Synthesis and Biological Evaluation of Novel Anti-Cancer Agents

A Thesis presented in part fulfillment
of the requirement for the
Degree of Doctor of Philosophy

by
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This thesis is dedicated to the memories of Christopher Henderson and Dione Henderson.

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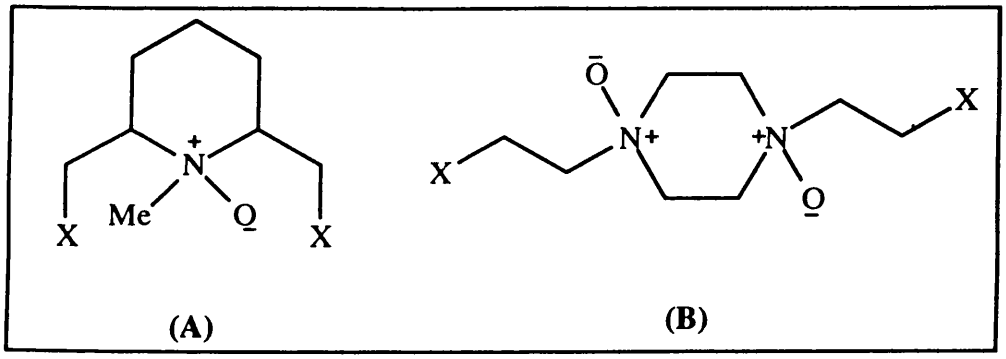
Summary

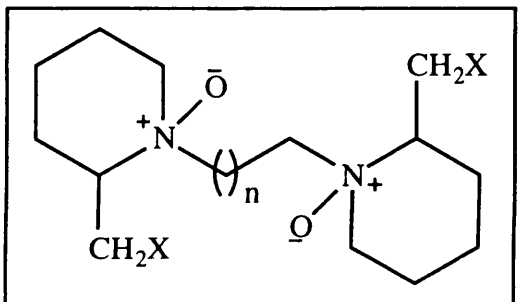
Bioreducible antitumour agents are prodrugs which are intended to be inactive in their own right, but are able to undergo metabolic reduction to produce toxic species which can damage biomolecules. This reduction may occur readily in solid tumours due to two factors which are unique to them, a) tumour hypoxia; and b) the expression of high levels of reductase enzymes. Each of these factors can promote specific cytotoxicity to the tumour and in principle could create a selective antitumour agent.

A series of *N*-oxides of heterocyclic aliphatic amines was prepared for evaluation as bioreducible drugs. *N*-oxides (A) of *cis*- and *trans*-2,6-disubstituted *N*-methyl-piperidine were prepared with X equal to carbamate or halogen. The free bases were designed to be bifunctional alkylating agents via aziridine ion formation. The *cis*-isomer was synthesised by hydrogenation of a derivative of dipicolinic acid followed by reduction to produce the diol from which the leaving groups were attatched. The *trans*-isomer was synthesised by ring closure of a pimelic acid derivative with methylamine followed by acidic epimerisation, from which the diol was obtained and derivatised. In total, 22 mostly novel compounds were synthesised and tested against two human colon carcinoma cell lines, HT 29 and BE cells under oxic and hypoxic conditions. HT 29 cells produce high amounts of DT-diaphorase which is a reductase enzyme that favours a 2e- reduction pathway, whilst BE cells contains no DT-diaphorase. IC_{50} values were also measured for selected compounds. The results showed that the free bases are toxic against both cell lines and the *N*-oxides are non-toxic. This suggested that the mono-*N*-oxides were not reduced under hypoxic conditions which implies that the reduction potentials of the compounds are not in the correct range for cellular reductants.



Bis-*N*-oxides (B) of 1,4-disubstituted piperazines were also prepared and 10 mainly novel compounds were synthesised and tested in the same biological systems. These *N*-oxides also did not give any evidence of being reduced under hypoxic conditions but did have reduced toxicity in comparison to the free bases. IC_{50} values were measured for these compounds and the values indicated that the free bases were up to 1000-fold more toxic than the corresponding bis-*N*-oxides.

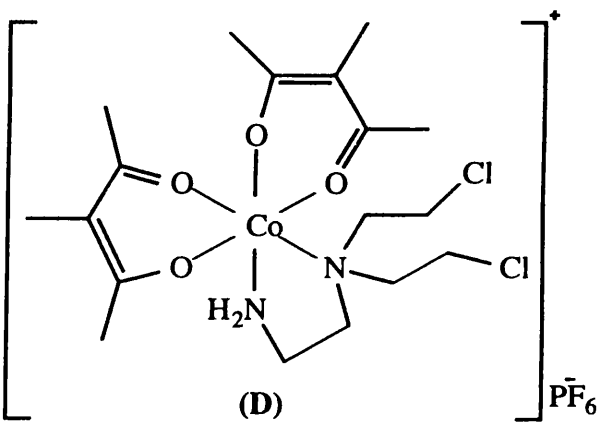
In attempts to modify the reduction potentials of the piperidine *N*-oxides, a series of bis-piperidine-bis-*N*-oxides (C) of varying carbon chain length were prepared by a general route by linking two methyl pipecolinate molecules with a selection of diacid chlorides followed by reduction and introduction of the X group. 20 predominantly novel compounds were prepared and evaluated for toxicity and selectivity. There was little evidence of hypoxic/oxic selectivity by the *N*-oxides although their toxicity was substantially reduced compared to the free bases. The toxicity of the free bases did seem affected by the length of the carbon chain, and the compounds with a four carbon chain were most toxic by several orders of magnitude.



n = 1, 2, 3.

