Synthesis of Polyamine Analogues as Antimalarial and Antifungal Agents.

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

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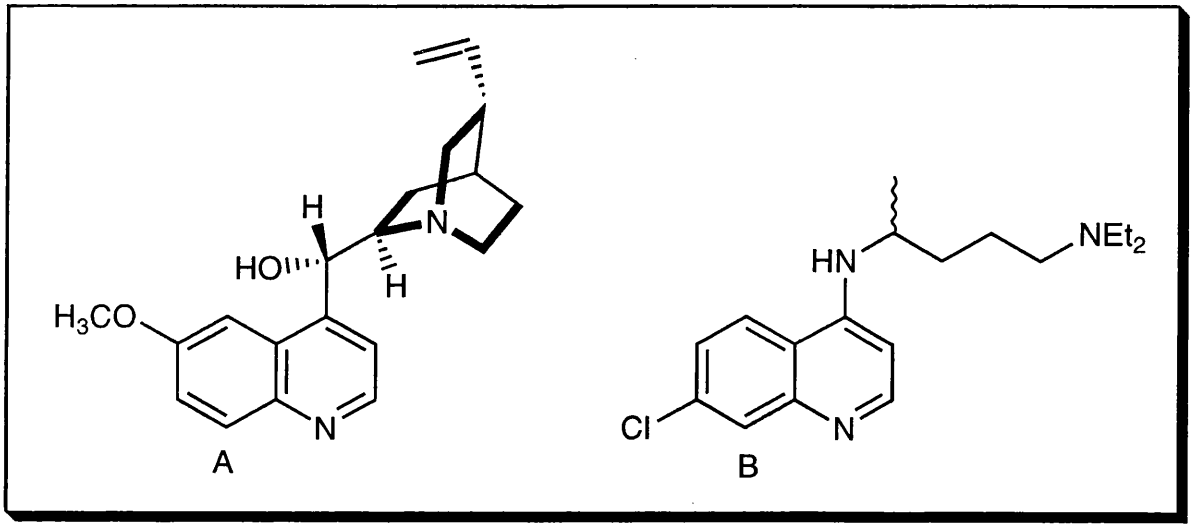
Summary

This research project was concerned with two areas, the synthesis of new antimalarial agents and the synthesis of new antifungal agents.

Malaria is spread to humans by parasites of the *Plasmodium* genus. The vector is the female *Anopheles* mosquito. It is the most important parasitic disease affecting mankind and kills about two million people every year. The parasites have developed resistance to the majority of the available treatments, including the naturally occurring compound quinine **(A)** and the synthetic drug chloroquine **(B)**. Hence there is an urgent need for new antimalarial agents.



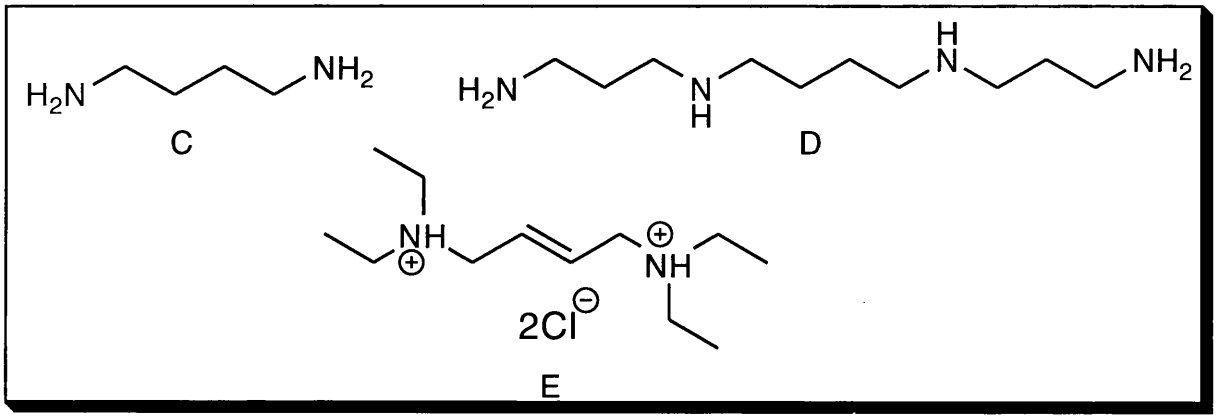
A B

The resistance of fungi which grow on important food crops to available fungicides has increased the need to find new antifungal agents.

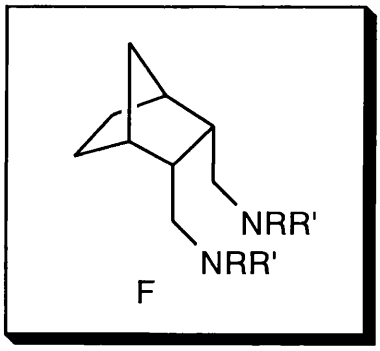
Polyamines such as putrescine **(C)** and spermine **(D)** are widespread in nature. They are important in cell development and are vital for normal cell growth. The study of these compounds, particularly their synthesis and biosynthetic enzymes, has stimulated the development of polyamines to treat a number of diseases including malaria and plant fungal diseases.

Previous workers within our group had synthesised a number of acyclic putrescine analogues such as **(E)** which was found to have

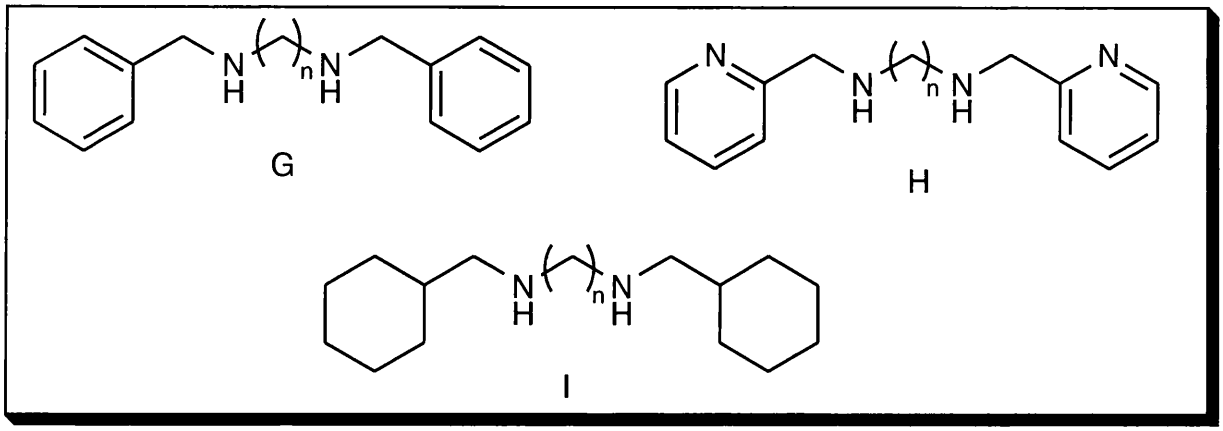
promising antimalarial and antifungal activity. We wanted to investigate the properties of a number of bicyclic putrescine analogues.



In this work we have developed a route to prepare novel symmetrical and unsymmetrical putrescine analogues of the general structure **(F)**.



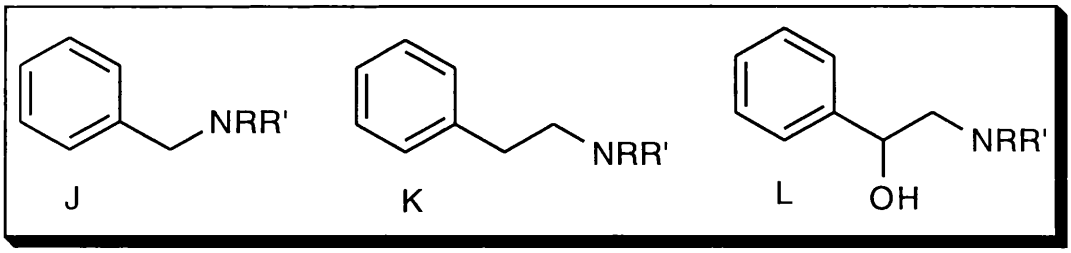
We have also synthesised a number of polyamine analogues **(G)**, **(H)** and **(I)**, varying the distance between the nitrogen atoms.



Antimalarial testing was carried out by Prof. Steven Phillips and Ms. Fiona McMonagle in the Division of Infection and Immunity, University of Glasgow. Antifungal tests were performed by Dr Dale Walters at the Scottish Agricultural College, Auchincruive.

Measurements of the *in vitro* antimalarial activity of **(G)** showed an improvement as the distance between the nitrogen atoms increased. The most active had activity comparable to chloroquine **(B)**. Unfortunately they showed no *in vivo* activity. Analogues **(H)** showed good *in vitro* activity which improved as the distance between the nitrogen atoms decreased. The most active compounds **(I)** showed similar activity to the corresponding analogues **(G)**. The *in vitro* antifungal results followed a similar pattern. The most active compounds substantially inhibit fungal growth, although not all the compounds have yet been evaluated.

A number of compounds containing pyridyl rings were prepared with general structures **(J)**, **(K)** and **(L)**. In each case those compounds bearing benzyl or cyclohexylmethyl substituents displayed the greatest antimalarial activity.



In vitro antimalarial activity increased from **(J)** to **(K)** but the results we have so far for **(L)** are disappointing. The most active analogue **(K)** *in vitro* was tested for *in vivo* activity, but was found to be inactive.

The *in vitro* antifungfal testing results for **(J)** were disappointing. None of the compounds showed appreciable activity at 0.1mM. We are awaiting the results for compounds **(K)** and **(L)**.

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Abbreviations

ADC	Arginine decarboxylase
AIDS	Acquired immune deficiency syndrome
CI	Chemical ionisation
DAO	Diamine Oxidase
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCU	*N,N'*-Dicyclohexylurea
DCM	Dichloromethane
DFMO	α–Difluoromethylornithine
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminium Hydride
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
EI	Electron impact
FPIX	Ferriprotoporphyrin IX
HEPT	Hexaethylphosphorus triamide
HMPT	Hexamethylphosphorus triamide
HOMO	Highest occupied molecular orbital
IC_2BCl	β-Chlorodiisopinocamphenylborane
IR	Infra-red
K_m	Michaelis Menten constant
LUMO	Lowest unoccupied molecular orbital
LEC	Lowest effective concentration
MDR	multidrug resistance
MS	Mass spectroscopy
NBS	*N*-bromosuccinimide
NMR	Nuclear magnetic resonance
ODC	Ornithine decarboxylase
PAO	Polyamine Oxidase
PLP	Pyridyl 5'-phosphate
RED-AL®	Sodium bis(2-methoxyethoxy)aluminium hydride
RNA	Ribonucleic acid
rt	Room temperature
THF	Tetrahydrofuran
V_{max}	Maximum rate

1

Malaria

1.1 A Brief History of Important Dates and Important People

Malaria is still the most important parasitic disease affecting mankind. Its symptoms have been known since time immemorial, although it has only been in the last 100 or so years that the actual causes have become well understood.

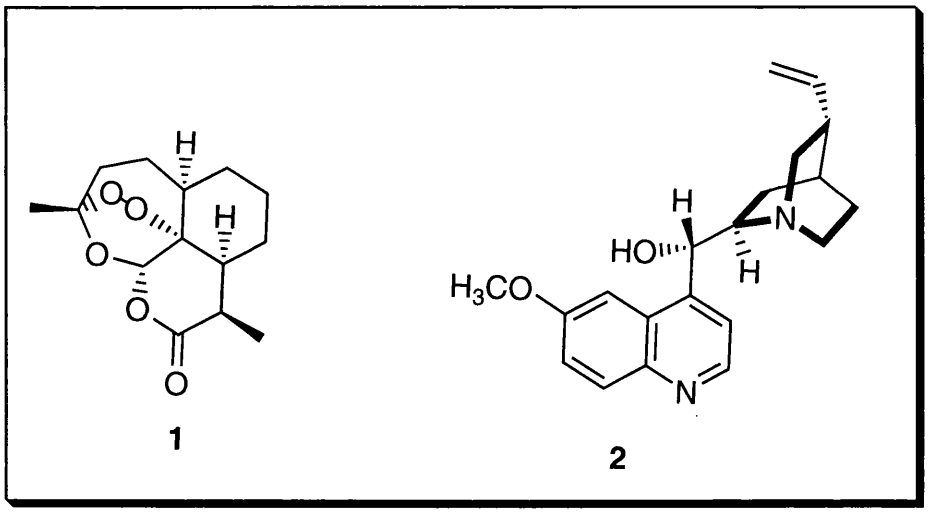
One hundred years ago it was established that malaria is caused by *Plasmodium* parasites with Anopheline mosquitoes as the vectors. Malaria parasites have been found in fossils of mosquitoes up to 30 million years old. The mosquito parasites are highly specific with man as the only natural vertebrate host. This specificity points to a long and adaptive relationship with man.[1]

Although in earlier times the causes of malaria were not known a number of treatments and remedies have been recorded to cure its fever-like symptoms and to prevent mosquito bites. It was thought that malaria was due to miasma (bad air or "mal aria") which came from swamps. Herodotus (485-425 BC) noted that in the swamps of Egypt, some people slept in tower-like structures out of the reach of mosquitoes, and others slept under nets.

Ancient remedies have proved remarkably effective and are still of immense value today.

An infusion of quighao has been used in China for at least 2000 years. Its active ingredient, qinghaosu (artemesinin **1**), was only recently identified.

The bark of the cinchona tree (*Cinchona ledgeriana*) was used by native Peruvians well before the 15th century as a treatment for recurring fevers. The alkaloid quinine **(2)** originates from the cinchona tree and has been and continues to be used to treat and cure millions of malaria sufferers, was isolated in 1820 by the French pharmacists Pelletier and Caventou.[2]



It was not until 1880 that the French army surgeon Laveran discovered in the blood of a patient the malaria parasite and suggested it as the causative agent. He observed some hair-like projections (flagella) which developed from a pigmented spherical body in a wet smear of fresh blood from a malaria patient. He was certain that this was from a living organism. A parasite was in the human blood. His work was greeted with scepticism for a number of years until the phenomenon was also observed by the Italians Marchiafava and Celli. Laveran was awarded the Nobel prize for medicine in 1907 for his pioneering work.[3]

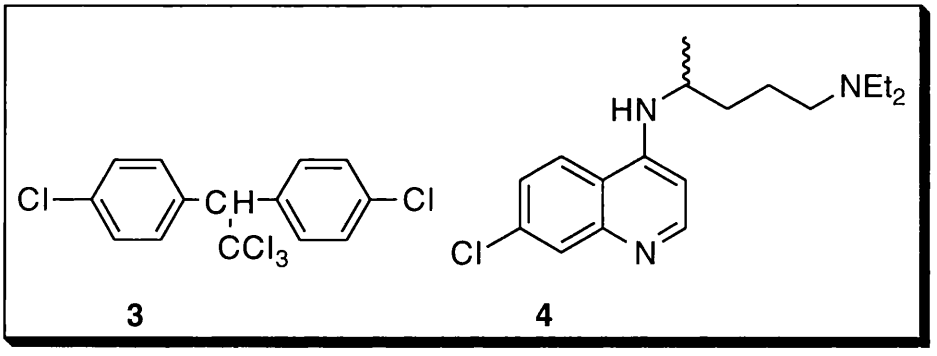
Almost exactly 100 years ago another army surgeon, this time the Scot Ronald Ross carried out a series of experiments that finally proved that the mosquito was the vector of malaria. On the 20th August 1897 while observing some Anopheles mosquitoes that had fed on a patient's blood he observed a pigmented malaria cyst on the stomach wall of the insect. A day later Ross dissected the last mosquito in the batch under study and found the same pigmented cells "now much larger". Ross had seen the malaria parasites of man and they were undergoing development in a mosquito.[3]

The discoveries of Lavernan and Ross led to the first systematic malaria control measures. Initial controls involved improved "sanitation". It was known that mosquitoes bred in ponds, marshes, swamps and standing water and that better drainage of these areas could provide outstanding results by reducing the vector population.[4] For instance the efforts of William Crawford Gorgas (1854-1920) saved the United States government some 80 million dollars in the building of the Panama canal. The malaria rate in the canal area showed a remarkable decline under the leadership of Gorgas. The number of canal workers admitted to

hospital suffering from malaria fell from a yearly rate of 821 per 1000 in 1906 to 76 per 1000 in 1913.[4]

In the second half of the twentieth century the treatments available for malaria increased greatly. The remarkable insecticidal properties of dichlorodiphenyltrichloroethane (DDT, **3**) were discovered by Paul Muller in 1939.[3] DDT was originally synthesised by Othmar Zeidler in 1874 but its effects on insects were not noted. After its discovery DDT was used in vast quantities and to great effect around the world as a general purpose insecticide. However by 1951 some species of *Anopheles* vectors had become resistant and when the major toxicity problems associated with DDT were discovered its widespread use was discontinued.[3,4] It is still used in Africa but banned in the U.S.A. and Europe.

Among a number of safe synthetic drugs developed around the time of the second world war was chloroquine (**4**).[3] It seemed to be free from the problem of resistance and it became the standby for prevention and treatment of malaria until the early 1960s when chloroquine resistance was reported in *Plasmodium falciparum* in highly endemic regions of South America and South-east Asia. Resistance has since spread throughout South America, and on to the remainder of South-east Asia, South Asia and reached East Africa in 1978. By 1985 chloroquine resistance had spread to West Africa.[5,6]



3 **4**

The resistance of malaria parasites to existing antimalarial agents, not just chloroquine, is the biggest problem being faced in the fight against malaria. This is why the search for new antimalarial agents is so vital. The topic of resistance will be discussed more fully in section 1.7.

1.2 Morphology of *Plasmodium* Parasites and *Anopheles* Mosquitoes

As indicated earlier malaria is caused by the protozoan parasites of the genus *Plasmodium*. The parasites are transmitted from one person to another by the female anopheline mosquito.[1] The males do not transmit the disease as they feed only on plant juices. The females feed on the blood of their victim in order to

propogate successfully. There are 380 species of mosquito but only 60 species are capable of transmitting the malaria parasites.[7] There are four species of *Plasmodium* which can infect and produce the disease in its various forms in man. These are *Plasmodium falciparum*, *P.vivax*, *P.ovale*, and *P. malariae*.

Of these four types *P. falciparum* is the most widespread and dangerous; untreated it can lead to severe and life threatening conditions such as cerebral malaria. Like all mosquitoes the Anophelines breed in water, each species having its preferred breeding grounds, feeding patterns and resting places. Sensitivity to insecticides and antimalarial drugs is also highly variable.[1,7]

1.2.1 The Life Cycle of the *Plasmodium* Parasite in Man and the Mosquito[1,7]

The parasite has a life cycle which is split between a vertebrate host and an insect vector. When the mosquito takes its blood meal, sporozoites from its salivary gland are injected into the victim.[1] The saliva must be injected as anticoagulant contained in it ensures a smooth-flowing meal. Once in the human bloodstream the sporozoites travel to the liver and penetrate hepatocytes, where they remain for 9-16 days. After multiplying within the cell they return to the blood and penetrate red blood cells in which they produce either merozoites or micro-gametocytes and macro-gametocytes.[1, 7] The merozoites reinfect the liver whereas micro-gametocytes and macro-gametocytes have no further activity within the host. When new mosquitoes arrive to feed on the victims blood they suck the gametocytes into their guts and exflagellation of the micro-gametocytes occurs while the macrogametocytes are fertilised. The result of this fertilisation is an ookinete. This penetrates the cell wall in the midgut and this develops into an oocyst. Sporogony within the oocyst produces many sporozoites when rupture of the oocyst occurs then sporozoites migrate to the salivary gland and can be injected into another host.[7]

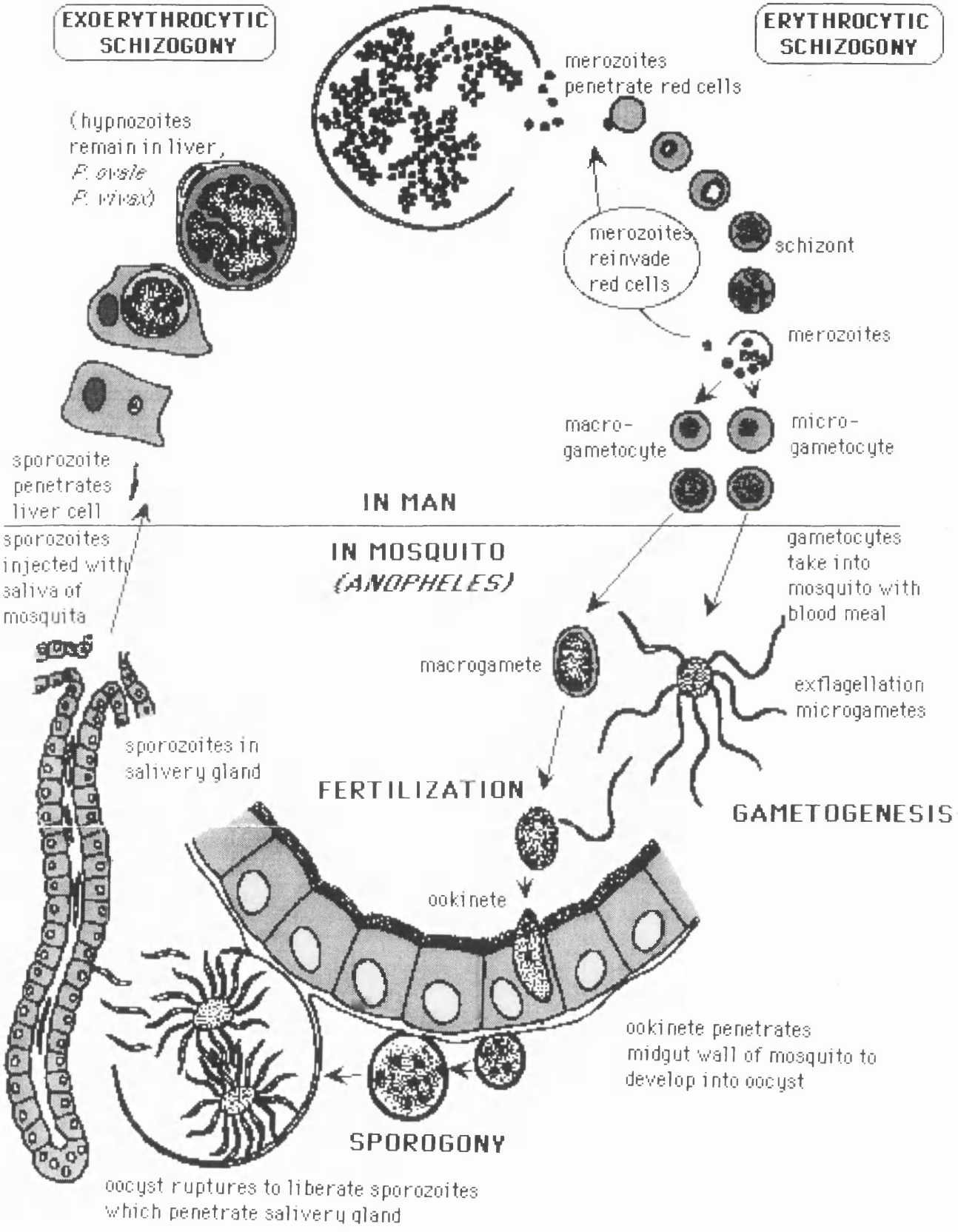
Sexual development of the *Plasmodium* begins as the merozoites invade the erythrocytes after their release from the liver. Schizogony occurs within the erythrocyte to produce more merozoites or the sexual micro and macrogametocytes. In *P. falciparum* erthyrocytic schizogony takes 48 hours and gametocytosis takes between 10-12 days.[7] A variable number of cycles of asexual erythrocytic schizogony takes place before any gametocytes are produced. At this stage the body's immune system may produce antibodies to the gametocytes.[7] Once drawn into the mosquito the gametocytes increase in

volume and escape from the erthyrocyte. Microgametes are formed by three mitotic divisions within the microgametocytes and are expelled explosively.[7]

Fertilisation of the female mosquito occurs where the outer membranes (plasmalemmas) of male and female gametes fuse and the nucleus of the microgamete and enters the female cytoplasm to produce a zygote. After about 18-24 hours the zygote which contains micronemes and a pellicle becomes elongated and motile. It invades the microvillus border, passes through the midgut cells, and lies beneath the basement membrane. The ookinete then becomes a static oocyst between the basal lamina and the basement cell membrane, and bounded by a thick plasmalemma. The main source of nutrients is the haemolymph in which the oocyst develops. Sporoblasts form and sporozoites bud off.

The oocyst ruptures and sporozoites escape into the haemocoele and migrate to and penetrate the salivary gland cells, where they can lie in vacuoles for up to 59 days. The sporozoites develop further and become 1000 times more infective than when they were in the oocyst.[1] These sporozoites are more antigenic, their motility is involved in their invasion of cells and escape from salivary glands. The sporozoites are about 12 mm long and 1mm across with a single nucleus and they posses a complex pellicle responsible for motility. The penetrating region contains extensions of the microneme ducts which release an agent which interacts with host cell plasma membrane during penetration.[7] The bite from a mosquito transfers almost 10% of its sporozoite load into the capillaries and the sporozoites begin their invasion of the host defences. This may be done binding serum proteins for "camouflage". Some are destroyed by macrophages or by antigen specific antibodies in immune individuals but not in non-immune individuals. They reach the hepatocytes and initiate schizogony or become hypnozoites. All sporozoites have left peripheral circulation within 45 minutes. The life cycle of *Plasmodium vivax* in man and the mosquito is shown diagramatically on page six.[1,7]

The life-cycle of *Plasmodium vivax* in man & the mosquito.[1] (after Vickerman and Cox, 1967)



1.3 The Diagnosis of Malaria

Malaria is diagnosed by its clinical symptoms and by detecting parasites in blood by microscopic examination of a blood smear. When the disease takes hold and the red blood cells break down the symptoms include fever, anaemia, shivering, pain in the joints and headaches. More severe forms of the disease include cerebral malaria where infected red blood cells obstruct blood vessels in the brain leading to hospitalisation and often death. Other vital organs including the kidneys, liver and lungs can be damaged.[8]

A definite diagnosis of malaria requires the finding of parasites in the blood because the symptoms of malaria are common to a number of ailments. The parasites are detected by making a blood smear on a slide and staining with Giemsa's stain and searching under the microscope for the parasite. Depending on the type of smear taken it can be possible to determine which particular parasite is responsible for the infection. [7,8]

1.4 The Current Situation

Malaria is a serious public health problem in more than 90 countries, inhabited by a total of 2.4 billion people, encompassing about 40% of the world's population. More than 90% of malaria cases are in sub-Saharan Africa. Other greatly affected countries include India and Brazil. It should be noted that outbreaks have recently been reported in Europe and in the United States 1000 to 1200 cases have been reported every year for the past few years.[9, 10]

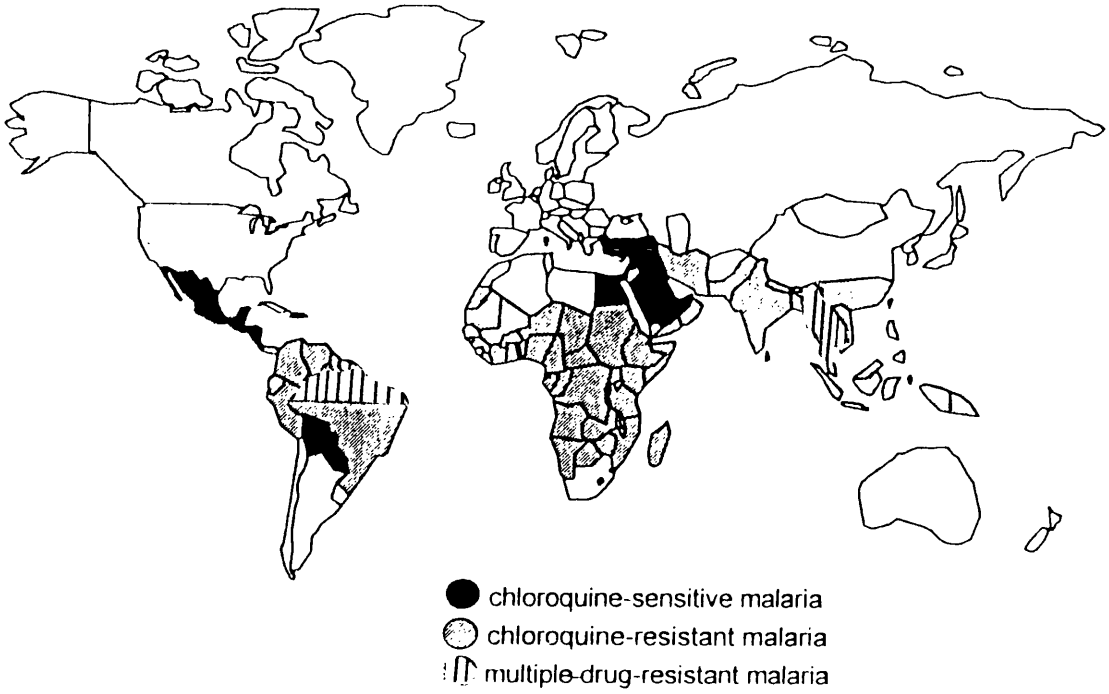
Worldwide the incidence of the disease has quadrupled in the past five years. The World Health Organisation (WHO) estimates that malaria is responsible for between 300-500 million clinical cases and 1.5 to 2.7 million deaths each year. It kills more people than any other communicable disease except tuberculosis and kills roughly twice as many people as AIDS each year.[9,10]

A number of factors are responsible for the large increase in malaria cases. In the Amazon road building, mining, logging and new agricultural and irrigation projects have been linked to the increase. In other parts of the world the disintegration of health services and armed conflicts with the mass movements of refugees worsen the situation.[11]

The main reason for the resurgence of malaria has been the resistance developed to common antimalarial drugs by the *Plasmodium* parasites. Resistance to chloroquine was noticed as long ago as 1961 but it is still used throughout most of that malaria endemic world as the first line of treatment.

Recent evidence indicates that it can no longer be considered an effective therapy.

The map below shows the world distribution of malaria in 1995 and how *P. falciparum* reacts to chloroquine. As well as resistance to chloroquine many strains of malaria are resistant to newer drugs such as mefloquine and halofantrine. Of more concern is the knowledge that many species of parasite are resistant to a number of antimalarial drugs. This is referred to as cross resistance. It is possible to treat some outbreaks of malaria by administering a combination of drugs but this is very expensive and already this has been shown to have a limited effect.[12]



World distribution of malaria in 1995, with delineation of areas where *P. falciparum* is sensitive to chloroquine, and resistant to numerous antimalarial drugs.[12]

The map indicates that malaria affects some of the poorest communities in the world, hence the need for cheap and affordable treatments. **Table 1** shows the cost of treatment for a single bout of malaria per patient.[12] Chloroquine was almost ideal due to its ease of administration, rapid action and it only cost $US 0.01 whereas the treatment with halofantrine costs $US 5.31.

Table 1 Cost of treatment for malaria.

$0.01	Chloroquine
$0.13	Pyrimethamine/sulfadoxine
$1.92	Mefloquine

$5.31 Halofantrine

As well as the terrible human suffering caused by malaria there are a number of social and economic costs that have to be paid. A single bout of malaria costs a sum equivalent to ten working days in Africa. This affects the ability of the sufferer to provide for his family and children lose out on education which could help them escape from the poverty trap. It has been estimated that in 1995 the global annual direct and indirect costs of malaria was $US 2 billion, whereas the estimated world-wide expenditure on malaria research, prevention and treatment in 1993 was a mere $US 84 million. In order to try and alleviate the situation WHO has formulated a global malaria strategy.[13,14]

1.4.1 The Malaria Control Strategy

The four basic elements of WHO's Global Malaria Strategy are:

1. Provision of early diagnosis and treatment for the disease;

2. Planning and implementation of selective and sustainable preventative measures, including vector control;

3. Early detection for the prevention of epidemics; and

4. Strengthening of local research capacities to promote regular assessment of a country's malaria situation; in particular the ecological, social and economic determinants of the disease.[2,10]

WHO based their fight against malaria on a number of fronts. This was a direct result of less than satisfactory results from their Global Malaria Eradication Plan between 1955 and 1969. They launched a series of campaigns to eradicate the mosquito by spraying homes with insecticides. This initiative was successful in North America, Southern Europe, the former Soviet Union and parts of Asia and South America. The results were disappointing in Latin America and most Asian countries where malaria persisted. Due to logistical problems large scale eradication was not attempted in African countries. In 1968 a massive epidemic broke out in Sri Lanka after malaria had been believed to be eradicated and 20 years later 25,000 people in Madagascar died from malaria. In 1994 there was a serious malaria outbreak in India and one reported in Azerbijan where it was thought the disease had been beaten.[15,16]

The ideal of global eradication of malaria was finally abandoned in 1969. It became obvious that an effective control plan had to be adapted to local

conditions and involve the whole community, the local health services and those involved in development of affected areas.[2]

The main objectives of the Global Malaria Control Strategies are:

1. To have at least 90% of affected countries or territories to be implementing appropriate control plans by 1997; and

2. To reduce malaria mortality by at least 20% in at least 75% of affected countries by 2000.[17]

One of the biggest achievements of the plan is in capacity building. This entails establishing a group of professionals in each endemic country who are responsible for the implementation and evaluation of national control programmes. For example in Africa 150 trainers have instructed 16,000 people on malaria courses with particular emphasis on the development of national drug policies, monitoring of drug efficacy, and the management of severe malaria.[17]

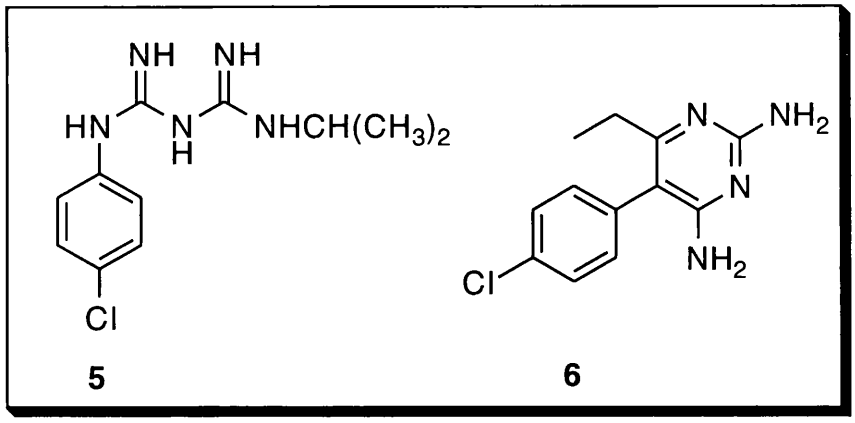
Significant strides have been made in the development of new tools for use in the control of malaria. One of the most promising and most successful has been the use of bed nets and curtains treated with insecticides. Controlled field trials by WHO suggest that in certain situations childhood mortality can be lowered by 15 to 35% by the use of impregnated bednets. Results have been very encouraging, but more work has to be done to optimise their effects in operational settings.[10]

The most important achievement of the Global Malarial Control Plan has been to complete successfully the first objective they set; at least 90% of affected countries have installed appropriate control plans. An accelerated control plan for Africa was started in 1998. However the global fight against malaria is still an enormous task.[17]

1.5 The Biological Classification of Antimalarial Drugs[18]

The biological classification of antimalarial drugs is based upon which part of the life cycle of the parasite they inhibit. The five groups are as follows.

1. **Tissue Schizontocides** - These inhibit the growth of the pre-erythrocytic stages of the parasite in the liver. The less toxic members of this group include proguanil **(5)** and pyrimethamine **(6)**. They are used singularly or in combination with sulfonamides as "casual prophylactics" to prevent infection.



2. **Hypnozoitocides** - These kill at the dormant liver stage known as the hypnozoites. They are used as "antirelapse drugs" in *P. vivax* and *P. ovale* infections. Primaquine **(7)**, an 8-aminoquinoline is one of the few hypnozoitocidal drugs found to be of any practical use in man.

3. **Blood Schizontocides** - These act rapidly and only at the erythrocytic stage. This group includes chloroquine and quinine. They can be used as "suppressive prophylactics" in the prevention of the disease. Both are ineffective against the mature gametocytes of *P. falciparum.*

4. **Gametocytocides** - These will destroy the mature gametocytes of *P. falciparum.* They act at the sexual stage of the parasites in the blood. Primaquine, an 8-aminoquinoline, has been used to good effect.

5. **Sporontocides** - These drugs or their metabolites have an inhibitory effect on the development of the oocyst and sporozoites in the mosquito. Agents of this group include pyrimethamine and proguanil.

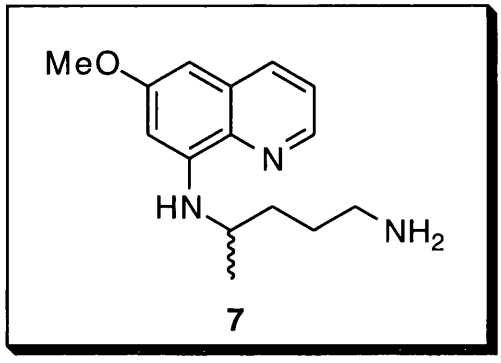
1.5.1 The Chemical/Biological Classification of Antimalarial Drugs.[18]

Another way which is also used to classify antimalarial drugs is based upon a mixed chemical/biological system. The three classes are outlined in brief below.

1. **8-Aminoquinolines** - They are most active on dormant parasite stages such as the gametocyte and the hypnozoite. They also have some effect at toxic concentrations on the asexual stages in the blood. Primaquine **(7)** is an example of this class of antimalarial drug.

2. **Antimetabolites** - These are active on all growing stages of the parasite. They include proguanil **(5),** pyrimethamine **(6)** and sulfonamides.

3. **Blood Schizontocides -** This class includes quinine **(2)**, chloroquine **(4)**, (and other aryl aminoalcohols). They are only active against growing stages of the parasite.



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1.6 Antimalarial Drugs - Past and Present

This section will contain a discussion on some of the more commonly used antimalarial drugs with particular emphasis on their mode of action and the possible ways in which resistance has become possible.

1.6.1 Chloroquine (4)

This is a synthetic compound which was first synthesised and developed in Germany in the late 1930s. It was the first choice for treatment and prophylaxis until the early 1960s. The spread of the chloroquine-resistant parasites has for the most part made it redundant for chemoprophylaxis. It is, however, used widely for uncomplicated *P. falciparum* infections (*i.e.* those infections not involving cerebral malaria).[19]

Chloroquine contains a quinoline heterocyclic ring, which is a feature common to many antimalarial agents. It is a diprotic weak base and accumulates to high concentrations in the acidic food vacuole of the parasite. This is the proposed site for drug action.[20]

The most widely accepted theory to explain the therapeutic affects of chloroquine was proposed by Slater and Cerami in 1992.[21] Their work indicates that chloroquine interferes with a haem detoxification enzyme that is essential to the malaria parasites in the red blood cell.

The rapid spread of the parasite produces a demand for nutrients that in part is met through the digestion of host cell haemoglobin in the acid food vacuole. This presents a problem for the parasite as one of the breakdown

products, ferriprotoporphyrin IX (FPIX) is toxic. The parasites, unlike their human host, cannot degrade these haem molecules. Instead they store FPIX within the food vacuoles as harmless dark brown granules called malaria pigment or haemozoin. The pigment is an insoluble, crystalline polymer of haem molecules linked between the central ferric ion of one haem and a carboxylate side-group oxygen of another. This is achieved by a haem polymerase enzyme that is unique to the malaria parasite.

Until Slater and Cerami proposed their theory in 1992, no one theory could explain all the experimental observations accumulated in the studies of chloroquine action. They showed that a catalytic activity in *P. falciparum* extracts polymerises haem in the form of FPIX hydroxide or haemoglobin into malaria pigment. Chloroquine and six other quinoline ring compounds block haem polymerisation in a manner directly related to the extent to which they stop the growth of malaria parasites. These data indicate that quinoline ring compounds like chloroquine act by inhibiting the haem polymerase enzyme that detoxifies FPIX, thus poisoning the acidic food vacuole and starving the parasite. This would explain the specific vulnerability of growing intraerythrocytic malaria parasites to quinoline-containing drugs such as chloroquine.[21,22]

Unfortunately the problem of resistance to chloroquine has arisen. The method by which resistance operates is not completely understood, although it is known to be associated with reduced concentrations of the drug in the acid food vacuole of the parasite. This is due to an increased efflux of chloroquine from the cell, not because of a reduced uptake of the drug. It has been calculated that resistant parasites can remove chloroquine between 40-50 times faster from the cell than sensitive parasites.[21, 22]

This process can be reversed or inhibited by a number of drugs including verapamil and desipramine. This has led to the suggestion that a parasite homologue of the P-glycoprotein responsible for the multidrug resistance (MDR) in mammalian tumour cells is responsible for the rapid efflux of chloroquine in resistant *P. falciparum.* This was suggested as these compounds also reverse MDR in mammalian tumour cells. Studies have yet to confirm this hypothesis.[23]

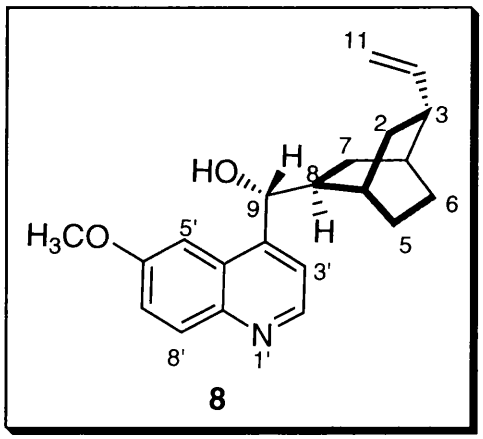
1.6.2 (-)-Quinine (2)

Since the discovery of chloroquine resistant *P. falciparum*, quinine has once again become an important therapeutic antimalarial drug.[24]

It is one of four major alkaloids found in cinchona bark. The others are (+)-quinidine **(8)**, cinchonidine and cinchonine. They all posses antimalarial activity. The same backbone structure is present; they all share a quinoline

heterocyclic nucleus and a tertiary amino aliphatic ring system, quinuclidine. These two moieties are linked *via* a hydroxymethylene bridge *para* to the aromatic nitrogen atom. In all cases the quinuclidine ring carries a vinyl group at position 3. In the case of quinine and quinidine the 6'-position bears a methoxy group which is missing in both cinchonidine and cinchonine. Each molecule possess four chiral centres, but in the naturally occurring alkaloids the configurations at C-3 and C-4 are the same. The configurations can vary at C-8 and C-9 giving rise to the four naturally occurring members of the family.[24]

Although it has been used to replace chloroquine in many endemic areas, quinine has also suffered problems with resistance. In Thailand it replaced chloroquine in 1978 but soon afterwards quinine resistance was reported in large areas of the country. It has now been replaced by its dextrarotatory diasteroisomer, quinidine, which is intrinsically more active than quinine but is also more cardiotoxic. Both agents reduce parasitaemia quickly, but they are generally used in combination with other drugs to prevent relapse.[25]



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Quinine and quinidine are both blood schizonticides. Their mechanism of action is to inhibit the haem polymerase enzyme in a manner similar to chloroquine.[18]

Resistance to both drugs is in part reversed by verapamil which leads people to believe that the rapid efflux mechanism shown in chloroquine resistance plays a part here.[25]

1.6.3 Proguanil (5) and Pyrimethamine (6)

Both will be considered here as they were synthesised around the same time and have a related mode of action. Proguanil, a biguanide and pyrimethamine, a diaminopyridine were introduced in the late 1940s and early

1950s. They destroy the early tissue stages, particularly in *P. falciparum* and so are used as "casual prophylactics" to prevent blood infection occurring. They also act as blood schizonticides, but work more slowly than chloroquine. Both have very few side effects; the only known side effect of proguanil to be reported is that of mouth ulcers. They both have excellent safety and tolerance records.

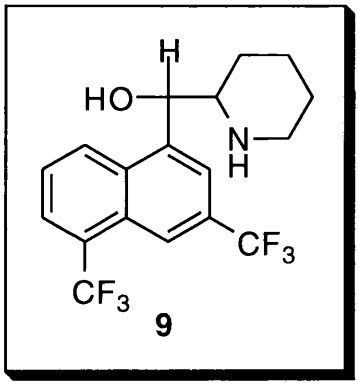
Both drugs act by interfering with folic acid metabolism in the parasite, and hence they are sometimes known as antifolates. Proguanil, or more correctly its metabolite cycloguanil, and pyrimethamine inhibit the bifunctional enzyme Dihydrofolate Reductase (DHFR)/Thymidylate Synthase. Other antifolate drugs, collectively known as the sulfonamides inhibit Dihydropteroate Synthetase.

Within a few years resistance to these drugs was observed. Resistance in both *P. falciparum* and *P. vivax* limited their usefulness. Resistance to the DHFR inhibitors results from reduced affinity of the DHFR-Thymidylate Synthase enzyme complex for the drug. This is due to point mutations in the DHFR gene. Studies show that several distinct base-pair mutations have been identified which result in affinities 100-1000 times less than that of the drug sensitive DHFR complex.

Resistance to one antifolate compound does not automatically mean resistance to another and so proguanil may be useful in some areas where pyrimethamine alone is not and vice versa. These compound are still very useful today, especially as chemoprophylactic drugs.[6,26]

1.6.4 Mefloquine (9)

The racemic drug was introduced in clinical trials in 1975 and was found to be an effective therapeutic agent against drug resistant *P. falciparum.*



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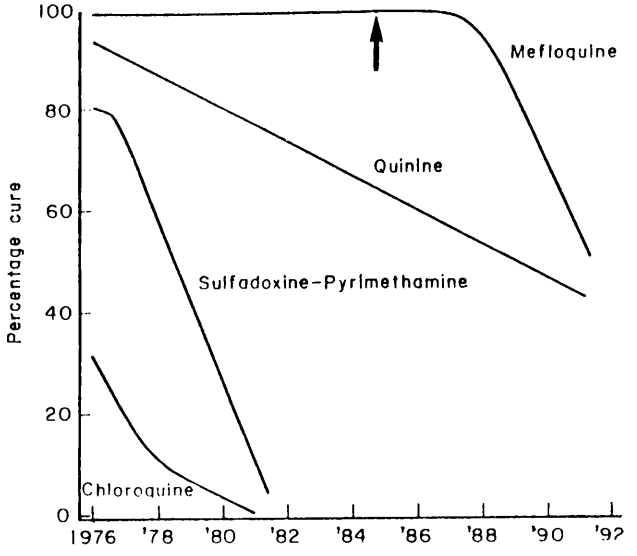
Mefloquine is a 4-quinolinemethanol and is structurally related to quinine and halofantrine. It is a blood schizontocide and like chloroquine it appears to work by inhibition of haem polymerase.

Resistance to mefloquine was first reported in parts of Thailand in 1982 and in other areas in the decade afterwards. The resistance mechanism is at present unknown. An added complication is the development of cross resistance. There is evidence that mefloquine resistance may drive halofantrine and possibly quinine resistance. At present, treatment with mefloquine is not a problem except in some parts of Thailand where cure rates have dropped from 98% in 1986 to 71% in 1990. The figure below shows the cure rates of some drugs in Eastern Thailand since 1976.[25] Note that all of the antimalarial agents in the study have seen a decrease in their effectiveness.[6,25]



Antimalarial cure rates in falciparum malaria in Eastern Thailand since 1976. The arrow marks the introduction of mefloquine to malaria clinics for the treatment of positive-slide *P. falciparum* infections.[25]

A bigger problem facing mefloquine is reports of its neuropsychiatric effects such as psychosis and convulsions. These have been reported in 1 out of 215 to 1 out of 1700 users. This coupled with the cross resistance with halofantrine limit its prescription; thus the future does not look good for mefloquine.[26]

1.7 A Summary of the Causes of Antimalarial Drug Resistance[, 27]

In the previous sections possible mechanisms of antimalarial drug resistance were discussed, but how do they develop? Resistance is considered to result from spontaneous chromosomal point mutations, a process which is

believed to be independent of drug pressure. In the case of DHFR inhibitors resistance can arise from single point mutations, but epidemiological and laboratory observations point to resistance to quinoline compounds requiring a series of unlinked additive mutations.

Resistant parasites are more likely to be selected if the population is exposed to a sub-therapeutic level of the drug. For resistance to spread, the resistant parasites must survive and produce gametocytes and these parasites must be transmitted. The female mosquitoes have varying degrees of receptiveness to different strains of the parasite. In some instances the vectors are more receptive and resistance can arise or they are less receptive to the new strain of parasite.

Resistance is known to spread in three circumstances:

1. Large scale antimalarial drug use;
2. Inadequate dosing; and
3. Adequate dosing with drugs that are expelled slowly from the body.

Inadequate dosing can arise in a number of ways, commonly due to the patients failing to comply with the prescribed regime of treatment and unregulated distribution of the drug. The greatest difficulties arise when a whole population is exposed to a low concentration which occurred in population experiments in South-east Asia when pyrimethamine or chloroquine was added to table salt. Many think this resulted in the development of chloroquine resistance in this region.

Drugs that are slowly eliminated from the body, such as chloroquine and halofantrine which have a half life of 1-2 months and 3 weeks, respectively, and can be detected in the blood months after treatment, will persist in sub-therapeutic levels and act as a selective pressure when the patient is reinfected. As the level of resistance rises, more parasites escape the first therapeutic actions of the drug and the chances of selecting resistant mutagenic strains from the initial infection increase and resistance quickens.

These lessons have been learned for treatment today and in the selection of new antimalarial drugs. Usage is more strictly controlled and people are educated on the need to follow the prescribed treatment methods. New potential antimalarial drugs like artemesinin have shorter half lives and are probably expelled from the body within hours.[6,27]

1.8 The Selection of Antimalarial Agents for Prophylaxis

This is a difficult area and in many cases there are no clear right or wrong answers. Any compromise that is reached must be under constant scrutiny and open to change when the need arises. One thing certain in all cases is that local knowledge of transmission areas and antimalarial drug resistance and sensitivity are vital in order to give the appropriate advice and treatment. However, some generalities can be made. Where *P. vivax* or sensitive strains of *P. falciparum* only are found, chloroquine on it own is an appropriate prophylactic. As resistance begins the weekly doses of chloroquine are increased from a base level of 5 to 10 mg/kg. This will "buy some time". Thereafter it is common to recommend chloroquine and proguanil combinations. This will not be totally effective everywhere, but it at least prevents severe symptoms arising. This combination is well tolerated and is still effective over large parts of Africa, Southern Asia and some of the Americas. Multidrug resistance is more wide-spread in south-east Asia but there are three options for prophylaxis. The first is weekly administration of mefloquine or daily use of the antibiotic, doxycycline. The final option is to administer mefloquine, halofantrine or quinine with tetracycline. An in depth knowledge of the local situation is essential when making a decision on which course of therapy to use.[6,27]

1.9 The Selection of Antimalarial Agents for Treatment

It is unfortunate but for most countries in the tropics, cost is the major factor determining what antimalarial drug is prescribed locally. Of course the sensitivity of parasites to antimalarial drugs is also taken into account. To treat infection successfully health workers need to know the *in vivo* response to antimalarial treatment. This information varies from the reliable and detailed assessments of drug efficacy in a particular community to the less than reliable observations from health workers that patients seem to be coming back for treatment in a few weeks. As there is a limited number of drugs available, their efficacy has to be monitored frequently and with a high degree of care and attention. The tolerance and effect of different drug therapies must also be assessed.[6]

Failure of a course of treatment can arise due to intrinsic resistance of the parasite to the drug in use, or host factors (*i.e.* pharmacokinetics) which will result in an insufficient concentration of the drug in the blood. These two features can be distinguished since assays exist to quantify antimalarial drug levels in the blood.[6, 27]

The classification of *in vivo* resistance most frequently used is derived for evaluation of aminoquinoline resistance **(table 2)**. With some limitations it can be applied to other drugs.

As resistance develops, increasing numbers of people with R_I recrudescence are observed. This means that the initial therapeutic response is satisfactory, but infection returns later. As resistance increases, more and more patients are seen whose infections are not cured. These are the R_{II} and R_{III} high grade resistances shown in **Table 2**.[6]

Table 2 The World Health Organisation grading of resistance of asexual parasites (*P. falcipraum*) to 'schizontocidal' drugs (4-aminoquinolines).

Response	Recommending grading	Evidence
Sensitivity	S	Clearance of asexual parasitaemia within seven days of initiation of treatment, without subsequent recrudescence.
Low-grade resistance	R_I	Clearance of asexual parasitaemia, as in sensitivity, followed by recrudescence[a].
High-grade resistance	R_{II}	Marked reduction (75%) of asexual parasitaemia, but no clearance, *i.e.* the parasitaemia remains patent for seven days.
	R_{III}	No marked reduction in parasitaemia, *i.e.* the parasite count does not fall by more than 75% within 48 h.

[a]The assessment is usually made 28 days after the last treatment, although it is known that recrudescences can occur up to ten weeks following drug administration. Reinfection cannot be excluded if the patient re-enters an area of malaria transmission.[6]

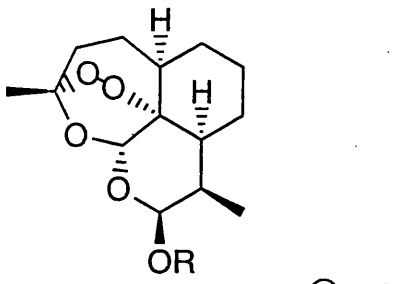
Current treatment strategies can be improved but the underlying problem is that not enough new drugs have been developed and not enough research is being conducted.

