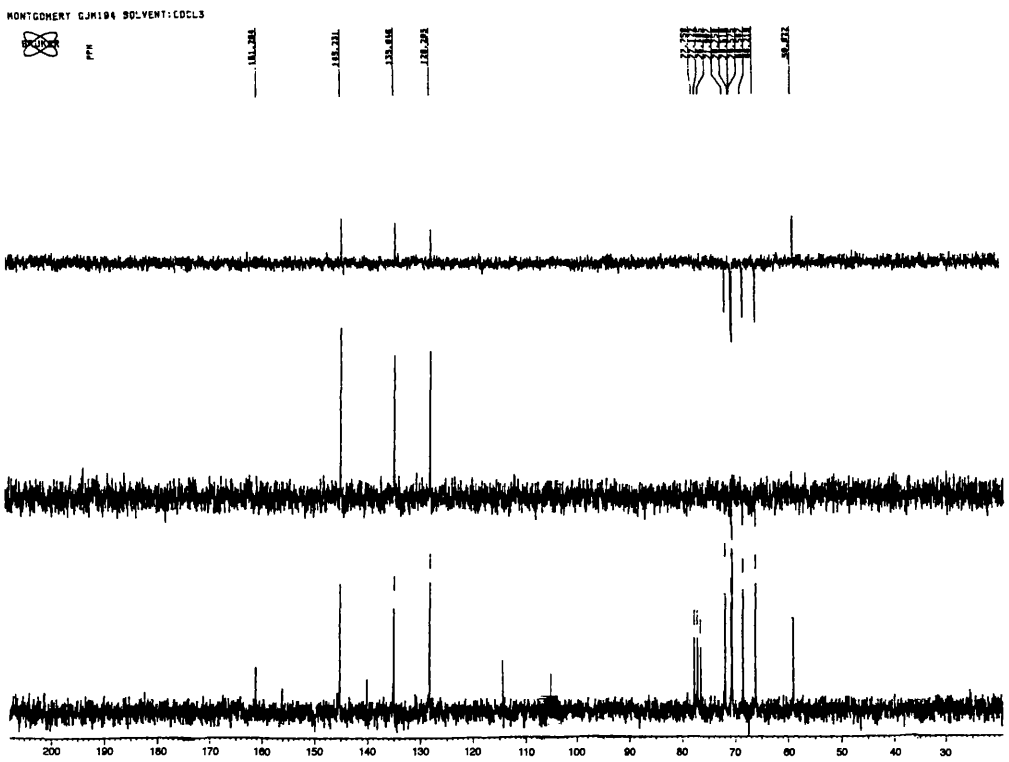


Figure 13

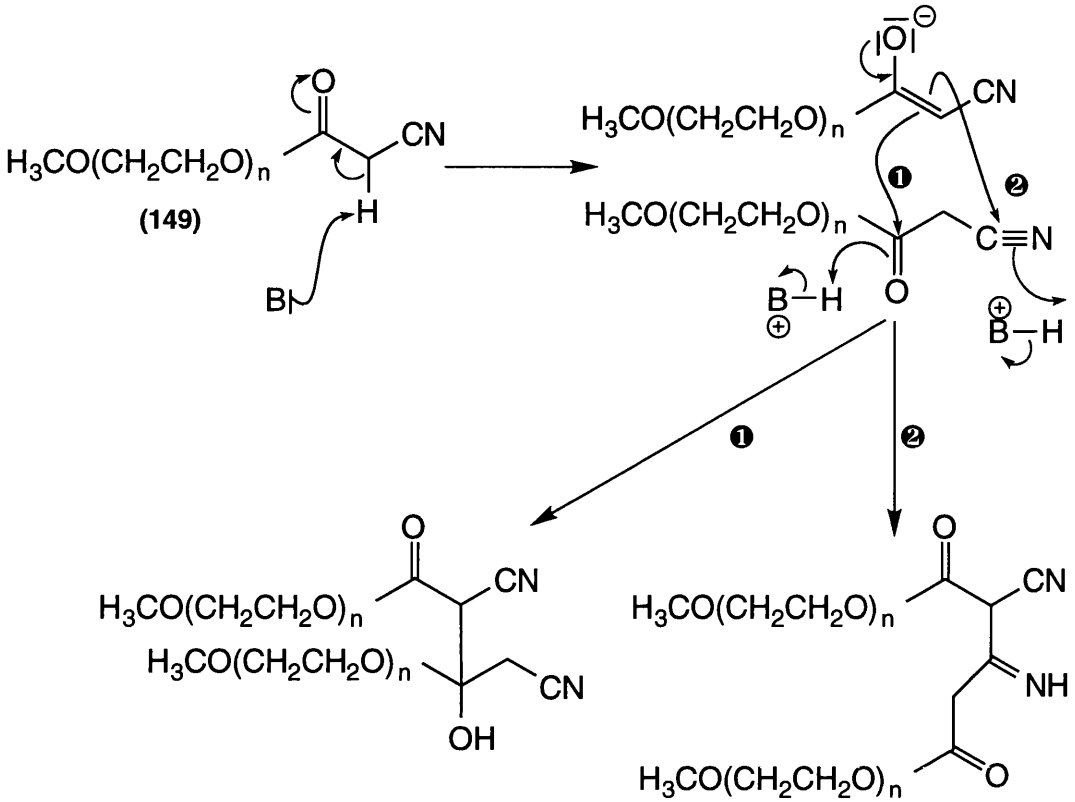
A second tri(ethyleneglycol) ester, **(119)**, was prepared next, from the corresponding cyanoacetate using piperidine as the base. After a reaction time of 14 hours, the product was isolated as a peach solid in 62 % yield. The olefinic proton was observed as a singlet in the ^1H NMR spectrum at δ 9.21. The aromatic protons came as broad singlets at δ 8.71 and 8.62. The six groups of methylene protons were seen as a broad multiplet at δ 4.71-3.38, and the terminal methyl group was a singlet at δ 3.26. In the ^{13}C NMR spectrum, the carbonyl carbon observed at δ 161.4. In the aromatic region, a quaternary carbon at δ 148.2 was assigned to the aromatic C-NO_2, and another signal at δ 146.6 corresponded to the ring carbon bearing the sidearm. Two CH signals at δ 147.9 and 136.0 corresponded to the ring carbons in the 5- and 4-positions. The methylene carbons came between δ 71.4-65.8, and the terminal methyl group was seen at δ 58.2. The IR spectrum had a medium signal at 2878 cm^{-1}, corresponding to a C-O-CH_3 bend. A weak signal at 2224 cm^{-1} was assigned to a nitrile group, and a sharp absorption at 1716 cm^{-1} corresponding to an αβ-unsaturated carbonyl was accompanied by signals at 1350 and 1272 cm^{-1} for an ester C-O stretch. There was a medium absorption at 1610 cm^{-1} for a C=C bond conjugated to an aromatic system, and a sharp absorption at 1536 cm^{-1} was assigned to a C-NO_2 stretch. A signal for the molecular ion at *m/z* 370 was seen in the mass spectrum. Other signals at *m/z* 295, 267 and 251 corresponded to fragmentation of the tri(ethyleneglycol) moiety.

The 2-nitro-4-thienyl tri(ethyleneglycol) ester derivative compound **(128),** was prepared from 2-nitro-4-thiophenecarbaldehyde **(84)** and tri(ethyleneglycol)cyanoacetate methyl ether **(95)** using piperidine as the base. The reaction gave a dark precipitate upon stirring at room temperature for ten minutes, and TLC showed the presence of three compounds with R_F values 0-0.17, 0.47 and 0.65. The two upper spots corresponded to unreacted aldehyde and 2-nitro-4-thiophenemethanol-1,2-diethyl ether, but as these spots were very faint it was decided to terminate the reaction and to purify the product by column chromatography. Despite careful collection of small fractions and after flushing the column with methanol to remove any residual amounts of product, only 10.5 % of **(128)** was isolated after crystallisation from ethanol. Purity was confirmed by accurate mass spectrometry and microanalysis, and the IR spectrum showed 2 sharp signals at 1730 and 1270 cm^{-1}, corresponding to the carbonyl and C-O stretches of an αβ-unsaturated ester. Other signals at 2826 and 2228 cm^{-1}, both weak in intensity, could be assigned to an O-CH_3 stretch and a CN group. Three downfield signals of equal intensity in the ^1H NMR spectrum consisting of two doublets at δ 8.54 and 8.39 with a coupling of 1.93 Hz , and a singlet at δ 8.16 were assigned to the aromatic protons and the olefinic proton respectively. A multiplet at δ 4.50-3.53 with a relative intensity

of 12 corresponded to the six groups of methylene protons, and the protons of the terminal methyl group were observed as a singlet of intensity three at δ 3.37. In the ^{13}C NMR spectrum, a signal for the carbonyl carbon was seen at δ 161.6, whilst the aromatic carbons came as two quaternary carbons and two CH groups between δ 153.2-133.0. The olefinic methine carbon was at δ 128.0, and the nitrile carbon and quaternary olefinic carbon were observed at δ 114.0 and 104.0. Six methylene groups were observed between δ 71.9-66.0, and the methyl carbon was seen at δ 59.0. The molecular ion and signals corresponding to fragmentation of the tri(ethyleneglycol) portion of the parent compound were observed in the mass spectrum.

3.8.4 Attempted Syntheses of Poly(ethyleneglycol) Ester Derivatives

A similar approach to the first step shown in **Scheme 22** was adopted to synthesise the poly(ethyleneglycol) monomethyl ether **(149)**. The product was isolated as a yellow waxy solid which was characterised by NMR spectroscopy. Both the ^{1}H and ^{13}C NMR spectra were dominated by a broad multiplet at δ 3.57 and δ 77.9-58.9 respectively, which were assigned to the poly(ethyleneglycol) groups. In the IR spectrum, sharp signals at 2950 and 2914 cm^{-1} were assigned to multiple methylene groups. Since the product had a molecular weight in excess of 2000, it was not feasible to characterise it by mass spectrometric methods, and microanalytical data were unhelpful owing to the wide range of molecular weights in the product mixture. We decided to treat **(149)** with 5-nitro-2-thiophenecarbaldehyde in the presence of a catalytic amount of piperidine in ethanol. Upon addition of the piperidine, the solution immediately turned black, and TLC showed the presence of the aldehyde with R_F 0.53 and a black spot on the baseline. This spot may represent the product of a nucleophilic addition reaction of the cyanoacetate to the carbonyl or nitrile carbons of another molecule of **(149)** **(Scheme 23)**.



Scheme 23

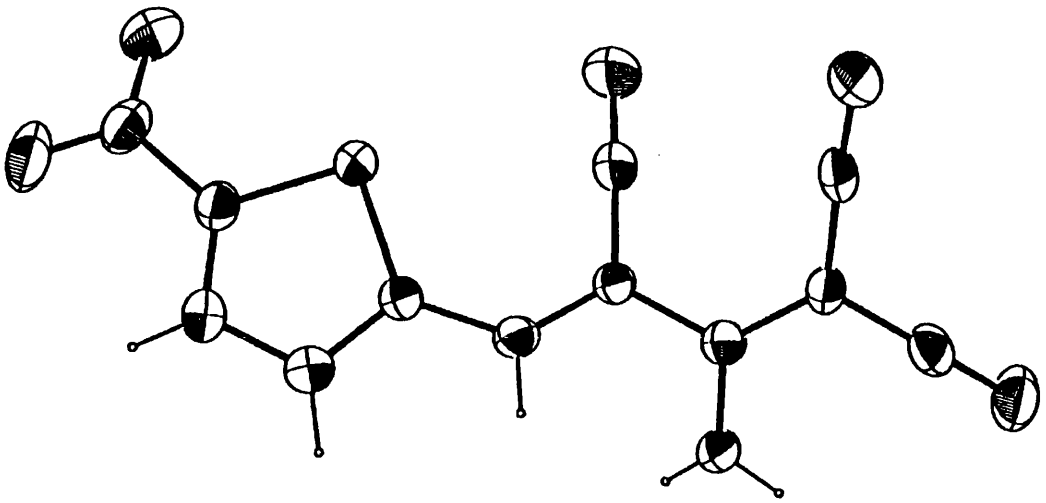
When the reaction was repeated in the presence of dilute piperidine solution both at 25 °C and at 0 °C, the same effect was observed. In the absence of base, only starting materials were recovered. Had this preparation succeeded, it might have been difficult to purify the product as it would consist of a number of species of differing masses, which would also result in a fairly substantial margin of error when calculating the molarities of solutions of the product to be used for biological evaluation. With this in mind, it was decided not to pursue this route further.

3.9 Crystal Structure of a Nitrothienyl Tyrphostin

2,4-Dicyano-3-amino-5-[2-(5-nitrothienyl)]-penta-2,4-dienonitrile **(100)** was recrystallised in a flat-bottomed flask from a 1:1 mixture of acetone/chloroform to give brown needles of about 5 mm length. These were submitted to Dr K.W. Muir (University of Glasgow) for X-ray crystallographic analysis.

The displacement ellipsoid structure of this compound is shown overleaf **(Figure 14)**. Several important structural features were confirmed from the analysis. Of particular importance is the *trans*-configuration of the large groups across the C(4)-C(5) double bond. The molecule is essentially planar, with the C(7)-C(4)-C(5)-C(1') torsion angle, ω, at -1.4 °. A slight distortion from the plane

of the molecule by the second extraanullar double bond was observed, and the C(6)-C(2)-C(3)-C(4) torsion angle was 7.3 °. In addition, a substantial out-of-plane distortion of the C(3)-C(4) bond [N(3)-C(3)-C(4)-C(5) torsion angle $\omega = 29.4$ °)] may arise from the repulsive effects of the nitrile groups [C(6)-N(6) and C(7)-N(7)]. The combined effects of the sidearm and nitro group had little effect on the C-C and C-S bond lengths in the aromatic ring compared to values obtained from microwave studies of 2-nitrothiophene[176] **(Table 7)**.



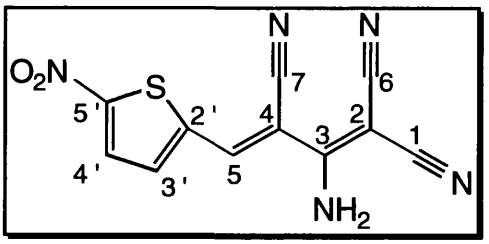


Figure 14

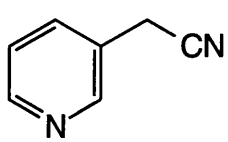
Parameter	Observed Length (Å)	Reported Length (Å)[176]
C(2')-C(3')	1.377	1.359
C(3')-C(4')	1.390	1.424
C(4')-C(5')	1.348	1.370
C(5')-S	1.712	1.689

Table 7

3.10 Synthesis of 'Hydrated' Tyrphostins

The Hann-Lapworth Mechanism for the Knöevenagel condensation proposes that an intermediate β-hydroxy compound is formed which undergoes *anti*-elimination of H_2O to form the Knöevenagel product. It has been suggested that this mechanism holds true when a tertiary amine is used to deprotonate the malonate derivative, although it is perfectly feasible for a reaction to proceed in this way when other bases are employed.

As part of our synthetic route to tyrphostins containing a heteroaromatic moiety on the sidearm, we treated 5-nitro-2-thiophenecarbaldehyde **(85)** with 3-pyridylacetonitrile **(150)**. The earlier attempts at this reaction involving the treatment of the reactants with a catalytic amount of piperidine or potassium carbonate[104] using ethanol as the solvent at room temperature failed to give any product, and only starting materials were recovered. However, when the reaction was performed at reflux temperature without the addition of base, a brown solid appeared after about 2.5 hours; TLC revealed this to be a polar material with R_F 0.17. The reaction was continued for a further 15 hours to give more brown solid, which was filtered off and washed using a little diethyl ether, and was dried under vacuum in a drying pistol for several hours. The ^1H NMR spectrum of the product was complicated **(Figure 15)**, but clearly showed that a mixture of two diastereoisomers was present in a 3:1 ratio. Of particular note were two doublets of doublets at δ 5.56 and 5.34, accompanied by doublets at δ 4.91 and 4.82. A very broad signal at δ 3.42 was also observed, which did not arise from water impurities in the sample since fresh DMSO had been used and an NMR spectrum of the solvent confirmed the absence of water. The doublets of doublets became two doublets upon D_2O exchange, and the broad singlet disappeared. The signals at δ 5.56, 5.34, 4.91 and 4.82 clearly corresponded to non-aromatic protons which were nonetheless in a deshielded environment. The aromatic region of this spectrum also appeared to be considerably more complicated than would be expected for the Knöevanagel product, and a doubling-up of the signals in this region **(Figure 16)** was clearly apparent.



(150)

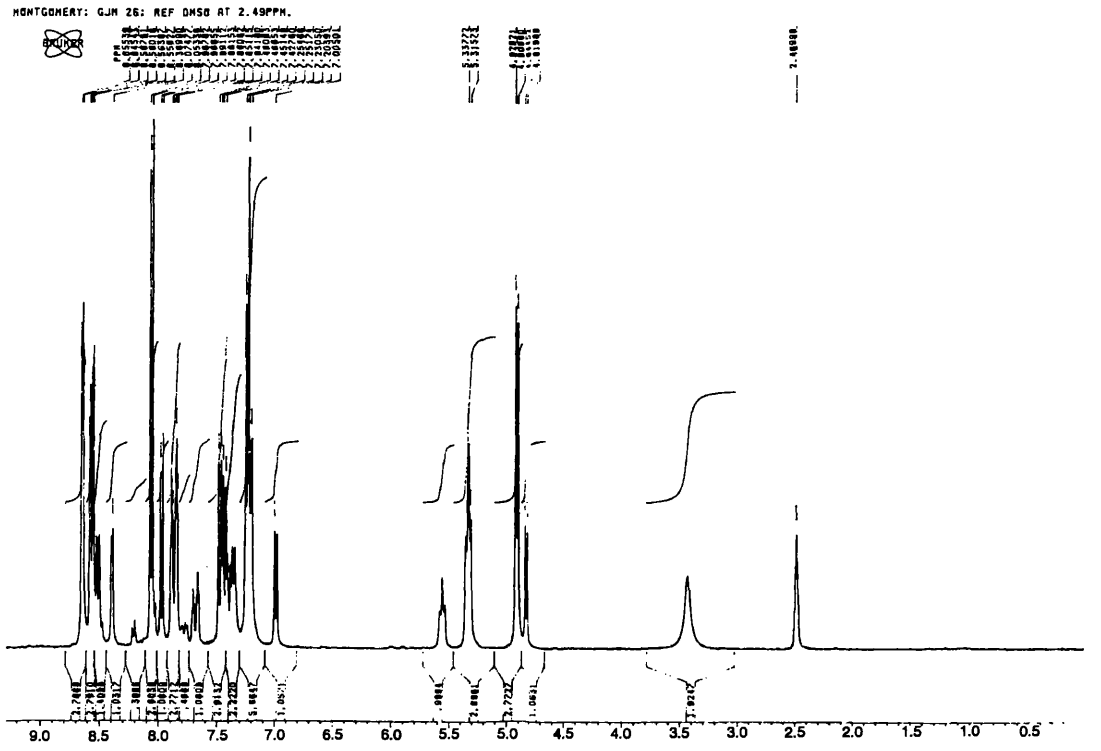


Figure 15

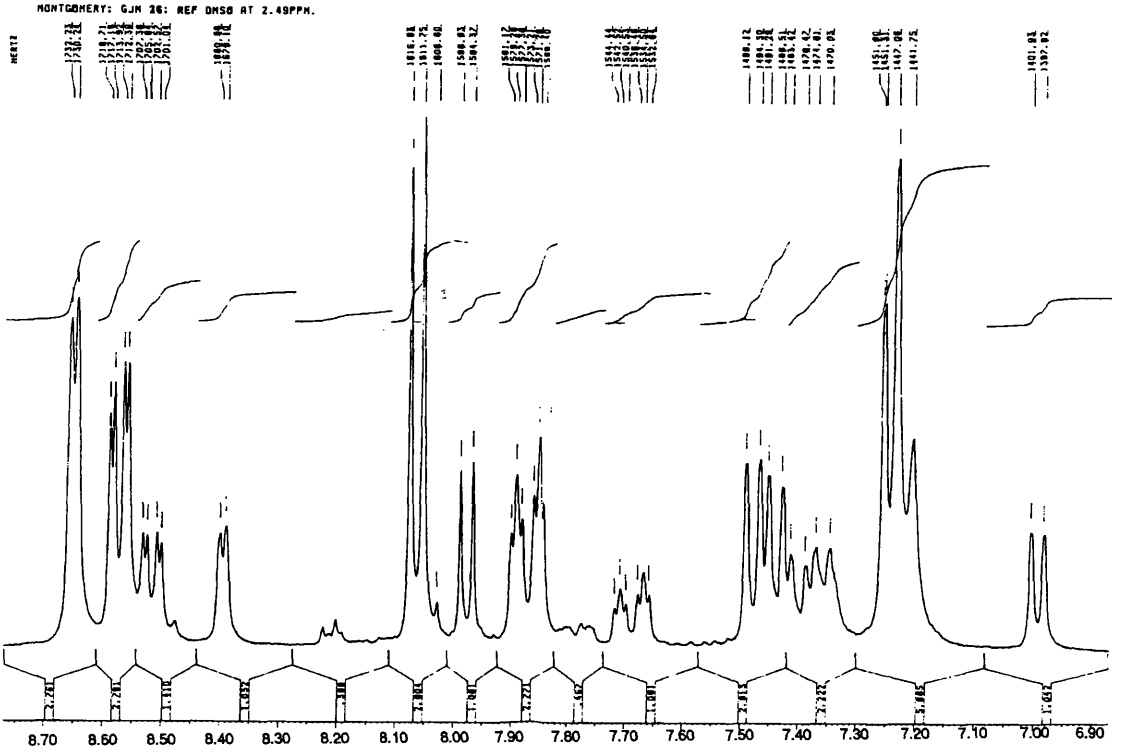
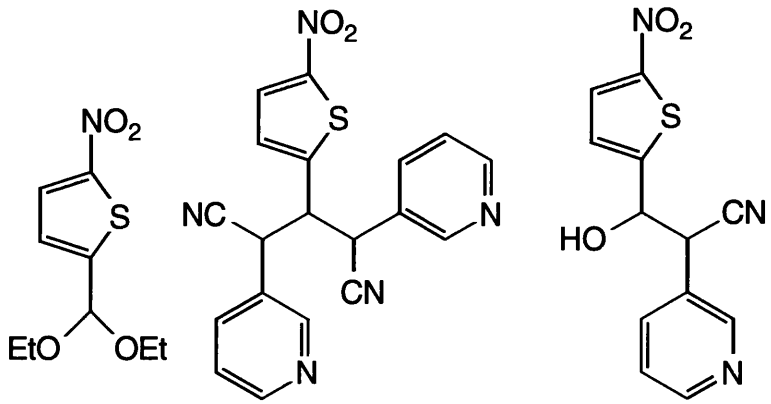


Figure 16

This led us to consider the possibility of either an intermediate of the reaction or a completely different product having been isolated **(Figure 17)**. Since no base had been employed, it was not possible for the reaction to have proceeded *via* the classical mechanism, and no signals for the diethyl ether **(151)** had been observed in the ^1H NMR spectrum, nor had a spot at R_F 0.71 been noted by TLC.

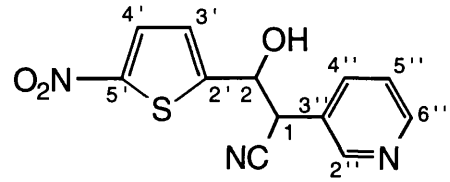


(151) **(152)** **(153)**

Figure 17

Another possibility was that a Michael addition to the expected Knöevenagel product had taken place to give **(152)**. This was overruled by the fact that a 2:1 ratio of the pyridyl and thienyl protons was not observed in the ^1H and ^{13}C NMR spectra, nor were three deshielded methine groups observed. In addition, the IR spectrum of the product showed absorptions at 3431 cm^{-1} and 1294 cm^{-1} corresponding to OH stretches and bends. A C-O stretch at 1161 cm^{-1} was also observed, suggesting that compound **(153)** was in fact the product. Further evidence from microanalytical and accurate mass data confirmed that the compound had a molecular formula of $C_{12}H_{10}N_3O_3S$, consistent with that of compound **(153)**. Detailed evaluation of the ^1H and ^{13}C spectra confirmed these findings **(Table 8)**.

Compound **(153)** does not appear to decompose, nor does it dehydrate to the Knöevenagel product. This was confirmed when **(153)** was dissolved in ethanol and heated to reflux temperature, either in the presence of piperidine or K_2CO_3 for extensive times of up to 72 hours. Only **(153)** was observed by TLC and NMR spectroscopy. NMR spectra of samples of **(153)** which were up to six months old showed no additional signals.



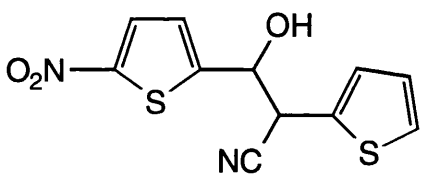
(153)

	Diastereoisomer 1		Diastereoisomer 2		
	δ_H	δ_C	δ_H	δ_C	**Notes**
1	4.91, d, ^3J 3.9 Hz[1]	70.0	4.82, d, ^3J 4.9 Hz	69.4	[1] Coupling between 1-H & 2-H
2	5.34, dd, ^3J 4.4, ^3J 4.3 Hz[1]	43.0	5.56, dd, ^3J 4.5, ^3J 4.4 Hz	42.2	not observed
2'	-	149.8	-	149.8	
3'	7.23, d, ^3J 4.3 Hz	123.7	7.00, d, ^3J 4.3 Hz	123.5	
4'	8.07, d, ^3J 4.3 Hz	149.1	7.98, d, ^3J 4.3 Hz	148.9	
5'	-	156.5	-	155.1	
2"	8.66, d, ^5J 1.9 Hz	149.5	8.40, d, ^5J 1.9 Hz	149.7	
3"	-	129.2	-	127.8	
4"	7.88, dd, ^3J 4.1, ^5J 1.9 Hz	130.1	7.69, dd, ^3J 4.1, ^5J 1.9 Hz[2]	129.7	[2] Coupling between 4"-H &
5"	7.46, dd, ^3J 7.8, ^3J 4.8 Hz	124.4	7.38, dd, ^3J 8.5, ^3J 4.8 Hz	124.8	5"-H not observed
6"	8.58, dd, ^3J 4.8, ^4J 1.5 Hz	136.4	8.52, d, ^3J 4.8, ^4J 1.5 Hz	136.6	
CN	-	118.2	-	118.9	
OH	3.42, v.br s	-	3.42, v. br s	-	

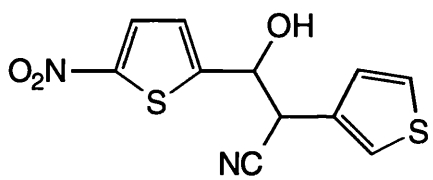
Table 8

Compound **(154)** was prepared by dissolving 2-nitro-4-thiophenecarbaldehyde **(84)** in ethanol and one drop of piperidine. 3-Pyridylacetonitrile was then added, as a solid, over a period of around five minutes. The mixture was stirred at room temperature for 6.5 hours until a yellow precipitate was observed. TLC revealed this to be a fairly polar compound with R_F 0.23. The precipitate was filtered off the liquors, and was washed and dried with a little diethyl ether, then placed in a drying pistol and heated under vacuum for several hours to remove traces of solvent and/or water. The ^1H NMR spectrum of this solid was similar to that for compound **(153)** in that two sets of deshielded doublets were observed at δ 5.61 and 4.96, and at δ 5.38 and 4.88, in a 3:2 ratio. These were accompanied by a complex set of broad multiplets in the aromatic region, and a very broad signal at δ 3.79 corresponding to the alcohol proton. DEPT analysis of the ^{13}C NMR spectrum allowed complete assignment of the signals. Details of the NMR spectra are given in **Table 9.**

We then considered it necessary to attempt a synthesis of a hydrated form of a nitrothienyl tyrphostin containing a 2- or 3-thienyl moiety (compounds **155** and **156**). 5-Nitro-2-thiophenecarbaldehyde **(85)** was treated with 2- and 3-thiopheneacetonitrile in the absence or presence of piperidine or pyridine, both at room temperature and under reflux conditions. Either a black tar formed or starting material was recovered in most of these cases (the exception being the reaction of 5-nitro-2-thiophenecarbaldehyde with 2-thiopheneacetonitrile **(98)** in the presence of piperidine at room temperature for eight days, which gave compound **(108)** as before). When K_2CO_3 was used, a very complex mixture of about ten products was observed by TLC, which could not be separated.

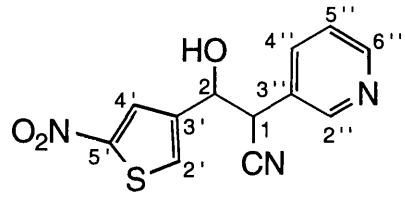


(155)



(156)

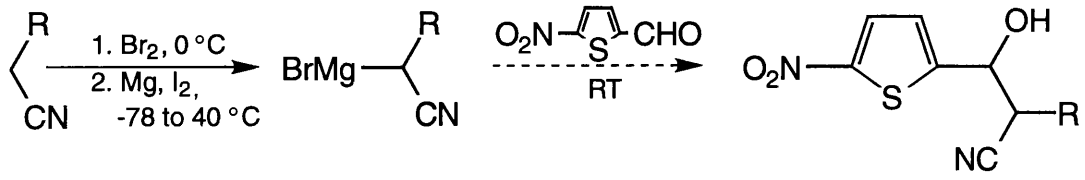
An alternative strategy involves the reaction of the aromatic aldehyde with a Grignard reagent as outlined in **Scheme 24**.



(154)

	Diastereoisomer 1		Diastereoisomer 2		
	δ_H	δ_C	δ_H	δ_C	Notes
1	5.61, d, ^3J 3.6 Hz	70.1	5.38, d, ^3J 4.9 Hz	69.5	
2	4.96, d, ^3J 3.6 Hz	43.1	4.88, d, ^3J 4.9 Hz	42.3	
2'	7.05, s	124.4	7.03, s	124.9	
3'	-	127.9	-	127.6	
4'	8.26, s	135.7, d	8.28, s	136.0	
5'	-	156.6, s	-	155.2	
2''	8.65-8.58, br m[1]	149.8/149.1[2]	8.65-8.58, br m[1]	149.9/149.5[2]	[1] Overlapping signals [2] Could not be assigned
3''	-	123.8	-	124.0	
4''	8.24-7.81, br m[3]	136.5	8.24-7.81, br m[3]	136.7	[3] Overlapping signals
5''	7.49-7.42, br m[4]	130.1	7.49-7.42, br m[4]	129.8, d	[4] Overlapping signals
6''	8.65-8.58, br m[1]	149.8/149.1[2]	8.65-8.58, br m[1]	149.9/149.5[2]	[1] Overlapping signals [2] Could not be assigned
CN	-	118.3	-	119.0	
OH	3.79, v. br[5]	-	3.42, v. br[5]	-	[5] Overlapping signals

Table 9

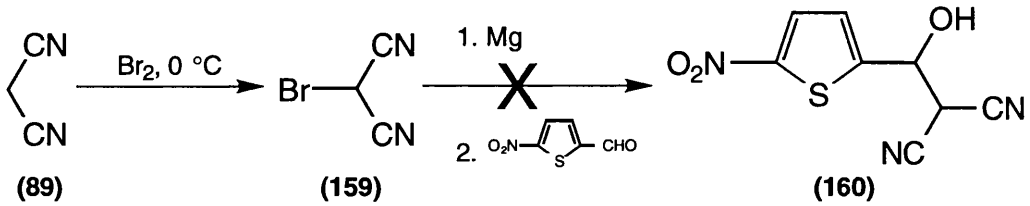


(157) **(158)**

Scheme 24

A suitable malonate derivative is treated with bromine to generate an α-bromo compound. Subsequent treatment of this compound with magnesium in an inert atmosphere should give the Grignard reagent **(157)**, which is reacted *in situ* with the aromatic compound to generate the β-hydroxy-β-thienyl malonate **(158)**.

A number of test reactions were performed to check the validity of this route. Malononitrile was successfully brominated in this manner to give 2-bromomalononitrile **(159)**.[177] This was then treated with magnesium turnings and then with 5-nitro-2-thiophenecarbaldehyde at a variety of temperatures, but only starting materials were recovered **(Scheme 25)**. Attempts to synthesise α-bromothiopheneacetonitrile and α-bromopyridylacetonitrile were unsuccessful.



(89) **(159)** **(160)**

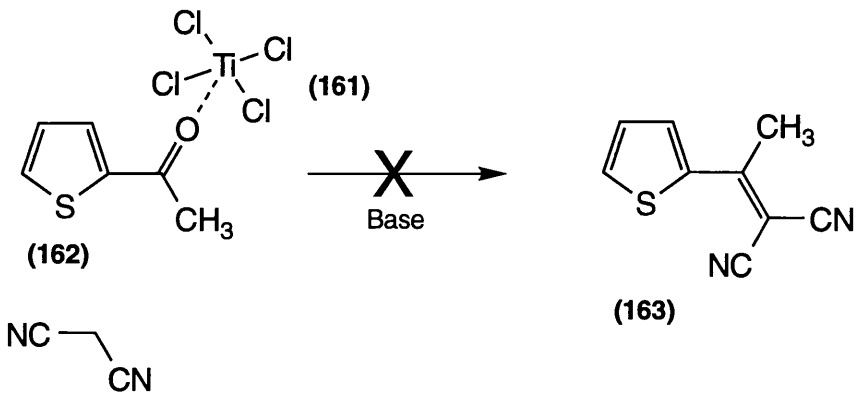
Scheme 25

An alternative approach would be to generate a lithium salt of the malonate derivative and to react it *in situ* with an aromatic aldehyde. This method was not attempted.

3.11 Attempted Synthesis of a β-Methyl Nitrothienyl Tyrphostin

It is possible to perform a variant of the Knöevenagel condensation on ketones using a strong Lewis acid such as $TiCl_4$ **(161)**, which co-ordinates with the carbonyl and therefore increases the δ^+ at the carbon. A strong base is then employed to deprotonate the malonate derivative, and reaction at the electropositive centre of the ketone then results **(Scheme 26)**. When an earlier attempt to react 2-acetylthiophene **(162)** with malononitrile using piperidine failed, we decided to attempt this synthesis using the methodology of

Lehnert.[178] 2-Acetylthiophene and malononitrile were dissolved in dry THF, and cooled to 0 °C before $TiCl_4$ was slowly added. The addition was extremely exothermic, and the mixture was not allowed to heat above 5 °C. After about two hours, pyridine was added to the mixture, giving a deep red solution instantly, and a beige oil separated from the solution. This solution solidified, giving a complex mixture of products (R_F 0.86-0.29, several spots). Attempts to crystallise the mixture from a range of solvent systems were unsuccessful, and the ^1H NMR spectrum did not show a signal corresponding to a deshielded methyl group.



Scheme 26

The same result was observed using a procedure in which $TiCl_4$ and 2-acetylthiophene were stirred at 0 °C for two hours before pyridine and malononitrile were added.

3.12 Conclusion

A total of 40 tyrphostins based upon different nitrothienyl moieties were prepared. In addition, two 'hydrated' tyrphostins were isolated. These were presented for biological evaluation, and the first 1-electron reduction potentials of a number of these compounds were measured (see Chapter 6). These data have been submitted for publication in the scientific press.[179]

4. Synthesis of Quinolyl Tyrphostins

4.1 Introduction

Only a few tyrphostins based upon a six-membered heteroaromatic nucleus have been reported in the literature.[98, 106] Of these, some tyrphostins based upon a 3-substituted pyridyl nucleus have shown a degree of inhibition of function of EGF receptor kinase activity. It may be possible that the electron-deficient character of the pyridine ring may, at least in part, contribute to the biological activity of these compounds compared with that of tyrphostins based upon the BMN nucleus (see Chapter 1). Tyrphostins such as RG13022 **(39)** and RG14620 **(40)** which contain pyridyl moieties in the sidearm have been shown to inhibit human squamous cell carcinoma *in vivo*.[100]

In order to investigate other electron-deficient systems, our group synthesised a series of tyrphostins containing a quinoline nucleus, and recent data[180, 181] have shown that quinolyl tyrphostins display a varied degree of antiproliferative and tyrosine kinase inhibition activity. In particular, 2,4-dicyano-3-amino-5-(2-quinolyl)-penta-2,4-dienonitrile **(165)** and 2,4-dicyano-3-amino-5-(4-quinolyl)-penta-2,4-dienonitrile **(183)** were the most potent compounds in the EGF receptor kinase assay, whereas the equivalent 3-substituted compound **(174)** had an activity at least 15 times lower than the other two. A number of other 2-, 3- and 4-substituted quinolyl tyrphostins prepared by Lear and McKeown[180] possessed moderate antiproliferative activity in an MCF-7 cancer cell assay (details given in Chapters 6 and 7) **(Table 8)**.

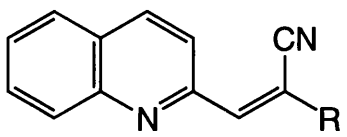
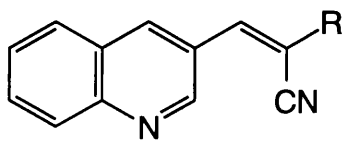
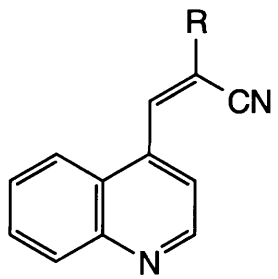
Structure	No.	R	IC_{50} (μM) EGFRK	IC_{50} (μM) MCF-7
	(164)	CN	58	>100
	(165)	$C(NH_2)=C(CN)_2$	1.7	77
	(166)	CO_2Me	200	59
	(167)	CO_2Et	240	17
	(168)	$CO_2{}^nBu$	60	57
	(169)	$CO_2{}^tBu$	145	14
	(170)	2-Pyridyl	>500	5
	(171)	3-Pyridyl	>500	8
	(172)	3-Thienyl	>500	28
	(173)	CN	400	76
	(174)	$C(NH_2)=C(CN)_2$	27	52
	(175)	CO_2Me	450	>100
	(176)	CO_2Et	>500	>100
	(177)	$CO_2{}^nBu$	>500	>100
	(178)	$CO_2{}^tBu$	>500	61
	(179)	$CONH_2$	>500	n.d.
	(180)	$CSNH_2$	300	>100
	(181)	CO_2H	350	>100
	(182)	CN	170	78
	(183)	$C(NH_2)=C(CN)_2$	4.7	55
	(184)	CO_2Me	250	16
	(185)	CO_2Et	330	n.d.
	(186)	$CO_2{}^nBu$	275	22
	(187)	$CO_2{}^tBu$	325	1.6
	(188)	$CONH_2$	140	49
	(189)	$CSNH_2$	38	41
	(190)	CO_2H	380	>100

Table 10 Details of the assays are given in Chapter 7
(reproduced by kind permission of the authors of reference 180).

Quinolines undergo nucleophilic attack at positions 2- and 4-, which are the most electropositive centres on the ring **(Figure 18)**. Taylor used a method involving the pyrolysis of 1-(*x*-quinolyl)ethyl acetate to obtain direct measurements of the electrophilic reactivity at position *x*.[182] He concluded that the order of reactivity to electrophilic attack was

N > 5 > 8 = 6 > 3 > 7 >> 2 > 4

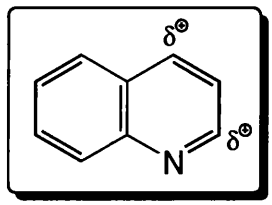


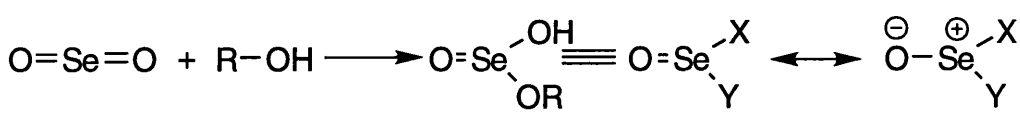
Figure 18

4-Substituted quinolyl tyrphostins are significantly more potent than the 2- or 3-substituted drugs. There is a clear structure-activity relationship (SAR) between the position of the sidearm relative to the ring and the electronegativity at that position.

In order to extend the evidence for the SAR, we considered it essential to synthesise a series of quinolyl tyrphostins. These were prepared from the appropriate methyl quinoline using the method outlined in Section 4.2. We would expect the 7-substituted compounds to have a slightly greater biological activity than the equivalent 3-substituted derivatives. The 6- and 8-substituted derivatives would be expected to have similar activities to each other, but should be substantially less active than the other compounds.

4.2 Synthesis of 6-, 7- and 8-quinolinecarbaldehyde

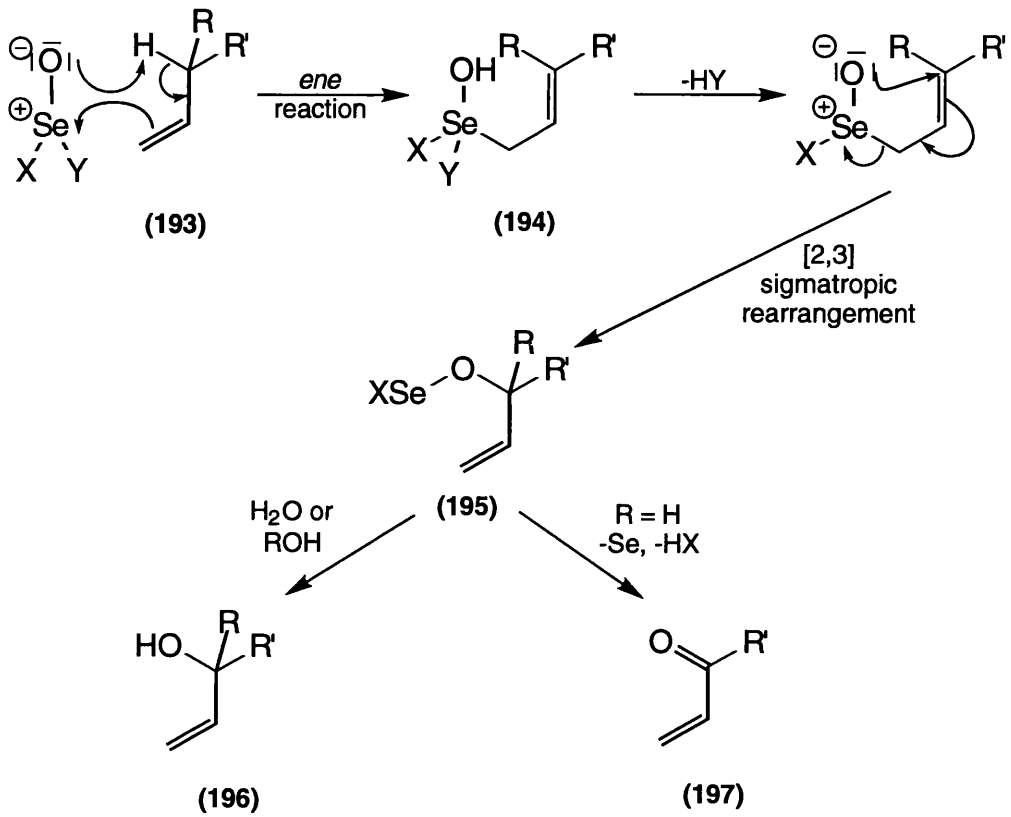
There have been many publications concerning the oxidation by selenium (IV) oxide **(191)** of aromatic methyl groups, and several reviews are devoted to this subject.[183-185] In 1972, the mechanism of these oxidations was clarified[186] and improvements to the experimental procedure have since been possible.



(191) **(192)**

Scheme 27

In the presence of a hydroxylated solvent such as water, alcohol or carboxylic acid, the reagent is in fact the selenious acid, an alkyl selenite or a mixed anhydride **(192) (Scheme 27)**. It is understood that the oxidation is in fact a three-step process. Owing to the basic character of the oxygen atom linked to the selenium atom, the reagent undergoes an *ene* reaction with the substrate **(193)** to give **(194)**. A [2,3]-sigmatropic rearrangement leads to a Se (II) ester **(195)** which is either hydrolysed, as a selenenate, to give the allylic alcohol **(196)**, or decomposes to give the enone **(197) (Scheme 28)**.



(193) **(194)** **(195)** **(196)** **(197)**

Scheme 28

Tadros *et al* reported the syntheses of 5-, 6-, 7- and 8-chloro- and 5-, 6-, 7- and 8-nitroquinolinacrylic acids from the corresponding aldehydes.[187] These had been prepared from the corresponding methylquinoline in moderate yields using SeO_2 as the oxidant.[188] Similarly, Johnson and Hamilton[189] had earlier reported the synthesis of 8-nitro-4-quinolinecarbaldehyde using this methodology.

There are, of course, other reagents which may be employed for the oxidation of methylaromatics. The use of chromic acid,[190] alkaline permanganate,[191] potassium hydrogen persulfate,[192] ruthenium tetroxide[193] and other reagents has been widely reported. The advantage of SeO_2 over these reagents is that the position of the methyl group and the additional presence of other functional groups appears to have little effect upon the yield of the reaction. The products of the reaction are also easily isolated by filtration followed by recrystallisation.

4.2.1 Synthesis of 6-Quinolinecarbaldehyde (198)

This was prepared by treating 6-methylquinoline neat with SeO_2 at reflux for 90 minutes. As an added precaution, the reaction vessel was covered with aluminium foil since the starting material was light-sensitive. Initiation of the reaction was indicated by an exotherm, which raised the temperature to 210 °C. The evolution of heat was allowed to subside before heating at reflux (140 °C) was continued. Thin layer chromatography was employed to check for the presence of the starting material, which had an R_F value of 0.60 compared to 0.38 for the product. After 90 minutes, no starting material was present; however some polymerisation was noted by the presence of some dark baseline material on the TLC. The most effective method for removing the product from selenium metal residues was by filtration through Celite, which also had the added advantage of making the toxic metal residues easier to handle for disposal. The product was then purified by column chromatography followed by recrystallisation.

The ^1H NMR spectrum of **(198) (Figure 19)** possessed a deshielded singlet at δ 10.09 arising from the aldehydic proton, and a set of signals in the aromatic region between δ 8.95-7.42. Some of these signals could be assigned **(Table 11)** whilst the remaining signals for the protons on C-4, C-5, C-7 and C-8 came as a multiplet in the middle of this group at δ 8.25-8.04. In the ^{13}C NMR spectrum, each signal was easily assigned. No signal for a methyl group at δ 19.2 in the ^{13}C NMR spectrum or at δ 1.19 in the ^1H NMR spectrum was observed.

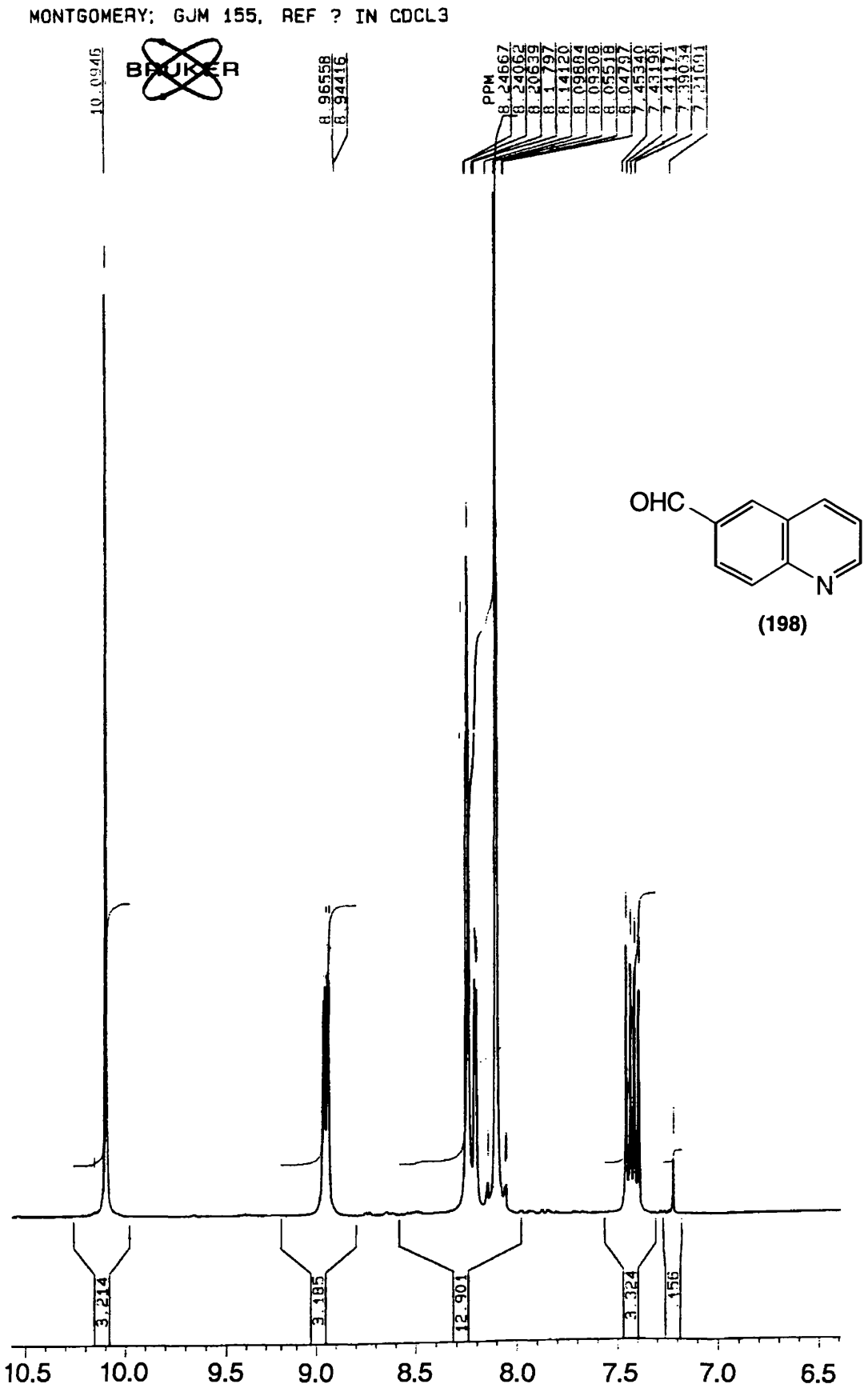
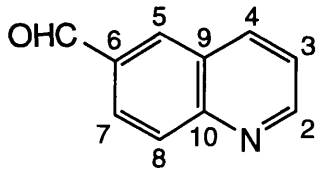

MONTGOMERY; GJM 155, REF ? IN CDCL3
BRUKER
10.0946
8.96558
8.94416
PPM
8.24667
8.24062
8.20639
8.1797
8.14120
8.09864
8.09308
8.05518
8.04797
7.45340
7.43198
7.41171
7.39034
7.21601
OHC
N
(198)
3.214
3.185
12.901
3.324
1.156
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5

Figure 19



(198)

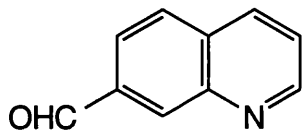
Ring Position	δ_H	δ_C
2	8.95, d, ^3J 4.28 Hz	153.0
3	7.42, dd, ^3J 8.34, 4.28 Hz	122.1
4	8.25-8.04, m	133.6
5	8.25-8.04, m	130.7
6	-	134.2
7	8.25-8.04, m	126.6
8	8.25-8.04, m	137.4
9	-	127.6
10	-	150.7
CHO	10.09, s	191.4

Table 11

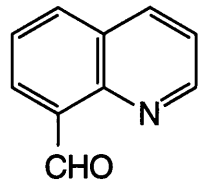
The IR spectrum of **(198)** contained absorbances for the main functional groups, namely a carbonyl stretch at 1692 cm^{-1}, signals arising from aromatic bonds at 1623, 1575 and 1501 cm^{-1} and two sharp absorbances at 838 and 801 cm^{-1} from out-of-plane aromatic C-H bends. In the mass spectrum, the molecular ion came as the largest peak at *m/z* 157, with a signal corresponding to loss of the aldehyde group at *m/z* 128.

