INHIBITION OF POLYAMINE BIOSYNTHESIS USING POLYAMINE ANALOGUES: A NOVEL APPROACH TO CONTROLLING FUNGAL PLANT DISEASES

A Thesis presented in part fulfilment of
the requirements for the Degree of
Doctor of Philosophy

by

Fiona McCallum Cook

Department of Organic Chemistry
University of Glasgow

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

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This thesis is dedicated to
my father and mother

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ABBREVIATIONS

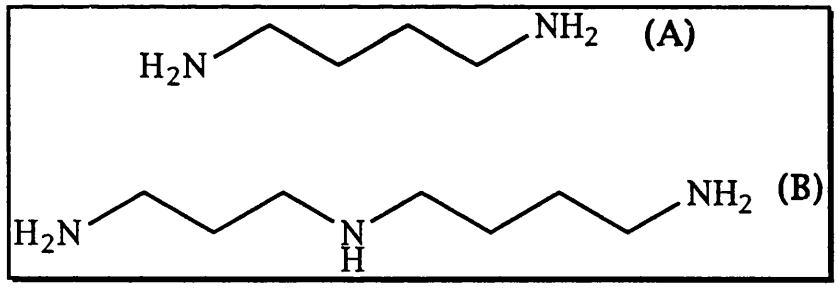
Ac	-	acetyl
ADC	-	arginine decarboxylase
AdoMetDC	-	*S*-adenosylmethionine decarboxylase
BAD	-	1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene
d	-	doublet
D	-	deuterium (^2H)
DCM	-	dichloromethane
DEAD	-	diethyl azodicarboxylate
DIAD	-	di-isopropyl azodicarboxylate
DIBAL	-	di-isobutyl aluminium hydride
DFMO	-	difluoromethylornithine
DMF	-	*N,N*-dimethylformamide
DNA	-	deoxyribonucleic acid
E-BED	-	1,4-diaminobut-2-ene
HMPT	-	hexamethylphosphorus triamide
IR	-	infra red
m	-	multiplet
MS	-	mass spectrometry
NMR	-	nuclear magnetic resonance
ODC	-	ornithine decarboxylase
PLP	-	pyridoxal phosphate
ptsa	-	*p*-toluenesulfonic acid
q	-	quartet
RNA	-	ribonucleic acid

s	-	singlet
TED	-	N,N,N',N'-tetraethyl-1,4-diaminobut-2-ene
t	-	triplet
THF	-	tetrahydrofuran
TLC	-	thin layer chromatography
UV	-	ultra violet

ABSTRACT

Plants are attacked by a wide range of fungi which are the cause of considerable losses to yield and quality of crops. The famines and economic catastrophies caused by fungal epidemics over the centuries are of huge proportions. Modern systemic and non-systemic fungicides are prone to fungal resistance and therefore there is a real need for novel fungicides with different modes of action to the ones currently in use.

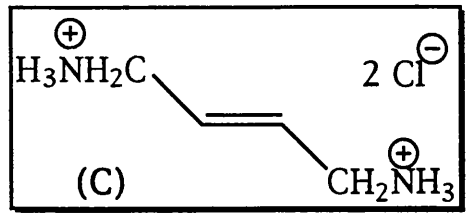
Polyamines are a group of simple aliphatic compounds some of which such as putrescine (A) and spermidine (B), are probably found in all cells. They are essential for the growth and development of all organisms including plants and fungi.



Preliminary studies by W. Martin in this research group showed that analogues of putrescine possess antifungal activity. The aim of this project was to synthesise analogues in several structure areas, in order to test for antifungal effects on various types of fungi.

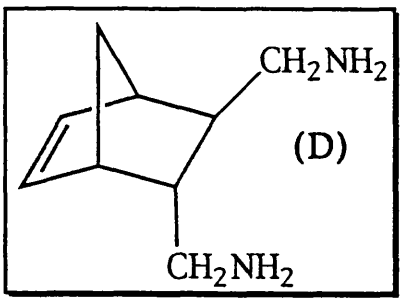
The first type of compounds was based on *(E)*-1,4-diaminobut-2-ene hydrochloride (E-BED) (C), a simple unsaturated diamine that is similar in size to putrescine and that displayed considerable antifungal activity, over a wide range of fungi. A

range of different salts of the free base of E-BED were synthesised. This work led to the filing of an initial patent by The British Technology Group (BTG).



N-Alkylated derivatives of E-BED were identified as the second area of target compounds. These were produced in an effort to modify and improve the antifungal activity shown by E-BED. An example of one of the several compounds that were made in this area is *(E)-N,N'*-dipropyl-1,4-diaminobut-2-ene. *(Z)-N,N,N'N'*-Tetraethyl-1,4-diaminobut-2-ene (Z-TED) was also synthesised and it was noted that *cis*-derivatives did not perform as well as *trans*-derivatives in antifungal testing. BTG filed a second patent with us for work done in this area.

The final set of compounds were all cyclic diamines. It was discovered that some of these alicyclic diamines exhibited very useful antifungal activity. Of these, initial interest was associated with 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene hydrochloride (BAD) made by W. Martin. BAD analogues prepared in this work included *N*-alkylated BAD, *trans*-BAD, four-, five- and six-membered ring systems, and bridged and heterocyclic systems. An example of a bridged system that was synthesised was *trans*-5,6-bis(aminomethyl)bicyclo[2.2.1]hept-2-ene (D). A third patent was filed as a result of the work completed in this area.


CH_2NH_2
(D)
CH_2NH_2

CHAPTER 1

INTRODUCTION

1.1 The Historical and Economic Perspective of Fungal Plant Diseases

Ever since man attempted to tame nature by cultivating the land fungal plant pathogens have caused him considerable problems. The development of agriculture through the centuries brought with it the threat of plant disease epidemics. Fungal diseases are mentioned in the Bible and have been around since antiquity. The catalogue of major epidemics caused by pathogens is evidence enough of the effect they have had on man (Table 1).[1] In 1760 up to half of the French wheat crop was lost due to bunt and much of the remaining crop was affected by smut.

From the middle of the nineteenth century major crops were severely attacked by fungal diseases and this had serious repercussions in many countries and societies. In 1845 the fungal pathogen potato blight made its debut in Europe and swept through Poland, Germany, Belgium, France, England and Ireland. The Irish potato famine starved about a million people to death and caused the emigration of another million to North America. Coffee rust disease destroyed the entire Ceylon (Sri Lankan) coffee industry between 1870 and 1880 and the economic result was very dramatic; it led to the disappearance of the coffee industry and its replacement by the Ceylon tea industry.

The economic losses caused by fungal epidemics are of huge proportions. In 1987 Europe suffered an estimated $23 billion

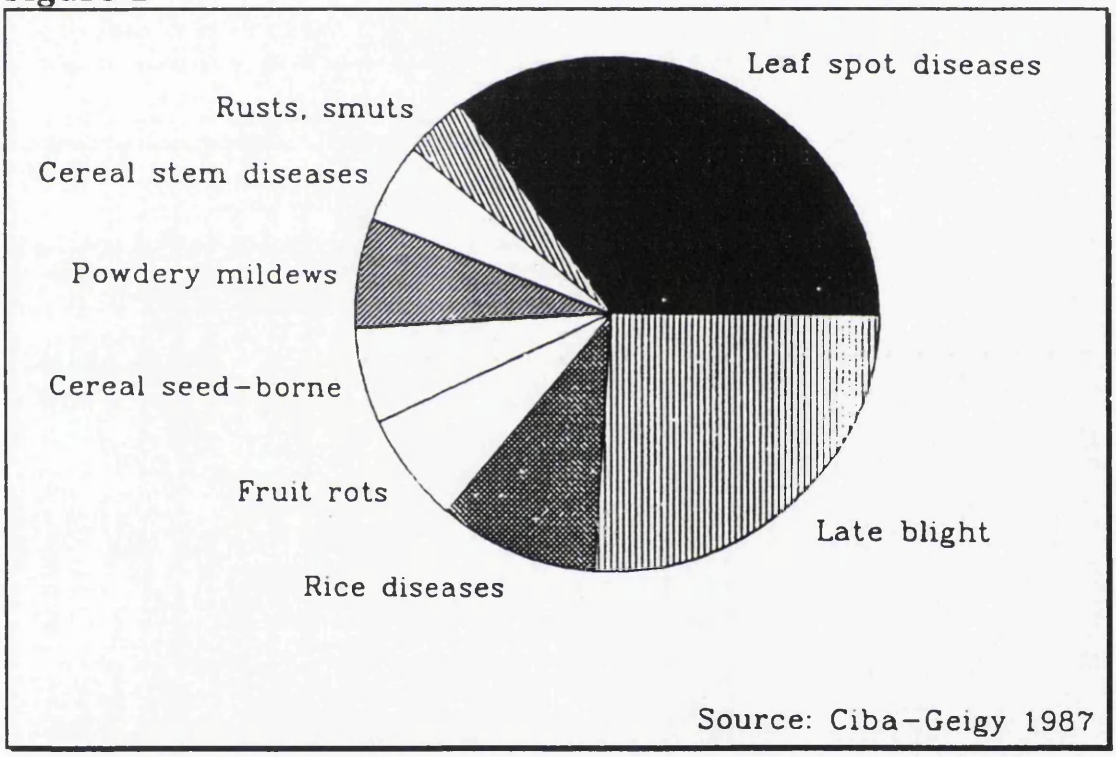
Table 1

DISEASE	REGION	EXAMPLES
Ergot of Rye, *Claviceps purpurea*	Europe, Africa	Epidemics 10th-20th century
Downy and Powdery mildews, *Plasmopara viticola*, *Uncinula necator*	Europe	Epidemics 1840-5 and 1870-80. Frequent outbreaks since then
Late blight of potato, *Phytophthora infestans*	Worldwide in humid climates	Irish famine 1845-46, German famine 1916-17 Frequent outbreak since
Coffee rust, *Hemileia vastatrix*	South East Brazil	Epidemics 1870-80 Since 1970
Leaf and stem rust of wheat, *Puccinia* spp.	Europe USA Mexico	Epidemics 1932, 1955 Epidemic 1935 Epidemic 1976-77
Brown spot of rice, *Drechslera oryzae*	Asia	Bengal famine, 1942-43
Maize rust, *Puccinia sorghi*	West Africa	Epidemic 1957
Blue mould of tobacco, *Peronospora tabacina*	Europe America	Epidemics 1960s Epidemic 1979-80
Rice blast, *Pyricularia oryzae*	Japan	Epidemics 1963, 1974
Cereal smuts, *Ustilago* spp.	Worldwide	Frequent severe epidemics
Chestnut blight, *Endothia parasitica*	USA	Destroyed most chestnut trees, 1904-1940
Sigatoka disease of bananas, *Mycosphaerella musicola*, *M. Fijiensis*	Worldwide	Great annual losses

worth of losses due to plant disease with Asia topping the global chart with an estimated $26 billion worth of losses. There is an

economic demand for both crop quantity and crop quality. Analysis of the relative importance of the major fungal plant disease groups (Figure 1)[1] shows that, on the basis of market potential for the chemical control, the leaf spot diseases are ranked highest followed by blights. This economic need for better and more reliable control measures to combat plant diseases has provided the incentive to find new fungicides in order to avoid the plant disease epidemics of the nineteenth century.

Figure 1



1.2 The Advent of Fungicides

In 1667 Robert Hooke examined fungal organisms but he did not realise what they were. It was not until 1767 that Felice Fontana recognised fungi in their own right and even later when, in the mid 1800s, wheat rust, bunt and potato blight were examined. The first fungicides were all inorganic compounds.[2] In 1752 copper compounds were used to control wheat bunt. Sulfur

was first used in France in 1845 and effectively controlled vine powdery mildew. By 1885 this had been developed into the Bordeaux mixture which was simply a copper sulphate and lime mixture.

The period between 1850 to 1950 is known as the 'Foundation Century' for crop protection and it was during this time that major advances in fungicidal technology occurred. Organic chemistry made its debut into the area of fungicides at the beginning of the twentieth century. The first wave of 'organic' fungicides were in fact organometallic compounds made from various metals combined with benzene or with organic dyestuff intermediates. 1915 saw the first in a long and successful line of organomercuric compounds. These compounds continued to dominate certain markets until mercurial compounds were banned by various governments in the 1970s and 1980s. By 1950 it had become established that organic chemistry could potentially provide a way forward for controlling the ever increasing fungal pests.

1.3 Fungi and Modern Fungicides

Fungi are the most diversified and important group of plant parasitic microorganisms covering 20,000 species. A crucial point is that fungi can almost limitlessly regenerate from only a few surviving spores; therefore successful fungicides must be extremely persistent. Clearly a knowledge of how fungi regenerate and the way in which they infect plants is essential for effective chemical control. To understand which fungi are affected by which fungicides it is necessary to use an approach based upon the means by which the infection is transmitted and a knowledge of

the lifestyle of the fungus. Table 2,[2] based on a scheme by Barnes (1972), classifies pathogenic fungi according to the method of reaching the plants and their reliance on water. Massive advances have been made in fungicidal performance, including quality, spectrum of action and duration of control.

Table 2

AIRBORNE INFECTIONS*

Group 1A. Water-dependent at infection stage—motile spores. Mostly Oomycetes (e.g. the downy mildews, *Pythium, Phytophthora, Peronospora, Bremia, Pseudoperonospora, Sclerospora*)
Fungicides: copper, dithiocarbamates, phthalimides, dichlofluanid (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, Pseudocercosporella*)
Fungicides: phthalimides, dichlofluanid, 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. Include some Basidiomycetes (e.g. *Ustilago*), some Ascomycetes (e.g. *Pyrenophora*) and some Deuteromycetes (e.g. *Septoria*)
Fungicides: dithiocarbamates, cuprous oxide, organomercury, some systemic compounds (e.g. carboxin, azoles)

SOILBORNE INFECTIONS
Fungi belong to many taxonomic groups, e.g. *Pythium* (Oomycetes), *Fusarium* (Deuteromycetes) and *Urocystis* (Basidiomycetes)
Fungicides: according to circumstances, correct choice from nabam, dichloran, chloroneb, copper, some systemic fungicides (e.g. thiophanate-methyl, thiabendazole)

* *Seed* or *soil* applications of systemic fungicides can sometimes control *airborne* infections.

An advance since the late 1960s has been the availability of xylem-systemic compounds. Up until that point fungicides could only kill superficial fungal infections as fungicides deposited on the leaf surface would not enter the plant tissue or move within the plant, *i.e.* they were non-systemic. This meant that treatment had to be preventative, before the disease had become established. The systemic fungicides reach the fungi within the plant tissue and protect new areas of growth. This obviously gives

better control and is effective for a longer duration as the fungicide cannot simply be washed away by rain. Non-systemic fungicides have, however, advantages over systemic fungicides in that the latter seem to be much more prone to fungal resistance. The reason for this could be because systemic fungicides are very site specific and they may select out existing mutants or even favour rapid gene mutation. Resistance persists for many generations after use of the compound has ceased and as the use of systemic compounds increase so do the potential disastrous consequences. Systemic and non-systemic fungicides are often used together or alternatively in order to reduce the chances of fungal resistance developing. Non-systemic fungicides tend to have low inherent toxicity and limited persistence and this probably, in part, accounts for the low incidence of resistance in these fungicides.

1.3.1 Non-Systemic Fungicides[2, 3]

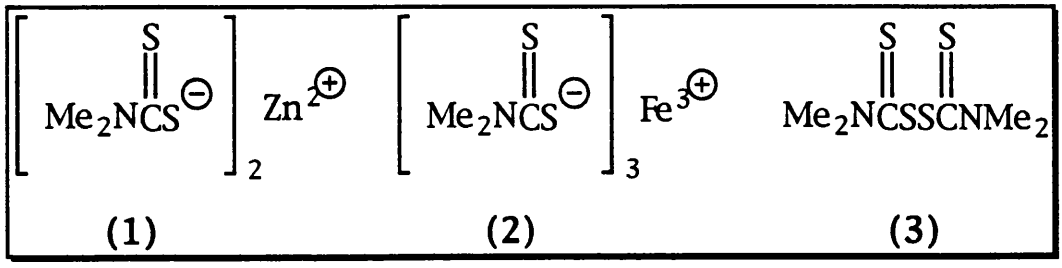
The majority of successful non-systemic fungicides are most effective when applied prior to the arrival of the infection.

Table 3

Group	Examples
Organosulphur compounds	
1. Dithiocarbamates	Thiram, zineb, mancozeb
2. Phthalimides	Captan, folpet, captafol
Dinitrophenol derivatives	Dinocap, binapacryl
Chlorinated aromatics	
1. Chlorinated nitro compounds	Quintozene
2. Chlorinated amino compounds	Dicloran
3. Chlorinated nitriles	Chlorothalonil
4. Chlorinated quinones	Dichlone
Other non-systemic or poorly systemic compounds	
1. Guanidine derivatives	Dodine acetate
2. Imidazoles (others are systemic)	Imazalil, prochloraz
3. Dicarboximides	Iprodione, vinclozolin

Most are insoluble in water and therefore have some degree of persistence. Table 3[2] shows the important non-systemic organic fungicides which are divided into groups of different molecular structure and mode of action. Some of these are now discussed in very rough chronological order.

Organosulfur Compounds. Since the early 1930s organosulfur compounds have been in use as fungicides and two compound groups of this type are the dithiocarbamates and the phthalimides. Both are still heavily used today and have been developed to produce numerous commercial fungicides. The dithiocarbamates can be separated into two main groups; those with one hydrogen on the nitrogen atom and those with none. Most of the commercial fungicides in this area are metallic complexes. Ziram (1) and ferbam (2) are zinc and iron complexes of dimethyldithiocarbamates and have limited protective fungicidal control. By contrast the disulfide oxidation product of the dimethyldithiocarbamates, thiram (3), has several areas of success including botrytis, rusts, wilt and root rots. It is produced by oxidising sodium dimethyldithiocarbamate and is very effective in seed treatment particularly as a protection against damping off in beans, peas and maize.

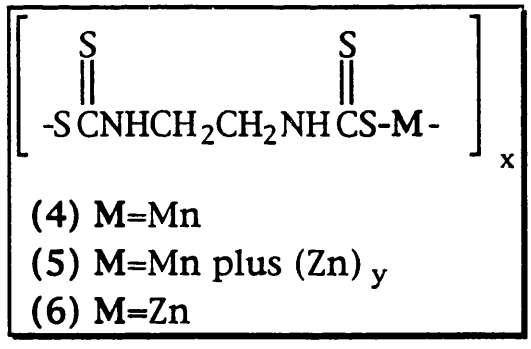


(1) (2) (3)

It has been suggested that the mode of action of these compounds may be that they form toxic complexes with copper, or alternatively, that they may sequester essential trace metals from

the fungi. The theory implies that free dithiocarbamate ions are formed within the cell and that these ions form a complex with internal heavy metals. Dithiocarbamate ions also have the ability to inactivate thiol groups and are therefore toxic in their own right.

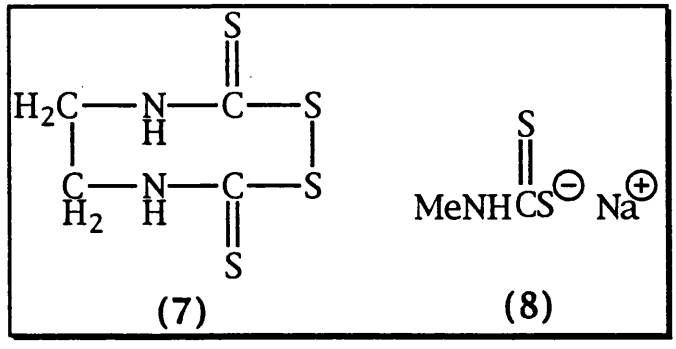
Bisdithiocarbamates and monomethyldithiocarbamates are types of compounds that only have one hydrogen atom on the nitrogen and have different fungicidal properties from the dimethyldithiocarbamates. The bisdithiocarbamates, maneb (polymeric manganese complex) (4), mancozeb (manganese and zinc) (5), and zineb (zinc) (6) are all very important in potato and tomato blight control. Mancozeb is often used in mixtures with systemic compounds and controls tulip fire and black spot on roses as well as cereal rust. Downey mildews, rusts and root rots are diseases that zineb is often used to combat.



(4) M=Mn
(5) M=Mn plus $(Zn)_y$
(6) M=Zn

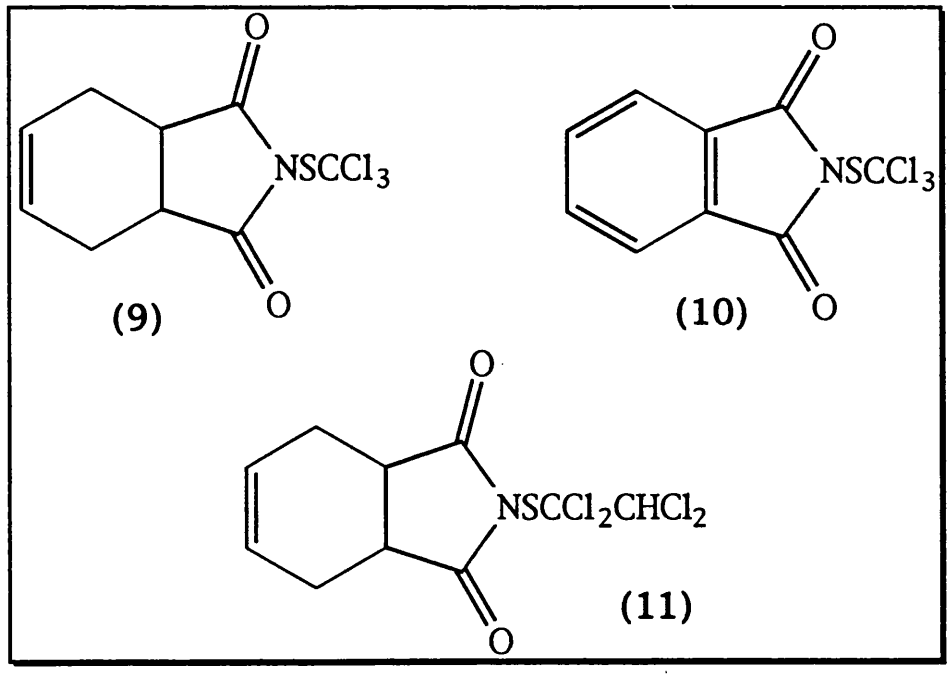
The mode of action of these fungicides is associated with the labile hydrogen on the nitrogen which renders the compounds unstable. They react with thiol groups in essential biological components and break down to give numerous products including isothiocyanates and thioureas which are known to react with enzymes and proteins. It has been proposed however that one breakdown product in particular, common to all these fungicides and called ethylenethiran disulfide (7), is in fact the actual toxic

agent. The monomethyldithiocarbamate metham-sodium (8) is a very potent soil sterilant.



It is a costly soil fungicide and is not widely used. It is made by the reaction of methylamine with carbon disulphide in the presence of sodium hydroxide and decomposes to give methyl isothiocyanate which is a highly volatile fumigant.

Around 1950 the phthalimides captan (1,2,3,6-tetrahydro-*N*-(trichloromethylthio)phthalimide) (9) and folpet (*N*-trichloromethylthio)phthalimide) (10) were introduced as fungicides.

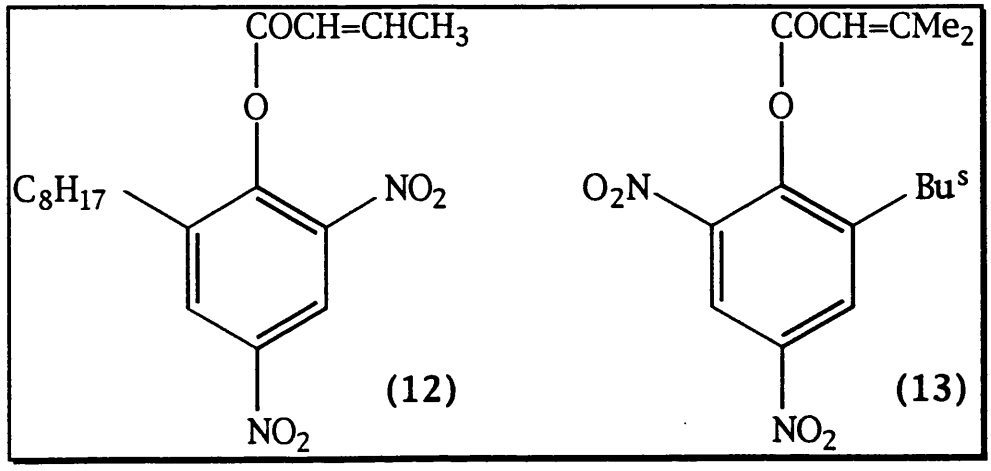


They are very similar and have a broad spectrum of preventative activity, although their main success is in fruit damage

prevention. Captan is produced by reacting maleic anhydride with buta-1,3-diene, and condensing this product with ammonia to give 1,2,3,6-tetrahydrophthalimide. This is then treated with trichloromethanesulphenyl chloride to produce captan. Folpet is made by the reaction of trichloromethanesulphenyl chloride with sodium phthalimide.

There are several theories as to the mode of action of captan. One of them is that, in the fungi, captan reacts with thiol groups and is converted into thiophosgene which can, in turn, react with thiol groups causing a mechanism which blocks enzyme activity. Captafol [1,2,3,6-tetrahydro-*N*-1,1,2,2-(tetrachloroethylthio)phthalimide] (11) was introduced in 1961 and is more expensive. However it has the advantage over the previous two in that it controls potato blight, leaf blotch of barley, glume blotch of wheat and white tip of leeks. It is also successful when mixed with systemic fungicides at controlling red core disease of strawberries.

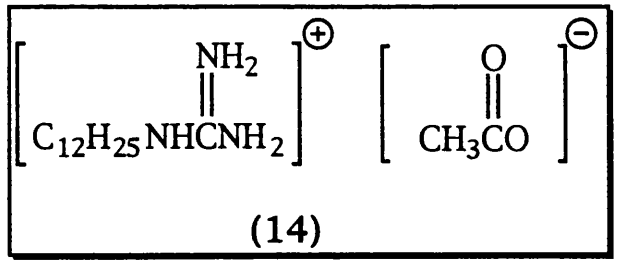
Dinitrophenol Derivatives. It was in 1946 that non-systemic organic fungicides first appeared in the form of dinitrophenol derivatives. Dinocap (12), a dark brown oil, is an isomeric mixture



of 2,4-dinitro-6-octylphenyl crotonates and 2,6-dinitro-4-octylphenyl crotonates and is produced by condensing phenol and octan-1-ol, nitrating this product and esterifying with crotonyl chloride. Dinocap controls powdery mildew on a variety of fruit, ornamentals and hops. Its analogue binapacryl (2-*sec*-butyl-4,6-dinitrophenyl-3-methylcrotonate) (13) was introduced in 1960 and also kills powdery mildews. However, both compounds are currently being phased out of use, for fear of toxicity to higher animals, and are being replaced by systemic fungicides.

Both dinocap and binapacryl are esters and their fungicidal activity is probably due to hydrolysis by enzymes in fungi to liberate free dinitrophenols which are then fungitoxic.

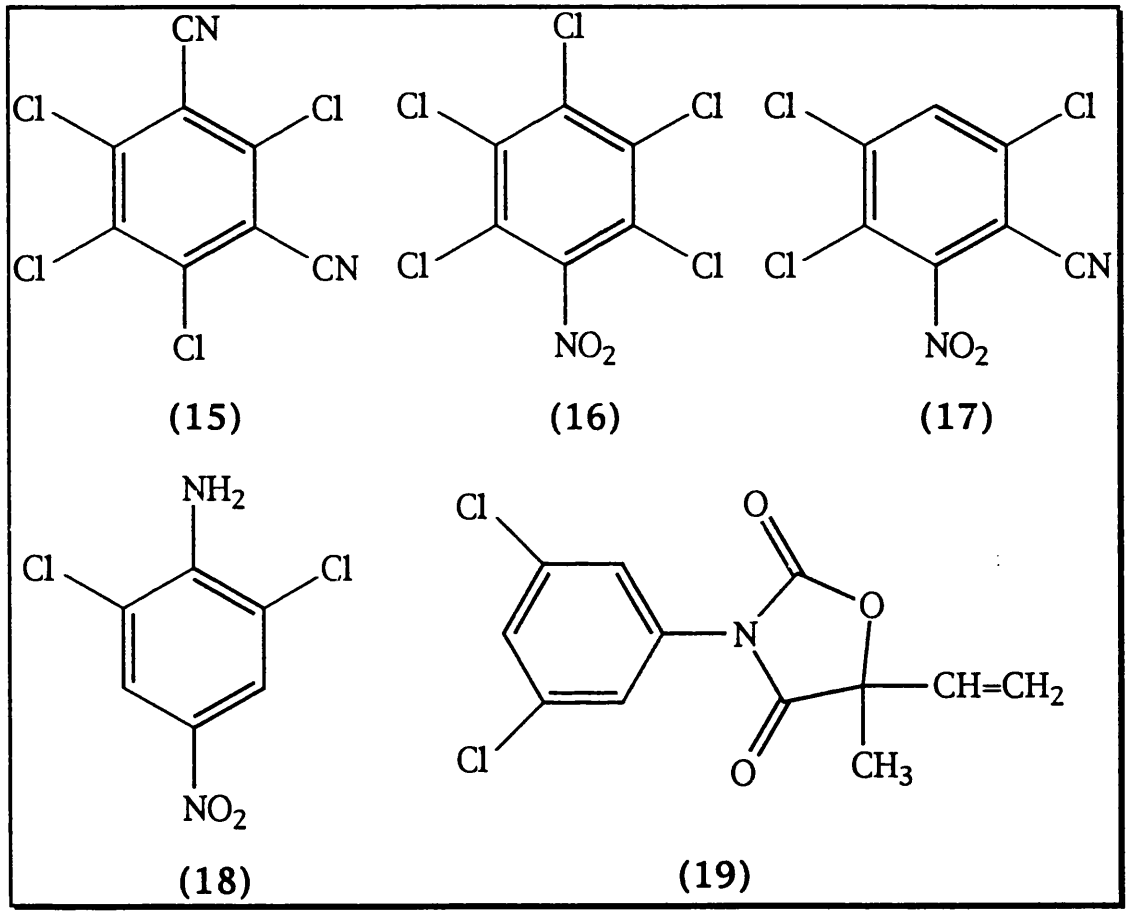
Guanidines. 1956 saw the arrival of the first guanidine derivative to be used as a fungicide. It had been known that cationic detergents had bactericidal properties and dodine acetate [1-dodecylguanidinium acetate] (14) was thus developed. It is made by reacting 1-dodecyl halide with sodium cyanamide and then treatment with ammonia. Dodine is particularly effective when used against apple scab, especially when infection incidence is high and it probably attacks lipoprotein membranes.



(14)

Chlorinated Aromatics. There are many types of aromatic chlorinated fungicides, all of which have limited use but some of them have had recent interest paid to them. The reason for this interest is the effect these non-systemic compounds can have

when mixed with systemic fungicides, in the effort to combat resistance. One such compound is chlorothalonil [tetrachloroisophthalonitrile] (15) which is made by the chlorination of isophthalonitrile and is used as a foliage spray. It has been very successful when mixed with the systemic compound benomyl (See 1.3.2) to control root rot and other fungal diseases.

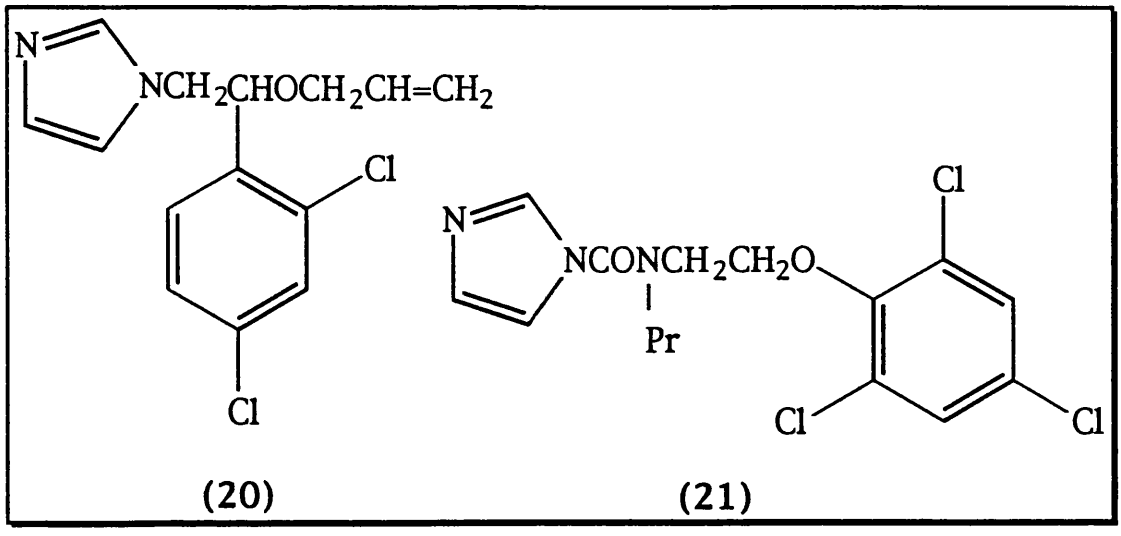


PCNB [pentachloronitrobenzene; common name - quintozene] (16) and TCNB [1,2,4,5-tetrachloro-3-nitrobenzene; common name - tecnazene] (17) were among the first chlorinated organic fungicides, having been introduced in the late 1930s and 1946 respectively. They are closely related and are used to control soil- and seed-borne fungi. They are capable of producing many

metabolites and it has been suggested that they in some way interfere with chitin synthesis in fungal cell walls. Dichloran [2,6-dichloro-4-nitroaniline] (18), introduced in 1959, is used both as a soil-applied fungicide and as foliar spray and can protect vegetable and ornamental plants from a range of fungi including botrytis.

Dicarboximides. In 1975 the dicarboximide vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-1,4-dione] (19) was established as of great use in controlling grey mould on grapevines in Europe. Generally the dicarboximides are weakly systemic when applied to roots and are very specifically toxic to grey mould. They have been used extensively to protect lettuce in the U.K., but no mode of action has, as yet, been ascertained.

Imidazoles. Imazalil [1-β-allyloxy-2,4-dichlorophenethyl)-imidazole] (20) and prochloraz [*N*-propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl]-imidazole-1-carboxamide] (21) are two compounds that belong to a group of imidazoles that inhibits steroid synthesis.



(20) (21)

Their fungicidal properties were discovered in 1972 and 1977 respectively. They are weakly systemic and are particularly useful against pathogens that are seed- or soil-borne. Prochloraz, the more recent of the two, has a wide spectrum of activity, controlling cereal diseases, powdery mildew, net blotch, and major fungal pathogens of mushrooms.

1.3.2 Systemic fungicides[2, 3]

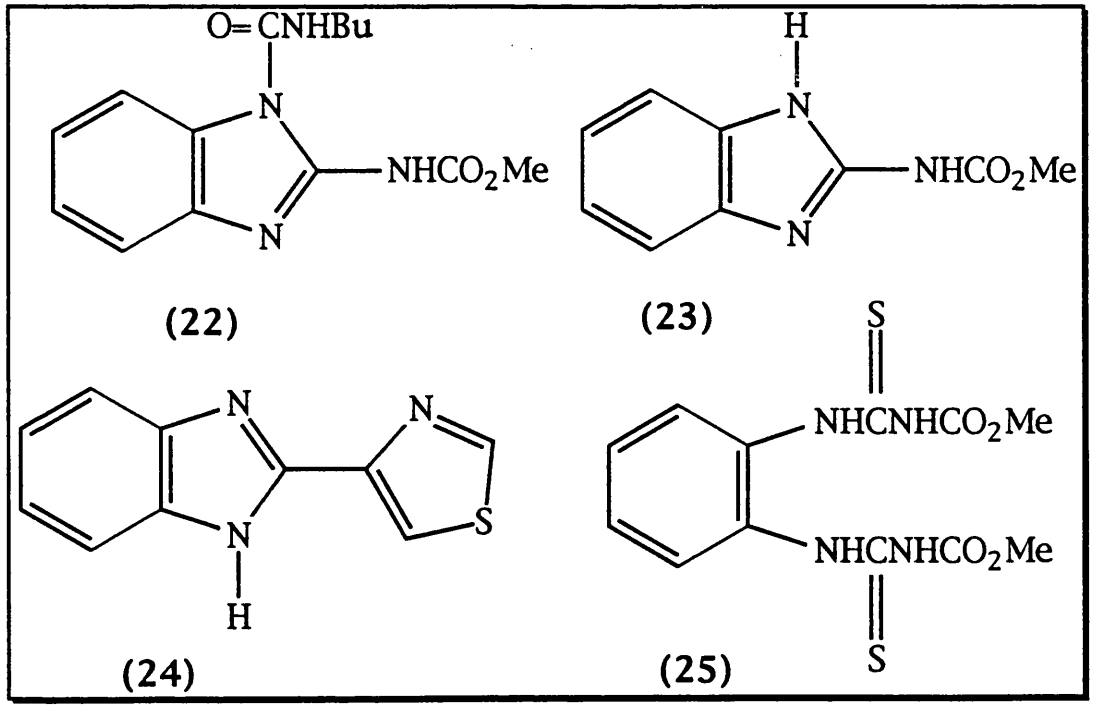
It was around 1965 that systemic fungicides became dominant in the battle against fungal pathogens and a huge development drive then ensued. As mentioned before, systemic fungicides have the advantage of being able to move within the plant and are therefore more persistent than non-systemics, but have the disadvantage of being more prone to fungal resistance. Most systemic fungicides appear to be site specific and attack very specific processes in fungi. Table 4[2] shows the important groups of systemic organic fungicides, which are discussed below.

Table 4

Group	Examples
Benzimidazoles	Benomyl, carbendazim, thiophanate, thiabendazole
Oxathiins or carboxamides	Carboxin, oxycarboxin
Morpholines	Tridemorph, dodemorph
Inhibitors of steroid C-14 demethylation	
(a) Triazoles	Triadimefon, triadimenol, propiconazole diclobutrazole
(b) Pyrimidines	Fenarimol, nuarimol
(c) Pyridines	Buthiobate
(d) Piperazines	Triforine
(e) Imidazoles	Imazalil, prochloraz
Hydroxyaminopyrimidines	Ethirimol, bupirimate, dimethirimol
Antibiotics	Kasugamycin, streptomycin
Phenylamides that target Oomycetes	Metalaxyl, ofurace, oxadixyl
Miscellaneous antifungal compounds	

Benzimidazoles. In the 1970s several benzimidazoles were recognized to be fairly broad-spectrum systemic fungicides. They are not, however, efficient enough to control fast spreading leaf diseases like rusts and mildews. The benzimidazoles can be split up into three groups, the carbamates, the non-carbamates, and the thiophanates (which become benzimidazoles upon uptake).

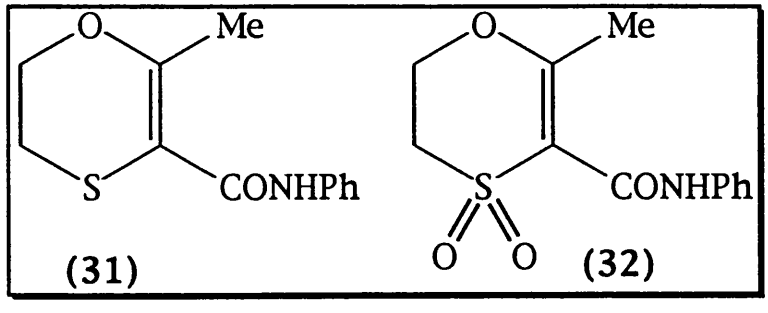
Benomyl [methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate] (22) was introduced in 1967 as an experimental fungicide and is produced by condensing *o*-phenylenediamine with $NCNHCOOCH_3$ and then treating the product with butyl isocyanate. It can be applied to foliage, used as a bulb dip and as a soil treatment. Benomyl is hydrolysed to carbendazim [methyl benzimidazol-2-ylcarbamate] (23) upon root uptake and moves up the plant as the latter. Carbendazim is fungitoxic and is, in fact, itself a commercial fungicide. It has been suggested that no matter



which of several benzimidazoles is used carbendazim is the active substance. Carbendazim has developed a widespread resistance problem with eyespot disease and suitable non-systemic fungicides are now being used. Thiabendazole [2-(thiazol-4-yl)benzimidazole] (24) is used against various fungal pathogens attacking stored potatoes and has been used in the U.S.A. to combat Dutch Elm disease. Not very much is known of the routes by which it is metabolised and therefore its mode of action is also still being debated.

Thiophanate-methyl [dimethyl 4,4'-(*o*-phenylene)bis(3-thioallophanate)] (25) cannot be classified as a benzimidazole. However it does cyclise to give carbendazim in plants and therefore presumably has the same mode of action as the other benzimidazoles. This mode of action seems to be to interfere with the division of cell nuclei.

Oxathiins or Carboxamides. In 1966 carboxin [5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide] (26) and oxycarboxin [2,3-dihydro-6-methyl-5-phenylcarbamoyl-1,4-oxathiin 4,4-dioxide] (27) were prepared and they are still used widely today.

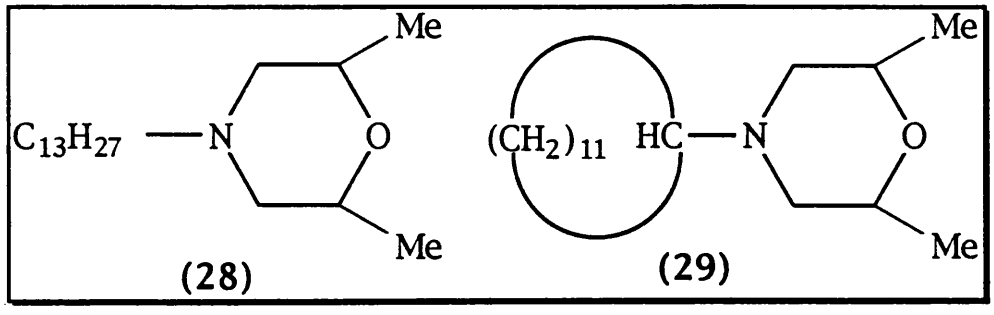


Carboxin is used for seed treatment of cereals against smuts and bunts while oxycarboxin, which is made from the oxidation of carboxin with hydrogen peroxide, is used for treatment of rust

diseases of vegetables and ornamentals. The mode of action appears to be that a molecule of carboxin fits into an active site of succinate dehydrogenase and therefore disorganises the flow of electrons to ubiquinone.

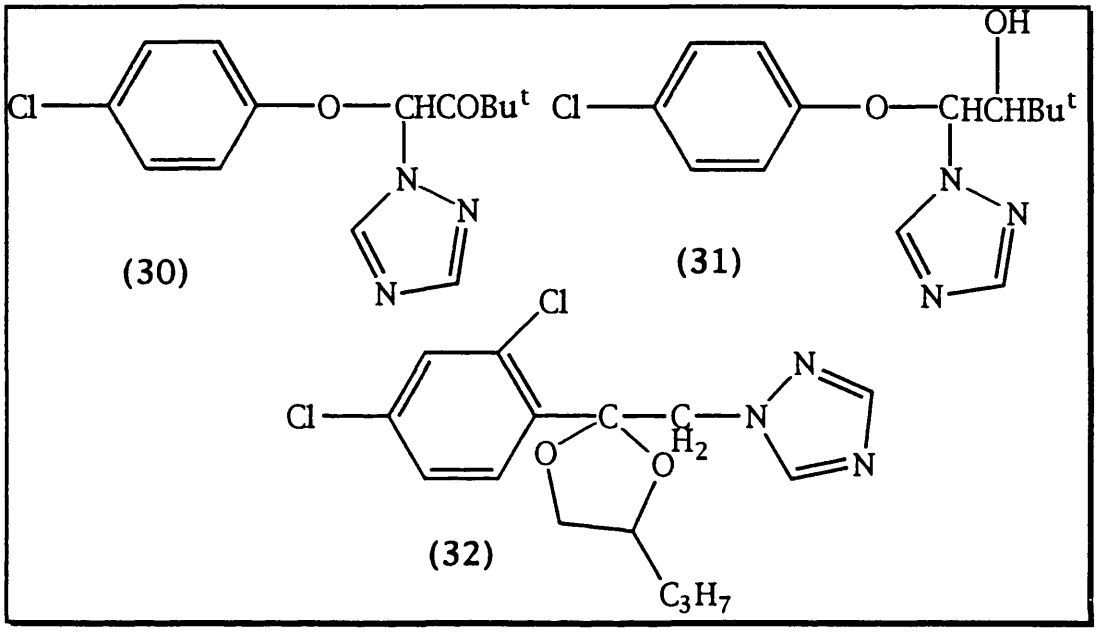
Inhibitors of Sterol and Steroid Synthesis. Inhibition of sterols and steriods is the mode of action for a great many fungicides. Without explaining the intricacies of sterol and steroid synthesis, except to give several references, the next two groups of fungicides have modes of action that interfere with one or other of these biosynthetic routes in fungi.

Morpholines are a group of fungicides that inhibit sterol synthesis. The two most important fungicides in this group are tridemorph [2,6-dimethyl-4-tridecylmorpholine] (28) (1969), which controls cereal mildews, and dodemorph [4-cyclododecyl-2,6-dimethylmorpholine] (29) (1967), which controls rose and cucumber powdery mildews. They are both commonly used in mixtures with non-systemics to increase their spectrum of activity. There is doubt as to exactly how the morpholines inhibit sterol synthesis simply because of the problems associated with sterol identification.



