Synthesis of Diamines for Testing as Antifungal Agents

A thesis presented in part fulfilment
of the requirement for the
Degree of Doctor of Philosophy

by
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To my mum, dad, family and old friends (too numerous to mention?), without whom this would not exist, I owe more than I can express.

Finally, this thesis is dedicated to Amanda, with love.

"Free at last, free at last!"

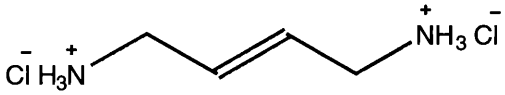
-MLK

Summary

Polyamines are thought to be essential to all living systems. The most common of these polyamines are based on 1,4-diaminobutane (putrescine) (A). The biosynthesis of putrescine and derivatives has been widely studied in plants, animals and microorganisms. In plants, two biosynthetic pathways appear to exist, whereas in mammals and fungi only one pathway occurs. It was believed to be possible to inhibit fungal putrescine biosynthesis, whilst allowing plant putrescine biosynthesis to continue via an alternative pathway. Thus, it was considered that inhibitors of the fungal pathway to putrescine would show selective toxicity towards plant-pathogenic fungi and be non-toxic to plants. The work in this project produced a variety of putrescine analogues for testing as antifungal agents.

(A)

Initial targets were straight-chain analogues of putrescine. Of the primary diamines produced, *E*-1,4-diaminobut-2-ene dihydrochloride (B) showed most widespread antifungal activity. A general trend of increasing activity with increasing alkyl substitution on the amino group of compounds of this type was observed with the synthesis and testing of *E*-1,4-bis(alkylamino)but-2-enes and *E*-1,4-bis(dialkylamino)but-2-enes. In particular, *E*-1,4-bis(diethylamino)but-2-ene dihydrochloride (C) showed high activity against powdery mildew.



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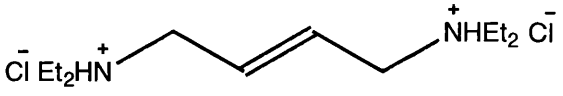
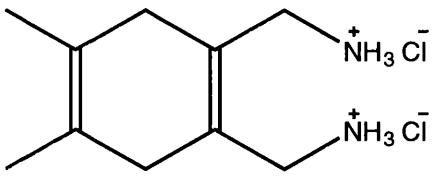


(C)

The synthetic methodology developed in the early targets was applied to the synthesis of several cyclic 1,4-diamines. In this range, high antifungal activity was seen with 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (D) and *trans*-4,5-bis(aminomethyl)-1,2-dimethylcyclohexene dihydrochloride (E).



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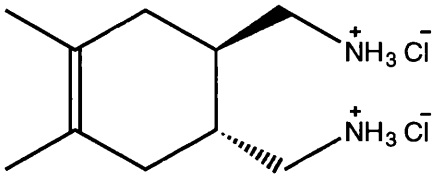


(E)

Some work was also carried out towards the synthesis of heterocyclic analogues of putrescine and also on alternative routes to the 1,4-diamine functionality. The ideas behind these schemes generate further possibilities for development of other antifungal and biologically active agents.

The synthesis recorded in this work and the biological testing of the targets produced, carried out by Dr. Dale Walters and Mr. Neil Havis at the Scottish Agricultural College, Auchincruive, formed the basis of two scientific papers and three patent applications.

Abbreviations

ADC:	argenine decarboxylase
α:	alpha
β:	beta
d:	days
DFMA:	α,α-difluoromethylargenine
DFMO:	α,α-difluoromethylornithine
DIBAL:	diisobutylaluminium hydride
DMF:	dimethylformamide
DNA:	deoxyribonucleic acid
DOPA:	3,4-dihydroxyphenylalanine
δ:	delta
EMGBG:	ethylmethylglyoxal bis(guanylhydrazone)
g:	grams
γ:	gamma
GABA:	γ-aminobutyric acid
h:	hours
IR:	infra-red
LDC:	lysine decarboxylase
M:	molar
MBC:	methyl benzimidazole-2-yl carbamate
MGBG:	methylglyoxal bis(guanylhydrazone)
MHz:	megahertz
min:	minutes
ml:	millilitres
mM:	millimolar

mmol:	millimoles
mol:	moles
μM:	micromolar
NMR:	nuclear magnetic reasonance
ODC:	ornithine decarboxylase
ppm:	parts per million
PTC:	phase transfer catalysis
SAMDC:	*S*-adenosylmethionine decarboxylase
THF:	tetrahydrofuran
TLC:	thin layer chromatography
tRNA:	transfer ribonucleic acid
UV:	ultraviolet
w/v:	weight per volume
w/w:	weight per weight

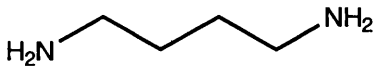
Contents

Chapter 1

Introduction

1.1 Preface

Polyamines such as putrescine (1), spermidine (2) and spermine (3) are probably present in all cells. Little is known for certain about their role and metabolism in plants and agriculturally-important fungi, although they have been more widely studied in mammalian tissue and other prokaryotes. They do appear to have a role in plant growth regulation, and increases in plant growth rate coincide with an increase in the rate of polyamine biosynthesis. Inhibition of polyamine biosynthesis retards growth. Polyamines are also thought to have a role in plant senescence and in response to certain environmental stress situations. Accumulations of putrescine have been observed under such circumstances.



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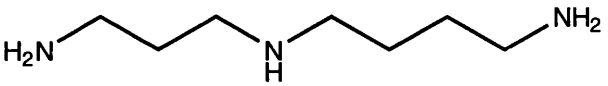

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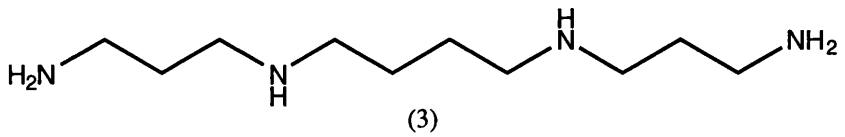
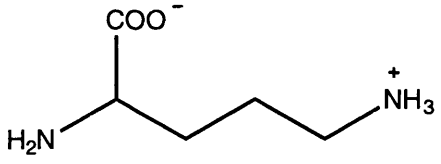


(3)

Putrescine can be formed biologically by two pathways. It is the direct product of the action of the enzyme ornithine decarboxylase (ODC)

on the amino acid ornithine (4). A less direct route follows the decarboxylation of arginine (5) by arginine decarboxylase (ADC). The higher-order polyamines spermidine and spermine are formed from putrescine. The aminopropyl units required for these conversions are provided by the action of *S*-adenosylmethionine decarboxylase (SAMDC). The addition of these aminopropyl units is controlled by spermidine and spermine synthases, respectively.



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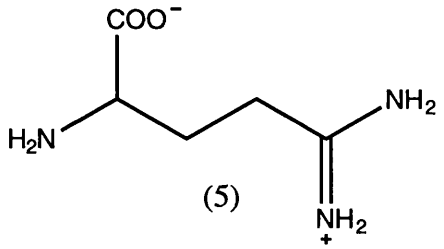

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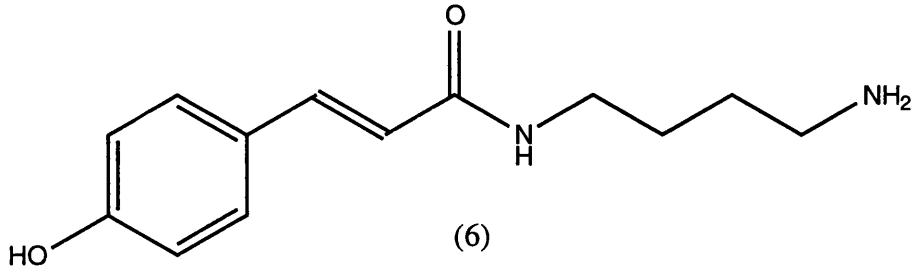
ADC is found only in cell cytosol, while ODC exists in the nucleus also. Initially this indicated that ADC played a part in stress response, whereas ODC was concerned with cell division. In some plant cells, ADC, ODC and SAMDC can be detected in certain organelles, revealing a more complex scenario.[1]

Uncertainty as to the nature of polyamine function has led to recent study of diseased plants. Increased polyamine concentrations are seen around plant tumours. However, this may be due only to increased cell division, and not a disease response. Nevertheless there is evidence of a response to viral infections, with increases in the activities of the enzymes involved with polyamine biosynthesis. This coincides with a rise in the concentration of the *p*-hydroxycinnamic acid amide conjugate (6) of putrescine. The polyamines are regularly found as such amides. When

applied exogenously, phenolic acid derivatives like these have been shown to reduce lesions in virally infected leaves.[1]



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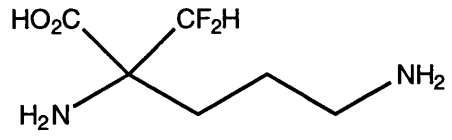
Study of plant polyamine production to counteract pathogenic fungal infection is a complex matter, due to the number and nature of possible biochemical interactions which can result. Ethylene, for example, is often produced following fungal infection of fruit. This process requires *S*-adenosylmethionine, with the result that polyamine production is reduced. This evidence alone might suggest that putrescine and its derivatives are not required as part of the plants' defence mechanism. Yet these compounds have been found to accumulate in 'green islands' around biotrophic fungal pustules on plant leaves. These areas remain healthy while the rest of the foliage senesces. 'Green islands' can also be induced by cytokinins, and the possibility exists that polyamines act as second messengers to delay the onset of ageing. The similarities between the areas induced by pathogens and those by cytokinins has led to the belief that inhibition of polyamine biosynthesis might prevent their formation.[1]

Fungi, and in particular plant pathogenic fungi, appear to possess only one route for polyamine synthesis with putrescine produced only *via* ODC. Higher plants have both the ODC and ADC pathways previously mentioned. As it seems that polyamines are essential to life, inhibition of enzymes in these routes should be fatal. This thinking led to the

development of ODC inhibitors like α-difluoromethylornithine (DFMO) (7) as anti-tumour agents by Merrell-Dow. This compound has also been shown to be a potent *in vitro* and *in vivo* fungicide. Furthermore, no evidence has been accumulated to suggest any adverse effect on plant growth or polyamine concentration.[2]



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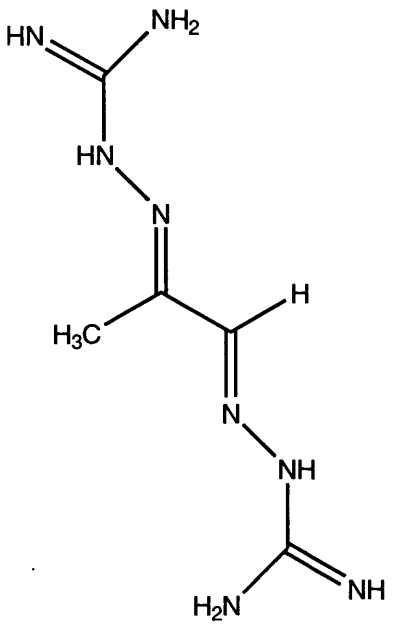
The main polyamine found in fungi is spermidine. Since its formation is dependent on aminopropyl units provided by SAMDC, it was postulated that inhibition of this enzyme would be fatal to the fungi in question. This has proved to be the case, although only in certain species. SAMDC inhibitors such as methylglyoxal bis(guanylhydrazone) (MGBG) (8) and ethylmethylglyoxal bis(guanylhydrazone) (EMGBG) (9) have been shown to be particularly active against biotrophic fungi.[3]

The aim of this work was to synthesise a range of putrescine analogues to be tested as antifungal agents on whole-plant systems. The main targets for these compounds were plant-pathogenic fungi which lead to yield loss in crops, in response to an ever-increasing market for effective control agents of less complexity. Putrescine analogues were chosen as the most promising synthetic goals as these were the most likely to show selective inhibition of ODC over ADC. It was hoped that any such selectivity could be conferred to *in vivo* situations, resulting in a specific, universal toxicity towards fungi.



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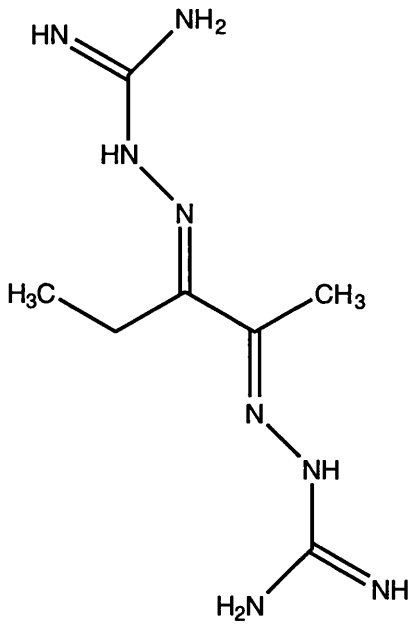

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Some precedent exists for this rationale,[4] although the area has been hitherto relatively unexplored. Thus, it was planned to follow initial results with the design of more particular targets in order to open up this comparatively new strategy against biotrophic fungi.

1.2 Plan of Thesis

A brief outline of polyamine function in plants, and the enzymes and mechanisms involved in their biosynthesis is given in Chapter 2. The most common agriculturally-important fungicides are surveyed in Chapter 3, together with development opportunities related to polyamine regulation. Known synthetic methodologies towards targets indicated in Chapter 3 are reviewed in Chapter 4, while synthetic studies carried out in this work are detailed in the following Chapters 5, 6 and 7. Chapter 8 contains a brief summary of the antifungal results obtained with the

compounds whose synthesis was discussed in the preceding chapters. Finally, the experimental details of the work contained in Chapters 5, 6 and 7 are recorded in Chapters 9, 10 and 11 respectively.

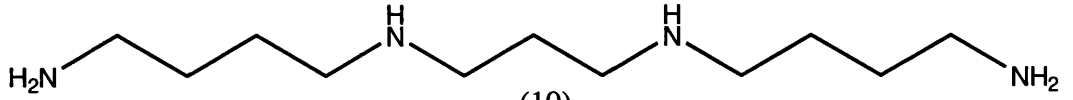
Chapter 2

Polyamines

2.1 Introduction

2.1.1 Occurrence

The polyamines spermidine, spermine, and their precursor putrescine occur ubiquitously in plants[5] in concentrations of approximately 10 micromolar to 1000 micromolar. They exist as free bases, or as amides, when combined with phenolic acids. These amides can be low molecular weight compounds or macromolecules.[6] Concentrations depend on the environment and vary with, for example, potassium deficiency, low external pH and osmotic stress.[6] Other polyamines have been identified which are related in structure and metabolism to those previously mentioned e.g. canavalamine (10) (from *Canavalia*), hirudonine (11) (found in leeches), cadaverine (12) (formed from lysine decarboxylation), and several others in algae.[5] Spermine occurs only in eukaryotes, where it exists exclusively in the nucleus. However, some thermophilic bacteria make other tetramines and even a pentamine. Such strict evolutionary conservation suggests the performance of important cell functions, and it is for this reason that much of the recent biological interest has been stimulated.



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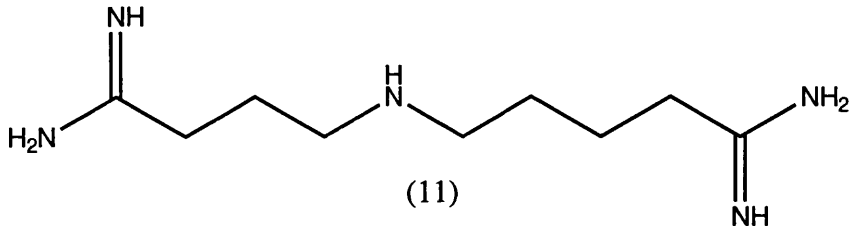

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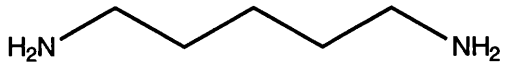

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2.1.2 History

The first evidence of polyamines occurring in nature was observed, albeit unwittingly, by van Leeuwenhoek in 1678. The stellate crystals which he discovered from ageing sperm were not named as spermine phosphate[7] until 1888.[8] Structure elucidation was accomplished during the 1920s, and it was also around this time that spermidine was discovered and identified.[6] The subject mainly attracted the interest of chemists over the next fifty years, probably because biologists were concerned more with the biochemistry of organic anions.[7] Under cellular conditions, polyamines are protonated, and their cationic nature is central to some of their functions.

2.1.3 General function

It is understood that spermine in the nucleus has a role in the conformation of chromatin.[7] With four protonated cationic amino groups, spermine has the potential to bind with four phosphate anionic moieties, applying an overall rigidity to the structure. It is thought that ionic binding to nucleic acids is part of the stimulation of growth. Certainly, increased polyamine synthesis promotes transcription and translation.[5] This effect is only replaceable in part by polyvalent cations

like magnesium,[7] which are normally associated with this process. Indeed, experimental evidence shows that removal of spermine from the genetic material converts DNA from its functional β-arrangement into its inactive Z-form *in vitro*. In addition, spermidine has been found associated with tRNA, and with the nucleic acid of a virus.[7]

Further evidence of polyamine involvement in growth processes are the visible effects produced from addition of exogenous polyamines to certain plant tissues. The onset of senescence is delayed in excised leaves, while growth and flowering have been induced from vegetative tissue cultures. It appears that at least part of the differentiation activity is promoted by these compounds.[6]

The amines also interact ionically with the phosphate anion heads of phospholipids in cell membranes, with the dual effect that the membrane is stabilised to prevent leakage,[5] and the cell is protected from lysis.[6]

In the field of biology polyamines first came under the scrutiny of medical oncologists. It was discovered that metastasizing tumours excrete acetylated putrescine, probably as a detoxification product.[7] It was also noted that the enzyme ODC was activated by partial hepatectomy, by certain hormones, and by carcinogens inducing rapid growth in tissues. A half life of 10-12 minutes was observed for ODC in liver tissues, the shortest observed for any eukaryotic enzyme. ODC activity was found to be essential for replication and cell division, and inhibition of the enzyme prevents cells from progressing from the G1 phase of this process.[7]

This behaviour was not limited to mammalian cells. Rapidly dividing cells of microorganisms and plant tumours showed increases in the synthesis of polyamines. Furthermore, genetically-engineered mutants of *Escherichia coli* and *Saccharomyces cerevisiae*, with the alteration of a

single gene locus blocking biosynthesis of polyamines, were only able to grow on addition of exogenous polyamines. It was also noted that the physiological consequences of the action of 'suicide' inhibitors could be reversed by the addition of polyamines.[7]

At present, polyamine research appears to mirror that of the now-accepted plant hormones. Current evidence, which points to their regulatory role, is similar to that of the hormones at the corresponding stage in the elucidation of their function in the plant. If this trend is to continue, it is expected that specific molecular mechanisms will follow, and that polyamines will be established in their correct physiological and biochemical importance.[6]

2.2 Biosynthesis

2.2.1 Enzymes

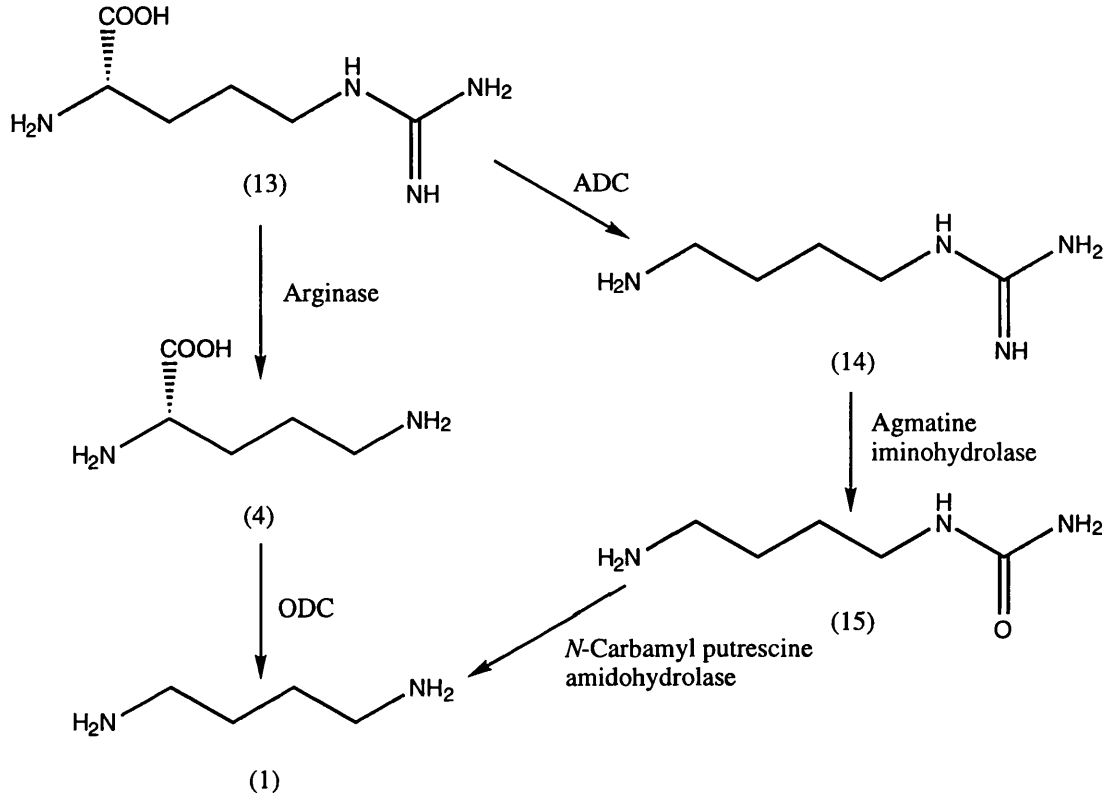
Three amino acids provide the essential groups for polyamine biosynthesis. L-Arginine (13) and L-lysine (16) contribute the four- and five-carbon main skeletons respectively, while L-methionine supplies the three-carbon side chains.[7]

The initial step of polyamine biosynthesis in fungi and mammals is the decarboxylation of L-ornithine (4) to give putrescine (1), performed by the enzyme ornithine decarboxylase (ODC)[1] (Scheme 1).

In plants and bacteria, this route is available alongside an alternative which involves decarboxylation of L-arginine (13) by arginine decarboxylase (ADC). The first product of this route, agmatine (14), is then converted into *N*-carbamylputrescine (15) by the enzyme agmatine iminohydrolase. Ultimately, *N*-carbamylputrescine is converted

enzymatically into putrescine (1) by *N*-carbamylputrescine amidohydrolase.[1]

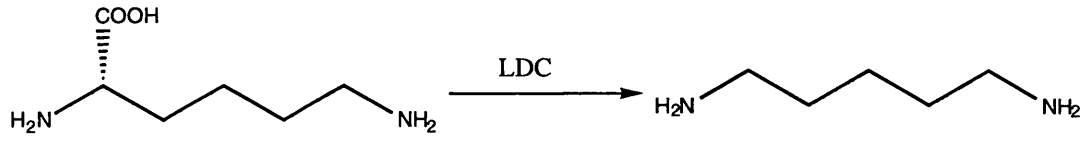
Scheme 1



It should also be noted that the enzyme arginase converts L-arginine (13) into L-ornithine (4), via citrulline as part of the urea cycle. Thus, arginine is ultimately the source of polyamine four-carbon units, regardless of whether the ADC or ODC route predominates.

Decarboxylation of L-lysine (16) furnishes cadaverine (12) (Scheme 2). This occurs by an independent enzymic pathway involving lysine decarboxylase (LDC),[7] although the mechanism is analogous to the ornithine and arginine decarboxylations.

Scheme 2



(16) (12)

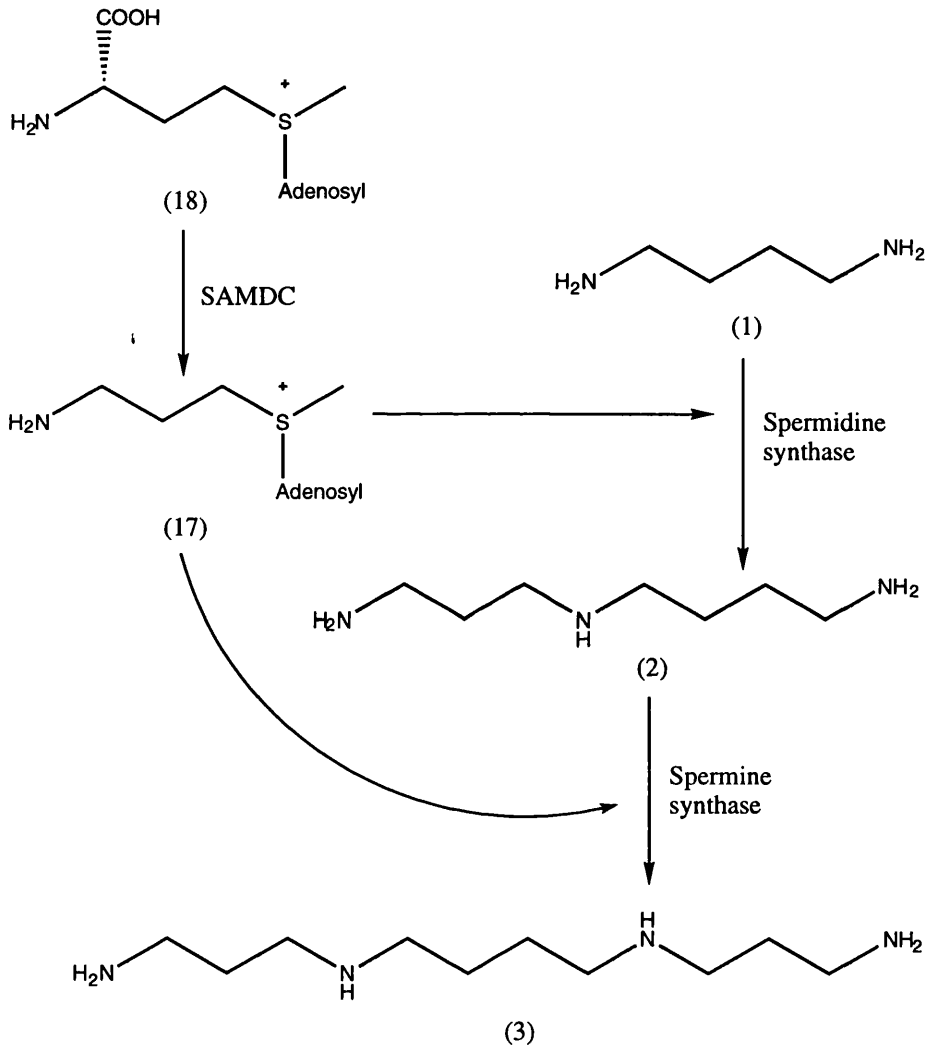
Spermidine (2) is produced from putrescine (1) from either arginine or ornithine using spermidine synthase (Scheme 3). This triamine can then be further modified to spermine (3) by way of spermine synthase.[1] It is at this point that the biosynthesis converges, and enzymes of these types occur in all cells producing these amines.

Each of these steps requires the addition of an aminopropyl group to the substrate. These groups are donated from decarboxylated *S*-adenosylmethionine (17), and this is the product of the action of *S*-adenosylmethionine decarboxylase on the corresponding modified amino acid (18).[1]

Arginine decarboxylase has been widely studied in higher plants, where it can show up to five times its normal activity in response to stresses like potassium deficiency. The enzyme has been isolated from oats, and appears to be a hexamer. It gave two fractions by electrophoresis, one of which was twice the molecular weight and twice as active as the other. An equivalent enzyme isolated from rice gave similar results. The more active fraction was stimulated by pyridoxal phosphate, and consequently was inhibited by NSD 1055, a known inhibitor of enzymes with this co-factor. Inhibition was also seen with D-arginine and canavanine, while the polyamines were only weak inhibitors. The enzyme showed absolute specificity for L-arginine as substrate. ADC has been

characterized in several other species and pyridoxal phosphate is a common co-factor in all ADC enzymes thus far studied.[5]

Scheme 3



Ornithine decarboxylase has been characterized more in animals (being their sole source of putrescine) than in higher plants. However, the enzyme is widespread in plants. It is often found in the cell nucleus, and sometimes up to 75% of the total cell content of ODC is associated with

chromatin. Both nuclear and cytosolic ODC have similar properties. Inhibition of the enzyme can be induced by both putrescine and spermidine via activation of a protein antizyme. A protein activator of ODC is also thought to exist.[5]

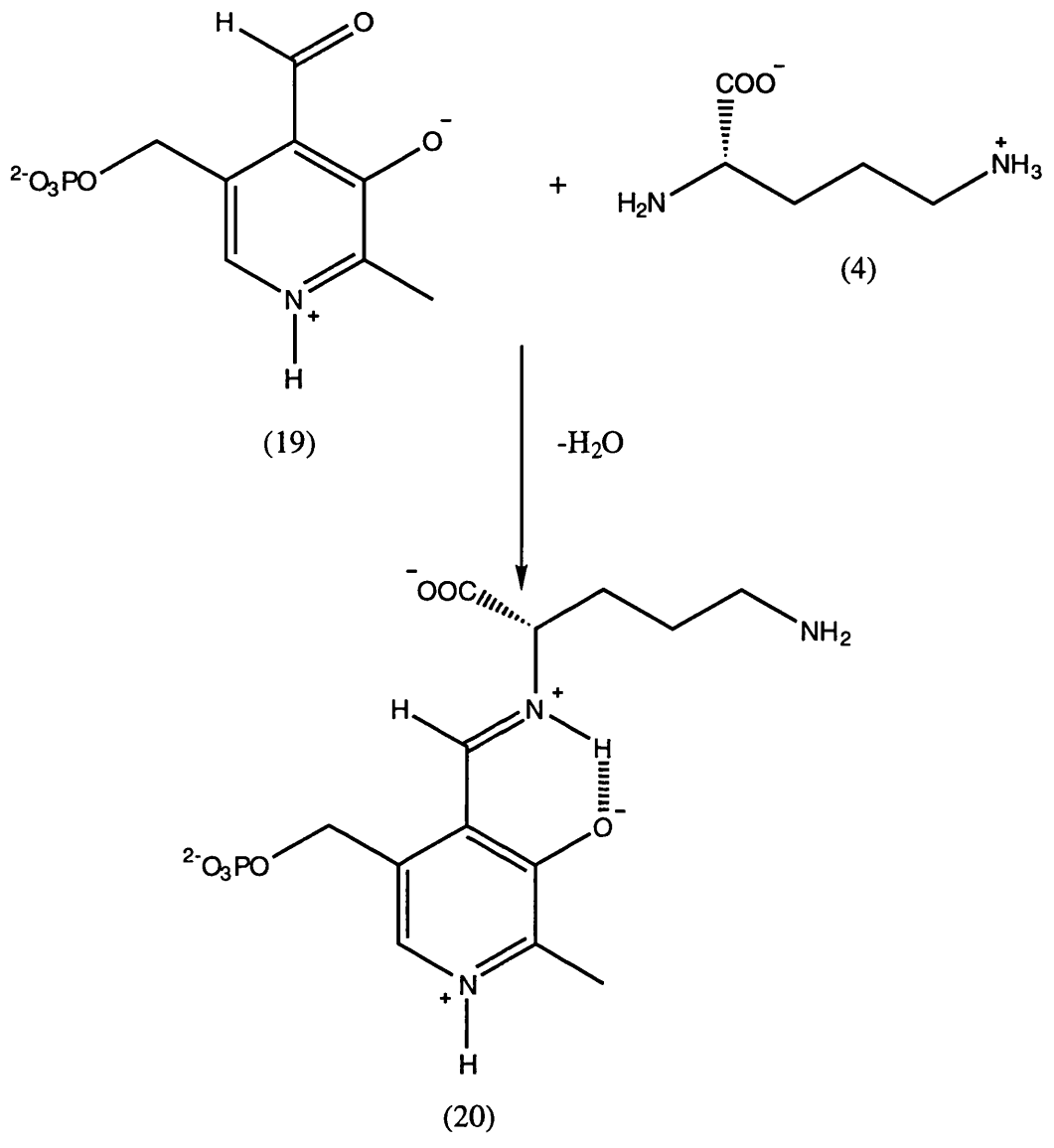
The cellular ratio of ADC to ODC in plants varies with species, type of tissue, and the physiological state in which these exist. Both enzymes are indistinguishable as the source of putrescine. However, in terms of function, it has been postulated that ODC may be involved in cell division, while ADC is concerned with cell elongation, and with responses to light, pH lowering, osmotic shock and potassium deficiency.[5]

S-Adenosylmethionine decarboxylase has been discovered in several plant species, and various characteristics have been observed. Isolated from *Lathyrus sativus*, the enzyme was found to be magnesium ion dependent. Putrescine was shown not to be a stimulant. The optimal pH for catalysis was 7.6. The enzyme was also isolated from corn, and was found to be unstimulated by magnesium or putrescine. In this case the optimal pH was 8.6. Both enzymes exhibited inhibition by MGBG.[5]

2.2.2 Mechanism

A general mechanism has been proposed for the decarboxylation of amino acids. The decarboxylase enzymes operate with a co-factor, pyridoxal phosphate (19). Pyridoxal is a 3-hydroxypyridine-4-carboxaldehyde, and exists in zwitterionic form. The aldehyde group plays a major role in a wide variety of enzymic reactions for which this compound is a co-factor.

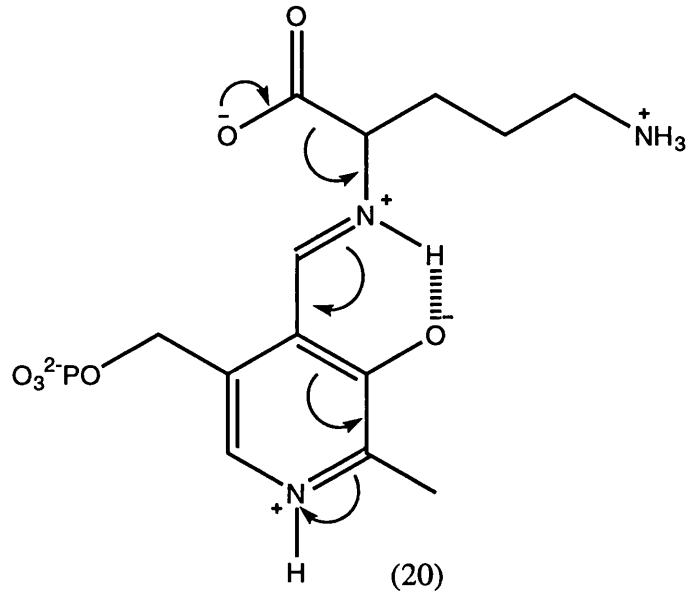
Scheme 4



The first step in decarboxylation reactions involves the condensation between the α-amino group of the amino acid and the aldehyde moiety with the elimination of water, shown here for ornithine (4) (Scheme 4). This forms a Schiff's base imine (20) with the pyridoxal prosthetic group, and allows the second step of decarboxylation to proceed. The release of carbon dioxide in this step generates a carbanion

on the α-carbon which is resonance-stabilised (21) by the pyridinium ring. This lowers the free energy of the reaction (Scheme 5). The third step is protonation of this carbanion, and finally hydrolysis of the imine to yield the appropriate decarboxylated amino acid with concomitant regeneration of the co-factor aldehyde group (Scheme 6).

Scheme 5



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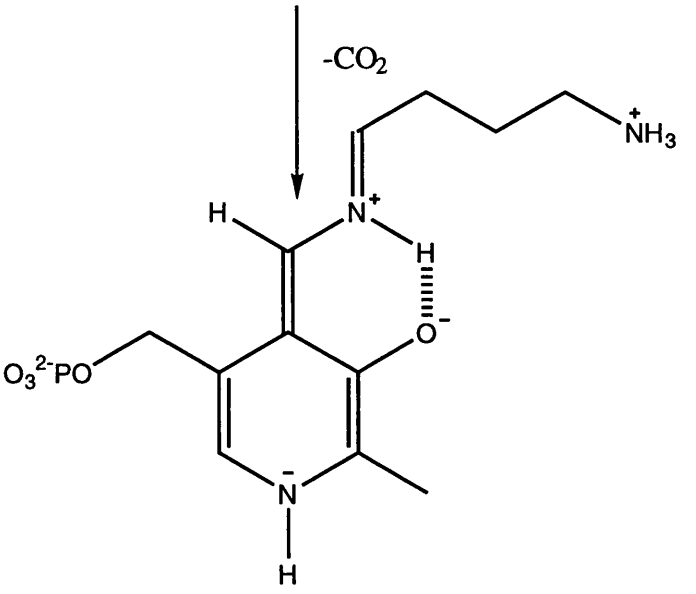

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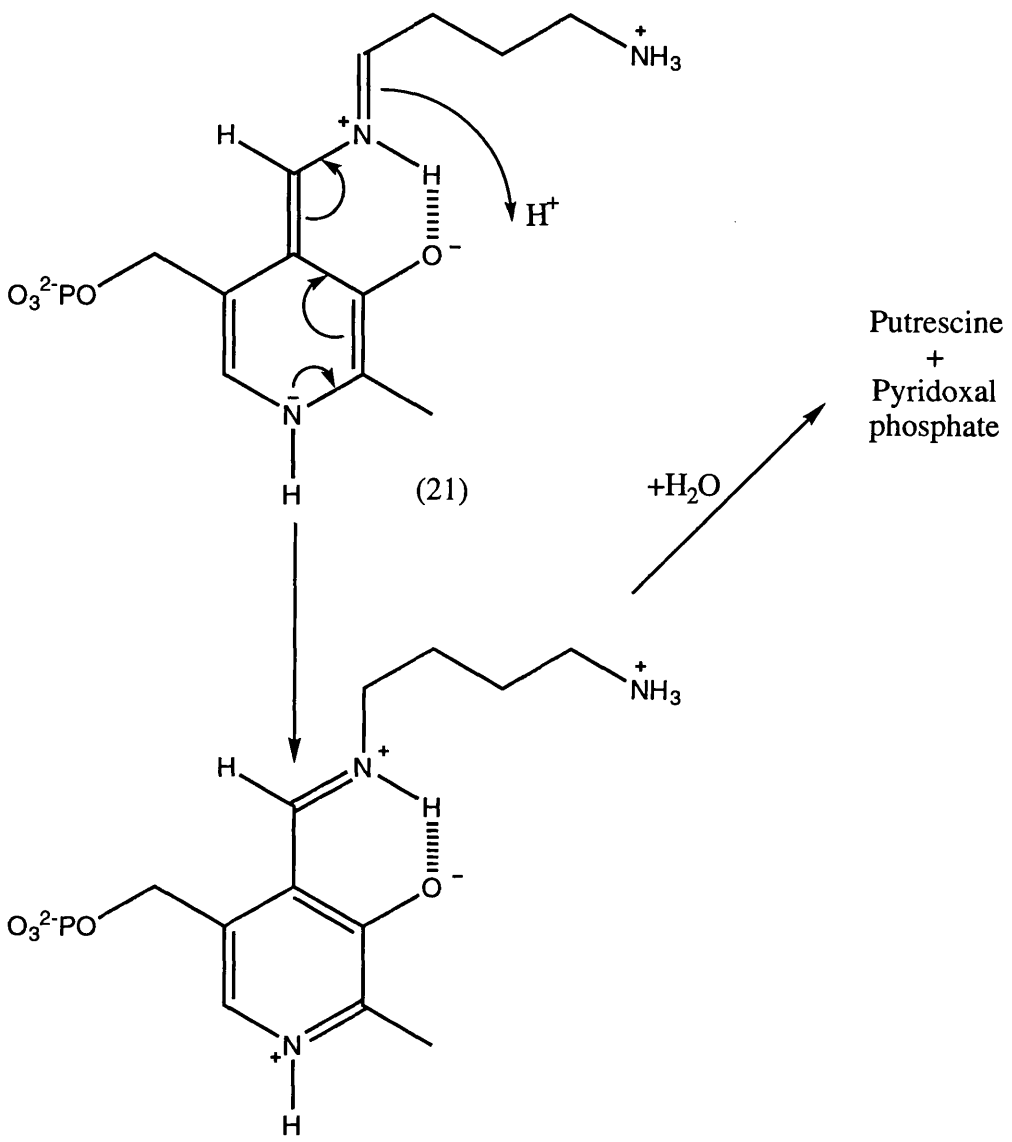
Scheme 6


NH3
H
N
H
H+
O
O3 2-PO
N
H
(21)
+H2O
Putrescine
+
Pyridoxal
phosphate
NH3
H
N
H
O
O3 2-PO
N
H

Chapter 3

Fungicides

3.1 Introduction

According to their actions, fungicides can be divided into two main categories, protectant and systemic. Protectant fungicides act at the site of application, and protect only the seeds, stalks or leaves present at that time. Systemic fungicides can be adsorbed and eliminate disease locally. Most fungicides however are taken up and translocated throughout the plant, with the result that eradication of infection can be effected remote from the site of application. Some fungicides have both preventative and curative capabilities, allowing both eradication of established infections and resistance to future attack from pathogens. The translocation of these fungicides can also allow their properties to be used by new shoots without need for further application.