4.2.2 Synthesis of 7-Quinolinecarbaldehyde (199)

The starting material was commercially available from Lancaster as a mixture of 5- and 7-methylquinoline in a 30:70 ratio. Since the boiling points and R_F values of these compounds were very similar, we decided to treat the mixture with selenium (IV) oxide and then purify the oxidation products. Using the procedure outlined in Section 4.2.1, heating the methylquinolines with SeO_2 at 70 °C resulted in a strong exotherm which raised the temperature to *ca.* 120 °C. When this had subsided, heating at 70 °C was continued for 5 hours until TLC showed the absence of starting materials. Extraction of the products and concentration gave an orange oil, which was purified by column chromatography to give 7-quinolinecarbaldehyde **(199)** as the major product. A minor impurity was tentatively assigned to 5-quinolinecarbaldehyde from similar R_F value to **(199)**.



(199)



(200)

The ^{1}H NMR spectrum of **(199)** showed a very deshielded singlet at δ 10.16 for the aldehydic proton. The carbonyl carbon in the ^{13}C NMR spectrum was not observed even when the experiment was run overnight, although a signal for an aldehydic carbonyl stretch was observed as a strong absorption at 1690 cm^{-1} in the IR spectrum. Other signals at 3022, 840 and 807 cm^{-1} arose from aromatic C-H bond vibrations, and were accompanied by a set of three signals of medium intensity corresponding to an aromatic system at 1623, 1572 and 1502 cm^{-1}. The largest signal in the mass spectrum corresponded to the molecular ion at *m/z* 157, with a signal at *m/z* 128 for loss of the aldehyde from the parent molecule.

4.2.3 Synthesis of 8-Quinolinecarbaldehyde (200)

8-Methylquinoline and SeO_2 were reacted in much the same manner as previously, although the reaction time was extended to 22 hours, after which there still remained a considerable quantity of starting material. When the reaction was repeated with up to two equivalents of SeO_2, the same effect was observed. Although the SeO_2 appeared to be pure (an off-white crystalline solid), it was purified by sublimation[194] and the reaction was repeated using one equivalent over the same period of time as before, but this did not give a marked increase in the yield (13%, as opposed to 11% before sublimation). The product **(200)** was isolated from the starting material by filtration and purification by column chromatography.

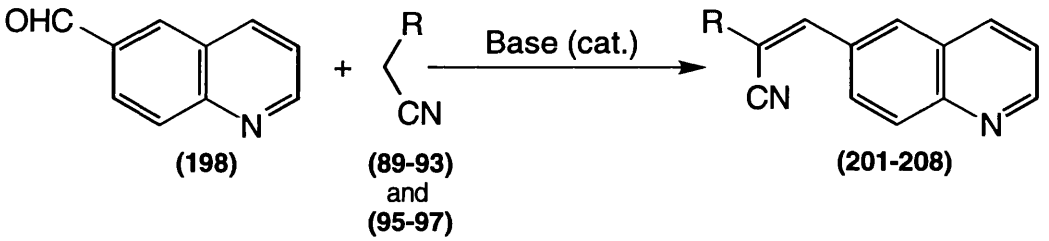
The most distinguishing features in the ^{1}H and ^{13}C NMR spectra were the signals corresponding to the aldehyde moiety. The presence of this functional group was further confirmed by IR spectroscopy, where a sharp absorption at 1709 cm^{-1} was observed. Unlike the mass spectra of the other two quinoline carbaldehydes, only a very small signal was observed for the molecular ion, although larger signals corresponding to fragmentation of the parent molecule were present.

4.2.4 Use of Oxidants to Regenerate SeO_2

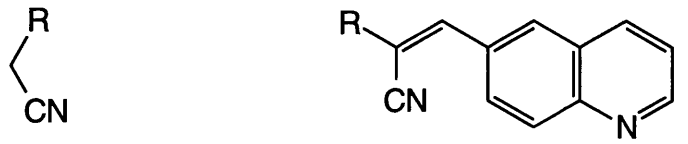
We were concerned about the generation of highly toxic selenium metal residues from these reactions. Sharpless reported[195] that such reactions can also be accomplished using *t*butyl hydroperoxide to oxidise $Se(OH)_2$ or selenium metal, which need only be present in catalytic amounts. However, significant amounts of side-products are formed when the double bond is in a ring system.[196] An alternative oxidant is 4-methylmorpholine-*N*-oxide, but when we attempted to repeat the above reactions using this reagent in the presence of a catalytic amount of SeO_2, the yields fell dramatically (from 57% to 15% in the case of 7-quinoline carbaldehyde **(199)**).

4.3 Synthesis of 6-Quinolyl Tyrphostins

These were prepared in the usual manner by Knöevenagel condensation of 6-quinolinecarbaldehyde **(198)** with a number of malonate derivatives **(Scheme 29** and **Table 12)**. As with these and other quinolyl tyrphostins, solubility problems prevented recrystallisation from organic solvents, but the products, which were normally powders, were pure by microanalysis and accurate mass spectrometry.



Scheme 29



R	No.	Base	No.	Yield (%)
CN	**(89)**	-	**(201)**	90
$C(NH_2)=C(CN)_2$	**(90)**	dilute piperidine	**(202)**	89
CO_2Me	**(91)**	-	**(203)**	61
CO_2Et	**(92)**	-	**(204)**	47
$CO_2{}^nBu$	**(93)**	piperidine	**(205)**	45
$CO_2(TEG)OMe$	**(95)**	piperidine	**(206)**	63
$CONH_2$	**(96)**	piperidine	**(207)**	58
$CSNH_2$	**(97)**	-	**(208)**	88

Table 12

Compound **(201)** was previously reported in a patent for electrophotographic photoreceptors,[197] although no characterisation of this compound was noted. In the ^{1}H NMR spectrum **(Figure 20)**, the most deshielded signal came at δ 9.09 as a broad doublet of doublets with couplings of 4.21 and 1.47 Hz. This signal corresponded to the proton in the 2-position on the quinoline ring. The couplings arose from an interaction with the protons in the 3- and 4-positions, which came as another doublet of doublets at δ 7.70 and a very broad signal at δ 8.54, respectively. The protons in the 5- and 7-positions came as very broad doublets at δ 8.21 and 8.32; the broadening of these signals may be a result of additional coupling with the proton in the 8-position, which was assigned to a very broad signal at δ 8.57. These data are summarised in **Table 13.**

The ^{13}C NMR spectrum **(Figure 21)** consisted of a signal at δ 160.7 (CH) and one at δ 82.6 for the olefinic carbons, accompanied by 9 signals between δ 154 and δ 123 for the aromatic carbons and two singlets at δ 114.3 and 112.2 for the nitrile groups. The IR spectrum contained the expected signals at 1617, 1577 and 1500 cm^{-1} for the aromatic ring, accompanied by other signals at 3034 cm^{-1} (aryl C-H), 2224 cm^{-1} (CN) and at 833 and 794 cm^{-1} (2 adjacent and 3 adjacent out-of-plane aryl C-H bends). The molecular ion was observed in the mass spectrum and signals corresponding to loss of fragments of the sidearm from the parent molecule were also present.

The derivative formed from malononitrile dimer, **(202)**, displayed two broad singlets at δ 9.28 and δ 9.18 in its ^{1}H NMR spectrum corresponding to the $-NH_2$ protons. The olefinic proton came at δ 8.26, whilst signals for the nitrile carbons were observed at δ 115.6, 114.9 and 114.7 in the ^{13}C NMR spectrum. In the IR spectrum, signals for out-of-plane aromatic C-H bends came at 826 and 794 cm^{-1}, with a vibration resulting from a C=C bond at 1656 cm^{-1}. The molecular ion came at *m/z* 271 in the mass spectrum.

Tyrphostins possessing an ester group in the sidearm, **(203-206)**, all displayed characteristic signals in the ^{1}H and ^{13}C NMR spectra. The IR spectra contained characteristically sharp absorptions around 1720 cm^{-1} arising from carbonyl stretches in an αβ-unsaturated system, accompanied by signals corresponding to aryl C-H stretches and bends. In all cases the molecular ion and signals corresponding to loss of fragments of the ester moiety were observed in the mass spectra.

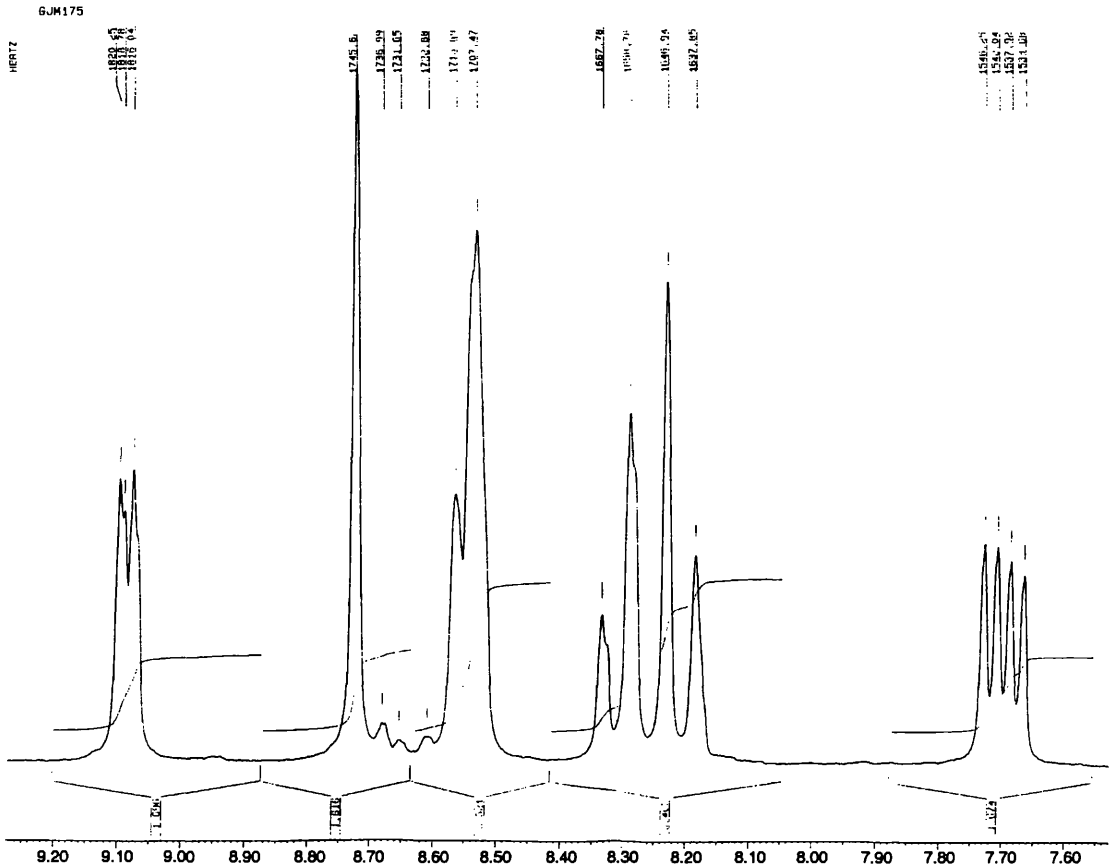
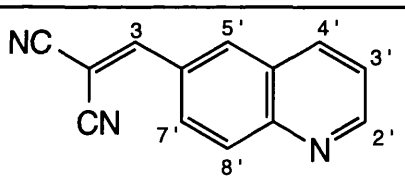


Figure 20



(201)

Position	δ_H	Multiplicity	Coupled to
3	8.73	s	-
2'	9.09	br dd	3' and 4'
3'	7.70	dd	2' and 4'
4'	8.54	v. br	2' (and 3'?)
5'	8.21	v. br d	7' (and 8'?)
7'	8.32	v. br d	8' (and 7'?)
8'	8.57	v. br	7' (and 5'?)

Table 13

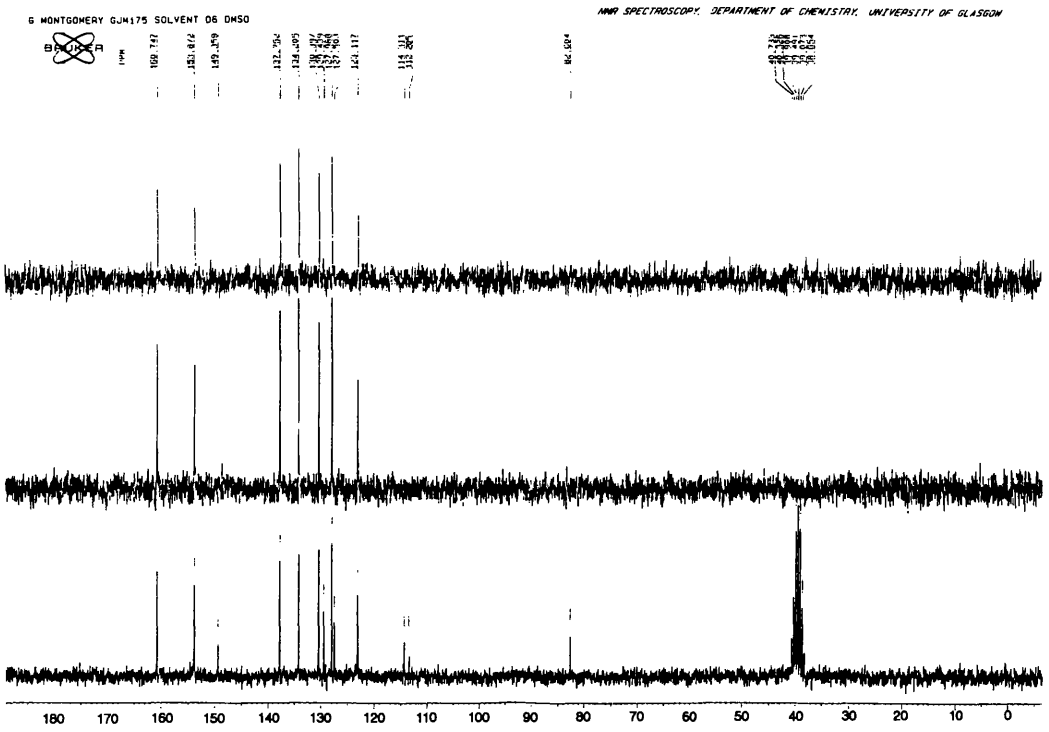
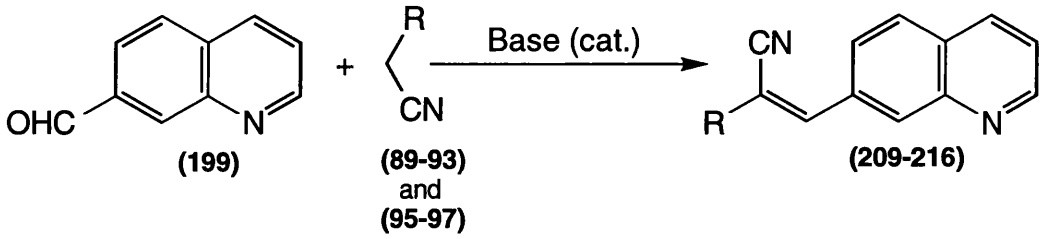


Figure 21

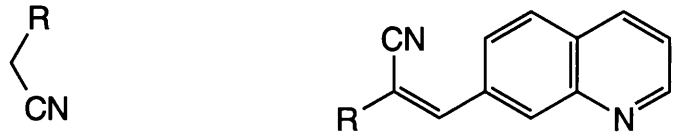
Compounds **(207)** and **(208)** were then synthesised. No catalytic amount of base was required in the preparation of the latter, and the products precipitated from solution within 15 minutes. Attempts to recrystallise the products from ethyl acetate were unsuccessful, although microanalysis confirmed their purity. Broadening of most of the signals in the aromatic region of the ^{1}H NMR spectra was observed, although doublets of doublets at δ 8.39 **(207)** and 7.65 **(208)** were assigned to the protons in position 3 of the quinoline rings. Two singlets around δ 7.8 for the amide and δ 9.8 for the thioamide were assigned to the $-NH_2$ protons. A singlet corresponding to the carbonyl or thiocarbonyl carbon was not observed in the ^{13}C NMR spectra, although sharp absorptions at 1699 cm^{-1} were assigned to a C=O stretch for **(207)**. Other signals in the ^{13}C NMR spectra could be assigned to the remaining carbons in the compounds. The molecular ions were accompanied by signals at *m/z* 179 for loss of $-CXNH_2$ from the parent molecules.

4.4 Synthesis of 7-Quinolyl Tyrphostins

These were all prepared in moderate to good yield in the usual manner **(Scheme 30** and **Table 14).**



Scheme 30



R	No.	Base	No.	Yield (%)
CN	**(89)**	-	**(209)**	79
$C(NH_2)=C(CN)_2$	**(90)**	-	**(210)**	86
CO_2Me	**(91)**	piperidine	**(211)**	46
CO_2Et	**(92)**	piperidine	**(212)**	67
$CO_2{}^nBu$	**(93)**	piperidine	**(213)**	42
$CO_2(TEG)OMe$	**(95)**	piperidine	**(214)**	78
$CONH_2$	**(96)**	piperidine	**(215)**	73
$CSNH_2$	**(97)**	-	**(216)**	45

Table 14

2-Cyano-3-(7-quinolyl)propenonitrile **(209)** was the first tyrphostin to be prepared in this series. The starting materials reacted within one minute and without the addition of base to give the product as a beige solid in good yield. In the ^1H NMR spectrum a total of three doublets of doublets, two doublets and two singlets were observed. The three dds corresponded to the protons in the 2-, 3- and 6-positions on the ring, coming at δ 9.06, 7.73 and 8.11 respectively. The doublets at δ 8.49 and 8.21 were from the protons in positions 4 and 5, whilst singlets at δ 8.63 and 8.75 corresponded to the proton on position 8 and the olefinic proton. The ^{13}C NMR contained signals at δ 161.0 (CH) and 83.3 for the olefinic carbons and two singlets at δ 114.2 and 113.2 for the nitrile groups. The IR spectrum did not give a great deal of information, although a sharp peak at 2228 cm^{-1} arose from two nitrile groups and signals were also observed for aromatic bonds and for aromatic C-H stretches and bends. The molecular ion was the largest signal in the mass spectrum, and was observed at *m/z* 205.

The ^1H NMR spectrum of **(210)** did not differ substantially from that of **(209)** except for the presence of two singlets at δ 9.30 and 9.25 for the $-NH_2$ protons.

Three signals for the nitrile carbons came at δ 115.6, 114.9 and 114.7 in the ^{13}C NMR spectrum, and additional signals at δ 165.4 and 103.6 were assigned to quaternary olefinic carbons. The more deshielded of these two corresponded to the terminal olefinic carbon bearing two nitrile groups. More information was obtained from the IR spectrum, since signals at 2366 and 1667 cm^{-1} were attributed to an N-H bend and a C=C in conjugation with an aromatic system. Absorptions arising from a nitrile group, and from aromatic C-C and C-H bonds were also observed. The molecular ion was accompanied by signals at *m/z* 245 for loss of one nitrile and at *m/z* 206 for loss of two nitriles and one carbon from the parent molecule in the mass spectrum.

Methyl 2-cyano-3-(7-quinolyl)propenoate **(211)** was then prepared. A yellow precipitate formed after one minute, and TLC showed the formation of a polar material with R_F 0.23. The yield for this reaction was fairly low at 46 % and despite repeated attempts at various temperatures and using other bases such as pyridine, this yield could not be increased. The ^{13}C NMR spectrum contained a deshielded signal at δ 159.3 for the carbonyl carbon, whilst the methyl group on the ester came at δ 54.0 (and at δ 3.95 in the ^{1}H NMR spectrum, which also contained a singlet at δ 8.76 from the olefinic methine proton). The IR spectrum had a sharp absorption at 1727 cm^{-1} arising from an αβ-unsaturated carbonyl stretch, a weak signal at 2219 cm^{-1} from a nitrile group, and weak signals at 833 and 780 cm^{-1} from two and three adjacent aromatic C-H out-of-plane bends. The largest signal in the mass spectrum corresponded to the molecular ion, and signals at *m/z* 223, 207 and 179 arose from loss of a methyl, a methoxy and a methyl ester moiety, respectively.

The NMR spectra of the ethyl **(212)** and ^{n}butyl **(213)** esters differed only in respect to the signals for the alkyl groups, which were observed as signals with the chemical shifts and multiplicity consistent with those expected. αβ-Unsaturated carbonyl stretches at around 1720 cm^{-1} and signals around 1600 cm^{-1} for an aromatic system were observed in the IR spectra. Additional signals arising from out-of plane aryl C-H bends came at ~830 and ~770 cm^{-1}. Both compounds gave signals for the molecular ion in their mass spectra.

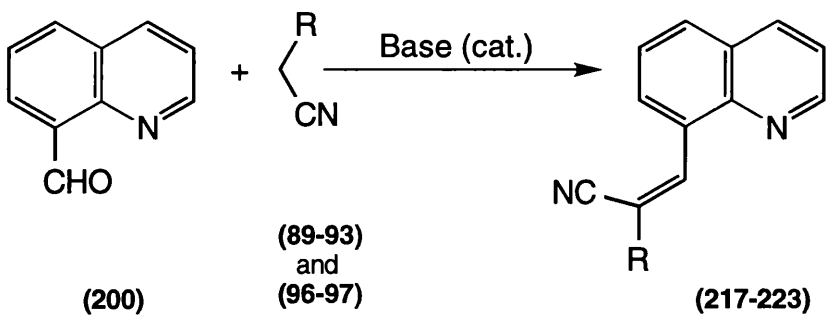
Preparation of the tri(ethyleneglycol) ester derivative **(214)** required a slightly longer reaction time of one hour to give the product, which was a brown oil at room temperature. The aromatic region of the ^{1}H NMR spectrum was very similar to that of the other ester derivatives, and a broad multiplet of relative intensity 12 between δ 3.62-2.92 was assigned to the six groups of methylene protons. The ^{13}C NMR spectrum had six signals between δ 71.8-57.7 for these groups, and the terminal methyl group was at δ 1.09 in the ^{1}H spectrum and at

δ 18.3 in the ^{13}C NMR spectrum. In the IR spectrum, signals consistent with functional groups in this molecule were assigned. Of particular note were two signals at 1452 and 730 cm^{-1} arising from C-H deformations and a $-CH_2-$ rocking, and another at 2878 cm^{-1} from an $O-CH_3$ vibration. The molecular ion came as a signal at *m/z* 371 in the mass spectrum. A signal at *m/z* 251 corresponded to loss of $(OCH_2CH_2)_2OCH_3$, whilst one at *m/z* 207 arose from the loss of $(OCH_2CH_2)_3OCH_3$ and a hydrogen from the molecular ion.

The synthesis of 2-cyano-3-(7-quinolyl)propenamide **(215)** required the presence of piperidine, whereas 2-cyano-3-(7-quinolyl)propenthioamide **(216)** did not. Signals arising from the $-NH_2$ protons were seen between δ 10.13 and 9.81 in the ^1H NMR spectra of the products, and the olefinic proton was seen at δ 8.73 in the spectrum of **(216)**. A complex multiplet in the ^1H NMR spectrum of **(215)** was assigned to the aromatic and olefinic protons. In the ^{13}C NMR spectra, the carbonyl and thiocarbonyl carbons came at δ 168.8 and 172.1. Signals for the olefinic carbons were observed in both these spectra. Amide and thioamide N-H stretches were observed in the IR spectra, whilst C=O and C=S vibrations came as sharp absorptions at 1690 and 1123 cm^{-1}. The molecular ions were observed in the mass spectra at *m/z* 222 and 239, respectively.

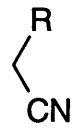
4.5 Synthesis of 8-Quinolyl Tyrphostins

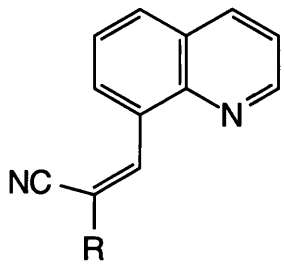
The final set of quinolyl tyrphostins was prepared as before **(Scheme 31** and **Table 15).**



(200) **(89-93)** and **(96-97)** **(217-223)**

Scheme 31





R	No.	Base	No.	Yield (%)
CN	**(89)**	piperidine	**(217)**	28
$C(NH_2)=C(CN)_2$	**(90)**	-	**(218)**	78
CO_2Me	**(91)**	piperidine	**(219)**	60
CO_2Et	**(92)**	piperidine	**(220)**	27
$CO_2{}^nBu$	**(93)**	piperidine	**(221)**	28
$CONH_2$	**(96)**	dilute piperidine	**(222)**	92
$CSNH_2$	**(97)**	dilute piperidine	**(223)**	49

Table 15

The derivatives **(217** and **218)** made from malononitrile monomer **(89)** and its dimer **(90)** were the first to be prepared. The ^1H NMR spectra contained singlets corresponding to the olefinic proton at *ca* δ 8.5, with two additional singlets at δ 9.31 and 9.25 for the $-NH_2$ protons on **(218)**. Signals corresponding to the nitrile moieties came at ~δ 115 in the ^{13}C NMR spectra. The IR spectra each contained absorptions corresponding to aromatic C-H stretches at ~3050 cm^{-1}, out-of-plane C-H bends around 800 cm^{-1} and a C=C bond conjugated to an aromatic system at 1620 cm^{-1}. The IR spectrum of **(218)** contained an additional sharp signal at 2208 cm^{-1} which was attributed to the three nitrile groups. Molecular ions were observed in the mass spectra.

Tyrphostins containing methyl **(219)**, ethyl **(220)** and ^{n}butyl **(221)** ester groups on the sidearm were then synthesised. Each required the addition of a catalytic amount of piperidine, and the methyl and ethyl esters were isolated as yellow solids within one hour, whereas the ^{n}butyl ester product took almost a full day to precipitate from solution, and even after this time the yield was low. However, microanalysis of the products confirmed their purity. The aromatic regions of the ^1H and ^{13}C NMR spectra were similar, and the alkyl fragments of the ester gave characteristic upfield signals and multiplicities. The IR spectra contained the usual signals consistent with this type of compound, namely carbonyl stretches at ~1710 cm^{-1} (indicative of an $\alpha\beta$-unsaturated C=O), aromatic C-C vibrations at 1580 and 1520 cm^{-1}, C=C conjugated to an aromatic system at ~1620 cm^{-1} and aromatic C-H stretches and bends coming at 3064 and 804-802 cm^{-1}. Although no signal around 2220 cm^{-1} from a nitrile stretch was observed in any of these spectra, all of the ^{13}C NMR spectra possessed

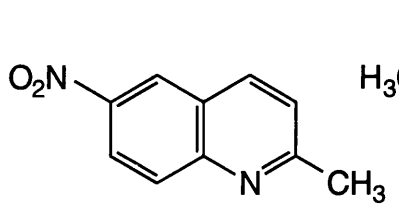
singlets around δ 115 corresponding to this moiety. Mass spectra contained somewhat small signals corresponding to the molecular ions.

We then attempted a synthesis of the tri(ethyleneglycol) ester derivative using standard conditions. Tri(ethylene glycol) cyanoacetate methyl ether **(95)** was treated with 8-quinolinecarbaldehyde **(200)** and piperidine, but no product was noted after several hours mixing. Sequential addition of pyridine, K_2CO_3 and triethylamine to the reaction failed to give any product, and when KF was added, a black tar formed instantly. It is clear that the choice of base is of extreme importance for this reaction, but we were unable to identify an appropriate base to be used for the synthesis of this compound.

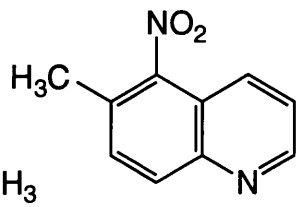
The series was completed with the syntheses of the amide and thioamide derivatives **(222** and **223)**. Extended reaction times of up to 24 hours and the use of piperidine was required, and the products were isolated in good yields. The $-NH_2$ protons were observed in each ^1H NMR spectrum, and the carbonyl and thiocarbonyl carbons came at δ 168.2 and 170.1 respectively in the ^{13}C NMR spectra. Aromatic C-H stretches and bends (~3060 and 800 cm^{-1}), nitrile stretches (~2200 cm^{-1}), C=C conjugated to an aromatic system (1618-1606 cm^{-1}) and N-H stretches at 3400-3300 cm^{-1} were observed in both IR spectra, and the IR spectrum of **(222)** also contained an absorption of medium intensity at 3058 cm^{-1} for an amide N-H stretch. The molecular ion was observed in both the mass spectra.

4.6 Nitroquinolinecarbaldehydes

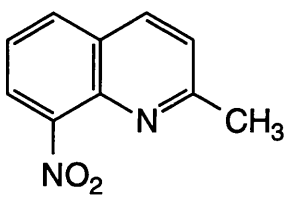
As a continuation of our investigation of potential bioreducible tyrphostins, we considered it desirable to undertake a synthesis of a nitroquinolinecarbaldehyde. Earlier synthetic methods involving the oxidation of 2- or 4-methylquinolines which had a nitro substituent in the 5-, 6-, 7- or 8-position had been reported, [187, 189] and we wished to develop this work further to investigate similar systems.



(224)

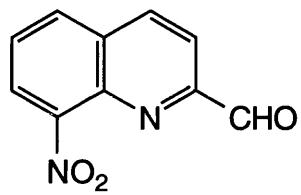


(225)



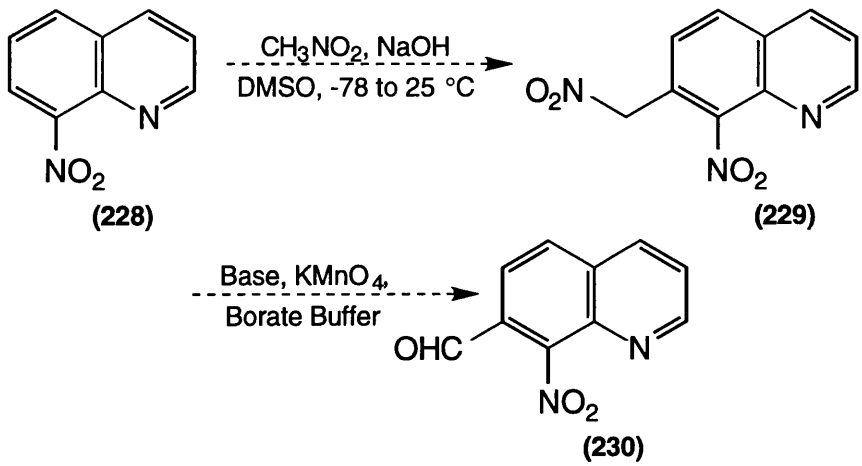
(226)

Several methylnitroquinolines such as **(224-226)** are commercially available. Following existing methodology, we attempted to oxidise the methyl group on these compounds using SeO_2.[188] Each compound is a solid at room temperature, so these compounds were dissolved in dioxan before addition of SeO_2. Despite repeated attempts, no reaction was observed after several hours. We then tried using toluene or xylene as solvent to allow an increase in the reaction temperature, but again no reaction was observed except for 8-nitro-2-methylquinoline, where the product **(227)** was isolated in 53 % yield when toluene was used. The ^1H NMR spectrum contained a deshielded signal at δ 10.11 for the aldehyde, which was also seen in the ^{13}C NMR spectrum at δ 192.8. No signals corresponding to the methyl group were observed in either of these spectra. The IR spectrum had a sharp signal from a carbonyl stretch at 1716 cm^{-1}.



(227)

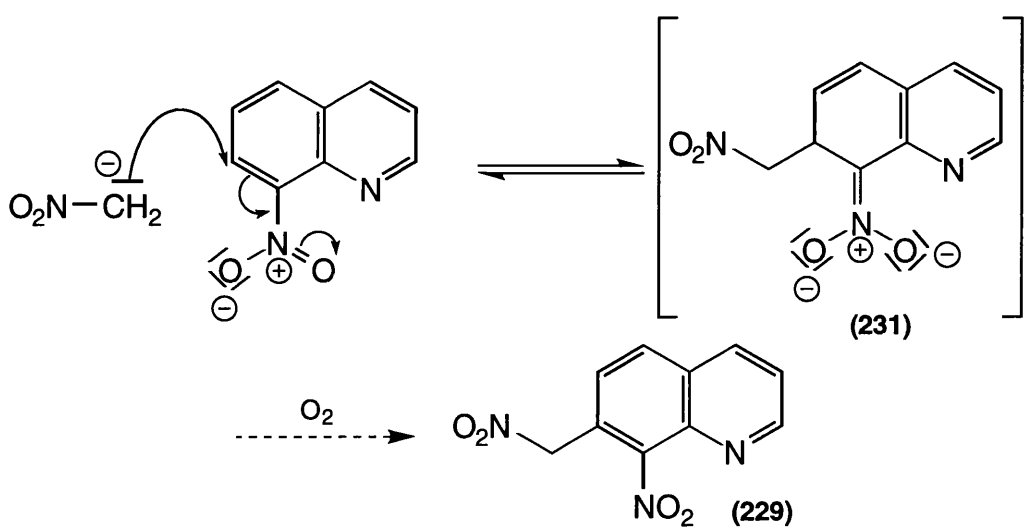
A second method involving the route shown in **Scheme 32** was also attempted to introduce an aldehyde moiety α to the nitro group.[198] Reaction of the nitroquinoline with the nitromethane anion generates the α-nitromethyl species such as **(229)**, which can then be oxidised using Nef methodology.[199] The overall process can be considered as a nucleophilic formylation of the nitroquinoline in which the nitromethyl anion serves as a formyl anion equivalent.



Scheme 32

We attempted the first step using the methods employed by Danikiewicz and Mąkosza[198] when they introduced a nitromethyl group onto 1-nitronaphthalene or 8-nitroquinoline. When a solution of 8-nitroquinoline **(228)** in DMSO was treated with a solution of nitromethane and sodium hydroxide (two equivalents) in DMSO at 0 °C, a red colouration of the mixture occurred instantly, and TLC revealed the presence of two very faint new spots (visible only by UV) with R_F 0.38 and 0.32 accompanied by spots corresponding to starting material at R_F 0.53 and baseline material, and even after several hours the intensity of these new spots did not increase. It was assumed that these corresponded to the *ortho*-product and some of the *para*-isomer, and so the reaction was terminated, and the excess NaOH was neutralised using acetic acid. Purification of the products by column chromatography over silica was attempted, but the only material to be isolated was starting material accompanied by a substantial amount of baseline material. We then repeated the reaction at a variety of temperatures (between -78 °C and room temperature), but the same result was observed.

Normally the rate-determining step of this reaction is the addition of the nitromethyl anion to the aromatic species, forming a *pseudo* Meisenheimer salt such as **(231)**. These are generally stable and some have been isolated, and a number of studies have been performed on these salts which have shown that the presence of additional nitro moieties on the aromatic system is generally associated with increased stability.[200] Whilst no reports of Meisenheimer salts based upon a quinoline moiety have been reported, Terrier showed that for nitronaphthyl and nitropyridyl Meisenheimer salts, stability was increased with additional nitro moieties irrespective of their position relative to the first nitro group.[200]

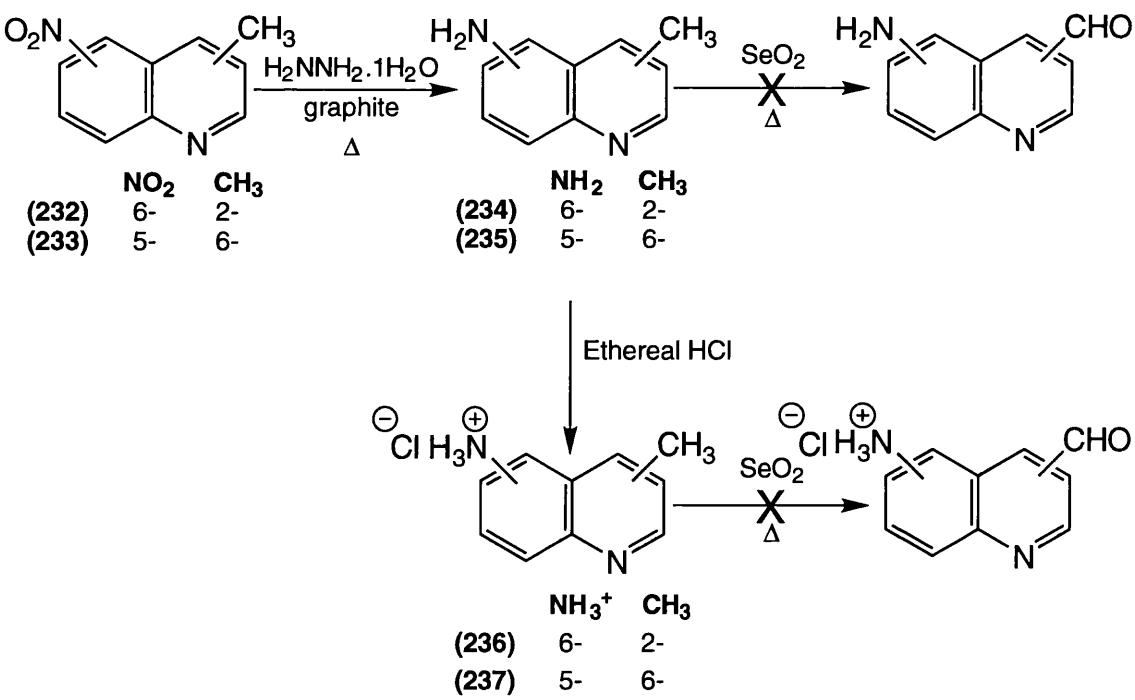


Scheme 33

In this case, it appears that the presence of a second extra-annular nitro group confers stability to the intermediate. This would account for the presence of baseline material. In this instance, the first step may not be rate-limiting, and the second step could be slower, accounting for the fact that no product was observed.

4.7 Aminoquinolinecarbaldehydes

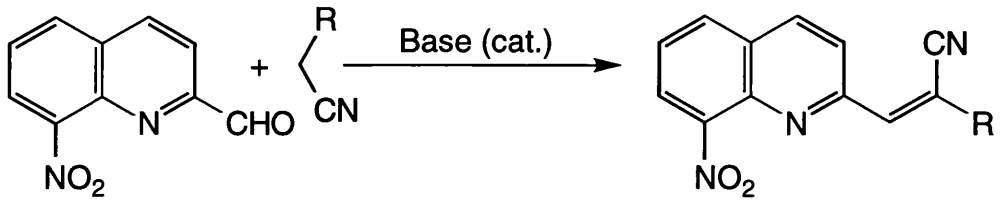
In order to improve water solubility of our compounds, we decided to attempt to synthesise a number of quinolinecarbaldehydes which possessed an amino group. Compounds **(232)** and **(233)** were reduced using hydrazine monohydrate **(Scheme 34)** in the presence of a catalytic amount of graphite powder.[201] It has been proposed that under these conditions, molecular hydrogen is adsorbed onto the surface of the graphite,[202] although it is generally accepted that reductions of nitro groups by Sn/HCl involves an electron transfer process. Signals arising from the -NH_2 moieties were observed in the ^1H NMR and IR spectra of these products. We then treated either of the amines **(234** or **235)** or its HCl salt **(236** or **237)** with SeO_2 in the usual manner, but in each case no reaction was observed.



Scheme 34

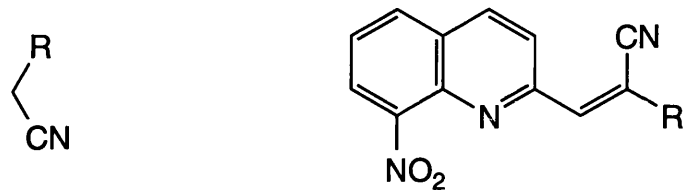
4.8 Synthesis of 8-Nitro-2-Quinolyl Tyrphostins

8-Nitro-2-quinoline carbaldehyde **(227)** was the only nitroquinolinecarbaldehyde that we could prepare. For comparison with the most active 2-quinolyl tyrphostins which were previously reported,[180] we decided to prepare the derivatives **(238)** and **(239)** from malononitrile **(89)** and its dimer **(90)** **(Scheme 35** and **Table 16)**.



(227) **(88-89)** **(238-239)**

Scheme 35



R	No.	Base	No.	Yield (%)
CN	**(89)**	dilute piperidine	**(238)**	69
$C(NH_2)=C(CN)_2$	**(90)**	-	**(239)**	30

Table 16

Both products were identified firstly from their ^1H NMR spectra, which differed from the starting material by the presence of a deshielded singlet at ~δ 8.3 for the olefinic proton, whilst the spectrum of **(239)** also contained two slightly broad singlets at δ 8.36 and 8.31 from the NH_2 protons. Nitrile signals came between δ 116 and 113 in the ^{13}C NMR spectra. C-NO_2 and nitrile stretches were observed in the IR spectra, and were accompanied by signals corresponding to aromatic C-H stretches and bends.

4.9 Conclusions

We have been able to develop a synthetic route to give series of 6-, 7- and 8-substituted quinolyl tyrphostins which involves oxidation of the equivalent methylquinoline using SeO_2. We were unable to modify the reactions by adding an oxidant to reoxidise the selenium and thereby reduce the production of highly toxic selenium metal residues, but recovery of the metal was complete and therefore could be reoxidised separately for repeated use. When the aromatic ring was modified by including either a nitro group or an amino moiety,

this methodology failed except for one instance, where a quantity of 8-nitro-2-quinolinecarbaldehyde was isolated and was subsequently treated with monomeric or dimeric malononitrile to give two nitroquinolyl tyrphostins.

The 25 quinolyl tyrphostins synthesised have been submitted for biological testing in cancer cells which have a high concentration of the EGF receptor. The measurement of the reduction potentials of compounds **(238)** and **(239)** are discussed in Chapter 6.

5. Attempted Synthesis of an ADEPT Prodrug

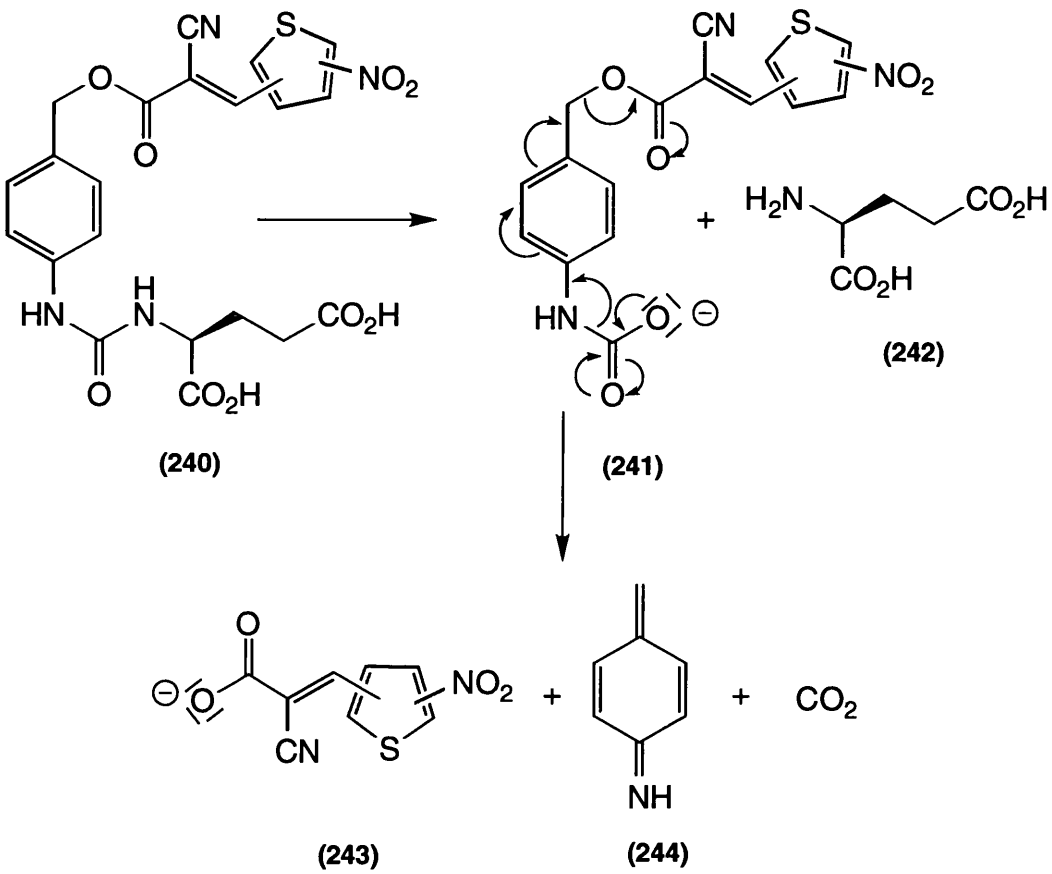
5.1 Introduction

Antibody-Directed Enzyme Prodrug Therapy (ADEPT) is a new approach which delivers potent anticancer agents to cancer cells. The patient is exposed to a monoclonal antibody-enzyme conjugate (AEC) which recognises surface antigens unique to the cancer cells before a prodrug is administered. The prodrug is hydrolysed by the AEC, and decomposes to release the active drug. The advantages of this approach are numerous (see Chapter 2).

A prodrug **(240)** containing an L-glutamate moiety was chosen since a suitable antibody-enzyme conjugate containing carboxypeptidase G_2 (CPG_2) was available. This enzyme hydrolyses amide bonds adjacent to an L-glutamic acid residue[154, 155] (see **Scheme 7** in Chapter 2). We intended to link a tyrphostin through an aromatic spacer to the glutamate, which is essential for recognition by the enzyme. It was anticipated that the prodrug would also act to increase the solubility of the tyrphostin fragment. Cleavage of the amide bond by the enzyme would result in the release of a free electron pair which would assist in the breakdown of the compound, and would subsequently release the drug **(Scheme 35)**. Sutherland[203] had previously reported the synthesis of an ADEPT prodrug fragment consisting of a glutamic acid attached to the aromatic spacer by a carbamate linkage, but we decided to attempt to join these groups *via* a urea linkage. This was chosen because of its stability and apparent ease of synthesis. It was also vital for us to choose tyrphostins which showed an acceptable degree of growth and/or tyrosine kinase inhibition (see Chapter 6), and which possessed functional groups which could be easily adapted to attach the drug to the spacer unit in the ADEPT prodrug. We therefore chose compounds **(141)** and **(144)**, in which the carboxylic acid moiety could be utilised to form either an ester or amide bond with the spacer group. However, as will be seen in Chapter 6, the biological activity of these compounds was much lower compared to other tyrphostins which had been synthesised. This

may be attributed to the low lipid solubility of carboxylate anions. The strategies discussed in this Chapter were therefore employed to develop the chemistry of synthesising prodrugs for ADEPT rather than to make a viable prodrug for use in this therapeutic approach.

When the glutamic acid is cleaved from the parent molecule **(240)** which has the tyrphostin moiety attached by an ester linkage, electrons should be donated from the resulting carbamate **(241)** to assist in the release of the drug. The breakdown products would be the tyrphostin carboxylate anion **(243)**, an iminoquinone methide **(244)** which may itself display toxicity by reacting with cellular nucleophiles such as glutathione,[204] and one mole of carbon dioxide.

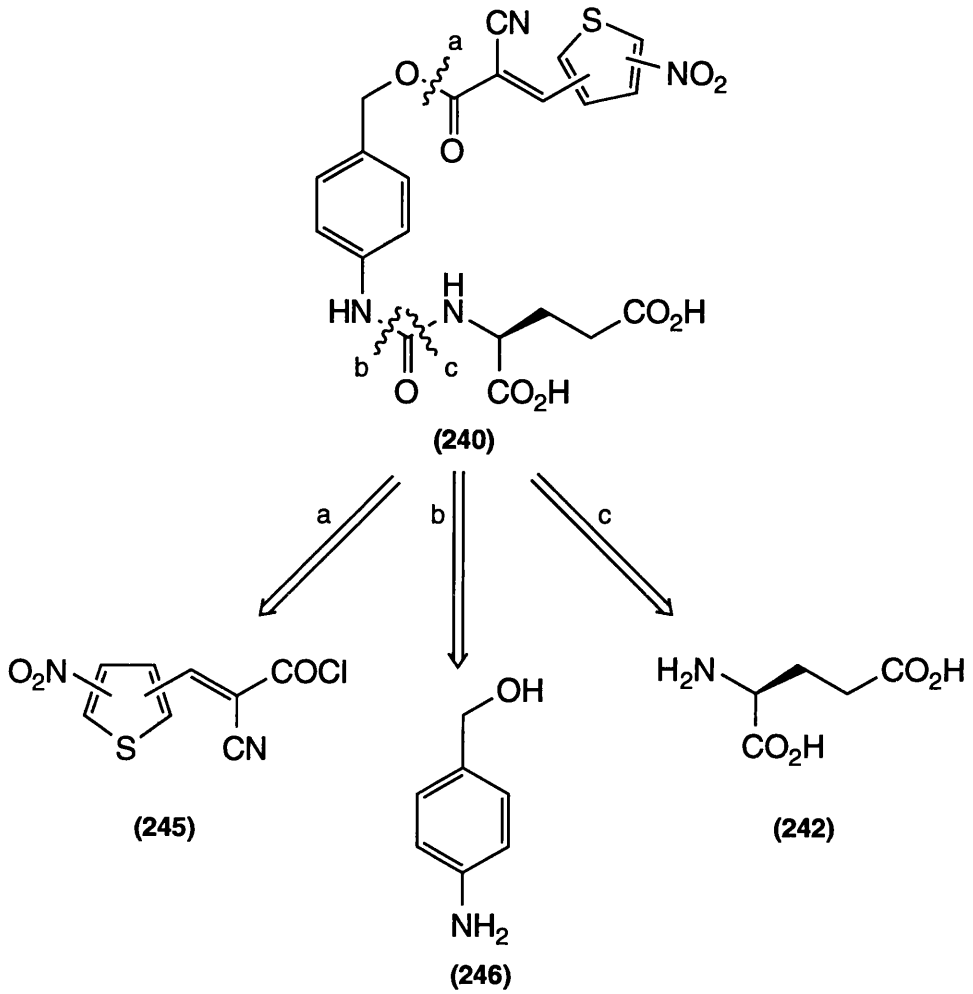


Scheme 35

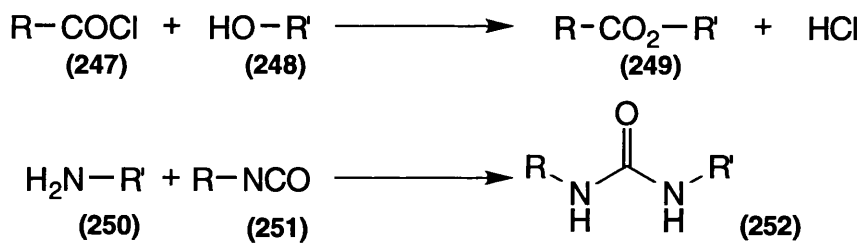
5.2 Retrosynthetic Analysis of the Prodrug

Disconnection of compound **(240)** at the urea linkage with the glutamate portion and at the ester linkage with the tyrphostin gives the drug fragment as an acid chloride **(245)**, 4-aminobenzyl alcohol **(246)** and L-glutamic acid **(242)** **(Scheme 36)**. General methods were sought which could be utilised to produce either the carbamate or ester linkages between these subunits. One possibility is the

reaction of an acid chloride **(247)** with an alcohol **(248)** to generate an ester **(249)**, and the treatment of an amine **(250)** with an isocyanate **(251)** to form a urea **(252)** **(Scheme 37)**.