1.10 Antimalarial Drugs - What Next?

Perhaps the future of antimalarial chemotherapy lies in the hands of a drug that has been prescribed for over 2000 years. Artemesinin **(1)**, which was briefly mentioned in section 1.1, and its derivatives are the most promising drugs undergoing development at present. It was isolated in 1972 as the active component of *Artemisia annua*, the Chinese wormwood plant which was prescribed as a herbal remedy for fevers. Artemesinin **(1)** itself is a sesquiterpene lactone with an endoperoxide bridge across one of the rings of the trioxane structure and is unrelated to any previously known antimalarial agent.[6]

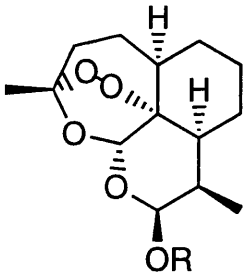
Three very potent semi-synthetic derivatives of artemesinin have been developed. These are a water soluble hemisuccinate salt known as artesunate **(10)**, and two compounds that are oil soluble, artemether **(11)** and arteether **(12)**. The latter was studied by WHO for its lipophilic properties which could be important when treating cerebral malaria. They all have faster clearance times and potency than any other antimalarial agent and are rapidly active against drug resistant *P. falciparum* but there is a high incidence of relapse in pre-clinical trials when it is used alone in therapy. This problem appears to be solved by administration of the drug in combination with mefloquine. The synthetic derivatives have greater potency and stability than artemesinin (sometimes 500% more potent), but artemesinin is much cheaper to produce.[27]

No completely convincing theories exist to account for the antimalarial effect of artemesinin and its derivatives. Some reports suggest that activity is in part due to activated oxygenated radicals which are toxic and which may react with the high concentration of haem within the parasite resulting in selective toxicity. What is known for sure is that they are all converted into a common biologically active metabolite dihydroartemesinin **(13)**.[25]

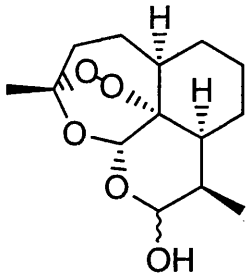


$R=CO(CH_2)_2CO_2^{\ominus}Na^{\oplus}$

10



11 R=Me
12 R=Et



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At present there is no licence for these drugs in Europe or the USA. However, artemether and artesunate have been available in China and Indochina for a number of years. Some are of the opinion that the widespread use of artemesinin and its derivatives is unlikely since a therapeutic dose has been seen to induce foetal resorption in rats and that the most probable application of these drugs will be in cerebral malaria, where its rapid effects make it superior to quinine and mefloquine. This is a worse case scenario, since it is clear that at the moment there is insufficient pre-clinical and toxicity data to meet the strict requirements necessary for the drugs to be licensed.[27]

1.11 Other Strategies to Combat Malaria

Two of the most innovative ideas owe their development to advances in molecular biology and genetic engineering. Much time and money is being spent on the development of transgenic mosquitoes and in the research for vaccines against malaria. A brief discussion of each will follow.

1.11.1 Transgenic Mosquitoes

This is a totally new idea in vector control. Past evidence shows that mosquitoes have been extraordinarily difficult to control, and have quickly developed resistance to most insecticides. Two options are being looked at. The first involves creating transgenic mosquitoes incapable of transmitting malaria, and finding ways to spread the genes involved in natural resistance throughout the indigenous population. The key behind this idea is that many of the 380 species of mosquito are resistant to *Plasmodium* parasites. Genes of this type

have been mapped in a resistant strain of *Anopheles gambie* at the European Molecular Biology Lab. in Heidelberg. The other approach using transgenic mosquitoes is to engineer mosquitoes carrying foreign genes that confer protection. A mammalian gene for an antibody that attacks an antigen in the ookinete phase of the parasite has been cloned successfully.[28]

The biggest problem in this approach is spreading these new genetically engineered traits in nature. Work with *Drosophila* has shown that releasing a relatively small number of transformed mosquitoes should result in spreading the "new" genes throughout the wild population.[28]

At present no one has transformed mosquitoes in a stable manner, but researchers believe they are close to achieving it.

Major ethical and safety problems exists with this work and it will undoubtedly be a long time, perhaps decades before any genetically engineered mosquito could be released into the environment.[28,29]

1.11.2 Malaria Vaccines

The potential for a malaria vaccine was built upon the observation that people who have been repeatedly bitten by infectious mosquitoes gradually acquire some form of immunity. This resistance to infection has arisen because the immune system has mounted an effective response.[7,30]

Immunity to infection can occur at three stages of the parasite's life cycle and is stage and species specific.

Three types of vaccine are currently under development.

1. **Antisporozoite Vaccines -** These are designed to prevent infection.

2. **Transmission Blocking Vaccines** - They retard the development of the parasite in the mosquito, in the hope of reducing or preventing transmission of the disease.

3. **Anti-asexual Blood Stage Vaccines -** These are designed to reduce severe and complicated symptoms of the disease.

SmithKline Beecham in conjunction with the US army have developed an antisporozoite vaccine which protected 6 out of 7 volunteers who had been repeatedly bitten by infected mosquitoes. The vaccine is based upon the major surface protein of the sporozoites, which helps them infect hepatocytes.

Previous potential vaccines in this area failed because they did not elicit a sufficient immune response. New studies indicate that this can be overcome by using adjuvants. These are compounds which enhance an immune response.

Vaccines of the third group have been given priority by WHO as such vaccines could lower morbidity and mortality in children under 5 years old in Africa, which is the main risk group. At present several such vaccine candidates are subject to clinical and field trials.[30]

This is an exciting area of work. One major drawback is the species specificity of any vaccine, This could mean that a vaccine that is effective against, for instance, *P. falciparum* would be useless against any other *Plasmodium* parasite.[10,30]

1.12 Malaria - Will it Affect Us in the UK?

This may seem like a rather selfish question but some reports claim that malaria could return to northern Europe early in the new millennium as a result of global warming. The hot and humid conditions are ideal for the spread of mosquitoes. A more immediate problem is that of cheaper international travel. [31]

Over the past 20 or 30 years there has been a large increase in the number of people taking holidays or going on business trips to malaria endemic areas such as the Far East, Africa and South America. The highest risk of infection by *Plasmodium falciparum* occurs in areas of West Africa, including Ghana and Nigeria. WHO reported in 1991 that the number of imported malaria cases had doubled over the past decade, with over 10,000 cases reported in Europe and 1000 in North America.[5]

This way of contracting malaria, can be avoided in most cases if a number of precautions are taken and the individual acts in a responsible manner.

Anyone travelling to a known endemic area of the world should see their doctor, who will prescribe the appropriate antimalarial drug. The patient must follow the prescribed doses and use it for up to 4 weeks after leaving the area.

All reasonable precautions should be taken to minimise contact with mosquitoes, such as sleeping under insecticide impregnated bednets and wearing clothes that cover arms and legs from dawn to dusk; the times when the mosquitoes are most active. It is also wise to apply mosquito repellents such as *N,N'*-diethyl-*m*-toluamide. Some people may object to these measures, but they are a small price to pay when considering the potential consequences.[27]

Finally no drug is 100% effective, so if symptoms of malaria are noticed then immediate medical advice must be sought.[5]

1.13 Conclusions

Malaria is still one of the most important public health problems in the world today and it is estimated that 1 in 17 of the people alive today will die from the disease or effects directly related to it. Science has learned the lessons of past mistakes and is fighting the problem on a number of fronts, with chemistry having a vital role to play.

2

Fungi, Fungicides and Polyamines

2.1 The Effect of Fungal Plant Diseases - Past, Present and Future

Fungal plant diseases have been a constant enemy in man's bid to cultivate the land and provide food for himself and the community at large. Books of the Bible refer to blights, blasts and mildews and as early as 700 B.C. the Romans were making attempts to find favour with Rubigo, their god of rusts.[32]

These diseases are more prevalent and more spectacular amongst farmed crops because they are grown as a single cultivar. They are all genetically identical so if one is affected they all will be.[32]

Effects of such diseases have ranged from minor annoyances to major famines that have been responsible for millions of deaths, and the movement of millions of people trying to escape from the resulting hunger and poverty. One well documented example is the Irish potato famine of the 1840s. The potato was the major source of food for Irish peasants but the potato blight *Phytophthora infestans* resulted in much lower than expected yields and premature rotting of the stored crop. The food shortage that resulted was responsible for around 1.5 million deaths and caused about 1 million people to emigrate.[32]

Just over 100 years later in 1942 a catastrophe of a similar magnitude struck in Bengal when favourable weather helped *Helminthosporium oryzae* to decimate the rice crop. The resulting price increase was too much for many and about 2 million people died of starvation.[32]

In more recent times in developed parts of the world plant diseases have been seen which did not result in famine but did cause severe economic hardship and a reduction in the quality of life of large numbers of people. In 1970 15% of the U.S. corn crop was destroyed by southern corn leaf blight, a disease induced by *Helminthosporium maydis.* This resulted in financial loss of about $1 billion.[32]

The economic effects of plant diseases on crops is enormous. It has been calculated that even with the use of crop protection products, around one third of the world's food is lost each year to pests and diseases. In 1987 it was estimated that plant diseases cost Europe $23 billion and Asia $26 billion.[33]

Apart from economic needs new antifungal compounds are needed for a number of reasons. Three of the most important are as follows

1. To avoid the famines caused by fungal disease epidemics of the nineteenth and twentieth centuries.

2. Urbanisation and the increasing population has put a strain on natural resources and decreased the amount of good quality farming land available. Farmers will have to increase yields from existing agricultural land. It has been estimated that in 1991 there was 0.33 hectares of land to feed each person which will shrink to 0.2 hectares per person in 2025 and by 2050 the figure may be as low as 0.15 hectares per person.

3. Many crop fungi have developed resistance to common antifungal agents. New antifungal agents need to be found to combat this problem.[33]

2.2 Fungi as Plant Pathogens

As well as fungal diseases, the growth of plants can be affected by viruses, bacteria or by a deficiency of essential minerals in the soil. However, it is fungi that are by far the most destructive parasites of plants.[34]

Fungi derive all their nutrients for living from the plant. They share a common life, termed a symbiotic relationship. They live together but only the fungus derives any benefit. The host is harmed and this relationship is termed parasitic symbiosis.[34]

The fungal kingdom includes the varied and numerous moulds found on decaying vegetation and the yeasts found on the surfaces of ripe fruit. Mildews, rusts, smuts and many other plant pathogens also belong to this group.

The classification of fungi is to many people subjective and often controversial, so any numbers quoted must be treated with caution. A recent estimate puts the number of species at over 64,000.[34]

At the simplest level a fungus consists of a feeding system of threads called the mycelium, which produces spores on specialised reproductive structures. These spores are liberated and dispersed. The production of spores is crucial for the existence of fungi as a fungus can almost limitlessly regenerate from only a few surviving spores. This means that if a fungicide is to be of any practical use it must be very persistent.[34]

To pick the correct fungicide for effective control is of course essential. To understand which fungi are affected by which fungicide requires a knowledge of how the fungus transmits the infection and the life style of the fungus.

One very useful system was developed by Barnes in 1972. He classified pathogenic fungi according to the method by which they reach the plant and their reliance on water. Three methods of spreading infection are known. These are by airborne pathogens, seedborne pathogens and soilborne pathogens. These groups are shown in more detail in the **table 3** below.[35]

Table 3 A simplified classification of pathogenic fungi according to method of reaching host plants and reliance on water; examples of effective fungicides are included.

AIRBORNE INFECTIONS[a]

Group 1A. Water dependent at infection stage-motile spores. Mostly Oomycetes (*e.g.* the downy mildews, *Pythium*, *Phytophthora*, *Peronospors*, *Bremia*, *Pseudoperonospora*)
Fungicides: copper, dithiocarbamates, phthalimides, Dichlorfluanid (most systemics are ineffective)

Group 1B Most need water to infect but spores move passively. Mycelium hydrophobic and lives under cuticle. Mostly members of Ascomycetes and Deuteromycetes (*e.g.Septoria, Venturia, Sclerotinia, Botrytis*)
Fungicides: phthalimides, Dichlorofluanid, some systemic fungicides (*e.g.* benzimidazoles)

Group 2 Not directly dependent on water at infection stage. Live, at least initially under the cuticle or deeper. Includes pathogenic Ascomycetes, especially all the powdery mildews. Also rusts.
Fungicides: hydroxypyrimidines, Triforine, azole derivatives

SEEDBORNE INFECTIONS

Fungi belong to many different orders. Some live on seeds, others within them. They Include some Basidiomycetes (*e.g. Ustilago*), some Ascomycetes (*e.g. Pyrenophora*) and some Deuteromycetes (*e.g. Septoria*)
Fungicides: dithiocarbamates, cuprous oxide, organomercury compounds, some systemic compounds (*e.g.* carboxin, azoles)

SOILBORNE INFECTONS

Fungi belong to many taxonomic groups *e.g. Pythium* (Oomycetes), *Fusarium* (Deuteromycetes) and *Urocystis* (Basidiomycetes)
Fungicides: according to the circumstances, need a correct choice from nabam, Dichloran, Chloroneb, copper, some systemics (*e.g.* Thiophanate-methyl thiabendazole).

[a] Seed or soil applications of some systemic fungicides can sometimes control airborne infections.

With the above knowledge in hand a decision on what fungicide to use can be made. There are two broad classes of fungicide; these are systemic and non-systemic.[35]

2.3 Non-Systemic and Systemic Fungicides

Non-systemic fungicides were the dominant form of fungicides until the late 1960s. They are often referred to as protectant fungicides. They were applied as a treatment to prevent or protect against infection. They may be applied to seeds, soils or the plant surface but cannot penetrate into the plant tissues in effective amounts, hence they have to be applied before infection. This is an example of prophylaxis.[35]

Systemic fungicides can be used as a form of therapy (*i.e.* they can be effective once infection has occurred) and as prophylaxis. These chemicals can penetrate the plant cuticle and move through the cell membrane, thus reaching fungi within the plant tissue. This movement, or translocation within the plant allows systemic fungicides to act at places remote from the site of application and to protect new areas of growth. This gives another clear advantages when compared to non-systemic compounds. When a non-systemic compound is applied to a plant there is no surface redistribution, so areas missed by the first application remain unprotected as does any subsequent growth. This means repeated applications are necessary to ensure continuous protection.[36]

Another advantage of the penetrative nature of systemic fungicides is that they can be effective for a longer duration as they cannot be washed away by rain.[35]

Non-systemic fungicides do have one big advantage over systemic fungicides, in that they appear to be less prone to fungal resistance. One reason for this could be due to the high site specificity of systemic fungicides resulting in fungi selecting out mutants or favouring rapid gene mutation. Resistance persists for many years after the use of the compound has ceased and as the use of systemic fungicides increases so does the potential for resistance.

The low inherent toxicity and limited persistence of non-systemic compounds may also account for in part the low incidence of resistance among these compounds.

Two strategies are commonly used in order to try and reduce the chances of resistance developing. The first is to apply a mixture of systemic and non-systemic fungicides together or use them in rotation.[35]

2.4 Non-Systemic Fungicides - Chemical Aspects

Non-systemic antifungal compounds are most effective when applied before infection. An important property of most of them is that they are insoluble in water. This gives them some degree of persistence, *i.e.* they are not washed away by rain water. **Table 4** shows that organic non-systemic compounds are divided into classes that are dependent on their molecular structure.[35]

This section will begin with a brief discussion of some of the inorganic non-systemic fungicides (the only type of fungicides used before 1930), followed by a more detailed discussion of some of the more important organic non-systemic fungicides.

Table 4 Classification of non-systemic organic fungicides other than organometallic compounds.

Group	Examples
Organosulfur compounds	
1. Dithiocarbamates	Thiram, Zuneb, Mancozeb
2. Phthalimides	Captan, Folpet, Captofol
Dinitrophenol derivatives	Dinocap, binapacryl
Chlorinated aromatics	
1. Chlorinated nitro compounds	Quinozene
2. Chlorinated amino compounds	Dichloran
3. Chlorinated nitriles	Chlorthalonil
4. Chlorinated quinones	Dichlone
Other non-systemic compounds	
1. Guanidine Derivatives	Dodine acetate
2. Dicarboximides	Iprodione, Vinclozolin

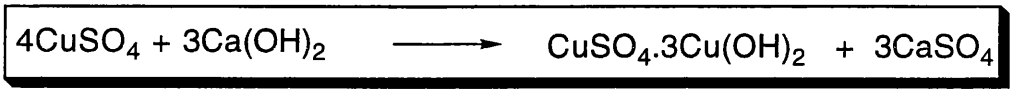
2.4.1 Sulfur

The fungicidal effects of sulfur were known to the ancient Greeks. They were forgotten during the middle ages only to be rediscovered again around 1800 when mixtures of sulfur lime were used to suppress powdery mildew on tree fruits. The efficacy of elemental sulfur was recognised in 1850 when it was used to protect grapes against powdery mildew. Sulfur was used to such an extent that even in the 1970s elemental sulfur was used more than any other fungicide in the U.S.A. This was because of its broad spectrum of activity (but it is most effective against powdery mildews) and its low cost. Usually it is dusted onto plants but it has been mixed with surfactants in sprays. For adequate effect it has to be used in large amounts. The mechanism by which sulfur displays its fungitoxicity is still uncertain and even the identity of the active form is unclear.[32]

2.4.2 Copper

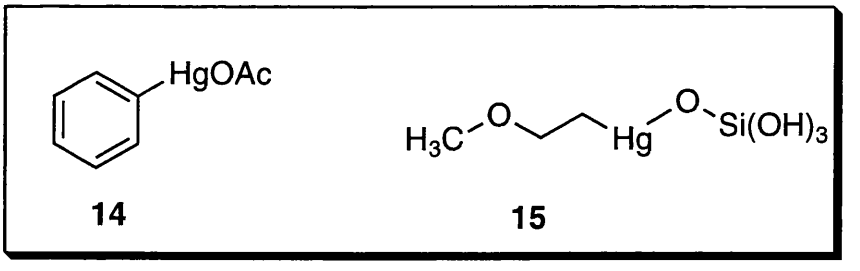
In the Medoc region of France in 1882 it was noticed by Millardet that a mixture of copper sulfate and lime on the surface of grapes suppressed powdery mildew. Publication of his results led to extensive testing of many related mixtures and the widespread use of Bordeaux mixture began. Until the middle of the twentieth century copper-based compounds accounted for the vast majority of fungicides. Bordeaux mix is used extensively in the tropics where other fungicides are quickly washed away.[32]

Bordeaux mixture is toxic because copper ions from water insoluble deposits are chelated by fungal metabolites and absorbed into fungal cells until toxic concentrations of copper are accumulated. The insoluble copper sulfate mixture is formed via the following reaction.



2.4.3 Organic Mercury Fungicides

Compounds of this type were first used in 1913 to control diseases of seeds which were spread by soil-borne pathogens. Compounds used include phenyl mercury acetate **(14)** and methyloxyethyl mercury silicate **(15)**.[37]



14 **15**

The use of mercury based compounds is now decreasing due to their general toxicity. Mercury concentrations have increased in the food chain and there were reports of human poisonings resulting from the consumption of treated seeds.[35, 38]

2.4.4 Other Organometallic Fungicides

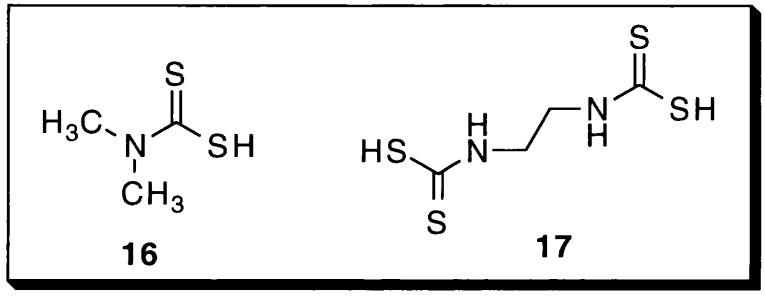
A variety of other organometallic fungicides have been used, mostly based on cadmium, copper and tin. They have been found to be more fungitoxic than inorganic metal but their use is limited due to fears about toxicity in the environment.[35]

2.5 Non-Systemic Organic Fungicides

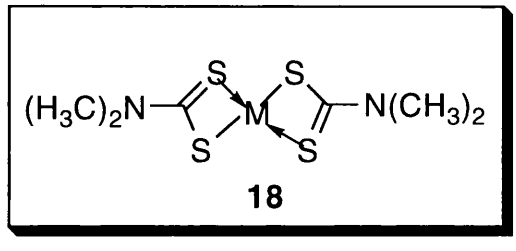
Organosulfur compounds are the most widely used fungicides on a tonnage scale today. There are two main classes of organosulfur compounds; the dithiocarbamates and the phthalimides.[35]

2.5.1 Dithiocarbamates

These compounds can be divided into two distinct groups. Type one compounds have no hydrogen atom attached to the carbamate nitrogen and are called dimethyldithiocarbamates. Type two compounds do have a hydrogen atom attached to the carbamate nitrogen and are referred to as bisdithiocarbamates. The general structures of dimethyldithiocarbamates and bisdithiocarbamates are represented below as **(16)** and **(17)** respectively.[35]

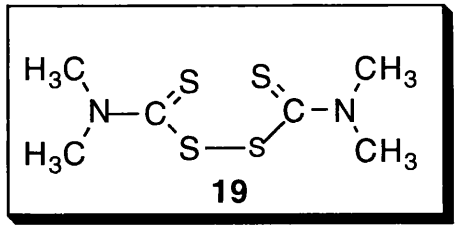


Most of the dimethyldithiocarbamates in commercial use are metallic complexes such as the iron complex, Febram **(18)** (M=Fe) and the zinc complex, Ziram **(18)** (M=Zn).



Both these compounds have limited use. For instance Febram is used successfully against apple scab mildew but it gives poor control of powdery mildew.

In contrast the disulphide oxidation product of dimethyldithiocarbamates Thiram **(19)** is successful in many areas.[35]

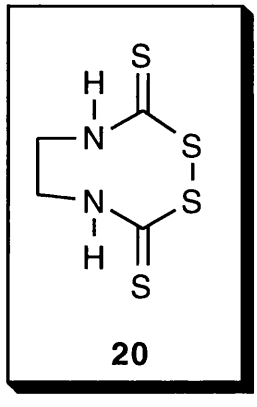


19

The mode of action of type 1 compounds is unclear. It has been suggested that they may form toxic complexes with copper or that they may sequester essential trace metals from the fungus (by co-ordination). Dithiocarbamate ions can inactivate thiol groups and so are toxic in their own right.

Type two dithiocarbamates have different fungicidal properties, and many of them are important in treating potato and tomato blight.

The mode of action of such fungicides is associated with the labile hydrogen on the nitrogen; it makes the compounds unstable. They react with thiol groups in essential biological components and then break down to give products including isothiocyanates and thioureas which react with enzymes and proteins. It has also been proposed that ethylenethiran disulfide **(20)**, a common breakdown product of all these fungicides is the actual fungitoxic agent.[35]



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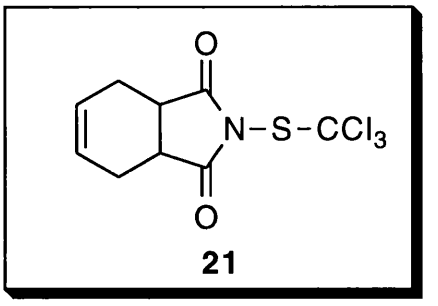
2.5.2 Phthalimide

The first phthalimide fungicide was introduced in 1952 as Captan **(21)**. Although it is a broad spectrum protective fungicide it has mainly been used to prevent fruit damage.[35, 39]

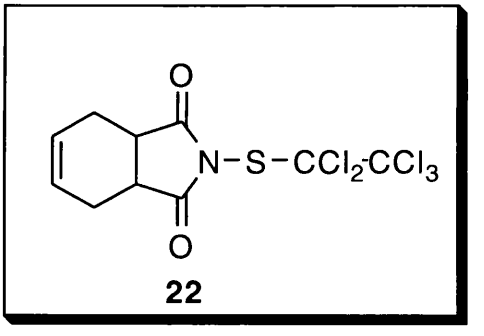


21

It is synthesised by reacting maleic anhydride with buta-1,3-diene and condensing the product with ammonia to produce 1,2,3,6-tetrahydrophthalimide. This is treated with trichloromethanesulfenyl chloride to give Captan.

Derivatives of Captan such as its analogue Captafol **(22)** have been developed and have found to be more effective.

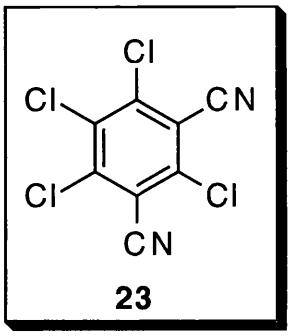
The exact details of the mode of action of captan are unclear. One theory is that Captan reacts with thiol groups in fungi and is converted into thiophosgene which can react with thiol groups starting a chain of events that blocks enzyme synthesis.[35]



22

2.5.3 Chlorine-Substituted Aromatic Fungicides

There are many examples of chlorinated aromatic fungicides. They are commonly used for soil or seed treatment and until recently they were of local but limited importance. Interest in this type of compound, such as Chlorthalonil **(23)** increased when it was used as a non-systemic component of a mixed formulation of systemic and non-systemic compounds in attempts to decrease the risk of systemic compounds causing resistance to develop. It was found to work well and widen its spectrum of action.[35]



23

Chlorthalonil is made by the addition of chlorine to isophthalonitrile. It is believed that this type of compound may display their fungicidal activity by interfering with chitin synthesis in fungi cell walls.[35, 40]

A more detailed review of non systemic fungicides has been published (see reference 35, chapter 11.)

2.6 Systemic Fungicides

Systemic fungicides were first developed in the mid 1960s because it was felt that they would be able to attack internal mycelium thus overcoming the problem that arises from small surviving pieces of mycelium regenerating. It was also expected that systemic fungicides would reduce the need for non-systemic fungicides which suffer the twin problems of being subject to weathering and being unable to protect new areas of growth after the plant has been sprayed. In many instances this proved to be the case but non-systemic fungicides are still widely used.[35]

The main types are divided into groups distinguished by their molecular structure and mode of action. Most of the main classes of plant pathogen are attacked in varying degrees by one or more of these groups. **Table 5** shows some of the commonly used systemic fungicides and their trivial names.[35]

Table 5 Classification of some systemic fungicides.

Group	Examples
Benzimidazoles	Benomyl, Carbendazim, Thiophanate, Thiabendazole
Oxathins or carboxamides	Carboxin, Oxycarboxin
Morpholines	Tridemorph, Dodemorph
Inhibitors of C-14 demethylation (a) Triazoles	Triadimefon, Triadimenol, Diclobutazole

(b) Pyrimidines	Fenarimol, Nuarimol
(c) Pyridines	Buthiobate
(d) Piperazines	Triforine
(e) Imidazoles	Imazalil, Prochloraz, Ketoconazole
Hydroxyaminopyridines	Ethirimol, Bupirimate, Dimethirimol
Antibiotics	Kasugamycin, Streptomycin
Phenylamides that target Oomycetes	Metalxyl, Ofurace, Oxadixyl

The majority of systemic fungicides seem to exhibit their fungitoxic activity by attacking specific processes in the fungi. Site specific activities such as these are usually influenced by either single gene selection, amplification or modification. Some of the specific activities that have been attributed to systemic compounds include interference with nucleotide base synthesis, and with polynucleotide and protein formation and the synthesis of steroids.[35]

Systemic compounds do have the disadvantage of being more prone to fungal resistance than non-systemic compounds. Possible reasons for this were discussed in section 2.3. More resistance was reported in the decade after they were introduced than in the previous 50 years when only non-systemic fungicides were used, hence the need for new systemic fungicides.[35, 36]

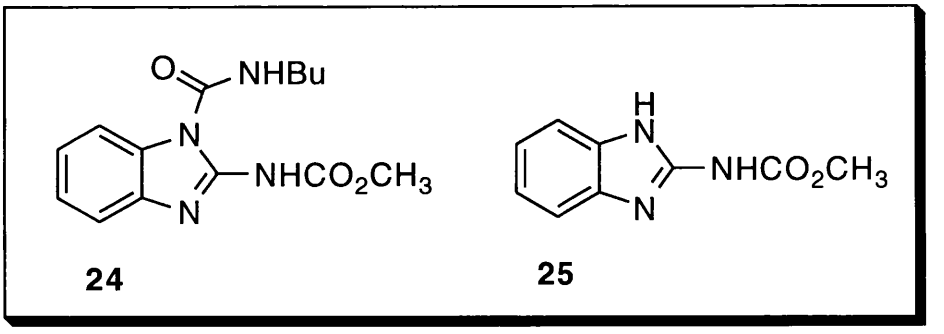
A brief discussion of some of the important systemic fungicides will follow. For a fuller review see reference 35 chapter 11 and reference 36 chapter 13.

2.6.1 Benzimidazoles

Systemic fungicides of this group have had a great effect on disease management. They all have a wide spectrum of activity, being particularly useful against Ascomycetes and Basidomycetes. The benzimidazoles can be subdivided into three groups: the carbamates, the non-carbamates, and thiophanates. The benzimidazole carbamates are formed by replacing one of the hydrogens of the amino group of carbamic acid with the benzimidazole radical. The thiophanates only become benzimidazoles after application.

Benomyl **(24)** is synthesised by condensing *o*-phenylenediamine with methyl cyanocarbamate and then treating the product with butyl isocyanate. Upon uptake into the root of the plant Benomyl is hydrolysed to Carbendazim **(25)** and this moves upwards through the plant. It has been suggested that no matter which of the benzimidazoles is used Carbendazim is the active antifungal agent. Carbendazim is itself a commercial fungicide; it has developed a

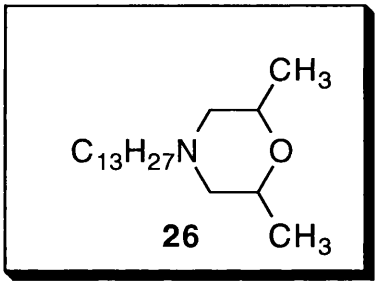
widespread resistance with several types of fungi, but it can still be effective in very large doses.[35]



24 **25**

2.6.2 Morpholines

One of the most important morpholine fungicides is Tridemorph **(26)**. It is effective against powdery mildew and various other fungi. It is often used in mixtures with non-systemic fungicides in order to increase its spectrum of activity.



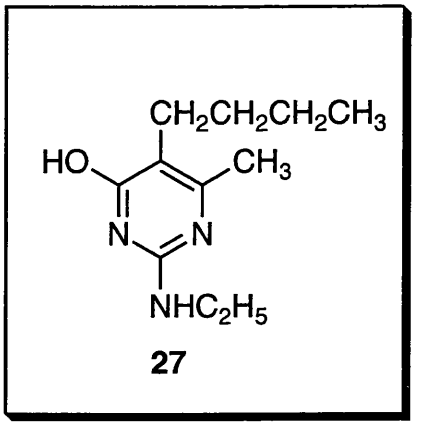
26

Like other morpholines it exhibits its fungicidal action by inhibiting sterol synthesis in the fungi but the exact mechanism is not known due to the problems with sterol identification.[35, 36]

A large number of sterol inhibitors have been used as antifungal agents, many of them based around nitrogen containing heterocycles such as pyridine, pyrimidine and triazole. They work by inhibiting the demethylation of sterols.[35]

2.6.3 Hydroxyaminopyrimidine

Compounds such as Ethirimol **(27)** were developed in the late 1960s specifically to control powdery mildews and consequently are of little use against other fungi.[35]



27

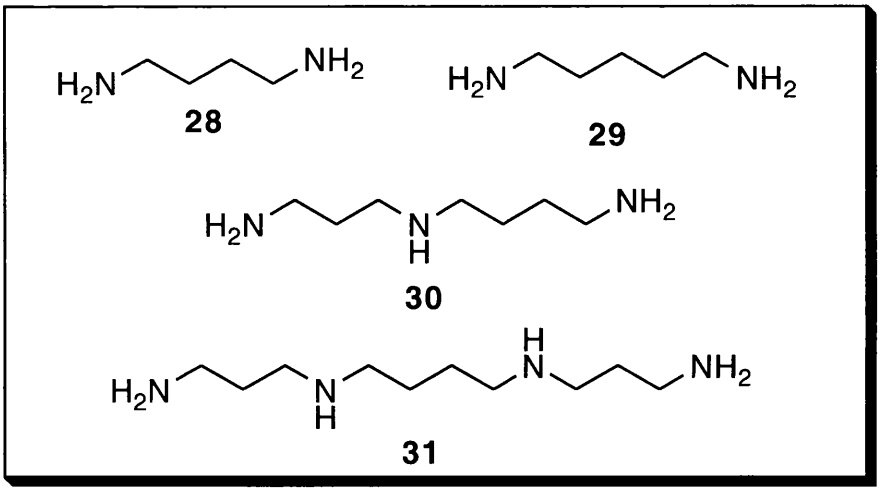
Their use has declined in recent years due to high levels of resistance that have developed against them. Many theories have been suggested to explain the mode of action of Ethirimol. The current theory is that it interferes with the enzyme adenosine deaminase.[35]

2.7 Polyamines

Polyamines are simple polycationic molecules, some of which are abundant in cells. These include the diamines putrescine **(28)** and cadaverine **(29)**, the triamine spermidine **(30)** and the tetraamine spermine **(31).**

Spermidine **(30)** and spermine **(31)** bearing three and four net positive charges respectively at physiological pH are the most cationic small molecules of cells which means that they bind to polyanionic molecules such as DNA, RNA and phospholipids. The distribution of charge in these basic polyamines may allow them to interact more flexibly with the acidic phosphate groups of nucleic acids than multivalent cations such as Mg^{2+}, where the charge is localised.[41, 42]

It is now clear that polyamines have a number of important physiological roles but in particular their involvement in growth and cell replication is gaining increasing attention. Their role in cancer development is one of a number of areas where the roles of polyamines has been explored.[43, 44] This section will be concerned with their relevance with fungal diseases and malaria.



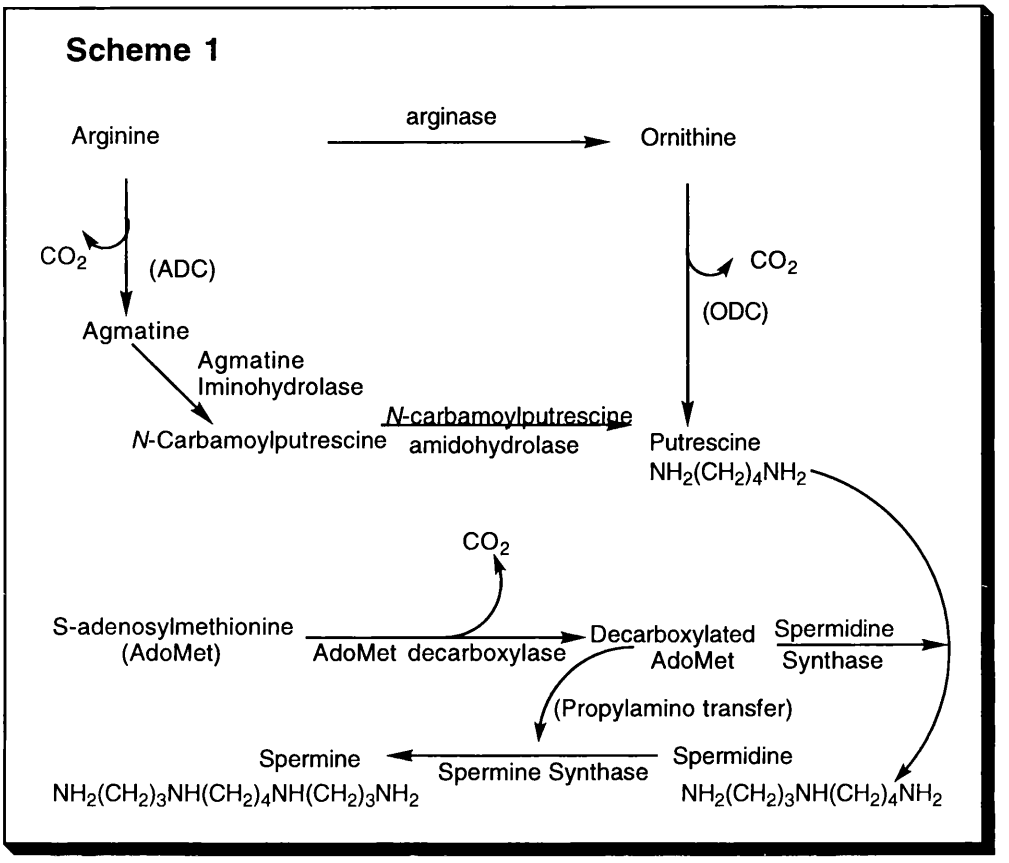
2.7.1 Polyamine Biosynthesis

It is now known that changes in the level of putrescine **(28)** and spermidine **(30)** in fungi are associated with a number of morphological events. For example, polyamine biosynthesis increased during zoospore germination in *Blastocladiella emersonii* and morphogenesis of *Mucor racemosus.* The need for polyamines has also been considered vital in the dimorphic transition of *Mucor* species. In the yeast to mycelium conversion of *M. racemosus*, polyamine biosynthesis increases, while monomorphic mutants of *M. baciliformis* possessing low levels of the polyamine biosynthetic enzyme ornithine decarboxylase **(ODC)** cannot grow mycelially.[41]

Numerous studies with mutants and inhibitors of polyamine biosynthesis have proved that polyamines are essential for normal growth.[41, 45, 46] To examine the possibilities of controlling fungal disease by interfering with polyamine metabolism it is necessary to discuss polyamine biosynthesis in plants and fungi.

In mammalian cells, protozoa and fungi the first step in the synthesis of polyamines is the decarboxylation of the amino acid, ornithine to form putrescine, catalysed by the enzyme ornithine decarboxylase. Putrescine is the precursor for further polyamine synthesis.[41, 46]

In plants and bacteria, putrescine can also be synthesised indirectly from arginine; decarboxylation with the enzyme, arginine decarboxylase gives agmatine which is converted into *N*-carbamoylputrescine by agmatine iminohydrolase. The final step is completed with the help of the enzyme, *N*-carbamoylputrescine amidohydrolase **(Scheme 1)**.[41]



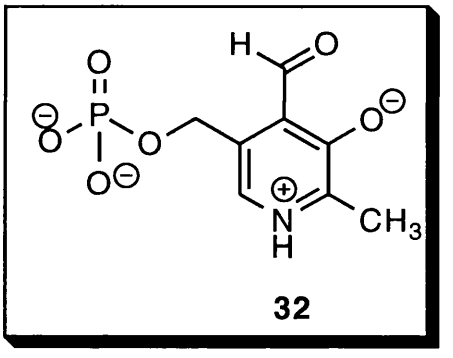
The polyamines spermidine and spermine are synthesised by the subsequent additions of aminopropyl groups donated by *S*-adenosylmethionine (AdoMet).The addition of the aminopropyl groups are catalysed by the enzymes spermidine and spermine synthase to give spermidine **(30)** and spermine **(31)** respectively.

The two pathways shown for the synthesis of putrescine and further polyamines are the basis for many approaches to the control of fungi. Plants and bacteria may synthesise polyamines essential for normal growth directly via the ODC pathway or indirectly via the ADC pathway. However the vast majority of fungi possess only the ODC pathway (it was believed until quite recently that all fungi synthesised putrescine via the ODC route, but there is evidence for the operation of the ADC route in a small number of fungi). Inhibition of the ODC route should be fatal to most fungi but plants should be unaffected as they have the alternative route to polyamine synthesis.[41, 46]

2.8 Ornithine Decarboxylase and its Mode of Action

The ODC enzyme is a dimer of molecular weight 80000 to 82000. Problems with the isolation and purification of a significant amount of ODC has meant that direct mechanistic studies of ODC have been limited. It has been shown to deplete rapidly the polyamines putrescine, spermidine and spermine and to slow the proliferation rate in a number of tumour cells and parasitic protozoa.

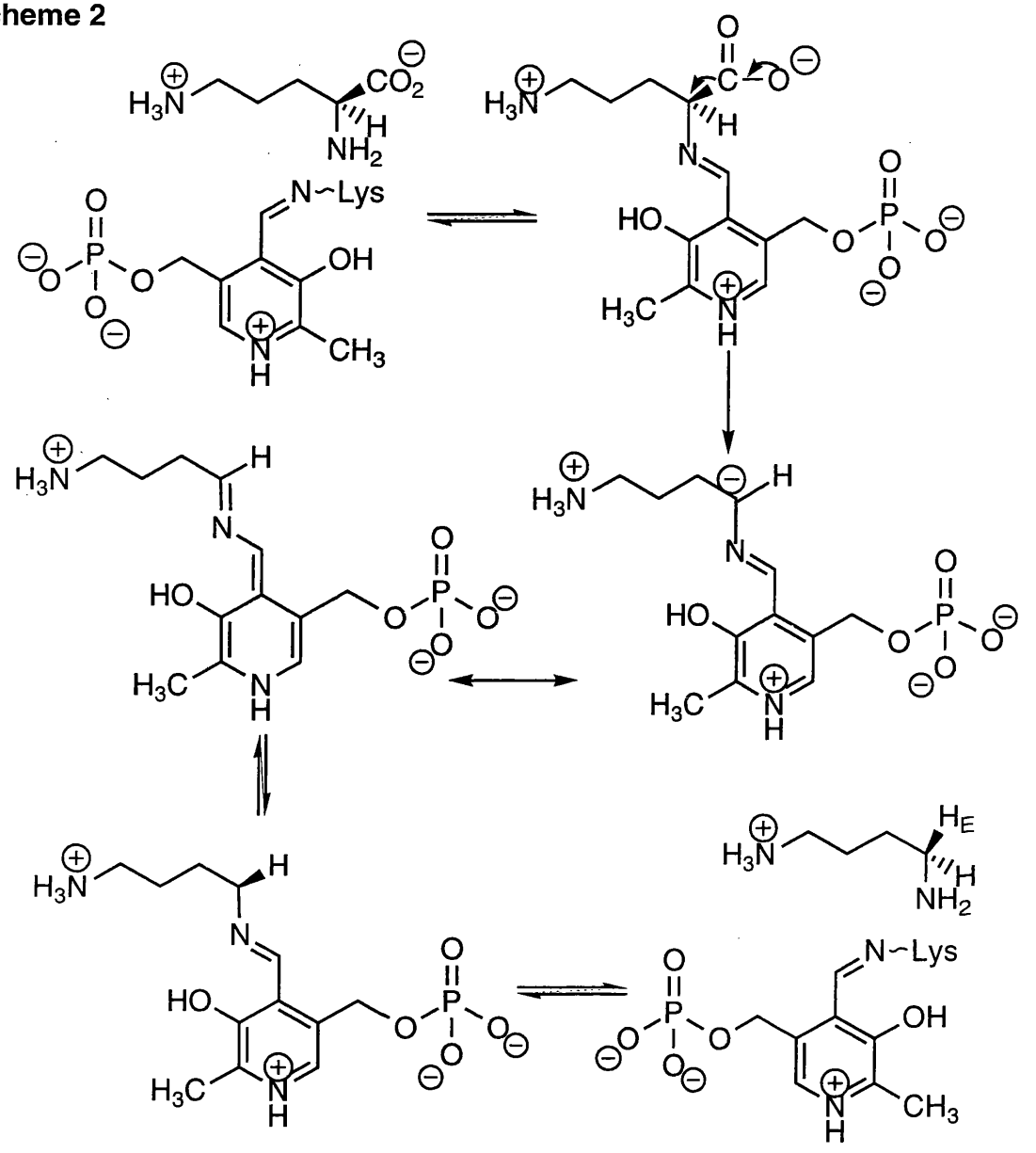
ODC activity has been found in plants, protozoa, fungi and mammalian tissue. The enzymes will differ in each case but all ODC enzymes that have been investigated require pyridoxal phosphate (PLP) **(32)** as a co-factor for activity. PLP is likely to sit in a 'pocket' of the enzyme so ornithine can approach and undergo decarboxylation with the minimum of hindrance. ODC exhibits a high degree of selectivity for ornithine as a substrate. Putrescine, the product of the reaction, spermidine and spermine are all weak competitive inhibitors of ODC.[47]



32

The mechanism for the action of ODC on ornithine is shown in **Scheme 2**.[47] The co-factor decarboxylates ornithine with retention of configuration. The hydrogen ($\mathbf{H_E}$) introduced by the conversion has the same stereochemical position as the carboxyl group. PLP is shown linked to the terminal amino group of a lysine residue of the enzyme. When a substrate appears the lysine detaches to leave the aldehyde and a Schiff's base is formed between the amino group ornithine and the aldehyde group of PLP. The bonds to the α-carbon of ornithine are weakened due to the attraction of the electrons towards the cationic nitrogen in the pyridoxal ring system and cleavage of the carboxyl group releases carbon dioxide; this reaction is irreversible. The resulting intermediate has extended conjugation. Protonation of the α-carbon gives an imine which is hydrolysed to give putrescine and regenerate PLP enzyme complex.[47]

Scheme 2



2.9 ODC Inhibitors

Since the first publication dealing with ODC inhibitors by Skinner and Johansson in 1972,[48] three approaches have been investigated in order to try to inhibit ODC.[41]

1. Synthesis of analogues of the substrate ornithine and the product putrescine as potential competitive inhibitors.

2. Synthesis of compounds capable of interacting or combining with the enzyme co-factor (PLP).

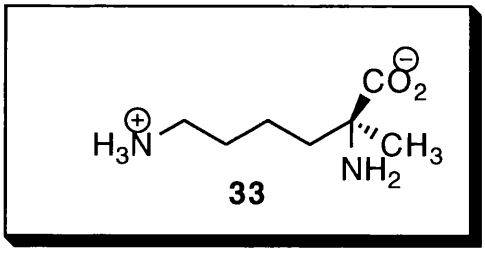
3. Design and synthesis of enzyme activated inhibitors.

The compounds of the first two areas are reversible inhibitors, while those in group three are irreversible inhibitors.

Most success has been achieved with the third group, enzyme activated inhibitors. Each group will be discussed briefly in turn.

2.9.1. Reversible Inhibition with Analogues of Ornithine and Putrescine

The first compound to be reported of this class was α-methylornithine **(33)**.[49] It is decarboxylated 6000 times slower than ornithine and also produces gradual inactivation of ODC. The inactivation of ODC is achieved by converting PLP Into pyridoxamine phosphate.



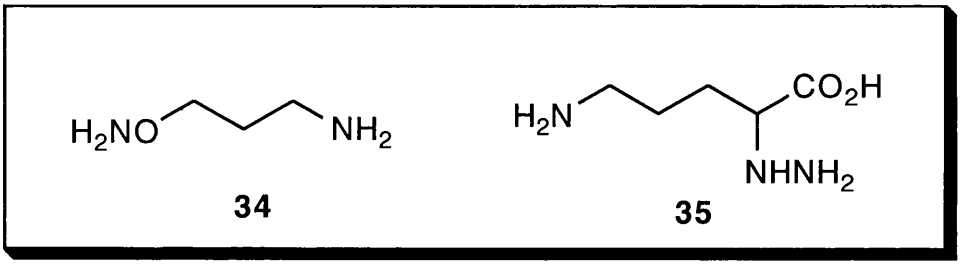
33

The only compounds of this class that have shown a greater inhibitory effect are (*E*)-dehydro-analogues of ornithine and putrescine.[41, 47, 50] The (*E*)-dehydro-analogues have the carbon -carbon double bond between carbon atom three and carbon atom four.

2.9.2 Reversible Inhibitors that Interact with the PLP Co-factor

The target for this area of work is the first step in **Scheme 2**, *i.e.* the reversible formation of a Schiff's base between the α-amino carbon of ornithine and the aldehyde group of PLP.

One of the most potent ODC inhibitors of rat liver ODC is 1-aminooxy-3-aminopropane **(34).** However, it also inactivates *S*-adenosylmethionine decarboxylase and spermidine synthase indicating a lack of specificity for ODC. α-hydrazinoornithine **(35)** is another potent ODC inhibitor but again it shows a lack of specificity as it also inhibits a number of other PLP dependent enzymes.[51]



The origin of the inhibitory effect of these compounds is unclear but it may be due to them forming a stable adduct with PLP and thereby mimicking some transition state along the reaction co-ordinate.[51]

The problems associated with lack of specificity and the inability of these compounds to block polyamine biosynthesis *in vivo* as well as they do in cell cultures means less attention is now being paid to reversible inhibitors. Most current work is being focused in the area of enzyme-activated inhibitors.[51]

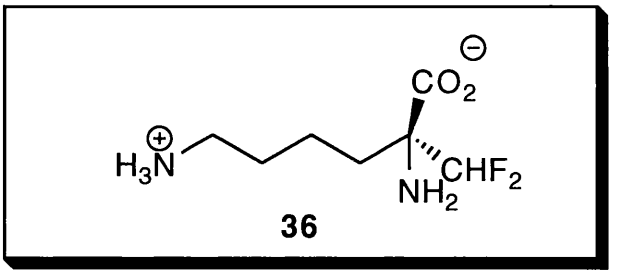
2.9.3. Irreversible Enzyme-Activated Inhibitors

The above nomenclature was coined by Merrell Dow chemists who took the initiative in this area. Enzyme-activated inhibitors are chemically inert pseudosubstrates of the target enzyme. Incorporated into their structure is a latent group that is transformed by catalytic turnover into a species that eventually inactivates the enzyme.[41]

Irrefutable proof that a compound is an enzyme-activated inhibitor requires the agreement of a number of pieces of evidence which are difficult, but not impossible to collect. However, reasonable evidence can be obtained from simple kinetic experiments on the crude enzyme.[41, 47]

Several enzyme-activated inhibitors of ODC have now been prepared, the most important being α-difluoromethylornithine (DFMO) **(36).** It was synthesised in the early 1970s and initially it was used in studies on animal tumours and it was also found to be a highly effective treatment of all stages of sleeping sickness caused by *Trypanosoma brucei gambinese.* It has been found to be an

effective antifungal agent in many situations. Most of the important studies on the inhibition of ODC over the past decade have been carried out using DFMO.[47]



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Both enantiomers of DFMO inhibit mammalian ODC, but (-)-DFMO is much more potent than the corresponding (+)-DFMO enantiomer.

The *in vitro* reduction of fungal growth by DFMO has been reported by a number of workers, but many fungi appeared to be relatively insensitive to it. A variety of explanations has been proposed to account for this insensitivity. Two of the most convincing are as follows.[41]

1. A large intracellular concentration of DFMO results in a reduced effectiveness of the inhibitor due to competition for ODC.

2. Alternatively a different route for putrescine biosynthesis exists. This has been suggested for the insensitivity of the fungus *Ceratocycstis ulmi* in Dutch Elm disease which has been attributed to ADC activity.

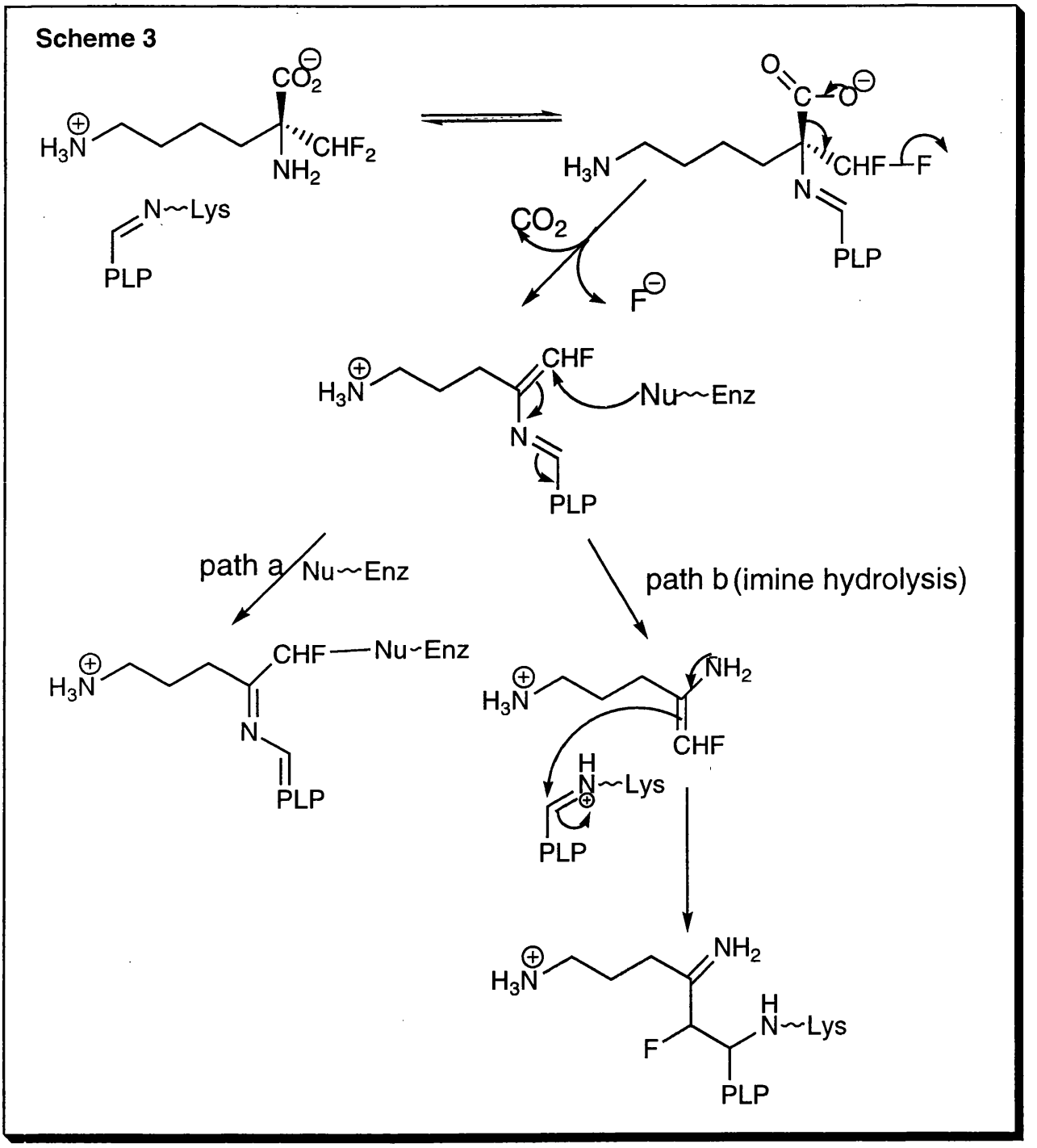
This can be misleading as *in vitro* growth of fungi on a cell culture and *in vivo* work on a plant are two very different situations. In *in vitro* work fungal mycelia are exposed to the inhibitors, on the plant. The inhibitor may affect germination and subsequent early development of the fungus on the plant.