Several groups of nitrogen-containing heterocyclic fungicides function by inhibiting steroid demethylation. These

nitrogen atoms can form a co-ordination complex with haem iron which blocks important active sites which impede ergosterol synthesis. These compounds are called ergosterol biosynthesis inhibitors (EBIs).

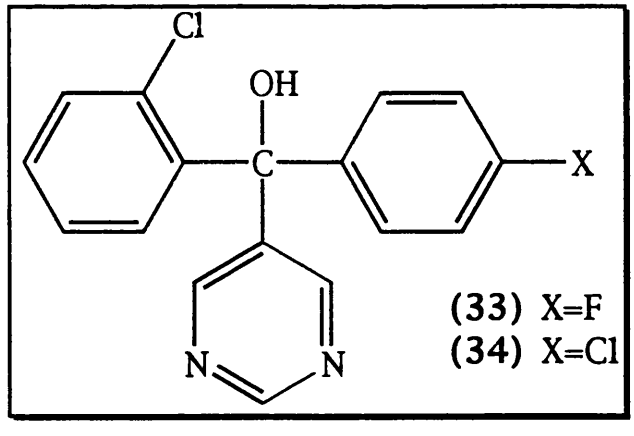


The triazoles, the largest of these groups, have produced many commercial fungicides and can be split into two types. Within the triazole group small changes to structure lead to large changes in toxicity of fungi. Two examples of the first type are triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butanone] (30) and tridimenol [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butan-2-ol] (31), the former being converted into isomers of the latter upon uptake. Both are effective against a large variety of mildews and rusts.

The second type of triazole has an extra carbon atom between the triazole nucleus and the 'T-junction' of the molecule. Propiconazole [1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxoan-2-yl]methyl]-1*H*-1,2,4-triazole (32) is one such example and

controls mildews and rusts on wheat and barley. This was used in this project by Dr. Dale Walters, as a commercial comparison when assessing the fungicidal properties of novel fungicides (See Section 3.4).

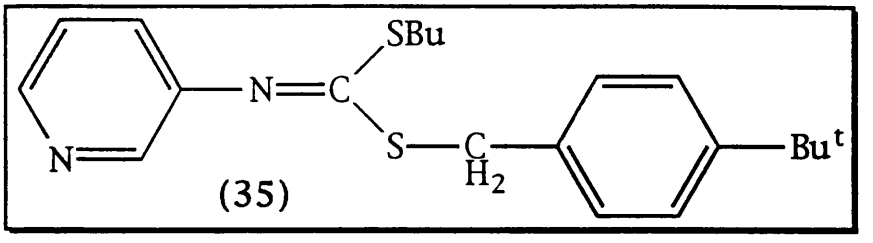
The pyrimidines nuarimol [2-chloro-4'-fluoro-α-(pyrimidin-5-yl)benzhydryl alcohol] (33) and fenarmol [2,4'-dichloro-α-(pyrimidin-5-yl)benzhydryl alcohol] (34) were both introduced in 1975 and have a wide spectrum of activity, in particular against powdery mildews and scab on fruit. The first step in the preparation of nuarimol and fenarmol is the Friedel-Crafts reaction of 2-chlorobenzoyl chloride with chlorobenzene or fluorobenzene respectively. The products at this stage are then condensed with pyrimidin-5-yllithium. In addition to being EBIs they may have a secondary mode of action altering the composition of plant lipoprotein membranes.



(33) X=F
(34) X=Cl

The pyridine buthiobate [butyl 4-*tert*-butylbenzyl *N*-(3-pyridyl)dithiocarbonimidate] (35) was brought onto the market in 1977 and is made by the reaction of butyl(3-pyridyl)dithiocarbamate with a 4-*tert*-butylbenzyl halide. It is a

preventative, curative and persistent fungicide which is effective against powdery mildews of many crops.



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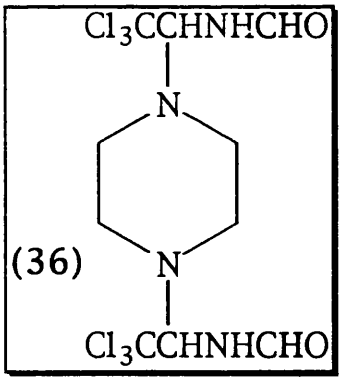
Triforine [1,4-bis(2,2,2-trichloro-1-formamidoethyl)-piperazine] (36) is an example of an important fungicide of the piperazine group and is one of the earlier EBIs, being synthesised in 1967. It is produced by the reaction of piperazine with *N*-(1,2,2,2-tetrachloroethyl)formamide and is effective against powdery mildew, scab and other diseases of fruits, vegetables and ornamentals.

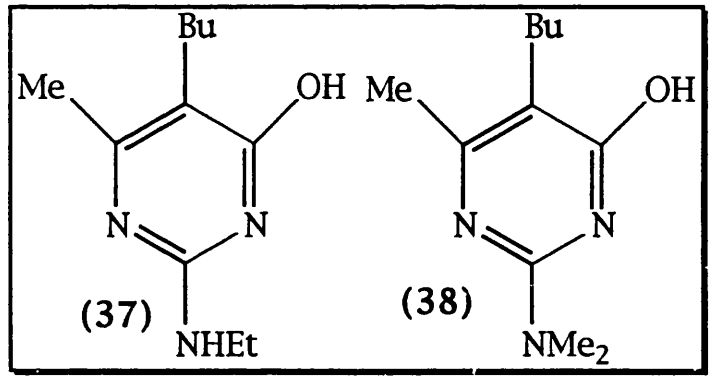


(36)

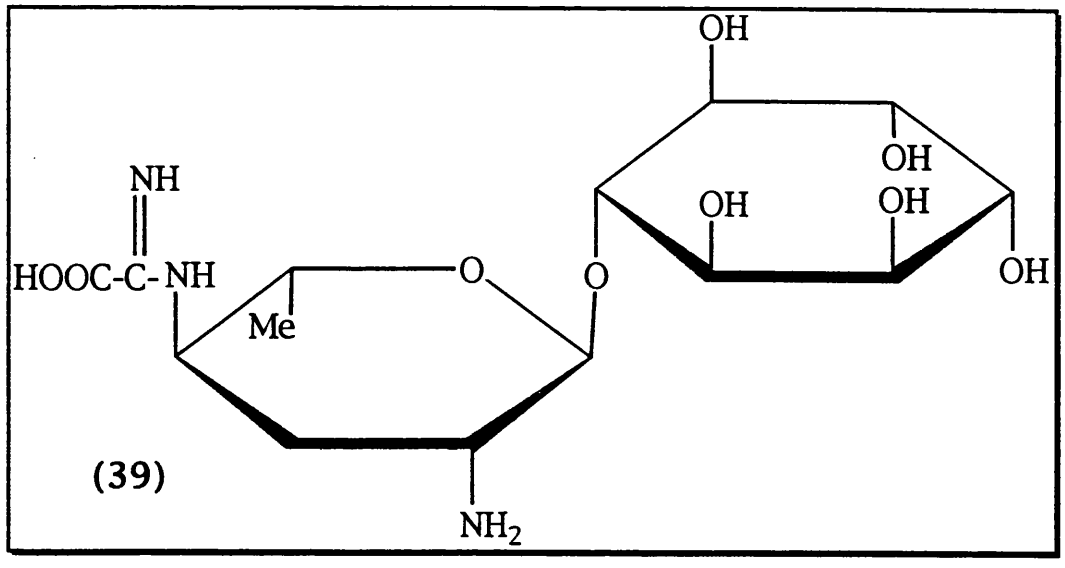
The imidazoles, imazil (20) and prochloraz (21) have already been discussed in the non-systemics section as they are only weakly systemic.

The hydroxyaminopyrimidines are compounds that were introduced specifically to control powdery mildews and, as such, are of little use against other fungi. Ethirimol [5-butyl-2-ethylamino-6-methylpyrimidin-4-ol] (37) is produced by

condensing 1-ethylguanidine with ethyl 2-acetylhexanoate and controls powdery mildew on a range of cereals. Dimethirimol [5-butyl-2-dimethylamino-6-methylpyrimidin-4-ol] (38) is an isomer of ethirimol and controls cucumber powdery mildew. This group of compounds seemed to cause large degrees of resistance in fungi and because of this their use is becoming much more limited. There have been several suggestions as to the mode of action of these compounds and it appears that ethirimol certainly interferes in some way with the action of the enzyme adenosine deaminase within fungi.



Antibiotics. There are some antibiotics that are marketed as fungicides but they tend to have very limited use. Kasugamycin (39), like other antibiotics, is only useful in controlling the rice blast fungus. It has taken over from blasticidin-S (40) as the major rice blast fungicide in Japan. The reason for this is that blasticidin-S can actually damage the rice even when used in only slight excess of the recommended concentration and kasugamycin is much less phytotoxic and is more persistent. The modes of action of these compounds are similar in that they selectively inhibit the initiation of bacterial protein synthesis.



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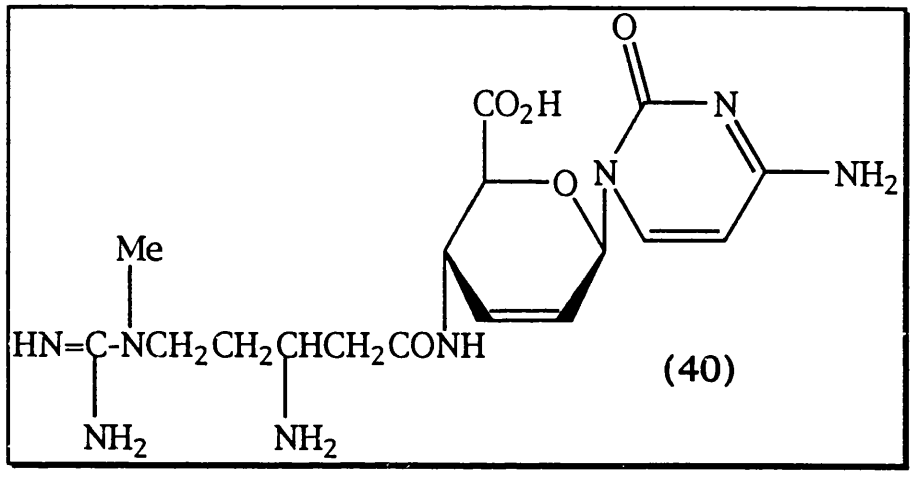
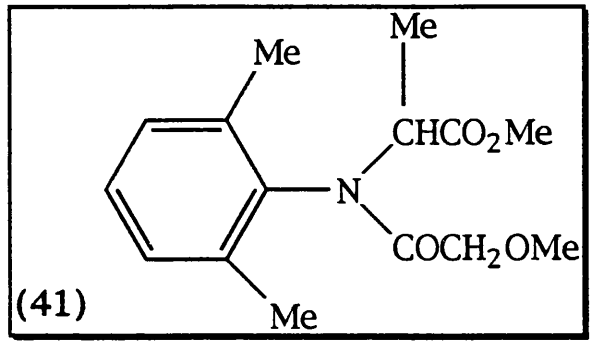


(40)

Phenylamides. Metalaxyl [methyl *N*-(2-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate] (41) is an example from the group of phenylamide fungicides. The compounds in this group control many Oomycetes, which are different from other fungi in several ways and have proved more difficult to control. The fungicidal properties of metalaxyl were first described in 1977. It has preventative and curative action on diseases caused by air- and soil-borne Oomycetes. It is particularly recommended when attempting to control late potato blight and is often used in a mixture with non-systemics in order to help reduce resistance. The non-systemic manab is often mixed with metalaxyl as they

have complimentary action, with the former preventing zoospores from entering cells of leaves and the latter affecting mycelial growth and subsequent sporulation.



(41)

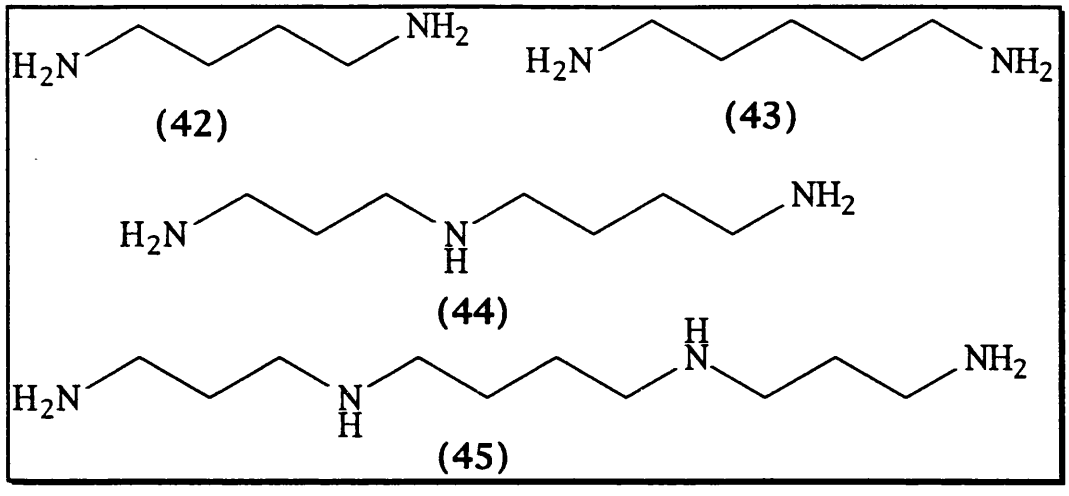
There are many more miscellaneous antifungal compounds that are too numerous to list but they are of limited value or interest.

An overview of the different types of fungi and the methods used to control them has been presented. In the next sections of this Chapter a review of the possible mechanisms used by certain fungicides to inhibit fungi is discussed.

1.4 Polyamines and Polyamine Biosynthesis

It is only fairly recently that there has been an increase in interest in polyamines in plants and fungi.

Polyamines are a group of simple aliphatic compounds, some of which, such as putrescine (42), cadaverine (43), spermidine (44), and spermine (45) are ubiquitous in all cells.[4] Polyamines appear to have many physiological roles but it is their involvement in growth and cell replication which is attracting increasing attention.[5]



It was in 1678 that Antoni van Leeuwenhoek first observed polyamine crystals but it was not for another 210 years that they were named as spermine. Again, it was not for a long time after this, in the mid 1920s that the correct chemical composition and structure were determined.[6] The growth promoting properties of polyamines were first observed in 1949 when a component in orange juice was found to be essential for the growth of the bacterium *Hemophilics parainfluenzae.* This component was later discovered to be putrescine. The first suggestion that polyamines had regulatory actions in plants was made in 1973 by Nello Bagni and his wife Donatella Serafini-Fracassini[6] and since that time more and more research has been done in this exciting area.

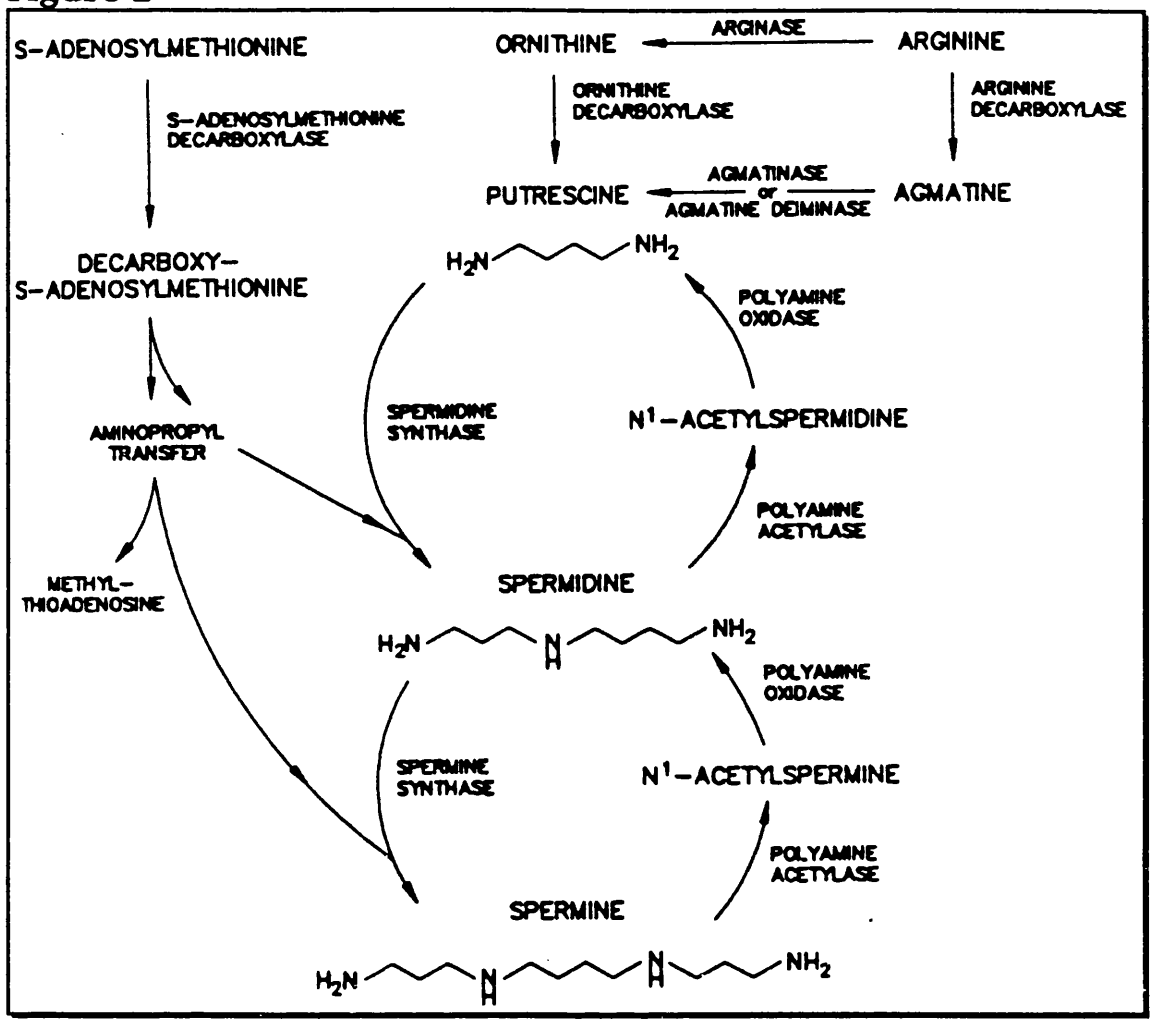
The chemistry and pathways of biosynthesis and metabolism of many polyamines in mammalian tissues are well characterised. However little is known about these aspects in plants and even less in fungi. It is clear that polyamines occur in cells as cations, but they are often conjugated to small molecules like phenolic acids and also to various macromolecules.[6] It is known that they play a prominent part in mechanisms for the control of growth and development.[7] Polyamine biosynthesis is greatly stimulated in rapidly growing tissues, and exogenous

polyamines promote growth. This seems to be due to their ionic binding to nucleic acids.[5] Spermidine and spermine, because of their basic nature, bind strongly to the acidic phosphate groups of nucleic acids and seem to stabilise DNA and RNA. They also appear to speed up every step in the transcription-translation sequence. There is also convincing evidence, from work done on the carrot embryogenesis system, that polyamines are essential for cell differentiation.[5]

In order to discover how to use polyamines to combat plant disease it is necessary to look at the biosynthesis of polyamines in plants and fungi.

In mammalian cells, in protozoa, and in fungi, the initial step in the synthesis of polyamines is the decarboxylation of the simple amino acid L-ornithine by the enzyme ornithine decarboxylase (ODC).[8, 9] This first step produces the diamine putrescine which is the precursor for further polyamine synthesis (Figure 2).[8] Spermidine and spermine are formed by the sequential addition of aminopropyl groups donated by decarboxylated *S*-adenosylmethionine (SAM). The enzymes spermidine synthase and spermine synthase catalyse the respective reactions that produce spermidine and spermine. This is the route by which polyamines are biosynthesised within fungal and mammalian cells, but there is a specific membrane transport system that enables some polyamines to be taken up from extracellular sources. The presence of serum oxidases may lead to the deconstruction of these extracellular amines but the possibility of polyamines arising from other cells or from external sources cannot be ruled out.

Figure 2

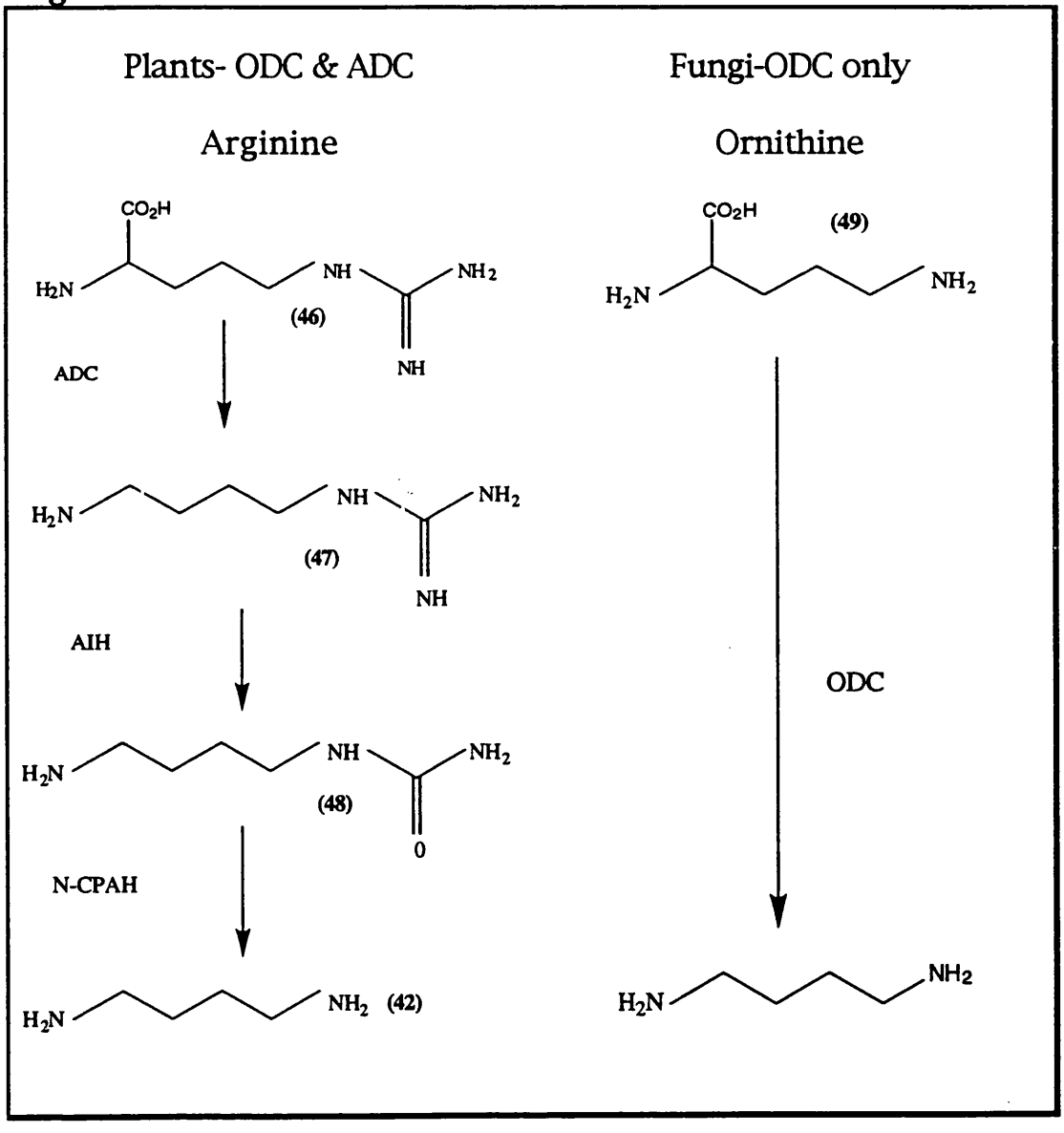


In plants and many bacteria two pathways may lead to putrescine synthesis, either via the ODC route as discussed earlier or indirectly via agmatine (47), the first product of arginine decarboxylation, in a reaction catalysed by arginine decarboxylase (ADC).[10] Spermidine and spermine are then synthesised from putrescine using the same route as before.

The two routes to putrescine, via ODC and via ADC, are looked at more at closely in Figure 3. L-Arginine (46) is decarboxylated in the presence of ADC to give agmatine (47). Agmatine iminohydrolase (AIH) then catalyses the conversion of agmatine into *N*-carbamoylputrescine (48), which is then

converted into putrescine via the enzyme *N*-carbamoylputrescine amidohydrolase. Putrescine is synthesised via a more direct route by the the action of ODC on L-ornithine (46). So, if fungi only possess the ODC pathway for making polyamines, the inhibition of this enzyme should prove lethal. Plants of course have an alternative route to polyamine biosynthesis and should therefore not be effected.

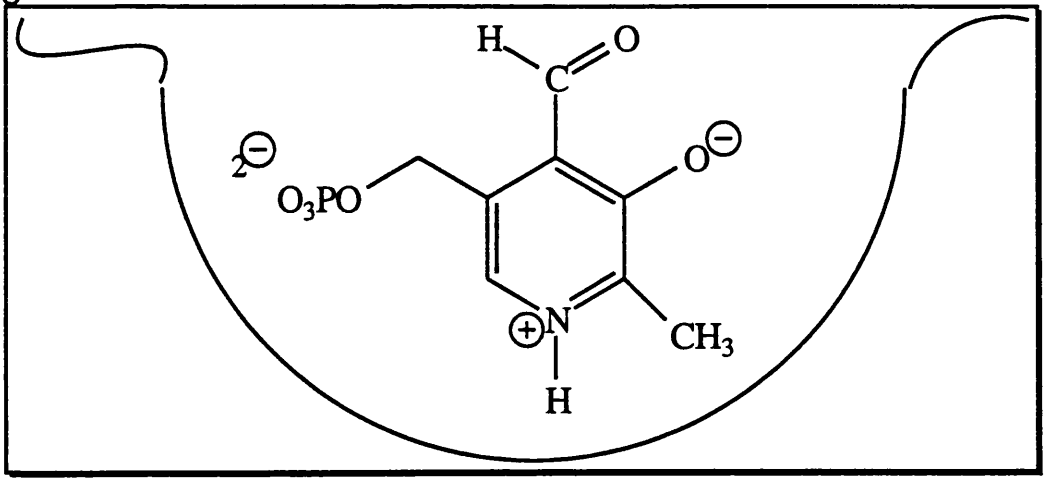
Figure 3



The ODC enzyme is a dimer of molecular weight 80,000 to 82,000.[11] The first ODC activity was detected in an extract of *Escherichia coli* in 1946.[12] There are in fact two different ODC enzymes in *E. coli*, namely a degradative one and a biosynthetic one. The biosynthetic ODC is responsible for putrescine synthesis while the degradative ODC is only active at low pH and appears to be involved in the regulation of the pH of the medium. The first attempt to purify ODC was in 1972 by Ono and co-workers.[13] This was indeed a formidable task as ODC is only present in very low amounts and only 1.02 mg was obtained from 7 kg of rat liver. As a result of the low levels of enzyme present in tissues and the considerable technical difficulties encountered in purification of the enzyme, direct mechanistic studies of ODC have been restricted.

ODC activity has been found in plants, protozoa, fungi and mammalian tissues.[11] Obviously the enzyme differs in each case but all known ODC enzymes require pyridoxal phosphate (PLP) for

Figure 5



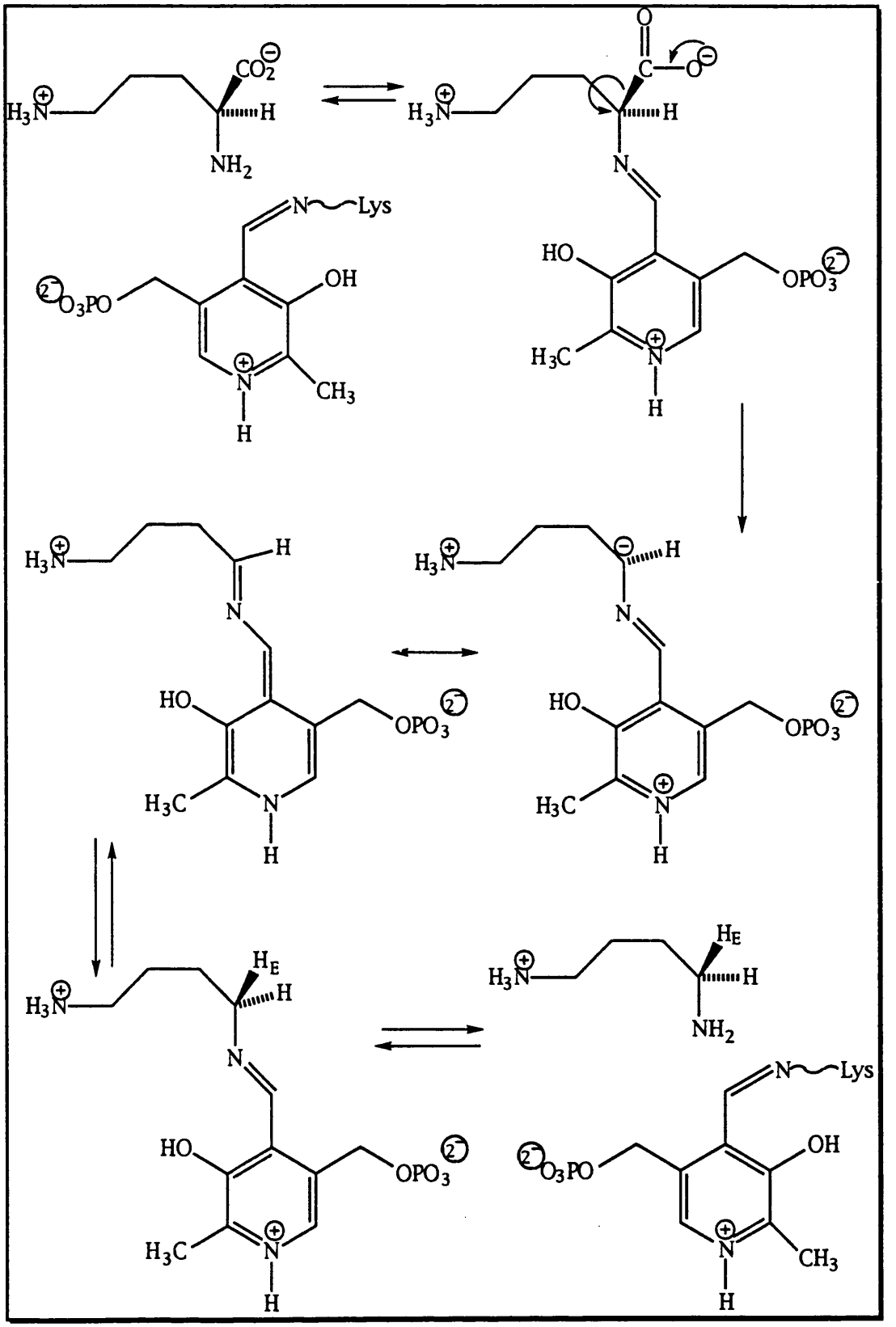
activity. PLP is known as the co-factor for ODC and probably sits in a 'pocket' of the enzyme where ornithine or any other substrate can easily approach and be decarboxylated. Figure 5 gives a rough

idea of the ODC 'pocket' with one of the forms of PLP being held in place by the various functional groups and the charges associated with them. There is evidence that ODC may exist in distinct forms which differ in their affinity for the co-factor and their overall activity. ODC shows a high degree of selectivity for L-ornithine as a substrate. Putrescine, the product of the reaction, and the higher polyamines spermidine and spermine, are usually weak competitive inhibitors for ODC.[14]

Specific PLP-dependent decarboxylases are known for many of the amino acids. The mechanism of action of ODC on L-ornithine to produce putrescine is shown in Scheme 1.[11] This scheme shows how the PLP co-factor decarboxylates L-ornithine with retention of configuration; *i.e.* the newly introduced hydrogen atom H_E in the putrescine product has the same stereochemical position as the departing carboxyl group. PLP is shown here covalently linked to the terminal amino group of a lysine residue of the apoenzyme. In the absence of a substrate this is how the co-factor appears. However, as soon as a substrate is present the lysine residue detaches to leave the aldehyde (see Figure 5) in that position. The first intermediate is a Schiff's base adduct formed between the α-amino group of ornithine and the aldehyde group of PLP. These Schiff base intermediates are common to all PLP-dependent enzymes. The three bonds to the α-carbon atom of ornithine become weaker due to a displacement of their electrons toward the cationic nitrogen in the pyridoxal ring system. Cleavage of the carboxyl group releases carbon dioxide in an irreversible reaction. The resulting intermediate has extended conjugation right through into the pyridine ring. Protonation on the α-carbon of the substrate gives an imine which undergoes hydrolysis to yield the

product, putrescine, and regenerates the original PLP with the terminal amino function of the lysine residue of the enzyme.

Scheme 1



1.5 ODC Inhibitors

The first publication dealing with inhibitors of ODC appeared in 1972.[15] Three main approaches have been used since that time to inhibit ODC.

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

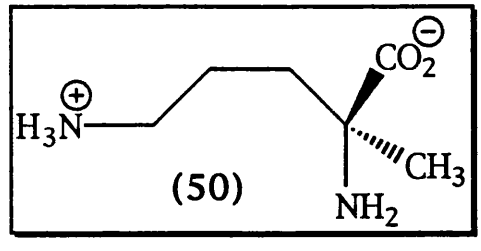
2. Synthesis of molecules capable of interacting or combining with the PLP co-factor.

3. Design of enzyme-activated inhibitors.

The enzyme-activated inhibitors seem to be the most successful ODC inhibitors although extensive work has been done in all three areas and each will accordingly be discussed.

1. Reversible Inhibitors that are Analogues of Ornithine and Putrescine.

One of the first reversible inhibitors to have been reported[16] for ODC is α-methylornithine (50) and since then, very few other



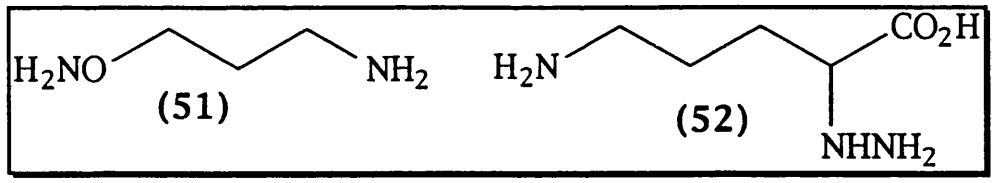
(50)

reversible inhibitors have been found to be as potent. α-Methylornithine is decarboxylated 6,000 times slower than ornithine and also produces a gradual inactivation of ODC.[17] This inactivation is caused by a transformation of PLP into pyridoxamine phosphate. *(E)*-Dehydro-analogues of ornithine and putrescine are the only compounds that have had a greater inhibitory affect than α-methylornithine. However, all the

negative results have been useful in providing important information on the active site of ODC and the structural requirements for substrate binding.

2. Reversible Inhibitors that Interact with the Co-factor.

Reversible formation of a Schiff base between the α-amino function of ornithine and the aldehyde group of PLP is an obligatory step not only in the case of ODC but for any of the α-amino acid decarboxylases. A variety of compounds with various functional groups could replace the natural substrate and form a more stable adduct with PLP. For example, 1-aminoxy-3-aminopropane (51), is one of the most potent ODC inhibitors of mouse kidney and of rat liver ODC.[18] Interestingly, introduction of a methyl group on the carbon atom bearing the amino function, or extentions of the chain by one carbon, only slightly reduces the inhibitory activity. 1-Amino-oxy-3-aminopropane also inactivates *S*-adenosylmethionine decarboxylase and spermidine synthase and therefore seems to lack specificity for ODC.



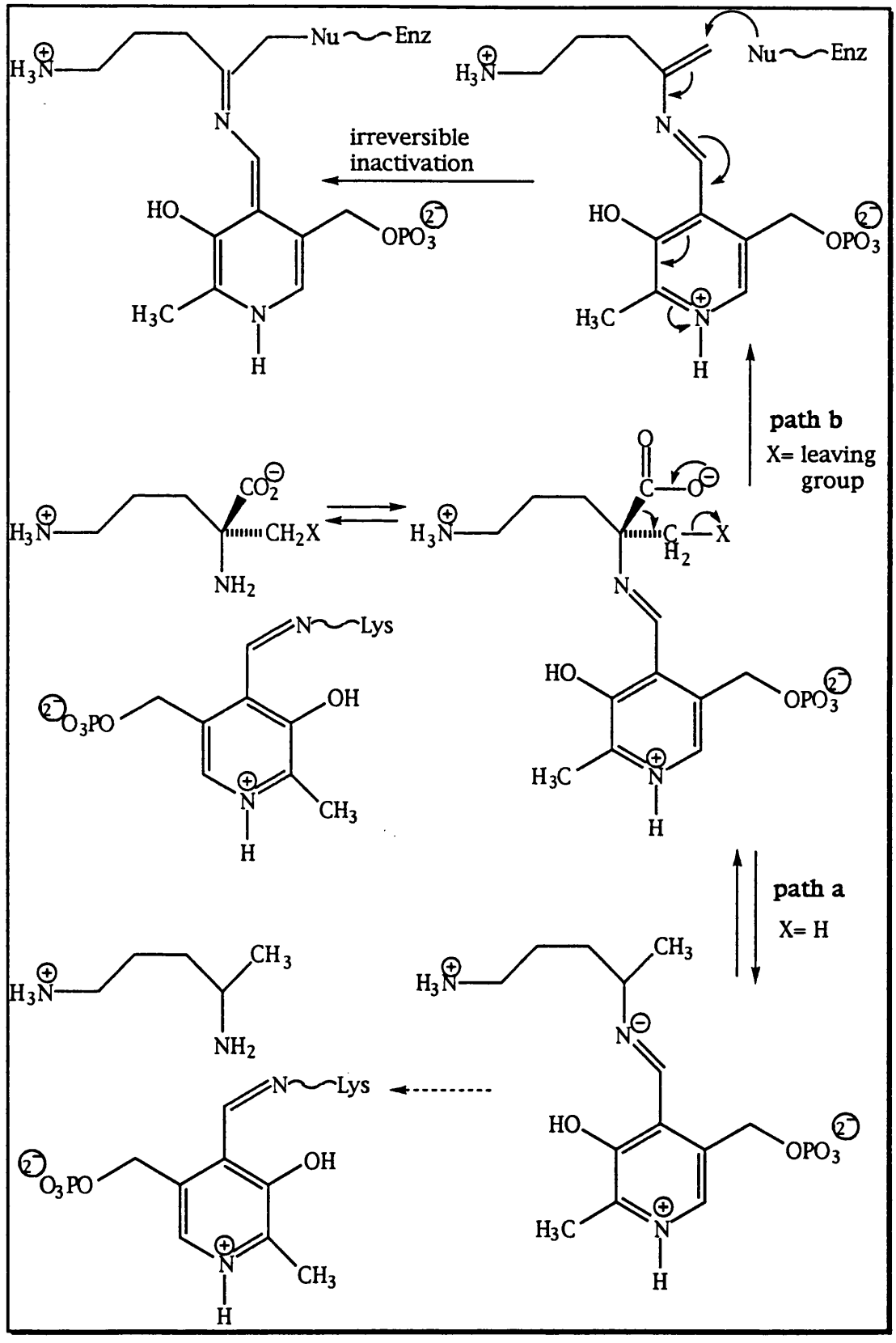
α-Hydrazinoornithine (5-amino-2-hydrazinopentanoic acid) (52) is a potent inhibitor of *E. coli* and rat prostrate ODC but also inhibits many other PLP-dependent enzymes, which, again shows lack of specificity.[19] The inhibitory effects of these compounds, which form stable adducts with PLP, possibly arise because they mimic some transition states along the reaction pathway. Unfortunately, although these reversible inhibitors block

biosynthesis of polyamines in cells in culture, they do not repeat these results *in vivo*. Here they seem to increase levels of ODC. When the concentration of the inhibitor in the body decreases due to metabolic clearance, reactivation of the enzyme results in an 'overshoot' of putrescine production. Due to the many problems associated with reversible inhibitors, the focus is now on the search for irreversible inhibitors.

3. Irreversible Enzyme-Activated Inhibitors.

The name enzyme-activated inhibitors was coined by Merrell scientists, who in the early 1970s initiated a lot of the work in this area.[8, 11] These inhibitors are chemically inert pseudosubstrates of the target enzymes, in this case ODC, which incorporate in their structure a group that is transformed into a species that eventually inactivates the enzyme. Enzyme-activated inhibitors should be very selective *in vivo* and have immense potential in the concept of drug design. In order to prove that a compound is an enzyme-activated inhibitor various pieces of evidence must agree. Purified enzyme must be used to determine the stoichiometry of the inhibition; to characterise the structure of the enzyme inhibitor adduct after inactivation; and to demonstrate that the inhibitor is transformed by the target enzyme before the inactivation takes place. However, reasonable evidence can be obtained from relatively simple kinetic experiments performed with a crude preparation of enzyme. In addition, if the ODC is inactivated then dialysis or passage through a Sephadex column should not lead to reactivation of the enzyme. This is compatible with formation of a covalent linkage between the enzyme and the inhibitor (N.B. not all enzyme-activated inhibitors lead to covalent bond formation).

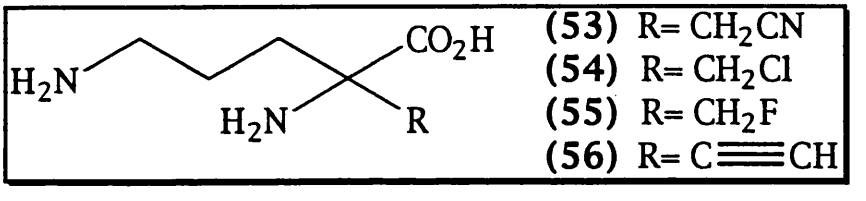
Scheme 2



PLP-dependent enzymes are good enzymes for the design of enzyme-activated inhibitors. In 1978 Merrell Dow scientists

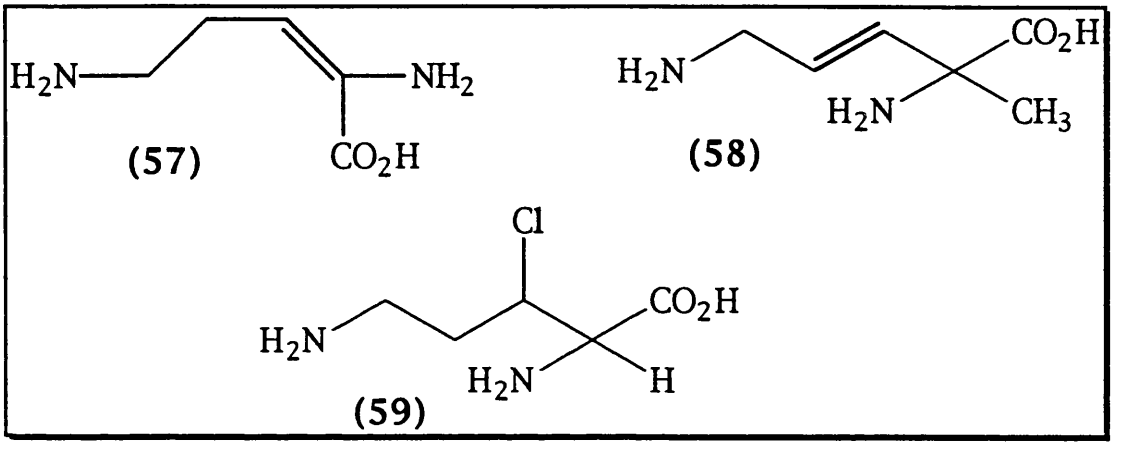
proposed that analogues of α-amino acids incorporating either a leaving group on an α-methyl substituent, or an acetylene or ethylene function in place of the α-hydrogen atom, could be potential irreversible inhibitors of the corresponding α-amino acid decarboxylase.[20,21] Scheme 2[11] shows the postulated mechanism for the irreversible inhibition of ODC by α-functionalised methylornithine analogues. **Path a** represents the decarboxylation of α-methylornithine to α-methylputrescine. However, when **X** is a leaving group (**path b**), the normal electron flow towards the PLP ring can be redirected to catalyse the elimination of X^- and generate an electrophilic imine which eventually could alkylate a residue (**Nu**) from the enzyme.