Scheme 36

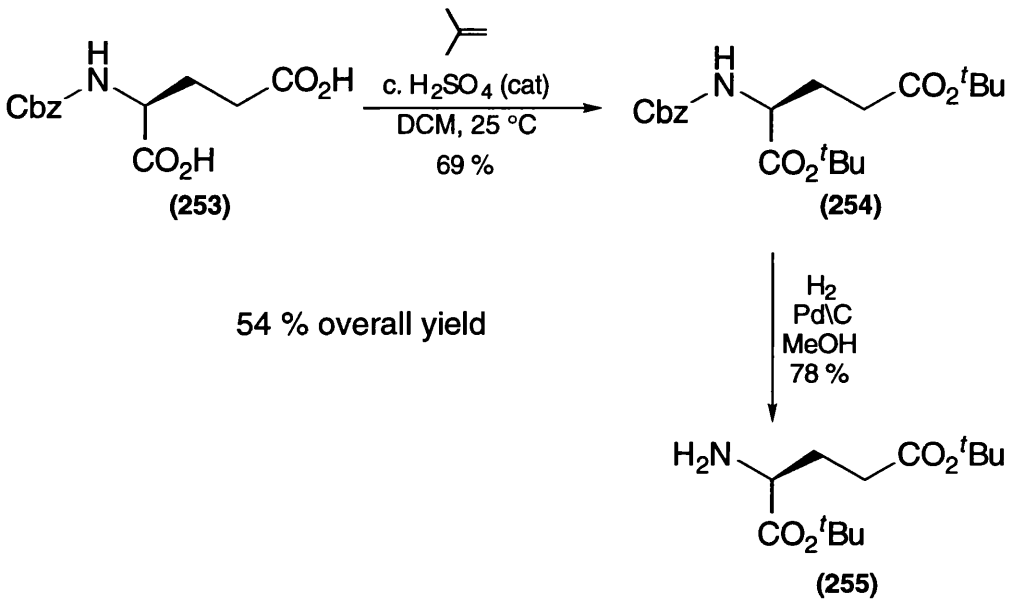


Scheme 37

5.3 Synthesis of the Specifier Fragment

Protection of L-glutamic acid **(242)** as its di-^{t}butyl ester **(255)** was carried out in the manner shown in **Scheme 38**. *N*-Benzyloxycarbonyl-L-glutamic acid **(253)** was treated with isobutylene and a few drops of concentrated H_2SO_4 in DCM in a sealed vessel using standard procedures.[205] The progress of the reaction

could be easily monitored since the product was much more soluble in DCM compared to the starting material, and after 96 hours all of the starting material had disappeared. The product was isolated as an amber oil in good yield, but a considerable amount of polymeric material was also observed. When the procedure was repeated using a single drop of conc. H_2SO_4, the reaction time had to be extended (10 days) and no noticeable increase in yield was observed.



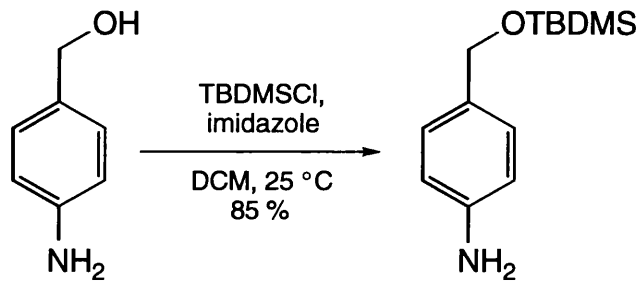
Scheme 38

In the ^1H NMR spectrum of the Cbz-protected diester **(254)**, a broad singlet corresponding to the two *t*butyl groups was observed. The presence of these groups was corroborated in the ^{13}C NMR spectrum by signals for two *t*butyl groups in magnetically distinct environments. No signals corresponding to an OH stretch or bend from the starting material was observed in the IR spectrum. The mass spectrum of **(254)** contained a signal for the molecular ion at *m/z* 393, and the product also gave acceptable microanalytical data.

Hydrogenolysis of the protected amine was carried out using palladium on carbon.[206] Contrary to Sutherland's findings,[203] we did not experience any difficulties when the reaction was carried out in methanol, and the product **(255)** was isolated as a yellow oil after a reaction time of 24 hours at room temperature. The product was characterised by ^1H and ^{13}C NMR spectroscopy, which did not show downfield signals in the aromatic region corresponding to the Cbz group. The signals associated with the aromatic ring of **(254)** were absent in the IR spectrum of **(255)**.

5.4 Synthesis of the Spacer Section

Our initial target molecule **(240)** contained a spacer unit derived from 4-aminobenzyl alcohol **(246)**. Since we were unsure as to whether the spacer or glutamic acid would be converted into an isocyanate, it was necessary to protect the alcohol group on **(246)** as a tertiarybutyldimethylsilyl (TBDMS) ether **(256)** using standard procedures **(Scheme 39)**.[207] Other protecting groups, such as trimethylsilyl ethers were discounted owing to their instability under basic conditions,[208] and Sutherland had unsuccessfully attempted to protect using triisopropylsilyl (TIPS) triflate and lutidine to generate the TIPS ether.[203] 4-Aminobenzyl alcohol **(246)** was treated with TBDMSCl and imidazole in a solution of dry DCM, to give the product **(256)** as a clear oil in high yield after distillation. The ^{13}C NMR spectrum of **(256)** contained a very highfield signal (δ -5.0) corresponding to the methyl groups directly attached to the silicon. Si-O stretches were observed at 1110 cm^{-1} in the IR spectrum, and the molecular ion and a signal corresponding to loss of OTBDMS from the parent molecule were observed in the mass spectrum.



(246) **(256)**

Scheme 39

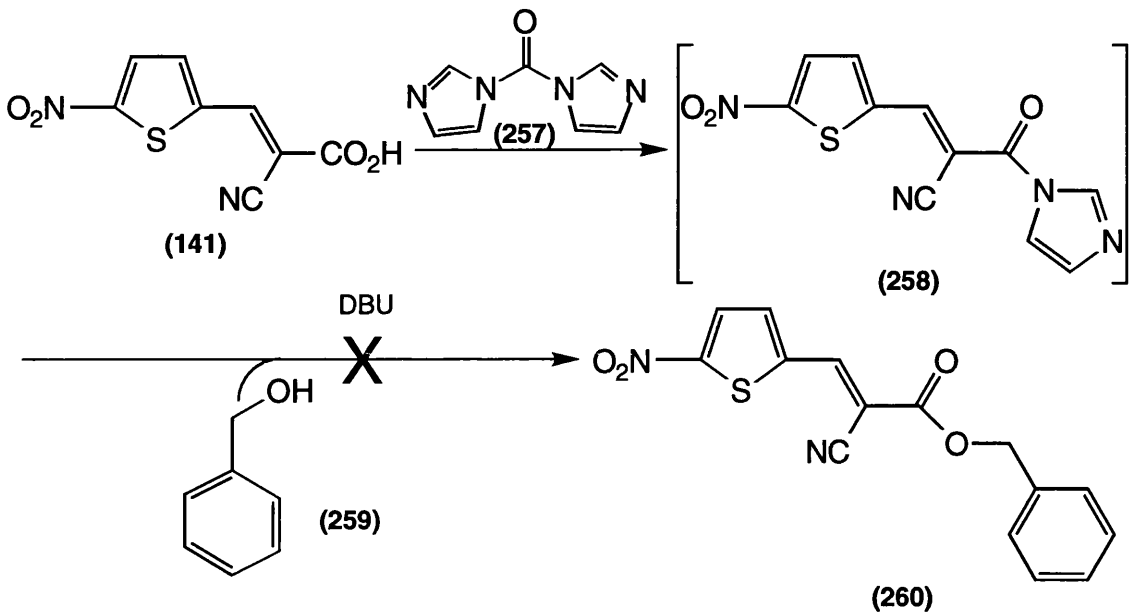
5.5 Coupling the Spacer Subunit to the Drug

It would be possible to attach a tyrphostin such as **(141)** *via* an ester linkage to the spacer unit, or by an amide bond. When the glutamate residue is hydrolysed by CPG_2, an intermediate such as **(241)** would be formed, and this would decompose in the manner shown **(Scheme 35)** to release the drug.

5.5.1 Ester Linkage

Carbonyldiimidazole (CDI) **(257)** has been employed as a coupling agent in the formation of esters from benzoic acid or $\alpha\beta$-unsaturated carboxylic acids which had an aromatic or heteroaromatic group in the β position.[209] When 2-cyano-3-[2-(5-nitrothienyl)]propenoic acid **(141)** was dissolved in dry DMF and was then

treated with CDI **(257)**, we observed an immediate colour change which implied formation of an *N*-acylimidazole intermediate **(258)**. Addition of benzyl alcohol **(259)** and DBU (which was previously shown to accelerate the reaction rate of this type of reaction[209]) and stirring at room temperature for an extended period of 43 hours gave a brown solid which was shown to consist of a mixture of starting materials by NMR spectroscopy. The procedure was repeated either at 80 °C, 120 °C or at reflux temperature, but in each case the same result was observed.

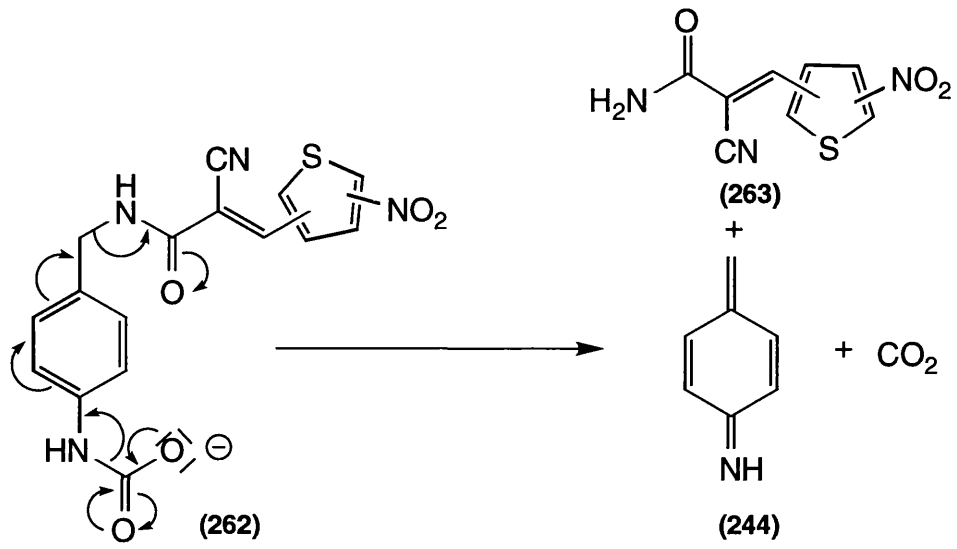


Scheme 40

We then attempted to treat benzyl alcohol with the acid chloride **(261)** of **(141)** which had been formed *in situ* using standard methodology. Again, no product was isolated, and starting materials (i.e. the carboxylic acid and benzyl alcohol) were recovered.

5.5.2 Amide Linkage

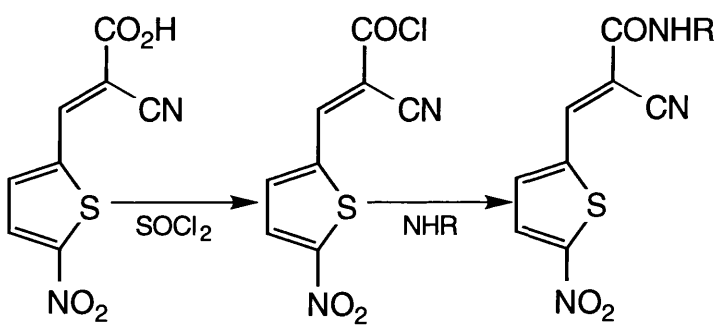
The tyrphostin fragment may alternatively be attached to the spacer subunit *via* an amide linkage. Hydrolysis of this bond would generate the carboxylate anion **(262)** which would decompose as before **(Scheme 41**; compare with **Scheme 35)**.



Scheme 41

We carried out a series of test reactions involving the treatment of 2-cyano-3-[2-(5-nitrothienyl)]propenoic acid **(141)** with thionyl chloride. This was then reacted *in situ* with either ethylamine or 4-bromobenzylamine to give the corresponding amides **(Scheme 42** and **Table 17)**.

The latter reaction with 4-bromobenzylamine gave the product **(265)** in 69 % yield. In the ^1H NMR spectrum, a broad singlet at δ 6.80-6.60 was assigned to the NH proton; an AA'BB' and an AB system in a 2:1 ratio between δ 8.29-7.00 arose from the phenyl and thienyl protons. The carbonyl stretch for an αβ-unsaturated carboxylic acid was replaced by an amide carbonyl stretch at 1700 cm^{-1} in the IR spectrum, together with an amide N-H stretch at 3448 cm^{-1}. The intensity of signals for aromatic C-H stretches and bends were substantially greater compared to the IR spectrum of **(141)**. Microanalytical and accurate mass spectrometric data confirmed the purity of **(265)**.



(141) **(261)** **(264-265)**

Scheme 42

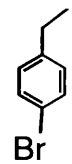
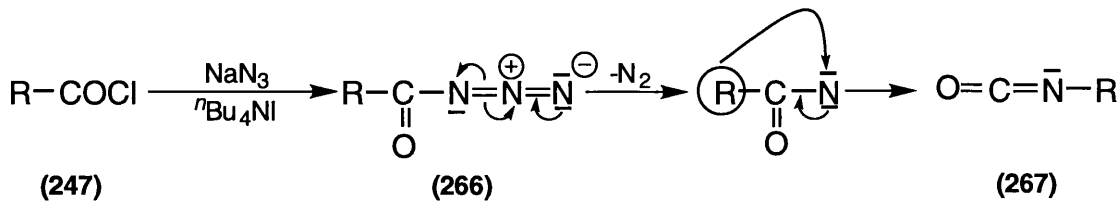
Product	R
(264)	Et
(265)	

Table 17

5.6 Coupling the Spacer Subunit with Glutamic Acid

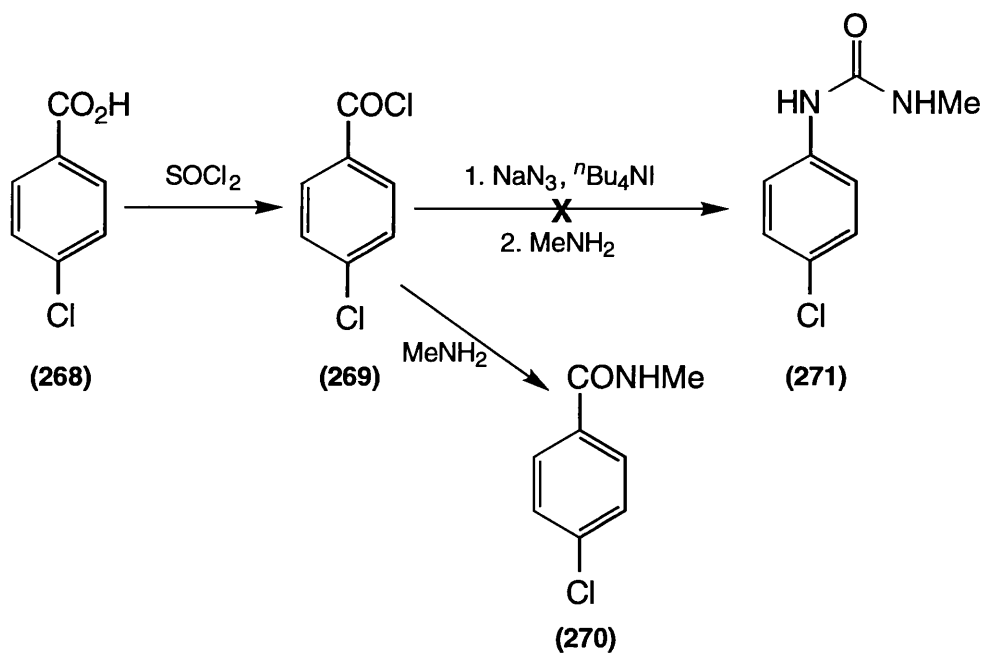
A method to generate isocyanates involves the treatment of an acid chloride **(247)** with sodium azide in the presence of a catalytic amount of tetrabutylammonium iodide to generate an acyl azide **(266)**, which undergoes Curtius rearrangement to give an isocyanate **(267)** in good yield,[210] since no water is present to hydrolyse the product to the amine **(Scheme 43)**.



(247) **(266)** **(267)**

Scheme 43

We performed a test reaction using 4-chlorobenzoic acid **(268)** as the substrate. This was treated for several hours with thionyl chloride to generate an acid chloride **(269)**, which was either quenched with methylamine to give an amide **(270)** in 78 % yield, or was treated sequentially *in situ* with sodium azide and $^{n}Bu_4NI$ followed by methylamine in an effort to form the *N*-methylurea **(271)**. The expected product was not isolated, and instead again the methylamide **(270)** was produced, clearly indicating that acyl azide formation had not occurred. Given the dangers associated with azide chemistry, we decided not to repeat the reaction at elevated temperature and this route was abandoned.

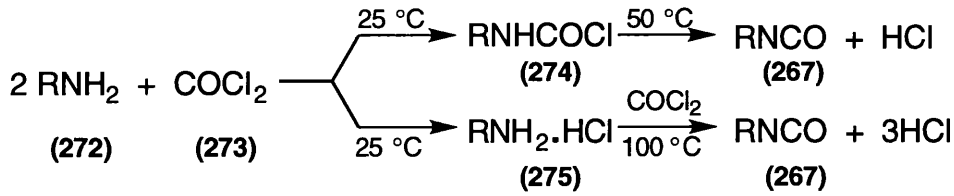


(268) **(269)** **(271)** **(270)**

Scheme 44

One of the most common methods for the preparation of isocyanates is the reaction of aliphatic and aromatic primary amines **(272)** with phosgene **(273)** to

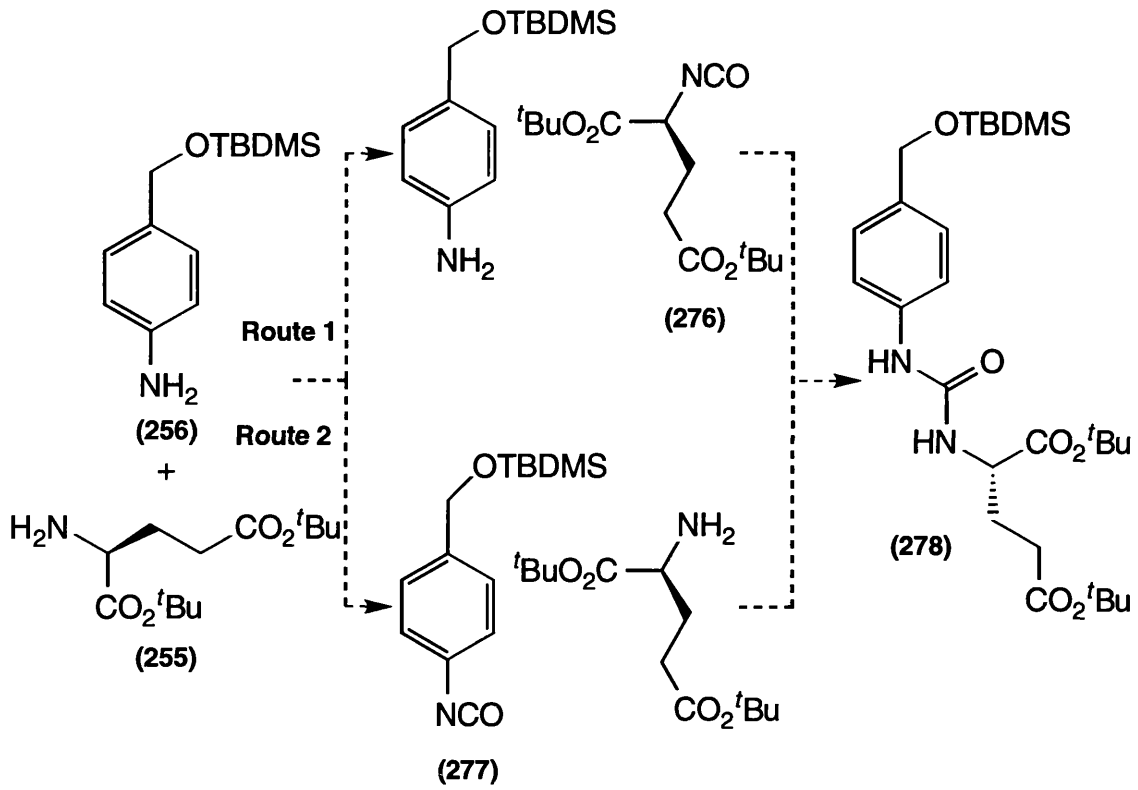
form chloroformates **(274)** or amine hydrochlorides **(275)**, which lose HCl upon heating to give isocyanates **(267)** in good yield **(Scheme 45)**.[211] As the reaction involves the use of highly toxic phosgene gas, alternative methods involving the use of triphosgene,[212] bis(trichloromethyl)carbonate **(276)** which is a solid at room temperature, or carbonyldiimidazole[213] **(257)** have been developed to transform amines into isocyanates in good yields. Modification of the reactions involving the use of phosgene or triphosgene by adding excess triethylamine results in the removal of the HCl produced as triethylamine hydrochloride.



Scheme 45

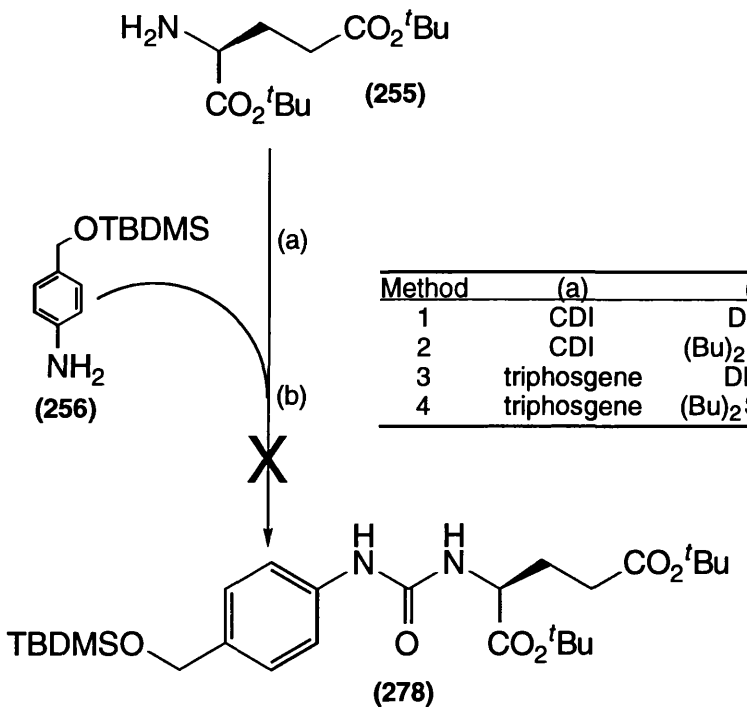
We were then faced with the problem as to which amine, either the protected glutamic acid **(255)** or the protected aniline **(256)** was to be treated with phosgene (or a phosgene equivalent) to give an isocyanate in good yield **(Scheme 46)**. Neither compound is a particularly strong base, given that the amino groups have similar pKa values.[214, 215] A literature search had generated a greater number of publications on the synthesis of phenylisocyanates. In either case, sensitivity of isocyanates to moisture necessitated a strategy which would involve generation of the isocyanate and reaction with an amine to give the urea **(278)** in a one-pot reaction.

Di-*t*butyl-L-glutamate **(255)** was heated with CDI **(257)** in dry THF until TLC showed the absence of starting material. 4-Hydroxymethylaniline *t*butyldimethylsilyl ether **(256)** dissolved in dry THF was then added, followed by 4-dimethylaminopyridine (DMAP, a coupling reagent). Heating at reflux for 24 hours gave a complex mixture, but none of these could be identified as the expected product. A substantial quantity of **(255)** and **(256)** were recovered by column chromatography.



Scheme 46

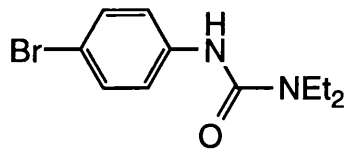
Alternative methods using triphosgene to generate an isocyanate or dibutyltin diacetate as the coupling agent under the same conditions were investigated **(Scheme 47)**. In each of these cases, complex mixtures were produced, and various amounts of starting materials were recovered.



Method	(a)	(b)
1	CDI	DMAP
2	CDI	$(Bu)_2Sn(OAc)_2$
3	triphosgene	DMAP
4	triphosgene	$(Bu)_2Sn(OAc)_2$

Scheme 47

A second strategy would be to generate an isocyanate from **(256)** using the above technique and to react it with **(255)**. In a test reaction, 4-bromoaniline was treated with CDI **(257)** in dry THF for 46 hours until TLC showed the absence of starting material. Diethylamine was then added, and gentle heating gave the diethylcarbamate **(279)** in moderate yield as large brown crystals. The ^{1}H NMR spectrum of **(279)** contained a very broad singlet at δ 12.21 (NH), an AA'BB' system between δ 7.81-7.13 and a triplet and quartet at δ 1.11 and 3.36 respectively arising from the ethyl groups. These were also observed in the ^{13}C NMR spectrum at around δ 13 and 41. Signals in the IR spectrum included an N-H stretching mode at 3264 cm^{-1} and a carbonyl stretch at 1633 cm^{-1}.

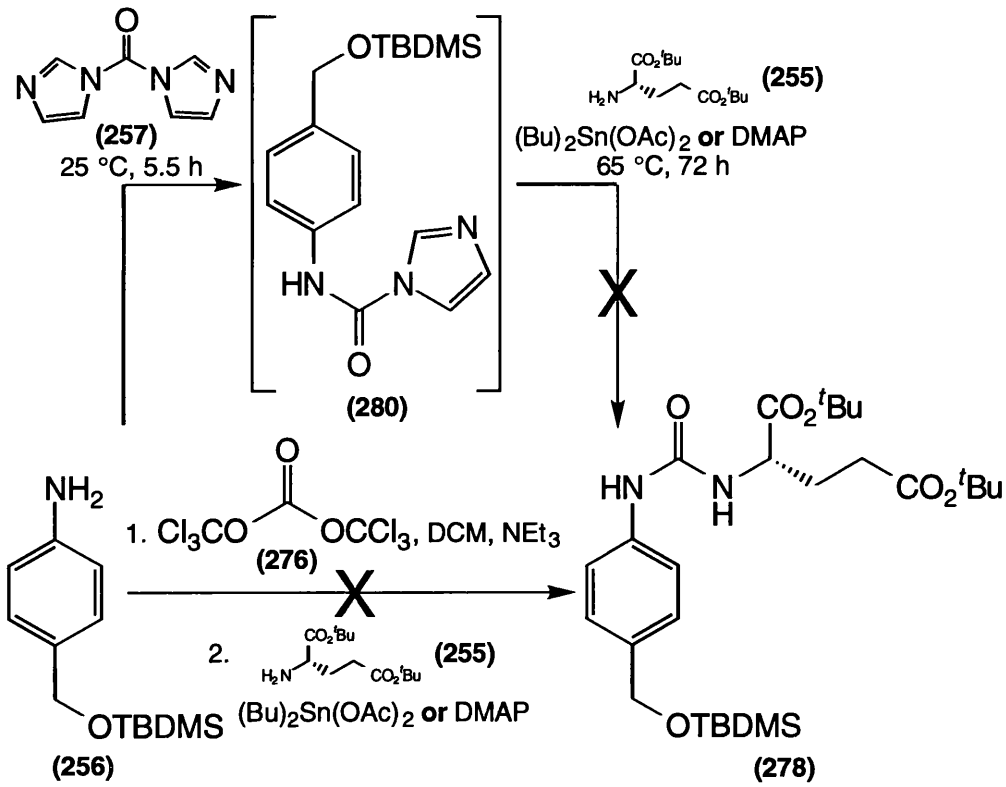


(279)

This procedure was repeated using 4-hydroxymethylaniline *t*butyldimethylsilyl ether **(256)** and CDI **(257)** **(Scheme 48)**. Precipitation of a white solid which was identified as imidazole by ^{1}H NMR spectroscopy occurred within six hours, and a new spot with R_F 0.19 which was assumed to be the *N*-acylimidazole **(280)** was observed by TLC. Stirring was continued for a further four hours, but this spot did not disappear, and although the isocyanate may perhaps not have formed, di-*t*butyl-L-glutamate **(255)** and dibutyltin diacetate were added, and stirring was continued at an elevated temperature (70 °C) for three days, by which time the presence of a beige crystalline solid was apparent. TLC and ^{1}H NMR spectroscopy showed this to consist of a mixture of **(255)** and **(256)**, although the spot with R_F 0.19 was no longer present.

We attempted to modify the procedure in the same manner as before using DMAP as the coupling reagent, but this gave the same result. CDI was then replaced with triphosgene, and the procedure was repeated using dibutyltin diacetate or DMAP, but again the product was not observed and starting materials were recovered.

We were unable to generate the diethylurea by trapping with diethylamine when either CDI or triphosgene was used.

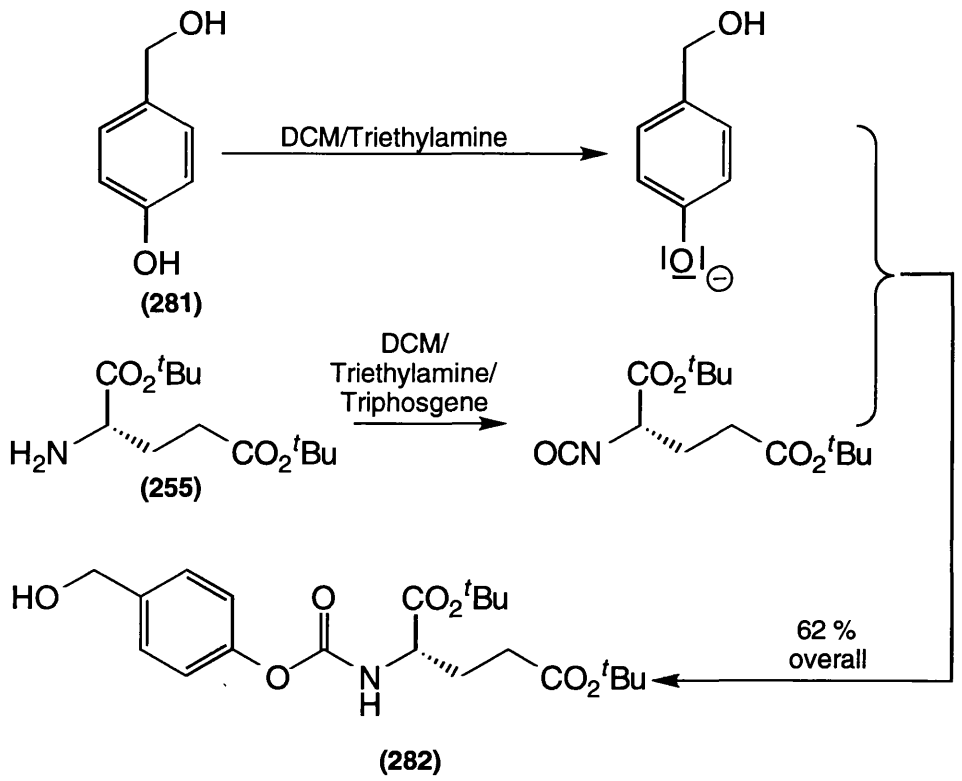


Scheme 48

5.7 Conclusions

Whilst the logic behind this synthetic route is sound, there appears to be little chance of its success. It is clearly apparent that the formation of a urea linkage between the spacer and specifier subunits is prevented by steric hindrance arising from the bulky *t*butyl groups. Sutherland unsuccessfully investigated the possibility of generating a urea from diphenyl L-glutamate and the protected spacer unit **(256)** using triphosgene.[203]

Considerably more success was apparently achieved to generate a specifier-spacer unit which contained a carbamate linkage **(282) (Scheme 49)**.[203] This was synthesised from 4-hydroxybenzyl alcohol and compound **(281)**, which was generated from di-*t*butyl glutamate **(254)** and triphosgene. This particular route is worthy of further investigation, and so the author would like to propose some other synthetic routes which may be useful in developing ADEPT prodrugs containing tyrphostins in the future.



Scheme 49

5.8 Future Work

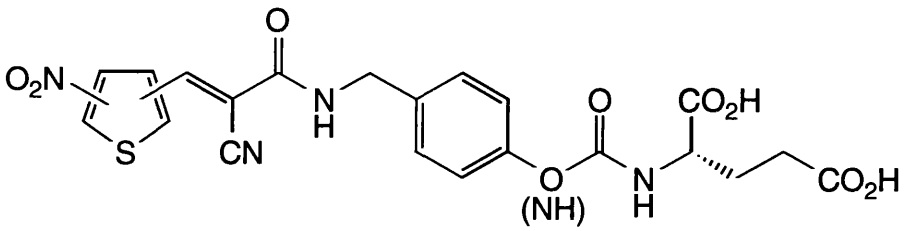
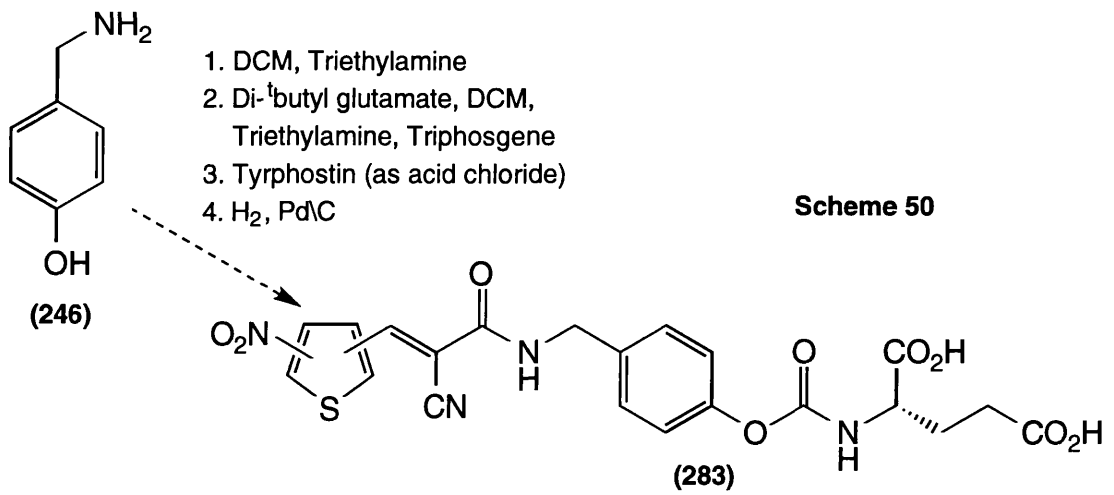


Figure 22

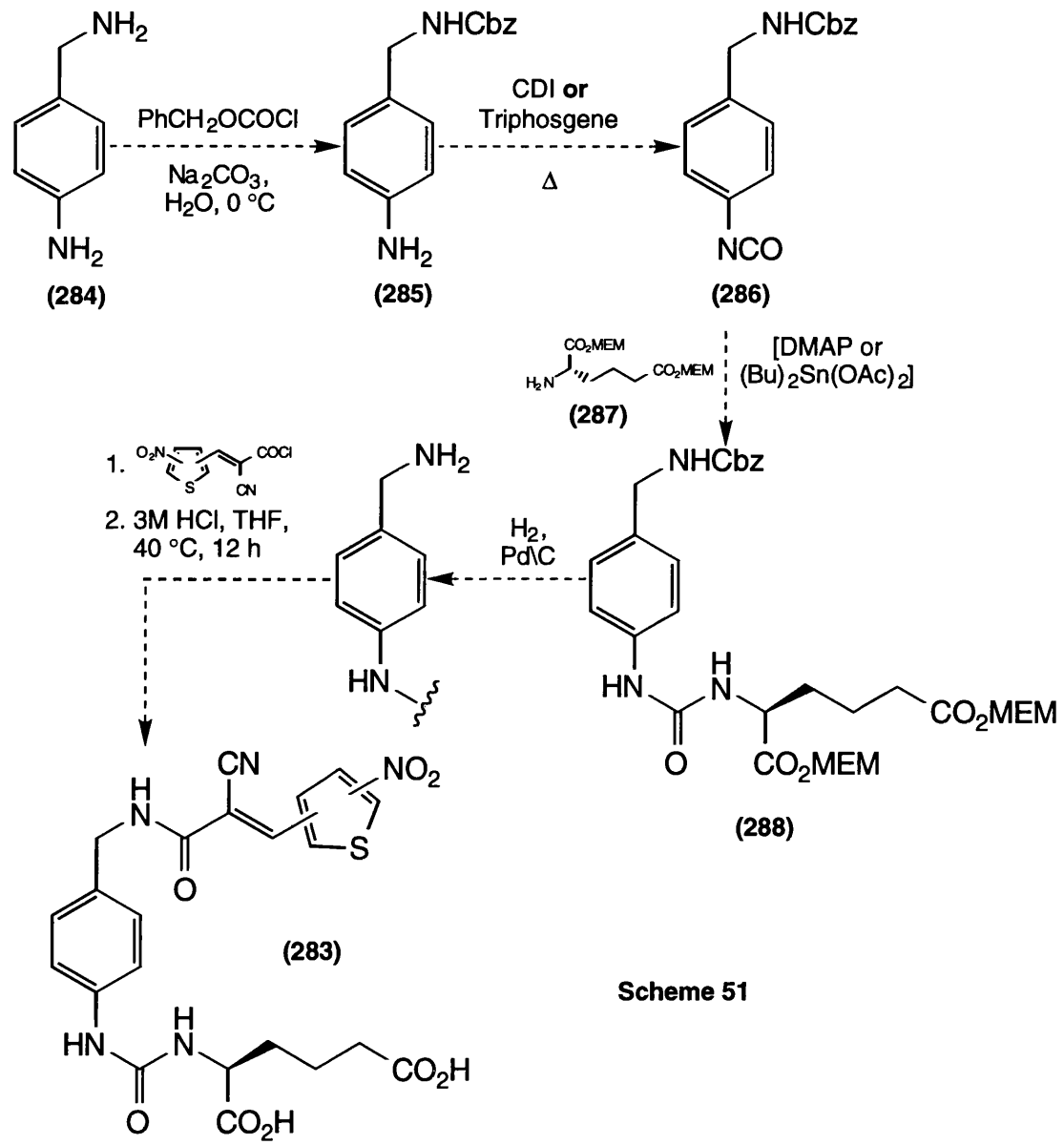
It is now clear that the preferred target is of the type shown in **Figure 22**. This consists of a tyrphostin linked to a spacer unit *via* an amide bond, and a specifier attached either through a urea or carbamate linkage, which may be achieved in the manner shown **(Scheme 50)**.



Scheme 50

It would, however, be preferable to synthesise an ADEPT prodrug whereby the specifier was attached to the spacer unit by a urea linkage. Since benzylamine

is a stronger base compared to aniline (pK_a 4.62 *vs.* pK_a 9.3),[216] the aliphatic amino group of 4-aminobenzylamine **(284)** would react more rapidly with benzylchloroformate to give the monoprotected species **(285) (Scheme 51)**.[217] The Cbz protecting group also has the advantage of being easily removed later by hydrogenolysis.[206, 218] Compound **(285)** could then be treated with either CDI **(257)** or triphosgene **(276)** to generate an isocyanate **(286)**, which could react with an L-glutamate such as **(287)** to give a urea **(288)**. This step may require the use of a coupling agent. It is likely that this reaction would proceed more readily than before, since earlier failures may be attributed to a steric effect caused by the bulky *t*butyl groups which were used to protect the glutamic acid, and in this case the straight-chain MEM groups (methoxyethoxymethyl ester)[219] would reduce the steric bulk around the amino moiety. Deprotection of the aliphatic amino group[206] and reaction with the acid chloride derivative of the tyrphostin of choice, followed by deprotection of the acid moieties under very mild conditions[206] would generate the target molecule **(283)**.



Scheme 51

6. Biological Evaluation and Electrochemical Data

6.1 Biology

The compounds discussed in Chapters 3 and 4 were presented for biological evaluation to the CRC Beatson Laboratories, Department of Medical Oncology, University of Glasgow, or to the Paterson Institute for Cancer Research, Christie Hospital, Manchester. Additional testing was carried out by the Drug Development Section at the Institute of Cancer Research (ICR), Sutton, Surrey.

IC_{50} values for growth inhibition in MCF-7 human breast adenocarcinoma cells were measured by myself (unless otherwise indicated) under the supervision of Dr Valerie Brunton at the Department of Medical Oncology, or by Dr Alan McGown and co-workers at the Paterson Institute. Dr Brunton carried out or supervised the assays which were used to determine the antiproliferative effect of the tyrphostins in HER14 fibroblasts which had been transfected with the human EGF receptor, the cytotoxicity assays in a number of squamous cell carcinoma cells, and the measurement of EGF receptor kinase activity in a cell-free system.

Alkaline elution studies to measure DNA single-strand breaks caused by compounds **(100)**, **(102)**, **(123)** and **(125)** were carried out by Dr Lloyd Kelland and co-workers at ICR Sutton.

6.2 Biological Data

6.2.1 Antiproliferative Assays

MCF-7 and MCF-7ADR breast adenocarcinoma cells. The growth stimulation of the MCF-7 breast cancer cell line is EGF-dependent, and antibodies to the EGF receptor have been shown to inhibit growth of breast cancer cells *in vitro*,[220, 221] suggesting that signalling *via* the EGF receptor plays an important

role in the proliferation of this tumour type. MCF-7ADR cells have a higher concentration of the EGF receptor.

HER14 Fibroblasts. Additionally, several compounds were tested in HER14 fibroblasts which had been transfected with the human EGF receptor. When quiescent, these cells undergo a further cycle of DNA synthesis after stimulation with EGF or 10 % Donor Calf Serum (DCS). Sequential exposure of these cells to either DCS or EGF followed by the compounds was carried out to determine whether the inhibition was EGF-dependant.

6.2.2 Measurement of EGF Receptor Kinase Activity

Other mitogens such as oestrogen and the insulin-like growth factors are also involved in the growth control mechanisms of cancer cells,[222] and it was important to determine whether the ability of these compounds to inhibit proliferation was a result of control of the EGF receptor function, or was through some other mechanism. To this end, compounds were also tested in a cell-free system.

EGF receptor tyrosine kinase activity was measured in membrane fractions from the HN5 cell line, which is derived from a human squamous cell carcinoma of the tongue.[223] These cells overexpress the EGF receptor, having 4.5 x 10^6 binding sites / cell (V.G. Brunton, unpublished observation). The EGF receptor kinase activity in the membrane fractions was determined by measuring the EGF-dependent phosphorylation of a synthetic substrate, poly(Glu, Ala, Tyr).

6.2.3 Cytotoxicity Assay

Compounds which displayed good activity in the cell-free assay were then screened against a panel of four squamous cell carcinoma cell lines derived from tumours of the vulva, tongue, cervix and uterus. These cell lines have varying levels of EGF receptor concentration **(Table 18)**, with the HN5 and A431 lines having high levels of receptor, and the SiHa cells having levels of EGF receptor similar to that found in normal keratinocytes. Low IC_{50} values in the A431, HN5 and CaSki cell lines, accompanied by a considerably higher (i.e. several orders of magnitude) figure for the SiHa cells would imply that the compound was exerting toxicity upon the cancer cells through inhibition of EGF receptor function.

	HN5	A431	CaSki	SiHa
Tumour Type	tongue	vulval	cervix	uterus
EGF Number (x 10^6 / cell)	5.195	1.544	0.785	0.114

Table 18

6.2.4 Alkaline Elution

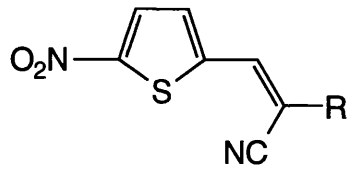
Bioreduction of nitroaromatic species *via* a 1-electron mechanism gives rise to a cytotoxic free radical **(47)**, which may induce radical-induced DNA single strand breaks. Compounds **(100)**, **(102)**, **(107)**, **(122)** and **(125)** are reducible by a 1-electron mechanism, and some of the reduction potential values for these [compounds **(100)** and **(102)**] fell within the range of -200 to -500 mV, which are required if they were to be reduced within a biological system (see Section 6.5.). By assaying for DNA strand-breaks *in vitro*, it is possible to infer whether the compound or its reduced species contributes to inhibition of DNA synthesis and thus prevents cellular proliferation.

6.2.5 General

This multi-stage screening process was designed to enable us to achieve early identification of compounds which either possessed potent EGF inhibition activity, or displayed a strong ability to inhibit cellular proliferation. By assaying for EGF receptor kinase activity and assessing the response of MCF-7 cells to the compounds, we were able to choose compounds which showed promising TK inhibition activity. We were then able to present these for further evaluation in the cytotoxicity and subsequently the alkaline elution studies.

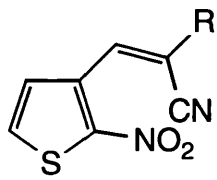
6.3 Biological Activity of Nitrothienyl Tyrphostins

Tables 19-24 show the results from the screening of the 40 nitrothienyl tyrphostins synthesised. For the MCF-7 assay, all but those containing tri(ethyleneglycol) ester groups on their sidearm [compounds **(GJM194)**, **(119)** and **(128)**] were tested at the Department of Medical Oncology by Dr Brunton and her colleagues. The remaining compounds were submitted for testing at the Paterson Institute, and the data from these sets of studies are dealt with separately. The data collected from all of these assays are discussed in Section 6.6.



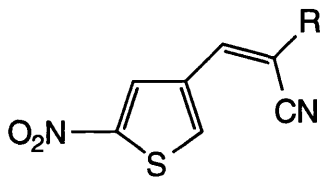
Compound Number	R	IC50 (μM) MCF-7	EGF-R	HER14 DCS	EGF	HN5	A431	CaSki	SiHa
(99)	CN	18.4	50			20.70	28.00	25.80	25.40
(100)	$C(NH_2)=C(CN)_2$	12.0	12.25	18.9	18.1	8.40	6.07	9.24	13.40
(101)	CO_2Me	>100	>500						
(102)	CO_2Et	<1	34.00	29.8	26.5	5.40	4.60	6.94	4.59
(103)	$CO_2{}^nBu$	17.8	>500						
(104)	$CO_2{}^tBu$	n.d.	75.00						
(106)	$CONH_2$	1.9	>500						
(107)	$CSNH_2$	<1	37.50	82.1	85.3	66.90	71.60	82.93	82.10
(108)	2-Thienyl	<0.001	>500						
(141)	CO_2H	n.d.	150.00						
(142)	$CO_2Na.\ 5H_20$	>100	>500						

Table 19



Compound Number	R	IC50 (μM) MCF-7	EGF-R	HN5	A431	CaSki	SiHa
(113)	CN	<0.001	15.33				
(114)	$C(NH_2)=C(CN)_2$	0.025	14.63	5.10	4.73	4.70	7.80
(115)	CO_2Me	6.0	50.00				
(116)	CO_2Et	<0.001	100				
(117)	$CO_2{}^nBu$	<0.001	>500				
(118)	$CO_2{}^tBu$	n.d.	9.00				
(120)	$CONH_2$	<0.001	400				
(121)	$CSNH_2$	0.8	22.67	0.27	0.11	0.32	0.35

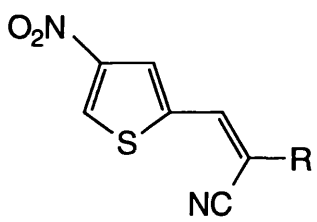
Table 20



		IC_{50} (μM)							
Compound Number	R	MCF-7¶	EGF-R	HER14 DCS	HER14 EGF	HN5	A431	CaSki	SiHa
(122)	CN	<0.001	23.00	82.9	71.5	40.40	28.41	40.00	44.56
(123)	$C(NH_2)=C(CN)_2$	<0.001	7.00			>100	>100	>100	>100
(124)	CO_2Me	>20	>500						
(125)	CO_2Et	<0.001	9.00	>100	>100				
(126)	$CO_2{}^nBu$	>20	>500						
(127)	$CO_2{}^tBu$	n.d.	150						
(129)	$CONH_2$	0.9	>500						
(130)	$CSNH_2$	<0.001	10.50	>100	>100	>100	>100	>100	>100
(144)	CO_2H	n.d.	>500						
(145)	$CO_2Na.\ 5H_20$	>100	>500						

¶ Tested by Miss Jillian Queen

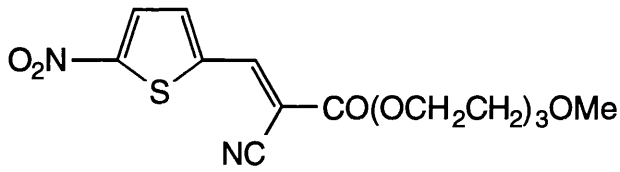
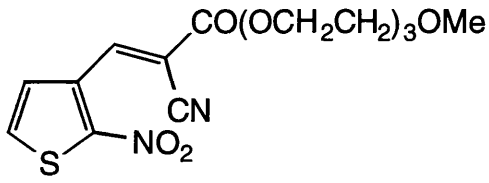
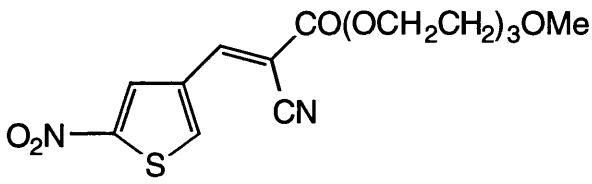
Table 21



		IC_{50} (μM)	
Compound Number	R	MCF-7¶	EGF-R
(132)	CN	0.91	2.00
(133)	$C(NH_2)=C(CN)_2$	<0.001	160
(134)	CO_2Me	<0.001	18.00
(135)	CO_2Et	<0.001	>500
(136)	$CO_2{}^nBu$	<0.001	>500
(137)	$CO_2{}^tBu$	<0.001	
(138)	$CONH_2$	<0.001	47.00
(139)	$CSNH_2$	<0.001	>500

¶ Tested by Martin Lear

Table 22

Compound Number	Structure	IC_{50} (μM) MCF-7	MCF-7ADR
(105)		1.76†	2.27†
(119)		20.79†	n.t.¶
(128)		42.12†	54.81†

† Tested by Dr Alan McGown, Paterson Institute.
¶ Not toxic.