3.2 Protectant fungicides

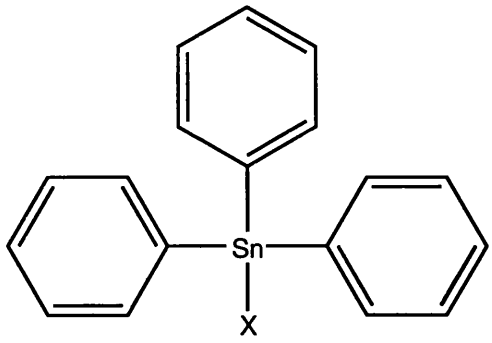
Protectant fungicides are applied to plant surfaces, where the cuticle prevents their uptake into plant tissues. Selectivity is attained because fungal spores or mycelia do not possess a cuticle, and therefore accumulation of the fungicide in the pathogen is not prevented. Although still the most important in terms of use, they have not been widely studied to determine how they execute their toxic effect. A generalised mode of action seems to be a non-specific denaturation of enzymes and proteins in

fungal cells leading to their death. This is achieved often by the complexation of the fungicide with thiol, hydroxyl or amino groups of these proteins.

3.2.1 Metal-based Fungicides: Copper, Tin and Mercury

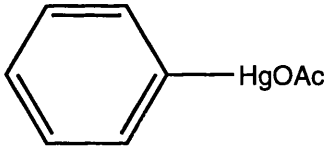
The earliest formulated protectant fungicides were metal-based. Bordeaux mixture has been used for over a hundred years to fight plant infection. It is a solution of copper sulfate and calcium hydroxide, developed by Millardet for protection of vines from attack by downy mildew. Phytotoxicity of Bordeaux mixture led to the development of other copper-based fungicides, such as copper oxychloride. This is less toxic to plants, and is used as a leaf spray. Copper accumulation can occur in sporangia (spore-producing structures) and zoospores (asexual reproductive cells) of blight and downy mildews, and may effect protein denaturation. This may result in the inability of spores to germinate, and this inhibition has been observed in some species. For example, Bordeaux mixture prevents the formation of zoospores in *Phytophthera infestans*. The plasmalemma (cell membrane) is the initial target within the cell, resulting in potassium losses.[9]

Tin-based compounds such as triphenyl tin acetate (fentin acetate) (22) and triphenyl tin hydroxide (fentin hydroxide) (23) offer the advantage of a much lower application dose than their copper counterparts. However, their leaf-surface persistence is not as great, and they are toxic to mammals to an extent. Despite this, they are used as foliar sprays to combat potato blight. The mode of action has not been fully elucidated, although membrane interference occurs in certain fungal species.



(22) X=OAc
(23) X=OH

Diseases of seeds have been controlled by mercury-based fungicides such as phenyl mercury acetate (24) and methoxyethyl mercury silicate (25) since 1913.[10] However, use of these compounds as seed dressings is decreasing due to the cumulative toxic effects of mercury in food chains. Nevertheless, mercurous chloride is still employed to treat club root.



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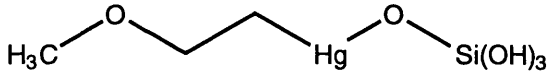


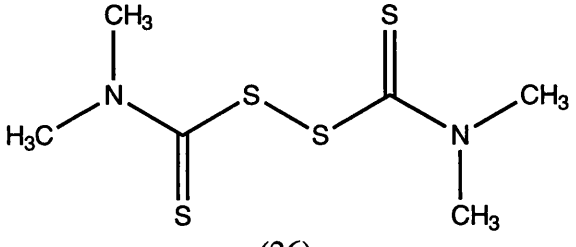
(25)

3.2.2 Sulfur-containing Fungicides: Thiocarbamates and Dithiocarbamates

Application of sulfur is probably the oldest known treatment of crop disease, being first described in 1802,[10] and still used particularly for the prevention of powdery mildew growth on fruit. Its longevity is probably due to its safety and cost efficiency, but it is not very persistent and requires regularly renewed application. Elemental sulfur may be fungicidal due to competition with oxygen as an electron acceptor in

fungal respiratory processes, producing hydrogen sulfide which chelates metals.[11] Its specificity for powdery mildew is due to its selective uptake by that pathogen.[10]

Many organic protectant fungicides contain thio-groups, mainly derivatives of dithiocarbamic acid. These are the most extensively employed fungicides worldwide. Tetramethylthiuram disulfide (26) is widely used as a seed dressing to protect against *Pythium* species, which cause 'damping-off' diseases. Second generation compounds were produced to combat potato blight, downy mildews and rusts. Among these were metal dithiocarbamates like the iron (27) and zinc (28) compounds shown here, first revealed in 1934.[10] These have since largely been superceded by structurally-similar ethylenebisdithiocarbamates (29) and (30), introduced in 1943.[10] It is thought that activities of the former compounds depend upon copper ions, with which they form water-soluble 1:1 complexes and insoluble 1:2 complexes. The enzyme-denaturing effect of these compounds is antagonised by histidine and imidazoles, which compete for them.[12] The ethylenebisdithiocarbamates are understood to break down following uptake into fungal hyphae, and one possible metabolite is ethylenediisothiocyanate. It is believed that this compound may react with protein thiol moieties, causing denaturation and death. The effects are inhibited by cysteine and thioglycolic acid.[12]



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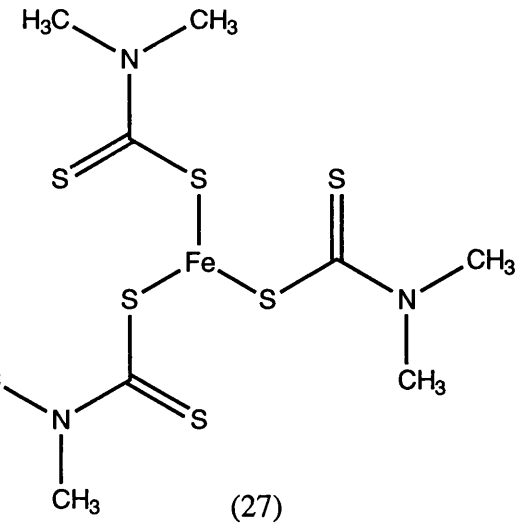

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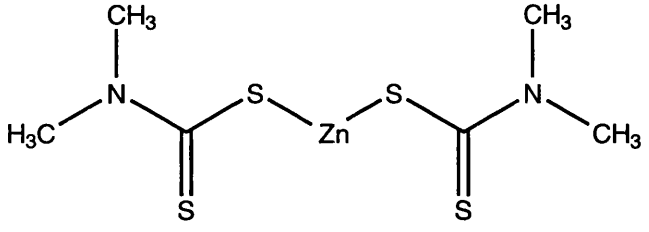

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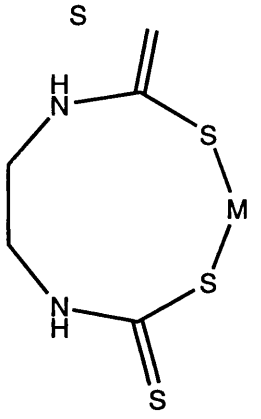
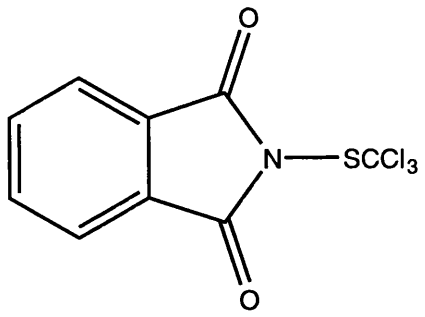


(29) M=Zn
(30) M=Mn

3.2.3 Dicarboximides

Dicarboximides form a major class of protectant fungicides. The earliest of these was captan (31), developed in the 1950s and employed against *Pythium* damping-off diseases and scab on apples. It is thought that captan and the related dichlofluanid (32) may produce thiophosgene in fungal cytoplasm, which would again cause non-specific protein denaturation through reaction with thiol groups. Dichlofluanid is very effective against *Botrytis cinerea* (grey mould), preventing spore germination.



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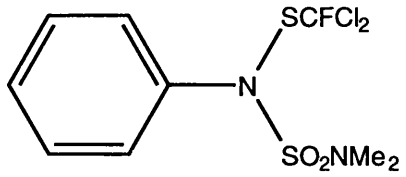
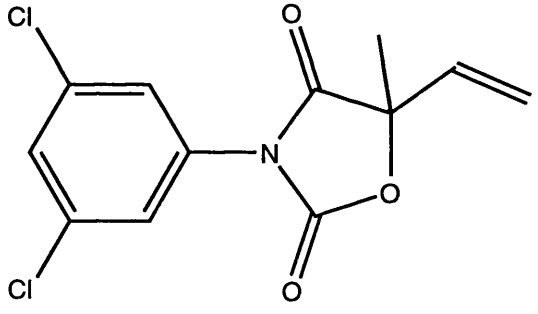


(32)

Other dicarboxamides are used against *Botrytis cinerea*, and oilseed rape infections. Among these are vinclozolin (33) and iprodione (34), introduced in the 1970s. Although mainly classed as protectant fungicides, some systemic nature has been noted. Allied to this, their activity seems to be more selectively restricted to sites in the cell nucleus.



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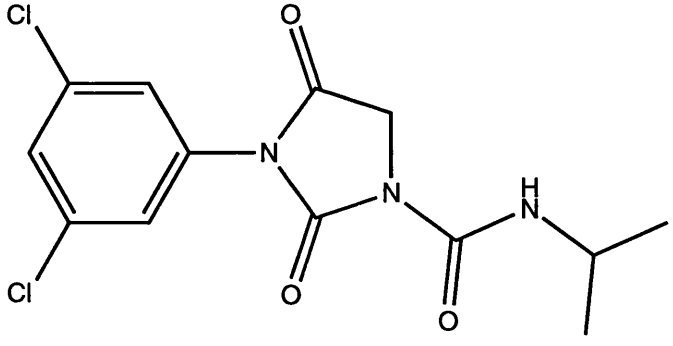


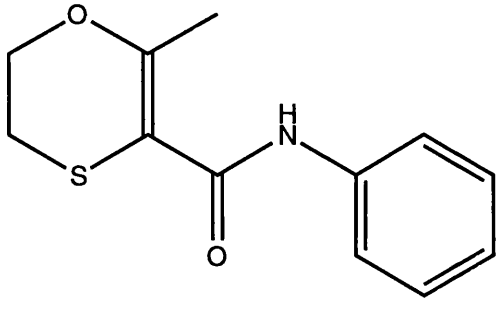
(34)

3.3 Systemic Fungicides

All systemic fungicides are site-specific. They inhibit particular biochemical processes, as opposed to the general toxicity of protectant fungicides. Whereas a barrier prevents protectant fungicides from entering plant tissue, this is not required with systemic antifungal agents due to their selectivity, and they are free to move within the xylem of plants to areas of infection. Early formulations gave a narrow band of activity as a consequence of this selectivity. More recently, a broader spectrum has been achieved with individual compounds.

3.3.1 Oxathiin-type Fungicides

Fungicides with the oxathiin-type structure, like carboxin (35), were produced to control cereal smuts and rusts. Employed as seed-dressings, they act specifically against these diseases by interfering with the citric acid cycle. They complex with a co-factor of succinate dehydrogenase, preventing formation of fumarate, with the physical effect being a swelling and rupturing of mitochondria in the fungal mycelia.



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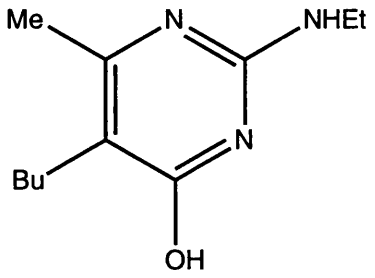
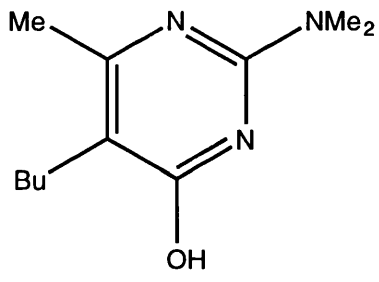


(36)

3.3.2 Hydroxypyrimidines

Powdery mildew can be controlled with the application of the hydroxypyrimidine fungicides. Ethirimol (36) has been used as a seed treatment on barley, while dimethirimol (37) functions as a cucumber root treatment. Bupirimate (38) is also used against this infection on apple. A variety of steps in mildew development are obstructed, such as germination, appressorium formation and sporulation. Powdery mildew haustoria accumulate these compounds, where they inhibit adenosine deaminase. This enzyme plays a role in RNA synthesis by converting adenosine into inosine. Ethirimol does not affect the corresponding step in other fungi.[13]



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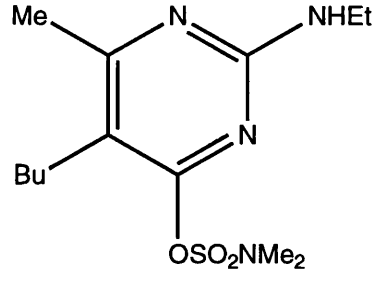
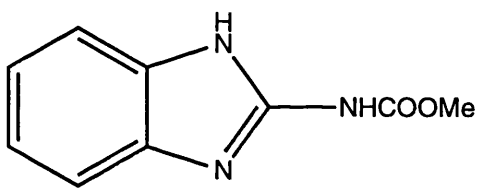


(38)

3.3.3 MBC-Generating Fungicides

Methyl benzimidazol-2-ylcarbamate (MBC) (39) complexes with b-tubulin and prevents its incorporation into microtubules, disturbing mitosis in sensitive fungi.[14] A marked selectivity is seen towards certain species e.g. ascomycete fungi, while in others and in plants spindle formation is totally unaffected. This is probably due to small structural differences in tubulin from varying sources.[10] MBC was originally marketed as carbendazim, and several related compounds have been produced which are hydrolysed or metabolised to MBC, both in plants

and fungi, known as MBC generators. These include thiophanate methyl (40), thiabendazole (41), benomyl (42) and fuberidazole (43), introduced throughout the 1960s.[10] *Botrytis cinerea* was the initial target of these compounds, but a broader spectrum of activity has been unveiled. Recently, eyespot on cereals has been controlled by MBC generators, while thiophanate-methyl, fuberidazole and benomyl have found application as cereal seed dressings, for control of powdery mildew and scald on barley, and *Leptosphaeria maculans* on oilseed rape stems. Benzimidazole fungicides also exhibit toxicity towards nematodes, earthworms, mites, algae and fish.[10]



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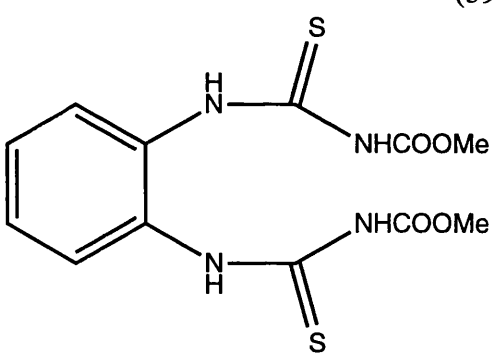

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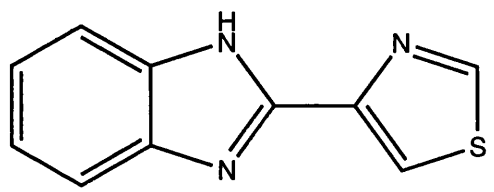

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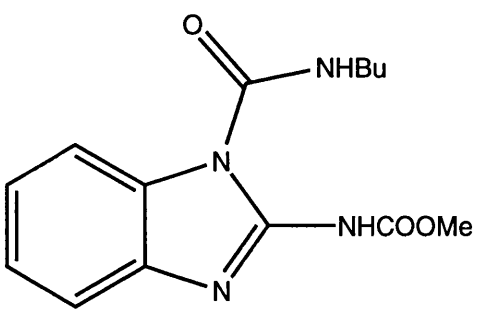

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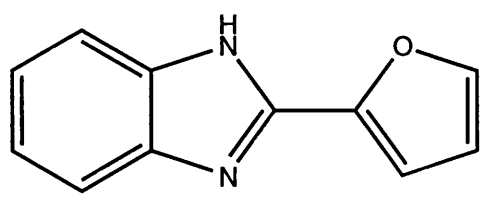
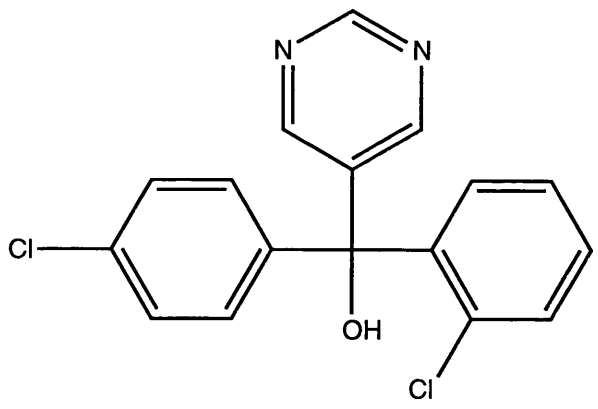


(43)

3.3.4 Ergosterol Biosynthesis Inhibitors

Many species of fungi contain an essential membrane component ergosterol, and therefore inhibition of the biosynthetic pathway to this sterol has proved an attractive and fruitful target, yielding several broad-range antifungal agents. Triazole and imidazole derivatives are now the most extensively used treatments, but initial compounds were diverse in structure, such as fenarimol (44) and triforine (45) which gave eradication of powdery mildew on fruit and cereals respectively. Triadimefon (46) was introduced to control mildews and rusts, and others of this class and activity spectrum followed, such as propiconazole (47) and prochloraz (48). These have additional selectivity, being active against net blotch and eyespot respectively. The area is still active in research and recent additions to compounds in production include flutriafol (49) and myclobutanil (50), which has found a market in apple scab treatment. The imidazole and triazole fungicides bind to the cytochrome P450 component of mixed function oxygenases, preventing oxidative demethylation of an ergosterol precursor. This leads to accumulation of C-4-methyl sterols (e.g. obtusifoliol), C-4,4-dimethyl sterols (e.g. methylenedihydrolanosterol), and 14α-methyl-$\Delta^{8,24}$-ergosta-dienol.[15,16] The nitrogen heterocycles can bind to the protohaem iron atom of cytochrome P450, and the lipophilic portions occupy the enzyme active site. This prevents entry of the 14α-methyl sterol substrate into the pocket, thus inhibiting the demethylation process.[17]



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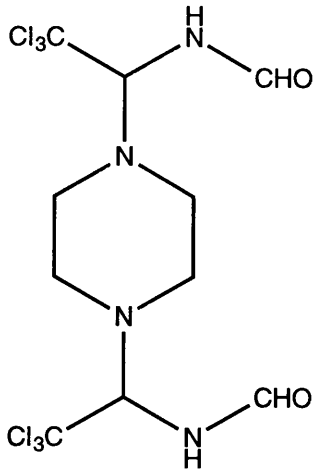

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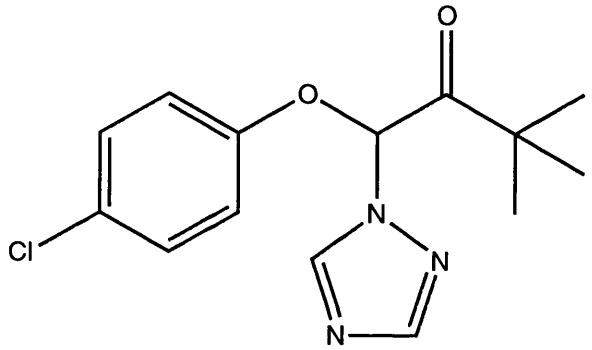

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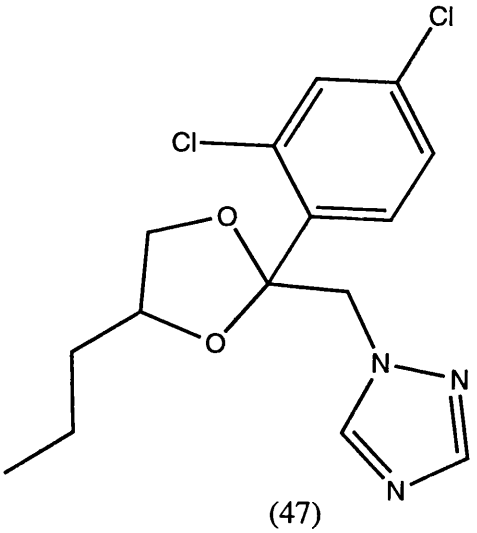

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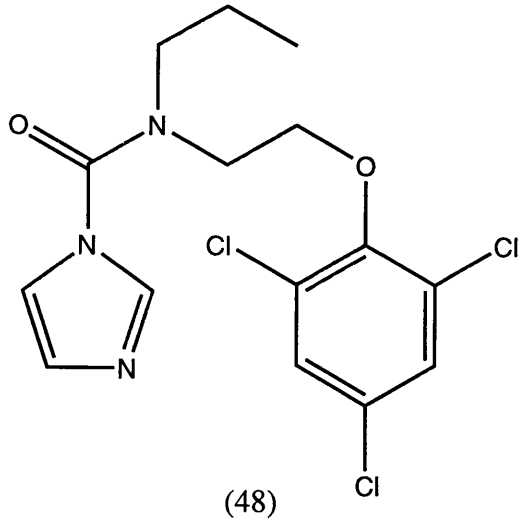

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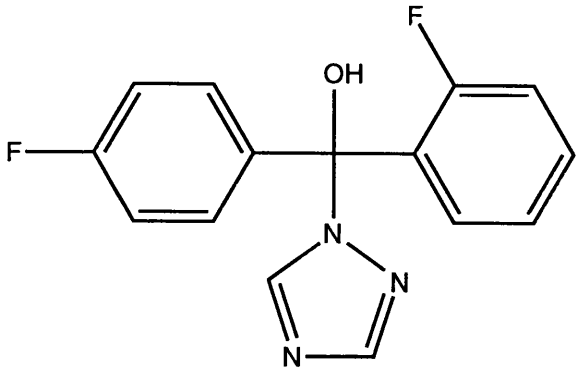
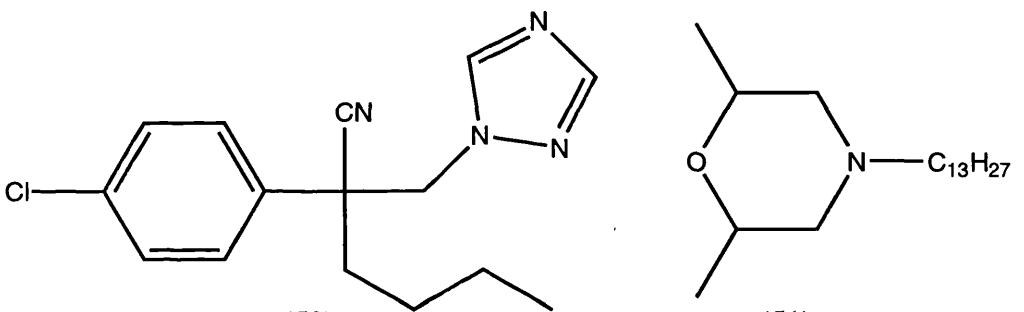


(49)

Of similar activity to this group are the six-membered nitrogen heterocyclic derivatives, for example tridemorph (51), fenpropimorph (52) and fenpropidin (53). Fungicides of this type inhibit the biosynthesis of ergosterol at a different point from those previously mentioned; in *Botrytis cinerea* they act at a later stage, preventing isomerisation of Δ^8 sterols,[18] and have thus found application where resistance to the imidazoles and triazoles is a problem. In some species, reduction of the C-14(15) sterol double bond is prevented, resulting in ignosterol accumulation.[19]



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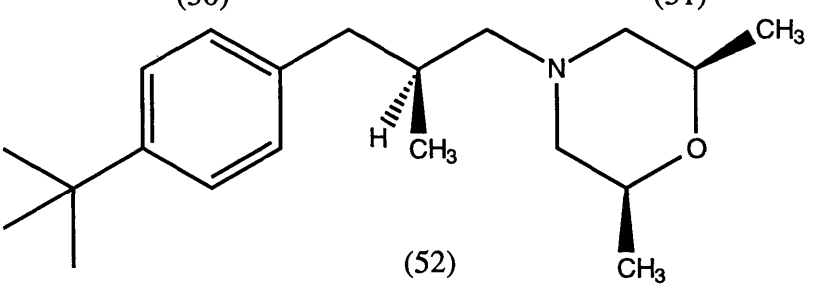

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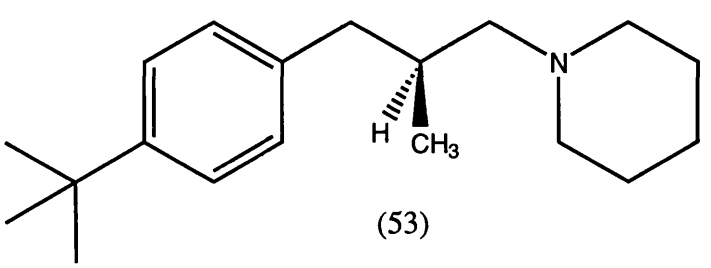
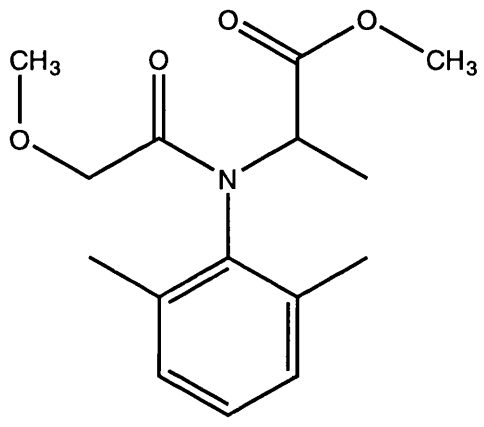


(53)

3.3.5 Phenylamide Fungicides

Control of downy mildews, as well as potato blight, is also achieved with phenylamide antifungal agents. Acylalanines such as metalaxyl (54), ofurace (55) and benalaxyl (56) interfere with RNA synthesis, although benalaxyl appears to have more than one site of growth inhibition. In particular, metalaxyl seemed to operate by inhibition of an RNA-polymerase-template complex in the nucleus.[20]



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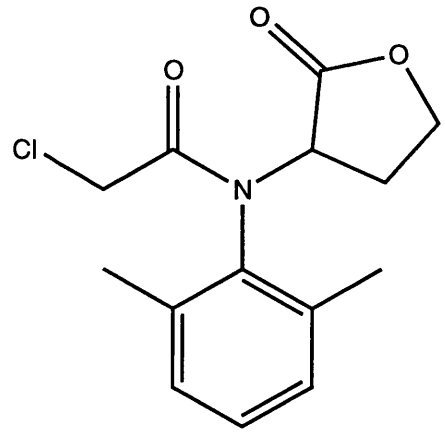

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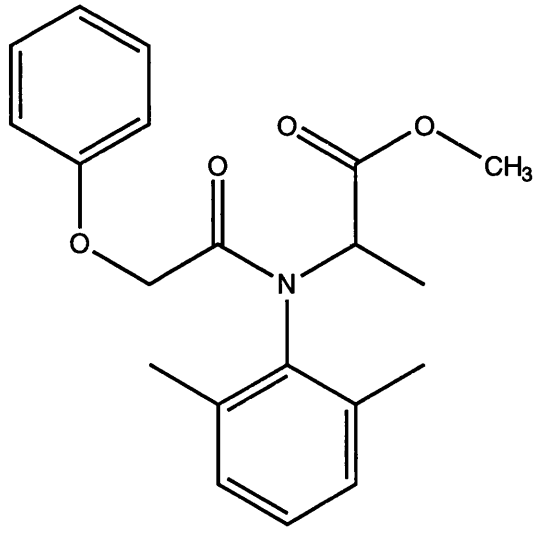
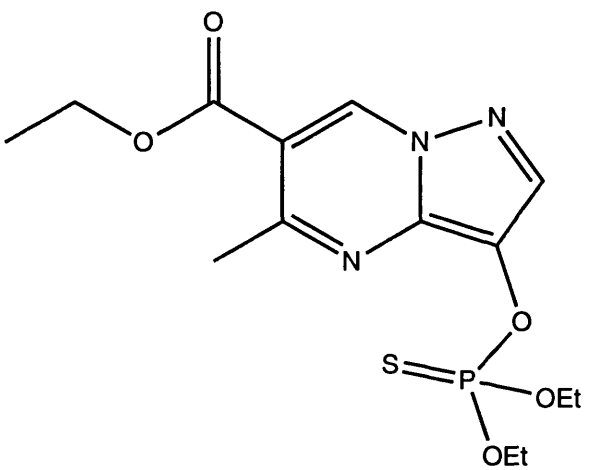


(56)

3.3.6 Other Fungicides

Other miscellaneous systemic fungicides include the phosphorus-containing pyrazophos (57) and fosetyl-aluminium (58). The latter compound is highly unusual in that it is translocated downwards to where its activity against root diseases is effected. Almost all other systemic fungicides are xylem-mobile.



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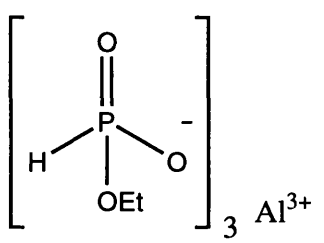


(58)

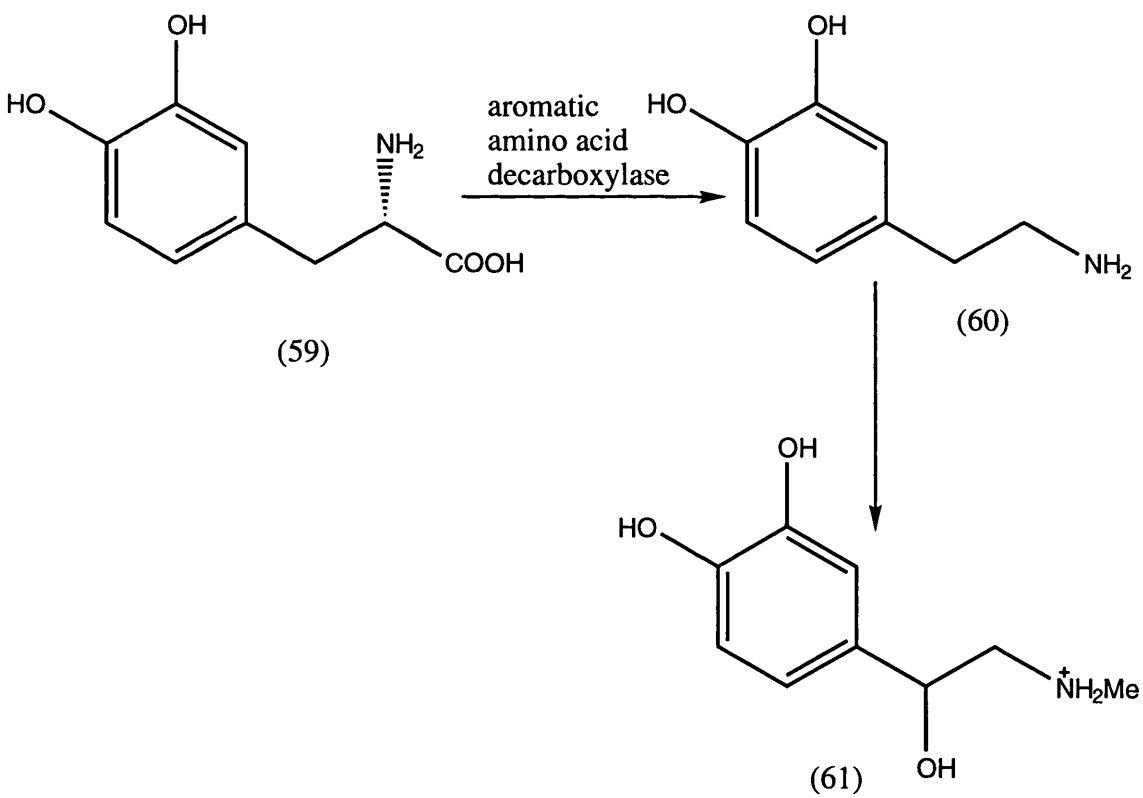
3.4 Proposed Design of New Antifungal Agents

3.4.1 Mechanism-Based Decarboxylase Inhibitors

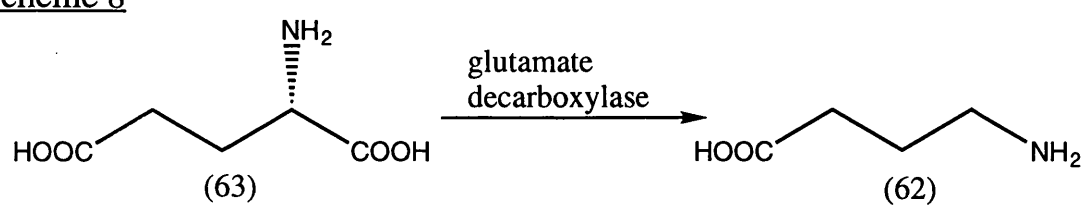
The amino acid decarboxylases make excellent targets for inhibition in many research areas, as they supply a number of diverse and essential biochemical intermediates. Their mechanism of action is, on the whole, well understood (Section 2.2) and is known to involve pyridoxal phosphate as a co-factor. Several 'suicide' inhibitors have been designed based on this mechanism, and have proved to be effective in inactivating their specific target enzyme.

In addition to the ornithine and arginine decarboxylases mentioned previously, 3,4-dihydroxyphenylethylamine (dopamine) (60) is produced by the action of aromatic amino acid decarboxylase, which decarboxylates 3,4-dihydroxyphenylalanine (DOPA) (59) (Scheme 7). Dopamine is a biosynthetic precursor to the neurotransmitter adrenaline (61). Also, the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (62) is formed from the decarboxylation of glutamic acid (63) by glutamate decarboxylase (Scheme 8).

Scheme 7



Scheme 8

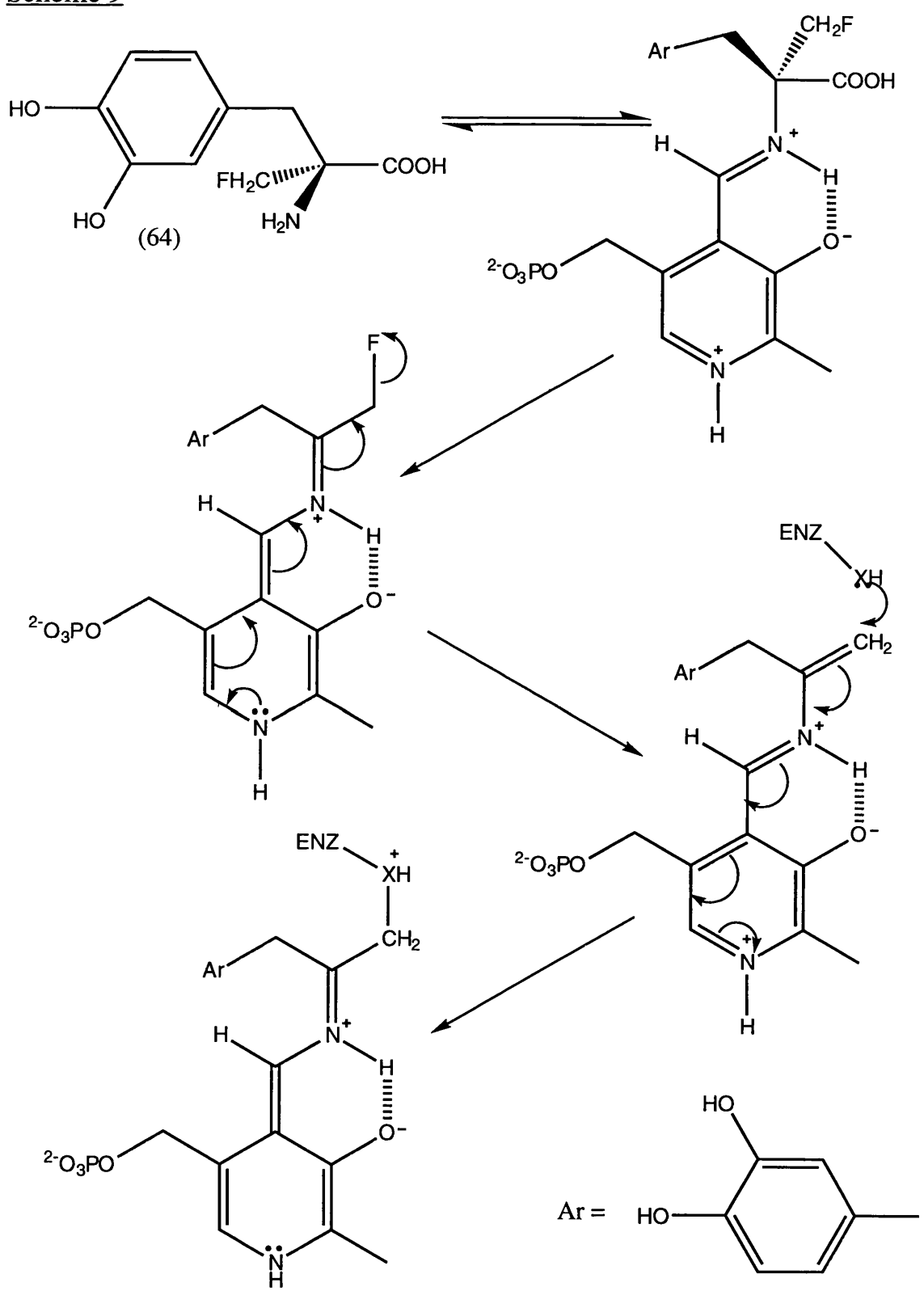


DFMO has been cited previously as a potent inhibitor of ornithine decarboxylase. In general, α-halomethyl analogues of amino acids have been shown to be inhibitors of the appropriate decarboxylases. Thus, a-fluoromethyl-DOPA (64) binds covalently to the aromatic amino acid decarboxylase enzyme, followed by a decarboxylation analogous to that which occurs with the structurally-related amino acid DOPA (Scheme 9).[21] The absolute configurations of the inhibitor and the natural substrate also show a correlation, and the mechanism is consistent with stereochemical labelling studies. ^{14}C-labelled carboxylate groups are lost from the *S* enantiomers in both cases, but not from the corresponding *R* enantiomers.

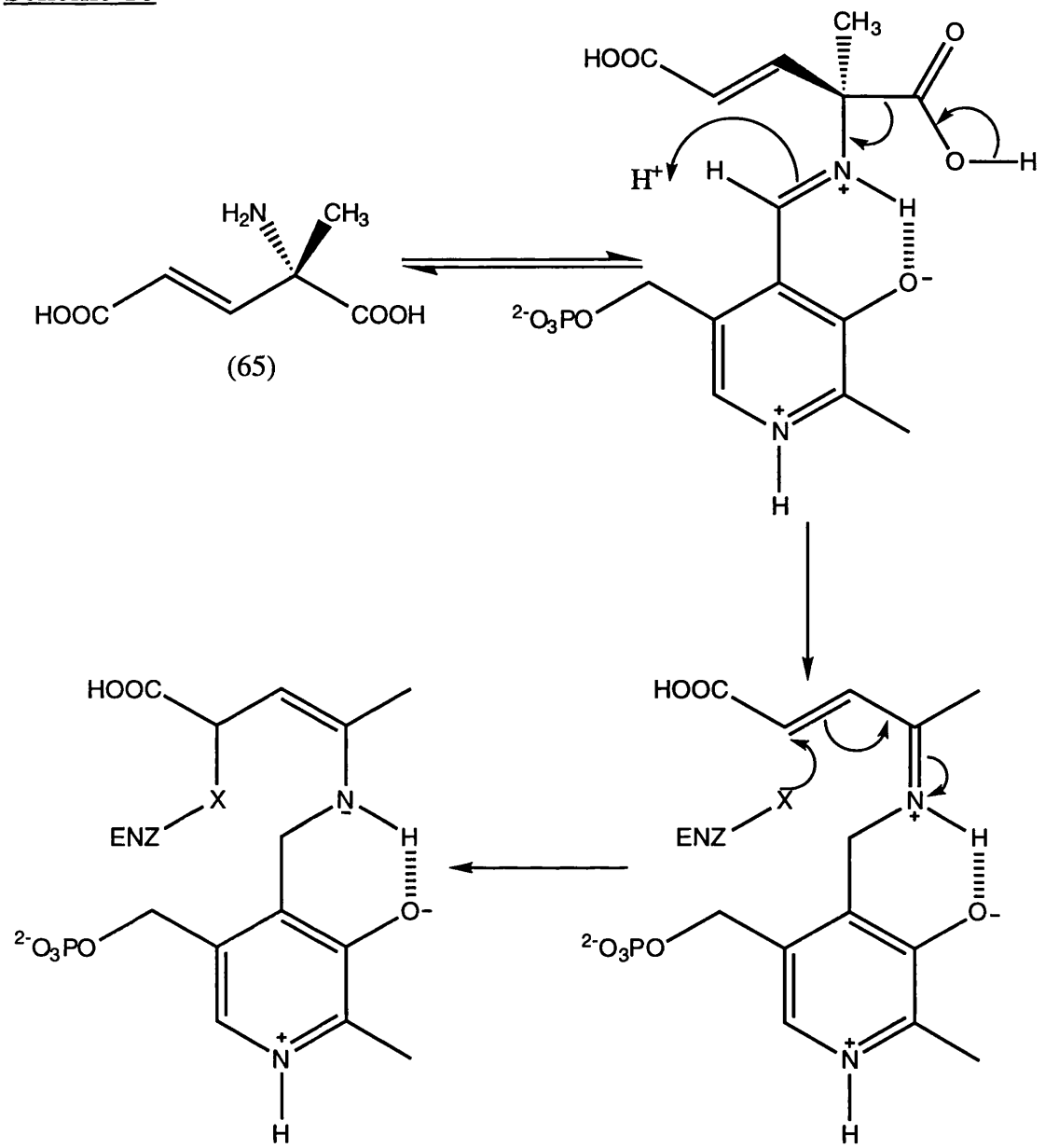
A further opportunity inferred from the mechanism is the possibility of introducing reactivity to a normally-unreactive group in the substrate or inhibitor. Such reactivity may then induce the irreversible formation of a bond between the enzyme and the latent functional group of the inhibitor, resulting in inactivation of the enzyme. Efforts in this region have mainly been focussed on bringing unsaturated groups in the inhibitor into conjugation, creating Michael acceptors.