The potency of these inhibitors depends on the nature of the functional groups. An increase in the bulkiness and/or a decrease in the nucleofugality of the leaving group on the α-methyl substituent results in a lowering of the inhibitory activity. For example, α-cyanomethylornithine (53) displays only weak inhibitory activity.[20] The fluorine atom, with its small size, high energy C-F bond, and good leaving group ability, makes the α-fluoromethylornithines excellent inhibitors of ODC. α-Chloromethylornithine (54) appears to be much less potent than α-fluoromethylornithine (55) and there is some doubt as to whether it is even an enzyme-activated inhibitor of ODC. The inhibitory activity of α-ethynylornithine (56) is similar to that of the α-fluoromethylornithines.[21]



(53) R= CH_2CN
(54) R= CH_2Cl
(55) R= CH_2F
(56) R= $C{\equiv}CH$

Ornithine analogues with changes at the β-carbon have also been looked at as potential ODC inhibitors. *(E)*-Dehydroornithine (57), *(E)*-α-methyldehydroornithine (58) and β-chloroornithine (59) showed no inhibition of mammalian ODC.[11] *(E)*-α-Methyldehydroornithine did, however, show inhibitory activity of

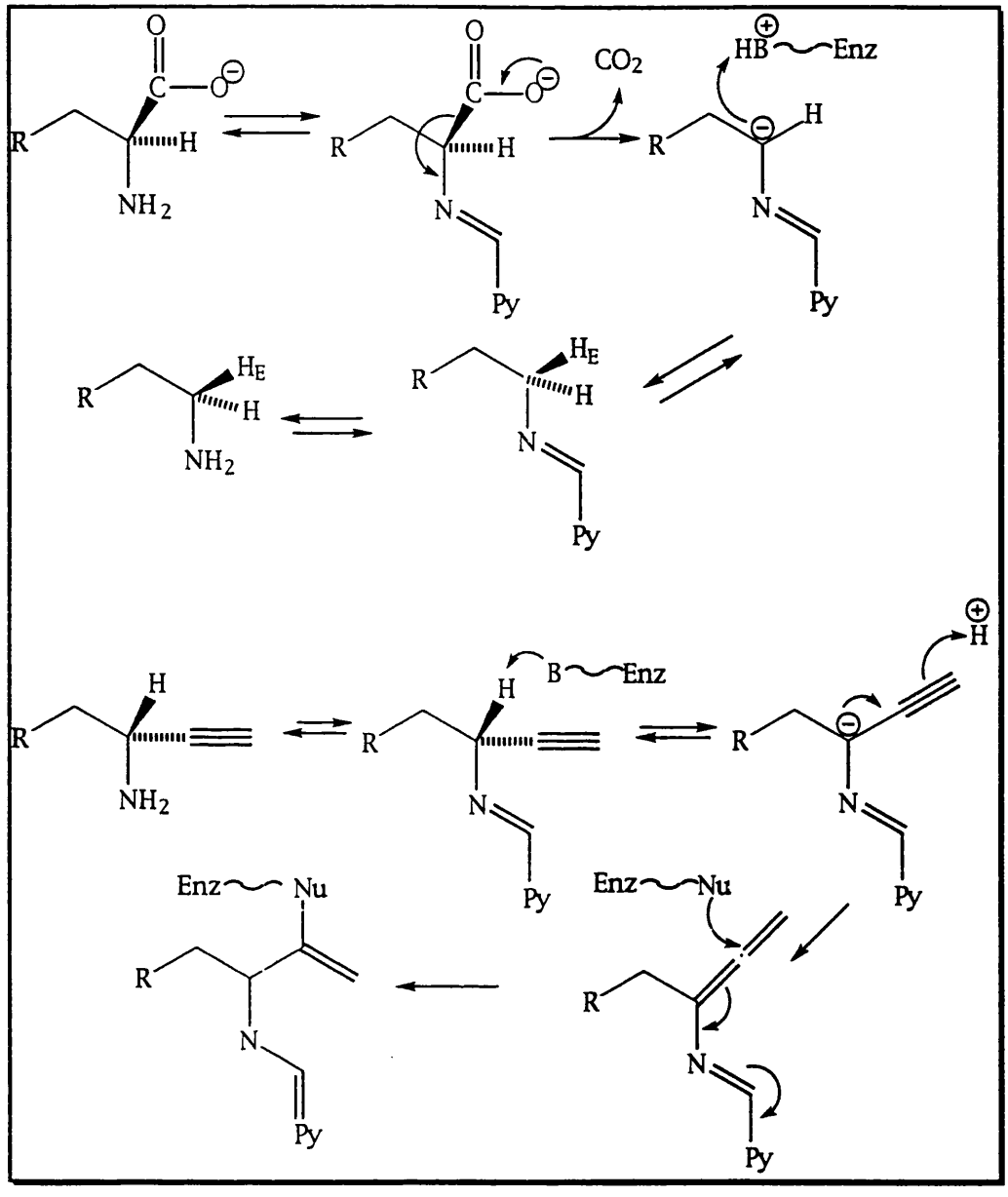


ODC in *E. coli*. The introduction of a *trans*-double bond in the side chain of mono- and di-fluoromethylornithines did not increase their inhibitory activity however.

Putrescine analogues with reactive groups in the α-position were also studied as potential ODC inhibitors. Introduction of a *trans*-double bond in the chain improved the inhibitory activity of these putrescine analogues.

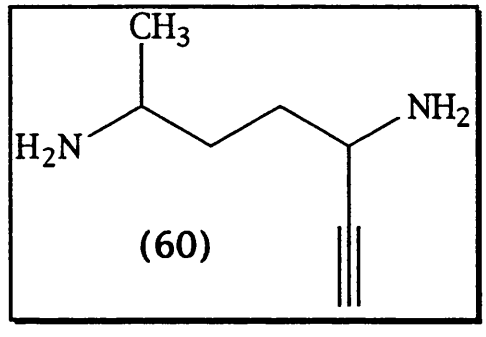
Scheme 3[11] shows the postulated mechanism for the irreversible inhibition of ODC by α-ethynylputrescine (R=$(CH_2)_2NH_2$). The same residue responsible for the protonation (with H_E) of the carbanionic intermediate in the normal decarboxylation reaction (top line) can abstract the α-hydrogen atom and generate a carbanion that can rearrange to an electrophilic allenyl imine. This allenyl imine can then alkylate another residue on the enzyme and irreversibly inactivate ODC.

Scheme 3



Although both the *(R)*- and *(S)*-enantiomers of α-ethynylputrescine were tested for ODC inhibition, it was found that only *(R)*-α-ethynylputrescine irreversibly inhibited mammalian ODC and that the *(S)*-isomer was only a weak competitive inhibitor.[22] *In vivo*, α-ethynylputrescine and α-fluoromethylputrescine inhibit ODC very efficiently, and also inhibit other enzymes which can have profound effects on other biological mechanisms. Monoamine oxidase is inhibited by these

putrescine analogues, but it is known that putrescine analogues α-methyl substituents are poor inhibitors of monoamine oxidase. The ODC active site can accommodate substituents on the δ-carbon atom, so α-ethynyl-δ-methylputrescine (60) was tested in the hope that ODC inhibitory activity would be retained and inhibition



(60)

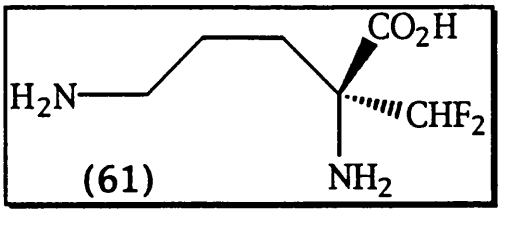
of monoamine oxidase would be prevented.[23] This was indeed the case, but the new problem of having two asymmetric centres, and therefore four possible isomers arose. In an attempt to avoid this stereochemical complication, the δ-*gem*-dimethyl analogue was tested but found to be only weakly active, indicating that the stereochemistry of the methyl substituent was important for activity. Out of the four isomers only the *(2R, 5R)*-isomer was found to have reasonable inhibitory activity.[24] This does not seem strange as enzymes are usually stereospecific in their mode of action and enzyme-activated inhibitors have to be substrates of the target enzyme. Interestingly, by contrast with mammalian ODC, bacterial ODC is inactivated by both enantiomers of α-ethynylputrescine. With *(E)*-monofluoromethyldehydroornithine both enantiomers have a similar inhibitory activity on mammalian ODC. An explanation for this apparent lack of stereospecificity could be that the enzyme-activated inhibitors are poor substrates for their target enzymes. It has been suggested that there is an

alternative mechanism to the previous ones put forward for the enzyme-activated inhibition of PLP-dependent enzymes such as ODC.[11] This suggestion states that these inhibitors might be activated through chemical transformations which do not correspond to the known reaction pathways catalysed by the target enzyme.

To conclude, ODC is the enzyme in the polyamine biosynthetic pathway which has attracted most attention as a target for inhibition. Of the various approaches that have been explored to inhibit ODC, the design of enzyme-activated irreversible inhibitors has been particularly successful. Although several postulated mechanisms exist, the exact mechanism of action of these inhibitors remains to be determined. The chemical structure of an enyme inhibitor adduct after inactivation of the enzyme has also yet to be established.

1.5.1 The Enzyme-Activated Irreversible Inhibitor DFMO

Several powerfui enzyme-activated irreversible inhibitors of ODC have now been prepared, notably difluoromethylornithine (DFMO) (61). This was made in the early 1970s by Merrell Dow



(61)

Pharmacueticals in the U.S.A.[8] The majority of the important studies on the topic of inhibition of ODC over the past decade have been carried out using DFMO. In 1985 Venkat Rajam and Arthur Galston at Yale University showed that DFMO effectively inhibited the growth of several fungi on artificial media.[5] But inhibiting

fungal growth in culture and on plants are two very different situations. After all, will the inhibitor stay on the surface long enough to be effective; will it enter the plants; and if so, will it be transported to other parts of the plant? Later on in 1985 Rajam *et al.* [5] showed that DFMO very effectively controlled rust infection of pinto beans. The ODC inhibitor was shown to be effective applied before or after inoculation with the fungus and furthermore, some systemic action was observed. Interestingly, DFMO and other ODC inhibitors have been tested as possible anti-tumour drugs. In the late 1970s Albert Sjoerdsma and colleagues at Merrell Dow began clinical studies of DFMO in patients with cancer.[5] DFMO is also a powerful inhibitor of certain protozoal parasites of animals (*e.g. Trypanosoma brucei brucei*, the cause of trypanosomiasis in cattle and *Plasmodium falciparum*, the malaria parasite) and the prospects for chemotherapy of such infections using ODC inhibitors seem very interesting.

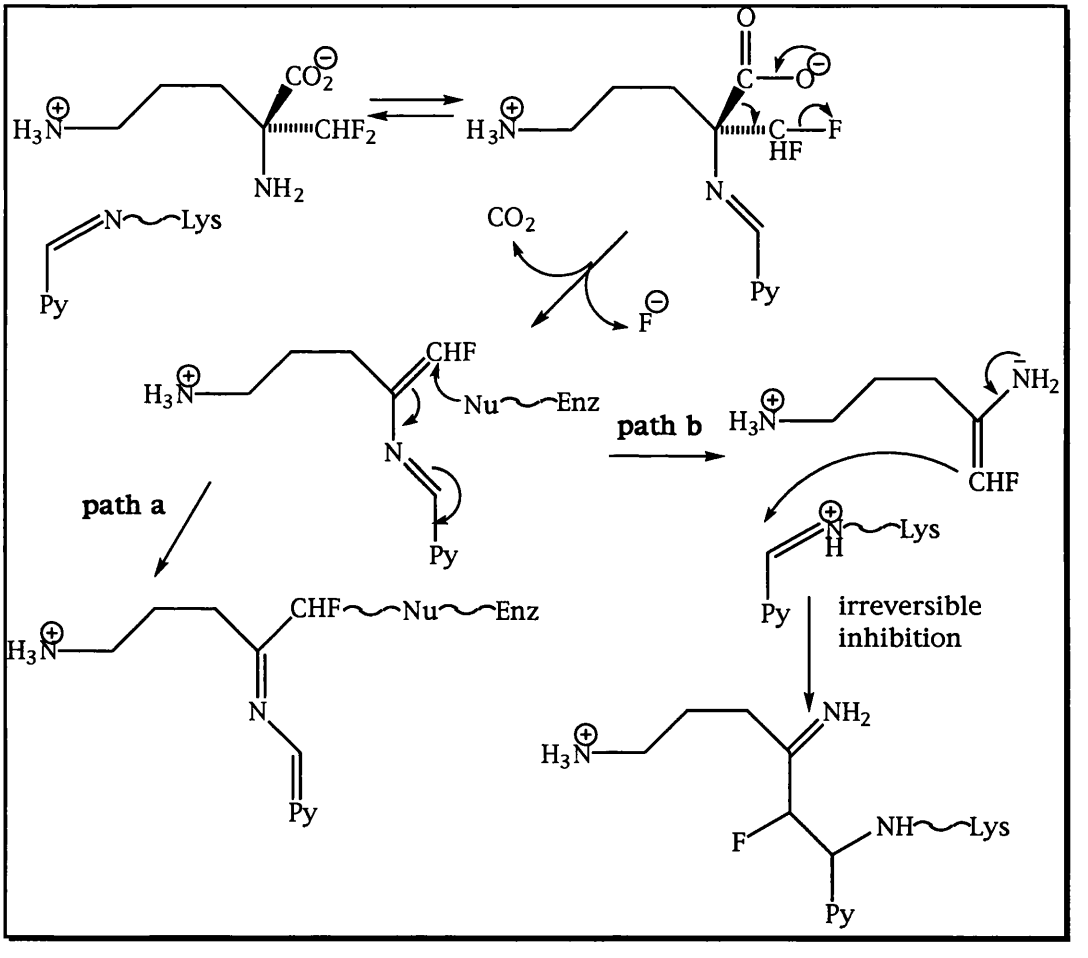
Experiments with DFMO labelled with ^{14}C in the 1-position or 5-position demonstrated that the inhibitor is decarboxylated by ODC with a partition ratio of decarboxylation to inactivation of about 2.5, and that one mole of inhibitor binds to one subunit of ODC through covalent linkage through the lysine residue at position 298.[25]

Both enantiomers of DFMO are found to inhibit mammalian ODC, although (-)-DFMO is markedly more potent than the corresponding (+)-enantiomer.

In 1982 Likos *et al.*[26] completed some work that suggested an alternative mechanism of ODC inhibition should be considered for inhibitors such as DFMO (and other putrescine and ornithine analogues).[30] This alternative mechanism is shown in Scheme 4,[11]

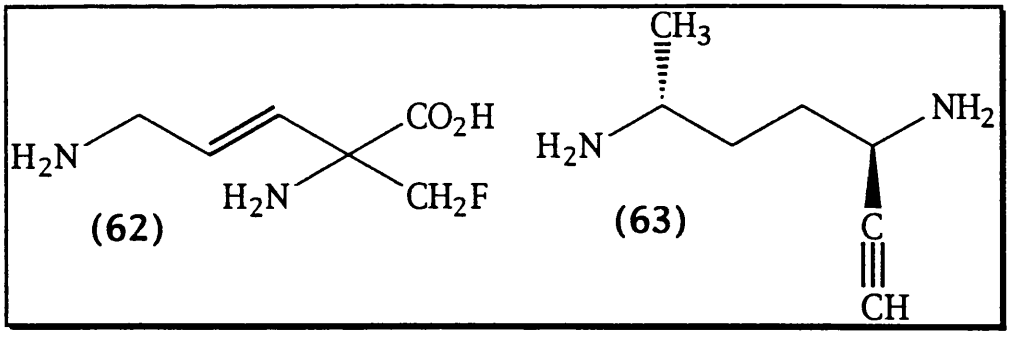
path b, with the original mechanism represented in **path a.** If this mechanism was to be operative in the inactivation of ODC by DFMO, then the enzyme-inhibitor adduct would be expected to be stable to dialysis conditions while **path a** would result in an enzyme-inhibitor adduct that should readily hydrolyse during dialysis.

Scheme 4



1.5.2 Recent Potential Enzyme-Activated Irreversible Inhibitors

Recently, two other enzyme-activated irreversible inhibitors of ODC which also show potential for *in vivo* applications have appeared. The first one is *(E)*-dehydrofluoromethylornithine (62) and it is used *in vivo* as its methyl ester in order to allow it to penetrate the cell membrane more efficiently.[28] The second one is



the *(2R,5R)*-isomer of δ-methyl-α-ethynylputrescine (63) and is the first analogue of putrescine to be of any real interest as an ODC inhibitor.

The rationale discussed above, in Sections 1.4 and 1.5, was used in this project for the design of potential fungicides. The next chapter discusses the compounds identified as targets and the synthetic chemistry utilised in their synthesis.

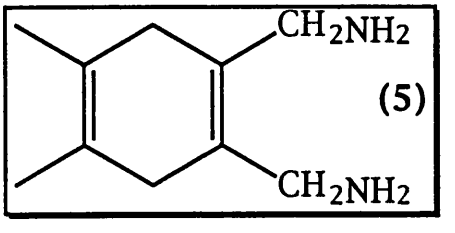
CHAPTER 2

DISCUSSION

The direction taken and choice of compounds synthesised in this project were a result of preliminary synthesis by Martin,[29] and antifungal studies by Dr. Dale Walters. The test data generated from this earlier work formed the basis from which this project grew. It was found that the 1,4-diamino-2-butene moiety was of particular interest and synthesis of compounds with this essential skeleton was then embarked upon.

N-Alkylated compounds with the 1,4-diamino-2-butene moiety were also found to possess fungicidal properties and this was another structure area in which analogues were synthesised.

Cyclic compounds with a variety of features was the third structural area in which compounds were made. Although compounds synthesised in this area varied a great deal, all had at least two nitrogen atoms four carbon atoms apart, and all were cyclic. The original compound which created interest in this area was 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene (BAD) (5). BAD was first made by Martin[29] and was extremely



(5)

successful in controlling the growth of mildew. The scope for using chemistry involving the formation of rings during syntheses of these compounds was of particular interest.

The common feature linking most of the compounds made in this project is that they are 1,4-diamines. It would then be beneficial to have a brief review of amines and the general methods used in their preparation.[30, 31, 32]

The fundamental properties associated with amines are their basicity and corresponding nucleophilic character. Their basicity depends on the number of alkyl groups bonded to the nitrogen atom, with tertiary amines being less basic than primary and secondary amines. Amines are polar compounds and with the exception of tertiary amines they can form intermolecular hydrogen bonds. All amines can form hydrogen bonds with water and as a result the smaller amines are quite soluble in water, with borderline solubility being reached with amines containing about six carbon atoms.

Salts of amines are easily prepared by addition of acid and are typical ionic compounds. They are non-volatile solids, and upon heating generally decompose before the high temperature for melting is reached. The halides, nitrates and sulfates are soluble in water but are insoluble in non-polar solvents.

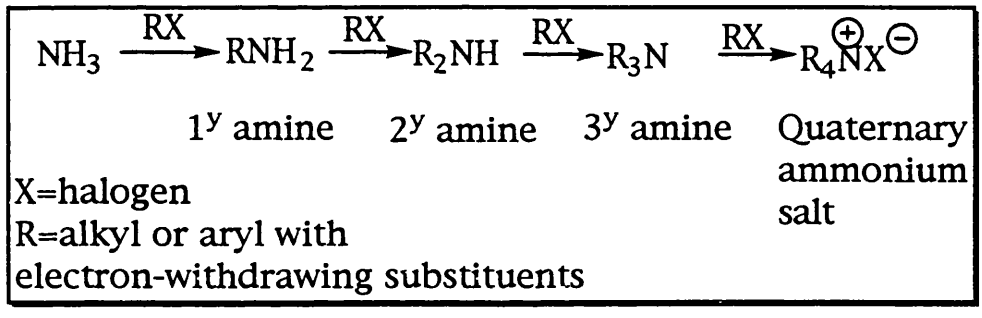
There are various techniques available for purification of amines. Generally amines can be separated from non-basic compounds by their solubility in acid. After separation the amine can be easily regenerated by making the aqueous solution alkaline and extracting the amine into an organic solvent.

2.1 Common Methods of Preparation of Amines

2.1.1 Amines from Alkyl Halides

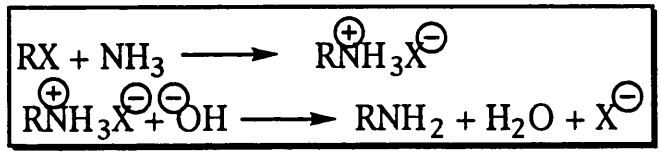
Reactions of Ammonia and Amines with Alkyl Halides. Since an amine is a compound in which one or more of the hydrogen atoms of an alkyl or aryl group have been replaced by an organic group it makes sense to look at this method of preparation first (Scheme 5). Ammonia reacts as a nucleophile with alkyl halides to form amines.

Scheme 5



It is difficult to prepare pure primary amines using this method. Displacement of the halogen atom by ammonia yields the amine salt, from which the free amine can be liberated by treatment with hydoxide ion or by use of excess ammonia to produce the free amine and ammonium halide (Equation 1). This nucleophilic substitution reaction suffers from the competition of

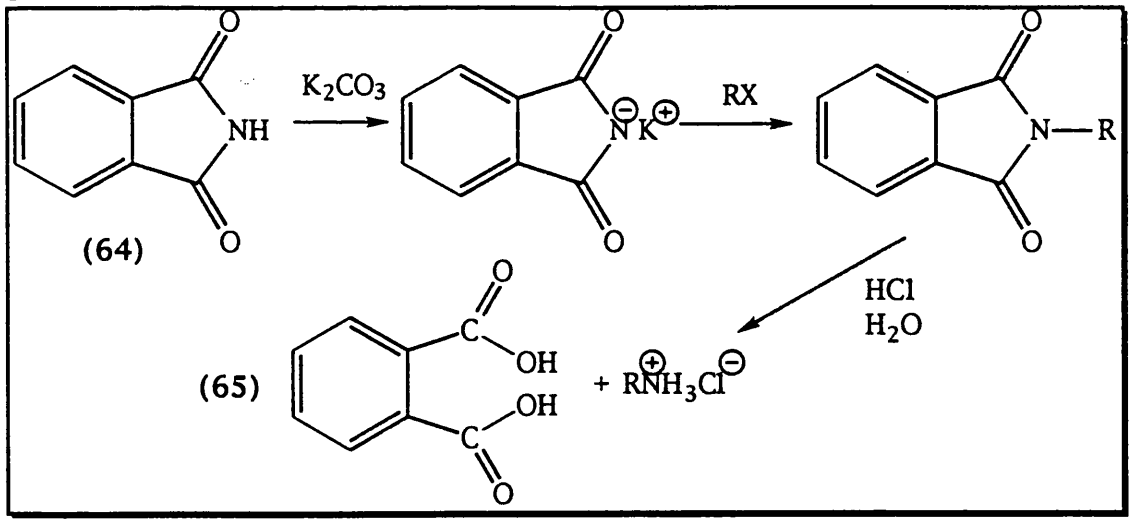
Equation 1



the elimination reaction in which ammonia also attacks hydrogen to form the alkene. As a result ammoniolysis is of practically no use with tertiary halides where the elimination reaction predominates.

The Gabriel Phthalimide Synthesis. Nucleophilic substitution reactions of ammonia with alkyl halides result in mixtures of primary, secondary and tertiary amines, unless a large excess of ammonia is used. Pure primary amines can be prepared more conveniently if the nitrogen atom is protected so that alkylation can take place only once. Such a protected nitrogen atom is present in phthalimide (64), which is acidic enough (pKa 7.4) to be deprotonated easily to give a nitrogen anion. The acidity of phthalimide can be rationalised by the possibilities for delocalisation of the negative charge in its conjugate base. The

Scheme 6

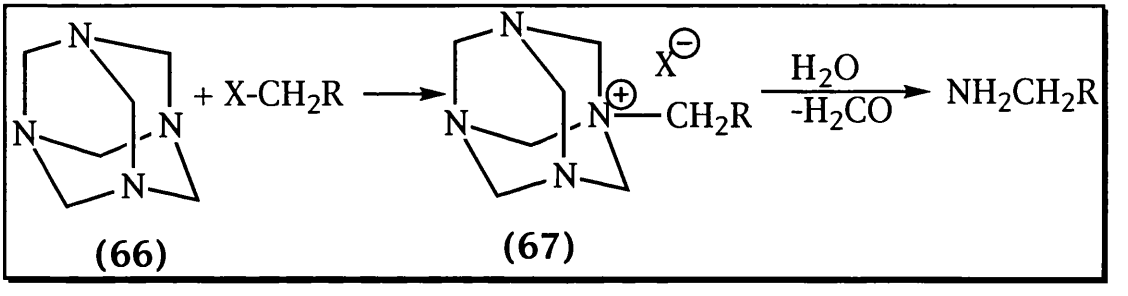


phthalimide anion is a good nucleophile and can effect an S_N2 reaction with an alkyl halide, then cleavage of the phthalimide is easily accomplished by use of acid to produce the required

primary amine and phthalic acid (65). This sequence of reactions is called the Gabriel synthesis (Scheme 6).

The Delépine Reaction. Another method of preparing primary amines from alkyl halides is via the Delépine reaction (Scheme 7). Alkyl halides react with hexamethylenetetramine (66)[33] to give a hexaminium salt (67). The introduction of the amino group is via these quaternary salts. Hexamethylenetetramine, readily obtainable from ammonia and

Scheme 7



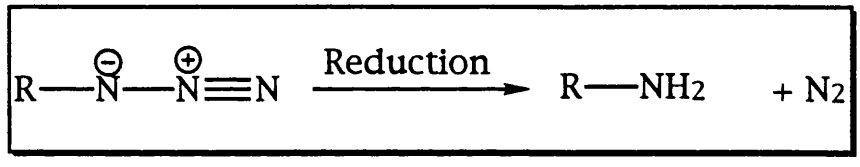
formaldehyde, has a symmetrical adamantane-like structure, which is quite stable although dihetero-substituted methylene groups are usually highly reactive. On quarternisation of one of the nitrogen atoms, the hexamethylenetetramine molecule loses its symmetry and forms a hexaminium salt (67) which can be isolated when the preparation is carried out in polar aprotic solvents. In strongly acidic ethanol, primary amines are formed with the formaldehyde being removed as volatile formaldehyde diethylacetal. The advantages of this process is that the reagent is cheap, the reaction conditions are simple, and there are short reaction times.

2.1.2 Reductions that Produce Amines

Reduction Of Azides. Various functional groups can be reduced in order to form amines. Reduction of azides gives

primary amines. This has the disadvantage of using highly toxic substances. The azide ion, N_3^-, is a good nucleophile and is used to create carbon-nitrogen bonds in S_N2 reactions. The azide is then reduced to the amine with retention of configuration at the carbon next to the nitrogen with the other two nitrogen atoms of the azide group being lost as nitrogen gas (Scheme 8).

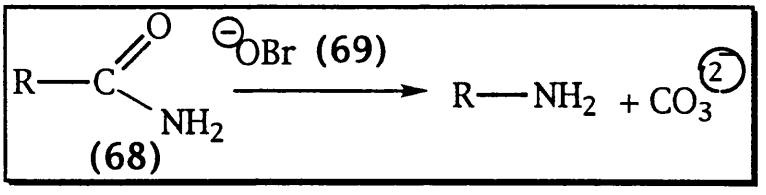
Scheme 8



Reduction of Nitriles. Synthesis via the formation and reduction of nitriles has the useful feature of increasing the length of the carbon chain, producing a primary amine that has one more carbon atom than the alkyl halide from which the nitrile was made. Nitriles can be reduced to amines by various metal halides or by hydrogen and a catalyst.

Reduction of Amides. Amines can be produced by the reduction of amides (68). The reductive reagents mentioned above can also be used to reduce amides to give amines containing the same number of carbon atoms. The Hofmann Degradation,[34] although not used in this project, should nevertheless be mentioned as a possible route to amines from amides (Scheme 9). It has the special feature of yielding a product containing one carbon atom less than the starting material. The conversion is carried out by the action of alkaline hypobromite (69) and involves a molecular rearrangement. During the reaction a migration of a group from the carbonyl carbon to the adjacent nitrogen atom occurs to form the amine.

Scheme 9

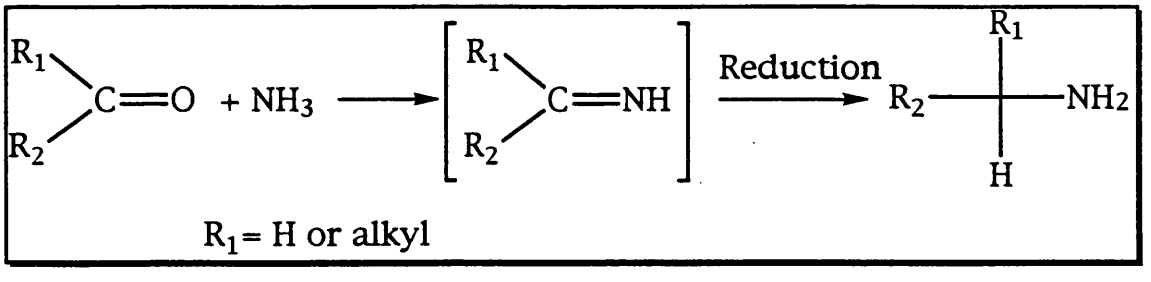


2.1.3 Reductive Amination and Reduction of Nitro Compounds

The following methods are popular routes to amines, however neither was used in this project. They are both general methods that are widely used and are discussed briefly in order to complete this section.

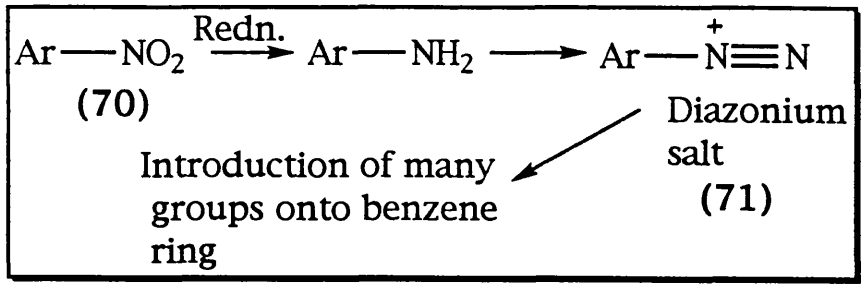
Reductive Amination. Many aldehydes and ketones are converted into amines by reductive amination. Ammonia or an amine can be used as the nitrogen source and reduction can be accomplished catalytically or by use of sodium cyanohydroborate. The reaction (Scheme 10) involves the reduction of an intermediate, an imine or an oxime, neither of which need be isolated in order to prepare the amine.

Scheme 10



Reduction of Nitro Compounds. This is one of the most useful methods of preparing amines. Alkyl nitro compounds and, more importantly, aromatic nitro compounds (70) can be reduced by catalytic hydrogenation or by chemical reduction using a metal

Scheme 11

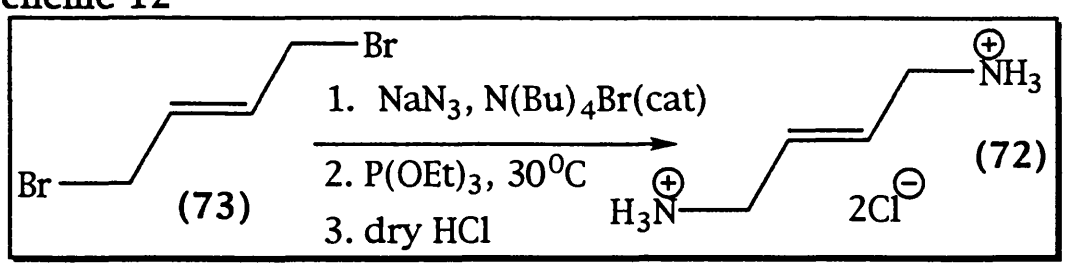


and an acid (Scheme 11). The primary aromatic amines that are readily accessible via this route can be converted into aromatic diazonium salts (71), which are among the most versatile classes of organic compounds known.

2.2 *(E)*-1,4-Diaminobut-2-ene Dihydrochloride (E-BED) and Analogues

Several procedures for the synthesis of E-BED (72), a simple unsaturated diamine that is similar in size to putrescine, were carried out by Martin.[29] All had a number of low yielding steps and some, for example shown in Scheme 12, involved the preparation and use of highly toxic substances. The route to E-BED

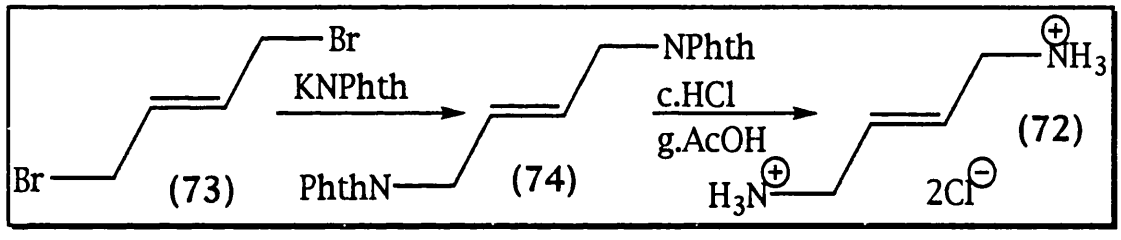
Scheme 12



shown in Scheme 12 could only be performed on a small scale as large scale reactions produced unsatisfactory yields (*ca.* 10-12%). This may be due to the heterogeneous nature of the first stage of the reaction. Methods which avoided the use of unstable organic

azides were investigated. The Gabriel synthesis[35] uses potassium phthalimide to convert alkyl halides into primary amines and allowed E-BED to be produced in high yield on a reasonable scale (Scheme 13). At the start of this project we wanted to improve the yields of the steps involved in converting the dibromo compounds into E-BED and to avoid the use of toxic reagents. Thus, reaction of two equivalents of potassium phthalimide with *(E)*-1,4-dibromobut-2-ene (73) in DMF gave yields of 80-90%. The cleavage step, using glacial acetic acid and concentrated HCl, usually took two days due to the insolubility of the diphthalimide

Scheme 13



compound (74) but when considering the yield (*c.a.* 62 %) and the quality of product this can be classified as a successful reaction. The Gabriel synthesis was successful at producing E-BED as a salt (72), but in order to prepare other compounds the free base was required. Bottini *et al*[36] used hexamethylenetetramine (66) in the Delépine reaction to prepare many aliphatic amines. This procedure was adapted to produce E-BED free base. The Delépine reaction was a good alternative to handling insoluble diphthalimides.

The phosphate (75), fumarate (76), propionate (77) and benzoate (78) salts of E-BED were prepared in order to obtain

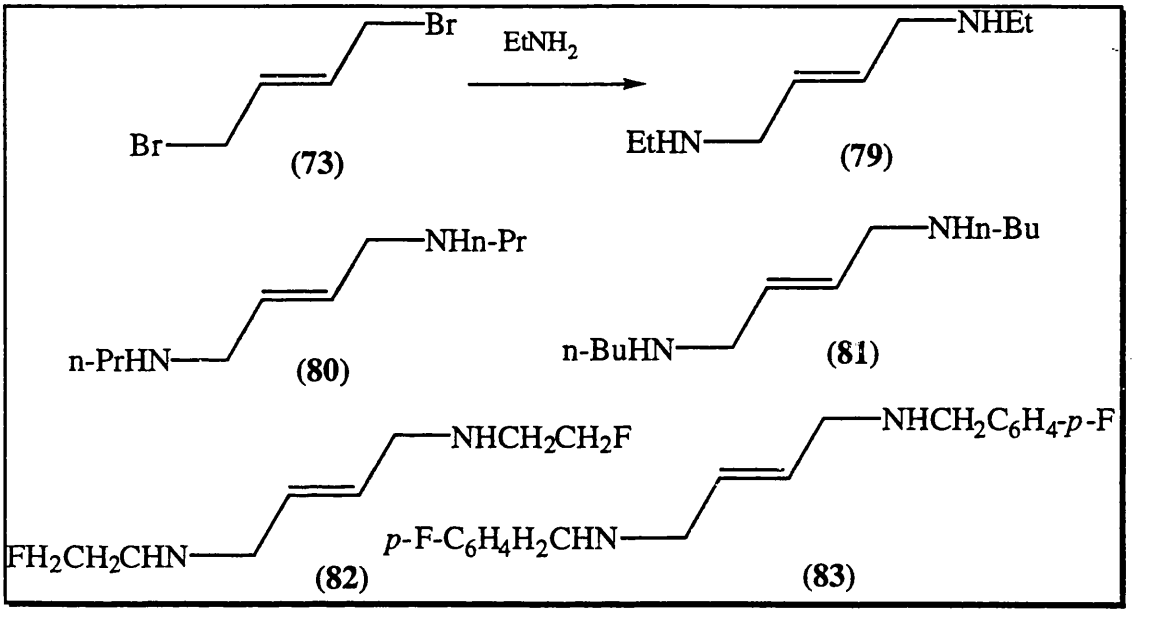
more information from test data and to satisfy patent requirements.[37]

2.2.1 *N*-Alkylated E-BED Analogues

As the next stage of this work *N*-alkylated analogues[39, 41] of E-BED were prepared in an effort to modify the antifungal activity. Analogues were prepared from ethylamine (79), n-propylamine (80) and n-butylamine (81) all in good yield (Scheme 14). They were isolated as their benzoate salts in an effort to produce solid material to be sent for testing. These compounds were identified using the usual techniques, including proton NMR spectroscopy. For example, the ^{1}H NMR spectrum of *(E)-N,N'*-diethyl-1,4-diaminobut-2-ene (79) dihydrobenzoate showed a triplet at δ 1.06 and a quartet at δ 2.83 indicating the ethyl groups, with the vinylic protons at δ 5.79. Another multiplet with a chemical shift of 3.48 represented the methylene protons α to the double bond. The usual multiplet in the aromatic region indicated the protons on the benzene rings. Experiments with the fluorinated alkylamines 2-fluoroethylamine and *p*-fluorobenzylamine failed to produce the required products (82) and (83). Fluorinated compounds are of interest when studying biological systems as the fluorine atom is a similar size to a hydrogen atom and because of this it is more likely to be able to fit into active sites of enzymes and may cause interesting chemical effects because of its electronegativity. 2-Fluoroethylamine could only be obtained commercially as the dihydrochloride salt and it was therefore necessary to generate its free base initially. Because of the low boiling point of 2-fluoroethylamine (lit.,[40] 35-42 °C) an attempt was made to liberate the free base *in situ* by addition of a base. ^{1}H and ^{13}C NMR spectra showed a mixture of products

which could not be easily identified. Although 4-fluorobenzylamine was available as the free base the reaction[41] with *(E)*-1,4-dibromobut-2-ene still produced a mixture by ^1H NMR spectroscopy and purification by distillation was unsuccessful.

Scheme 14

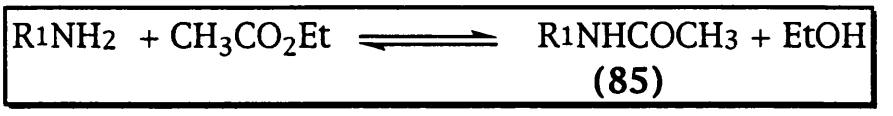


2.2.2 *N*-Acetyl-*(E)*-1,4-diaminobut-2-ene Hydrochloride

N-Acetylputrescine[42] can be made by a chemical method, where putrescine is treated with acetic anhydride and acid under carefully controlled conditions, or by the use of the enzyme porcine pancreatic lipase (PPL). The enzymatic route appeared to give better yields[42] and an attempt to make *N*-acetyl-E-BED hydrochloride (84) via this route was made. PPL is stable in non-polar organic solvents and has been reported to have the ability to accommodate a diverse range of substrate structures. Acylation of amines is catalysed by PPL using ethyl acetate. Ethyl acetate acts as both acylating agent and solvent. In this reaction the amine

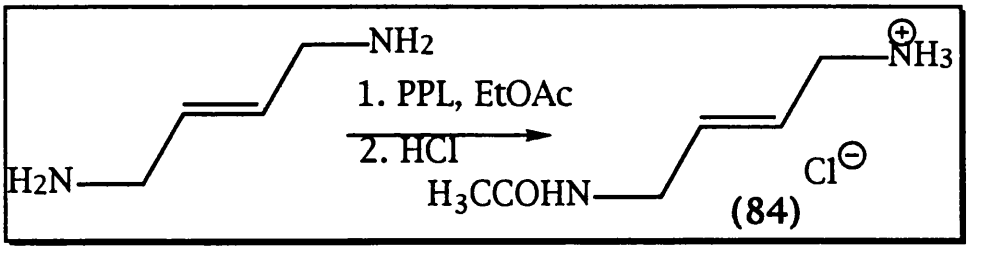
reacts with the ester (ethyl acetate) to form an amide (85) and releases an alcohol (ethanol) (Equation 2).

Equation 2



The reaction is reversible, but since ethyl acetate is present in such a large excess the reverse reaction should be suppressed. Only very low yields were obtained (12%) for the synthesis of *N*-acetyl-E-BED (Scheme 15) due to the difficulty in minimising the amounts of diacylated product and in separating this and starting material from the desired product. The free base of E-BED, *i.e.* starting material, can easily be distinguished from the acylated products as there is no methyl proton signals in the ^{1}H NMR spectrum. ^{13}C NMR spectroscopy clearly shows the different carbon environments, allowing the differentiation between the monoacylated and diacylated compounds.

Scheme 15

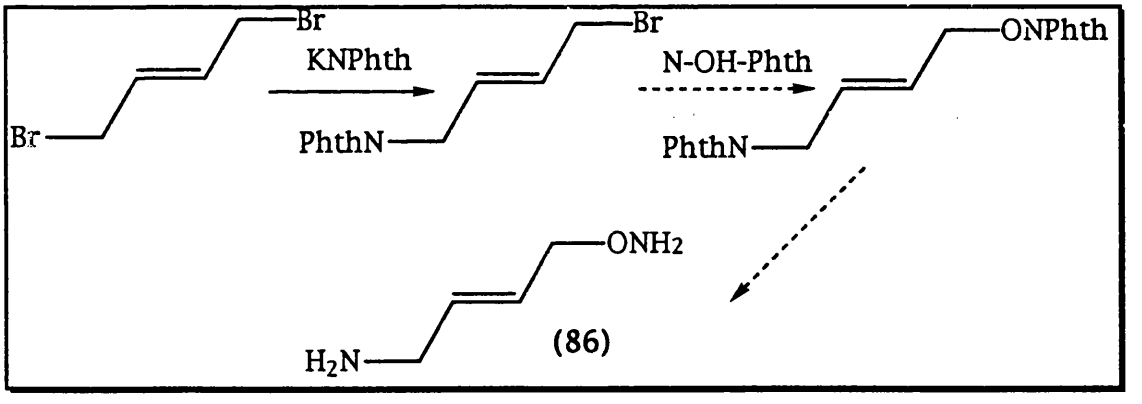


2.2.3 *(E)*-1-Amino-oxy-4-aminobut-2-ene (Oxy E-BED)

The synthesis and testing of cadaverine analogues was an area that was identified as being of interest. With this in mind, an attempt to synthesis *(E)*-1-amino-oxy-4-aminobut-2-ene (86) was made. Obviously the two key structural moieties are the *trans*-

double bond, which by this time had become the dominant characteristic of most analogues, and the insertion of a heteroatom, *i.e.* oxygen in place of the fifth carbon atom of cadaverine. The phthalimide group was put on one end of the dibromo compound with relative ease, the solvent used being acetone.[43] The addition of *N*-hydroxyphthalimide (N-OH-Phth) to this monobromo compound was attempted many times but insolubility of the *N*-hydroxyphthalimide was a problem which was never satisfactorily solved (Scheme 16).[43] Attempts to dissolve *N*-hydroxyphthalimide in triethylamine, chloroform and acetonitrile were all unsuccessful, even after heating to reflux for several hours. Acetone appeared to be the most successful solvent although the reaction failed to give the desired product.

Scheme 16

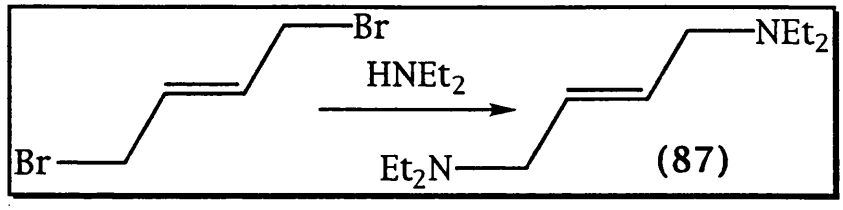


2.3 *(E)-NNN'N'*-Tetraethyl-1,4-diaminobut-2-ene (TED) and Analogues

The synthesis of *(E)-NNN'N'*-tetraethyl-1,4-diaminobut-2-ene (TED) (87) was achieved by dropping diethylamine into 1,4-dibromobut-2-ene to afford the free base TED (Scheme 17).[41] The ^{1}H NMR spectrum showed a triplet and a quartet indicating

the four ethyl groups, with a doublet for the four protons α to the double bond and a multiplet representing the two vinylic protons.

Scheme 17

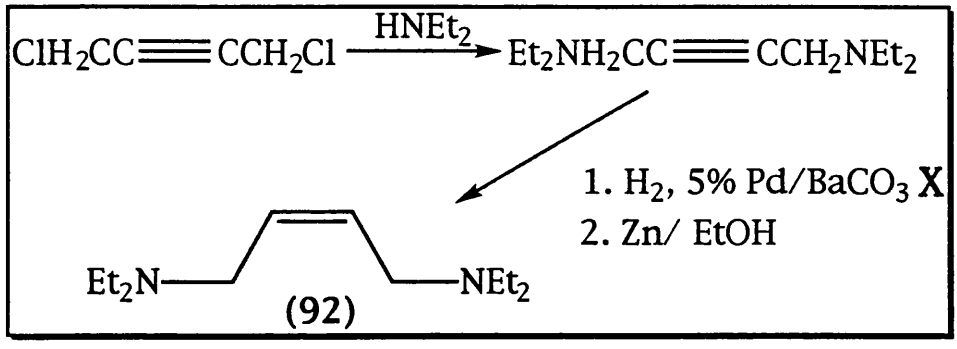


Again, four salts of TED namely benzoate (88), phosphate (89), fumarate (90) and propionate (91) were synthesised for evaluation as antifungal agents.