Table 23

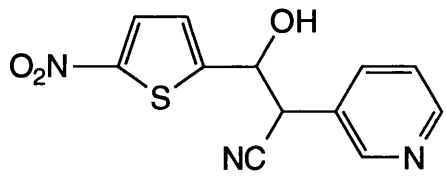
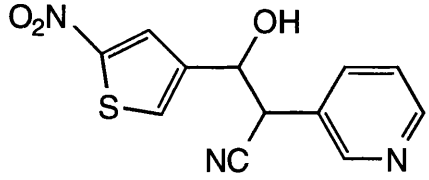
Compound Number	Structure	IC_{50} (μM) MCF-7	EGF-R
(153)		<0.001	>500
(154)		<0.001	>500

Table 24

6.4 Biological Activity of Quinolyl and Nitroquinolyl Tyrphostins

After the departure of Professor Workman, the facilities at the Beatson Institute were unavailable for the biological evaluation of the 25 quinolyl and nitroquinolyl tyrphostins synthesised (Chapter 4). Provisions were made for these compounds to be tested in MCF-7 and MCF-7ADR cell lines (the latter has a higher concentration of the EGF receptor) at the Paterson Institute for Cancer Research, Manchester. These compounds have been submitted for testing, and results are eagerly awaited.

6.5 Reduction Potentials

6.5.1 Cyclic Voltammetry

Cyclic voltammetry is a well-established technique which has been employed to measure the value and nature of the reduction and oxidation of compounds in solution. In cyclic voltammetry, a wide variety of electrodes and solvents may be used, and the ionic conductivity of a solvent may be enhanced by the addition of a supporting electrolyte.

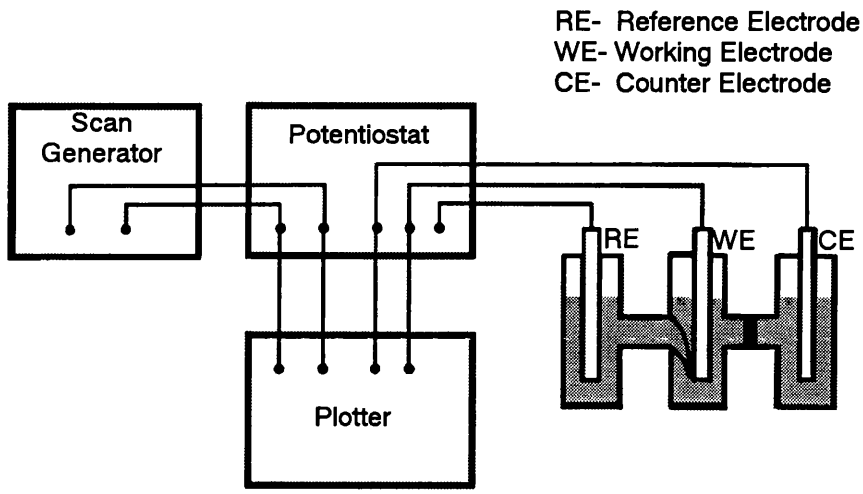


Figure 23 Modified from reference 224.

A schematic representation of the instrumental set-up for cyclic voltammetric experiments is given in **Figure 23**. Three electrodes are attached to the cell containing the redox species in solution. A scan generator controls the voltage applied to the cell, which is scanned linearly to the working electrode, W, from an initial level, E_1 to a predetermined level E_2, when the direction of the scan is reversed. The current response is plotted as a function of the applied potential. Figure X shows a typical CV obtained from the reversible electron transfer of an oxidisable material, R, for the reaction

$$R + ne^{\ominus} \rightleftharpoons O$$

In order to validate measurements obtained by cyclic voltammetry, it is necessary to repeat scans and to vary the scan rate, which also helps to identify the nature of the redox process. In cases where the reduction is completely reversible (such as the first 1-electron reduction of a nitro group, see **Scheme 5** in Chapter 2), there is usually little difference between the values recorded for different scans or scan rates.

Reversible electron transfer processes are diffusion-controlled. A schematic representation of a cyclic voltammogram (CV) of a wholly reversible redox couple is shown in **Figure 24**. When one of the redox species is a radical [such as **(47)**], it is often the case that this species will give an intense colour at the working electrode. Removal of these radicals from the surface of the working electrode is achieved between scans by means of bubbling nitrogen through the solution, which also removes air bubbles (which may affect the readings) from the surface of the electrode.

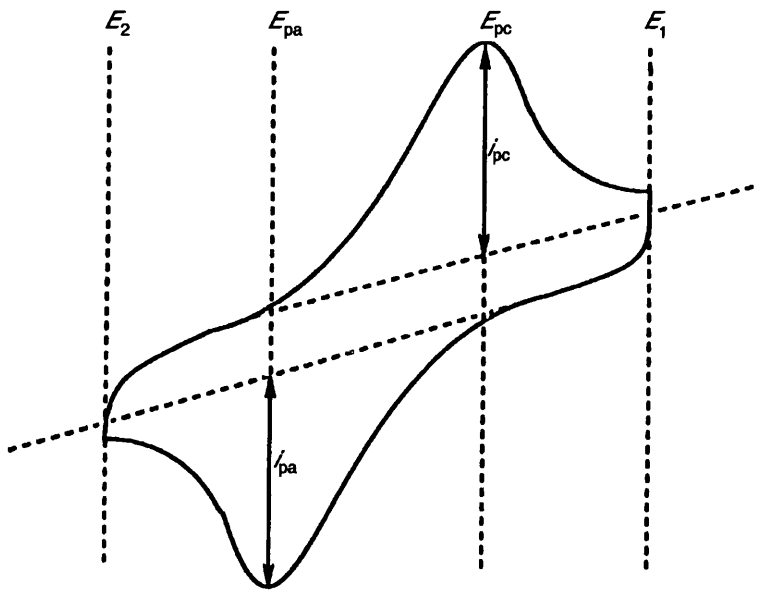


Figure 24 Adapted from references 225 and 226.

The wave for a reversible 1-electron transfer exhibits the following characteristics.

1. The cathodic (E_{pc}) and anodic (E_{pa}) peak potentials are independent of scan rate.

2. The separation (ΔE_p) between E_{pc} and E_{pa} is constant irrespective of scan rate.

3. The cathodic (i_{pc}) and anodic (i_{pa}) peak currents are such that

$$\frac{i_{pc}}{i_{pa}} \approx 1$$

4. The square root of the scan rate is directly proportional to i_{pa}.

Irreversible or quasi-reversible electrochemical reactions may occur when electron transfer and diffusion of the reactants occur at similar rates, or when the rate of electron transfer is much slower than diffusion. In such cases, at

least some of the properties listed above may not hold true. The CVs of such processes are more complicated, but essentially the return signal is not a mirror image of the forward peak, and is, instead, normally a horizontal line.

It is common practice to report the average value of the forward and reverse peaks [(E_{pc} + E_{pa})/2] as the formal reduction potential for the redox couple. This approximation is most accurate when the process is a reversible one where the diffusion co-efficients for the oxidised and reduced species are the same.

6.5.2 Experimental Conditions

Details of the general conditions for measuring the reduction potentials are given in Chapter 7. Atmospheric humidity had a noticeable effect on the values of ΔE_p, which increased when humidity was high. CVs were obtained on three separate dates, and on each occasion the reduction potential of ferrocene was measured as an internal standard. Whilst the value of ΔE_p fluctuated substantially (see **Table 25**), the reduction potential remained constant at around -10 mV. Measurements were carried out by myself under the supervision of Dr Robert D. Peacock and Dr Stephen M. Lacy (University of Glasgow).

6.5.3 Reduction Potentials of Selected Nitrothienyl and Nitroquinolyl Tyrphostins

Compounds which displayed good biological activities in the assays noted in Section 6.4 were tested. The results are given in **Table 25**. The observed reduction potential (E^{1}_{obs}) was corrected by +580 mV (a value which represents the difference in potential between the $Ag/AgNO_3$ reference electrode and the Normal Hydrogen Electrode, NHE)[227] to give the reduction potential (E^{1}_{7}) with respect to NHE, to which these values are normally referred.

Samples were scanned at 20, 50, 200 and 500 mVs^{-1}, and in some cases the margins of error of the measurements were quite high. This was attributed to internal resistance in the equipment. **Figure 25** is the cyclic voltammogram for **(99)**, which shows the reduction of this compound to be a reversible process. As can be seen, two one-electron reductions occurred within the parameters of the scan; only the values of the first reduction are recorded in **Table 25**. The cyclic voltammogram of an irreversible electron transfer involving compound **(132)** is shown in **Figure 26**. Both CVs also possess a large signal around +150 mV corresponding to oxidation of the solvent.

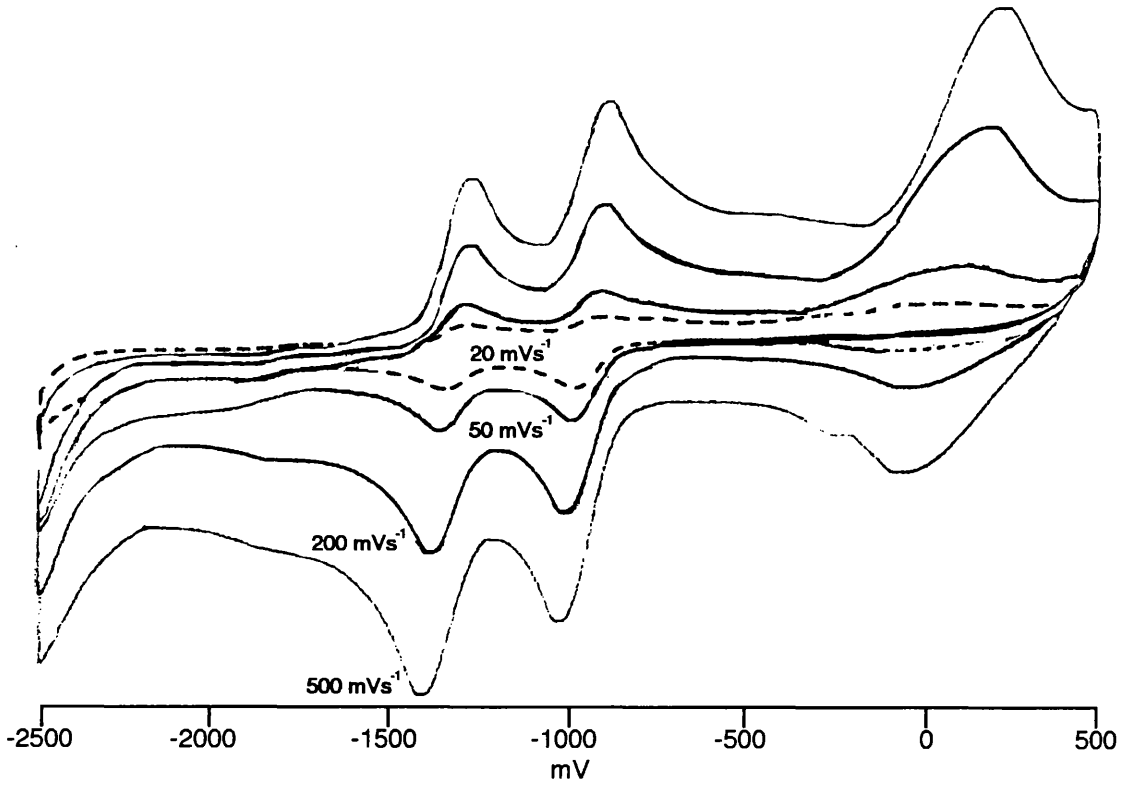


Figure 25

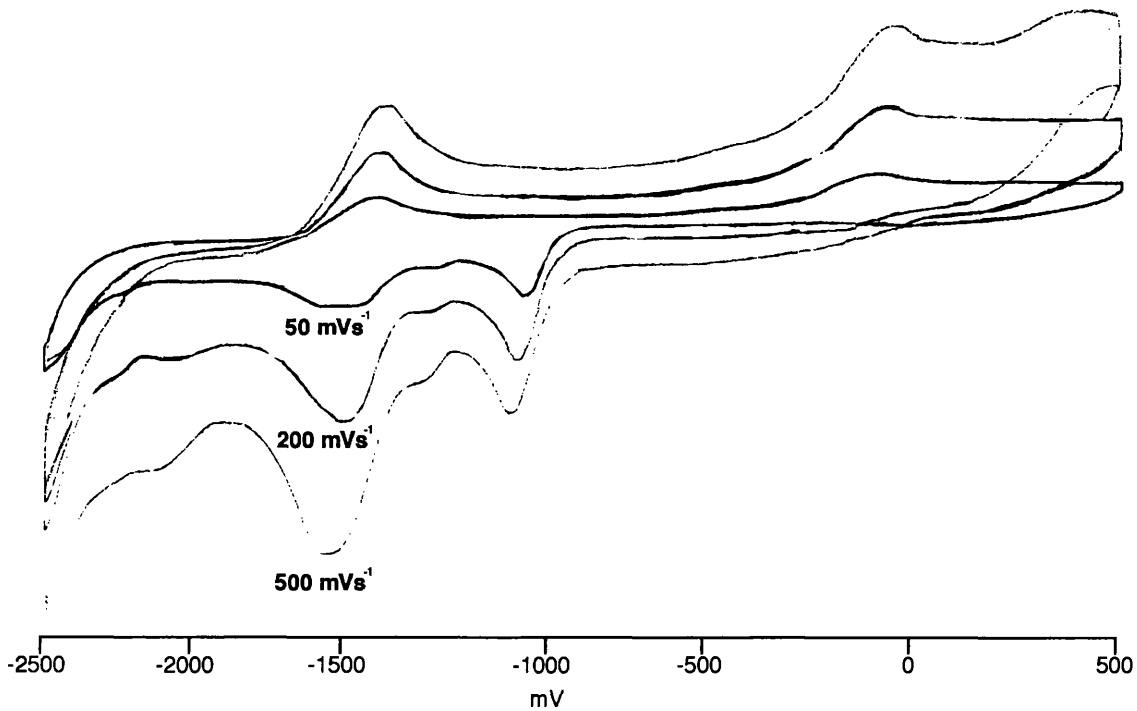


Figure 26

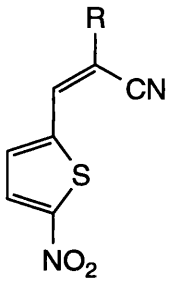
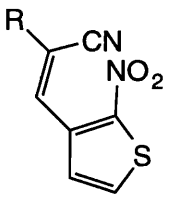
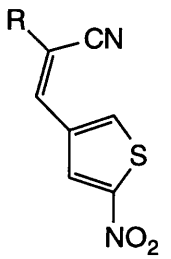
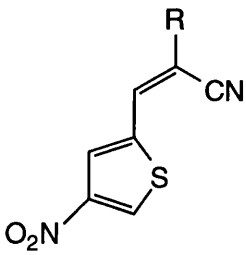
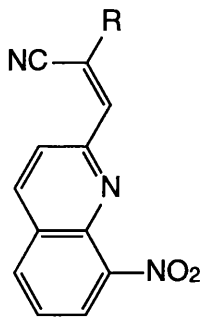
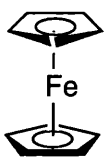
Ar	R	No.	Run No.	E^1_{obs} (mV)	ΔE_p (mV)	E^1_7 (mV)	Nature of Reduction: Reversible	Nature of Reduction: Irreversible
	CN	**(99)**	1	-1050	100	-470 ± 50	✗	
	$C(NH_2)=C(CN)_2$	**(100)**	1	-1000	70	-420 ± 20	✗	
	CO_2Me	**(101)**	1	-950	120	-370 ± 60	✗	
	CO_2Et	**(102)**	2	-910	227	-330 ± 50	✗	
	$CONH_2$	**(106)**	1	-1140	116	-560 ± 20	✗	
	$CSNH_2$	**(107)**	1	-1340	-	-760 ± 10		✗
	$C(NH_2)=C(CN)_2$	**(114)**	3	-960	121	-380 ± 10	✗	
	CO_2Et	**(116)**	3	-930	120	-350 ± 50	✗	
	$CSNH_2$	**(121)**	3	-1090	-	-510 ± 30		✗
	CN	**(122)**	2	-1260	-	-680 ± 20		✗
	$C(NH_2)=C(CN)_2$	**(123)**	2	-1790	-	-1210 ± 30		✗
	CO_2Et	**(125)**	2	-1300	-	-720 ± 10		✗
	$CSNH_2$	**(130)**	2	-1380	-	-800 ± 30		✗
	CN	**(132)**	3	-1120	-	-540 ± 20		✗
	CO_2Me	**(134)**	3	-1220	-	-760 ± 20		✗
	CN	**(238)**	3	-1120	-	-540 ± 30		✗
	$C(NH_2)=C(CN)_2$	**(239)**	3	-1227	-	-643 ± 20		✗
		-	1		100	-10 ± 5	✗	
	Ferrocene	-	2		220	-8 ± 6	✗	
		-	3		120	-12 ± 4	✗	

Table 25

6.6 Discussion

A total of 40 nitrothienyl tyrphostins have been synthesised, 36 of which are novel, as well as 25 quinolyl tyrphostins, of which only one has been reported in the existing literature (this information was obtained by means of a Chemical Abstracts System on-line substructure search). It was anticipated that these compounds would selectively inhibit the growth of cancer cells which possessed a high concentration of the EGF receptor by inhibiting a key stage in the molecular cascade leading to cellular proliferation. It has been proposed that some tyrphostins are selective inhibitors of specific TKs, but the precise mode of inhibition of these compounds is as yet unknown. Indeed, evidence is emerging which suggests that tyrphostins do not inhibit TKs at all, but perhaps act upon some other key molecule in the signalling pathway.

To this end, we considered it important to test the compounds both *in vitro* in intact cells and in a cell free system which specifically measures EGF-dependent phosphorylation of a known substrate. A good correlation between both these data sets would suggest that a compound's antiproliferative effect arose through inhibition of the EGF receptor.

A number of compounds tested in this manner displayed biological activities in the nanomolar range in the MCF-7 cell assay, which is several orders of magnitude better than previously reported tyrphostins.[93, 97, 98, 99] In particular, **(99)**, **(113)**, **(122)** and **(132)** which contained two nitrile groups in the sidearm were extremely active in both test systems. Similarly, when the conjugation was extended by the inclusion of a 2-aminoethene-1,1-dinitrile moiety [compounds **(100)**, **(114)** and **(123)**] the activity remained extremely high.

In the case of all the ester derivatives, activity generally decreased with ester chain length or bulk, and almost without exception the ethyl esters were the most active in all series, although the low measured activity of the methyl ester derivatives may be related to the fact that these compounds were often so insoluble that they would precipitate out of solution whilst they were being prepared for administration to the biological system. The two carboxylic acid derivatives **(141)** and **(144)** were inactive in the cell-free assays. A similar result was observed for their sodium salts **(142)** and **(145)**, which were also inactive *in vitro*. This is probably due to the fact that anions are extremely lipophobic, and are not readily taken up by the cell.

No clear structure-activity relationship has emerged from our studies. Indeed, it may be true to say that the data contradicts earlier predictions regarding the effect of the substitution pattern of the sidearm and nitro groups around the

aromatic ring with respect to the observed biological activity.[93] Activity seems to arise solely from the nature of the substituents on the sidearm.

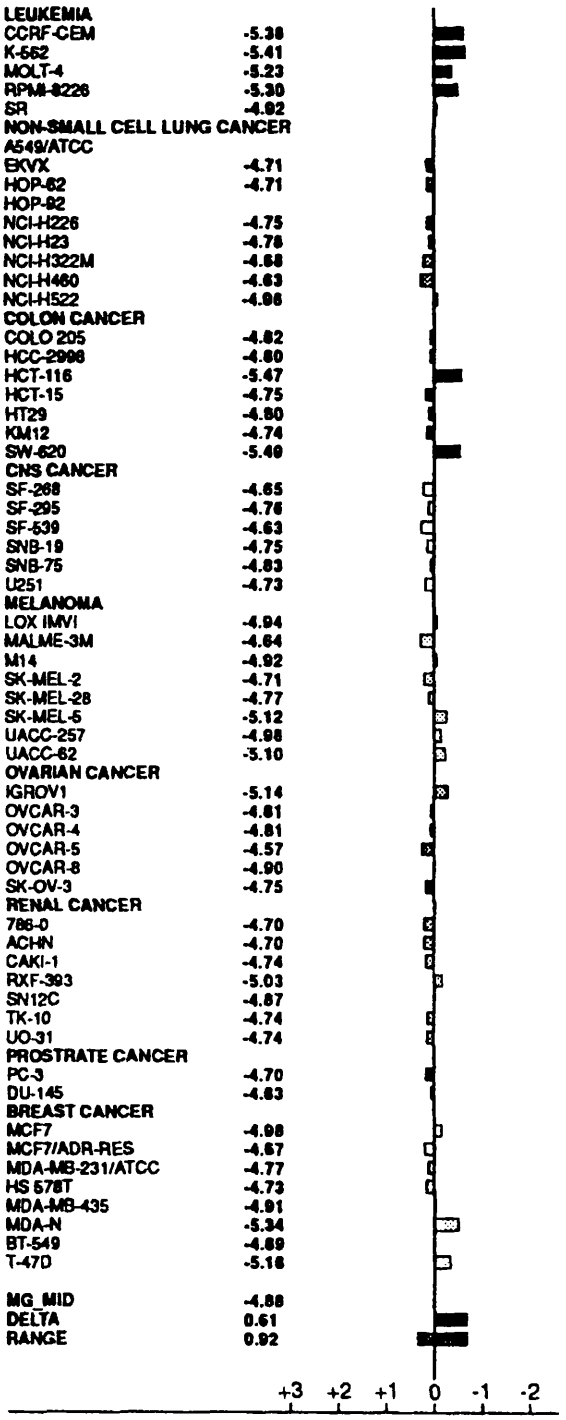
A substantial difference in the activities of many of the compounds across both assays was observed. **(117)**, **(135)** and **(136)** all exerted antiproliferative effects at nM concentration, but failed to display any inhibition at the EGF receptor.

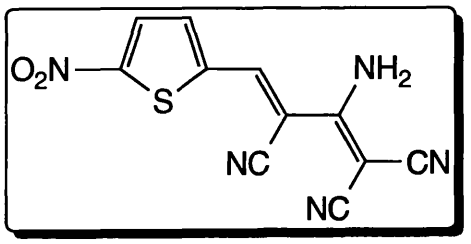
If nitrothienyl tyrphostins are not necessarily inhibitors of EGF receptor kinase function, what is their mode of action? Testing of selected compounds in HER14 fibroblasts showed that the inhibition was not EGF-dependent, and suggests that these compounds may be antiproliferative agents. Testing of compounds in squamous cells showed that cytotoxicity was independent of EGF receptor concentration.

Further evidence for cytotoxicity came from testing carried out at the US National Cancer Institute (NCI), where selected compounds were screened against an extensive panel of 60 human tumour cell lines **(Figures 27 and 28)**. Compounds **(100)** and **(122)** appeared to show a very narrow range of GI_{50} values (50 % growth inhibition) in different cell lines, indicating no selectivity against particular cell types. However, compound **(102)** showed significant variation in the sensitivity of cell types, with leukaemic tumour cells being particularly sensitive, as well as the majority of colon cancer, melanoma and breast cancer cell lines. Conversely, the CNS-derived and non-small cell lung cancer lines showed a degree of resistance to these compounds. A computerised analysis called COMPARE indicated that these three compounds had unique sensitivity profiles in the 60 cell lines compared to a panel of agents whose mechanism of action is well understood.

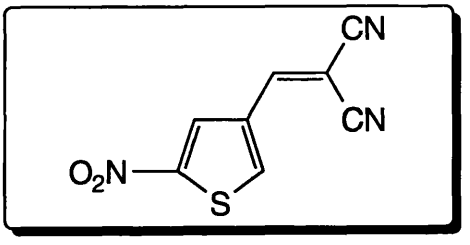
Investigation of compound **(102)** *in vivo* against leukaemic, renal tumour and melanoma xenografts was then carried out at NCI. This compound had no significant antitumour activity within the treatment schedule, and did not prolong the lives of the test animals.

Alkaline elution studies of compounds **(100)**, **(102)**, **(123)** and **(125)** showed that **(102)** and **(100)** exerted significant damage to DNA at 50 μM concentration, whereas compounds **(123)** and **(125)** were less effective. These data are extremely significant given that the first one-electron reduction potentials of **(102)** and **(100)** are both reversible and also fall within the limits required for reduction to occur within a biological system. The other two compounds had much more negative reduction potential values, which were also irreversible.



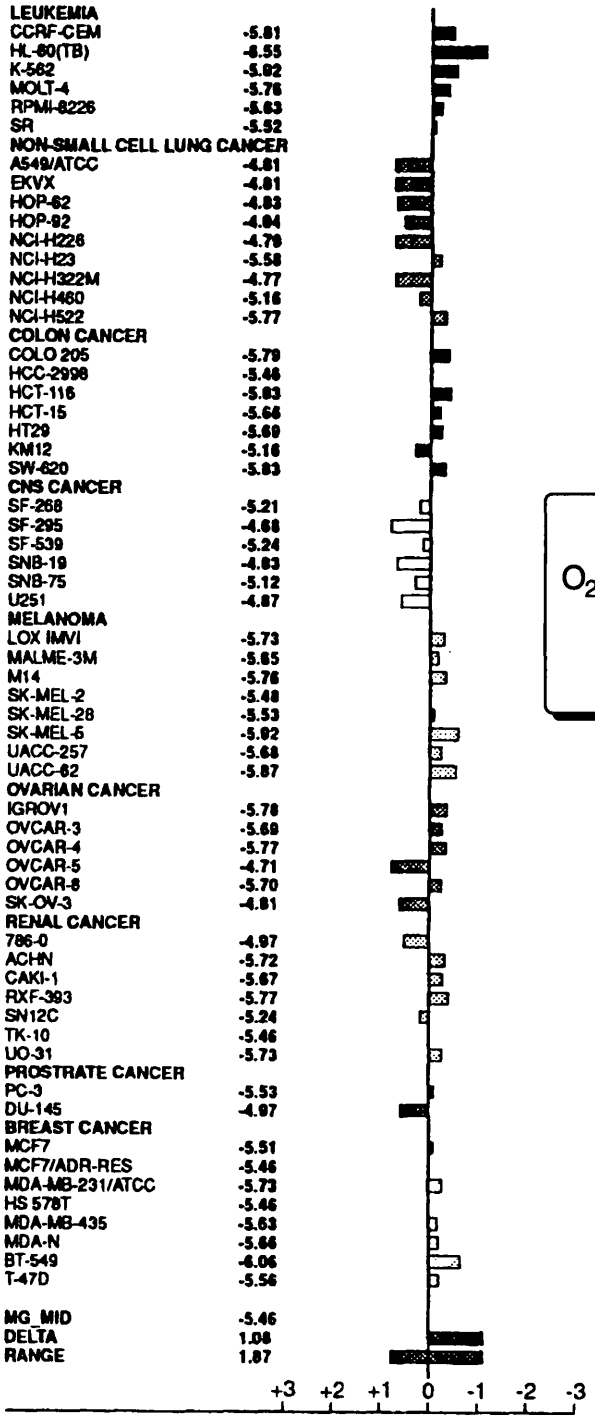


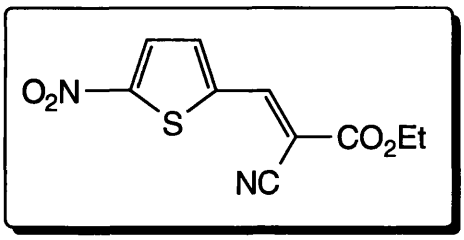
(100)



(122)

Figure 27 NCI Test Data. For each graph, the vertical line represents the log of the mean GI_{50} value based on all the 60 cell lines. Bars to the right represent activity greater than the mean [lower log (GI_{50})] whilst bars to the left represent activity lower than the mean [higher log (GI_{50})].





(102)

Figure 28

Our reduction potential data show that for nitrothienyl tyrphostins which have the sidearm and the nitro group *ortho* or *'para'*, the reduction is both within the desired parameters and is normally reversible (the only exception being compounds **(107)** and **(121)**, which have a thioamide moiety on the sidearm). Conversely, *meta*-substituted nitrothienyl tyrphostins are irreversibly reduced by a 1-electron mechanism at values which are much more negative. Clearly, a number of the compounds under investigation have reduction potentials of the order which would allow them to be reduced *in vivo* to an extremely toxic cytotoxic free radical, but to date none of the compounds reported in this thesis

have been tested under hypoxic conditions, nor have they been tested in normal cells to determine whether or not the cytotoxicity is universal or is only with respect to cancer cells.

The three tri(ethyleneglycol) ester derivatives, **(105)** **(119)** and **(128)** gave IC_{50} values in the micromolar region, and all but one compound displayed very good activities in MCF-7ADR cells, which have a higher concentration of the EGF receptor (IC_{50} 2.27 μM). These are extremely promising results.

It is not possible to comment upon the biological activity of the quinolyl and nitroquinolyl tyrphostins synthesised since they are still undergoing evaluation. We would expect that the most active series would be based upon the 7-substituted quinoline nucleus, and that the 6- and 8-substituted quinolines would have a relatively low biological activity. The two nitroquinolyl tyrphostins which were synthesised displayed extremely negative 1-electron reductions, which were irreversible.

The mode of action of tyrphostins is still unknown. We have shown that some tyrphostins based upon a nitrothienyl nucleus appear to inhibit EGF receptor tyrosine kinase activity in a cell-free assay, but little evidence exists for specific inhibition of EGF receptor function in intact cells. Nevertheless, a number of compounds do show interesting antiproliferative and cytotoxic effects, and, in particular, compound **(102)** displayed a significant degree of tumour type selectivity in the US NCI *in vitro* panel of human cell lines, although this effect was not repeated *in vivo*. This may simply be due to a problem of solubility, given that this compound (and nearly all of the others reported) are only soluble in polar aprotic solvents such as DMSO, which is itself highly toxic. Nonetheless, these are still highly potent compounds which may yet be further developed, either as free drugs, or, more likely, utilising new technologies such as ADEPT as a method of increasing specificity and solubility. Further investigation, particularly of the method of action of these compounds, is required if they are to be exploited as anticancer agents.

7. Experimental

7.1 Instrumentation

All chemicals were purchased from Aldrich or MTM Chemicals. Organic solvents were dried with anhydrous Na_2SO_4 or $MgSO_4$ and concentrated under reduced pressure using a rotary evaporator. Unless otherwise stated, ether refers to diethyl ether and petroleum ether refers to the fraction which distils between 40-60 °C. R_F values were obtained by performing qualitative thin-layer chromatography (TLC) using Merck GF_{254} silica gel plates of 0.25 mm thickness. Compounds were detected by viewing under UV.

Melting points (m.p.) were determined on a Reichert-Kofler hot stage apparatus and are uncorrected. IR spectra were recorded as KBr discs or Nujol Mulls on a Philips PU9800 Fourier Transform IR spectrometer. IR frequencies, ν, are given in wave numbers (cm^{-1}) and the relative intensities (weak, medium and strong) are denoted by w, m and s respectively. Ultraviolet spectra were recorded as solutions in dimethylsulfoxide using a Perkin-Elmer Lambda 9 UV/Visible/Near IR spectrometer. Nuclear magnetic resonance spectra were recorded on Brücker AM200SY or WP200SY instruments operating at 200 MHz (δ_H) and 50 MHz (δ_C). The multiplicities of the ^{13}C NMR spectra were determined using DEPT spectra with pulse angles of ϕ=90° and ϕ=135°. Multiplicities (singlet, doublet, triplet, quartet, multiplet and broad) are denoted by s, d, t, q, m and br respectively. The numbering systems refer to the appropriate structures. Mass Spectra (MS) were performed on an AEI/Kratos MS12 (low resolution) and an AEI/Kratos MS902S (high resolution) instrument. Elemental compositions for C, H, N and S were determined on a Carlo Erba Strumentazione Elemental Analyzer-MOD1106 with a Hewlett-Packard 3394A Integrator.

7.2 General Procedures

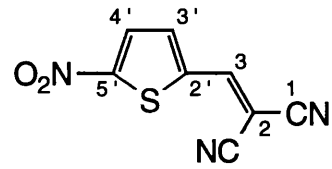
General Procedure A. A round-bottomed flask was charged with a magnetic stirring bar and 1-6 mmol of aryl carbaldehyde in absolute ethanol (1-5 ml). The solution was gently heated to obtain a clear liquid. To this was added an equimolar amount of malonate derivative dissolved in absolute ethanol (1-5 ml) and piperidine (1-5 drops from a pasteur pipette). The mixture was stirred at room temperature until precipitation of the product had occurred (10 min - 24 h) or until TLC (silica gel/1:1 ethyl acetate/petroleum ether unless otherwise stated) showed completion. The precipitate was filtered, washed (hexane) and dried (diethyl ether) under suction and needed no further purification.

General Procedure B. A round-bottomed flask was charged with a magnetic stirring bar and 1-6 mmol of aryl carbaldehyde in absolute ethanol (1-5 ml). The solution was gently heated to obtain a clear liquid. To this was added an equimolar amount of malonate derivative dissolved in absolute ethanol (1-5 ml) and piperidine (1-5 drops of an approx. 1:100 v/v solution of piperidine in absolute ethanol from a pasteur pipette; referred to as *dilute piperidine solution* in the text). The mixture was stirred at room temperature until precipitation of the product had occurred (10 min - 24 h) or until TLC (silica gel/1:1 ethyl acetate/petroleum ether unless otherwise stated) showed completion. The precipitate was filtered, washed (hexane) and dried (diethyl ether) under suction and needed no further purification.

General Procedure C. A round-bottomed flask was charged with a magnetic stirring bar and 1-6 mmol of aryl carbaldehyde in absolute ethanol (1-5 ml). The solution was gently heated to obtain a clear liquid. To this was added an equimolar amount of malonate derivative dissolved in absolute ethanol (1-5 ml). The mixture was stirred at room temperature until precipitation of the product had occurred (10 min - 24 h) or until TLC (silica gel/1:1 ethyl acetate/petroleum ether unless otherwise stated) showed completion. The precipitate was filtered, washed (hexane) and dried (diethyl ether) under suction and needed no further purification.

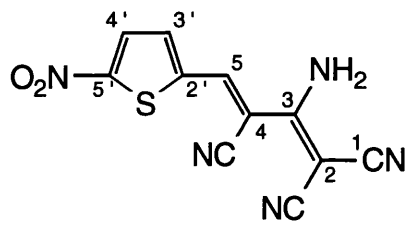
7.3 Experimental Procedures for Chapter 3

2-Cyano-3-[2-(5-nitrothienyl)]propenonitrile (99)[168, 228-230]



Using General Procedure A, 5-nitro-2-thiophenecarbaldehyde (0.5002 g, 3.2 mmol) was treated with malononitrile (0.2986 g, 4.5 mmol) in ethanol (15 ml) and piperidine (5 drops) at 0 °C to give a precipitate after 10 min which was dissolved in propan-2-ol and slowly formed brown crystals (0.2512 g, 38 %), m.p. 146-148 [lit.: 147-148][230] °C. R_F 0.54 (ethyl acetate); (Found: C 47.02; H 1.59; N 20.52 %; M^+, 204.9949. $C_8H_3N_3O_2S$ requires C 46.83; H 1.47; N 20.48 %; *M*, 204.9946); ν_{max}/cm^{-1} (KBr disc) 3050s (Ar-H), 2240w (CN), 1590s (extended conjugation to aromatic system), 1485s (C-NO_2), 815w (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.79 (br s, 1H, 3-H), 8.23 (d, ^3J 4.4 Hz, 1H, 4'-H), 7.89 (d, ^3J 4.4 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) [lit.: 25 MHz, DMSO-d_6][168] 155.9 [155.8] (s, 5'-C), 152.5 [152.3] (d, 3-CH), 139.2 [139.1] (s, 2'-C), 137.9 [137.8] (d, 3'-CH), 129.6 [129.3] (d, 4'-CH), 114.9 and 112.9 [113.0 and 112.5] (2 x s, 2 x CN), 102.8 [82.4] (s, 2-C); *m/z* 205 (M^+, 67 %), 159 (M^+ - NO_2).

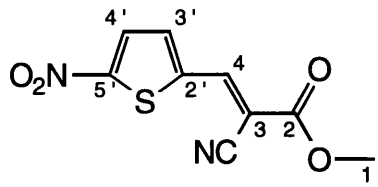
2,4-Dicyano-3-amino-5-[2-(5-nitrothienyl)]penta-2,4-dienonitrile (100)



5-Nitro-2-thiophenecarbaldehyde (0.7949 g, 5.06 mmol) was treated with 2-aminopropene-1,1,3-tricarbonitrile (1.0946 g, 8.28 mmol) in ethanol (20 ml) and piperidine (1 drop) using General Procedure A, to give brown crystals after 10 min (0.5459 g, 40 %), m.p. 212-214 °C (1:1 acetone:chloroform). R_F 0.50 (ethyl acetate); (Found: C 48.52; H 1.91; N 25.96; S 12.06 %; M^+, 271.0164. $C_{11}H_5N_5O_2S$ requires C 48.71; H 1.86; N 25.82 S 11.82 %; *M*, 271.0170); ν_{max}/cm^{-1} (KBr disc) 3092w (Ar-H), 2250w (NH_2), 2222w (CN), 1637s (C=C), 1518s (C-NO_2); δ_H (200 MHz, DMSO-d_6) 9.17 (s, 2H, -NH_2), 8.41 (d, ^4J 0.4 Hz, 1H, 5-H), 8.23 (d, ^3J 4.4 Hz, 1H, 4'-H), 7.88 (dd, ^3J 4.4 Hz, ^4J 0.4 Hz, Hz, 1H,

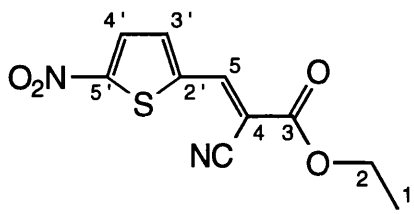
3'-H); δ_C (50 MHz, DMSO-d_6) 163.8 (s, 5'-C), 154.9 (s, 2'-C), 145.3 (d, 4'-CH), 139.9 (d, 3'-CH), 136.7 (d, 5-CH), 129.7 (s, 2-C), 115.5 (s, 7-CN), 115.2 (s, 6-CN), 114.4 (s, 1-CN), 103.0 (s, 4-C), 50.4 (s, 3-C); *m/z* 271 (M^+, 34 %), 225 ($M^+ - NO_2$), 153 ($M^+ - [NC\text{-}C(NH_2)=C(CN)_2]$), 92 ($[C(NH_2)=C(CN)_2]^+$).

Methyl 2-cyano-3-[2-(5-nitrothienyl)]propenoate (101)[168]



Using General Procedure B, 5-nitro-2-thiophenecarbaldehyde (0.4646 g, 2.96 mmol) was treated with methyl cyanoacetate (0.50 ml, 5.68 mmol) and dilute piperidine solution (1 drop) to give yellow crystals after 10 min (0.6440 g, 94 %), m.p. 170-172 [lit.: 136-137][168] °C. R_F 0.60 (ethyl acetate); (Found: C 45.54; H 2.50; N 11.81; S 13.66 %; M^+, 238.0088. $C_9H_6N_2O_4S$ requires C 45.38; H 2.54; N 11.76; S 13.44 %; *M*, 238.0048); ν_{max}/cm^{-1} (KBr disc) 3100s (Ar-H), 2950s (CH_x), 1720s ($\alpha\beta$-unsaturated carbonyl), 1500s (C-NO_2), 1340b (-OCOR), 835s and 815s (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) [lit.: 80 MHz, $CDCl_3$] 8.67 [8.56] (s, 1H, 4-H), 8.22 [8.12] (d, ^3J 4.5 Hz, 1H, 3'-H), 8.02 [8.04] (d, ^3J 4.5 Hz, 1H, 4'-H), 3.85 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.5 (s, C=O), 155.2 (s, 5'-C), 146.4 (d, 4'-CH), 140.2 (s, 2'-C), 138.1 (d, 3'-CH), 129.6 (d, 4-CH), 115.1 (s, CN), 103.2 (s, 3-C), 53.4 (t, CH_3); *m/z* (M^+, 38 %), 192 ($M^+ - NO_2$), 179 ($M^+ - CO_2Me$).

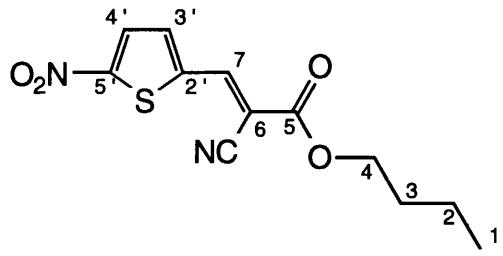
Ethyl 2-cyano-3-[2-(5-nitrothienyl)]propenoate (102)



Using General Procedure A, 5-nitro-2-thiophenecarbaldehyde (0.50 g, 3.2 mmol) was treated with ethyl cyanoacetate (0.40 g, 4.5 mmol) in ethanol (15 ml) and piperidine (5 drops) to give yellow crystals after 30 min (0.40252. g, 49 %), m.p. 170-172 °C. R_F 0.52 (ethyl acetate); (Found: C 47.48; H 3.14; N 11.02 %; M^+, 252.0211. $C_{10}H_8N_2O_4S$ requires C 47.62; H 3.20; N 11.11 %;

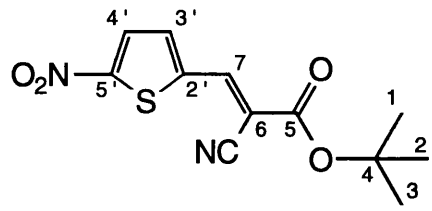
M, 252.0205); ν_{max}/cm^{-1} (KBr disc) 3100s (Ar-H), 2220w (CN), 1710s (αβ-unsaturated carbonyl), 1600s (extended conjugation to aromatic system), 1500s (C-NO_2); λ_{max} (DMSO)/nm 368; δ_H (200 MHz, DMSO-d_6) 8.64 (s, 1H, 5-H), 8.22 (d, ^3J 4.3 Hz, 1H, 3'-H), 8.03 (d, ^3J 4.3 Hz, 1H, 4'-H), 4.35 (q, ^3J 7.1 Hz, 2H, CH_2), 1.33 (t, ^3J 7.1 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.0 (s, C=O), 155.2 (s, 5'-C), 146.3 (d, 4'-CH), 140.34 (s, 2'-C) 138.1 (d, 3'-CH), 129.6 (d, 5-CH), 115.0 (s, CN), 103.5 (s, 4-C), 62.9 (t, CH_2), 14.0 (q, CH_3); *m/z* 252 (M^+, 32 %), 206 (M^+ - NO_2), 179 (M^+ - CO_2Et).

^{n}Butyl 2-cyano-3-[2-(5-nitrothienyl)]propenoate (103)



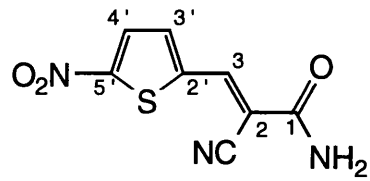
5-Nitro-2-thiophenecarbaldehyde (0.48 g, 3.05 mmol) was treated with ^{n}butylcyanoacetate (1.0 ml, 7.08 mmol) in propan-2-ol (2.5 ml) and dilute piperidine solution (1 drop) using General Procedure B, to give a yellow precipitate after 10 min (0.56 g, 65 %), m.p. 110-112 °C. R_F 0.71 (ethyl acetate); (Found: C 51.45; H 4.30; N 9.96 %; M^+, 280.0513. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.31; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 3100s (Ar-H), 2950s (CH_x), 2120w (CN), 1720s (αβ-unsaturated carbonyl), 1500s (C-NO_2), 1340b (-OCOR),835s and 810s (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.65 (br s, 1H, 7-H), 8.22 (d, ^3J 4.5 Hz, 1H, 4'-H), 8.03 (d, ^3J 4.5 Hz, 1H, 3'-H), 4.26 (t, ^3J 6.4 Hz, 2H, 4-H_2), 1.72-1.58 (tt, ^3J 7.2 Hz, ^3J 6.4 Hz, 2H, 3-H_2), 1.48-1.29 (tq, ^3J 7.2 Hz, ^3J 7.2 Hz, 2H, 2-H_2), 0.91 (t, ^3J 7.2 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.0 (s, C=O), 155.1 (d, 4'-C), 146.4 (s, 5'-C), 140.3 (s, 2'-C), 138.1 (d, 3'-C), 129.6 (d, 7-CH), 115.0 (s, CN), 103.4 (s, 6-C), 66.3 (t, 4-CH_2), 30.0 (t, 3-CH_2), 16.6 (t, 2-CH_2), 13.6 (q, CH_3); *m/z* 280 (M^+, 18 %), 234 (M^+ - NO_2), 179 (M^+ - $CO_2{}^nBu$).

^{t}Butyl 2-cyano-3-[2-(5-nitrothienyl)]propenoate (104)



5-Nitro-2-thiophenecarbaldehyde (0.4513 g, 2.87 mmol) was treated with ^{t}butyl cyanoacetate (0.4155 g, 2.94 mmol) in ethanol (7.5 ml) and piperidine (2 drops) using General Procedure A, to give a beige precipitate after 1 h (0.4893 g, 61 %), m.p. 167-170 °C. R_F 0.73 (1:1 ethyl acetate/petroleum ether); (Found: C 51.21; H 4.35; N 9.96 %; M^+, 280.0522. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.31; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 3105s (Ar-H), 2986s and 2355s (CH_x), 2225w (CN), 1728s (C=C), 1605s (extended conjugation to aromatic system), 1532s and 1343s (C-NO_2), 1396s and 1373s (C-H), 839s and 824s (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.57 (s, 1H, 7-H), 8.23 (d, ^3J 4.0 Hz, 1H, 4'-H), 8.04 (d, ^3J 4.0 Hz, 1H, 3'-H), 1.58 (s, 9H, ^{t}Bu); δ_C (50 MHz, DMSO-d_6, 333K) 159.6 (s, C=O), 154.7 (s, 5'-C), 145.5 (d, 4'-CH), 140.2 (s, 2'-C), 137.1 (d, 3'-CH), 129.4 (d, 7-CH), 115.0 (s, CN), 105.0 (s, 6-C), 83.9 (s, $C(CH_3)_3$), 27.3 (s, 3 x CH_3); *m/z* 280 (M^+, 100 %), 234 (M^+ - NO_2), 224 (M^+ - ^{t}Bu, + H), 191 (M^+ -$CO_2{}^t$Bu), 57 (^{t}Bu$^+$).

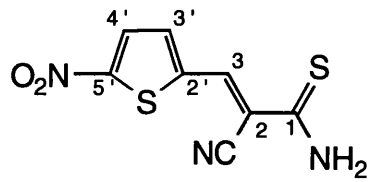
2-Cyano-3-[2-(5-nitrothienyl)]propenamide (106)[169]



Using General Procedure A, 5-nitro-2-thiophenecarbaldehyde (0.50 g, 3.2 mmol) was treated with 2-cyanoacetamide (0.30 g, 3.5 mmol) in ethanol (15 ml) and 5 drops piperidine to give a brown precipitate after 30 min (4.8 g, 15 %), m.p. 230-232 °C. R_F 0.58 (ethyl acetate); (Found: C 43.26; H 2.43; N 18.66 %; M^+, 223.0041. $C_8H_5N_3O_3S$ requires C 43.05; H 2.25; N 18.82 %; *M*, 223.0052); ν_{max}/cm^{-1} (KBr disc) 3350s and 3250m (-$CONH_2$), 2920s (Ar-H), 2200w (CN), 1690s (extended conjugation to aromatic system), 1500m (C-NO_2), 821m (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.46 (s, 1H, 3-H), 8.24 (d, ^3J 4.4 Hz, 1H, 4'-H), 8.03 and 7.96 (2 br s, 2H, NH_2), 7.90 (d, ^3J 4.4 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) [lit.: 25 MHz, DMSO-d_6] 161.5 [161.3] (s, C=O),

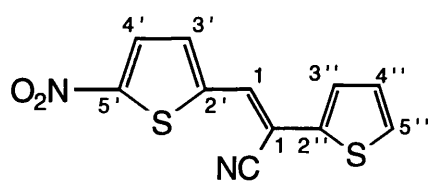
154.1 [153.9] (s, 5'-C), 142.6 [142.8] (d, 3-CH), 141.1 [141.0] (s, 2'-C), 136.7 [135.8] (d, 3'-CH), 129.7 [129.5] (d, 4'-CH), 115.9 [115.7] (s, CN), 107.5 [107.4] (s, 2-C); *m/z* 223 (M^+, 11 %), 179 (M^+ - $CONH_2$), 177 (M^+ - NO_2).