Studies have shown that DFMO has powerful fungicidal activity. It is most active against rusts and powdery mildews and it has also shown good activity against *Botrytis cinera*. It has been shown that as little as 25 g of DFMO per acre would be sufficient to protect bean plants from the rust fungus *Uromcyces phaseoli*.[41]

The mechanism by which DFMO inactivates ODC is unclear. Two alternative mechanisms have been suggested **(Scheme 3)**. The original mechanism is shown in path a. If this was the correct mechanism then the enzyme-inhibitor adduct should be stable under dialysis conditions. The alternative pathway, path b was suggested by Likos in 1982. If inactivation of

ODC occurred by this mechanism the enzyme-inhibitor adduct formed should easily be hydrolysed during dialysis.[52]

Scheme 3

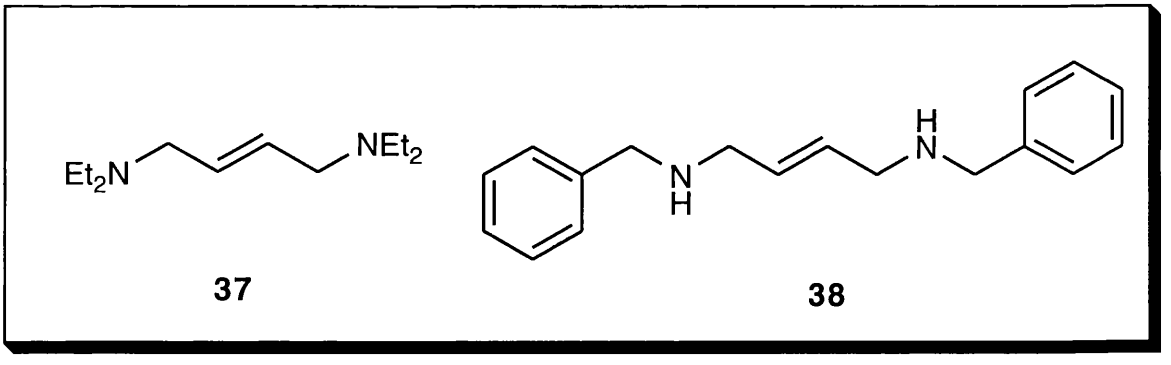


2.10 Use of Polyamine Analogues to Inhibit Fungal Growth

Some of the physiological effects of polyamines were outlined in section **2.7.** These led some to believe that polyamine analogues varying slightly from the naturally occurring compounds might be able to reduce cell growth by one or more of the following mechanisms: (1) inhibiting polyamine biosynthesis; (2) regulating biosynthetic enzymes; (3) competing for polyamine binding sites related to cell proliferation; and (4) binding at polyamine sites with the resultant disruption of macromolecular structure and proliferation.[41]

A number of papers have reported polyamine analogues which possess antitumour and antiprotozoal activity.[43, 44, 53] Of more relevance here are reports of polyamine analogues inhibiting fungal growth.

Compounds based on putrescine have shown considerable fungicidal activity. It was noted that the commercially available compound keto-putrescine controlled a number of economically important plant pathogens. The synthetic putrescine analogues (*E*)-*N, N, N', N'*-tetraethyl-1,4-diamino-2-butene (*E*-TED) **(37)** and the (*Z*) isomer (Z-TED) and BED **(38)** were among a group of polyamine analogues that controlled at least five important crop fungi including *Erysiphe graminis*[54] and *Phytophthora infestans*[55]in both glasshouse and field conditions. A number of alicyclic diamine analogues of putrescine have shown excellent results in reducing mildew infections on barley seedlings.[41]



37 **38**

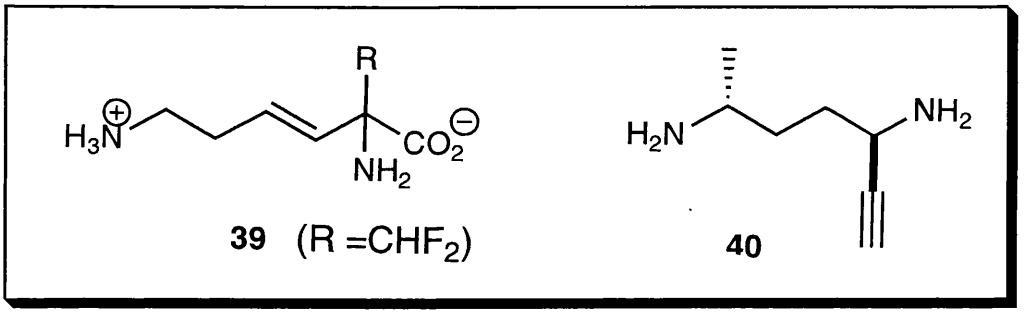
2.11 Polyamines in Antimalarial Chemotherapy

The discovery of classes of active chemical compounds radically different from those used in the past to treat malaria is an exciting prospect. They may prevent the problems caused by cross-resistance between antimalarial drugs. The strategies described in sections **2.9** and **2.10** to control fungi have also been

investigated for applications in malarial chemotherapy. Each will be discussed briefly.

2.11.1 The Use of Inhibitors of Polyamine Biosynthesis and Function as Antimalarial Agents

ODC inhibitors are being investigated as potential antimalarial agents. It was found that DFMO, α-monofluoromethyl-3,4-dehydroornithine methyl ester **(39)**, its ethyl ester and (1*R*, 4*R*)-δ–methyl–α–ethynyl putrescine **(40)** could all inhibit erythrocytic schizogony of *P. falciparum in vivo*. Only DFMO was effective at limiting erythrocyctic schizogony of *P. berghi in vivo.* The effects were due to the rapid depletion of putrescine, spermidine and spermine which slowed proliferation rates in these cells. Results showed that the greater the reduction in spermine, the greater the reduction in parasitaemia. Although parasitaemia levels could be decreased by as much as 55% there was no increase in the survival times of the mice used in the experiments.[56]



39 (R =CHF_2) **40**

Gillet *et al.* examined the effects of DFMO on exoerythrocytic schizogony using *P. berghei* as a model. They found that it could inhibit exoerythrocytic schizogony in mice and would also completely inhibit the sporogonous life cycle in the mosquito.[57]

The inhibition of other polyamine biosynthetic enzymes is being looked at as potential targets for antimalarial chemotherapy. Irreversible inhibitors of *S*-adenosylmethionine can inhibit the growth of chloroquine-sensitive and chloroquine-resistant *P. falciparum* in equal measures. These drugs worked during the erythrocytic cycle.[58]

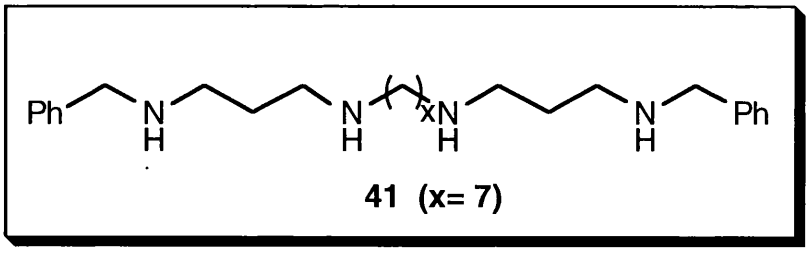
Some very encouraging results have been obtained in these areas, which suggest that agents that deplete the parasite's polyamine levels may be a viable form of chemotherapy. Work in this area has led to the development of novel treatments for *Trypanosoma gambiense* infections in humans.[58]

2.11.2 Polyamine Analogues as Potential Antimalarial Agents

Some excellent results have been obtained from the screening of a number of polyamines against *P. falciparum* and *P. berghei.* Perhaps the most important leads have been synthesised by Bitonti *et al.* at the Merrell Dow research institute, Cincinnati. They have shown that a number of bis(benzyl)polyamine analogues are potent inhibitors of both chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum* and *P. berghei in vitro* and *in vivo.* [59, 60]

All the compounds tested were spermine analogues that have elongated central polymethylene chains and either benzyl substituents or terminal free amines. The activity of the analogues against *P. falciparum* increased as the length of the central polymethylene chain was lengthened from 4 to 12 carbon atoms. The general structure of the spermine bis(benzyl)analogues is shown below, the greatest activity was shown when X = 7; this compound **(41)** was as potent as quinine.

Importantly it was found that combination therapy with **(41)** and DFMO resulted in cures for 47 out of 54 mice infected with *P. berghei.* Cured mice were found to be immune to reinfection with the same strain of parasite four months after the initial infection and drug-induced cure. The combined treatment was also found to be effective against severe and established infections of *P. berghei* in mice.[60]



41 (x= 7)

Studies involving DNA and RNA synthesis indicated that the major cytotoxic event may be the direct binding of the compound to DNA with the subsequent disruption of macromolecular synthesis and cell death. Inhibition of ODC activity may also in part account for the action of compounds such as **(41)**.

It was found that the more water soluble polyamine analogues with free amine ends are at least 1000 times less potent than their bis(benzyl) counterparts. The increased lipophilic nature of the bis(benzyl) compounds may explain their greater efficacy as antimalarial agents as they will be more able to be carried into the human erythrocytes.[61]

A great deal of work has to be completed before compounds of this type could be used in the treatment of malaria. They are, however, producing a number of exciting leads.

3

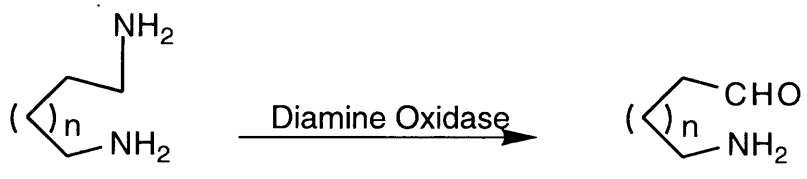
Synthesis of 2-(Aminomethyl)pyridines and 2-(Aminoethyl)pyridines.

3.1 Introduction

The direction taken in this work was mainly as a consequence of the encouraging antifungal testing results obtained by Dr Dale Walters. Initial leads were established from a selection of diamines synthesised by Dr Steven Gavin, who was a past worker in the polyamine group.[62]

Gavin's work involved investigations of the enzyme diamine oxidase (DAO). This enzyme catalyses the oxidative deamination of diamines to their corresponding aminoaldehydes **(Scheme 4)**.

Scheme 4



Two areas of his work were of particular relevance to this project:

1. oxidation of aromatic compounds with amine side chains by pea seedling diamine oxidase; and
2. inhibition of pea seedling diamine oxidase.

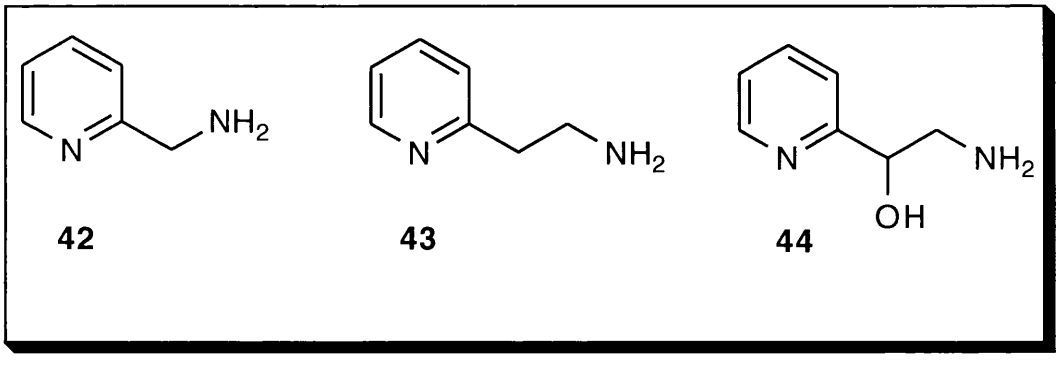
The first area involved the synthesis of a range of pyridine, quinoline, thiophene and pyrrole derivatives with amine side chains and testing them as substrates for pea seedling diamine oxidase. From a spectrophotometric assay values of V_{max} and K_M were obtained for the oxidation of each substrate using pea seedling DAO. The value of V_{max} is the maximal rate of oxidation and is an indicator of the ease of oxidation rate of various substrates. The K_M is a measure of the strength of the enzyme-substrate complex and indicates the binding efficiency of the substrate to the enzyme. This gave information on

the enzymatic process and the nature of the enzyme's binding site. More importantly for our work was that a comparison of the kinetic data obtained from the various aromatic substrates allowed for studies on how the binding affinity and the rate of oxidation changed with the structures of the substrates.[62] This led into work in the second area and consequently to the synthesis of the compounds discussed in this chapter.

As indicated in chapter 2 polyamines are known to be essential for normal growth and replication and diamine oxidase is vital in polyamine metabolism. Inhibition of DAO could therefore have a dramatic effect on polyamine metabolism and hence cell growth.

A number of the compounds that were shown to bind efficiently to pea seedling diamine oxidase were tested as inhibitors. The majority of the compounds tested were found to inhibit the DAO-catalysed deamination of putrescine **(Scheme 4, (n=1))** and were shown to be competitive inhibitors.[62]

Of the compounds screened for antifungal activity by Dr Dale Walters the following were chosen as lead compounds: 2-(aminomethyl)pyridine **(42)**; 2-(aminoethyl)pyridine **(43);** and (±)–2-hydroxy-2-(2'-pyridyl)ethylamine **(44)**. All of Gavin's samples were tested as their dihydrochloride salt .



42 **43** **44**

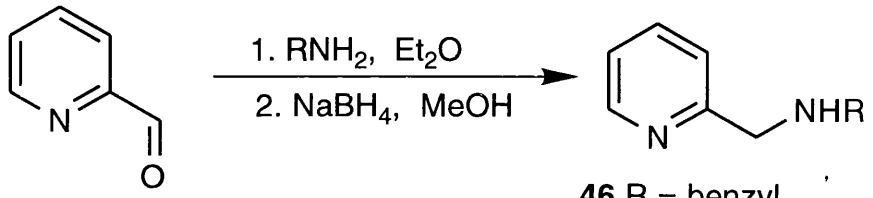
In this chapter substitution of the terminal nitrogen of each of the lead compounds will be discussed together with future work to be considered in this area.

3.2 Synthesis of 2-Substituted Aminomethylpyridines

The choice of method for the synthesis of the above compounds was straightforward. Earlier experience within our group and numerous examples in the literature suggested that reductive amination would give the desired compounds easily and in high yield and this proved to be the case. [63, 64]

Four compounds were synthesised by the route shown below in **scheme 5**.

Scheme 5



45

46 R = benzyl
47 R = cyclohexylmethyl
48 R = ^{t}butyl,
49 R = ^{n}butyl

All starting materials were commercially available and needed no further purification. It was found that the reaction went in highest yield when dry ether was used as the solvent for intermediate imine formation. The reaction also proceeded in dry benzene using a Dean and Stark apparatus to remove the water formed in the reaction. However, the reaction was considerably slower in this solvent.[64]

The yield of free base isolated after working up the reaction was typically between 78-85%. When the intermediate imine was dried with Drierite™ before treatment with sodium borohydride the yield rose to 92%. Drierite™ is anhydrous calcium sulfate which is available from the Aldrich Chemical company.

It was envisaged that purification could best be achieved by forming the corresponding dihydrochloride salts and recrystallising them to remove impurities. This however was not possible as the dihydrochlorde salts were very hygroscopic. In order to avoid this problem the oxalate salts were formed by treating the appropriate diamine with one equivalent of oxalic acid. One

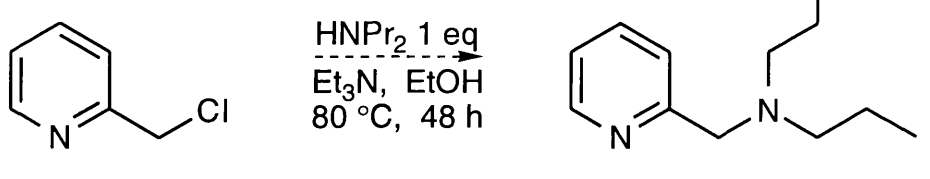
recrystallisation from water or ethanol gave the oxalate salts in an analytically pure form.

The compounds synthesised were screened for antifungal and antimalarial activity. To increase the chances of observing antimalarial activity we chose to synthesise diamines **(46)** and **(47)** as earlier work within the group and the literature suggested that optimal *in vivo* antimalarial activity was displayed when the diamine contained these large hydrophobic moieties. It is believed that the lipophilic character of these groups allows the compounds to pass into the parasitised erythrocyte more easily.

3.3 Attempted Synthesis of 2-(*N,N*-Dialkylamino)methylpyridines

Attempts were made to synthesise the corresponding diamines with two alkyl groups instead of one, *i.e.* 2-(*N,N*-dialkylamino)methylpyridines. A literature precedent suggested we could use commercially available 2-(chloromethyl)pyridine **(50)** to make the desired product **(51)** by a simple S_N2 displacement by heating one equivalent of the appropriate secondary amine and an excess of triethylamine in ethanol at 80 °C for 48 hours.[65] This reaction was tried a number of times with the reagents used in the original paper, but each time the reaction ended in failure, even after correction of the errors in the quantities made in the paper. 2-(Chloromethyl)pyridine **(50)** was used as its hydrochloride salt and as soon as base was added a red colour developed in the flask which persisted. TLC of the reaction mixture and ^1H NMR spectra of the crude products indicated a large number of products had been formed on each occasion. An example of the reactions attempted is shown in **Scheme 6** below.

Scheme 6



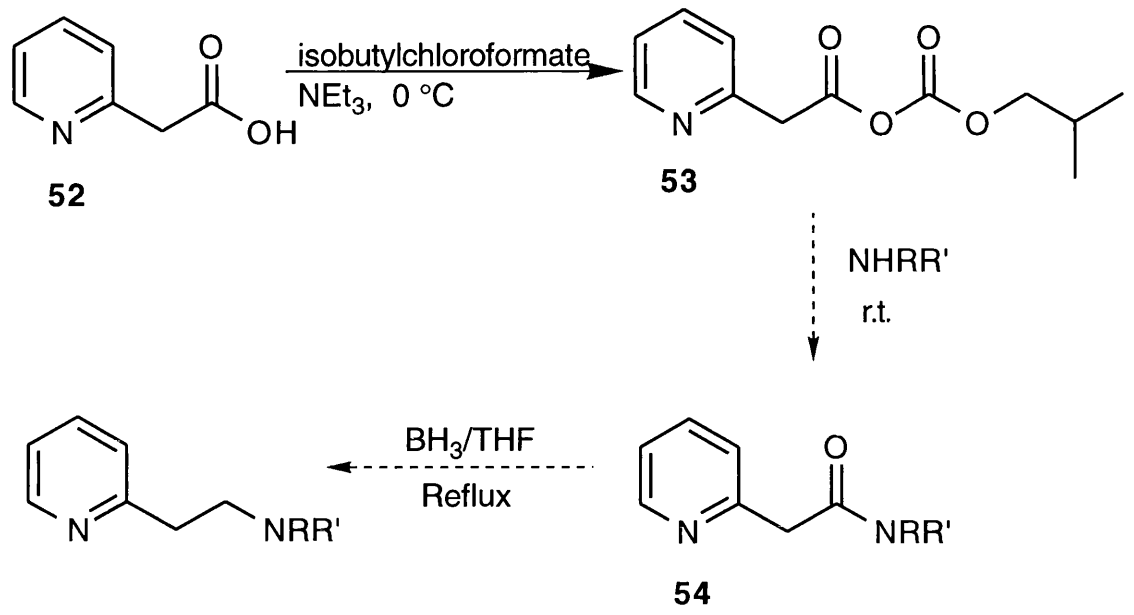
50 **51**

3.4 Synthesis of 2-Substituted Aminoethylpyridines

Initial investigations in this area were carried out by Kieron Brear, another PhD student in our group.[66] His strategy to synthesise compounds of

this class was to start from 2-pyridylacetic acid and convert it into the mixed anhydride which was then cleaved by an amine to form the amide, which after reduction with BH_3 yielded the desired diamine. This is shown in **Scheme 7**.

Scheme 7



Brear experienced difficulty with this route, most commonly at the second stage, namely the conversion of the mixed anhydride **(53)** into the corresponding amide. This could have been due to conducting this reaction at room temperature. With greater experience we found it was best to carry out this reaction at 0 °C.

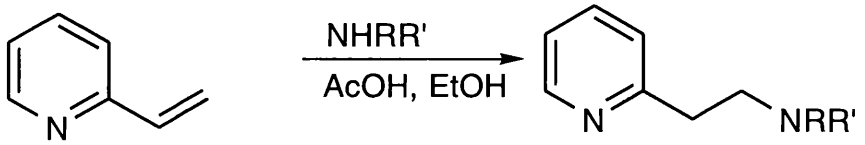
Brear tried two further methods to make amide **(54)**. Both routes involved making the corresponding acid chloride. Using thionyl chloride as the chlorinating agent led to a complex mixture of products after the amidation step and using freshly distilled oxalyl chloride as the chlorinating agent also proved unsuccessful.

The solution to this problem lay in a reaction that is well known in the literature, namely the pyridylethylation of active hydrogen compounds.[67, 68] The reaction has been shown to work for the condensation of 2-vinylpyridine with ketones. This is a Michael condensation reaction.

The reaction is also possible between 2-vinylpyridine and primary or secondary amines using acetic acid as the condensing agent **(Scheme 8)**.

All three reactions were first reported by Levine and co-workers in the mid 1950s.[67] It was found that amines such as pyrrole and 2,5-dimethypyrrole were too weakly basic to be pyridylethylated under acidic conditions but the condensation reaction with 2-vinylpyridine can be achieved by using sodium.

Scheme 8

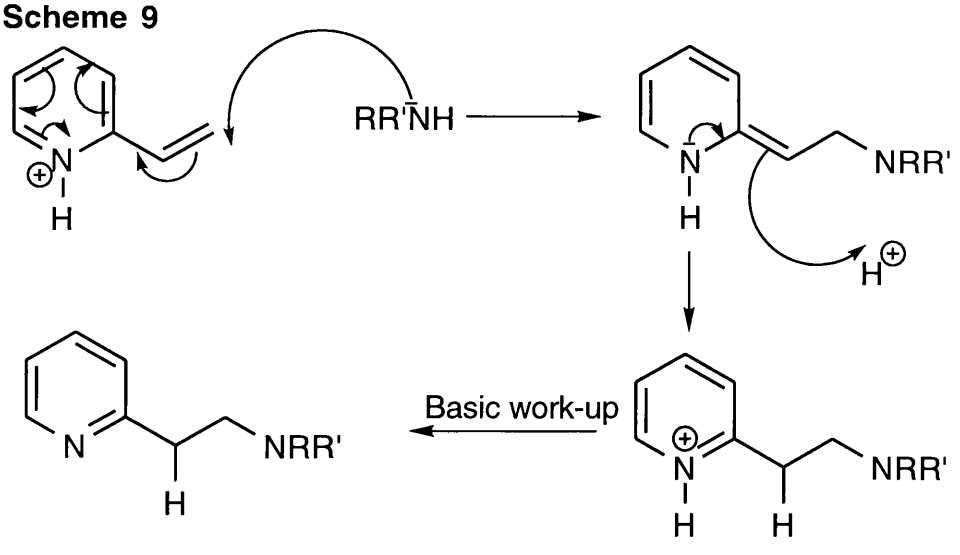


55

56 R = benzyl, R' = H
57 R =cyclohexylmethyl,R'= H
58 R = ^{t}butyl, R' = H
59 R = ^{n}butyl, R' =H
60 R = ethyl, R' = ethyl

We believe that the reaction follows the mechanism outlined in **Scheme 9**. Initially 2-vinylpyridine is protonated on nitrogen by acetic acid then undergoes the Michael reaction with either a primary or secondary amine thereby reducing the aromatic character of the molecule. Full aromaticity is regenerated by a proton transfer reaction. A basic work-up affords the compound as the free base.

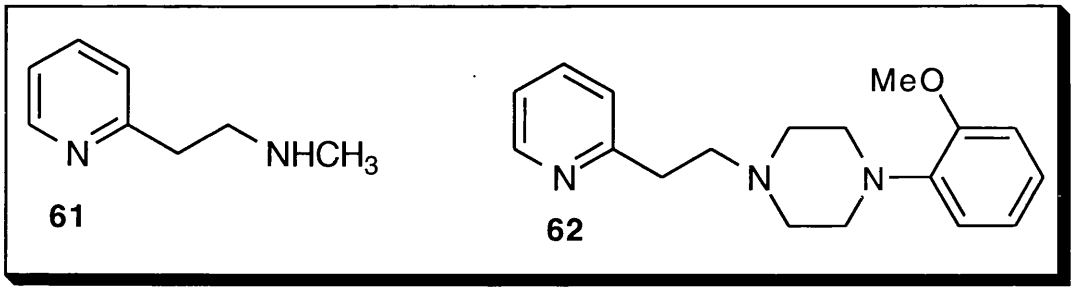
Scheme 9



R = alkyl, benzyl or cyclohexylmethyl
R' = alkyl or hydrogen

In order to carry out this reaction we decided to use the method of Shiozawa and co-workers.[69] They were investigating analogues of betahistidine **(61)** as potential antivertigo agents. Betahistidine has a histamine like action and has found clinical use as an antivertigo agent. A number of analogues of betahistidine have been synthesised to investigate their biological effects. Some analogues have also been found to possess tranquillising or a antihypertensive action.

Shiozawa and co-workers found that the 4-(2-methoxyphenyl) piperazine **(62)** group was the most effective amine moiety for activity in their study. A number of structure-activity relationships are discussed in their paper.



61 **62**

A typical procedure by Shiozawa and co-workers involved heating an equimolar solution of 2-vinylpyridine, amine, and acetic acid in ethanol at reflux for 5 hours. The reaction was worked up by evaporation of the solvent *in vacuo* followed by basification of the residue by treatment with 5 M NaOH and extraction with chloroform. After drying and removal of the solvent the product was purified by recrystallisation from an appropriate solvent.

When attempting this reaction we found that five hours was not long enough to allow the reaction to reach completion. TLC analysis indicated the presence of product after five hours but not all of the 2-vinylpyridine had been consumed and excess of primary or secondary amine remained. An older reference suggested heating the reactants at reflux for a period of eight hours, whether using a primary or secondary amine.[67] However TLC again indicated that the reaction had not reached completion and even heating overnight at reflux did not consume all the reactants.

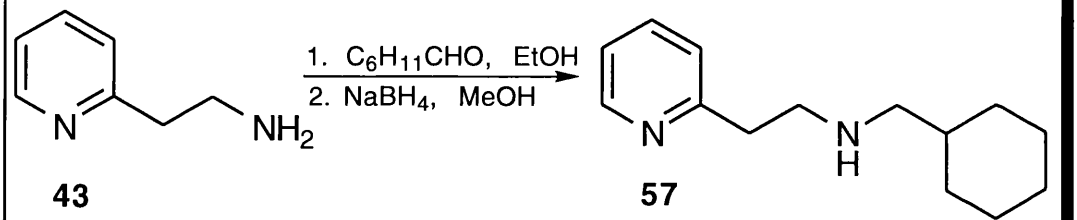
It was decided to allow the reaction to proceed for an appropriate length of time, as judged by TLC, which was typically 18-20 hours and any unreacted starting material was removed by flash column chromatography.

Obviously as the reaction did not go to completion a lower yield was expected. The work of Shiozawa and co-workers indicated that the yield of the reaction was highly dependent on the structure of the amine to be pyridylethylated, with a range between 41-73%. Our work would also suggest this to be the case. A yield of 54% was achieved for the pyridylethylation of benzylamine to give **(56)** and the lowest yield was 35% in the preparation of **(60)** using the secondary amine, diethylamine. A brief survey of the literature suggests that the pyridylethylation reaction works best for primary rather than secondary amines and our work supports that observation.

It should be noted here that in going from 2-substituted aminomethylpyridines **(46-49)**, via a reductive amination reaction to 2-substituted aminoethylpyridines **(56-60)**, via a Michael reaction, *i.e.* the addition of one methylene unit we see a decrease in yields from over 90% to less than 40% in some cases. Purification is also more time consuming and expensive as compounds **(56-60)** have to be subjected to column chromatography, whereas compounds **(46-49)** can be purified by crystallisation.

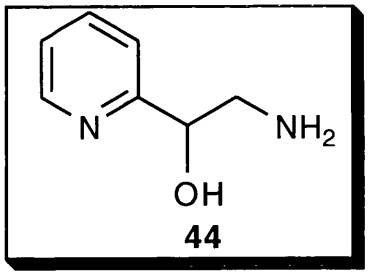
Compounds such as **(56)** and **(57)** can be made by the reductive amination route using commercially available 2-(2-aminoethyl)pyridine and the appropriate aldehyde. As a comparison of the Michael type reaction with the reductive amination, compound **(57)** was synthesised by both routes. The yield achieved by the reductive amination route **(Scheme 10)** was 95% as compared to 36% from the Michael reaction.

Scheme 10



43 **57**

3.5 Synthesis of (±)-2-Hydroxy-2-(2'-pyridyl)ethylamine (44)



44

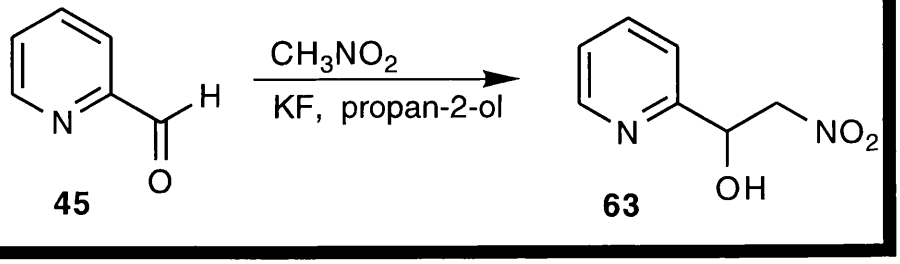
After initial evaluation of the antifungal properties of (±)-2-hydroxy-2-(2'-pyridyl)ethylamine hydrochloride **(44)** larger quantities were required by Dr. Dale Walters for further assays and testing. We also devised a route to prepare substituted analogues.

We decided to synthesise **(44)** by much the same method as Gavin as he found the reaction reasonably efficient with an overall yield of 54% for two steps.

3.6 Synthesis of (±)-2-Hydroxy-2-(2'-pyridyl)nitroethane (63)

The first step in our synthesis involved the condensation of 2-pyridinecarbaldehyde **(45)** with nitromethane, using potassium fluoride as a base to give (±)-2-hydroxy-2-(2'-pyridyl)nitroethane.[70] The reactants were stirred at room temperature for three hours, then at 40 °C for one hour. After purification by silica gel chromatography and crystallisation, the product, a β-nitroalcohol was isolated in 60% yield **(Scheme 11)**.

Scheme 11



45 **63**

The above reaction is an example of the Henry reaction, or as it is sometimes known the nitroaldol addition reaction.[71] Deprotonation at the carbon adjacent to the nitro group can be achieved with a number of bases

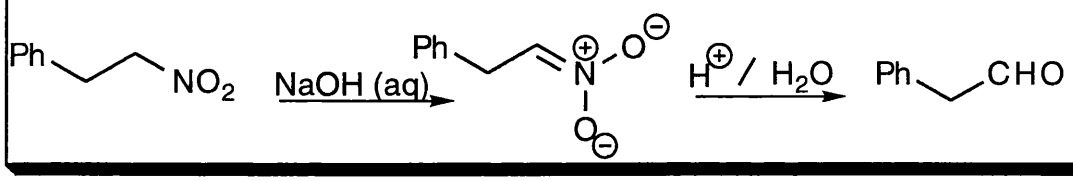
(the pK_a of nitromethane is 10.2). The resultant anion is nucleophilic and hence will add to an aldehyde or ketone. This is one of the classical C-C bond forming processes.

Often the corresponding nitroalkene can be generated *in situ* under the reaction conditions.[72] There was no evidence of this reaction occurring under our conditions. This dehydration reaction can also be facilitated by a number of reagents including *N,N'*-dicyclohexylcarbodiimide (DCC)[73], pivaloyl chloride,[74] and phthalic anhydride.[75] A combination of the Henry reaction followed by dehydration to form the alkene is known as a Knoevenagel reaction.[72]

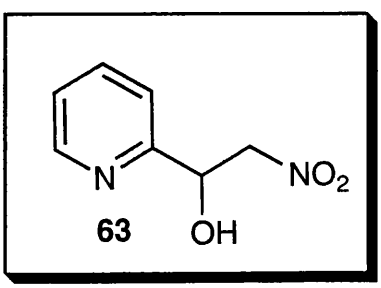
As well as being a classical C-C bond forming reaction the nitro group introduced via a Henry reaction is extremely versatile in synthesis as it can be transformed into a number of other functional groups. [76]

The nitro group can be replaced by hydrogen in a denitration reaction or converted into a carbonyl group by the classical Nef reaction **(Scheme 12)**.[76] This reaction requires vigorous conditions and reductive hydrolysis with $TiCl_3$ (aq) is usually a better way of generating the aldehyde or ketone. [76] Nitro groups can be reduced to generate oximes,[77] ketones,[72] hydroxylamines[78] or amines.[76] Primary nitro groups can be dehydrated to produce nitrile oxides, which are important compounds in cycloaddition reactions. [79]

Scheme 12



3.7 Reduction of (±)-2-Hydroxy-2-(2'-pyridyl)nitroethane (63)



63

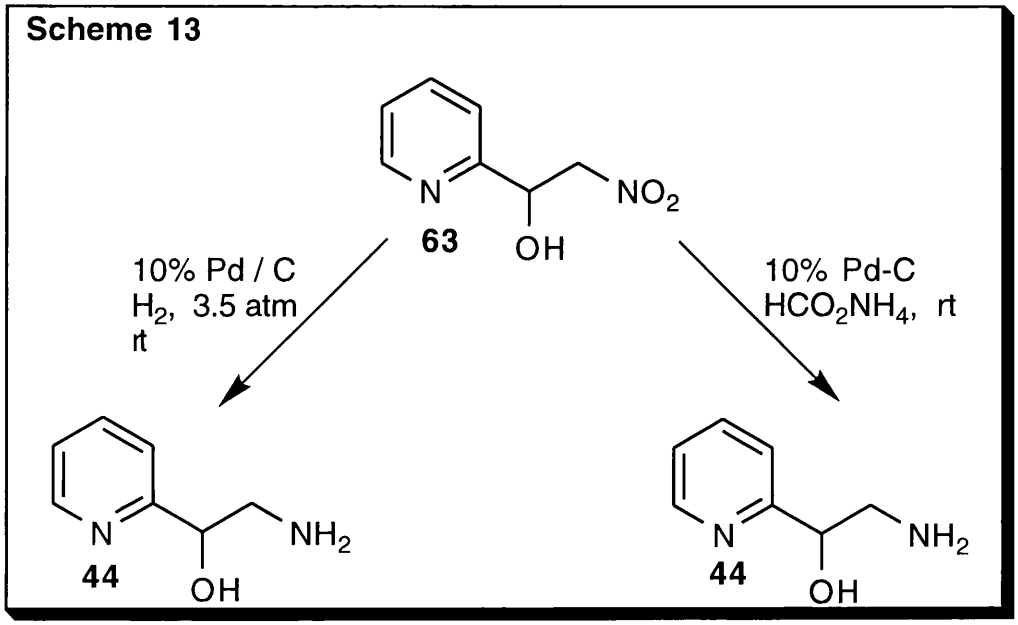
The conversion of the β–nitroalcohol **(63)** to the corresponding β-aminoalcohol **(44)** could have been achieved by a number of reagents including lithium aluminium hydride.[76] We chose to use a method based on catalytic hydrogenation. We investigated two possibilities: catalytic hydrogenation of the substrate at medium pressure;[80] and transfer hydrogenation at atmospheric pressure using ammonium formate as the source of hydrogen.[81] These reactions are shown in **Scheme 13**.

Initially transfer hydrogenation using ammonium formate found use in peptide synthesis for the rapid removal of hydrogenolysable protecting groups under mild conditions. A variety of protecting groups including benzyl, ^{t}butyl and nitro were removed in under 10 minutes in yields above 90%.[82]

This work was exploited by a number of people including Barrett and Spilling who converted a series of β–nitroalcohols into the corresponding β–aminoalcohols with retention of configuration by transfer hydrogenation using ammonium formate and palladium on carbon.[83] Reductions of this type had usually been carried out with hydrogenation over Raney nickel or platinum. In most cases the reaction was complete well within an hour.[82]

The major advantage of this method for reducing a nitro group is that it is rapid and selective in the presence of other functional groups such as nitriles and carbonyl groups. Ammonium formate is inexpensive, stable and non-toxic and can be used with either palladium-carbon or Raney-nickel catalysts.[81]

Scheme 13

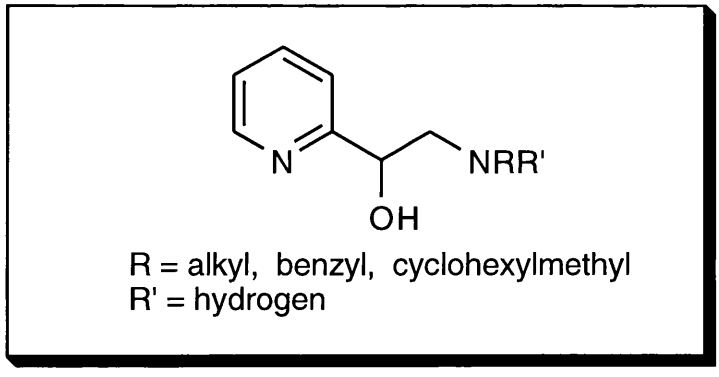


It is believed that the active species in the process is the formate anion and the various systems developed use ammonium formate as the hydrogen donor and palladium on carbon as the heterogeneous catalyst.[84]

We attempted the reaction a number of times on **(63)** using ammonium formate with palladium-carbon for various time periods between 1-48 hours without success. A typical procedure involved dissolving the nitroalcohol **(63)** in methanol and then adding 50 mg of palladium on carbon followed by 5 equivalents of ammonium formate and stirring the resultant mixture at ambient temperature. We expected the reaction if successful to be complete within a few minutes, but TLC indicated only the presence of starting material. This was unexpected as examples of the reaction with benzyl compounds are found in the literature.[85] However, we could not find any examples with pyridyl substrates.

As Gavin had completed the transformation successfully using catalytic hydrogenation with palladium on carbon at 3.5 atm we decided to synthesise the desired β-aminoalcohol by this method. This reaction is operationally simple. A mixture of the β-nitroalcohol and palladium on carbon dissolved in methanol was hydrogenated for 17 hours at ambient temperature. The product was separated from the palladium on carbon by filtering the reaction mixture through a plug of Celite.[62] Purification of the product was achieved by formation of the corresponding oxalate salt and its subsequent crystallisation.

3.8 Synthesis of Substituted (±)-2-Hydroxy-2-(2'-pyridyl)ethylamines

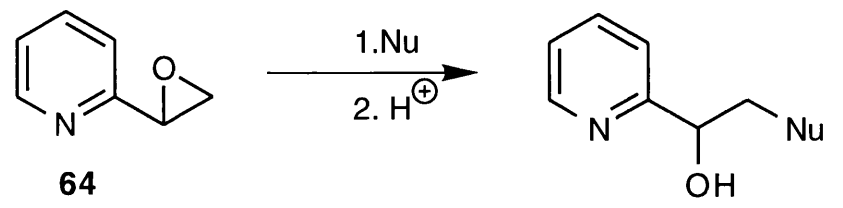


R = alkyl, benzyl, cyclohexylmethyl
R' = hydrogen

The route used to synthesise **(44)** gave us the desired compound in reasonable yield. However, it was not amenable to the synthesis of the analogues we required for evaluation as antifungal and antimalarial agents.

We of course wanted a route that would allow us to synthesise a range of analogues. We believed this could be best achieved by the regioselective opening of the epoxide **(64)** with the appropriate nucleophile **(Scheme 14)**.

Scheme 14



64

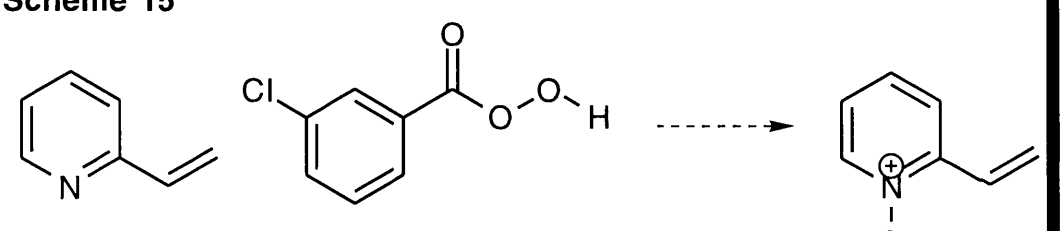
3.8.1 Synthesis of 1-(2-Pyridyl)oxirane

A number of methods exist for the synthesis of epoxides some of which could be applied to synthesise **(64)** and others which could not. A brief discussion of possible methods will follow.

3.8.2 Electrophilic Reagents

In the laboratory epoxides are usually prepared by the treatment of an alkene with a peroxyacid such as *m*-chloroperoxybenzoic acid, *m*-cpba **(65)**.[86] The alkene, 2-vinylpyridine **(55)**, is available but we believed that the reaction between it and electrophilic reagents such as *m*-cpba **(65)** would result in *N*-oxide formation.

Scheme 15



55 **65**

3.8.3 Sulfonium Methylides

Corey and Chaykovsky showed that the dimethyloxosulfonium methylide and the dimethylsulfonium methylide are nucleophiles that transfer a methylene moiety to a number of electrophilic unsaturated linkages such as C=N, C=S and in some cases C=O.[87]

The oxosulfonium methylide is less reactive and interacts with the carbonyl group of aromatic and non-conjugated aldehydes and ketones to form oxiranes. It reacts with α,β-unsaturated Michael acceptors to form cyclopropyl ketones.[87]

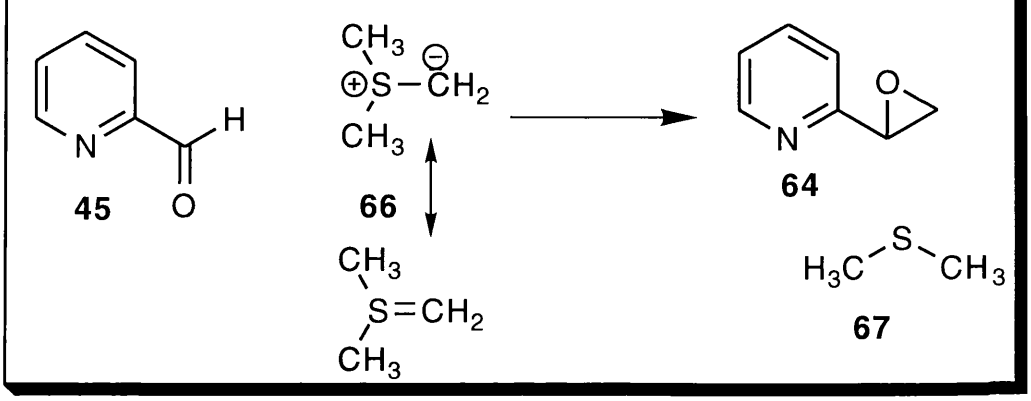
The sulfonium methylide reacts with both types of substrate to give oxiranes exclusively.[87]

Our desired epoxide **(64)** has been synthesised using either methylide and 2-pyridinecarbaldehyde. With the dimethylsulfonium methylide **(66)** a yield of 3% was reported **(Scheme 16)**. The dimethyloxosulfonium methylide gave an improved yield of epoxide, but it was still relatively poor at 17%.[88]

Both methylides can be purchased commercially or synthesised from the corresponding chloride or iodide. Dimethyloxosulfonium methylide can be synthesised by treatment of trimethylsulfonium chloride or iodide in dry dimethyl sulfoxide with sodium hydride. The reaction is carried out under nitrogen and proceeds rapidly with the evolution of hydrogen to give the ylide and sodium halide. [87]

The poor yields and the possible problems controlling odorous by-products such as dimethylsufide **(67)** meant other routes were examined.

Scheme 16



3.8.4 Lithiation of 2-Chloromethylpyridine

A short paper appeared in the literature detailing the synthesis of oxiranyl pyridines by lithiation of 2-chloromethylpyridines and their subsequent reaction with carbonyl compounds. We did not follow this procedure as experimental detail was sparse and the reaction appeared only to be applicable to the synthesis of oxiranes substituted with bulky groups.[89]

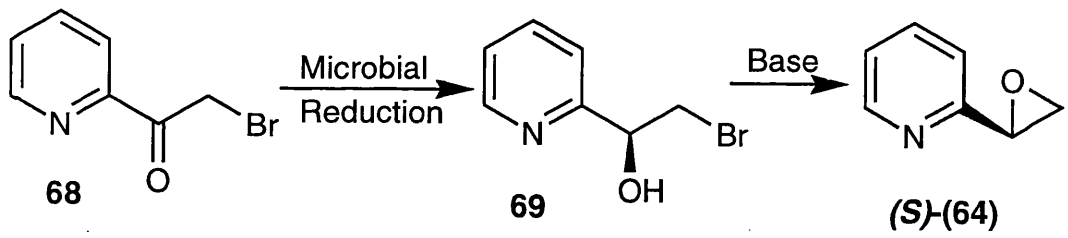
3.8.5 Enzymatic Methods

Imiuta and co-workers synthesised epoxide **(64)** in high enantiomeric excess while examining the product stereoselectivity in the microbial reduction of α–haloaryl ketones. [90]

The α–haloaryl ketone **(68)** was shaken with a two-day-old culture of *Cryptococcus macaerans* to give the bromohydrin **(69)** in enantiomeric excess of 80% or greater. Treatment of the bromohydrin with base gave the (*S*)-(-) enantiomer **(Scheme 17)**.

At this point we wanted to develop a synthesis of racemic β–aminoalcohols. However, the synthesis we did use to make racemic 1-(2-pyridyl)oxirane did rely on intermediate bromhydrin formation.

Scheme 17

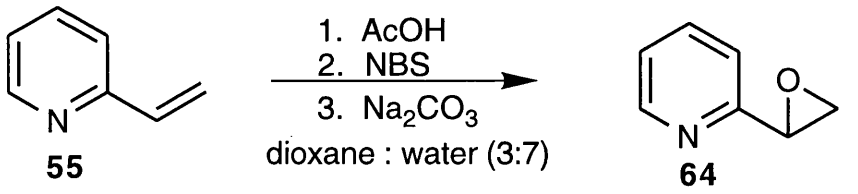


3.8.6 Epoxide Synthesis *via* a Bromhydrin

Bromohydrins can be prepared by the electrophilic addition of HO-Br to alkenes using Br_2 and water but in practice very few alkenes are soluble in water.[91]

We chose to synthesise **(64)** by adapting the methods of Hanzlik and co-workers[92] and those of Thurkauf and co-workers[93] who both synthesised 1-(2-pyridyl)oxirane. The reaction is shown below in **Scheme 18**.

Scheme 18



55 **64**

We synthesised the epoxide by treating 2-vinylpyridine with acetic acid in a 3:7 mixture of dioxane and water and then adding *N*-bromosuccinimide in small portions. The bromohydrin was not isolated, but reacted with sodium carbonate and the reaction was stirred overnight during which time the mixture became very viscous. The reaction was worked-up in a similar manner to that of Thurkauf and co-workers. When purifying the product by distillation we found it was better not to precipitate excess succinimide beforehand as this led to problems. The crude epoxide was very viscous and stuck to the sinter and to the precipitated succinimide. Distillation gave a clear oil that yellowed on standing after a few days, even when refrigerated. ^{1}H NMR spectroscopy indicated that the purity was unaffected. Thurkauf and co-workers claimed to achieve a yield of 66% for this synthesis. Typically our yield was between 45 and 55%. Our lower yield may have been a consequence of not removing the succinimide before distillation.

Synthesis of **(64)** by this method allows us to overcome the problems associated with the poor solubility of alkenes. We carried out the reaction in aqueous dioxane (other solvents commonly employed with water in this type of reaction include tetrahydrofuran[92] and dimethylsulfoxide[94]) using *N*-bromosuccinimide (NBS) as the bromine source. NBS slowly decomposes in water to generate Br_2 at a controlled rate. [96, 96] Another advantage of using NBS is that it is stable and easy to handle. Bromine could be used to form the intermediate bromonium ion but as a reagent bromine is more dangerous and difficult to handle.

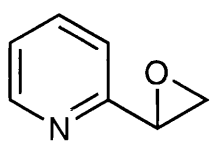
The bromonium ion is attacked by water to generate the bromohydrin. Treatment of the bromohydrin with a base effects an intramolecular substitution to afford the epoxide. [96] This final step, the synthesis of epoxides by base treatment of halohydrins, is in fact an intramolecular Williamson ether synthesis. The nucleophilic oxygen atom and the electrophilic carbon atom are in the same molecule.[96]

Having found a reliable method to synthesise large amounts of epoxide **(64)**, its reaction with the appropriate amine gave the β–aminoalcohols we desired.

3.8.7 Regiochemistry of Epoxide Ring Opening

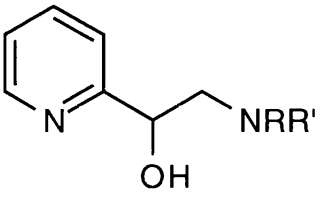
The direction in which unsymmetrical epoxides are opened is dependent on the conditions used. We wanted to open the oxide with a basic, nitrogen nucleophile and under these conditions a typical S_N2 type reaction takes place at the less hindered epoxide carbon.[97] This is shown in **Scheme 19**.

Scheme 19



64

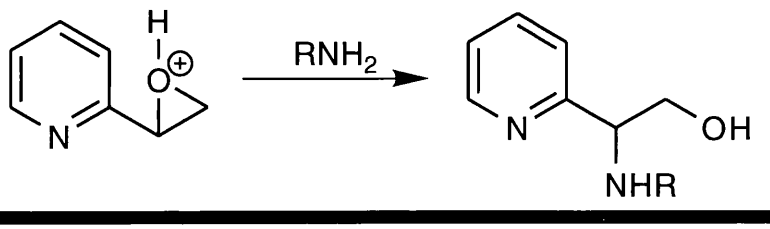
RR'NH ⟶



70 R = benzyl, R'= H
71 R =cyclohexylmethyl, R' =H
72 R =nbutyl, R'=H

In contrast if acidic conditions are employed attack of the nucleophile occurs primarily at the more substituted carbon atom. The mechanism for acid catalysed opening of an epoxide is interesting as it appears to be midway between an S_N1 and an S_N2 reaction. Studies have shown that the transition state has an S_N2 like geometry but a high degree of S_N1 carbocation character. The protonated epoxide is not a full carbocation but it is strongly polarised so that the positive charge is shared by the more highly substituted carbon atom. Therefore, the nucleophile attacks this more highly substituted site **(Scheme 20)**.[98]

Scheme 20



We wanted to synthesise β–aminoalcohols so we chose to use basic conditions to open the epoxide. We found it best to heat a solution of the epoxide at reflux in methanol with the appropriate secondary amine until all the epoxide had been consumed. Typically this took about eight hours and then the mixture was stirred at room temperature overnight. TLC indicated that an excess of secondary amine remained at completion of the reaction.