2.3.1 *Z-N,N,N',N'*-Tetraethyl-1,4-diaminobut-2-ene (Z-TED)

Although *Z*-1,4-diaminobut-2-ene (Z-BED) dihydrochloride, which was prepared by Martin, gave poor antifungal test results, in the light of the positive results achieved with TED, it was felt that *Z-N,N,N',N'*-tetraethyl-1,4-diaminobut-2-ene (Z-TED) (92) should be synthesised and data collected. Initial attempts at synthesising Z-TED were based on work done by Biel and DiPierro.[44]

Scheme 18



Biel and DiPierro carried out research in the area of formation and reactions of acetylenic diamines.[45] Readily available

1,4-dichlorobut-2-yne and diethylamine gave the corresponding *N,N'*-dialkylated but-2-yne in good yield (96%) (Scheme 18).

Triple bonds can be reduced either by catalytic hydrogenation or by a variety of other reagents.[46] Some of these other reagents include zinc and acids, sodium in ethanol, lithium and aliphatic amines, chromous ion and diisobutylaluminium hydride (DIBAL). Catalytic hydrogenation of triple bonds and the reaction with DIBAL usually give the *cis*-alkene. Whether they are heterogeneous or homogeneous reactions most of these have been shown to proceed with *syn*-stereochemistry, with the hydrogens entering from the less hindered side of the molecule. The stereochemistry of heterogeneous catalytic hydrogenation is very complicated and is not thoroughly understood. Most of the other methods of triple bond reduction lead to the more thermodynamically stable *trans*-alkene.

Various palladium catalysts are available and in order to stop the reduction at the alkene these catalysts are partially poisoned. In this case a 5% palladium-on-barium carbonate catalyst was used with two drops of synthetic quinoline.[47] The failure of the reaction to take place could be attributed to either a poor quality of catalyst or that 4 bar of hydrogen was insufficient. An attempt using greater than 4 bar was made however only starting material was recovered.

A second attempt at reduction used activated zinc. Aerssens and co-workers[47] demonstrated that regio- as well as stereo-specific *cis*-reductions of a wide variety of acetylenic derivatives could be carried out in absolute ethanol using zinc powder activated with 1,2-dibromoethane or with zinc powder treated successively with dibromoethane and lithium bromocuprate. The

first reagent is less powerful and more selective than the second one. The advantages of this method over the reduction with hydrogen and poisoned palladium catalysts are the short reaction times and the experimental simplicity. Analar grade zinc powder activated by treatment only with 1,2-dibromoethane was not sufficient, therefore the addition of a homogeneous solution of copper (I) bromide and anhydrous lithium bromide (lithium bromocuprate) in THF to the suspension of zinc and dibromoethane was done in order to give highly reactive zinc. The resulting one to three ratio of the *trans*- and *cis*- (92) products was thought to be due to the reflux conditions. It was felt that although the *cis*-product should be specifically formed the fact that the reaction is done under reflux conditions allows some of the thermodynamically stable *trans*-product (TED) to be formed. ^{13}C NMR spectra clearly showed the difference in the carbon environments of the vinylic carbons of the *trans*- and *cis*-products. The latter being further downfield than the former.

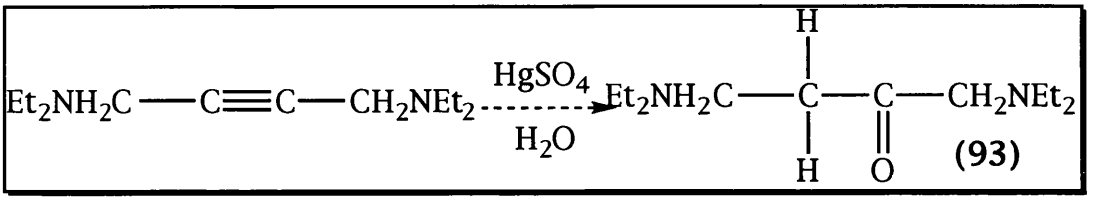
2.3.2 *N,N,N',N'*-Tetraethylketoputrescine [1,4-Bis(diethylamino)-butan-2-one]

Commercially available ketoputrescine was tested for antifungal activity by Dr. Dale Walters and was found to inhibit the growth of various fungi in culture and to control mildew on plants. These results prompted an investigation into ketoputrescine derivatives. As TED (87) had been so successful in testing, *N,N,N',N'*-tetraethylketoputrescine (93) was an appropriate target.

As mentioned in section 2.3.1 Biel and DiPierro[44, 45] had investigated the synthesis of acetylenes and their derivatives.

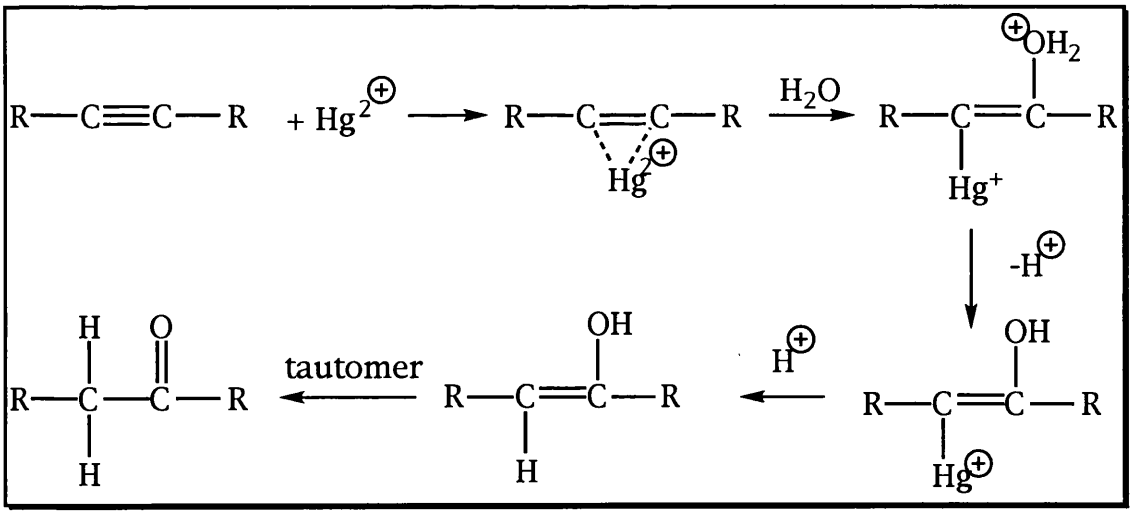
Accordingly 1,4-bis(diethylamino)but-2-yne was synthesised and attempts at hydration of the triple bond were made (Scheme 19).

Scheme 19



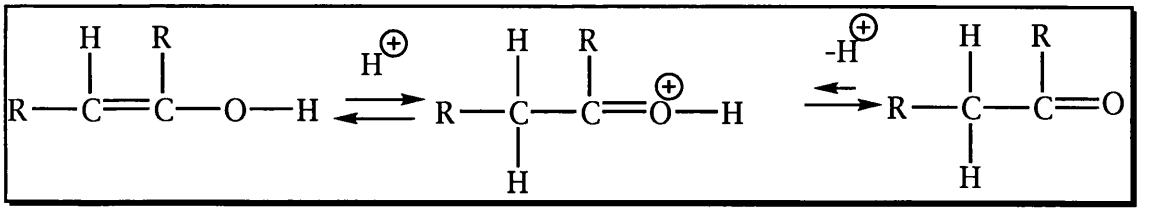
Hydrations of alkynes were reviewed by Khan and Martell.[48] Hydrations of acetylene diamines with dilute aqueous sulphuric acid in the presence of a mercuric sulphate catalyst are electrophilic addition reactions and proceed by way of carbocations.[49] The hydration or dihydro-oxo-biaddition proceeds via the mechanism shown in Scheme 20.

Scheme 20



The keto-enol tautomeric equilibrium shown in Scheme 21 generally favours the structure in which hydrogen is bonded to carbon rather than to a more electronegative atom.

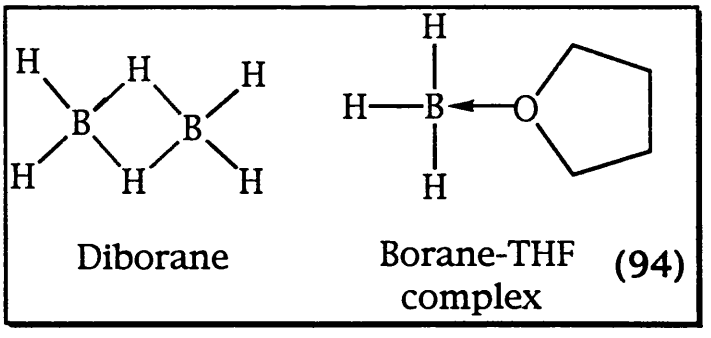
Scheme 21



The use of Nafion-H was identified as a convenient method for the hydration of alkynes.[50] One of the serious problems arising from mercury (II) salts as catalysts is the formation of a precipitate of an inactive sludge consisting of finely divided mercury (II)-organic compounds. Apart from the difficulties in the work-up stage, this side reaction also causes the loss of the catalyst and environmental problems.

Nafion-H is a superacidic perfluorinated resin sulfonic acid and once impregnated with mercury, can be used in the hydration of alkynes. The advantage of using a heterogeneous catalyst is that it can easily be separated from the reaction mixture and re-used. This method of hydration was also unsuccessful although in hindsight there are some obvious reasons for failure. Since only about 25% of the protons are substituted by mercury (II) ions around 75% of the Nafion-H would be able to complex with the nitrogens on the acetylenic diamine substrate. It was hoped that by washing the Nafion-H catalyst with conc. ammonia solution the desired bis-aminoketone would be released. This unsatisfactory method of hydration was, not surprisingly, unsuccessful.

The hydroboration of acetylenes involves the addition of a boron-hydrogen bond to the carbon-carbon triple bond.[51, 53, 54] This organoborane can then undergo rapid and essentially quantitative oxidation with alkaline hydrogen peroxide. In the attempted



Diborane Borane-THF complex (94)

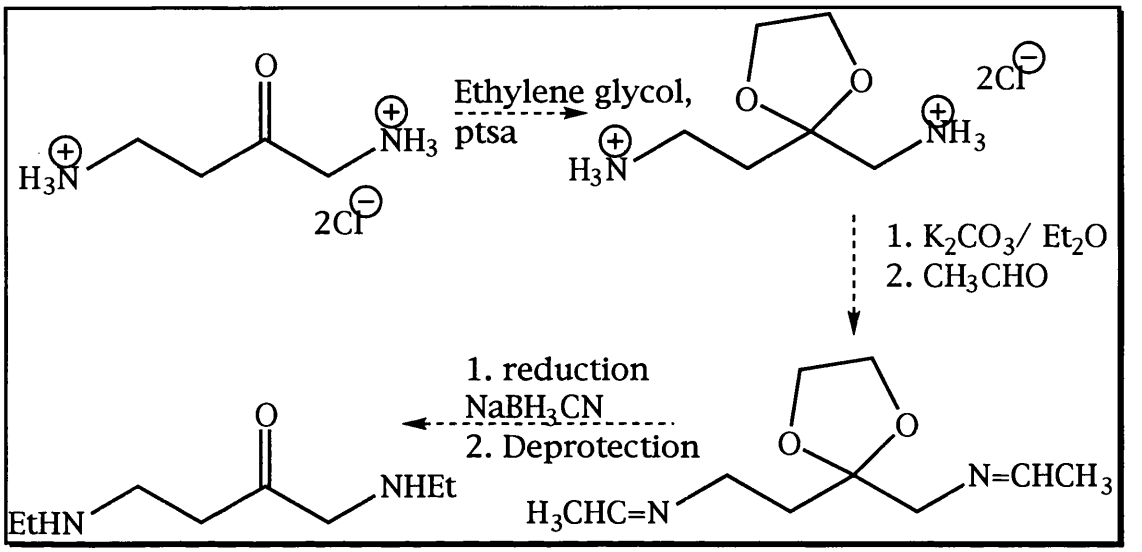
hydroboration of 1,4-bis(diethylamino)but-2-yne the reaction was done in THF, where borane exists as the monomer in the form of an acid-base complex with solvent (94).[52] Addition is highly regioselective and the process gives products corrosponding to anti-Markovnikov addition of water to the carbon-carbon triple bond. Rearrangement does not occur in hydroboration, evidently because carbocations are not intermediates and the method is therefore not complicated by other addition reactions. No carbonyl signals were detected when this reaction was carried out with 1,4-bis(diethylamino)but-2-yne and the hydroboration-oxidation was concluded to be unsuccessful.

Future Work. Having approached the synthesis of 1,4-bis(diethylamino)butan-2-one (93) by attempted hydration of 1,4-bis(diethylamino)but-2-yne and having little success it was felt that any future work in this area should approach the synthesis in a different way. Perhaps 1,4-dichlorobut-2-yne would be a more receptive substrate for hydration. If successful the ketone could then be protected and the chlorine displaced by diethylamine as a route to 1,4-bis(diethylamino)butan-2-one.

Since ketoputrescine is commercially available as a starting material a route was devised beginning with it.[55] Scheme 22 shows the synthetic pathway which could be a potential method

for forming 1,4-bis(ethylamino)butan-2-one. This route is not optimised but could form the basis of future work.

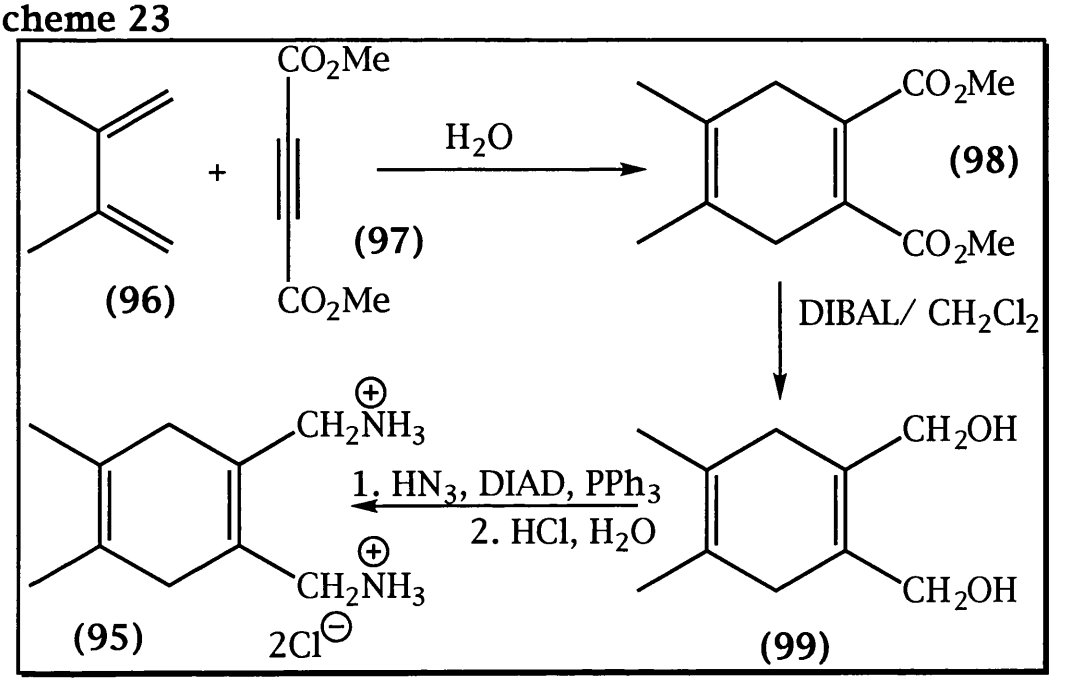
Scheme 22



2,4 1,2-Bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene Dihydrochloride (BAD) and BAD Derivatives and Analogues

Martin[29] carried out initial work which resulted in the synthesis of BAD (95). Indications from preliminary test data (see

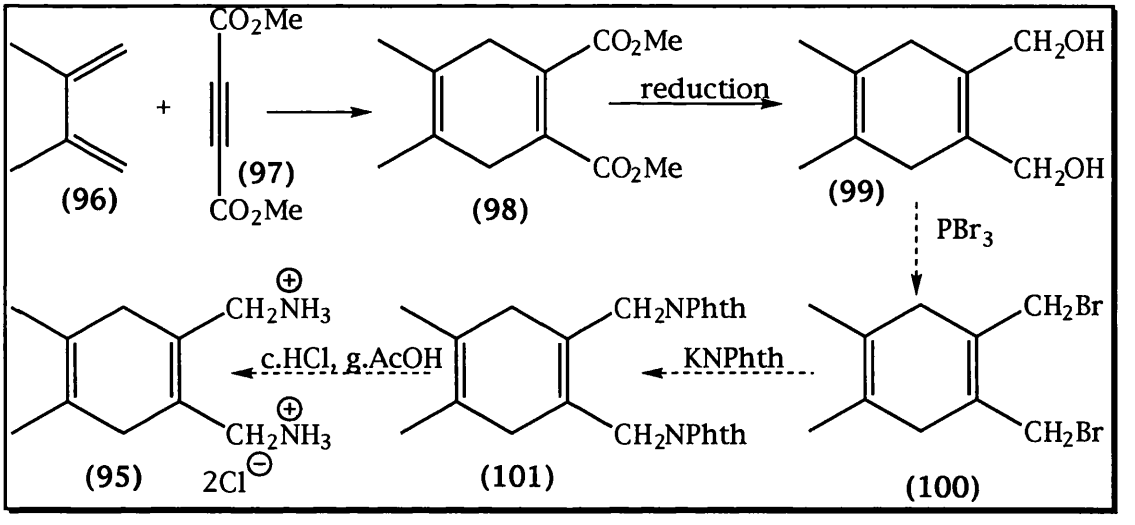
Scheme 23



Chapter 3) were that BAD showed very powerful antifungal activity. The route shown in Scheme 23, although successful, used hydrazoic acid for the conversion of a diol (99) into the diamine and a more convenient alternative for this final step was required.

A new synthetic strategy was devised in which the last step in Scheme 23 was replaced by conversion of the diol (99) into the dibromo compound (100) and introduction of the amine groups was via the Gabriel phthalimide route (Scheme 24).

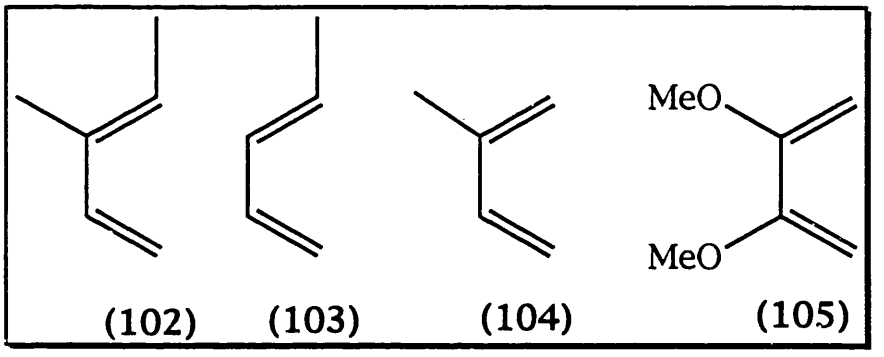
Scheme 24



A Diels Alder reaction with the readily available starting materials 2,3-dimethyl-1,3-butadiene (96) and dimethyl acetylenedicarboxylate (97) produced dimethyl 4,5-dimethylcyclohexa-2,4-diene-1,2-dicarboxylate (98). The Diels Alder reaction was a key first step in the synthesis of many of the cyclic compounds involved in our work and more detail of this reaction is discussed in Section 2.7.

Dimethyl 4,5-dimethylcyclohexa-2,4-diene-1,2-dicarboxylate (98) crystallised out of the aqueous solution upon cooling and was recrystallised from acetone giving on average 90% yields.

The positioning of the methyl groups around the ring and the affect this would have on the antifungal activity of the compounds was of interest. To this end 2,3-dimethyl-1,3-butadiene (96) was replaced as the diene by either 3-methylpenta-1,3-diene (102), penta-1,3-diene (103), isoprene (104) or 2,3-dimethoxybuta-1,3-diene (105) in the Diels Alder reaction with dimethyl acetylenedicarboxylate and the resulting cyclic diesters were isolated in varying yields (see Section 2.7). It was intended that these diesters could be taken through the route shown in Scheme 24.[56]

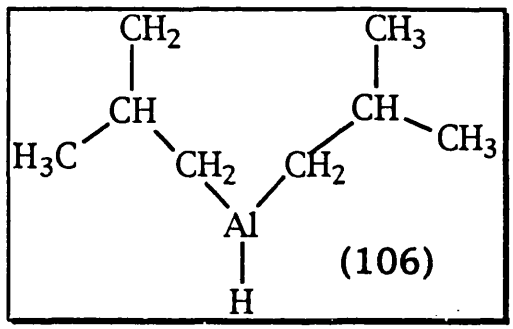


(102) (103) (104) (105)

The second step of the BAD synthesis involved the reduction of the diester (98) to the corresponding diol (99). This was very problematic and a great deal of work went into trying to improve this step. Three different reducing reagents were used and with each, different reaction conditions and work-up methods were applied in order to try to improve yields (Table 5). Obviously it was necessary in this case to make use of a reducing agent which had a degree of selectivity. We required to reduce two groups (the esters) in the compound without affecting any other reducible groups (the double bonds). The most common broad spectrum chemoselective reducing agents are the metal hydrides. A review of hydride reducing agents that were used until 1957 is discussed

by Gaylord.[57] The two most common hydride reducing agents are lithium aluminium hydride ($LiAlH_4$) and sodium borohydride ($NaBH_4$). Many different metal hydride systems have been investigated in order to find conditions under which a given group will be reduced without reducing another group in the same molecule.[58] All three of the reducing agents used were metal hydrides.

1. Diisobutylaluminium Hydride (DIBAL). DIBAL (106) is generated by refluxing triisobutylaluminium in heptane.[59] Its synthetic utility lies in three areas; reduction of esters, reduction of lactones, and reduction of nitriles. This reducing agent was used



(106)

initially as previous work completed by Martin using this reagent had generated reasonable yields. As seen from Table 5 various reaction conditions were used for the reduction of (98) into (99) but all resulted in low yields. The work-up was particularly messy due to the generation of aluminium salts. A great deal of the product could have been trapped in these salts giving the low yields. An attempt to reduce the amount of Celite used in the work-up did not change yields considerably. The choice of a reducing agent is often dictated by the relative ease of isolation of a given product. Yields achieved by Martin could not be repeated and it was felt that perhaps the reactivity of the DIBAL /

dichloromethane solution used had depleted during storage. However, fresh DIBAL was used and still only low yields were accomplished.

2. Lithium Aluminium Hydride (LiAlH4). The synthesis and initial application of this reagent for reduction of organic compounds was reported by Schlesinger and co-workers[60] and Nystrom and Brown[61] in 1947. $LiAlH_4$ is prepared by reaction of lithium hydride (LiH) with aluminium chloride ($AlCl_3$)[62] as shown in Equation 3. This is a well known and powerful reducing agent

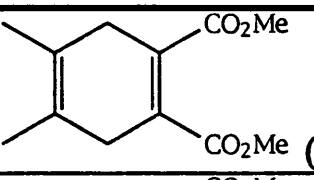
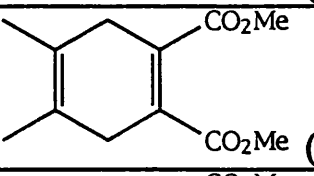
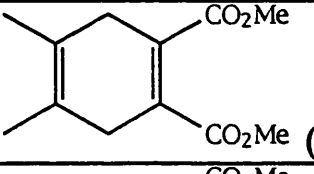
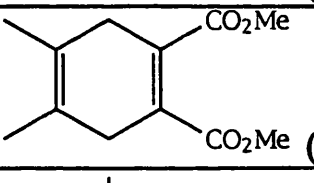
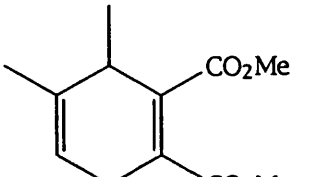
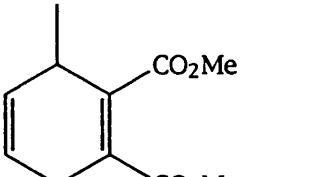
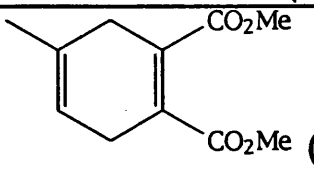
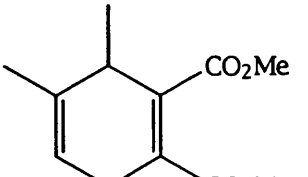
Equation 3

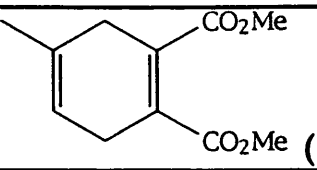
$$4LiH + AlCl_3 \xrightarrow{\text{ether}} LiAlH_4 + 3LiCl$$

which is much less chemoselective than most other metal hydrides. It is, however, usually inert with respect to most double bond reductions and was consequently used in an attempt to reduce two of the BAD analogues, dimethyl 3,4-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (107) and dimethyl 4-methylcyclohexa-1,4-diene-1,2-dicarboxylate (108). Both attempted reductions gave thick oils which could not be purified.

3. Diisobutylaluminium Hydride and n-Butyllithium Ate Complex. The development of hydride reducing agents, capable of achieving stereo- and chemo-selective reductions is an area in which much research has been done. Such selective reductions have been accomplished by modifying the steric and electronic effects of substituents on the aluminium atom. The ate complex

Table 5

Reduction Reagent	Reaction Conditions	Substrate	Outcome/ Yield
DIBAL/ CH_2Cl_2	-20°C, 1.5 h	 (98)	Impure prod. 3%
DIBAL/ CH_2Cl_2	-20°C, 2 h	 (98)	Impure prod. 10%
DIBAL/ Toluene	-20°C, 1.5 h	 (98)	No product
DIBAL/ CH_2Cl_2	-20°C, 6 h, celite reduced	 (98)	Impure prod. 5%
DIBAL/ BuLi	5 h, DCM continuous extraction	 (107)	40%
DIBAL/ BuLi	5 h, DCM continuous extraction	 (109)	Impure prod.
DIBAL/ BuLi	5 h, DCM continuous extraction	 (108)	Impure prod.
LiAlH4	THF, 2 h, R.T.	 (107)	No product

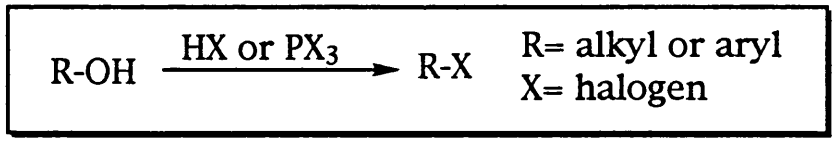
LiAlH4	THF, 2 h, R.T.	 (108)	Impure prod.

from DIBAL and methyllithium was originally utilised for the facile *trans*-hydroalumination of disubstituted alkynes by Zweifel and Steele,[63] but its reducing property had been unexplored. Kim and Ahn[64] investigated the reducing properties of the ate complex generated from DIBAL and n-butyllithium in the reduction of a series of selected organic compounds containing various functional groups. Since the exact nature of the reducing agent was not known they described the reducing agent as the "ate" complex rather than lithium diisobutyl-n-butylaluminium hydride. The reagent was made from DIBAL and n-butyllithium (n-BuLi) in an equimolar ratio in THF-hexane and has unique and unusual reducing properties different from those obtained with lithium aluminium hydride, DIBAL and other hydride reducing agents. Müller and Rodriguez[65] used this reagent to convert diesters that were connected by a *cis*-double bond successfully into *cis-1*,4-diols with preservation of the *cis*-double bond.[66] In addition to this, they incorporated a 24 hour continuous dichloromethane extraction into their work-up procedure. As mentioned previously there were two possible reasons for failure of the DIBAL reductions: **a)** the unsuitability of DIBAL for *cis*-1,2-diester reduction; and **b)** the extraction of the diols from the aluminium salts. By using the DIBAL / *n*-BuLi reducing agent and the continuous extraction work-up procedure, both problems incurred using DIBAL should be minimised.

The diol extraction problems were reduced by the use of continuous extraction. Although the amounts of material extracted were increased due to this technique the problem of purification of these oils was significant. Attempts at drying under vacuum conditions and purification via crystallisation proved to be inadequate. These diols did not give good chromatographic results and so a method that would convert them into compounds that could be purified using chromatography was devised. Greene[67] refers to a convenient method of acetylating diols, which could then be easily purified using column chromotography, before removing the acetyl groups. This work was not undertaken as our initial six step synthesis would be converted into a less desirable eight step synthesis.

The third step in the synthesis is a dibromination. It was necessary to convert the diol into a dihalo compound in order to facilitate introduction of the diamino moiety via the Gabriel synthesis (as discussed in section 2.1.1). In fact alkyl halides are

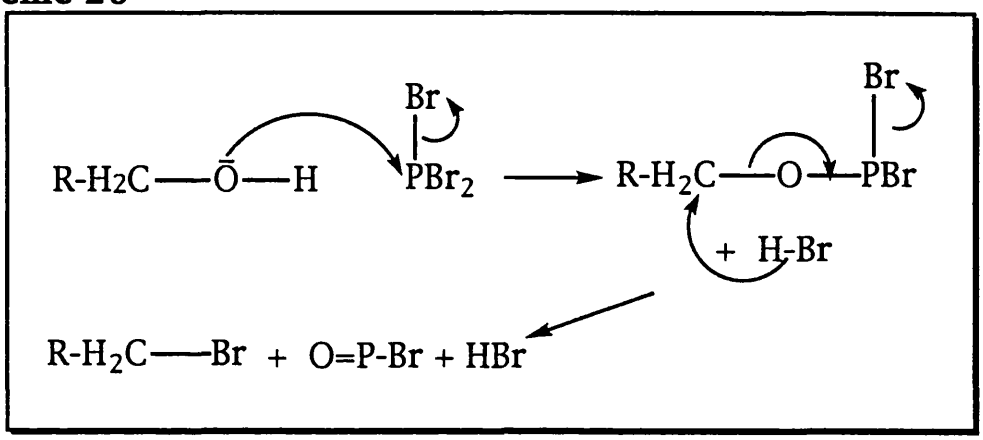
Scheme 25



nearly all prepared from alcohols. This is done by the use of hydrogen halides or phosphorus halides (Scheme 25). Some alcohols (excluding most primary alcohols) tend to undergo rearrangements during replacement of -OH by -X, but this tendency can be minimised by use of phosphorus halides. Whether rearrangements occur or not depends on whether the reaction is of the S_N1 or S_N2 type. A search of the literature

resulted in the discovery of a method by Feigenbaum and Lehn[68] that used phosphorus tribromide to dibrominate the diol (Scheme

Scheme 26



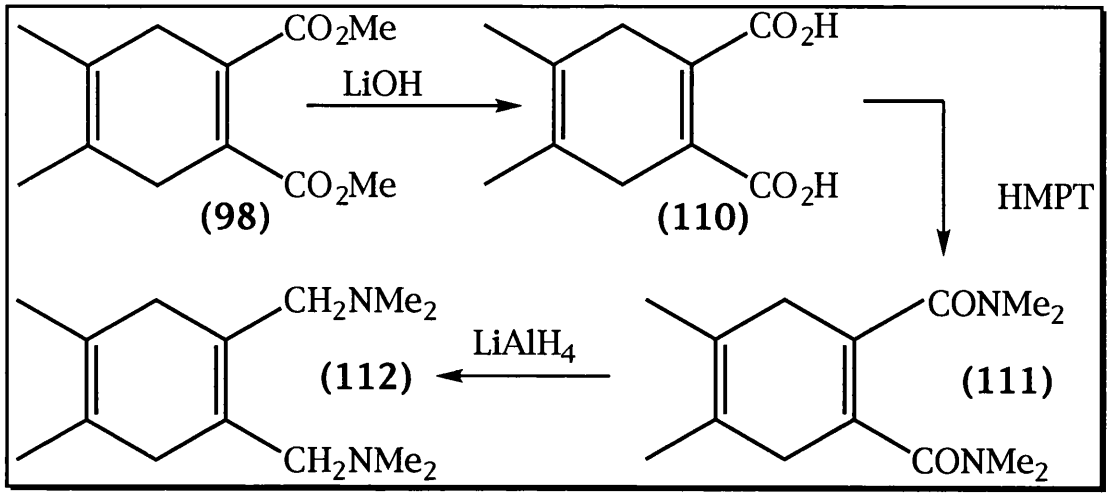
26). The diol could then be converted into the corresponding diphthalimido compound and then cleavage should yield the diamine. A discussion of conditions for introduction of the phthalimide groups and cleavage conditions used has already been mentioned in Section 2.2.

Because no satisfactory method could be found for reducing the cyclic diesters to the corresponding diols this area of work was abandoned.

2.4.1 *N*-Alkylated BAD Derivatives

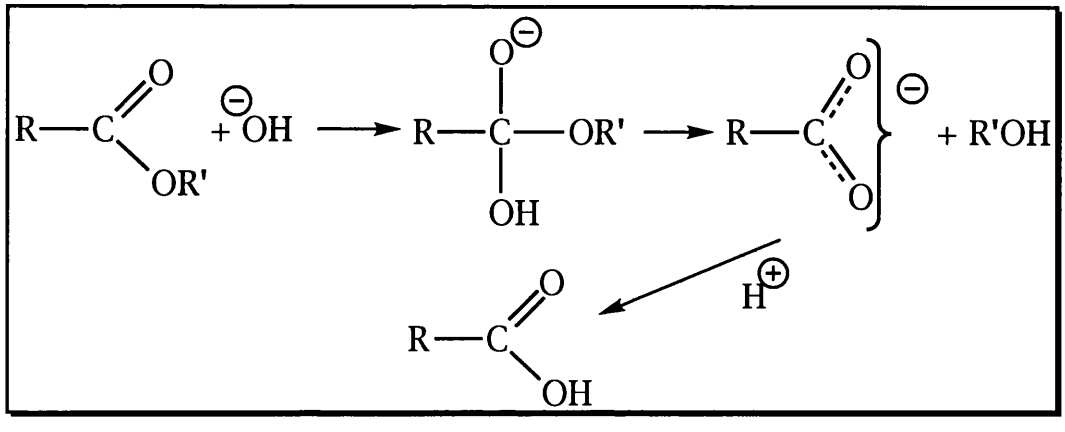
N-Alkylated BAD derivatives were of obvious interest considering the antifungal success of TED (87) and BAD (95). The synthetic routes to 1,2-bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene (112) and the equivalent tetraethyl derivative are similar and both involve going through the relevant diamides.

Scheme 27



The synthesis of tetramethyl-BAD (112) shown in Scheme 27 started from a Diels Alder reaction to produce the diester (98) as discussed in 2.7. Two methods were used in an attempt to convert the diester (98) into the diacid (110). Greene and Wuts[69] refers to a method devised by Corey *et al.*[70] which uses lithium hydroxide monohydrate in methanol/water to accomplish hydrolysis. Under alkaline conditions (Scheme 28) the acid was obtained as its salt, from which it could be liberated by addition of

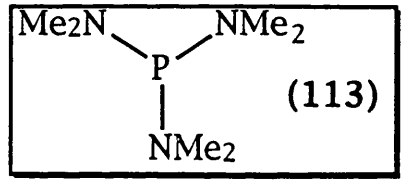
Scheme 28



acid. This reaction, unlike acidic hydrolysis, is essentially irreversible since a resonance-stabilised carboxylate anion shows

little tendency to react with an alcohol. An insoluble white solid was recovered. Diacids of this sort are extremely insoluble in water. Another method using 5% sodium hydroxide also gave the product though in lower yield.

Hexamethylphosphorus triamide (HMPT) (113) was used in several reaction schemes throughout this work. The use of HMPT as a reagent for the one step conversion of acids into dimethyl amides proceeds in very good yield and gives a product of such high purity that no further purification is usually required. Quin *et al.*[71] used HMPT in their research and developed successful reaction conditions for its use.

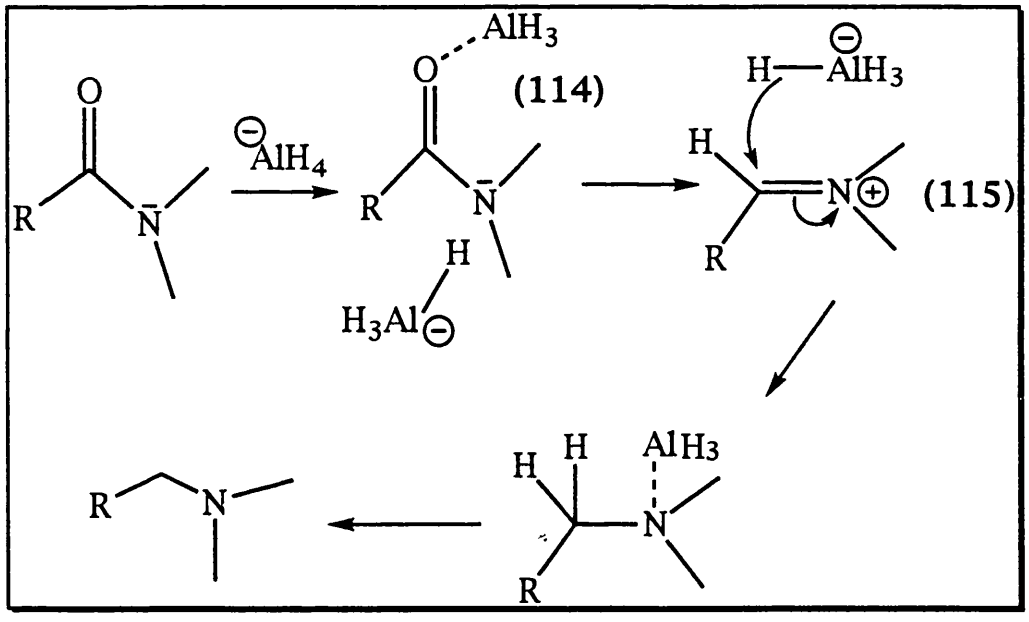


(113)

Lithium aluminium hydride ($LiAlH_4$) was used by Quin *et al.*[71] to accomplish the reduction of the amide (111) to the corresponding amine (112). $LiAlH_4$ is a selective reducing agent which did not reduce the double bonds in the substrate. As discussed earlier, isolation of the desired product from $LiAlH_4$ reductions can be difficult. If excess water is added in the work-up, the alumina salts formed during the hydrolysis can form a paste, which is difficult to filter. The basic work-up procedure of Micovic and Mihailovic[72] (first reported by Amundsen[73]) was followed and has several advantages. It produces a granular precipitate which can be easily filtered. Amides do not react with $LiAlH_4$ in the same way as other carbonyl derivatives do. The reaction gives an intermediate iminium salt, which is further

reduced to an amine as the final product. Initial transfer of hydride to the amide forms a complex as represented by (114) (Scheme 29).[58] Subsequent elimination of the alkoxyaluminate leads to formation of the intermediate iminium salt (115). Addition of another equivalent of hydride gives the amine. As the steric hindrance on nitrogen increases, initial coordination and delivery of hydride is less facile.

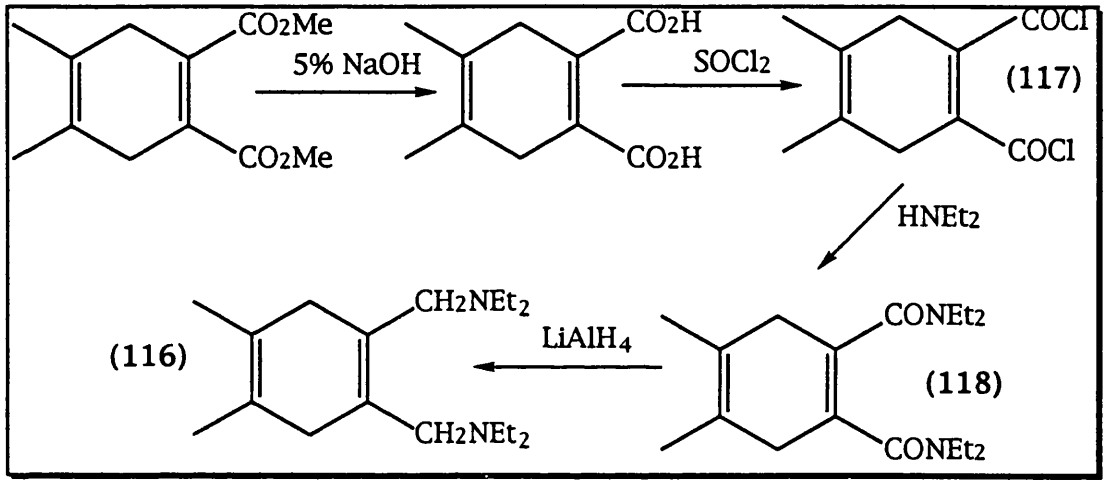
Scheme 29



The amide *N',N',N,N*-tetraethyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxamide (116) was made via the more conventional method shown in Scheme 30. Acid chlorides are prepared by substitution of -Cl for the -OH of carboxylic acids. Three reagents are commonly used for this purpose; thionyl chloride ($SOCl_2$), phosphorus trichloride (PCl_3) and phosphorus pentachloride (PCl_5). Thionyl chloride is particularly convenient since the by-products formed are gases and are easily separated from the acid chloride product.[74] As illustrated in our work any

excess low boiling thionyl chloride (79 °C) can be easily removed by distillation. Acid chlorides are highly reactive and once (117) was generated it was added almost immediately to diethylamine to give the amide (118). $LiAlH_4$ reduction of this amide was attempted several times but the product, which was a glutinous solid, could not be easily purified.

Scheme 30

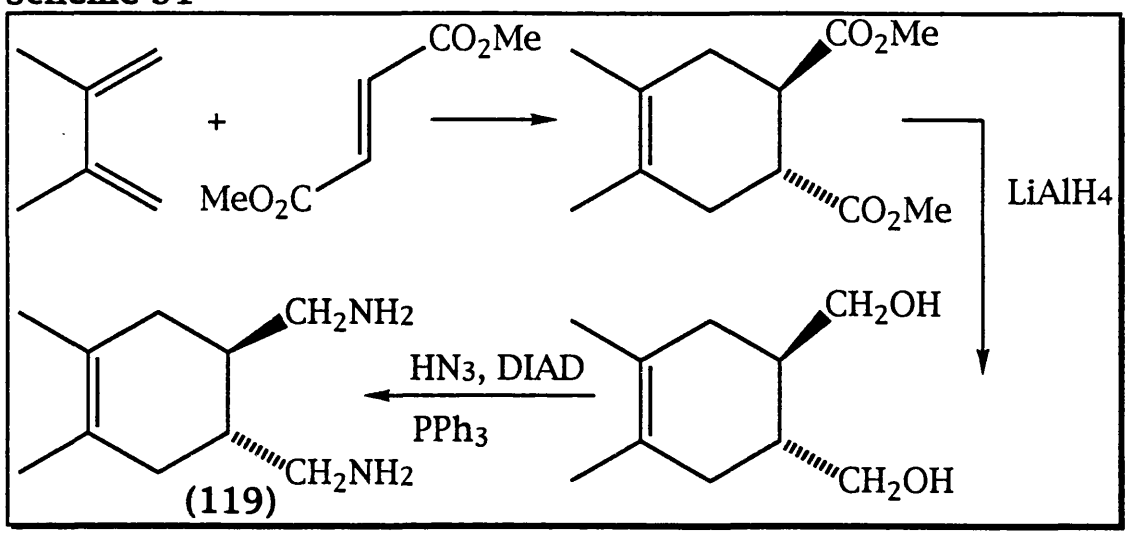


2.4.2 *trans*-4,5-Bis(aminomethyl)-1,2-dimethylcyclohex-1-ene dihydrochloride

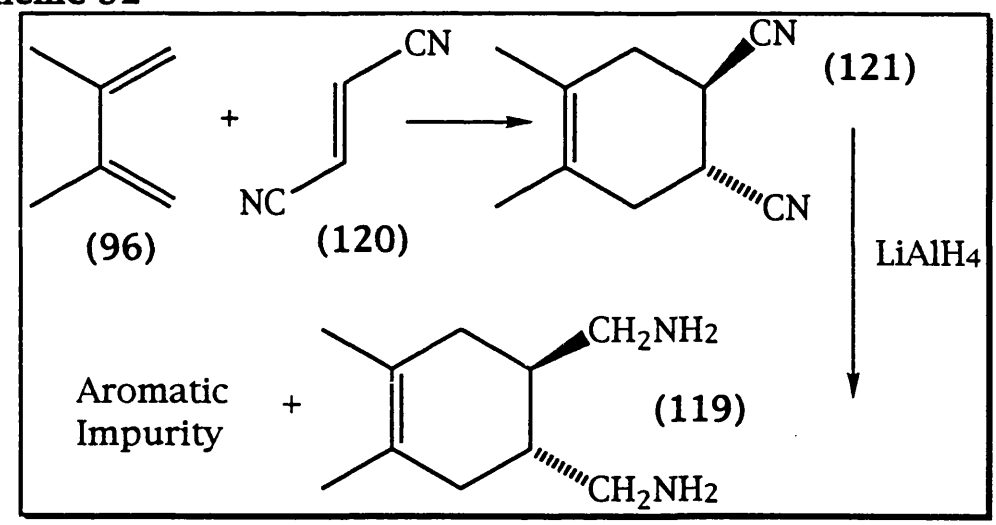
Martin successfully made *trans*-4,5-bis(aminomethyl)-1,2-dimethylcyclohex-1-ene dihydrochloride (119) via the route shown in Scheme 31.[29]

An attempt in this project to shorten this route resulted in the synthesis of (119) and an aromatic impurity. Scheme 32 shows the route which was followed.