(C)

Cobalt is known to form stable octahedral Co(III) complexes with a range of aliphatic amines and the reduction potentials of such complexes fall within an appropriate range so that bioreduction to the Co(II) species is likely to occur. This reduction results in release of the ligands and it has been suggested that this may be incorporated into the design of novel bioreducible antitumour agents. A range of diamines, triamines and tetraamines with alkylating functionality were synthesised and complexed to Co(III). The polyamines were formed by a general route starting with amino acids and using mixed anhydride methodology to attach the nitrogen mustard functionality.



(D)

The amines were obtained as hydrochloride salts and IC_{50} values were obtained. All were reasonably toxic, the most toxic having IC_{50} values in the μ molar region. Five cobalt complexes, such as D, were isolated and tested for oxic/hypoxic selectivity, two of which had been synthesised before. Of these, two showed promising selectivity, exhibiting greater toxicity under hypoxic conditions than under oxic conditions, although further testing is required.

Chapter 1

Introduction

1.1 Cancer

A cancer represents a population of cells within the body which escapes from normal control mechanisms and continues to increase until, unless treated, it leads to the death of the host. Once an individual cell, or group of cells, escapes from these normal constraints the resulting uncontrolled replication leads to the formation of a tumour mass. As this increases in size and age, the cells tend to resemble the parent tissue less and less. They become undifferentiated and lose the specific characteristics which initially distinguished them. The growth will invade and destroy surrounding normal tissues and clumps of cancer cells may break off into the lymphatic vessels or the veins and be carried to distant sites, forming secondary growths or metastases (It is these two properties - invasion of adjacent structures, resulting in tissue damage, and the ability to produce metastases - that distinguishes cancers from benign growths). Eventually, if left untreated, involvement of vital organs or general debility will lead to death.[1]

The tumour may be controlled by surgical removal, by killing the growing cells with ionising radiation or by chemical means. Surgery and radiotherapy are only successful if the lesion is still localised so that it may either be completely excised or covered by a field of radiation. These local methods of treatment have only limited value once the disease has disseminated, offering symptom control in certain circumstances, but having no potential for cure at this late stage. Chemical methods of treatment may be used for both local and metastastic lesions. Such drug treatment involves either endocrine therapy, altering the hormonal environment of certain tumours and thereby slowing their growth, or the administration of cytotoxic drugs. Cytotoxic drugs act against the tumour by interfering with cell division thereby slowing down or reversing the growth of the cancer.

1.2 Cancer Chemotherapy[2]

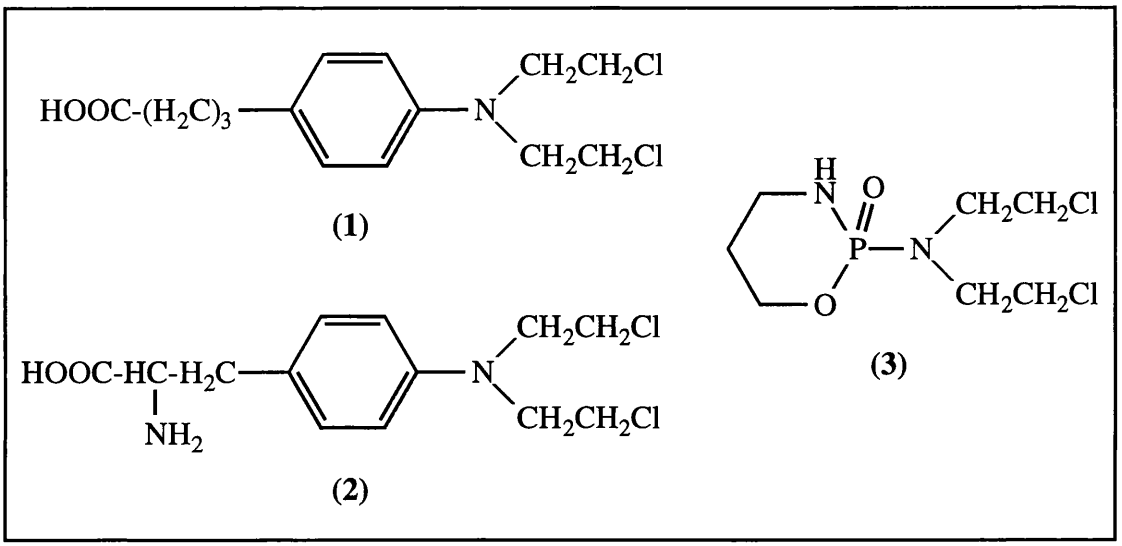
Cancer chemotherapy made its initial tangible thrust in 1941 when Huggins and Hodges reported that the sex hormone oestrogen was useful in the treatment of prostatic cancer in men. Later, Farber and associates noticed that folic acid treatment of anaemic children with acute leukaemia led to a worsened leukaemia condition. This suggested the use of antifolic acid compounds for the treatment of leukaemia.

Wartime research on chemical warfare agents led to the discovery of the nitrogen mustards, although it had first been noted in the First World War that mustard gas produced a profound fall in white cell counts with damage to marrow and lymphatic tissue. In 1946, the first studies of cytotoxic therapy for the treatment of leukaemias and lymphomas were reported as the underlying pathological process of these cancers is excessive white cell formation by either the bone marrow or the lymphatic system. The agent used was nitrogen mustard as this is less irritant to normal tissue compared to mustard gas. The initial results were encouraging and can be seen as the first steps on the path of the development of cytotoxic chemotherapy.[3]

Throughout the last 40 years, the search for effective small-molecule anticancer agents has been one of the significant goals of medicinal chemistry. Somewhat less than 40 agents are in clinical use today and can be classified as follows.

a) Alkylating agents - The alkylating agents used in cancer therapy form covalent bonds with a number of biologically active molecules including nucleic acids, proteins, amino acids and nucleotides and have the potential to damage cell membranes, deplete amino acid stores and inactivate enzymes. Although alkylation of these enzymes will eventually inhibit mitosis, it is generally considered that the formation of cross-linkages between DNA chains is the more important action. Alkylating agents are chemically diverse drugs which have the common property that they may undergo transformation to produce reactive intermediates which are electron deficient.