The first of the β–aminoalcohols synthesised by this method was the benzylated analogue **(70).** We worked up the reaction by dissolving the residue in ether and extracting with acid, washing the solution with ether and then adding sodium hydroxide pellets until the solution was basic. This solution was extracted with chloroform and dried with potassium carbonate. ^{1}H NMR spectra of the concentrated extract indicated that an excess of benzylamine remained. We removed this excess by distillation. The benzylamine distilled first and our desired β–aminoalcohol **(70)** remained. After recovery of the free base we converted it into the corresponding oxalate salt by dissolving it in a small amount of methanol and treating it with one equivalent of oxalic acid dissolved in methanol. We expected precipitation of the salt to occur rapidly, but this was only achieved by vigorous scratching of the solution with a glass rod. The product was purified by crystallisation from water.

Compounds **(71)** and **(72)** were synthesised in the same manner but in these cases it was found that the excess of amine was removed during the work-up of the reaction and there was no need for further purification before salt formation, except for **(72)** which unfortunately was destroyed when we attempted purification by distillation.

The ^{1}H NMR spectra of compounds **(70)**, **(71)** and **(72)** were as expected for compounds with a typical ABX system. For instance the diastereotopic methylene group of **(70)** appeared as two doublets of doublets at δ 3.25 (*J* 9.3 and 3.7) and δ 3.42 (*J* 9.7 and 3.4). The proton on the carbon of the secondary alcohol, at the chiral centre couples differently to each of the diastereotopic protons of the methylene, and it comes into resonance at δ 5.20 (*J* 5.9 and 3.3).

3.9 Studies Towards an Enantioselective Synthesis of 2-Hydroxy-2-(2'-pyridyl)ethylamines

While awaiting both antifungal and antimalarial evaluation of our racemic β–aminoalcohols, we turned our attention towards developing a route to synthesise these compounds in an enantiomerically pure form.

Biological systems are usually homochiral. So, it is probable that enantiomers will have different biological effects. In general three types of activity are observed.[98]

1. All the desired activity belongs to one enantiomer and the other enantiomer is inactive or has a different activity. This is commonly observed. This means that separation of the racemic mixture into a single enantiomer should give us a more potent agent, which should also be less toxic than using the racemate.

2. The enantiomers differ in potency. In this case most of the activity is in one enantiomer and the other displays weaker activity. The less potent enantiomer has some binding with the biological site but it is a weaker interaction. This is also a common occurrence.

3. Both enantiomers have the same activity. This is rarely seen and not expected since activity depends on the agent interacting with a homochiral biological site.

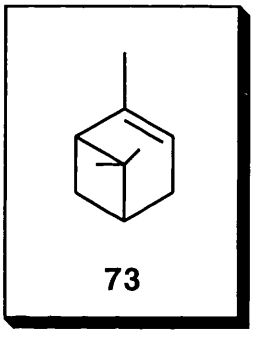
In terms of potency and selectivity and reducing toxicity there is a strong case to develop drugs as single enantiomers.[98]

We believed that we could resolve the racemic mixtures of β–aminoalcohols by classical techniques. This could be achieved by making diastereoisomeric salts, separating them by fractional crystallisation and then regenerating each enantiomer. So, ideally any route we considered should have a small number of high yielding steps.

3.9.1 Synthesis via Regioselective Opening of Chiral Epoxides

Our original idea was to utilise the powerful regioselectivity displayed when opening epoxide **(64)** with an amine. We needed a method to synthesise both enantiomers of the epoxide selectively.

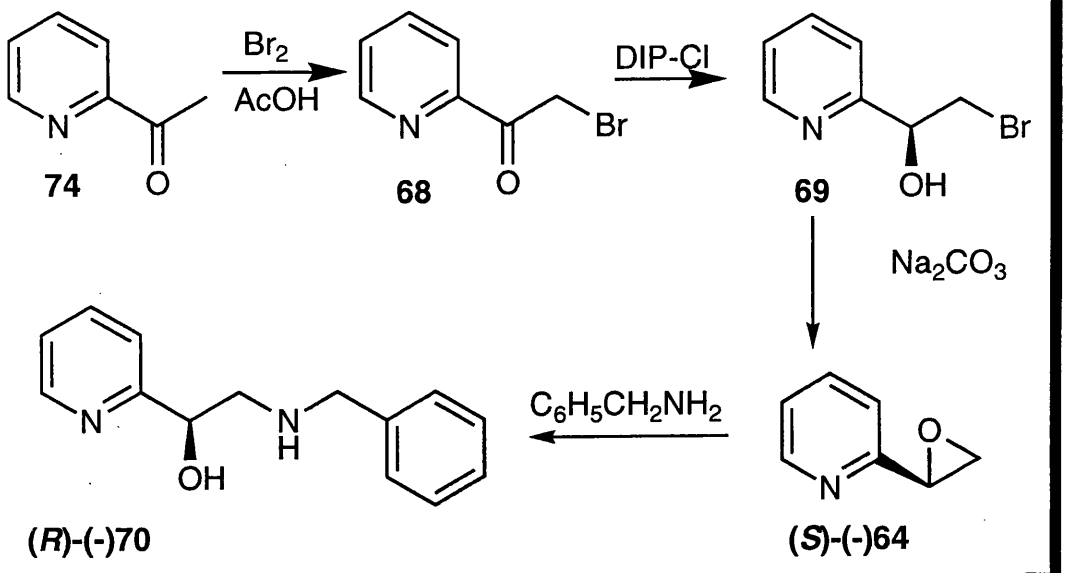
We knew that the α–bromoketone **(68)** could be subjected to a microbial reduction to give the enantiomerically enriched bromohydrin **(69)**, which in turn could be cyclised to give the (*R*)-(-)-enantiomer of epoxide **(64)**. However, this would only give us access to one enantiomer. Fortunately a number of reagents for reducing α–bromoketones to either enantiomer of the corresponding bromohydrin are commercially available. Many are homochiral organoboranes based on α–pinene **(73)**.[99] Other reagents that can perform such reductions include Corey's oxazaborolidine,[100] and its variants[101] as well as binaphthyl-modified lithium aluminium hydride reagents.[102]



73

Our first idea involved bromination of the appropriate ketone **(74)** to form α–bromoketone **(68)** and reduce it to either enantiomer of the corresponding bromohydrin **(69)** using the appropriate organoborane reagent. Treatment of the bromohydrin with base should give an enantiomerically enriched epoxide which should be opened regioselectively with an amine such as benzylamine, in the manner discussed in section 3.8.7. The overall method is shown in **Scheme 21**.

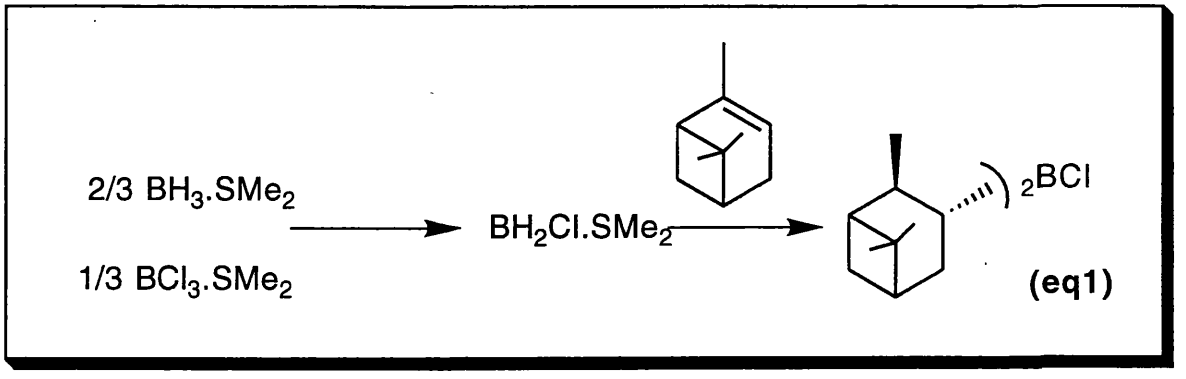
Scheme 21



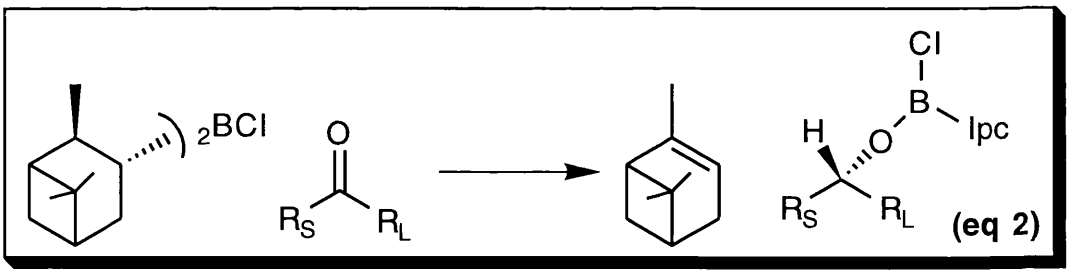
The reagent we proposed to use for the reduction of the bromoketone **(68)** was β–chlorodiisopinocamphenylborane (Ic_2BCl) which is commercially available in both of its enantiomeric forms, known as DIP-Cl™. It has been found to reduce substituted aryl alkylketones with "extraordinary" efficiency, with enantiomeric excesses of greater than 97% not being uncommon. Another advantage of using this reagent is that the stereochemistry of the product is highly predictable.[99]

The reduction sequence, including the synthesis of the reagent involves four steps.[99]

Step 1 - Formation of the reagent by treatment of $BH_3.SMe_2$ and α–pinene with $H_2BCl.SMe_2$ or $H_2BCl.OEt_2$ **(eq 1)**.

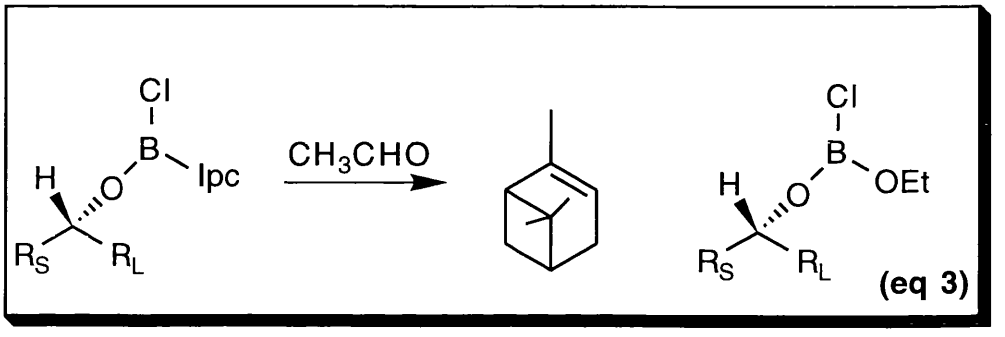


Step 2 -Asymmetric reduction of the ketone **(eq 2)**.



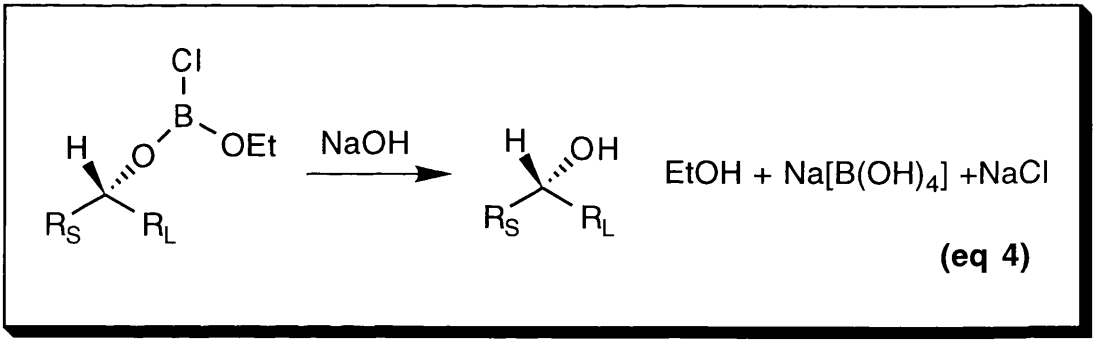
(eq 2)

Step 3- Treatment with acetaldehyde with the subsequent elimination of the other unit of α–pinene **(eq 3)**.



(eq 3)

Step 4-Hydrolysis of the product mixture **(eq 4)**.



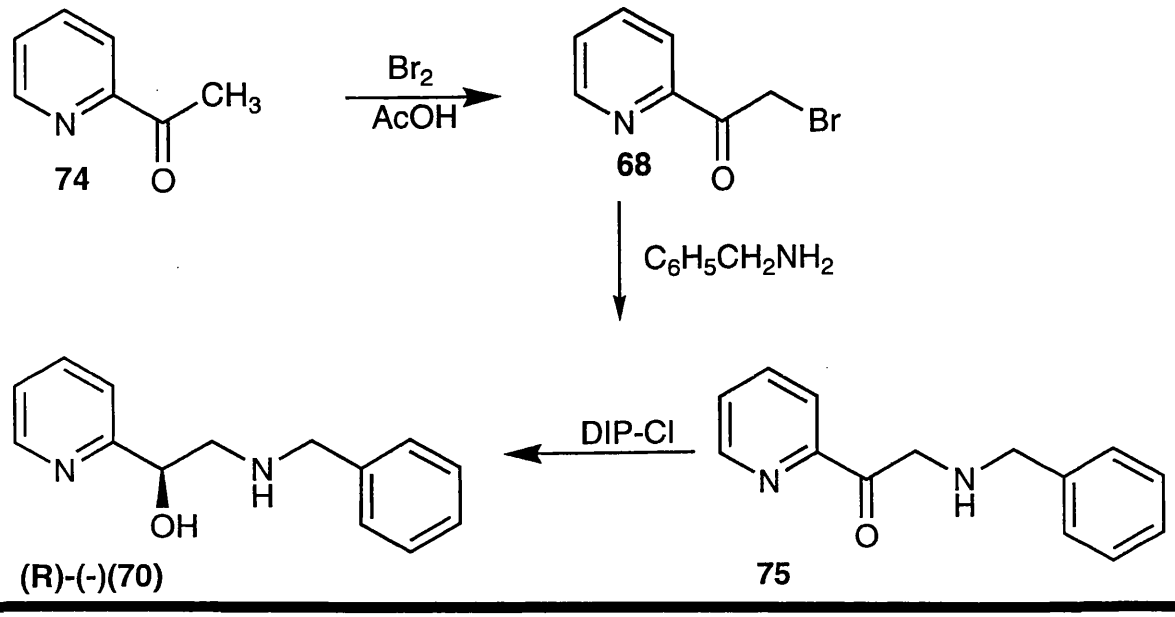
(eq 4)

Overall one equivalent of the ketone is reduced by two equivalents of α–pinene and one equivalent of $BHCl_2$. The α–pinene can be recovered without loss of optical activity and can be re-used. 1.5 moles of ketone are reduced per mole of BH_3.[99]

The route shown in **Scheme 21** appeared a good choice as it was relatively short; there was a literature precedent for the first step: and it took advantage of chemistry we already had shown worked. However, on further investigation we believed we could shorten the route by using the set of

reactions shown in **Scheme 22**. By reacting the α-bromoketone **(68)** directly with an amine such as benzylamine to make the keto-diamine **(75)** and then reduction with DIP-Cl™ we believed that we could synthesise our desired product without the need to synthesise the epoxide. Another advantage of this scheme was that we would isolate ketodiamines such as **(75)**. Foster and Walters showed that ketoputrescine controlled a number of economically important plant pathogens.[41]

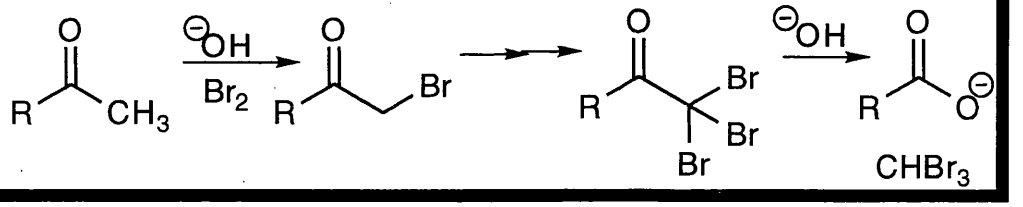
Scheme 22



3.9.2 α–Bromination of Ketones

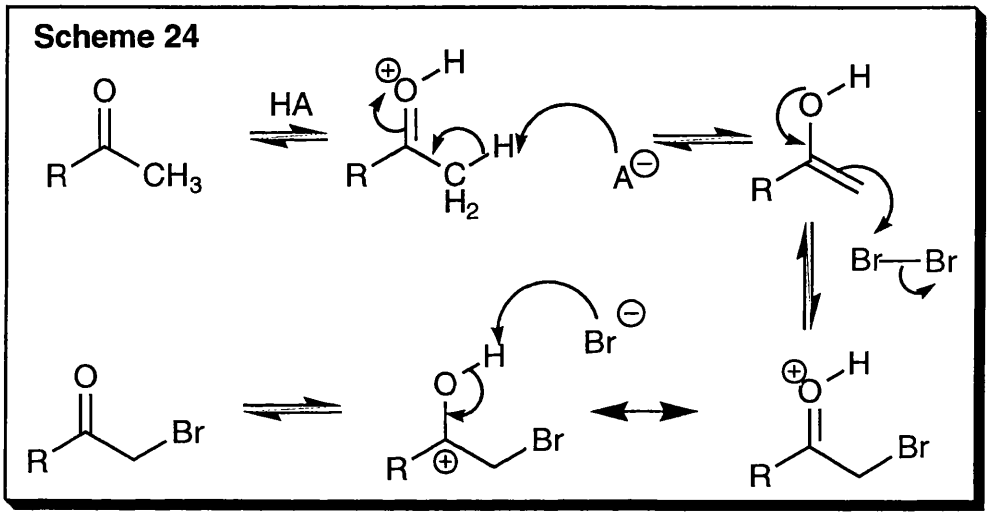
The reagent of choice for the α–bromination of ketones is molecular bromine. Two sets of conditions can be employed, base catalysed[103] bromination and acid catalysed bromination.[104] Base catalysis is rarely used as the reaction is very difficult to stop after mono-bromination. Typically the product of the reaction is bromoform and the salt of the corresponding carboxylic acid. This reaction is known as the bromoform reaction and is often used to produce carboxylic acids with one less carbon atom than the starting ketone **(Scheme 23)**.[103]

Scheme 23



α–Bromination of ketones is usually achieved under conditions of acid catalysis **(Scheme 24)**. The reaction proceeds via enol intermediates. The first step is an acid-base reaction between the catalyst and the carbonyl group of the ketone to form a protonated carbonyl compound. This loses an acidic proton from the α–carbon to yield an enol intermediate, which attacks the positively polarised bromine atom to give an intermediate cation. The cation loses a proton to form the halogenated adduct.[104]

Scheme 24



Commonly used catalysts for the bromination reaction are acetic acid[104] and hydrobromic acid.[105] In our attempts to synthesise **(68)** we investigated the use of them separately and together.

We purchased our ketone **(74)** from Lancaster synthesis Ltd. It was a light brown liquid and ^1H NMR spectroscopy indicated that it was impure. We purified it by distillation to afford a colourless starting material.

We attempted the reaction using acetic acid as the catalyst a number of times without success. A typical procedure involved the dropwise addition of a small excess of neat bromine to a stirred solution of 2-acetylpyridine **(74)** and acetic acid containing 48% HBr at 0°C and then raising the temperature to 40°C for 1.5 hours and to 75°C for 1 hour, cooling, filtering and washing the

product.[106] These conditions were unsatisfactory. The ^{13}C NMR spectrum of the product indicated a number of products had been produced and low resolution mass spectrometry indicated the presence of the dibrominated ketone.

Alterations were made to the experimental procedure such as adding the bromine as a solution in acetic acid, carrying out the entire reaction at 0°C, room temperature and 75°C as well as varying the reaction times, but all proved unsuccessful.

Applying the method of Taurins and Blaga[105] we obtained the compound in a yield of 75%. Operationally this method was very simple. One equivalent of the ketone was dissolved in 48% hydrobromic acid at 70 °C a solution of one equivalent of bromine in the same acid was added slowly with vigorous stirring. The temperature and the vigorous stirring was maintained for three hours. Upon cooling a crystalline product dropped out of solution which was washed, filtered and dried *in vacuo.* Crystallisation from 20% hydrobromic acid gave the α–bromoketone as the pyridinium salt.

3.9.3 Nucleophilic Substitution of 2-Bromoacetylpyridine

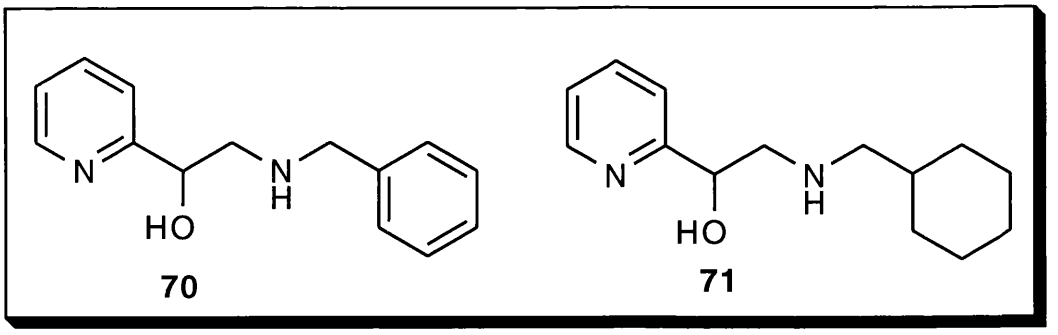
We believed that the displacement of bromide by a nitrogen nucleophile in a typical S_N2 reaction would be straightforward. Unfortunately this did not prove to be the case. Despite repeated efforts we failed to isolate the desired product. Much is known about the S_N2 reaction as it is important in organic synthesis. We don't intend to cover the topic in great depth, but **Table 6** details the favourable conditions for a successful S_N2 nucleophilic displacement.[107]

Table 6 Effect of reaction variables on nucleophilic substitution

Solvent	Nucleophile/base	Leaving group	Substrate structure
Strong effect	Strong effect	Strong effect	Strong effect
Reaction favoured by polar aprotic solvents	Favoured by good nucleophile/weak base	Favoured by good leaving group	favoured by 1° substrates

When carrying out the reaction we took each of these factors into account where possible. The structure of the substrate was fixed and we had

in mind two products in particular, those being the enantiomerically pure forms of **(70)** and **(71)**, so this limited the choice of nucleophiles we could employ.



If we consider the substrate the ideal structure should allow for the easy approach of an incoming nucleophile. The transition state of the S_N2 reaction involves partial bonding between the attacking nucleophile and the substrate and the transition state is co-planar. Bulky substrates which shield the carbon atom to be attacked by the incoming nucleophile react more slowly than less hindered substrates because the transition state is sterically hindered and consequently of a higher energy. The presence of the large pyridine ring of the α–bromoketone may contribute to a degree of steric congestion, but there should be sufficient room to allow the nucleophile, substrate and leaving group to reach a co-planar arrangement.[108]

The leaving group is another very important consideration in an S_N2 reaction. The leaving group in most reactions is expelled with a negative charge, so, it follows that the best leaving groups are those that best stabilise the negative charge. The most stable anions (weak bases) also make the best leaving groups. This can be rationalised by again looking at the transition state of an S_N2 reaction. The charge is distributed over both the attacking nucleophile and the leaving group. This means that the transition state is more stable and thus the reaction is faster. The bromide ion is an excellent leaving group, the pK_a of the conjugate acid is -9 and it has a relative reactivity of 10,000. The iodide ion is a better leaving group as the pK_a of the conjugate acid is -9.5 and it has a relative activity of 30,000. The chloride ion is the strongest base with the pK_a of the conjugate acid being -7 and is the poorest nucleophile of the three and it has a relative reactivity of 200.[109]

Another important variable in the S_N2 reaction is the nature of the attacking nucleophile. As we mentioned earlier we wanted to synthesise the benzyl **(70)** and cyclohexylmethyl analogues **(71)**. This limited our choice of

nucleophiles to benzylamine and cyclohexylmethylamine. In our studies we exclusively investigated the reaction with benzylamine.

When trying to explain the role of the nucleophile in the S_N2 reaction it is best to think of it in terms of the nucleophilicity of the attacking species. Nucleophilicity has been defined as the relative attraction of the attacking species in the S_N2 reaction for the carbon atom bearing the leaving group. Under a set of standard conditions, the stronger the nucleophile the faster the reaction.[110]

No single factor can be used to explain nucleophilicity, however, we can make a number of broad generalisations. It must be remembered that the following rules are not invariant but depend on a number of factors including the substrate, solvent and leaving group.

With the above proviso there are four main principles that govern nucleophilicity for the S_N2 reaction in solution.[111]

1. A nucleophile with a negative charge is a more powerful nucleophile than its conjugate acid.

2. In comparing nucleophilicites of species whose attacking atom is in the same row of the periodic table, nucleophilicity is approximately in order of basicity.

3. Going down the periodic table, nucleophilicity increases. For instance a sulfur nucleophile is more powerful than its oxygen analogue and any phosphorus nucleophile is more potent than its nitrogen counterpart.

4. The freer the nucleophile, the greater the rate of reaction. Solvents that poorly solvate the nucleophile lead to faster reaction and solvent free or gas phase reactions are even faster.

This is only a very basic description of a very complex topic For more detail see reference 111 pages 348-352 and references therein.

The first solvent we looked at for the reaction was DMF. As a polar aprotic solvent this should be an ideal solvent for an S_N2 reaction. Unfortunately when we attempted the reaction we isolated a brown oil which ^1H NMR spectra and TLC indicated was a complex mixture of products and a

white solid which ^1H NMR spectroscopy proved to be benzylamine hydrobromide. The reaction was attempted a number of times varying the amount of benzylamine and temperature, but the results were always similar.

Other solvents investigated were ethanol, water, acetone and DMSO, all without success. With each of the other solvents, under various conditions a similar outcome to that described above was achieved, namely the isolation of a brown oil which could not be purified and benzylamine hydrobromide.

We decided to investigate the number of equivalents of benzylamine used in the reaction. Initially we used two equivalents, one to act as nucleophile and one to mop up the hydrogen bromide by-product formed. We increased the number of equivalents of benzylamine from two through to five but without success. Instead of using benzylamine as a base as well as nucleophile in the reaction we used various amounts of triethylamine but again this proved fruitless. Again a complex brown oil was isolated, this time with solid triethylamine hydrobromide.

We can give no reason for the failure of this reaction, but it is possible that contrary to our initial belief the large pyridyl group of the substrate prevented the nucleophile approaching the substrate with the correct geometry.

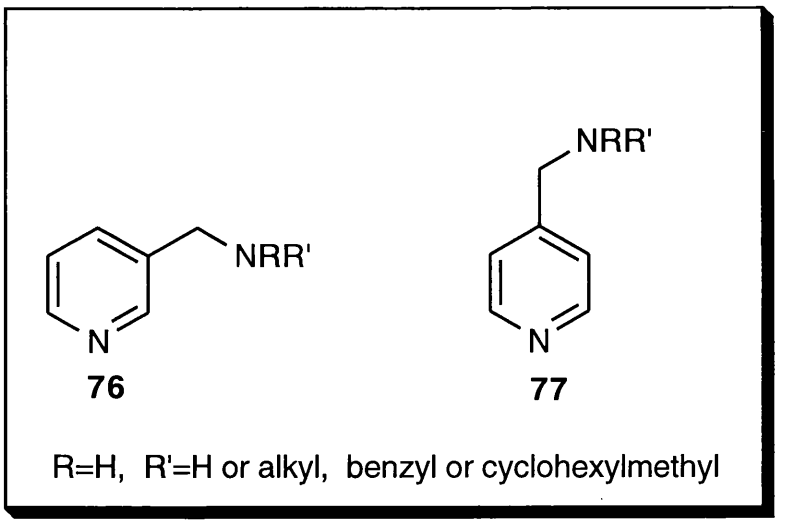
Unfortunately time constraints meant we did not investigate this area any further.

3.10 Future Work

The work presented in this chapter could be extended in a number of ways. The most obvious and straightforward way would be to alter the position of the substituent on the aromatic ring of the most active compounds.

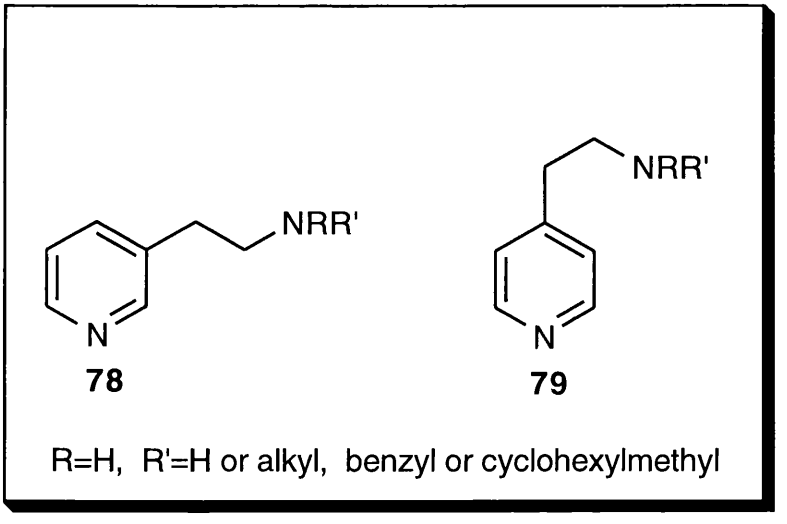
3.10.1 Synthesis of 3- and 4- Substituted Aminomethylpyridines

3-Pyridinecarbaldehyde and 4-pyridinecarbaldehyde are both commercially available. By using the reaction described in section 3.12 we would get compounds such as **(76)** and **(77)**. These are putrescine analogues.



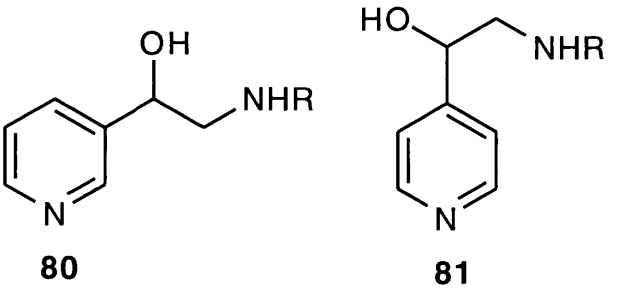
3.10.2 Synthesis of 3- and 4- Substituted Aminoethylpyridine

3-Vinylpyridine and 4-vinylpyridine are commercially available. Using the Michael type reaction described in section 3.14 we would be able to examine compounds of the type **(78)** and **(79)**. Compounds **(78)** are putrescine analogues whereas compounds such as **(79)** are cadaverine analogues.



3.10.3 Synthesis of 3- and 4-substituted (±)-Hydroxy-2-(2'-pyridyl)ethylamines

The availability of 3-vinylpyridine and 4-vinylpyridine also makes it viable to make compounds such as the putrescine analogues **(80)** and the cadverine analogues **(81)** by the reactions discussed in section 3.17.



80 **81**

R=H, R'=H or alkyl, benzyl or cyclohexylmethyl

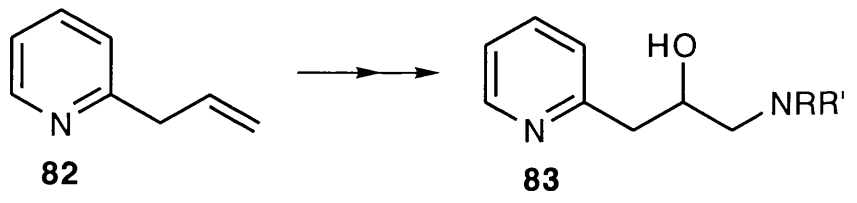
3.10.4 Synthesis of Analogues of (±)-2-Hydroxy-2-(2'-pyridyl)ethylamines

A number of structural alterations could be investigated and a few are indicated below.

1. Extension of the carbon chain by one unit **(Scheme 25)**.

Literature precedent exists for the synthesis of 2-allylpyridine **(82).**[112] from which we could synthesise the corresponding epoxide. This would give rise to a new class of homo-putrescine analogues **(83)**.

Scheme 25

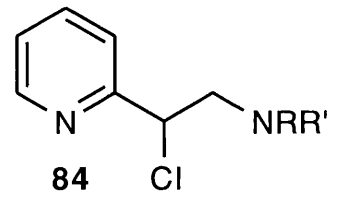


82 **83**

R=H, R'=H or alkyl or benzyl or cyclohexylmethyl

2. Replacement of the hydroxy group by chlorine or bromine.

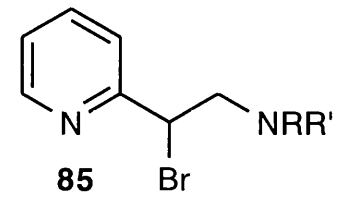
Treatment of the hydroxy compound with phosphorus trichloride[113] or thionyl chloride[114] should give rise to the chlorinated analogue **(84)**.



84

R=H, R'=H or alkyl, benzyl or cyclohexylmethyl

In an analogous fashion treatment of the alcohol with phosphorus tribromide[115] or thionyl bromide[116] would give the corresponding bromide **(85)**.



85

R=H, R'=H or alkyl, benzyl or cyclohexylmethyl

Further investigations into the synthesis of enantiomerically pure 2-hydroxy-2-(2'-pyridyl)ethylamines could be undertaken. It may be more viable to synthesise them via the route outlined in **Scheme 22** which does not involve an S_N2 reaction on a 2-bromoalkylpyridine.

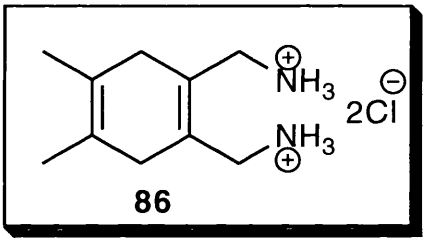
4

Investigations Towards Cyclic and Bicyclic Putrescine Analogues

This chapter will be split into two sections; the first will deal with our experiences in the area of six-membered cyclic putrescine analogues, and the second will detail our work in the field of bicyclic putrescine analogues.

4.1.1 Cyclic Putrescine Analogues

The lead compound for work in this area was 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride **(86)** which is known as BAD.



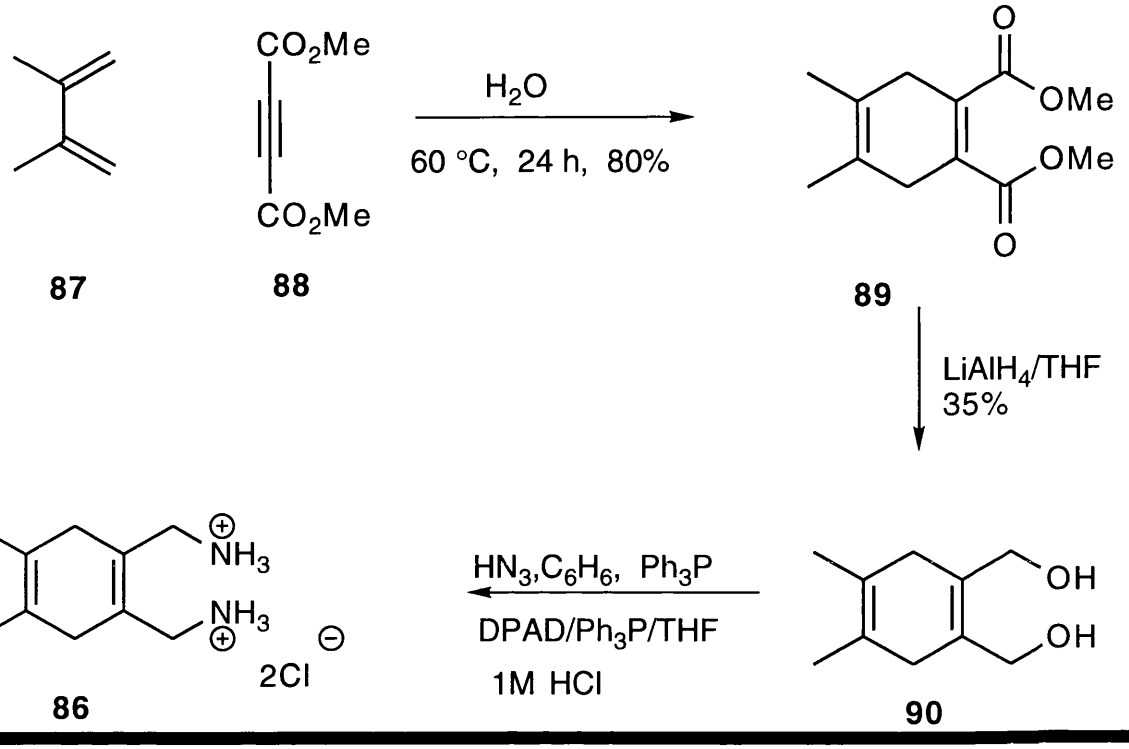
86

This compound had previously been synthesised within the group and was found to have excellent antifungal properties and indeed had been patented for this purpose.[117] It was found to reduce powdery mildew infection of barley seedlings by 93%. As was shown in chapters one and two polyamines can show antifungal and antimalarial activity by similar mechanisms. The co-sponsors of this work required a sample of BAD to test for *in vivo* antimalarial activity.

4.1.2 Synthesis of BAD

We synthesised BAD from commercially available starting materials in an overall yield of 12%, as indicated in **Scheme 26**. A brief discussion of the reactions employed follows.

Scheme 26



4.1.3 Diels-Alder Cycloaddition

The first stage in the synthesis was a Diels-Alder reaction between 2,3-dimethyl-1,3-butadiene **(87)** and dimethyl acetylenedicarboxylate **(88)** to give the cyclic diester **(89)**. The reaction proceeds smoothly in a yield of 80%.[118] The electron withdrawing substituents on the dienophile and the electron donating substituents on the diene are responsible for an accelerated reaction rate.

The Diels-Alder reaction is also influenced by the choice of solvent. Many have been used successfully in the reaction, including benzene, toluene, ether and ethanol. We carried out this Diels-Alder reaction in water to take advantage of the hydrophobic effect to increase the rate of reaction.[119]

The hydrophobic effect was first explored by Breslow and co-workers, although it had previously been noted that water can have a remarkable effect on the rate of the Diels-Alder reaction.[119] Breslow *et al.* conducted a number of experiments and concluded that the rate enhancement was not due to solvent polarity effects. Breslow proposed that the rate acceleration displayed in water was due to the promotion of hydrophobic packing of the reactants.[119]

Evidence for the hydrophobic effect was gathered by conducting a number of experiments in which reaction rates were increased in the presence of additives such as lithium chloride which increase hydrophobic effects and guanidinium salts that decrease the hydrophobic effect.[119]

A variety of other methods have been used to accelerate the Diels-Alder reaction such as the use of microwave radiation[120] and ultracentrifugation.[121]

4.1.4 Synthesis of Diol (90)

The second stage of the synthesis was the reduction of diester **(89)** to the corresponding diol **(90)**. This reaction proved more testing than the previous one. A number of reducing conditions had been investigated by a previous worker within the group in an attempt to achieve this transformation. Most were based on diisobutylaluminium hydride (DIBAL-H) in either DCM or toluene.[122] These were either unsuccessful or gave the product in an impure form and in no better than 10% yield.

We found that it was best to do the reaction in THF with four equivalents of lithium aluminium hydride. The yield of the reaction was a significant improvement at 35% and after one recrystallisation from ethyl acetate the diol **(90)** was isolated in an analytically pure form.

No attempt was made to optimise the yield of the reaction. We could easily make large amounts of the diester **(89)** and so could synthesise a reasonable amount of the diol by carrying out the reduction on a large scale.

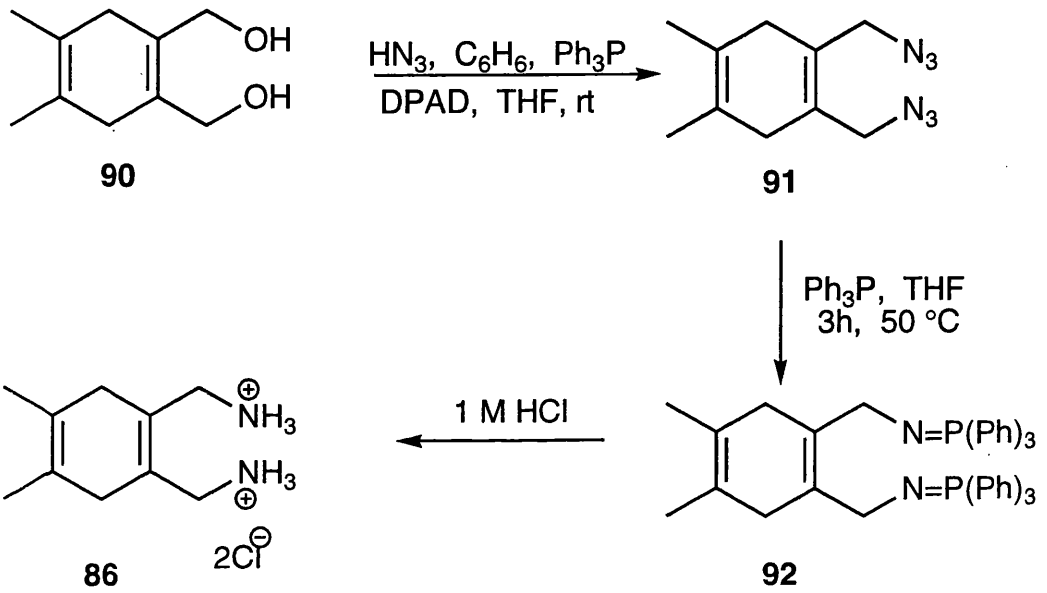
4.1.5 Conversion of Diol (90) into Diamine (86)

The conversion of the diol **(90)** into the diamine **(86)** involved the combination of three known reactions; the Mitsunobu,[123] the Staudinger[124] and acid hydrolysis as shown in **Scheme 27**. Our procedure was adapted from that of Fabiano and co-workers.[125]

The first reaction in **Scheme 27**, the Mitsunobu, was developed in the early 1980s by Oyo Mitsunobu and has found a number of synthetic applications, and is used routinely to invert the stereochemistry at a chiral

centre containing a hydroxyl group.[123] It has also found widespread use in the transformation of natural products as it can be carried out under mild, neutral conditions and exhibits stereospecificity, regioselectivity and functional selectivity.[123]

Scheme 27



90 **91** **92** **86**

The Mitsunobu reaction converts the diol **(90)** into the diazide **(91)** via a stabilisation process. Triphenylphosphine is oxidised to triphenylphosphine oxide and diisopropyl azodicarboxylate (DPAD) is reduced to diisopropyl hydrazinedicarboxylate.[123]

The second reaction in the synthesis, the Staudinger reaction involves an *in situ* reaction of the diazide with triphenylphosphine produces the iminophosphorane intermediate **(92)**. This reaction occurs via a 3-membered transition state and the loss of diatomic nitrogen and is therefore irreversible.[124]

The final step is a simple hydrolysis of **(92)** by dilute hydrochloric acid to give the dihydrochloride salt **(86)**.

The yield for the conversion of the diol **(90)** into the diamine dihydrochloride of **(86)** was 43%. We did not try and optimise the yield for two reasons. We had made the product before and work within the group and in

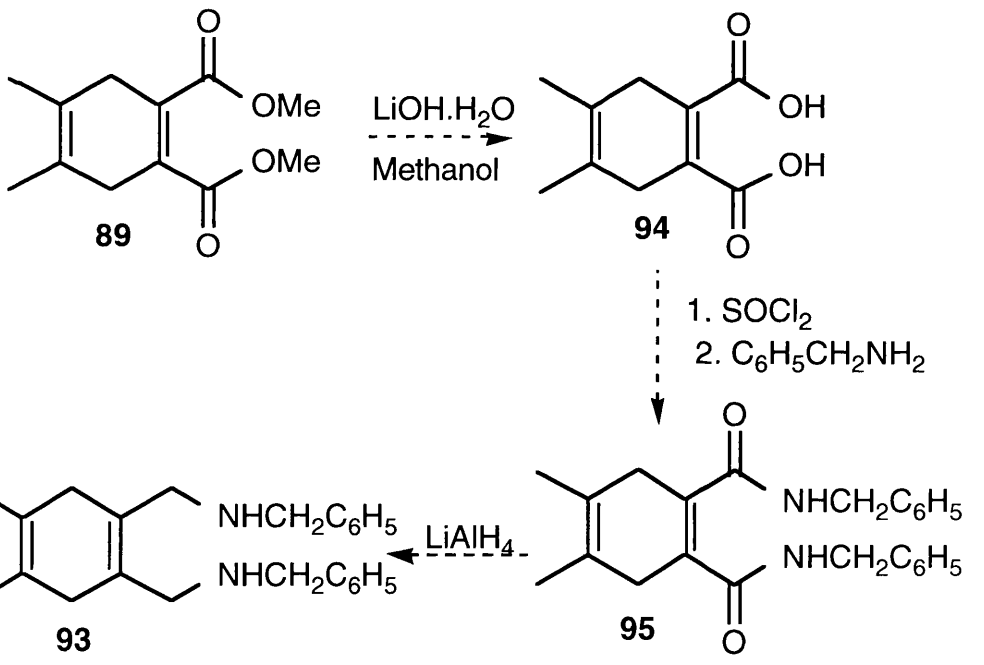
the literature suggested that simple dihydrochloride salts such as **(86)** would not exhibit good antimalarial properties. [59, 60] It was felt that the dibenzyl derivative of **(86)** with the lipophilic benzyl groups would show better antimalarial activity. Possible reasons for this were discussed in chapter 2.

A sample of **(86)** was sent to the World Health Organisation (W.H.O.), who assessed it for *in vitro* antimalarial activity. As expected no activity was observed. We then turned our attention to substituted BAD derivatives, in particular dibenzyl BAD **(93)**.

4.1.6 Approaches towards Dibenzyl BAD (93)

Our initial plan to synthesise dibenzyl BAD is outlined in **Scheme 28**. Successful hydrolysis of diester **(89)** would yield the corresponding diacid **(94)**, activation of the diacid followed by reaction with benzylamine would produce the diamide **(95)**. Reduction of **(95)** would give the desired diamine **(93)**.

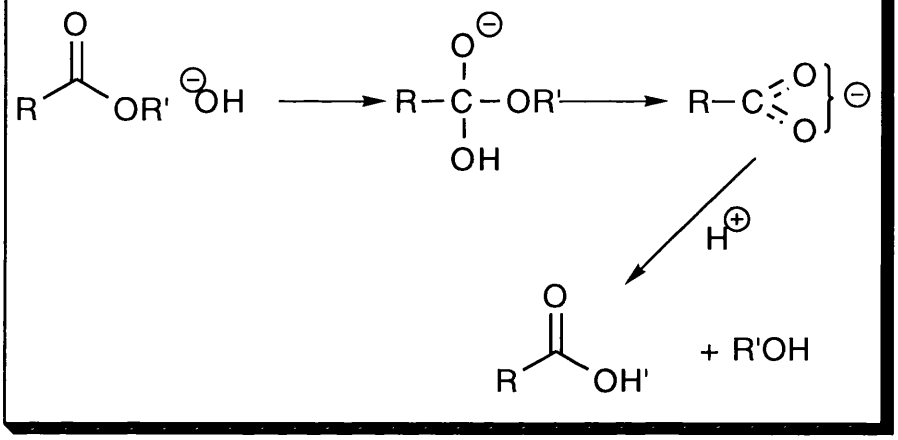
Scheme 28



4.1.7 Investigations towards the Synthesis of Diacid (94)

Being able to produce diester **(89)** easily and in high yield meant that reactions involving its hydrolysis to give the diacid **(94)** were important. The traditional methods of hydrolysing methyl esters are with acid or alkali. Of these two methods alkaline hydrolysis is most often favoured as it is essentially irreversible as the resonance stabilised carboxylate anion does not react with the alcohol formed.[126] Alkaline hydrolysis gives the acid as its salt, from which it can be liberated by the addition of acid **(Scheme 29)**.

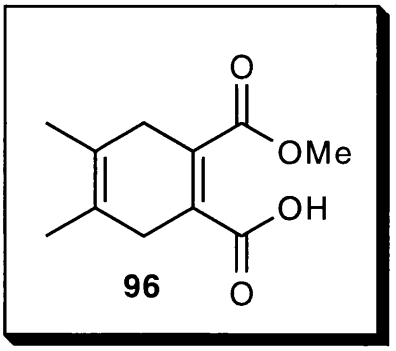
Scheme 29



The first method we investigated for alkaline hydrolysis of the diester was an adaptation of that reported by Corey and co-workers.[127] This method employed lithium hydroxide monohydrate in a methanol : water (3:1) system.

Initial attempts were all done at room temperature using between five and ten equivalents of $LiOH.H_2O$. A typical procedure involved dissolving the diester in methanol and adding $LiOH.H_2O$ in methanol : water with stirring. As the addition proceeded the solution became yellow in colour. The resulting solution was stirred at room temperature for 24 hours after which time TLC in methanol : DCM (4:1) and two drops of acetic acid indicated all the starting material had been consumed and a single more polar product had been formed. After acidification, extraction and drying, a sparingly soluble white solid was obtained. A ^1H NMR spectrum strongly suggested the presence of ester functionality, the desired acidic protons as well as aromatic and olefinic protons.

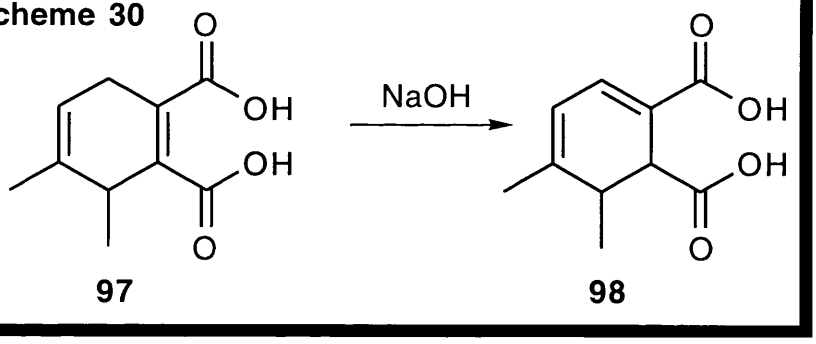
This situation can be explained by partial hydrolysis of the ester to give the half ester **(96)**.



96

In addition, oxidation of the 1,4-diene to give a more stable aromatic system is not an unexpected reaction. Isomerisation of double bonds with alkali to give the conjugated diacid has been reported for the similar diacid **(97)** to give **(98)** as shown in **Scheme 30**,[118] and this may also have happened within our system.

Scheme 30



97 **98**

As well as carrying out the reaction at room temperature we tried to achieve the transformation at 6°C by the use of a cyclohexene/dry ice bath. This was in order to mimic the conditions employed by Corey and co-workers as closely as possible. Unfortunately this proved to be no more successful than when carrying out the reaction at ambient temperature or 0 °C.

4.1.8 Attempted Hydrolysis of (89) with Sodium Hydroxide

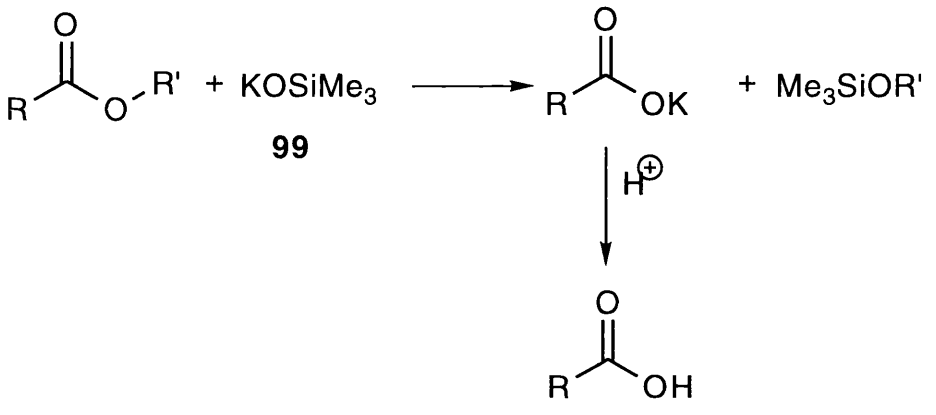
We also attempted to synthesise diacid (**94**) from diester **(89)** by hydrolysis with aqueous sodium hydroxide solutions of various concentrations at either room temperature or reflux. All the methods gave similar results to those described above for $LiOH.H_2O$.

Two other methods were investigated to hydrolyse the diester **(89)**. These are briefly discussed.

4.1.9 Alkali Metal Silanolates

Metal silanolates such as potassium trimethylsilanolate **(99)** and lithium trimethyl silanolate have been found to convert carboxylic acid derivatives including esters into their corresponding anhydrous acid salts under mild conditions **(Scheme 31)**.[128]

Scheme 31



Metal silanolates are organic soluble equivalents for O^{2-}. They have two advantages over other oxygen anions; they have appreciable solubility in organic solvents, and the silicon oxygen bond may be cleaved under mild reaction conditions. The reagents are soluble in solvents such as ether, THF, toluene and dichloromethane.

We adapted the procedure of Laganis and Chenard with diester **(89)** in dry THF using 2.2 equivalents of potassium trimethylsilanolate under nitrogen at room temperature.[128] TLC of the reaction mixture at regular intervals over 24 hours indicated only starting material was present.