Scheme 31



Scheme 32

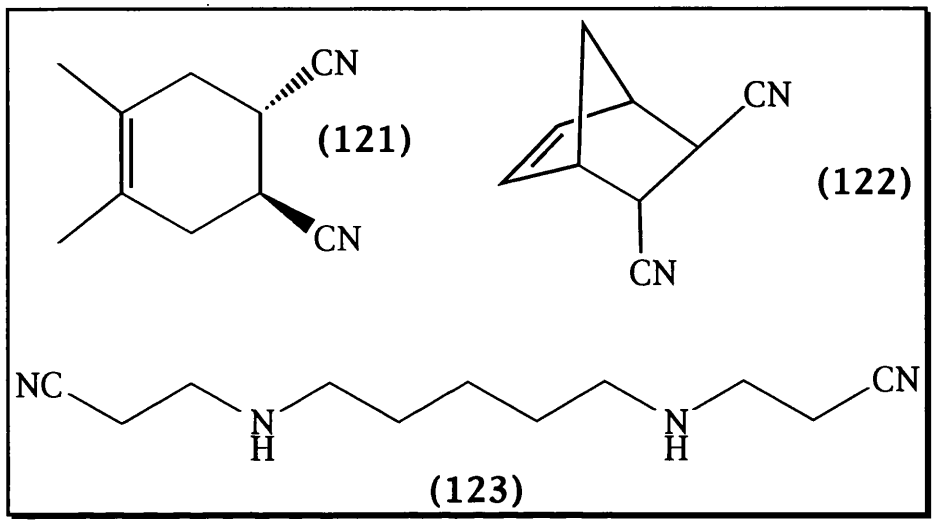


The Diels Alder conditions for the reaction of 2,3-dimethyl-1,3-butadiene (96) and fumaronitrile (120) are discussed in Section 2.7.

Amines are the usual final reduction products of nitriles. In this work, three compounds (121), (122) and (123) containing nitrile groups were prepared and required to be reduced to primary amines.

The literature suggested several reagents including lithium aluminium hydride,[75, 76] lithium aluminium hydride/ aluminium chloride,[77, 78] catalytic hydrogenation,[79] and borane reductions.[80, 81]

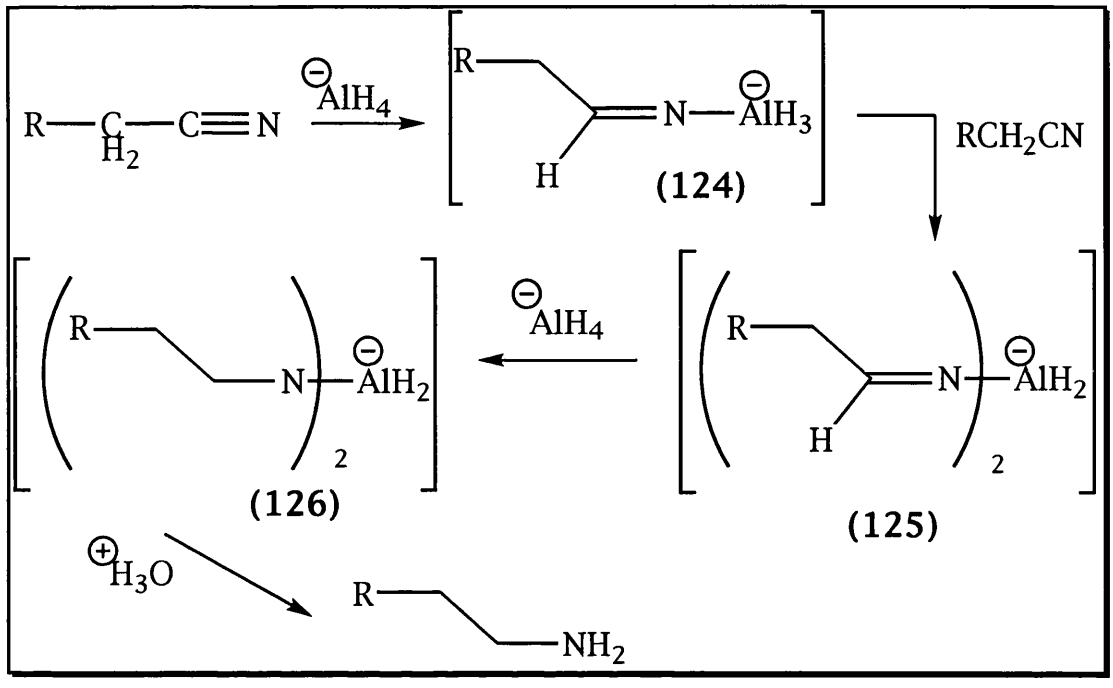
Reductions of (122) and (123) were completed successfully (discussed in Sections 2.5.2 and 2.6.2 respectively) while reduction of (121) resulted in the formation of a major aromatic side product.



Reduction of the cyano group using $LiAlH_4$ is a general reduction method which has been adequately reviewed.[58] The reaction proceeds via initial reduction to an iminium salt (124), which is converted into a bis-imino aluminate (125) (Scheme 33).[58] The literature is vague concerning the specific structure of this intermediate but reduction of (125) with additional hydride leads to (126) as reported by Soffer and Katz.[83] It is likely that the hydrogen atoms in the final product come from hydrolysis. The absence of secondary amines in the product is a highly advantageous feature of this method.

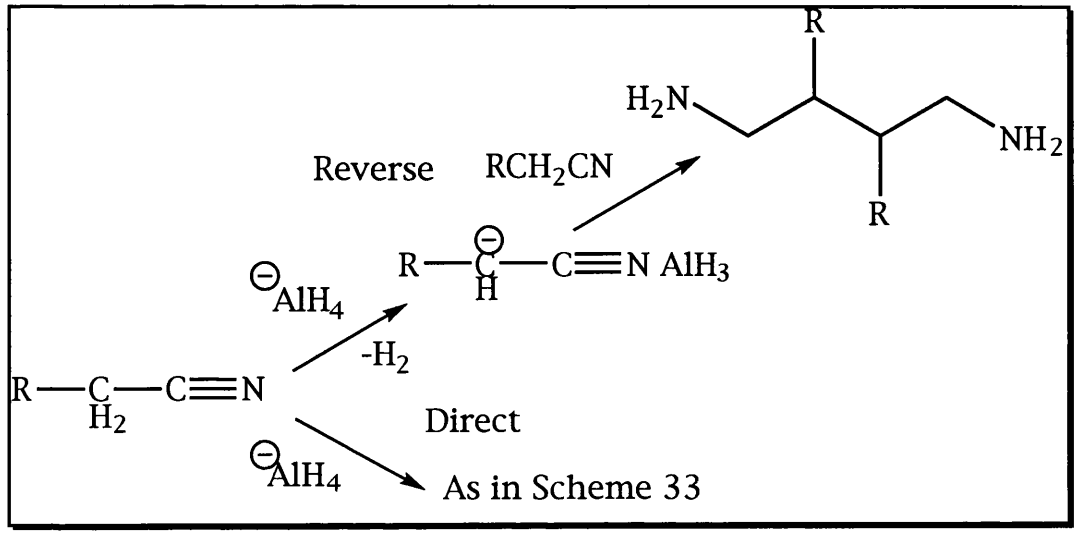
The solvent and method of addition used in $LiAlH_4$ reductions of nitriles is an area which has had great attention paid to it. Soffer and Katz[83] studied the effects of using ether or THF as solvents. The aluminium in (126) may be coordinated by either of these solvents. There are two methods of addition;[82] addition of

Scheme 33



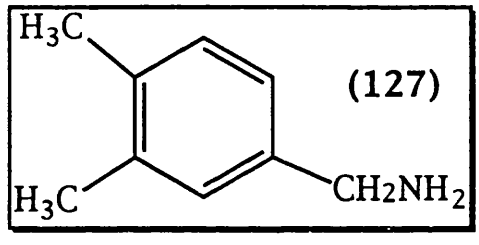
the nitrile to the hydride (direct) and addition of the hydride to the nitrile (reverse). When the direct method of addition is used the proposed sequence of reactions is as illustrated in Scheme 33. In the reverse addition, diamines were produced as well as the required primary amine. Scheme 34 shows the two competing reactions which could explain these experimental results.

Scheme 34



The Thorpe condensation[84] of an intermediate nitrile carbanion competes with the mechanism shown in Scheme 33. Results were comparable when both THF and ether were used as solvents.

The reduction of (121) was attempted in both THF[75] and ether[76] and in both cases an aromatic impurity appeared. The mechanism by which this could be formed and the reasons for its appearance are an unknown, however the proton and carbon NMR spectra indicate that it could have the structure (127), as illustrated.



(127)

The evidence for the structure of this impurity is that the ^{1}H NMR spectrum shows signals in the aromatic region equivalent to three protons, the number of CH signals is confirmed in the ^{13}C NMR spectrum. A singlet at δ 2.09 in the ^{1}H NMR spectrum indicates the six protons in the two methyl groups, with another singlet at δ 3.93 representing the two protons next to the amine group.

2.5 Various Ring Sizes and Bridged Systems

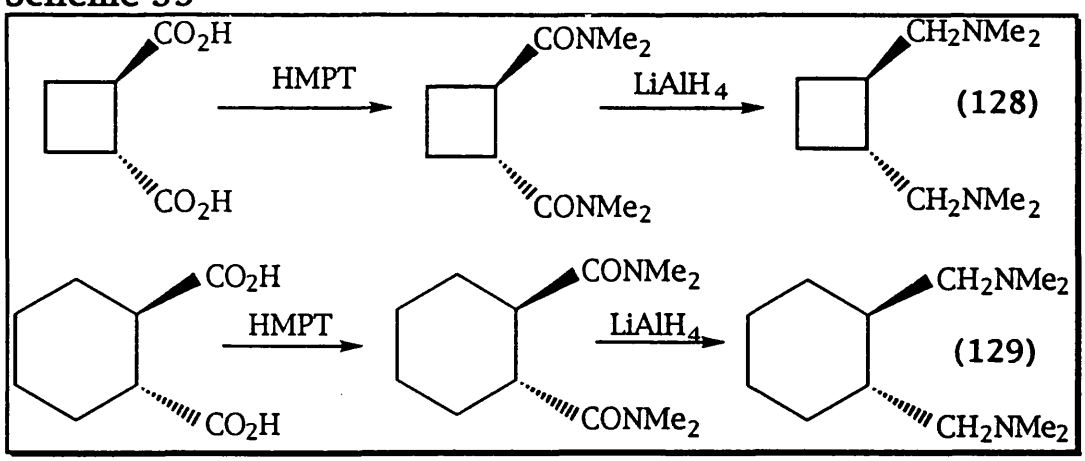
The purpose of this project was not only to synthesise relevant compounds for testing as antifungal agents but also to incorporate some creative and interesting chemistry into the synthesis. Varying the ring size and creating bridged systems

made use of slightly different chemical reactions and widened the range of compounds available for testing.

2.5.1 Four- Five- and Six-Membered Rings

The cyclic compounds synthesised in this project up until this point had all contained six-membered rings. Routes to a variety of *N,N'*-dialkylated saturated cyclic compounds with four-, five- and six-membered rings were designed. Two routes, starting from commercially available diacids and illustrated in Schemes 35 and 36 were felt to be viable.[85, 86, 87, 88] The syntheses of three *trans*-1,2-bis(dimethylaminomethyl)cycloalkanes were completed successfully. Two of these, the four- (128) and the six-membered (129) rings, were made using HMPT[33] as the source of the dimethylamino moiety. The five-membered ring (130)[88] was formed using the route shown in Scheme 36, using dimethylamine instead of diethylamine.

Scheme 35

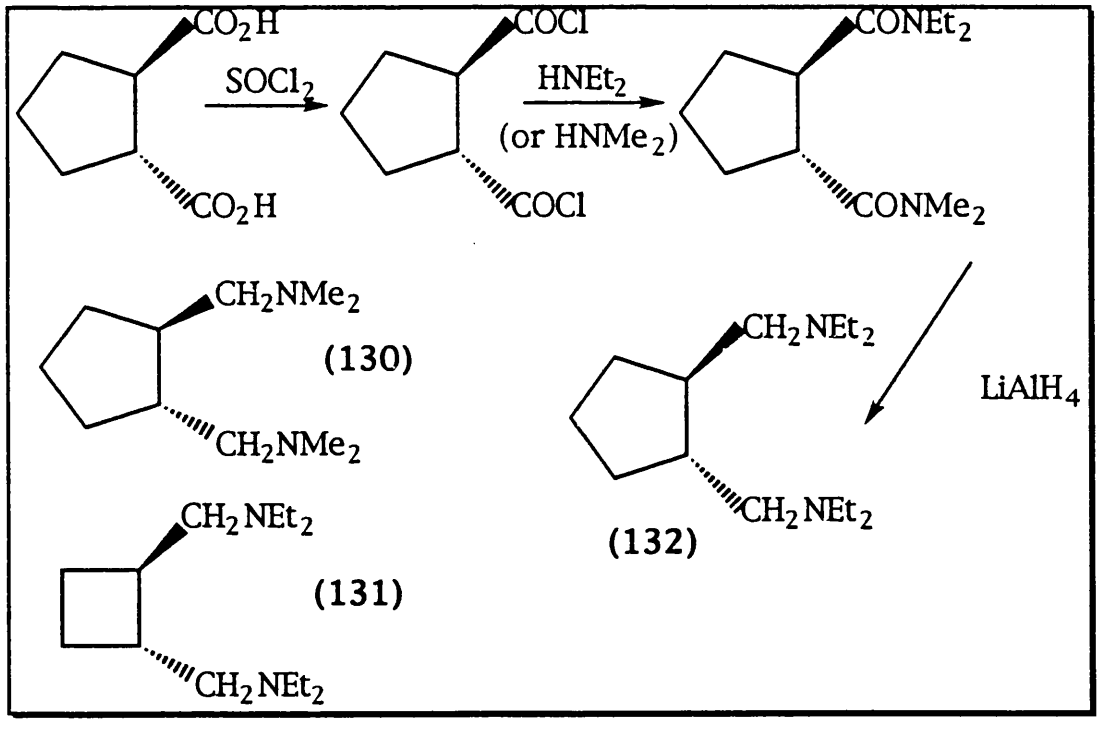


Due to the low boiling point of dimethylamine a large excess of this reagent was used. This was not a satisfactory situation and, therefore, *trans*-1,2-bis(dimethylaminomethyl)cyclopentane (130) was the only *N,N,N'N'*-tetramethyl diamine that was made via this

route. The use of HMPT to form an amide which can then be reduced to an amine has already been discussed in Section 2.4.1.

Diethylamine has a higher boiling point and, although a large excess of it was used in the reaction, there was no other convenient way of producing the required *N,N,N'N'*-tetraethyl diamines. *trans*-1,2-Bis(diethyl-aminomethyl)cyclobutane (131) and *trans*-1,2-bis(diethylaminomethyl)-cyclopentane (132) were synthesised following the route shown in Scheme 36.

Scheme 36



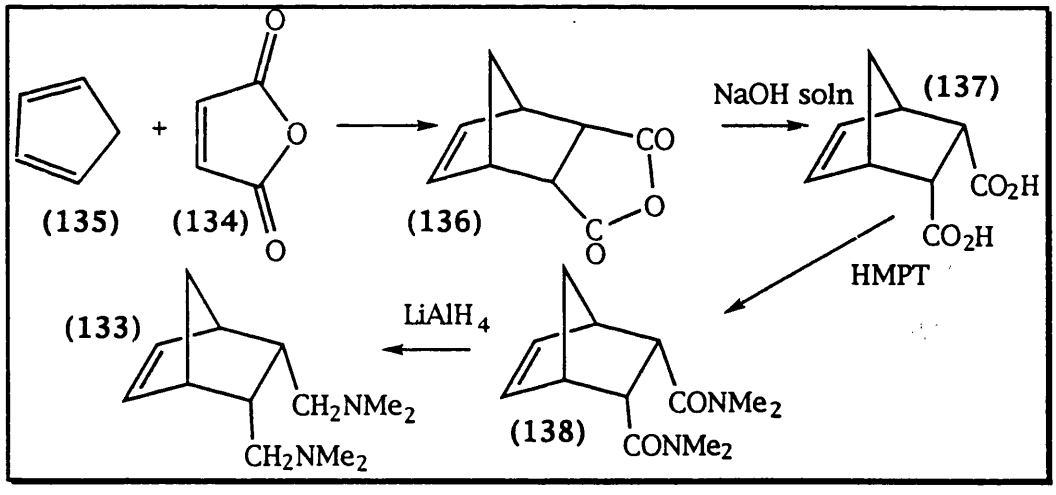
In general, the reductions of the diamides to the diamines using LiAlH4 were not very successful.[72] In a few cases yields were difficult to establish due to the glutinous nature of these diamines and some were converted into their dihydrochloride salts in an effort to purify them.

2.5.2 Bridged Systems

Attempts to synthesise three bridged compounds were made during this project. Two of the compounds were norbornenes and the third was a [2.2.2] bridged compound.

Scheme 37 illustrates the route taken in the synthesis of *cis*-2,3-bis(dimethylaminomethyl)norborn-5-ene (133). Maleic anhydride (134) and cyclopentadiene (135) readily react in the Diels Alder reaction (see Section 2.7) to generate the bridged compound 136.[89, 90] The anhydride was opened up using alkali, and the product acidifed, to give the diacid (137) which, as

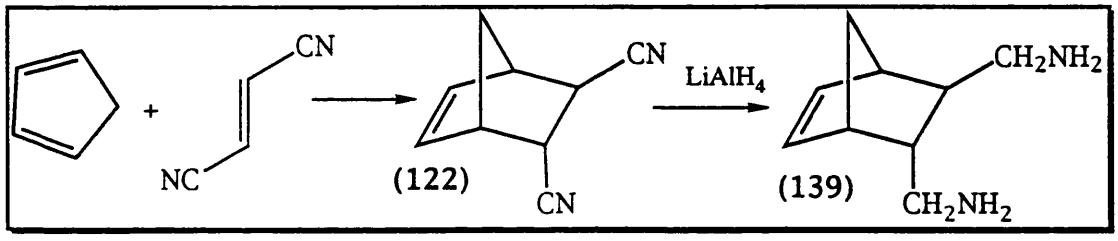
Scheme 37



previously discussed, reacts with HMPT to give *N',N',N,N*-tetramethylbicyclo[2.2.1]hept-2-ene-5,6-endo-dicarboxamide (138). $LiAlH_4$ in dry THF reduced the diamide (138) to the diamine product (133). The mass spectrum of (133) showed a molecular ion corresponding to the required product but high molecular weight impurities were also present. There was insufficient material to allow purification.

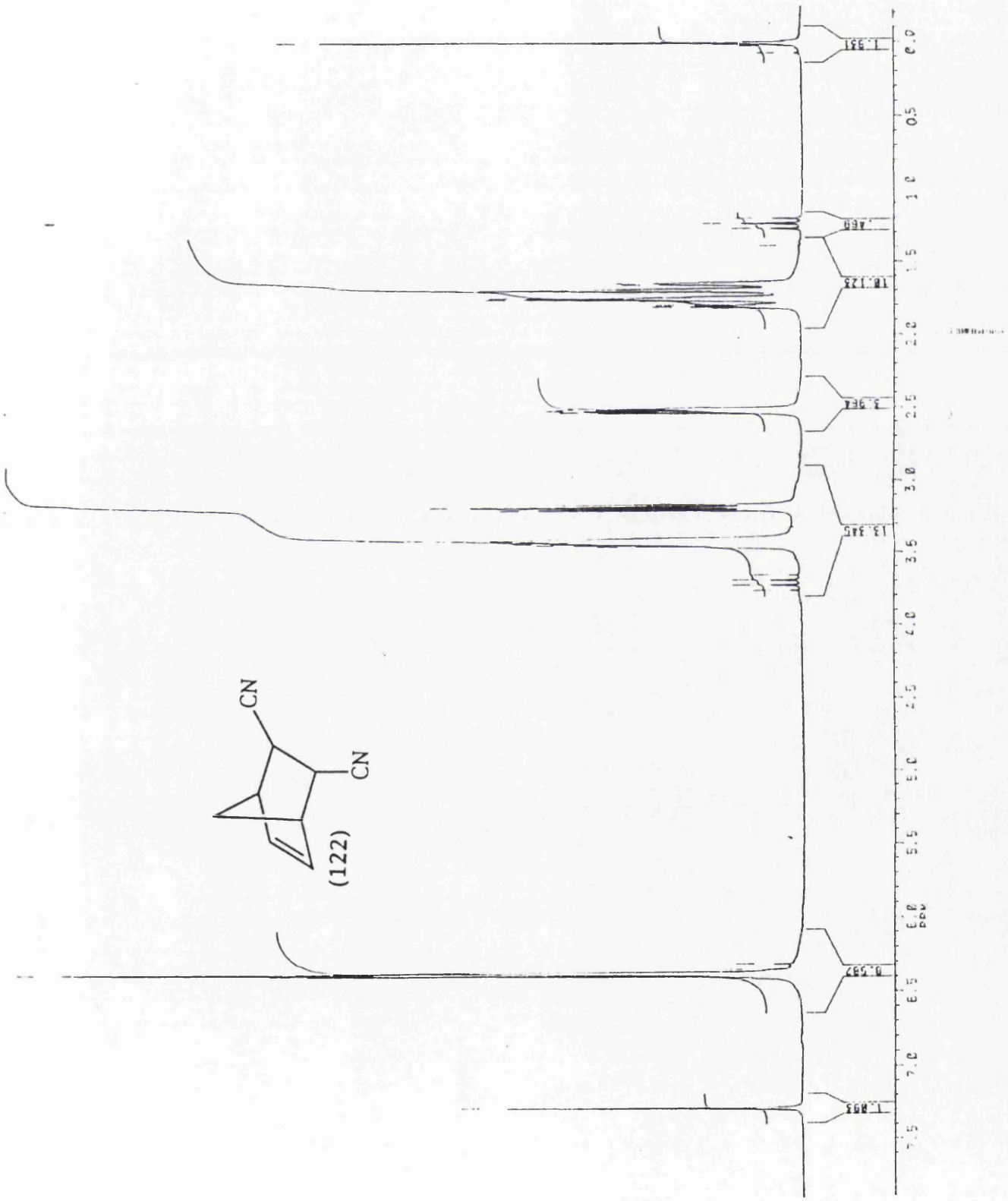
trans-5,6-Bis(aminomethyl)bicyclo[2.2.1]hept-5-ene (139) was the second norbornene synthesised in this project. Cummins *et al.*[76] followed a simple and high yielding route for the synthesis of *trans*-5,6-bis(aminomethyl)bicyclo[2.2.1]hept-5-ene as shown in Scheme 38. This route was adopted in this project and both steps gave high yields of 75% and 93% respectively.

Scheme 38



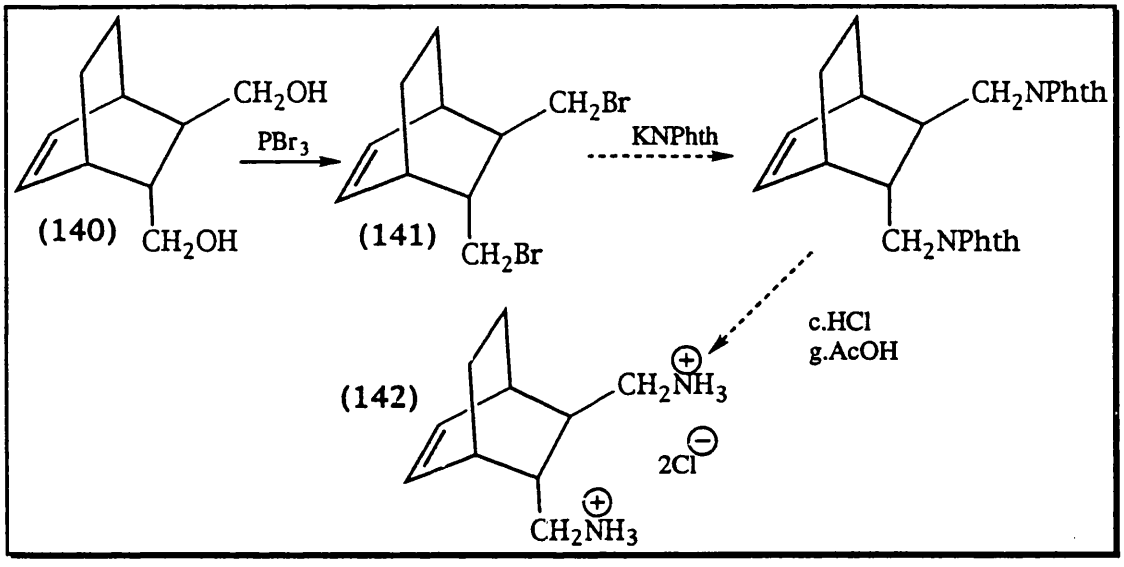
The key features in the proton NMR spectrum are the two bridgehead (δ 3.40-3.45) and the two olefinic (δ 6.37) protons which are clearly shown, as illustrated in Spectrum 1. The peak for one of the apex protons is overlapped by the signal for the *endo*-methine (δ 1.64-1.81). The other apex proton can be seen as a multiplet at δ 2.50-2.53, while the *exo*-methine proton appears at δ 3.16-3.19. The LiAlH4 reduction gave a particularly high yield of 93% and was the most successful reduction completed in this project.

Spectrum 1 The ^{1}H NMR Spectrum (200 MHz, $CDCl_3$) of *trans*-Bicyclo[2.2.1]hept-2-ene-5,6-dinitrile (122).



An attempt to synthesise the third bridged compound was unsuccessful. The starting material (140) was commercially available and the potential was seen for this diol to be brominated, and the dibromo compound (141) that was generated to be taken through the phthalimide route to the diamine *trans*-2,3-bis(aminomethyl)bicyclo[2.2.2]oct-5-ene (142) as shown in Scheme 39.

Scheme 39



The bromination using phosphorus tribromide was unsuccessful and as the rest of the route was proving difficult to follow for simpler, less sterically hindered compounds, further work on the use of other potential brominating agents was not thought to be appropriate. Scheiner and Vaughan[91] suggest an alternative route, via a dicarbonitrile, to form diamines similar to (142), but this was not attempted.

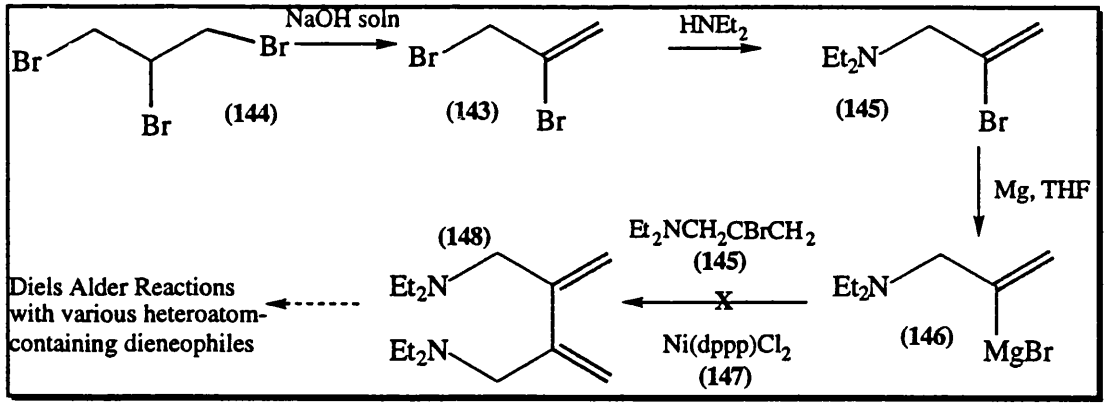
2.6 Heterocyclic Systems and Other Work

A synthetic route which has the potential to produce a range of cyclic and heterocyclic compounds was commenced in this project. The synthesis of a number of other compounds, which do not fall under any of the other headings have been gathered together and described in Section 2.6.2- Other Work.

2.6.1 Heterocyclic Systems

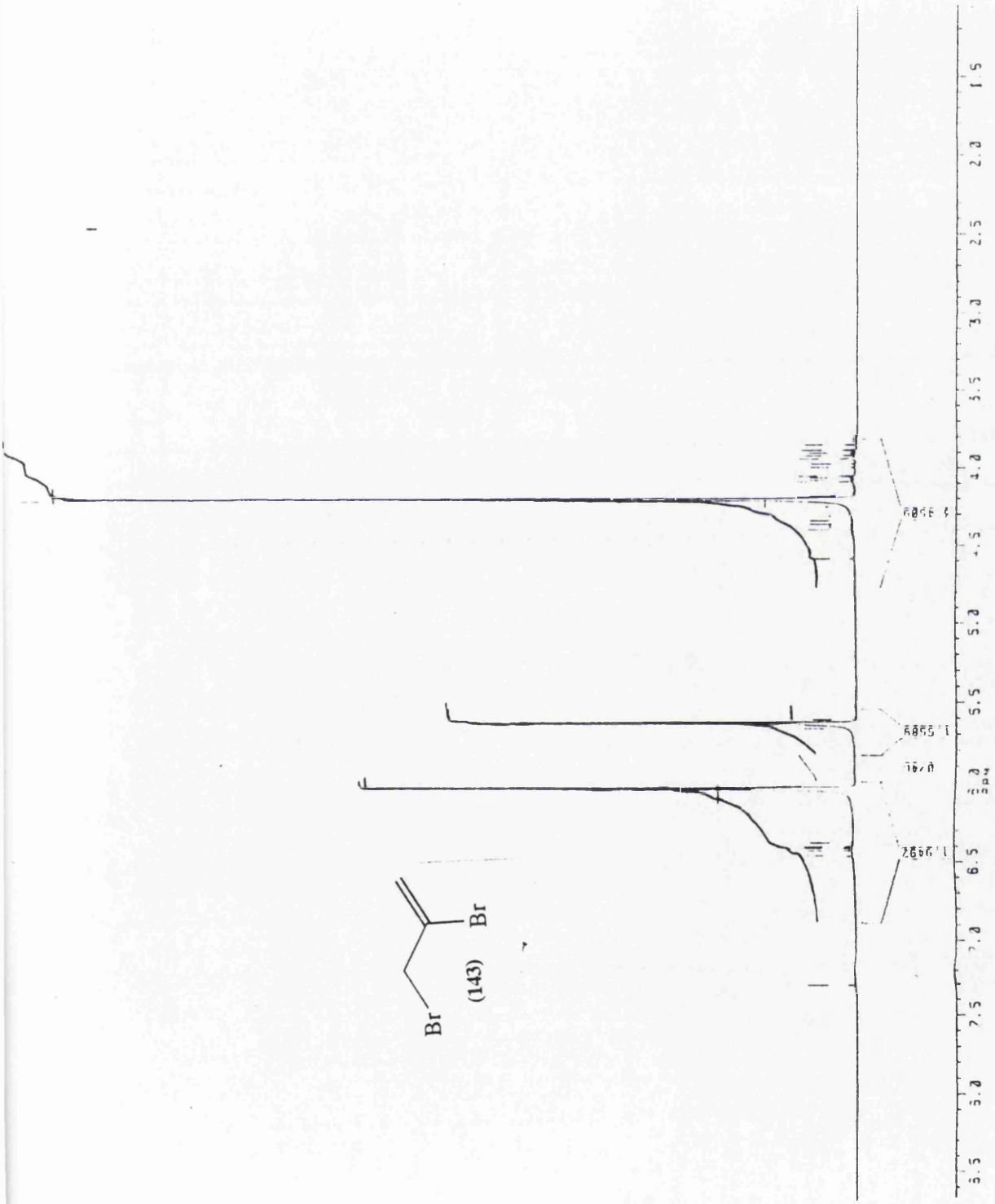
A new approach to the synthesis of the required target cyclic *N*-diethyl diamines was devised by Martin. Instead of starting the synthetic route with a Diels Alder reaction and then manipulating the functional groups attached to give the diamine, Martin investigated a way of producing a diene which already had the required diamine moiety in place. Obviously this route

Scheme 40



(Scheme 40) was restricted to making *N,N,N',N'*-tetraalkyl cyclic diamines. The possibility of using heteroatom-containing dienophiles opened up a new area of compounds that have heterocyclic moieties.

Spectrum 2 The ^{1}H NMR Spectrum (200 MHz, $CDCl_3$) of 2,3-Dibromopropene (143).



In 1932 Lespieau and Bourguel[92] reported that 2,3-dibromopropene (143) could be easily made from 1,2,3-tribromopropane (144) and sodium hydroxide with spontaneous distillation of the product. Spectrum 2 illustrates the proton NMR spectrum recorded upon purification by distillation. The two olefinic protons have quite different chemical shifts of δ 5.63 and 6.04 respectively, while the methylene protons appear as a singlet at δ 2.40. 2,3-Dibromopropene was then reacted with diethylamine to give 2-bromo-3-diethylaminopropene (145).[93]

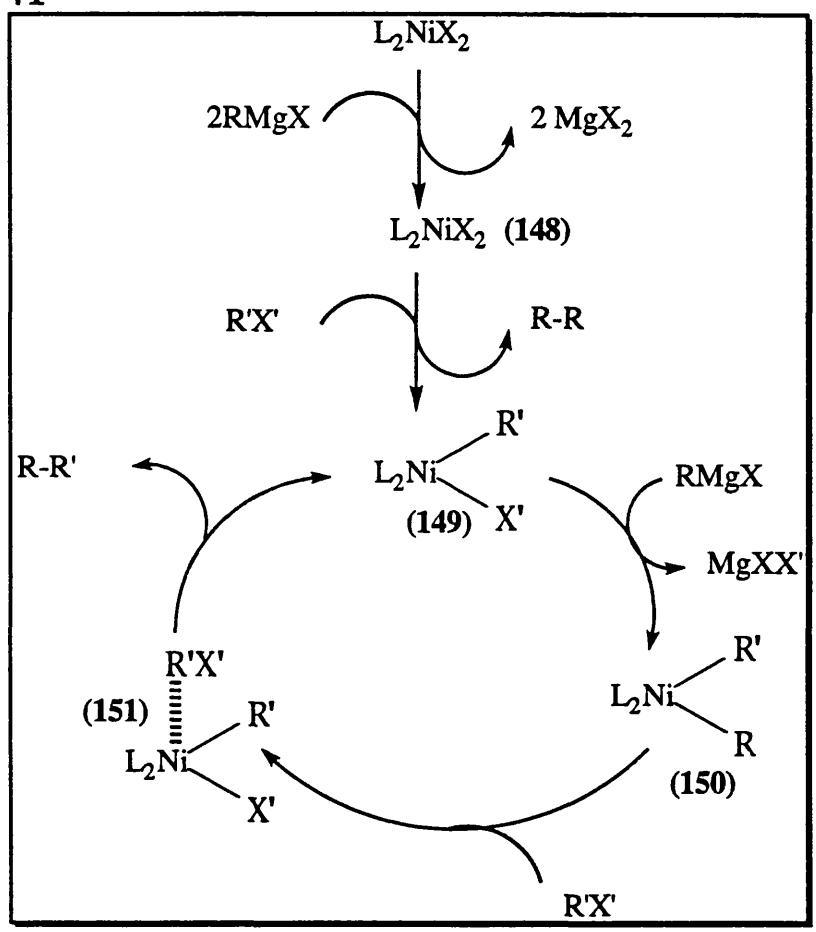
Hosomi *et al.*[93, 94] wrote several papers on the synthesis of various 1,3-dienes according to the general route shown in Scheme 40. The dienes were prepared by the cross-coupling of the Grignard reagent, in this case (146) with the original vinylic bromo compound, in this case (145), using different catalysts. Here the catalyst dichloro[1,3-bis(diphenylphosphino)-propane]nickel was used in an attempt to synthesise 2,3-bis(diethylaminomethyl)-1,3-butadiene (148).

Grignard Reaction. The Grignard reaction[95, 96] is one of the most attractive and useful procedures for formation of the carbon-carbon sigma bond. It has been known for thirty years that catalytic amounts of transition metal salts induce the coupling reaction of Grignard reagents with organic halides. In 1972 Tamao *et al.*[97] reported that nickel-phosphine complexes catalyse the selective cross-coupling of Grignard reagents with aryl and alkenyl halides.

Tamao *et al.*[97] investigated the catalytic activity of nickel complexes and found that it depends strongly upon the nature of the ligands. Bidentate phosphine ligands exhibited much higher activity than unidentate ones. Dichloro[1,3-

bis(diphenylphosphino)-propane]nickel (147) was discovered to be the most useful catalyst when compared to a large range of other nickel catalysts.

Scheme 41



It has been proposed that the dihalodiphosphine nickel catalysts react with a Grignard reagent to form the intermediate diorgano nickel complex (148). Scheme 41[97] illustrates this theory. Compound (148) is converted into the halo(organo) nickel complex (149) by an organic halide. The complex (149) reacts with the Grignard reagent and forms a new diorgano complex (150) from which the cross-coupling product is released by the attack of the organic halide. Tamao *et al.* propose that the reaction proceeds via

a penta-coordinated intermediate (151). The original complex (149) is regenerated to complete the catalytic cycle.

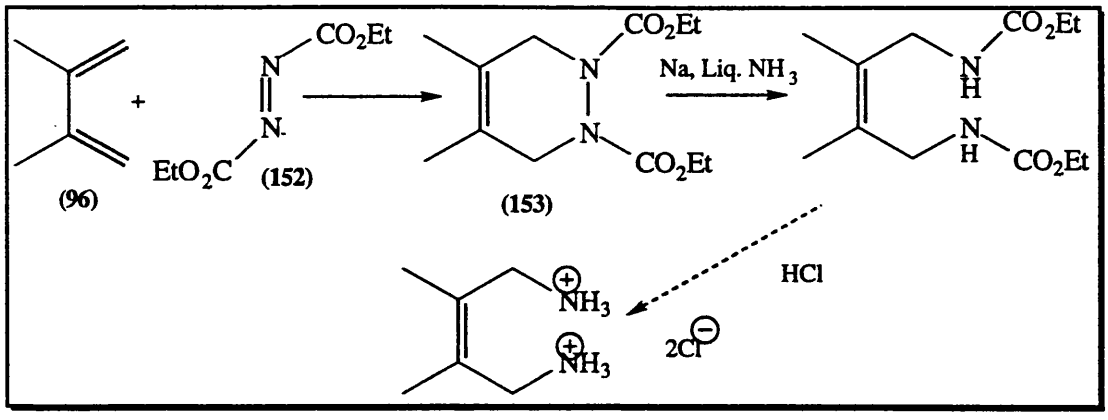
In this project several attempts were made at preparing the Grignard reagent. The only evidence that suggested that the Grignard reagent had been generated was that the reaction mixture spontaneously heated and a brown precipitate appeared. The Grignard reagent was then transferred to the coupling reaction vessel where there were no noticeable changes in colour or temperature. Mass spectroscopy indicated only starting material and from all the above evidence it was assumed that the coupling stage of the reaction had been unsuccessful. The reaction mixture was heterogeneous and the lack of reactivity could in part be attributed to this.

2.6.2 Other Work

The possibility of creating more examples of *cis*-1,4-diaminobut-2-enes was seen by reductively cleaving the nitrogen-nitrogen bond in hydrazine derivatives.[98, 99] Scheme 42 shows the proposed synthetic route where variations of the diene in the first step could extend the final compound range considerably. 2,3-Dimethylbuta-2-diene (96) and diethyl azodicarboxylate (DEAD) (152)[100] were used in a Diels Alder reaction to give the six-membered ring (153) in 84% yield.

Mellor and Smith[98] studied reductive cleavage of the nitrogen-nitrogen bond in several hydrazine derivatives using sodium in liquid ammonia. Initially the reaction was stirred for about one hour while sodium balls were being added and blue colour remained. The reaction was then worked up yielding only starting material. In a second attempt the reaction was stirred for a further one hour after the sodium had all been added. The final

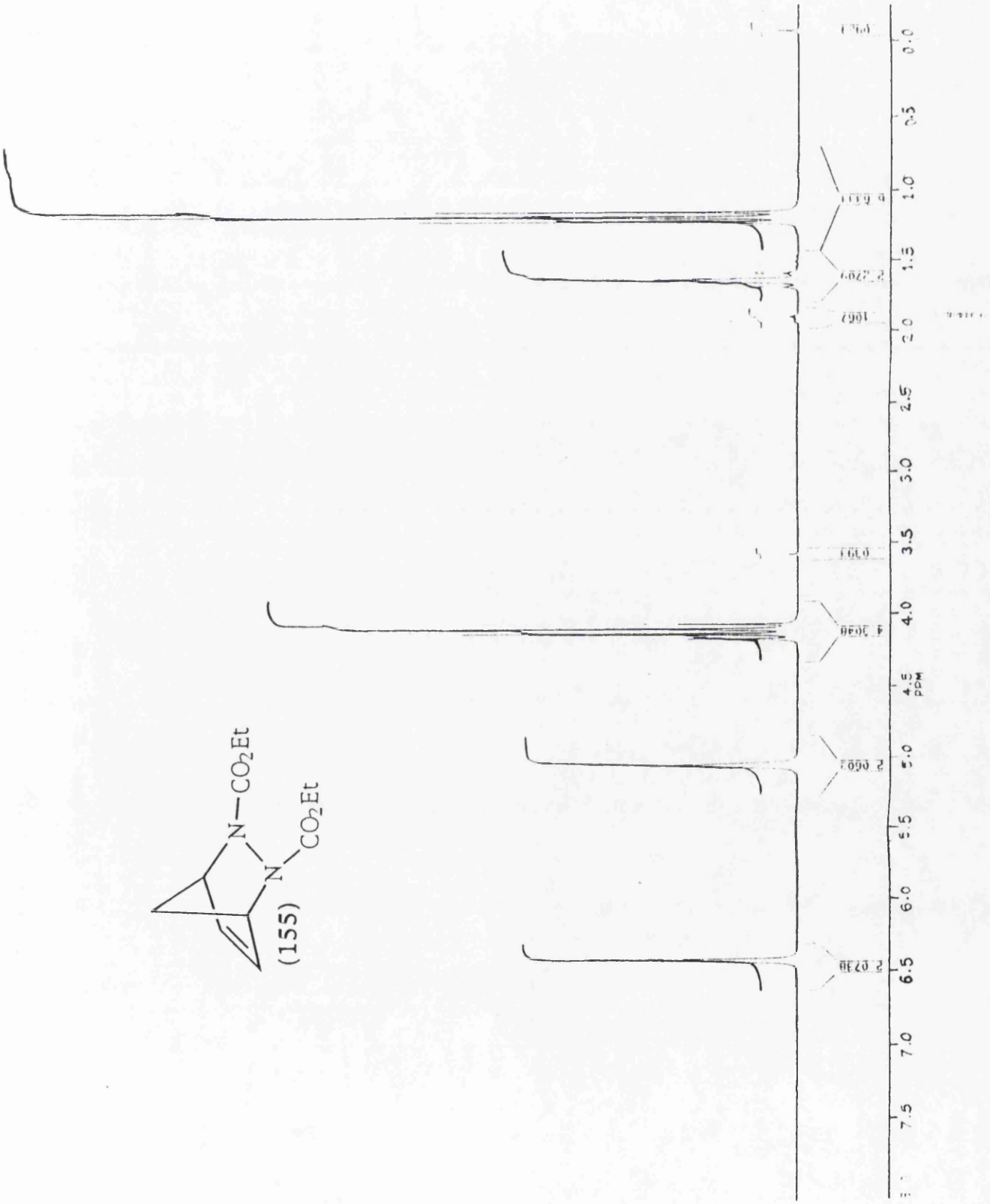
Scheme 42



attempt had a two hour stirring time after the sodium addition was complete. Obviously the cleavage of a nitrogen-nitrogen bond and the addition of two hydrogen atoms does not drastically change the end product from starting material in terms of analysis. It was hoped that the opening of the hydrazine ring would enable detection of the product by NMR spectroscopy. NMR spectroscopy indicated a mixture of starting material and product while mass spectroscopy showed a molecular ion of *m/z* 258 which corresponds to that of the product. Time constraints did not allow this work to continue, either in repeating the reaction with a longer stirring time or in attempting what could be a difficult separation of starting material and product. As mentioned above, this technique has wide scope and many variations on the initial target could potentially be produced.