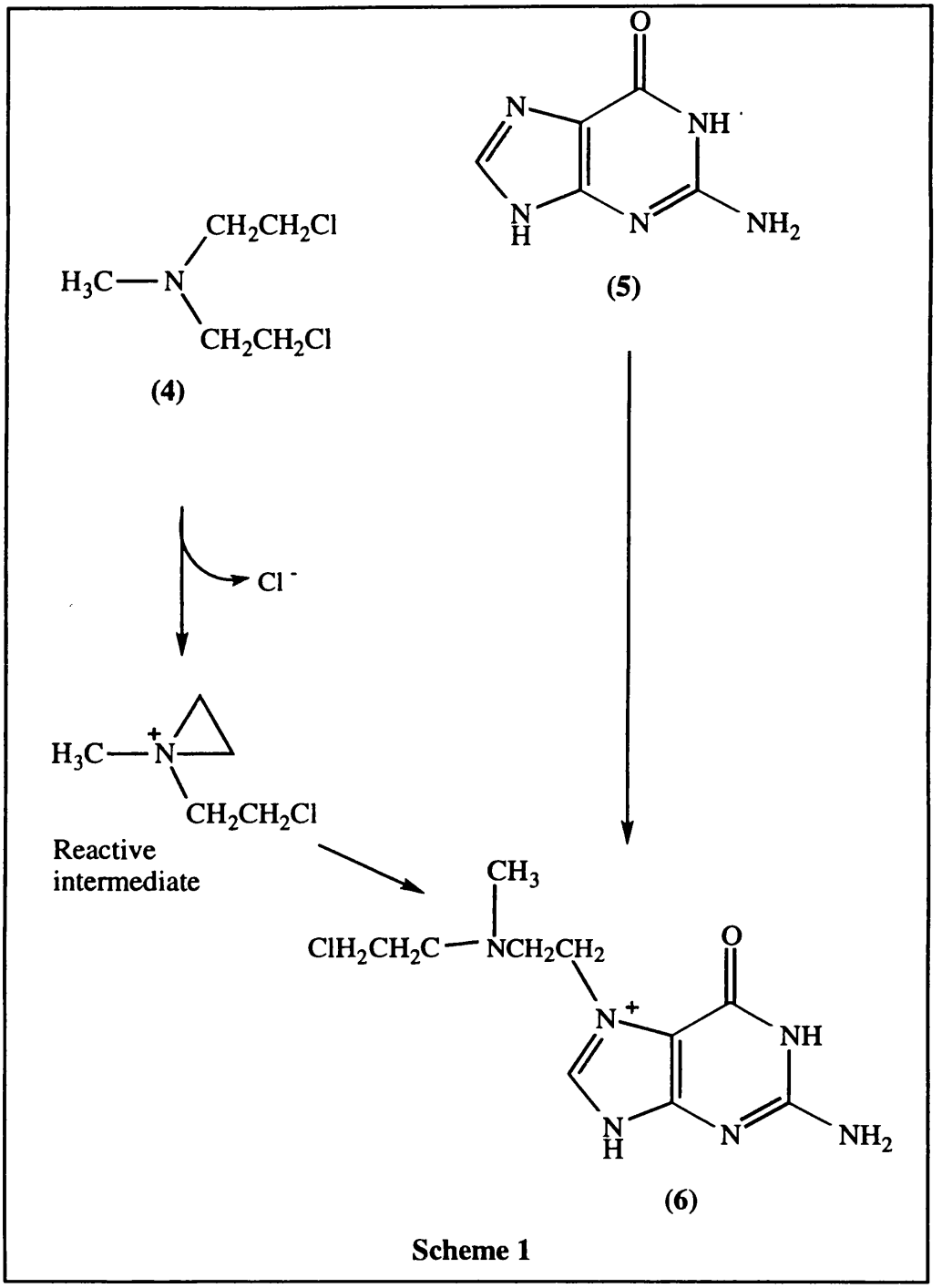
The family of nitrogen mustards contains several drugs which are in common clinical use, such as chlorambucil (1), melphalan (2) and cyclophosphamide (3).



Each of these are bifunctional with two chloroethyl groups which form the reactive electron-deficient groups responsible for alkylation of DNA.

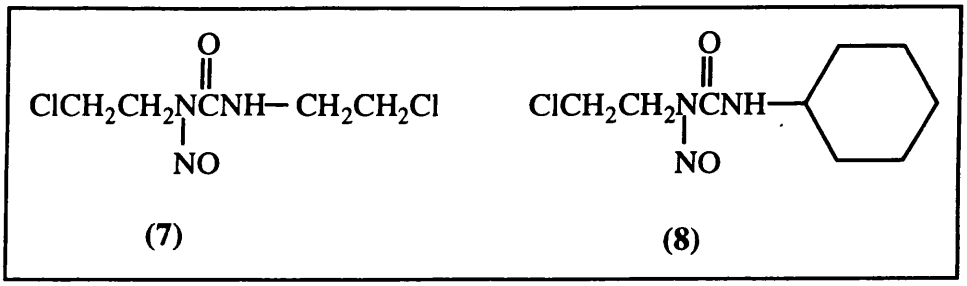
The most common site of alkylation of DNA is the N-7 position on the base guanine. The reactions leading to alkylation of guanine (5) are illustrated in Scheme 1 for nitrogen mustard (4). First, formation of the highly reactive positively charged aziridine ring occurs. This intermediate may then bind covalently with the electronegative N-7 group on a guanine base, resulting in alkylation (6). This may lead to mispairing with thymine or to strand breakage. The second chloroethyl side chain may undergo a similar reaction leading to covalent binding with another base on the opposite strand of DNA, leading to formation of a cross-link.