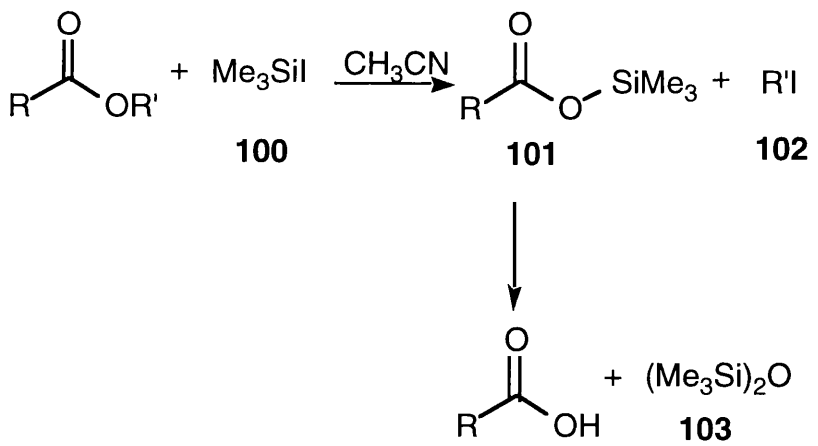
4.1.10 Neutral Conditions with Trimethylsilyl Iodide

As well as hydrolysis of alkyl carboxylic esters under acidic or basic conditions it is also possible to achieve hydrolysis under neutral conditions. Many of these procedures require the use of strong nucleophiles and high temperatures to achieve dealkylation.

Treatment of alkyl carboxylic esters with trimethylsilyl iodide **(100)** followed by aqueous hydrolysis has been shown to be a very mild method for the dealkylation under neutral conditions in almost quantitative yield **(Scheme 32)**.[129]

As suggested above the high bond energy of the silicon-oxygen bond makes it thermodynamically very favourable to use a reagent with a weak Si-I bond and react it with an appropriate oxygen-containing organic compound to form a silicon-oxygen bonded intermediate such as **(101)** which can be transformed to another product in a subsequent step.[130] The by-products of this reaction are the alkyl iodide **(102)** and hexamethyldisiloxane **(103)**.

Scheme 32



As well as being used to cleave esters, trimethylsilyl iodide has been shown to cleave lactones to carboxylic acids, ethers to alcohols and carbamates to amines under various conditions in high yield.[130]

Trimethylsilyl iodide is a commercially available reagent but it is extremely air sensitive due to the hydrolytic susceptibility of the Si-I bond. We

chose to synthesise it *in situ* by the reaction of sodium iodide with the more stable trimethylsilyl chloride.[130]

We attempted the reaction on diester **(89)** by adapting the procedures of two papers published by Olah and co-workers.[129, 130] Typically the reaction is carried out in a polar aprotic solvent such as acetonitrile or carbon tetrachloride. We carried out the reaction by treating diester **(89)** with 2.4 equivalents of dry sodium iodide and 2 equivalents of trimethylsilyl chloride in acetonitrile. The reaction time was decided upon by comparison with the time given to hydrolyse similar esters that were investigated by Olah and co-workers. After work-up we only succeeded in isolating a small amount of starting material.

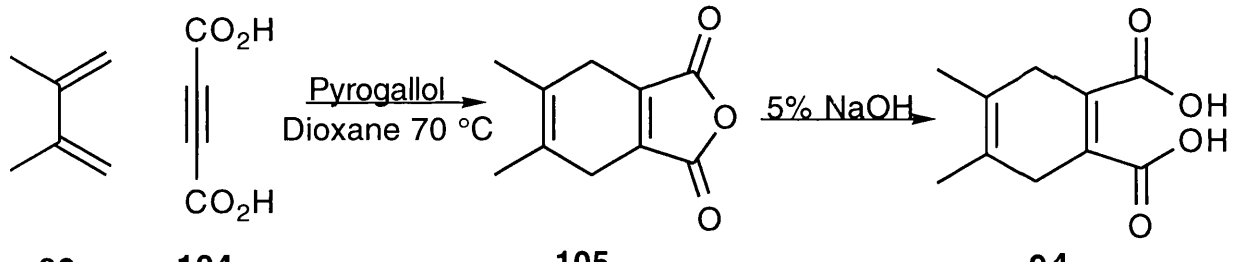
We were unsuccessful in all the methods we employed to hydrolyse diester **(89)** to the corresponding diacid. Aromatisation and double bond shifts were problems that could be expected. In all the methods employed we feel that the 1,2-relationship of the diester functionality may have contributed to a degree of steric hindrance which ultimately prevented the desired reaction.

The desired diacid was synthesised by a return to the Diels-Alder reaction.

4.1.11 Synthesis of Diacid (94) via the Diels-Alder Reaction

The diacid **(94)** was synthesised by the Diels-Alder reaction reported by Kucherov and co-workers in 1960 as illustrated in **Scheme 33**.[118]

Scheme 32



89 **104** **105** **94**

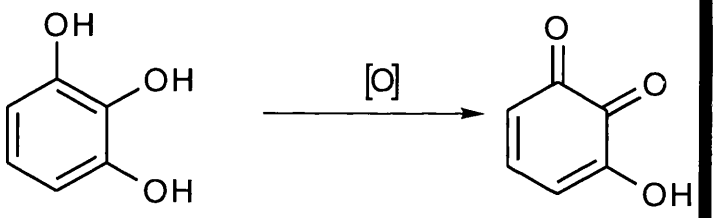
The reaction involved heating acetylenedicarboxylic acid **(104)** and 2.1 equivalents of diene **(87)** and 0.1 equivalent of pyrogallol in dioxane at 65-75 °C. After removal of the dioxane and excess diene we should have been left

with the crude anhydride **(105)** which can be converted into the diacid **(94)** with dilute sodium hydroxide solution. However, when we carried out the cyclisation we always isolated a mixture of the anhydride and the diacid even when using rigorously dried dioxane and glassware and under nitrogen.

Carboxylic acids are notorious for there ability to absorb water. We obtained our reagent from the Aldrich chemical company. It may be that we have to sublime the material to get it completely dry. At this stage this was not a problem as we desired the diacid **(92)** and the mixture could be converted exclusively into this by treatment with a dilute sodium hydroxide solution. We obtained the diacid in an overall yield of 60%.

Presumably the pyrogallol **(106)** is used as an anti-oxidant. It is oxidised to the *ortho*-quinone **(107)** in preference to oxidation of the anhydride **(105)** or diacid **(94)** **(Scheme 34)**.

Scheme 34



106 **107**

4.1.12 Attempted Synthesis of Diamide (95)

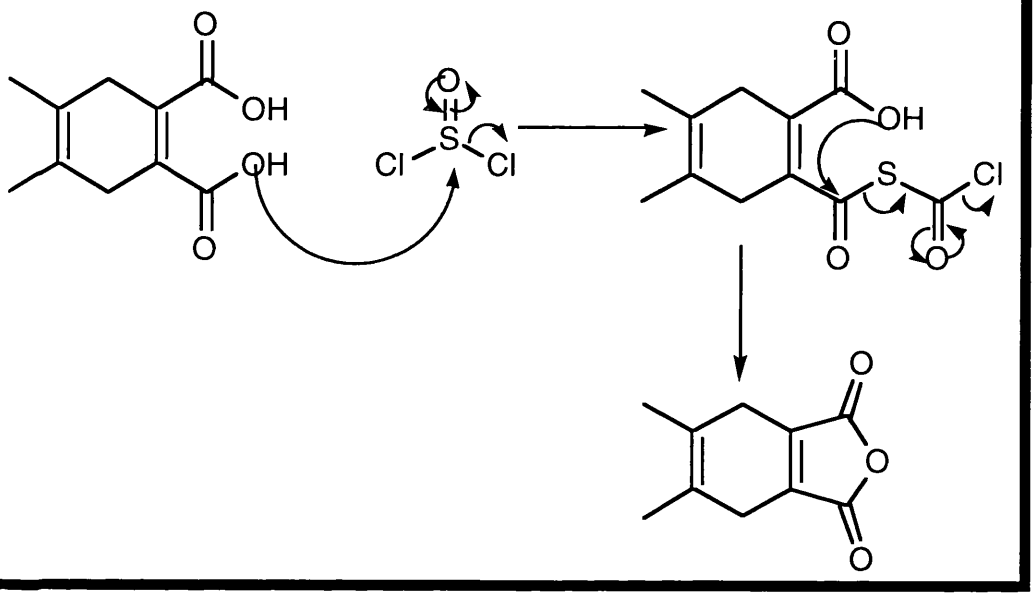
We believed we could synthesise diamide **(95)** by activating diacid **(94)** with thionyl chloride to yield the corresponding diacid chloride which after reaction with benzylamine would furnish us with our target compound.

The first procedure attempted had been successfully applied to a number of other diacids our group had investigated.[122] Typically it involved heating the appropriate diacid at reflux in six equivalents of thionyl chloride for six hours. After removal of the excess thionyl chloride the oil obtained was dissolved in an organic solvent such as ether and stirred at 0 °C and eight equivalents of the appropriate amine was added dropwise. After removal of the precipitated amine hydrochloride by-product the desired diamide was obtained.

When we applied this method to this diacid we encountered a problem. Heating the diacid at reflux in thionyl chloride proceeded normally. However, when the excess reagent was evaporated instead of being left with an oil a white solid was obtained which was insoluble in common organic solvents.

^{1}H NMR spectroscopy indicated that the solid was a mixture of the starting diacid and anhydride **(105)**. We believe that the diacid was formed by the mechanism shown in **Scheme 35**.

Scheme 35



The reaction conditions were varied in an attempt to achieve a more favourable outcome. The number of equivalents of thionyl chloride used were reduced and the reaction mixture was stirred at ambient temperature instead of reflux temperature. It soon became apparent that this was not a viable method to synthesise diamide **(95)**.

4.1.13 Coupling Reaction with *N,N'*-Dicyclohexylcarbodiimide (DCC)

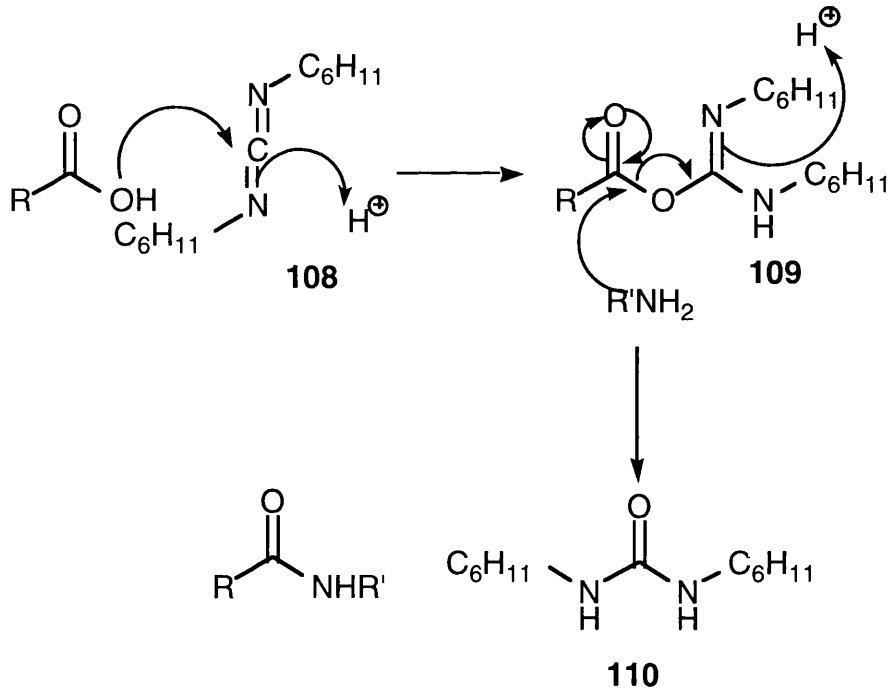
We briefly looked at the possibility of using DCC **(108)** as a reagent to couple diacid **(94)** with benzylamine to generate the corresponding diamide.[131]

DCC is one of the most widely used reagents for preparing amides/peptides and it can also be used to make esters.[131]

The reaction has been studied in depth and the mechanism for amide formation is shown below in **Scheme 36**. It follows a very similar path to that typically shown when thionyl chloride is used to form amides. The carboxylic acid is activated by the addition of DCC. The adduct **(109)** is attacked by the amine to generate an amide and *N,N'*-dicyclohexylurea (DCU) **(110)**. The DCU can be precipitated out of the reaction mixture and the amide recovered relatively easily.[131]

When this reagent was applied to our substrate a white solid precipitated out almost immediately, but we only succeeded in protonating the benzylamine. The order of addition of the reagents was altered but the same result occurred. We quickly abandoned this idea.

Scheme 36

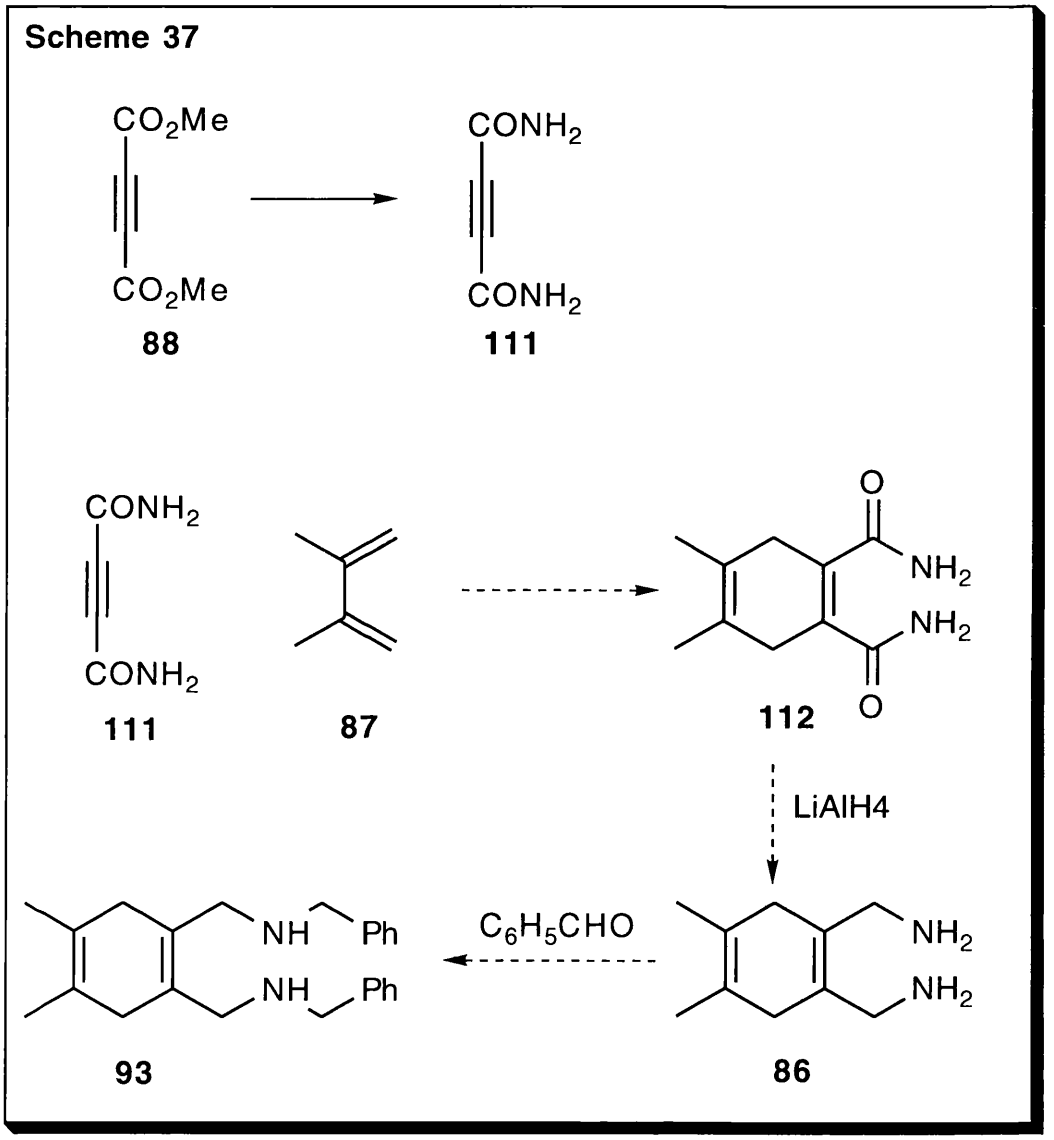


We felt the close proximity of the acid functionality in our diamide adversely affected the outcome of both these attempts and we looked at other methods to synthesise dibenzyl BAD. The one we felt most likely to succeed is discussed briefly below.

4.1.14 Attempted Synthesis of (93) via Acetylenedicarboxamide

In our earlier work we had shown that we could carry out the Diels Alder reaction and reductive amination in high yield. We thought it might have been feasible to incorporate these reactions into the synthesis of dibenzyl BAD by the route shown in **Scheme 37**.

Scheme 37



The procedure of Saggiomo to synthesise acetylenedicarboxamide was followed. This procedure involved treating an excess of aqueous ammonia solution at -10 °C with the diester **(88)**, then filtering and washing the precipitated solid **(111)**.[132]

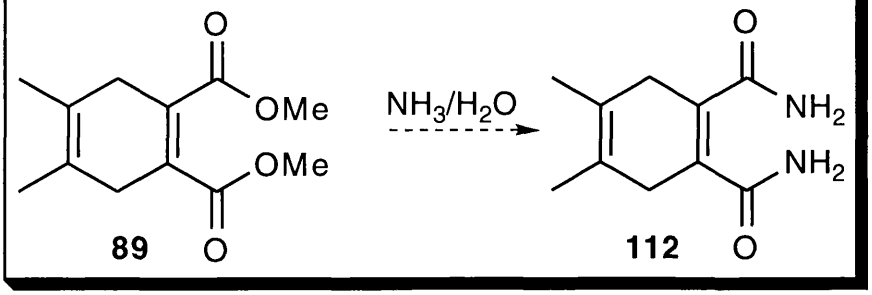
The reaction was also carried out under anhydrous conditions by condensing gaseous ammonia, cooling it to -78 °C and adding to it a solution of diester **(88)** in methanol. This method also proved successful but the quality of the product was inferior to that produced by the first method and it was operationally more involved.

The diamide **(111)** produced by this method is an antibiotic substance produced by *Streptomcyes chibaensis* from soil collected in Japan.[133]

Unfortunately when the Diels Alder reaction between the butadiene **(87)** and acetylenedicarboxamide **(111)** was attempted to form the cyclic diamide **(112)** the diamide **(111)** was very insoluble in all common organic solvents investigated and consequently the proposed cyclisation could not be carried out. We also investigated the solubility of diamide **(109)** in water, but as expected it was insoluble.

Instead of converting diester **(86)** into diamide **(111)** and then into the cyclic diamide **(112)** we investigated the possibility of converting the cyclic diester **(89)** into the cyclic diamide **(112)**. This would mean the Diels Alder reaction with the insoluble diamide would no longer be required. We thought it would be possible to do this in an analogous way to that used to convert diester **(88)** into diamide **(111)** as shown in **Scheme 38**.

Scheme 38

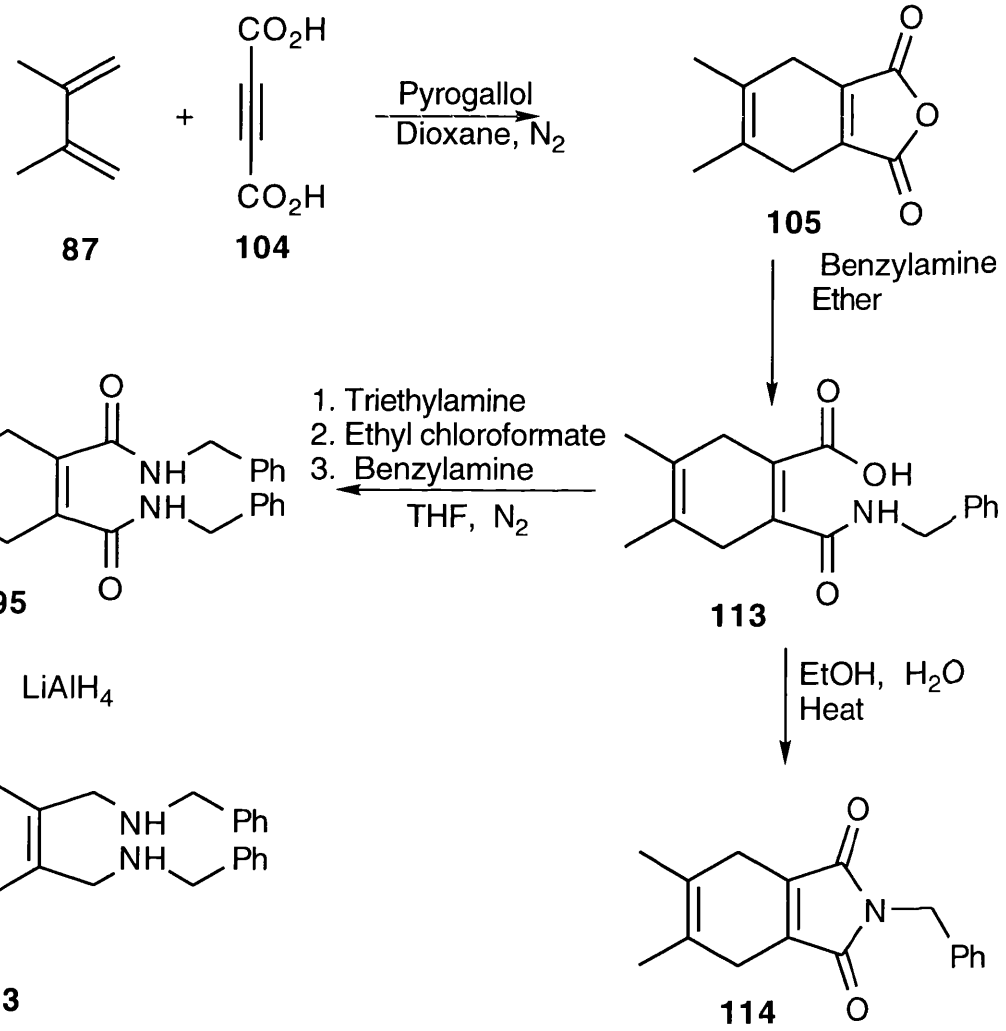


Unfortunately when this reaction was tried on the cyclic diester we were unsuccessful. Regular TLC analysis of the reaction mixture showed that only unreacted diester **(89)** was present. We tried the reaction again under anhydrous conditions but the results were the same.

4.1.15 Synthesis of Diamide (95)

By applying some of the experience gained in trying to synthesise anhydride **(105)** and that obtained in our work with bicyclic compounds, which we will discussed later, it was felt that it would have been possible to synthesise diamide **(95)** and thence dibenzyl BAD **(93)** by the reactions shown in **Scheme 39**.

Scheme 39



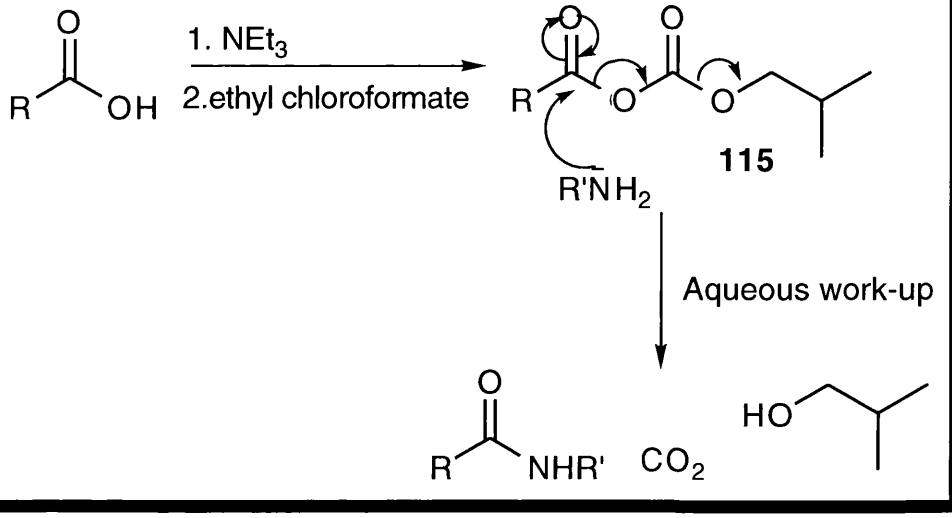
As was documented earlier our attempts to synthesise anhydride **(105)** by the Diels Alder reaction of **(87)** with **(104)** in the presence of pyrogallol **(106)** resulted in isolation of a mixture of the anhydride and the corresponding diacid.[118] This problem may have been due to moisture present in the reaction or in the subsequent work-up. In order to try and minimise this

problem the cycloaddition was carried out under under a dry nitrogen atmosphere using dry solvent and the product was isolated without purification. The subsequent opening of the anhydride was performed with 1.1 equivalents of benzylamine as soon as the crude product was isolated from the first step. The analogous reaction was carried out on a similar anhydride in high yield. With this substrate the desired product **(113)** was isolated but in a much lower yield of 19%. This was almost certainly a consequence of not purifying the product of the first step.

When we attempted to purify the crude mixture isolated in the synthesis of **(113)** by crystallisation in ethanol - water a cyclisation reaction took place to give a mixture of **(95)** and the diimide **(114)**, **Scheme 39**. As the crude product containing **(113)** was very insoluble in most organic solvents, (it was sparingly soluble in DMSO) we chose not to purify it by column chromatography.

We chose to convert a crude sample of **(113)** into the diamide **(95)** via a mixed anhydride. This involves activation of the carbonyl group to make it more susceptible to nucleophilic attack. The mechanism is well know and shown in **Scheme 40**. Deprotonation of the carboxylic acid with a non-nucleophilic base such as triethylamine generates the carboxylate anion which reacts with an alkyl chloroformate such as ethyl chloroformate to generate the mixed anhydride **(115)**. The anhydride is cleaved by a nucleophile, in this case an amine, to generate the amide.[134] The reaction is irreversible as one of the by-products is carbon dioxide. The reaction is best carried out at 0 °C, and the desired amide was isolated in a yield of 39%.

Scheme 40



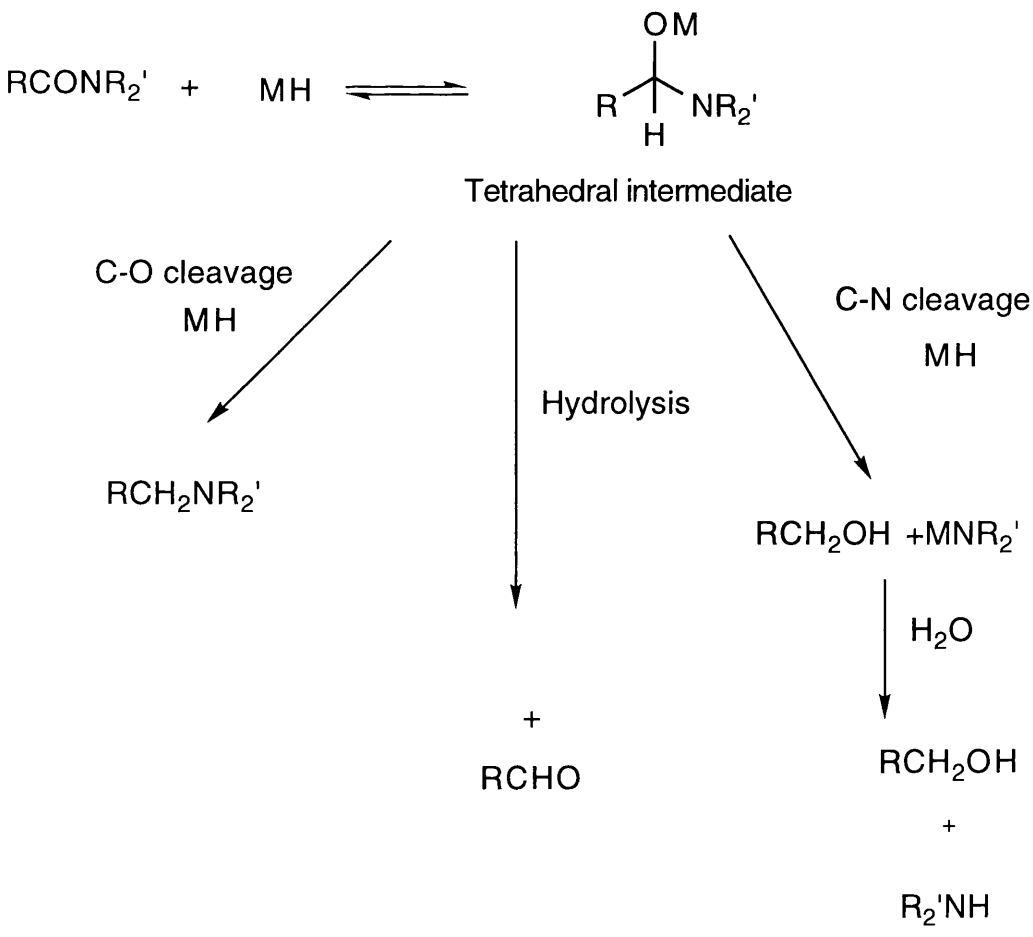
4.1.16 Attempted Reduction of Diamide (95) to Diamine (93)

The reduction of diamide **(95)** with lithium aluminium hydride was attempted without success and due to a shortage of **(95)** the reaction could not be repeated. It is possible that lithium aluminium hydride is too harsh a reagent to complete this transformation. It is known that reduction of carboxylic amides with metal hydride reagents are prone to problems, the main one being cleavage of the C-N bond to form the alcohol as opposed to the desired C-O bond cleavage to generate the amide (**Scheme 41**).[135]

It may be possible to achieve the desired reduction using a milder reducing agent such as sodium bis(2-methoxyethoxy)aluminium hydride **(116)**, which is commercially available as a solution in toluene and is known as Red-Al®.[136]

116 $[(CH_3OCH_2CH_2O)_2AlH_2]Na$

Scheme 41



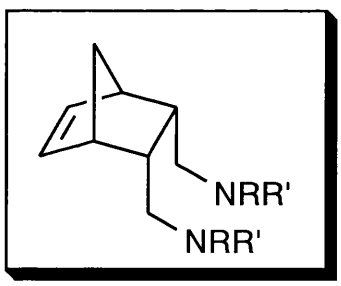
4.2.1 Studies towards the Synthesis of Bicyclic Putrescine Analogues

Our studies in this area arose for two main reasons. Firstly, a study of the literature indicated that compounds containing the bicyclo-[2.2.1]-heptane skeleton had been found to a have a wide range of biological activity[137] and a number had been incorporated in pharmaceutical agents[138] As far as we were aware they had not been investigated as antimalarial agents.

Secondly, we were having difficulty synthesising dibenzyl BAD and we could easily synthesise a number of norbornyl compounds which had similar structures to some of the possible precursors of dibenzyl BAD. This gave us the chance to try and synthesise a number of relevant compounds for antimalarial and antifungal evaluation and the chance to develop methods that we might be able to use in the synthesis of dibenzyl BAD.

4.2.2 Disubstituted Bis-(aminomethyl)-bicyclo-[2.2.1]-*endo-cis*-5-heptenes

When we began our work in this area the compounds we desired to make were disubstituted bis-(aminomethyl)-bicyclo-[2.2.1]-*endo-cis*-5-heptenes, and their general structure is shown below.



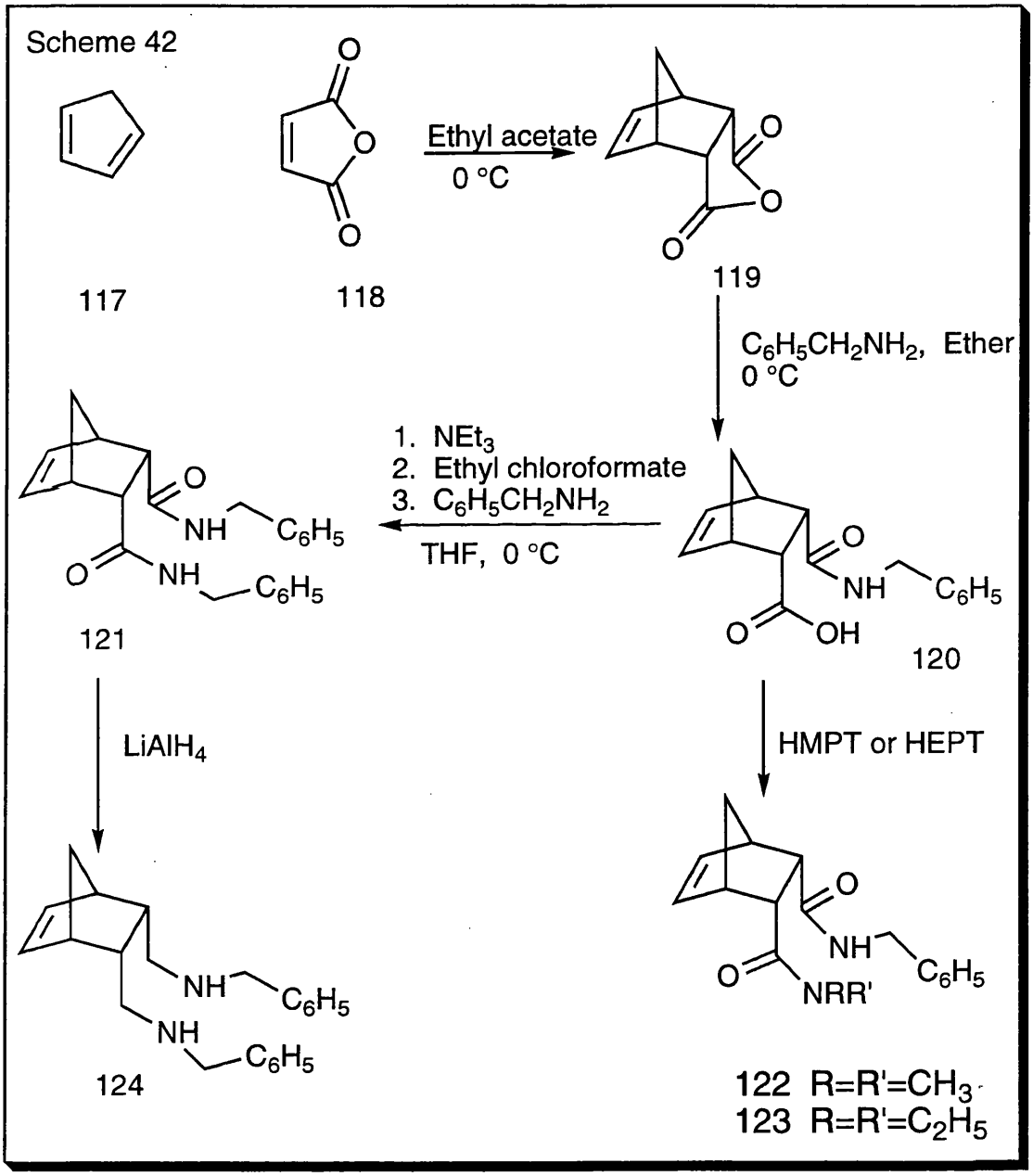
This type of compound was chosen for four main reasons.

1. The carbon framework can be constructed easily, cheaply and in high yield.

2. They are similar to dibenzyl BAD and related compounds.
3. They contain a putrescine portion within the molecule.

4. The method we selected to synthesise them should have made it possible to synthesise diamines bearing different amino substituents in the same molecule. There is evidence within the literature that suggests that putrescine analogues bearing different amino substituents are more potent antimalarial agents than their symmetrical counterparts.[139]

The method chosen for the synthesis of these compounds is shown in **Scheme 42**.



4.2.3 Synthesis of *endo-cis*-anhydride (119)

The first reaction in **Scheme 42**, the synthesis of the *endo-cis*-anhydride **(119)** via the Diels Alder reaction between cyclopentadiene **(117)** and maleic anhydride **(118)** is well documented in the literature.[140] It is a classic example of the effect of secondary orbital overlap on the cycloaddition reaction.[141]

There are two possible modes of addition of diene **(117)** to dienophile **(118)**; *endo* and *exo*, of these the *endo* geometry predominates by far.

For *endo* overlap there are two possibilities; the highest occupied molecular orbital (HOMO) of the diene overlaps with the lowest unoccupied molecular orbital (LUMO) of the dienophile, or the LUMO of the diene overlaps with HOMO of the dienophile. In both cases the *endo* orientation is stabilised by additional overlap. There is no such stabilisation when addition is from the *exo* position.[142]

The anhydride **(119)** is commercially available but as it is quick and easy to synthesise it was made in the laboratory. The reaction proceeded smoothly in a yield of 85%.

4.2.4 Reactions of Anhydride (119)

The opening of the anhydride **(119)** with the appropriate amine to give **(120)** was one we had already shown to have carried out on an impure sample of anhydride **(105).** As we could isolate the anhydride **(119)** in a pure form the yield and purity of **(120)** was significantly higher than that for **(113)**. The yield was 72% .

There was a choice of methods to convert **(120)** into the diamides **(121)**, **(122)** and **(123)**. To prepare the dibenzyl diamide **(121)** the mixed anhydride method was selected, and this gave the desired product in a yield of 71 %.

To synthesise compounds **(122)** and **(123)** the reagents hexamethylphosphorus triamide, HMPT **(125, R'=CH_3)**[143] and the analogous reagent hexaethylphosphorus triamide, HEPT **(126, R'=C_2H_5)**[144], were chosen respectively.

HMPT is prepared by the reaction of phosphorus (III) chloride with anhydrous dimethylamine and then purified by distillation.[145] The same procedure can be used to prepare the higher alkyl homologues. Both HMPT and HEPT are commercially available.

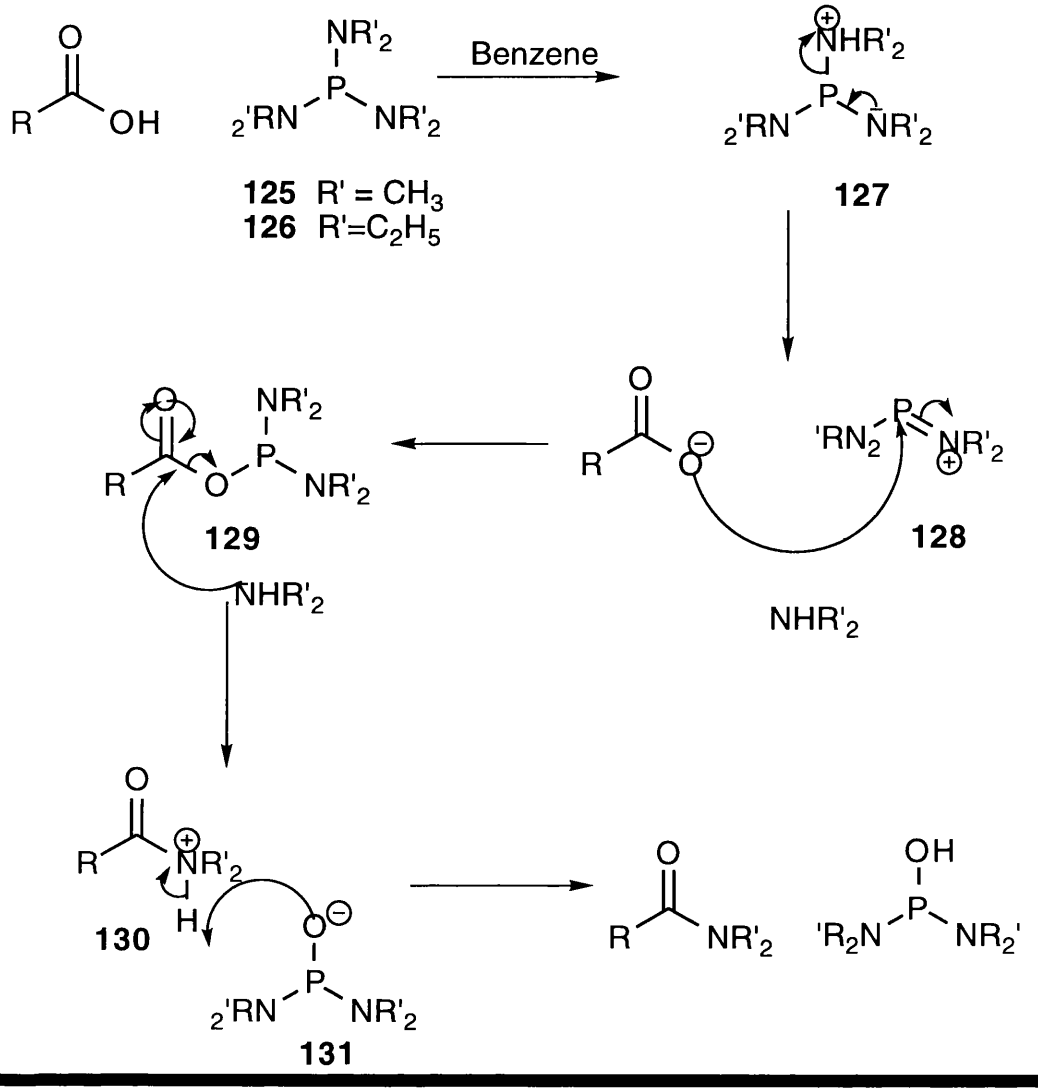
HMPT has found most of its applications in the synthesis of epoxides from aldehydes and arene oxides from aryldialdehydes.[143] It has also been used to replace triphenylphosphine in the Wittig and the Horner-Wittig reaction.[143] In all cases one of the main benefits of the reaction is that the by-product, hexamethylphosphoric triamide is water soluble and hence easily removable.[143]

The method reported by Quin *et al.* was followed to take advantage of the short reaction time and simple isolation and purification of the product.[146]

We believe the reaction to proceed by the mechanism outlined in **Scheme 43.** Protonation of HMPT by the carboxylic acid gives the intermediate **(127)** which eliminates a molecule of diethylamine to form the charged species **(128)** which reacts with the carboxylic acid or its carboxylate equivalent to form the ester **(129)** which is attacked by a molecule of diethylamine released in a previous step to give the protonated amide **(130)** which is neutralised by **(131)** to give the desired product. The mechanism for HMPT would follow an analogous path.

Both **(122)** and **(123)** as expected exist as rotamers and the ^{1}H NMR spectrum of **(122)** shows the two inequivalent methyl groups as two distinct singlets. The ^{1}H NMR spectrum also clearly shows the two doublets of doublets of the diastereotopic protons of the methylene carbon of the benzyl group.

Scheme 43

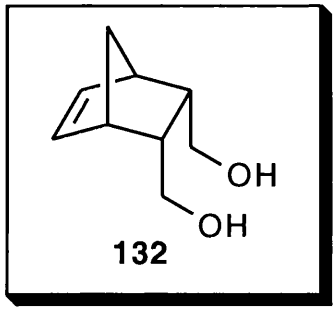


4.2.5 Attempted Reduction of (121)

The reduction of the diamides **(121)**, **(122)** and **(123)** to the corresponding diamines was investigated by working with **(121)** as this compound was available in the largest quantity and the diamine that would result from its successful reduction was of most interest.

The reduction was attempted by heating **(121)** with four equivalents of lithium aluminium hydride at reflux in THF and monitoring the reaction by TLC.[146] The procedure was carried out a number of times and on most occasions either starting material, or a complex mixture of products were

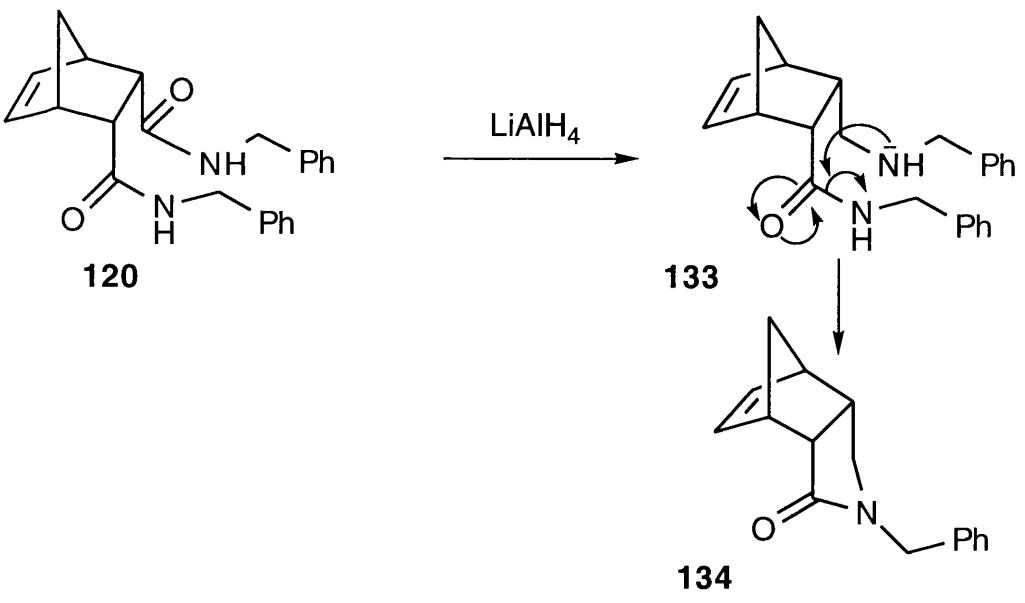
isolated, among which seemed to be the diol **(132)**. This would be the result of C-N bond cleavage as opposed to the desired C-O bond cleavage as shown by the method outlined in **Scheme 41**.



132

On one occasion TLC indicated the consumption of all the starting diamide and the appearance of a single product spot. ^{1}H NMR and ^{13}C NMR spectra of the isolated material suggested that instead of the desired product we may have obtained the half-reduced product **(133)** or possibly the lactam **(134)**. They are presumably formed by the mechanism shown in **Scheme 44**. This material was subjected to further reduction with lithium aluminium hydride and a brown oil was isolated which ^{1}H NMR spectroscopy showed to be a complex mixture.

Scheme 44

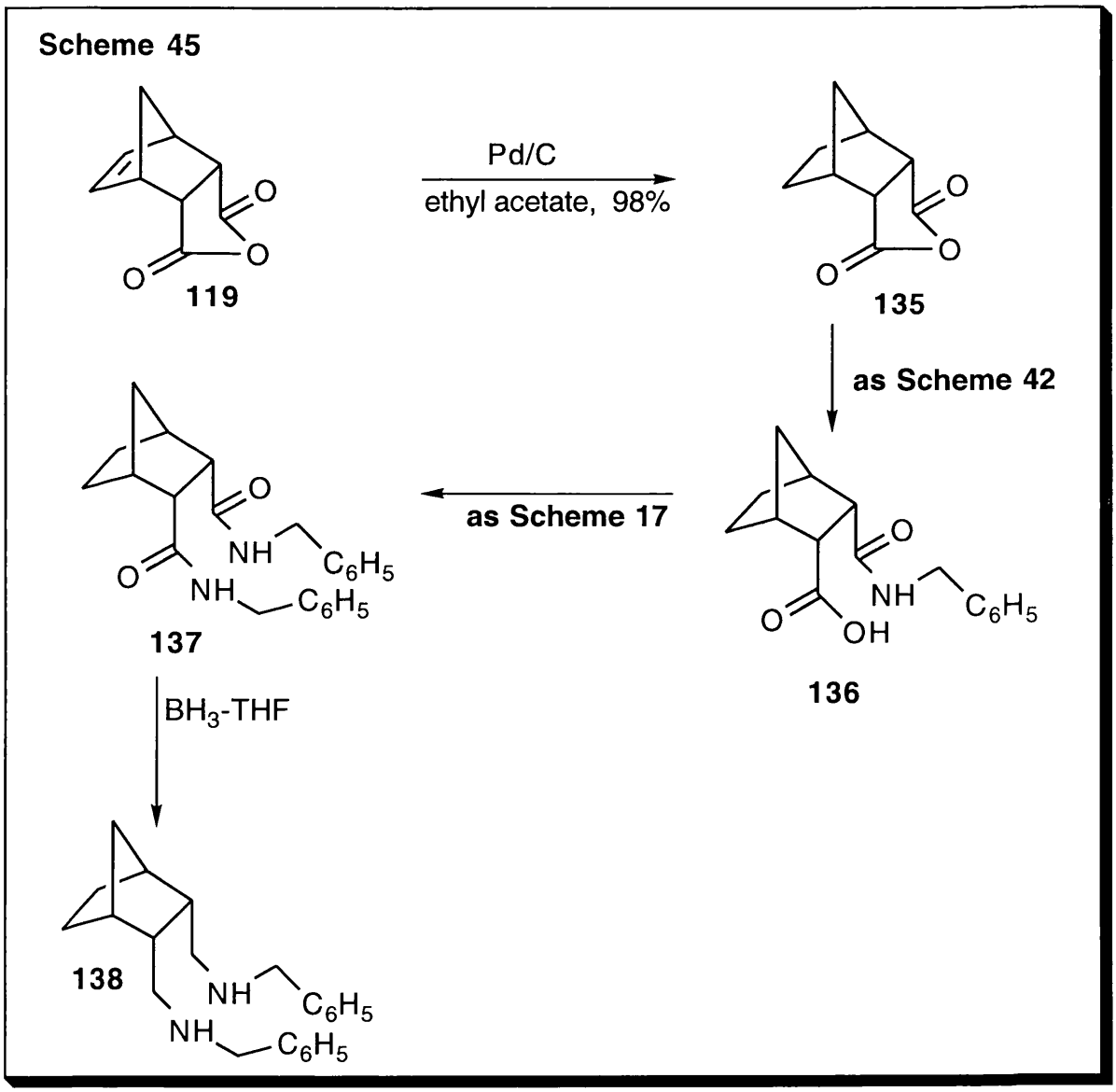


120 **133** **134**

4.2.6 Synthesis of *N,N'*-Dibenzyl-bicyclo[2.2.1]heptane-2,3-*endo*-*cis*-diamine Dihydrochloride (138)

After considering the problems encountered in synthesising dibenzyl BAD **(93)** it was decided to remove the double bond from the norbornyl ring to allow the use of borane-THF as the reducing agent.[135] We could not use borane-THF on the alkene as hydroboration would almost certainly result. We went back to the anhydride **(119)** and subjected it to hydrogenation at one atmosphere using 5% palladium on charcoal, by the method of Ohtani *et al.*[147] The reaction went smoothly in a yield of 98%. At this point we decided to concentrate on the synthesis of the dibenzylated diamine **(138)** and synthesised it by the method outlined in **Scheme 45**.

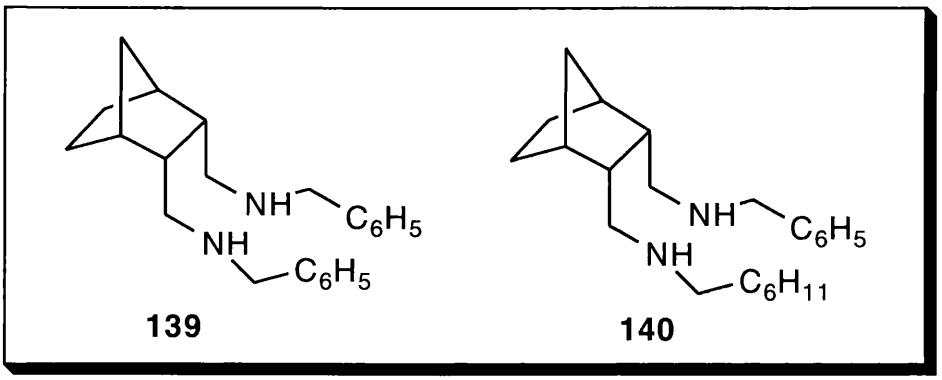
Scheme 45



The conversion of anhydride **(135)** into the dibenzylated diamide **(137)** went smoothly and in a similar yield to that recorded for the conversion of **(119)** into **(121).** Reduction of diamide **(137)** into diamine **(138)** went in 75% yield after we dissolved the crude dihydrochloride salt in ethanol and precipitated it out with ethereal hydrochloric acid.

4.2.7 Future work

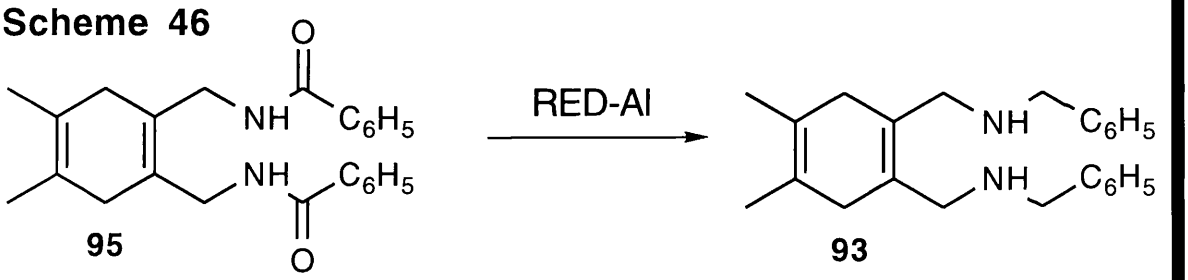
Now that we have developed a route to synthesis bicyclic putrescine analogues it would be prudent to investigate the antimalarial and antifungal activity of a carefully chosen selection of analogues. The two compounds that we would be most interested in are the dicyclohexylmethyl analogue **(139)** and the unsymmetrical analogue containing benzyl and cyclohexylmethyl moieties **(140)**. These groups have displayed the most potent antimalarial and antifungal activity in our work so far.