In such a case, glutamate decarboxylase is inhibited in mammals by 2-methyl-3,4-didehydroglutamate (65). The double bond at the 3-position is brought into conjugation upon decarboxylation, and the inhibitor eventually binds to the enzyme at the now-activated 4-position (Scheme 10).[21]

Scheme 9



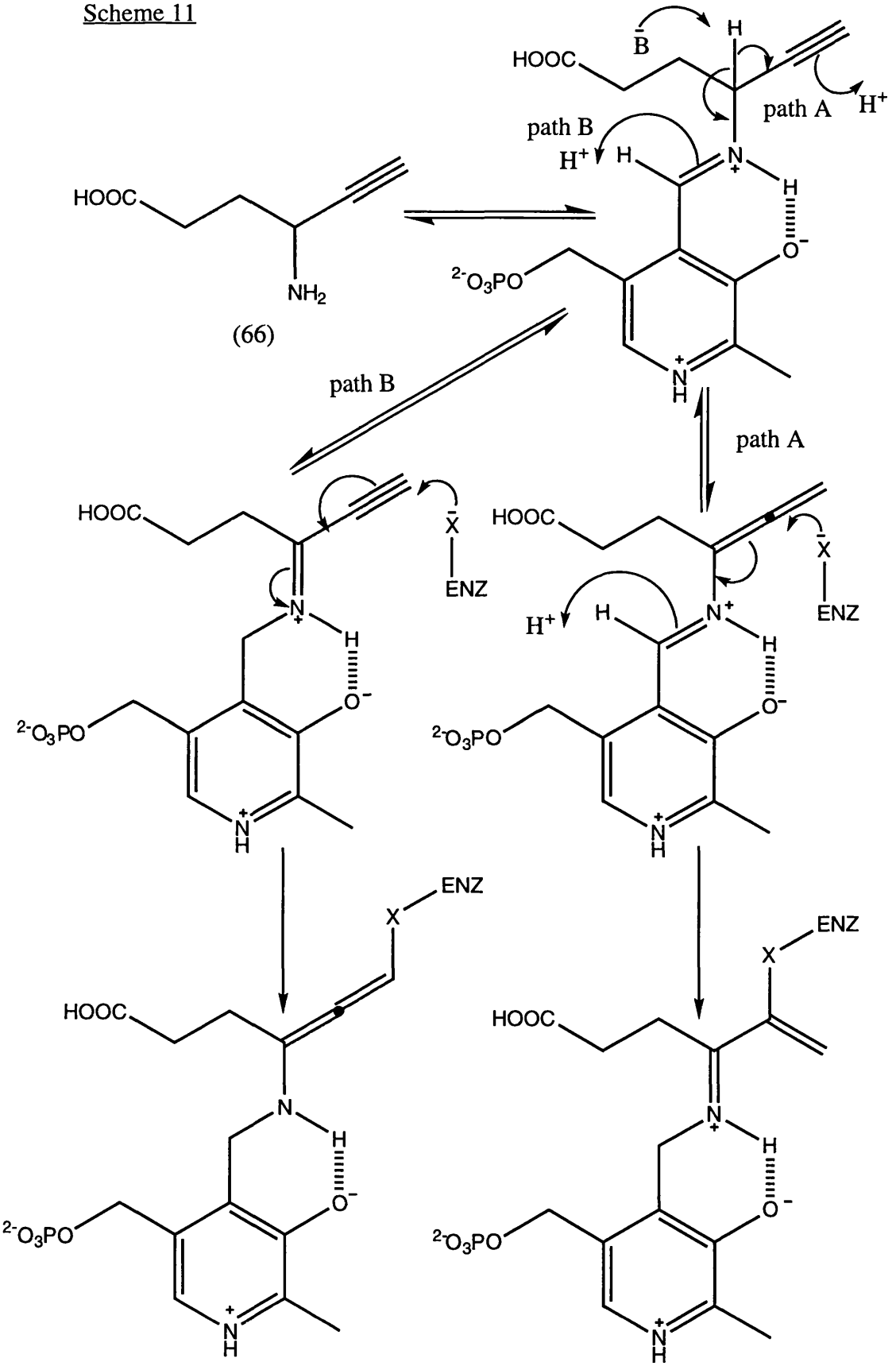
Scheme 10



An important alternative is the possibility of inhibitory product analogues, utilizing the principle of microscopic reversibility. This forms the basis of the approach to polyamine biosynthesis inhibition adopted within this project. Bacterial glutamate decarboxylase has been inactivated by *R*-4-aminohex-5-ynoic acid (66), a GABA analogue.[21] The mechanism may follow either of two pathways, both of which involve proton transfer

(Scheme 11). One leads to an enzyme-bound allene compound, and the other to a covalently-bonded alkyne, but both are achieved through conjugated intermediates. Only the R-isomer of this inhibitor inactivates the bacterial decarboxylase. However, the *S*-isomer inhibits the mammalian equivalent, due to a difference in enzyme activity. Apparently the mammalian enzyme has retained some transaminase activity, with the result that the proton from the 4-position of the *S*-isomer can be abstracted. The result is that the pyridoxal aldehyde group is converted into an amine group, and the enzyme complex is inactivated.

Scheme 11



3.4.2 Design of ODC Inhibitors

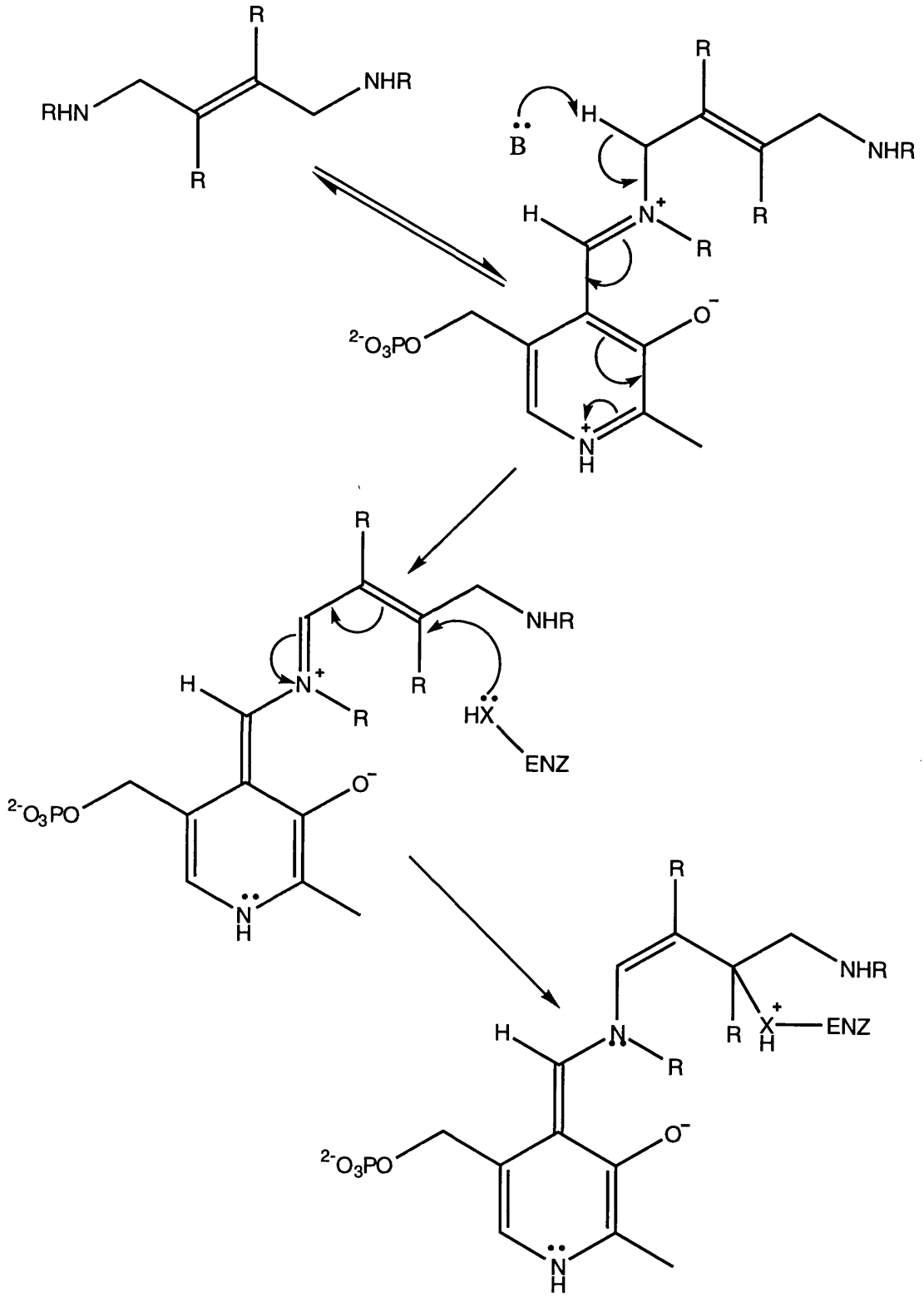
The mechanisms of other pyridoxal phosphate-dependent enzymes are known. The inhibition of these enzymes by substrate and product analogues has also been studied, and appears to follow similar pathways to those already discussed. Importantly, decarboxylation of substrates and substrate-type inhibitors is replaced by deprotonation at the same position in the product-type inhibitors. In each case, a carbanion is formed which is delocalised in the same way, and the inhibitor eventually becomes bound to the enzyme.

From the generality of these mechanisms, the design of specific inhibitors can be inferred. This has implications in the eradication of plant-pathogenic fungal diseases. Specifically-designed mechanism-based inhibitors have been a phenomenon only recently addressed in this area. The difference in polyamine biosynthesis in plants and fungi gives the ideal scenario for this approach (Chapter 2), and this opportunity was exploited in this work.

It was proposed that initial targets for inhibition of ODC could be putrescine analogues, as product analogues had been shown to be feasible inactivators of glutamate decarboxylase and other enzymes. It was also believed that incorporation of unsaturation into the design would increase the likelihood of inhibitory properties, using the previous examples as models. Thus, this approach is distinct from DFMO and DFMA inhibition of ODC and ADC, respectively, as these are substrate analogues, with accordingly different mechanisms.

A proposed mechanism of action for such a designed inhibitor is shown (Scheme 12). It was hoped that further targets could then be based on modifications of these initial compounds.

Scheme 12


R
RHN
NHR
R
B
H
R
NHR
H
N+
R
R
O-
2-O3PO
N
H
R
NHR
H
N+
R
R
HX
ENZ
O-
2-O3PO
N
H
R
NHR
H
N
R
R
X+
H
ENZ
O-
2-O3PO
N
H

Chapter 4

Synthetic Methods Towards Unsaturated Amines

4.1 Introduction

Primary, secondary and tertiary allylic amines can be synthesised in a number of ways. General strategies include the formation of the carbon-nitrogen bond from functionalised allylic precursors; formation of the C-1 to C-2 carbon-carbon bond, usually from organometallic reagents and imines or iminium ions; carbon to carbon multiple bond formation; rearrangement reactions; and manipulation of pre-formed allylic or propargylic amine systems.

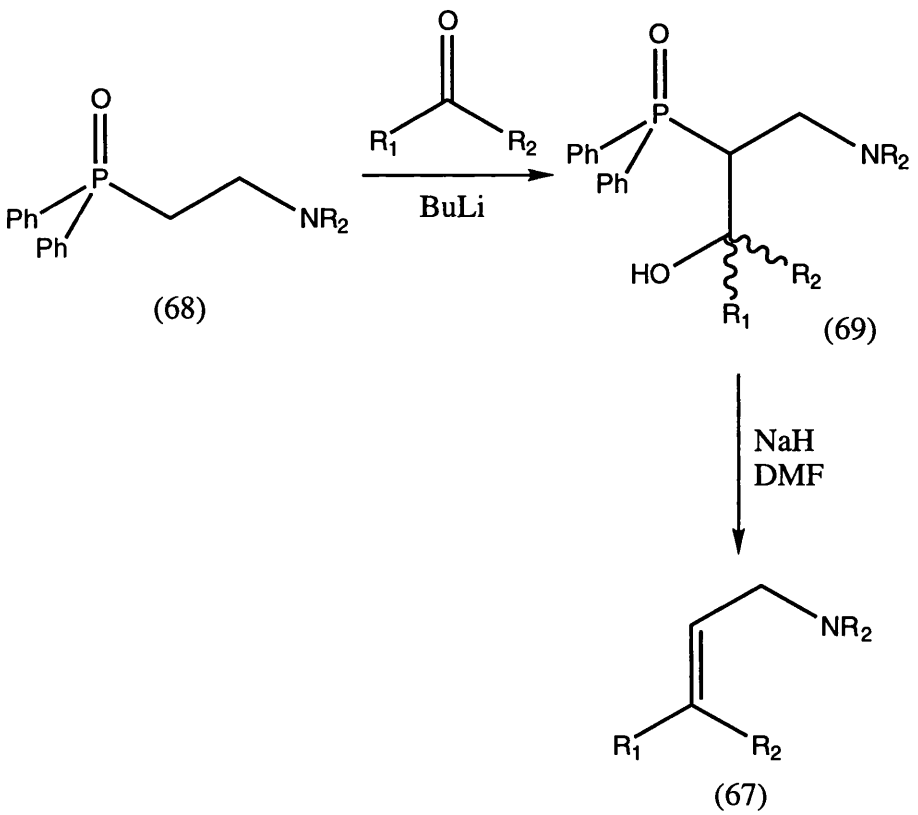
4.2 Synthesis of Unsaturated Amines by Formation of Carbon-Carbon Multiple Bonds

There are a great many reactions which give rise to functionalised olefins which can be further manipulated to yield allylamines. Consideration is given here only to those reactions which yield allylamines directly.

Tertiary allylic amines (67) were synthesised by Cavalla *et al.*,[22] who obtained pure *E*- and *Z*-isomers of each example. Using the Horner modification of the Wittig reaction, 2-(aminoalkyl)diphenylphosphine oxides (68) were added to aldehydes and ketones to give the corresponding alcohols (69) as diastereoisomers, which could be

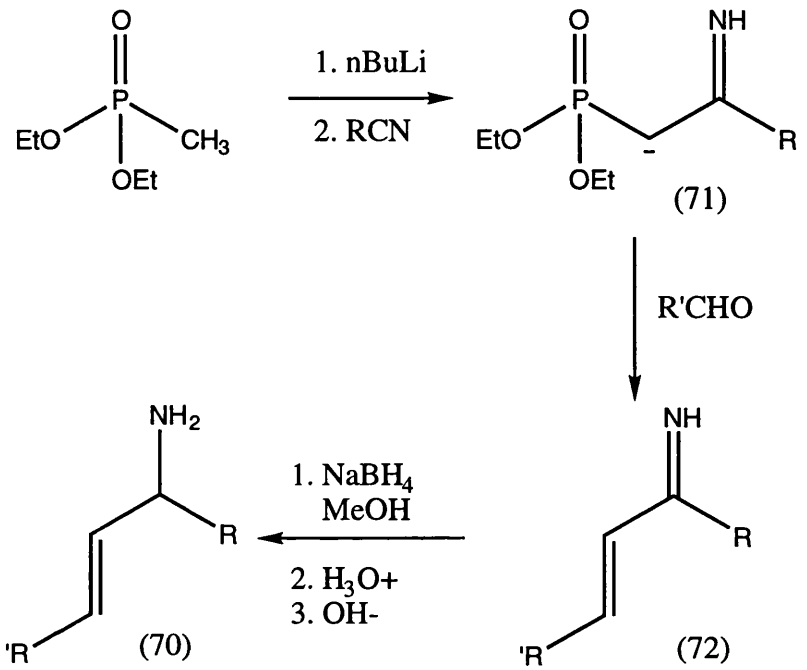
separated. An elimination process from each isomer gave *E*- and *Z*-allylamines, respectively (Scheme 13).

Scheme 13



Selective synthesis of primary allylic amines (70) with the *E*-configuration was carried out by Shin and co-workers,[23] also utilising Wittig-type chemistry. The sequence involves the addition of diethyl methylphosphonate anion to a nitrile, followed by a reaction of an aldehyde with the iminophosphonate anion intermediate (70), and finally reduction of the unsaturated imine (71) formed to furnish the *E*-allylic amine (72) (Scheme 14). The entire sequence can be carried out in one pot.[23]

Scheme 14



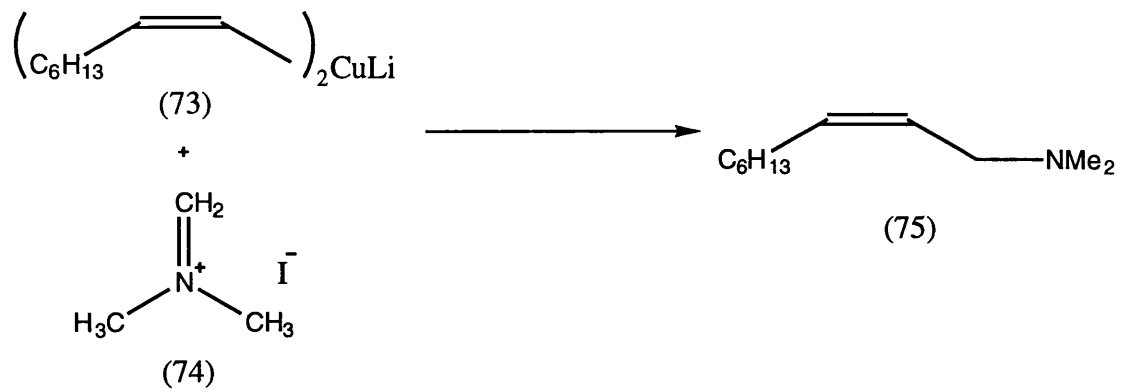
4.3 Preparation of Allylic Amines by Formation of a Bond Between C-1 and C-2

Synthesis of allylamines from single-carbon amine units and alkenyl or alkynyl units is generally manifested in the reaction between organometallic reagents and aminoethers or iminium salts.

4.3.1 Synthesis of Tertiary Allylamines

Addition of organocopper reagents such as lithium divinyl cuprates (73) to Eschenmoser's salt (74) gave good yields of the resultant allylamines (75), with the *Z*-configuration of the double bond conferred from the way in which the cuprate is first formed (Scheme 15).[24]

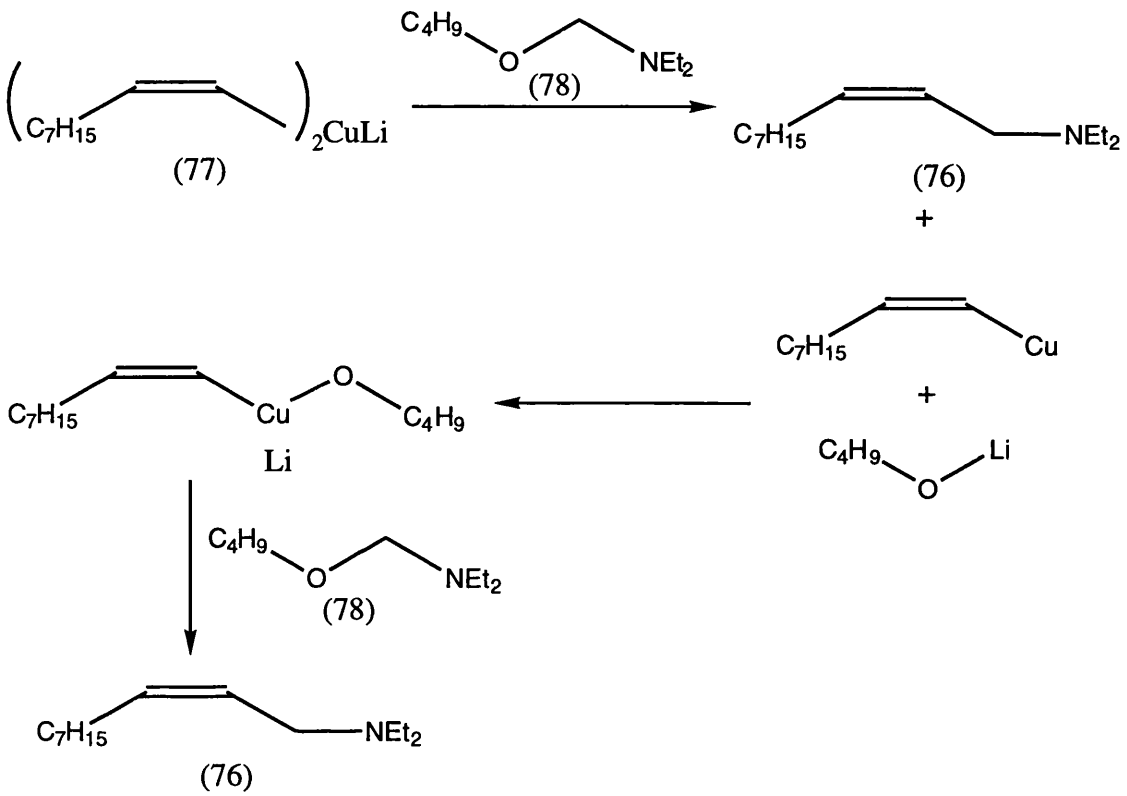
Scheme 15



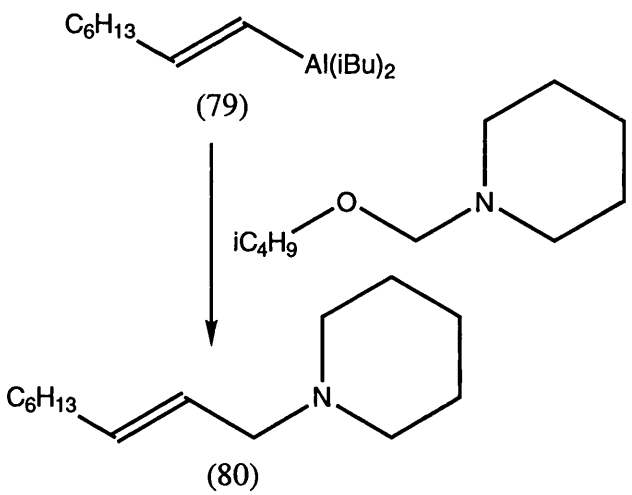
Similarly, the work of Germon *et al.* [24,25] demonstrated that formation of allylic amines (76) is achieved in good yield from the reaction of the lithium vinyl cuprates (77) with aminomethyl ethers (78). Both alkenyl groups are used in this reaction, the second being transferred from a mixed alkoxy-alkenyl cuprate, a side product from the first addition (Scheme 16). Magnesium alkenyl copper reagents performed the same reaction, but in lower yields.[24]

When *E*-allylic amines were desired, a better starting reagent was the alkenyl alane (79). A good yield of the *E*-allylamine (80) was obtained with the aminoether shown (Scheme 17), in high stereoisomeric purity. The alkenylalane obtains its *E*-stereochemistry from the hydroalumination of the corresponding alkyne, a *syn*-addition process.

Scheme 16



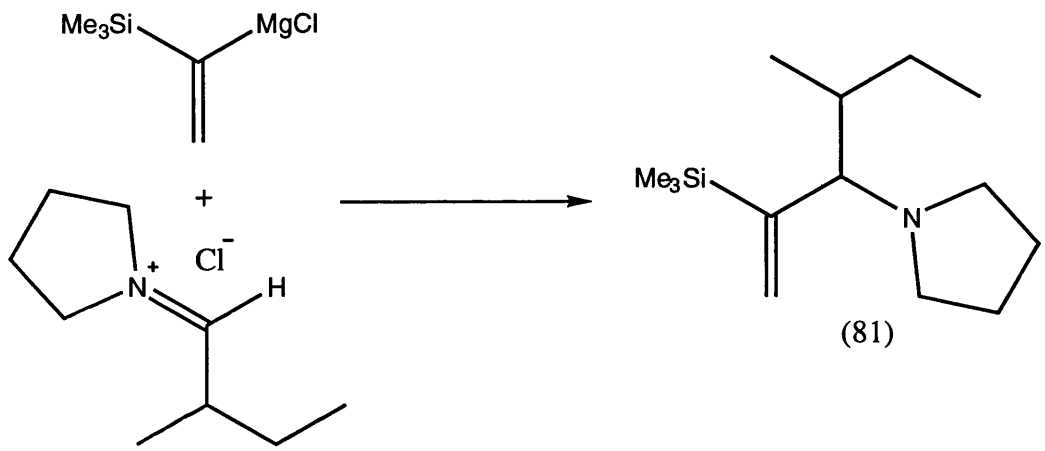
Scheme 17



When amino-thioethers were used as the aminomethyl-donating agent, higher yields and faster reaction times were observed.[24,25]

Tertiary allylamines (81) were also constructed from vinyl Grignard reagents and iminium salts (Scheme 18), although yields were modest.[26]

Scheme 18



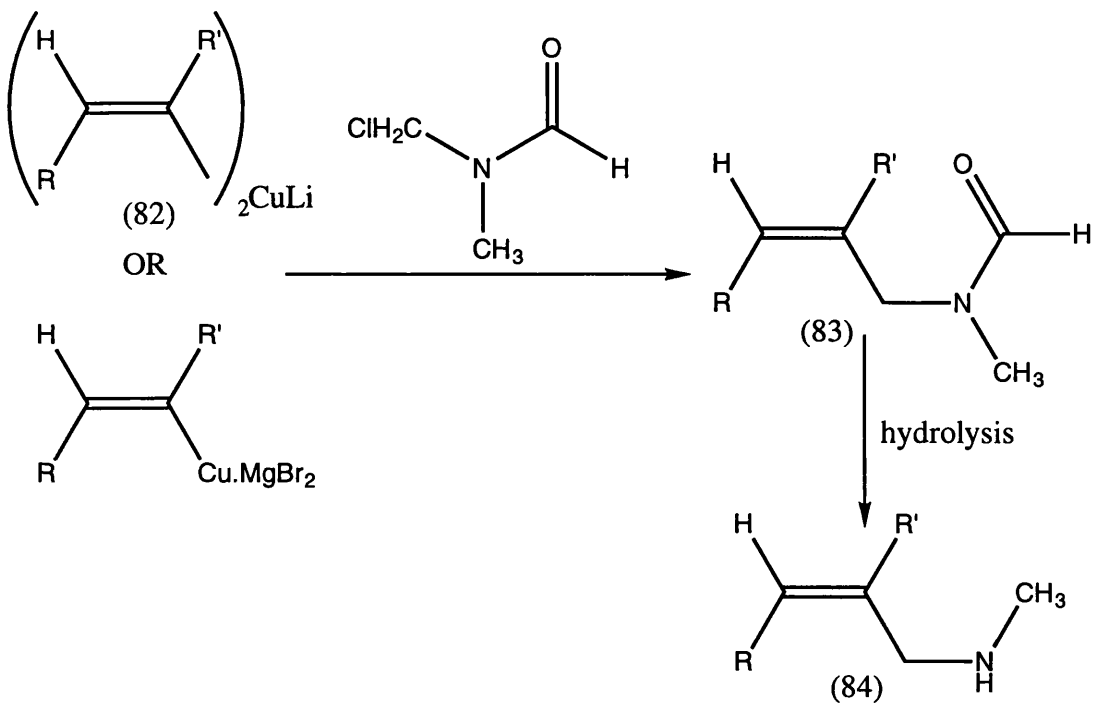
(81)

4.3.2 Synthesis of Secondary Allylamines

When secondary allylic amines were required, alternative aminomethyl-transfer reagents were used.

Gemon *et al.* [27] found that lithium dialkenyl cuprates (82) combine with *N*-chloromethyl-*N*-methylformamide to give the corresponding *Z*-configured allylic amides (83) in high yields (Scheme 19). The magnesium halide/cuprous alkene complexes also react in this way.[27] The resultant allylic formamides can be easily hydrolysed to secondary allylamines (84).

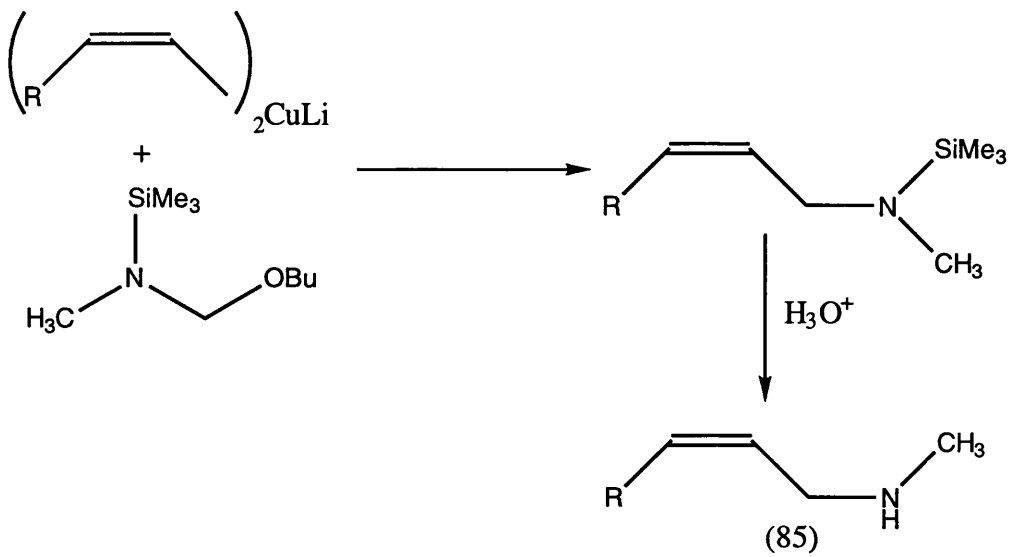
Scheme 19



Courtois and Miginiac[28] adapted the procedure of synthesising tertiary allylamines from aminoethers to produce secondary allylamines. Replacement of one of the alkyl groups on the dialkylaminoether by a trimethylsilyl moiety allows the synthesis of silyl-protected secondary allylamines, which can be deprotected *in situ* to give secondary allylamines (85) (Scheme 20).

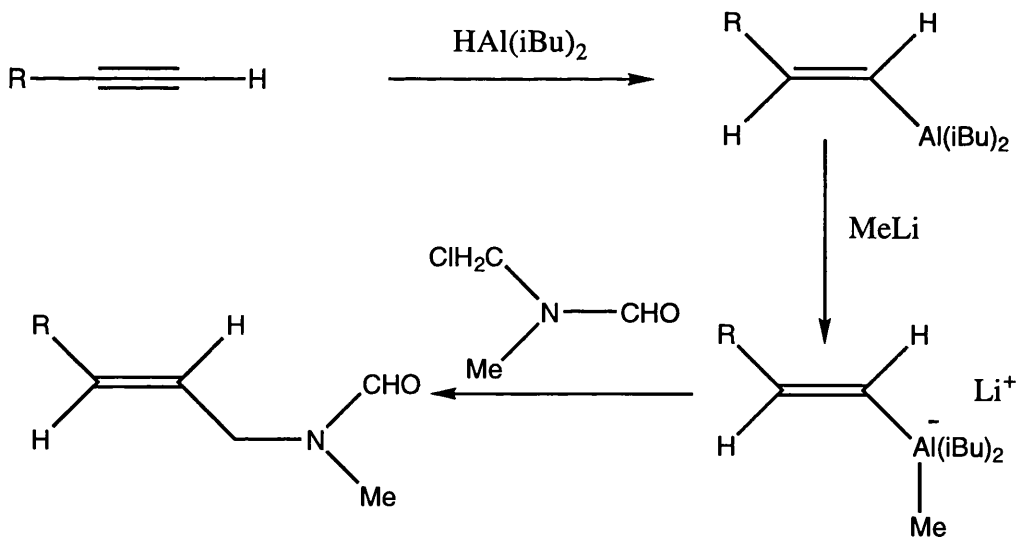
Secondary allylamines with the *E*-configuration can be formed, again using aluminoalkenes.[27] However, conversion of the organoaluminium compounds into the more-nucleophilic lithium tetraalkylalanates with methyl lithium was required, before reaction with the appropriate amidomethylating agent (Scheme 21).

Scheme 20



(85)

Scheme 21

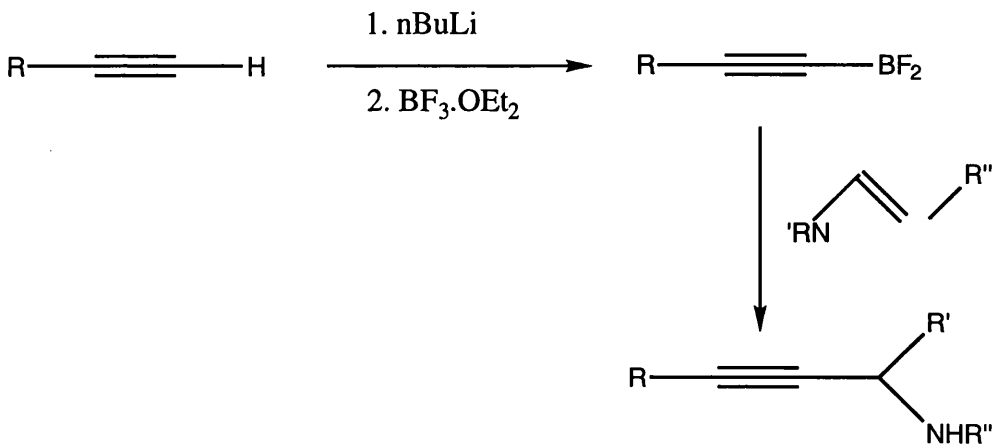


4.3.3 Synthesis of Propargylamines

Secondary and tertiary propargylamines have been formed previously from Lewis-acid coordinated acetylide anions.[29,30] Direct

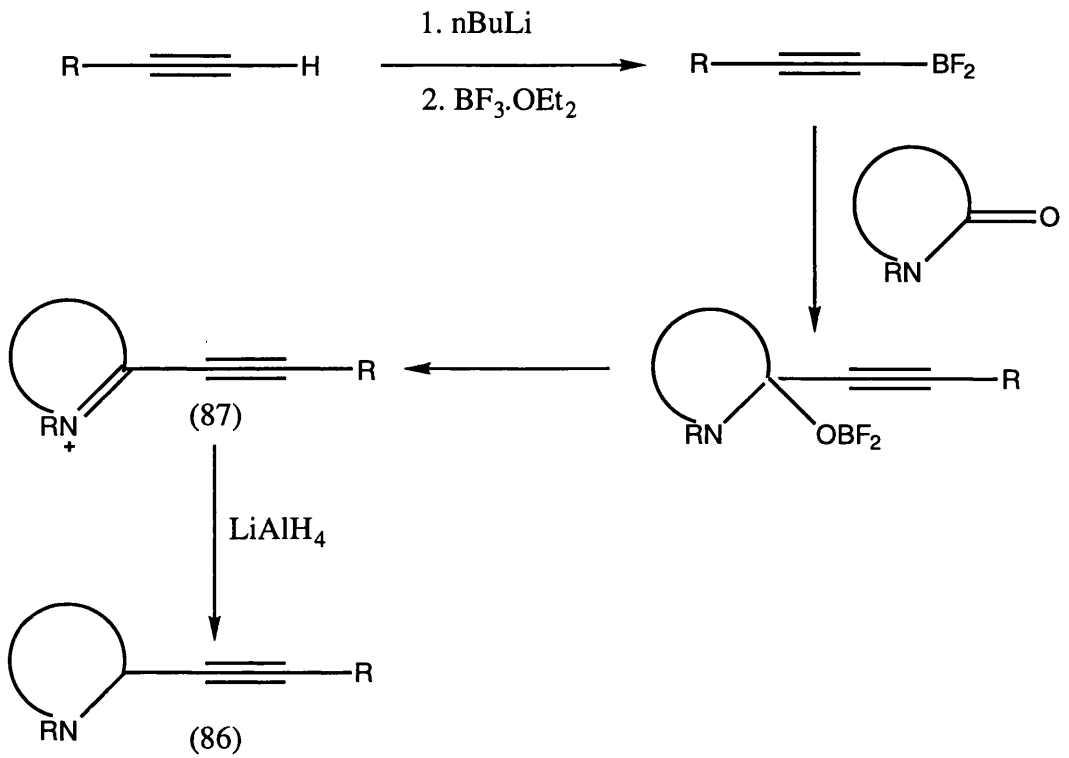
reaction of these species with aldimines gave rise to secondary propargylamines (Scheme 22), when attempted reaction of uncoordinated lithium acetylides did not occur at all.[29] This was thought to be due to polarisation of the aldimine by the boron trifluoride present, making it more reactive towards the acetylide nucleophile.

Scheme 22



Boron trifluoride/acetylide complexes were also used to synthesise tertiary propargylamines (86) by reaction with lactams (Scheme 23).[30] Again, uncoordinated organometallics did not give the reaction. The mechanism is thought to follow a Lewis acid-assisted formation of an iminium salt intermediate (87), which can be reduced *in situ* with lithium aluminium hydride.[30]

Scheme 23



When the acetylide already contains an aminomethyl functionality, 1,4-diaminobut-2-ynes are formed, which can be further converted into *E*- and *Z*-1,4-diaminoalkenes by appropriate manipulation of the triple bond.[31]

4.4 Preparation of Allylamines by Formation of the Carbon-Nitrogen Bond

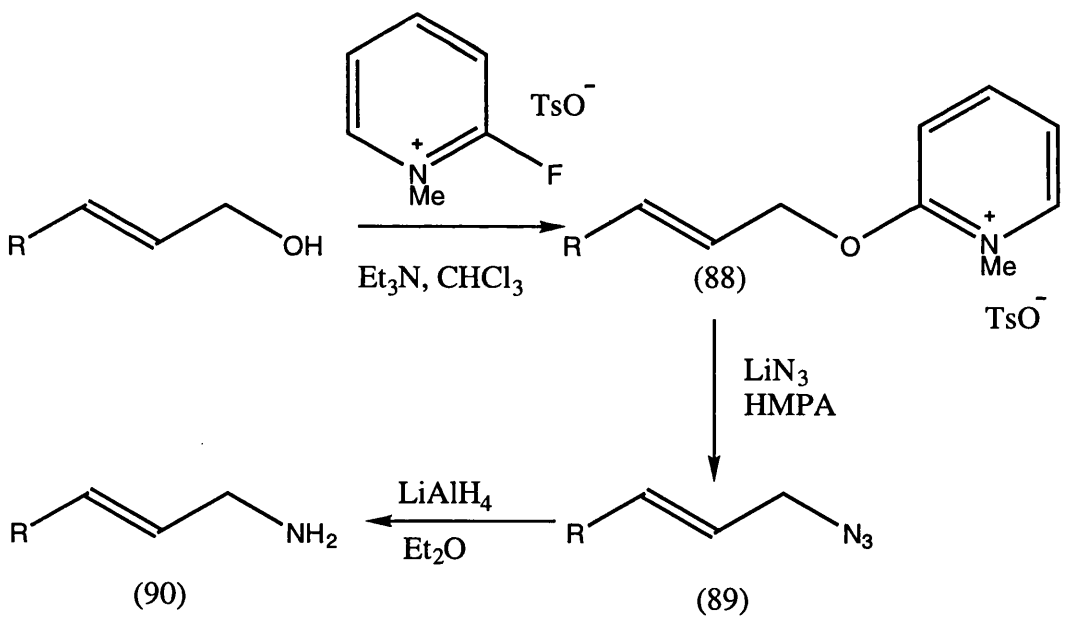
Formation of the carbon-nitrogen bond in allylamines can be considered as alkylation of an appropriate nitrogen nucleophile. In this case, the alkylating agents are generally activated allylic alcohols or

allylic halides. Only pertinent examples of this extensive area are presented in this section.

4.4.1 Synthesis of Primary Allylamines

Allylic alcohols have been transformed into allylic amines by a variety of processes. Conversion into 2-allyloxypyridinium salts (88), which are converted *in situ* into allylic azides (89), was effected by Hojo *et al.*[32] The resultant azides were then reduced with lithium aluminim hydride to the corresponding primary allylic amines (90) in high yield (Scheme 24).

Scheme 24



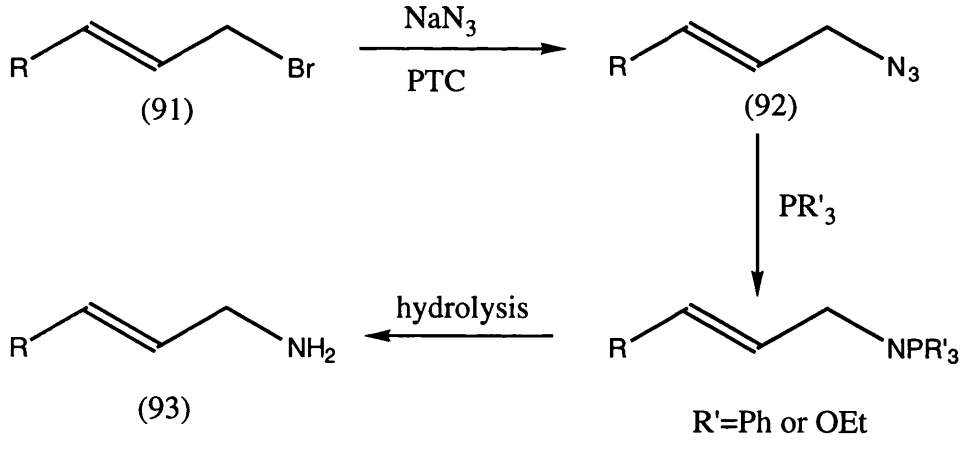
The Mitsunobu method of alcohol activation has been widely employed in synthesis. Golding *et al.*[33] used the method to convert allylic

alcohols directly into primary allylamines via allyl azide intermediates. The reaction was extended to produce 1,4-diaminoalkenes and alkynes from the appropriate diols.[33]

Allylic azides have been synthesised from the corresponding alcohols under Mitsunobu-type conditions, using zinc azide/bis(pyridine) complex as the nucleophile provider.[34] No rearranged products were observed in this case, and it was deemed that no S_N2' reaction had occurred.