2-Cyano-3-[2-(5-nitrothienyl)]propenethioamide (107)



A round-bottomed flask was charged with 5-nitro-2-thiophenecarbaldehyde (0.50 g, 3.2 mmol) in absolute ethanol (10 ml). The solution was cooled to -78 °C. To this was added 2-cyanothioacetamide (0.40 g, 4.0 mmol) dissolved in absolute ethanol (5 ml) and diethylamine (5 drops from a pasteur pipette). The mixture was stirred at -78 °C for 30 min until precipitation of the product had occurred. The precipitate was filtered, washed (hexane) and dried (diethyl ether) under suction to give a dark brown product (0.33 g, 44 %) which needed no further purification, m.p. 134-137 °C. R_F 0.47 (ethyl acetate); (Found: C 40.32; H 2.14; N 17.40 %; M^+, 238.9849. $C_8H_5N_3O_2S_2$ requires C 40.16; H 2.10; N 17.56 %; *M*, 238.9823); ν_{max}/cm^{-1} (KBr disc) 3145s (Ar-H), 2190w (CN), 1470s (C-NO_2), 1460b ($CSNH_2$); λ_{max} (DMSO)/nm 365; δ_H (200 MHz, DMSO-d_6) 8.36 (s, 1H, 3-H), 8.22 (d, ^3J 4.4 Hz, 1H, 4'-H), 7.98 and 7.95 (2 br s, 2H, NH_2), 7.90 (d, ^3J 4.4 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 162.4 (s, C=S), 148.8 (s, 5'-C), 141.8 (d, 4'-CH), 140.7 (s, 2'-C), 134.2 (s, 3'-C), 127.3 (d, 3-CH), 112.4 (s, CN), 104.3 (s, 2-C); *m/z* 239 (M^+, 24.0 %), 193 (M^+ - NO_2), 179 (M^+ - $CSNH_2$).

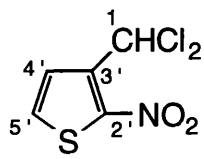
2-Cyano-3-[4-(2-nitrothienyl)]-1-(2-thienyl)propene (108)



2-Nitro-4-thiophenecarbaldehyde (0.1984 g, 1.26 mmol) was treated with 2-thiopheneacetonitrile (0.1678 g, 1.51 mmol) in ethanol (3 ml) and piperidine (5 drops) using General Procedure A to give dark crystals after 8 d (0.0469 g, 15 %), m.p. 245 °C. R_F 0.45 (1:1 ethyl acetate/petroleum ether); (Found:

C 50.40; H 2.28; N 10.65 %; M^+, 261.9875. $C_{11}H_6N_2O_2S_2$ requires C 50.37; H 2.30; N 10.68 %; *M*, 261.9871); ν_{max}/cm^{-1} (KBr disc) 2970s (Ar-H), 2220w (CN), 1615s (extended conjugation to aromatic system), 1500 (C-NO_2), 819m (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.43 (s, 1H, 2-H), 8.30-7.45 (m, 5H, 2', 4', 3", 4" and 5"-H); δ_C (50 MHz, DMSO-d_6) 152.1 (s, 5'-C), 147.2 (d, 4'-CH), 139.0 (d, 2'-CH), 133.5 (s, 3'-C), 133.2 (s, 2"-C), 130.1 (d, 2-CH), 127.3 and 127.0 (2 x d, 3" and 5"-CH), 125.6 (d, 4"-CH), 116.4 (s, CN), 106.2 (s, 1-C); *m/z* 262 (M^+, 15 %), 216 (M^+ -NO_2), 179 (M^+ -thiophene + H), 82 (thiophene$^+$).

3-Dichloromethyl-2-nitrothiophene (112)[170]

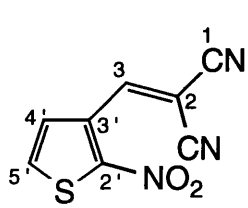


A flame-dried, 3-necked round-bottom flask, equipped with a magnetic stirrer, low-temperature thermometer, nitrogen bubbler, pressure-equilibrated dropping funnel and acetone/dry ice bath was set up, containing a dispersion of potassium ^{t}butoxide (11.5107 g, 102.6 mmol) in dry DMF (25 ml) and distilled THF (25 ml). 2-Nitrothiophene (4.4146 g, 34.18 mmol) dissolved in dry dimethyl formamide (6 ml) and chloroform (6 ml, 74.9 mmol) was added to the flask over a period of about 2 h. The temperature during addition was not allowed to exceed -50 °C. Upon completion of addition of this solution, the reaction mixture was immediately quenched with a 1:1 solution of acetic acid in methanol (22 ml). Excess acid was neutralised using aqueous $NaHCO_3$, and the thick brown slurry which formed was allowed to warm to room temperature and was washed with water (101 ml) and extracted into ether (3 x 100 ml). The ethereal extracts were dried ($MgSO_4$) and concentrated *in vacuo* to a black viscous tar. Column chromatography over silica (5:1 hexane/ethyl acetate) yielded the product as large brown crystals upon recrystallisation from methanol. (6.8223 g, 94 %), m.p. 70-72 °C, R_F 0.75 (1:1 ethyl acetate/petroleum ether). δ_H (200 MHz, acetone-d_6) 8.04 (d, ^3J 5.7 Hz, 1H, 5'-H), 7.75 (s, 1H, 1-H), 7.70 (d, ^3J 5.7 Hz, 4'-H) ;δ_C (50 MHz, acetone-d_6) 140.9 (s, 2'-C), 130.0 (d, 5'-CH), 128.9 (d, 4'-CH), 125.0 (s, 3'-C), 63.9 (d, $CHCl_3$).

2-Nitro-3-thiophenecarbaldehyde (83)[170]

3-Dichloromethyl-2-nitrothiophene **(112)** (6.8223 g, 32.17 mmol) and an 85 % w/v aqueous solution of formic acid (101 ml) were heated at reflux temperature under a nitrogen atmosphere for 22 h. The mixture was cooled to room temperature, and the excess formic acid was neutralised using aqueous sodium bicarbonate solution. The mixture was extracted with diethyl ether (5 x 100 ml), and the combined ethereal extracts were concentrated *in vacuo*. Flash column chromatography over SiO_2 using 3:1 hexane/ethyl acetate, followed by concentration *in vacuo* gave a brown oil, which solidified at room temperature. The product was recrystallised from ethyl acetate to give fine brown needles (1.4391 g, 28 %), m.p. 59 [lit.: 54][170] °C, R_F 0.58 (1:1 ethyl acetate/petroleum ether); (Found: C 38.06; H 1.87; N 11.12 %; M^+, 156.9846. $C_5H_3NO_3S$ requires C 38.21; H 1.92; N 11.22 %; *M*, 156.9834); ν_{max}/cm^{-1} (KBr disc) 3096s (Ar-H), 3080s (Ar-H) 1694s (Ar-CHO), 1534 (C-NO_2); δ_H (200 MHz, DMSO-d_6) [lit.: 90 MHz, DMSO-d_6] 10.61 [10.70] (s, 1H, -CHO), 7.95 (d, ^3J 5.4 Hz, 5'-H), 7.78 (d, ^3J 5.4 Hz, 4'-H); δ_C (50 MHz, DMSO-d_6) 183.9 (d, -CHO), 133.0 (s, 2'-C), 131.0 (d, 5'-CH), 126.7 (d, 4'-CH), 126.0 (s, 3'-C); *m/z* 157 (M^+, 2 %), 111 (M^+ - NO_2).

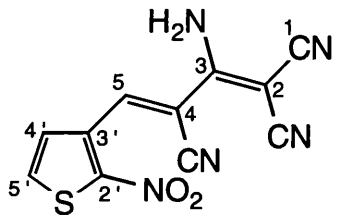
2-Cyano-3-[2-(3-nitrothienyl)]propenonitrile (113)



Using General Procedure C, 2-nitro-3-thiophenecarbaldehyde **(83)** (0.2295 g, 1.46 mmol) was treated with malononitrile (0.1041 g, 1.57 mmol) in ethanol (7 ml) to give a brown precipitate after 12 h (0.2288 g, 77 %), m.p. 192-196 °C. R_F 0.67 (1:1 ethyl acetate/petroleum ether); (Found: C 46.67; H 1.48; N 20.69 %; M^+, 204.9962. $C_8H_3N_3O_2S$ requires C 46.83; H 1.47; N 20.48 %; *M*, 204.9946); ν_{max}/cm^{-1} (KBr disc) 3038s (Ar-H), 2230w (CN), 1636s (extended conjugation to aromatic system), 1580s (aromatic C=C), 1522s (C-NO_2), 870w and 834w (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.72 (s, 1H, 3-H), 8.22 (d, ^3J 5.7 Hz, 1H, 5'-H), 7.76 (d, ^3J 5.7 Hz, 1H, 4'-H); δ_C (50 MHz,

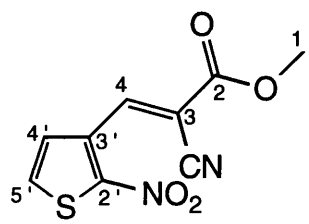
DMSO-d_6) 152.2 (d, 3-CH), 134.3 (d, 5'-CH), 131.6 and 131.5 (2 x s, 2'-C and 3'-C), 127.3 (d, 3-CH), 125.7 (s, 5'-C), 113.3 and 112.2 (2 x s, 2 x CN), 88.3 (s, 2-C); *m/z* 205 (M^+, 46 %), 159 (M^+ - NO_2), 82 (thiophene$^+$).

2,4-Dicyano-3-amino-5-[3-(2-nitrothienyl)]penta-2,4-dienonitrile (114)



2-Nitro-3-thiophenecarbaldehyde **(83)** (0.1046 g, 0.67 mmol) was treated with 2-aminopropene-1,1,3-tricarbonitrile (0.0914 g, 0.69 mmol) in ethanol (6 ml) and dilute piperidine solution (2 drops) using General Procedure B, to give brown crystals after 48 h (0.1351 g, 75 %), m.p. 209-211 °C. R_F 0.58 (1:1 ethyl acetate/petroleum ether); (Found: C 48.86; H 1.91; N 25.91, S 11.84 %; M^+, 271.0146. $C_{11}H_5N_5O_2S$ requires C 48.71; H 1.82; N 25.82, S 11.81 %; *M*, 271.0164); ν_{max}/cm^{-1} (KBr disc) 3052s (Ar-H), 2726s (NH_2), 2223m and 2209m (2 x CN), 1647s (external conjugation to aromatic system), 1607 (aromatic C=C), 1489s (C-NO_2), 820w (Ar-H); δ_H (200 MHz, DMSO-d_6) 9.27 (br s, 2H, -NH_2), 8.40 (s, 1H, 5-H), 8.23 (d, ^3J 5.6 Hz, 1H, 5'-H), 7.78 (d, ^3J 5.6 Hz, 1H, 5'-H); δ_C (50 MHz, DMSO-d_6) 164.0 (s, 2'-C), 151.2 (s, 3'-C), 144.5 (d, 5'-CH), 134.3 (d, 4'-CH), 132.2 (s, 3-C), 127.2 (d, 5-CH), 115.1, 114.4 and 114.0 (3 x s, 3 x CN), 108.8 (d, 4-CH), 108.2 (s, 2-C); *m/z* 270 (M^+, 2 %), 245 (M^+ - CN), 225 (M^+ - NO_2), 82 (thiophene$^+$), 92 ($C(NH_2)=C(CN)_2^+$).

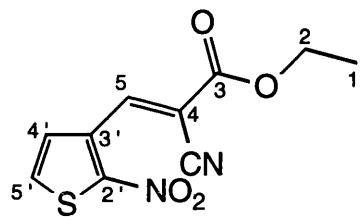
Methyl 2-cyano-3-[3-(2-nitrothienyl)]propenoate (115)



Using General Procedure A, 2-nitro-3-thiophenecarbaldehyde **(83)** (0.1650 g, 1.05 mmol) was treated with methyl cyanoacetate (0.1164 g, 1.17 mmol) in methanol (1 ml) and piperidine (5 drops) to give brown crystals after 10 min (0.1921 g, 77 %), m.p. 119-121 °C. R_F 0.46 (1:1 ethyl acetate/petroleum ether);

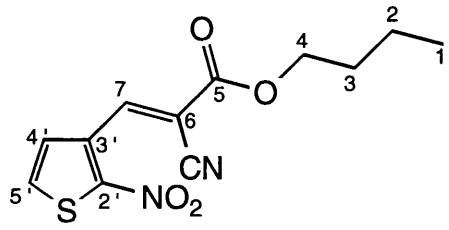
(Found: C 45.28; H 2.57; N 11.59 %; M^+, 238.0056. $C_9H_6N_2O_4S$ requires C 45.38; H 2.54; N 11.76 %; *M*, 238.0048); ν_{max}/cm^{-1} (KBr disc) 3125m (Ar-H), 2250w (CN), 1717s (αβ-unsaturated carbonyl), 1530m (C-NO_2) 820m (Ar-H); δ_H (200 MHz, DMSO-d_6) 9.00 (s, 1H, 4-H), 8.28 (d, ^3J5.7 Hz, 1H, 5'-H), 7.83 (d, ^3J5.7 Hz, 1H, 4'-H), 3.86 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.6 (s, C=O), 145.7 (d, 5'-CH), 143.0 (s, 2'-C), 133.9 (d, 4'-CH), 132.5 (s, 3'-C), 127.5 (d, 4-CH), 114.6 (s, CN), 108.2 (s, 3-C), 53.9 (q, CH_3); *m/z* 238 (M^+, 9 %), 192 (M^+ - NO_2), 179 (M^+ - CO_2Me), 59 (CO_2Me).

Ethyl 2-cyano-3-[3-(2-nitrothienyl)]propenoate (116)



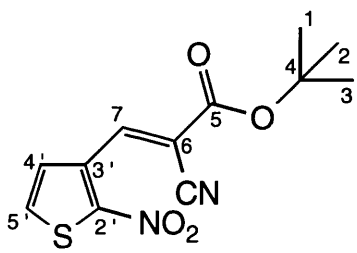
2-Nitro-3-thiophenecarbaldehyde **(83)** (0.2245 g, 1.43 mmol) was treated with ethyl cyanoacetate (0.1694 g, 1.49 mmol) in ethanol (7 ml) and dilute piperidine solution (1 drop) using General Procedure B to give a brown precipitate after 10 min (0.1650 g, 45 %), m.p. 89 °C. R_F 0.63 (1:1 ethyl acetate/petroleum ether); (Found: C 47.55; H 3.14; N 11.09 %; M^+, 252.0221. $C_{10}H_8N_2O_4S$ requires C 47.62; H 3.20; N 11.11 %; *M*, 252.0205); ν_{max}/cm^{-1} (KBr disc) 3115m (Ar-H), 1725s (αβ-unsaturated carbonyl), 1607s (extended conjugation to aromatic system), 1532s (C-NO_2), 1501s (aromatic C=C), 819m (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.72 (s, 1H, 5-H), 8.20 (d, ^3J5.6 Hz, 1H, 5'-H), 7.83 (d, ^3J5.6 Hz, 1H, 4'-H), 8.36 (q, ^3J 7.1 Hz, 2H, CH_2), 1.36 (t, ^3J 7.1 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.0 (s, C=O), 145.3 (d, 5'-CH), 142.7 (s, 2'-C), 133.9 (d, 4'-CH), 132.4 (s, 3'-C), 127.4 (d, 5-CH), 114.6 (s, CN), 108.4 (s, 4-C), 63.0 (t, CH_2), 14.0 (s, CH_3); *m/z* 252 (M^+, 7 %), 206 (M^+ - NO_2), 179 (M^+ - CO_2Et).

^{n}Butyl 2-cyano-3-[3-(2-nitrothienyl)]propenoate (117)



2-Nitro-3-thiophenecarbaldehyde **(83)** (0.2209 g, 1.41 mmol) was treated with ^{n}butyl cyanoacetate (0.1992 g, 1.41 mmol) in ethanol (3 ml) and piperidine (1 drop) using General Procedure A, to give yellow crystals after 10 min (0.1328 g, 33 %), m.p. 67-69 °C. R_F 0.53 (1:1 ethyl acetate/petroleum ether); (Found: C 51.36; H 4.35; N 9.87 %; M^+, 280.0517. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.31; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 3120w (Ar-H), 2250w (CN), 1725s (C=O), 1608s (extended conjugation to aromatic system), 1530s (C-NO_2), 1522s (aromatic C=C), 822s (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.82 (s, 1H, 7-H), 8.20 (d, ^{3}J 5.6 Hz, 1H, 5'-H), 7.86 (d, ^{3}J 5.6 Hz, 1H, 4'-H), 4.35 (t, ^{3}J 6.4 Hz, 2H, 4-H_2), 1.80-1.66 (tt, ^{3}J 7.8 Hz, ^{3}J 6.4 Hz, 2H, 3-H_2), 1.55-1.37 (tq, ^{3}J 7.8 Hz, ^{3}J 7.1 Hz, 2H, 2-H_2), 0.93 (t, ^{3}J 7.1 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.0 (s, C=O), 145.4 (d, 5'-CH), 133.9 (d, 4'-CH), 132.4 (s, 2'-C), 127.4 (d, 7-CH), 125.7 (s, 3'-C), 114.6 (s, CN), 106.4 (s, 6-C), 66.5 (t, 4-CH_2), 30.1 (t, 3-CH_2), 18.7 (t, 2-CH_2), 13.6 (q, CH_3); *m/z* 280 (M^+, 4 %), 234 (M^+ - NO_2), 181 (M^+ - $CO_2{}^{n}Bu$, - 2H).

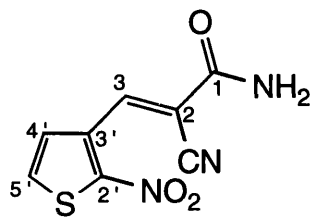
^{t}Butyl 2-cyano-3-[3-(2-nitrothienyl)]propenoate (118)



2-Nitro-3-thiophenecarbaldehyde **(83)** (0.0757 g, 0.48 mmol) was treated with ^{t}butyl cyanoacetate (0.10 ml, 0.70 mmol) in propan-2-ol (2 ml) and piperidine (1 drop) using General Procedure A to give brown crystals after 24 h (0.1077 g, 80 %), m.p. 171-173 °C. R_F 0.69 (1:1 ethyl acetate/petroleum ether); (Found: C 51.69; H 4.26; N 9.95%; M^+, 280.0546. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.31; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 3105s (Ar-H), 2222m (CN), 1724s (αβ-unsaturated carbonyl), 1640s (αβ-unsaturated ester), 1614s (extended conjugation to aromatic system), 1540m (C-NO_2), 886w and 860w

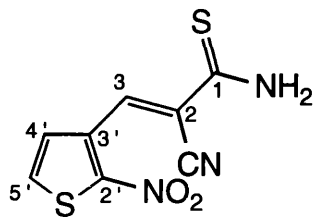
886w and 860w (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.92 (s, 1H, 7-H), 7.92 (d, ^3J 5.6 Hz, 1H, 5'-H), 8.18 (d, ^3J 5.6 Hz, 1H, 4'-H), 1.47 (s, 9H, ^{t}Bu); δ_C (50 MHz, DMSO-d_6) 163.4 (s, C=O), 140.1 (s, 2'-C), 134.1 (d, 5'-CH), 128.2 (d, 4'-CH), 126.4 (d, 7-CH), 125.5 (s, 6-C), 115.4 (s, CN), 27.6 (q, 3 x CH_3), 25.6 (s, $C(CH_3)_3$); *m/z* 280 (M^+, 15%), 234 (M^+ - NO_2), 223 (M^+ - ^{t}Bu), 179 (M^+ -$CO_2{}^t$Bu), 57 (^{t}Bu).

2-Cyano-3-[3-(2-nitrothienyl)] propenamide (120)



2-Nitro-3-thiophenecarbaldehyde **(83)** (0.1586 g, 1.01 mmol) was treated with 2-cyanoacetamide (0.0849 g, 1.01 mmol) in ethanol (4 ml) and piperidine (1 drop) using General Procedure A, to give a brown precipitate after 50 h (0.0718 g, 31 %), m.p. 216-217 °C. R_F 0.56 (1:1 ethyl acetate/petroleum ether); (Found: C 43.04; H 2.31; N 19.01 %; M^+, 223.0059. $C_8H_5N_3O_3S$ requires C 43.05; H 2.25; N 18.82 %; *M*, 223.0052); ν_{max}/cm^{-1} (KBr disc) 3455s and 3380s (-$CONH_2$), 3077b (Ar-H), 2200m (CN), 1618s (extended conjugation to aromatic system), 1516w (-$CONH_2$), 1495m (aromatic C=C), 824s (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.20 (s, 1H, 3-H), 8.19 (d, ^3J 5.6 Hz, 1H, 5'-H), 8.08 (br s, 2H, NH_2), 7.79 (d, ^3J 5.6 Hz, 1H, 4'-H); δ_C (50 MHz, DMSO-d_6) 161.3 (s, C=O), 141.9 (d, 5'-CH), 139.5 (s, 2'-C), 133.8 (d, 4'-CH), 131.7 (s, 3'-C), 127.7 (d, 3-CH), 115.3 (s, -CN), 112.3 (s, 2-C); *m/z* 223 (M^+, 5 %), 179 (M^+ - $CONH_2$), 177 (M^+ - NO_2).

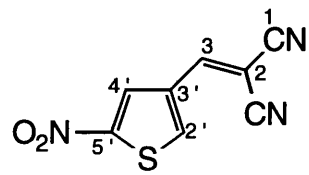
2-Cyano-3-[3-(2-nitrothienyl)]propenethioamide (121)



Using General Procedure C, 2-nitro-3-thiophenecarbaldehyde **(83)** (0.1226 g, 0.78 mmol) was treated with 2-cyanothioacetamide (0.0801 g, 0.80 mmol) in

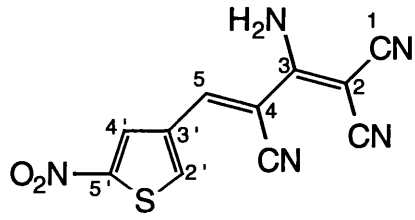
propan-2-ol (4 ml) to give beige crystals after 10 min (0.1594 g, 85 %), m.p. 89-91 °C. R_F 0.55 (1:1 ethyl acetate/petroleum ether); (Found: C 40.23; H 2.15; N 17.66 %; M^+, 238.9787. $C_8H_5N_3O_2S_2$ requires C 40.16; H 2.10; N 17.56 %; *M*, 238.9823); ν_{max}/cm^{-1} (KBr disc) 3096s (Ar-H), 2188m (CN), 1625s (extended conjugation to aromatic system), 1507s (C-NO_2), 1459s ($CSNH_2$), 818w (Ar-H); δ_H (200 MHz, DMSO-d_6) 9.84 and 9.55 (2 x br s, 2H, NH_2), 8.05 (d, ^3J 5.6 Hz, 1H, 5'-H), 7.73 (s, 1H, 3-H), 7.62 (d, ^3J 5.6 Hz, 1H, 4'-H); δ_C (50 MHz, DMSO-d_6) 168.2 (s, C=S), 143.1 (d, 5'-CH), 140.2 (s, 2'-C), 138.1 (d, 4'-CH), 134.2 (s, 3'-C), 128.4 (d, 3-CH), 116.1 (s, CN), 111.8 (s, 2-C); *m/z* 239 (M^+, 8 %), 192 (M^+ - NO_2 - H), 178 (M^+ - $CSNH_2$ - H).

2-Cyano-3-[4-(2-nitrothienyl)]propenonitrile (122)[116, 118]



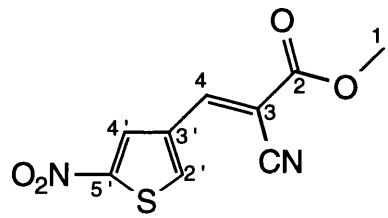
Using General Procedure C, 2-nitro-4-thiophenecarbaldehyde (0.4985 g, 3.17 mmol) was treated with malononitrile (0.5680 g, 8.60 mmol) in ethanol (5 ml) to give a pink precipitate after 10 min (0.4096 g, 63 %), m.p. 104-106 °C. R_F 0.60 (ethyl acetate); (Found: C 46.75; H 1.52; N 20.54; S 15.70 %; M^+, 204.9926. $C_8H_3N_3O_2S$ requires C 46.83; H 1.47; N 20.48; S 15.61 %; *M*, 204.9946); ν_{max}/cm^{-1} (nujol) 3103s (Ar-H), 2233s (CN), 1597s (C=C), 1508s (C-NO_2), 819s (isolated Ar-H); δ_H (200 MHz, DMSO-d_6) 8.76-8.74 (dd, ^4J 1.9 Hz, ^4J 0.5 Hz, 1H, 3-H), 8.48-8.47 (dd, ^4J 0.9 Hz, ^4J 0.5 Hz, 1H, 4'-H), 8.46-8.44 (dd, ^4J 1.9 Hz, ^4J 0.9 Hz, 1H, 2'-H); δ_C (50 MHz, DMSO-d_6) 153.4 (d, 3-CH), 152.8 (s, 5'-C), 143.5 (d, 4'-CH), 132.0 (s, 3'-C), 127.5 (d, 2'-CH), 113.6 and 112.9 (2 x s, 2 x CN), 82.0 (s, 2-C); *m/z* 205 (M^+, 67 %), 159 (M^+ - NO_2).

2,4-Dicyano-3-amino-5-[4-(2-nitrothienyl)]penta-2,4-dienonitrile (123)



Using General Procedure A, 2-nitro-4-thiophenecarbaldehyde (0.5205 g, 3.32 mmol) was treated with 2-aminopropene-1,1,3-tricarbonitrile (0.4415 g, 3.34 mmol) in ethanol (15 ml) and piperidine (3 drops) to give large brown crystals after 8 d (0.6750 g, 75 %), m.p. 153-155 °C. R_F 0.50 (1:1 ethyl acetate/petroleum ether); (Found: C 48.98; H 1.99; N 26.04 %; M^+, 271.0168. $C_{11}H_5N_5O_2S$ requires C 48.71; H 1.84; N 25.82 %; *M*, 271.0164); ν_{max}/cm^{-1} (KBr disc) 3038w (Ar-H), 2230s (CN), 1636s (extended conjugation to aromatic system), 1522s (C-NO_2), 870s and 834s (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 9.10 (br, 2H, -NH_2), 8.58 (d, ^4J2.3 Hz, 1H, 4'-H), 8.70 (d, ^4J2.3 Hz, 1H, 2'-H), 8.15 (s, 1H, 5-H); δ_C (50 MHz, DMSO-d_6) 164.6 (s, 5'-C), 152.9 (s, 3'-C), 145.8 (d, 4'-CH), 141.6 (d, 2'-CH), 132.4 (s, 4-C), 127.6 (d, 5-CH), 115.4, 114.7, 114.6 (3 x s, 3 x CN), 102.6 (s, 2-C), 50.1 (s, 3-C); *m/z* 271 (M^+, 65 %), 225 (M^+ - NO_2), 141 (M^+ - [C(CN)-C(NH_2)=C$(CN)_2$]), 92 ([C(NH_2)=C$(CN)_2$]$^+$).

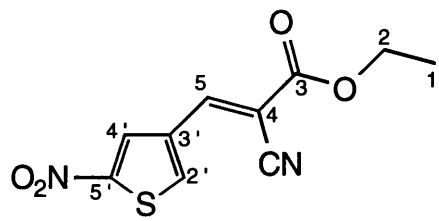
Methyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate (124)



Using General Procedure B, 2-nitro-4-thiophenecarbaldehyde (0.1361 g, 0.86 mmol) was treated with methyl cyanoacetate (0.1248 g, 1.26 mmol) in methanol (2.5 ml) and dilute piperidine solution (20 drops) to give orange crystals after 10 min (0.1764 g, 86 %), m.p. 88 °C. R_F 0.49 (1;1 ethyl acetate/petroleum ether); (Found: C 45.42; H 2.49; N 11.80 %; M^+, 238.0026. $C_9H_6N_2O_4S$ requires C 45.38; H 2.54; N 11.76 %; *M*, 238.0048); ν_{max}/cm^{-1} (nujol) 3100s (Ar-H), 2950s (CH_x), 1720s (αβ-unsaturated carbonyl), 1500s (C-NO_2), 1340b (-OCOR), 835s and 815s (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.86 (d, ^4J0.5 Hz, 1H, 4'-H), 8.62 (d, ^4J0.5 Hz, 1H, 2'-H), 8.45 (s, 1H, 4-H), 3.85 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 207.4 (s, C=O), 162.0 (s, 5'-C), 152.6 (d, 4'-CH), 147.2 (d, 2'-CH), 142.9 (s, 3'-C), 132.5 (d, 4-CH), 115.5 (s, CN),

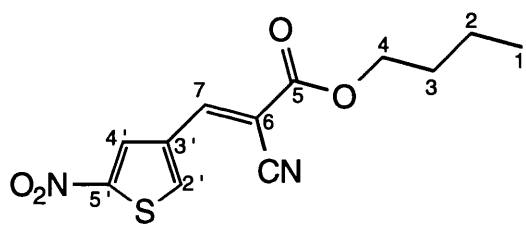
102.3 (s, 3-C), 53.5 (q, CH_3); *m/z* 238 (M^+, 21 %), 192 (M^+ - NO_2), 179 (M^+ - CO_2Me), 59 (CO_2Me^+).

Ethyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate (125)



2-Nitro-4-thiophenecarbaldehyde (0.1651 g, 1.05 mmol) was treated with ethyl cyanoacetate (0.1831 g, 1.62 mmol) in methanol (2 ml) and dilute piperidine solution (20 drops) using General Procedure B to give orange crystals after 10 min (0.2536 g, 95 %), m.p. 140-141 °C. R_F 0.45 (1:1 ethyl acetate/petroleum ether); (Found: C 47.49; H 3.20; N 10.94 %; M^+, 252.0195. $C_{10}H_8N_2O_4S$ requires C 47.62; H 3.17; N 11.11 %; *M*, 252.0205); ν_{max}/cm^{-1} (KBr disc) 3100s (Ar-H), 2240s (CN), 1450s (C-NO_2), 1300s (-OCOR); δ_H (200 MHz, DMSO-d_6) 8.86 (d, ^4J 1.9 Hz, 1H, 4'-H), 8.63 (d, ^4J 1.9 Hz, 1H, 2'-H), 8.42 (s, 1H, 5-H), 4.30 (q, ^3J 7.1 Hz, 2H, CH_2), 1.29 (t, ^3J 7.1 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.5 (s, C=O) , 152.5 (s, 5'-C), 147.1 (d, 4'-CH), 142.8 (d, 2'-CH), 132.6 (s, 3'-C), 128.3 (d, 5-CH), 115.5 (s, CN), 103.0 (s, 4-C), 62.6 (t, CH_2), 14.0 (q, CH_3); *m/z* 252 (M^+, 19 %), 206 (M^+ - NO_2), 179 (M^+ - CO_2Et).

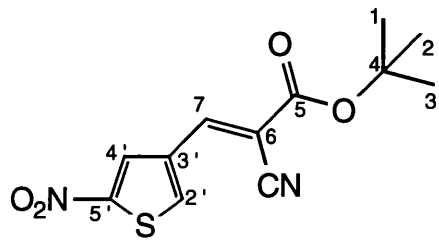
^{n}Butyl 2-cyano-3-[4-(2-nitrothienyl)]pentanoate (126)



2-Nitro-4-thiophenecarbaldehyde (0.1713 g, 1.09 mmol) was treated with ^{n}butyl cyanoacetate (0.22 ml , 1.61 mmol) in propan-2-ol (3 ml) and dilute piperidine solution (1 drop) using General Procedure B to give a pink crystalline solid after 10 min (0.1733 g, 57 %), m.p. 142-144 °C. R_F 0.70 (1:1 ethyl acetate/petroleum ether); (Found: C 51.28; H 4.32; N 9.97 %; M^+, 280.0517. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.31; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 3100s (Ar-H), 2200w (CN), 1718w (αβ-unsaturated carbonyl), 1610w (external

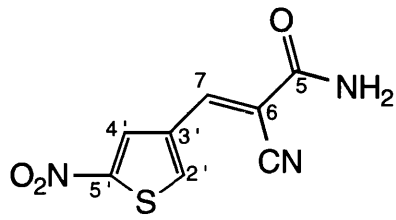
conjugation to aromatic system), 1491s (C-NO_2), 817w (isolated Ar-H); δ_H (200 MHz, DMSO-d_6) 8.86 (m, 1H, 4'-H), 8.64 (m, 1H, 2'-H), 8.42 (m, 1H, 7-H), 4.26 (t, ^3J 6.5 Hz, 2H, 4-H_2), 1.69-1.58 (tt, ^3J 6.4 Hz, ^3J 6.5 Hz, 2H, 3-H_2), 1.44-1.34 (tq, ^3J 7.2 Hz, ^3J 6.4 Hz, 2H, 2-H_2), 0.94-0.87 (t, ^3J 7.2 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.5 (s, C=O), 152.5 (s, 5'-C), 147.1 (d, 4'-CH), 142.7 (d, 2'-CH), 132.6 (s, 3'-C), 128.4 (d, 7-CH), 115.5 (s, CN), 103.0 (s, 6-C), 66.1 (t, 4-CH_2), 30.0 (t, 3-CH_2, 18.6 (t, 2-CH_2), 13.6 (q, CH_3); *m/z* 280 (M^+, 8 %), 234 (M^+ - NO_2), 69 ([C(CN)-$CO_2{}^n$Bu]$^{2+}$), 57 (^{n}Bu$^+$).

^{t}Butyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate (127)



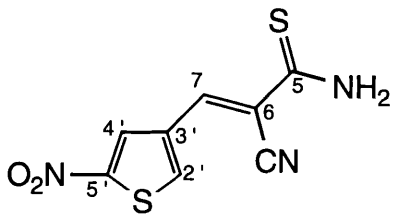
Using General Procedure A, 2-nitro-4-thiophenecarbaldehyde (0.4030 g, 2.56 mmol) was treated with ^{t}butyl cyanoacetate (0.36 ml, 2.56 mmol) in ethanol (6 ml) and piperidine (1 drop) to give a red precipitate after 20 min (0.4475 g, 62 %), m.p. 165-167 °C. R_F 0.60 (1:1 ethyl acetate/petroleum ether); (Found: C 51.39; H 4.03; N 10.00 %; M^+, 280.0516. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.31; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 2226m (CN), 1772s (C=C), 1726s (αβ-unsaturated carbonyl), 1636s (αβ-unsaturated ester), 1614s (extended conjugation to aromatic system), 1350m (C-NO_2), 886w and 860w (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.87 (d, ^4J 1.9 Hz, 1H, 4'-H), 8.67 (d, ^4J 1.9 Hz, 1H, 2'-H), 8.38 (s, 1H, 7-H), 1.57 (s, 9H, ^{t}Bu); δ_C (50 MHz, DMSO-d_6) 160.3 (s, C=O), 152.4 (s, 5'-C), 148.5 (d, 4'-CH), 142.3 (d, 2'-CH), 132.6 (s, 3'-C), 128.3 (d, 7-CH), 115.5 (s, CN), 104.4 (s, 6-C), 83.6 (s, *C*(CH_3)$_3$), 27.5 (s, 3 x CH_3); *m/z* 280 (M^+, 5 %), 234 (M^+ - NO_2), 223 (M^+ - ^{t}Bu).

2-Cyano-3-[4-(2-nitrothienyl)]propenamide (129)



2-Nitro-4-thiophenecarbaldehyde (0.1997 g, 1.27 mmol) was treated with 2-cyanoacetamide (0.1241 g, 1.47 mmol) in ethanol (4 ml) using General Procedure C to give brown crystals after 10 min (0.1971 g, 69 %), m.p. 223-225 °C. R_F 0.44 (1:1 ethyl acetate/petroleum ether); (Found: C 43.02; H 2.22; N 18.90 %; M^+, 223.0069. $C_8H_5N_3O_3S$ requires C 43.05; H 2.25; N 18.82 %; *M*, 223.0052); ν_{max}/cm^{-1} (KBr disc) 3422w, 3335w and 3289w (-$CONH_2$), 3125s (Ar-H), 2849w (NH_2), 2200w (CN), 1686s (external conjugation to aromatic system), 1597s (amide N-H), 1526s (C-NO_2), 1491s (aromatic C=C); δ_H (200 MHz, DMSO-d_6) 8.63 (d, ^4J 1.7 Hz, 1H, 4'-H),8.50 (d, ^4J 1.7 Hz, 1H, 2'-H), 8.13 (s, 1H, 3-H), 7.84 and 7.80 (2 br s, 2H, -NH_2); δ_C (50 MHz, DMSO-d_6) 162.0 (s, C=O), 152.5 (s, 5'-C), 143.2 (d, 4'-CH), 140.8 (d, 2'-CH), 133.1 (s, 3'-C), 128.0 (d, 3-CH), 116.3 (s, CN), 106.9 (s, 2-C); *m/z* 223 (M^+, 25 %), 179 (M^+ - $CONH_2$), 177 (M^+ - NO_2).

2-Cyano-3-[4-(2-nitrothienyl)]propenthioamide (130)



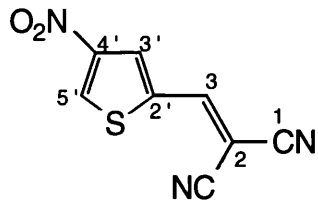
Using General Procedure C, 2-nitro-4-thiophenecarbaldehyde (0.2113 g, 1.34 mmol) was treated with 2-cyanothioacetamide (0.1687 g, 1.68 mmol) in ethanol (3 ml) to give brown crystals after 10 min (0.1989 g, 62 %), m.p. 197-199 °C. R_F 0.50 (1:1 ethyl acetate/petroleum ether); (Found: C 40.13; H 2.08; N 17.40 %; M^+, 238.9813. $C_8H_5N_3O_2S_2$ requires C 40.15;H 2.10; N 17.56 %; *M*, 238.9823); ν_{max}/cm^{-1} (KBr disc) 3135s (Ar-H), 2215w (CN), 1480b ($CSNH_2$), 1470s (C-NO_2); δ_H (200 MHz, DMSO-d_6) 8.69 and 8.61 (2 x s, 2H, NH_2), 8.17 (d, ^4J 1.9 Hz, 1H, 4'-H), 8.11 (s, 1H, 3-H), 8.10 (d, ^4J 1.9 Hz, 1H, 2'-H); δ_C (50 MHz, DMSO-d_6) 156.8 (s, C=S), 152.5 (s, 5'-C), 140.9 (d, 4'-CH), 139.3 (d, 2'-CH), 133.0 (s, 3'-C), 128.2 (d, 3-CH), 116.2 (s, CN), 112.8 (s, 2-C);

m/z 239 (M^+, 22 %), 192 (M^+ - NO_2), 178 (M^+ - $CSNH_2$), 82 (thiophene$^+$), 60 ($CSNH_2^+$).

4-Nitro-2-thiophenecarbaldehyde (87)[171]

2-Thiophenecarbaldehyde (1.3236 g, 11.91 mmol) and concentrated sulfuric acid (23 ml, 0.43 mol) were placed in a conical flask, which was cooled to -15 °C by means of a salt/ice bath. To this was slowly added a mixture of concentrated sulfuric acid (17.5 ml, 0.33 mol) and fuming nitric acid (25.2 ml, 0.63 mol) such that the temperature of the reaction mixture did not exceed 5 °C. The resulting black solution was neutralised using aqueous sodium bicarbonate solution, poured onto a slurry of ice and water and left to warm to room temperature overnight. The reaction was extracted with hot ethyl acetate (3 x 1000 ml), and the organic fractions were dried ($MgSO_4$) and concentrated *in vacuo* to give a brown tar. Column chromatography (silica gel, 1:1 ethyl acetate/petroleum ether) gave the product, which was recrystallised from diethyl ether/methanol to give large orange crystals (1.4972 g, 80 %), m.p. 50 [lit.: 35-37] °C. R_F 0.73 (1:1 ethyl acetate/petroleum ether); ν_{max}/cm^{-1} (KBr disc) 1734s (CHO), 1508s and 1366s (NO_2); δ_H (200 MHz, DMSO-d_6) 9.26 (s, 1H, -CHO), 8.65 (s, 1H, 5'-H), 8.29-8.12 (br s, 1H, 3'-H); *m/z* 157 (M^+, 100 %), 111 (M^+ - NO_2).

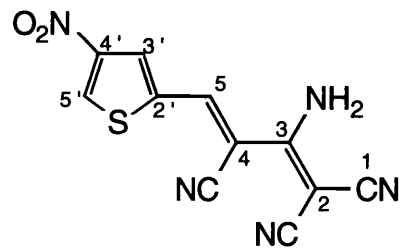
2-Cyano-3-[2-(4-nitrothienyl)]propenonitrile (132)



4-Nitro-2-thiophenecarbaldehyde **(87)** (0.1005 g, 0.64 mmol) was treated with malononitrile (0.0430 g, 0.65 mmol) in ethanol (1.5 ml) and dilute piperidine solution (1 drop) using General Procedure B to give a red precipitate after 20 min (0.0920 g, 70 %), m.p. 169-171 °C. R_F 0.81 (diethyl ether); (Found: C 46.56; H 1.53; N 20.53 %; M^+, 204.9938. $C_8H_3N_3O_2S$ requires C 46.83;

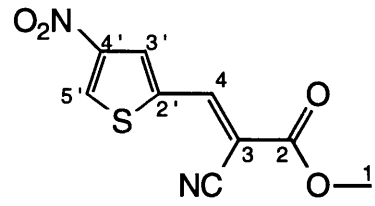
H 1.47; N 20.48 %; *M*, 204.9946); ν_{max}/cm^{-1} (KBr disc) 3038s (Ar-H), 2230w (CN), 1636s (extended conjugation to aromatic system), 1580s (aromatic C=C), 1522s (C-NO_2), 870w and 834w (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.72 (s, 1H, 3-H), 8.22 (d, ^4J 1.7 Hz, 1H, 5'-H), 7.76 (d, ^4J 1.7 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 151.9 (d, 3-CH), 149.2 (s, 4'-C), 136.1 (d, 5'-CH), 135.8 (s, 2'-C), 132.1 (d, 3'-CH), 117.3 (s, 2-C), 112.6 and 113.0 (2 x s, 2 x CN); *m/z* 205 (M^+, 61 %), 159 (M^+ - NO_2), 82 (thiophene$^+$).

2,4-Dicyano-3-amino-5-[2-(4-nitrothienyl)]penta-2,4-dienonitrile (133)



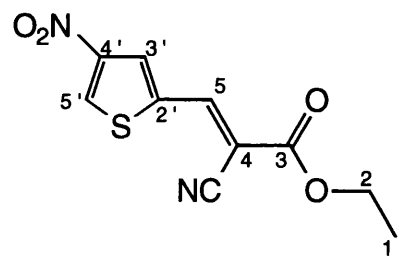
Using General Procedure B, 4-Nitro-2-thiophenecarbaldehyde **(87)** (0.2106 g, 1.34 mmol) was treated with 2-aminopropene-1,1,3-tricarbonitrile (0.1776 g, 1.34 mmol) in ethanol (3 ml) and dilute piperidine solution (2 drops) to give a yellow precipitate after 30 min (0.2005 g, 55 %), m.p. 271-272 °C. R_F 0.16 (diethyl ether); (Found: C 48.79; H 1.82; N 25.81 %; M^+, 271.0169. $C_{11}H_5N_5O_2S$ requires C 48.71; H 1.86; N 25.82 %; *M*, 271.0164); ν_{max}/cm^{-1} (KBr disc) 3052s (Ar-H), 2726s (NH_2), 2223m and 2209m (2 x CN), 1647s (external conjugation to aromatic system), 1607m (aromatic C=C), 1489s (C-NO_2), 820w (Ar-H); δ_H (200 MHz, DMSO-d_6) 9.19 and 9.17 (2 x br s, 2H, NH_2), 9.18 (s, 1H, 5'-H), 8.42 (s, 2H, 5-H and 3'-H); δ_C (50 MHz, DMSO-d_6) 164.3 (s, 3-C), 147.9 (s, 4'-C), 145.1 (d, 5-CH), 136.8 (d, 3'-CH), 130.9 (d, 5'-CH), 130.7 (s, 2'-C), 115.4 (s, 7-CN), 114.6 and 114.5 (2 x s, 1-CN and 6-CN), 101.2 (s, 4-C), 50.2 (s, 2-C); *m/z* 271 (M^+, 28 %), 225 (M^+ - NO_2), 245 (M^+ - CN), 82 (thiophene$^+$), 92 ($C(NH_2)=C(CN)_2^+$).

Methyl 2-cyano-3-[2-(4-nitrothienyl)]propenoate (134)



4-Nitro-2-thiophenecarbaldehyde **(87)** (0.2164 g, 1.37 mmol) was treated with methyl cyanoacetate (0.12 ml, 1.37 mmol) in methanol (0.5 ml) and dilute piperidine solution (1 drop) using General Procedure B to give a brown precipitate after 20 min (0.2807 g, 86 %), m.p. 172-174 °C. R_F 0.81 (diethyl ether); (Found: C 45.15; H 2.47; N 11.90 %; M^+, 238.0031. $C_9H_6N_2O_4S$ requires C 45.38; H 2.54; N 11.76 %; *M*, 238.0048); ν_{max}/cm^{-1} (KBr disc) 3310m and 3025m (=C–H), 2958w and 2854m (CH_3), 2225m (CN), 1716s (C=O), 1604s (Ar, C=C), 1533s (NO_2), 1266s (C–O), 762m and 742m (=C–H); δ_H (200 MHz, DMSO-d_6) 9.18 (d, 1H, ^4J 1.4 Hz, 5'-H), 8.69 (s, 1H, 4-H), 8.59 (d, ^4J 1.4 Hz, 3'-H), 3.90 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.9 (s, C=O), 147.8 (s, 4'-C), 146.4 (d, 4-CH), 136.4 (d, 5'-CH), 136.0 (s, 2'-C), 132.3 (d, 3'-CH), 115.3 (s, CN), 101.4 (s, 3-C), 53.6 (q, CH_3); *m/z* 238 (M^+, 40 %), 222 ($M^+ - CH_3$, - H), 207 ($M^+ - OCH_3$), 191 ($M^+ - NO_2$, – H), 179 ($M^+ - CO_2CH_3$), 160 ($M^+ - NO_2$, – S), 133 ($M^+ - NO_2$, – CO_2CH_3).

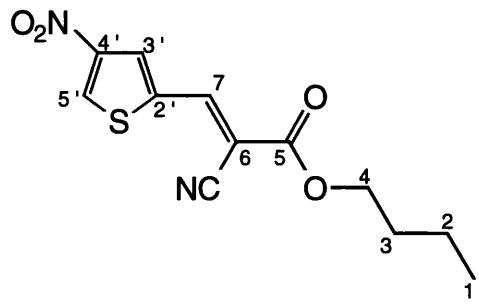
Ethyl 2-cyano-3-[2-(4-nitrothienyl)]propenoate (135)



Using General Procedure B, 4-Nitro-2-thiophenecarbaldehyde **(87)** (0.1833 g, 1.16 mmol) was treated with ethyl cyanoacetate (0.125 ml, 1.16 mmol) in ethanol (0.5 ml) and dilute piperidine solution (1 drop) to give brown crystals after 3 h (0.117 g, 40 %), m.p. 169-170 °C. R_F 0.84 (diethyl ether); (Found: C 47.38; H 3.20; N 11.28 %; M^+, 252.0221. $C_{10}H_8N_2O_4S$ requires C 47.62; H 3.20; N 11.11 %; *M*, 252.0205); ν_{max}/cm^{-1} (KBr disc) 3102m and 3026w (=C-H), 2991w and 2942m (CH_2CH_3), 2225m (CN), 1718s (C=O), 1607s (Ar, C=C), 1539s (NO_2), 1259s (C–O), 762m and 736m (=C–H); δ_H (200 MHz, DMSO-d_6) 9.16 (d, ^4J 0.8 Hz, 1H, 5'-H), 8.69 (s, 1H, 5-H), 8.61 (d, ^4J 0.8 Hz,

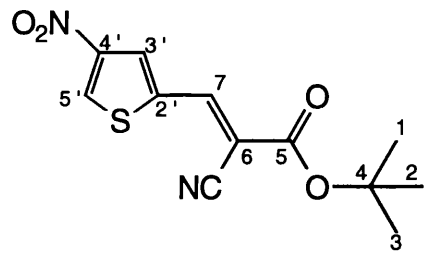
1H, 3'-H), 4.39 (q, ^3J 7.11 Hz, 2H, CH_2), 1.38 (t, ^3J 7.11 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.2 (s, C=O), 148.0 (s, 4'-C), 145.8 (d, 5-CH), 136.1 (s, 2'-C), 135.3 (d, 5'-CH), 131.5 (d, 3'-CH), 114.9 (s, CN), 102.0 (s, 4-C), 62.4 (t, CH_2), 13.4 (q, CH_3); *m/z* 252 (M^+, 80 %), 236 ($M^+ - CH_3$, - H), 207 ($M^+ - OCH_2CH_3$), 179 ($M^+ - CO_2CH_2CH_3$), 133 ($M^+ - NO_2$, $- CO_2CH_2CH_3$).

^{n}Butyl 2-cyano-3-[2-(4-nitrothienyl)]propenoate (136)



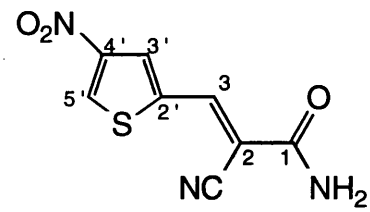
Using General Procedure B, 4-Nitro-2-thiophenecarbaldehyde **(87)** (0.1230 g, 7.8 mmol) was treated with ^{n}butyl cyanoacetate (0.11 ml, 7.8 mmol) in propan-2-ol (0.5 ml) and dilute piperidine solution (1 drop) to give a purple precipitate after 20 min (0.0658 g, 30 %), m.p. 150-151 °C. R_F 0.89 (diethyl ether); (Found: C 51.36; H 4.10; N 10.15 %; M^+, 280.0519. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.31; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 3097m and 3024w (=C–H), 2937m and 2875w (CH_2CH_3), 2223w (CN), 1713s (C=O), 1608s (Ar, C=C), 1540s (NO_2), 1278s (C–O), 761m and 736s (=C–H); δ_H (200 MHz, DMSO-d_6) 9.20 (q, ^4J 1.7 Hz, 1H, 5'-H), 8.71 (s, 1H, 7-H), 8.63 (dd, ^4J 1.7 Hz, ^4J 0.4 Hz, 1H, 3'-H), 4.31 (t, ^3J 6.4 Hz, 2H, 4-H_2), 1.71 (tt, ^3J 6.4 Hz, ^3J 6.8 Hz, 2H, 3-H_2), 1.44 (tq, ^3J 7.3 Hz,^3J 6.8 Hz, 2H, 2-H_2), 0.97 (t, ^3J 7.3 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.4 (s, C=O), 147.9 (s, 4'-C), 146.4 (d, 7-CH), 136.5 (5'-CH), 136.1 (2'-C), 132.3 (d, 3'-CH), 115.4 (s, CN), 101.7 (s, 6-C), 66.2 (t, 4-CH_2), 30.1 (t, 3-CH_2), 18.6 (t, 2-CH_2), 13.7 (q, CH_3); *m/z* 280 (M^+, 20 %), 224 ($M^+ - {}^n$Bu, +H), 207 ($M^+ - O^n$Bu), 180 ($M^+ - CO_2{}^n$Bu, +H), 133 ($M^+ - NO_2$, $- CO_2{}^n$Bu).