139 **140**

As discussed in section 4.1.16 it may be possible to reduce diamide **(95)** to dibenzyl BAD **(93)** with RED-Al®. We may investigate that possibility in the future **(Scheme 46)**.

Scheme 46



95 **93**

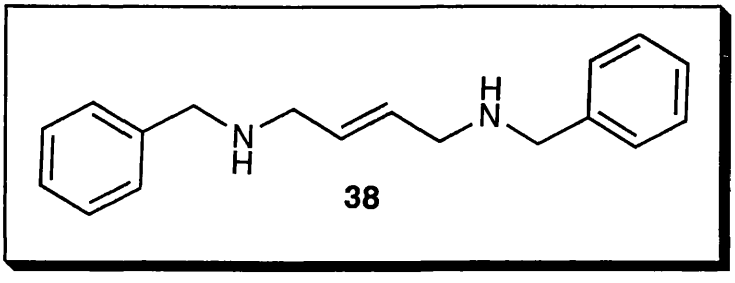
5

Polyamine Analogues

5.1 Synthesis of Polyamine Analogues

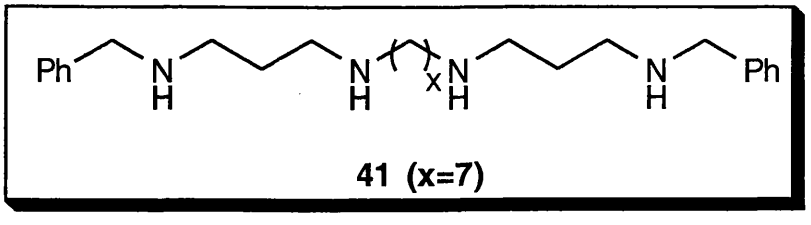
As we discussed in section 2.10 polyamine analogues have been shown to inhibit fungal growth and have a possible use in the area of antimalarial chemotherapy.

Past workers within our group had shown that synthetic putrescine analogues such as (*E*)-BED **(38)** could control important harmful fungi on crops.[41] This compound became one of our lead compounds into the area of antimalarial chemotherapy.[41]



38

We have also discussed in sections 2.11 to 2.11.2 how polyamine analogues can be used in antimalarial chemotherapy and in particular the effectiveness of the bis(benzyl)polyamine analogues, such as **(41)** developed by Bitonti *et al.* at the Merrell Dow institute in Cincinnati, USA.[59, 60]



41 (x=7)

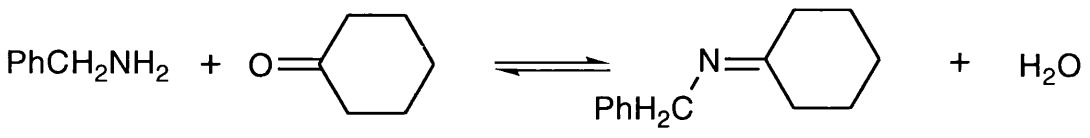
In this area of work it was decided to synthesise a number of polyamine analogues by simple, high yielding reactions to investigate how the spacing between the nitrogen atoms affected both the antimalarial and antifungal activity. The imine formation reaction and subsequent reduction

was used and a brief discussion of these reactions will be given in the following two sections.

5.2 Synthesis of Imines

Imines can be synthesised by a large variety of methods including the addition of Grignard reagents to nitriles[148] and the reduction of oximes.[149] By far the most common and simple method is by the addition of ammonia or amines to aldehydes[150,151] and ketones [152]. An example with cyclohexanone and benzylamine is shown below **(Scheme 47)**.

Scheme 47



The C=N π bond is weak and so the equilibrium of the above reaction lies to the left if there is excess water present. This does not present much of a problem when coupling aldehydes and amines as the reaction is fast, but the reaction is much slower with the corresponding amine and ketone and often longer reaction times and higher temperatures are required.[76] When using a ketone, in order to obtain a good yield of the imine it is often necessary to remove the water formed in the reaction. This can be done by azeotropic distillation, the use of molecular sieves[153] or drying agents such as titanium (IV) chloride.[154]

If the imine is conjugated to an aromatic ring then it is known as a Schiff's base and is more stable than a simple aliphatic imine. However, in most cases the imines are used immediately as they have a limited stability on storage. The diimines made in the synthesis of compounds discussed in section 5.4 are Schiff bases.

5.3 Reduction Of Imines

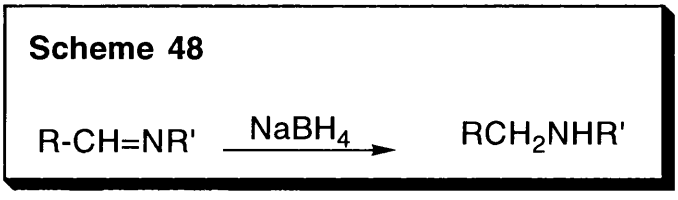
Imines can be reduced to the corresponding amines with a large number of reducing agents including aluminium hydride[155] and

diborane.[156] The two most common reagents are catalytic hydrogenation (H_2/Pd/C)[157] or sodium borohydride.[151]

We were unable to use catalytic hydrogenation with the dibenzyl diimines generated in section 5.4 as cleavage of the benzyl group would have resulted to regenerate starting material.

Sodium borohydride was used as this reagent is known to be easy to use and effective in high yield, and this proved to be the case in our studies. Our target compounds were prepared in essentially quantitative yields **(Scheme 48)**.

Scheme 48



5.4 Synthesis of *N,N'*-Bis(benzyl)diaminoalkane Dihydrochlorides

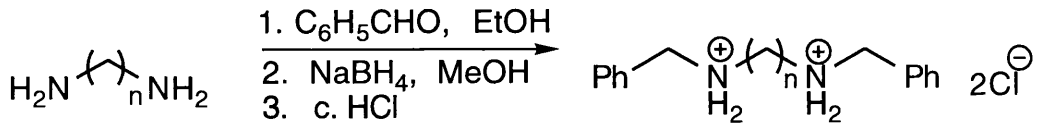
The synthesis of these compounds is very straightforward and goes in almost quantitative yield **(Scheme 49)**. The appropriate diamine was coupled with benzaldehyde to give the diimine which was reduced with sodium borohydride to give the corresponding diamine which was converted into the dihydrochloride salt upon the addition of concentrated hydrochloric acid.

The diimine from each reaction was not purified. It is possible to crystallise the diimine but this was only done on one occasion. In general they were isolated as oils and treated with sodium borohydride almost immediately. These compounds were prepared by the method of Lee *et al.*[151]

As well as the method reported by Lee *et al.* we also investigated the procedure reported by Sclafani *et al.* [152] This method involved reacting the diamine and aldehyde in chloroform in the presence of molecular sieves. The molecular sieves are there to absorb the water given off in the formation of the imine in order to drive the reaction to completion. We found

that this gave no benefit in terms of yield and made the work up of the reaction more complex.

Scheme 49



141 n=4
142 n=5

143 (n=2), **144** (n=3), **145** (n=4)
146 (n=5), **147** (n=6), **148** (n=7)
149 (n=8), **150** (n=9) and **151** (n=10)

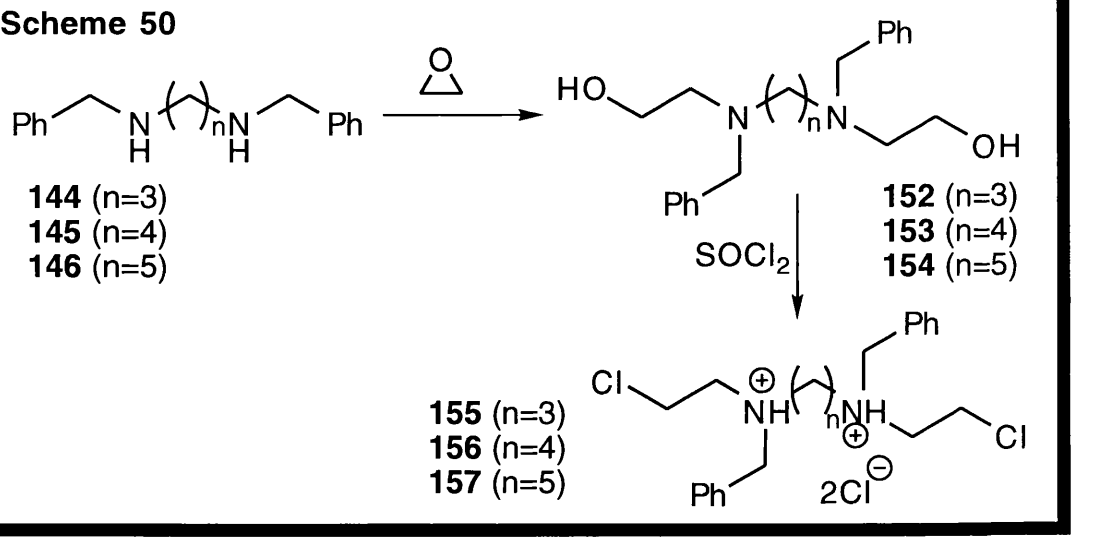
Bitonti worked mainly with spermidine analogues but we decided to work with commercially available diamines. We began our studies with putrescine **(141)** (n=4) and cadaverine **(142)** (n=5) **(Scheme 49)**. Both the *in vitro* antimalarial and antifungal testing results of these compounds were very encouraging and we decided to extend the chain length in order to see if this would increase the biological activity. We synthesised *N,N'*-bis(benzyl)diaminoalkanes with a chain length from 2 to 10 to give a complete set of compounds.

5.5 Synthesis of *N,N'*-Bis(2-chloroethyl)-*N,N'*-dibenzylaminoalkane Dihdrochlorides

Fiona Anderson, a co-worker in our group had obtained some encouraging *in vivo* antifungal testing results with a number of nitrogen mustards incorporating an *N,N'*-bis(2-chloroethyl) moiety.[158] We prepared a small number of *N,N'*-bis(2-chloroethyl)-*N,N'*-dibenzylaminoalkanes as they would be easily accessible and might provide us with further leads.

We chose to extend compounds **(144)**, **(145)** and **(146)** by the method reported by Lee *et al.* as shown in **Scheme 50**.

Scheme 50



Hydroxyethylation of these compounds presented two difficulties. Initially each *N,N'*-bis(benzyl)diaminoalkane was treated with 20 equivalents of ethylene oxide at 0 °C. The desired product was obtained each time but yields were lower than those quoted and the purity was poor. These problems were solved by carrying out the reaction at -78 °C. Twenty equivalents of ethylene oxide was used and the corresponding *N,N'*-bis(2-hydroxyethyl)-*N,N'*-bis(benzyl)diaminoalkane was isolated in a yield of about 75% and used in the subsequent step without purification.

The final step was also problematic. Firstly the thionyl chloride must be distilled from quinoline in order to remove any acidic impurities otherwise the starting material precipitates out of solution as the hydrochloride salt. Isolation of the pure final product was a problem in some cases. TLC indicated that each reaction gave one product but initially they were extremely sticky and getting them pure was time consuming. The problem was believed to be due to the large excess of thionyl chloride used and the hygroscopic nature of the compounds. Another method using only 2.2 equivalents of thionyl chloride has been found.[159]

It is difficult to distinguish between an *N,N'*-bis(2-hydroxyethyl)-*N,N'*-bis(benzyl)diaminoalkane and its corresponding *N,N'*-bis(2-chloroethyl)-*N,N'* -bis(benzyl)aminoalkane by ^{1}H NMR spectroscopy but a difference can be distinguished in the ^{13}C NMR spectra. The methylene attached to the hydroxyl group has a chemical shift δ of about 40 whereas the methylene attached to a chlorine atom comes into resonance at about δ

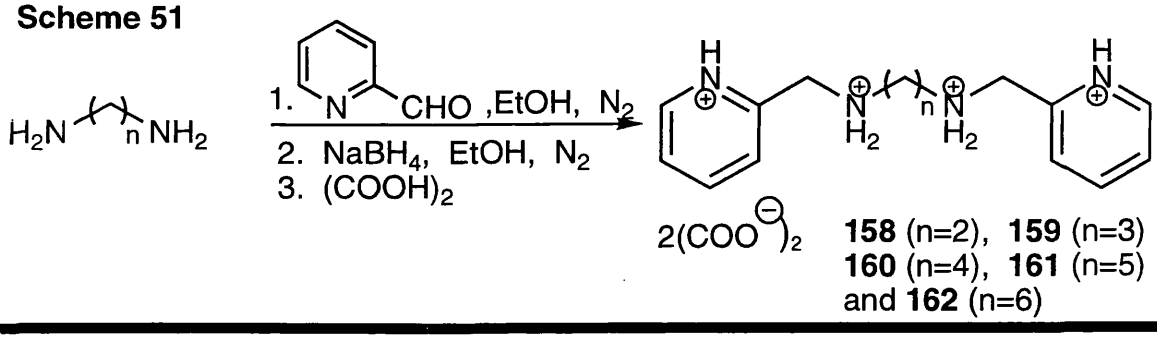
64.[160] This difference is apparent in the ^{13}C NMR spectra of our compounds.

5.6 Synthesis of *N,N'*-Bis-(2-pyridylmethyl)diaminoalkane Oxalates

To investigate the effect of introducing more nitrogen atoms into the compounds and hence increase the charge on each molecule the *N,N'*-bis-(2-pyridylmethyl)diaminoalkanes were prepared.

These compounds were synthesised in a similar route to that shown in **Scheme 51** except that the formation of the intermediate imine had to be done in rigorously dry ethanol under an inert nitrogen atmosphere. The reduction of the imine was achieved using $NaBH_4$ in ethanol. The hydrochloride salts were converted into the corresponding dioxalate salts for the purpose of testing as the pyridyl compounds we made previously had been converted into their oxalate salts due to their hygroscopic nature.

Scheme 51



158 (n=2), **159** (n=3)
160 (n=4), **161** (n=5)
and **162** (n=6)

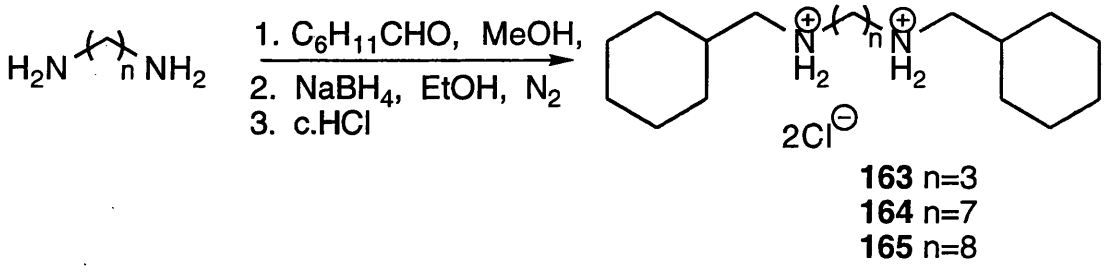
5.7 Synthesis of *N,N'*-Bis(cyclohexylmethyl)diaminoalkane Dihydrochlorides

The literature suggested that polyamines bearing cyclohexylmethyl groups exhibited good antiparasitic activity[161] and this was confirmed with the antimalarial testing results of some of the compounds synthesised by Brear.[66]

We decided to identify the most active polyamines we had synthesised and make the cyclohexylmethyl analogues. These compounds were made by coupling cyclohexanecarbaldehyde with the appropriate amine to form the imine which was reduced with $NaBH_4$ to give the desired compound as the free base. Subsequent treatment with concentrated

hydrochloric acid generated the dihydrochloride salt for biological testing. **(Scheme 52)**.

Scheme 52



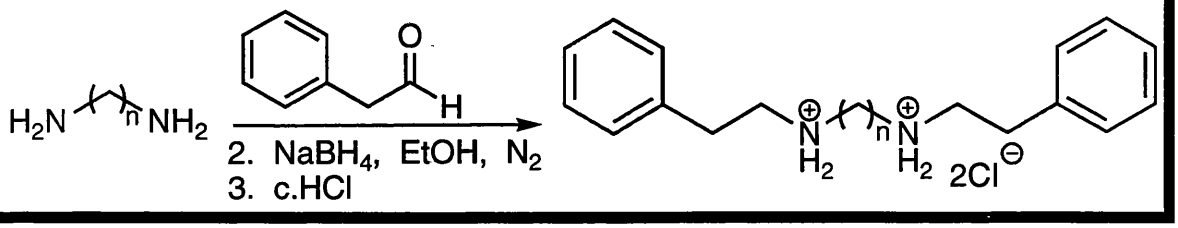
163 n=3
164 n=7
165 n=8

5.8 Future Work

Future work in this area is governed by the results of biological testing. Our testing results which are documented in chapter 6 suggest that an important factor in the antimalarial activity of our compounds is the distance between the nitrogen atoms. If this is the case then it would be prudent to continue with the synthesis of *N, N'*-bis(benzyl)diaminoalkane dihydrochlorides, increasing the chain length until we observe a drop in activity.

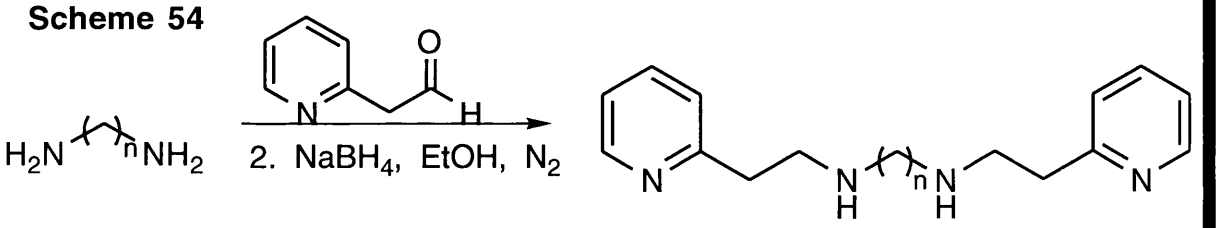
It may also be worthwhile to examine the introduction of an extra methylene group into the most active bis(benzyl) analogues to make the corresponding aminoethylbenzenes **(Scheme 53)**.

Scheme 53



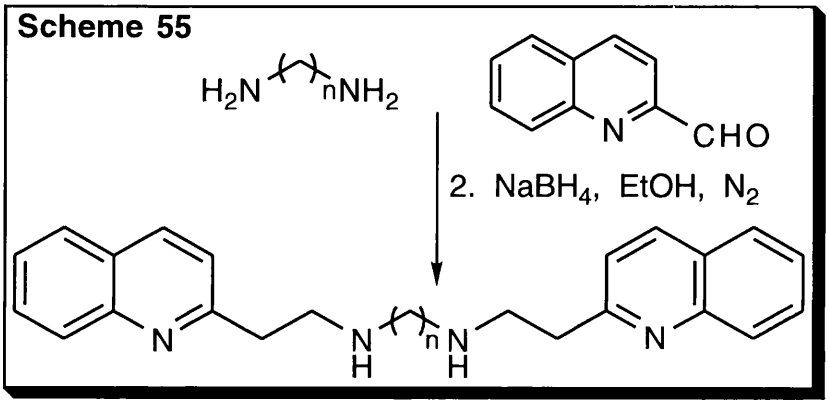
A similar reaction can be used to prepare the corresponding aminoethylpyridines. This introduces another methylene group between the nitrogen of the pyridyl ring and those of the main chain **(Scheme 54)**.

Scheme 54



Examining other ring systems is another area worth investigating. The most obvious starting point would be the introduction of the quinoline ring system **(Scheme 55)**.

Scheme 55



6

Antimalarial and Antifungal Testing Results

6.1 Biological Evaluation

This chapter will be split into two sections. The first will detail the testing regime and results of the testing of a number of those compounds for antimalarial activity. The second will deal with antifungal evaluation.

6.2 Antimalarial Evaluation

This work was carried out by Professor Stephen Phillips and Fiona McMonagle in the Institute of Biomedical and Life Sciences, University of Glasgow. Initially compounds were tested *in vitro* and if appropriate *in vivo* tests were carried out.

6.3 In vitro Testing against *P. falciparum* Asexual Erythrocytic Stages

Two *P. falciparum* culture adapted stains were used; these were FCR (Gambia) and JS (Zimbabwe). Both of these strains are chloroquine sensitive and were grown as stock cultures *in vitro* in petri dishes by modifying the candle jar technique of Trager and Jensen.[162] In culture the parasites were asynchronous in their growth and therefore all stages in the 48 hour asexual erythrocytic cycle were represented at any one time. The assay system for screening compounds for activity against *P. falciparum* was that described by Desjardins *et al.*[163] Each test was carried out as a minimum in triplicate. After 24 hours incubation in a candle jar 100 µl were removed from each well and replaced with 100 µl of fresh medium containing 1 µCi ^{3}H-hypoxanthine[164] The plate was incubated for a further 18 hours after which the parasites were harvested and parasite growth was measured as the incorporation of the radioisotope, using liquid scintillation counting. The mean and standard deviation of the counts per minute for each dilution of the test compounds and the control culture were calculated and the results displayed using Cricket Graph. The results were analysed using a standard t-test.

The activity of the compounds was shown as the lowest concentration in $\mu g\ ml^{-1}$ where the compound under testing showed any significant activity. For a further comparison this lowest activity was converted into a lowest effective concentration **(LEC)** which in μM takes into account the molecular weight of the test compound. The LEC is not an accurate IC_{50} value but it does represent a good guide to the compound's activity. We assessed the data using an activity scale (**Table 7**) which was designed to help determine whether certain analogues were appropriate for *in vivo* testing and/or further structural modifications. Chloroquine is greater than nine on this scale. Any compound that had an activity of less than four was not pursued.

Activity Scale	1	2	3	4	5	6	7	8	9
Lowest Activity ($mgml^{-1}$)	1000	500	100	50	10	5	1	0.5	0.05

(Table 7 *in vitro* antimalarial evaluation scale)

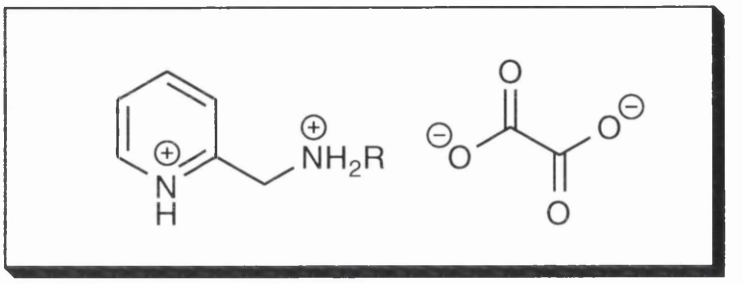
6.4 In vivo activity against *P. chabaudi*

Plasmodium chabaudi AS strain, in inbred NIH mice, was handled as described by McDonald and Phillips.[165] In brief three months old male inbred NIH mice were infected intravenously with $1x10^5$ infected erythrocytes and groups of five mice immediately afterwards were injected with either the test compound or chloroquine at 50mg/kg body weight. The compounds were dissolved in physiological saline and injected intraperitoneally. A control group of mice were given saline only.

6.5 *In vitro* Activity of Compounds Discussed in chapter 3

6.5.1 2-Substituted Aminomethylpyridine Oxalates

These are compounds of the general structure shown below.



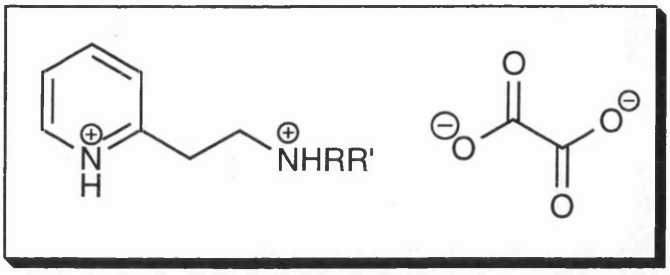
The activities of compounds **(46)**, **(47)**, **(48)** and **(49)** were measured *in vitro* against one of the *P. falciparum* chloroquine sensitive cultures named in section 6.3. Chloroquine was used as a positive control **(Table 8)**.

compound	R	Activity ($\mu g\ ml^{-1}$)	LEC (μM)
46	benzyl	5-10	17-35
47	cyclohexylmethyl	5-10	17-34
48	^{t}butyl	50	197
49	^{n}butyl	50	197
control	Chloroquine	<1	<1.9

(**Table 8** *in vitro* results for 2-substituted aminomethylpyridine oxalates)

The biological activity for these four compounds was mixed but none of the compounds came near to the activity of chloroquine. The ^{t}butyl and the ^{n}butyl analogues showed reasonable activity at 50 $\mu g\ ml^{-1}$. The benzyl and cyclohexylmethyl analogues showed promising *in vitro* activity and in fact have slightly lower LEC values than (*E*)-BED **(38)** (LEC 18-36 μM) which was one of the lead compounds when our group moved into the area of antimalarial chemotherapy.

6.5.2 2-Substituted Aminoethylpyridine Oxalates

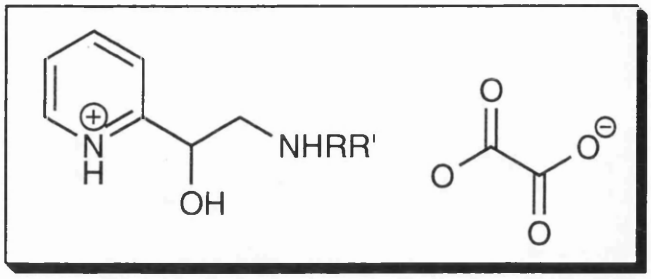


By inserting an additional methylene group into the compounds we had increased the distance between the nitrogen atoms. This resulted in an improvement in the activity of the most potent compounds discussed in section 6.5.1 above. The most marked effect was seen in **(57)**, the analogue containing the cyclohexylmethyl moiety. The *in vitro* results for this compound showed it to have comparable activity and LEC value to those of chloroquine. We also recorded a large improvement in the performance of the benzyl analogue **(56)**.The ^{t}butyl and ^{n}butyl analogues were disappointing and at this point we decided not to investigate these groups any further **(Table 9)**.

compound	R	R'	Activity ($\mu g\ ml^{-1}$)	LEC (μM)
56	benzyl	H	5	16
57	cyclohexylmethyl	H	0.1-10	0.32-32
58	^{t}butyl	H	50-100	187-383
59	^{n}butyl	H	10-100	37-186
60	ethyl	ethyl	to go in	to go in
control	Chloroquine		<1	<1.9

(**Table 9** *in vitro* results for 2-substituted aminoethylpyridine oxalates)

6.5.3 (±)–2–Hydroxy–2–(2'-pyridyl)ethylamine Oxalates



We were mainly interested in these compounds as potential antifungal compounds, but we also screened then for antimalarial activity. Results from these compounds were disappointing **(Table 10)**. The unsubstituted amino compound **(44)** showed no activity. This was expected and helps to confirm the hypothesis that to exhibit activity any potential antimalarial compound should possess hydrophobic groups to enable it to have sufficient lipophillicity to pass through the red cell membrane. This observation is backed up with the result obtained for the benzyl analogue **(70)**. Although this compound had reasonable activity it was signifcantly less active than the corresponding benzyl analogues **(46)** and **(56)**. At present we have no results for the cyclohexylmethyl analogue **(71).**

compound	R, R'	Activity ($\mu g\ ml^{-1}$)	LEC (μM)
44	H, H	no activity	N/A
70	benzyl, H	10	31
71	cyclohexylmethyl, H	not tested	not tested
control	Chloroquine	<1	<1.9

(**Table 10** *in vitro* results for (±)–2–Hydroxy–2–(2'-pyridyl)ethylamines)

6.6 Antimalarial Activity of Compounds discussed in Chapter 4

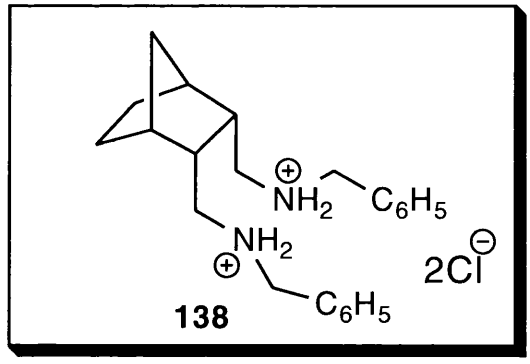
Due to the problems encountered in this area of our work we have very little to report in the way of antimalarial testing results.

6.6.1 BAD (86)

A sample of this was sent to the WHO and it was shown to have no *in vivo* activity. For the reason outlined earlier this result was not unexpected. It became apparent to us from a survey of the literature and from our own work that to exhibit antimalarial activity compounds need to possess hydrophobic moieties such as the benzyl group to give them sufficient lipophillicity to pass through parasitized red blood cell walls. This result backs up this hypothesis.

6.6.2 *N,N'*-Dibenzyl-bicyclo[2.2.1]heptane-2,3-*endo-cis*-diamine Dihydrochloride (138)

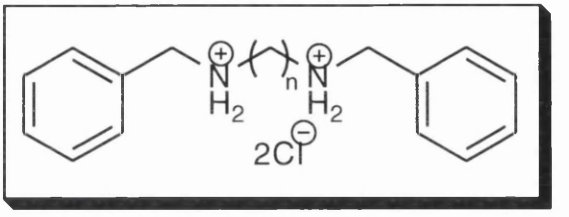
The dibenzyl compound **(138)** has been synthesised and has been made available for antimalarial evaluation.



138

6.7 *In vitro* Activity of Compounds Discussed in Chapter 5

6.7.1 *N,N'*-Bis(benzyl)diaminoalkane Dihydrochlorides

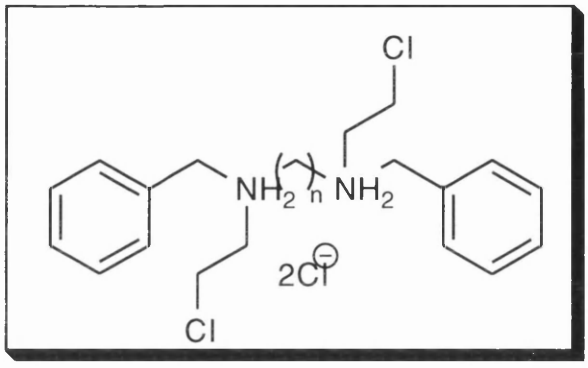


This set of compounds gave us our most promising results. It is clear that as the distance between the nitrogen atoms increases the LEC decreases and from n=7 to n=10 the activity and LEC are better than those for chloroquine. From n=2 to n=6 the activity is also very promising and this is matched by a more gradual decrease in the LEC **(Table 11)**.

compound	n	Activity ($\mu g\ ml^{-1}$)	LEC (μM)
143	2	10	32
144	3	10	30
145	4	10	29
146	5	5	14
147	6	1	2.7
148	7	0.1	0.26
149	8	<0.1-0.5	<0.25-1.26
150	9	0.1	0.24
151	10	0.1	0.23
Control	Chloroquine	<1	<1.9

(**Table 11** *in vitro* results for *N,N'*-Bis(benzyl)diaminoalkane dihydrochlorides)

6.7.2 *N,N'-Bis*(2-chloroethyl)-*N,N'*-dibenzyldiaminoalkane dihydrochlorides

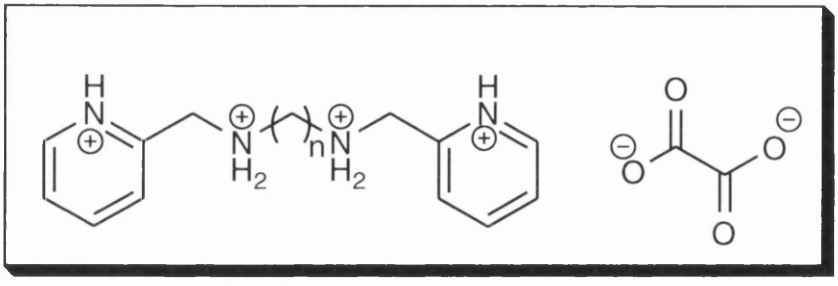


The results obtained for these compounds are also very promising and those tested showed good activity down to 5 μg ml^{-1} **(Table 12)**. We have not exploited this work any further.

compound	n	Activity (μg ml^{-1})	LEC (μM)
155	3	not tested	N/A
156	4	5	11
157	5	5	11
control	Chloroquine	<1	<1.9

(**Table 12** *in vitro* results for *N,N'-Bis*(2-chloroethyl)-*N,N'*-dibenzyldiaminoalkane dihydrochlorides)

6.7.3 *N,N'*-Bis(2-pyridylmethyl)diaminoalkane oxalates

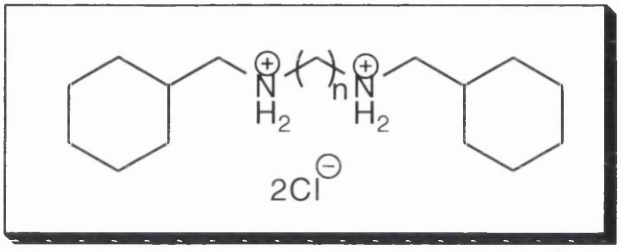


Like their bisbenzyl counterparts these compounds also showed a relationship between the value of n and activity. However, on this occasion as n decreased the activity of the compound increased. When the distance between the two nitrogen atoms was at its smallest the compound exhibited greater activity and a lower LEC *in vitro* than those values for chloroquine **(Table 13)**.

compound	n	Activity (μg ml^{-1})	LEC (μM)
158	2	0.1	0.28
159	3	0.5	1.34
160	4	10	26
161	5	10	25
162	6	10	24
control	Chloroquine	<1	<1.9

(**Table 13** *in vitro* results for *N,N'*-Bis(2-pyridyl)diaminoalkane oxalates)

6.7.4 *N,N'*-Bis(cyclohexylmethyl)diaminoalkane dihydrochlorides



We prepared these compounds to compare them with the most active *N,N'*-bis(2-pyridyl)diaminoalkane oxalates and *N,N'*-bisbenzyldiaminoalkanes we had at that time. Subsequent testing would uncover more potent analogues. We prepared **(163)** with n=3 to compare with the analogous dipyridyl compounds **(159)**, **(164)** and **(165)** to compare them with the corresponding dibenzyl compounds **(148)** and **(149)**. At present we have no data for the cyclohexylmethyl analogue **(163)**. Compounds **(164)** and **(165)** have a similar activity profile to their bisbenzyl counterparts **(Table 14)**.

compound	n	Activity (μg ml^{-1})	LEC (μM)
163	3	not tested	N/A
164	7	0.1	0.25
165	8	0.1	0.24
control	Chloroquine	<1	<1.9

(**Table 14** *in vitro* results for *N,N'*-Bis(cyclohexylmethyl)diaminoalkane dihydrochlorides).

6.7.5 Results of *in vivo* Testing against *P. chabaudi*

Three of our compounds were considered good enough candidates to be investigated further and were screened form *in vivo* activity. The compounds screened were the cylclohexylmethyl analogue **(57)** and the bis(benzyl) analogues **(146)** and **(147)**.

Each of the compounds were tested in mice infected with the rodent malaria *P. chabaudi.* Administering a 50 mg kg^{-1} dose of chloroquine was curative and no parasitaemia was detected in mice over 14 days. Our compounds showed no significant antimalarial activity at doses of 100 mg kg^{1} and 300 mg kg^{-1}. Unfortunately none of these compounds showed *in vivo* antimalarial activity.

6.7.6 Summary of Antimalarial Testing Results

Our antimalarial work gave us some extremely active compounds *in vitro*, some with activity comparable to chloroquine. We have identified a number of promising new leads. It also supports the hypothesis that large hydrophobic moieties such as benzyl groups are required for antimalarial activity.[59, 60]

The *in vitro* results also strongly suggest a relationship between the distance separating nitrogen atoms in polyamines and activity. This was seen most dramatically with the *N,N'*-bis(benzyl)aminoalkane dihydrochlorides where activity increased 10 fold and the LEC decreased more than 100 fold in moving from the compound with the smallest N-N distance **(143)** to that with the longest N-N distance **(151)** in our study.

As we indicated in section 2.11.2 the action of bis(benzyl)polyamines has been attributed to the direct binding of the compound to DNA with the subsequent disruption of macromolecular synthesis and cell death. It is possible that this mechanism is occurring with our compounds. Inhibition of ODC activity has also been suggested as a reason for the action of compounds similar to ours.

The dependence on nitrogen-nitrogen distance was also clearly seen in the 2-substituted aminomethylpyridine oxalates and the 2-substituted aminoethylpyridine oxalates, where the most potent analogues with the extra

methylene between the nitrogen atoms was substantially more active. It would appear that the introduction of a hydroxyl group into the 2-substituted aminoethylpyridines to produce (±)-2-hydroxy-2-(2'-pyridyl)ethylamines resulted in a decrease in activity.

It has also become clear that polyamines bearing cyclohexylmethyl substituents can show excellent *in vitro* activity. It would be interesting to examine them for *in vivo* activity.

In vivo testing results so far have been disappointing, unfortunately this is a common occurrence in the area of antimalarial research.

6.8 Antifungal Testing Results

Antifungal testing was carried out by Dr Dale Walters, Department of Plant Sciences, The Scottish Agricultural College, Auchincruive.

The first group of compounds supplied to Walters for *in vitro* evaluation were the 2-substituted aminomethylpyridine oxalates **(46)**, **(48)** and **(49)** , the *N, N*-'bis(benzyl)diaminoalkane dihydrochlorides **(144)** - **(147)**, the *N,N'*-bis(2-chloroethyl)-*N,N'*-dibenzylaminoalkane dihydochlorides **(155)** - **(157)** and finally the *N,N'*-bis(2-pyridyl)diaminoalkane oxalates **(159)** and **(160).**

Each of the compounds was assessed for its ability to inhibit *in vitro* mycelial growth of *Pyrenophora avenae*. Only compounds **(146)**, **(147)** and **(159)** at 0.1 mM concentration were effective at reducing mycelial growth. The reduction of mycelial growth after seven days is shown in **Table 15**.

Compound	% inhibition of mycelial growth
146	45
147	36
159	55

(**Table 15** *in vitro* inhibition of mycelial growth for selected polyamines)

These compounds all significantly reduced the *in vitro* growth of *Pyrenophora avenae* on solid media although interestingly they all affected enzyme activities and polyamine concentrations in different ways. A brief

description of the results and a discussion of some of their implications will follow.

6.8.1 ODC and AdoMetDC Actvity

The activity of the enzymes ODC and AdoMetDC was measured in the presence of compounds **(146)**, **(147)** and **(159)**. Compounds **(147)** and **(159)** were assayed in a different group from **(146)** hence the need for two controls **(Table 16)**.

	Enzyme Activity (pmol CO_2) (mg protein)$^{-1}$ hr^{-1}	**Enzyme Activity (pmol CO_2) (mg protein)$^{-1}$ hr^{-1}**
Treatment	**ODC Activity**	**AdometDC Activity**
control	906.8 ±105.8	110.6±19.8
159	1043.6±342.5	70.6±19.8
147	907.2±106.6	80.4±33.6
Control	801.6±377.0	49.1±9.5
146	1897.3±294.0*	51.4±6.0

(**Table 16** Effects of **(159)**, **(147)** and **(146)** on ODC and AdoMetDC activities in *P. avenae* values are means of 4 replicates ± standard errors)

Statistical differences shown at $^{*}P \leq 0.05$

We can see from the table that ODC activity in **(147)** and **(159)** was not increased but in the presence of **(146)** there was a 2.4 fold increase in its activity. AdoMetDC activity was reduced by both **(147)** and **(159)** but there was no difference in AdoMetDC activity in the presence of **(146)**.

6.8.2 DAO and PAO Activity

DAO activity was increased by 33% by **(146)** but not at all by **(147)** and only a slight increase was observed in the presence of **(159)**. PAO activity doubled in the presence of **(158)**, while a 66% increase was noted in the presence of **(146)** no effect was observed with **(146)** **(Table 17)**.

	Enzyme Activity (pmol CO_2) (mg protein)$^{-1}$ hr^{-1}	Enzyme Activity (pmol CO_2) (mg protein)$^{-1}$ hr^{-1}
Treatment	**DAO Activity**	**PAO Activity**
control	100.1±32.1	32.8±5.8
146	133.2±17.6	54.5±9.9**
147	103.2±12.4	32.6±2.9
159	114.9±15.8	68.3±5.0

(**Table 17** Effects of **(146)**, **(147)** and **(159)** on DAO and PAO activities in *P. avenae* values are means of 4 replicates ± standard errors).
Statistical differences shown at $^{**}P \leq 0.01$

6.9 Polyamine Concentrations

The effects of our compounds on polyamine concentrations are shown in **Table 18**. Spermine concentration was reduced by 30-40% in the presence of all the test compounds. Compounds **(147)** and **(159)** did not alter spermidine levels but **(146)** led to a 32% decrease in spermidine concentration. All three of the compounds caused a reduction in the level of cadaverine recorded in the fungus while **(146)** and **(147)** reduced the putrescine levels, and **(159)** caused a slight increase.

Polyamine Concentration (μM/gf.wt)

Treatment	Spermine	Spermidine	Cadaverine	Putrescine
Control	210.0±12.3	182.3±4.4	314.1±21.4	355.9±19.0
159	143.1±11.6**	186.1±6.4	258.9±27.6	410.0±77.9
147	145.9±23.9	188.3±13.1	221.6±68.9	317.3±47.1
Control	157.7±20.1	173.3±21.1	546.7±28.1	683.3±83.6
146	93.1±10.9*	117.4±9.7	268.4±19.7***	416.8±29.1*

(**Table 18** Effects of **(159)**, **(147)** and **(146)** on polyamine concentrations in *P. avenae* values are means of 4 replicates ± standard errors).
statistical differences shown at $^{*}P \leq 0.05$. $^{**}P \leq 0.01$ and $^{***}P \leq 0..001$

6.10 Discussion of Biological Testing Results

As we said in section 6.8 **(146)**, **(147)** and **(159)** all significantly reduced *in vitro* mycelical growth of *P. avenae* and as we have shown with a selection of our biological testing results they all affected enzyme activities and polyamine activities in different ways.

Perhaps the most interesting results were demonstrated by **(146)**. In our studies ODC activity more than doubled in the presence of **(146)**. This should have led to an increase in the polyamine concentrations, but there were significant reductions in the levels of putrescine, cadaverine, spermine and spermidine **(Table 18)**. AdometDC activity was not altered and this coupled with the reduction in putrescine levels could account for the reduction in spermine and spermidine levels. One possible reason for the reduced putrescine levels is that there may have been an increased efflux of putrescine from the cells, or putrescine may have been acetylated to form *N*-acetylputrescine.[166] Similar results have been seen when the *in vitro* growth of *P. avenae* was inhibited by (*E*)-TED **(38)**. The reasons outlined above may also account for the large reduction in cadaverine concentration.

It is clear that for whatever reason polyamine concentrations were reduced by **(146)** and this correlates with the observed reduction in mycelial growth. It is well established that polyamines are essential for normal growth so reducing their levels should repress the growth of fungi. The results discussed show that it is possible that perturbation of polyamine biosynthesis in the presence of **(146)** could be the cause of the observed fungicidal effect.[167]

Compound **(147)** differs from **(146)** by the addition of one methylene group in the diamine chain. The effects shown for **(147)** were not as pronounced as those for **(146)** and **(159)**. It induced insignificant reductions in amounts of putrescine and cadaverine. No change was observed in spermidine levels, but there was a reduction in spermine levels. The reduction of putrescine levels was not matched by a decrease in ODC activity and there was no increase in DAO. The inhibition of mycelial growth by **(147)** cannot be correlated with inhibition of polyamine biosynthesis.

The tetraamine **(159)** induced putrescine levels higher than the control, even though there was only a small increase in ODC activity. The

increased putrescine levels may be due to the substantial increase in PAO activity. Although spermine levels were significantly reduced, spermidine levels were similar to control levels. The large increase in PAO activity may account for the fungicidal activity displayed.

It is probable that closely related compounds, particularly **(146)** and **(147)** are displaying powerful antifungal activity by different mechanisms. Further work, such as *in vivo* studies, is required to determine if perturbation of polyamine metabolism and/or function is responsible for the antifungal activities displayed.

At present we are awaiting *in vitro* results for twenty compounds.

Further Antifungal Testing Results

Preliminary data are presented for the following compounds

The effects of a 0.25mM solution of the following compounds on powdery mildew infection of barley seedlings were recorded. Measurements were taken seven days after inoculation and are displayed in the table below.

Compound	% Infection
control	34 ± 1.9
70	11 ±1.2
71	16 ± 1.3
143	10 ± 1.2
151	6 ± 0.9
163	15 ± 1.2
164	10 ± 1.2
165	12 ± 1.9

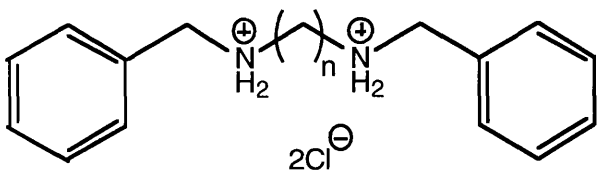
It is clear from these results that all of the compounds substantially inhibited fungal growth.

The *N,N'*-bis(benzyl)diaminoalkanes **(143)** and **(151)** showed a possible correlation with activity and the distance between the nitrogen atoms. The activity of the compounds increased as the distance between the nitrogen atoms increased. The % infection nearly halved when going from **(143)** n=2 to **(151)** n=10. This is in agreement with our previous work.

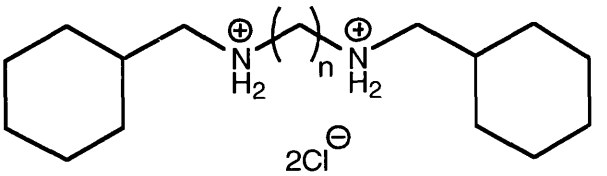
The *N,N'*-bis(cyclohexylmethyl)diaminoalkanes **(163)**, **(164)** and **(165)** also showed encouraging antifungal activity although the activity does not appear to related to the distance between the nitrogen atoms.

The final group of compounds in this section are the (±)-2-hydroxy-2-(2'-pyridyl)ethylamines. These compounds showed good activity at 0.25 mM. The benzyl derivative **(70)** was slightly more active than the cyclohexylmethyl derivative **(71)**.

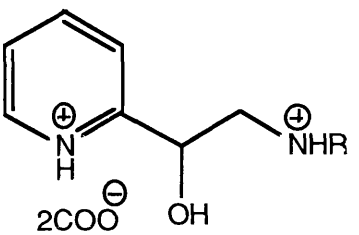
All the compounds in this section are being investigated for their effects on enzyme activity and polyamine concentrations.



(143) n=2
(151) n=10



(163) n=3
(164) n=7
(165) n=8



70 R =benzyl
71 R =cyclohexylmethyl

7

Experimental to Chapters 3 - 5

7.1 General Experimental Section

All chemicals were purchased either from the Aldrich Chemical Co. (Gillingham, Dorset, U.K.) or Lancaster Synthesis Ltd (Eastgate, White Lund, Morecambe, U.K.). The chemicals were used without further purification except where stated.

Commercially available solvents were used without further purification except those detailed below. Methanol and ethanol were dried by distilling from magnesium filings. Ether, tetrahydrofuran (THF), dioxane and benzene were dried by distillation from sodium and benzophenone.

All melting points (mp) were measured with a Gallencamp apparatus and are uncorrected.

All NMR spectra were obtained either on Bruker AM200-SY, AM360-SY or AM400-SY spectrometers operating at 200 MHz for ^{1}H NMR spectra and 50 MHz for ^{13}C NMR spectra, 360 MHz for ^{1}H NMR spectra and 90 MHz for ^{13}C NMR spectra and 400 MHz for ^{1}H NMR spectra and 100 MHz for ^{13}C NMR spectra, respectively. All coupling constants quoted are given in Hz. The numbering system used is solely to aid NMR characterisation, and does not reflect IUPC rules.

Mass spectra were recorded on AEI MS12 or a VG updated MS 902 mass spectrometer.

Infrared spectra were recorded using a Perkin Elmer 500 spectrophotometer.

Thin layer chromatography (TLC) was carried out on silica gel G plates of 0.25 mm thickness. Compounds were visualised by UV, iodine or with the dragendorff reagent. Column chromatography was carried out on silica gel, 70-230 mesh, 60 Å.

Section 7.2 Experimental to Chapter 3

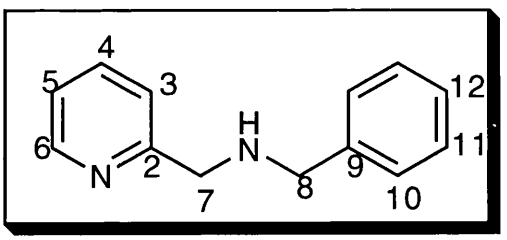
General Procedure A - Synthesis of 2-substituted aminomethylpyridines

Dry ether was added to a dry 3-necked flask fitted with a condenser, stirring bar, septum and N_2 balloon. With stirring 2-pyridinecarbaldehyde was added and the resulting solution was stirred for 10 min. One equivalent of the appropriate primary amine was added dropwise and the solution became cloudy. The solution was stirred at room temperature for a period of 3-16 h. Solvent was removed under reduced pressure to give the imine as a yellow oil.

The imine was dissolved in dry ethanol and added to a dry flask as above and 2 equivalents of $NaBH_4$ were added slowly. After addition was complete the solution was stirred at room temperature overnight. The reaction mixture was washed with brine (1 x 15 cm^3) and extracted with chloroform (3 x 50 cm^3). The organic extracts were dried with anhydrous sodium sulfate, filtered and concentrated to leave the crude free base. A sample of the free base was converted into its oxalate salt by dissolving it in methanol (5 cm^3) and 1 equivalent of oxalic acid dissolved in methanol (5 cm^3) was added. The precipitate was dried and recrystallised from water or 95% aqueous ethanol.

The yields are based on the crude free base as not all of each sample was converted into its oxalate salt.

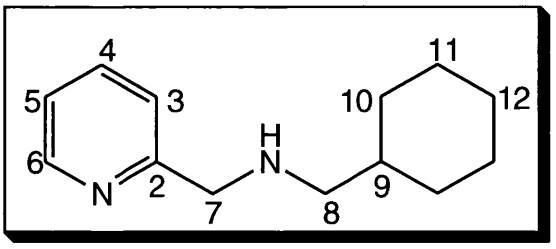
2-Benzylaminomethylpyridine (46)[168]



General procedure A was used on a 14 mmol scale to give **(46)** as a yellow oil (1.81 g, 11 mmol, 78%); oxalate salt (white crystals) mp 202-204 °C; δ_H (200 MHz, D_2O);[168] 4.11 (2 H, s, 8-H_2), 4.16 (2 H, s, 7-H_2), 7.25 (7 H, m, 3-H, 5-H, 10-H, 11-H and 12-H), 7.69 (1 H, dt, *J* 7.8, 1.7, 4-H) and 8. 38 (1H, d, *J* 4.0, 6-H); Oxalate salt, new data: ν_{max}(KBr)/cm^{-1}; 3436, 3036, 2849, 1716, 1590, 1445

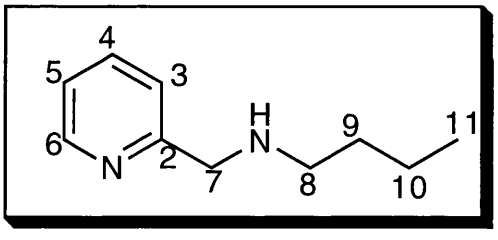
and 942; (*m /z*)(+CI/NH_3) 198 (M +H)$^+$(%) 199 (100), 197 (12), 96 (30) and 79 (20).

2-Cyclohexylmethyaminoethylpyridine (47)



The title compound was prepared by general procedure A on a 28 mmol scale (5.2g, 25.5 mmol, 91%); (Oxalate Salt) mp 207-210 °C; ν_{max} (KBr disc/cm^{-1}) 3438, 3049, 2926, 1719, 1593, 1473, 1200, 764 and 720; (Found: C, 61.26; H, 7.53; N, 9.61 $C_{15}H_{22}N_2O_4$ requires C, 61.22; H, 7.48; N, 9.52); δ_H (400 MHz, D_2O); 0.96 (2 H, m, 11-H_2), 1.15 (4 -H, m, 10-H_2), 1.53 (1 H, m, 9-H), 1.65 (4 H, m, 12-H_2), 2.88 (2 H, d, *J* 8.0, 8-H_2), 4.28 (2 H, s, 7-H_2), 7.44 (2 H, m, 3 and 5-H_2), 7.88 (1 H, m, 4-H_2) and 8.50 (1 H, d, *J* 4.0, 6-H); δ_C (100 MHz, D_2O); 25.3 (CH_2), 25.8 (CH_2), 30.1 (CH_2), 34.8 (CH), 51.3 (CH_2), 53.8 (CH_2), 124.9 (CH), 125.1 (CH), 139.6 (CH), 149.2 (CH), 150.0 (C) and 166.4 (C=O); (*m/z*) (+CI/NH_3) 204 (M+H)$^+$ (%) 205 (100), 121 (2.5) and 93 (2.5); (Found (M+H)$^+$ 205.1705 $C_{13}H_{20}N_2$ requires 205.1705).