Allyl azides (92) are easily accessible from the corresponding bromides (91). Koziara *et al.*[35] performed this transformation under phase transfer conditions, and converted the azides *in situ* into the corresponding amines (93) with a variation of the Staudinger reaction[36] (Scheme 25).

Scheme 25



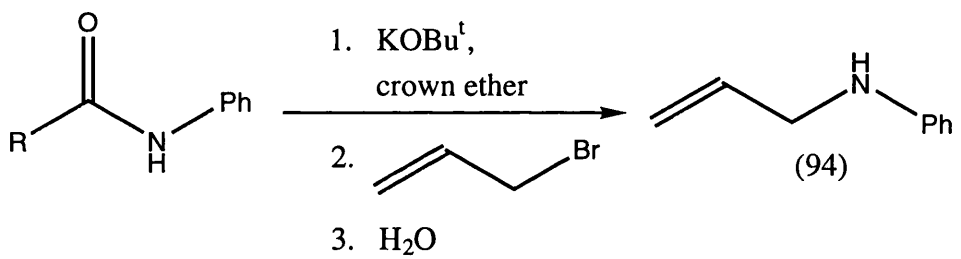
Other classical methods have been used to produce primary allylamines from suitable allylating agents, including the Gabriel synthesis with potassium phthalimide,[37] and other variants,[38] and the Delepine

reaction with hexamethylene tetramine.[39] Many of these will be discussed in the Chapters to follow.

4.4.2 Synthesis of Secondary and Tertiary Allylamines

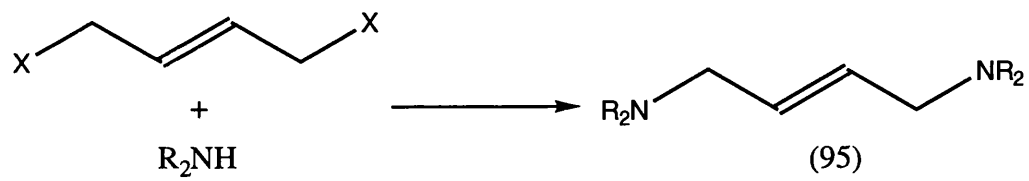
N-Allylanilines (94) were produced by nucleophilic attack on allylic bromides by benzanilides in the presence of base, followed by hydrolysis, according to the procedure of Luh and Fung (Scheme 26).[40]

Scheme 26



A large range of 1,4-bis(dialkylamino)alkenes (95) was produced by Roberts and Ross,[41] from nucleophilic substitution by an appropriate dialkylamine on the corresponding 1,4-dihalogenobut-2-ene (Scheme 27). The reaction was extended to produce 1,4-bis(alkylamino)alkenes also. Biel and DiPierro[42] carried out similar transformations on 1,4-dihalogenobut-2-ynes to furnish 1,4-bis(dialkylamino)-alkynes (96) (Scheme 28).

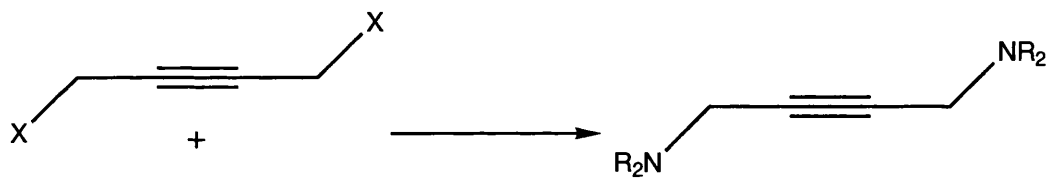
Scheme 27



R=alkyl, X=halogen

(95)

Scheme 28



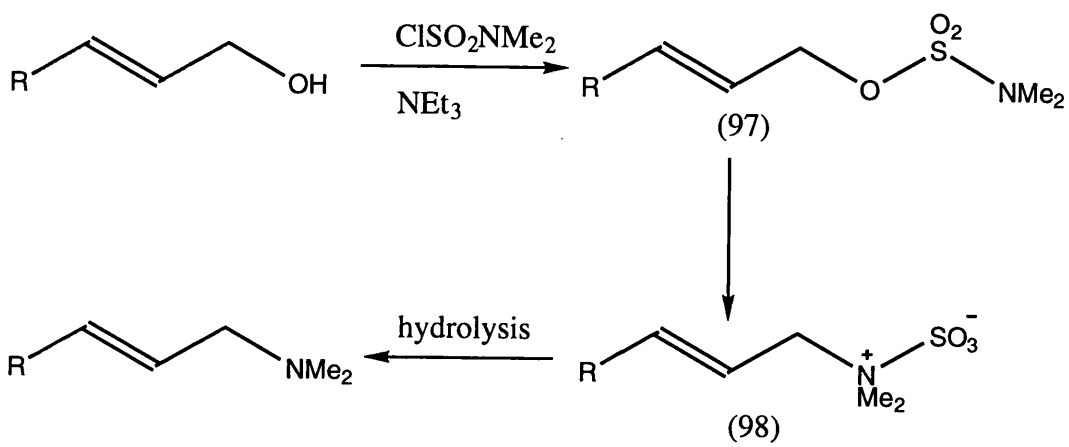
R=alkyl, X=halogen

(96)

White and Elliger[43] prepared allyl sulfamate esters (97), which rearranged with migration of the allyl group from oxygen to nitrogen by an S_Ni process, giving betaine analogues (98) (Scheme 29). These compounds could then be easily hydrolysed to the corresponding allylamines. When *E*-allylic alcohols were used, allylic-rearranged products predominated, formed by an S_Ni' mechanism.

Some of the methods presented in this Chapter are discussed in more detail in the following Chapters.

Scheme 29


R
OH
ClSO2NMe2
NEt3
R
O
O2
S
NMe2
(97)
hydrolysis
R
NMe2
R
N
Me2
SO3
(98)

Chapter 5

Synthesis of 1,4-Diaminobut-2-enes as Potential Antifungal Agents

5.1 Introduction

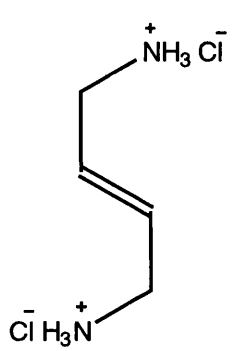
For the reasons outlined in Chapters 2 and 3, the earliest synthetic targets in this work were putrescine analogues, i.e. 1,4-diamines and derivatives. In particular, compounds with unsaturation incorporated between the 2- and 3-positions were of most interest. The parent compound targetted was therefore 1,4-diaminobut-2-ene, and thereafter simple derivatives of this structure. It was hoped that test results of the antifungal capabilities of these early targets would lead to the design of other related compounds to be synthesised.

This chapter examines the synthesis of these initial straight chain putrescine derivatives and their analogues. The relative effectiveness of these compounds to prevent infection of crops by plant pathogens is recorded in Chapter 8.

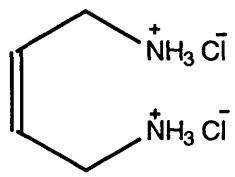
5.2 Synthesis of Initial Targets - Straight Chain Primary 1,4-Diamines

The simplest group of straight chain targets in terms of structure included *E*-1,4-diaminobut-2-ene dihydrochloride (99), *Z*-1,4-diaminobut-2-ene dihydrochloride (100) and 1,4-diaminobut-2-yne dihydrochloride (101). Retrosynthetically, it would appear that these

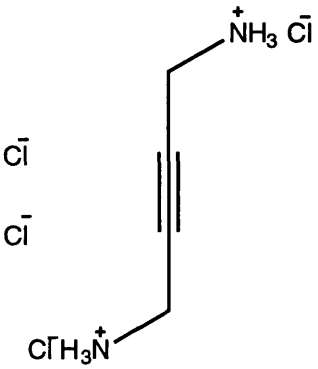
compounds could be prepared from the corresponding 1,4-diols. For the latter two targets, these precursors are readily available. *E*-But-2-ene-1,4-diol is not commercially available, but could be prepared from fumarate diesters. However, a viable alternative strategy was to employ *E*-1,4-dibromobut-2-ene (106) as the starting point, as this compound is available from commercial suppliers.



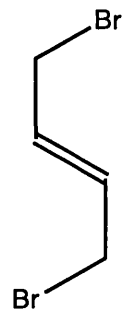
(99)



(100)



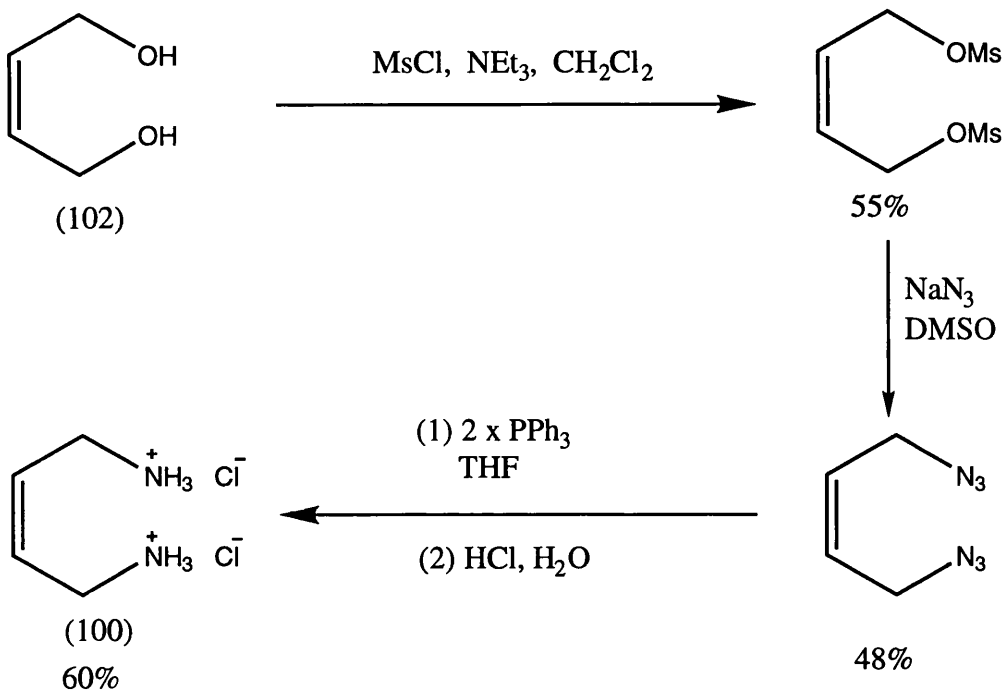
(101)



(106)

In Chapter 4, several methods were outlined for the conversion of the alcohol functionality into the primary amine group. Indirect means include transformation of the hydroxy moiety into a suitable leaving group [e.g. methanesulfonate (mesylate), trifluoromethanesulfonate (triflate), or halogen], followed by displacement with an appropriate nitrogen nucleophile which could be converted into the primary amine group. This strategy was used at an early stage in this work to transform *Z*-but-2-ene-1,4-diol (102) into *Z*-1,4-diaminobut-2-ene dihydrochloride (100) via the corresponding dimesylate and diazide intermediates (Scheme 30).

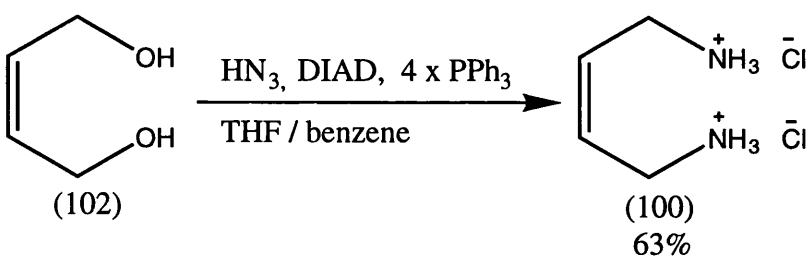
Scheme 30



However this route to 1,4-diamines did not prove satisfactory. Each stage involved isolation of a reactive intermediate which was often unstable. Side reactions were possible, and yields for each stage were modest and variable. A more convenient process was the Mitsunobu reaction[44] in which the alcohol is activated with a triphenyl phosphine/dialkyl azodicarboxylate couple, followed by *in situ* substitution with appropriate nucleophiles. This method has been used in the preparation and alkylation of amines on numerous occasions,[45,46] and where primary amines were desired nucleophilic substitution has often been accomplished by azide from hydrazoic acid.[33] As indicated in Chapter 4, several methods also exist for the reduction of the azide functionality to yield primary amines.

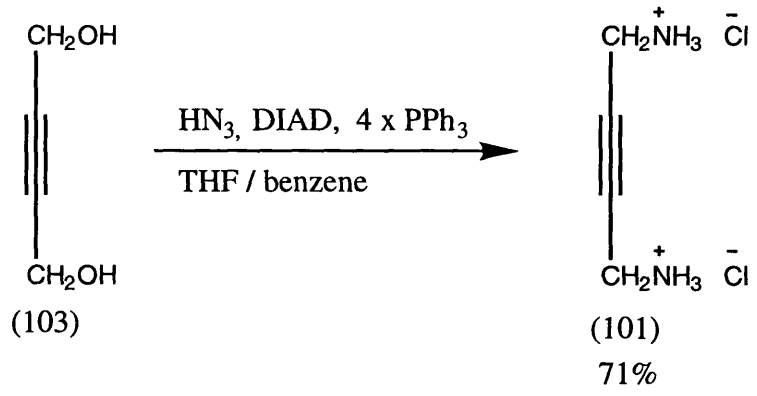
An added attraction to this route is the opportunity for concomitant reduction *in situ* of the alkyl azide formed with triphenylphosphine. The iminophosphorane thus produced can then be hydrolytically cleaved to yield the primary amine. This process can be combined with the Mitsunobu reaction in a one-step synthesis of primary amines from alcohols which avoids the problematic isolation of azide intermediates. This system facilitated the conversion of *Z*-but-2-ene-1,4-diol (102) into *Z*-1,4-diaminobut-2-ene dihydrochloride (100) (Scheme 31) in 63% yield. But-2-yne-1,4-diol (103) was also converted into 1,4-diaminobut-2-yne dihydrochloride (101) (Scheme 32) by this route, and *E*-2,3-dibromobut-2-ene-1,4-diol (104) afforded *E*-1,4-diamino-2,3-dibromobut-2-ene dihydrochloride (105) (Scheme 33) in yields of 71% and 58% respectively. Quaternary carbons were observed in the ^{13}C NMR spectra of compounds (101) and (105), at 79.2 and 119.0 ppm respectively. This is consistent with the different environments of the quaternary carbons in each compound.

Scheme 31



(102) (100) 63%

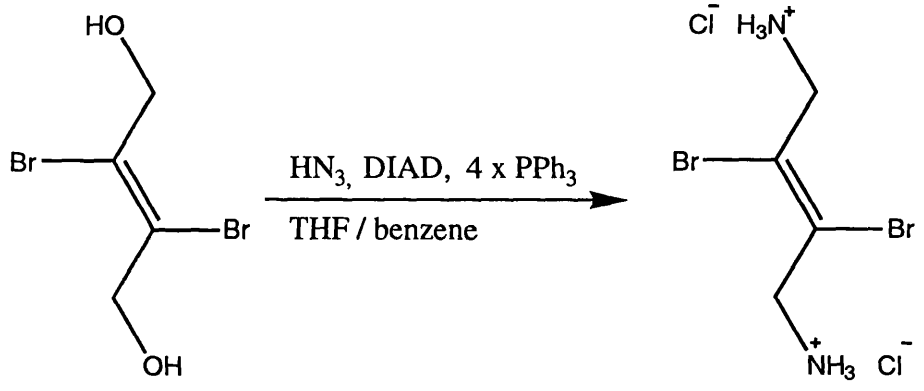
Scheme 32



(103) (101)

71%

Scheme 33

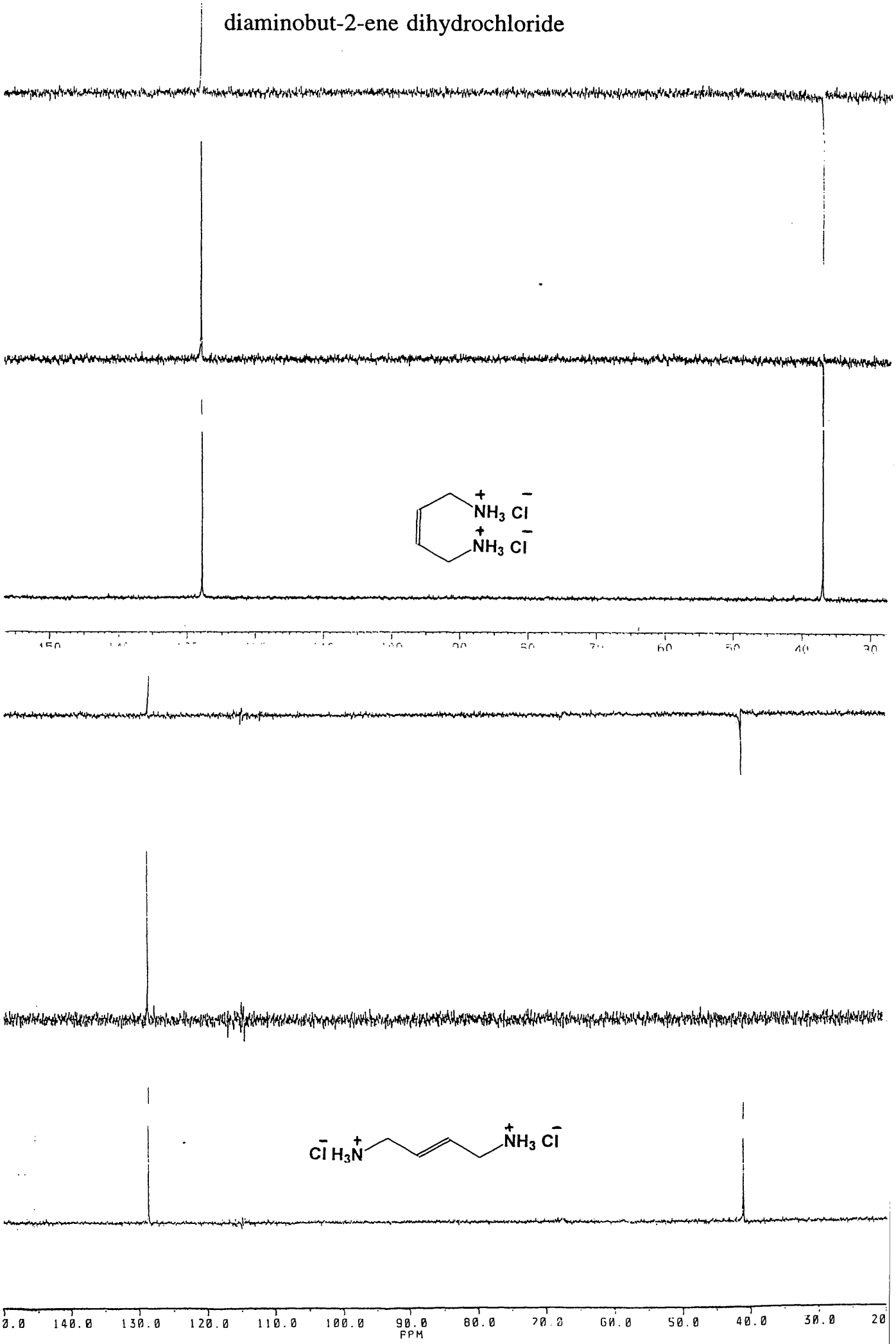


(104) (105)

58%

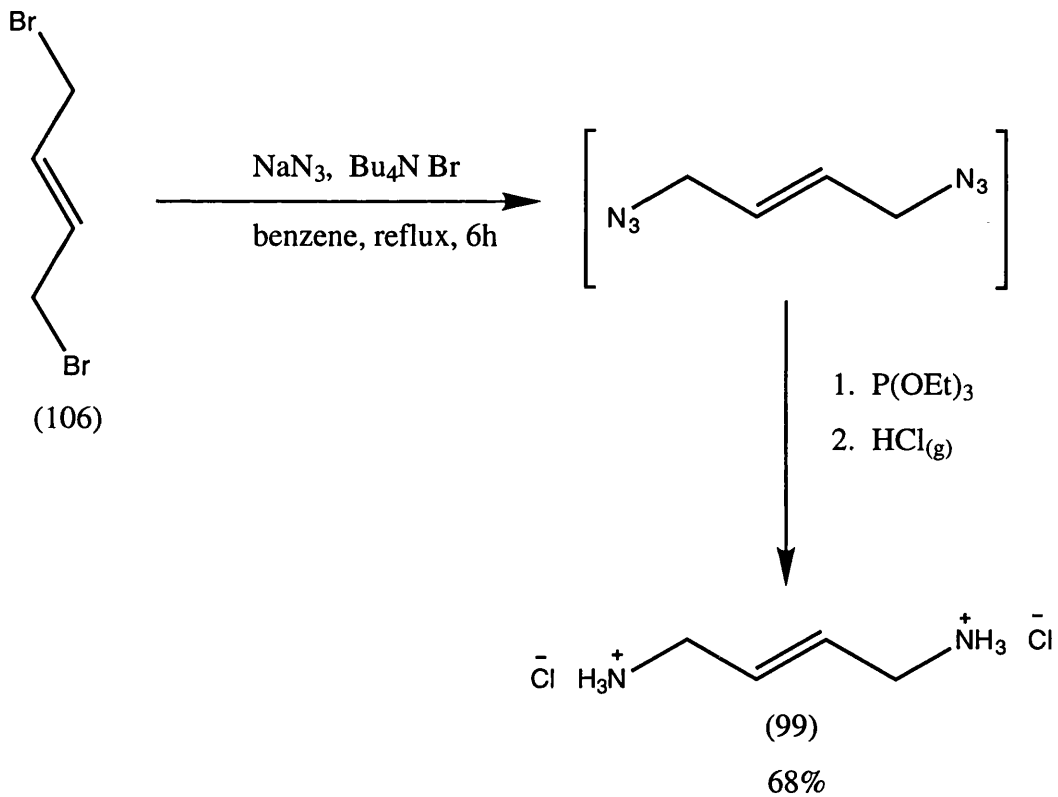
It was believed that *E*-1,4-diaminobut-2-ene (99) could be prepared similarly from *E*-but-2-ene-1,4-diol. However, previous attempts to furnish this diol by DIBAL reduction of dimethyl fumarate did not give desirable yields. An alternative route was available, starting from the conversion of *E*-1,4-dibromobut-2-ene (106) into the corresponding diazido derivative by heating with sodium azide at reflux in benzene under phase-transfer catalysis conditions.[35] Again in this case, *E*-1,4-

Figure 1: ^{13}C NMR Spectra of *Z*- and *E*-1,4-diaminobut-2-ene dihydrochloride



diazidobut-2-ene was not isolated, but treated *in situ* with triethyl phosphite. The phosphimidate ester so formed was cleaved by passing hydrogen chloride gas through the organic solution, which deposited the desired diamine as its dihydrochloride salt (Scheme 34). The ^{13}C NMR spectrum showed the methylene carbons at 41.1 ppm (cf. 36.9 ppm for the *Z*-isomer) (fig. 1).

Scheme 34



A detailed account of the relative antifungal activities of these compounds is contained in Chapter 8. To summarise, *E*-1,4-diaminobut-2-ene dihydrochloride (99) gave consistently better results than the other compounds. On this basis, it was decided to conduct field trials with this

compound, and therefore a route was required which would provide high yields of *E*-1,4-diaminobut-2-ene dihydrochloride on multiple gram scales.

Conversion of *E*-1,4-dibromobut-2-ene into the desired diamine dihydrochloride *via* the diazide as indicated previously (Scheme 34) repeatedly failed to give satisfactory yields, frequently less than 10% on a 20 g scale. A much more consistent route was to convert the starting dibromide into the corresponding *E*-1,4-diphthalimidobut-2-ene (107), using potassium phthalimide in DMF at 25 °C. The diphthalimido derivative formed was then subjected to a concentrated hydrochloric acid/glacial acetic acid mixture heated at reflux, which removed the phthalimide protecting groups to give *E*-1,4-diaminobut-2-ene dihydrochloride (99) on work up. This modification of the Gabriel synthesis gave the desired compound in an overall yield of 80% for the two steps, even during large-scale preparation (Scheme 35). This work has been published,[47] and formed the basis of a patent.[48]

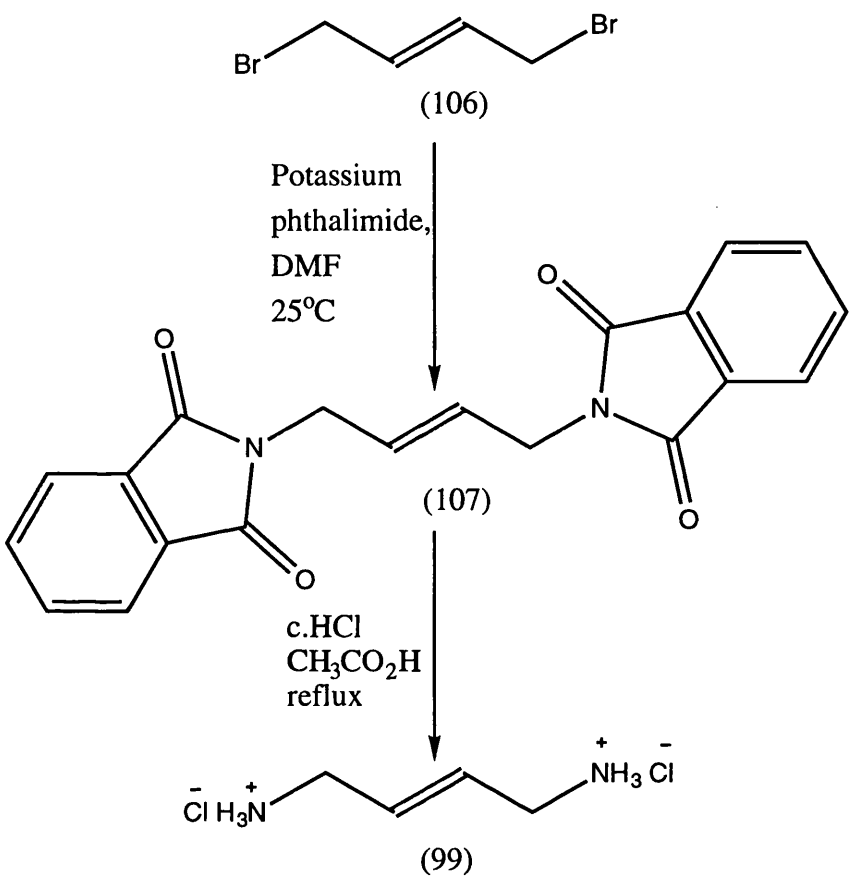
5.3 Secondary and Tertiary *E*-1,4-Diaminobut-2-enes

In following up the initial test results, it was of interest to investigate the effect of alkyl substitution on the amine groups of *E*-1,4-diaminobut-2-ene dihydrochloride. Thus, studies were undertaken into the synthesis of *E*-1,4-bis(dialkylamino)but-2-enes and *E*-1,4-bis-(alkylamino)but-2-enes.

Tertiary *E*-1,4-(diamino)but-2-enes were the simpler of these targets in terms of synthesis. Compounds of this structure were readily

available from alkylation of appropriate dialkyl amines, again utilising *E*-1,4-dibromobut-2-ene as the alkylating agent.

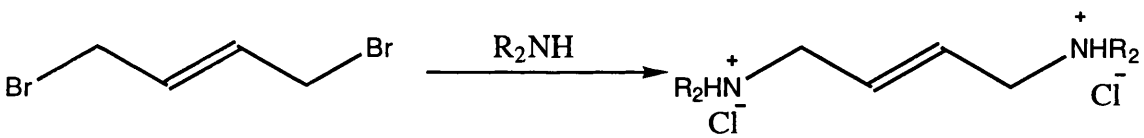
Scheme 35



In fact, *E*-1,4-bis(dialkylamino)but-2-enes could be produced directly as dihydrobromide salts, when two equivalents of, for instance, diethylamine were added to a solution of *E*-1,4-dibromobut-2-ene. Precipitation of the product was effected when non-polar solvents were used, and filtration of the crude product was carried out directly from the reaction mixture. However, this method suffered from incomplete reaction and mixtures of products. Faster reactions were achieved using

the procedure of Roberts and Ross,[41] which involved adding the alkylating agent to a solution of a large excess of the dialkylamine. Distillation removed solvents and excess dialkylamine, leaving the *E*-1,4-bis(dialkylamino)but-2-enes as free bases. These were then taken up into hydrochloric acid, and the solution was concentrated *in vacuo* to leave the dihydrochloride salt derivatives. *E*-1,4-Bis(diethylamino)but-2-ene dihydrochloride (108), *E*-1,4-bis(dimethylamino)but-2-ene dihydrochloride (109) and *E*-1,4-bis(piperidin-1-yl)but-2-ene dihydrochloride (110) were synthesised in this manner (Scheme 36). Allylic methylene protons for all these compounds had ^{1}H NMR chemical shifts of around 3.66 ppm (fig. 2).

Scheme 36



(106)

(108) R=Et
(109) R=Me
(110) R_2=-$(CH_2)_5$-

This method was also extended to the preparation of secondary *E*-1,4-diaminobut-2-enes. However, alkylation of a primary amine to give secondary amines suffers from over-alkylation side reactions, due to the greater nucleophilicity of the product secondary amines over the starting primary amines. Thus, attempted synthesis of *E*-1,4-bis(methylamino)but-2-ene dihydrochloride (111) gave polymeric side products, even when vast excesses of methylamine were employed. These side products were only removed by using hot filtration and multiple crystallisation

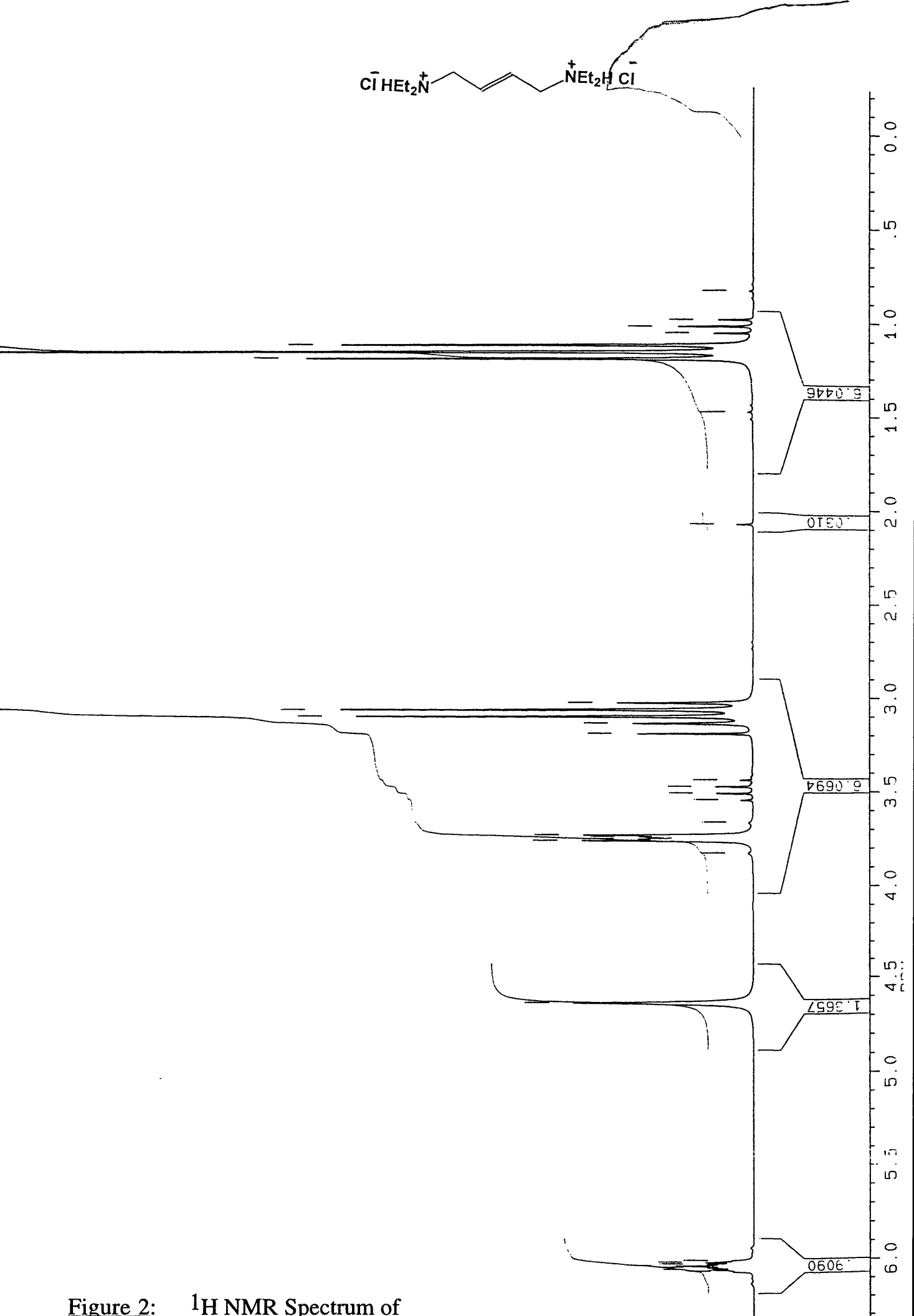
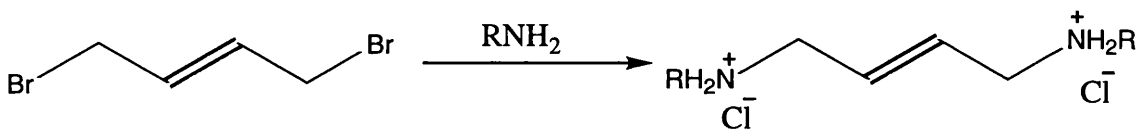


Figure 2: ^{1}H NMR Spectrum of

E-1,4-bis(diethylamino)but-2-ene dihydrochloride

techniques. Synthesis of *E*-1,4-bis(cyclohexylamino)but-2-ene dihydrochloride (112) did not give rise to this problem to the same extent. This may be due to the secondary amine produced from the initial alkylation being relatively less nucleophilic, because of steric factors (Scheme 37).

Scheme 37



(106)

(111) R=Me
(112) R=cyclohexyl

Of this group of derivatives, *E*-1,4-bis(diethylamino)but-2-ene dihydrochloride showed the greatest antifungal activity against a wide range of plant pathogens, and this is documented in Chapter 8. Some of this work has been published,[49] and formed the basis of a second patent.[50]

5.4 Synthesis of *E*-1-Amino-4-diethylaminobut-2-ene Dihydrochloride

As both the primary amino group and diethylamino group had shown antifungal activity when placed as 1,4-substituents on the *E*-but-2-ene chain, it was of interest to observe the effect that both of these groups would have as part of one structure. Therefore, synthetic studies were carried out to furnish *E*-1-amino-4-diethylaminobut-2-ene

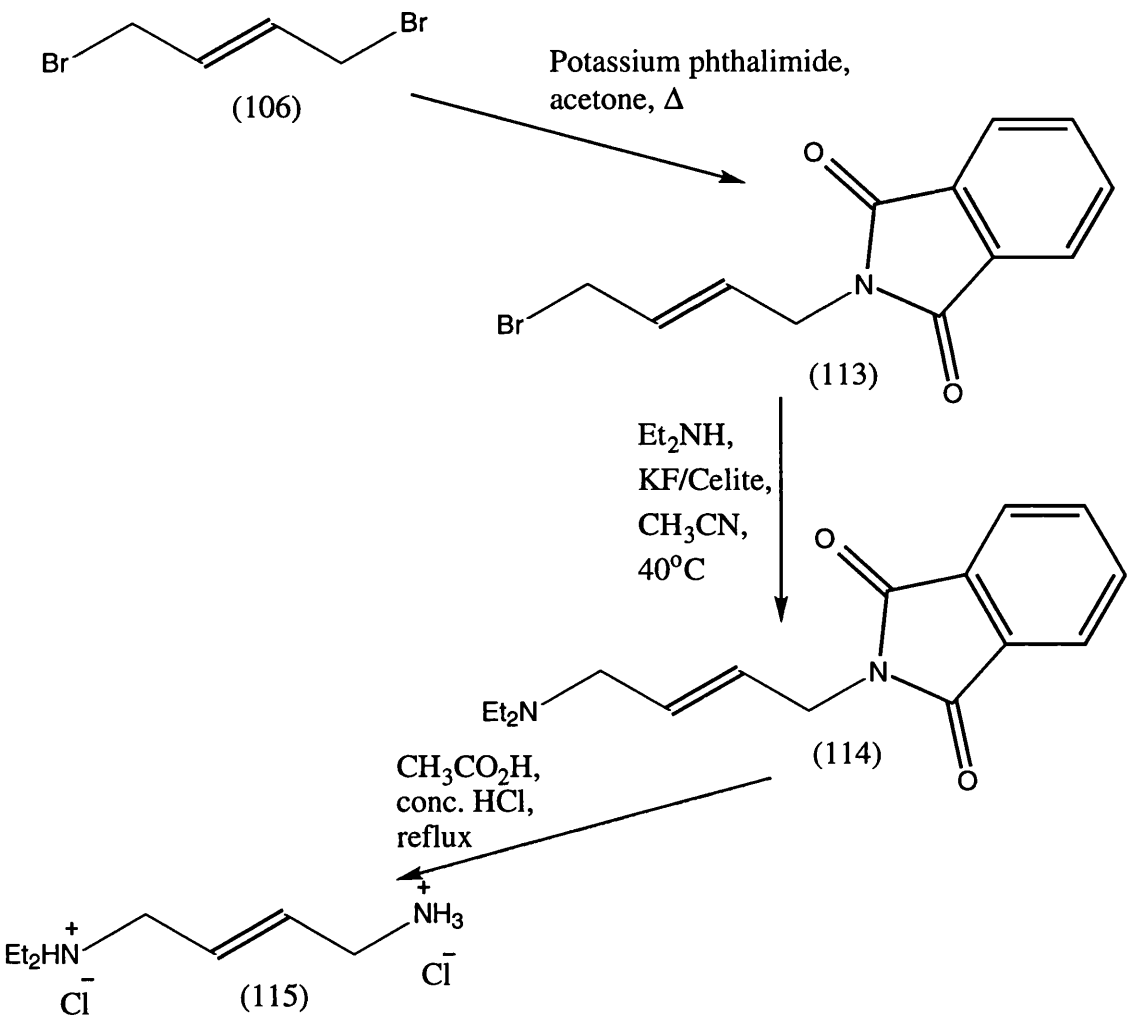
dihydrochloride (115), with a view to producing a strategy that could be adapted to other unsymmetrical targets of this type.

The Gabriel synthesis, involving alkylation of phthalimide anion, had proved fruitful in the incorporation of the primary amine group on *E*-1,4-dibromobut-2-ene before. It was deemed therefore that *E*-1-bromo-4-phthalimidobut-2-ene (113) was a desirable intermediate, which would allow displacement of the bromine atom with diethylamine, followed by deprotection of the phthaloyl group to yield the primary amine. This strategy was akin to that of Samejima *et al.*[51] who used 1-bromo-3-phthalimidopropane as an aminopropyl unit precursor for amine alkylations.

To produce 1-bromo-3-phthalimidopropane, these authors used the action of a large excess of 1,3-dibromopropane on potassium phthalimide. Similarly, addition of potassium phthalimide to a 10-fold excess of *E*-1,4-dibromobut-2-ene gave a mixture of *E*-1-bromo-4-phthalimidobut-2-ene (113) and the starting material, which was easily separated by flash chromatography. However, this process was wasteful in the use of *E*-1,4-dibromobut-2-ene. A more efficient conversion was achieved using equimolar amounts of this dibromide and potassium phthalimide in refluxing acetone. The high reactivity of *E*-1,4-dibromobut-2-ene (106) in this medium allowed nucleophilic displacement of one bromide by phthalimide. The low solubility of the resultant *E*-1-bromo-4-phthalimidobut-2-ene (113) in refluxing acetone prevented further reaction, and thus a clean and high-yielding reaction was achieved. The product showed an IR band at 1686 cm^{-1}, consistent with an unsymmetrically substituted double bond.

E-1-Bromo-4-phthalimidobut-2-ene (113) was then used to alkylate diethylamine, utilizing potassium fluoride supported on Celite as a catalyst.[51] The resultant *E*-1-diethylamino-4-phthalimidobut-2-ene (114) underwent hydrolysis in glacial acetic acid and concentrated hydrochloric acid to give *E*-1-amino-4-diethylaminobut-2-ene dihydrochloride (115) (Scheme 38), giving an IR band at 1664 cm^{-1}.