Nitrogen mustard (4) is still used clinically (e.g. as part of the four-drug "MOPP" protocol for Hodgkins' disease), but its reactivity makes it unstable and liable to cause irritation at the injection site.



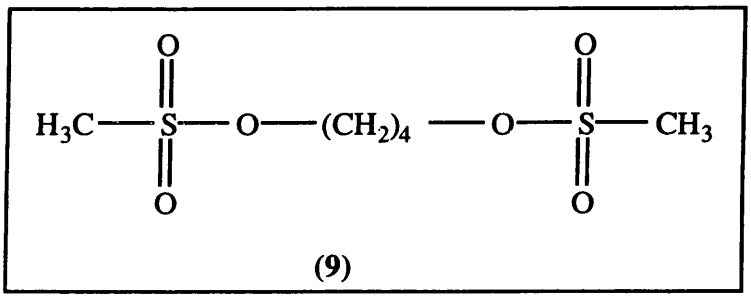
Scheme 1

The nitrosoureas BCNU (7) and CCNU (8) are lipid-soluble drugs which have the potential for penetration into the central nervous system for treatment of intracranial tumours. These drugs are effective for treatment of experimental tumours in mice, but have only limited clinical application. They tend to cause prolonged myelosuppression, probably because of direct effects on bone marrow stem cells.



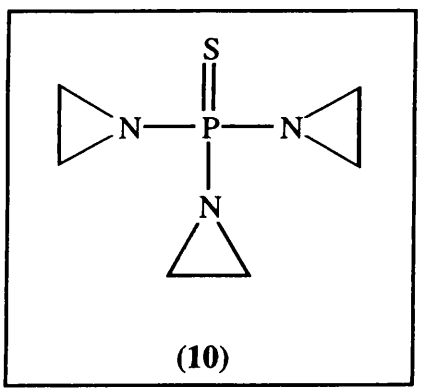
(7) (8)

Busulfan (9) is used for the treatment of chronic myelogenous leukaemia. The electron-deficient groups of bulsulfan are sulfonates and these can form covalent bonds to alkylate DNA and may form cross-links.



(9)

Thio-TEPA (triethylenethiophosphoramide) (10) is an example of the aziridine group of drugs and is now used primarily for local instillation in the bladder for treatment of patients with superficial bladder cancer.



(10)

b) Antimetabolites - Antimetabolites are compounds that have a similar structure to essential metabolites which are required in the cell for synthesis of nucleic acids and proteins so that the cell can divide. The cell may then be duped into incorporating the mimic compound and as a result either the new nuclear material will be unable to fulfil its function or enzyme inhibition will prevent protein synthesis. One example is methotrexate which is a folic acid antagonist.

c) Natural Products - This heterogenous group of compounds includes doxorubicin, bleomycin, the vinca alkaloids (such as vincristine), epipodophyllotoxins, mitomycin C and anthracyclines. Doxorubicin appears to intercalate DNA which can lead to partial unwinding of the DNA helix, although the mechanisms of the underlying toxicity remain variable, complex and only partially understood; probably mainly by involving inhibition of topoisomerase 2. Bleomycin causes DNA strand breakage through a complex sequence of reactions that involves the binding of a bleomycin-ferrous iron complex to DNA. The vinca alkaloids principle mode of action is to bind to tubulin, an intracellular protein. This prevents microtubule formation and hence halts mitosis. Microtubules are involved in a number of functions, including mitosis, transport of solutes, cell movement and maintaining the structural integrity of the cell. Mitomycin C is derived from a *Streptomyces* species and requires activation to an alkylating metabolite by reductive metabolism. This compound is discussed further in Chapter Two. The group of anthracyclines act as antimitotic antibiotics. Antibiotics are beneficial in the treatment of infections because they inhibit the multiplication of bacteria. In the 1950s, it became apparent that some of the compounds had a similar inhibitory effect on dividing tumour cells.

e) Miscellaneous Compounds - These include non-classical alkylating agents, such as cisplatin, and enzyme therapy.

Most of the major developments in cancer therapy over the last 25 years have been in the field of drug treatment. The advances have frequently been dramatic and impressive, with cure now possible in a number of previously fatal conditions, such as acute leukaemia, advanced lymphoma, a number of childhood malignancies, metastatic

testicular cancer and choriocarcinoma. Unfortunately, however, cancers curable with chemotherapy form only a small proportion, less than 10%, of all malignant tumours and it is the older modalities of surgery and radiotherapy which still account for the great majority of cancer cures today. This is especially true in the solid tumours, the carcinomas and the sarcomas, which account for over 90% of cancers. In these instances, with the possible exception of early breast cancer and a few uncommon conditions, chemotherapy has yet to make any significant impact on cure rates or survival times.

1.3 Drug Selectivity

Selectivity of existing anticancer agents is still most unsatisfactory. Almost all agents in clinical use owe their limited use to a selective toxicity towards proliferating cells, and, as a consequence, are toxic to normal tissues with high rates of cell turnover.