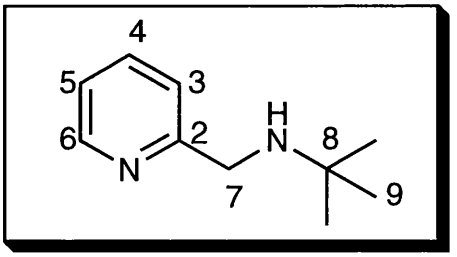
2-Butylaminomethylpyridine (48)[168]



General procedure A was used on a 14 mmol scale except that the imine was dried with Drierite® before treatment with sodium borohydride to give **(48)** as a yellow oil (2.1 g, 12.9 mmol, 92%); oxalate salt data (white crystals); mp 198-200 °C. (lit.[168] 199-200 °C); δ_H (400 MHz, D_2O) 0.74 (3 H, t , *J*, 7.4, 11-H_3), 1.21 (2 H, m, 10-H_2), 1.52 (2 H, m, 9-H_2), 2.96 (2 H, m, 8-H_2), 4.21 (2 H, s, 7-H_2), 7.37 (2 H, m, 3 and 5-H_2), 7.80 (1 H, td, *J* 7.8, 1.7, 4-H) and 8.43 (1 H, d, *J* 4.3, 6-H); New data, ν_{max}(KBr)/cm^{-1} 3436, 3051, 2870, 1716, 1654, 1473 and

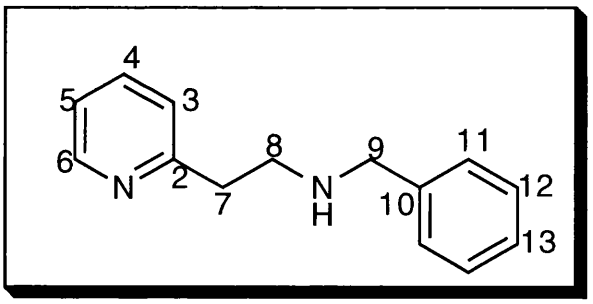
865; δ_C (100 MHz, D_2O); 13.0 (CH_3), 19.4 (CH_2), 27.7 (CH_2), 47.6 (CH_2), 51.0 (CH_2), 124.7 (CH), 125.0 (CH), 139.5 (CH), 149.1 (CH), 150.1 (C) and 166.4 (C=O); (*m/z*) (+CI/NH_3) 164 $(M+H)^+$ (%) 165 (100), 163 (5), 96 (5) and 79 (5).

2-tert-Butylaminomethylpyridine (49)[169]



General procedure A was used on a 14 mmol scale to give **(49)** as a yellow oil (1.9 g, 11.6 mmol, 83%); Free base; δ_H (400 MHz, $CDCl_3$); 1.00 (9 H, s, 9-H_3) 3.70 (2 H, s, 7-H_2); 6.96 (1 H, m, 5-H); 7.16 (1 H, m, 3-H), 7.40 (1 H, m, 4-H) and 8.35 (1 H, m, 6-H); oxalate salt data (white crystals) mp 119-120 °C; New data, ν_{max}(KBr)/cm^{-1} 3345, 2771, 2462, 1719, 1609, 1383 and 980; δ_C (100 MHz, D_2O) 25.1 (CH_3), 45.5 (CH_2), 58.0 (C), 124.6 (CH), 125.0 (CH), 139.5 (CH), 149.0 (CH), 150.5 (C) and 166.5 (C=O); (*m/z*)(+CI/NH_3) 164 $(M+H)^+$ (%) 165 (100), 149 (5), 96 (22) and 79 (15)

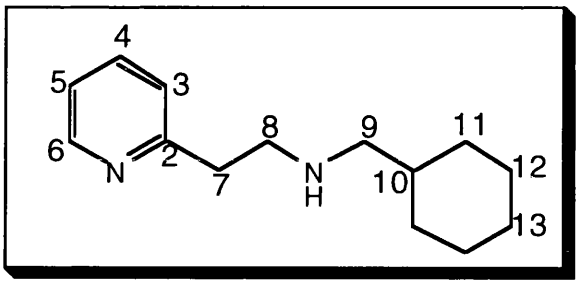
2-Benzylaminoethylpyridine (56)[170]



Dry ethanol (20 cm^3) was added to an oven-dried 3-necked flask fitted with a condenser, septum and nitrogen balloon. 2-Vinylpyridine (2 g, 19 mmol) and acetic acid (1.14 g, 19 mmol) was added and the solution was stirred. Benzylamine (1.38 g, 19 mmol) was added and the solution brought to reflux temperature which was maintained for 5 h. The solution was allowed to cool and stirred at room temperature for 14 h. The solvent was removed *in vacuo* to give 3.2 g of a white solid which was dissolved in 5 M NaOH (50 cm^3) and extracted with chloroform (3 x50 cm^3). The organic extracts were dried (K_2CO_3), filtered and evaporated *in vacuo* to give a brown oil. The title compound was obtained by silica gel

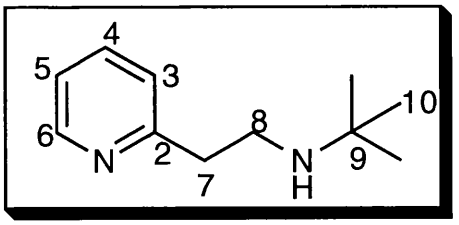
chromatography of the oil (hexane - ethyl acetate, 7:2). The free base was converted into the oxalate salt in a manner similar to that outlined in general procedure A. The precipitate was filtered, dried and recrystallised from 95% aqueous ethanol.(1.61 g, 7.6 mmol, 54%); (Found : C, 63.69; H, 6.10; N, 9.27% requires C, 63.58; H, 5.96; N, 9.27%); δ_H (400 MHz, D_2O); [170] 3.27 (2 H, m, 7-H_2), 3.38 (2 H, m, 8-H_2), 4.18 (2 H, s, 9-H_2), 7.36 (5 H, m, 11, 12 and 13 -H), 7.60 (2 H, m, 3 and 5-H), 7.60 (2 H, m, 3 and 5-H), 8.13 (1 H, dt, *J* 8.0, 1.7, 4-H), 8.49 (1 H, d, *J* 4.0, 6-H); *(m/z)* (+CI/NH_3), 212 $(M+H)^+$(%) 213 (100), 211 (2.8) and 121 (2).

2-Cyclohexylmethylaminoethylpyridine (57)



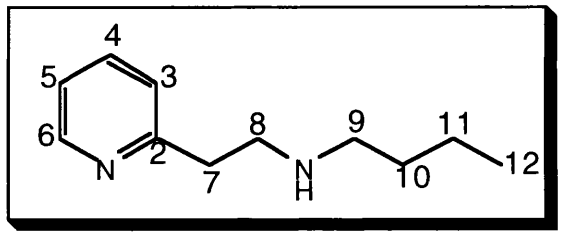
Compound **(57)** was prepared on a 15 mmol scale and purified in a similar manner to that of **(56)** (1.18 g, 5.4 mmol, 36%); oxalate salt data; mp 178-180°C; (Found: C, 58.23; H, 7.58; N, 10.45. $C_{13}H_{20}N_2O_4$ requires C, 58.21; H, 7.46; N, 10.45); ν_{max} (KBr)/cm-^{1}3059, 3013, 2927, 2852, 2810, 1718, 1701, 1475, 1217, 770, 720 and 709; δ_H (400 MHz, D_2O); 0.88 (2 H, m, 13-H_2), 1.08 (4 H, m, 12-H_2), 1.52 (1 H, m, 10-H), 1.59 (4 H, broad signal, 11-H_2), 2.84 (2 H, d, *J* 6.6, 9-H), 3.33 (4 H, m, 7 and 8-H_2), 7.67 (2 H, m, 3 and 5-H), 8.22 (1 H, distorted dd, *J* 7.8, 4-H), 8.52 (1 H, d, *J* 5.4, 6-H); δ_C (100 MHz, D_2O); 25.2 (CH_2), 25.7 (CH_2), 30.1 (CH_2), 30.7 (CH_2), 34.9 (CH), 46.4 (CH_2), 54.0 (CH_2), 125.4 (CH), 126.7 (CH), 144.0 (CH), 144.9 (CH) 152.8 (C), 168.9 (C=O); (*m/z*) 218 (%) (+CI/NH_3) $(M+H)^+$ 219 (100), 135 (5) and 14 (5); (Found: $(M+H)^+$ 219.1860 $C_{14}H_{22}N_2$ requires 219.1861).

2-tert-Butylaminoethylpyridine (58)[171]



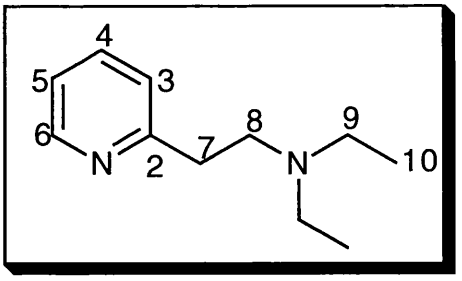
The title compound **(58)** was prepared on a 19 mmol scale and purified in a manner similar to that described for **(56)**, (1.33 g, 7.4 mmol, 39%); Oxalate salt data; ν_{max} (KBr)/cm^{1}; 3421, 3036, 2977, 2794, 1590, 794, 706 and 486; δ_H (400 MHz, D_2O); 1.29 (9-H, m, 10-H_3), 4.19 (6-H, s, 7 and 8-H_2), 7.33 (1 H, dd, *J* 7.0, 5.3, 5-H), 7.39 (1 H, d, *J* 8.0, 3-H); 8.18 (1 H, dt, *J*, 7.9, 1.6, 4-H); 8.41 (1 H, d, *J* 4.0, 6-H); δ_C (100 MHz, D_2O); 25.1 (CH_3), 31.6 (CH_2), 40.2 (CH_2), 57.9 (C), 125.3 (CH), 126.7 (CH), 144.4 (CH), 144.6 (CH), 153.1 (C) and 170.2 (C=O); (*m/z*) (+CI/NH_3) 179, $(M+H)^+$ (%) 179 (100).

2-Butylaminomethylpyridine (59)[67]



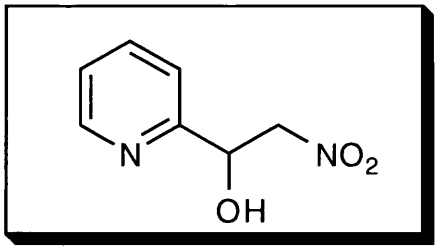
The title compound **(59)** was prepared on an 19 mmol scale and purified in a manner similar to that described for **(56)** (1.43 g, 42%, 8 mmol), Oxalate salt data; (Found: C, 58.23; H, 7.58; N, 10.45%. $C_{13}H_{20}N_2O_4$ requires C, 58.21; H, 7.46; N, 10.45%) New data: ν_{max} (KBr)/cm-^{1}, 3078, 2964, 2934, 1719, 1578, 1214, 751 and 705; δ_H (400 MHz, D_2O), 0.80 (3 H, t, *J* 7.4, 12-H_3), 1.26 (2 H, m, 11-H_2), 1.55 (2 H, m, 10-H_2), 2.99 (2 H, t, *J* 7.4, 9-H_2), 3.31 (2 H, m, 8-H_2), 3.36 (2 H, m, 9-H_2), 7.65 (2 H, m, 3 and 5-H), 8.18 (1 H, dt, *J*, 7.9, 1.6, 4-H) and 8.51 (1 H, d, *J* 0.5, 6-H); δ_C (100 MHz, D_2O) 13.1 (CH_3), 19.5 (CH_2), 27.8 (CH_2), 31.2 (CH_2), 46. 1 (CH_2), 48.0 (CH_2), 125.2 (CH), 126.7 (CH), 144.4 (CH), 144.6 (CH), 153.2 (C) and 170. 5 (C=O); mp 176-177 °C (*m/z*) (+CI/NH_3) 178, $(M + H)^+$(%) 179 (100).

2-Diethylaminoethyl pyridine (60)[172]



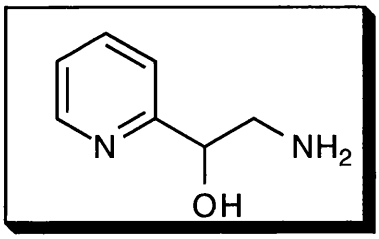
The title compound **(60)** was prepared on a 19 mmol scale and purified in a manner similar to that described for **(56)** (1.05 g, 5.9 mmol, 31%); Oxalate salt data, mp 93-95 °C. (Found : C, 57.06; H, 7.22; N, 10.15 %. $C_{13}H_{20}N_2O_4$ requires C, 57.21; H, 7.46; N, 10.45 %); ν_{max} (KBr)/cm-^{1}, 3049, 2853, 1718, 1475, 1209, 789, 754 and 701; δ_H (400 MHz, D_2O); 1.21 (6 H, t, *J* 7.3, 10-H_3), 3.19 (4 H, q, *J* 7.3, 9-H_2), 3.31 (2 H, m, 8-H_2), 3.45 (2 H, m, 7-H_2), 7.63 (2 H, m, 3 and 5-H), 8.16 (1 H, td, *J* 8.0, 1.6, 4-H) and 8.50 (1 H, d, *J* 4.0, 6-H); δ_C (100 MHz, D_2O) 8.4 (CH_3), 29.3 (CH_2), 47.8 (CH_2), 50.2 (CH_2), 125.2 (CH), 126.6 (CH), 144.2 (CH), 144.8 (CH), 153.0 (C) and 170.2 (C=O); (*m/z*) (+CI/NH_3) 178 $(M+H)^+$ (%) 179 (100) and 86 (12).

(±)–2–(1-Hydroxy-2-nitroethyl)pyridine (63)[173]



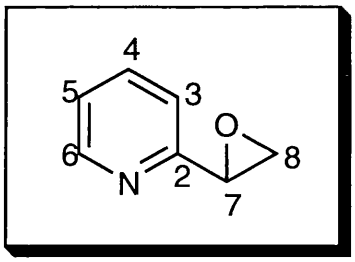
The title compound **(63)** was prepared on a 23 mmol scale by the method of Gavin and purified by silica gel chromatography (hexane - ethyl acetate, 2:3), (1.69 g, 9.9 mmol, 43%) The ^{1}H NMR, ^{13}C NMR, IR and MS spectral data were in good agreement with those reported.[66] (Found : C, 50.20; H, 4.83; N, 16.62 % requires C, 50.00; H, 4.76; N, 16.67 %).

(±)–2–(1-Hydroxy-2-aminoethyl)pyridine (44)[174]



The title compound was prepared by the method of Gavin on an 7.7 mmol scale, (80%). ^{1}H NMR and ^{13}C NMR data were in good agreement with those reported.[66]

1-(2-pyridyl)oxirane (64)[93]



The title compound was made by adapting the procedures of Thurkauf *et al*[93] and Hanzlik *et al.*[92]

To a 3-necked flask at 0 °C containing a solution of dioxane - water (3:7, 148 cm^3) was added 2-vinylpyridine (5.5 g, 52.3 mmol) and acetic acid (2.75 g, 45.8 mmol) and the solution was stirred for 10 min. *N*-Bromosuccinimide* (9.9 g, 55.6 mmol, 1.1 eq) was added in portions over 30 min and the resulting solution was stirred at 0 °C for 1 h then sodium carbonate (11 g, 103.8 mmol) was added in portions over 45 min and the solution was stirred for 15 h.

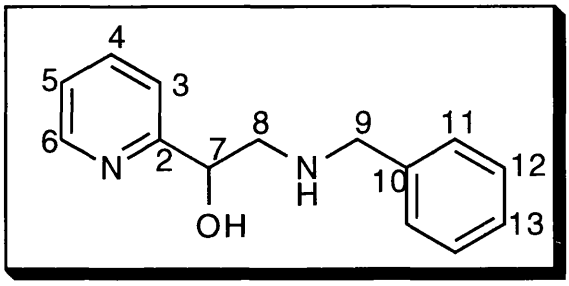
The solution which had become very viscous was extracted with diethyl ether (4 x30 cm^3). The ether extracts were dried with sodium sulfate, filtered and the solvent evaporated *in vacuo* to give a dark brown oil.

The residue was distilled (bp 58 °C, 0.2 Torr)[93] to give the title compound as a colourless oil, which darkened on standing after two d (2.48 g, 20.5 mmol, 39%). New data ν_{max} (thin film)/cm^{-1} 3404, 3057, 3012, 2992, 1593, 14378, 878, 780, 581 and 541; δ_C (50 MHz, $CDCl_3$) 50.2 (CH_2), 52.7 (CH), 119.6 (CH),

123.0 (CH), 136.7 (CH), 149.2 (CH) and 157.0 (C); (*m/z*) (+EI) 121 $(M+H)^+$ (%) 122 (10), 121 (30), 120 (100).

* *N*-bromosuccinimde was purified by the method outlined by D.D. Perrin and W.L.F. Armarego, Purification of Laboratory Chemicals, 3rd Edition, Pergamon Press, 1992.

(±)-2-(Benzylamino-1-hydroxyethyl)pyridine (70)



The following procedure was adapted from that published by that of Backvall and Bjorkmann.[175]

To a dry 3-necked flask fitted with a condenser, septum and stopper was added methanol (5 cm^3) and benzylamine (1.71 g, 16 mmol). With stirring, epoxide **(64)** (0.5 g, 4.1 mmol) was added and the solution was brought to reflux temperature which was maintained for 8 h. The solution was stirred at ambient temperature for 14 h.

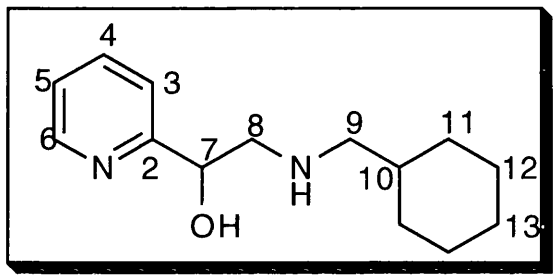
The reaction mixture was poured into ether (12 cm^3) and extracted with 2 M HCl (3x10 cm^3). The aqueous extracts were washed with ether (2 x 15 cm^3).. NaOH pellets were added until the solution was basic (pH =14) and the solution was extracted with chloroform (3 x 50 cm^3) and extracts dried with K_2CO_3. Filtration and evaporation of the solvent afforded 1.62g of a brown oil. Distillation gave **(70)** (0.5g, 2.21 mmol, 54%).

The free base was converted to the corresponding oxalate salt by dissolving the free base in methanol (3 cm^3) and adding it to a solution of 1 equivalent of oxalic acid in methanol (3 cm^3). Vigorous scratching of the solution gave a white precipitate of the salt, which was crystallised from water

mp 186-187°C (decomp); ν_{max} (KBr)/cm^{-1} 3402, 3062, 2851, 1772, 1595, 1474, 1213, 1116, 998, 758 and 700; (Found : C, 60.35; H, 5.77, N, 8.81%

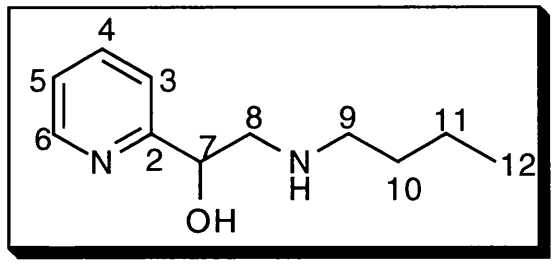
$C_{16}H_{18}N_2O_5$ requires C, 60.38, H, 5.66; N, 8.80%); δ_H (400 MHz, D_2O); 3.25 (1H, dd, *J* 9.3, 3.7, 8-H), 3.42 (1 H, dd, *J* 9.7, 3.4, 8-H'), 4.25 (2 H, s, 9-H_2), 5.20 (1 H, dd, *J* 5.9, 3.3, 7-H), 7.40 (5 H, m, 11, 12 and 13-H), 7.58 (1 H, overlapping dd, *J* 8.0, 5-H), 7.65 (1 H, d, *J* 8.0, 3-H), 8.12 (1 H, m, 4-H) and 8.51 (1 H, d, *J* 5.0, 6-H); δ_C (100 MHz, D_2O); 51.1 (CH_2), 51.5 (CH_2), 67.8 (CH), 123.2 (CH), 125.7 (CH), 129.6 (CH), 130 .1 (CH), 130.3 (CH), 130.6 (C), 142.7 (CH), 145.9 (CH), 156.1 (C) and 168.6 (C=O); (*m/z*) (+CI/NH_3), 228, $(M + H)^+$ (%) 229 (100), 227 (5), 108 (15), 96 (5) and 79 (5); (Found $(M+H)^+$ 229.1340 $C_{14}H_{17}N_2O$ requires 229.1341).

(±)-2-(Cyclohexylamino-1-hydroxyethyl)pyridine (71)



The title compound **(71)** was prepared as described for **(70)**, except that **(71)** was not purified by distillation. mp 178-180°C; ν_{max} (KBr)/cm^{-1} 3410, 2927, 2853, 1596, 1439, 1216, 1080, 720 and 498; δ_H (400 MHz, D_2O); 0.92 (2 H, m, 13-H_2), 1.12 (4 H, m, 12-H_2), 1.54 (1 H, broad signal, 10-H), 1.63 (4 H, broad signal, 11-H_2), 2.89 (2 H, d, *J* 6.2, 9-H_2), 3.23 (1 H, dd, *J* 9.4, 3.6, 8-H), 3.39 (1 H, dd, *J* 9.8, 3.3, 8'-H), 5.10 (1 H, dd, *J* 6.1, 3.2, 7-H), 7.56 (1 H, m, 3-H), 7.67 (1 H, m, 5-H), 8.09 (1 H, td, *J* 7.8, 1.5, 4-H) and 8.51 (1 H, d, *J* 5.1, 6-H); δ_C (100 MHz, D_2O); 25.3 (CH_2), 25.8 (CH_2), 30.1 (CH_2), 34.6 (CH), 52.0 (CH_2), 54.0 (CH_2), 67.4 (CH), 123.5 (CH), 125.9 (CH), 143.4 (CH), 145.4 (CH), 156.0 (C) and 168.1 (C=O); (*m/z*) (+CI/NH_3), 234 $(M+H)^+$(%) 235 (100), 217 (2), 126 (9) and 109 (12) (Found $(M+H)^+$ 235.1809 $C_{14}H_{23}N_2O$ requires 235.1810).

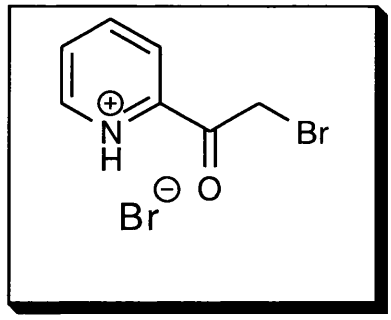
(±)-2-(Butylamino-1-hydroxyethyl)pyridine (72)



The title compound **(72)** was prepared as described for **(70)**, except that **(72)** decomposed upon distillation. δ_H (400 MHz, $CDCl_3$); 0.89 (3 H, t, *J* 7.3, 12-H_3), 1.37-1.41 (2 H, m, 11-H_2), 1.44-1.55 (2 H, m, 10-H_2), 2.67-2.71 (2 H, m, 9-H_2), 2.78 (1 H, dd, *J* 7.0, 3.3 8-H), 2.97 (1 H, dd, *J* 9.9, 4.3, 8'-H), 4.82 (1 H, dd, *J* 4.7, 3.6, 7-H), 7.18 (1H, m, 3-H or 5-H), 7.43 (1H, m, 3-H or 5-H), 7.63 (1H, m, 4-H) and 8.52 (1H, d, *J* 4.8, 6-H); δ_C(100 MHz, $CDCl_3$); 14.3 (12-CH_3), 20.7 (11-CH_2), 32.5 (10-CH_2), 49.7 (9-CH_2), 56.5 (8-CH_2), 72.1 (7-CH), 121.0 (3CH or 5CH), 122.7 (3-CH or 5-CH), 137.0 (6-CH) 148.8 (4-CH) and 162.5 (2-C). Plus impurities.

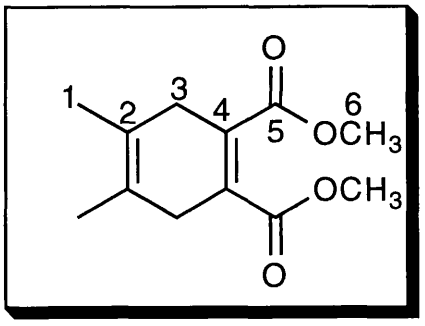
2-Bromoacetylpyridine Hydrobromide (74)

The title compound was prepared on a 20 mmol scale and isolated in a yield of 85% following the method of Taurins and Blaga.[105] The ^1H NMR spectrum and melting point are in good agreement with the published data.[106]



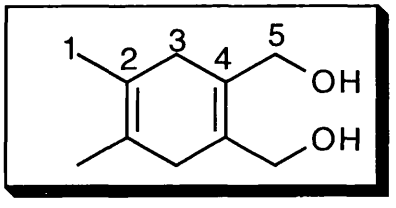
Section 7.3 Experimental to chapter 4

Dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (89)[118]



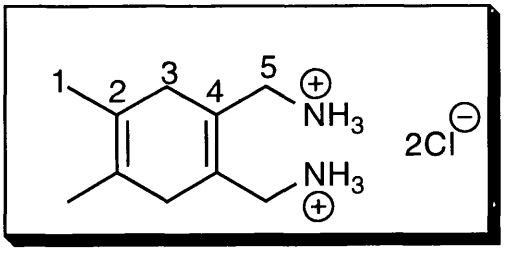
Compound **(89)** was prepared on a 30 mmol scale by the method of Kucherov and Grigovera.[118] (80%); mp 75-76°C (lit[118] 75-76°C); δ_H (200 MHz, $CDCl_3$); 1.66 (3 H, s, 1-H_3); 2.92 (2 H, s, 3-H_2) and 3.78 (3 H, s, 6-H_3).

1,2-Bis(hydroxymethyl)-4,5-dimethylcyclohexa-1,4-diene (90)[176]



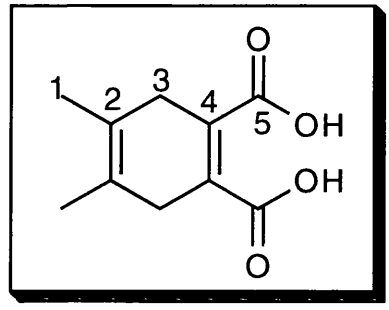
Compound **(90)** was prepared on a 50 mmol scale by the method of Martin[176] and was recrystallised from ethyl acetate (35%); mp 147-149°C (lit.[177] 146-148 °C); δ_H (200 MHz, D_6 DMSO); 1.65 (3 H, s, 1-H_3); 2.68 (2 H, s, 3-H_2); 4.00 (2 H, s, 5-H_2) and 4.53 (2 H, s, OH).

1,2-Bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (86)[176]



The title compound was prepared on a 40 mmol scale by the method of Fabiano *et al.*[125] (43%); δ_H (200 MHz, D_2O)[(176)]; 1.50 (3 H, s, 1-H_3); 2.58 (2 H, s, 3-H_2) and 3.57 (2 H, s, 5-H_2).

Attempted synthesis of 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (94)



The following procedure was adapted from that of Corey *et al.* [127] Diester **(89)** (1.0 g, 4.5 mmol) was dissolved in methanol (3 cm^3). Lithium hydroxide monohydrate (0.1g, 22.5 mmol) was dissolved in a 3:1 methanol - water mixture (40 cm^3) and was added in slowly over 1.5 h. The solution was stirred at ambient temperature for 24 h after which time it was a pale yellow colour. TLC (methanol:DCM, 4:1, acetic acid) of the reaction mixture indicated that all of **(89)** had been consumed and a spot appeared for a more polar product. The solution was stirred for a further 6 h after which time it was colourless.

The reaction mixture was washed with ether (3 x 10 cm^3) and 3 M HCl was added until the solution reached pH 2. The acid solution was extracted with ethyl acetate (3x 10 cm^3). The extracts were dried with anhydrous sodium sulfate, filtered and concentrated to give 0.50 g of a white solid. ^1H NMR spectroscopy indicated the presence of aromatised and hydrolysed products.

Attempted synthesis of 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (94)

To a round-bottom flask fitted with a condenser was added diester **(1)** and a 5% sodium hydroxide solution. The mixture was stirred and heated at reflux for 4 h. The reaction was worked up in the manner previously described to afford 0.3 g of a white solid which was insoluble in all deuterated solvents investigated.

Further attempts to complete the reaction with sodium hydroxide solutions of different concentrations proved unsuccessful.

Attempted synthesis of 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (94)

The following procedure was adapted from Olah *et al*.[130]

To a dry 3-necked flask fitted with a nitrogen balloon, condenser and septum was added diester **(89)** (1.1 g, 6.4 mmol) followed by dry acetonitrile and the solution was stirred. Sodium iodide (2.9 g, 19.7 mmol) was added and stirring was continued. Trimethylsilyl chloride (1.4 g, 13.1 mmol) was added dropwise and the solution turned a yellow colour. The reaction was heated at reflux for 1 h after which a brown colour persisted in the flask. The reaction was stirred at ambient temperature for 14 h and then taken up in ether (30 cm^3) and washed sequentially with water (10 cm^3); 10% sodium thiosulfate (10 cm^3) and brine (10 cm^3). Acidification of the combined sodium bicarbonate extracts with 6 M hydrochloric acid did not produce the expected precipitate.

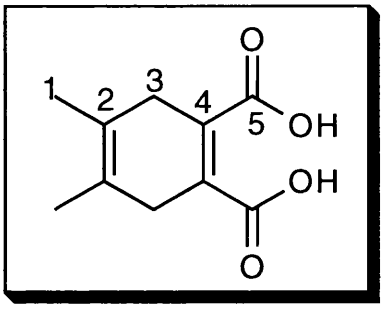
Drying and evaporation of the ether extracts yielded 40 mg of the starting diester **(89)**.

Attempted synthesis of 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (94)

The following procedure was adapted from that of Laganis and Chenard[128].

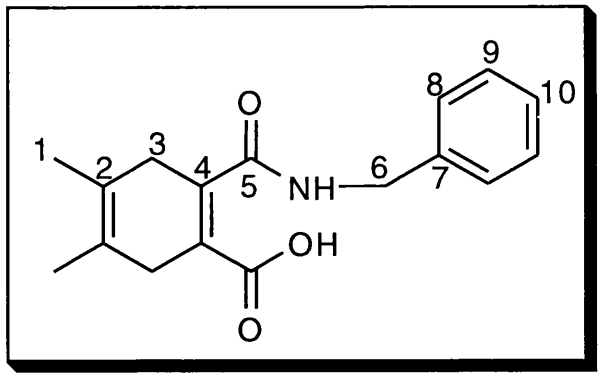
To a dry 3-necked flask fitted with a nitrogen balloon, condenser and septum was added diester **(89)** (0.56 g, 2.5 mmol) and dry THF (50 cm^3) and the solution was stirred. Potassium trimethylsilanolate was added and the resultant mixture was stirred at ambient overnight, after which time TLC (1:1 hexane - ethyl acetate) indicated the presence of starting material and two more polar products. Concentrated hydrochloric acid (5 cm^3) was added followed by water (40 cm^3). The reaction mixture was then washed with ethyl acetate (3 x 40 cm^3); dried, filtered and concentrated to give a thin oily film. Drying of the ethyl acetate extracts and subsequent filtering and concentration yield 400 mg of starting material.

Synthesis of 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (94)[118]



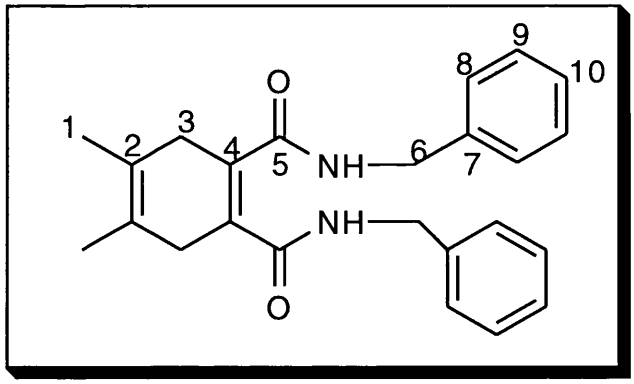
The following procedure was adapted from that of Kucherov and Grigovera[118]. To a dry 3-necked flask fitted with a nitrogen balloon, condenser and septum was added acetylenedicarboxylic acid (2.0 g, 17.6 mmol) and dry dioxane (30 cm^3). With stirring 2,3-dimethyl-1,3-butadiene (3.0 g, 36.6 mmol) and pyrogallol (0.2g, 1.56 mmol) were added and the reaction mixture was stirred and heated at 75 °C for 15 h. Dioxane and the excess diene were distilled off under vacuum and the resulting brown residue was washed with a 1:1 mixture of diethyl ether and pet ether (bp 60 - 80 °C) to give a white solid. ^1H NMR spectroscopy indicated this to be a mixture of the desired diacid **(94)** and the corresponding anhydride. The mixture was dissolved in 5% NaOH solution (25 cm^3) and the flask shaken until almost all the solid had dissolved. The volume of the solvent was reduced *in vacuo* to 5 cm^3 and then the flask was cooled to 0 °C. Concentrated hydrochloric acid was added to precipitate out a white solid **(94)** which could not be recrystallised from the solvents investigated (2.1 g, 10.7 mmol, 60%); mp 157-161°C (lit[118] 158.5-160.5°C); δ_H (200 MHz, D_2O); 1.66 (3 H, s, 1-H_3) and 2.87 (2 H, s, 3-H_2).

4,5-Dimethylcyclohexane-1,2-dicarboxylic acid, mono *N*-benzylamide (113)



To an ice cooled stirred suspension of crude **(105)** (1.68 g, 9.4 mmol) in dry ether (10 cm^3) was added benzylamine (1.1 g, 10.5 mmol) in dry ether (10 cm^3) dropwise. Stirring was continued at 0 °C under a dry nitrogen atmosphere for 4 h. The white precipitate that formed was filtered, dried and crystallised to give a crude sample of **(113)** (Found $(M+H)^+$ 286.1445. $C_{17}H_{20}NO_3$ requires 286.1443).

N,N'-Dibenzyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxamide (95)

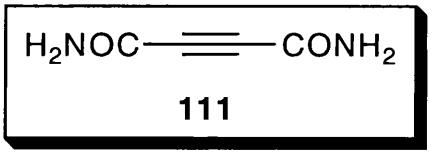


To an ice cooled solution of crude **(113)** (0.5 g, 1.7 mmol) and dry triethylamine (0.3 g, 2.3 mmol) in dry THF (15 cm^3) was added ethyl chloroformate (0.22 g, 2.1mmol) and the resulting solution was stirred for 20 min. A solution of benzylamine (0.22 g, 2.1 mmol) in THF (10 cm^3) was added dropwise and stirring was continued for 15 h. Water (10 cm^3) was added and the reaction mixture was extracted with chloroform (2 x 10 cm^3). The combined organic layers were washed with water (2 x 15 cm^3), dried with anhydrous

magnesium sulfate, filtered and concentrated. The white solid was crystallised from ethyl acetate to give the title compound **(95)** (250 mg, 0.67 mmol, 39%); R_f (1:1 hexane - ethyl acetate); mp 197.5 -198.5 °C (ethyl acetate). ν_{max}(KBr)/cm^{-1}, 3409, 3288, 1697, 1529, 1453, 1027, 690 and 650; δ_H (200 MHz, $CDCl_3$); 1.61 (6 H, s, 1-H_3); 2.87 (4 H, s, 3-H_2); 4.20 (4 H, d, 6-H_2); 6.87 (2 H, broad, NH) and 7.26 (10 H, m, 8, 9 and 10-H). δ_C (50 MHz, $CDCl_3$); 17.9 (CH_3); 34.3 (CH_2); 43.2 (CH_2); 121.8 (C); 127.4 (CH); 127.8 (CH); 128.6 (CH); 132.3 (C), 137.8 (C) and 169.6 (C). (*m/z*) 374 (+EI) (%) (M^+)(1%); 269 (85), 240 (30), 133 (25), 119 (30), 91 (100), 77 (15) and 65 (12).

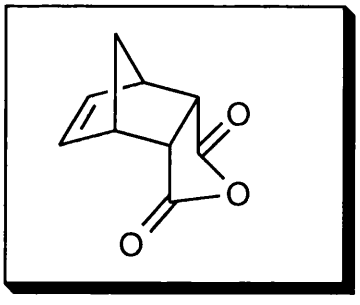
Acetylenedicarboxamide (111)[132]

The title compound was synthesised in a yield of 78% in the manner reported by Saggiomo[132] mp 214 -218 °C (decomp) from ethanol, (lit[133] 216-218 °C, ethanol).



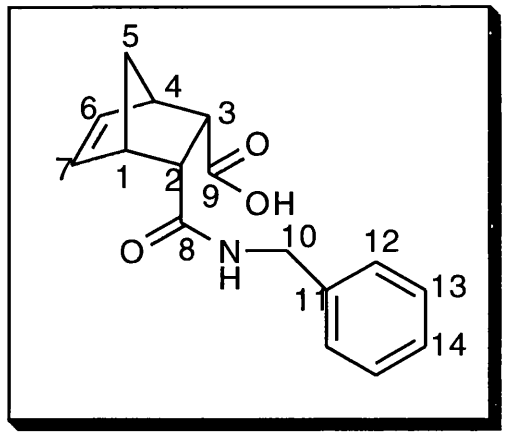
111

Norborn-5-ene-2-*endo*,3-*endo*-dicarboxylic acid anhydride (119)



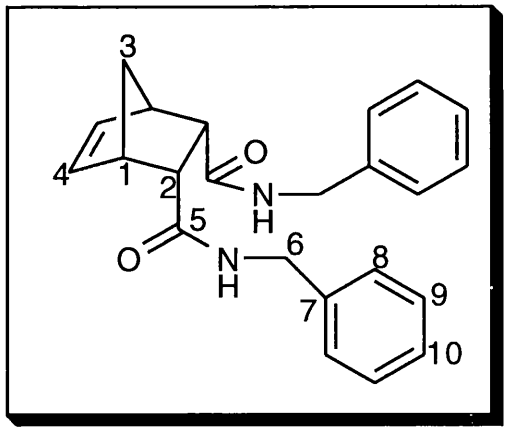
The title compound was synthesised on a 30 mmol scale by the method of Sadukin *et al.* [140] (85%); mp 165 -167 °C (Lit[178] 165 -167 °C).

Bicyclo[2.2.1]hept-5-ene-*endo-cis*-1,2-dicarboxylic acid mono *N*-benzylamide (120) [179]



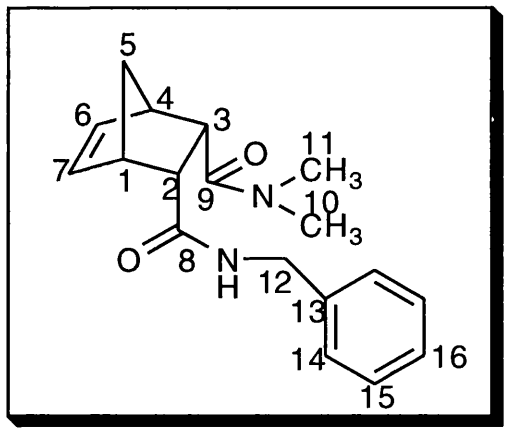
To an ice cooled stirred suspension of the *endo-cis*-anhydride **(119)** (2 g, 12.2 mmol), was added benzylamine (1.4 g, 13.4 mmol) in dry ether (10 cm^3) dropwise. Stirring was continued at 0 °C for 4 h. The white precipitate that formed was filtered, dried and crystallised from chloroform-hexane and recrystallised from 95% aqueous ethanol to give **(120)** (2.71 g, 10.0 mmol, 82%); mp 123-130° C; ν_{max} (KBr disc)/cm^{-1} 3314, 3030, 1635, 1496, 1267 and 749; δ_H (200 MHz, D_6 DMSO); 1.30 (2 H, m, 5-H_2), 3.04 (2H, broad 1-H and 4-H), 3.17 (2 H, m, 2-H and 3-H), 4.32 (2-H, m, 10-H), 5.9 (1 H, m, 7-H), 6.25 (1 H, m, 6-H), 7.36 (5 H, m, 12, 13 and 14-H), 8.37 (1 H, t, *J* 6.0, NH) and 11.77 (1 H, b.s., OH); δ_C (50 MHz, D_6 DMSO); 42.2 (CH_2), 45.4 (1-CH), 47.1 (4-CH), 48.4 (CH_2 and CH,), 48.6 (CH) 126.7 (CH), 127.2 (CH), 128.3 (CH), 133.6 (CH), 135.3 (CH), 171.3 (C) and 140.0 (C); (*m/z*) (+EI) 271 (M^+, 2.7%), 266, 253, 187, 106, 91 and 79 (Found M^+. 271.1203 $C_{16}H_{17}N0_3$ requires 271.1208).

_N,N'_-Dibenzyl-bicyclo[2.2.1]hept-2-ene-2,3-_*endo-cis*_-dicarboxamide (121)



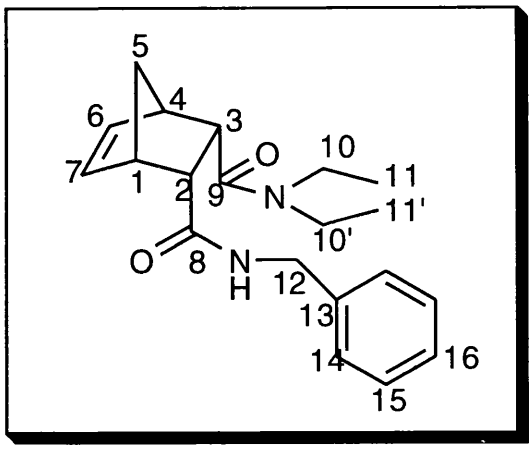
To an ice cooled, stirred solution of **(120)** (2 g, 7.38 mmol) and dry triethylamine (1.33 cm^3, 9.5 mmol) in dry THF (15 cm^3) was added ethyl chloroformate (0.97 g, 8.90 mmol). The resulting solution was stirred for 20 min. A solution of benzylamine (0.97 g, 8.90 mmol) in dry THF (10 cm^3) was added dropwise. Stirring was continued for 5 h at room temperature and the mixture was left overnight. Water (25 cm^3) was added and the reaction mixture was extracted with chloroform (2 x 30 cm^3). The combined organic layers were washed with water (2 x 20 cm^3) dried with anhydrous magnesium sulphate, filtered and concentrated to give (2.42 g) of a white solid, crystallisation of the solid from ethyl acetate gave the title compound (1.9g, 5.23 mmol, 71%); mp 140-141°C; ν_{max} (KBr disc)/ cm^{-1} 3331, 3030, 1656, 1548, 731 and 698; δ_H (200MHz, $CDCl_3$); 1.22 (2 H, m, 3-H_2); 2.98 (2 H , broad singlet, 1-H), 3.16 (2 H, broad singlet, 2-H), 4.17 (4 H, m, 6-H_2); 6.23 (2 H, broad singlet, 4-H), 7.22 (10 H, m, 8, 9, and 10-H); δc (200 MHz, D_6-DMSO); 43.4 (3-CH_2); 47.3 (1-CH), 49.9 (6-CH_2), 51.8 (2-CH), 127.3 (10-CH), 127.6 (8-CH), 128.6 (9-CH), 135.7 (4-CH), 138.2 (7-CH) and 173.2 (5-C); (m/z)(+EI) 360, (M^+, 26 %), 342, 253, 226, 185, 106, 91, 66 and 65; (Found M^+ 360.1831. $C_{16}H_{17}N0_3$ requires 360.1838).

N-Benzyl-*N',N'*-dimethylbicyclo[2.2.1]hept-5-ene-*endo-cis*-1,2-dicarboxamide (122)



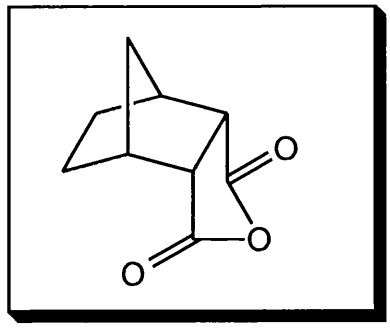
To a suspension of **(120)** (1 g, 3.7 mmol) in benzene (10 cm^3) was added hexamethylphosphorus triamide (0.31 g, 1.9 mmol) over 5 min. The solution was then heated to reflux for 20 min, cooled to room temperature then saturated sodium carbonate (10 cm^3) was added. The aqueous layer was extracted with DCM (3 x 20 cm^3). The organic extracts were filtered and the solvent evaporated *in vacuo* to give a yellow oil which crystallised on standing. The solid was recrystallised from a small amount of ethanol (0.68 g, 2.3 mmol, 62%); mp 164 -166 °C; ν_{max} (solution)/ cm^{-1} 3022, 2397, 1651, 1524, 1209 and 915; δ_H (200MHz, $CDCl_3$); 1.34 - 1.48, (2 H, m, 5-H_2), 2.58 (3 H, s, 10 or 11-H_3), 2.93 (3 H, s, 10 or 11-H_3) , 3.08 (1 H, broad signal, 1 or 4-H), 3.27 (1 H, broad signal, 1 or 4-H), 3.36 -3.46 (2 H, m, 2 and 3-H); 4.18 (1 H, dd, *J* 14 and 6, 12-H_2), 4.36 (1 H, dd, *J* 16, 6, 12-H'$_2$) 5.98 (2 H, dd, *J* 10.8, 6.3, 6 and 7-H), 6.73 (1 H, dd, *J* 10.8, 6.3, NH); and 7.17 -7.36 (5 H, m, 14, 15 and 16-H); δ_C (50 MHz, $CDCl_3$); 35.1 (CH_3), 36.7 (CH_3), 43.3 (CH_2), 47.0 (CH), 47.6 (CH), 49.4 (CH_3), 49.6 (CH), 51.9 (CH), 127.2 (CH), 127.8 (CH), 128.5 (CH), 131.8 (CH), 138.5 and 139.7; (*m/z*)(+EI) 297 $(M+H)^+$ (%) 298 (10), 253 (86), 187 (100), 106 (40), 91 (82), 66 (68) and 44 (20). (Found $(M+H)^+$ 298.1678 $C_{18}H_{22}N_2O_2$ requires 298.1681).

N-Benzyl-*N',N'*-diethylbicyclo[2.2.1]hept-5-ene-*endo*-*cis*-1,2-dicarboxamide (123)



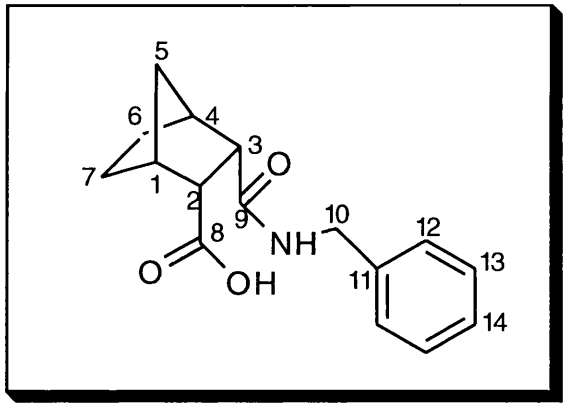
The title compound was prepared in a manner similar to that **(122)** using **(120)** (1 g, 3.7 mmol) and hexaethylphosphorus triamide (0.47 g 1.9 mmol). The desired compound was recrystallised from ethanol (0.54 g, 1.67 mmol, 45%); mp 128 -130 °C; ν_{max} (nujol)/cm^{-1} , 3024, 2394, 1645, 1265, 1206 and 930; δ_H (400 MHz, $CDCl_3$); 0.96 (3 H, t, *J* 6, 11 or 11'-H_3), 1.17 (3 H, t, *J* 7.1, 11 or 11'-H_3), 1.37 (1 H, d, *J* 8, 5-H), 1.48 -1.51 (1 H, m, 5-H), 2.92 -3.57 (8 H, m, 1, 2, 3 and 4-H and 10 and 10'H_2) 5.96 (1 H, dd, *J* 3.1 and 5.4, 6-H or 7-H); 4.43 (1 H, dd, *J* 14.7, 6.7, 12-H), 4.05 (1 H, dd, *J* 14.7, 6.7, 12'-H), 6.12 (1 H, broad signal, NH), 6.70 (1 H, dd, *J* 3.2, 5.4, 6-H or 7-H) 7.19 - 7.32 (5 H, m, 14, 15 and 16-H); δ_C (100 MHz, $CDCl_3$); 13.3 (CH_3), 14.5 (CH_3), 40.5 (CH_2), 41.9 (CH_2), 43.9 (CH_2), 47.9 (CH), 48.2 (CH), 49.4 (CH), 50.3 (CH_2), 53.0 (CH), 127.6 (CH), 128.1 (CH), 128.9 (CH), 132.3 (CH), 138.7 (CH), 140 .1 (CH), 172.5 (C=O) and 172.5 (C=O); (*m/z*)(+EI) 297 $(M + H)^+$ (%) 298 (10), 253 (86), 187 (100), 106 (40), 91 (82), 66 (68) and 44 (20). (Found $(M+H)^+$ 326.1995 $C_{20}H_{26}N_2O_2$ requires 326.1994).

Norbornane-2-*endo*,3-*endo*-dicarboxylic acid anhydride (135)[147]



The title compound was made on a 30 mmol scale using the procedure of Ohanti [147] and was recrystallised from ethyl acetate (26 mmol, 98 %). The ^{1}H NMR spectrum is in good agreement with that published.[147]

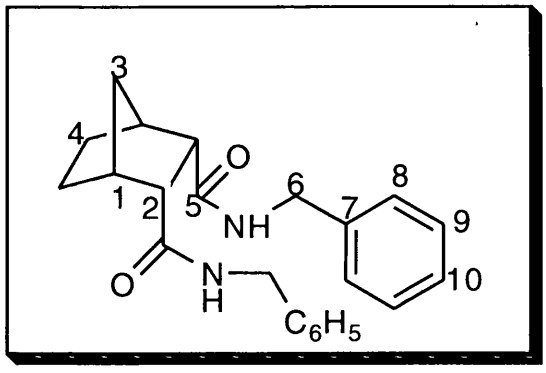
Bicyclo[2.2.1]heptane-*endo*-*cis*-1,2-dicarboxylic acid mono *N*-benzylamide (136)



The title compound was made on a 5 mmol scale in a similar manner to that already described for **(120).** Recrystallisation from ethanol gave **(136)** (1.04 g, 3.8 mmol, 76%); mp 170 - 174 °C; (Found: C, 70.47; H, 6.96; N, 5.27 $C_{16}H_{19}NO_3$ requires C, 70.33; H, 6.96; N, 5.13); δ_H (400 MHz, D_6 DMSO); 1.17 - 1.31 (4 H, m, 6 and 7-H_2), 1.34 - 1.55 (2 H, m, 5-H_2), 2.30 (1 H, m, 1 or 4-H), 2.51 (1 H, m, 1 or 4-H), 2.68 (1 H, m, 2 or 3-H), 3.00 (1 H, dd, *J* 11.7, 4.1, 2 or 3-H), 3.51 (1 H, broad signal, OH), 4.28 (1 H, dd, *J* 6.0, 15.4, 10-H_2), 4.23 (1 H, dd, *J* 6.1, 15.4, 10'-H_2) 7.24 (5 H, m, 12, 13 and 14-H), 8.28 (1 H, t, *J* 5.9, NH); δ_C (100 MHz, D_6 DMSO); 22.9 (CH_2), 25.1 (CH_2), 40.3 (CH), 40.5 (CH_2), 41.8 (CH), 42.3 (CH_2), 46.8 (CH), 46.8 (CH), 126.9 (CH), 127.4 (CH), 128.5 (CH), 140.2 (C), 172.6 (C=O) and 174.1

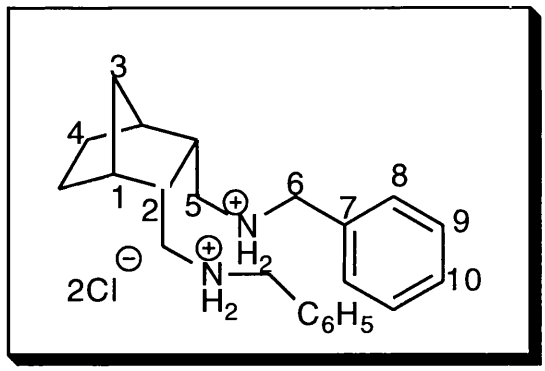
(C=O); ν_{max} (KBr disc) /cm^{-1} 3310, 3032, 1600, 1496, 1235 and 739; (*m/z*) (+EI) 273 (M^+) (%) 273 (7), 255 (14), 227 (5), 189 (7), 106 (14), 91 (46), 79 (35) and 66 (100); (Found $(M+H)^+$ 273.1368 requires 273.1365).

N,N'-Dibenzyl-bicyclo[2.2.1]heptane-2,3-*endo-cis*-dicarboxamide (137)



The title compound was made on a 10 mmol scale in a similar manner to that already described for **(121).** Recrystallisation from ethyl acetate gave **(137)** (2.3 g, 6.5 mmol, 65%); mp 158-161 °C; ν_{max} (KBr)/cm^{-1} 3327, 3285, 2948, 1650, 1549, 1229, 760 and 701; δ_H (400 MHz, $CDCl_3$); 1.44 (4 H, m, 4-H_2), 1.95 (2 H, m, 3-H_2), 2.50 (2 H, broad signal, 1-H), 2.83 (2 H, broad signal, 2-H), 4.19 (1 H, dd, *J* 15.2, 5.8, 6-H_2), 4.28 (1 H, dd, *J* 15.2, 5.9, 6'-H_2),7.28 (10 H, m, 8, 9 and 10-H), 7.95 (2 H, t, *J* 5.6, NH); δ_C (100 MHz, $CDCl_3$); 24.5 (CH_2), 40.9 (CH_2), 41.9 (CH), 44.0 (CH), 49.4 (CH_2), 127.7 (CH), 128.2 (CH), 129.0 (CH), 138.9 (C), 172.8 (C=O) (*m/z*)(+EI) 362 (M^+) (%) 362 (7), 344 (7), 296 (20), 255 (68), 228 (14), 188 (10), 162 (14), 106 (40), 91 (100), 79 (7) and 65 (5); (Found $(M+H)^+$ 362.1994 $C_{20}H_{26}N_2O_2$ requires 362.1994).