Scheme 38



5.5 Studies Towards Synthesis of 2,3-Substituted Straight Chain 1,4-Diamine Derivatives

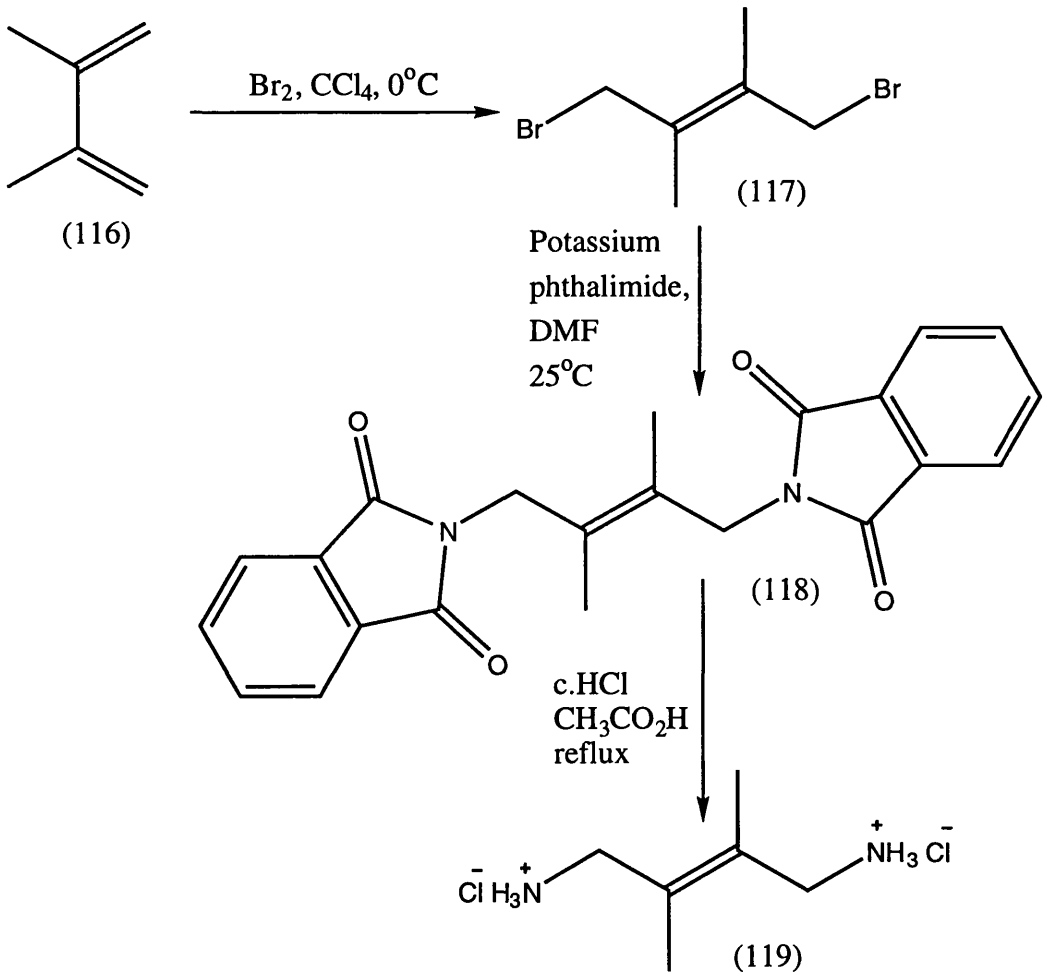
An interesting extension to this work was to investigate the effect of altering the carbon backbone of these targets. 2,3-Dialkyl substituted derivatives of *E*-1,4-diaminobut-2-enes had not previously been studied as amino acid decarboxylase inhibitors or as antifungal agents. Therefore, a programme of work concerned with the synthesis of such analogues was executed. The simplest compound formed, *E*-1,4-diamino-2,3-dimethylbut-2-ene dihydrochloride, is documented here. More complex targets are discussed in the following chapter.

Initial results had indicated that 1,4-diols and 1,4-dibromides were suitable precursors to the 1,4-diamine system, but neither was readily available for this target. However, of interest was the fact that reaction of bromine with conjugated dienes occurs via 1,4-addition to yield 1,4-dibromo-2-alkenes.[52] Where possible, the new double bond formed in the product adopts the *trans*-configuration. This type of functionalisation was highly desirable, as conversion of these types of dibromides into the corresponding diamines proceeded without significant problems previously. Where suitable 1,3-dienes were available, this provided an alternative, and in many ways preferable, route to *E*-1,4-diaminobut-2-enes.

Thus 2,3-dimethyl-1,3-butadiene (116) was treated with one equivalent of bromine in carbon tetrachloride at 0 °C to give *E*-1,4-dibromo-2,3-dimethylbut-2-ene (117) almost exclusively, which was crystallized from the *Z*-isomer and other by-products. The dibromide was then stirred with potassium phthalimide in DMF for 72 hours to yield *E*-

2,3-dimethyl-1,4-diphthalimidobut-2-ene (118) showing the characteristic IR band at 1769 cm^{-1}. Hydrolytic cleavage of the phthaloyl protecting groups was effected as before with concentrated hydrochloric acid and glacial acetic acid to give the desired *E*-1,4-diamino-2,3-dimethylbut-2-ene dihydrochloride (119) with a ^1H NMR spectrum showing methylene protons at 3.60 ppm (Scheme 39).

Scheme 39



5.6 Summary

Several 1,4-diaminobut-2-enes were synthesised. Of these, *E*-1,4-diaminobut-2-ene dihydrochloride and *E*-1,4-bis(diethyl-amino)but-2-ene dihydrochloride showed greatest general fungicidal activity. Large-scale routes were developed to both targets. This work formed the basis of two publications and two patents.

Chapter 6

Synthesis of Unsaturated Carbocyclic Diamines as Antifungal Agents

6.1 Introduction

In the previous chapter, success with the synthesis and antifungal evaluation of initial unsaturated putrescine analogues led to further study. An extension of this work was the synthesis of cycloalkenes with a 1,2-bis(aminomethyl) substitution pattern. Compounds of this type were novel, and had not been tested previously as antifungal agents.

6.2 Synthesis of Cyclohexene Derivatives

The ease of construction of six-membered ring made compounds with this substructure the most attractive initial targets. As conversion of diols into diamines had appeared to proceed relatively smoothly in the case of the straight chain compounds, it was logical to assume that key intermediates were carbocycles with 1,2-bis(hydroxymethyl) groups in place. An obvious precursor to this type of system is the cyclic 1,2-dicarboxylic acid ester moiety. Reduction of these ester groups was expected to provide the desired diols.

The Diels-Alder reaction is often the method of choice for six-membered ring formation. It has been widely exploited, mainly due to two factors. Firstly, the reaction can be used to give the concurrent formation of up to four stereocentres with desired relative configurations.

Secondly, the products of the reaction often allow further manipulation by the introduction of functional groups in key positions around the ring.

The criteria adopted for these cyclic targets remained as it was for the straight chain analogues, with unsaturation in place between the two amine groups. From the Diels-Alder reaction, this could be achieved from both the diene and the dienophile component. Formation of the desired 1,2-bis(aminomethyl)cyclohexene system via the butadiene component would require a 2,3-bis(aminomethyl)-1,3-butadiene precursor. Studies towards compounds of this type are discussed later.

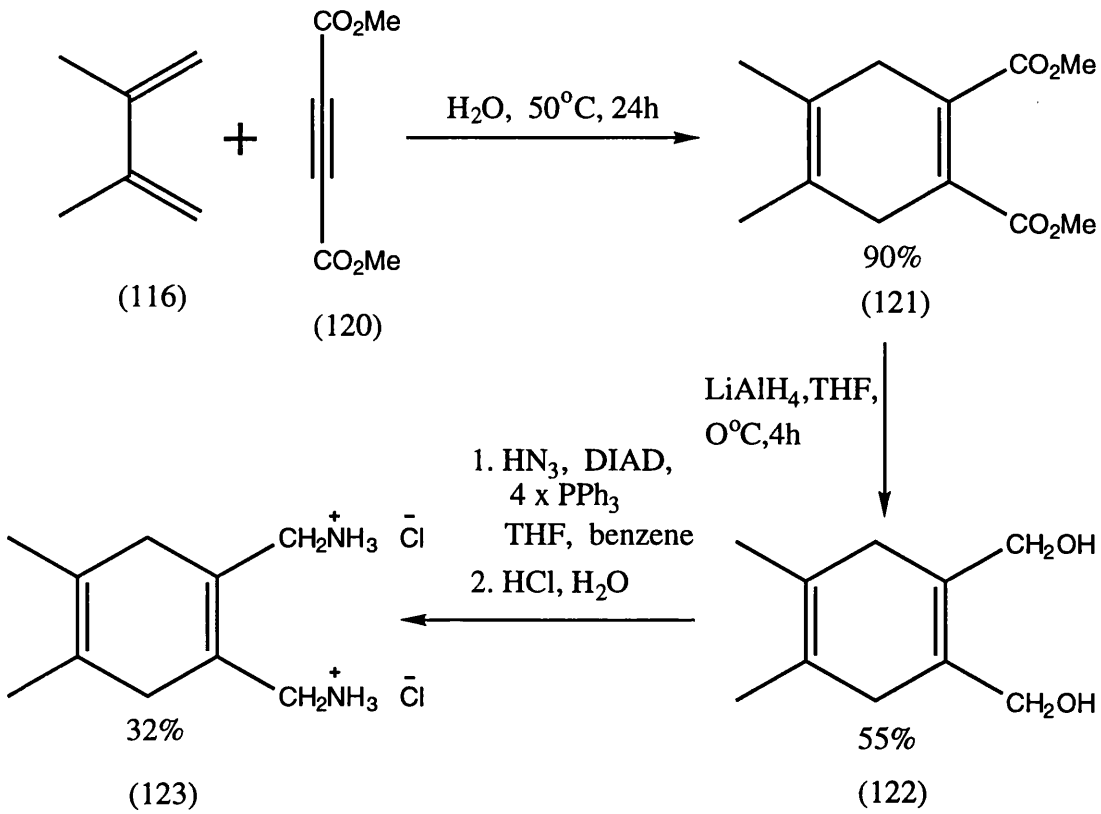
The direct use of but-2-yne-1,4-diol or the corresponding diamine as the dienophile component was not feasible due to the requirement of carbocyclic Diels-Alder reactions for electron-poor dienophiles. However, acetylenedicarboxylate derivatives have found widespread use in the reaction, and the 1,2-dicarboxylate products formed can be converted into diamines.

6.2.1 Synthesis of 1,2-Bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene Dihydrochloride

The first series of cyclic targets was planned using 2,3-dimethylbuta-1,3-diene, as this diene was available and easy to handle. Therefore, reaction of equal mole equivalents of 2,3-dimethylbuta-1,3-diene (116) and dimethyl acetylenedicarboxylate (120) as an aqueous emulsion at 50 °C gave dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (121) in high yield. Reduction of the resultant diester to afford 1,2-bis(hydroxymethyl)-4,5-dimethylcyclohexa-1,4-diene (122) was effected with lithium aluminium hydride at low temperature. Finally, transformation of the hydroxy groups to yield 1,2-bis(aminomethyl)-4,5-

dimethylcyclohexa-1,4-diene dihydrochloride (123) was carried out under Mitsunobu conditions as before with the structurally similar *Z*-but-2-ene-1,4-diol (Chapter 5) (Scheme 40). The product (123) showed a singlet at 3.76 ppm in the ^1H NMR spectrum for the exocyclic methylene protons.

Scheme 40



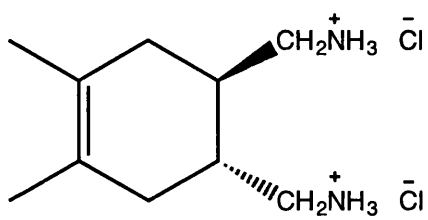
This compound showed antifungal activity mainly against powdery mildew and led to further study.

Yields for the latter two steps of this, and subsequent similar syntheses, were low in relation to similar reactions in the literature. Reductions of α,β-unsatuarated esters to allylic alcohols has received much attention from synthetic chemists, and several reagents are proposed

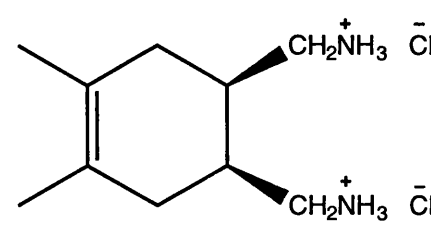
in the literature to effect this transformation.[53,54] Diisobutyl aluminium hydride (DIBAL) appears to be the reagent of choice,[55] but commercially available forms in dichloromethane and in toluene failed to give the diol (122) in reproducible yield. High levels of impurities could not be separated from the product. Similar results were obtained when the reagent was modified with butyllithium or boron trifluoride. The major drawback with lithium aluminium hydride was over-reduction of the allylic double bond, but this reagent gave the best results in temperature-controlled reactions.

6.2.2 Synthesis of *cis* and *trans* 1,2-Dimethyl-4,5-bis(aminomethyl)cyclohexene Dihydrochloride

Synthesis of other cyclic diamine derivatives was required to give a clearer indication of structure-activity relationships, for example by assigning to the amine groups rigidly held stereochemical positions. To this end, other possible cyclic targets were identified, taking into account their ease of construction. These targets included *trans*-1,2-dimethyl-4,5-bis(aminomethyl)cyclohexene dihydrochloride (124) and *cis*-1,2-dimethyl-4,5-bis(aminomethyl)cyclohexene dihydrochloride (125). The former would be formed as a racemate initially and the latter is a *meso* compound.



(124)



(125)

Again, the cyclohexene skeleton of these structures was thought to be easily constructed by Diels-Alder reactions. These compounds essentially change the position of the amine groups in space with respect to the cyclohexadiene analogue, 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (123). It is important to note here that these compounds do not have the designated unsaturation at the 2- and 3- carbons between the amine groups of the 1,4-diamine substructure. It should be stressed that while this functionality was justifiably included in early targets to promote the possibility of inhibition of vital polyamine biosynthesis enzymes, the mode of action of the fungicidal agents produced with the 1,4-diamino-2-butene structure need not necessarily be attributed to the mechanism hypothesised previously (Chapter 3). Indeed, the putrescine analogues manufactured in this work may not interfere adversely with fungal polyamine biosynthesis at all. The testing of these compounds gives only an indication of their effect on whole fungal systems, and their ability to stop proliferation and to stop the spread of infection. Therefore, replacement of the unsaturation by dihydro derivatives may not result in loss of activity. This appears to be borne out in the test data (Chapter 8).

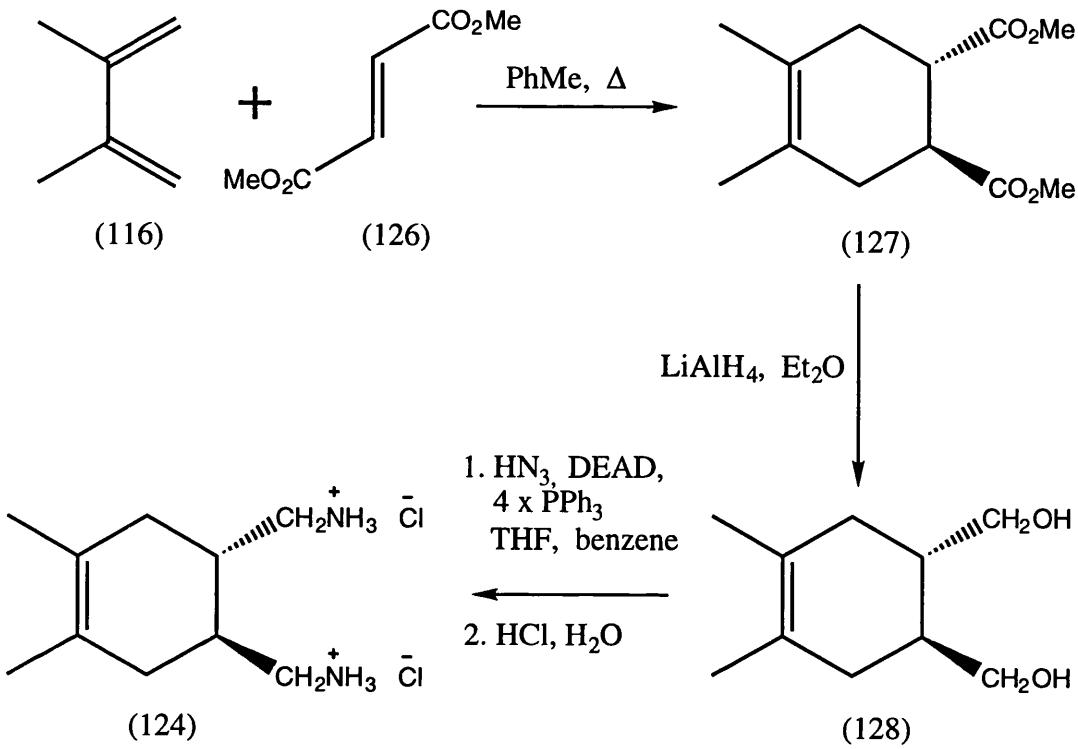
The routes to *trans*-1,2-dimethyl-4,5-bis(aminomethyl)-cyclohexene dihydrochloride (124) and to *cis*-1,2-dimethyl-4,5-bis(aminomethyl)-cyclohexene dihydrochloride (125) were perceived to follow a similar pathway to the formation of 1,2-bis(aminomethyl)-4,5-dimethylcyclohexene-1,4-diene dihydrochloride (123). The stereochemistry of the final compounds could be put in place in the initial Diels-Alder cyclisation, by the choice of dienophile component. Utilizing the principle that the stereochemistry of the starting materials is retained in

the product of a Diels-Alder reaction, it was expected that reaction of 2,3-dimethylbutadiene with dimethyl fumarate and maleic anhydride would generate the *trans*-disubstituted and *cis*-disubstituted cyclohexene ring systems respectively. The stereochemistry could then be transferred into the final products, being unaffected by subsequent reactions. This was found to be the case.

Reaction of 2,3-dimethylbutadiene (116) with dimethyl fumarate (126) in toluene at 50 °C produced dimethyl *trans*-1,2-dimethyl-cyclohexene-4,5-dicarboxylate (127) in excellent yield. Reduction of this diester to the corresponding diol with lithium aluminium hydride in ether proceeded smoothly in 36% yield. The resultant *trans*-1,2-dimethyl-4,5-bis(hydroxymethyl)cyclohexene (128) was subjected to treatment with hydrazoic acid under Mitsunobu conditions. The diazide formed was reacted with triphenylphosphine *in situ*, and the product was hydrolysed with aqueous acid to give *trans*-1,2-dimethyl-4,5-bis(aminomethyl)cyclohexene dihydrochloride (124) (Scheme 41). The aminomethyl hydrogens came into resonance in the ^{1}H NMR spectrum at 2.85 ppm (fig. 3).

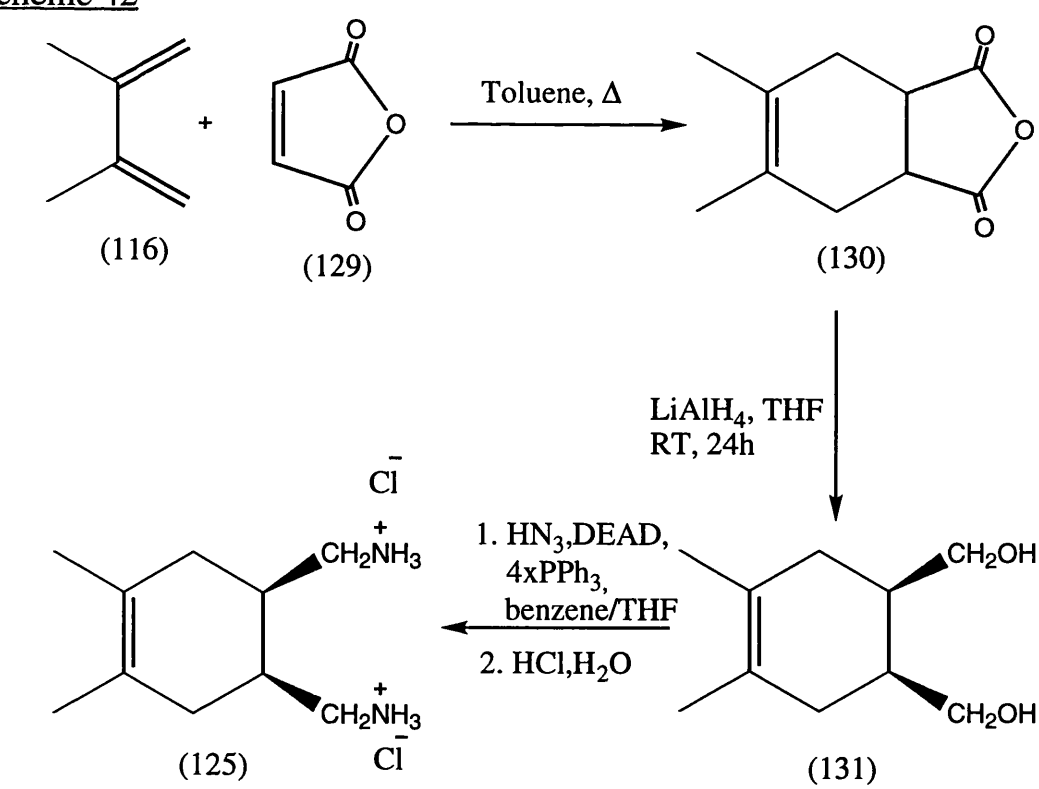
Diels-Alder reaction of 2,3-dimethylbuta-1,3-diene (116) with maleic anhydride (129) in toluene at 50 °C gave *cis*-1,2-dimethylcyclohexene-4,5-dicarboxylic anhydride (130). Reduction of this anhydride was effected with lithium aluminium hydride in ether at reflux to give *cis*-1,2-dimethyl-4,5-bis(hydroxymethyl)cyclohexene (131). The Mitsunobu reaction with hydrazoic acid was carried out as outlined previously, followed by iminophosphorane formation and hydrolysis steps to give the desired *cis*-1,2-dimethyl-4,5-bis(aminomethyl)cyclohexene dihydrochloride (125) (Scheme 42).

Scheme 41



(116) (126) (127) (124) (128)

Scheme 42



(116) (129) (130) (125) (131)

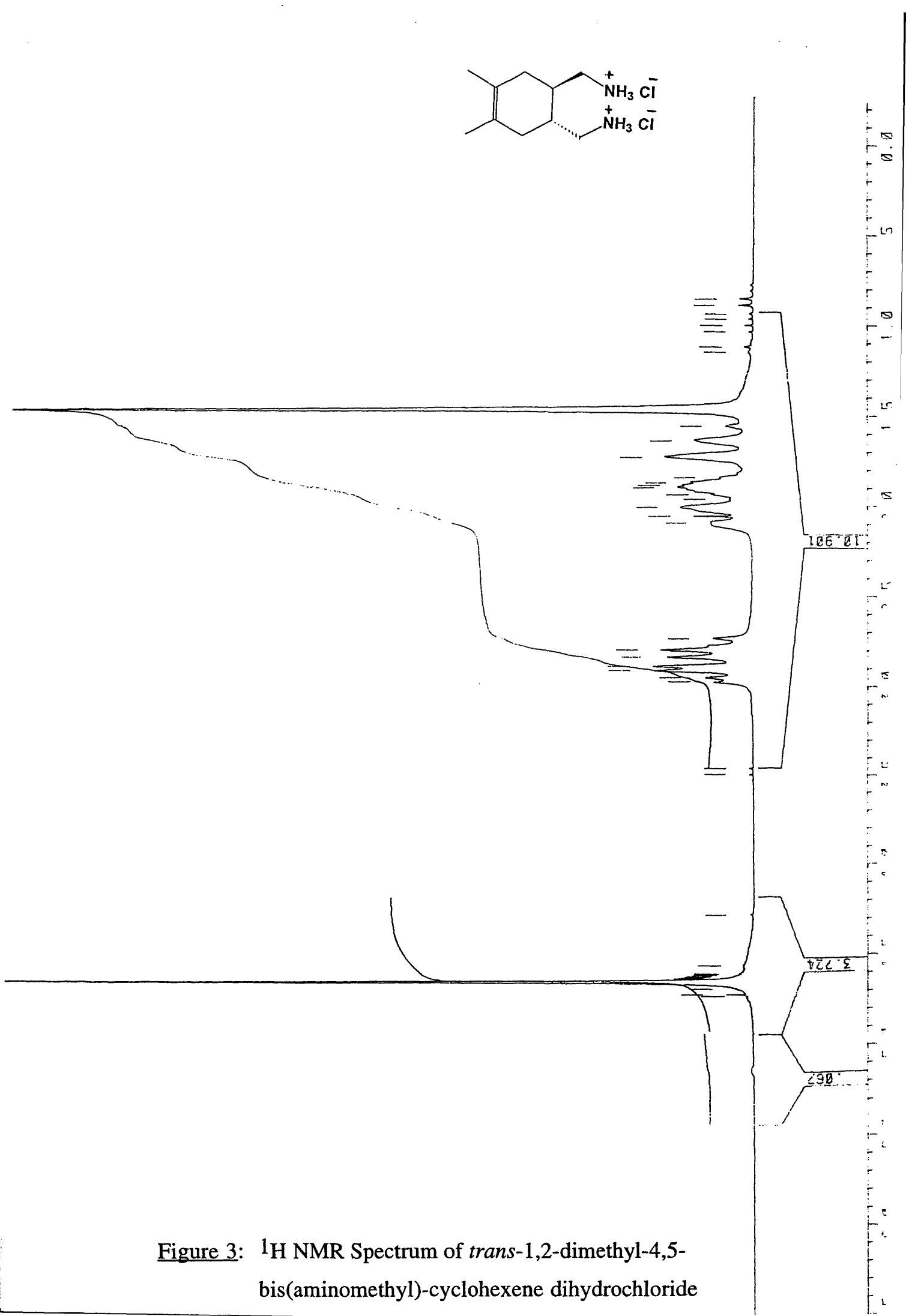


Figure 3: ^{1}H NMR Spectrum of *trans*-1,2-dimethyl-4,5-bis(aminomethyl)-cyclohexene dihydrochloride

6.2.3 Synthesis of Other Six-Membered Ring Diamines

1,2-Bis(aminomethyl)cyclohexene dihydrochloride (132) was also a target compound, mainly due to the commercial availability of 3,4,5,6,-tetrahydrophthalic anhydride. This compound leads to a diamine with different substituents on the 6-membered ring, giving a comparison with those compounds derived from Diels-Alder reaction of 2,3-dimethylbuta-1,3-diene.

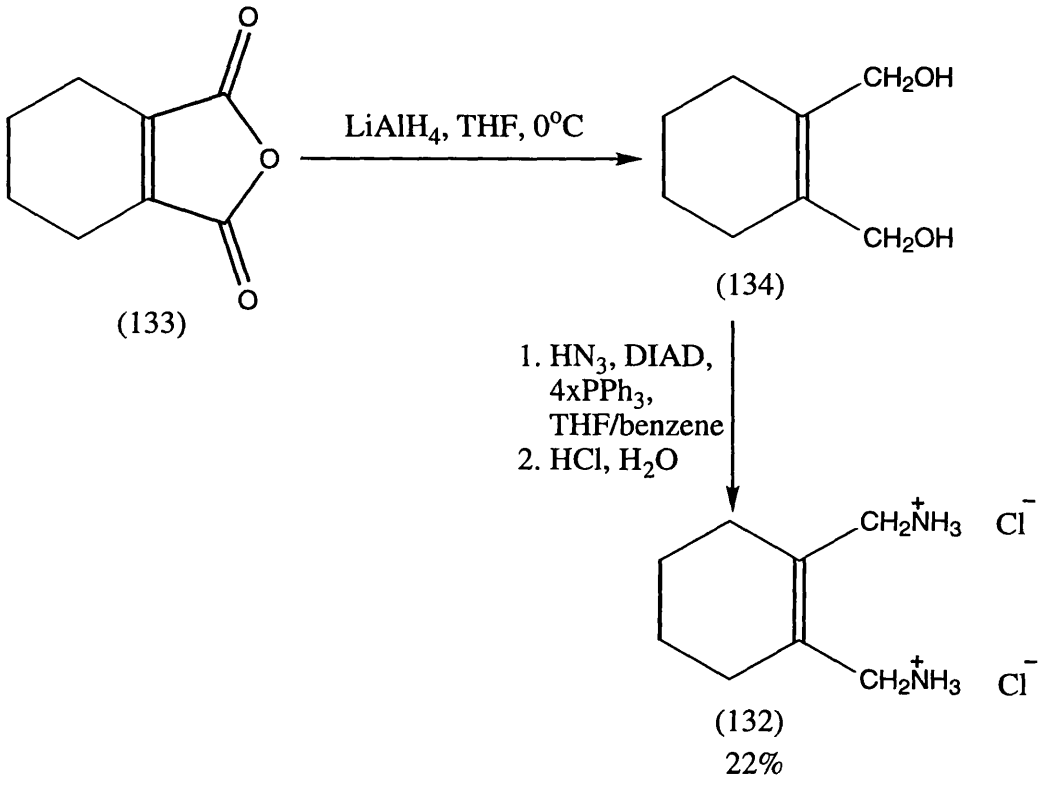
3,4,5,6-Tetrahydrophthalic anhydride (133) was converted into 1,2-bis(hydroxymethyl)cyclohexene (134) via reduction with lithium aluminium hydride in THF at low temperature. This diol underwent transformation to the corresponding 1,2-bis(aminomethyl)cyclohexene dihydrochloride (132) via the intermediate diazide by successive Mitsunobu, Staudinger and hydrolysis reactions as indicated (Scheme 43), with the ^{1}H NMR spectrum showing a singlet at 3.82 ppm for the exocyclic methylene protons.

6.3 Synthesis of Other 1,2-Bis(aminomethyl)cycloalkenes

Studies towards the synthesis of compounds with other ring sizes were undertaken in order to investigate the effect of ring size on the activity of the diamines as antifungal agents. Incorporation of unsaturation between the aminomethyl groups in these targets was again a prominent feature of the synthetic routes chosen. Once more a general route was sought which would give 4-, 5- and 7- membered rings containing the cycloalkene-1,2-dicarboxylic ester functionality. Such compounds were produced previously by McDonald *et al.*,[56] who

required dimethyl cyclobutene-1,2-dicarboxylate, dimethyl cyclopentene-1,2-dicarboxylate and dimethyl cycloheptene-1,2-dicarboxylate as part of the syntheses of substituted bicyclic [2.1.0] systems.

Scheme 43



(133) (134) (132)

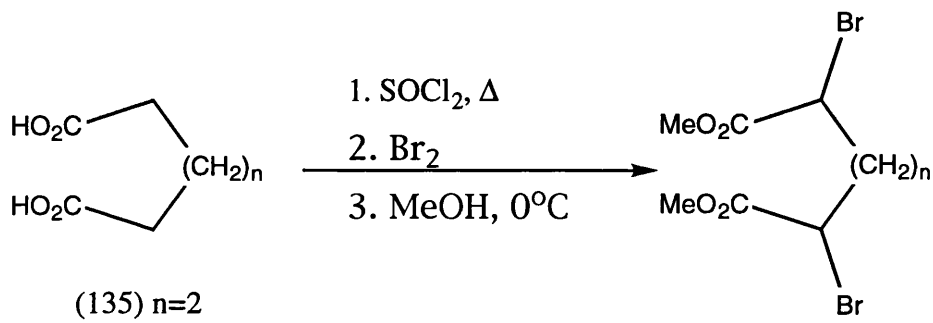
22%

In this work, dimethyl cyclobutene-1,2-dicarboxylate was prepared from adipic acid, by conversion of this diacid into the corresponding α,α'-dibromodimethyl ester (Scheme 44). This was followed by sodium hydride-induced cyclisation and elimination. Dimethyl cyclopentene-1,2-dicarboxylate was similarly synthesised from pimelic acid, and dimethyl cycloheptene-1,2-dicarboxylate from azelaic acid.

It was hoped that these diesters would then undergo reduction to the corresponding diols, followed by the Mitsunobu conditions to give, ultimately, the diamines.

Conversion of the above diesters into the appropriate diols had some precedent. Butina and Sondheimer[57] had previously prepared 1,2-bis(hydroxymethyl)cyclobutene and 1,2-bis(hydroxymethyl)cyclopentene from the corresponding dimethyl diesters by reduction using DIBAL, but found yields were modest and purification laborious.

Scheme 44



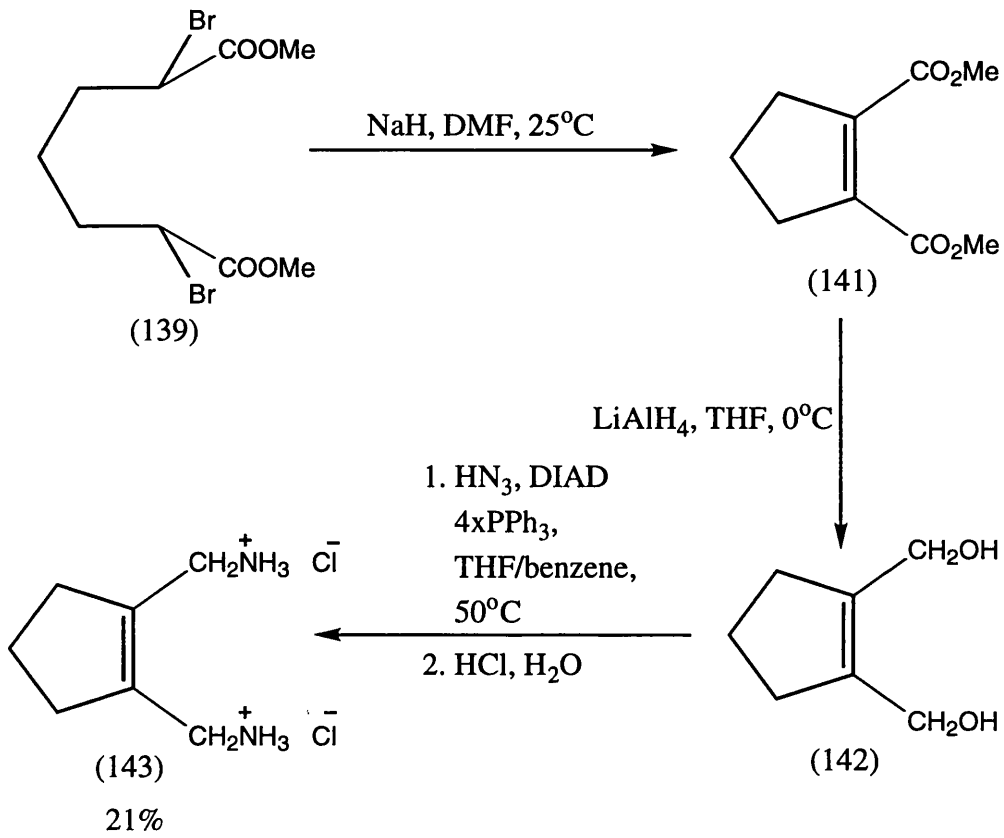
(135) n=2
(136) n=3
(137) n=5

(138) n=2
(139) n=3
(140) n=5

It was found in our work that cyclisation of dimethyl 2,5-dibromoadipate (138) was unsuccessful, and none of the desired product could be isolated from the reaction mixture. This was also true of the attempt at formation of dimethyl cycloheptene-1,2-dicarboxylate from dimethyl 2,8-dibromoazeleate (140). However, dimethyl 2,6-dibromopimelate (139) was converted into dimethyl cyclopentene-1,2-dicarboxylate (141) with sodium hydride in DMF. Low temperature reduction with lithium aluminium hydride in ether then produced 1,2-bis(hydroxymethyl)cyclopentene (142) in good yield. Conversion of this diol into the corresponding diazide and finally diamine dihydrochloride (143) was achieved with triphenylphosphine, diisopropyl azodicarboxylate

and aqueous acid work-up (Scheme 45). The ^1H NMR spectrum showed a singlet at 3.63 ppm for the aminomethyl protons.

Scheme 45



6.4 Summary

Several putrescine analogues with cyclohexene structures were synthesised, although the reactions generally suffered from low yields. *trans*-1,2-Dimethyl-4,5-bis(aminomethyl)cyclohexene dihydrochloride (124) showed antifungal activity over a range of species, while 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (123)

displayed particular activity towards powdery mildew. Further work is required in the synthesis of diamines with other ring sizes.

Chapter 7

Studies Towards the Synthesis of Heterocyclic 1,4-Diamines as Potential Antifungal Agents

7.1 Introduction

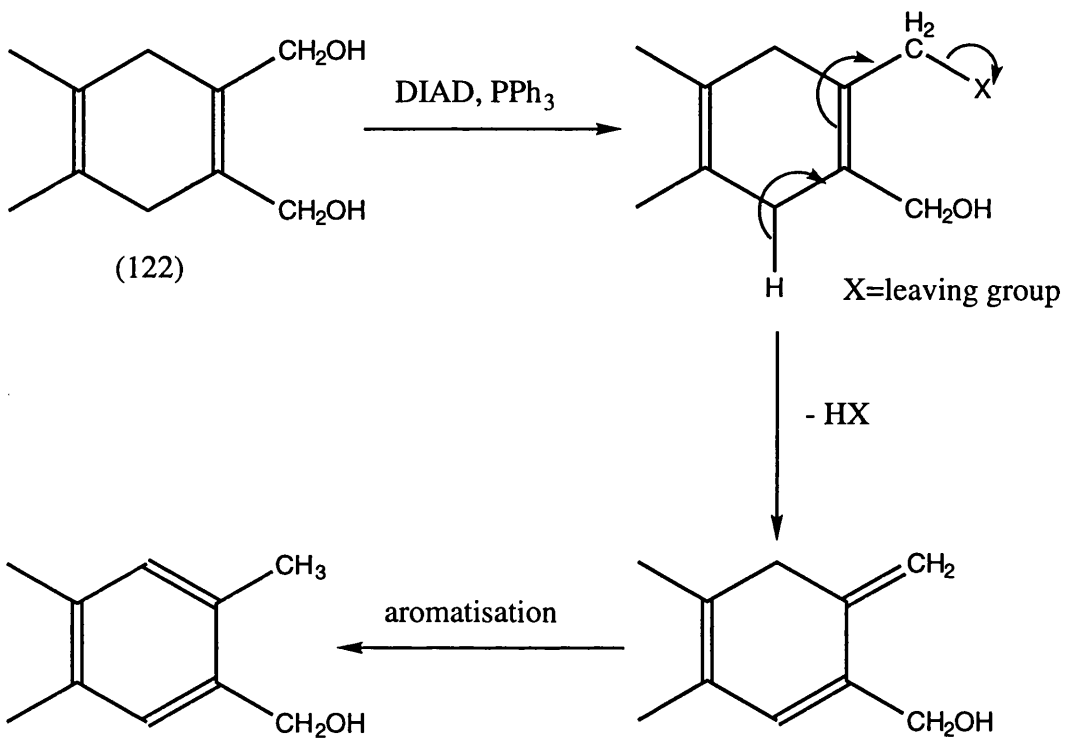
In our attempts to furnish the carbocylic diamines described in Chapter 6, low yields were encountered both for the formation of the appropriate diol precursors, and for their conversion into the corresponding diamine dihydrochlorides. It was felt that that one of the causes of these low yields may have been the spacial proximity of the functional groups concerned. It seemed feasible that intramolecular cyclisations and other side reactions, such as that indicated (Scheme 46), would compete with the desired substitution and reduction reactions. It was also reasoned that this would be a problem with the production of heterocyclic compounds with 1,2-bis(aminomethyl) groups from heterocyclic compounds with 1,2-bis(hydroxymethyl) groups, as these compounds would have a similar functional group arrangement. Alternative methods of introducing the 1,4-diamine functionality into compounds were investigated. It was hoped that these methods would avoid any such side-reactions and also give higher yields of diamines.