Achieving selectivity for tumour cells, along with the development of drug resistance, has always been the limiting factor in the development of cytotoxic substances as anticancer drugs. Recent research in tumour biology has shown that solid tumours are structurally very complex. Cells in solid tumours exist in a number of distinct microenvironments, which can be defined largely by the accessibility of the cells to oxygen. In most solid tumours, this is dictated by the limitation of tumour vascular networks, which, although stimulated to develop by angiogenesis factors secreted by tumour cells,[4] remain growth-limiting on the tumour.[5] This microenvironmental heterogeneity provides a level at which to seek tumour selectivity. Unique and potentially exploitable microenvironmental factors which cells in solid tumours experience include poor venous drainage, low intracellular pH due to increased glycolysis, a primitive vasculature and chronic hypoxia.[6,7]

1.4 Tumour Hypoxia

For a solid tumour to reach a diameter greater than a few millimetres, it must develop its own vascular network.[4] This vasculature is not efficient and results in many cells existing in a state of chronic (irreversible) hypoxia because of their relatively long distance from the nearest blood vessel. Another type of hypoxia, acute hypoxia, caused by the spasmodic closing off of a blood vessel due to compression by the growing tumour, has also been demonstrated in animal tumours.[8] The two forms of hypoxia are shown schematically in Figure 1.[9]

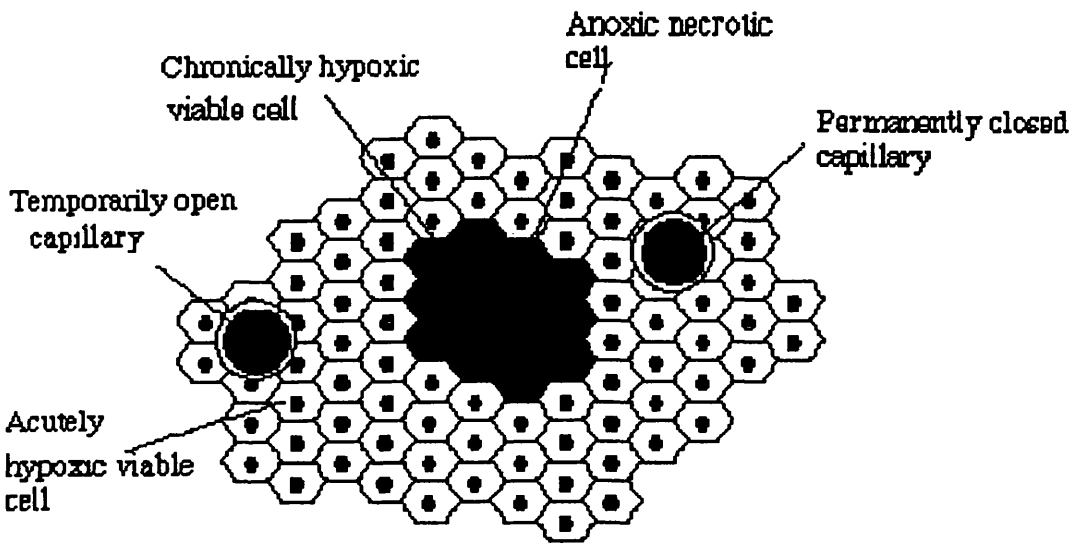


Figure 1

Hypoxic cells have been demonstrated to exist in human solid tumours[4,10] and have been shown to be resistant to radiation treatment and chemotherapy.[11] Despite these difficulties, the hypoxic microenvironment is an attractive target since nearly all normal tissue is well perfused, and drugs which could be activated only in hypoxic regions offer the possibility of being truly selective for solid tumours.[12]

To gain selectivity in this way, it is necessary to design a prodrug which is inactive in its own right, but can undergo metabolic reduction in hypoxic cells to release a toxic species which can then damage biomolecules *in situ*. These prodrugs are termed

hypoxia-selective cytotoxins and because they are activated by reductive biotransformations, they are commonly referred to as bioreductive drugs. They would in fact be more correctly termed bioreducible drugs.

1.5 Reductase Enzymes

A second dimension of selectivity can be introduced as it is known that a number of reductase enzymes are hyperexpressed in some solid tumours. This approach has been termed 'Enzyme-directed Bioreductive Drug Development'.[13] The enzymes concerned are able to catalyse reduction of bioreducible agents. Some examples include cytochrome P-450, cytochrome P-450 reductase, xanthine oxidase, aldehyde oxidase and DT-diaphorase. Since different enzymes participate to different extents with various bioreducible agents, and even catalyse activation in one case and protection in another, it follows that if an enzyme profile is known for an individual tumour, then a complementary drug can be chosen for treatment. The enzyme specificity data could also be used to modify the structure of a bioreducible agent to suit the catalytic preference of a particular activating reductase which might be hyperexpressed in a particular tumour. Conversely, it should be possible to design out activity with respect to an activating enzyme that predominates in normal tissue.

1.6 Bioreducible Agents

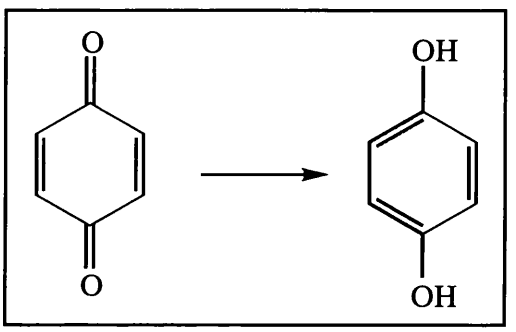
Bioreducible agents are thought to act *in vivo* and *in vitro* through their initial activation upon enzymatic one electron reduction to produce metabolites, which are considerably more cytotoxic than the parent compound. Such bioreduction is critically dependent upon such factors as oxygen tension, the redox potential of the agent and temperature, the latter reflecting the rate of enzymatic steps. Oxygen acts by protecting against formation of reduced metabolites of the agents through 'futile cycling', a process which generates superoxide at the expense of the one electron reduced intermediate of the

bioreducible agent. Therefore their preferential cytotoxicity towards hypoxic cells is as a consequence of the protective role of oxygen under aerobic conditions. Agents which exhibit such bioreducible properties may have potential application as chemotherapeutic agents of hypoxic cells commonly found in tumours. There are currently four functional groups which are under investigation for use as bioreducible agents, all of which are known to be reduced metabolically via pathways which can be inhibited by oxygen. The reduction potential of the prodrug must lie within the range required for cellular reductants i.e. between -200 and -400 mV *vs* normal hydrogen electrode.