2,3-*Endo-cis*-bis(benzylaminomethyl)bicyclo[2.2.1]heptane Dihydrochloride (138)



To a stirred solution of **(137)** (0.5 g, 1.4 mmol) in dry THF (20 cm^3) was added a 1 M THF-borane solution (5.6 cm^3, 5.6 mmol). The solution was stirred for 1 h at room temperature, then heated to reflux and maintained at this temperature for 17 hours. The reaction was cooled to 0 °C and 6 M HCl was added cautiously until hydrogen evolution had ceased. The THF and water was evaporated to leave a glassy solid which was recrystallised from ethanol. The solid was added to a small amount of ethanol and ethereal HCl was added and the solution was scratched until a white precipitate of **(138)** formed (0.44 g, 1.1 mmol, 75%); mp 266-268 °C decomp; ν_{max} (KBr)/ cm^{-1}, 3447, 2956, 2743, 2673, 2589, 2049, 1587, 1458, 1211, 1016, 752, 697 and 488; δ_H (400 MHz, D_2O); 1.11 -1.99 (2 H, m, 3-H_2) 1.36 - 1.46 (4 H, m, 4-H_2), 2.31 (2 H, Broad, 2-H), 2.24 (2 H broad signal, 1-H), 2.88 (4 H, broad, 5-H_2); 4.16 (4 H, s, 6-H_2), 7.42 (10 -H, m, 8, 9 and 10-H); δ_C (100 MHz, D_2O); 21.6 (CH_2), 37.7 (CH), 38.5 (CH), 39.1 (CH_2), 44.4 (CH_2), 51.7 (CH_2), 129.7 (CH), 130.2 (CH), 130.4 (CH) and 130.6 (C); (*m/z*)(+Cl/Isobutane) 334 $(M+H)^+$ (%) 335 (100), 270 (5), 243 (18), 228 (28), 120 (14), 108 (10) and 91 (18); (Found $(M+H)^+$ 335.2487 $C_{23}H_{30}N_2$ requires 335. 2487).

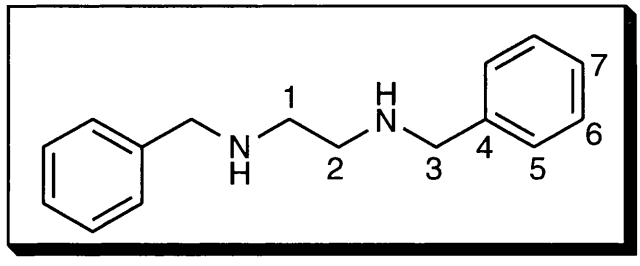
Section 7.4 Experimental to Chapter 5

General Procedure B - Synthesis of *N, N'*-Dibenzyldiaminoalkanes

To a stirred solution of the aldehyde (2 eq) in absolute ethanol was added the appropriate diamine dropwise. The solution was stirred at room temperature for 20 min, at reflux for 25 min, allowed to cool and the solvent evaporated *in vacuo* to give a syrup which solidified on cooling. The residue was dissolved in methanol and the solution was stirred at 0°C. Excess sodium borohydride was dissolved in methanol and added cautiously. The solution was stirred for 40 min more, heated at reflux for 15 min, then stirred at room temperature overnight.

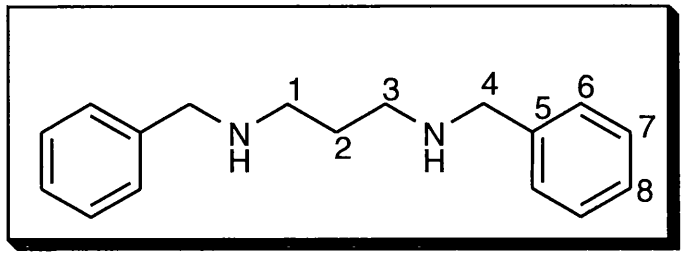
The solution was taken up in water and extracted with dichloromethane (2 x 30 cm^3). The organic extracts were dried with anhydrous sodium sulfate, filtered and concentrated to give the appropriate dibenzyldiamine as the free base. For the purposes of microanalysis, melting point analysis and biological testing a sample or all of the free base was converted into the corresponding dihydrochloride salt in almost quantitative yield by dissolving it in a small amount of chloroform, adding concentrated hydrochloric acid and crystallising the precipitated solid from water.

N,N'-Dibenzyl-1,2-diaminoethane (143)[170]



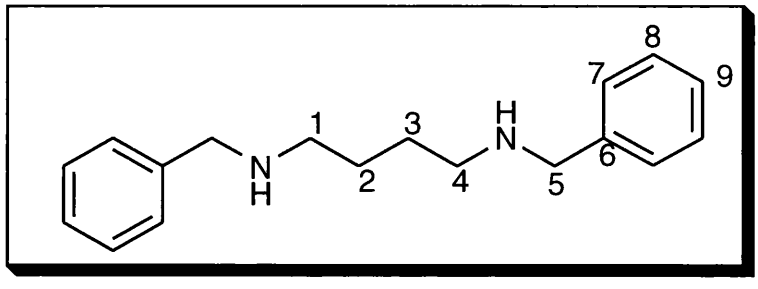
General procedure B was used with benzaldehyde (3.5 g, 33.2 mmol), 1,2-diaminoethane (1 g, 16.6 mmol) and sodium borohydride (2.1 g, 54.8 mmol) to give **(143)** as the free base (3.8 g, 15.9 mmol, 96%); hydrochloride salt δ_H (400 MHz, D_2O),[170] 3.35 (4H, s, 1 and 2-H_2), 4.18 (4 H, s, 3-H_2) and 7.26 (10 H, m, 5-H, 6-H and 7-H).

N,N'-Dibenzyl-1,3-diaminopropane (144)[180]



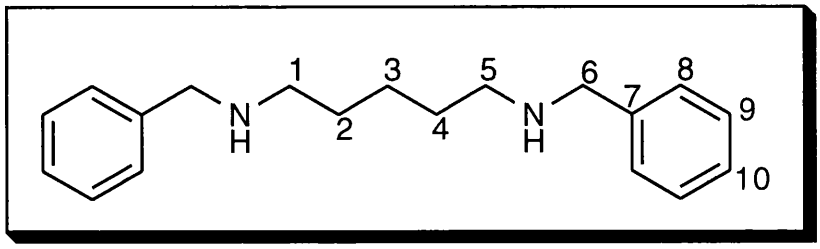
The title compound was made by general procedure B using benzaldehyde (5.73 g, 54.0 mmol), 1,3-diaminopropane (2 g, 27 mmol) and excess sodium borohydride (3.8g, 0.1 mol) to give **(144)** as a clear oil (6.5 g, 25.7 mmol, 95%). δ_H (200 MHz, $CDCl_3$);[180] 1.75 (2H, quin, *J* 6.0, 2-H_2), 2.41 (2H, bs, NH), 2.65 (4H, t, *J* 6.0, 1 and 3-H_2), 3.76 (4 H, s, 4-H_2) and 7.31 (10 H, m, 6-H, 7-H and 8-H). Hydrochloride salt (Found: C, 62.15, H, 7.49, N, 8.59, Cl, 21.63 % $C_{17}H_{24}N_2Cl_2$ 0.2 H_2O requires:C, 61.89, H, 7.40, N, 8.49, Cl, 21.34 %).

N,N'-Dibenzyl-1,4-diaminobutane (145)[151]



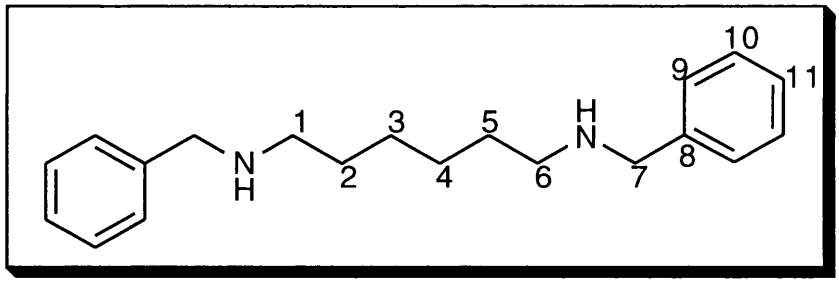
Compound **(145)** was prepared using benzaldehyde (4.81 g, 45.4 mmol), 1,4-diaminobutane (2 g, 22.7 mmol) and excess sodium borohydride (3.8 g, 0.1 mol) according to general procedure B. The title product was isolated as a clear oil. (5.60 g, 20.1 mmol, 92%). δ_H (200 MHz, $CDCl_3$); free base 1.55 (4 H, broad, 2 and 3-H_2), 2.89 (4 H, broad, 1 and 4-H_2), 4.02 (4 H, s, 5-H_2) and 7.31 (10 H, broad. 6-H, 7-H and 8-H). Hydrochloride salt (Found: C, 62.87, H, 7.98, N, 8.31, Cl, 20.84%, $C_{18}H_{26}N_2Cl_2$..0.2 H_20 requires:C, 62.86, H, 7.68, N, 8.15, Cl, 20.37%).

N,N'-Dibenzyl-1,5-diaminopentane (146)[151]



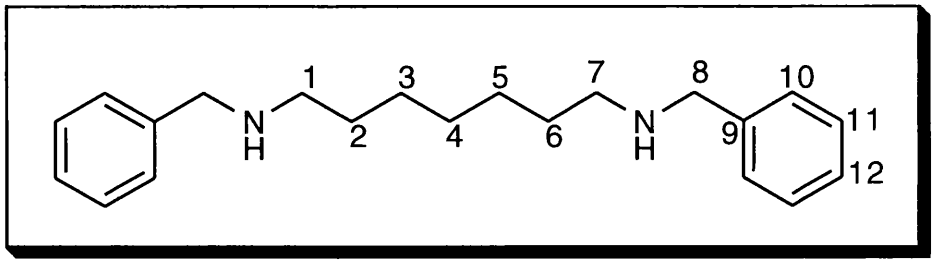
Compound **(146)** was synthesised by general procedure B using benzaldehyde (4.24 g, 40.0 mmol), 1,5-diaminopentane (2.0 g, 20.0 mmol) and excess sodium borohydride (3.8 g, 0.1 mol). **(146)** was isolated as a yellow oil. (5.4 g, 19.0 mmol, 95%). δ_H (200 MHz, $CDCl_3$); 1.21 (2 H, quin, *J* 8.0, 3-H_2), 1.52 (4 H, quin, *J* 8.0, 2 and 4-H_2), 2.86 (4 H, t, *J* 8.0, 1 and 5-H_2), 4.01 (4 H, s, 6-H_2) and 7.28 (10 H, broad, 8-H, 9-H and10-H).

N,N'-Dibenzyl-1,6-diaminohexane (147)[181]



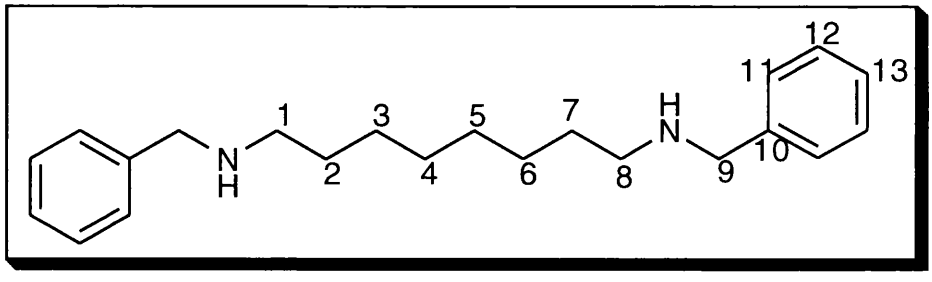
General procedure B using benzaldehyde (1.1 g, 10 mmol), 1,6-diaminohexane (0.58 g, 5 mmol) and sodium borohydride (0.62 g, 16.5 mmol) gave compound **(147)** as the free base (1.3 g, 4.4 mmol, 88%). The compound was converted into the dihydrochloride salt. δ_H (400 MHz, D_2O); 1.22 (4 H, m, 3-H_2 and 4-H_2), 1.53 (4 H, m, 2-H_2 and 5-H_2), 2.89 (4 H, m, 1-H_2 and 6-H_2), 4.06 (4 H, s, 7-H_2) and 7.32 (10 H, 9, 10 and 11-H), δ_C (100 MHz, D_2O); 25.5 (2 x CH_2), 47.1 (CH_2), 51.2 (CH_2), 129.5 (CH), 129.9 (CH) and 130.1 (CH) and 131.0 (C).

***N,N'*-Dibenzyl-1,7-diaminoheptane (148)**[182]



General procedure B was employed using benzaldehyde (3.0 g, 27.6 mmol), 1,7-diaminoheptane (1.8 g, 13.8 mmol) and sodium borohydride (1.7 g, 45.5 mmol) to give **(148)** as the free base. (3.82 g, 12.3 mmol, 89%). The compound was converted into the dihydrochloride salt. (Found: C, 65.81, H, 8.40, N, 7.23, Cl, 18.57 % $C_{21}H_{32}N_2Cl_2$ requires: C, 65.97, H, 8.38, N, 7.33, Cl 18.32 %; δ_H (400 MHz, D_2O); 1.20 (6 H, broad, 3-H, 4-H and 5-H_2), 1.52 (4 H, m, 2 and 6-H_2), 2.90 (4 H, t, *J* 8.0, 1 and 7-H_2), 4.08 (4 H, s, 8-H_2), and 7.34 (10 H, broad, 10-H, 11-H and 12-H), δ_C (100 MHz, D_2O); 25.6 (CH_2), 25.8 (CH_2), 47.2 (N-CH_2), 51.2 (N-CH_2), 129.5 (CH), 129.9 (CH), 130.1 (CH), 131.1 (C); (*m/z*) (+Cl/Isobutane)(%) 310, $(M+H)^+$ (%) 311(100) and 219 (10); (Found: $(M+H)^+$ 311.2486 $C_{21}H_{30}N_2$ requires 311.2487).

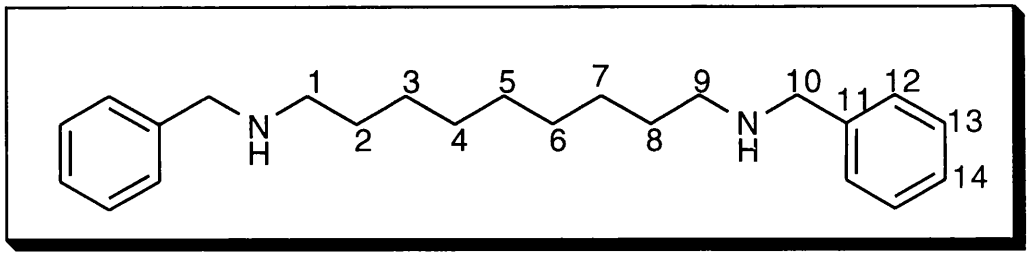
***N,N'*-Dibenzyl-1,8-diaminooctane (149)**[183]



General procedure B was employed using benzaldehyde (3.0 g, 27.6 mmol), 1,8-diaminooctane (2.0 g, 13.9 mmol) and sodium borohydride (1.7 g, 44.9 mmol) to give **(149)** as the free base (3.96 g 12.3 mmol, 89%). The compound was converted into the dihydrochloride salt, mp (water) 278-280°C (lit [183] 276 -280°C, EtOH); δ_H (400 MHz, D_2O); 1.17 (8 H, broad, 3, 4,5 and 6-H_2), 1.53 (4 H, m, 2 and 7-H_2), 2.90 (4 H, t, *J* 8.0, 1 and 8 -H_2), 4.08 (4 H, s, 9-H_2) and 7.35 (10 H, m, 11-H, 12-H and 13-H), δ_C (100 MHz, D_2O); 25.6 (1 and 8-CH_2), 25.9 (CH_2), 28. 2 (CH_2), 47.3 (CH_2), 51.2 (CH_2), 129. 5

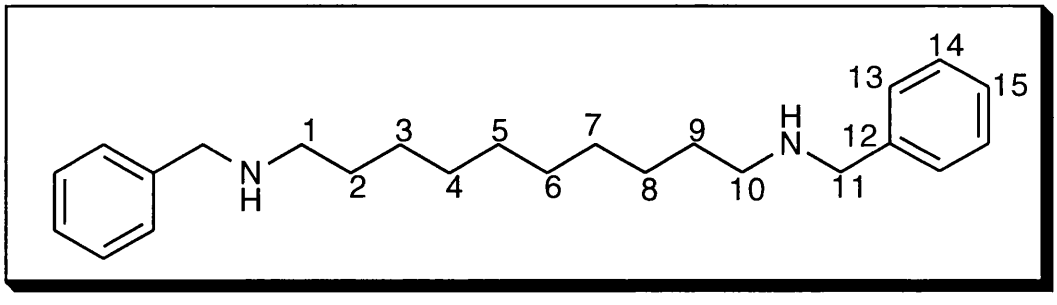
(CH), 129.9 (CH) 130.1 (CH) and 131.1 (C); (*m/z*) (+Cl/Isobutane) (%)325 (100) and 235 (5); (Found: 325.2645. $C_{22}H_{32}N_2$ requires 325.2644).

N,N'-Dibenzyl-1,9-diaminononane (150)[184]



General procedure B was employed using benzaldehyde (2.70g, 25.2 mmol), 1,9-diaminononane (2.0 g, 12.6 mmol) and sodium borohydride (1.6 g, 41.2 mmol) to give **(150)** as the free base. (3.9 g, 11.6 mmol, 92%). The compound was converted into the dihydrochloride salt, mp (water) 268-269°C (lit[184] 269-270°C); δ_H (400 MHz, D_2O); (Found: C, 67.32, H, 8.78, N, 6.83, Cl, 17.07 % $C_{23}H_{36}N_2Cl_2$ requires: C, 67.32, H, 8.71, N, 6.78, Cl 17.27 %; δ_H (400 MHz, D_2O); 1.15 (10 H, b.s. 3, 4, 5, 6 and 7-H_2), 1.52 (4 H, m, 2 and 8-H_2), 2.90 (4 H, t, *J* 8, 1 and 9-H_2), 4.08 (4 H, s, 10-H_2) and 7.36 (10 H, m, 12, 13 and 14-H), δ_C (100 MHz, D_2O); 25.7 (CH_2), 25.9 (CH_2), 28.3 (CH_2), 28.5 (CH_2), 47.3 (N-CH_2), 51.2 (N-CH_2), 129.5 (Ar-CH), 129.9 (Ar-CH), 130.1 (Ar-CH) and 131.1 (Ar-C), (*m/z*) (+Cl/Isobutane)(%) 339 (100), 337 (15) and 247 (10); (Found: 339.2799 $C_{23}H_{34}N_2$ requires 339.2800).

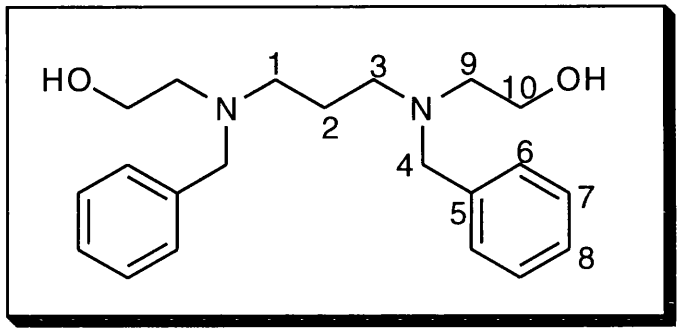
N,N'-Dibenzyl-1,10-diaminodecane (151)[185]



General procedure A was employed using benzaldehyde (2.46 g, 23.2 mmol), 1,10-diaminodecane (2.0 g, 11.6 mmol) and sodium borohydride (1.6 g, 41.2 mmol) to give **(151)** as the free base. (2.9 g, 8.2 mmol, 71%). The compound was converted into the dihydrochloride salt; mp (water) 270-272°C; (Found: C, 67.72, H, 8.96, N, 6.58, Cl, 16.72 % $C_{24}H_{38}N_2Cl_2$

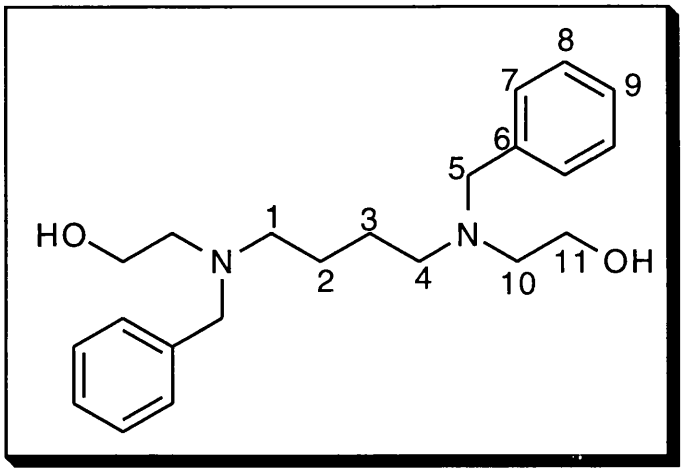
requires: C, 67.92, H, 8.96, N, 6.60, Cl 16.51 %); δ_H (400 MHz, D_2O); 1.02 (12-H, m, 3, 4, 5, 6, 7 and 8-H_2), 1.42 (4-H, m, 2 and 9-H_2), 2.52 (4-H, m, 1 and 10-H_2), 3.70 (4-H, s, 11-H_2) and 7.20 (10-H, m, 13, 14 and 15-H); δ_C (100 MHz, D_2O); 25.7 (CH_2), 26.0 (CH_2), 28.4 (CH_2), 28.6 (CH_2), 47.3 (N-CH_2), 51.2 (N-CH_2), 129.5 (Ar-CH), 129.5 (Ar-CH) and 130.0 (Ar-CH); 130.1 (Ar-CH) and 131.1 (Ar-C); *m/z* (+CI/Isobutane)(%) 353 (100), 351 (15) and 261 (7); (Found 353.2958 $C_{24}H_{36}N_2$ requires 353.2957).

N,N'-Bis(2-hydroxyethyl)-*N,N'*-dibenzyl-1,3-diaminopropane (152)[151]



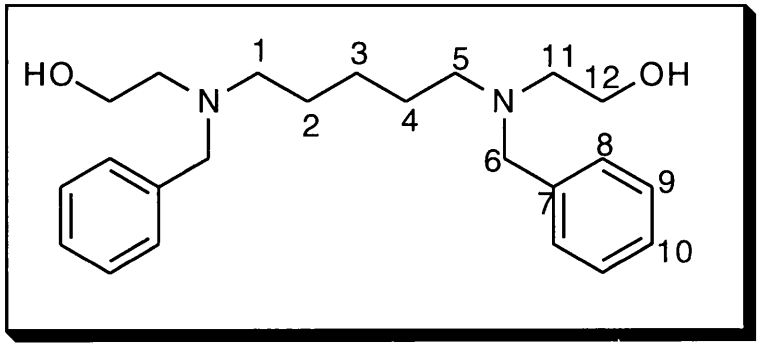
To a stirred solution of **(144)** (1 g, 3.94 mmol) in methanol (15 cm^3) at -78 °C was added freshly distilled ethylene oxide (4 cm^3, 0.08 mol). The flask was stoppered and the solution allowed to return to room temperature and was stirred overnight. The solvent was evaporated *in vacuo* to leave 1.6 g of a clear oil which was dissolved in dichloromethane (10 cm^3) and washed with water (3 x 10 cm^3). The organic layer was dried with anhydrous sodium sulfate, filtered and concentrated to give **(152)** with sufficient purity for the next step (1.01 g, 2.95 mmol, 75 %). New data; δ_H (200 MHz, $CDCl_3$); 1.67 (2 H, quin, *J* 8.0, 2-H_2), 2.48 (4 H, t, *J* 8.0, 1,3-H_2), 2.53 (4 H, t, *J* 6.0, 9-H_2), 3.55 (8 H, m, overlapping signals, 4-H_2 and 10-H_2), 7.26 (10 H, m, 6-H, 7-H and 8-H); δ_C (50 MHz, $CDCl_3$); 24.3 (CH_2), 51.4 (CH_2), 55.2 (CH_2), 55.7 (CH_2), 58.6 (CH_2), 127.2 (CH), 128.3 (CH), 128.9 (CH), 138.4 (C).

N,N'-Bis(2-hydroxyethyl)-*N,N'*-dibenzyl-1,4-diaminobutane (153)[151]



(153) was prepared as that described for **(152)** using **(145)** (1.0 g, 3.73 mmol) in methanol (15 cm^3) with ethylene oxide (4 cm^3, 0.08 mol) to give **(153)** with sufficient purity for the next step (0.92 g, 2.57 mmol, 69 %), ν_{max} (thin film)/cm^{-1} 3093, 3084, 1601, 1549, 1493, 1049 and 737, δ_H (200MHz, $CDCl_3$); 1.44 (4 H, m, 2 and 3-H_2), 2.43 (4 H, m, 1-H_2 and 4-H_2), 2.60 (4 H, t, *J* 6.0, 10-H_2), 2.80 (2 H, b.s., OH), 3.57 (8 H, m, overlapping signals, 4-H_2 and 11-H_2), 7.30 (10H, m, 7, 8, 9-H); δc (50 MHz, $CDCl_3$); 24.6 (CH_2), 53.3 (CH_2), 55.1 (CH_2), 58.3 (CH_2), 58.4 (CH_2), 127.1 (CH), 128.3 (CH), 128.7 (CH) and 138.9 (CH); (*m/z*)(+EI) (%) 356, 357 (< 0.1), 338 (3), 325 (5), 308 (8), 296 (5), 265 (7), 204 (55), 190 (25), 174 (60), 148 (100), 114 (65), 91(100) and 85 (25).

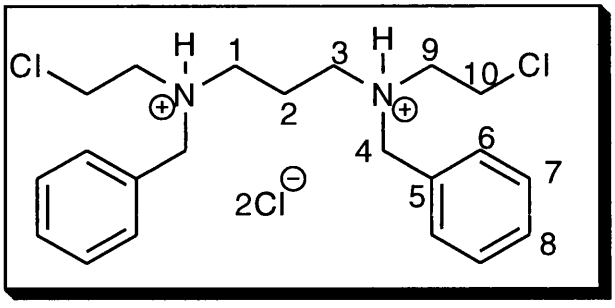
N,N'-Bis (2-hydroxyethyl)-*N,N'*-dibenzyl-1,5-diaminopentane (154)[151]



Using **(146)** (1 g, 3.55 mmol) in methanol (15 cm^3) and ethylene oxide (4 cm^3, 0.08 mol) yielded **(154)** with sufficient purity for the next step

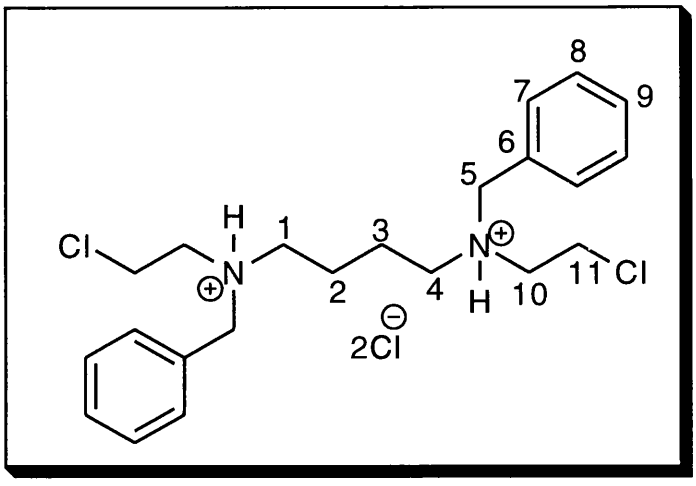
(0.97 g, 2.63 mmol, 74 %). New data; δ_H (200 MHz, $CDCl_3$); 1.11 (2H, m, 3-H_2), 1.25 (4 H, distorted quintet, *J* 4.0, 2 and 4-H_2), 2.38 (4 H, t, *J* 6.0, 1-H_2 and 5-H_2), 2.54 (4 H, t, *J* 6.0, 11-H_2), 2.79 (2 H, b.s., OH), 3.46 (8 H, overlapping signals, 6-H_2 and 12-H_2) and 7.20 (10 H, m, 8-H , 9-H and 10-H); δ_C (50 MHz, $CDCl_3$); 24.8 (CH_2), 26.8 (CH_2), 53.4 (CH_2), 55.1 (CH_2), 58.3 (CH_2), 58.4 (CH_2), 127.1 (CH), 128.3 (CH), 128.9 (CH) and 139.0 (C).

N,N'-Bis(2-chloroethyl)-*N,N'*-dibenzyl-1,3-diaminopropane Dihydrochloride (155)[151]



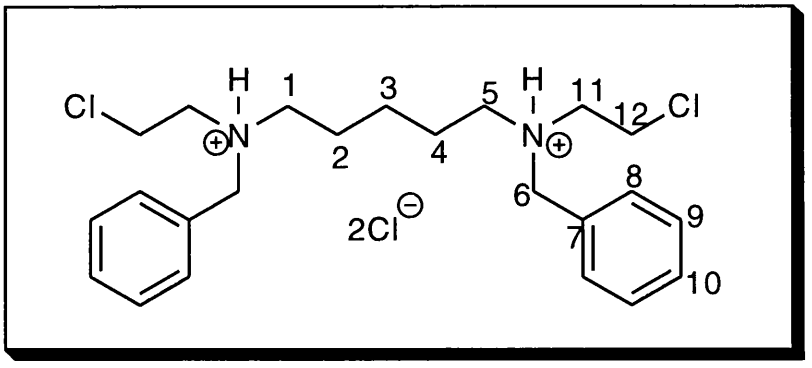
To a stirred solution of the bishydroxyamine **(152)** (0.9 g, 2.63 mmol) in chloroform (3 cm^3) was added freshly distilled thionyl chloride (6.0 g, 0.05 mol). The mixture was heated at reflux for 3 h and then poured into petroleum ether (bp. 40-60 °C) (40 cm^3). The precipitate that formed was extremely sticky and was stirred in hexane for 2 d which had no affect on the nature of the product. Water (30 cm^3), followed by 1 M KOH (20 cm^3) was added and a white precipitate formed which dissolved in the water layer. The water layer was separated, acidified then extracted with chloroform - ethyl acetate (1:1), (3 x 30 cm^3). The combined organic extracts were dried with anhydrous sodium sulfate, filtered and concentrated to give **(155)** as a brown oil (0.40 g, 0.88 mmol, 31%). ν_{max} (thin film)/ cm^{-1} 3384, 3060, 1956, 1600, 1252, 830 and 720. δ_H (200 MHz, $CDCl_3$); 1.67(2 H, quin, *J* 8.0, 2-H_2), 2.57 (4 H, t, *J* 8.0, 1,3-H_2), 2.81(4 H, *J* 6.0, 9-H_2), 3.54 (4 H, t, *J* 6.0, 10-H_2), 3.64 (4 H, s, 4-H_2) and 7.35 (10 H, m, 6-H, 7-H and 8-H); δ_C (50 MHz, $CDCl_3$); 25.2 (CH_2), 42.0 (CH_2), 52.2 (CH_2), 55.8 (CH_2), 59.1 (CH_2), 127.2 (CH), 128.3 (CH), 128.7 (CH) and 139.3 (C).

N,N'-Bis(2-chloroethyl)-*N,N'*-dibenzyl-1,4-diaminobutane Dihydrochloride (156)[151]



To a stirred solution of the bishydroxyethylamine **(153)** (0.9 g, 2.53 mmol) in chloroform (3 cm^3) was added freshly distilled thionyl chloride (6.0 g, 0.05 cm^3) The mixture was heated at reflux for 1h, allowed to cool to room temperature and then poured on to petroleum ether (bp 40-60 °C) (40 cm^3). The precipitate was collected, washed with pet. ether then benzene to give crude product (1.1 g). Crystallisation from 95% aqueous ethanol gave the title product (0.79 g, 1.7 mmol, 67%), mp 231- 233 °C (lit. [151] 230-232°C); ν_{max} (KBr disc)/ cm^{-1} 3424, 3007, 2475, 1624, 1497, 1066 and 735; δ_H (200 MHz, D_2O); 1.64 (4 H, broad, 2-H_2 and 3-H_2), 3.05 (4 H, broad , 1-H_2 and 4-H_2) 3.41(4 H, t, *J* 6.0, 10-H), 3.72 (4 H, t , *J* 6.0, 11-H_2), 4.26 (4 H, s, 5-H_2) and 7.33 (10 H, broad , 7, 8 and 9-H); δ_C (50 MHz, D_2O); 21.0 (CH_2), 38.3 (CH_2), 52.8 (CH_2), 54.5 (CH_2), 58.3 (CH_2), 129.0 (CH_2), 130.3 (CH), 131.3 (CH) and 132.0 (CH); (*m/z*) (+EI) (%) 392, (M^+ +2) 394 (< 0.1%), 357 (2), 222 (20), 187 (35), 182 (30), 160 (25), 148 (95), 132 (50), 120 (15), 91 (100) and 85 (10).

N,N'-Bis(2-chloroethyl)-*N,N'*-dibenzyl-1,5-diaminopentane Dihydrochloride (155)[151]



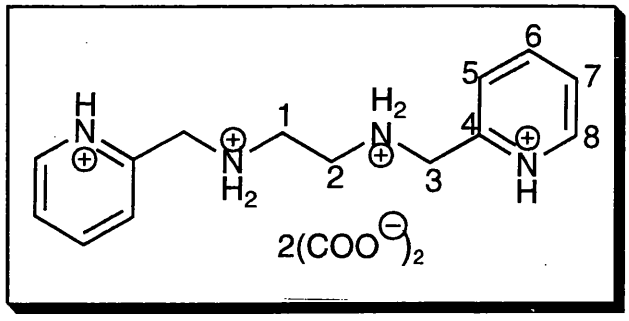
To a stirred solution of the bishydroxyethylamine **(154)** (0.5 g, 1.35 mmol) in chloroform (3 cm^3) was added freshly distilled thionyl chloride (3.0 g, 25 mmol). The mixture was heated at reflux for 3 h then allowed to cool to room temperature and was then poured onto petroleum-ether (bp 40-60 °C), (20 cm^3). The precipitate that formed was washed with pet-ether. The resulting sticky brown solid was stirred in hexane for 1 week after which time a white solid **(157)** was obtained, which was crystallised from ethanol-hexane, (0.3 g, 0.61 mmol, 45 %), mp 180-181°C (lit.[151] 180-181.5 °C), $\nu_{max.}$ 3424, 3056, 3004, 2483, 1499, 1002, 923 and 736; δ_H (200 MHz, D_2O); 1.15 (2 H, m, 3-H_2), 1.62 (4 H, broad, 2-H_2 and 4-H_2), 2.99 (4 H, t, *J* 8.0, 1-H_2 and 5-H_2), 3.40 (4 H, t, *J* 6.0, 11-H_2), 3.71 (4 H, t, *J* 6.0, 12-H_2), 4.25 (4 H, s, 6-H_2) and 7.32 (10 H, broad signal, 8, 9 and 10-H); δ_C (50 MHz, D_2O), 23.2 (CH_2), 23.6 (CH_2), 38.3 (CH_2), 53.4 (CH_2), 54.3 (CH_2), 58.3 (CH_2), 129.1 (C), 130.2 (CH), 131.2 (CH) and 132.0 (CH).

General Procedure C- Synthesis of *N, N'*-dipyridylmethyldiamino alkanes

General procedure B was followed to synthesise solutions of the appropriate free base.

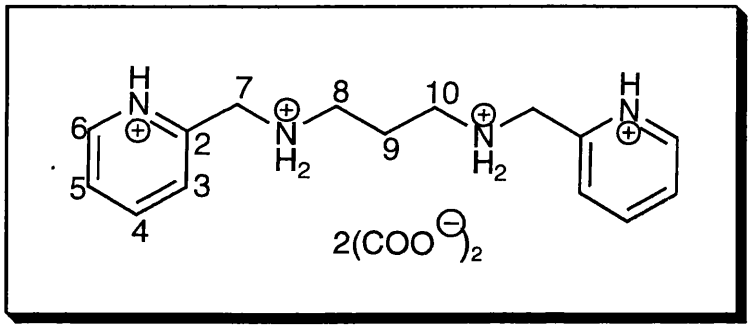
The solution was taken up in chloroform and washed with 1M NaOH. The organic extracts were dried with anhydrous sodium sulfate, filtered and concentrated to give the appropriate dipyridyldiamine as the crude free base. The free base was dissolved in a small amount of methanol. Oxalic acid (2 eq) was dissolved in methanol and added to give a white precipitate of the dioxalate which was recrystallised from 95% aqueous ethanol or water.

N,N'-Bis(2-pyridylmethyl)-1,2-diaminoethane Dioxalate (158)[186]



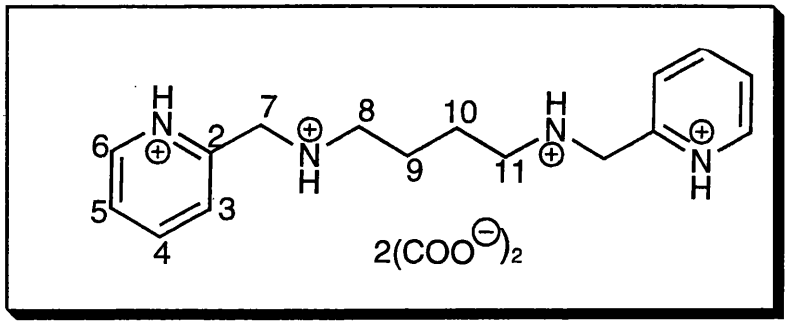
General procedure C was applied using pyridine-2-carbaldehyde (3.5 g, 33.3 mmol), 1,2-diaminoethane (1.0 g, 16.7 mmol) and sodium borohydride (2.2 g, 58 mmol) to give the free base (2.6 g, 10.8 mmol, 65%) which was treated with oxalic acid and recrystallised from water to give **(158)**; mp 236-240 °C decomp; δ_H (400 MHz, D_2O); 3.36 (4 H, s, 1 and 2-H_2), 4.18 (4 H, s, 3-H_2) and 7.38 (8 H, m, 5-H, 6-H, 7-H and 8-H_2).

N,N'-Bis(2-pyridylmethyl)-1,3-diaminopropane Dioxalate (159)[186]



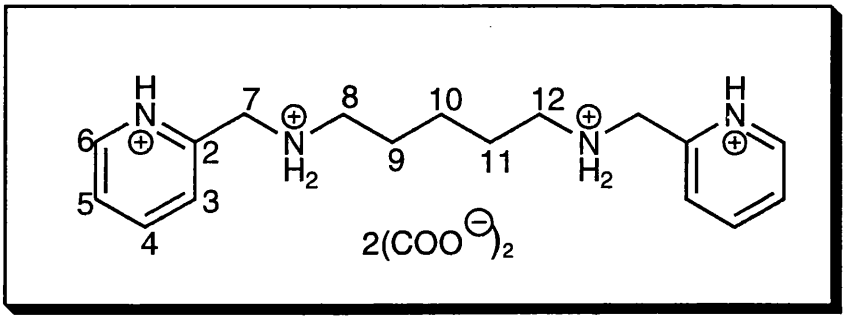
General procedure B was applied using pyridine-2-carbaldehyde (0.41 g, 3.8 mmol), 1,3-diaminopropane (0.14 g, 1.9 mmol) and sodium borohydride (0.24 g, 6.7 mmol) to give **(159)** as white crystals (0.98 g, 1.12 mmol, 59%), mp 214-215 °C, ν_{max}(KBr)/cm^{-1} 3567, 3052, 2858, 1718, 1646, 1476 and 708; δ_H (400 MHz, D_2O); 2.05 (2H, b.s., 9-H_2), 3.09 (2 H, t, *J* 8.0, 8 and 10-H_2), 4.24 (4 H, s, 7-H_2), 7.35 (4 H, m, 3 and 5-H), 7.78 (4 H, m, 4-H), 8.41 (2 H, d, *J* 4.0, 6-H); δ_C (100 MHz, D_2O); 22.9 (CH_2), 44.6 (CH_2), 51.2 (CH_2), 124.7 (CH), 152.1 (CH), 139.4 (CH), 149.2 (CH), 149.7 (C) and 166.6 (C=O); (*m/z*) (+CI/NH_3) 256 $(M+H)^+$ (%) 257 (50), 166 (45), 147 (50), 109 (100), 107 (85), 94 (57) and 80 (30).

N,N'-Bis(2-pyridylmethyl)-1,4-diaminobutane Dioxalate (160)[186]



The reported procedure was applied using pyridine-2-carbaldehyde (0.32 g, 3.0 mmol), (0.13 g, 1.5 mmol) and sodium borohydride (0.20 g, 5 mmol) to give **(160)** as tan crystals (0.82 g, 61%), mp 226-228 °C, ν_{max} (KBr)/cm^{-1} 3437, 3061, 2781, 1720, 1637, 1217, 720; δ_H (400 MHz, D_2O); 1.66 (4 H, quintet, *J* 4.0, 9-H_2 and 10-H_2), 3.08 (4 H, b.s., 8-H_2 and 11-H_2), 4.23 (4 H, s, 7-H_2), 7.36 (4 H, m, 3-H and 5-H), 7.74 (2 H, m, 4-H) and 8.43 (2 H, d, *J* 4.0, 6-H); δ_C (100 MHz, D_2O); 23.0 (CH_2), 47.0 (CH_2), 51.0 (CH_2), 124.8 (CH), 125.1 (CH), 139.7 (CH), 149.0 (CH), 149.8 (C) and 166.5 (C=O); (*m/z*) (+CI /NH_3) (%) 270 (100), 269 (10) 180 (50), 161 (70), 109 (87), 107 (65), 94 (55) and 80 (17).

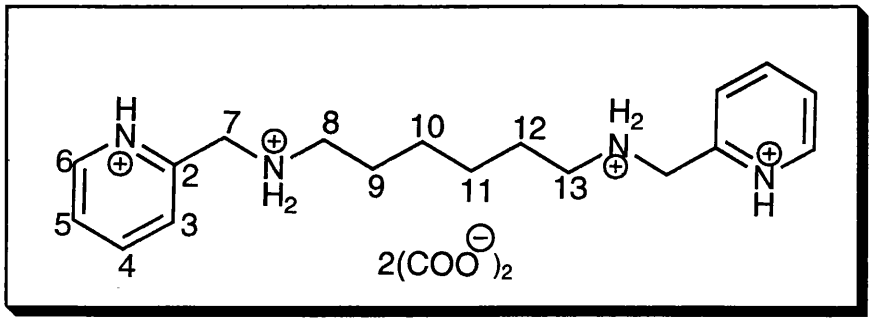
N,N'-Bis(2-pyridylmethyl)-1,5-diaminopentane Dioxalate (161)



The procedure was repeated using pyridine-2-carbaldehyde (0.32 g, 3.0 mmol), 1,5-diaminopentane (0.15 g, 1.5 mmol) and sodium borohydride (0.20 g, 5 mmol) to give **(161)** as a white powder (1.03 g, 74%), mp 223-224 °C; (Found:C, 55.14, H, 5.76, N, 12.29 %, $C_{21}H_{28}N_4O_8$ requires: C, 55.33, H, 5.78 N, 12.44 %); δ_H (400 MHz, D_2O); 1.28 (2 H, quintet, *J* 8.0, 10-H_2), 1.57 (4 H, quintet, *J* 8.0, 9-H_2 and 11-H_2), 2.96 (4 H, t, *J* 8.0, 8-H_2 and 12-H_2), 4.20 (4 H, s, 7-H_2), 7.33 (4 H, m, 3-H and 5-H), 7.78 (2 H, m, 4-H) and 8.39 (2 H, d, *J* 4.0, 6-H); δ_C (100 MHz, D_2O); 23.1 (CH_2), 25.2

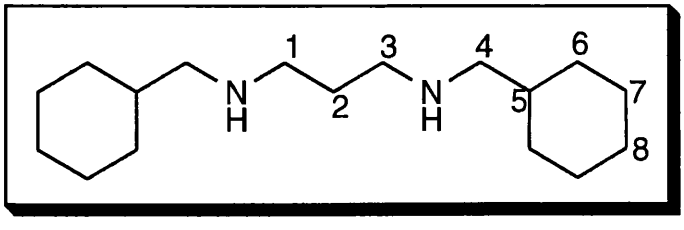
(CH_2), 47.4 (CH_2), 50.9 (CH_2), 124.8 (CH), 125.1 (CH), 139.7 (CH), 148.9 (CH), 166.5 (C) and 167.0 (C=O oxalate); (*m/z*)(+CI/NH_3) 284 $(M+H)^+$ (%) 285 (100), 283 (15), 194 (30), 175 (55), 122 (18), 108 (50) and 80 (8); (Found $(M+H)^+$ 285.2077. $C_{17}H_{24}N_4$ requires 285.2079).

N,N'-Bis(2-pyridylmethyl)-1,6-diaminohexane Dioxalate (162)



The procedure was applied using pyridine-2-carbaldehyde (0.32 g, 3.0 mmol), 1,6-diaminopentane (0.17 g, 1.5 mmol) and sodium borohydride (0.20 g, 5 mmol) to give **(162)** as a white powder (1.28 g, 91%), mp 248-249 °C; (Found : C, 55.13, H, 6.24, N, 11.60 %, $C_{22}H_{30}N_4O_8$ requires: C, 55.23, H, 5.28 N, 11.59 %); ν_{max} (KBr disc)/cm^{-1}, 3434, 3050, 1720, 1593, 1475, 710 and 499, δ_H (400 MHz, D_2O); 1.23 (4-H, b.s., 10-H_2 and 11-H_2), 1.56 (4 H, m, 9-H_2 and 12-H_2), 2.95 (4 H, t, *J* 8.0, 8-H_2 and 13-H_2), 4.20 (4 H, s, 7-H_2), 7.36 (4 H, m, 3-H and 5-H), 7.81 (2 H, m, 4-H) and 8.42 (2 H, d, *J* 4.0, 6-H), δ_C (100 MHz, D_2O); 25.4 (CH_2), 25.5 (CH_2), 47.6 (CH_2), 50.9 (CH_2) 124.7 (CH), 125.0 (CH), 139.5 (CH), 149.1 (CH), 150.0 (C) and 166.4 (C=O oxalate); (*m/z*) (+CI/NH_3) 298 $(M+H)^+$ (%) 299 (100), 297 (12), 208 (10), 189 (30), 155 (17), 109 (37) and 79 (40); (Found: $(M+H)^+$ 299.2236 $C_{18}H_{24}N_4$ requires 299.2236).

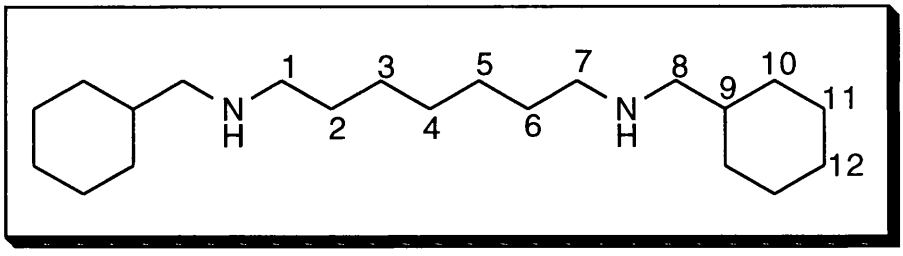
N,N'-Bis(cyclohexylmethyl)-1,3-diaminopropane (163)



Compound **(163)** was prepared by general procedure B, using cyclohexanecarbaldehyde (1.0 g, 8.9 mmol), 1,3-diaminopropane (0.33 g, 4.5 mmol) and sodium borohydride (0.56g, 14.9 mmol) as the free base (1.1 g, 4.1 mmol, 91%) which was converted into the dihydrochloride salt and

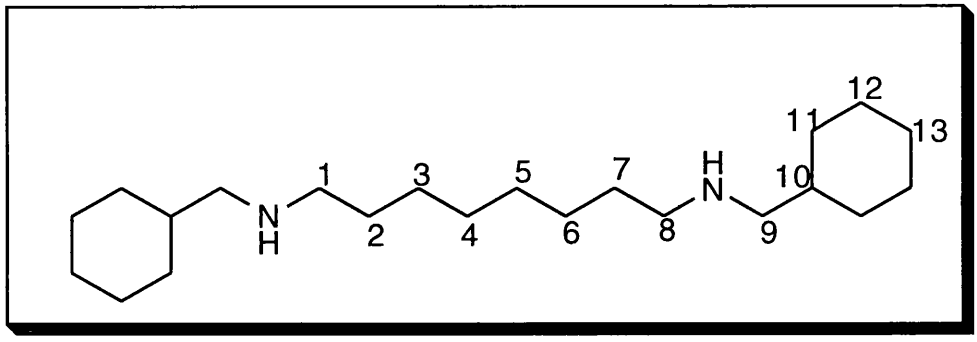
recrystallised from 95% aqueous ethanol; mp 294 -297°C decomp; ν_{max} (KBr disc)/ cm^{-1} 2852, 2776, 2360, 2341, 1455 and 668; δ_H (400 MHz, D_2O); 0.89 - 1.04 (4 H, m), 1.07 - 1.23 (7 H, m), 1.56 -1.66 (12 H. m), 2.03 (2 H, m, 5-H), 2.84 (4 H, m, 3-H_2), 3.04 (4 H, t, *J* 8, 4-H_2); δ_C (100 MHz, D_2O); 22.7 (CH_2), 25.3 (CH_2), 25.8 (CH_2), 30.1 (CH_2), 35.0 (CH), 45.2 (CH_2) and 54.1 (CH_2); (*m/z*) (+Cl/isobutane) 336 $(M+H)^+$ (%) 337 (100).

N,N'-Bis(cyclohexylmethyl)-1,7-diaminoheptane (164)



Compound **(164)** was prepared by general procedure B, except that the crude product was washed with 1M NaOH, using cyclohexanecarbaldehyde (3.3 g, 30.8 mmol), 1,7-diaminoheptane (2.0 g, 15.4 mmol) and sodium borohydride (1.9 g, 50.8 mmol), as the free base (4 g, 13.7 mmol, 89%) which was converted into the dihydrochloride salt and recrystallised from 95% aqueous ethanol; mp 288-292 °C decomp; (Found: C, 63.86, H, 10.98, N, 6.92 %, $C_{22}H_{44}N_2Cl_2$ requires: C, 63.96, H, 11.17, N, 7.11 %); ν_{max} (KBr disc)/ cm^{-1} 3423, 2853, 2473, 2417, 1593, 1447, 1000 and 888; δ_H (400 MHz, D_2O) 0.83 - 0.92 (4 H, m), 1.06 - 1.16 (8 H, m), 1.22 (8 H, broad signal), 2.76 (4 H, m, 7-H_2) and 2.89 (4 H, m, 8-H_2); δ_C (100 MHz, D_2O), 25.3 (CH_2), 25.6 (CH_2), 25.8 (CH_2), 25.9 (CH_2), 28.3 (CH_2), 30.2 (CH_2), 34.9 (CH), 48.3 (CH_2) and 53.7 (CH_2); (*m/z*) (+Cl/Isobutane) (%) 323 (100) and 239 (20); (Found: $(M+H)^+$ 323.3426. $C_{22}H_{42}N_2$ requires 323.3426).

N,N'-Bis(cyclohexylmethyl)-1,8-diaminooctane (165)



Compound **(165)** was prepared by general procedure B, except that the crude product was washed with 1M NaOH, using cyclohexanecarbaldehyde (3.1 g, 27.8 mmol), 1,8-diaminooctane (2.0 g, 13.9 mmol) and sodium borohydride (51 mmol) as the free base (4.1 g, 12.2 mmol, 88%) which was converted into the dihydrochloride salt and recrystallised from 95% aqueous ethanol; mp. 278 -280°C decomp; ν_{max} (KBr disc)/ cm^{-1} 3431, 2938, 2473, 2419, 1595, 1149 and 1033. δ_H (400 MHz, D_2O) 0.83 - 0.99 (4 H, m), 1.08 - 1.15 (7 H, m), 1.24 (7 H, broad signal), 1.51 - 1.61 (16 H, m), 2.75 (4 H, d, *J* 6.8, 1 and 8-H_2) and 2.90 (4 H, m); δ_C (100 MHz, D_2O), 25. 3 (CH_2), 25.5 (CH_2), 25.8 (CH_2), 25.8 (CH_2), 28.0 (CH_2), 30.2 (CH_2), 34.9 (CH), 48.3 (CH_2) and 53.7 (CH_2); (*m/z*)(+CI/NH_3) (%) 337 (100) and 253 (20); (Found: $(M+H)^+$ 337.3582. $C_{23}H_{44}N_2$ requires 337.3583).

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