7.2 Concurrent Introduction of the Amine Groups

A possible solution was to introduce the amine groups concurrently, to avoid intramolecular reactions which may have been

associated with stepwise introduction of the functional groups. One such method involved the Diels-Alder reaction using dialkyl azodicarboxylates as dienophiles[58]. Cycloaddition of these compounds with 1,3-dienes gives rise to tetrahydropyridazines.[59]

Scheme 46

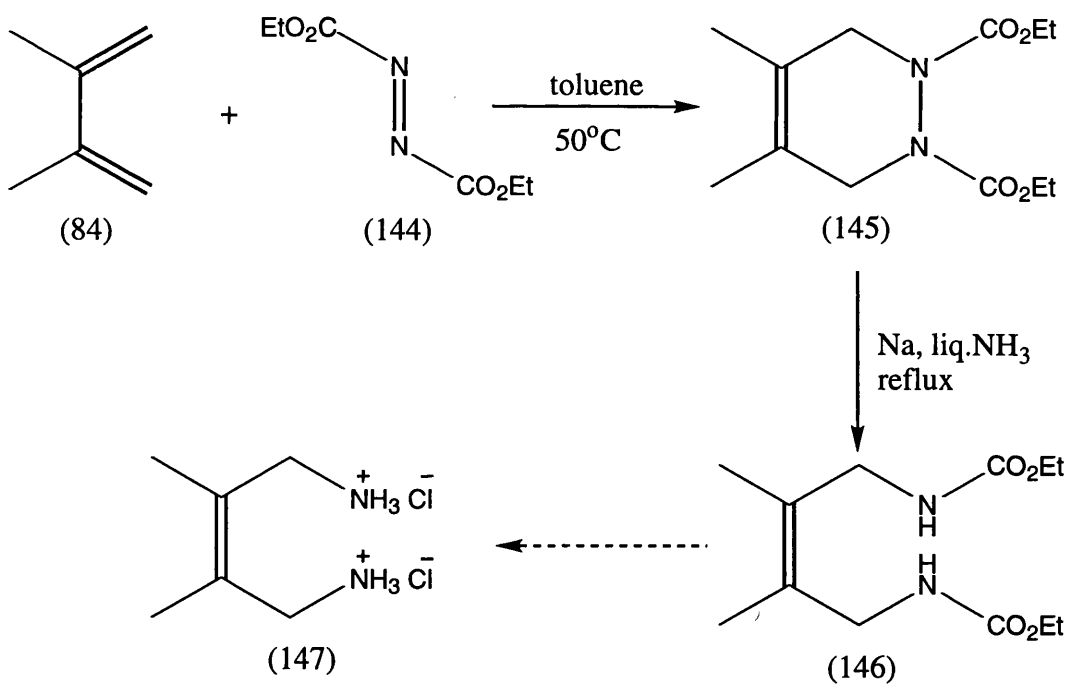


(122)

It seemed possible that the nitrogen-nitrogen bond in such compounds could be cleaved to yield a derivatised *Z*-1,4-diaminobut-2-ene structure. Synthesis of diamine derivatives could be carried out using the reductive cleavage of hydrazo derivatives reported by Johnston and Moody.[60] This was found to be the case. 2,3-Dimethylbuta-1,3-diene (84) underwent a Diels-Alder cyclisation with diethyl azodicarboxylate (144), giving diethyl 4,5-dimethyl-1,2,3,6-tetrahydropyridazine-1,2-

dicarboxylate (145). This was then treated with sodium in liquid ammonia[58] which cleaved the hydrazo bond to give *Z*-1,4-bis(ethoxycarbonylamino)-2,3-dimethylbut-2-ene (146) in a yield of 39% after flash chromatography (Scheme 47). The ^1H NMR spectrum of (146) showed a doublet 3.82 ppm for the allylic methylene protons. An attempt at deprotection of the amine groups by refluxing (146) in aqueous acid to give *Z*-2,3-dimethyl-1,4-diaminobut-2-ene (147) was not successful, but in principle it was demonstrated that tetrahydropyridazine compounds were suitable precursors to *Z*-1,4-diaminobut-2-enes, and their ease of construction made this route desirable.

Scheme 47



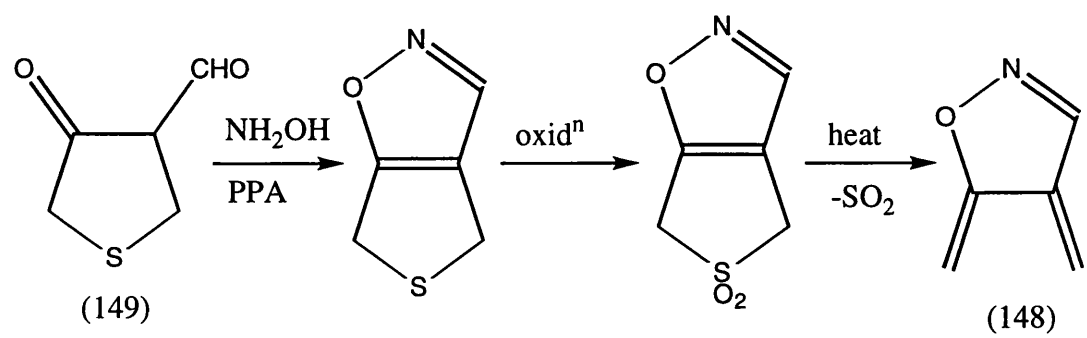
It was believed that this could facilitate the synthesis of several heteroaromatic 1,4-diamines. Chou and Chang[61] had shown the

accessibility of a wide range of heterocyclic compounds containing the 2,3-dimethylene functionality, and these compounds made excellent dienes for Diels-Alder reactions. It was possible to construct tetrahydropyridazine rings onto these structures using diethyl azodicarboxylate. However, the harshness of the sodium/liquid ammonia reducing conditions was likely to destroy the aromatic rings fused to the tetrahydropyridazine, and so alternative strategies were required.

7.2.1 Routes to Ketoputrescine Derivatives

Heterocyclic compounds containing *o*-dimethylene groups have often been constructed from appropriately functionalised butane derivatives. For example, *o*-dimethylene isoxazole (148) was synthesised in several steps from 4-formyl-3-oxosulpholane (149) (Scheme 48).[61] The heterocyclic portion was built on to the sulpholane before oxidation of the sulphur and [4+2] cycloelimination unveiled the latent diene functionality (148). By analogy, it was deduced that heterocyclic systems could be constructed onto the backbone of 2-ketoputrescine derivatives, thereby avoiding the need for harsh reducing conditions at the end of the synthesis. Routes to this key intermediate were therefore identified.

Scheme 48



(149) (148)

Using the Diels-Alder reaction of diethyl azodicarboxylate to put the 1,4-diamine functionality in place, it was thought that 2-substituted butadienes would be suitable precursors to the 2-ketone moiety. 2-(Trimethylsilyloxy)buta-1,3-diene (150) seemed an ideal candidate.[63]

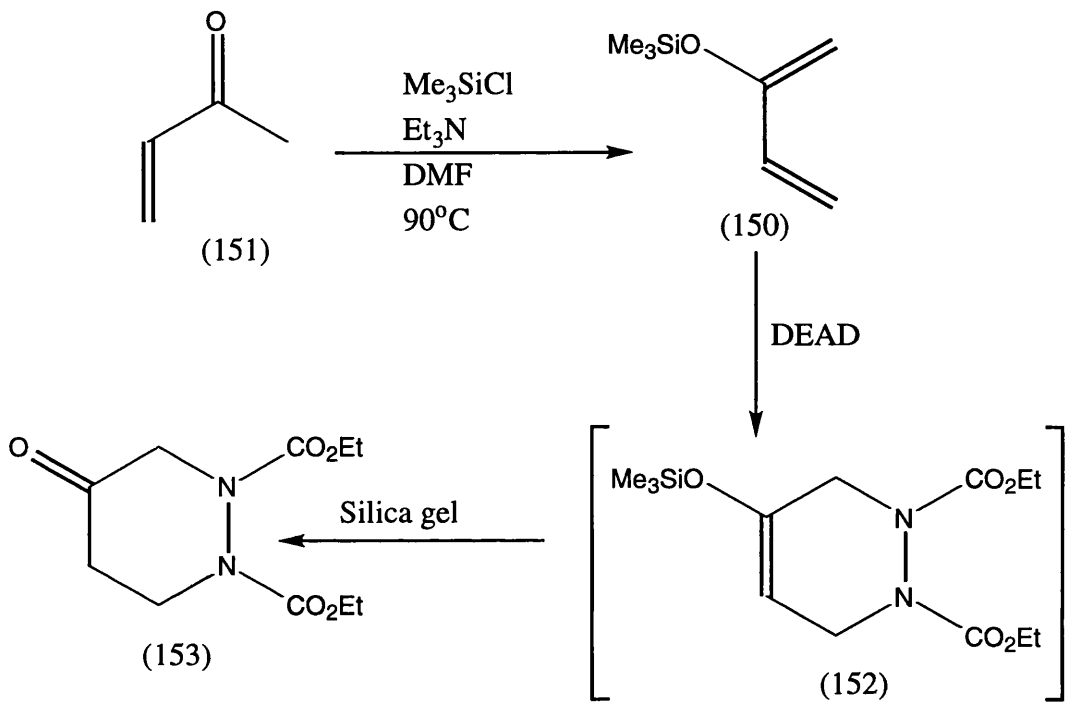
Methyl vinyl ketone (151) was reacted with chlorotrimethylsilane in the presence of triethylamine in hot DMF. The resultant 2-(trimethylsilyloxy)buta-1,3-diene (150) underwent Diels-Alder cyclisation with diethyl azodicarboxylate to give diethyl 4-(trimethylsilyloxy)-1,2,3,6-tetrahydropyridazine-1,2-dicarboxylate (152) (Scheme 49), but upon purification by flash column chromatography, it would appear that this compound was converted to the corresponding ketone (153), giving the correct molecular ion of 244 in a mass spectrum, and showing a chemical shift of 201.6 ppm for the carbonyl carbon atom in a ^{13}C NMR spectrum.

It was thought feasible to formylate the trimethylsilyl enol ether (152) regioselectively at the 5-position on the ring. However attempts to purify (152) by distillation were not successful, and the route was not pursued further.

Other approaches were made to oxygenated 1,4-diaminobutanes. It was thought that the acyloin condensation of appropriately substituted acetate esters would provide oxygenated butanes onto which heterocyclic rings could be constructed.[64] α-Aminoacetate esters would therefore give precursors to heterocyclic 1,4-diamines. Treatment of ethyl bromoacetate (154) with potassium phthalimide in DMF furnished ethyl phthalimidoacetate (155) in high yield (Scheme 50), but reaction of this compound under acyloin condensation conditions gave complex mixtures,

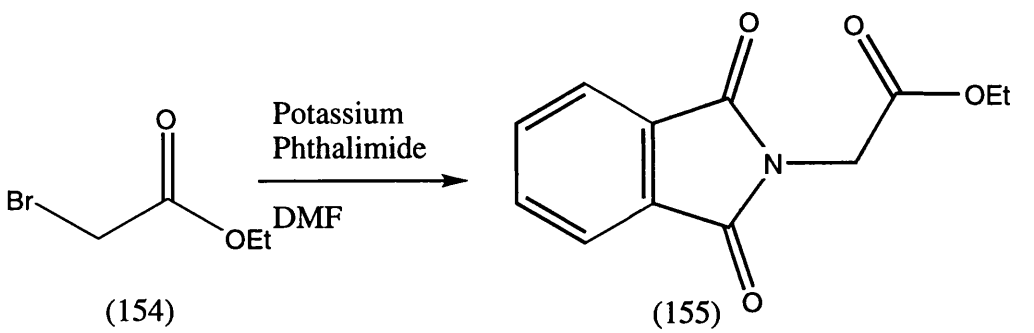
and no evidence of the desired product. It was believed that the aromatic ring systems did not survive these conditions.

Scheme 49



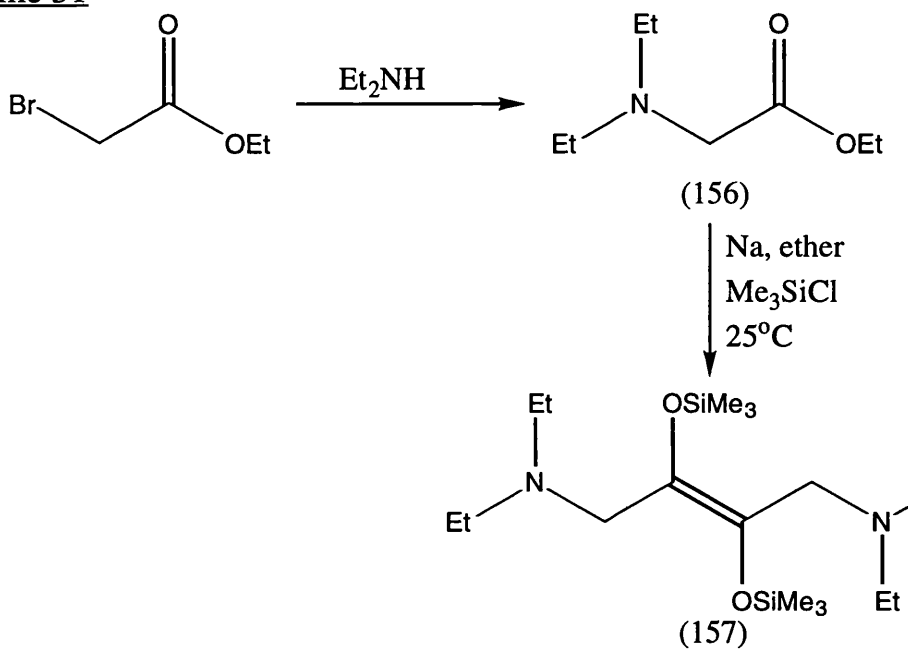
However, ethyl bromoacetate was treated with excess diethylamine to produce ethyl diethylaminoacetate (156) in quantitative yield. This product was then treated with sodium sand in toluene at reflux, under nitrogen and in the presence of chlorotrimethylsilane,[64] to give the acyloin condensation product 1,4-bis(diethylamino)-2,3-bis-(trimethylsilyoxy)but-2-ene (157) (Scheme 51), with the allylic protons appearing at 3.60 ppm in a ^{1}H NMR spectrum.

Scheme 50



(154) (155)

Scheme 51



(156)

(157)

It appeared that the enediol diether (157) was not stable as accurate microanalysis could not be obtained, and no attempts were made at further reactions.

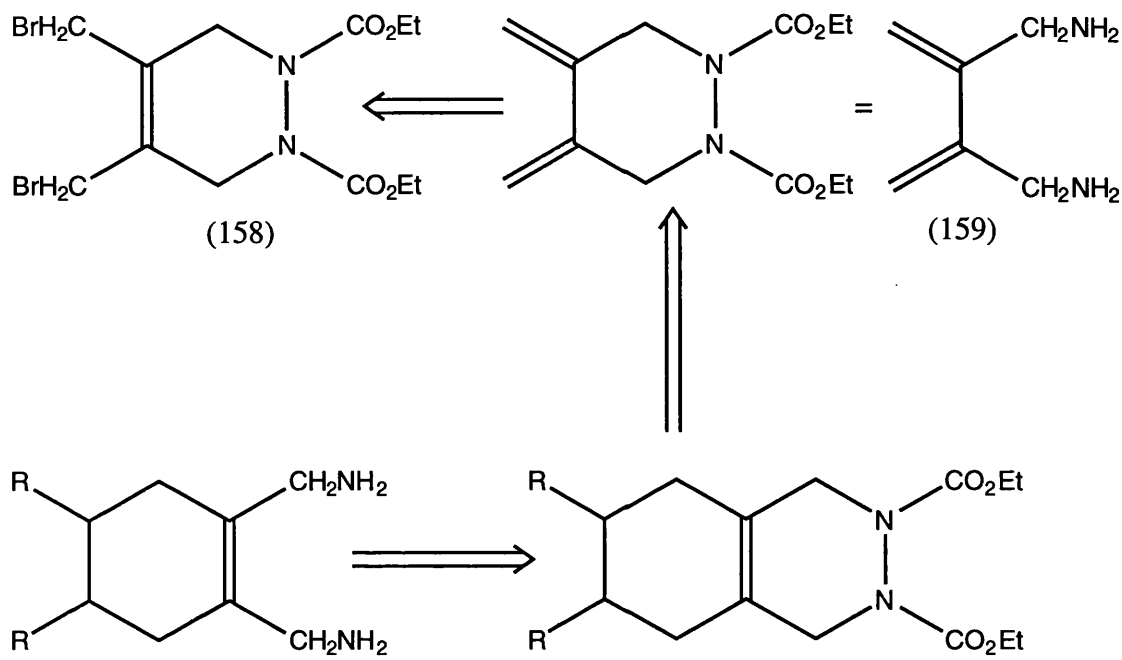
7.3 Routes to 5- and 6-Membered Ring Non-Aromatic Heterocycles

It was believed that 5- and 6-membered non-aromatic heterocyclic 1,4-diamines could be constructed from an appropriately substituted 1,2,3,6-tetrahydropyridazine compound. In particular, diethyl 4,5-bis(bromomethyl)-1,2,3,6-tetrahydropyridazine-1,2-dicarboxylate (158) was an attractive intermediate target. It was thought that this compound could act as a 2,3-bis(aminomethyl)buta-1,3-diene synthon (159) for the construction of 6-membered ring diamines from Diels-Alder reactions (Scheme 52), and also as a 2,3-bis(bromomethyl)-1,4-diaminobut-2-ene synthon (160) for the synthesis of 5-membered ring diamines from cyclisation reactions (Scheme 53).

The dimethyl diester analogue of (158) was prepared by Gaoni and Sadeh[59] from 2,3-bis(bromomethyl)-1,4-dibromobut-2-ene (161). This compound was also shown in the literature to be a precursor for heterocyclic compounds containing 3,4-dimethylene groups[65,66] and other 2,3-substituted dienes,[67] all of which were of interest to this work. Therefore, synthesis of 2,3-bis(bromomethyl)-1,4-dibromobut-2-ene[68] was undertaken.

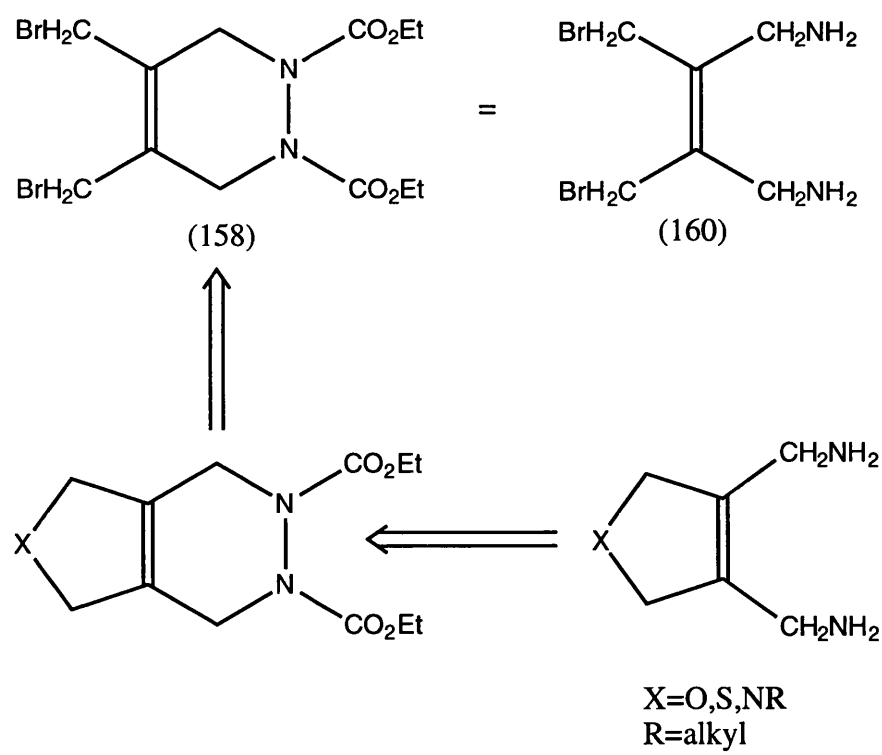
Bromine was added to a cooled solution of 2,3-dimethylbuta-1,3-diene (84) to form 1,4-dibromo-2,3-dimethylbut-2-ene (85). This compound was not isolated on this occasion, but the carbon tetrachloride solution was subjected to the action of *N*-bromosuccinimide at reflux, in the presence of a catalytic amount of dibenzoyl peroxide as a radical initiator. This allylic bromination afforded 2,3-bis(bromomethyl)-1,4-dibromobut-2-ene (161) as yellow crystals[68] (Scheme 54).

Scheme 52



R=CO_2Me, etc.

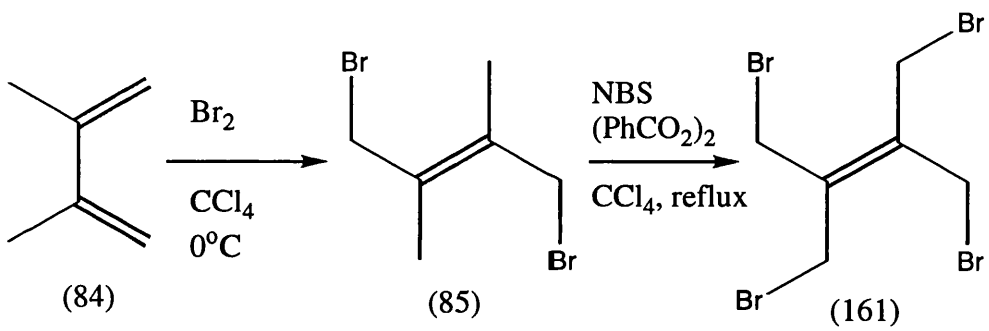
Scheme 53



X=O,S,NR
R=alkyl

Attempts to convert 2,3-bis(bromomethyl)-1,4-dibromobut-2-ene into 2,3-bis(bromomethyl)buta-1,3-diene proved troublesome. A solution of the tetrabromide in ether was treated with an activated zinc-copper couple.[59] Upon isolation of the resultant 2,3-bis(bromomethyl)buta-1,3-diene, insoluble solids were obtained, thought to be polymeric materials suggested in the literature. Reaction of a more stable form of the diene in ether solution with equivalents of both diethyl azodicarboxylate and dimethyl acetylenedicarboxylate did not give the desired product.

Scheme 54



(84) (85) (161)

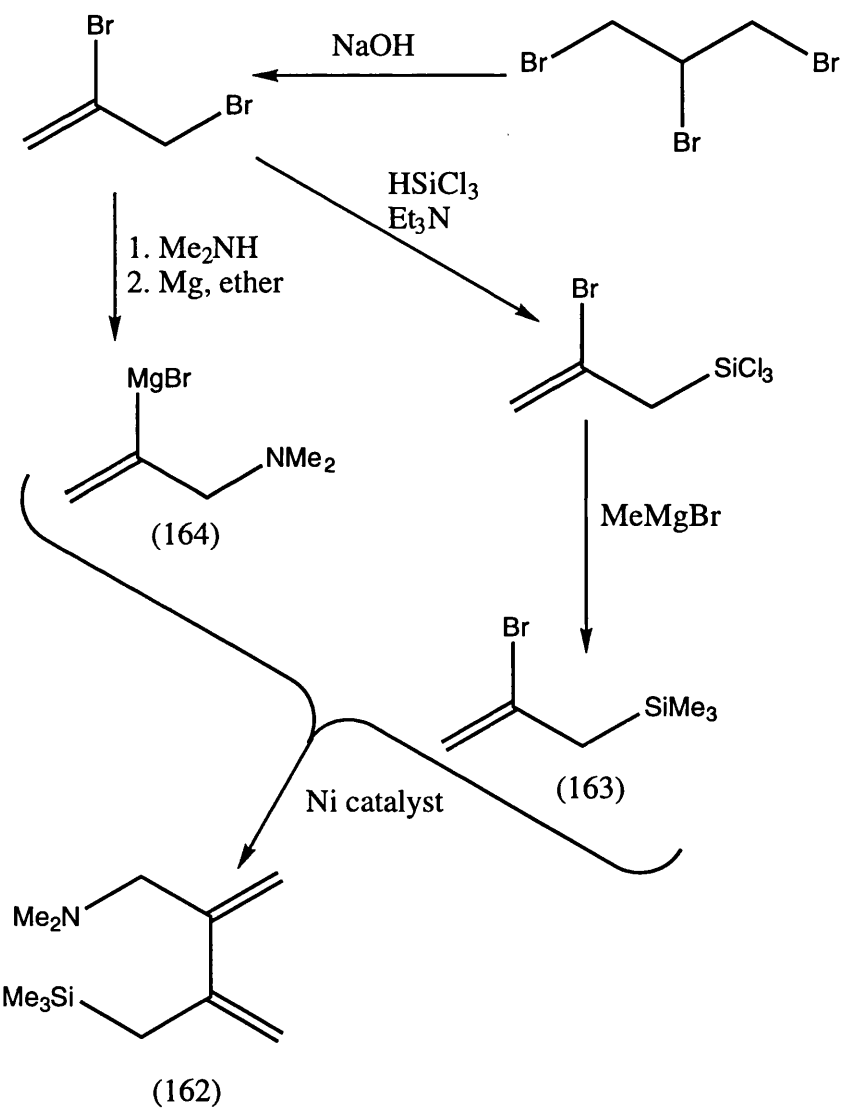
7.3.1 Recommendations for Future Work

It is possible that a milder method of preparation may give a more-easily handled form of 2,3-bis(bromomethyl)buta-1,3-diene for further reaction. Reductive elimination of iodine from a bis-iodo derivative has been suggested in the literature.[67]

An alternative approach to give dienes suitable for further conversion into heterocyclic diamines is seen in the synthesis of 2-(dimethylaminomethyl)-3-(trimethylsilylmethyl)buta-1,3-diene (162). The route of synthesis of this compound is laborious (Scheme 55).[69,70,71] However, the last stage involving a nickel catalysed coupling of a vinyl

Grignard reagent (163) and a vinyl bromide (164)[72] has been adapted to produce 2,3-bis(aminomethyl)buta-1,3-diene compounds in a more concise synthesis.[73] It is recommended that further work should be carried out on this route.

Scheme 55



Chapter 8

Summary of Antifungal Activities of Diamines Synthesised in Previous Chapters

8.1 Introduction

In this Chapter, the results of the testing of the compounds synthesised in Chapters 5 and 6 for selective fungicidal activity are summarised. The work carried out to achieve these results does not form part of this thesis, and a full account of the summary presented here is contained elsewhere.[74] These results have been published[47,49] and also form part of three patents[48,50,75]

8.2 Activities Against Powdery Mildew

8.2.1 Straight-chain Primary Diamines

E-1,4-Diaminobut-2-ene dihydrochloride (99) demonstrated widespread antifungal activity. For example, it prevented mildew infection on barley when applied as a pre-inoculation treatment. Moreover, even better curative action was shown against mildew on barley particularly when it was applied 1 or 2 days post-inoculation.

E-1,4-Diaminobut-2-ene dihydrochloride performed better than *E*-2,3-dibromo-1,4-diamino-2-butene dihydrochloride (105), which, as a 1 mM post-inoculation spray, gave little control of powdery mildew on barley. Ten days after application the average leaf area infected was 28.2 % in the treated plant against 30.7 % in the untreated control.

8.2.2 Straight-chain Secondary and Tertiary Diamines

N-Alkylated derivatives of *E*-1,4-diaminobut-2-ene dihydrochloride appeared to have increased activity against powdery mildew on barley. *E*-1,4-Bis(diethylamino)but-2-ene dihydrochloride (108) reduced the pathogen infection when applied as a 1 mM spray at a variety of times after inoculation, although the greatest control was achieved when the formulation was applied two days post-inoculation.

E-1,4-Bis(methylamino)but-2-ene dihydrochloride (111) had a similar profile, giving its best reduction in infection (70 %) when applied one day post-inoculation.

However, large variations in fungicidal capabilities were observed when these compounds were applied differently. *E*-1,4-Bis(methylamino)but-2-ene dihydrochloride (111) gave small reductions in barley mildew infection when applied as a root drench at various times prior to and after inoculation. When similarly applied, *E*-1,4-bis(diethylamino)but-2-ene dihydrochloride (108) did not reduce mildew infection on barley at all.

In addition to these findings, other *N*-alkylated derivatives such as *E*-1,4-bis(cyclohexylamino)but-2-ene dihydrochloride (112) and *E*-1,4-bis(*N*-piperidyl)but-2-ene dihydrochloride (110) gave little control of mildew on barley. *E*-1,4-Bis(dimethylamino)but-2-ene dihydrochloride (109) gave some control of powdery mildew as a post-inoculation 1 mM spray, but the effect was less than that observed with *E*-1,4-diaminobut-2-ene dihydrochloride (99).

8.2.3 Carbocyclic Compounds

Both pre- and post-inoculation applications of 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (123) reduced mildew infection, although considerably better curative action (post-inoculation) than prevention (pre-inoculation) was observed. However, when applied pre- and post-inoculation as 1 mM or 5 mM sprays against apple powdery mildew (*Podosphaera leucotricha*), both powerful curative and preventative actions were seen.

1,2-Bis(aminomethyl)cyclohexene dihydrochloride (132) and *cis*-1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (125) were not significantly active against mildew, but 1,2-bis(aminomethyl)cyclopentene dihydrochloride (143) did give significant reductions in infection on barley when applied as a post-inoculation spray at 1 mM concentration. After 6 days, there was only 1.17% mildew infection in treated plants, against 3.36% in the untreated control.

8.2.4 Combinations

When used in combination with *trans*-4,5-bis(aminomethyl)-1,2-dimethylcyclohexene dihydrochloride (124), and *E*-1,4-bis(diethylamino)-but-2-ene dihydrochloride (108), *E*-1,4-diaminobut-2-ene dihydrochloride increased the antifungal activity of the above compounds against powdery mildew on barley. However, these combinations, and others with *E*-1,4-bis(dimethylamino)but-2-ene dihydrochloride (109) and 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (123) respectively, did not outperform *E*-1,4-diaminobut-2-ene dihydrochloride (99) alone in reducing infection.

8.2.5 Comparison with Commercial Formulations

E-1,4-Diaminobut-2-ene dihydrochloride (99), *E*-1,4-bis(diethylamino)but-2-ene dihydrochloride (108) and 1,2-bis(aminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride (123), applied as 1 mM post-inoculation sprays, gave control of powdery mildew on barley which was as good as commercial standards such as flutriafol, propiconazole, tridemorph and fenpropidin. These agents were applied at 1% concentrations of the active ingredient.

8.3 Activities Against *Pyrenophora avenae*

E-1,4-Diamino-2-butene dihydrochloride (99) and *Z*-1,4-diamino-2-butene dihydrochloride (100) gave relatively higher levels of inhibition than DFMO of both soluble and bound ODC from *Pyrenophora avenae*. The results reflect the relative antifungal capabilities of these compounds against *Pyrenophora avenae*.

Against SAMDC from the same source, both *E*- and *Z*-1,4-diamino-2-butene dihydrochloride gave variable results. However, it is likely that reduced enzyme activity would be conferred *in vivo* as a result of reduced putrescine concentrations from the ODC-inhibiting activity of the synthetic diamines. DFMO increases the SAMDC activity by increasing the stability of the enzyme.

E-1,4-Bis(diethylamino)but-2-ene dihydrochloride (108) at 0.1 mM reduced cellular putrescine and spermidine concentrations in *Pyrenophora avenae*, while cadaverine and spermine concentrations were unaltered. This could explain, at least in part, the antifungal nature of this compound to *Pyrenophora avenae*.

8.4 Activities Against *Pyricularia oryzae*

Against ODC from *Pyricularia oryzae* (rice blast), none of the potential inhibitors DMFO, *E*-1,4-diamino-2-butene dihydrochloride or *Z*-1,4-diamino-2-butene dihydrochloride showed consistent reductions in enzyme activity. This is in line with their lack of antifungal activity against the rice blast pathogen.

Against SAMDC from the same source, all three compounds demonstrated an increase in activity of the soluble enzyme, while the bound enzyme gave variable results.

8.5 Activities Against *Uromyces viciae-fabae*

Both *E*-1,4-diamino-2-butene dihydrochloride and *Z*-1,4-diamino-2-butene dihydrochloride caused increases in the activity of ODC from *Uromyces viciae-fabae*, particularly in the bound enzyme, and also stimulated the activity of SAMDC, particularly soluble SAMDC. By comparison, DMFO decreased soluble ODC activity from the same source by 48 % at 1 mM concentration, and had little effect on SAMDC.

Two main possibilities exist to explain the fungicidal nature of *E*-1,4-diamino-2-butene dihydrochloride towards rusts. Either it may cause feedback inhibition by causing an accumulation of the products of one or both activities of ODC and SAMDC *in vivo*, or it replaces the respective polyamines at the active sites, without the ability to perform the same physiological functions.

E-1,4-Bis(diethylamino)but-2-ene dihydrochloride (108) gave a 61 % reduction in *Uromyces viciae-fabae* infection when applied as a 1 mM

solution spray, three days after inoculation. *E*-1,4-Bis(dimethylamino)but-2-ene dihydrochloride (109) also gave substantial reductions under similar conditions, whereas *trans*-1,2-dimethyl-4,5-bis(aminomethyl)cyclohexene dihydrochloride (124) had little effect.

8.6 Activities Against *Phytophthora infestans*

E-1,4-Bis(diethylamino)but-2-ene dihydrochloride (108) reduced the infection of potato leaves infected with *Phytophthora infestans* (blight fungus) at 1 mM concentrations, whereas at 5 mM concentration, infection was prevented.

The dihydrochlorides (124) and (109) of *trans*-4,5-bis-(aminomethyl)-1,2-dimethylcyclohexene and *E*-1,4-bis(dimethylamino)-but-2-ene gave reductions in infection by blight on potato leaves when applied as 5 mM solutions.

8.7 Field Trials

Field trials were constructed to compare the antifungal capabilities of *E*-1,4-diamino-2-butene dihydrochloride (99) and *E*-1,4-bis(diethylamino)but-2-ene dihydrochloride (108) with some commercial products, 'Folicur', 'Corbel' and 'Radar'. All compounds were applied as spray formulations against powdery mildew on the spring barley variety Golf. Applications were carried out at two strategic growth periods, and results were analysed after seven and fourteen days.

Neither *E*-1,4-diamino-2-butene dihydrochloride nor *E*-1,4-bis(diethylamino)but-2-ene dihydrochloride gave better control of

powdery mildew infection than 'Corbel'. For example, after seven days, *E*-1,4-diamino-2-butene dihydrochloride had reduced mildew infection by 14%, with respect to an untreated control; after fourteen days, the reduction of infection had risen to 34% against the control. Over the same periods, *E*-1,4-bis(diethylamino)but-2-ene dihydrochloride had reduced the infection by 23% and 36% respectively. However, 'Corbel' gave reductions of 52% and 79% respectively. It should be noted, though, that the applications were late due to bad weather, and were therefore essentially post-inoculation.

In terms of the harvest, *E*-1,4-diamino-2-butene dihydrochloride outperformed the other antifungal agents, giving greater plant height, weight and grain yield. Formulated *E*-1,4-diamino-2-butene dihydrochloride reduced mildew infection under glasshouse conditions to a greater extent than the non-formulated equivalent, and also proved more effective than the commercial products 'Folicur' and 'Corbel'.

General Experimental

All melting points were measured with a Gallenkamp Melting Point Apparatus and are uncorrected. Infrared spectra were obtained on a Phillips analytical PU9800 FTIR spectrometer. Nuclear magnetic resonance spectra were recorded with a Perkin Elmer R32 spectrometer operating at 90 MHz (δ_H) or with a Bruker WP-200 SY operating at 200 MHz (δ_H) or 50 MHz (δ_C). Low resolution mass spectra were determined with a VG updated A.E.L. MS spectrometer and high resolution mass spectra were determined with a VG updated MS 902 spectrometer.

Analytical TLC was carried out on Kieselgel 60 F_{254} plastic sheets of 0.25 mm thickness. Spots were viewed under a UV lamp and were developed by iodine vapour. Column chromatography was carried out using 70-230 mesh silica gel.

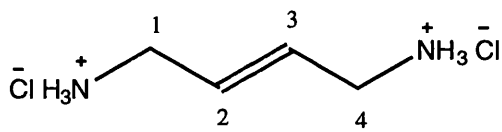
All solvents and reagents were of analytical grade unless otherwise stated. Organic solvents were dried with magnesium sulphate and evaporated on a Buchi rotary evaporator under water pump vacuum with slight heating. Dichloromethane was distilled from calcium hydride; ethanol was distilled from magnesium turnings and iodine; triethylamine was distilled from potassium hydroxide; tetrahydrofuran was distilled from sodium pieces and benzophenone; dimethylformamide was distilled from silica gel; and acetonitrile was dried with calcium hydride.

Chapter 9

Experimental To Chapter 5

E-1,4-DIAMINOBUT-2-ENE DIHYDROCHLORIDE

METHOD A



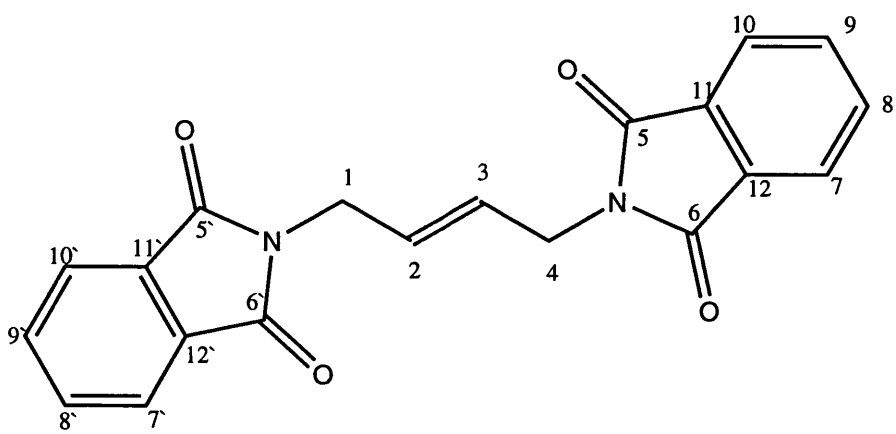
Prepared following the procedure of Koziara *et al.*[35]

To a solution of *E*-1,4-dibromo-2-butene (2.14 g, 10 mmol) in benzene (20 ml) was added sodium azide (1.30 g, 20 mmol) and tetrabutylammonium bromide (322 mg, 1 mmol) with vigorous stirring. The suspension was heated to reflux for 6h, during which time it turned dark red. The resultant mixture was allowed to cool to room temperature, filtered and the solids were washed with benzene (2 x 10 ml). The filtrate was washed with water (2 x 10 ml), and the organic layer was separated, dried ($MgSO_4$) and filtered. Triethylphosphite (1.66 g, 10 mmol) was then added to the benzene solution dropwise with stirring at 0-10 °C, with concurrent evolution of nitrogen. The solution was stirred at room temperature for 18 h. Hydrogen chloride gas was passed through the solution at ambient temperature until saturation (2 h), and the resultant suspension was allowed to stand overnight. The solvents were then

removed *in vacuo,* ether was added to the residue and the mixture refridgerated for 24 h. The precipitate was filtered and washed with ether to give *E*-1,4-diamino-2-butene dihydrochloride in 68% yield, m.p.159-161°C (dec.). δ_H (200 MHz, D_2O) 5.77 (m, 2H, H-2, H-3), 3.47 (m, 4H, H-1, H-4); δ_C (50 MHz, D_2O) 128.7 (C-2, C-3), 47.1 (C-1, C-4); ν_{max}(KBr disc) 3440, 3070, 3004, 2330, 2280, 2240, 1610, 1587, 1451, 1398, 1370, 1335, 1153, 1130, 1099, 987, 929, 880 and 519 cm^{-1}; *m/z* 86 (M^+-2HCl, 12%), 71, 70, 69, 68, 59, 58, 57, 56, 44, 43, 42, 41, 39, 38, 37, 36 (100%); found: C 31.31%, H 7.96%, N 17.10%; calc. for $C_4H_{12}N_2Cl_2$: C 30.21%, H 7.60%, N 17.61%

METHOD B

E-1,4-DIPHTHALIMIDOBUT-2-ENE

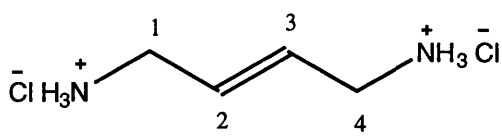


Prepared following the procedure of Macholan *et al.*[76]

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 DMF (100 ml) at room temperature. Stirring was continued for

a further 72 h at this temperature and the suspension was then poured into water (100 ml). The mixture was then extracted with dichloromethane (5 x 100 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo*. What remained consisted of residual DMF (*ca.* 30 ml), and a white solid, which was filtered off and washed with ether (3 x 10 ml) to give *E*-1,4-diphthalimidobut-2-ene (15.75 g, 91%), m.p. 227-229°C (lit.[84] 228-230 °C). δ_H (200 MHz, $CDCl_3$) 7.83 (m, 4H, H-7, H-10), 7.70 (m, 4H, H-8, H-9), 5.79 (m, 2H, H-2, H-3), 4.26 (m, 4H, H-1, H-4); δ_C (50 MHz, $CDCl_3$) 167.7 (C-5, C-6), 133.9 (C-8, C-9), 132.0 (C-11, C-12), 127.2 (C-2, C-3), 123.3 (C-7, C-10), 38.7 (C-1, C-4); ν_{max}(KBr disc) 3463, 2930, 1765, 1716, 1709, 1705, 1701, 1612, 1597, 1462, 1429, 1394, 1360, 1340, 1323, 1284, 1269, 1184, 1149, 1095, 1084, 1064, 997, 937, 729, 711, and 532 cm^{-1}; *m/z* 199 (M^+-$C_6H_4(CO)_2NH$, 5%), 186, 160, 133, 130, 114, 105, 104, 103, 102, 90, 77, 76 (100%), 75, 51, 50, 39; found: C 69.10%, H 4.12%, N 8.03%; calc. for $C_{20}H_{14}N_2O_4$: C 69.36%, H 4.05%, N 8.09%.

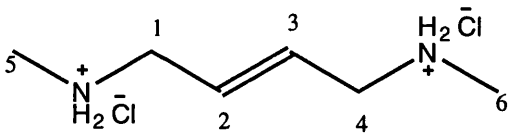
E-1,4-DIAMINOBUT-2-ENE (E-BED) DIHYDROCHLORIDE



E-1,4-Diphthalimidobut-2-ene (15.72 g, 45 mmol) was suspended in glacial acetic acid (160 ml), and concentrated hydrochloric acid (160 ml) was added. The mixture was heated to reflux until all of the *E*-1,4-diphthalimido-2-butene had dissolved, and then heating was continued for

a further 24 h. The solution was allowed to cool to room temperature, and precipitated phthalic acid was removed by filtration. The filtrate was concentrated *in vacuo* to *ca.* 10 ml, upon which a further amount of phthalic acid precipitated. The solid was again removed by filtration, and the filtrate was treated with acetone (*ca.* 15 ml). The white precipitate was collected and washed with ether (2 x 10 ml) to afford *E*-1,4-diamino-2-butene dihydrochloride (6.48 g, 90%). δ_H (200 MHz, D_2O) 5.77 (m, 2H, H-2, H-3), 3.47 (m, 4H, H-1, H-4); δ_C (50 MHz, D_2O) 128.7 (C-2, C-3), 47.1 (C-1, C-4); ν_{max}(KBr disc) 3440, 3070, 3004, 2330, 2280, 2240, 1610, 1587, 1451, 1398, 1370, 1335, 1153, 1130, 1099, 987, 929, 880 and 519 cm^{-1}; *m/z* 86 (M^+-2HCl, 10%), 71, 70, 69, 68, 59, 58, 57, 56, 44, 43, 42, 41, 39, 38, 37, 36 (100%); found: C 31.11%, H 7.98%, N 17.04%; calc. for $C_4H_{12}N_2Cl_2$: C 30.21%, H 7.60%, N 17.61%.