a) Nitro to amine.

$$R\text{-}NO_2 \longrightarrow R\text{-}NH_2$$

b) Quinone to hydroquinone.



c) *N*-Oxide to tertiary amine.

$$R_3N^+\text{-}O^- \longrightarrow R_3N$$

d) Transition metal complexes.

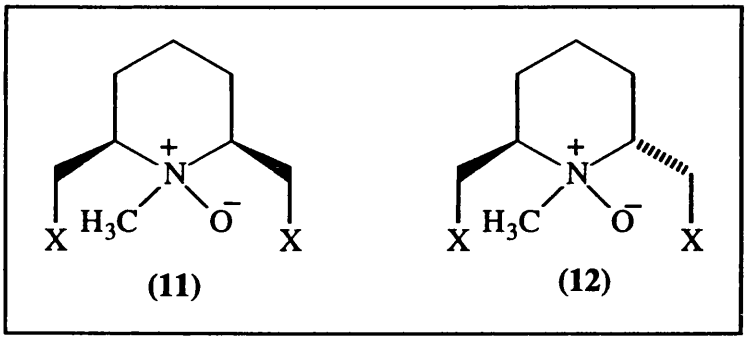
$$M^{(III)}L_6 \longrightarrow M^{(II)}(H_2O)_6 + 6L$$

1.7 Aims of This Project

Achieving selectivity between cancer cells and normal cells is of fundamental interest. The area of bioreducible drugs is still a relatively new area of cancer research and this field is reviewed in Chapter 2. The potential to develop new hypoxia selective cytotoxins which could act as bioreducible drugs is considerable. Accordingly, this research project was directed primarily to the synthesis of novel heterocyclic prodrugs

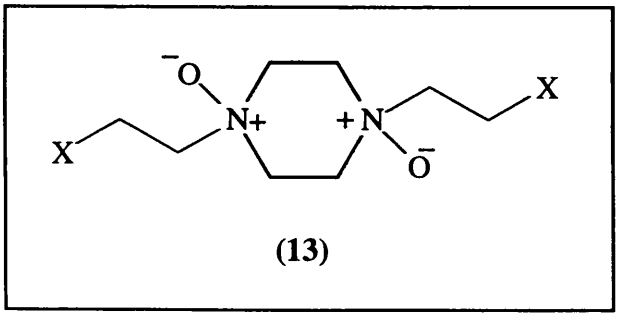
containing *N*-oxides which might be activated under hypoxic conditions to release toxic species. These compounds can be divided into 3 groups.

i) Mono *N*-oxides :- *cis*- (11) and *trans*- (12) 2,6-disubstituted *N*-methylpiperidine-*N*-oxides in which X is a leaving group such as a halogen or a carbamate. It was hoped that the non-toxic *N*-oxide would be reduced in the tumour cell to form a toxic alkylating species.

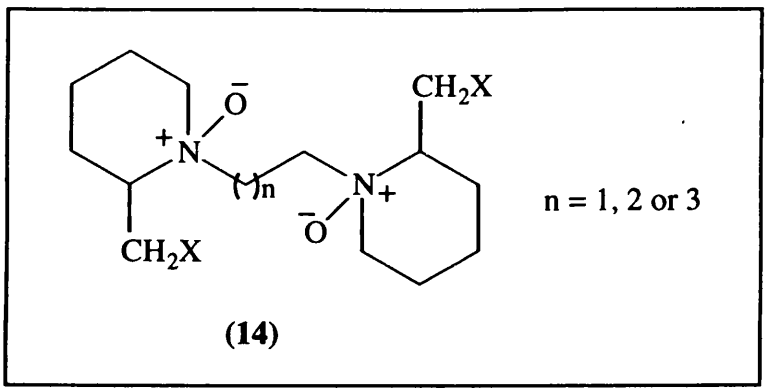


Both the *cis*- and *trans*-isomers were to be synthesised as the difference in spatial arrangements may be important in cross-linking of DNA. The rationale for choosing these compounds and the work related is described in Chapters 3 and 4.

ii) Incorporation of another *N*-oxide to produce a bis-*N*-oxide can be achieved by making piperazine derivatives (13) and this work is discussed in Chapter 5.

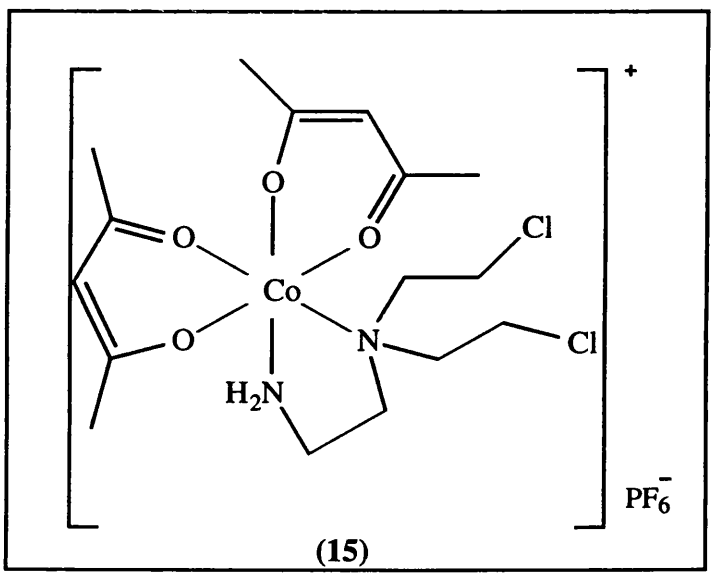


iii) By joining together two piperidine rings with a carbon chain of varying length (14), a series of derivatives containing bis-*N*-oxides can be obtained and their biological activity compared. Their synthesis is described in Chapter 6.