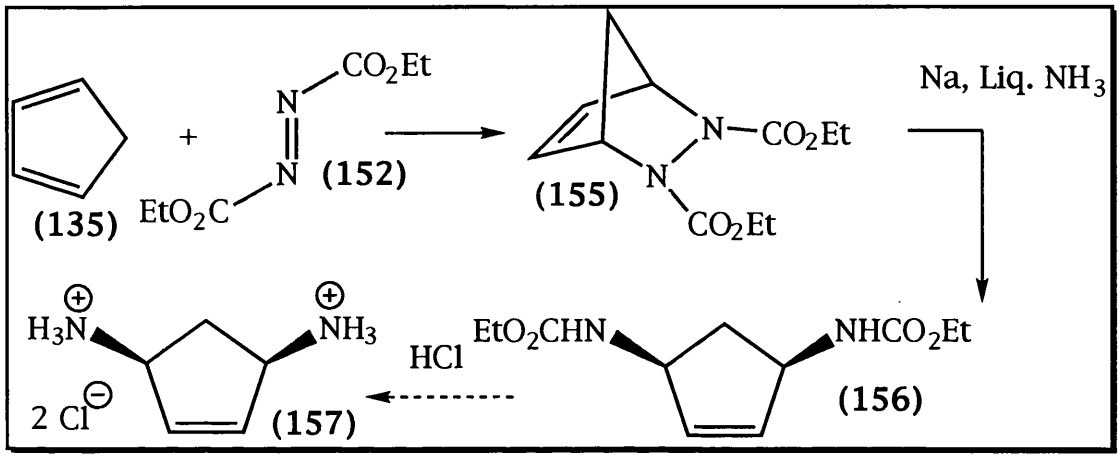
The Diels Alder reaction between cyclopentadiene (135) and DEAD (152) was completed with a yield of 96%.[101] As illustrated by Spectrum 3, the proton NMR spectrum of (155) clearly shows the two ethyl groups, *i.e.* the triplet at δ 1.19 and the quartet at δ 4.11. The two apex protons appear as a multiplet at δ 1.63, while the other two multiplets further up field correspond

Spectrum 3 The ^{1}H NMR Spectrum (200 MHz, $CDCl_3$) of Diethyl 2,3-Diazobicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate (155).



to the bridgehead protons (δ 5.05) and the olefinic protons (δ 6.42). The nitrogen-nitrogen cleavage in Scheme 43 was attempted and failed.

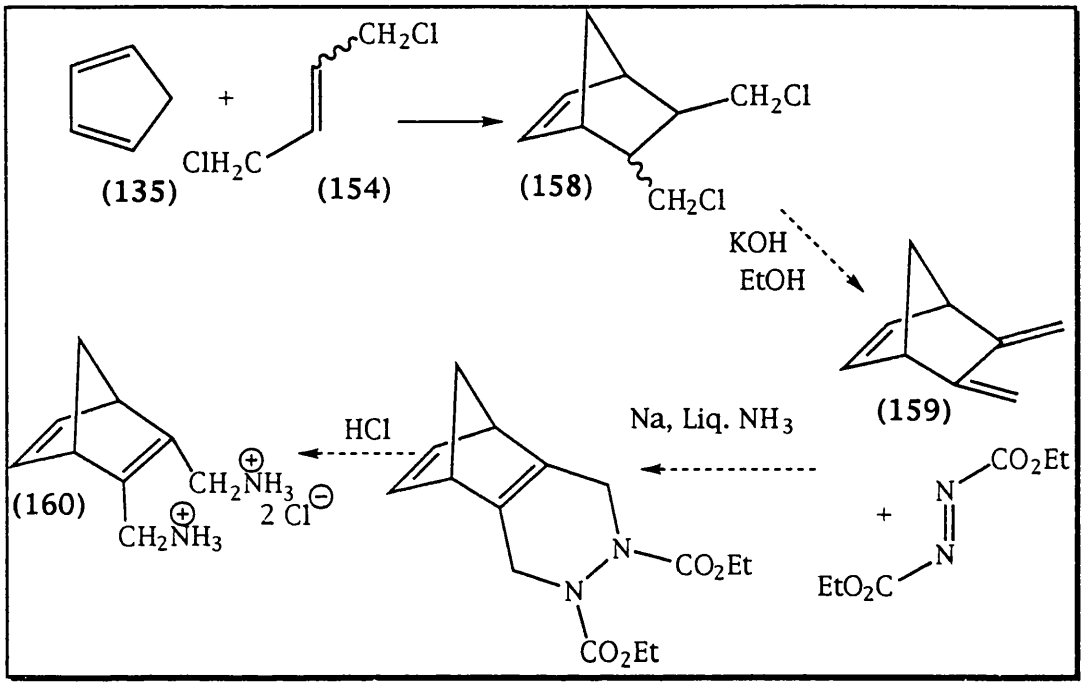
Scheme 43



The route shown in Scheme 44 was prevented from being taken to the cleavage step by the initial failure of the Diels Alder[102] reaction between cyclopentadiene (135) and 1,4-dichlorobut-2-ene (154) (see Section 2.7). The first attempt at this Diels Alder reaction involved heating compounds (135) and (154) at reflux for 4 hours. The resulting mixture was distilled and the clear liquid which came off was identified as starting material (154). The reaction was repeated with a reaction time of 16 hours but only a black solid could be crystallised out of the glutinous black material left upon distillation. Analysis was unsuccessful with no evidence to suggest that (158)[102, 103] had been formed. Future work on this route would entail (158) being heated at reflux for 4 h with ethanol and potassium hydroxide to produce (159). Compound (159) would then be treated with DEAD in a Diels Alder reaction[104] to give the tricyclic diazo compound shown in Scheme 44. The subsequent hydrolysis of the carbamate groups

and decarboxylation would complete the synthesis of the diamine (160). This route warrants more work in the future.

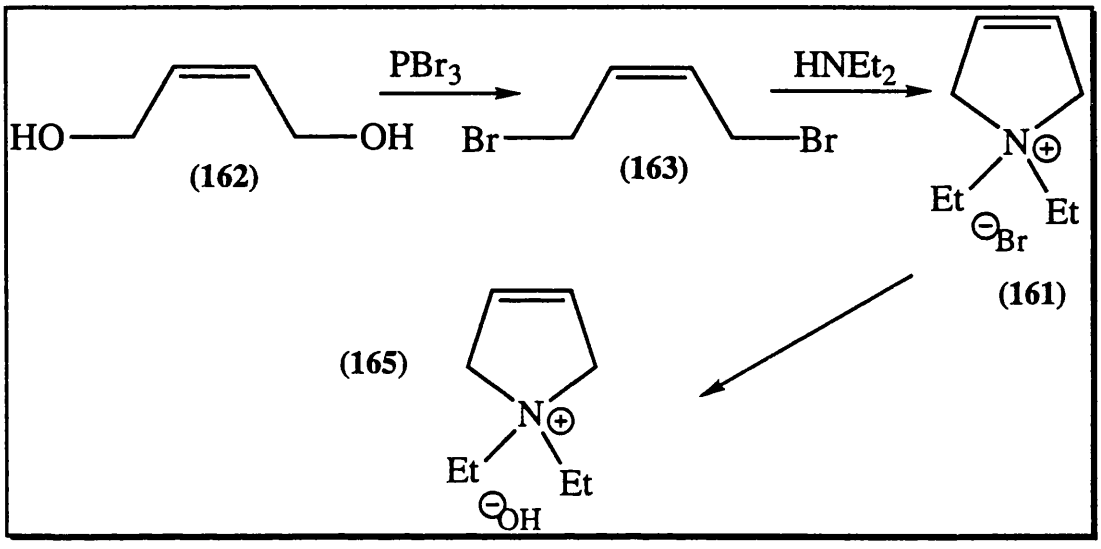
Scheme 44



An early attempt, in this project, at synthesising *(Z)*-*N,N,N',N'*-tetraethyl-1,4-diaminobut-2-ene (Z-TED) (92) resulted in a ring closing reaction producing the quaternary salt *N,N*-diethyl-2,5-dihydropyrrolium (161) (Scheme 45). The *(Z)*-1,4-but-2-ene diol (162) was commercially available and a procedure by Feigenbaum and Lehn[68] was followed, using phosphorus tribromide to brominate the diol (162). *cis*-Dibromobut-2-ene (163) was yielded as a light coloured oil which, after time, changed into the crystalline solid *trans*-dibromobut-2-ene. Due to this conversion the dibromo compound (163) was freshly prepared for each reaction. The ring forming reaction, which seems quite obvious in retrospect, produced (161) and diethylamine hydrobromide. The first attempt at separating these

salts was via crystallisation. Several solvent systems were tried but none gave the separation required. The use of an ion exchange column was more successful. The general literature

Scheme 45

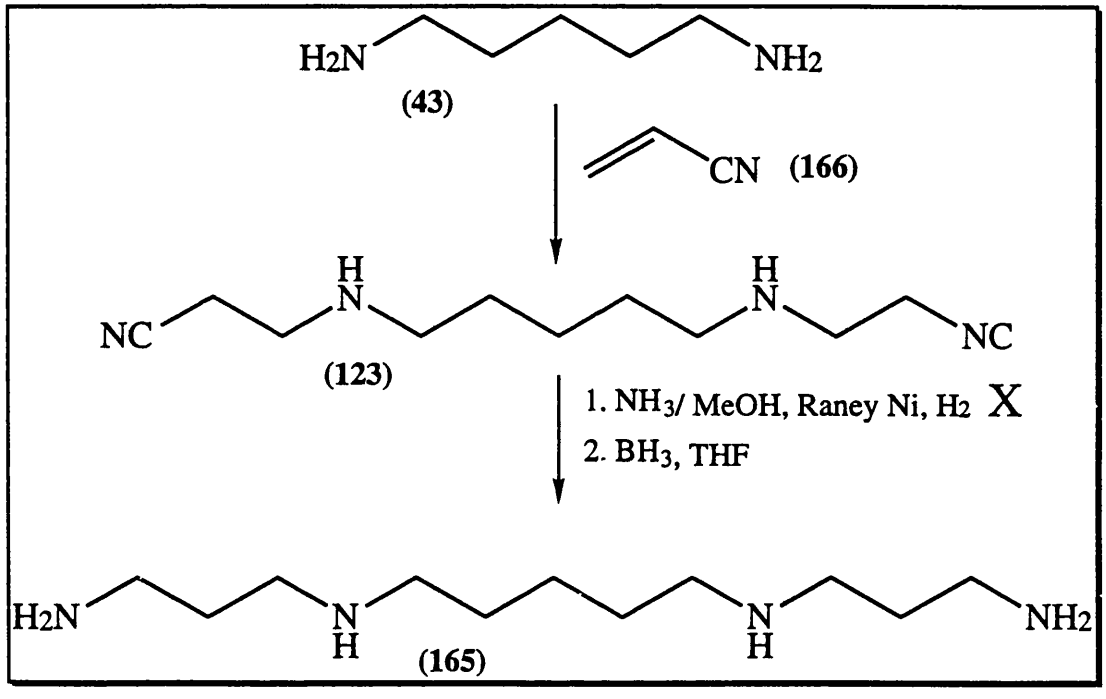


recommended Amberlite IRA400 resin treated with sodium hydroxide in order to exchange the bromide ions with the hydroxide ions. The yellow oil produced could not be further purified or solidified possibly due to the hygroscopic nature of *N,N*-diethyl-2,5-dihydropyrrolium hydroxide (164).

Scheme 46 illustrates the synthesis of *N,N'*-bis(3-propylamino)-1,5-diaminopentane (165). This compound was requested by Dr. Dale Walters in order to obtain evidence that it was produced by one of the fungi he had in culture. The first step is a simple cyanoethylation via a Michael type reaction using acrylonitrile (166) and cadaverine (43).[79] The reduction of the dinitrile (123) in the second step was unsuccessful at the first attempt when methanol saturated with ammonia and Raney nickel and hydrogen were used.[79] Raney nickel is sometimes known to be unreliable and it was for this reason that another

method was attempted. Borane in THF[81] was found to be a more effective alternative although the optimal reaction conditions were not found and the product (165) was contaminated with starting material (123). Chromatography was unsuccessful in purifying the product.

Scheme 46



2.7 The Diels Alder Reaction

In 1928, Diels and Alder[105] first reported what has now become one of the most important methods for constructing cyclic compounds in all of organic chemistry. They were awarded the Nobel Prize for it in 1950, and since that time countless variations have been reported.[106, 107, 108]

In the Diels Alder reaction a double bond (dienophile) adds 1,4 to a conjugated diene (a [4 + 2] cycloaddition) so that the product is always a six-membered ring. This can be explained in terms of molecular orbital theory.[109] Analysis of a reaction by

frontier orbital theory can predict reactivity and stereochemistry. There are four rules for Diels Alder reactions based on molecular orbital arguments.[109,110] These rules are as follows.

1. Electron withdrawing substituents (Z) on dienophiles and electron donating substituents (X) on dienes increase the rate of reaction.

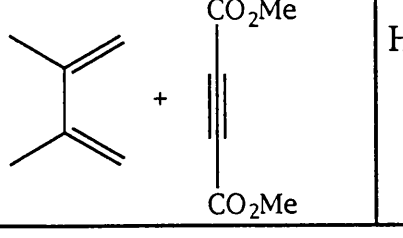
2. The diene and dienophile configurations are retained in the adduct (the *cis*-principle).

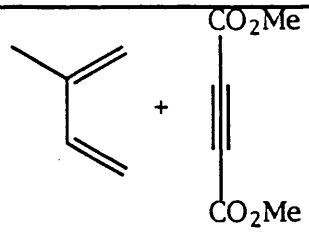
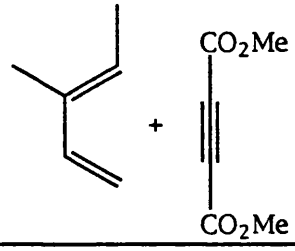
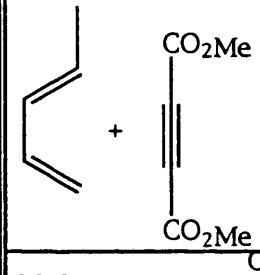
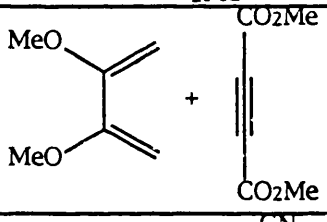
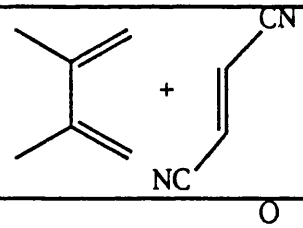
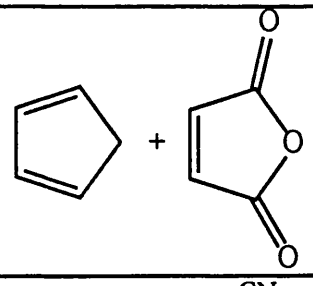
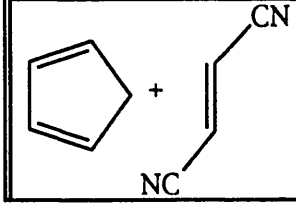
3. The *endo*-transition state is favoured over the *exo*-transition state (the *endo*-rule).

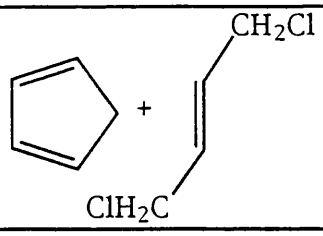
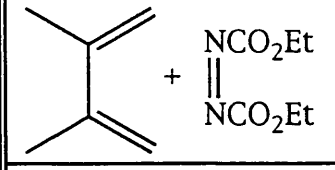
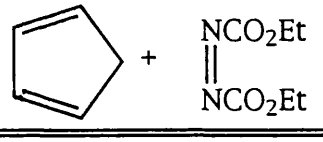
4. (Z)-Substituted dienophiles react with 1-substituted butadienes (in normal electron demand Diels Alder reactions) to give 3,4-disubstituted cyclohexenes, independent of the nature of the diene substituents (the *ortho*-effect).

The Diels Alder reaction takes place very readily for a wide variety of reactants. Table 6 summarises the Diels Alder reactions completed in this project and includes the outcome of the experiments.

Table 6

Diels Alder Product	Reaction Conditions	Outcome/ Yields
	H_2O, 60°C, 24 h	74% Yield

	a) neat, R.T., 16 h b) sealed tube, 60°C, 24 h c) benzene, 60°C, 24 h	65% Yield
	sealed tube, 60°C, 24 h	40% Yield
	benzene, sealed tube, 60°C, 48 h	26% Yield
	sealed tube, 70°C, 24 h	Mixture
	toluene, 100°C, 16h	53% Yield
	ether, room temp.	60% Yield
	ethanol, 0°C-room temp.	75% Yield

	neat, reflux, 2h	Black oil
	benzene, room temp., 14 h	84 % Yield
	neat, reflux, 0.5 h	96% Yield

For the purposes of this work, all the dienophiles were symmetrical and therefore the possibility of having a mixture of products was not a concern. Reaction is favoured by electron-withdrawing substituents in the dienophile. Briefly, when considering the dienophiles used in this project, most have electron withdrawing substituents; -CO_2Me, -CN, and maleic anhydride which has the equivalent of two electron withdrawing substituents. The lack of reactivity of the -CH_2Cl substituent is not surprising because of the weaker inductive effect of chlorine substituents. Most of these dienophiles would be predicted, on the basis of FMO theory, to be very reactive under Diels Alder conditions. The yields of reactions where dimethyl acetylenedicarboxylate (97) was the dienophile varied and obviously the reactivity of the diene used in each case must effect the progress of the reaction. Table 6 clearly shows that the position of an electron donating methyl group influences the yields of the reactions. It is perhaps surprising that the yields should vary so much for this set of reactions. The reaction between 2,3-dimethoxybuta-1,3-diene (105) and dimethyl acetylenedicarboxylate (97)[111, 112] gave a mixture of products

which could not be purified and perhaps needed harsher conditions for complete reaction.

When substituents are attached to a diene, effects on the frontier orbitals are expected to be similar to those observed in alkenes. Cyclopentadiene (135) was used as a diene with three different alkenes. In the Diels Alder reaction with 1,4-dichlorobut-2-ene (154) the alkene, although more stable in the *trans*-position, should still react. Again, the reason for failure could be that reaction conditions were not strong enough. Cyclopentadiene gave reasonable yields of cyclic products with maleic anhydride (134)[89] and fumaronitrile (120).[113]

Two hetero-Diels Alder reactions reactions were completed in this project, both with very good yields. Diethyl azodicarboxylate (DEAD) (160) is a useful reagent when used as a dienophile in hetero-Diels Alder reactions particularly when the target compound is a pyridazine.

CHAPTER 3

ANTIFUNGAL TEST RESULTS

3.1 Introduction

All the compounds synthesised in this project were sent for antifungal testing to Dr Dale Walters (Department of Plant Sciences, West of Scotland Agricultural College, Auchincruive, Ayrshire). The testing regime consisted of preliminary studies *in vitro* and *in vivo*. If a compound was deemed of further interest then work would continue in the glasshouse or in field trials.

The *in vivo* testing dealt with the following range of variables.

1) Type of fungi
2) Type of plant
3) Location of plant
4) Timing of spray pre-inoculation
5) Timing of spray post-inoculation
6) Concentration of active material in spray
7) Type of application (*e.g.* spray or root treatment)
8) Method of measurement of infection

Not all variables were able to be tested for every compound but the effects of a few compounds were investigated in great depth. Some of the types of fungi involved and their common names are listed below;

Main Fungus Used:
Erysiphe graminis DC f.sp. *hordei* Marchal
(Powdery mildew on barley)

Other Fungi Used:

Uromyces viciae-fabae (Pers.) Schroet

(Rust on broad bean)

Botrytis fabae Sardina

(Chocolate spot on broad bean)

Podosphaera leucotricha (Ell.& Ev.) Salm.

(Powdery mildew on apple)

Phytophthora infestans (Mont) De Bary

(Late blight on potato)

The range of different types of plant used in these studies included barley (*Hordeum vulgare* L. cv. Golden Promise), broad bean (*Vicia faba* L. cv. Express Long Pod) and apple (*Malus bitenfelder*). A review of the historical and economic effects that some of these fungi have had on society is discussed in Section 1.1.

Whether a compound has protective or curative action can be assessed by applying the compound before or after the fungal pathogen has been inoculated. Varying the timing of the application can give information on a) the persistence of the compound and b) at which stage of fungal growth the compound is active.

The method of measuring the amount of fungal infection is not precise. As with many biological systems, accurate quantitative results can be hard to obtain. Infection intensity was assessed as follows.[114]

For mildew and rust: 6-10 days or 17 days respectively, after inoculation the percentage leaf area with postules was estimated using a standard area diagram.

For chocolate spot; 7 days after inoculation the percentage leaf area infected was estimated using a standard area diagram.

The second type of testing, *in vitro*, was completed in a sterile environment and allowed the measurement of key parameters.[114, 115]

The *in vitro* testing parameters include the following.

1) Type of fungi

2) Type of medium

3) Procedure used

Several fungi were used in this *in vitro* work; *P. infestans* (potato blight), *Pyrenophora avenae* Ito & Kuribay (oat patogen), *Botrytis cinerea* Pers. ex Fr. (grey mould) and *Pyricularia oxyzae* Br. & Cav. (rice blast). The selected results which are quoted here are all for work done on the fungi *Pyrenophora avenae* Ito & Kuribay (oat patogen). Sterile medium containing the analogue, with a plug of mycelium in the center, were placed in a Petri dish. The colony growth was measured radially after 3, 6 and 8 days. In order to measure the enzyme and polyamine activities, flasks were prepared with sterile liquid potato dextrose agar, the analogue being assessed and a disc of mycelium. After 4 days the mixture was washed and centrifuged in order to obtain material for analysis.[10]

The resulting effects on the following were assessed.

1) ODC levels

2) AdoMet levels

3) Polyamine levels

A further range of parameters were investigated on only a few compounds. These included the following.

1) Comparison to commercial fungicides

2) Formulation of spray

3) Transport within plant

The test results are extensive and go into great detail, covering many different aspects. For the purpose of this chapter only a summary of all the fungicidal effects is detailed. This covers the results of importance and of interest but is no means an exhaustive account of all the studies completed.

All these results should help give some idea of the mode of action of the analogue against the fungi.

3.2 Fungicidal Activity *In Vivo*

The fungicidal effects of compounds synthesised in this project were determined by applying them to plants in aqueous solution via a spray,[114] unless otherwise stated. All compounds were initially tested against *Erysiphe graminis* DC f.sp. *hordei* Marchal so that a comparable overall picture of relative fungicidal activity could be drawn up (Tables 7, 8 and 9). Obviously this is a very limited view as every fungi on different types of plant will be affected in a different way by each compound. Powdery mildew on barley was chosen as both plant and fungi grow well at Auchincruive where there is a good knowledge of both. The barley seedlings (*Hordeum vulgare* L. cv. Golden Promise) were grown in trays in a glasshouse and, once at the stage of the second leaf

unfolding, were sprayed before or after inoculation with the powdery mildew fungus.[114] All compounds were applied as 1mM concentrations unless otherwise stated.

The following three tables: Table 7, E-BED and analogues; Table 8, E-TED and analogues; and Table 9, BAD and cyclic compounds, show the disease % control that compounds had on powdery mildew on barley. Some of the compounds were tested pre-inoculation and also over various time periods post-inoculation, however, for the purpose of this summary these tables show only the best of the post-inoculation results.[114, 115, 116, 117]

Table 7 Effects of E-BED, Salts and Analogues on Powdery Mildew on Barley.

Compound	Disease % Control
E-BED.2HCl	75
E-BED benzoate	57
E-BED phosphate	42
E-BED fumarate	65
E-BED propionate	69
Z-BED.2HCl	42
(E)-N,N'-Diethyl-1,4-diaminobut-2-ene dihydrobenzoate	20
(E)-N,N'-Dipropyl-1,4-diaminobut-2-ene dihydrobenzoate	20
(E)-N,N'-Dibutyl-1,4-diaminobut-2-ene dihydrobenzoate	14

Table 8 Effects of E-TED, Salts and Z-TED on Powdery Mildew on Barley.

Compound	Disease % Control
E-TED.2HCl	80
E-TED benzoate	54
E-TED phosphate	68
E-TED fumarate	65
E-TED propionate	48
Z-TED	66

Table 9 Effects of BAD and Cyclic compounds on Powdery Mildew on Barley.

Compound	Disease % Control
BAD	93
1,2-bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene	76
(E)-1,2-Bis(dimethylaminomethyl)-cyclobutane	87
(E)-1,2-Bis(dimethylaminomethyl)-cyclopentane	22
(E)-1,2-Bis(dimethylaminomethyl)-cyclohexane[b]	52
(E)-1,2-Bis(diethylaminomethyl)-cyclobutane	54
(E)-1,2-Bis(diethylaminomethyl)-cyclopentane	76
(E)-2,3-Bis(aminomethyl)norborn-5-ene	88
(Z)-2,3-Bis(dimethylaminomethyl)norborn-5-ene dihydrochloride	32
N,N-Diethyl-2,5-dihydropyrrolium hydroxide[a]	15

[a] Mildew on apple seedling instead of barley.

[b] Concentration of 1.23mM was used.

It is difficult to do a direct comparison when experiments were done on different days, however these results indicate that

BAD was of exceptional antifungal activity against mildew with compounds such as E-BED, E-TED, E-1,2-bis(dimethylaminomethyl)-cyclobutane and E-2,3-bis(amino-methyl)norborn-5-ene also having good disease control.

In a time-course experiment against powdery mildew (Table 10), E-BED gave greatest control 5 days after inoculation which suggests that it acts as a curative fungicide as well as having preventative properties. BAD, as indicated above, possesses substantial fungicidal activity, although this seems to be most effective when it is applied after inoculation of the fungi. E-TED gave the best control when applied two days after inoculation. In fact all analogues seemed to be much more active when applied as post-inoculation sprays.

Table 10 Effect of Timing and Determination of curative and/or preventative action of a Range of Compounds on Powdery Mildew on Barley.

Compound	% control Pre-inoc[a]	% control Post-inoc[b]
E-BED.2HCl	64 (1d)[c] 53 (2d) 65 (5d)	67 (1d) 74 (2d) 75 (5d) 75 (8d)
Z-BED.2HCl	53 (3h)	42 (3h)
E-TED.2HBr	26 (1d) 46 (2d) 34 (5d)	80 (3h) 39 (1d) 82 (2d) 70 (5d)
Z-TED	40 (3h)	66 (3h)
BAD	61 (3h)	93 (3h)
1,2-Bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene	43	76 73 (6d) 61 (8d) 76 (10d)

(E)-1,2-Bis(diethylaminomethyl)-cyclopentane	12	76 73 (6d) 67 (8d) 76 (10d)
(E)-2,3-Bis(aminomethyl)norborn-5-ene	52	87 88 (6d) 84 (8d) 87 (10d)

[a] Disease % control when analogue applied pre-inoculation

[b] Disease % control when analogue applied post-inoculation

[c] The time periods in brackets refer to the timing of the spray either before or after inoculation.

In an effort to see the effects on other plants and fungi, some of our compounds were tested on *Uromyces viciae-fabae* (Pers.) Schroet (Rust on broad bean) and *Botrytis fabae* Sardina (Chocolate spot on broad bean). The preparation of plants and fungi for this experiment are described by Havis and Walters.[114] Table 11 shows the results.

Table 11 Effect of a Range of Compounds on rust and chocolate spot on broad bean.

Compound	% control Rust	% control Choc. spot
E-BED.2HCl	72	91
Z-BED.2HCl	56	79
E-TED.2HBr	60	45
Z-TED	71	-
1,2-Bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene	0	62
(E)-1,2-Bis(diethylaminomethyl)-cyclopentane	22	58
(E)-2,3-Bis(aminomethyl)norborn-5-ene	0	40

Growth of both rust and chocolate spot were significantly reduced in the presence of E-BED. Chocolate spot was especially affected with a disease percentage control of 91 %. The cyclic compounds were much less active, with only one of them, *(E)*-1,2-bis(diethylaminomethyl)cyclopentane, having any antifungal activity at all against rust. BAD did not reduce the growth of other fungi to any extent (data not shown).

3.3 *In vitro* Results

The *in vitro* testing can be split into two categories. The first is a measure of the effects that various compounds had on the mycelial growth of *Pyrenophora avenae* Ito & Kuribay (oat pathogen).

In Table 12 all compounds were applied as 1mM concentrations unless otherwise stated.

Table 12 Effect of a Range of Compounds on oat pathogen *in vitro*.

Compound	Disease % Control
E-BED.2HCl	58
Z-BED.2HCl	10
E-TED.2HBr[a]	59
1,2-Bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene	59
(E)-1,2-Bis(diethylaminomethyl)-cyclopentane	26
(E)-2,3-Bis(aminomethyl)norborn-5-ene	29

[a] 0.5 mM was used

E-BED, E-TED and 1,2-bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene all possess significant antifungal activity against oat pathogen *in vitro.*

The second set of results from *in vitro* testing measures the enzyme activities and polyamine concentrations in *Pyrenophora avenae* (oat pathogen). The activities of the two enzymes ornithine decarboxylase (ODC) and *S*-adenosyl methionine decarboxylase (AdoMetDC) were assayed. The concentration of putrescine, spermidine and spermine were determined and results shown in Table 13.

Table 13[a] Effect of a Range of Compounds on ODC and AdoMetDC Activity.

Compound	ODC Activity	AdoMetDC Activity
E-BED.2HCl	87 dec	82 dec
Z-BED.2HCl	31 dec	82 dec
E-TED.2HBr	81 inc	62 dec
1,2-Bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene	18 dec	>100 inc
(E)-1,2-Bis(diethylaminomethyl)-cyclopentane	34 dec	45 inc
(E)-2,3-Bis(aminomethyl)norborn-5-ene	60 inc	7 dec

[a] Results in Tables 12 and 13 are given as percentages of the control.

Table 14 Effect of a Range of Compounds on Polyamine Concentrations

Compound	Putres. Conc.[a]	Spermid. Conc.[b]	Spermine Conc.[c]
E-BED.2HCl	>100 inc	32 dec	61 inc
Z-BED.2HCl	no effect	41 dec	21 inc

E-TED.2HBr	49 dec	19 dec	4 dec
1,2-Bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene	68 dec	no effect	12 dec
(E)-1,2-Bis(diethylaminomethyl)-cyclopentane	84 dec	55 dec	64 dec
(E)-2,3-Bis(aminomethyl)norborn-5-ene	47 dec	>100 inc	54 dec

[a] Concentration of putrescine.

[b] Concentration of spermidine.

[c] Concentration of spermine.

All of the analogues tested were shown to alter polyamine biosynthesis in some way. Table 14 illustrates that all the compounds significantly reduced the concentration of putrescine, apart from E-BED and Z-BED. Interestingly, although E-BED greatly decreased ODC and AdoMetDC levels in oat pathogen, the levels of putrescine within the cells accumulated in vast quantities. It is also perhaps surprising that two putrescine analogues, such as E-BED and E-TED should effect polyamine levels in such different ways.

3.4 The Commercial Aspect

It can be seen from all the results in the two preceding sections that several compounds displayed very impressive antifungal activity. However, in order to quantify these results in a wider sense it was necessary to do a direct comparison of our analogues with commercial fungicides already on the market. In a glasshouse evaluation, several commercial fungicides including Propiconazole (37), Tridemorph and Flutriafol were compared to

our compounds (see Section 1.3.2 for commercial fungicide review). Briefly, E-TED compared favourably to Propiconazole, a triazole systemic fungicide, in reduction of mildew. E-BED and BAD gave similar results as Propiconazole. However both E-BED and BAD out-performed Flutriafol.

Due to these encouraging results, E-BED was formulated through British Technology Group (BTG) and field trials initiated. It was found that E-BED significantly reduced mildew infection early in the season, but had no effect on mildew later on in the season.

A package has been prepared by BTG for distribution to interested agrochemical companies.

CHAPTER 4

EXPERIMENTAL

4.1 General

Melting points were measured on a Gallenkamp melting point apparatus. Infra red spectra were obtained using a Perkin Elmer 500 spectrometer. Nuclear magnetic resonance spectra were recorded with a Perkin Elmer R 32 spectrophotometer operating at 90 MHz (δ_H), or a Bruker WP200-SY spectrophotometer operating at 200 MHz (δ_H) or 50.3 MHz (δ_C). The multiplicities of the ^{13}C NMR spectra were determined using DEPT spectra with pulse angles of $\theta = 90°$ and $\theta = 135°$. Spectra were recorded with either tetramethylsilane at 0 ppm or the NMR solvent as the internal standard. Mass spectra were obtained using A.E.I. MS 12 or 902 spectrometers. Elemental analysis were performed with a Carlos-Erba 1106 elemental analyser.

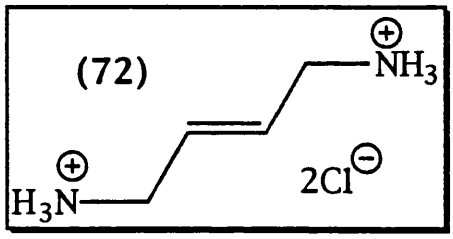
Thin layer chromatography (TLC) was carried out on Merck GF_{254} silica gel plates of 0.25 mm thickness. UV was used to detect compounds on the plates. Chromatographic purification was carried out by dry column flash chromatography using Merck 70-230 mesh.

All solvents were purified by standard techniques.[118] Tetrahydrofuran (THF) and diethyl ether were dried by distillation from sodium/benzophenone under nitrogen immediately before use. Organic solvents were dried using either anhydrous sodium sulphate or anhydrous magnesium sulphate.

Solvents were evaporated off under reduced pressure below 50 °C.

4.2 E-BED and Analogues

Synthesis of *(E)*-1,4-Dibromobut-2-ene Dihydrochloride (E-BED)



Procedure A

This synthesis was carried out by adapting the procedure of Macholan.[35] Potassium phthalimide (20 g, 108 mmol) was added in portions over 2 h to a stirred solution of *(E)*-1,4-dibromobut-2-ene (10.7 g, 50 mmol) in dimethylformamide (DMF) (100 ml) at room temperature. The mixture was stirred for a further 3 d at this temperature, then poured into water (100 ml), and the mixture was extracted with dichloromethane (DCM) (5 × 100 ml). The organic extracts were dried, filtered and concentrated *in vacuo* to leave DMF (*ca.* 30 ml), and a white solid, which was washed with ether (3 × 10 ml) to give *(E)*-1,4-diphthalimidobut-2-ene (14.96 g, 86%), ν_{max}/cm^{-1} (KBr disc) 3461, 2929, 1765, 1708, 1613 and 1462; δ_H (200 MHz, $CDCl_3$) 4.27 (4H, m), 5.80 (2H, m), and 7.79 (8H, m); δ_C (50MHz, $CDCl_3$) 38.7 (CH_2), 123.3 (CH), 127.3 (CH, olefinics), 132.1 (C), 134.0 (CH), and 167.8 (CO); *m/z* 347 (M^++1), 346 (M^+, 5.1%), 199 (100%), 186, 160, 133 and 104 (Found: C, 69.22; H, 4.13; N, 8.10%; M^+, 346.0943. $C_{20}H_{14}N_2O_4$ requires C, 69.36; H, 4.04; N, 8.09%; M^+, 346.0953).

(E)-1,4-Diphthalimidobut-2-ene (14 g, 40mmol) was suspended in glacial acetic acid (160 ml), and concentrated hydrochloric acid (160 ml) was added. The mixture was heated at reflux until all the *(E)*-1,4-diphthalimidobut-2-ene had dissolved, then for a further 24 h. The solution was cooled, filtered, and the solvents were concentrated *in vacuo* to *ca.* 10 ml. The precipitate was collected and washed with ether to afford (*E*)-1,4-diaminobut-2-ene dihydrochloride (3.98 g, 62%), δ_H (200 MHz, D_2O) 3.58 (4H, d) and 5.89 (2H, m); δ_C (50 MHz, D_2O) 41.2 (CH_2) and 128.8 (CH); (Found: C, 30.01; H, 7.35; N, 17.53%; $C_4H_{12}N_2Cl_2$ requires C, 30.17; H, 7.60; N, 17.60%).

Procedure B

This synthesis was carried out by adapting the procedure of Bottini *et al.*[36] Hexamethylenetetramine (7.19 g, 51.39 mmol) in chloroform (175 ml) was stirred under reflux while *(E)*-1,4-dibromobut-2-ene (5 g, 23.36 mmol) was added dropwise over a period of 45 min. Precipitation of the product was noted soon after the first addition of *(E)*-1,4-dibromobut-2-ene. After the addition was complete, the reaction mixture was stirred under reflux for 3 h and was allowed to stand overnight. The mixture was cooled in an ice bath and the solid was filtered off to give the crude hexaminium bromide salt (10.6 g, 92%), ν_{max}/cm^{-1} (KBr disc) 3458, 3388, 2976 and 2894; δ_H (200 MHz, D_2O) 3.56 (4H, m), 4.64 (12H, s), 5.00 (12H, m) and 6.16 (2H, m); δ_C (50 MHz, D_2O) 58.2 (CH_2), 70.8 (CH_2), 79.2 (CH_2) and 128.8 (CH).

The hexaminium bromide salt (10 g, 20 mmol) was dissolved in a warm solution prepared from water (15 ml), ethanol (75 ml), and 10 M hydrochloric acid (23 ml). A white

precipitate of ammonium chloride formed within 1 h and the precipitate was removed. The mother liquor was evaporated to dryness and the residue was dissolved in water (15 ml). The solution was cooled in an ice bath and made strongly alkaline (pH 13) with sodium hydroxide solution (6 M, 20 ml). This solution was extracted with DCM (5 × 30 ml) and the extracts were combined, dried ($MgSO_4$), and concentrated *in vacuo* to yield E-BED free base (0.43 g, 25%), δ_H (200 MHz, $CDCl_3$) 0.91 (4H, bs), 2.73 (4H, m) and 5.14 (2H, m); δ_C (50 MHz, $CDCl_3$) 43.2 (CH_2) and 130.69 (CH); *m/z* 86, 83, 69 (100%), 56, 41 and 30.

Synthesis of *(E)*-1,4-Diaminobut-2-ene and Salts

(E)-1,4-Diaminobut-2-ene dihydrochloride (1.21 g, 7.6 mmol) was dissolved in the minimum amount of water (10 ml), added to ether (100 ml) and stirred vigorously. Potassium carbonate (40 g) was added and the stirring was continued for 0.5 h. The solid was filtered off, and the filtrate was concentrated *in vacuo* to give *(E)*-1,4-diaminobut-2-ene as an oil (0.46 g, 70%), δ_H (90 MHz, $CDCl_3$) 1.80 (4H, s), 3.3 (4H, m) and 5.7 (2H, m).

Preparation of *(E)*-1,4-Diaminobut-2-ene Benzoate (78)

(E)-1,4-Diaminobut-2-ene (1 equiv.) was stirred with benzoic acid (2 equiv.) in benzene for 1 h. The precipitate was filtered and washed with ether to afford a white solid (0.79 g, 68%), ν_{max}/cm^{-1} (KBr disc) 3061, 3032, 1695, 1624, 1547, 1523, 1398; δ_H (200 MHz, D_2O) 3.46 (4H, m), 5.78 (2H, m), and 7.38 (10H, m); δ_C (50 MHz, D_2O) 41.1 (CH_2), 106.3 (CH), 129.0 (CH), 129.3 (CH), 129.8 (CH) and 132.8 (CO); *m/z* 122 (M^+, 64.5%), 105, 77, 51, 28 and 18.

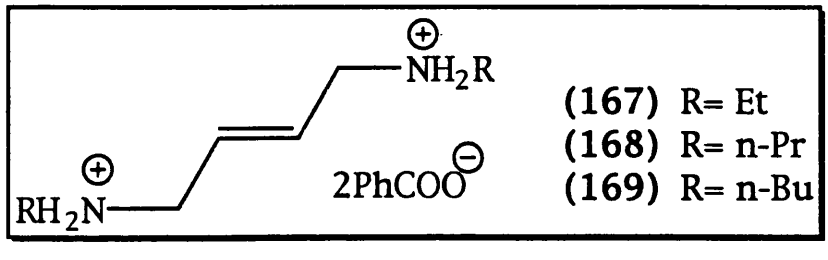
The following three compounds were prepared by this method.

(E)-1,4-Diaminobut-2-ene Phosphate (75) (2.81 g, 85%), ν_{max}/cm^{-1} (KBr disc) 3069, 3007, 2926, 2872, 1635, 1601, 1466, 1450, 1332, 983 and 924; δ_H (200 MHz, D_2O) 3.42 (4H, m) and 5.73 (2H, m) ppm; δ_C (50 MHz, D_2O) 41.3 (CH_2) and 128.9 (CH); *m/z* 98, 81, 28 and 18 (100%).

(E)-1,4-Diaminobut-2-ene Fumarate (76) (0.75 g, 70%), ν_{max}/cm^{-1} (KBr disc) 3430, 3071, 3009, 1680, 1560, 1450, 1275 and 983; δ_H (200 MHz, D_2O) 3.48 (4H, m), 5.80 (2H, m) and 6.51 (4H, m); δ_C (50 MHz, D_2O) 41.1 (CH_2), 128.7 (CH), 135.5 (CH) and 172.2 (CO); *m/z* 116, 98, 88, 45 (100%), 27 and 18.

(E)-1,4-Diaminobut-2-ene Propionate (77) (0.41 g, 83%), ν_{max}/cm^{-1} (KBr disc) 2970, 2934, 1647, 1549, 1406 and 976; δ_H (200 MHz, D_2O) 0.84 (6H, t), 1.99 (4H, q), 3.41 (4H, m), and 5.72 (2H, m); δ_C (50 MHz, D_2O) 10.2 (CH_3), 29.9 (CH_2), 41.0 (CH_2), 128.7 (CH) and 183.5 (CO); *m/z* 74, 56, 45, 28(100%) and 18.

Synthesis of *(E)-NN'*-Diethyl-1,4-diaminobut-2-ene Dihydrobenzoate (167)



To a stirred solution of ethylamine (1.35 g, 30 mmol) in chloroform (25ml) was added (*E*)-1,4-dibromobut-2-ene (1.07 g, 5 mmol) in chloroform (75 ml) dropwise at room temperature, and the solution was stirred for 24 h. The solvents were removed *in vacuo* to give an oil (0.2 g, 28%). This oil was then dissolved in warm ether (5 ml) and excess benzoic acid (in benzene) was added dropwise. The mixture was stirred for 1 h and the precipitate was filtered off and washed with ether to afford a hygroscopic white solid (1.8 g, 33%), ν_{max}/cm^{-1} (KBr disc) 2945, 2799, 1701, 1686, 1655, 1581, 1448 and 1380; δ_H (200MHz, D_2O) 1.06 (6H, t), 2.83 (4H, q), 3.48 (4H, m), 5.79 (2H, m) and 7.58 (10H, m); δ_C (50MHz, D_2O) 11.3 (CH_3), 43.1 (CH_2), 48.2 (CH_2), 129.1 (CH), 129.3 (CH), 130.0 (CH), 133.1 (CH) and 174.4 (CO); *m/z* 122, 105, 97, 77 (100%), 51, 44, 28 and 18.

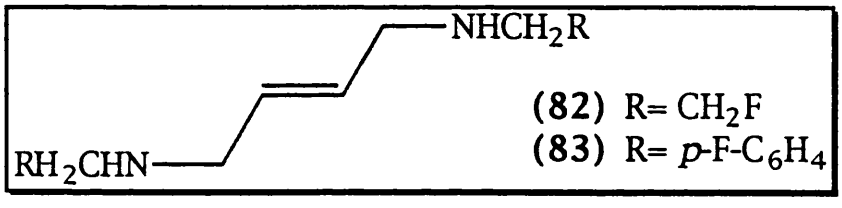
Two other compounds were prepared by this method.

(E)-NN'-Dipropyl-1,4-diaminobut-2-ene Dihydrobenzoate (168)
Free base 0.73 g, (85%); salt (hygroscopic) 1.25 g, (68%), ν_{max}/cm^{-1} (KBr disc) 2964, 2941, 1709, 1583, 1448 and 1381; δ_H (200 MHz, D_2O) 0.71 (6H, t), 1.41 (4H, m), 2.66 (4H, t), 3.42 (4H, m), 5.77 (2H, m) and 7.50 (10H, m); δ_C (50 MHz, D_2O) 11.2 (CH_3), 20.0 (CH_2), 48.7 (CH_2), 49.3 (CH_2), 129.1 (CH), 129.3 (CH), 130.1 (CH), 135.6 (CH) and 174.2 (CO); *m/z* 122, 112, 105, 82, 77 (100%), 51, 39, 28 and 18.

(E)-NN'-Dibutyl-1,4-diaminobut-2-ene Dihydrobenzoate (169)
Free base 0.45 g, (45%); salt (hygroscopic) 2.03 g, (91%). ν_{max}/cm^{-1} (KBr disc) 2955, 2930, 1628, 1599, 1560, 1375; δ_H (200 MHz,

D_2O) 0.68 (6H, t), 1.12 (4H, m), 1.40 (4H, m), 2.78 (4H, t), 3.47 (4H, m), 5.78 (2H, m) and 7.42 (10H, m); δ_C (50 MHz, D_2O) 13.5 (CH_3), 19.9 (CH_2), 28.3 (CH_2), 47.6 (CH_2), 48.7 (CH_2), 129.0 (CH), 129.1 (CH), 129.5 (CH), 132.0 (CH) and 174.0 (CO); *m/z* 126, 122, 105 (100%), 82, 77, 72, 55, 51, 28 and18 (Found: C, 70.54; H, 8.86; N, 6.21%. $C_{26}H_{38}N_2O_4$ requires C, 70.58; H, 8.59; N, 6.33%).