<u>*E*-1,4-BIS(METHYLAMINO)BUT-2-ENE DIHYDROCHLORIDE</u>

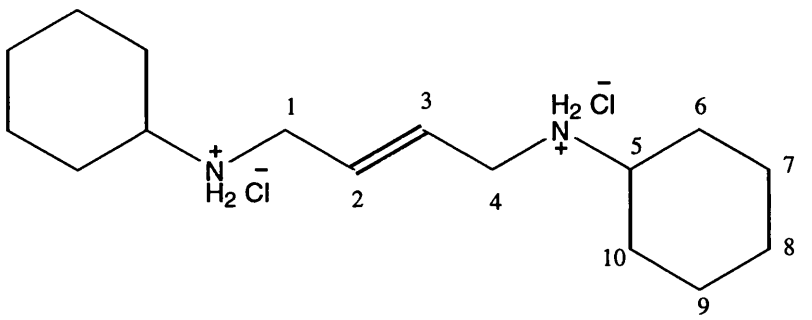


Prepared following the procedure of Roberts and Ross.[41]

E-1,4-Dibromobut-2-ene (2.14 g, 10 mmol) in benzene (50 ml) was added dropwise to a stirred solution of methylamine (30% w/v in industrial methylated spirits, 21 ml, 200 mmol) in benzene (50 ml) at room temperature. The resulting solution was stirred for 24 h at room temperature, then chloroform (100 ml) was added and the solution was

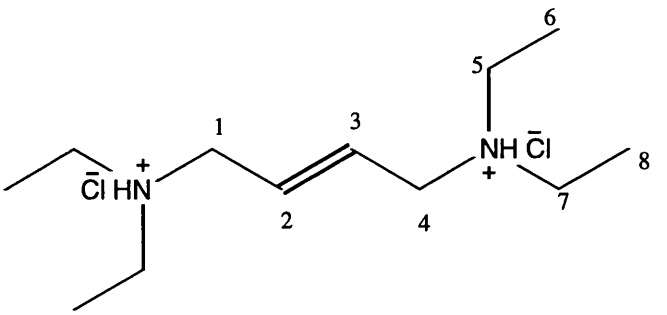
washed with water (3 x 100 ml). The organic layer was separated and concentrated *in vacuo* to leave an oily residue which was partitioned between chloroform (50 ml) and hydrochloric acid (2 M, 50 ml). The aqueous layer was decanted, washed with chloroform (2 x 25 ml) and concentrated to dryness *in vacuo* to afford an off-white solid. Absolute alcohol/water (95:5, *ca* 10 ml) was added to the residue, and the suspension was heated to reflux. The hot solution was subjected to hot filtration to remove some polymeric material. The filtrate was concentrated *in vacuo* to give crude *E*-1,4-bis(methylamino)but-2-ene dihydrochloride, which was recrystallised from ethanol/acetone (2.26 g, 42%), m.p. 195°C (dec.). δ_H (200 MHz, D_2O) 5.95 (m, 2H, H-2, H-3), 3.68 (m, 4H, H-1, H-4), 2.63 (s, 6H, H-5, H-6); δ_C (50 MHz, D_2O) 127.5 (C-2, C-3), 50.2 (C-1, C-4), 33.2 (C-5, C-6); ν_{max}(KBr disc) 3590, 3567, 3432, 3007, 2967, 2942, 2701, 2631, 2483, 2396, 2348, 1655, 1647, 1638, 1630, 1624, 1618, 1612, 1468, 1437, 1425, 1398, 1076, 980 and 924 cm^{-1}; *m/z* 96, 94, 84 (M^+-2HCl, -NHMe, 2%), 83, 82, 70, 68, 57, 55, 44, 42 (100%), 41, 38, 36, 35, 30, 28; found: C 38.62%, H 8.57%, N 15.03%; calc. for $C_6H_{16}N_2Cl_2$: C 38.51%, H 8.62%, N 14.97%.

E-1,4-BIS(CYCLOHEXYLAMINO)BUT-2-ENE DIHYDROCHLORIDE



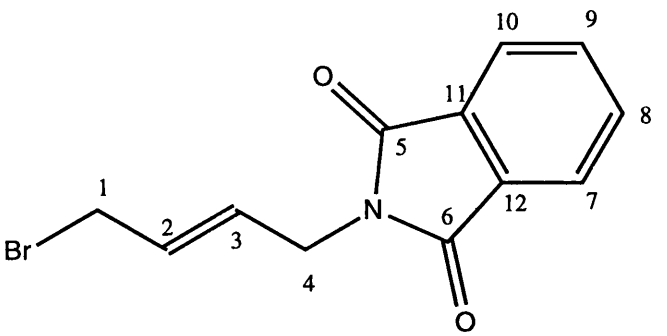
To a stirred solution of cyclohexylamine (8.91 g, 90 mmol) in chloroform (25 ml) was added *E*-1,4-dibromobut-2-ene (2.14 g, 10 mmol) in chloroform (75 ml) dropwise at room temperature, and the solution was stirred for 24 h. The solution was washed with water (3 x 40 ml), dried ($MgSO_4$) and filtered. The solvent was removed from the filtrate *in vacuo* and the residue was dissolved in hydrochloric acid (2 M, 100 ml).The solvents were removed *in vacuo* to give a white solid, which was recrystallised from methanol to yield the title compound (2.31 g 56%), m.p.>250°C. δ_H (200 MHz, D_2O) 5.90 (m, 2H, H-2, H-3), 3.62 (m, 4H, H-1, H-4), 3.00 (m, 2H, H-5), 0.70-2.11 (m, 20H, H-6-H-10). δ_C (50 MHz, D_2O) 129.3 (C-2, C-3), 57.6 (C-5), 45.9 (C-1, C-4), 29.7 (C-6, C-10), 25.4 (C-7, C-9), 24.8 (C-8); ν_{max}(KBr disc) 3450, 2940, 2850, 2810, 2705, 2530, 2500, 1640, 1635, 1455, 990, 970 and 910 cm^{-1}; *m/z* 207, 152, 151 (M+-2HBr,-$C_6H_{11}NH$, 4.5%), 138, 122, 108, 94, 82, 70. found C 46.90%, H 7.66%, N 6.72%; calc. for $C_{16}H_{32}N_2Br_2$ C 46.62%, H 7.82%, N 6.80%.

E-1,4-BIS(DIETHYLAMINO)BUT-2-ENE DIHYDROCHLORIDE



Diethylamine (14.6 g, 200 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) over 30 min at room temperature, and the solution was stirred for 4 h. The white precipitate formed was filtered off and the solvent removed from the filtrate *in vacuo*. The residue was dissolved in hydrochloric acid (2 M, 100 ml) and the solvent removed *in vacuo*. The solid residue was dissolved in hot aqueous ethanol. The solution was allowed to cool and acetone was added. The precipitate was filtered and washed with acetone to yield *E*-1,4-bis(diethylamino)amino-2-butene dihydrochloride (2.16 g, 60%), m.p.>250°C. δ_H (200 MHz, D_2O) 6.03 (m, 2H, H-2, H-3), 3.74 (d, 4H, J=8.6 Hz, H-1, H-4), 3.08 (q, 8H, J=7 Hz, H-5, H-7), and 1.15 (t, 12H, J=7 Hz, H-6, H-8). δ_C (50 MHz, D_2O) 129.6 (C-2, C-3), 53.2 (C-5, C-7), 48.3 (C-1, C-4), 9.2 (C-6, C-8); ν_{max}(KBr disc) 3590, 3569, 3499, 3428, 2974, 2934, 2731, 2644, 2494, 2348, 1655, 1630, 1624, 1618, 1612, 1560, 1508, 1468, 1389, 1363, 1173, 1130, 1109, 1072, 1043, 1026, 991 and 808 cm^{-1}; *m/z* 126 (M^+-2HCl, -NEt_2, 16%), 125, 112, 110, 96, 86, 72, 58, 56, 55, 42, 41, 36, 35, 30, 29 (100%), 28, 27; found: C 53.26%, H 10.33%, N 10.17%; calc. for $C_{12}H_{28}N_2Cl_2$: C 53.13%, H 10.40%. N 10.37%.

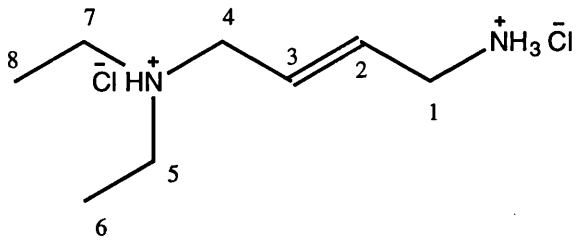
E-1-BROMO-4-PHTHALIMIDOBUT-2-ENE



Prepared following the procedure of Robins[77].

Potassium phthalimide (18.5 g, 0.1 mol) was added in portions over 5 h to a stirred solution of *E*-1,4-dibromobut-2-ene (21.4 g, 0.1 mol) in acetone (200 ml) heated at reflux. The suspension was stirred for 24 h at reflux, then cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* to afford a white solid, which was recrystallized three times from acetone to yield *E*-1-bromo-4-phthalimidobut-2-ene (15.76 g, 56.2%), m.p.210-214°C. δ_H (200 MHz, $CDCl_3$) 7.78 (m, 4H, H-7-H-10), 5.86 (m, 2H, H-2, H-3), 4.29 (d, 2H, J=5.6Hz, H-1), 3.90 (d, 2H, J=7Hz, H-4); δ_C (50 MHz, $CDCl_3$) 167.7 (C-5, C-6), 134.1 (C-8, C-9), 132.0 (C-11, C-12), 129.9 (C-3), 128.3 (C-2), 123.4 (C-7, C-10), 38.6 (C-4), 31.3 (C-1); ν_{max}(KBr disc) 3459, 1769, 1717, 1709, 1686, 1466, 1422, 1393, 1368, 1323, 1206, 1115, 955, 735, 721, 712, 577 and 529 cm^{-1}; *m/z* 200 (100%, M^+-Br), 182, 160, 154, 133, 130, 127, 105, 104, 102, 77, 76, 75, 74, 54, 53, 51, 50, 41, 39, 28, 27; found: C 51.54%, H 3.66%, N 5.12%; calc. for $C_{12}H_{10}NO_2Br$: C 51.43%, H 3.57%, N 5.00%.

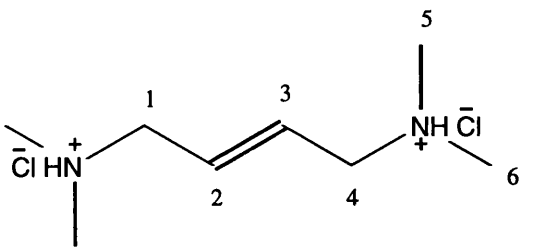
E-1-AMINO-4-DIETHYLAMINOBUT-2-ENE DIHYDROCHLORIDE



Prepared following the procedure of Samejima *et al*[51].

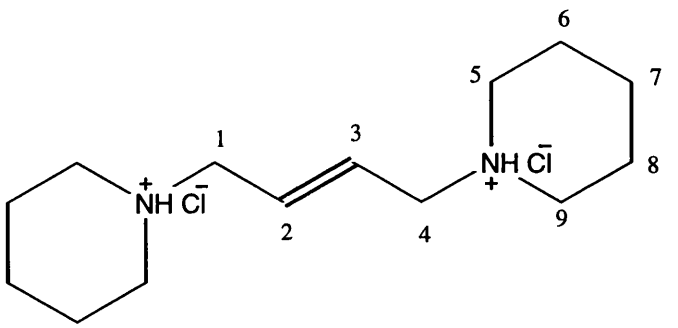
E-1-Bromo-4-phthalimidobut-2-ene (4.2 g, 15 mmol), diethylamine (1.1 g, 15 mmol) and potassium fluoride supported on Celite (7.5 g) were stirred together in acetonitrile (75 ml) at 40 °C for 18 h. The solution was filtered, and the filtrate was concentrated *in vacuo* to afford an oil, which was dissolved in glacial acetic acid (30 ml) and concentrated hydrochloric acid (30 ml). The mixture was heated at reflux for 30 h. The solution was cooled, filtered, and the solvents were removed *in vacuo* to afford *E*-1-amino-4-diethylaminobut-2-ene dihydrochloride as a solid (1.32 g, 41%), m.p. 231-234°C. δ_H (200 MHz, D_2O) 5.95 (m, 2H, H-2, H-3), 3.65 (m, 4H, H-1, H-4), 3.14 (q, 4H, J=7Hz, H-5, H-7), 1.17 (t, 6H, J=7Hz, H-6, H-8); δ_C (50 MHz, D_2O) 129.7 (C-2), 129.0 (C-3), 53.6 (C-5, C-7), 49.5 (C-1), 42.3 (C-4), 9.4 (C-6, C-8); ν_{max}(KBr disc) 3579, 3456, 3008, 2970, 2725, 2350, 2331, 2275, 1664, 1623, 1618, 1612, 1584, 1455, 1388, 1367, 1130, 1101, 989 and 840 cm^{-1}; *m/z* 142 (M^+-2HCl, 9.5%), 125 (100%), 96, 87, 86, 70, 69, 68, 30, 29, 28; found: C 44.79%, H 9.12%, N 12.88%; calc. for $C_8H_{20}N_2Cl_2$: C 44.66%, H 9.37%, N 13.02%.

E-1,4-BIS(DIMETHYLAMINO)BUT-2-ENE DIHYDROCHLORIDE



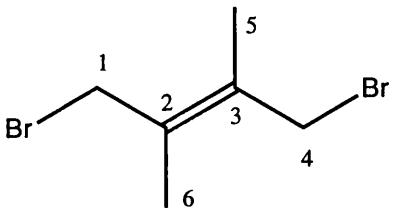
To a stirred solution of dimethylamine (40% solution in water, 33 ml, 300 mmol) was added *E*-1,4-dibromobut-2-ene (2.14 g, 10 mmol) in chloroform (70 ml) dropwise at room temperature, and the solution was stirred for 4 h. The solvents were removed *in vacuo* to give a white solid, which was recrystallised from methanol to yield the title compound, (2.52 g, 83%); δ_H (200 MHz, D_2O) 5.99 (m, 2H, H-2, H-3), 3.69 (m, 4H, H-1, H-4), 2.70 (s, 12H, H-5, H-6); δ_C (50 MHz, D_2O) 130.2 (C-2, C-3), 58.9 (C-5, C-6), 43.2 (C-1, C-4); ν_{max}(KBr disc) 3439, 3017, 2961, 2791, 2666, 2633, 2606, 2585, 2506, 2477, 1479, 1466, 1427, 1404, 1389, 1383, 1327, 1267, 1169, 1140, 1020, 1001, 960 and 949 cm^{-1}; *m/z* 97 (M^+-2HCl,-Me_2NH, 22%). 82, 68, 58 (100%), 53, 45, 44, 42, 36, 35, 30, 28, 15; found: C 44.63%, H 9.12%, N 13.17%; calc. for $C_8H_{20}N_2Cl_2$: C 44.65%, H 9.30%, N 13.02%.

E-1,4-BIS(*N*-PIPERIDYL)BUT-2-ENE DIHYDROCHLORIDE



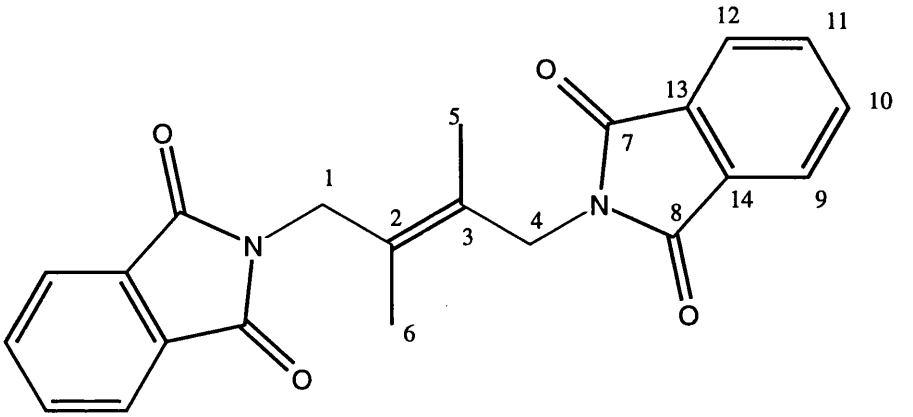
To a stirred solution of piperidine (8.16 g, 100 mmol) in chloroform (30 ml) was added *E*-1,4-dibromobut-2-ene (2.14 g, 10 mmol) in chloroform (70 ml) dropwise at room temperature, and stirring was continued for another 24 h. The solution was then washed with water (3 x 25 ml), and the organic layer was decanted, dried ($MgSO_4$) and filtered. The solvents were removed from the filtrate *in vacuo* to give an oil. The residue was taken up in ether (25 ml), and to the resultant solution was added ether saturated with hydrogen chloride gas (20 ml), slowly and with cooling. A precipitate formed immediately and was filtered. Recrystallisation from methanol (5 ml) yielded the title compound. (2.30 g, 60%), m.p. 198-200°C; δ_H (200 MHz, D_2O) 5.93 (m, 2H, H-2, H-3), 3.62 (m, 4H, H-1, H-4), 3.24 (m, 4H, H-5, H-9), 2.80 (m, 4H, H-5, H-9), 1.64 (bs,12H, H-6-H-8); δ_C (50 MHz, D_2O) 129.8 (C-2, C-3), 58.2 (C-1, C-4), 53.7 (C-5, C-9), 23.7 (C-6, C-8), 21.8 (C-7); ν_{max}(KBr disc) 3433, 2980, 2943, 2860, 2687, 2619, 2583, 2488, 2421, 2401, 2345, 2288, 1462, 1444, 1429, 1404, 1381, 1151, 1078, 1066, 982, 957, 949 and 590 cm^{-1}; *m/z* 137 (M+,-2HCl,-$C_6H_{10}NH$, 64%), 122, 98, 85 (100%), 65; found: C 56.76%, H 9.45%, N 9.21%; calc. for $C_{14}H_{28}N_2Cl_2$: C 56.95%, H 9.49%, N 9.49%.

E-1,4-DIBROMO-2,3-DIMETHYLBUT-2-ENE



To a stirred solution of 2,3-dimethyl-1,3-butadiene (20.05 g, 0.25 mol) in chloroform (100 ml), cooled to 0 °C, was added bromine (40.0 g, 0.25 mol) in chloroform (75 ml) dropwise over 5 h. The resultant colourless solution was stirred for a further 2 h at 0 °C, after which the solvent was removed *in vacuo* to leave a liquid residue which rapidly crystallised. The solid was recrystallised twice from petroleum ether (40-60 °C), and filtered to yield *E*-1,4-dibromo-2,3-dimethylbut-2-ene (48.2 g, 79.7%), m.p. 45-47°C (lit.[52] 47°C); δ_H (200 MHz, $CDCl_3$) 3.99 (s, 4H, H-1, H-4), 1.87 (s, 6H, H-5, H-6); δ_C (50 MHz, $CDCl_3$) 131.7 (C-2, C-3), 34.1 (C-1, C-4), 14.0 (C-5, C-6); ν_{max}(KBr disc) 3441, 2978, 2920, 2863, 1445, 1377, 1206, 1167, 1109, 1055, 922, 854, 640, 617 and 598 cm^{-1}; *m/z* 244, 242 (M^+, 18%), 240, 163, 161, 82, 81, 79, 67, 53, 41, 40, 39 (100%).

E-2,3-DIMETHYL-1,4-DIPHTHALIMIDOBUT-2-ENE

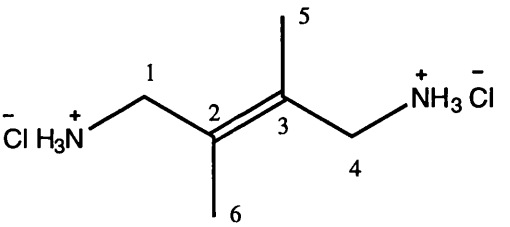


Prepared following the procedure of Macholan.[76]

Potassium phthalimide (20 g, 108 mmol) was added in portions over 2 h to a stirred solution of *E*-1,4-dibromo-2,3-dimethylbut-2-ene (12.1 g, 50 mmol) in 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 (5 x 100 ml). The organic extracts were dried, filtered and concentrated *in vacuo* to leave DMF (*ca.* 30 ml), and a white solid, which was filtered off and washed with ether (3 x 10 ml) to give *E*-2,3-dimethyl-1,4-diphthalimidobut-2-ene (17.02 g, 91%); δ_H (200 MHz, $CDCl_3$) 7.71 (m, 8H, H-9-H-12), 4.28 (s, 4H, H-1, H-4), 1.66 (s, 6H, H-5, H-6); δ_C (50 MHz, $CDCl_3$) 168.4 (C-7, C-8), 133.9 (C-13, C-14), 132.0 (C-10, C-11), 128.0 (C-2, C-3), 123.2 (C-9, C-12), 40.7 (C-1, C-4), 16.1 (C-5, C-6); ν_{max}(KBr disc) 3456, 1769, 1713, 1466, 1428, 1397, 1341, 1335, 1165, 1071, 945, 727, 710, 642 and 530 cm^{-1}; *m/z* 374 (M^+, 52%), 227, 214 (100%), 212, 184, 160, 133, 130, 105, 104, 77, 75, 50, 41; found: C

53.45%, H 3.97%, N 5.94%; calc. for $C_{22}H_{18}O_4N_2$: C 70.59%, H 4.81%, N 7.49.

E-1,4-DIAMINO-2,3-DIMETHYLBUT-2-ENE DIHYDROCHLORIDE



E-2,3-Dimethyl-1,4-diphthalimidobut-2-ene (16.83 g, 45 mmol) was suspended in glacial acetic acid (160 ml), and concentrated hydrochloric acid (160 ml) was added. The mixture was heated to reflux until all of the *E*-2,3-dimethyl-1,4-diphthalimidobut-2-ene had dissolved, and continued for a further 24 h. The solution was allowed to cool to room temperature, and precipitated phthalic acid was removed by filtration. The filtrate was concentrated *in vacuo* to *ca.* 10 ml, upon which a precipitate formed. The solid was collected and washed with ether to afford *E*-1,4-diamino-2,3-dimethyl-2-butene dihydrochloride (7.57 g, 90%); δ_H (200 MHz, D_2O) 3.60 (s, 4H, H-1, H-4), 1.75 (s, 6H, H-5, H-6); δ_C (50 MHz, D_2O) 129.6 (C-2, C-3), 42.3 (C-1, C-4), 16.8 (C-5, C-6); ν_{max}(KBr disc) 3569, 3439, 3214, 3009, 2969, 2897, 2700, 2614, 2044, 2017, 1638, 1618, 1603, 1512, 1481, 1391, 1381, 1170, 1113, 972, 544 and 438 cm^{-1}; *m/z* 97 (M^+-2HCl, -NH_3, 3%), 84, 82, 67, 57, 56, 43, 42, 41, 36, 30 (100%), 28, 18; found: C 20.55%, H 4.27%, N 7.37%; calc. for $C_6H_{16}N_2Cl_2$: C 38.09%, H 8.46%, N 14.81%.

HYDRAZOIC ACID

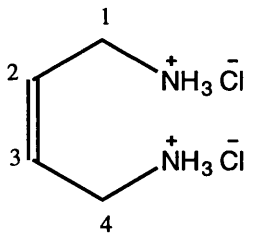
Sodium azide (32.5 g) and water (32.5 ml) were mixed to a thick paste with vigorous stirring. Benzene (100 ml) was added in one portion, and the resultant slurry cooled to 0-5 °C. To the cooled mixture was added sulphuric acid (98% w/w, 13.6 ml) slowly dropwise, while maintaining the temperature of the slurry at less than 10 °C. The slurry was stirred for a further 1 hour at 0-5 °C, filtered, and the filtrate dried (Na_2SO_4) and filtered. The resultant benzene solution of hydrazoic acid was sealed and could be stored for 1-2 days at 0 °C. The molarity of the solution was determined by titration before each use.

TITRATION

The hydrazoic acid solution (1.0 ml) and water (1.0 ml) were mixed together with phenolphthalein (10-20 mg). The mixture was titrated against a dropwise addition of sodium hydroxide solution (1.0 M) until a deep blue colour persisted.

Solutions of hydrazoic acid prepared and titrated this way had molarities typically *ca.* 1.0 M

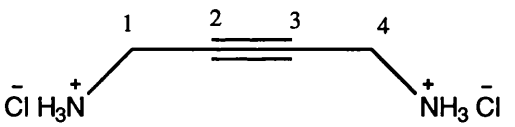
Z-1,4-DIAMINOBUT-2-ENE DIHYDROCHLORIDE



To a stirred solution of *Z*-but-2-ene-1,4-diol (1.41 g, 16 mmol) in dry THF (20 ml) under a nitrogen atmosphere was added a solution of hydrazoic acid in benzene (1.1 M, 30 ml, 33 mmol). To this was added a solution of diisopropyl azodicarboxylate (7.0 g, 35 mmol) in dry THF (10 ml) with stirring. Triphenylphosphine (18.4 g, 70 mmol) in dry THF (60 ml) was added dropwise to the mixture, maintaining the reaction temperature below 40 °C by cooling with an ice-bath. The reaction mixture was stirred for 1 h at room temperature, then heated to 50 °C for 3 h. Water (2 ml) was then added via syringe, and the solution was stirred at 50 °C for a further 3 h. The reaction was allowed to cool to room temperature, and the solvents were removed *in vacuo*. The residual solids were partitioned between hydrochloric acid (1 M, 100 ml) and dichloromethane (100 ml). The aqueous layer was further washed with dichloromethane (2 x 80 ml), then evaporated to dryness *in vacuo* to give a light brown solid. Recrystallisation from aqueous ethanol/acetone gave the title compound as an off-white powder in 63% yield, m.p.185-187°C (dec.). δ_H (200 MHz, D_2O) 5.73 (m, 2H, H-2, H-3), 3.59 (d, 4H, J=8.2Hz, H-1, H-4); δ_C (50 MHz, D_2O) 127.7 (C-2, C-3), 36.9 (C-1, C-4); ν_{max}(KBr disc) 3432, 3027, 2866, 2718, 2664, 2585, 2484, 2042, 1608, 1589, 1485, 1470, 1456, 1420, 1346, 1271, 1134, 1105, 1096, 903,

893 and 758 cm^{-1}; *m/z* 69 (100%, M^+-NH_3), 68, 56, 54, 43, 42, 41, 39, 38, 36, 35, 30, 28; found: C 30.32%, H 7.61%, N 17.76%; calc. for $C_4H_{12}N_2Cl_2$: C 30.19%, H 7.55%, N 17.61%.

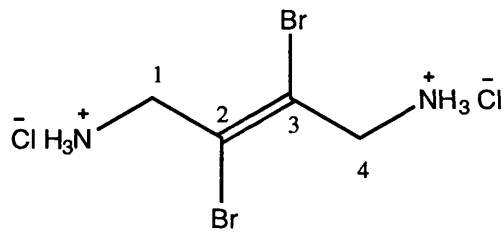
1,4-DIAMINOBUT-2-YNE DIHYDROCHLORIDE



To a solution of but-2-yne-1,4-diol (1.29 g, 15 mmol) in dry THF (20 ml) under a nitrogen atmosphere was added hydrazoic acid (1.0 M in benzene, 30 ml, 30 mmol) with stirring. A solution of diisopropyl azodicarboxylate (6.78 g, 33.9 mmol) in dry THF (10 ml) was then introduced. To this mixture was added triphenyl-phosphine (17.8 g, 67.8 mmol) in dry THF (60 ml) dropwise, maintaining the reaction temperature below 40 °C by cooling. The reaction mixture was stirred for 1 h at room temperature, then heated to 50 °C for 3 h. Water (2 ml) was added, and the solution stirred at 50 °C for a further 3 h. The solvents were removed *in vacuo* and the residue was partitioned between hydrochloric acid (1 M, 80 ml) and dichloromethane (80 ml). The aqueous layer was further washed with dichloromethane (2 x 80 ml), then evaporated to dryness *in vacuo* to give a light brown solid. Recrystallisation from aqueous ethanol/acetone gave the title compound in 71% yield, m.p. 235-237 °C. δ_H (200 MHz, D_2O) 3.80 (s, 4H, H-1, H-4); δ_C (50 MHz, D_2O) 79.2 (C-2, C-3), 29.9 (C-1, C-4); ν_{max}(KBr disc) 3630, 3621, 3440, 2982, 2926, 1665, 1655, 1647, 1638, 1624, 1618,

1613, 1579, 1560, 1508, 1500, 1371, 1171, 1078 and 519 cm^{-1}; *m/z* 84 (M^+, 6.5%), 83, 77, 69, 68, 67, 66, 57, 56 (100%), 55, 54, 52, 43, 42, 41; found: C 27.64%, H 6.80%, N 16.18%; calc. for $C_4H_{10}N_2Cl_2$: C 30.57%, H 6.36%, N 17.83%.

E-1,4-DIAMINO-2,3-DIBROMOBUT-2-ENE DIHYDROCHLORIDE



To a solution of *E*-2,3-dibromobut-2-ene-1,4-diol (3.69 g, 15 mmol) in dry THF (20 ml) under nitrogen was added hydrazoic acid (1.0 M in benzene, 30 ml, 30 mmol) with stirring. To this mixture was added a solution of diisopropyl azodicarboxylate (6.78 g, 33.9 mmol) in dry THF (10 ml) with stirring. To this mixture was added triphenylphosphine (17.8 g, 67.8 mmol) in dry THF (60 ml), maintaining the reaction temperature at 40 °C by cooling. The reaction mixture was stirred for 1 h at room temperature, then heated to 50 °C for 3 h. Water (2 ml) was added, and the solution was stirred at 50 °C for a further 3 h. The solvents were removed *in vacuo* and the residue was partitioned between hydrochloric acid (1 M, 80 ml) and dichloromethane (80 ml). The aqueous layer was further washed with dichloromethane (2 x 80 ml), then evaporated to dryness *in vacuo* to give a light brown solid. Recrystallisation from aqueous ethanol/acetone gave the title compound in

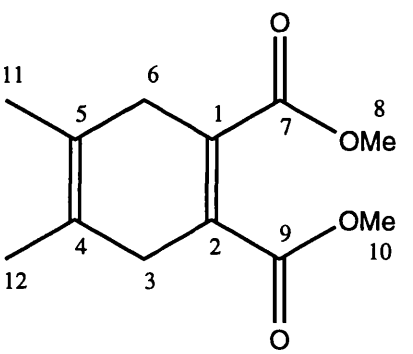
58% yield, m.p.>250°. δ_H (200 MHz, D_2O) 4.15 (s, 4H, H-1, H-4); δ_C (50 MHz, D_2O) 119.0 (C-2, C-3), 47.3 (C-1, C-4); ν_{max}(KBr disc) 3005, 2963, 2905, 2855, 2828, 2716, 2662, 2621, 2581, 1994, 1601, 1518, 1425, 1371, 1155, 1124, 1078, 953, 901, 638 and 534 cm^{-1}; *m/z* 228 (M^+-NH_3, 0.5%), 227, 226, 148, 146 (100%), 136, 134, 119, 117, 84, 83, 82; found: C 15.04%, H 3.33%, N 8.69%; calc. for $C_4H_{10}N_2Br_2Cl_2$: C 15.14%, H 3.15%, N 8.83%.

Chapter 10

Experimental To Chapter 6

1,2-BIS(AMINOMETHYL)-4,5-DIMETHYL-1,4-CYCLOHEXADIENE DIHYDROCHLORIDE

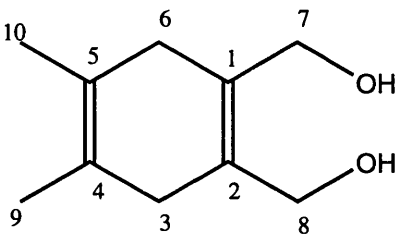
DIMETHYL-4,5-DIMETHYL-1,4-CYCLOHEXADIENE-1,2-DICARBOXYLATE



2,3-Dimethylbutadiene (9.84 g, 0.12 mol) and dimethyl acetylene-dicarboxylate (14.2 g, 0.1 mol) were stirred together in water (50 ml) at 60 °C for 24 h. The emulsion was cooled to room temperature and filtered. The filtered solid was recrystallised from ether to give the title compound in 78% yield, m.p. 74-75 °C (lit.[78] 75-76 °C). δ_H (200 MHz, $CDCl_3$) 3.76 (s, 6H, H-8, H-10), 2.91 (s, 4H, H-3, H-6), 1.66 (s, 6H, H-11, H-12); δ_C (50 MHz, $CDCl_3$) 168.3 (C-7, C-9), 132.7 (C-1, C-2), 121.6 (C-4, C-5), 52.1 (C-8, C-10), 34.1 (C-3, C-6), 17.9 (C-11, C-12);

ν_{max}(KBr disc) 3480, 1740, 1720, 1450, 1295, 1285, 1140, and 1080 cm^{-1}; *m/z* 224 (M^+, 4%), 193, 192, 191, 177 (100%), 133, 119, 117, 105, 103, 91, 77, 75, 65, 59, 51, 39, 28, 18; found: C 64.18%, H 7.16%; calc. for $C_{12}H_{16}O_4$: C 64.29%, H 7.14%.

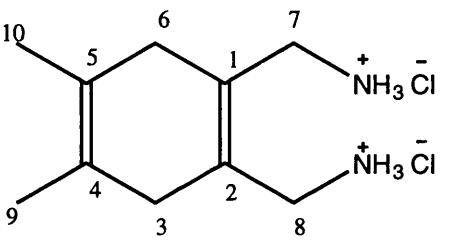
1,2-BIS(HYDROXYMETHYL)-4,5-DIMETHYL-1,4-CYCLOHEXADIENE[79]



To a slurry of lithium aluminium hydride (1.71 g, 0.045 mol) in THF (45 ml) cooled to ice bath temperature under nitrogen was added dimethyl 4,5-dimethyl-1,4-cyclohexadiene-1,2-dicarboxylate (2.24 g, 0.01 mol) in dry THF (30 ml) over 30 min. The resultant solution was stirred for a further 60 min at this temperature, after which methanol (10 ml) was added. The mixture was allowed to warm to room temperature, filtered through a Celite pad, and the filtrate was concentrated *in vacuo* to give an oil. Crystallisation from ethyl acetate/petroleum ether (b.p. 40 °C-60 °C) gave the title compound in 52% yield, m.p. 146-148 °C. δ_H (200 MHz, $CDCl_3$) 4.16 (s, 4H, H-7, H-8), 2.76 (s, 4H, H-3, H-6), 1.65 (s, 6H, H-9, H-10); δ_C (50 MHz, $CDCl_3$) 132.4 (C-1, C-2), 122.8 (C-4, C-5), 62.0 (C-7, C-8), 36.7 (C-3, C-6), 18.0 (C-9, C-10); ν_{max}(KBr disc) 3280, 2900, 2870, 2700, 1050, 1010, and 990 cm^{-1}; *m/z* 168 (M^+, 23%),

121, 120, 119, 107, 105, 93, 91 (100%), 79, 77, 67, 65, 55, 53, 51, 43, 41, 39, 31, 29, 27; found: C 70.67%, H 9.43%; calc. for $C_{10}H_{16}O_2$: C 71.43%, H 9.52%.

1,2-BIS(AMINOMETHYL)-4,5-DIMETHYL-1,4-CYCLOHEXADIENE DIHYDROCHLORIDE



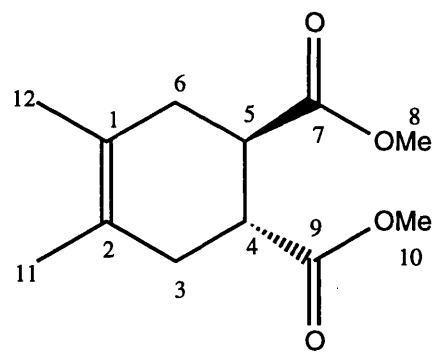
CAUTION: This procedure must be carried out in a good fumehood

To a solution of hydrazoic acid (1.0 M) in benzene (24 ml) was added 1,2-bis(hydroxymethyl)-4,5-dimethyl-1,4-cyclohexadiene (1.68 g, 0.01 mol) in dry THF (20 ml), and diisopropyl azodicarboxylate (2.52 g, 0.025 mol) in dry THF (10 ml). To this stirred solution was added triphenylphosphine (10.48 g, 0.05 mol) in dry THF (35 ml) over 1 h. The mixture was then stirred at 50 °C for a further 7 h, then water (5 ml) was added. The mixture was allowed to cool, then partitioned between hydrochloric acid (1 M, 100 ml) and dichloromethane (100 ml). The aqueous layer was further washed with dichloromethane (2 x 80 ml), and the water was removed *in vacuo* to give a brown solid, which was dissolved in ethanol and precipitated with ether to give the title compound, yield 32%. δ_H (200 MHz, D_2O) 3.76 (s, 4H, H-7, H-8), 2.76 (s, 4H, H-3, H-6), 1.64 (s, 6H, H-9, H-10); δ_C (50 MHz, D_2O) 140.2 (C-

1, C-2), 123.2 (C-4, C-5), 40.2 (C-7, C-8), 36.0 (C-3, C-6), 19.6 (C-9, C-10); ν_{max}(KBr disc) 3420, 3010, 2920, 1660, 1640, 1620, 1507, 1480, 1450, 1380, and 1110 cm^{-1}; *m/z* 166 (M^+-2HCl, 1.5%), 147, 145, 134, 132, 120, 119, 117, 107, 105, 91, 79, 77, 65, 53, 51, 41, 39, 36, 31, 30 (100%), 28; found: C 50.46%, H 8.17%, N 11.56%; calc. for $C_{10}H_{20}N_2Cl_2$: C 50.21%, H 8.37%, N 11.72%.

trans-4,5-BIS(AMINOMETHYL)-1,2-DIMETHYLCYCLOHEXENE DIHYDROCHLORIDE

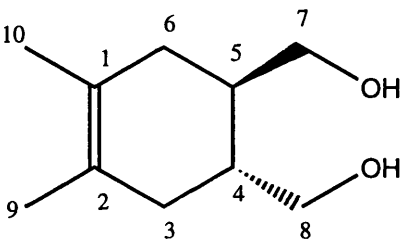
DIMETHYL *trans*-1,2-DIMETHYLCYCLOHEXENE-4,5-DICARBOXYLATE



2,3-Dimethylbuta-1,3-diene (9.84 g, 0.12 mol) and dimethyl fumarate (14.4 g, 0.1 mol) were stirred together in toluene (40 ml) at 50 °C for 24 h. The solvent was removed *in vacuo* and the solid residue was recrystallised (ether) to give the title compound in 85% yield, m.p. 52-54°C (lit.[80] 53.5-54.5°C). δ_H (200 MHz, $CDCl_3$) 3.56 (s, 6H, H-8, H-10), 2.47 (t, 2H, J=3Hz, H-4, H-5), 2.06 (m, 4H, H-3, H-6), 1.53 (s, 6H, H-11,

H-12); δ_C (50 MHz, $CDCl_3$) 174.6 (C-7, C-9), 123.5 (C-1, C-2), 51.2 (C-8, C-10), 41.5 (C-4, C-5), 33.8 (C-3, C-6), 18.0 (C-11, C-12); ν_{max}(KBr disc) 3007, 2959, 2943, 2847, 1736, 1437, 1323, 1242, 1197, and 1157 cm^{-1}; *m/z* 226 (M^+, 14%), 166, 151, 107, 106, 105, 93, 91, 79, 77, 65, 56, 41, 39, 32, 28 (100%), 18; found: C 63.64%, H 8.07%; calc. for $C_{12}H_{18}O_4$: C 63.72%, H 7.96%.