^{t}Butyl 2-cyano-3-[2-(4-nitrothienyl)]propenoate (137)



Using General Procedure B, 4-Nitro-2-thiophenecarbaldehyde **(87)** (0.3392 g, 2.16 mmol) was treated with ^{t}butyl cyanoacetate (0.31 ml, 2.16 mmol) in ethanol (4 ml) and dilute piperidine solution (1 drop) to give a brown precipitate after 20 min (0.2419 g, 40 %), m.p. 320-324 °C. R_F 0.61 (1:1 ethyl acetate/petroleum ether); (Found: C 51.36; H 4.31; N 10.10 %; M^+, 280.0516. $C_{12}H_{12}N_2O_4S$ requires C 51.42; H 4.21; N 9.99 %; *M*, 280.0518); ν_{max}/cm^{-1} (KBr disc) 3114s (Ar-H), 2224w (CN), 1730s (αβ-unsaturated carbonyl), 1350m (C-NO_2); δ_H (200 MHz, DMSO-d_6) 9.30 (s, 1H, 5'-H), 8.82 (s, 1H, 7-H), 8.60 (s, 1H, 3'-H), 1.62 (s, 9H, ^{t}Bu); δ_C (50 MHz, DMSO-d_6) 162.1 (s, C=O), 148.2 (s, 4'-C), 147.1 (d, 7-CH), 137.2 (s, 2'-C), 136.8 (d, 5'-CH), 133.4 (d, 3'-CH), 115.8 (s, CN), 107.1 (s, 6-C), 85.4 (s, 4-C), 28.4 (q, 3 x CH_3); *m/z* 280 (M^+, 18 %), 234 (M^+ - NO_2), 223 (M^+ - ^{t}Bu), 179 (M^+ - $CO_2{}^t$Bu).

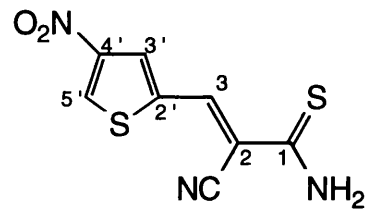
2-Cyano-3-[2-(4-nitrothienyl)]propenamide (138)



Using General Procedure B, 4-Nitro-2-thiophenecarbaldehyde **(87)** (0.2112 g, 1.34 mmol) was treated with 2-cyanoacetamide (0.1148 g, 1.36 mmol) in ethanol (3 ml) and dilute piperidine solution (1 drop) to give a brown precipitate after 5 min (0.0452g, 15 %), m.p. 201-204 °C. R_F 0.34 (diethyl ether); (Found: C 43.06; H 2.16; N 18.64 %; M^+, 223.0096. $C_8H_5N_3O_3S$ requires C 43.05; H 2.25; N 18.82 %; *M*, 223.0072); ν_{max}/cm^{-1} (KBr disc) 3423m and 3181m (NH_2), 3089s and 3048m (=C–H), 2216s (CN), 1690s (C=O), 1599s and 1542m (Ar), 1541s (NO_2), 781m and 738m (=C–H); δ_H (200 MHz, DMSO-d_6) 9.16 (br s, 1H, 5'-H), 8.62 (s, 1H, 3-H), 8.41 (br s, 1H, 3'-H), 7.92 (v.br s, 2H, NH_2); δ_C (50 MHz, DMSO-d_6) 161.7 (s, C=O), 147.8 (s, 4'-C), 142.6 (d, 3-CH), 136.6 (s, 2'-C), 134.9 (d, 5'-CH), 130.0 (d, 3'-CH), 116.2 (s, CN), 105.6 (s, 2-C); *m/z*

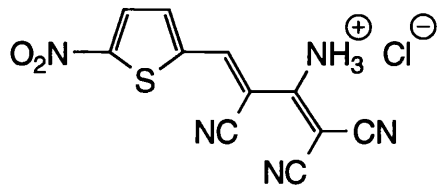
223 (M^+, 5 %), 207 ($M^+ - NH_2$), 193 ($M^+ - H, - H\text{-}N{=}O$), 179 ($M^+ - CONH_2$), 176 ($M^+ - NO_2, - H$).

2-Cyano-3-[2-(4-nitrothienyl)]propenthioamide (139)



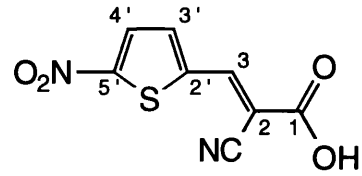
4-Nitro-2-thiophenecarbaldehyde **(87)** (0.1463 g, 0.93 mmol) was treated with 2-cyanothioacetamide (0.0940g, 0.94 mmol) in ethanol (1 ml) and dilute piperidine solution (2 drops) using General Procedure B to give a brown precipitate after 15 min (0.0223 g, 10 %), m.p. 210 °C (decomp.). R_F 0.62 (diethyl ether); (Found: C 39.98; H 2.14; N 17.00 %; M^+, 238.9810. $C_8H_5N_3O_2S_2$ requires C 40.16; H 2.10; N 17.56 %; *M*, 238.9823); ν_{max}/cm^{-1} (KBr disc) 3277m and 3170m (NH_2), 3084s and 3012m (=C–H), 2209m (CN), 1628s (C=S), 1596s and 1541m (Ar), 1540s (NO_2), 780m and 732m (=C–H); δ_H (200 MHz, DMSO-d_6) 10.22 and 9.66 (2 x br s, 2H, NH_2), 9.15 (d, ^4J 1.2 Hz, 1H, 5'-H), 8.40 (d, ^4J 1.2 Hz, 1H, 3'-H), 8.36 (s, 1H, 3-H); δ_C (50 MHz, DMSO-d_6) 191.0 (s, C=S), 148.8 (s, 4'-C), 147.9 (d, 3-CH), 136.5 (s, 2'-C), 135.0 (d, 5'-CH), 130.0 (d, 3'-CH), 116.1 (s, CN), 111.3 (s, 2-C); *m/z* 239 (M^+, 72 %), 192 ($M^+ - NO_2, - H$), 161 ($M^+ - NO_2, - S$).

Attempted synthesis of 2,4-dicyano-3-amino-5-[2-(5-nitrothienyl)] penta-2,4-dienonitrile hydrochloride (140)



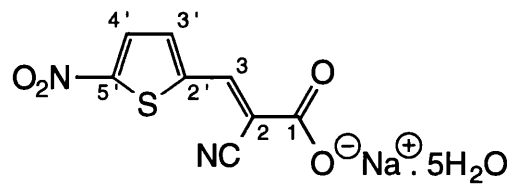
To a solution of 2,4-dicyano-3-amino-5-[2-(5-nitrothienyl)]penta-2,4-dienonitrile **(100)** in acetone (5 ml) was added 1.0 M hydrochloric acid (1 ml). The mixture was stirred at room temperature for 3 h. TLC showed only the presence of starting material. The procedure was repeated using ethereal hydrochloric acid to give the same result.

2-Cyano-3-[2-(5-nitrothienyl)]propenoic acid (141)



*t*Butyl-2-cyano-3-[2-(5-nitrothienyl)]propenoate **(104)** (0.2181 g, 0.78 mmol) was dissolved in formic acid (10 ml) and stirred at room temperature for 3 h. The mixture was concentrated *in vacuo* and neutralised with 6 % w/v aqueous sodium bicarbonate solution. This was washed with ethyl acetate (4 x 50 ml) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to a green powder, which was recrystallised from ethyl acetate/hexane to give a green solid, 0.1099 g (63 %), m.p. 189-191 °C. R_F 0.0 (1:1 ethyl acetate/petroleum ether); (Found: C 42.65; H 1.87; N 12.24 %; M^+, 223.9890. $C_8H_4N_2O_4S$ requires C 42.86; H 1.78; N 12.50 %; *M*, 223.9892); ν_{max}/cm^{-1} (KBr disc) 3100m and 3048m (Ar-H), 2280w (CN), 1694s (C=O, αβ-unsaturated acid), 1600s (extended conjugation to aromatic system), 1528s and 1339s (C-NO_2), 818m (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.50 (s, 1H, 3-H), 8.21 (d, ^3J 4.0 Hz, 1H, 4'-H), 8.03 (d, ^3J 4.0 Hz, 1H, 3'-H), signal for acidic proton observed along baseline between δ 7.80-7.00; δ_C (50 MHz, DMSO-d_6) 162.4 (s, C=O), 154.8 (s, 5'-C), 145.5 (d, 4'-CH), 140.8 (s, 2'-C), 137.3 (d, 3'-CH), 129.7 (d, 3-CH), 115.8 (s, CN), 105.5 (s, 2-C); *m/z* 224 (M^+, 19 %), 177 (M^+ - NO_2), 141 (M^+ - NC-CH_2CO_2H).

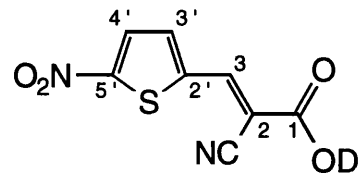
Sodium 2-cyano-3-[2-(5-nitrothienyl)]propenoate pentahydrate (142)



To 2-cyano-3-[2-(5-nitrothienyl)]propenoic acid **(141)** (0.0587 g, 0.26 mmol) was added sodium bicarbonate (0.0207 g, 0.29 mmol) and water (5 ml). The mixture was stirred at 25 °C for 8 h until all of the starting material was fully dissolved. Freeze drying for 24 h gave yellow crystals which were identified as the pentahydrate (0.0613 g, 70 %), m.p. >300 °C. R_F 0.0 (1:1 ethyl acetate/petroleum ether); (Found: C 28.50; H 3.76; N 8.48 %; M, 222.9812. $C_8H_3N_2O_4S.5H_2O$ requires C 28.58; H 3.90; N 8.33 %; *M*, 2232.9814; ν_{max}/ cm^{-1} (KBr disc) 3164 (Ar-H), 2260w (CN), 1629s (extended conjugation to

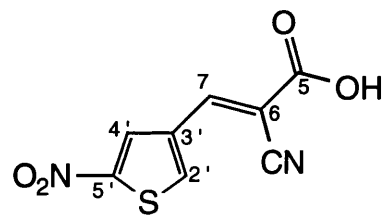
aromatic system), 1534s and 1399s (C-NO_2), 819m (Ar-H); δ_H (200 MHz, DMSO-d_6) 8.17 (d, ^3J 4.4 Hz, 1H, 4'-H), 8.09 (s, 1H, 3-H), 7.74 (d,^3J 4.4 Hz, 1H, 3'-H); *m/z* 223 (M, 20 %), 178 (M^+ - NO_2).

Deutero 2-cyano-3-[2-(5-nitrothienyl)]propenoic acid (143)



Sodium 2-cyano-3-[2-(5-nitrothienyl)]propenoate pentahydrate **(142)** (0.0110 g, 0.033 mmol) was dissolved in methanol (1 ml). Upon addition of 1 drop of deuterium chloride (1M in D_2O) a yellow precipitate formed which was insoluble in water (0.074 g, 100 %), m.p. >300 °C. R_F 0.0 (1:1 ethyl acetate/petroleum ether); δ_H (91 MHz, DMSO-d_6) 8.5 (s, 1H, 3-H), 8.2 (d, ^3J 4 Hz, 1H, 4'-H), 8.0 (d, ^3J 4 Hz, 1H, 3'-H).

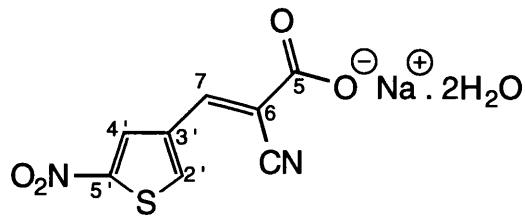
2-Cyano-3-[4-(2-nitrothienyl)]propenoic acid (144)



^{t}Butyl 2-cyano-3-[4-(2-nitrothienyl)]propenoate **(127)** (0.1634 g, 0.58 mmol) was dissolved in formic acid (10 ml) and stirred at room temperature for 2.5 h. The mixture was concentrated *in vacuo* and neutralised with 6 % w/v aqueous sodium bicarbonate solution. This was washed with ethyl acetate (4 x 50 ml) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to a beige solid (0.1118 g, 100 %) which needed no further purification; m.p. 230-231 °C. R_F 0.0 (1:1 ethyl acetate/petroleum ether); (Found: C 42.85; H 1.57; N 12.25 %; M^+, 223.9885. $C_8H_4N_2O_4S$ requires C 42.86; H 1.78; N 12.50 %; *M*, 223.9892); ν_{max}/cm^{-1} (KBr disc) 3098m (OH), 3060m (Ar-H), 2232m (CN), 1750s (-CO_2H, C=O stretch), 1606s (extended conjugation to aromatic system), 1534m (NO_2), 1388s (C-NO_2); δ_H (200 MHz, DMSO-d_6) 8.78 (d, ^4J 1.5 Hz, 1H, 4'-H), 8.65 (d, ^4J 1.5 Hz, 1H, 2'-H), 8.22 (s, 1H, 3-H), signal for acidic proton observed along baseline between δ 8.0-7.0;

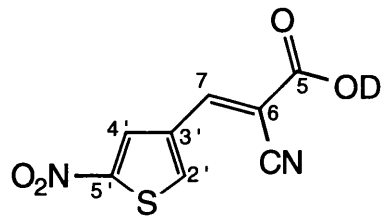
δ_C (50 MHz, DMSO-d_6) 163.1 (s, C=O), 152.3 (s, 5'-C), 143.5 (d, 4'-CH), 140.5 (d, 2'-CH), 133.6 (s, 3'-C), 128.2 (d, 3-CH), 116.4 (s, CN), 108.8 (s, 2-C); *m/z* 224 (M^+, 47 %), 177 (M^+ - NO_2), 57 (C-CO_2H).

Sodium 2-cyano-3-[4-(2-nitrothienyl)]propenoate dihydrate (145)



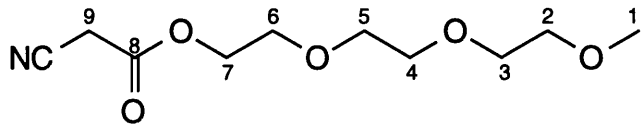
To 2-cyano-3-[4-(2-nitrothienyl)]propenoic acid **(144)** (0.1017 g, 0.45 mmol) was added sodium bicarbonate (0.0333 g, 0.46 mmol) and water (5 ml). The mixture was stirred for 2.5 h at 25 °C to give a clear yellow solution. Freeze drying for 6 h gave yellow crystals which were identified as the dihydrate (0.0910 g, 71 %), m.p. 71 °C. R_F 0.0 (1:1 ethyl acetate/petroleum ether); (Found: C 37.38; H 2.47; N 10.83 %; M, 222.9808. $C_8H_3N_2O_4S.2H_2O$ requires C 37.07; H 2.72; N 10.81 %; *M* , 222.9814; ν_{max}/cm^{-1} (KBr disc) 3108s (Ar-H), 2214m (CN), 1734s (C=C), 1542m (NO_2), 1378s (C-NO_2), 889w and 816w (2 x Ar-H); δ_H (200 MHz, DMSO-d_6) 8.69 (d, ^4J 1.6 Hz, 1H, 4'-H), 8.59 (d, ^4J 1.6 Hz, 1H, 2'-H), 8.18 (s, 1H, 3-H); δ_C (50 MHz, DMSO-d_6) 162.3 (s, C=O), 152.1 (s, 5'-C), 139.4 (d, 4'-CH), 137.9 (d, 2'-CH), 134.8 (s, 3'-C), 128.3 (d, 3-CH), 119.2 (s, CN), 114.9 (s, 2-C); *m/z* 223 (M, 10 %), 177 (M^+ - NO_2).

Deutero 2-cyano-3-[4-(2-nitrothienyl)]propenoic acid (146)



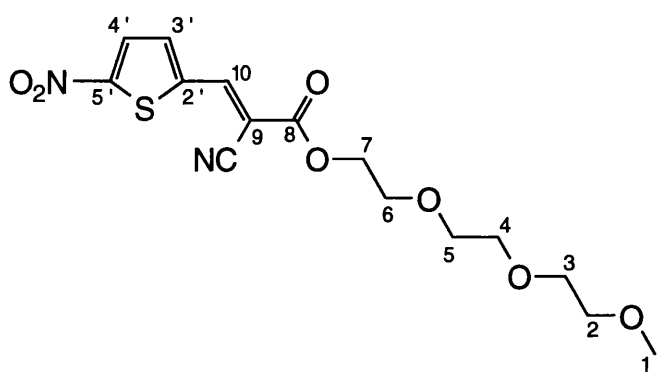
To sodium 2-cyano-3-[4-(2-nitrothienyl)]propenoate dihydrate **(145)** (0.0104 g, 0.037 mmol) in water (5 ml) was added 1 drop deuterium chloride (1M in D_2O). A white precipitate formed immediately, which was insoluble in water (0.0083 g, 100 %), m.p. >300 °C. R_F 0.0 (1:1 ethyl acetate/petroleum ether); δ_H (90 MHz, DMSO-d_6) 8.7 (d, ^4J 1.6 Hz, 1H, 4'-H), 8.6 (d, ^4J 1.6 Hz, 1H, 2'-H), 8.2 (s, 1H, 3-H).

Tri(ethylene glycol) cyanoacetate methyl ether (95)



To a 100 ml round bottom flask were added 2-cyanoacetic acid **(147)** (10.1319 g, 119.1 mmol), tri(ethylene glycol) monomethyl ether **(148)** (20 ml, 125.0 mmol), 4-toluenesulfonic acid (20 mg) and toluene (50 ml). The mixture was heated at reflux temperature under Dean-Stark conditions for 24 h. Upon allowing to cool to room temperature, the mixture was concentrated *in vacuo*, and column chromatography over silica (ethyl acetate) gave the product as an amber oil (17.5766 g, 64 %), d 1.117 g ml^{-1}. R_F 0.30 (1:1 ethyl acetate/petroleum ether); (Found: C 51.94; H 7.26; N 6.11 %; M^+, 231.1205. $C_{10}H_{17}NO_5$ requires C 51.94; H 7.41; N 6.06 %; *M*, 231.1205); ν_{max}/cm^{-1} (KBr disc) 2885m (C-O-CH_3), 2262m (CN), 1750s (C=O), 1105s (C-O-C); δ_H (200 MHz, DMSO-d_6) 4.01 (m, 2H, 9-H_2), 3.42-3.29 (m, 12H, 2, 3, 4, 5, 6, 7-H_2), 3.03 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 162.9 (s, C=O), 113.0 (s, CN), 70.6 (t, 9-CH_2), 69.2 (t, 7-CH_2), 69.1 (t, 6-CH_2), 69.0 (t, 5-CH_2), 67.2 (t, 4-CH_2), 64.4 (t, 3-CH_2), 57.4 (t, 2-CH_2), 23.2 (q, CH_3); *m/z* 231 (M^+, 1 %), 186 (M^+ - CH_2OCH_3), 156 (M^+ - $OCH_2CH_2OCH_3$), 112 [M^+ - $(OCH_2CH_2)_2OCH_3$)].

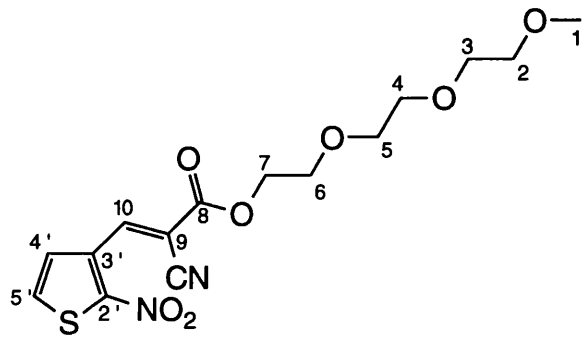
Tri(ethylene glycol) 2-cyano-3-[2-(5-nitrothienyl)]propenoate methyl ether (105)



5-Nitro-2-thiophenecarbaldehyde (0.6717 g, 4.27 mmol) was treated with tri(ethylene glycol) cyanoacetate methyl ether **(149)** (906.5 µl, 4.33 mmol) in ethanol (10 ml) and piperidine (1 drop) using General Procedure A to give an orange precipitate after 10 min (1.4060 g, 89 %), m.p. 84-87 °C. R_F 0.13-0.24 (1:1 ethyl acetate/petroleum ether); (Found: C 48.58; H 5.11; N 7.59 %; M^+ - CH_3, 355.0651. $C_{15}H_{18}N_2O_7S$ requires C 48.64; H 4.90; N 7.56 %; *M* - CH_3,

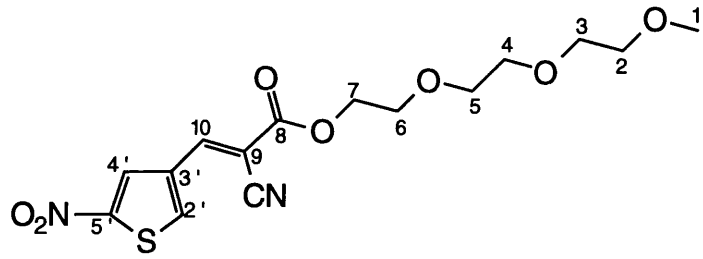
355.0835); ν_{max}/cm^{-1} (KBr disc) 2892m (C-O-CH_3), 2222w (CN), 1725m ($\alpha\beta$-unsaturated carbonyl), 1599m (extended conjugation to aromatic system), 1504m (C-NO_2), 1338s and 1271s (ester, C-O stretch); δ_H (200 MHz, DMSO-d_6) 8.29 (s, 1H, 10-H), 7.97 (d, ^3J 4.4 Hz, 1H, 4'-H), 7.76 (d, ^3J 4.4 Hz, 1H, 3'-H), 3.86-3.39 (m, 12H, 2, 3, 4, 5, 6, 7-H_2), 3.37 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.2 (s, C=O), 156.0 (s, 5'-C), 145.2 (d, 10-CH), 140.5 (s, 2'-C), 135.0 (d, 4'-CH), 128.2 (d, 3'-CH), 114.2 (s, CN), 104.8 (s, 9-C), 71.9 (t, 7-CH_2), 70.8 (t, 6-CH_2), 70.6 (t, 5-CH_2), 70.5 (t, 4-CH_2), 68.6 (t, 3-CH_2), 66.2 (t, 2-CH_2), 59.0 (q, CH_3); *m/z* 355 (M^+ - CH_3, 5 %), 324 (M^+ - NO_2), 252 (M^+ - [$(OC_2H_4)_2OCH_3$], + H), 235 (M^+ - [$(OC_2H_4)_2OCH_3$], - O).

Tri(ethylene glycol) 2-cyano-3-[3-(2-nitrothienyl)]propenoate methyl ether (119)



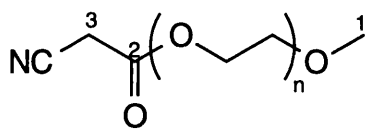
2-Nitro-3-thiophenecarbaldehyde **(83)** (0.1317 g, 0.84 mmol) was treated with tri(ethylene glycol) cyanoacetate methyl ether **(149)** (173.5 µl, 0.84 mmol) in ethanol (1 ml) and dilute piperidine solution (1 drop) using General Procedure B to give a peach precipitate after 14 h (0.1933 g, 62 %), m.p. 67-69 °C. R_F 0.10 (1:1 ethyl acetate/petroleum ether); (Found: C 48.69; H 5.05; N 7.50 %; M^+, 370.0783. $C_{15}H_{18}N_2O_7S$ requires C 48.64; H 4.90; N 7.56 %; *M*, 370.0835); ν_{max}/cm^{-1} (KBr disc) 2878m (C-O-CH_3), 2224w (CN), 1716s ($\alpha\beta$-unsaturated carbonyl), 1610m (extended conjugation to aromatic system), 1536s (C-NO_2), 1350m and 1272s (ester, C-O stretch); δ_H (200 MHz, DMSO-d_6) 9.21 (s, 1H, 10-H), 8.71 (br, 1H, 5'-H), 8.62 (br, 1H, 4'-H), 4.71-3.38 (br m, 12H, 2, 3, 4, 5, 6, 7-H_2), 3.26 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.4(s, C=O), 148.2 (s, 2'-C), 147.9 (d, 5'-CH), 146.6 (s, 3'-C), 136.0 (d, 4'-CH), 132.4 (d, 10-CH), 115.3 (s, CN), 101.5 (s, 9-C), 71.4 (t, 7-CH_2), 70.0 (t, 6-CH_2), 69.9 (t, 5-CH_2), 69.7 (t, 4-CH_2), 68.1 (t, 3-CH_2), 65.8 (t, 2-CH_2), 58.2 (q, CH_3); *m/z* 370 (M^+, 5 %), 295 (M^+ - $OC_2H_4OCH_3$), 267 (M^+ - [$(OC_2H_4)_2OCH_3$],) 251 (M^+ - [$(OC_2H_4)_2OCH_3$, -O).

Tri(ethylene glycol) 2-cyano-3-[4-(2-nitrothienyl)]propenoate methyl ether (128)



Using General Procedure A, 2-nitro-4-thiophenecarbaldehyde (0.2146 g, 1.37 mmol) was treated with tri(ethylene glycol) cyanoacetate methyl ether **(149)** (290.0 µl, 1.40 mmol) in ethanol (25 ml) and piperidine (1 drop) to give a dark precipitate after 10 min. Column chromatography (silica gel/1:1 ethyl acetate/petroleum ether) gave the product which was recrystallised from ethanol to give small brown needles (0.0534 g, 10.5 %), m.p. 72-74 °C. R_F 0-0.17 (1:1 ethyl acetate/petroleum ether); (Found: C 48.32; H 5.06; N 7.45 %; M^+, 370.0861. $C_{15}H_{18}N_2O_7S$ requires C 48.64; H 4.90; N 7.56 %; *M*, 370.0834); ν_{max}/cm^{-1} (KBr disc) 2826w (C-O-CH_3), 2228w (CN), 1730s (αβ-unsaturated carbonyl), 1616s (extended conjugation to aromatic system), 1512s (C-NO_2), 1270s (ester, C-O stretch); δ_H (200 MHz, DMSO-d_6) 8.54 (d, ^4J 1.9 Hz, 1H, 4'-H), 8.39 (d, ^4J 1.9 Hz, 1H, 2'-H), 8.16 (s, 1H, 10-H), 4.50-3.53 (m, 12H, 2, 3, 4, 5, 6, 7-H_2), 3.37 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.6 (s, C=O), 153.2 (s, 5'-C), 146.0 (d, 4'-CH), 138.5 (d, 2'-CH), 133.0 (s, 3'-C), 128.0 (d, 10-CH), 114.0 (s, CN), 104.4 (s, 9-C), 71.9 (t, 7-CH_2), 70.7 (t, 6-CH_2), 70.6 (t, 5-CH_2), 70.5 (t, 4-CH_2), 68.6 (t, 3-CH_2), 66.0 (t, 2-CH_2), 59.0 (q, CH_3); *m/z* 372 (M^+, 6 %), 324 (M^+ - NO_2), 297 (M^+ - $OC_2H_4OCH_3$ + 2H), 252 (M^+ - [$(OC_2H_4)_2OCH_3$)], + H), 235 (M^+ - [$(OC_2H_4)_2OCH_3$)], - O)..

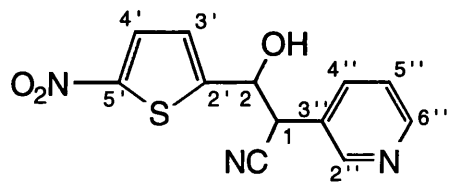
Poly(ethylene glycol) cyanoacetate methyl ether (149)



To a round-bottomed flask was added cyanoacetic acid (0.5717 g, 6.72 mmol), poly(ethylene glycol) monomethyl ether (13.60 g, *ca* 6.80 mmol), 4-toluenesulfonic acid (101 mg) and toluene (50 ml). The mixture was refluxed under Dean-Stark conditions for 72 h. Upon cooling to room temperature, the solvent was removed *in vacuo* to leave a yellow liquid which solidified to give a waxy solid which needed no further purification (12.1789 g, *ca* 90 %), m.p.

58-61 °C. R_F 0 (1:1 ethyl acetate/petroleum ether); ν_{max}/cm^{-1} (KBr disc) 2888s (OCH_x), 2230w (CN), 1750w (C=O), 2950s and 2914s (-CH_2-); δ_H (200 MHz, DMSO-d_6) 4.28 (m, 2H, 3-H), 3.57 (br, 140 H, poly [CH_2CH_2]), 3.30 (s, 3H, CH_3) (^{1}H NMR spectrum was dominated by large signal for PEG; relative intensities are only estimated); δ_C (50 MHz, DMSO-d_6) 13.4 (s, C=O), 137.4 (t, 3-CH_2), 108.0 (s, CN), 77.9-58.9 (multiplet, poly[CH_2CH_2]), 24.6 (t, CH_3).

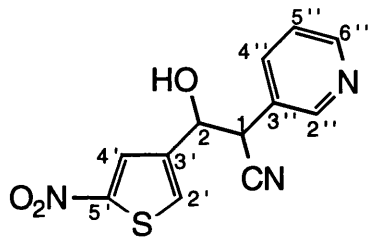
1-(3-Pyridyl)-1-cyano-2-[2-(5-nitrothienyl)ethan-2-ol (153)



5-Nitro-2-thiophene carbaldehyde (2.0687 g, 13.16 mmol) was dissolved in ethanol (15 ml). To this was added 3-pyridylacetonitrile (1.5722 g, 13.31 mmol). The mixture was heated at reflux temperature for 17.5 h until a precipitate formed. Filtration followed by washing with diethyl ether gave the product as a brown solid which was observed to be a mixture of diastereoisomers in a 1:3 ratio by NMR spectroscopy. (1.6619 g, 46 %), m.p. 170-172 °C. R_F 0.18 (1:1 ethyl acetate/petroleum ether); (Found: C 51.95; H 3.84; N 15.55 %; M^+, 276.0428. $C_{12}H_{10}N_3O_3S$ requires C 52.16; H 3.64; N 15.18 %; *M*, 276.0443); ν_{max}/cm^{-1} (KBr disc) 3431m (OH), 3106 (aryl-H), 2223w (CN), 1537 (aromatic C=C), 1499s (C-NO_2), 1294m (OH bend), 1161m (C-O stretch), 814m (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) **Diastereoisomer 1 (relative intensity 3):-** 8.66 (d, ^5J 1.9 Hz, 1H, 2"-H), 8.58 (dd, ^3J 4.8 Hz, ^4J 1.5 Hz, 1H, 6"-H), 8.07 (d, ^3J 4.3 Hz, 1H, 4'-H), 7.88 (dd, ^3J 4.1 Hz, ^5J 1.9 Hz, 1H, 4"-H), 7.46 (dd, ^3J 7.8 Hz, ^3J 4.8 Hz, 1H, 5"-H), 7.23 (d, ^3J 4.3 Hz, 1H, 3'-H), 5.34 (dd, ^3J 4.4 Hz, ^3J 4.3 Hz, 1H, 2-H), 4.91 (d, ^3J 3.9 Hz, 1H, 1-H), 3.42 (v. br s, 1H, OH); **Diastereoisomer 2 (relative intensity 1):-** 8.52 (dd, ^3J 4.8 Hz, ^4J 1.5 Hz, 1H, 6"-H), 8.40 (d, ^5J 1.9 Hz, 1H, 2"-H), 7.98 (d, ^3J 4.3 Hz, 1H, 4'-H), 7.69 (dd, ^3J 4.1 Hz, ^5J 1.9 Hz, 1H, 4"-H), 7.38 (dd, ^3J 8.5 Hz, ^3J 4.8 Hz, 1H, 5"-H), 7.00 (d, ^3J 4.3 Hz, 1H, 3'-H), 5.56 (dd, ^3J 4.5 Hz, ^3J 4.4 Hz, 1H, 2-H), 4.82 (d, ^3J 4.9 Hz, 1H, 1-H), 3.42 (v. br s, 1H, OH); δ_C (50 MHz, DMSO-d_6) **Diastereoisomer 1 (relative intensity 3):-** 156.5 (s, 5'-C), 149.8 (s, 2'-C), 149.5 (d, 2"-CH), 149.1 (d, 4'-CH), 136.4 (d, 6"-CH), 130.1 (d, 4"-CH), 129.2 (s, 3"-C), 124.4 (d, 5"-CH), 123.7 (d, 3'-CH), 118.2 (s, CN), 70.0 (d, 1-CH), 43.0 (d, 2-CH); **Diastereoisomer 2 (relative intensity 1):-** 155.1 (s, 5'-C), 149.8 (s, 2'-C), 149.7 (d, 2"-CH), 148.9 (d, 4'-CH), 136.6 (d, 6"-CH), 129.7 (d, 4"-CH),

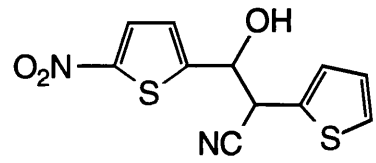
127.8 (s, 3"-C), 124.8 (d, 5"-CH), 123.5 (d, 3'-CH), 118.9 (s, CN), 69.4 (d, 1-CH), 42.2 (d, 2-CH); *m/z* 259 (M^+- OH, 2 %), 198 (M^+ - [pyridine]$^+$), 160 (M^+ - [NC-C-pyridine]$^+$), 128 ([O_2N-thiophene]$^+$).

1-(3-Pyridyl)-1-cyano-2-[4-(2-nitrothienyl)ethan-2-ol (154)



To a solution of 2-nitro-4-thiophene carbaldehdye (0.5596 g, 3.56 mmol) in ethanol (20 ml) and piperidine (1 drop) was added 3-pyridylacetonitrile (0.3946 g, 3.34 mmol) over a period of 5 min. The mixture was stirred at room temperature for 6.5 h until precipitation of solid was observed. This was filtered, washed (hexane) and dried (diethyl ether) to yield the product as a yellow solid which was observed to be a mixture of diastereoisomers in a 2:3 ratio by NMR spectroscopy. (0.6211 g, 63 %), m.p. 175 °C. R_F 0.23 (1:1 ethyl acetate/petroleum ether); (Found: C 52.06; H 3.35; N 15.18 %; M^+, 276.0469. $C_{12}H_{10}N_3O_3S$ requires C 52.16; H 3.64; N 15.20 %; *M*, 276.0443); ν_{max}/cm^{-1} (KBr disc) 3432m (OH), 2250w (CN), 684m (external conjugation to aromatic system), 1534s (C-NO_2), 1484m (aromatic C=C), 1290s (O-H bend), 1134w (C-O stretch), 820m (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) **Overlapping Signals:-** 8.65-8.58 (br m, 2H, 2" and 6"-H), 8.24-7.81 (br m, 1H, 4"-H), 7.49-7.42 (br m, 1H, 5"-H), 3.79 (v.br, 1H, OH); **Diastereoisomer 1 (relative intensity 3):-** 8.26 (s, 1H, 4'-H), 7.05 (s, 1H, 2'-H), 5.61 (d, ^3J 3.6 Hz, 1H, 1-H), 4.96 (d, ^3J 3.6 Hz, 1H, 2-H); **Diastereoisomer 2 (relative intensity 2):-** 8.28 (s, 1H, 4'-H), 7.03 (s, 1H, 2'-H), 5.38 (d, ^3J 4.9 Hz, 1H, 1-H), 4.88 (d, ^3J 4.9 Hz, 1H, 2-H); δ_C (50 MHz, DMSO-d_6) **Diastereoisomer 1 (relative intensity 3):-** 156.6 (s, 5'-C), 149.8 and 149.1 (2 x d, 2" and 6"-CH), 136.5 (d, 4"-CH), 135.7 (d, 4'-CH), 130.1 (d, 5"-CH), 127.9 (s, 3'-C), 124.4 (d, 2'-CH), 123.8 (s, 3"-C), 118.3 (s, CN), 70.1 (d, 1-CH), 43.1 (d, 2-CH); **Diastereoisomer 2 (relative intensity 2):-** 155.2 (s, 5'-C), 149.9 and 149.5 (2 x d, 2" and 6"-CH), 136.7 (d, 4"-CH), 136.0 (d, 4'-CH), 129.8 (d, 5"-CH), 127.6 (s, 3'-C), 124.9 (d, 2'-CH), 124.0 (s, 3"-CH), 119.0 (s, CN), 69.5 (d, 1-CH), 42.3 (d, 2-CH); *m/z* 258 (M^+ - OH, - H, 2 %), 198 (M^+ - [pyridine]), 160 (M^+ - [NC-C-pyridine]), 127 ([O_2N-thiophene]$^+$, - H), 116 ([NC-C-pyridine]$^+$).

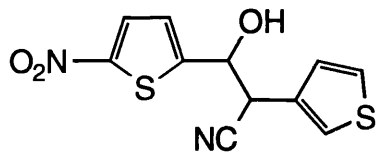
Attempted syntheses of 1-(2-thienyl)-1-cyano-2-[5-(2-nitrothienyl)] ethan-2-ol (155)



Method 1. Using General Procedure A, 5-nitro-2-thiophenecarbaldehyde and 2-thiopheneacetonitrile were mixed in ethanol. To this was added sequentially catalytic amounts of pyridine and piperidine. The reaction was monitored by TLC, which showed only starting materials 12 h after the addition of piperidine. At this point, 1 molar equivalent of piperidine was added, and the mixture was heated at reflux temperature. However, TLC showed only starting materials after 72 h.

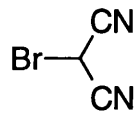
Method 2. A round-bottomed flask was charged with a magnetic stirring bar and 5-nitro-2-thiophenecarbaldehyde (0.3460 g, 2.20 mmol) in absolute ethanol (5 ml). The solution was gently heated to obtain a clear liquid. To this was added 2-thiopheneacetonitrile (0.2777 g, 2.25 mmol) and potassium carbonate (10 mg) in absolute ethanol (5 ml). The mixture was heated at reflux temperature for 1 h to give a complex mixture of products which could not be separated by column chromatography.

Attempted synthesis of 1-(3-thienyl)-1-cyano-2-[5-(2-nitrothienyl)] ethan-2-ol (156)



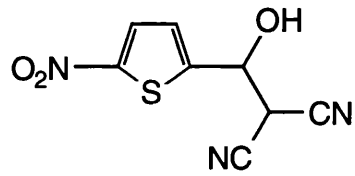
Using General Procedure A, 5-nitro-2-thiophenecarbaldehyde and 3-thiopheneacetonitrile were mixed in ethanol. To this was added sequentially catalytic amounts of pyridine and piperidine. The reaction was monitored by TLC, which showed the presence of starting materials 12 h after the addition of piperidine. At this point, 1 molar equivalent of piperidine was added, and the mixture was heated at reflux temperature. However, TLC showed only starting materials after 72 h.

Bromomalononitrile (159)[177]



To a solution of malononitrile (1.9655 g, 29 mmol) in water (20 ml) at 0 °C was added bromine (1.50 ml, 29 mmol). After stirring at 0 °C for 3.5 h, a white precipitate was observed; neutralisation with aqueous sodium bicarbonate followed by extraction into ethyl acetate (4 x 100 ml) and drying ($MgSO_4$) gave a brown solution after filtration. This was concentrated to a brown oil, which slowly crystallised to give beige crystals (0.8520 g, 20 %), m.p. 55-57 [lit.: 64-65][177] °C, R_f 0.0. δ_H (91 MHz, DMSO-d_6) 3.0 (s, 1H); *m/z* 145 (M^+, 15 %), 79 (Br^+), 66 (M^+ - Br).

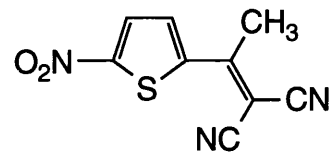
Attempted syntheses of 1,1-dicyano-2-[2-(5-nitrothienyl)ethan-2-ol (160)



To a solution of bromomalononitrile **(160)** (0.5072 g, 3.52 mmol) in dry ethanol (50 ml) was added magnesium (0.0840 g, 3.45 mmol). The mixture was stirred at -78 °C under an atmosphere of nitrogen for 2.5 h, then was allowed to warm to 25 °C. To this was added 5-nitro-2-thiophene carbaldehyde (0.4216 g, 2.68 mmol) in dry ethanol (7 ml). The mixture was stirred at 25 °C for 48 h, then was heated at reflux temperature for 24 h. Filtration and concentration *in vacuo* gave a beige precipitate, which was shown to be starting material by NMR spectroscopy.

This procedure was repeated at various temperatures and gave the same result.

Attempted syntheses of 2-Cyano-3-methyl-3-[4-(2-nitrothienyl)] propenonitrile (163)

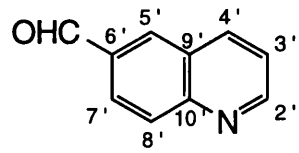


Method 1. 2-Acetylthiophene (1.4558 g, 11.53 mmol) and malononitrile (0.7694 g, 11.65 mmol) were mixed in ethanol (2 ml) and piperidine (2 drops) under the conditions of General Procedure A, to give a complex mixture of products (R_F 0.24-0.54) which could not be separated by column chromatography.

Method 2.[178] To a flame-dried 3-necked round bottom flask, equipped with a low temperature thermometer, nitrogen balloon and septum cap, was added 2-acetylthiophene (6.3129g 50.0 mmol) and malononitrile (3.3243 g, 50.32 mmol) in dry THF (150 ml). The solution was cooled to 0 °C, and $TiCl_4$ (11 ml, 100 mmol) was slowly added such that the temperature of the reaction mixture did not exceed 5 °C. The mixture was stirred at 0 °C for 2 h, and pyridine (16 ml, 197. 8 mmol) was added to give a red solution instantly. Stirring at room temperature for 20 min gave a very viscous beige oil which separated from the solution and solidified to give a complex mixture of products which could not be separated.

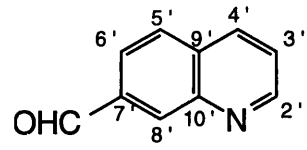
7.4 Experimental Procedures for Chapter 4

6-Quinolinecarbaldehyde (198)



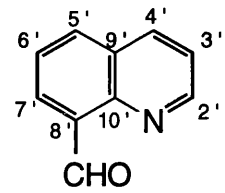
This was prepared using the method of V.M. Rodionov and M.A Berkengeĭm.[183] A 3-necked round bottom flask, equipped with a water condenser and thermometer was charged with 6-methylquinoline (5.40 g, 37 mmol) and selenium (IV) oxide (4.59 g, 41 mmol). The mixture was heated to 140 °C, whereupon an exothermic reaction occurred, raising the temperature to 210 °C. When the exotherm had subsided, the mixture was heated at reflux for 90 min. Upon cooling to room temperature, the mixture was filtered through Celite, washed with water (50 ml) and extracted with diethyl ether (4 x 50 ml). The combined ethereal extracts were dried ($MgSO_4$) and concentrated *in vacuo* to a yellow oil. Column chromatography over silica gel (1:1 ethyl acetate/petroleum ether) isolated the product, which was recrystallised from diethyl ether to give fine yellow needles (2.85 g, 49 %), m.p. 65-66 [lit.: 75-76][198] °C. R_F 0.38 (1:1 ethyl acetate/petroleum ether); (Found: C 76.32; H 4.49; N 8.82 %; M^+, 157.0516. $C_{10}H_7NO$ requires C 76.42; H 4.49; N 8.91 %; *M*, 157.0528); ν_{max}/cm^{-1} (KBr disc) 3039m (Ar-H), 1692s (C=O, aromatic aldehyde), 1623m, 1575m and 1501m (aromatic ring), 838s (2 adjacent Ar-H), 801s (3 adjacent Ar-H); δ_H (200 MHz, $CDCl_3$) 10.09 (s, 1H, -CHO), 8.95 (d, ^3J 4.3 Hz, 1H, 2'-H), 8.25-8.04 (m, 4H, 4', 5', 7', 8'-H), 7.42 (dd, ^3J 8.3 Hz, ^3J 4.3 Hz, 1H, 3'-H); δ_C (50 MHz, $CDCl_3$) 191.4 (d, CHO), 153.0 (d, 2'-CH), 150.7 (s, 10'-C), 137.4 (d, 8'-CH), 134.2 (s, 6'-C), 133.6 (d, 4'-CH), 130.7 (d, 5'-CH), 127.6 (s, 9'-C), 126.6 (d, 7'-CH), 122.1 (d, 3'-CH); *m/z* 157 (M^+, 100 %), 128 (M^+ - CHO), 115 (M^+ - CHO, -CH), 100 (M^+ - CHO, - C_2H_2).

7-Quinolinecarbaldehyde (199)



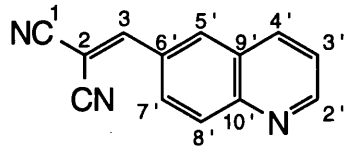
This was prepared using the method of V.M. Rodionov and M.A Berkengeĭm.[183] To a 3-necked round bottomed flask equipped with a water condenser and thermometer was added 7-methylquinoline (4 ml of a mixture consisting of 70 % 7-methyl quinoline and 30 % 5-methyl quinoline, *i.e.* 20.92 mmol 7-methylquinoline) and selenium (IV) oxide (3.3716 g, 30.39 mmol). Heating to *ca.* 70 °C resulted in an exothermic reaction; once this had subsided, the mixture was heated at 70 °C for 5 h. The reaction was allowed to cool to room temperature, and was partitioned between ethyl acetate (50 ml) and water (50 ml), then filtered through Celite to remove residual selenium. After extracting with a further quantity of ethyl acetate (3 x 100 ml), the combined organic extracts were dried (Na_2SO_4), filtered and concentrated to an orange oil which solidified on standing at room temperature. Column chromatography over silica (1:1 ethyl acetate/petroleum ether) resulted in the isolation of 7-quinolinecarbaldehyde, which was recrystallised from ethyl acetate to yield fine orange needles (1.8827 g, 57 %), m.p. 87-89 [lit.: 85-86][183] °C. R_F 0.35 (1:1 ethyl acetate/petroleum ether); (Found: C 76.27; H 4.61; N 8.87 %; M^+, 157.0519. $C_{10}H_7NO$ requires C 76.42; H 4.49; N 8.91 %; *M*, 157.0528); ν_{max}/cm^{-1} (KBr disc) 3022w (Ar-H), 1690s (aldehydic C=O), 1623m, 1572m and 1502m (aromatic ring), 840s (2 adjacent Ar-H), 807w (3 adjacent Ar-H); δ_H (200 MHz, $CDCl_3$) 10.16 (s, 1H, CHO), 8.95 (d, ^3J 4.2 Hz, ^7J 1.8 Hz, 1H, 2'-H), 8.49 (d, ^7J 1.0 Hz, 1H, 8'-H), 8.16 (dd, ^3J 8.3 Hz, ^7J 1.0 Hz, 1H, 4'-H), 7.97 (dd, ^3J 8.8 Hz, ^7J 1.8 Hz, 1H, 6'-H), 7.84 (d, ^3J 8.8 Hz, 1H, 5'-H), 7.47 (d, ^3J 8.3 Hz, ^3J 4.2 Hz, 1H, 3'-H); δ_C (50 MHz, $CDCl_3$) 151.6 (d, 2'-CH), 147.7 (s, 10'-C), 136.9 (s, 7'-C), 136.1 (d, 8'-CH), 135.5 (d, 4'-CH), 131.8 (s, 9'-C), 129.0 (d, 6'-CH), 123.4 (d, 5'-CH), 123.1 (d, 3'-CH), signal for C=O not observed; *m/z* 157 (M^+, 100 %), 128 (M^+ - CHO), 101 (M^+ - CHO, $-C_2H_2$, + H).

8-Quinolinecarbaldehyde (200)



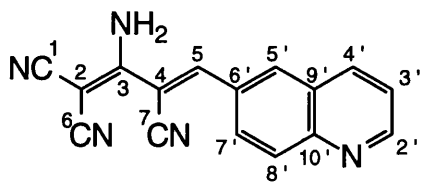
This was prepared using the method of V.M. Rodionov and M.A Berkengeĭm.[183] A 3-necked round bottomed flask equipped with a water condenser and thermometer was charged with 8-methylquinoline (10 ml, 73.5 mmol) and selenium (IV) oxide (14.0399 g, 126.5 mmol). The mixture was heated at reflux for 22 h, then the condenser was removed and heating was continued until all of the remaining 8-methylquinoline had evaporated. The remaining brown solid was dissolved in ethyl acetate (50 ml) and water (50 ml) and was heated at reflux for 2 h. On cooling to room temperature this was filtered through Celite and extracted with ethyl acetate (3 x 50 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to a beige solid. Column chromatography over silica gel (1:1 ethyl acetate/petroleum ether) gave a product which was recrystallised from methanol/water to yield a yellow solid (1.4983 g, 13 %), m.p. 96-99 [lit.: 94-95][183] °C. R_F 0.13 (1:1 ethyl acetate/petroleum ether); (Found: C 76.58; H 4.92; N 8.57 %; M^+, 157.0514. $C_{10}H_7NO$ requires C 76.42; H 4.49; N 8.91 %; *M*, 157.0528); ν_{max}/cm^{-1} (KBr disc) 3064w (Ar-H), 1709s (C=O, aromatic aldehyde), 1617m , 1583s and 1524s (aromatic ring), 842m (2 adjacent Ar-H), 804m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 11.26 (s, 1H, CHO), 9.04 (dd, ^3J 3.9 Hz, ^4J 1.0 Hz, 1H, 2'-H), 8.62 (d, ^3J 8.0 Hz, 1H, 7'-H), 8.45 (dd, ^3J 7.3 Hz, ^3J 1.0 Hz, 1H, 4'-H), 7.63 (d, ^3J 7.8 Hz, 1H, 5'-H), 7.16 (m, 1H, 6'-H), 7.10 (dd, ^3J 7.3 Hz, ^3J 3.9 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 167.1 (s, CHO), 149.8 (d, 2'-CH), 144.3 (s, 10'-C), 139.1 (d, 4'-CH), 134.9 (s, 8'-C), 133.7 (d, 5'-CH), 133.0 (d, 7'-CH), 128.1 (s, 9'-C), 127.0 (d, 6'-CH), 122.2 (d, 3'-CH); *m/z* 157 (M^+, 1%), 128 (M^+ - CHO), 102 (M^+ - CHO, $-C_2H_2$, + 2H).