Attempted Synthesis of Fluorinated 1,4-diaminobut-2-enes



(82) R= CH_2F
(83) R= *p*-F-C_6H_4

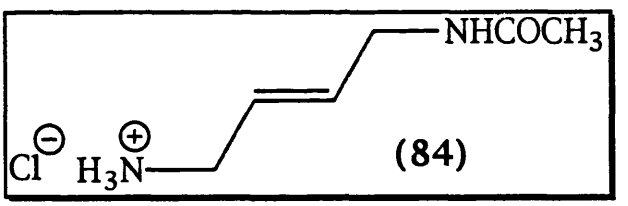
Attempted Synthesis of *NN'*-Bis(2-fluoroethyl)-1,4-diaminobut-2-ene (82)

A suspension of 2-fluoroethylamine hydrochloride (0.25 g, 2.25 mmol) in tetrahydrofuran (THF) (10 ml) was stirred at 0 °C, and 5% sodium hydroxide solution was added dropwise (0.1 g, 2 ml). The solid salt dissolved and stirring was continued for 0.5 h. *(E)*-1,4-Dibromobut-2-ene (0.22 g, 2 mmol) in THF (10 ml) was stirred at room temperature and the 2-fluoroethylamine/THF solution was added dropwise, and the mixture was stirred for 2 h. Water (5 ml) and 5% sodium hydroxide (NaOH) (5 ml) were added and the free base was extracted with ether (5 × 20 ml). The ether was reduced in volume to approximately 20 ml and ethereal hydrochloric acid was added dropwise (20 ml). This mixture was stirred overnight, concentrated *in vacuo* and the resulting brown oil, which was a mixture of compounds by NMR spectroscopy, could not be purified.

Attempted Synthesis of *NN'*-Bis(4-fluorobenzyl)-1,4-diaminobut-2-ene (83)

This synthesis was carried out by adapting the general procedure of Roberts and Ross.[41] 4-Fluorobenzylamine (2.1 g, 16 mmol) was added during 15 min to a cooled solution (O °C) of *(E)*-1,4-dibromobut-2-ene (0.4 g, 1.8 mmol) in benzene (5 ml). A white solid precipitated immediately and the reaction was stirred for 0.5 h. The product was diluted with chloroform (10 ml) and washed with water (4 × 15 ml). The chloroform layer was dried (K_2CO_3), filtered, and concentrated *in vacuo* to give an oil which ^1H NMR spectroscopy showed contained starting material and other products. Distillation of the oil was unsuccessful.

Synthesis of *N*-Acetyl-*(E)*-1,4-diaminobut-2-ene Hydrochloride (N-Acetyl-E-BED) (84)

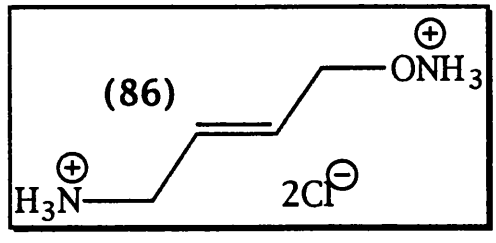


(84)

The free base (E-BED) was prepared as described earlier in Procedure B. The free base (0.30 g, 3.5 mmol)) was then placed in a conical flask with ethyl acetate (10 ml) and to this was added porcine pancreatic lipase (PPL) (0.30 g, 13.3 units of activity per mg of solid). This mixture was shaken at 100 rpm in a constant temperature bath at 25 °C. The reaction was monitored by TLC using ethyl acetate/ isopropanol/ conc. ammonia (9:7:4) as the solvent system. After several attempts it was found that the

optimum reaction time, when the maximum amount of monoacetylated product was formed and the least diacetylated material was 4 d. At this time the solvent was decanted and the coagulated enzyme was stirred for 1 h with chloroform/methanol (9:1) (10 ml). This mixture was filtered through Celite and the filtrate was combined with the reaction solvent. This was then dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield an oil. This oil was dissolved in a mixture of hot water (5 ml) and 6M hydrochloric acid (3.3 ml). The solution was evaporated to dryness to leave a solid. Extraction of this solid with isopropanol (20 ml) dissolved the desired product and the dihydrochloride salt of the starting material was filtered off. The filtrate was reduced in volume to *ca.* 1 ml and left at 0 °C overnight. N-Acetyl-E-BED hydrochloride was recrystallised from isopropanol/diethyl ether (0.07 g, 12%), δ_H (200 MHz, D_2O) 1.81 (3H, s), 3.39 (2H, d), 3.60 (2H, d) and 5.47-5.75 (2H, m); δ_C (50 MHz, D_2O) 22.6 (CH_3), 41.1 (CH_2), 41.3 (CH_2), 122.7 (CH), 127.6 (CH) and 174.9 (CO); *m/z* 129, 128, 111, 86, 69, 56 and 43.

Attempted Synthesis of *(E)*-1-Amino-oxy-4-amino-but-2-ene Dihydrochloride (86)



Preparation of *(E)*-1-Phthalimido-4-bromobut-2-ene

Potassium phthalimide (4.32 g, 23.3 mmol) was added in portions over 3 h to a stirred solution of *(E)*-1,4-dibromobut-2-ene (5 g, 23.3mmol) in acetone (60 ml) at 80 °C. The suspension was stirred for 24 h at 85 °C, then cooled and filtered. The filtrate was concentrated *in vacuo* to afford a white solid which was recrystallised from acetone to give the product (3.88 g, 60%), ν_{max}/cm^{-1} (KBr disc) 3462, 2935, 1774, 1710, 1612 and 1392; δ_H (200 MHz, $CDCl_3$) 3.92 (2H, d), 4.30 (2H, d), 5.89 (2H, m) and 7.79 (4H, m); δ_C (50 MHz, D_2O) 31.2 (CH_2), 38.5 (CH_2), 123.3 (CH), 128.3 (CH), 129.8 (CH), 131.9 (C), 134.0 (CH) and 167.7 (CO); *m/z* 201, 200 (100%), 102, 160, 130, 104, 76 and 53 (Found C, 50.89; H, 3.55; N, 5.03%; M^+ (-Br) 200.0718. $C_{12}H_{10}NO_2Br$ requires C, 51.43; H, 3.60; N, 5.00%; M^+ (-Br) 200.0712).

Attempted Preparation of *(E)*-1-Phthalimido-oxy-4-phthalimido-but-2-ene

Initially *(E)*-1-phthalimido-4-bromobut-2-ene (1 g, 3.57 mmol) and triethylamine (40 ml) were stirred and *N*-hydroxyphthalimide (0.58 g, 3.57 mmol) was added in portions, however, even after heating at reflux overnight, the *N*-hydroxyphthalimide still had not dissolved. Various solvents were tried and it was found that *(E)*-1-phthalimido-4-bromo-but-2-ene dissolved in acetonitrile and *N*-hydroxyphthalimide partially dissolved after heating in acetonitrile. Starting materials (amounts as above) and acetonitrile (25 ml) were heated to reflux for 48 h then allowed to cool. The acetonitrile and excess triethylamine were removed *in vacuo* and water (20 ml) was added. The resulting orange precipitate was filtered off and initial NMR

analysis showed a variety of compounds was present. Attempts at purification by recrystallisation all failed to give pure product.

Attempted Preparation of *(E)*-1-Amino-oxy-4-amino-but-2-ene Dihydrochloride

Although pure starting material was not available several attempts at phthalimide cleavage were tried.

1. Using Hydrazine[43]

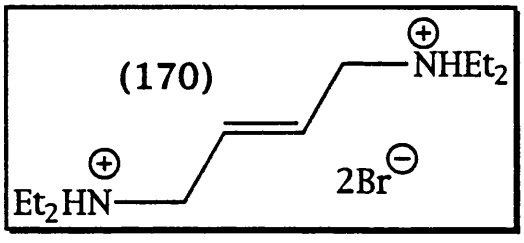
Hydrazine monohydrate (0.11 g, 2.2 mmol) in ethanol (20 ml) was added dropwise to a stirred solution of impure*(E)*-1-phthalimido-oxy-4-phthalimidobut-2-ene (0.4 g, 1.1 mmol) in ethanol (10 ml). This mixture was heated at reflux overnight and allowed to cool. The mixture was then poured into ether (50 ml) and washed with 2M hydrochloric acid (3 × 50 ml). The acid portions were collected and concentrated *in vacuo* to give a light brown solid which could not be purified.

2. Using Acid

Impure *(E)*-1-phthalimido-oxy-4-phthalimidobut-2-ene (0.15 g, 0.41 mmol) was suspended in glacial acetic acid (15 ml), and conc. hydrochloric acid (15 ml) was added slowly. The mixture was heated at reflux until all the solid had dissolved, then for a further 24 h. The solution turned dark brown, was cooled, and was concentrated *in vacuo* to *ca.* 5 ml. Crystallisation of the brown solid remaining (methanol/ether) was unsuccessful. The residue was a mixture of products by ^{1}H NMR spectroscopy and when the time of reflux was varied to 48 h the desired product was still not produced.

4.3 E-TED and Analogues

(E)-NNN'N'-Tetraethyl-1,4-diaminobut-2-ene (E-TED) Dihydrobromide (170)[38]



Diethylamine (1.5 g, 20 mmol) in toluene (50 ml) was added to a stirred solution of *(E)*-1,4-dibromobut-2-ene (2.14 g, 10 mmol) in toluene (50 ml) at room temperature, and the solution was stirred for 4 h. The white precipitate formed was filtered off, washed with ether (2 × 20 ml), and dissolved in hot aqueous ethanol. The solution was allowed to cool and acetone was added. The white precipitate formed was filtered off and washed with acetone to yield *(E)-NNN'N'*-tetraethyl-1,4-diaminobut-2-ene dihydrobromide (2.16 g, 60%), ν_{max}/cm^{-1} (KBr disc) 3450, 2950, 2820 and 1610; δ_H (200 MHz, D_2O) 1.15 (12H, t), 3.08 (8H, q), 3.74 (4H, d) and 6.03 (2H, m); δ_C (50 MHz, D_2O) 9.2 (CH_3), 48.3 (CH_2), 53.2 (CH_2) and 129.6 (CH); *m/z* 200, 199, 165, 73, 58 (100%) and 44 (Found: C, 53.26; H, 10.33; N, 10.17%. $C_{12}H_{28}N_2Cl_2$ requires C, 53.13; H,10.40; N, 10.37%).

Synthesis of *(E)-NNN'N'*-Tetraethyl-1,4-diaminobut-2-ene Dihydrobenzoate (88)

The synthesis of the free base was carried out by the general procedure of Roberts and Ross.[41] Diethylamine (3.285 g,

0.045 mol) was added during 15 min to a cooled solution (O °C) of *(E)*-1,4-dibromobut-2-ene (1.07 g, 5 mmol) in benzene (5 ml). The product was diluted with chloroform (25 ml) and the organic layer was washed with water (4 × 25 ml). The chloroform layer was dried, filtered, and concentrated *in vacuo* to give *(E)-NNN'N'*-tetraethyl-1,4-diaminobut-2-ene as an oil (0.82 g, 82%), δ_H (90 MHz, $CDCl_3$) 1.10 (12H, t), 2.60 (8H, q), 3.20 (4H, m) and 5.75 (2H, m).

(E)-NNN'N'-Tetraethyl-1,4-diaminobut-2-ene (0.72 g, 3.6 mmol) was stirred with benzoic acid (0.87 g, 7.13 mmol) in benzene (5 ml) for 1 h. The precipitate was filtered and washed with ether to afford *(E)-NNN'N'*-tetraethyl-1,4-diaminobut-2-ene dihydrobenzoate as a hygroscopic white solid (1.11 g, 69%), ν_{max}/cm^{-1} (KBr disc) 3443, 2986, 1637, 1599 and 1379; δ_H (200 MHz, D_2O) 1.05 (12H, t), 2.94 (8H, q), 3.58 (4H, m), 5.88 (2H, m), and 7.34 (10H, m); δ_C (50 MHz, D_2O) 9.1 (CH_3), 48.0 (CH_2), 53.0 (CH_2), 129.1 (CH), 129.5 (CH), 129.6 (CH), 132.1 (CH) and 176.4 (CO); *m/z* 198, 122, 105 (100%), 86, 77, 72, 51, 28, and18 (Found: C, 70.71; H, 8.84; N, 6.33%. $C_{26}H_{38}N_2O_4$ requires C, 70.58; H, 8.59; N, 6.29%).

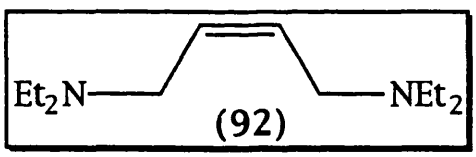
Three other compounds were prepared by this method.

E-TED phosphate (89) (0.56 g, 72%), ν_{max}/cm^{-1} (KBr disc) 3440, 2970, 2820, 2470, 1625, 1480 and 1390; δ_H (200 MHz, D_2O) 1.07 (12H, t), 2.98 (8H, q), 3.64 (4H, m), and 5.93 (2H, m); δ_C (50 MHz, D_2O) 9.1 (CH_3), 48.1 (CH_2), 53.0 (CH_2) and 129.5 (CH); *m/z* 126, 86, 73, 58, 44 and 30 (100%).

E-TED fumarate (90) (1.21 g, 68%); ν_{max}/cm^{-1} (KBr disc) 3493, 3429, 2932, 2652, 2496, 1686, 1458, 1431 and 1296; δ_H (200 MHz, D_2O) 1.09 (12H, t), 2.99 (8H, q), 3.66 (4H, m), 5.95 (2H, m) and 6.51 (2H, s); δ_C (50 MHz, D_2O) 9.1 (CH_3), 48.1 (CH_2), 53.1 (CH_2), 129.5 (CH), 135.4 (CH) and 171.7 (CO); *m/z* 126, 98, 86, 72, 55, 45, 28 and 27 (100%).

E-TED propionate (91) (0.72 g, 58%), ν_{max}/cm^{-1} (thin film) 3420, 2980, 2940, 2500, 1720, 1575, 1460 and 1290; δ_H (200 MHz, D_2O) 0.82 (6H, t), 1.06 (12H, t), 1.97 (4H, q), 2.98 (8H, q), 3.63 (4H, m), and 5.92 (2H, m); δ_C (50 MHz, D_2O) 9.1 (CH_3), 10.7 (CH_3), 30.9 (CH_2), 48.1 (CH_2), 53.0 (CH_2), 129.5 (CH) and 184.0 (CO); *m/z* 199, 126, 86, 72, 56, 42 and 28 (100%).

Synthesis of *(Z)-NNN'N'*-Tetraethyl-1,4-diaminobut-2-ene (92)



(92)

The first step in the synthesis of the free base was carried out by modifying the general procedure of Biel and DiPierro.[45] 1,4-Dichloro-2-butyne (2 ml, 20 mmol) was cooled to 0 °C and diethylamine (7.48 ml, 80 mmol) was added dropwise with stirring. The mixture was allowed to warm to room temperature and stirred for 1 h, then left to stand for 1 h. Water (10 ml) was added and this solution was then saturated with potassium hydroxide solution. The mixture was extracted with ether (5 x 20 ml) and the organic extracts were dried, filtered and concentrated *in vacuo*, to yield 1,4-bis(diethylamino)but-2-yne (2.81g, 72%), ν_{max}/cm^{-1} (thin film) 3406, 2970, 2936, 2818, 1744 and 1681;

δ_H (200 MHz, $CDCl_3$) 1.07 (12H, t), 2.54 (8H, q) and 3.45 (4H, s) ppm; δ_C (50 MHz, D_2O) 12.5 (CH_3), 40.6 (CH_2), 47.1 (CH_2) and 78.8 (C); *m/z* 197 (M^+ +1), 196 (M^+, 1.3%), 125, 108, 94, 86, 73, 58 (100%), 42 and 31; (Found: C, 73.16; H, 12.24; N, 14.27%; M^+, 196.1944. $C_{12}H_{24}N_2$ requires C, 73.46; H, 12.24; N, 14.28%; M^+, 196.1939).

1. Attempted Reduction Using a Palladium Catalyst[44]

An ethanolic solution (*ca.* 15 ml) containing *NNN'N'*-tetraethyl-1,4-diaminobut-2-yne (21 g, 10 mmol) and 2 drops of synthetic quinoline was subjected to hydrogenation at room temperature and 4 bar of hydrogen in the presence of 5% palladium-on barium carbonate catalyst. After being shaken overnight the catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give starting material.

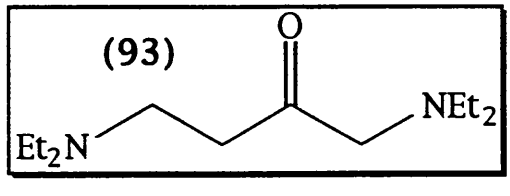
2. Reduction Using Activated Zinc

The second stage in this synthesis was adapted from a procedure of Aerssens *et al.*[47] The reduction is accomplished using activated zinc.

Activation of zinc powder. In a 250 ml round-bottomed, 3-necked flask, equipped with a gas inlet, a mechanical stirrer and a reflux condenser were placed Analar grade zinc powder (17.5 g) and dry ethanol (35 ml). The air in the flask was completely replaced by nitrogen gas, after which 1,2-dibromoethane (1 ml) was added. The stirred mixture was heated until a vigorous reaction started. When the intensity of the reaction subsided more 1,2-dibromoethane (2 ml) was added. After the exothermic reaction had ceased, the suspension was heated for 10 min under

reflux, while maintaining an atmosphere of nitrogen. After cooling the stirred suspension to about 50 °C, a solution of copper(I) bromide (4 g) and anhydrous lithium bromide (6 g) in THF (20 ml) was added over 2 min with efficient stirring. *NNN'N'*-Tetraethyl-1,4-diaminobut-2-yne (2.81 g, 14 mmol) was added to the activated zinc and this stirred suspension was heated under reflux for 4 h. During this period a slow stream of nitrogen was passed through the flask. After cooling to room temperature, the suspension was poured into an aqueous solution (100 ml) of ammonium chloride (25 g). The suspension was then basified with potassium hydroxide solution and extracted with DCM (7 × 100ml). The combined extracts were dried, filtered and concentrated *in vacuo* to yield *(Z)-NNN'N'*-tetraethyl-1,4-but-2-ene (with *ca.* 25% *(E)-NNN'N'*-tetraethyl-1,4-but-2-ene) (0.22 g, 8%), ν_{max}/cm^{-1} (KBr disc) 2972, 2936, 2501 and 1456; δ_H (200MHz, $CDCl_3$) 1.04 (12H, t), 2.52 (8H, q), 3.08 (4H, m), 5.64 (2H, m). δ_C(50 MHz, D_2O) 11.6 (CH_3), 46.5 (CH_2), 54.8 (CH_2) and 130.4 (CH); *m/z* 198 (M^+, 0.3%), 126, 110, 86 (100%), 72, 58 and 30.

Attempted Synthesis of 1,4-Bis(diethylamino)butan-2-one (93)



Synthesis of 1,4-Bis(diethylamino)but-2-yne

1,4-Bis(diethylamino)but-2-yne was prepared as described on page 125.

1. Attempted Hydration Using Mercury Impregnated Nafion-H Perfluorinated Resin Sulfonic Acid

Preparation of Mercury Impregnated Nafion-H Catalyst (Hg/Nafion-H). To a suspension of mercury (II) oxide (0.173 g, 0.8 mmol) in deionised water (25 ml) was added sulfuric acid (0.5 ml). Nafion-H NR 50 beads 10-35 mesh (1 g) were then stirred with this solution for 5 h at room temperature. The catalyst was filtered off, washed with deionised water (5 × 10 ml) and dried to constant weight (24 h). The catalyst should now have 25% of the protons substituted by mercury (II) ions.

Attempted Hydration of 1,4-Bis(diethylamino)but-2-yne. 1,4-Bis(diethylamino)but-2-yne (5 g, 32 mmol) and water (2.88 ml, 60 mmol) were dissolved in ethanol (25 ml). The Hg/Nafion-H catalyst (1.29 g) was added, and the mixture was stirred at room temperature for 1.5, 2, 5 and 24 h on four different occasions. The mixture was filtered, and the solid catalyst was washed with ethanol (2 × 10 ml) followed by ether (2 × 20 ml). Water (50 ml) was added and the mixture was extracted with ether (2 × 40 ml). The combined ether extracts were dried ($MgSO_4$) and concentrated *in vacuo* but no product could be detected (no carbonyl signals in the IR spectra). The work-up was modified by washing the Hg/Nafion-H catalyst with conc. ammonia solution. The Hg/Nafion-H catalyst beads went black and no product was extracted.

2. Attempted Hydroboration

A solution of 1,4-bis(diethylamino)but-2-yne (0.5 g, 2.55 mmol) in THF (5 ml) was added to borane-THF (1 M, 10.2 mmol, 10.2 ml) under nitrogen and stirred at room temperature for 2 h

and 24 h on two different occasions. The product was oxidised at 0 °C with 30% aqueous hydrogen peroxide (3 ml, 26 mmol), maintaining the pH at *ca.* 8. The mixture was stirred for 0.5 h, then 20% sodium hydroxide solution (*ca.* 3 ml) was added and the mixture was extracted with ether (4 × 20 ml). The ether extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give an oil which showed no carbonyl signal in the ^{13}C NMR spectrum.

3. Attempted Hydration Using Mercuric Oxide[44]

To 50% aqueous sulfuric acid (10 ml) was added mercuric (II) oxide (0.2 g, 0.9 mmol). The mixture was heated to 60 °C and 1,4-bis(diethylamino)but-2-yne (1 g, 5.1 mmol) was added dropwise with stirring. The temperature was held at 60 °C for 3 h, 24 h and 48 h on three different occasions, (During one experiment the temperature was raised to 90 °C and the mixture turned black.) The solution was then poured onto crushed ice. After the addition of sufficient solid potassium hydroxide pellets to produce two distinct layers, the alkaline mixture was extracted with ether (3 × 10 ml). The combined ether extracts were dried (K_2CO_3) and concentrated *in vacuo* to give an oil which showed no carbonyl signal in the ^{13}C NMR spectrum.

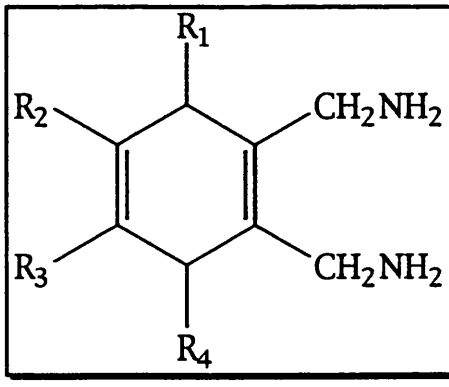
4. Attempted Hydration of 1,4-Dichlorobut-2-yne Using Mercuric Oxide

Mercuric (II) oxide (0.08 g, 0.4 mmol) was added to water (4 ml) and conc. sulfuric acid (1.5 ml) was added dropwise with stirring until all the mercuric oxide dissolved. This solution was then added dropwise to a stirred solution of 1,4-dichlorobut-2-yne (0.5 g, 4.06 mmol) in methanol (20 ml) at 50 °C for 24 h and

48 h on two different occasions. The solution was poured onto crushed ice (*ca.* 20 ml) and this mixture was extracted with ethyl acetate (3 × 20 ml). The combined ethyl acetate extracts were washed with water (3 × 10 ml), brine (3 × 20 ml), dried (K_2CO_3) and concentrated *in vacuo* to give an oil which could not be purified. This oil contained starting material (SM) and another product (0.37 g), δ_H (200 MHz, $CDCl_3$) 2.80 (2H, t), 3.32 (2H, s), 3.66 (2H, t) and 4.21 (4H, s, SM); δ_C (50 MHz, $CDCl_3$) 30.1 (CH_2, SM), 39.9 (CH_2), 48.9 (CH_2), 67.2 (CH_2), 80.9 (C, SM) and 200.6 (CO); *m/z* 158 (M^++ OH 0.2%), 122, 105, 87, 72, 45 (100%), 37 and 35.

4.4 BAD Analogues and Derivatives

General Procedure for Attempted Synthesis of 1,2-Bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene (BAD) Analogues



(171) $R_1 = R_2 = Me$; $R_3 = R_4 = H$
(172) $R_2 = Me$; $R_1 = R_3 = R_4 = H$
(173) $R_1 = Me$; $R_2 = R_3 = R_4 = H$
(174) $R_2 = R_3 = Me$; $R_1 = R_4 = H$
(175) $R_1 = R_4 = H$; $R_2 = R_3 = OMe$

Preparation of Dimethyl 3,4-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylate

3-Methylpenta-1,3-diene (2.5 g, 30 mmol) and dimethyl acetylenedicarboxylate (4.0 g, 28 mmol) were placed in a sealed

tube at 60 °C for 24 h. The mixture was cooled and initial TLC analysis showed that starting material was still present. The mixture was put down a silica column (hexane-ethyl acetate, 4:1) and the product was collected as an oil (2.51 g, 40%), δ_H (200 MHz, $CDCl_3$) 1.18 (3H, d), 1.75 (3H, m), 2.89-3.07 (1H and 2H, m), 3.75 (3H, s), 3.79 (3H, s) and 5.40 (1H, m); δ_C (50 MHz, $CDCl_3$) 18.5 (CH_3), 20.7 (CH_3), 27.7 (CH_2), 36.5 (CH), 51.9 (CH_3), 116.8 (CH), 130.6 (C), 134.9 (C), 139.6 (C) and 167.8 (CO); *m/z* 224 (M^+, 0.1%), 209, 194, 191, 179, 164, 133, 119, 105, 59 and 31(100%) (Found: M^+, 224.1042. $C_{12}H_{16}O_4$ requires M^+, 224.1048).

Preparation of 1,2-Bis(hydroxymethyl)-3,4-dimethylcyclohexa-1,4-diene

Method A. To a solution of lithium aluminium hydride (1.52 g, 40.1 mmol) in dry THF (25 ml) under an atmosphere of nitrogen at 0 °C was added dropwise a solution of dimethyl 3,4-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (3 g, 13.39 mmol) in dry THF (5 ml). When the addition was complete, the mixture was stirred for 2 h. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of a saturated solution of sodium sulfate (20 ml). After vigorous stirring for 10 min the aluminium salts were broken up by addition of 3 M sulfuric acid (30 ml). The mixture was washed with ether (3 × 25 ml) and these combined ether extracts were washed once with saturated sodium bicarbonate solution and once with brine, dried (Na_2SO_4) and concentrated *in vacuo* to afford an oil which contained a number of impurities by ^1H NMR spectroscopy which could not be purified.

Method B. Butyllithium (1.5 M, 35 ml, 53 mmol) in hexane was added to diisobutylaluminium hydride (DIBAL-H) (1.0 M, 53 ml, 53 mmol) in hexane at 0 °C under an atmosphere of nitrogen. After stirring for 0.5 h, the solution was cooled to -78 °C and dimethyl 3,4-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (2 g, 8.92 mmol) in THF (10 ml) was added dropwise. When the addition was complete, the mixture was stirred for a further 5 h. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of 10% sodium bisulfate solution (25 ml). Water (10 ml) was added to the mixture and this was then continuously extracted with DCM for 24 h. The DCM was dried ($MgSO_4$) and concentrated *in vacuo* to give an oil (0.6 g, 40%), ν_{max}/cm^{-1} (thin film) 3352, 3329, 2963, 2878 and 1050; δ_H (200 MHz, $CDCl_3$) 1.10 (3H, d), 1.71 (3H, m), 2.67-2.73 (1H and 2H, m), 3.84-4.26 (4H and 2H (OH), m) and 5.04 (1H, m); δ_C (50 MHz, $CDCl_3$) 19.0 (CH_3), 21.2 (CH_3), 36.2 (CH_2), 38.3 (CH), 60.3 (CH_2), 61.9 (CH_2), 118.4 (CH), 132.6 (C), 136.8 (C) and 137.7 (C); *m/z* 168 (M^+, 1.6%), 147, 131, 119, 105 (100%), 91, 79 and 41 (Found: M^+, 168.1154. $C_{10}H_{16}N_2O_2$ requires M^+, 168.1150).

Preparation of 1,2-Bis(bromomethyl)-3,4-dimethylcyclohexa-1,4-diene

Phosphorus tribromide (10 ml) was cooled to 0 °C in an ice bath and 1,2-bis(hydroxymethyl)-3,4-dimethylcyclohexa-1,4-diene (0.5 g, 2.97 mmol) in benzene (2 ml) was then added slowly with stirring. This gave a dark brown solution which was stirred overnight (15 h). Ice cold water (5 ml) was added slowly to hydrolyse excess reactant. The two layers were separated and

ether (5 ml) was added to the organic layer. This was then washed with saturated sodium bicarbonate solution (3 × 5 ml), dried (Na_2SO_4) and concentrated *in vacuo* to afford a dark coloured oil (0.1 g) which could not be purified.

Preparation of Dimethyl 4-Methylcyclohexa-1,4-diene-1,2-dicarboxylate

Isoprene (6.8 g, 100 mmol) and dimethyl acetylenedicarboxylate (7.1 g, 50 mmol) were placed in a sealed tube **a)** neat at room temperature for 16 h; **b)** neat at 60 °C for 24 h; and **c)** in benzene (5 ml) at 60 °C for 48 h.[56] The third set of conditions proved most successful and were thereafter used. The mixture was cooled and concentrated *in vacuo* to afford an oil (6.79 g, 65%), δ_H (200 MHz, $CDCl_3$) 1.70 (3H, s), 2.82-3.05 (4H, m), 3.77 (6H, s), and 5.39 (1H, m); δ_C (50 MHz, $CDCl_3$) 22.3 (CH_3), 28.3 (CH_2), 31.8 (CH_2), 53.4 (CH_3), 116.4 (CH), 128.9 (C), 132.0 (C), 132.6 (C), 166.0 (CO) and 166.3 (CO).

Preparation of 1,2-Bis(hydroxymethyl)-4-methylcyclohexa-1,4-diene

Two different methods were used in an attempt to increase the purity and yields of the reduction.

Method A. To a solution of lithium aluminium hydride (1.70 g, 44 mmol) in dry THF (50 ml) under an atmosphere of nitrogen at 0 °C was added dropwise a solution of dimethyl 4-methylcyclohexa-1,4-diene-1,2-dicarboxylate (2 g, 9.52 mmol) in dry THF (20 ml). When the addition was complete, the mixture was stirred for 2 h. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of saturated solution of

sodium sulfate (20 ml). After vigorous stirring for 10 min the aluminium salts were broken up by addition of 3 M sulfuric acid (30 ml). The mixture was washed with ether (3 × 25 ml) and these combined ether extracts were washed once with saturated sodium bicarbonate solution and once with brine, dried (Na_2SO_4) and concentrated *in vacuo* to afford an oil (1.1 g) which contained a number of impurities and could not be purified.

Method B. As described for the preparation of 1,2-bis(hydroxymethyl)-3,4-dimethylcyclohexa-1,4-diene (Method B), an oil was obtained with impurities as shown by ^1H NMR spectroscopy (0.54 g), δ_H (90 MHz, $CDCl_3$) 1.65 (3H, s), 2.72 (2H and 2H, m), 4.10 (4H, m) and 5.35 (1H, m), plus other signals.

Preparation of 1,2-Bis(bromomethyl)-4-methylcyclohexa-1,4-diene

As described for the preparation of 1,2-bis(bromomethyl)-3,4-dimethylcyclohexa-1,4-diene, an oil was obtained (1.59 g, 93%), *m/z* 281 (M^+, 17.7%), 280, 201, 200, 199, 119 (100%), 118, 105, 91, 79 and 39. ^1H and ^{13}C NMR spectra showed a great number of impurities and the product could not be purified.

Preparation of 1,2-Bis(phthalimidomethyl)-4-methylcyclohexa-1,4-diene

Potassium phthalimide (0.19 g, 1.05 mmol) was added to a stirred solution of impure 1,2-bis(bromomethyl)-4-methylcyclohexa-1,4-diene (0.14 g, 0.5 mmol) in DMF (5 ml) at room temperature. The mixture was stirred for a further 2 d at this temperature, then poured into water (10 ml), and the mixture was extracted with DCM (5 × 20 ml). The organic extracts were

dried ($MgSO_4$), filtered and concentrated *in vacuo* to leave DMF (*ca.* 5 ml), and a white solid, which was washed with ether (3 × 5 ml) and dried by suction (0.12 g). ^1H and ^{13}C NMR spectra showed signals that corresponded to the required product and a great number of impurities. Purification proved impossible.

Preparation of Dimethyl 3-Methylcyclohexa-1,4-diene-1,2-dicarboxylate

Penta-1,3-diene (1.36 g, 20 mmol) and dimethyl acetylenedicarboxylate (2.84 g, 20 mmol) were placed in a sealed tube with benzene (2 ml) at 60 °C for 48 h. The mixture was cooled and concentrated *in vacuo* to afford an oil (1.11 g, 26%), δ_H (200 MHz, $CDCl_3$) 1.12 (3H, d), 1.15 (3H, m), 2.77-3.25 (1H and 2H, m), 3.72 (3H, s), 3.77 (3H, s) and 5.64 (2H, m); δ_C (50 MHz, $CDCl_3$) 20.3 (CH_3), 26.8 (CH_2), 32.2 (CH), 51.8 (CH_3), 51.9 (CH_3), 121.2 (CH), 128.7 (CH), 134.3 (C), 140.1 (C), 167.4 (CO) and 169.6 (CO); *m/z* 210 (M^+, 0.6%), 177, 163, 151, 119, 105, 91(100%) and 59 (Found: M^+, 210.0870. $C_{11}H_{14}O_4$ requires M^+, 210.0891).

Preparation of 1,2-Bis(hydroxymethyl)-3-methylcyclohexa-1,4-diene

As described for the preparation of 1,2-bis(hydroxymethyl)-3,4-dimethylcyclohexa-1,4-diene (Method B), an oil (0.35 g) was obtained with ^1H and ^{13}C NMR spectra showing impurities.

Preparation of Dimethyl 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylate

2,3-Dimethyl-1,3-butadiene (1.64 g, 20 mmol), dimethyl acetylenedicarboxylate (2.84 g, 20 mmol) and water (50 ml) were

heated at 60 °C for 24 h. The reaction mixture was then cooled and the precipitate was filtered off and washed with water. The product was obtained as a white solid which was recrystallised from acetone to give colourless crystals (4.48 g, 73%), m.p. 62-65 °C (lit.,[119] 75-76 °C); ν_{max}/cm^{-1} (KBr disc) 3446, 2956, 2859, 1740, 1724 and 1696; δ_H (200 MHz, $CDCl_3$) 1.66 (6H, s), 2.92 (4H, s) and 3.77 (6H, s); δ_C (50 MHz, $CDCl_3$) 17.8 (CH_3), 33.9 (CH_2), 52.0 (CH_3), 121.4 (C), 132.6 (C) and 168.2 (CO); *m/z* 225 (M^++1), 244 (M^+, 4.6%), 191, 177 (100%), 133, 105, 91, 77 and 59. (Found: C, 64.13; H, 7.02 %; M^+, 224.1055. $C_{12}H_{16}O_4$ requires C, 64.25; H, 7.19 %; M^+, 224.1049).

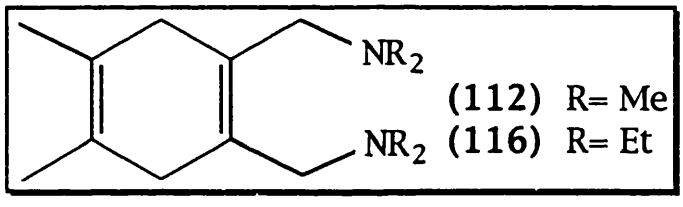
Preparation of 1,2-Bis(hydroxymethyl)-4,5-dimethylcyclohexa-1,4-diene

To a solution of DIBAL (1.0M, 26 ml, 26 mmol) in DCM at -20 °C under an atmosphere of nitrogen, was added dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (1.43 g, 6.38 mmol) in dry DCM (20 ml) over 15 min. After stirring at this temperature for 2 h, the solution was allowed to warm to room temperature. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of ethyl acetate (20 ml). The mixture was then poured into a solution of Celite (12.5 g) in acetone (60 ml). Methanol (20 ml) was added slowly until the mixture formed a gel. More methanol (20 ml) was added until the gel broke up then the mixture was filtered and washed with methanol. The filtrate was dried (Na_2SO_4) and concentrated *in vacuo* to give an oil (0.12 g, 10%) which showed impurities by ^1H NMR spectroscopy.

Preparation of Dimethyl 3,4-dimethoxycyclohexa-1,4-diene-1,2-dicarboxylate

2,3-Dimethoxy-1,3-butadiene (1.88 ml, 17.5 mmol) and dimethyl acetylenedicarboxylate (2.15 ml, 17.5 mmol) were heated in a sealed tube at 70 °C for 16 h. The reaction was monitored by TLC [ethyl acetate/pet. ether (1:1)] and left for another 24 h at 70 °C. The oil that was produced could not be purified.

General Procedure for *N,N,N',N'*-Tetramethyl- and *N,N,N',N'*-Tetraethyl-BAD Syntheses



(112) R= Me
(116) R= Et

Preparation of Dimethyl 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylate

Dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate was prepared as described on page 135.

Preparation of 4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylic Acid

Method A. Dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (2 g, 8.9 mmol) was dissolved in methanol (5 ml) and lithium hydroxide monohydrate (2 g, 47.6 mmol) in methanol-water (3:1) (40 ml) was dropped in slowly. A yellow colour appeared and stirring was continued for 24 h. After this time the yellow colour disappeared and the mixture was washed

with ether (3 × 20 ml) in order to remove any unreacted diester. 3 M Hydrochloric acid (10 ml) was added dropwise and a cloudy suspension appeared. Another portion of 3 M hydrochloric acid (5 ml) was added dropwise and the solution went clear (pH 2) and was extracted with ethyl acetate (3 × 20 ml). The combined ethyl acetate extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid (1.00 g, 57 %), which was insoluble in all solvents tried.

Method B. Dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (1 g, 4.46 mmol) was stirred in 5% sodium hydroxide solution (20 ml) and heated at reflux for 4 h. Water (15 ml) was added and the mixture was washed with ether (3 × 20 ml) in order to remove any unreacted diester. The remaining aqueous solution was then acidified with 6 M hydrochloric acid (6 ml) and extracted with ethyl acetate (3 × 20 ml). The combined ethyl acetate extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid (0.2 g, 23 %) which was very insoluble, m.p. 153-156 °C (lit.,[119] 158.5-160.5 °C); ν_{max}/cm^{-1} (KBr disc) 3421, 2935, 2632 and 1694; δ_H (200 MHz, D_2O and D_6-DMSO) 1.59 (6H, s) and 2.84 (4H, s); δ_C (50 MHz, D_2O and DMSO) 18.2 (CH_3), 34.5 (CH_2), 122.7 (C), 133.8 (C) and 172.8 (CO); *m/z* 197 (M^++1), 196 (M^+, 10.8 %), 178, 151, 133, 107, 91 (100%), 77 and 51 (Found: M^+, 196.0732. $C_{10}H_{12}O_4$ requires M^+, 196.0735).

Preparation of N,N,N',N'-Tetramethyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxamide

To a suspension of 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (1 g, 5.1 mmol) in benzene (10 ml) was added hexamethylphosphorus triamide (0.52 ml, 5.1 mmol) dropwise.

The mixture was heated to reflux temperature for 20 min and then allowed to cool to room temperature. Saturated sodium bicarbonate solution (10 ml) was added and the layers were separated. The aqueous layer was extracted with DCM (3 x 20 ml) and the extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give a yellow oil (0.63 g). The ^1H NMR spectrum contained starting material and product, δ_H (90 MHz, $CDCl_3$) 1.75 (6H and 6H (SM), m), 2.30-2.80 (4H and 4H (SM), m), 2.95 (6H, s) and 3.10 (6H, s).

Preparation of 1,2-Bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene (112)

To a solution of lithium aluminium hydride (0.36 g, 9.47 mmol, 4 equiv.) in dry ether under an atmosphere of nitrogen was added dropwise a solution of the *N,N,N',N'*-tetramethyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxamide (0.6 g, 2.4 mmol, 1 equiv.) in dry ether (5 ml). When the addition was complete, the mixture was heated at reflux for 1 h. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of water (0.5 ml), followed by 15% sodium hydroxide solution (1 ml), then water (*ca.* 2 ml) again. After vigorous stirring for 10 min the mixture was filtered with suction. The granular precipitate was washed thoroughly with ether (3 x 20 ml), and the filtrate was dried ($MgSO_4$) and concentrated *in vacuo* to give an oil. This oil was dissolved in a mixture of hot water (5 ml) and 6 M hydrochloric acid (3.3 ml). The solution was evaporated to dryness to leave a glutinous solid (0.12 g, 53 %), ν_{max}/cm^{-1} (thin film) 3802, 3422, 2918, 2866, 2523 and 1468; δ_H (200 MHz, D_2O) 1.29 (3H, s), 1.32 (3H, s) 2.00-2.34 (4H, m),

2.15 (6H, s), 2.16 (6H, s) and 2.71-3.02 (4H, m); δ_C (50 MHz, D_2O) 17.2 (CH_3), 19.5 (CH_3), 34.8 (CH_2), 43.2 (CH_3), 44.4 (CH_3), 62.2 (CH_2), 63.6 (CH_2), 123.9 (C) and 129.1 (C); *m/z* 223 (M^++1 -2HCl), 222 (M^+ -2HCl, 3.8 %), 164, 119, 105, 91, 77, 58 (100%), 42 and 30 (Found: M^+ -2HCl, 222.2108. $C_{14}H_{26}N_2$ requires M^+ -2HCl, 222.2096).

Preparation of *N,N,N',N'*-Tetraethyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxamide

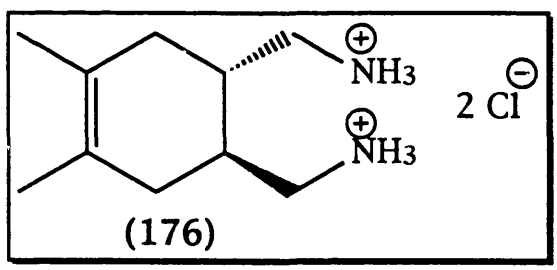
4,5-Dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (1 g, 5.1 mmol) was heated under reflux with thionyl chloride (2.23 ml, 30.5 mmol, 6 equiv.) for 6 h. The excess thionyl chloride was then removed under reduced pressure. The crude acid chloride was dissolved in ether and was added dropwise to a stirred solution of diethylamine (2.97 g, 40.8 mmol, 8 equiv.) in ether cooled to 0 °C. The mixture was allowed to warm to room temperature and stirred for 24 h. The precipitated amine hydrochloride was filtered off and washed several times with ether. The combined organic layers were concentrated *in vacuo* to give an oil (0.63 g, 40 %), δ_H (90 MHz, $CDCl_3$) 1.15 (12H, m), 2.2 (6H, s), 3.00 (8H, m) and 3.50 (4H, m).

Preparation of 1,2-Bis(diethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene (116)

To a solution of lithium aluminium hydride (0.29 g, mmol, 4 equiv.) in dry ether under an atmosphere of nitrogen was added dropwise a solution of the *N,N,N',N'*-tetraethyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxamide (0.6 g, 1.96 mmol) in dry ether (5 ml). When the addition was complete, the

mixture was heated at reflux for 1 h. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of water (0.5 ml), followed by a 15% solution of sodium hydroxide (1 ml), then water (*ca.* 2 ml) again. After vigorous stirring for 10 min the mixture was filtered with suction. The granular precipitate was washed thoroughly with ether (3 × 20 ml), and the filtrate was dried ($MgSO_4$) and concentrated *in vacuo* to give an oil. This oil was dissolved in a mixture of hot water (5 ml) and 6M hydrochloric acid (3.3 ml). The solution was evaporated to dryness to leave a glutinous solid which could not be purified.

Synthesis of *trans*-4,5-Bis(aminomethyl)-1,2-dimethylcyclohex-1-ene Dihydrochloride (176)



(176)

Preparation of *trans*-1,2-Dimethylcyclohex-1-ene-4,5-dinitrile

2,3-Dimethyl-1,3-butadiene (1.05 g, 12.8 mmol), fumaronitrile (1 g, 12.8 mmol) and toluene (10 ml) were heated for 16 h at 100 °C. A white precipitate appeared upon cooling and the mixture was further cooled (0 °C) for 30 min in an ice bath. The white solid was filtered off and recrystallised from chloroform to give 1,2-dimethylcyclohex-1-ene-4,5-dinitrile (1.1 g, 53%), m.p. 119-121 °C (lit.,[120] 120.5-121.5 °C); ν_{max}/cm^{-1} (KBr disc) 3426, 2922, 2845, 2244 and 1453; δ_H (200 MHz, $CDCl_3$) 1.68

(6H, s), 2.45 (4H, dd), and 3.09 (2H, m); δ_C (50 MHz, $CDCl_3$) 18.7 (CH_3), 28.2 (CH), 32.2 (CH_2), 119.0 (CN), and 122.9 (C, olefin); *m/z* 161 (M^++1), 160 (M^+, 48.9%), 145, 133, 110, 82, 67 (100%), 53 and 39 (Found: C, 74.86; H, 7.51; N, 17.53%; M^+, 160.0983. $C_{10}H_{12}N_2$ requires C, 75.00; H, 7.50; N, 17.50%; M^+, 160.1001).