(14)

The possibility of using metal complexes as bioreducible drugs has recently been demonstrated.[14] A number of diamines with alkylating functionality have been synthesised and complexed to cobalt(III) such as compound (15).



(15)

Synthesis of cobalt complexes containing nitrogen mustards is discussed in Chapter 7.

Detailed study of the activation of these prodrugs by reductase enzymes and cytotoxicity tests of the potential toxic species are presented in Chapter 8.

Chapter 2

Bioreducible Drugs

2.1 Hypoxia and Resistance

Hypoxic cells are those existing at an oxygen concentration which is suboptimal for cell growth and metabolism, but sufficient to maintain viability. Cells that are severely hypoxic at the time of irradiation are about three times more resistant to ionising radiation than aerobic ones. Hypoxia protects against radiation because molecular oxygen is a potent radiosensitiser. When cells are irradiated with X-rays or γ-rays, only a small fraction of the lethal damage results from direct interactions between radiation photons and DNA. Instead, most damage is produced indirectly. Absorption and scattering of the photons by atoms results in the ejection of high energy electrons. As these fast moving electrons travel through the cell, they interact with nearby atoms, producing a track of ion pairs. These short lived ions react with each other and nearby molecules to produce a wide variety of free radicals and reactive species. Chemical reactions between these species and critical biological molecules (such as DNA) produce biologically important damage that can kill, injure or mutate the cell. When oxygen molecules are present at the time of irradiation, these extremely electron-affinic molecules participate in the chemical reactions that lead to the production of biologically significant damage, causing an increase in the number and severity of the lesions over those produced in the absence of oxygen.

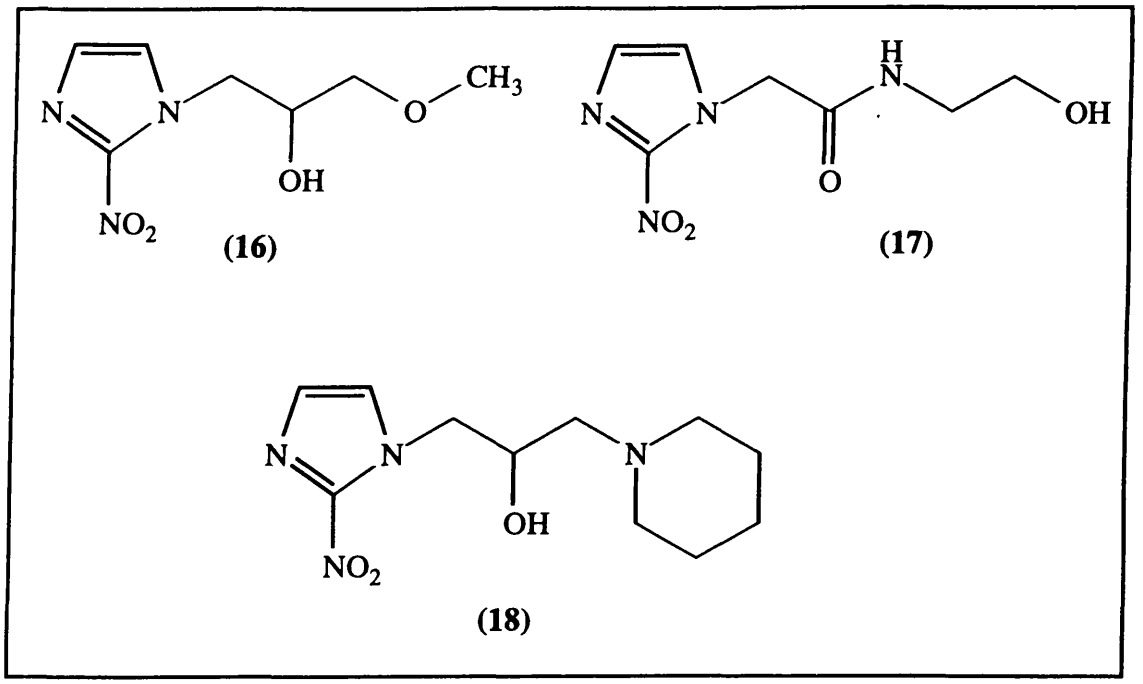
In addition to exhibiting resistance to radiation, hypoxic cells can be more resistant to various chemotherapeutic agents, since hypoxic cells may be blocked or slowly moving through the cell cycle, and can also be too distant from the nearest blood vessel to allow sufficient concentrations of the drug to reach the cells.