2-Cyano-3-(6-quinolyl)propenonitrile (201)



Using General Procedure C, 6-quinolinecarbaldehyde (0.1419 g, 0.90 mmol) was treated with malononitrile (0.0606 g, 0.91 mmol) in ethanol (1 ml) to give a yellow precipitate after 12 h which was recrystallised from 5:1 acetone/methanol (0.1661 g, 90 %), m.p. 196-197 °C. R_F 0.23 (1:1 ethyl acetate/petroleum ether); (Found: C 76.06; H 3.39; N 20.55 %; M^+, 205.6405. $C_{13}H_7N_3$ requires C 76.09; H 3.44; N 20.48 %; *M*, 205.6400); ν_{max}/cm^{-1} (KBr disc) 3034w (Ar-H), 2224m (CN), 1617w, 1577s and 1500w (aromatic ring), 833w (2 adjacent Ar-H), 794m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.09 (br dd, ^3J 4.2 Hz, ^4J 1.5 Hz, 1H, 2'-H), 8.73 (s, 1H, 3-H), 8.57 (v.br, 1H, 8'-H), 8.54 (v. br, 1H, 4'-H), 8.32 (v.br d, ^3J 9.0 Hz, 1H, 7'-H), 8.21 (v. br d, ^3J 9.0 Hz, 1H, 5'-H), 7.70 (dd, ^3J 8.4 Hz, ^3J 4.1 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 160.7 (d, 3-CH), 153.9 (d, 2'-CH), 149.4 (s, 10'-C), 137.7 (d, 8'-CH), 134.2 (d, 4'-CH), 130.4 (d, 5'-CH), 129.4 (s, 6'-C), 128.0 (s, 7'-CH), 127.5 (s, 9'-C), 123.1 (d, 3'-CH), 114.3 and 112.2 (2 x s, 2 x CN), 82.6 (s, 2-C); *m/z* 205 (M^+, 100%), 178 (M^+ - CN, - H), 154 (M^+ - 2 x CN, + H), 129 (M^+ - [C=C(CN)$_2$]).

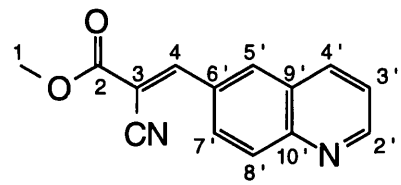
2,4-Dicyano-3-amino-5-(6-quinolyl)penta-2,4-dienonitrile (202)



6-Quinolinecarbaldehyde (0.1482 g, 0.94 mmol) and 2-aminopropene-1,1,3-tricarbonitrile (0.1269 g, 0.94 mmol) were treated with ethanol (1 ml) and dilute piperidine solution (1 drop) using General Procedure B to give a yellow solid after 2.5 h (0.2286 g, 89 %), m.p. 251-254 °C. R_F 0.28 (1:1 ethyl acetate/petroleum ether); (Found: C 70.92; H 3.52; N 25.56 %; M^+, 271.0837. $C_{16}H_9N_5$ requires C 70.84; H 3.34; N 25.82 %; *M* 271.0858); ν_{max}/cm^{-1} (KBr disc) 3016w (Ar-H), 2225w (CN), 1656s (C=C), 1618m, 1579s and 1505m (aromatic ring), 1536s (C-NO_2), 826m (2 adjacent Ar-H), 794m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.28 and 9.18 (2 x br s, 2H, NH_2), 9.05 (d, ^3J 4.2 Hz, 1H, 2'-H), 8.57-8.14 (m, 4H, 4', 5', 7', 8'-H), 8.26 (s, 1H, 5-H), 7.68 (dd, ^3J 8.3 Hz, ^3J 4.2 Hz, 3'-H); δ_C (50 MHz, DMSO-d_6) 165.4 (s, 3-C), 153.2

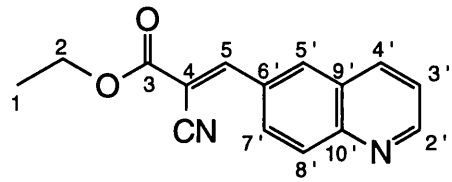
(d, 5-CH), 152.8 (d, 2'-CH), 148.9 (s, 10'-C), 137.5 (d, 8'-CH), 132.7 (d, 4'-CH), 130.3 (d, 5'-CH), 129.8 (s, 6'-C), 128.3 (d, 7'-CH), 127.7 (s, 9'-C), 122.9 (d, 3'-CH), 115.6 and 114.9 (2 x s, 1-CN and 6-CN),114.7 (s, 7-CN), 104.8 (s, 2-C), 103.2 (s, 4-C); *m/z* 271 (M^+, 71 %), 245 (M^+ - CN), 206 (M^+ - $C(CN)_2$, - H), 179 (M^+ - $[C(NH_2)=C(CN)_2]$).

Methyl 2-cyano-3-(6-quinolyl)propenoate (203)



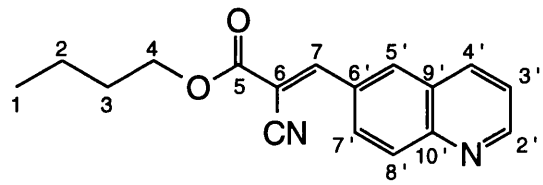
Using General Procedure C, 6-quinolinecarbaldehyde (0.3052 g, 1.94 mmol) and methyl cyanoacetate (0.20 ml , 2.27 mmol) were treated in methanol (2 ml) to give a yellow precipitate after 24 h (0.2987 g, 61 %), m.p. 144-146 °C. R_F 0.25 (1:1 ethyl acetate/petroleum ether); (Found: C 70.64; H 4.37; N 11.79 %; M^+, 238.0741. $C_{14}H_{10}N_2O_2$ requires C 70.60; H 4.20; N 11.76 %; *M*, 238.0743); ν_{max}/cm^{-1} (KBr disc) 3034w (Ar-H), 2215w (CN), 1728s (αβ-unsaturated C=O), 1601s and 1566w (aromatic ring), 842m (2 adjacent Ar-H), 779w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6, 40 °C) 9.08 (dd, ^3J 4.1 Hz, ^4J 1.6 Hz, 1H, 2'-H), 8.60 (br, 1H, 8'-H), 8.50 (s, 1H, 4-H), 8.47-8.35 (m, 2H, 4'-H and 7'-H), 8.20 (br, 1H, 5'-H), 7.60 (dd, ^3J 4.1 Hz, ^3J 8.4 Hz, 1H, 3'-H), 0.95 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6, 40 °C) 163.2 (s, C=O), 155.0 (d, 2'-CH), 153.7 (s, 10'-C), 150.2 (d, 4-C), 137.6 (d, 8'-CH), 135.0 (d, 4'-CH), 130.6 (d, 5'-CH), 129.7 (s, 6'-C), 129.5 (d, 7'-CH), 128.2 (s, 9'-C), 123.5 (d, 3'-CH), 118.2 (s, CN), 103.8 (s, 3-C), 55.2 (q, CH_3); *m/z* 238 (M^+, 100 %), 223 (M^+ - CH_3), 207 (M^+ - OCH_3), 179 (M^+ - CO_2CH_3), 152 (M^+ - CO_2CH_3, - CN, + H^+).

Ethyl 2-cyano-3-(6-quinolyl)propenoate (204)



6-Quinolinecarbaldehyde (0.1389 g, 0.88 mmol) was treated with ethyl cyanoacetate (0.1 ml, 0.94 mmol) in ethanol (1 ml) using General Procedure C for 12 h. Concentration *in vacuo* gave a solid which was recrystallised from acetone to give yellow crystals (0.1051 g, 47%), m.p. 138-140 °C. R_F 0.32 (1:1 ethyl acetate/petroleum ether); (Found: C 71.50; H 4.73; N 11.09%; M^+, 252.0889. $C_{15}H_{12}N_2O_2$ requires C 71.44; H 4.80; N 11.11%; *M*, 252.0899); ν_{max}/cm^{-1} (KBr disc) 3035w (aryl-H), 2221w (CN), 1720s (αβ-unsaturated carbonyl), 1570m and 1496m (aromatic ring), 846m (2 adjacent Ar-H), 797w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.06 (dd, ^3J 4.2 Hz, ^4J 1.7 Hz, 1H, 2'-H), 8.60 (d, ^3J 2.0 Hz, 1H, 8'-H), 8.54 (s, 1H, 5-H), 8.49-8.39 (m, 2H, 4'-H and 7'-H), 8.18 (br d, ^3J 8.8 Hz, 5'-H), 7.65 (dd, ^3J 8.3 Hz, ^4J 4.2 Hz, 1H, 3'-H), 4.43-4.33 (q, ^3J 7.1 Hz, 2H, CH_2), 1.38 (t, ^3J 7.1 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.8 (s, C=O), 154.1 (d, 2'-CH), 153.2 (s, 10'-C), 149.1 (s, 4-C), 137.3 (d, 8'-CH), 134.0 (d, 4'-CH), 130.1 (d, 5'-CH), 129.5 (s, 6'-C), 128.5 (d, 7'-CH), 127.6 (s, 9'-C), 122.7 (s, 3'-CH), 117.9 (s, CN), 103.6 (s, 5-C), 62.5 (t, CH_2), 13.9 (q, CH_3); *m/z* 252 (M^+, 100 %), 223 (M^+ - C_2H_5), 207 (M^+ - OC_2H_5), 179 (M^+ - $CO_2C_2H_5$), 153 (M^+ -$CO_2C_2H_5$, - CN).

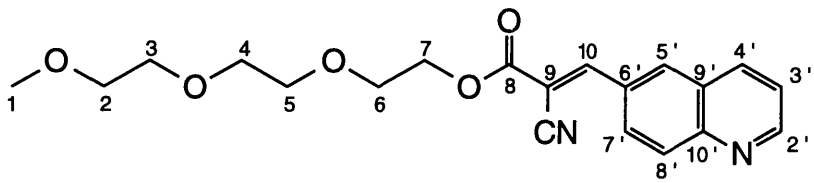
^{n}Butyl 2-cyano-3-(6-quinolyl)propenoate (205)



Using General Procedure A, 6-quinolinecarbaldehyde (0.3112 g, 1.98 mmol) was treated with ^{n}butyl cyanoacetate (0.28 ml, 1.97 mmol) in butan-1-ol (1 ml) and piperidine (10 drops) to give a yellow solid after 10 min which was filtered and washed (diethyl ether). After 24 h under oil pump vacuum, this yielded a yellow solid which needed no further purification (0.2666 g, 45 %), m.p. 71-72 °C. R_F 0.33 (1:1 ethyl acetate/petroleum ether); (Found: C 72.56; H 5.71; N 10.05 %; M^+, 280.1202. $C_{17}H_{16}N_2O_2$ requires C 72.86; H 5.75; N 10.00 %; *M*, 280.1212); ν_{max}/cm^{-1} (KBr disc) 3075w (Ar-H), 2226w (CN),

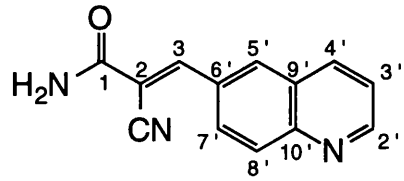
1720s (αβ-unsaturated carbonyl), 1632m (extended conjugation to aromatic ring), 1580s and 1500m (aromatic ring), 1370m (CH_3), 840m (2 adjacent Ar-H), 800w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.07 (dd, ^3J 4.3 Hz, ^3J 1.4 Hz, 1H, 2'-H), 8.62 (d, ^3J 2.0 Hz, 1H, 8'-H), 8.63 (s, 1H, 7-H), 8.47-8.39 (m, 2H, 4' and 7'-H), 8.20 (br, 1H, 5'-H), 7.64 (dd, ^3J 8.2 Hz, ^3J 4.3 Hz, 1H, 3'-H), 4.30 (t, ^3J 6.4 Hz, 2H, 4-H_2), 1.95-0.74 (m, 7H, 2 and 3-H_2, 1-H_3); δ_C (50 MHz, DMSO-d_6) 162.4 (s, C=O), 154.2 (d, 2'-CH), 153.4 (d, 7-CH), 149.2 (s, 10'-C), 137.4 (s, 8'-CH), 134.3 (d, 4'-CH), 130.2 (5'-CH), 129.5 (s, 6'-C), 128.5 (d, 7'-CH), 127.6 (s, 9'-C), 122.8 (d, 3'-CH), 115.6 (s, CN), 103.5 (s, 6-C), 66.1 (t, 4-CH_2), 30.1 (t, 3-CH_2), 18.5 (t, 2-CH_2), 13.6 (q, CH_3); *m/z* 280 (M^+, 75 %), 223 (M^+ - ^{n}Bu), 180 (M^+ - $CO_2{}^n$Bu, + H), 154 (M^+ - $CO_2{}^n$Bu, - CN, + H).

Tri(ethylene glycol) 2-cyano-3-(6-quinolyl)propenoate methyl ether (206)



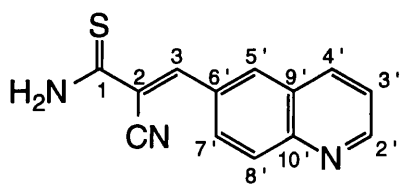
Using General Procedure A, 6-quinolinecarbaldehyde (0.0815 g, 0.52 mmol) was treated with tri(ethylene glycol) cyanoacetate methyl ether (52 μl, 0.52 mmol) in ethanol (2.5 ml) and piperidine (1 drop) for 12 h. Filtration, followed by concentration of the mother liquors *in vacuo* gave the product as an orange oil (0.1264 g, 63 %). R_F 0-0.14 (1:1 ethyl acetate/petroleum ether); (Found: C 64.79; H 5.97; N 7.58 %; M^+, 371.1505. $C_{20}H_{22}N_2O_5$ requires C 64.85; H 5.99; N 7.56 %; *M*, 371.1529); ν_{max}/cm^{-1} (thin film) 3048m (Ar-H), 2884s and 1434s (-CH_2-), 2224w (CN), 1716s (αβ-unsaturated carbonyl), 1620s (extended conjugation to aromatic system), 1586s, 1574s and 1504m (aromatic ring), 802w (3 adjacent Ar-H), 838m (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.01 (dd, ^3J 4.1 Hz, ^4J 1.5 Hz, 1H, 2'-H), 8.46 (m, 1H, 4'-H), 8.43 (s, 1H, 10 H), 8.41 (m, 1H, 8'-H), 8.32 (m, 1H, 5'-H), 8.23 (m, 1H, 7'-H), 7.51 (dd, ^3J 8.3 Hz, ^3J 4.3 Hz, 1H, 3'-H), 3.84-3.38 (m, 12H, 2, 3, 4, 5, 6, 7-H_2), 1.42 (t, ^3J 7.1 Hz, CH_3); δ_C (50 MHz, DMSO-d_6) 154.1 (d, 10-CH), 153.0 (d, 2'-H), 149.7 (s, 10'-C), 137.2 (d, 8'-CH), 133.1 (d, 4'-CH), 132.9 (s, 6'-C), 130.6 (d, 5'-CH), 129.4 (d, 7'-CH), 127.8 (s, 9'-C), 115.0 (s, CN), 103.8 (s, 9-C), 70.4 (t, 7-CH_2), 70.3 (t, 6-CH_2), 70.1 (t, 5-CH_2), 66.6 (t, 4-CH_2), 65.8 (t, 3-CH_2), 61.5 (t, 2-CH_2), 14.0 (q, CH_3), signal for C=O not observed ; *m/z* 371 (M^+, 43 %), 294 (M^+ - $OCH_2CH_2OCH_3$ - H), 268 (M^+ - [$(CH_2CH_2O)_2CH_3$]), 224 (M^+ - [$(CH_2CH_2O)_3CH_3$]), 179 (M^+ - [$CO(OCH_2CH_2)_3OCH_3$], -H).

2-Cyano-3-(6-quinolyl)propenamide (207)



6-Quinolinecarbaldehyde (0.1094 g, 0.70 mmol) was treated with 2-cyanoacetamide (0.0650 g, 0.77 mmol) in ethanol (5 ml) and piperidine (1 drop) using General Procedure A to give an off-white precipitate after 40 min (0.0925 g, 58 %), m.p. 236-239 °C. R_F 0.24 (1:1 ethyl acetate/petroleum ether); (Found: C 69.84; H 4.12; N 18.61 %; M^+, 223.0726. $C_{13}H_9N_3O$ requires C 69.95; H 4.06; N 18.82 %; *M*, 223.0746); ν_{max}/cm^{-1} (KBr disc) 3054m (Ar-H), 2214W (CN), 1699s (amide C=O), 1636m and 1603m (aromatic ring), 839m (2 adjacent Ar-H), 790w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.05 (br, 1H, 2'-H), 8.54 (s, 1H, 3-H), 8.41-8.06 (m, 4H, 4', 5', 7' and 8'-H), 7.90 and 7.74 (2 x s, NH_2), 8.39 (dd, ^3J 8.3 Hz, ^3J 4.2 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 154.1 (d, 3-CH), 152.3 (s, 2'-CH), 150.3 (s, 10'-C), 138.9 (d, 8'-CH), 131.6 (d, 4'-CH), 130.8 (d, 5'-CH), 130.5 (s, 6'-C), 127.9 (d, 7'-CH), 127.0 (s, 9'-C), 124.2 (d, 3'-CH), 117.3 (s, CN), 112.9 (s, 2-C), signal for C=O not observed; *m/z* 223 (M^+, 71 %), 207 (M^+ - NH_2), 179 (M^+ -$CONH_2$), 153 (M^+ - $CONH_2$, - CN).

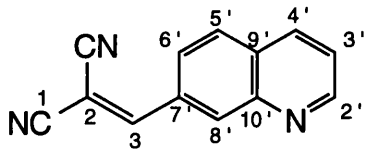
2-Cyano-3-(6-quinolyl)propenethioamide (208)



Using General Procedure C, 6-quinolinecarbaldehyde (0.3104 g, 1.97 mmol) and 2-cyanothioacetamide (0.2006 g, 2.00 mmol) were treated in ethanol (6 ml) to give an orange precipitate after 5 min (0.4147 g, 88 %), m.p. 223-225 °C. R_F 0.19 (1:1 ethyl acetate/petroleum ether); (Found: C 65.06; H 4.05; N 17.18 %; M^+, 239.0500. $C_{13}H_9N_3S$ requires C 65.25; H 3.79; N 17.56 %; *M*, 239.0517); ν_{max}/cm^{-1} (KBr disc) 2997m (Ar-H), 2211w (CN), 1602s (aromatic ring), 835m (2 adjacent Ar-H), 774w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.97 and 9.77 (2 x s, NH_2), 9.09 (br, 1H, 2'-H), 8.52 (s, 1H, 3-H), 8.35 (br, 1H, 8'-H), 8.30 (s, 1H, 5'-H), 8.20 (br, 1H, 4'-H), 8.16 (br, 1H, 7'-H), 7.65 (dd, ^3J 8.2 Hz, ^3J 4.2 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 148.7 (s, 10'-H), 152.9 (d, 3-CH), 146.3 (d, 2'-CH), 137.2 (d, 8'-CH), 132.4 (d, 4'-CH), 130.2 (s, 6'-C),

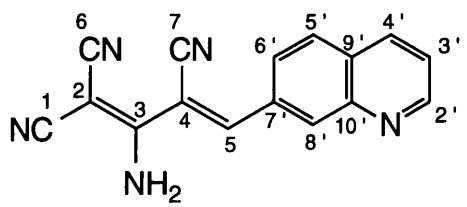
130.0 (d, 5'-CH), 128.9 (d, 7'-CH), 126.7 (s, 9'-C), 122.7 (d, 3'-CH), 116.3 (s, CN), 113.4 (s, 2-C), signal for C=S was not observed; *m/z* 239 (M^+, 84 %), 207 (M^+ - S), 179 (M^+ - $CSNH_2$), 152 (M^+ - $CSNH_2$, - CN, - H).

2-Cyano-3-(7-quinolyl)propenonitrile (209)



7-Quinolinecarbaldehyde (0.1775 g, 1.13 mmol) and malononitrile (0.0770 g, 1.16 mmol) were treated with ethanol (1 ml) using General Procedure C to give a beige precipitate after 1 min (0.1825 g, 79 %), m.p. 116-118 °C. R_F 0.25 (1:1 ethyl acetate/petroleum ether); (Found: C 76.00; H 3.50; N 20.20 %; M^+, 205.6411. $C_{13}H_7N_3$ requires C 76.09; H 3.44; N 20.48 %; *M*, 205.6400); ν_{max}/cm^{-1} (KBr disc) 3037m (Ar-H), 2228s (CN), 1617w, 1559m and 1507w (aromatic ring), 837s (2 adjacent Ar-H), 770m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.06 (dd, ^3J 4.2 Hz, ^7J 1.7 Hz, 1H, 2'-H), 8.75 (s, 1H, 3-H), 8.63 (s, 1H, 8'-H), 8.49 (d, ^3J 8.4 Hz, 1H, 4'-H), 8.21 (d, ^3J 8.6 Hz, 1H, 5'-H), 8.11 (dd, ^3J 8.6 Hz, ^7J 1.7 Hz, 1H, 6'-H), 7.73 (dd, ^3J 8.4 Hz, ^3J 4.2 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 161.0 (d 3-CH), 152.6 (d, 2'-CH), 146.8 (s, 10'-C), 136.3 (d, 8'-CH), 133.4 (d, 4'-CH), 132.1 (s, 7'-C), 130.8 (s, 9'-C), 129.7 (d, 6'-CH), 125.5 (d, 5'-CH), 124.3 (d, 3'-CH), 114.2 and 113.3 (2 x s, 2 x CN), 83.3 (s, 2-C); *m/z* 205 (M^+, 100 %), 179 (M^+ - CN), 154 (M^+ - 2 x CN, + H).

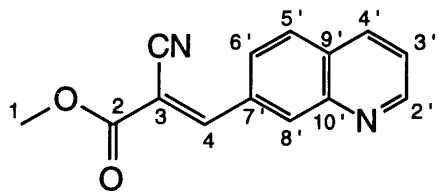
2,4-Dicyano-3-amino-5-(7-quinolyl)penta-2,4-dienonitrile (210)



Using General Procedure C, 7-quinolinecarbaldehyde (0.1604 g, 1.02 mmol) was treated with 2-aminopropene-1,1,3-tricarbonitrile (0.1350 g, 1.02 mmol) in ethanol (1 ml) to give a yellow precipitate after 1 min (0.2402 g, 86 %), m.p. 202-204 °C. R_F 0.05 (1:1 ethyl acetate/petroleum ether); (Found: C 70.79; H 3.73; N 25.55%; M^+, 271.0854. $C_{16}H_9N_5$ requires C 70.84; H 3.34; N 25.82 %; *M*, 271.0858); ν_{max}/cm^{-1} (KBr disc) 2366w (NH_2), 2219s (CN),

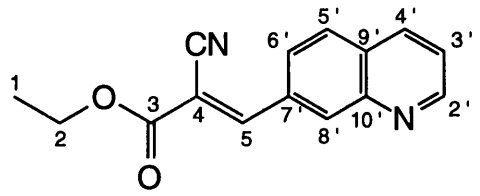
1667s (C=C conjugated to aromatic system), 1595s and 1543s (aromatic ring), 841m (2 adjacent Ar-H), 800w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.30 and 9.25 (2 x s, NH_2), 9.06 (m, 1H, 2'-H), 8.63 (s, 1H, 5-H), 8.47 (d, ^3J 8.3 Hz, 1H, 4'-H), 8.38 (s, 1H, 8'-H), 8.19-8.12 (m, 2H, 5'-H and 6'-H), 7.70 (dd, ^3J 8.3 Hz, ^3J 4.2 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 165.4 (s, 3-C), 152.9 (d, 5-CH), 152.3 (d, 2'-CH), 147.1 (s, 10'-C), 136.1 (d, 8'-CH), 132.5 (d, 4'-CH), 130.5 (s, 9'-C), 130.1 (s, 7'-C), 129.6 (d, 6'-CH), 125.6 (d, 5'-CH), 123.7 (d, 3'-CH), 115.6 (s, 7-CN), 114.9 and 114.7 (2 x s, 1-CN and 6-CN), 103.6 (s, 2-C), 50.2 (s, 4-C); *m/z* 271 (M^+, 100 %), 245 (M^+ - CN), 206 (M^+ - [$C(CN)_2$], - H), 179 (M^+ - [$C(NH_2)=C(CN)_2$]).

Methyl 2-cyano-3-(7-quinolyl)propenoate (211)



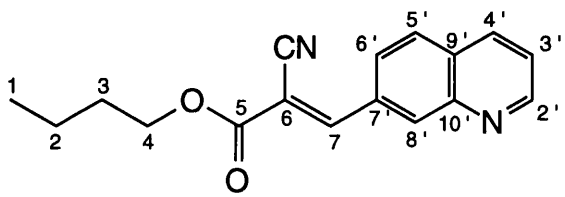
7-Quinolinecarbaldehyde (0.0895 g, 0.57 mmol) was treated with methyl cyanoacetate (52 µl, 0.57 mmol) in methanol (1 ml) and piperidine (1 drop) using General Procedure A to give a yellow precipitate after 1 min (0.0629 g, 46 %), m.p. 208-209 °C. R_F 0.23 (1:1 ethyl acetate/petroleum ether); (Found: C 70.59; H 4.26; N 11.73%; M^+, 238.0731. $C_{14}H_{10}N_2O_2$ requires C 70.60; H 4.20; N 11.76 %; *M*, 238.0742); ν_{max}/cm^{-1} (KBr disc) 3030w (Ar-H), 2219w (CN), 1727s (αβ-unsaturated carbonyl), 1599s (aromatic ring), 833m (2 adjacent Ar-H), 780w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.06 (m, 1H, 2'-H), 8.76 (s, 1H, 4-H), 8.67 (s, 1H, 8'-H), 8.48 (d, ^3J 8.2 Hz, 1H, 4'-H), 8.27-8.16 (m, 2H, 5'-H and 6'-H), 7.21 (dd, ^3J 8.2 Hz, ^3J 4.1 Hz, 1H, 3'-H), 3.95 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 159.3 (s, C=O), 152.9 (d, 4-CH), 152.4 (d, 2'-CH), 147.6 (s, 10'-C), 135.8 (d, 8'-CH), 132.9 (d, 4'-CH), 131.2 (s, 9'-C), 130.8 (d, 6'-CH), 130.2 (s, 7'-C), 125.8 (d, 5'-CH), 123.6 (d, 3'-CH), 116.2 (s, CN), 102.7 (s, 3-C), 54.0 (q, CH_3); *m/z* 238 (M^+, 100 %), 223 (M^+ - CH_3), 207 (M^+ - OCH_3), 179 (M^+ - CO_2CH_3), 152 (M^+ - CO_2CH_3, - CN, + H).

Ethyl 2-cyano-3-(7-quinolyl)propenoate (212)



7-Quinolinecarbaldehyde (0.1524 g, 0.97 mmol) and ethyl cyanoacetate (0.12 ml, 1.12 mmol) were treated in ethanol (1 ml) and piperidine (1 drop) using General Procedure A to give a yellow precipitate after 1 min (0.1723 g, 67 %), m.p. 162-164 °C. R_F 0.27 (1:1 ethyl acetate/petroleum ether); (Found: C 71.16; H 4.85; N 11.03%; M^+, 252.0887. $C_{15}H_{12}N_2O_2$ requires C 71.44; H 4.80; N 11.11 %; *M*, 252.0899); ν_{max}/cm^{-1} (KBr disc) 3047W (Ar-H), 2224w (CN), 1724s (αβ-unsaturated C=O), 1635w, 1600s and 1534w (aromatic ring), 836s (2 adjacent Ar-H), 784w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.06 (m, 1H, 2'-H), 8.70 (s, 1H, 8'-H), 8.64 (s, 1H, 5-H), 8.48 (d, ^3J 8.3 Hz, 1H, 4'-H), 8.24-8.15 (m, 2H, 5' and 6'-H), 7.72 (dd, ^3J 8.3 Hz, ^3J 4.1 Hz, 1H, 3'-H), 4.34 (q, ^3J 7.05 Hz, 2H, CH_2), 1.28 (t, ^3J 7.0 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 160.8 (s, C=O), 153.4 (d, 5-CH), 152.5 (d, 2'-CH), 147.2 (s, 10'-C), 136.0 (d, 8'-CH), 133.3 (d, 4'-CH), 131.8 (s, 7'-C), 130.8 (s, 9'-C), 129.6 (d, 6'-CH), 127.0 (d, 5'-CH), 123.7 (d, 3'-CH), 115.8 (s, CN), 103.5 (s, 4-C), 63.4 (t, CH_2), 15.2 (q, CH_3); *m/z* 252 (M^+, 100%), 223 (M^+ - C_2H_5), 207 (M^+ - OC_2H_5), 180 (M^+ - $CO_2C_2H_5$, + H), 152 (M^+ - $CO_2C_2H_5$ -CN, + H).

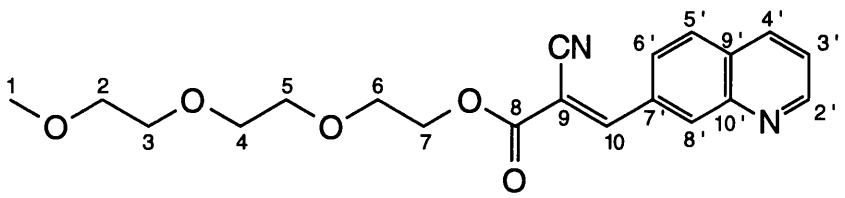
^{n}Butyl 2-cyano-3-(7-quinolyl)propenoate (213)



7-Quinolinecarbaldehyde (0.0812 g, 0.52 mmol) and ^{n}butyl cyanoacetate (73 µl, 0.52 mmol) were treated with butan-1-ol (2 ml) and piperidine (1 drop) using General Procedure A to give a beige precipitate after 1 min (0.0636 g, 42 %), m.p. 104-105 °C. R_F 0.38 (1:1 ethyl acetate/petroleum ether); (Found: C 72.66; H 5.65; N 9.96 %; M^+, 280.1215. $C_{17}H_{16}N_2O_2$ requires C 72.86; H 5.75; N 10.00 %; *M*, 280.1212); ν_{max}/cm^{-1} (KBr disc) 3026w (Ar-H), 2222w (CN), 1718s (αβ-unsaturated C=O), 1600s (aromatic ring), 832m (2 adjacent Ar-H), 760w (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.05 (m, 1H, 2'-H), 8.72 (s, 1H, 8'-H), 8.67 (s, 1H, 7-H), 8.48 (d, ^3J 8.3 Hz, 1H, 4'-H), 8.23-8.16 (m, 2H, 5'-H

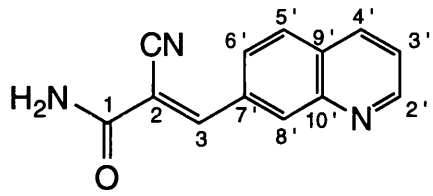
and 6'-H), 7.21 (dd, ^3J 8.3 Hz, ^3J 4.2 Hz, 1H, 3'-H), 4.35 (t, ^3J 6.4 Hz, 2H, 4-H_2), 1.74 (dd, ^3J 7.6 Hz, ^3J 6.4 Hz, 2H, 3-H_2), 1.46 (dd, ^3J 7.6 Hz, ^3J 7.2 Hz, 2H, 2-H_2), 0.98 (t, ^3J 7.2 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 161.8 (s, C=O), 154.6 (d, 7-CH), 152.2 (d, 2'-CH), 147.1 (s, 10'-C), 136.0 (d, 8'-CH), 133.5 (d, 4'-CH), 132.3 (s, 7'-C), 130.4 (s, 9'-C), 129.4 (d, 5'-CH), 126.3 (d, 6'-CH), 123.8 (d, 3'-CH), 115.7 (s, CN), 104.2 (s, 6-C), 66.2 (t, 4-CH_2), 30.2 (t, 3-CH_2), 18.7 (t, 2-CH_2), 13.7 (q, CH_3); *m/z* 280 (M^+, 10 %), 235 (M^+ - ^{n}Bu, + 2H), 180 (M^+ - $CO_2{}^n$Bu, + H), 152 (M^+ - $CO_2{}^n$Bu, - CN, - H).

Tri(ethylene glycol) 2-cyano-3-(7-quinolyl)propenoate methyl ether (214)



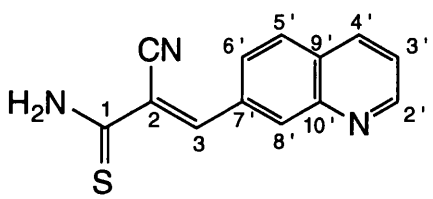
Using General Procedure A, 7-quinolinecarbaldehyde (0.1410 g, 0.90 mmol) was treated with tri(ethylene glycol) cyanoacetate methyl ether (186 µl, 0.90 mmol) in ethanol (2 ml) and piperidine (1 drop) for 1 h. Filtration and concentration *in vacuo* gave the product as a brown oil (0.2613 g, 78 %) R_F 0-0.38 (1:1 ethyl acetate/petroleum ether); (Found: C 64.98; H 6.02; N 7.54 %; M^+, 371.1534. $C_{20}H_{22}N_2O_5$ requires C 64.85; H 5.99; N 7.56 %; *M*, 371.1529); ν_{max}/cm^{-1} (KBr disc) 3054m (Ar-H), 2878s, 1452s and 730w (-CH_2-), 2244w (CN), 1720s (αβ-unsaturated carbonyl), 1626s (extended conjugation to aromatic system), 1598m, 1570m and 1502s (aromatic ring), 808m (3 adjacent Ar-H), 842s (2 adjacent Ar-H), 896w (isolated Ar-H); δ_H (200 MHz, DMSO-d_6) 8.74 (m, 1H, 2'-H), 8.09-7.24 (m, 6H, 10, 3', 4', 5', 6', 8'-H), 3.62-2.92 (m, 12H, 2, 3, 4, 5, 6, 7-H), 1.09 (s, CH_3); δ_C (50 MHz, DMSO-d_6) 151.1 (d, 10-CH), 150.7 (d, 2'-CH), 147.7 (s, 10'-C), 136.1 (d, 8'-CH), 128.9 (d, 4'-CH), 128.6 (s, 7'-C), 128.4 (s, 9'-C), 128.1 (d, 6'-CH), 127.9 (d, 5'-CH), 121.5 (d, 3'-CH), 115.2 (s, CN), 105.2 (s, 9-C), 71.8 (t, 7-CH_2), 70.4 (t, 6-CH_2), 70.3 (t, 5-CH_2), 70.1 (t, 4-CH_2), 68.3 (t, 3-CH_2), 57.7 (t, 2-CH_2), 18.3 (q, CH_3), signal for C=O not observed; *m/z* 371 (M^+, 35 %), 294 (M^+ - $OCH_2CH_2OCH_3$ - H), 268 (M^+ - [$(CH_2CH_2O)_2CH_3$]), 251 (M^+ - [$(OCH_2CH_2)_2OCH_3$], - H), 207 (M^+ - [$O(CH_2CH_2)_3OCH_3$], - H), 179 (M^+ - [$CO(OCH_2CH_2)_3OCH_3$], - H).

2-Cyano-3-(7-quinolyl)propenamide (215)



Using General Procedure A, 7-quinolinecarbaldehyde (0.1651 g, 1.05 mmol) and 2-cyanoacetamide (0.0932 g, 1.11 mmol) were treated with ethanol (2 ml) and piperidine (1 drop) to give a pink powder after 10 min (0.1696 g, 73 %), m.p. 185-187 °C. R_F 0.27 (1:1 ethyl acetate/petroleum ether); (Found: C 69.84; H 4.86; N 18.82%; M+, 223.0732. $C_{13}H_9N_3O$ requires C 69.95; H 4.06; N 18.82 %; *M*, 223.0746); ν_{max}/cm^{-1} (KBr disc) 2681w and 1598m (amide NH_2), 2253w (CN), 1579m (aromatic ring), 1690s (amide C=O), 840m (2 adjacent Ar H), 777m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 10.13 (br s, 2H, NH_2), 8.91 (m, 6H, 3, 2', 4', 5', 6' and 8'-H), 7.54 (dd, ^3J 8.3 Hz, ^3J 4.2 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 168.8 (s, C=O), 152.1 (d, 3-C), 150.8 (d, 2'-CH), 147.2 (s, 10'-C), 135.4 (d, 8'-CH), 132.9 (d, 4'-CH), 130.6 (s, 7'-C), 129.8 (s, 9'-C), 128.9 d, 6'-CH), 125.8 (d, 5'-CH), 124.2 (d, 3'-CH), 116.2 (s, CN), 102.8 (s, 2 C); *m/z* 222 (M^+, 100 %), 205 (M^+ - NH_2 - 2H), 179 (M^+ - $CONH_2$), 128 (M^+ - [C=C(CN)$CONH_2$] + H).

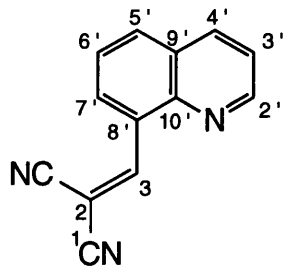
2-Cyano-3-(7-quinolyl)propenethioamide (216)



Using General Procedure C, 7-quinolinecarbaldehyde (0.1153 g, 0.73 mmol) was treated with 2-cyanothioacetamide (0.0763 g, 0.76 mmol) with ethanol (1 ml) to give an orange precipitate after 1 h (0.0779 g, 45 %), m.p. 194-196 °C. R_F 0.26 (1:1 ethyl acetate/petroleum ether); (Found: C 65.27; H 3.73; N 17.58 %; M+, 239.0512. $C_{13}H_9N_3S$ requires C 65.25; H 3.79; N 17.56 %; *M*, 239.0517); ν_{max}/cm^{-1} (KBr disc) 3347s (thioamide NH_2), 2211w (CN), 1587s and 1566m (aromatic ring), 1123s (C=S), 837s (2 adjacent Ar-H), 771m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.96 and 9.81 (2 x s, 2H, NH_2), 9.07 (m, 1H, 2'-H), 8.73 (s, 1H, 3-H), 8.63 (s, 1H, 8'-H), 8.48 (d, 1H, ^3J 8.3 Hz, 4'-H), 8.32-8.17 (m, 2H, 5' and 6'-H), 7.70 (dd, ^3J 8.3 Hz, ^3J 4.2 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 172.1 (s, C=S), 154.8 (d, 3-CH), 151.2 (d, 2'-CH), 148.5

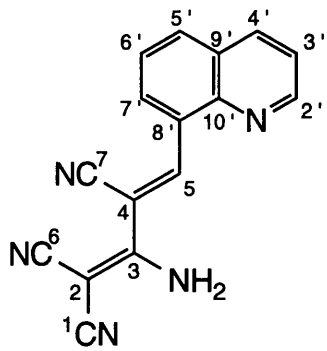
(s, 10'-C), 135.8 (d, 8'-CH), 133.2 (d, 4'-CH), 131.2 (s, 7'-C), 129.7 (s, 9'-C), 128.9 (d, 6'-CH), 126.1 (d, 5'-CH), 124.8 (d, 3'-CH), 118.4 (s, CN), 101.5 (s, 2-C); *m/z* 239 (M^+, 71 %), 223 ($M^+ - NH_2$), 211 ($M^+ - CNH_2$), 179 ($M^+ - CSNH_2$), 154 ($M^+ - CSNH_2$, - CN + H).

2-Cyano-3-(8-quinolyl)propenonitrile (217)



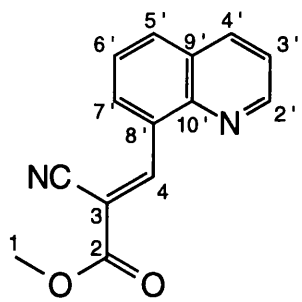
8-Quinolinecarbaldehyde (0.1101 g, 0.70 mmol) was treated with malononitrile (0.0480 g, 0.73 mmol) in methanol (5 ml) and piperidine (1 drop) using General Procedure A to give a beige solid after 24 h (0.0460 g, 28 %), m.p. 175-176 °C. R_F 0.17 (1:1 ethyl acetate/petroleum ether); (Found: C 76.14; H 3.49; N 20.68 %; M^+, 205.6408. $C_{13}H_7N_3$ requires C 76.09; H 3.44; N 20.48%; *M*, 205.6400); ν_{max}/cm^{-1} (KBr disc) 3048w (Ar-H), 1620m (extended conjugation to aromatic system), 1580s and 1520s (aromatic ring), 805m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.06 (dd, ^3J 3.9 Hz, ^4J 1.0 Hz, 1H, 2'-H), 8.71 (d, ^3J 8.1 Hz, 1H, 7'-CH), 8.52 (s, 3-H), 8.51 (dd, ^3J 7.4 Hz, ^4J 1.0 Hz, 1H, 4'-H), 7.72 (d, ^3J 7.8 Hz, 1H, 5'-H), 7.31 (m, 1H, 6'-H), 7.25 (dd, ^3J 7.4 Hz, ^3J 3.9 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 158.3 (d, 3-C), 150.2 (d, 2'-CH), 143.1 (s, 10'-C), 138.2 (d, 4'-CH), 134.2 (d, 7'-CH), 133.5 (d, 5'-CH), 132.8 (s, 8'-C), 127.1 (s, 9'-C), 126.8 (d, 6'-CH), 122.1 (d, 3'-CH), 84.4 (s, 2-C), 114.8 and 115.6 (2 x s, 2 x CN); *m/z* 205 (M^+, 14 %), 179 (M^+ - CN), 153 (M^+ - 2 x CN).

2,4-Dicyano-3-amino-5-(8-quinolyl)penta-2,4-dienonitrile (218)



Using General Procedure C, 8-quinolinecarbaldehyde (0.1987 g, 1.26 mmol) was treated with 2-aminopropene-1,1,3-tricarbonitrile (0.1677 g, 1.27 mmol) in ethanol (5 ml) to give a brown powder after 10 min (0.2671 g, 78 %), m.p. 107-110 °C. R_F 0.20 (1:1 ethyl acetate/petroleum ether); (Found: C 70.56; H 3.39; N 25.68 %; M^+, 271.0849. $C_{16}H_9N_5$ requires C; 70.84 H 3.34; N 25.82 %; *M*, 271.0856); ν_{max}/cm^{-1} (KBr disc) 3056m (Ar-H), 2208s (CN), 1620s (extended conjugation to aromatic ring), 798m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.31 and 9.25 (2 x s, NH_2), 9.04 (dd, ^3J 3.9 Hz, ^4J 1.0 Hz, 1H, 2'-H), 8.72-8.49 (m, 2H, 4' and 7'-H), 8.46 (s, 1H, 5-H), 7.75 (d, ^3J 7.9 Hz, 1H, 5'-H), 7.55 (m, 1H, 6'-H), 7.45 (dd, ^3J 7.4 Hz, ^3J 3.9 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 163.1 (s, 3-C), 156.2 (d, 5-CH), 150.4 (d, 2'-CH), 145.2 (s, 10'-C), 136.9 (d, 4'-CH), 134.8 (d, 7'-CH), 133.6 (s, 8'-C), 132.9 (d, 5'-CH), 127.6 (s, 4-C), 127.2 (d, 6'-CH), 122.4 (d, 3'-CH), 115.3, 114.9 and 114.6 (3 x s, 3 x CN), 104.3 (s, 2-C); *m/z* 271 (M^+, 10 %), 206 (M^+ - $[C(CN)_2]$, - H), 180 (M^+ - $[C(NH_2)=C(CN)_2]$, + H).

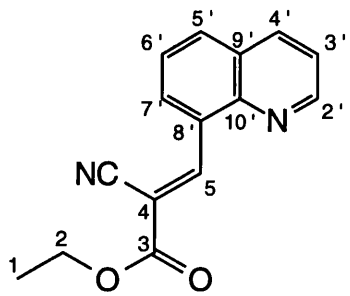
Methyl 2-cyano-3-(8-quinolyl)propenoate (219)



Using General Procedure A, 8-quinolinecarbaldehyde (0.2011 g, 1.28 mmol) and methyl cyanoacetate (115 µl, 1.28 mmol) were treated in methanol (7.5 ml) and piperidine (1 drop) to give a yellow solid after 45 min (0.1836 g, 60 %), m.p. 122-124 °C. R_F 0.21 (1:1 ethyl acetate/petroleum ether); (Found: C 70.42;

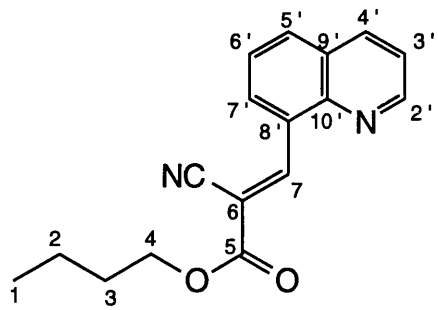
H 4.31; N 11.84 %; M^+, 238.0764. $C_{14}H_{10}N_2O_2$ requires C 70.60; H 4.20; N 11.76 %; *M*, 238.0743); ν_{max}/cm^{-1} (KBr disc) 3064w (Ar-H), 1708s (αβ-unsaturated carbonyl), 1616m (extended conjugation to aromatic ring), 1582s and 1524s (aromatic system), 804m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.07 (dd, ^3J 3.9 Hz, ^4J 0.9 Hz, 1H, 2'-H), 8.69 (d, ^3J 8.1 Hz, 1H, 7'-H), 8.55 (dd, ^3J 7.4 Hz, ^4J 0.9 Hz, 1H, 4'-H), 8.51 (s, 1H, 4-H), 7.82 (d, ^3J 7.9 Hz, 1H, 5'-H), 7.60 (m, 1H, 6'-H), 7.55 (dd, ^3J 7.4 Hz, ^3J 3.9 Hz, 1H, 3'-H), 3.41 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 167.2 (s, C=O), 153.1 (d, 4-CH), 152.2 (d, 2'-CH), 146.3 (s, 10'-C), 138.2 (d, 4'-CH), 136.4 (d, 7'-CH), 135.2 (s, 8'-C), 132.8 (d, 5'-CH), 128.9 (s, 9'-C), 128.2 (d, 6'-CH), 123.2 (d, 3'-C), 117.1 (s, CN), 104.8 (s, 3-C), 53.6 (q, CH_3); *m/z* 238 (M^+, 6 %), 223 (M^+ - CH_3), 179 (M^+ -CO_2CH_3), 153 (M^+ - CO_2CH_3, - CN).

Ethyl 2-cyano-3-(8-quinolyl)propenoate (220)



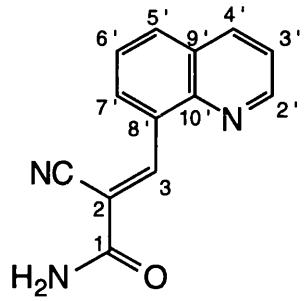
Using General Procedure A, 8-quinolinecarbaldehyde (0.1722 g, 1.09 mmol) and ethyl cyanoacetate (117.6 µl, 1.09 mmol) were treated in ethanol (5 ml) and piperidine (1 drop) to give a yellow solid after 23 h (0.0756 g, 27 %), m.p. 176-178 °C. R_F 0.11 (1:1 ethyl acetate/petroleum ether); (Found: C 71.63; H 4.90; N 11.05 %; M^+, 252.0888. $C_{15}H_{12}N_2O_2$ requires C 71.44; H 4.80; N 11.11%; *M*, 252.0899); ν_{max}/cm^{-1} (KBr disc) 3064w (Ar-H), 2924w (C-H stretch), 1710s (αβ-unsaturated carbonyl), 1618m (extended conjugation to aromatic system), 1582s and 1524s (aromatic ring), 804m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.06 (dd, ^3J 3.9 Hz, ^4J 0.9 Hz, 1H, 2'-H), 8.53 (dd, ^3J 7.4 Hz, ^4J 0.9 Hz, 1H, 4'-H), 8.50 (s, 1H, 5-H), 8.32 (d, ^3J 8.0 Hz, 1H, 7'-H), 7.79 (d, ^3J 7.9 Hz, 1H, 5'-H), 7.32 (m, 1H, 6'-H), 7.23 (dd, ^3J 7.4 Hz, ^3J 3.9 Hz, 1H, 3'-H), 3.42 (q, ^3J 7.1 Hz, 2H, CH_2), 1.28 (t, ^3J 7.1 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 166.8 (s, C=O), 154.0 (d, 5-CH), 152.8 (d, 2'-CH), 148.2 (s, 10'-C), 138.6 (d, 4'-CH), 138.1 (d, 7'-CH), 136.2 (s, 8'-C), 134.2 (d, 5'-CH), 129.8 (d, 6'-CH), 127.7 (s, 9'-C), 120.1 (d, 3'-CH), 116.9 (s, CN), 103.9 (s, 4-C), 62.8 (t, CH_2), 15.2 (q, CH_3); *m/z* 252 (M^+, 15 %), 223 (M^+ - C_2H_5), 208 (M^+ -OC_2H_5, + H), 179 (M^+ - COC_2H_5).

^{n}Butyl 2-cyano-3-(8-quinolyl)propenoate (221)



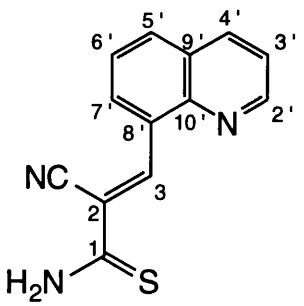
Using General Procedure A, 8-quinolinecarbaldehyde (0.1421 g, 0.90 mmol) and ^{n}butyl cyanoacetate (300 μl, 2.11 mmol) were treated in butan-1-ol (5 ml) and piperidine (1 drop) to give a brown solid after 22.5 h (0.0695 g, 28 %), m.p. 180-182 °C. R_F 0.11 (1:1 ethyl acetate/petroleum ether); (Found: C 72.64; H 5.85; N 10.04 %; M^+, 280.1204. $C_{17}H_{16}N_2O_2$ requires C 72.86; H 5.75; N 10.00 %; *M*, 280.1212); ν_{max}/cm^{-1} (KBr disc) 3064w (Ar-H), 2926w (C-H stretch), 1714s (αβ-unsaturated carbonyl), 1618w (extended conjugation to aromatic ring), 1582s and 1522s (aromatic ring), 1462m (C-H deformations), 1382m (-CH_3 symmetric deformation), 802m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 9.06 (d, ^{3}J 3.9 Hz, ^{4}J 0.9 Hz, 1H, 2'-H), 8.53 (s, 1H, 7-H), 8.72-8.50 (m, 2H, 4' and 7'-H), 7.84 (d, ^{3}J 8.0 Hz, 1H, 5'-H), 7.64 (m 1H, 6'-H), 7.47 (d, ^{3}J 7.4 Hz, ^{3}J 3.9 Hz, 1H, 3'-H), 4.32 (t, ^{3}J 6.5 Hz, 2H, 4-H_2), 1.81 (tt, ^{3}J 7.6 Hz, ^{3}J 6.5 Hz, 2H, 3-H_2), 1.51 (tt, ^{3}J 7.6 Hz, ^{3}J 7.2 Hz, 2H, 2-H_2), 0.95 (t, ^{3}J 7.2 Hz, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 165.8 (s, C=O), 155.7 (d, 7-CH), 155.2 (d, 2'-CH), 149.2 (s, 10'-C), 139.6 (d, 4'-CH), 137.2 (d, 7'-CH), 136.1 (s, 8'-C), 134.1 (d, 5'-CH), 128.4 (d, 6'-CH), 128.2 (s, 9'-C), 124.8 (d, 3'-CH), 116.1 (s, CN), 106.1 (s, 6-C), 68.1 (t, 4-CH_2), 34.3 (t, 3-CH_2), 18.2 (t, 2-CH_2), 15.6 (q, CH_3); *m/z* 280 (M^+, 6 %), 206 (M^+ - O^{n}Bu, + H), 180 (M^+ - $CO_2{}^{n}$Bu, + H), 151 (M^+ - $CO_2{}^{n}$Bu, -CN, - 2H).