Preparation of 4,5-Bis(aminomethyl)-1,2-dimethylcyclohex-1-ene Dihydrochloride

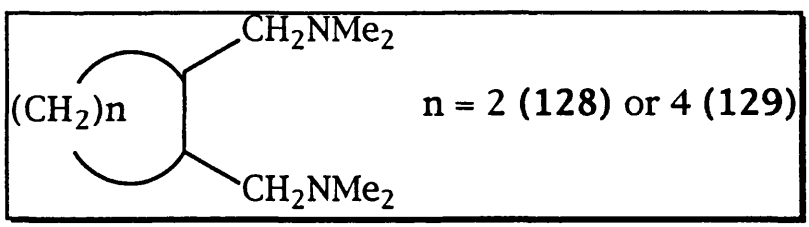
Lithium aluminium hydride (0.95 g, 25mmol, 4 equiv.) was placed in the reaction flask, under a nitrogen atmosphere, with dry ether (20 ml). 1,2-Dimethylcyclohex-1-ene-4,5-dinitrile (1 g, 6.25 mmol, 1 equiv.) in dry THF (15 ml) was added dropwise over 20 min. The reaction mixture went green and was stirred at room temperature for 24 h. The reaction was cooled to 0 °C and water (2 ml), 15% sodium hydroxide solution (2 ml) and further water (2 ml) were added sequentially and cautiously. The resulting white, granular precipitate was filtered and washed with ether (3 × 20 ml). These extracts were combined and dried (K_2CO_3). The white granular solid was stirred in ether overnight and filtered. The filtrate was then dried (K_2CO_3) and added to the previous extracts to be concentrated *in vacuo* to produce a light orange oil. The oil was dissolved in chloroform (5 ml) and washed well with water (3 × 5 ml). The chloroform was removed under reduced pressure and the resulting oil was dissolved in a solution of hot water (5 ml) and 6 M hydrochloric acid (5 ml). The solution was evaporated to as near dryness as possible to leave a thick oil. This oil was dissolved in dry ethanol and dry ether added until a white precipitate appeared. Attempts to recrystallise the highly hygroscopic product resulted in a white sticky solid (0.31 g).

Spectra also showed signs of an aromatic impurity (AI), δ_H (200 MHz, D_2O) 1.46 (6H, s), 1.63 (2H, m), 1.72-1.87 (4H, m), 2.09 (6H, s, AI), 2.77-2.94 (4H, m), 3.93 (2H, s, AI) and 6.90-7.10 (3H, m, AI); δ_C (50 MHz, D_2O) 18.9 (CH_3), 19.4 (CH_3, AI), 19.6 (CH_3, AI), 30.8 (CH_2), 33.8 (CH), 42.7 (CH_2), 43.6 (CH_2, AI), 123.3 (C olefin), 127.0 (CH, AI), 130.7 (CH, AI), 130.9 (C, AI), 131.0 (CH, AI), 138.8 (C, AI) and 139.0 (C, AI); *m/z* 168 (1.6%), 138, 135, 134, 123. 122, 108, 107, 93, 91 (100%), 77, 78 and 30.

The same procedure was followed except that the reaction was completed in dry diethyl ether, with no THF. The same impurity was formed under these conditions.

4.5 Ring and Bridged Systems

General Procedure for *trans*-1,2-Bis(dimethylaminomethyl)cycloalkane Synthesis



Preparation of *trans*-*N,N,N',N'*-Tetramethylcycloalkane-1,2-dicarboxamide

To a suspension of the appropriate *trans*-1,2-cycloalkanedicarboxylic acid in benzene was added hexamethylphosphorus triamide dropwise. The mixture was heated to reflux temperature for 20 min and then allowed to cool to room temperature. Saturated sodium bicarbonate solution was added and the layers were separated. The aqueous layer was

extracted with dichloromethane and the extracts were combined, dried ($MgSO_4$) and concentrated *in vacuo* to give a crude product.

Preparation of *trans*-1,2-Bis(dimethylaminomethyl)cycloalkane

To a solution of lithium aluminium hydride (4 equiv.) in dry ether in an atmosphere of nitrogen was added dropwise a solution of the appropriate diamide in dry ether. When the addition was complete, the mixture was heated at reflux for 1 h. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of water, followed by a 15% solution of sodium hydroxide, then water again. After vigorous stirring for 10 min the mixture was filtered with suction and the granular precipitate was washed thoroughly with ether. The filtrate was dried and concentrated *in vacuo* to give the crude cycloalkane.

trans-1,2-Bis(dimethylaminomethyl)cyclobutane (128)

trans-N,N,N',N'-Tetramethylcyclobutane-1,2-dicarboxamide was obtained as a white solid (0.61 g, 89%), ν_{max}/cm^{-1} (KBr disc) 3432, 2970, 2938, 2868 and 1631; δ_H (90 MHz, $CDCl_3$) 2.1 (4H, m), 2.85 (6H, s), 2.95 (6H, s), and 3.7 (2H, m); *m/z* 198 (M^+, 23%), 154, 126, 100, 72 (100%), 55 and 44 (Found: M^+, 198.1369. $C_{10}H_{18}N_2O_2$ requires M^+, 198.1368).

trans-1,2-Bis(dimethylaminomethyl)cyclobutane was a colourless oil (0.4 g, 77%), ν_{max}/cm^{-1} (thin film) 3404, 2966, 2939, 2855, 2814, 2764 and 1643; δ_H (200 MHz, $CDCl_3$) 1.58 (2H, m), 2.06 (4H, m), 2.18 (12H, s) and 2.32-2.50 (4H, m); δ_C (50 MHz, $CDCl_3$) 25.2 (CH_2), 38.5 (CH), 45.6 (CH_3), 65.4 (CH_2); *m/z* 170 (M^+, 0.1%), 125,

84, 58(100%) and 42 (Found: M^+, 170.1784. $C_{10}H_{22}N_2$ requires M^+, 170.1783).

trans-1,2-Bis(methylaminomethyl)cyclobutane was tested as its dihydrochloride salt, made by dissolving the product in ether and adding ethereal hydrochloric acid dropwise to precipitate the dihydrochloride.

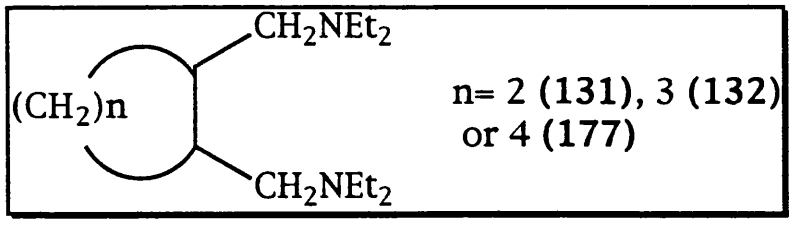
trans-1,2-Bis(dimethylaminomethyl)cyclohexane (129)

trans-N,N,N',N'-Tetramethylcyclohexane-1,2-dicarboxamide was obtained as an oil (4.72 g, 90%), ν_{max}/cm^{-1} ($CHCl_3$) 2935, 2859 and 1634; δ_H (90 MHz, $CDCl_3$) 1.27-1.37 (4H, m), 1.82 (4H, m), 2.89 (6H, s), 2.93-3.02 (2H, m) and 3.11 (6H, s); δ_C (50 MHz, $CDCl_3$) 25.6 (CH_2), 28.6 (CH_2), 35.7 (CH_3), 37.3 (CH_3), 42.6 (CH) and 175.6 (CO); *m/z* 226 (M^+, 7.0%), 182, 154 and 72 (100%) (Found: M^+, 226.1681. $C_{12}H_{22}N_2O_2$ requires M^+, 226.1681).

trans-1,2-Bis(dimethylaminomethyl)cyclohexane was an oil (1.03 g, 47%), ν_{max}/cm^{-1} (thin film) 2969, 2919 and 2814; δ_H (90 MHz, $CDCl_3$) 0.66-0.76 (2H, m), 0.86-1.02(4H, m), 1.27-1.65 (4H, m), 1.87 (12H, s) and 2.00-2.08 (4H, m); δ_C (50 MHz, $CDCl_3$) 24.9 (CH_2), 29.9 (CH_2), 38.3 (CH), 45.7 (CH_3) and 63.9 (CH_2); *m/z* 198 (M^+, 4.9%), 183, 154 and 58 (100%) (Found: C, 72.76; H, 12.99; N, 14.25%; M^+, 198.2077. $C_{12}H_{26}N_2$ requires C, 72.72; H, 13.13; N, 14.14%; M^+, 198.2095).

trans-1,2-Bis(dimethylaminomethyl)cyclohexane was tested as its dihydrochloride salt.

General Procedure for *trans*-1,2-Bis(diethylaminomethyl)cycloalkane Synthesis



Preparation of *trans*-*N,N,N',N'*-Tetraethylcycloalkane-1,2-dicarboxamide and *trans*-*N,N,N',N'*-Tetramethylcyclopentane-1,2-dicarboxamide

The appropriate *(E)*-1,2-cycloalkanedicarboxylic acid was heated under reflux with thionyl chloride (6 equiv.) for 6 h. The excess thionyl chloride was then removed under reduced pressure. The crude acid chloride was dissolved in ether and added dropwise to a stirred solution of diethylamine (8 equiv.) (or dimethylamine, as in the case of *trans*-*N,N,N',N'*-tetramethylcyclopentane-1,2-dicarboxamide) in ether cooled to 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The precipitated amine hydrochloride was filtered off and washed several times with ether. The combined organic layers were concentrated *in vacuo* to give the diamide product.

Preparation of *trans*-1,2-Bis(diethylaminomethyl)cycloalkane

To a solution of lithium aluminium hydride (4 equiv.), in dry ether, in an atmosphere of nitrogen, was added dropwise a solution of the appropriate diamide in dry ether. When the addition was complete, the mixture was heated at reflux for 1 h. The mixture was then cooled in an ice bath and excess hydride

was decomposed by the dropwise addition of water, followed by a 15% solution of sodium hydroxide, then water again. After vigorous stirring for 10 min the mixture was filtered with suction and the granular precipitate was washed thoroughly with ether. The filtrate was dried ($MgSO_4$) and concentrated *in vacuo* to give the crude cycloalkane.

trans-1,2-Bis(diethylaminomethyl)cyclobutane (131) dihydrochloride

trans-N,N,N',N'-Tetraethylcyclobutane-1,2-dicarboxamide was obtained as an oil (0.12 g, 71%), ν_{max}/cm^{-1} (thin film) 3419, 2978, 2485 and 1622; δ_H (200 MHz, $CDCl_3$) 1.13 (12H, m), 2.13 (4H, m), and 3.15-3.61 (8H and 2H, m); δ_C (50 MHz, $CDCl_3$) 12.8 (CH_3), 14.4 (CH_3), 22.4 (CH_2 on the ring), 38.2 (CH), 39.8 (CH_2), 41.2 (CH_2) and 172.4 (CO); *m/z* 254 (M^+, 2.1%), 154, 128, 100, 72 (100%), 58, 44 and 30 (Found: M^+, 254.1988. $C_{14}H_{26}N_2O_2$ requires M^+, 254.1994).

trans-1,2-Bis(diethylaminomethyl)cyclobutane was obtained as an oil, and initial analysis showed that it contained impurities. This impure product was dissolved in ether and ethereal hydrochloric acid was added dropwise. A white glutinous solid precipitated and many attempts at crystallisation only partially purified the product. *trans*-1,2-Bis(diethylaminomethyl)cyclobutane (free base); δ_H (200 MHz, $CDCl_3$) 1.00 (12H, m), 1.56 (2H, m), 2.03 (4H, m), and 2.32-2.57 (8H and 4H, m); δ_C (50 MHz, $CDCl_3$) 11.4 (CH_3), 25.8 (CH_2), 38.6 (CH), 46.9 (CH_2) and 58.6 (CH_2); *m/z* (dihydrochloride salt) 154, 140, 86 (100%), 72 and 58.

trans-1,2-Bis(diethylaminomethyl)cyclopentane (132)

trans-*N,N,N',N'*-Tetraethylcyclopentane-1,2-dicarboxamide was obtained as a light yellow oil (0.73 g, 89%), δ_H (90 MHz, $CDCl_3$) 1.13 (12H, m), 1.75 (4H, m), 2.00 (2H, m) and 3.20-3.55 (8H and 2H, m).

trans-1,2-Bis(diethylaminomethyl)cyclopentane was produced as a light coloured oil (0.71 g, 87%), ν_{max}/cm^{-1} (thin film) 2968, 2936, 2871 and 2796; δ_H (200 MHz, $CDCl_3$) 0.99 (12H, t), 1.31 (2H, m) 1.46-1.82 (4H and 2H, m), 2.32 (4H, m) and 2.48 (8H, q); δ_C (50 MHz, $CDCl_3$) 11.5 (CH_3), 24.2 (CH_2), 31.1 (CH_2), 41.9 (CH), 47.0 (CH_2) and 58.7 (CH_2); *m/z* 241 (M^++1), 240 (M^+, 7.7%), 211, 152, 138, 112, 99, 86 (100%), 72 and 58 (Found: C, 74.84; H, 13.10; N, 11.79%; M^+, 240.2564. $C_{15}H_{32}N_2$ requires C, 74.91; H, 13.42; N, 11.66%; M^+, 240.2565).

trans-1,2-Bis(dimethylaminomethyl)cyclopentane (130)[88]

trans-*N,N,N',N'*-Tetramethylcyclopentane-1,2-dicarboxamide was obtained as an oil (0.56 g, 77%), ν_{max}/cm^{-1} (thin film) 3478, 2946, 2872 and 1636; δ_H (200 MHz, $CDCl_3$) 1.26-1.53 (4H, m), 1.72-1.83 (2H, m), 2.64 (6H, s), 2.81 (6H, s) and 3.15-3.31 (2H, m); δ_C (50 MHz, $CDCl_3$) 25.2 (CH_2), 30.1 (CH_2), 35.3 (CH_3), 37.0 (CH_3), 44.8 (CH) and 174.5 (CO); *m/z* 213 (M^++1), 212 (M^+, 7.1%), 168, 140, 95 and 72 (100%) (Found: M^+, 212.1528. $C_{11}H_{20}N_2O_2$ requires M^+, 212.1525).

trans-1,2-Bis(dimethylaminomethyl)cyclopentane was obtained as an oil (0.26 g, 60%), ν_{max}/cm^{-1} (thin film) 2943, 2856, 2814 and 2763; δ_H (200 MHz, $CDCl_3$) 1.18-1.33 (2H, m), 1.43-1.80 (4H and 2H, m), 2.06-2.11 (4H, m) and 2.13 (12H, s); δ_C (50 MHz, $CDCl_3$) 24.3 (CH_2), 31.2 (CH_2), 42.2 (CH), 45.9 (CH_3) and 65.7 (CH_2); *m/z*

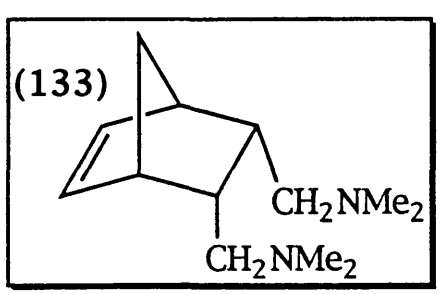
185 (M^++1), 184 (M^+, 1.1%), 124, 84 (100%), 71 and 58 (Found: M^+, 184.1935. $C_{11}H_{24}N_2$ requires M^+, 184.1939).

Attempted Preparation of *trans*-1,2-Bis(diethylaminomethyl)-cyclohexane dihydrochloride.

trans-N,N,N',N'-Tetraethylcyclohexane-1,2-dicarboxamide was obtained as a thick oil (1.17 g, 71%), ν_{max}/cm^{-1} (thin film) 2976, 2932, 2857, 1636 and 1452; δ_H (200 MHz, $CDCl_3$) 0.71 (12H, m), 1.39-1.48 (4H, m), 1.71-2.20 (4H, m) and 2.72-3.25 (8H and 2H, m); δ_C (50 MHz, $CDCl_3$) 12.6 (CH_3), 14.2 (CH_3), 24.9 (CH_2), 29.2 (CH_2), 40.0 (CH_2), 41.7 (CH_2), 42.1 (CH) and 174.0 (CO); *m/z* 283 (M^++1), 282 (M^+, 1.1%), 254, 210, 100, 72 (100%), 58 and 44 (Found: M^+, 282.2305. $C_{16}H_{30}N_2O_2$ requires M^+, 282.2307).

trans-1,2-Bis(diethylaminomethyl)cyclohexane was obtained as an oil and initial ^1H NMR spectral analysis showed that it contained a large number of impurities. This impure product was dissolved in ether and ethereal hydrochloric acid was added dropwise. A dark brown glutinous solid precipitated and many attempts at crystallisation failed to purify the product.

Synthesis of *cis*-2,3-Bis(dimethylaminomethyl)norborn-5-ene (133)



Preparation of Bicyclo[2.2.1]hept-2-ene-5,6-*endo*-dicarboxylic Anhydride[89]

Maleic anhydride (7.42 g, 75.7 mmol) was dissolved in ether (150 ml) and freshly distilled cyclopentadiene (5 g, 75.7 mmol) was added. A white precipitate appeared immediately and the mixture was stirred overnight. The solid was filtered and washed well with ether (7.35 g, 60%), m.p. 148-150 °C (lit.,[121] 147-148 °C); ν_{max}/cm^{-1} (KBr disc) 2981, 2956, 2880, 1855 and 1773; δ_H (200 MHz, $CDCl_3$) 1.68 (2H, dd), 3.54 (2H, split m), 3.58 (2H, m) and 6.31 (2H, m); δ_C (50 MHz, $CDCl_3$) 46.1 (CH), 47.1 (CH), 52.8 (CH_2), 135.5 (CH, olefin) and 171.4 (CO); *m/z* 165 (M^++1), 164 (M^+, 3.4%), 120, 91 and 66 (100%) (Found: C, 65.36; H, 4.81%; M^+, 164.0467. $C_9H_8O_3$ requires C, 65.83; H, 4.91%; M^+, 164.0473).

Preparation of Bicyclo[2.2.1]hept-2-ene-5,6-*endo*-dicarboxylic Acid

Bicyclo[2.2.1]hept-2-ene-5,6-*endo*-dicarboxylic anhydride (1 g, 6 mmol) was dissolved in 10 % sodium hydroxide solution (15 ml) and stirred for 10 min. The mixture was then cooled to 0 °C and 10 % citric acid was added dropwise until a white precipitate appeared. This white solid was filtered off and washed well with dry ether (0.7 g, 63%), δ_H (200 MHz, $CDCl_3$) 1.27 (2H, dd), 3.15 (2H, m), 3.26 (2H, m) and 6.26 (2H & 2H, bs); δ_C (50MHz) 46.3 (CH), 48.3 (CH), 48.7 (CH_2), 134.8 (CH) and 174.8 (CO).

Preparation of *N,N,N',N'*-Tetramethylbicyclo[2.2.1]hept-2-ene-5,6-*endo*-dicarboxamide

To a suspension of bicyclo[2.2.1]hept-2-ene-5,6-*endo*-dicarboxylic acid (0.4 g, 2.19 mmol) in benzene (10 ml) was added

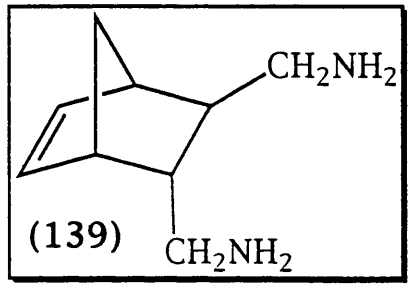
hexamethylphosphorus triamide (2 ml, 11 mmol) dropwise. The mixture was heated to reflux temperature for 20 min and then allowed to cool to room temperature. Saturated sodium bicarbonate solution (10 ml) was added and the layers were separated. The aqueous layer was extracted with DCM and the extracts were combined, dried (Na_2SO_4) and concentrated *in vacuo* to give a sticky solid. This was washed with hexane to give a white solid (0.37 g, 71%), ν_{max}/cm^{-1} (KBr disc) 3468, 2969, 2942, 1636 and 1632; δ_H (200 MHz, $CDCl_3$) 1.41 (2H, dd), 2.90 (12H, d), 3.10 (2H, m), 3.31 (2H, m) and 6.26 (2H, m); δ_C (50 MHz, $CDCl_3$) 35.6 (CH_3), 36.8 (CH_3), 46.9 (CH), 48.0 (CH), 48.2 (CH_2), 134.6 (CH) and 171.9 (CO); *m/z* 237 (M^++1), 236 (M^+, 2.9%), 191, 164, 126 (100%), 98 and 72 (Found: M^+, 236.1525. $C_{13}H_{20}N_2O_2$ requires M^+, 236.1525).

Preparation of *cis*-2,3-Bis(dimethylaminomethyl)norborn-5-ene

To a solution of lithium aluminium hydride (0.2 g, 5.2 mmol) in dry THF (10 ml) in an atmosphere of nitrogen was added a solution of *N,N,N',N'*-tetramethylbicyclo[2.2.1]hept-2-ene-5,6-*endo*-dicarboxamide (0.3 g, 1.3 mmol) in dry chloroform (1 ml). The mixture was heated at reflux for 1 h. The mixture was then cooled in an ice bath and excess hydride was decomposed by the dropwise addition of water (0.5 ml), followed by a 15% solution of sodium hydroxide (0.5 ml), then water (1 ml) again. After vigorous stirring for 10 min the mixture was filtered with suction and the granular precipitate was washed thoroughly with ether. The filtrate was dried and concentrated *in vacuo* to give a slightly yellow oil (0.06 g, 23%), Rf 0.75(1:1 pet. ether: ethyl acetate); δ_H (200 MHz, $CDCl_3$) 1.35 (2H, dd), 1.93 (4H, m), 2.20 (12H, s), 2.35

(2H, m), 2.91 (4H, m) and 6.14 (2H, m); δ_C (50 MHz, $CDCl_3$) 39.2 (CH), 45.9 (CH and CH_3), 48.5 (CH_2), 59.3 (CH_2) and 135.3 (CH); *m/z* 209, 208, 164, 150, 97, 82 and 58 (100%) (high molecular weight impurities present).

Synthesis of *trans*-5,6-Bis(aminomethyl)bicyclo[2.2.1]hept-2-ene (139)[76]



(139)

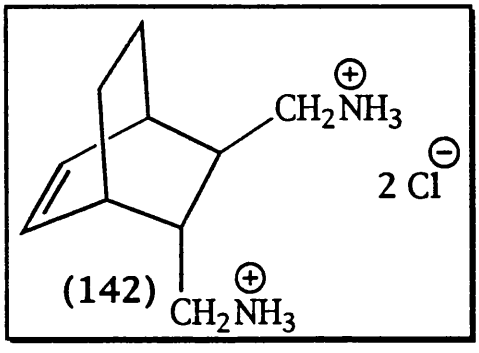
Preparation of *trans*-Bicyclo[2.2.1]hept-5-ene-5,6-dinitrile

To a cooled solution of fumaronitrile (2.73 g, 35 mmol) in ethanol (20 ml) was added, with stirring, freshly distilled cyclopentadiene (2.55 g, 38 mmol). When the dropwise addition was complete the solution was concentrated *in vacuo* to half its original volume. The solution was cooled to 0 °C and an ice crystal was used to seed crystallisation. The product was recrystallised from ethanol (3.81 g, 75%), m.p. 92-94 °C (lit.,[113] 95.5-96 °C); ν_{max}/cm^{-1} (KBr disc) 3448, 3072, 2998, and 2242; δ_H (200 MHz, $CDCl_3$) 1.64-1.81 (2H, m), 2.50-2.53 (1H, m), 3.16-3.19 (1H, m), 3.40-3.45 (2H, m) and 6.37 (2H, m); δ_C (50 MHz, $CDCl_3$) 34.5 (CH), 34.6 (CH), 46.2 (CH), 47.2 (CH), 48.2 (CH), 119.5 (CN), 119.9 (CN), 135.6 (CH) and 137.1 (CH); *m/z* 144 (M^+, 0.3%), 117, 104, 90, 77, 66 (100%), 51 and 39 (Found: C, 75.10; H, 5.45; N, 19.44%; M^+, 144.0674. $C_9H_8N_2$ requires C, 75.00; H, 5.55; N, 19.44%; M^+, 144.0678).

Preparation of *trans*-5,6-Bis(aminomethyl)bicyclo[2.2.1]hept-2-ene (139)

A three-necked flask was equipped with stopper, septum and condenser with nitrogen balloon. Lithium aluminium hydride (1.06 g, 27.9 mmol) was put into the flask and dry ether (15 ml) was added. *trans*-Bicyclo[2.2.1]hept-5-ene-5,6-dinitrile (1 g, 6.94 mmol) in dry ether (25 ml) was added dropwise over 20 min. Towards the end of the reaction the mixture became glutinous and difficult to stir. After addition was complete the reaction was chilled to 0 °C and water (2 ml), 15% sodium hydroxide solution (2 ml) and more water (5 ml) were added sequentially and cautiously. The resulting white precipitate was filtered off and washed well with dry ether (3 × 20 ml). The product was immiscible with ether and a fine emulsion resulted. The emulsion was dried (Na_2SO_4) and concentrated *in vacuo* to give a colourless oil (0.98 g, 93%), ν_{max}/cm^{-1} (thin film) 3291, 2959, 2909, 2870, 2432, 1570 and 1458; δ_H (200 MHz, $CDCl_3$) 0.90 (1H, ddd), 1.36 (1H, d), 1.44 (1H, d), 1.60 (1H, ddd), 2.26 (1H, dd), 2.45 (1H, dd), 2.57 (1H, dd), 2.61(1H, bs), 2.73 (1H, dd), 2.84 (1H, bs), 6.06 (1H,dd), 6.23 (1H, dd); δ_C (50 MHz, $CDCl_3$) 44.6 (CH), 45.5 (CH), 46.0 (CH_2), 46.9 (2 × CH_2), 47.7 (CH), 48.3 (CH), 135.0 (CH), 138.0 (CH). *m/z* 153 (M^++1), 152 (M^+, 1.0%), 135, 122, 106, 91, 78, 69, 66 (100%) and 56 (Found: M^+, 152.1314. $C_9H_{16}N_2$ requires M^+, 152.1314).

Attempted Synthesis of *trans*-2,3-Bis(aminomethyl)bicyclo-[2.2.2]oct-5-ene (142)

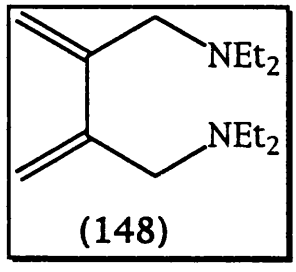


Attempted Preparation of *trans*-2,3-Bis(bromomethyl)bicyclo-[2.2.2]oct-5-ene

trans-2,3-Bicyclo[2.2.2]oct-5-ene-2,3-methanol (0.2 g, 1.19 mmol) was dissolved in DCM (1 ml) and the solution was cooled to 0 °C in an ice bath. Phosphorus tribromide (0.22 ml, 2.38 mmol) was added dropwise and the resulting dark brown mixture was stirred for 16 h. Ice water (2 ml) was added slowly to hydrolyse excess reactant and the organic and aqueous layers were separated. The organic layer was mixed with ether (10 ml) and this was washed well with saturated sodium bicarbonate solution (3 × 10 ml). This was then dried (Na_2SO_4) and concentrated *in vacuo* to give a light coloured oil which could not be purified.

4.6 Other Work

Attempted Synthesis of 2,3-Bis(diethylaminomethyl)-1,3-butadiene (148)



(148)

Preparation of 2,3-Dibromopropene[92]

1,2,3-Tribromopropane (5 g, 17.8 mmol) and water (1 ml) were placed in a round-bottomed flask and sodium hydroxide (1.25 g) in small lumps was added, at once, with shaking. The mixture warmed up and the flask was immediately connected to a distillation apparatus and was heated directly by means of a Bunsen burner held by hand. The mixture was shaken occasionally and the alkaline layer soon became partly emulsified. Heat was applied until vigorous boiling occurred whereupon spontaneous distillation of the reaction product took place. Water (10 ml) was added to the distillate and the two layers were separated. The lower layer was distilled (70-75°C, 75 mm) to give a clear oil (1.1 g, 30.8 %), ν_{max}/cm^{-1} ($CHCl_3$ solution) 3000, 1750 and 1625; δ_H (200 MHz, $CDCl_3$) 4.20 (2H, s), 5.63 (1H, m), and 6.04 (1H, m); δ_C (50 MHz, $CDCl_3$) 37.1 (CH_2), 121.3 (CH_2) and 127.7 (C, olefin); *m/z* 202 (M^++2), 201 (M^++1), 200 (M^+, 6.3%), 199, 198, 121, 119, 81, 79, 39 (100%) and 28 (Found: M^+, 200.8723. $C_3H_4Br_2$ requires M^+, 200.8739).

Preparation 2-Bromo-3-diethylaminopropene

2,3-Dibromopropene (1 g, 5 mmol) was stirred in chloroform (10 ml) at 0 °C and diethylamine (1.03 ml, 10 mmol) was added dropwise. The resulting dark orange mixture was stirred for 1 h, then washed with water (3 × 10 ml). The organic layer was dried (K_2CO_3) and concentrated *in vacuo* to produce an oil (0.2 g, 21%), ν_{max}/cm^{-1} ($CHCl_3$) 3000, 2750, 1625 and 1100; δ_H (200 MHz, $CDCl_3$) 1.02 (6H, t), 2.57 (4H, q), 3.23 (2H, m), 5.54 (1H, m) and 5.89 (1H, m); δ_C (50 MHz, $CDCl_3$) 11.7 (CH_3), 46.7 (CH_2), 61.5 (CH_2), 117.4 (CH_2, olefinic) and 132.7 (C); *m/z* 193 (M^++1), 192 (M^+, 1.4%), 178, 176, 119, 113, 112, 98, 97, 86 (100%), 84, 82, 58, 56, 47 and 42 (Found: C, 43.66; H, 7.35; N, 7.15%; M^+, 193.0294. $C_7H_{14}NBr$ requires C, 43.75; H, 7.29; N, 7.29%; M^+, 193.0290).

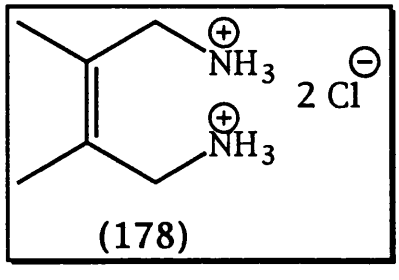
Attempted Preparation of 2,3-Bis(diethylaminomethyl)-1,3-butadiene

Preparation of the Grignard reagent. Into a three-necked flask, fitted with a condenser and under an atmosphere of nitrogen, were put dry, crushed, magnesium turnings (1.1 g) and dry THF (20 ml). A small portion of 2-bromo-3-diethylaminopropene (4 g, 20 mmol) in dry THF (5 ml) was added dropwise and the mixture stirred for 0.5 h. An iodine crystal was added to try to initiate the reaction and then 1,2-dibromoethane (1 drop) was added and the reaction heated up spontaneously. The rest of the 2-bromo-3-diethylaminopropene and THF solution were added dropwise and stirring was continued for another 1 h. When the reaction appeared to cool a heat gun was used to heat the reaction which once again began to reflux. This mixture was

stirred for a final 0.5 h and it was noted that a brown precipitate had appeared.

The coupling reaction. A three-necked flask under an atmosphere of nitrogen was charged with dichloro[1,3-bis(diphenylphosphino)propane]nickel catalyst (0.02 g) and dry THF (10 ml). The flask was cooled to 0 °C and 2-bromo-3-diethylaminopropene (2.14 g, 11.15 mmol) in dry THF (5 ml) was added slowly. The previously prepared Grignard reagent was transferred from its flask using a wide bore needle and added dropwise to the coupling reaction flask. After stirring for 17 h at room temperature, the reaction mixture was hydrolysed using 2M hydrochloric acid (*ca.* 5 ml) at 0 °C. The organic layer was separated and the aqueous layer was basified with sodium hydroxide pellets (pH 13) and extracted with ether (3 × 20 ml). The combined extracts were concentrated *in vacuo* to give a small amount of an oil (*ca.* 0.05 g). Initial ^{1}H NMR analysis indicated only starting material, however MS gave *m/z* 224, 193, 192, 178, 119, 97, 86 (100%), 56 and 42.

Attempted Synthesis of *cis*-1,4-Bis(aminomethyl)-2,3-dimethylbut-2-ene (178)



(178)

Preparation of Diethyl 4,5-Dimethyl-1,2,3,6-tetrahydro-pyridazine-1,2-dicarboxylate[100]

2,3-Dimethylbuta-1,3-diene (1 g, 12 mmol) was dissolved in benzene (5 ml) and diethyl azodicarboxylate (DEAD) (1.91 ml, 12 mmol) was added dropwise. The mixture was stirred at room temperature for 14 h at which time no starting material could be detected by TLC. The mixture was concentrated *in vacuo* to yield diethyl 4,5-dimethyl-1,2,3,6-tetrahydro-pyridazine-1,2-dicarboxylate as an oil (2.63 g, 84%), δ_H (200 MHz, $CDCl_3$) 1.05 (6H, t), 1.42 (6H, s), 3.71 (4H, m) and 4.00 (4H, q); δ_C (50 MHz) 14.3 (CH_3), 15.2(CH_3), 46.9 (CH_2 in ring), 62.0(CH_2), 122.6 (C) and 155.3 (CO); *m/z* 257 (M^++1), 256 (M^+, 27.3%), 183, 167, 111 (100%), 94, 55 and 41 (Found: M^+, 256.1415. $C_{12}H_{20}N_2O_4$ requires M^+, 256.1422).

General Procedure for Nitrogen-Nitrogen Bond Cleavage

Preparation of *cis*-1,4-Bis(ethoxycarbonylamino)-2,3-dimethyl-but-2-ene

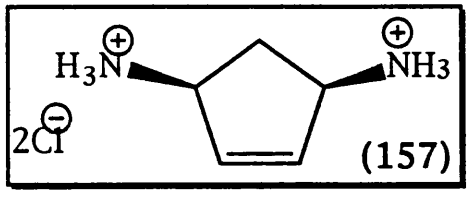
Liquid ammonia (*ca.* 100 ml) was fed from its pressurised container into a 500 ml three-necked flask, with cold finger, and stirred. Diethyl 4,5-dimethyl-1,2,3,6-tetrahydro-pyridazine-1,2-dicarboxylate (1.25 g, 5 mmol) was added slowly and allowed to mix well. Sodium balls (*ca.* 8) were added one by one, over *ca.* 1 h until the blue colour remained. Stirring was continued for a further 2 h and then excess ammonium chloride (*ca.* 2.5 g) was added slowly. The ammonia was allowed to evaporate off and the white solid was washed with ethyl acetate (4 × 30 ml). The extracts were combined, dried (Na_2SO_4) and concentrated *in*

vacuo to yield a clear oil which was a mixture of product and starting material (0.64 g), δ_H (200 MHz, $CDCl_3$) 1.00 (6H, m, SM), 1.25 (6H and 2NH, m), 1.58 (6H, m, SM), 1.69 (6H, m), 3.80 (4H (SM) and 4H, m) and 4.11 (4H (SM) and 4H , q); δ_C (50 MHz) 14.3 (CH_3, SM), 14.4 (CH_3), 15.3 (CH_3, SM), 21.1 (CH_3), 42.5 (CH_2), 44.9 (CH_2, SM), 60.4 (CH_2), 60.8 (CH_2, SM), 121.9 (C, SM), 129.1 (C), 154.1 (CO, SM) and 156.9 (CO); *m/z* 259 (M^++1), 258 (M^+, 0.4%), 256, 169, 156 (100%), 96, 84 and 43 (Found: M^+, 258.1581. $C_{12}H_{22}N_2O_4$ requires M^+, 258.1580).

Attempted Preparation of *cis*-1,4-Bis(aminomethyl)-2,3-dimethylbut-2-ene

Crude *cis*-1,4-bis(ethoxycarbonylamino)-2,3-dimethylbut-2-ene (0.52 g) was stirred in a hydrobromic acid and acetic acid solution at 25 °C for 18 h. The mixture was washed with DCM (4 × 10 ml). The extracts were combined, dried (Na_2SO_4) and concentrated *in vacuo* to produce a black solid which could not be purified.

Attempted Synthesis of *cis*-3,5-diaminocyclopentene



(157)

Preparation of Diethyl 2,3-Diazobicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate[101]

Freshly distilled cyclopentadiene (2 g, 30 mmol) was added dropwise via a pressure equilibrated dropping funnel to a stirred

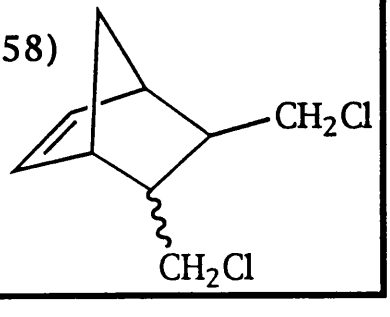
solution of diethyl azodicarboxylate (DEAD) (5 g, 28 mmol). A gentle reflux was maintained for 30 min and then the reaction mixture was allowed to stand overnight. The ether and unreacted cyclopentadiene were removed under reduced pressure to leave the product as a clear oil (6.91 g, 96%), ν_{max}/cm^{-1} (thin film) 2982, 1748, 1704 and 1317; δ_H (200 MHz, $CDCl_3$) 1.19 (6H, t), 1.63 (2H, m), 4.11 (4H, q), 5.05 (2H, m) and 6.42 (2H, m); δ_C (50 MHz) 14.1 (CH_3), 47.7 (CH_2), 62.0 (CH_2), 65.1 (CH), 136.2 (CH, olefin) and 158.6 (CO); *m/z* 241 (M^++1), 240 (M^+, 34%), 168, 123 and 66 (100%) (Found: C, 54.87; H, 6.52; N, 11.55%; M^+, 240.1104. $C_{11}H_{16}N_2O_4$ requires C, 55.00; H, 6.66; N, 11.66%; M^+, 240.1110).

Attempted Preparation of *cis*-(ethoxycarbonylamino)cyclopentene

The same procedure was followed as described above for the preparation of *cis*-1,4-bis(ethoxycarbonylamino)-2,3-dimethyl-but-2-ene. An oily material was recovered from the DCM extracts. TLC showed starting material was still present and two other compounds; *m/z* 243 (M^++1, 0.3%), 176, 130, 104 and 67 (100%).

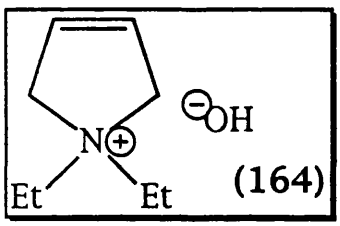
Attempted preparation of 5,6-Di(chloromethyl)bicyclo[2.2.1]hept-2-ene[102]

(158)



Cyclopentadiene (2 g, 30 mmol) and 1,4-dichlorobuta-diene (7.5 g, 60 mmol) were stirred under reflux conditions for 16 h. The mixture was distilled (0.3 mbar 40 °C) and ^1H NMR showed only starting material present in the distillate. The residue left after distillation was a black glutinous tar. Attempts at crystallising product out of this resulted in a dark brown solid which could not be purified.

Synthesis of *N,N*-Diethyl-2,5-dihydropyrrolium hydroxide (164)



(164)

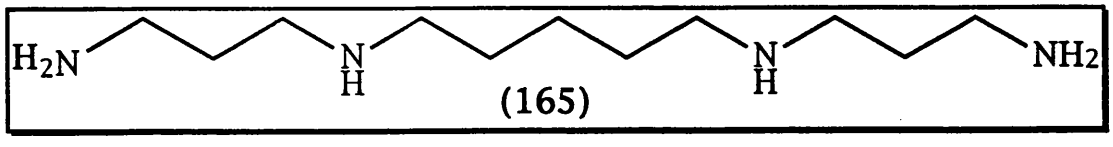
Preparation of *(Z)*-1,4-dibromobut-2-ene

Phosphorus tribromide (10 ml) was cooled to 0 °C in an ice bath and 1,4-but-2-enediol (12 g, 0.136 mol) was added slowly over 3 h. This gave a dark brown solution which was stirred overnight (15 h). Water (0 °C, 50 ml) was added slowly to hydrolyse excess reactant. The two layers were separated and ether (50 ml) was added to the organic layer. This was then washed with saturated sodium bicarbonate solution (3 × 20 ml), dried (Na_2SO_4) and concentrated *in vacuo* to afford a light coloured oil (24.25 g, 83%), ν_{max}/cm^{-1} (thin film) 3987, 3412, 1973 and 1452; δ_H (200 MHz, $CDCl_3$) 4.02 (4H, m) and 5.89 (2H, m); δ_C (50 MHz, $CDCl_3$) 25.4 (CH_2) and 129.8 (CH); *m/z* 216 (M^++2), 215 (M^++ 1), 214 (M^+, 3.4%), 135, 133 (100%), 81 and 53 (Found: M^+, 215.8787. $C_4H_6Br_2$ requires M^+, 215.8797).

Preparation of *N,N*-1,4-Diethyl-2,5-dihydropyrrolium hydroxide

(Z)-1,4-Dibromobut-2-ene (10.7 g, 50 mmol) was dissolved in toluene (25 ml) and was added dropwise to a stirred, cooled (0 °C) solution of diethylamine (7.3 g, 100 mmol) in toluene (25 ml). This was stirred overnight and the precipitate was filtered off, washed with dry ether and immediately stored in a desiccator as it is very hygroscopic. NMR analysis of the crude product showed it to be contaminated with diethylamine hydrobromide. As a method of purification the crude mixture was put down an ion exchange column. The column was prepared by washing Amberlite IRA400 resin (*ca.* 35 ml) with 1M sodium hydroxide solution (250 ml). The pH was monitored using pH paper, and when the eluate returned to neutral the *N,N*-1,4-diethyl-2,5-dihydropyrrolium bromide/diethylamine hydrobromide mixture (2 g) was run down the column in water (250 ml). The eluate was concentrated *in vacuo* to give a thick yellow oil. The product is very hygroscopic and only limited purification was achieved, ν_{max}/cm^{-1} (thin film) 3400, 1610, 1450 and 1400; δ_H (200 MHz, $CDCl_3$); δ_C (50 MHz, $CDCl_3$) ; *m/z* 144 (M^++1), 143 (M^+, 0.6%), 125, 110, 96, 82, 68, 56 and 44 (Found: M^+ (-OH), 126.1253. $C_8H_{16}N$ requires M^+ (-OH), 126.1282).

Synthesis of *N,N'*-Bis(3-aminopropyl)-1,5-diaminopentane (165)



(165)

Preparation of *N,N'*-Bis(2-cyanoethyl)-1,5-diaminopentane

Acrylonitrile (2.66 ml, 40.5 mmol) was added dropwise using a syringe pump (flow rate 5 ml/h) with stirring during 30 min to 1,5-diaminopentane (2.07 g, 20.3 mmol), and the temperature was kept below 30 °C by a cold water bath. Stirring was continued for 5 h after the addition was complete. The mixture was then heated at reflux for 90 min and allowed to stand overnight. The mixture was concentrated *in vacuo* to give a clear oil (3.91 g, 92%), ν_{max}/cm^{-1} ($CHCl_3$) 2900, 2850 and 2250; δ_H (200 MHz, $CDCl_3$) 1.03 (6H, m), 1.33 (2H, s), 2.15 (8H, m) and 2.45 (4H, m); δ_C (50 MHz) 18.1 (CH_2), 24.4 (CH_2), 29.3 (CH_2), 44.7 (CH_2), 48.6 (CH_2) and 119.0 (CN); *m/z* 208 (M^+, 3.9%), 137, 90, 83 (100%), 54 and 42 (Found: C, 63.54; H, 9.57; N, 27.01%; M^+, 208.1686. $C_{11}H_{20}N_4$ requires C, 63.46; H, 9.61; N, 26.92%; M^+, 208.1688).

Attempted Preparation of *N,N'*-Bis(3-aminopropyl)-1,5-diaminopentane Using Raney Nickel

Methanol (20 ml) was saturated with ammonia for 15 min and the resulting solution was put into a three-necked flask. *N,N'*-Bis(2-cyanoethyl)-1,5-diaminopentane (3.9 g, 18.75 mmol) was dissolved in the methanol/ammonia solution which was flushed through with hydrogen gas from a balloon. Raney nickel (1 ml) was added and the mixture was stirred at room temperature for 48 h. The mixture was filtered through a sintered funnel packed with a pad of Celite and washed well with methanol. The filtrate was concentrated *in vacuo* to give a clear oil. This was identified as starting material.

Preparation of *N,N'*-Bis(3-aminopropyl)-1,5-diaminopentane using Borane

A solution of *N,N'*-bis(2-cyanoethyl)-1,5-diaminopentane (1.22 g, 5.86 mmol) in THF (10 ml) was added to borane-THF (1M, 46.88 ml, 46.88 mmol) under nitrogen and heated at reflux for 2.5 h. The reaction mixture was cooled, 6 M hydrochloric acid (10 ml) was added, and the bulk of the THF was removed. The aqueous residue was basified (sodium hydroxide pellets) to pH 13 and extracted with chloroform (4 × 75 ml). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo* to give an oil. TLC (DCM-methanol-conc. ammonia, 2:2:1) showed that starting material was still present. Purification by silica column chromatography was unsuccessful. Further analysis confirmed the presence of a product/starting material mixture, δ_H (200 MHz, $CDCl_3$) 1.19-1.52 (10H, m) and 2.49-2.71 (12 H, m); δ_C (50 MHz) 18.5 (CH_2, SM), 24.8 (CH_2 and CH_2, SM), 29.7 (CH_2 and CH_2 SM), 33.4 (CH_2), 40.3(CH_2), 44.9 (CH_2, SM), 47.7 (CH_2), 48.9 (CH_2, SM), 49.8 (CH_2) and 118.7 (CN, SM).

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