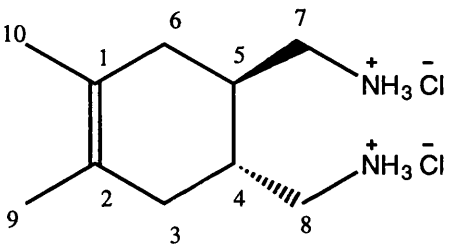
trans- 4,5-BIS(HYDROXYMETHYL)-1,2-DIMETHYLCYCLOHEXENE



To a suspension of lithium aluminium hydride (1.52 g, 0.04 mol) in dry diethyl ether (60 ml) at 0 °C was added dimethyl *trans*-1,2-dimethyl-cyclohexene-4,5-dicarboxylate (2.26 g, 0.01 mol) in dry diethyl ether (40 ml) over 30 min. The resultant suspension was stirred for another 1 h at 0 °C, and then saturated sodium sulfate solution (*ca.* 5 ml) was added dropwise. The solution was filtered and the filtrate was concentrated to give an oil, which on purification gave the title compound in 36% yield, m.p. 102-104 °C (lit.[81] 104.5-105 °C). δ_H (200 MHz, $CDCl_3$) 3.61 (m, 4H, H-7, H-8), 3.48 (m, 2H, H-4, H-5), 1.76 (m, 4H, H-3, H-6), 1.53 (s, 6H, H-9, H-10); δ_C (50 MHz, $CDCl_3$) 124.6 (C-1, C-2), 66.2 (C-7, C-8), 40.7 (C-4, C-5), 35.2 (C-3, C-6), 18.6 (C-9, C-10); ν_{max}(thin film) 3300, 2890, 2880, 1410, 1380, 1080, and 1030 cm^{-1}; *m/z* 170 (M^+, 6%),

152, 122, 121 (100%), 120, 119, 107, 105, 95, 93, 91, 81, 79, 77, 69, 67, 65, 55, 53, 51, 43, 41, 39, 31, 29, 27; found: C 70.65%, H 10.68%; calc. for $C_{10}H_{18}O_2$: C 70.59%, H 10.59%.

trans-4,5-BIS(AMINOMETHYL)-1,2-DIMETHYLCYCLOHEXENE DIHYDROCHLORIDE

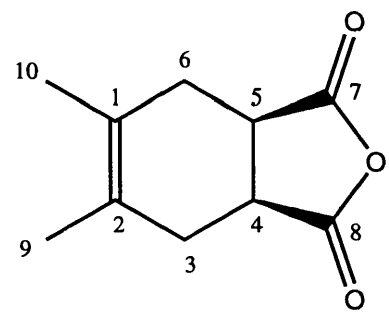


To a solution of hydrazoic acid (1.25 M) in benzene (19 ml) was added *trans*-4,5-bis(hydroxymethyl)-1,2-dimethylcyclohexene (1.70 g, 0.01 mol) in dry THF (20 ml), and diisopropyl azodicarboxylate (2.52 g, 0.025 mol) in dry THF (10 ml). To this stirred solution was added triphenylphosphine (10.48 g, 0.05 mol) in dry THF (35 ml) over 1 h. The mixture was then stirred at 50 ^{o}C for a further 16 h, then water (5 ml) was added. The mixture was allowed to cool, then partitioned between hydrochloric acid (1 M, 100 ml) and dichloromethane (100 ml). The aqueous layer was further washed with dichloromethane (2 x 80 ml), and the water was removed *in vacuo* to give a brown solid, which was recrystallised twice (ethanol/acetone) to give the title compound, yield 22%. δ_H (200 MHz, D_2O) 2.85 (m, 4H, H-7, H-8), 1.96 (m, 4H, H-3, H-6), 1.60 (m, 2H, H-4, H-5), 1.47 (s, 6H, H-9, H-10); δ_C (50 MHz, D_2O) 123.3 (C-1, C-2), 42.8 (C-7, C-8), 33.7 (C-4, C-5), 30.8 (C-3, C-6), 19.0

(C-9, C-10); ν_{max}(KBr disc) 3450, 3030, 2900, 2850, 1610, and 1500 cm^{-1}; *m/z* 168 (M^+-2HCl, 21%), 151, 134, 122, 121, 119, 107, 106, 105, 93, 91, 79, 77, 56, 41, 39, 36, 30 (100%), 28; found: C 49.58%, H 9.15%, N 11.52%; calc. for $C_{10}H_{20}N_2Cl_2$: C 49.79%, H 9.13%, N 11.62%.

cis-4,5-BIS(AMINOMETHYL)-1,2-DIMETHYLCYCLOHEXENE DIHYDROCHLORIDE

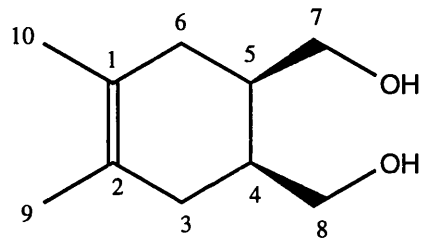
cis-1,2-DIMETHYLCYCLOHEXENE-4,5-DICARBOXYLIC ANHYDRIDE



2,3-Dimethylbutadiene (9.84 g, 0.12 mol) and maleic anhydride (9.8 g, 0.1 mol) were stirred together in toluene (50 ml) at 50 °C for 24 h. The solvent was removed *in vacuo* and the solid residue was recrystallised (ether) to give the title compound in 89% yield, m.p.76-77 °C (lit.[82] 77-78 °C). δ_H (200 MHz, $CDCl_3$) 3.29 (m, 2H, H-4, H-5), 2.30 (q, 4H, H-3, H-6), 1.64 (s, 6H, H-9, H-10); δ_C (50 MHz, $CDCl_3$) 174.6 (C-7, C-8), 127.3 (C-1, C-2), 40.3 (C-4, C-5), 30.4 (C-3, C-6), 19.3 (C-9, C-10); ν_{max}(KBr disc) 3420, 2950, 2890, 1795, 1790, 1430, 1220,

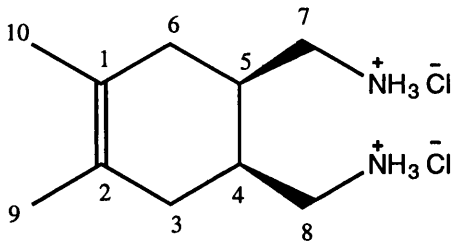
1140, 995, 930, and 920 cm^{-1}; *m/z* 180 (M^+, 18%), 152, 108, 107 (100%), 93, 91, 79, 77, 65, 57, 53, 51, 41, 39, 28, 27; found: C 66.43%, H 6.62%; calc. for $C_{10}H_{12}O_3$: C 66.59%, H 6.66%.

cis-4,5-BIS(HYDROXYMETHYL)-1,2-DIMETHYLCYCLOHEXENE



To a suspension of lithium aluminium hydride (1.52 g, 0.04 mol) in dry THF (60 ml) at 0 °C was added *cis*-1,2-dimethylcyclohexene-4,5-dicarboxylic anhydride (1.80 g, 0.01 mol) in dry THF (40 ml) over 30 min. The resultant suspension was stirred for another h at 0 °C, and then saturated sodium sulfate solution (ca. 5 ml) was added dropwise. The solution was filtered and the filtrate was concentrated to give a yellow oil, which on purification gave the title compound in 36% yield, m.p.72-72 °C (lit.[83] 74 °C). δ_H (200 MHz, $CDCl_3$) 4.71 (s, 2H, OH), 3.57 (m, 4H, H-7, H-8), 1.97 (m, 6H, H-3-H-6), 1.59 (s, 6H, H-9, H-10); δ_C (50 MHz, $CDCl_3$) 123.9 (C-1, C-2), 63.5 (C-7, C-8), 38.4 (C-4, C-5), 33.3 (C-3, C-6), 19.0 (C-9, C-10); ν_{max}(thin film) 3310, 2900, 1440, 1400, 1080, 1010, and 690 cm^{-1}; *m/z* 152 (M^+-H_2O, 3%), 121, 119, 107, 106, 105, 95, 94, 93, 91 (100%), 81, 79, 77, 69, 67, 65, 55, 53, 43, 41, 39, 31, 29, 27; found: C 70.76%, H 10.76%; calc. for $C_{10}H_{18}O_2$: C 70.59%, H 10.59%.

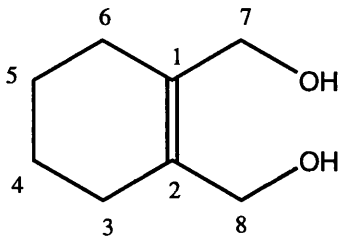
cis-4,5-BIS(AMINOMETHYL)-1,2-DIMETHYLCYCLOHEXENE DIHYDROCHLORIDE



To a solution of hydrazoic acid (1.10 M) in benzene (22 ml) was added *cis*-4.5-bis(hydroxymethyl)-1,2-dimethylcyclohexene (1.70 g, 0.01 mol) in dry THF (20 ml), and diisopropyl azodicarboxylate (2.52 g, 0.025 mol) in dry THF (10 ml). To this stirred solution was added triphenylphosphine (10.48 g, 0.05 mol) in dry THF (35 ml) over 1 h. The mixture was then stirred at 50 °C for a further 10 h, then water (5 ml) was added. The mixture was allowed to cool, then partitioned between hydrochloric acid (1 M, 100 ml) and dichloromethane (100 ml). The aqueous layer was further washed with dichloromethane (2 x 80 ml), and the water was removed *in vacuo* to give a brown solid, which was recrystallised (ethanol/acetone) to give the title compound, yield 22%. δ_H (200 MHz, D_2O) 3.95 (m, 4H, H-7, H-8), 1.99 (m, 2H, H-4, H-5), 1.93 (m, 4H, H-3, H-6), 1.52 (s, 6H, H-9, H-10); δ_C (50 MHz, D_2O) 122.6 (C-1, C-2), 40.1 (C-7, C-8), 31.4 (C-4, C-5), 28.9 (C-3, C-6), 19.4 (C-9, C-10); ν_{max}(KBr disc) 3450, 3030, 2900, 2850, 1610, and 1500 cm^{-1}; *m/z* 168 (M^+-2HCl, 2%), 151, 134, 119, 107, 106, 105, 93, 91, 79, 77, 67, 56, 53, 41, 39, 36, 30 (100%); found: C 49.85%, H 9.05%, N 11.25%; calc. for $C_{10}H_{20}N_2$: C 49.79%, H 9.13%, N 11.62%.

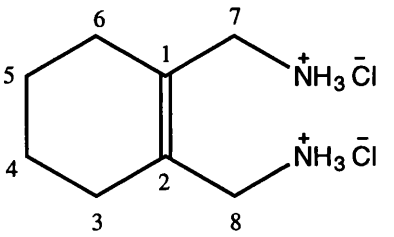
1,2 - BIS(AMINOMETHYL)CYCLOHEXENE DIHYDROCHLORIDE

1,2-BIS(HYDROXYMETHYL)CYCLOHEXENE[57]



To a suspension of lithium aluminium hydride (3.04 g, 0.08 mol) in dry THF (60 ml) at 0 °C was added 3,4,5,6-tetrahydrophthalic anhydride (3.04 g, 0.02 mol) in dry THF (40 ml) over 30 min. The resultant suspension was stirred for 1 h at 0 °C, and then saturated sodium sulfate solution (10 ml) added dropwise. The precipitate formed was removed by filtration and the filtrate evaporated to give an oil. Vacuum distillation (112-118 °C, 0.01 mm) gave the title compound in 36% yield. δ_H (200 MHz, $CDCl_3$) 4.57 (bs, 2H, OH), 4.06 (s, 4H, H-7, H-8), 2.12 (m, 4H, H-3, H-6), 1.60 ppm (m, 4H, H-4, H-5); δ_C (50 MHz, $CDCl_3$) 134.0 (C-1, C-2), 61.6 (C-7, C-8), 28.0 (C-3, C-6), 22.6 (C-4, C-5); ν_{max}($CHCl_3$) 3590, 3410, 3010, 2940, 2890, 2880, 1440, 1430, 1240 and 990 cm^{-1}; m/z 124 (M^+-H_2O, 23%), 111, 109, 96, 95 (100%), 93, 91, 81, 79, 77, 67, 55, 41, 39, 29, 27; found C 63.44%, H 9.35%; calc. for $C_8H_{14}O_2$: C 67.60%, H 9.86%

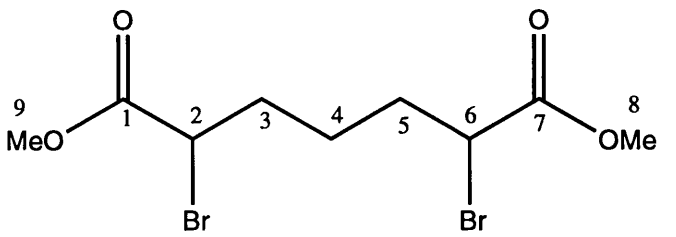
1,2-BIS(AMINOMETHYL)CYCLOHEXENE DIHYDROCHLORIDE



To a stirred solution of hydrazoic acid (1.10 M) in benzene (33 ml) at 0 °C was added 1,2-bis(hydroxymethyl)cyclohexene (2.13 g, 15 mmol) in dry THF (20 ml), and diisopropyl azodicarboxylate (3.83 g, 38 mmol) in dry THF (10 ml). To this stirred solution was added triphenylphosphine (15.72 g, 75 mmol) in dry THF (50 ml) over 1 h. The mixture was then stirred at 50 °C for a further 16 h, then water (5 ml) added. The mixture was allowed to cool, then partitioned between hydrochloric acid (1 M, 100 ml) and dichloromethane (100 ml) The aqueous layer was further washed with dichloromethane (2 x 80 ml), and the water was removed *in vacuo* to give a brown solid. The solid was dissolved in ethanol and then precipitated with acetone to give the title compound, yield 22%. δ_H (200 MHz, D_2O) 3.82 (s, 4H, H-7, H-8), 2.08 (t, 4H, J=6.4Hz, H-3, H-6), 1.58 (m, 4H, H-4, H-5); δ_C (50 MHz, D_2O) 141.0 (C-1, C-2), 39.8 (C-7, C-8), 28.4 (C-3, C-6), 22.3 (C-4, C-5); ν_{max}(KBr disc) 3350, 3030, 2450, 2115, 1610, 1520, 1406, 1385, 1145 and 988 cm^{-1}; *m/z* 123 (M^+-2HCl,-NH_3, 1%), 106, 105, 91, 90, 89, 77 (100%), 76, 75, 57, 55, 45, 44, 43, 32, 31, 28; found: C 44.61%, H 8.32%, N 12.88%; calc. for $C_8H_{18}N_2Cl_2$: C 45.07%, H 8.45%, N 13.15%.

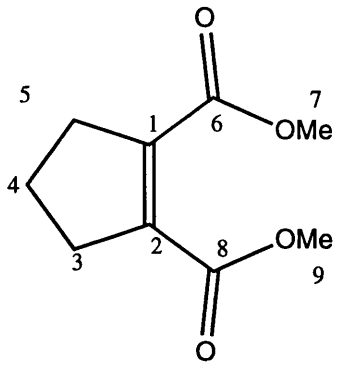
1,2-BIS(AMINOMETHYL)CYCLOPENTENE DIHYDROCHLORIDE

DIMETHYL-2,6-DIBROMOPIMELATE[56]



To pimelic acid (40 g, 0.25 mol) was added thionyl chloride (59.5 g, 0.5 mol) in four equal portions over 3 h, and the mixture heated to 80 °C with stirring for 8 h. Bromine (88 g, 0.55 mol) was added dropwise over 4 h and the reaction mixture was maintained at 80 °C for a further 4 h. The mixture was allowed to cool to room temperature and added slowly to methanol (50 ml) cooled to 0 °C. The solvents were removed *in vacuo* to give a yellow oil. Distillation gave the title compound (150 °C, 0.05 mm Hg) in 78% yield. δ_H (200 MHz, $CDCl_3$) 4.25 (t, 2H, J=7Hz, H-2, H-6), 3.79 (s, 6H, H-8, H-9), 2.07 (m, 4H, H-3, H-5), 1.54 (m, 2H, H-4); δ_C (50 MHz, $CDCl_3$) 169.8 (C-1, C-7), 53.0 (C-8, C-9), 44.9 (C-2, C-6), 33.8 (C-3, C-5), 24.9 (C-4) ; ν_{max}(thin film) 3003, 2955, 2868, 2847, 1741, 1437, 1356, 1281, 1258, 1194, and 1155 cm^{-1}; *m/z* 315, 313, 311, 266, 234, 232, 206, 204, 194, 192, 153, 151, 125, 114, 98, 93, 81, 79, 68, 67, 65, 59 (100%), 55, 53, 41, 39; found: C 40.29%, H 5.27%; calc. for $C_9H_{14}O_4Br_2$: C 40.60%, H 5.26%.

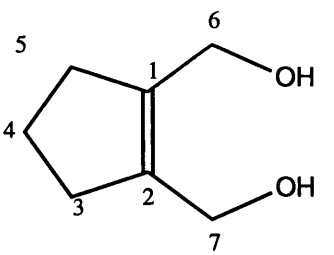
DIMETHYL CYCLOPENTENE-1,2-DICARBOXYLATE



Prepared following the procedure of McDonald *et al.*[56]

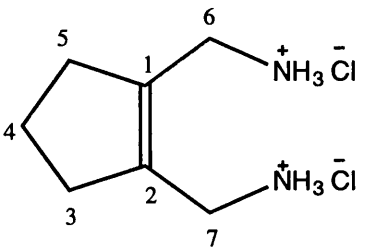
To a stirred solution of dimethyl-2,6-dibromopimelate (17.3 g, 0.05 mol) in DMF (200 ml) was added sodium hydride (3.6 g, 0.15 mol) in portions over 1 h. Stirring was continued for a further 3 h, and to the resultant yellow solution was added ether (200 ml). The precipitate formed was removed by filtration, and the filtrate was washed with saturated brine (4 x 200 ml). The organic phase was concentrated *in vacuo* to give a yellow oil, which was used without further purification. δ_H (200 MHz, $CDCl_3$) 3.75 (s, 6H, H-7, H-9), 2.70 (t, 4H, J=6Hz, H-3, H-5), 1.95 (q, 2H, J=6Hz, H-4); δ_C (50 MHz, $CDCl_3$) 166.5 (C-6, C-8), 134.0 (C-1, C-2), 54.3 (C-7, C-9), 33.2 (C-3, C-5), 23.8 (C-4); ν_{max}(thin film) 2910, 2880, 1725, 1650, 1450, and 1380 cm^{-1}; m/z 184(M^+, 10%), 169, 153, 152, 125, 124, 123, 110, 109, 79, 78, 65 (100%), 64, 63, 49, 38, 30; found C 58.60%, H 6.58%; calc. for $C_9H_{12}O_4$: C 58.70%, H 6.52%

1,2-BIS(HYDROXYMETHYL)CYCLOPENTENE



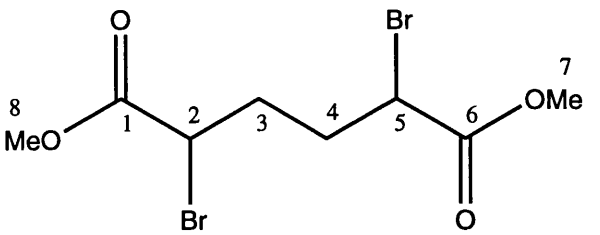
To a suspension of lithium aluminium hydride (3.04 g, 0.08 mol) in dry THF (80 ml) at 0 °C was added dimethyl cyclopentene-1,2-dicarboxylate (3.68 g, 0.02 mol) in dry THF (40 ml) over 30 min. The resultant suspension was stirred for another 1 h at 0 °C, and then saturated sodium sulfate solution (10 ml) was added dropwise. The solid precipitate was removed by filtration and the filtrate evaporated to give an oil, which on vacuum distillation (120-130 °C, 0.05 mm Hg) gave the title compound in 31% yield (m.p. lit.[57] 41-42 °C). δ_H (200 MHz, $CDCl_3$) 4.20 (s, 4H, H-6, H-7), 3.64 (bs, 2H, OH), 2.47 (t, 4H, J=6Hz, H-3, H-5), 1.85 ppm (q, 2H, J=6Hz, H-4); δ_C (50 MHz, $CDCl_3$) 129.6 (C-1, C-2), 62.6 (C-6, C-7), 36.7 (C-3, C-5), 23.0 (C-4); ν_{max}(thin film) 3300, 2885, 2875, 1645, 1445, 1190cm^{-1}; m/z 110 (M^+-H_2O, 9%), 94, 80, 79, 55, 54, 46, 45, 44, 37, 36, 31, 30 (100%), 28; found C 65.60%, H 9.31%; calc. for $C_7H_{12}O_2$: C 65.63%, H 9.38%

1,2-BIS(AMINOMETHYL)CYCLOPENTENE DIHYDROCHLORIDE



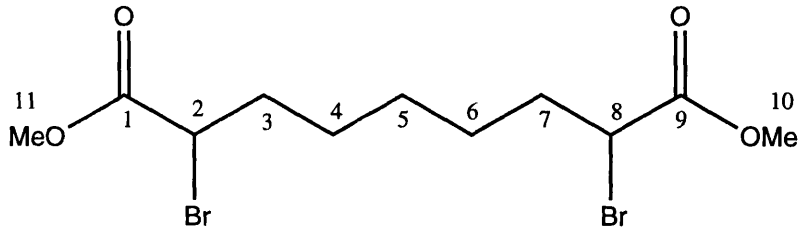
To a solution of hydrazoic acid (1.25 M) in benzene (19 ml) was added 1,2-bis(hydroxymethyl)cyclopentene (1.28 g, 0.01 mol) in dry THF (20 ml), and diisopropyl azodicarboxylate (2.52 g, 0.025 mol) in dry THF (10 ml). To this stirred solution was added triphenylphosphine (10.48 g, 0.05 mol) in dry THF (35 ml) over 1 h. The mixture was then stirred at 50 °C for a further 16 h, then water (5 ml) added. The mixture was allowed to cool, then partitioned between hydrochloric acid (1 M, 100 ml) and dichloromethane (100 ml) The aqueous layer was further washed with dichloromethane (2 x 80 ml), and the water was removed *in vacuo* to give a brown solid, which was recrystallised twice (ethanol/acetone) to give the title compound, yield 21%. δ_H (200 MHz, D_2O) 3.63 (s, 4H, H-6, H-7), 2.35 (t, 4H, J=6.1Hz, H-3, H-5), 1.79 (m, 2H, H-4); δ_C (50 MHz, D_2O) 136.0 (C-1, C-2), 37.3 (C-6, C-7), 34.8 (C-3, C-5), 22.3 (C-4); ν_{max}(KBr disc) 3434, 2951, 2264, 2212, 1601, 1473, 1406, 1385, 1120 and 937 cm^{-1}; *m/z* 109 (M^+-2HCl,-NH_3, 1.5%), 94, 81, 80, 79, 67, 57, 56, 44, 43, 41, 38, 37, 36 (100%), 30; found: C 42.13%, H 8.17%, N 13.91%; calc. for $C_7H_{16}N_2Cl_2$: C 42.21%, H 8.04%, N 14.07%.

DIMETHYL-2,6-DIBROMOADIPATE[56]



To adipic acid (36.5 g, 0.25 mol) was added thionyl chloride (59.5 g, 0.5 mol) in four equal portions over 3 h, and the mixture heated to 80 °C with stirring for 8 h. Bromine (88 g, 0.55 mol) was added dropwise over 4 h and the reaction was maintained at 80 °C for a further 4 h. The mixture was allowed to cool to room temperature and added slowly to methanol (50 ml) cooled to 0 °C. The solvents were removed *in vacuo* to give a yellow oil. Distillation gave the title compound (180 °C, 0.2 mm Hg) in 65% yield. δ_H (200 MHz, $CDCl_3$) 4.20 (m, 2H, H-2, H-5), 3.74 (s, 6H, H-7, H-8), 2.25 (m, 4H, H-3, H-4); δ_C (50 MHz, $CDCl_3$) 169.6 (C-1, C-6), 53.1 (C-7, C-8), 44.2 (C-2, C-5), 32.3 (C-3, C-4); ν_{max}(thin film) 3443, 3003, 2959, 1728, 1690, 1441, 1433, 1371, 1267, 1242, 1190, 1161, 1124, 1051, 970, 898, 810, 743, 723, 555, and 455 cm^{-1}; *m/z* 301 (M^+-OMe, 35%), 273, 242, 222, 189, 159, 142, 139, 111, 101; found: C 29.20%, H 3.86%; calc. for $C_8H_{12}O_4Br_2$: C 28.92%, H 3.61%.

DIMETHYL-2,6-DIBROMOAZELEATE[56]

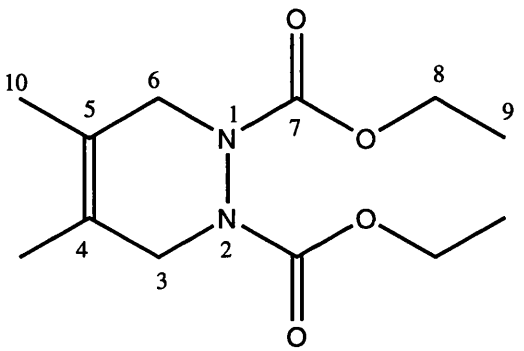


To azeleic acid (47 g, 0.25 mol) was added thionyl chloride (59.5 g, 0.5 mol) in four equal portions over 3 h, and the mixture heated to 80 °C with stirring for 8 h. Bromine (88 g, 0.55 mol) was added dropwise over 4 h and the reaction was maintained at 80 °C for a further 4 h. The mixture was allowed to cool to room temperature and added slowly to methanol (50 ml) cooled to 0 °C. The solvents were removed *in vacuo* to give a yellow oil. Distillation gave the title compound (140 °C, 0.1 mm Hg) in 81% yield. δ_H (200 MHz, $CDCl_3$) 4.23 (t, 2H, J=7Hz, H-2, H-8), 3.79 (s, 6H, H-10, H-11), 2.03 (m, 4H, H-3, H-7), 1.40 (m, 6H, H-4-H-6); δ_C (50 MHz, $CDCl_3$) 170.3 (C-1, C-9), 53.0 (C-10, C-11), 45.5 (C-2, C-8), 34.6 (C-3, C-7), 28.0 (C-4, C-6), 26.9 (C-5); ν_{max}(thin film) 3468, 3001, 2951, 2860, 1743, 1435, 1359, 1273, 1215, 1155, 1016, 731, 714 and 673 cm^{-1}; *m/z* 262, 260 (M^+-OMe,-Br, 56%), 234, 232, 230, 228, 222, 220, 154, 152, 121, 93, 81, 67, 59, 55, 41; found: C 35.11%, H 4.79%; calc. for $C_{11}H_{18}O_4Br_2$: C 35.29%, H 4.81%.

Chapter 11

Experimental To Chapter 7

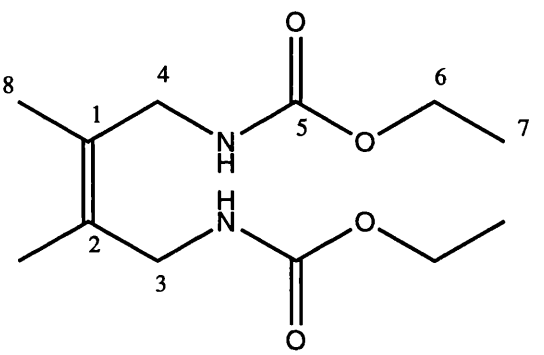
DIETHYL 4,5-DIMETHYL-1,2,3,6-TETRAHYDROPYRIDAZINE-1,2-DICARBOXYLATE



To a solution of 2,3-dimethylbutadiene (9.84 g, 0.12 mol) in toluene (20 ml) was added diethyl azodicarboxylate (9.8 g, 0.1 mol) in one portion at ambient temperature with stirring. After a few min a slight exotherm was observed and the temperature of the mixture rose to *ca.* 40 °C. The reaction was allowed to cool to room temperature and then heated to 50 °C with stirring for 4 h. The solvent was removed *in vacuo* to leave a yellow oil, in 85% yield. δ_H (200 MHz, $CDCl_3$) 4.26 - 3.51 (m, 8H, H-3, H-6, H-8), 1.54 (s, 6H, H-10), 1.17 (t, 6H, J=6.9Hz, H-9); δ_C (50 MHz, $CDCl_3$) 155.2 (C-7), 123.0 (C-4, C-5), 62.2 (C-8), 47.0 (C-3, C-6), 15.4 (C-10), 14.4 (C-9); ν_{max}($CHCl_3$) 3005, 2990, 2913, 2907, 2861, 2379, 1690, 1501, 1490, 1415, 1389, 1341, 1285, 1220, 1189,

1149, 1071, 1060, 1016, and 845 cm^{-1}; *m/z* 256 (M^+, 3%), 183, 167, 137, 111, 95, 94, 82, 69, 67, 55, 43, 41 (100%); found: C 55.64%, H 8.07%; calc. for $C_{12}H_{20}N_2O_4$: C 56.25%, H 7.81%.

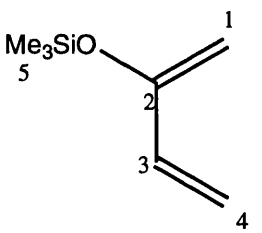
cis-2,3-DIMETHYL-1,4-BIS(ETHOXYCARBONYLAMINO)-2-BUTENE



Diethyl 4,5-dimethyl-1,2,3,6-tetrahydropyridazine-1,2-dicarboxylate (9.84 g, 0.12 mol) was dissolved in stirred, refluxing liquid ammonia (50 ml) at -33 °C. Sodium was dissolved in the solution in small portions until a deep blue colour persisted. Further portions were added at regular 10 min intervals to maintain the colour until no more portions were required, and then the solution was stirred for a further 1 h at reflux. Ammonium bromide (25 g) was added in one portion to the mixture. The solution was warmed to room temperature and the ammonia was evaporated. Ethyl acetate (100 ml) was added to the solid residue and the resultant suspension was filtered. The filtrate was treated with hot, activated charcoal (2 g) and the mixture was filtered through a Celite bed. The solvents were removed from the filtrate *in vacuo*, and the resultant

oil subjected to flash column chromatography (silica gel, ethyl acetate eluent) to give the title compound in 39% yield as a gum, which was not purified further. δ_H (200 MHz, $CDCl_3$) 5.91 (m, 2H, NH), 4.12 (q, 4H, J=7Hz, H-6), 3.82 (d, 4H, J=7.2Hz, H-3, H-4), 1.69 (s, 6H, H-8), 1.23 (t, 6H, J=7Hz, H-7); δ_C (50 MHz, $CDCl_3$) 157.0 (C-5), 129.1 (C-1, C-2), 62.6 (C-6), 60.5 (C-3, C-4), 14.4 (C-7), 14.3 (C-8); ν_{max}(KBr disc) 3444, 3098, 3072, 3004, 2921, 2870, 1712, 1505, 1470, 1409, 1395, 1334, 1215, 1182, 1081, and 1035 cm^{-1}; *m/z* 260 (M^+, 0.5%) 185, 170, 169, 156, 140, 129, 117, 102, 96 (100%), 85, 82, 69, 57, 56, 55, 45, 43, 41, 30; found: C 54.96%, H 8.79%, N 10.95%; calc. for $C_{12}H_{22}N_2O_4$: C 55.81%, H 8.53%, N 10.85%.

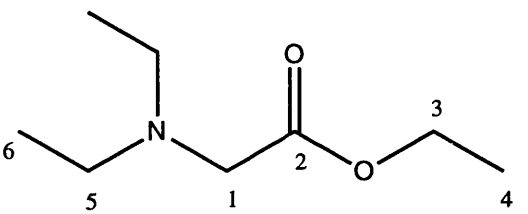
2-TRIMETHYLSILYLOXYBUTADIENE



To a stirred solution of triethylamine (20.2 g, 0.2 mol) in DMF (200 ml) at 90 °C under a nitrogen atmosphere was added methyl vinyl ketone (12.5 g, 0.18 mol) in DMF (15 ml) and chlorotrimethylsilane (21.7 g, 0.2 mol) in DMF (15 ml) dropwise over 30 min. The reaction was stirred at 90 °C for a further 20 h, during which time the solution turned dark red and a precipitate formed. The mixture was cooled to room temperature and the precipitate was removed by filtration. To the

filtrate was added pentane (150 ml), and the solution was washed with sodium bicarbonate solution (5% w/v, 500 ml). The aqueous layer was washed with pentane (2 x 150 ml), and the organic layers were combined, washed with water (100 ml) and dried (Na_2SO_4). The solution was filtered, and the solvent was removed from the filtrate by distillation at atmospheric pressure in an oil bath at 70 °C. The residue was subjected to fractional distillation to yield the title compound (50%). δ_H (200 MHz, $CDCl_3$) 6.13 (dd, 1H), 5.40 (dd, 1H), 5.00 (dt, 1H), 4.28 (s, 2H), 0.17 (s, 9H); δ_C (50 MHz, $CDCl_3$) 154.9 (C-2), 134.7 (C-3), 114.5 (C-4), 96.4 (C-1), 2.0 ($Si(CH_3)_3$); ν_{max}(thin film) 3432, 3117, 3101, 3024, 2961, 2901, 1715, 1684, 1672, 1636, 1586, 1408, 1373, 1279, 1254, 1186, 1167, 1059, 1008, 983, 916, 879, 845, and 756 cm^{-1}; *m/z* 142 (M^+, 6%), 127, 99, 86, 85, 75 (100%), 73, 69, 66, 62, 59, 47, 45, 43; found: 142.0813; calc. for $C_7H_{14}OSi$: 142.0814.

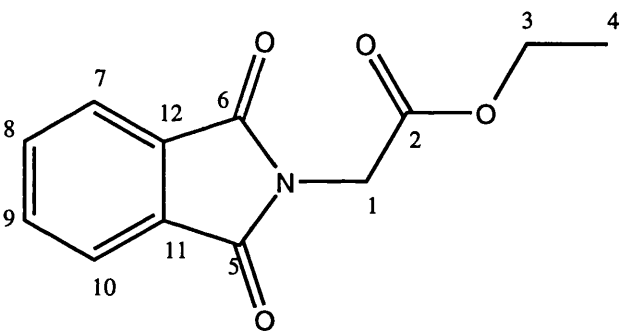
ETHYL (DIETHYLAMINO)ACETATE



To a magnetically stirred solution of diethylamine (7.3 g, 0.1 mol) in chloroform (45 ml) cooled to 0 °C was added ethyl bromoacetate (3.34 g, 20 mmol) in chloroform (25 ml) dropwise over 1 h. The resultant yellow solution was stirred for a further 6 h at room temperature, then washed with water (3 x 30 ml). The organic layer was dried (Na_2SO_4), filtered and the filtrate concentrated *in vacuo* to give a colourless oil,

which slowly became yellow on standing. Fractional distillation of this oil (b.p. 165-170°C) gave the title compound in 95% yield. δ_H (200 MHz, $CDCl_3$) 4.16 (q, 2H, J=6.8Hz, H-3), 3.29 (s, 2H, H-1), 2.64 (q, 4H, J=7Hz, H-5), 1.26 (t, 6H, J=7Hz, H-6), 1.05 (t, 3H, J=6.8Hz, H-4); δ_C (50 MHz, $CDCl_3$) 171.2 (C-2), 60.0 (C-3), 54.0 (C-1), 47.5 (C-5), 14.0 (C-4), 11.9 (C-6); ν_{max}(KBr disc) 3005, 2996, 2909, 2851, 2760, 1735, 1455, 1449, 1444, 1400, 1396, 1389, 1285, 1246, 1199, 1174, 1097, 1089, 1078, 1035, 790, 785, 780, 756, 746, 735, and 680 cm^{-1}; *m/z* 159 (M^+, 12%), 87, 86 (100%), 72, 58, 56, 43, 42, 30, 29, 28, 27; found: C 63.64%, H 8.07%; calc. for $C_{12}H_{18}O_4$: C 63.72%, H 7.96%.

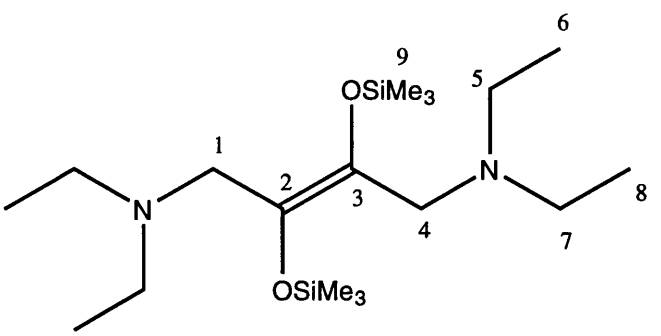
ETHYL PHTHALIMIDOACETATE



Potassium phthalimide (10 g, 54 mmol) was added in portions over 2 h to a stirred solution of ethyl bromoacetate (8.35 g, 50 mmol) in DMF (100 ml) at room temperature. Stirring was continued for a further 24 h at this temperature and the suspension was then poured into water (100 ml). The mixture was then extracted with dichloromethane (3 x 100 ml) and the dichloromethane layer was washed with saturated brine (5 x 100

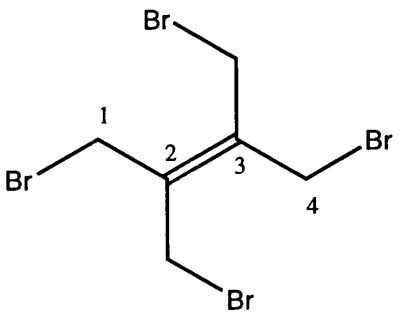
ml) and water (100 ml). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated *in vacuo* to a volume of *ca.* 20 ml, whereupon the solution became a thick slurry. A white solid was isolated by filtration and washed with ether (3 x 10 ml) to give ethyl phthalimidoacetate (15.75 g, 91%), m.p. 158-161°C. δ_H (200 MHz, $CDCl_3$) 7.77 (m, 2H, H-7, H-10), 7.68 (m, 2H, H-8, H-9), 4.35 (s, 2H, H-1), 4.14 (q, 2H, J=6.8Hz, H-3), 1.20 (t, 3H, J=6.8Hz, H-4); δ_C (50 MHz, $CDCl_3$) 167.4 (C-6, C-5), 167.2 (C-2), 134.2 (C-7, C-10), 131.9 (C-11, C-12), 123.5 (C-8, C-9), 61.8 (C-3), 38.8 (C-1), 14.0 (C-4); ν_{max}(KBr disc) 3455, 2965, 1794, 1736, 1724, 1466, 1411, 1393, 1388, 1383, 1300, 1219, 1199, 1106, 1010, 950, 741, and 706 cm^{-1}; *m/z* 233 (M^+, 65%), 188, 161, 160 (100%), 147, 133, 117, 104, 77, 76, 51, 50; found: C 61.75%, H 4.64%, N 5.97%; calc. for $C_{12}H_{11}NO_4$: C 61.80%, H 4.72%, N 6.00%.

<u>*E*-2,3-BIS(TRIMETHYLSILYLOXY)-1,4-BIS(DIETHYLAMINO)BUT-2-ENE</u>



Sodium (2.3 g, 0.1 mol) was stirred vigorously in toluene (70 ml) and heated to reflux under a nitrogen atmosphere. Heating was continued until the molten metal had become divided into small droplets, at which point the suspension was cooled rapidly to 0 °C (ice bath). Stirring was stopped and the sodium sand was allowed to settle. Most of the toluene was then removed by syringe, and dry ether (70 ml) was added via syringe. The solvent was again removed by syringe and was replaced with dry ether (70 ml). Stirring was recommenced and chlorotrimethylsilane (12.0 g, 0.11 mol) in dry ether (20 ml) was added dropwise via syringe. Ethyl diethylaminoacetate (7.9 g, 0.05 mol) in dry ether (10 ml) was added dropwise by syringe to the stirred suspension over 1 h, maintaining the reaction temperature at less than 5 °C during the addition. The reaction was allowed to come to room temperature and was then stirred for a further 18 h. The sodium chloride formed was removed by filtration, and the filtrate was concentrated *in vacuo* to give an oil. This oil gave correct spectroscopic analysis, but it appeared to decompose when stored at room temperature, and accurate microanalytical data could not be obtained. δ_H (200 MHz, $CDCl_3$) 3.60 (s, 4H, H-1, H-4), 2.63 (q, 8H, J=6.8Hz, H-5, H-7), 1.08 (t, 12H, J=6.8Hz, H-6, H-8), 0.00 (s, 18H, H-9); δ_C (50 MHz, $CDCl_3$) 118.1 (C-2, C-3), 47.6 (C-1, C-4), 43.4 (C-5, C-7), 14.5 (C-6, C-8); ν_{max}(KBr disc) 3684, 3292, 3020, 2963, 2874, 2434, 2401, 2338, 1736, 1718, 1711, 1664, 1649, 1637, 1624, 1618, 1603, 1577, 1572, 1560, 1522, 1518, 1475, 1458, 1425, 1253, 1209, 1057, 928, 848, and 787 cm^{-1}; *m/z* 222, 221, 166, 153, 150, 149, 144, 139, 131, 126, 122, 110, 100, 95, 86 (100%).

1,4-DIBROMO-2,3-BIS(BROMOMETHYL)-2-BUTENE



To a stirred solution of 2,3-dimethyl-1,3-butadiene (20.5 g, 0.25 mol) in carbon tetrachloride (250 ml) at 0 °C was added bromine (40 g, 0.25 mol) in carbon tetrachloride (100 ml) dropwise over 4 h. To the resultant colourless solution was added *N*-bromosuccinimide (89 g, 0.5 mol) and dibenzoyl peroxide (2 g,) in carbon tetrachloride (100 ml). The mixture was then heated to reflux for 5 min, after which the exothermic reaction maintained reflux without requirement for external heat input for 1 h. The reaction was cooled to room temperature, then heated at reflux for a further 1 h. The precipitated succinimide was removed by filtration of the hot reaction mixture, and the filtrate was cooled to 0 °C. A yellow precipitate formed and was removed by filtration. Recrystallisation of this solid from ethyl acetate gave the title compound as yellow needles in 57.5% yield, m.p. 156-158 °C (lit.[64] 158-159 °C). δ_H (200 MHz, $CDCl_3$) 4.15 (s, 8H, H-1, H-4); δ_C (50 MHz, $CDCl_3$) 137.1 (C-2, C-3) 27.8 (C-1, C-4); ν_{max}(KBr disc) 3590, 3569, 3441, 3036, 2976, 1701, 1460, 1437, 1233, 1200, 1096, 894, 862, 737, 662, 565, 544, and 496 cm^{-1}; *m/z* 399 (M^+, 31%), 320, 318, 240, 238, 236, 161, 159, 157, 81, 80, 79, 78, 77, 65, 63, 53, 52, 51, 50, 41, 40, 39

(100%); found: C 17.85%, H 2.03%; calc. for $C_6H_8Br_4$: C 18.00%, H 2.00%.

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