2-Cyano-3-(8-quinolyl)propenamide (222)



8-Quinolinecarbaldehyde (0.1024 g, 0.65 mmol) and 2-cyanoacetamide (0.0555 g, 0.66 mmol) were treated with ethanol (7.5 ml) and dilute piperidine solution (1 drop) using General Procedure B to give a beige precipitate after 24 h (0.1346 g, 92 %), m.p. 98-100 °C. R_F 0.14 (1:1 ethyl acetate/petroleum ether); (Found: C 69.74; H 4.00; N 18.77 %; M^+, 223.0724. $C_{13}H_9N_3O$ requires C 69.95; H 4.06; N 18.82%; *M*, 223.0746); ν_{max}/cm^{-1} (KBr disc) 3424w (NH_2), 3058m (Ar-H), 2230w (CN), 1606w (extended conjugation to aromatic ring), 1584w (amide N-H stretch); δ_H (200 MHz, DMSO-d_6) 10.02 and 9.98 (2 x s, NH_2), 9.07 (dd, ^3J 3.9 Hz, ^4J 0.9 Hz, 1H, 2'-H), 8.53 (s, 1H, 3-H), 8.45-8.76 (m, 2H, 4' and 7'-H), 7.89 (d, ^3J 8.0 Hz, 1H, 5'-H), 7.40 (m, 1H, 6'-H), 7.24 (dd, ^3J 7.4 Hz, ^3J 3.9 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 168.2 (s, C=O), 154.3 (d, 2'-CH), 153.1 (d, 3-CH), 148.8 (s, 10'-C), 137.1 (d, 4'-CH), 136.3 and 136.2 (2 x d, 5' and 7'-CH), 134.2 (s, 8'-C), 129.4 (d, 6'-CH), 126.2 (s, 9'-C), 123.6 (d, 3'-CH), 118.2 (s, CN), 110.3 (s, 2-C); *m/z* 223 (M^+, 14 %), 207 (M^+ - NH_2), 179 (M^+ - $CONH_2$), 153 (M^+ - $CONH_2$, - CN).

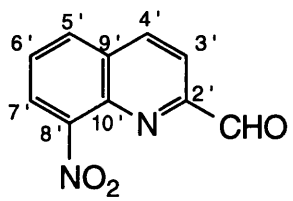
2-Cyano-3-(8-quinolyl)propenethioamide (223)



8-Quinolinecarbaldehyde (0.1022 g, 0.65 mmol) was treated with 2-cyanothioacetamide (0.0690 g, 0.68 mmol) in ethanol (5 ml) and dilute piperidine solution (1 drop) using General Procedure B to give an orange precipitate after 18.5 h (0.0767 g, 49 %), m.p. 139-142 °C. R_F 0.14 (1:1 ethyl acetate/petroleum ether); (Found: C 64.97; H 3.82; N 17.48 %; M^+, 239.0534.

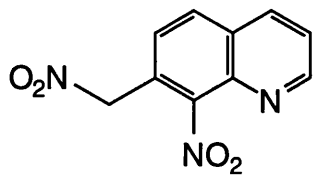
$C_{13}H_9N_3S$ requires C 65.25; H 3.79; N 17.56 %; *M*, 239.0.517); ν_{max}/cm^{-1} (KBr disc) 3314m (NH_2), 3066m (Ar-H), 2186w (CN), 1618m (extended conjugation to aromatic system), 800s (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 10.09 and 10.06 (2 x s, NH_2), 9.06 (dd, ^3J 3.9 Hz, ^4J 1.0 Hz, 1H, 2'-H), 8.55 (s, 1H, 3-H), 8.50-8.77 (m, 2H, 4' and 7'-H), 7.92 (d, ^3J 8.1 Hz, 1H, 5'-H), 7.62 (m, 1H, 6'-H), 7.23 (dd, ^3J 7.5 Hz, ^3J 3.9 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 170.1 (s, C=S), 155.3 (d, 2'-CH), 153.8 (d, 3-CH), 149.6 (s, 10'-C), 137.4 (d, 4'-CH), 135.8 (d, 5'-CH), 134.2 (d, 7'-CH), 133.1 (s, 8'-C), 128.0 (d, 6'-CH), 126.3 (s, 9'-C), 124.0 (d, 3'-CH), 118.2 (s, CN), 113.3 (s, 2-C); *m/z* 239 (M^+, 18 %), 223 ($M^+ - NH_2$), 211 ($M^+ - CNH_2$), 179 ($M^+ - CSNH_2$), 154 ($M^+ - CSNH_2$, - CN, + H).

8-Nitro-2-quinolinecarbaldehyde (227)



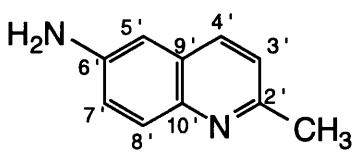
To a 3-necked round bottomed flask equipped with a water condenser was added 2-methyl-8-nitroquinoline (2.1482 g, 11.41 mmol) and toluene (10 ml). The mixture was heated to 50 °C until fully dissolved, and selenium (IV) oxide (1.3266 g, 11.96 mmol) was added. After the initial exotherm had subsided the reaction was heated at reflux temperature for 24 h. The mixture was then cooled and the solvent was removed *in vacuo* to give a solid which was washed with water (50 ml) and extracted with diethyl ether (3 x 100 ml). The combined ethereal extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to a red solid. Column chromatography over silica (1:1 ethyl acetate/petroleum ether) gave the product as an orange solid which was recrystallised from methanol (1.2815 g, 53 %), m.p. 136-137 °C. R_F 0.26 (1:1 ethyl acetate/petroleum ether); (Found: C 59.41; H 3.05; N 13.85%; M^+, 202.0364. $C_{10}H_6N_2O_3$ requires C 59.41; H 2.99; N 13.86%; *M*, 202.0378); ν_{max}/cm^{-1} (KBr disc) 3070w (Ar-H), 1716s (C=O, aromatic aldehyde), 1622m, 1590w and 1530s (aromatic ring), 854m (2 adjacent Ar-H), 798m (3 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 10.11 (s, 1H, CHO), 8.37 (d, ^3J 7.9 Hz, 1H, 4'-H), 8.18-8.04 (m, 3H, 5', 6' and 7'-H), 7.71 (d, ^3J 7.9 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 192.8 (d, CHO), 163.3 (s, 8'-C), 153.8 (s, 2'-C), 148.6 (s, 10'-C), 137.7 (d, 7'-CH), 131.8 (d, 4'-CH), 130.4 (s, 9'-C), 127.8 (d, 5'-CH), 124.4 (d, 6'-CH), 118.9 (d, 3'-CH); *m/z* 202 (M^+, 58 %), 174 (M^+ - CO, + H), 157 ($M^+ - NO_2$ + H), 127 (M^+ - CHO, - NO_2), 101 (M^+ - CHO, - NO_2, - C_2H_2 + 2H).

Attempted synthesis of 7-nitromethyl-8-nitroquinoline (238)



This experiment followed the procedure of Danikiewicz and Mąkosza[198]. A 250 ml 3-necked round bottom flask was cooled to 0 °C, and was charged with powdered sodium hydroxide (0.23 g, 5.75 mmol) dispersed in DMSO (7 ml). To this was added dropwise 8-nitroquinoline **(228)** (0.1745 g, 1.00 mmol) in DMSO (2 ml), followed by nitromethane (0.15 ml, 3.40 mmol). An immediate colour change, giving a red solution, was observed upon addition of the latter, and TLC showed that the spot at R_F 0.53 (8-nitroquinoline) was accompanied by two new spots (very faint) at R_F 0.38 and 0.32. The mixture was allowed to warm to room temperature, and was stirred at this temperature for 12 h, during which time the intensities of the three spots did not change. The excess NaOH was neutralised using aqueous HCl, and the mixture was washed with water (50 ml). The products were extracted (ethyl acetate, 3 x 50 ml), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Column chromatography (SiO_2, 1:1 ethyl acetate/petroleum ether) was then attempted, but only 8-nitroquinoline (identified by TLC) and a considerable quantity of baseline material were recovered.

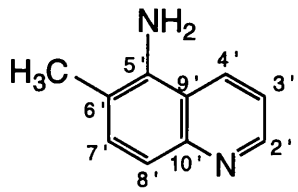
6-Amino-2-methylquinoline (234)



2-Methyl-6-nitroquinoline (0.9222 g, 4.90 mmol), hydrazine monohydrate (5 ml, 103.08 mmol), graphite (*ca.* 0.5 g) and ethanol (50 ml) were heated at reflux for 24 h. The mixture was allowed to cool to room temperature and was filtered through Celite then concentrated *in vacuo* to a brown solid which needed no further purification (0.7167 g, 68 %), m.p. 182-184 °C. R_F 0.15-0.32 (1:1 ethyl acetate/petroleum ether); (Found: C 76.08; H 6.11; N 17.81 %; M^+, 158.0841. $C_{10}H_{10}N_2$ requires C 75.92; H 6.37; N 17.70 %; *M*, 158.0844); ν_{max}/cm^{-1} (KBr disc) 3468m (>N-H), 2918w (Ar-H), 1624s, 1560w & 1502s (aromatic ring), 1380 (CH_3), 836s (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 8.71 (br, 1H, 3-H), 8.52-8.16 (m, 3H, 5', 7' & 8'-H), 7.21-6.94 (br d, 2H, NH_2), 7.42 (br, 1H,

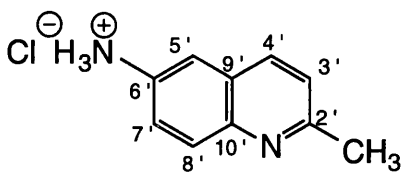
3'-H), 2.90 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 158.4 (s, 10'-C), 150.8 (s, 2'-C), 137.2 (d, 8'-CH), 135.8 (s, 6'-C), 132.4 (d, 4'-CH), 125.8 (s, 9'-C), 125.2 & 124.7 (2 x d, 5' & 6'-CH), 121.9 (d, 3'-CH), 28.4 (q, CH_3); *m/z* 158 (M^+, 23 %), 142 ($M^+ - CH_3$, - H), 112 ($M^+ - NO_2$), 98 ($M^+ - CH_3$, - NO_2, + H).

5-Amino-6-methylquinoline (235)



To 6-methyl-5-nitroquinoline (1.0010 g, 5.37 mmol) was added hydrazine monohydrate (1.60 ml, 32.98 mmol), graphite powder (*ca.* 1 g) and ethanol (5 ml). The mixture was heated at reflux for 48 h. Upon cooling to room temperature, the solution was filtered through Celite and concentrated under reduced pressure to a brown solid. This was dissolved in ethyl acetate and was placed under water pump vacuum until a few yellow crystals were observed. The solution was then allowed to stand at room temperature and atmospheric pressure to yield 5-amino-6-methylquinoline as fine yellow crystals (0.3908 g, 46 %), m.p. 179-180 °C. R_F 0.18 (1:1 ethyl acetate/petroleum ether); (Found: C 75.74; H 6.26; N 18.00 %; M^+, 158.0841. $C_{10}H_{10}N_2$ requires C 75.92; H 6.37; N 17.70 %; *M*, 158.0844); ν_{max}/cm^{-1} (KBr disc) 3414m (N-H), 3020w (Ar-H), 1574m & 1515m (aromatic ring), 802w (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 8.81 (d, ^3J 4.2 Hz, 1H, 2'-H), 8.15 (d, ^3J 8.2 Hz, 1H, 8'-H), 7.52 (d, ^3J 8.6 Hz, 1H, 4'-H), 7.43 (d, ^3J 8.2 Hz, 1H, 7'-H), 7.30 (dd, ^3J 8.6 Hz, ^3J 4.2 Hz, 1H, 3'-H), 4.16 (br s, 2H, NH_2), 2.34 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 149.2 (d, 2'-CH), 148.2 (s, 10'-C), 139.0 (s, 9'-C), 133.0 (d, 8'-CH), 129.0 (d, 4'-CH), 119.5 (d, 7'-CH), 119.3 (d, 3'-CH), 118.2 (s, 6'-C), 106.8 (s, 5'-C); *m/z* 158 (M^+, 15 %), 143 ($M^+ - CH_3$), 112 ($M^+ - NO_2$).

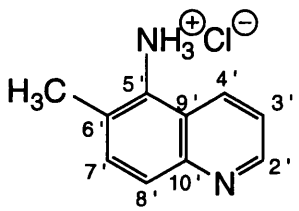
6-Amino-2-methylquinoline hydrochloride (236)



6-Amino-2-methylquinoline (0.4293 g, 2.71 mmol) was dissolved in diethyl ether (*ca.* 100 ml). To this was added ethereal hydrochloric acid (2 ml) to give a bright

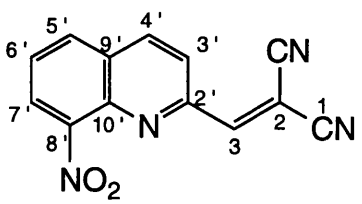
orange precipitate instantly (0.4055 g, 94 %), m.p. 251-253 °C. ν_{max}/cm^{-1} (KBr disc) 3034m (Ar-H), 2928m and 1394m (CH_3); δ_H (200 MHz, DMSO-d_6) 8.80 (br, 1H, 3-H), 8.60-8.25 (m, 3H, 5', 7' and 8'-H), 7.50 (br, 1H, 3'-H), 7.42-6.98 (v br, 3H, NH_3^+); δ_C (50 MHz, DMSO-d_6) 159.2 (s, 10'-C), 152.0 (s, 2'-C), 137.5 (d, 8'-CH), 136.2 (s, 6'-C), 132.9 (d, 4'-CH), 126.4 (s, 9'-C), 125.9 and 125.2 (2 x d, 5' and 6'-CH), 122.2 (d, 3'-CH), 30.1 (q, CH_3).

5-Amino-6-methylquinoline hydrochloride (237)



5-Amino-6-methylquinoline (0.0797 g, 0.50 mmol) was dissolved in diethyl ether (5 ml). To this was added ethereal hydrochloric acid (*ca.* 2 ml), whereupon a precipitate formed immediately. This was filtered and washed (hexane) to give the title compound as an orange solid (0.7165 g, 90 %), m.p. 256-258 °C. R_F 0.0 (ethyl acetate); δ_H (200 MHz, DMSO-d_6) 8.92 (d, ^3J 4.3 Hz, 1H, 2'-H), 8.19 (d, ^3J 8.2 Hz, 1H, 8'-H), 7.62 (d, ^3J 8.6 Hz, 1H, 4'-H), 7.48 (d, ^3J 8.2 Hz, 1H, 7'-H), 7.39 (dd, ^3J 8.6 Hz, ^3J 4.3 Hz, 1H, 3'-H), 4.19 (br s, 3H, NH_3), 2.40 (s, 3H, CH_3); δ_C (50 MHz, DMSO-d_6) 151.2 (d, 2'-CH), 149.8 (s, 10'-C), 141.0 (s, 9'-C), 135.0 (d, 8'-CH), 129.8 (d, 4'-CH), 120.5 (d, 7'-CH), 119.9 (d, 3'-CH), 119.2 (s, 6'-C), 107.1 (s, 5'-C).

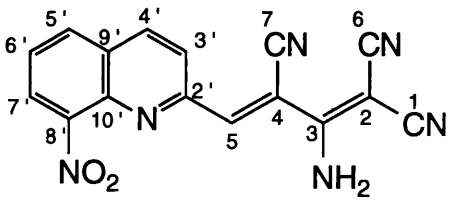
2-Cyano-3-[2-(8-nitroquinolyl)]propenonitrile (238)



8-Nitroquinoline-2-carbaldehyde (0.1200 g, 0.59 mmol) was treated with malononitrile (0.0413 g, 0.63 mmol) in ethanol (7 ml) and dilute piperidine solution (1 drop) using General Procedure B to give an orange solid after 3 h (0.1029 g, 69 %), m.p. 240-243 °C. R_F 0.25 (1:1 ethyl acetate/petroleum ether); (Found: C 63.01; H 2.39; N 22.45%; M^+, 250.0492. $C_{13}H_6N_4O_2$ requires C 62.40; H 2.42; N 22.39%; *M*, 250.0491); ν_{max}/cm^{-1} (KBr disc) 3072w (Ar-H),

2200w (CN), 1620m (extended conjugation to aromatic system), 1574s and 1520s (aromatic ring), 822s (3 adjacent Ar-H), 1510s (C-NO_2); δ_H (200 MHz, DMSO-d_6) 8.74 (2 x overlapping d, ^3J 8.4 Hz, ^3J 7.8 Hz, 2H, 4' and 7'-H), 8.39 (d, ^3J 8.0 Hz, 1H, 5'-H), 8.34 (s, 1H, 3-H), 7.97 (dd, ^3J 8.4 Hz, ^3J 8.0 Hz, 1H, 6'-H), 7.90 (d, ^3J 7.8 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 156.7 (d, 3-CH), 150.5 (s, 8'-C), 147.5 (s, 2'-C), 138.0 (d, 7'-CH), 136.0 (s, 10'-C), 131.1 (d, 4'-CH), 129.3 (s, 9'-C), 127.6 (d, 5'-CH), 125.1 (d, 6'-CH), 124.0 (d, 3'-CH), 114.1 and 111.9 (2 x s, 2 x CN), 87.3 (s, 2-C); *m/z* 250 (M^+, 71 %), 204 (M^+ - NO_2, -H), 174 (M^+ - [CH=C$(CN)_2$], + H).

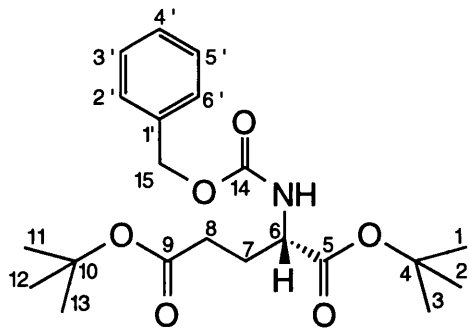
2,4-Dicyano-3-amino-5-[2-(8-nitroquinolyl)]penta-2,4-dienonitrile (239)



Using General Procedure C, 8-nitroquinoline-2-carbaldehyde (0.1164 g, 0.57 mmol) and 2-aminopropene-1,1,3-tricarbonitrile (0.0760 g, 0.57 mmol) were treated with ethanol (1 ml) to give an orange precipitate after 30 min which was filtered, dried and recrystallised from diethyl ether to give brown crystals (0.0556 g, 30 %), m.p. 100 °C (decomp.). R_F 0.11 (1:1 ethyl acetate/petroleum ether); (Found: C 60.82; H 2.61; N 26.23%; M^+, 316.0711. $C_{16}H_8N_6O_2$ requires C; 60.69 H 2.55; N 26.54 %; *M*, 316.0709); ν_{max}/cm^{-1} (KBr disc) 2367w (NH_2), 2217m (CN), 1645s (extended conjugation to aromatic ring), 1533s (aromatic ring), 1505s (C-NO_2), 847w (3 adjacent Ar-H), 797w (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 8.36 and 8.31 (2 x s, 2H, NH_2), 8.86-8.78 (overlapping d, ^3J 8.45 Hz, 1H, 7'-H and d, ^3J 7.8 Hz, 1H, 4'-H), 8.40 (d, ^3J 8.5 Hz, 1H, 5'-H), 8.29 (s, 1H, 5-H), 8.03 (dd, ^3J 8.5 Hz, ^3J 8.4 Hz, 1H, 6'-H), 7.91 (d, ^3J 7.8 Hz, 1H, 3'-H); δ_C (50 MHz, DMSO-d_6) 165.5 (s, 3-C), 164.5 (s, 8'-C), 151.4 (s, 2'-C), 149.1 (d, 5-CH), 147.8 (s, 10'-C), 138.9 (d, 7'-CH), 131.9 (d, 4'-CH), 129.0 (s, 9'-C), 128.0 (d, 5'-CH), 125.5 (d, 6'-CH), 124.6 (d, 3'-CH), 115.5, 114.4 and 113.7 (3 x s, 3 x CN), 109.9 (s, 4-C), 108.2 (s, 2-C); *m/z* 316 (M^+, 5 %), 251 (M^+ - [$C(CN)_2$], - H), 174 (M^+ - [CH=C(CN)-C(NH_2)=C$(CN)_2$)], + H), 143 ([CH=C(CN)-C(NH_2)=C$(CN)_2$)]$^+$), 128 ([quinoline]$^+$).

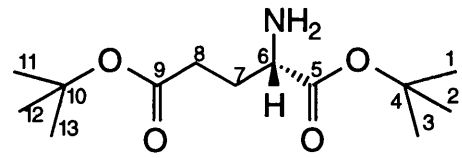
7.5 Experimental Procedures for Chapter 5

Di-^{t}butyl *N*-benzyloxycarbonyl-L-glutamate (254) [203, 205, 231]



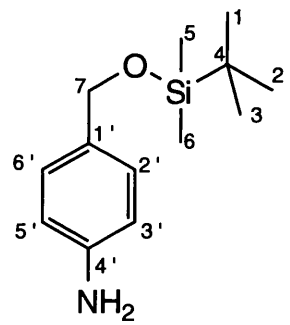
In a cold finger equipped with a rotovap tap was placed *N*-benzyloxycarbonyl-L-glutamic acid (4.2167 g, 14.99 mmol), dichloromethane (30 ml) and fuming sulfuric acid (0.3 ml, *ca.* 2 eq.). The mixture was cooled to -78 °C, and isobutene (*ca.* 20 ml) was added. The mixture was slowly warmed to 25 °C and was then sealed and left standing at this temperature for 96 h until starting material was no longer visible. Upon cooling to -78 °C, the vessel was opened and allowed to come to atmospheric pressure, then allowed to warm to room temperature. The contents were poured into water (100 ml) and brought to pH 7 by addition of aqueous $NaHCO_3$. Extraction with dichloromethane (2 x 50 ml) and subsequent drying ($MgSO_4$), filtration and concentration under reduced pressure of the combined organic extracts gave the product as a clear brown oil (4.0854 g, 69 %). R_F 0.72 (1:2 ethyl acetate/petroleum ether); (Found: C 64.36; H 7.70; N 3.53 %; M^+, 393.2158. $C_{21}H_{31}NO_6$ requires C 64.10; H 7.94; N 3.56 %; *M*, 393.2151); ν_{max}/cm^{-1} ($CHCl_3$) 3428w (CONH), 3030w (Ar-H), 1720s (ester and carbamate C=O), 766s and 698m (monosubstituted benzene); δ_H (200 MHz, DMSO-d_6) 8.60-8.50 (br s, 1H, NH), 7.29 (br s, 5H, 2', 3', 4', 5' and 6'-H), 5.11 (s, 2H, 15-H), 2.37-2.22 (m, 1H, 6-H), 1.41 (s, 18H, 2 x ^{t}Bu), 1.06-0.83 (m, 4H, 7 and 8-H); δ_C (50 MHz, DMSO-d_6) 171.8 and 171.2 (2 x s, 5 and 9 C), 156.0 (s, 14-C), 145.0 (s, 1'-C), 128.3, 128.0 and 127.8 (3 x d, 2', 3', 4', 5' and 6'-CH), 53.8 (d, 6-CH), 32.0, 31.9, 31.8, 31.7, 31.6 and 31.5 (6 x q, 1, 2, 3, 11, 12, 13-CH_3), 27.9 and 27.8 (2 x s, 2 x $C(CH_3)_3$); *m/z* 393 (M^+, 48 %), 292 (M^+ - $CO_2{}^t$Bu), 286 (M^+ - $PhCH_2$), 258 (M^+ - $PhCH_2OCO$), 192 (M^+ - 2 x $CO_2{}^t$Bu), 135 ($PhCH_2OCO^+$), 91 ($PhCH_2^+$), 58 (CH_2OCO^+).

Di-^{t}butyl L-glutamate (255)[203, 205, 231]



A 3-necked round bottom flask, charged with di-^{t}butyl *N*-benzyloxycarbonyl-L-glutamate (4.0854 g, 10 mmol), Methanol (10 ml) and a catalytic amount of palladium on carbon (*ca.* 20 mg) was placed under an atmosphere of hydrogen and stirred at 25 °C for 24 h. This was then filtered through Celite, washed with methanol and concentrated under reduced pressure to yield the title compound as a yellow oil (1.9062 g, 78 %). R_F 0.28 (1:4 ethyl acetate/petroleum ether); δ_H (200 MHz, DMSO-d_6) [lit.: 200 MHz, $CDCl_3$][203] 3.20-3.15 [3.33] (br, 1H, 6-H), 1.60-2.10 [1.60-2.10] (br m, 4H, 7 and 8-H_2), 1.28 [1.47 and 1.44] (s, 18H, 2 x ^{t}Bu); δ_C (50 MHz, DMSO-d_6) 175.5 and 173.0 (2 x s, 2 x C=O), 81.0 and 80.3 (2 x s, 4-C and 10-C), 54.0 (d, 6-CH), 32.1 and 22.8 (2 x t, 7-CH_2 and 8-CH_2), 30.4 and 27.6 (2 x q, 2 x CH_3).

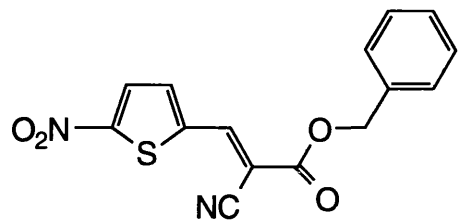
4-Hydroxymethylaniline tertiarybutyldimethylsilyl ether (256)



Prepared using the method of Corey and Venkateswarlu.[232]A flask was charged with 4-aminobenzyl alcohol (2.6361 g, 21.40 mmol) and imidazole (2.1378 g, 31.40 mmol). The system was placed under a nitrogen atmosphere and the solids were dissolved in distilled dichloromethane (7 ml). Addition of a solution of tertiarybutyldimethylsilylchloride (3.2368 g, 21.47 mmol) in distilled dichloromethane (5 ml) was added, and the reaction was stirred for 10 min until TLC showed the absence of starting materials. The mixture was then washed with water (3 x 30 ml), filtered and concentrated to give a pale yellow oil (7.5669 g). Purification by distillation gave a colourless oil (4.1998 g, 20.06 mmol, 94 %), (Found: C 65.58; H 9.71; N 5.95 %; M^+,237.1568. $C_{13}H_{23}NOSi$ requires C 65.77; H 9.76; N 5.90 %;M, 237.1549); R_F 0.50 (1:2 EtOAc/pet. ether); ν_{max}/cm^{-1} ($CHCl_3$) 3468 and 3390 (NH), 3015 (Ar-H), 2985

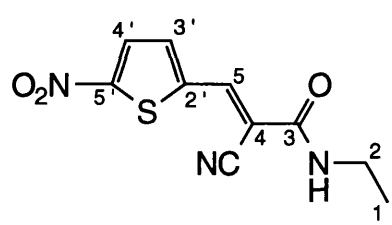
and 2965 (CH_3), 2900 (CH), 1630 (NH), 1260 (C-O), 1100 (Si-O); δ_H (200 MHz, $CDCl_3$) 7.12 (m, 2H, 3'-H and 5'-H), 6.62 (m, 2H, 2'-H and 6'-H), 3.54 (br s, 2 H, NH_2), 4.58 (s, 2H, CH_2), 0.90 (s, 9 H, ^{t}Bu), 0.05 (s, 6H, $Si(CH_3)_2$); δ_C (50 MHz, $CDCl_3$) 145.8 (s, 1'-C), 131.0 (s, 4'-C), 127.7 (d, 2'-C and 6'-C), 115.2 (d, CH, 3'-C and 5'-C), 65.2 (t, CH_2), 25.7 (q, $C(CH_3)_3$), 18.6 (s, $C(CH_3)_3$), -5.0 (q, $Si(CH_3)_2$); m/z 237 (M^+, 15 %), 222 (M^+ -CH_3), 180 (M^+ -^{t}Bu), 106 (M^+ -OTBDMS).

Attempted synthesis of Benzyl 2-cyano-3-[2-(5-nitrothienyl)]propenoate (260)[209]



A flame-dried 3-necked round bottom flask equipped with a nitrogen balloon was charged with a slurry of 2-cyano-3-[2-(5-nitrothienyl)] propenoic acid (0.1997 g, 0.88 mmol) in dry DMF (5 ml). To this was added carbonyl diimidazole (0.1472 g, 0.90 mmol) in dry DMF (5 ml). An immediate colour change giving a brown solution was observed. The mixture was stirred at room temperature for 1.5 h, before the addition of benzyl alcohol (0.09 ml, 0.87 mmol) in DBU (0.07 ml, 0.46 mmol). Stirring was continued at 25 °C for 43 h, then the mixture was washed with 10 % HCl (10 ml), 10 % $NaHCO_3$ (10 ml) and ethyl acetate (3 x 50 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to a beige solid. NMR spectroscopy revealed the presence of starting materials.

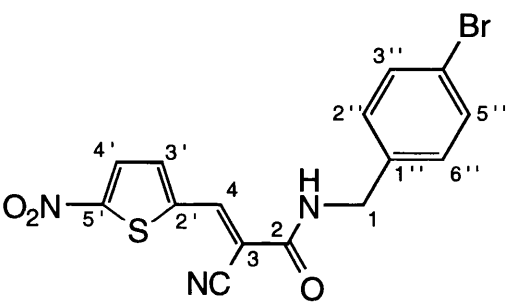
***N*-Ethyl-2-cyano-3-[2-(5-nitrothienyl)] propenamide (264)**



2-Cyano-3-[2-(5-nitrothienyl)]propenoic acid **(141)** (0.3913 g, 2.03 mmol) and thionyl chloride (2 ml, 10.30 mmol) were heated at reflux temperature for 2.5 h.

The excess thionyl chloride was removed *in vacuo* to give a green solid, which was dissolved in dry THF (100 ml), and the solution was flushed with nitrogen and cooled to 0 °C. To this was added ethylamine (0.25 ml, 3.74 mmol). Precipitation of a beige solid was observed after stirring at 0 °C for 2 h. The solvent was removed *in vacuo*, and the residue was recrystallised from ethanol to give beige crystals (0.0643 g, 13 %), m.p. 190-192 °C. R_F 0.0 (1:1 ethyl acetate/petroleum ether); (Found: C 47.46; H 3.64; N 16.78 %; M^+, 251.0389. $C_{10}H_9N_3O_3S$ requires C 47.77; H 3.61; N 16.78 %; *M*, 251.0365); ν_{max}/cm^{-1} (KBr disc) 3440m and 1670s (-CONHR), 2220w (-CN), 3106s (Ar-H), 1676m (external conjugation to aromatic system), 1534s (C-NO_2), 1472m (aromatic C=C); δ_H (200 MHz, DMSO-d_6) 8.66 (br. s, 1H, NH), 8.44 (s, 1H, 5-H), 8.26 (d, ^3J 4.4 Hz, 1H, 3'-H), 7.95 (d, ^3J 4.4 Hz, 1H, 2'-H), 3.42 (q, ^3J 7.0 Hz, 2H, 2-H_2), 1.05 (t, ^3J 7.0 Hz, 3H, 1-H_3); δ_C (50 MHz, DMSO-d_6) 162.1 (s, C=O), 153.7 (s, 4'-C), 142.7 (d, 3'-CH), 142.0 (s, 1'-C), 135.8 (d, 2'-CH), 129.8 (d, 5-CH), 117.1 (s, CN), 110.0 (s, 4-C), 40.4 (d, 2-CH), 11.2 (s, 1-C); *m/z* 251 (M^+, 3 %), 224 (M^+ - C_2H_3), 208 (M^+ - NC_2H_5), 179 (M^+ - $CONHC_2H_5$,+ H).

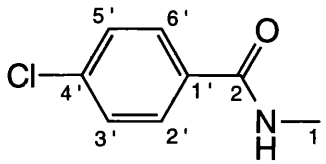
_N_-4-Bromobenzyl-2-cyano-3-[2-(5-nitrothienyl)]-propenamide (265)



To a solution of 2-cyano-3-[2-(5-nitrothienyl)]propenoyl chloride (0.4630 g, 2.19 mmol) in 10 ml dry THF was added 4-bromoaniline (0.3811 g, 2.20 mmol). The mixture was stirred at room temperature for 12 h to form a red precipitate. The solution was washed with water (25 ml) and ethyl acetate (2 x 25 ml) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to a brown crystalline solid (0.5123 g, 69 %), m.p. 169-170 °C. R_F 0.46 (1:1 ethyl acetate/petroleum ether); (Found: C 45.99; H 2.54; N 10.85 %; M^+, 390.9623. $C_{15}H_{10}N_3O_3SBr$ requires C 45.93; H 2.57; N 10.71 %; *M*, 390.9626); ν_{max}/cm^{-1} (KBr disc) 3448m (amide, NH stretch), 2210w (CN), 1700w (C=O), 1632s (extended conjugation to aromatic system), 1590s (amide N-H), 828m (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 8.66 (s, 1H, 4-H), 8.29 (d, ^3J 4.4 Hz, 1H, 4'-H), 8.06 (d, ^3J 4.4 Hz, 3'-H), 7.45 (d, ^3J 8.7 Hz, 2H, 3"-H and 5"-H), 7.00 (d, 2H, ^3J 8.7 Hz, 2"-H and 6"-H), 6.80-6.60 (br s, 1H, NH), 4.31 (br s, 2H, 1-

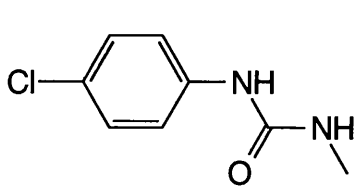
H_2); δ_C (50 MHz, DMSO-d_6) 162.4 (s, C=O), 154.9 (s, 4'-C), 145.9 (d, 3'-CH), 140.7 (s, 1'-C), 139.6 (s, 1"-C), 137.6 (d, 3'-CH), 132.1 (d, 3"-CH and 5"-CH), 129.8 (d, 4-CH), 121.0 (d, 6"-CH and 2"-CH), 115.8 (s, CN), 113.8 (s, 4"-C), 105.0 (s, 3-C), 50.5 (t, 1-CH_2); *m/z* 391 (M^+, 5 %), 264 (M^+ - Br, - NO_2, -H), 262 (M^+ - nitrothiophene), 237 (M^+ - PhBr), 184 (M^+ - [HC=C(CN)CONHPh).

4-Chloro-*N*-methylbenzamide (270)



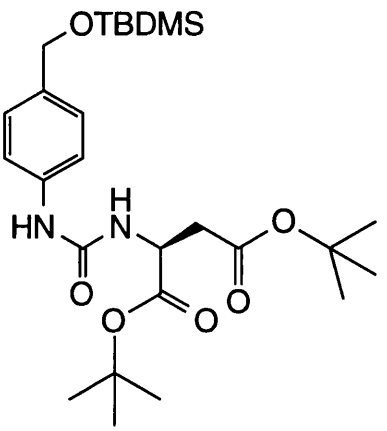
4-Chlorobenzoic acid (0.8194 g, 5.2 mmol) was reacted with thionyl chloride (0.39 ml, 5.3 mmol) at 0 °C using the method of Munch-Petersen.[233] After 4 h, TLC showed the absence of starting materials. A 40 % w/v solution of methylamine in industrial methylated spirits (0.5 ml) was then added, and stirring was continued at 0 °C for 1 h until formation of a white precipitate. This was filtered, washed (hexane) and dried (diethyl ether) to give the title compound (0.6924 g, 78 %), m.p. 176-176 °C, R_F 0.42 (1:1 ethyl acetate/petroleum ether). δ_H (90 MHz, DMSO-d_6) 8.1 (br s, 1H, NH), 7.6 (d, ^3J 6 Hz, 2H, 2' and 6'-H), 7.4 (d, ^3J 6 Hz, 2H, 3' and 5'-H), 3.9 (s, 3H, CH_3).

Attempted synthesis of *N*-methyl-*N'*-(4-chlorophenyl) urea (271)



4-Chlorobenzoic acid (1.6074 g, 10.3 mmol) was reacted with thionyl chloride (3 ml, 41.1 mmol) in CCl_4 (10 ml) at 0 °C for 4 h using the same procedure as above. To this was added tetrabutylammonium iodide (*ca* 10 mg), followed by the slow addition of sodium azide (1.0972 g, 16.8 mmol) in water (10 ml) to immediately give a pale pink solution. This was gently heated for 4 h, then cooled to -78 °C before the addition of a 40 % w/v solution of methylamine in industrial methylated spirits (0.8 ml). Upon stirring at -78 °C for 1.5 h, a white precipitate was observed, which was filtered, washed and dried. NMR spectroscopy showed this to be 4-chloro-*N*-methylbenzamide **(270)**.

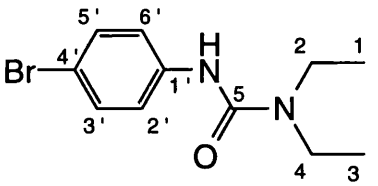
Attempted syntheses of *N*-(di-^{t}butyl-L-glutamate)carbonylamino-4-hydroxymethylbenzene ^{t}butyldimethylsilyl ether (278)



Method 1. In a flame dried 3-necked round bottom flask equipped with a nitrogen balloon was placed a solution of 4-aminobenzyl alcohol (0.2462 g, 1.99 mmol) in dry ethanol (5 ml). To this was slowly added carbonyldiimidazole (0.3244 g, 2.00 mmol) in dry ethanol (3 ml). The reaction was stirred at 25 °C until the precipitation of a white solid, whereupon di-^{t}butyl-L-glutamate (0.4962 g, 2.00 mmol) and dibutyltin diacetate (5 drops from a pasteur pipette) were added. The mixture was heated to 50 °C for 72 h until TLC showed the absence of the L-glutamate, and the presence of a yellow precipitate was observed. Filtration and drying (diethyl ether) gave a beige crystalline solid, which was shown by NMR spectroscopy to be a mixture of starting materials.

Method 2. To a flame-dried 3 necked flask, equipped with a water condenser and nitrogen balloon was added carbonyl diimidazole (0.1794 g, 1.11 mmol) in dry THF (5 ml) and di-^{t}butyl-L-glutamate (0.3109 g, 1.19 mmol) in dry THF (5 ml). The mixture was heated to 50 °C for 26.5 h. 4-Aminobenzyl alcohol (0.1386 g, 1.15 mmol) in THF (5 ml) was added, followed by 4-dimethylamino pyridine (0.2002 g, 1.64 mmol) in dry THF (5 ml). The reaction was heated at reflux temperature for 24 h. Upon cooling to room temperature, the solvent was removed under reduced pressure, and a silica plug of the mixture was prepared. Column chromatography (silica, 1:1 ethyl acetate/petroleum ether) resulted in the complete recovery of starting materials.

1,1-Diethyl-2-(4-bromophenyl) urea (279)[213]



To a flame-dried 3-necked round-bottom flask equipped with a nitrogen balloon and water condenser was added 4-bromoaniline (3.5652 g, 20.7 mmol) in dry THF (10 ml) and carbonyldiimidazole (3.3649 g, 20.6 mmol) in dry THF (50 ml). The mixture was gently heated, then stirred at 25 °C for 46 h. This was followed by addition of diethylamine (2.15 ml, 20.8 mmol) and gentle heating at 50 °C for 1 h. The mixture was concentrated *in vacuo* to a brown solid, which was redissolved in 1:1 methanol/water and recrystallised to give the title compound as large brown crystals (3.7216 g, 66 %), m.p. 280 °C (decomp.), R_F 0.48. (1:1 ethyl acetate/petroleum ether); (Found: C 48.91; H 5.64; N 10.42 %; M^+, 271.0354. $C_{11}H_{15}N_2OBr$ requires C 48.72; H 5.58; N 10.33 %; *M*, 270.0368); ν_{max}/cm^{-1} (KBr disc) 3264m (N-H, secondary amide), 3060w (Ar-H), 1633m (C=O), 1600s and 1514m (benzene ring), 835s (2 adjacent Ar-H); δ_H (200 MHz, DMSO-d_6) 12.21 (v br, 1H, NH), 7.81 (br, 2H, 3'-H and 5'-H), 7.13 (br, 2H, 2'-H and 6'-H), 3.36 (q, ^3J 7.1 Hz, 4H, 2-H_2 and 4 H_2), 1.11 (t, ^3J 7.1 Hz, 6H, 1-H_3 and 3-H_3); δ_C (50 MHz, DMSO-d_6) 154.6 (s, C=O), 140.3 (s, 1'-C), 131.6 and 131.4 (2 x d, 3' and 5'-CH), 122.1 and 121.8 (2 x d, 2' and 6'-CH), 113.4 (s, 4'-C), 42.4 and 40.7 (2 x t, 2 and 4-CH_2), 13.8 and 12.9 (2 x q, 1 and 3-CH_3); *m/z* 271 (M^+, 38 %), 242 (M^+ - CH_2CH_3), 198 (M^+ - NEt_2, -H), 191 (M^+ - Br), 171 (M^+ - $CONEt_2$), 143 (M^+ - $NHCONEt_2$, - H).

7.6 Experimental Procedures for Chapter 6

Antiproliferative Assays

1. MCF-7 breast adenocarcinoma cells. MCF-7 cells (American Type Culture Collection, Rockville, MD) were maintained in RPMI 1640 supplemented with 2 mM glutamine and 10 % (v/v) foetal calf serum (all obtained from Gibco BRL, Paisley, UK). [^{3}H]-Thymidine (sp. act. 5 Ci / mmol; Amersham Ltd, Amersham, UK) incorporation was used as an indicator of DNA synthesis. Cells were plated at a concentration of 1 x 10^3 / well in 96 well plates and grown in a humidified atmosphere of 5 % (v/v) CO_2:air at 37 °C for 72 hours. A range of inhibitor concentrations (0.001-500 μM) was then added and after 24 hours [^{3}H]-thymidine (0.25 μCi / well) was added to each well for a one hour pulse prior to harvesting. The medium was removed and the cell sheet was washed twice with ice-cold phosphate-buffered saline. The cells were harvested using trypsin / EDTA (0.25 % / 1 mM) and transferred onto filter mats using an LKB Cell Harvester. They were then washed three times with distilled water, allowed to dry and 10 ml Ecoscint A (National Diagnostics) was added. The radioactivity was determined using a Packard Liquid Scintillation Analyser.

2. HER14 Fibroblasts. [^{3}H]-Thymidine (sp. act. 5 Ci / mmol; Amersham Ltd, Amersham, UK) incorporation was used as an indicator of DNA synthesis. HER14 cells were plated at a concentration of 1 x 10^3 / well in 96 well plates and grown in a humidified atmosphere of 2 % (v/v) CO_2:air at 37 °C to confluence (72 hours). The medium was then replaced with fresh medium supplemented with 0.1 % (v/v) DCS. After a further 48 hours a range of inhibitor concentrations (0.01 μM - 50 μM) was added in either medium containing 10 % (v/v) DCS or medium containing 0.1 % (v/v) DCS plus 1 % bovine serum albumin and 10 ng / ml EGF. [^{3}H]-Thymidine (0.25 μCi / well) incorporation was measured over a 16 hour exposure to the drugs. The medium was then removed and the cell sheet was washed twice with ice-cold phosphate buffered saline. The cells were harvested using trypsin / EDTA (0.25 % / 1 mM) and transferred onto filter mats using a LKB Cell Harvester. They were then washed three times with distilled water, allowed to dry and 10 ml Ecoscint A (National Diagnostics) added. The radioactivity was determined using a Packard Liquid Scintillation Analyser.

Measurement of EGF Receptor Kinase Activity

The membrane fractions (0.5 μg protein) were incubated with kinase buffer [45 mM HEPES (pH 7.4), 100 μM sodium orthovanadate, 0.05 % Triton X-100, 2 % glycerol], 25 mM $MgCl_2$, 2 mg / ml poly(Glu, Ala, Tyr) and inhibitors at a range of concentrations. Incubations were carried out in the presence or absence of 0.25 μM EGF. The inhibitors were prepared as stock solutions in DMSO and diluted in distilled water. The final concentration of DMSO in the incubations (1 %) had no effect on the reaction rate. The reaction was initiated by the addition of [γ-^{32}P]-ATP (0.5 μCi, 20 μM, sp. act. 3000 Ci / mmol; Amersham Ltd, Amersham, UK). After 20 min at 30 °C, the reaction was stopped by the addition of 1 mM ATP on ice. The products of the reaction were spotted onto Whatman 3MM paper and the peptide precipitated with 10 % trichloroacetic acid containing 0.01 M sodium pyrophosphate. The filters were then washed extensively with the trichloroacetic acid solution, dried and transferred to scintillation vials. Ten millilitres of Ecoscint A (National Diagnostics) were added and the radioactivity determined using a Packard Liquid Scintillation Analyser.

Cytotoxicity Assay

This was carried out using the method of Plumb and co-workers.[234] Squamous cell carcinoma cells were seeded at the appropriate cell density in 96 well plates and grown for 72 hours in a humidified atmosphere of 2 % (v/v) CO_2:air at 37 °C before addition of a range of inhibitor concentrations in 200 μl of medium. Four replicate wells were used for each drug concentration. Cells alone were used as a control and medium alone was used as a blank. After 24 hours the inhibitors were removed and fresh medium added. The medium was replaced every 24 hours for a further three days allowing the cells to pass through two to three doublings. This allows the assay to be used as a measure of cell kill. On the third day 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) (5 μg / ml) was added to each well. The plates were then incubated in the dark at 37 °C for 4 hours. Medium and MTT were then removed and the formazan crystals (formed following reduction of MTT by live cells) were dissolved in 200 μl dimethylsulfoxide and 25 μl Sorensen's glycine buffer (0.1 M glycine plus 0.1 M NaCl adjusted to pH 10.5 with 0.1 M NaOH) then added. The absorbance was read at 570 nm in an enzyme-linked immunosorbent assay plate reader (Model 2550; Bio-Rad Laboratories). Log-concentration response curves were generated, from which IC_{50} values were determined as the dose to inhibit MTT absorbance by 50 %.

Alkaline Elution

DNA single strand breaks were measured by alkaline filter elution as previously described.[235] In brief DNA from control cells and cells treated for 2 hours with inhibitor was labelled with 0.03 μCi / ml [^{14}C]-thymidine (sp. act. 51 mCi / mmol, Amersham Ltd, Amersham, UK) for 24 hours. Cells labelled with 0.17 μCi / ml [^{3}H]-thymidine (sp. act. 5 Ci / mmol, Amersham Ltd, Amersham, UK) and γ-irradiated were used as an internal control. Cells were loaded onto 2 μm pore size polycarbonate filters and lysed. DNA was eluted at pH 12.2 with 0.1 M tetrapropylammonium hydroxide containing 0.1 % sodium dodecyl sulphate and 0.02 M EDTA over a 15 hour period at an elution rate of 0.011 ml / min. The radioactivity was determined in each fraction taken at 90 minute intervals and the results expressed as fraction of [^{14}C] retained versus fraction [^{3}H] (internal standard) retained.

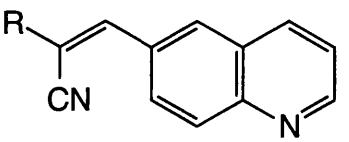
Cyclic Voltammetry

CVs were recorded using an EG & G Parc model 175 Universal Programmer, model 173 Potentiostat/Galvanostat and model 176 Current Follower to generate the signal, and were plotted using a Lloyd PL3 Plotter. Compounds were dissolved at 1 mM concentration in dry, double-distilled DMSO which contained tetraethylammonium tetrafluoroborate at 100 mM concentration. The DMSO was stored over 4Å molecular sieves under a nitrogen atmosphere for a maximum of 12 hours[236] prior to use (storage for longer times generally resulted in additional signals in the CVs corresponding to reduction and oxidation of water which had been acquired by the solvent), and the solvent was filtered using Millex PTFE-F syringe filters (0.5 μM pore size; Aldrich) to remove residues of the sieves. The CVs were measured using a 0.5 cm^2 gold working electrode, a platinum gauze counter electrode and a standard Ag/$AgNO_3$ reference electrode. The gold electrode was polished using Hyprez Five-Star Diamond Compound (Engis Ltd., England) before the reduction potential of each compound was measured. All samples were scanned between -2.5 V and +0.5 V , with scans commencing and terminating at 0 V.

Appendix: Additional Biological Data

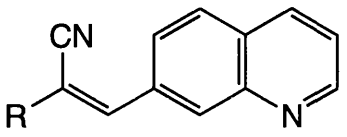
The biological evaluation of the quinolyl tyrphostins reported in the thesis has recently been completed, and the author wishes to record these values here. As can be seen, there are still a number of reasonably active compounds in these series, and, as predicted, the 7-substituted quinolines proved to be the most active, although a number of these compounds displayed no activity in the MCF-7ADR cytotoxicity assay. There was not quite such a dramatic difference in activities across these series compared to the case of the 2, 3 and 4-substituted compounds synthesised by Lear and McKeown.

The data does not give any clear evidence for the SAR of these compounds, and further biological evaluation will be necessary before any conclusions may be drawn.



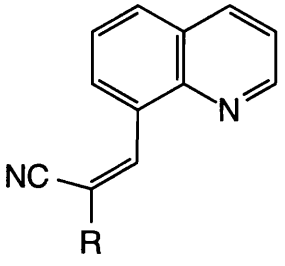
		IC_{50} (μM)	
Compound Number	**R**	**MCF-7¶**	**MCF-7ADR¶**
(201)	CN	>98	n.t.†
(202)	$C(NH_2)=C(CN)_2$	52	96
(203)	CO_2Me	47	56
(204)	CO_2Et	65	44
(205)	$CO_2{}^nBu$	59	106
(206)	CO_2TEG-OMe	n.t.†	n.t.†
(207)	$CONH_2$	65	91
(208)	$CSNH_2$	49	89

¶ Tested by Dr Alan McGown, Paterson Institute.
† Not toxic.



		IC_{50} (μM)	
Compound Number	**R**	**MCF-7¶**	**MCF-7ADR¶**
(209)	CN	17	23
(210)	$C(NH_2)=C(CN)_2$	46	75
(211)	CO_2Me	63	n.t.†
(212)	CO_2Et	2	n.t.†
(213)	$CO_2{}^nBu$	28	n.t.†
(214)	CO_2TEG-OMe	13	70
(215)	$CONH_2$	81	2
(216)	$CSNH_2$	68	79

¶ Tested by Dr Alan McGown, Paterson Institute.
† Not toxic.



		IC_{50} (μM)	
Compound Number	**R**	**MCF-7¶**	**MCF-7ADR¶**
(217)	CN	n.t.†	n.t.†
(218)	$C(NH_2)=C(CN)_2$	57	75
(219)	CO_2Me	169	n.t.†
(220)	CO_2Et	n.t.†	n.t.†
(221)	$CO_2{}^nBu$	146	n.t.†
(222)	$CONH_2$	n.t.†	n.t.†
(223)	$CSNH_2$	19	136

¶ Tested by Dr Alan McGown, Paterson Institute.
† Not toxic.

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