To overcome this problem of tumour hypoxia, fractionated treatment is used in clinical radiotherapy, where the tumour is re-oxygenated between treatments. A number

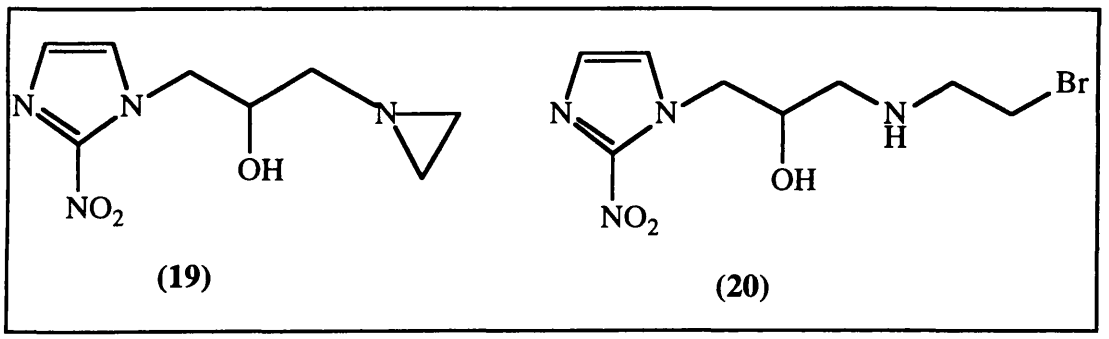
of ways to improve delivery of oxygen to tumours have been explored[15] but most interest has been in the development of chemicals which can take the place of oxygen in radiosensitising hypoxic cells. These have been termed electron-affinic radiosensitisers. These electron affinic compounds take the place of oxygen by transferring an electron to the initially formed DNA radicals, but are not so actively metabolised, so that they can reach the hypoxic interior of solid tumours.

2.2 Nitroimidazole Radiosensitisers

Following on from early work with the 5-nitroimidazole metronidazole, the first radiosensitiser to show some clinical activity was the 2-nitroimidazole derivative, misonidazole (16).[16] However, this compound had limited potency and efficacy, and the high concentrations required caused neurotoxicity. This led to developing analogues which focused on improving the pharmacokinetics of misonidazole (16),[17] to improve cell uptake (and thus potency) while lowering the relative uptake into the brain. Two analogues currently in clinical trials are etanidazole and pimonidazole. Etanidazole (17) has the advantage of relative exclusion from nervous tissues,[18] and doses three times that of misonidazole can be given to patients before neurotoxicity is observed. Pimonidazole (18) is a more potent agent, and accumulates in tissue by virtue of the basic piperidine function.[19,20] It was predicted that its basicity would enhance its excretion in acid urine, leading to a shorter elimination half-life and reduced normal tissue exposure.[21] It was further hoped that its basicity might lead to concentration in areas of low pH such as are commonly thought to occur in tumours. This, together with its higher electron affinity, would be expected to confer greater potency than misonidazole.



One strategy for improving the radiosensitisation of the 2-nitroimidazoles was to attach to the molecule an alkylating agent which would bind the drug covalently to DNA. The most well studied compound is the aziridine analogue, RSU-1069 (19),[22] which does show greater radiosensitisation. Unfortunately, clinical trials showed dose-limiting gastrointestinal toxicity,[23] but the derivative RB 6145 (20)[24] which is a prodrug of RSU 1069 and contains a masked aziridine moiety is less toxic and is now in preclinical development.



2.3 Bioreducible Cytotoxins

During the development of nitroimidazoles it was recognised that these agents express the ability to kill radiobiologically hypoxic cells to a greater extent than oxic cells.[25] This hypoxia-selective cytotoxicity is now recognised as offering an alternative strategy for eliminating hypoxic cells. Indeed, it may be preferable to kill hypoxic cells with a cytotoxin rather than sensitising them to radiation, so optimisation of hypoxia-selective cytotoxicity is a key objective in the design of drugs to overcome the oxygen problem in radiotherapy. Reasons which support this view include:

a) Hypoxia selective cytotoxins (HSCs) can turn hypoxia to their advantage if a high proportion of tumour cells spend some of their time in a hypoxic state and can thus be eliminated by frequent treatment with a HSC.[26]

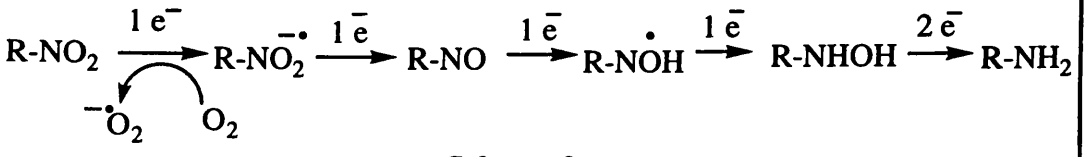
b) Bioactivation of the HSC in hypoxic tumour microenvironments may produce an active species which can diffuse locally to attack surrounding oxic cells.

d) HSCs have potential application in chemotherapy as well as radiotherapy if hypoxic cells are commonly resistant to available chemotherapeutic agents.

Two broad mechanisms for selective toxicity against hypoxic cells can be distinguished. The first is the exploitation of hypoxic cell biochemistry, such as with glucose analogues which are selectively toxic because of the dependence of hypoxic cells on high rates of anaerobic glycolysis.[27] The second involves the use of prodrugs which are activated under hypoxia or by high levels of reductase enzymes found in tumour cells.[28] HSCs of this class are widely referred to as bioreducible drugs since the biotransformation by which they are activated is a reductive one. The original idea[29] suggested that hypoxia in tumours might generate a reducing environment conducive to such reactions. Existing bioreducible agents make use of oxygen-sensitive biotransformation of four different types of functionality : nitro, quinone, *N*-oxide and transition metal complexes. These will now be discussed separately in the following sections.

2.4 Nitroaromatics and Nitroheterocyclics

Many nitroaromatics are selectively toxic under hypoxic conditions because the first reduction of the nitro group (Scheme 2) to more toxic products is inhibited by oxygen, resulting in preferential metabolism in hypoxic cells.[30]

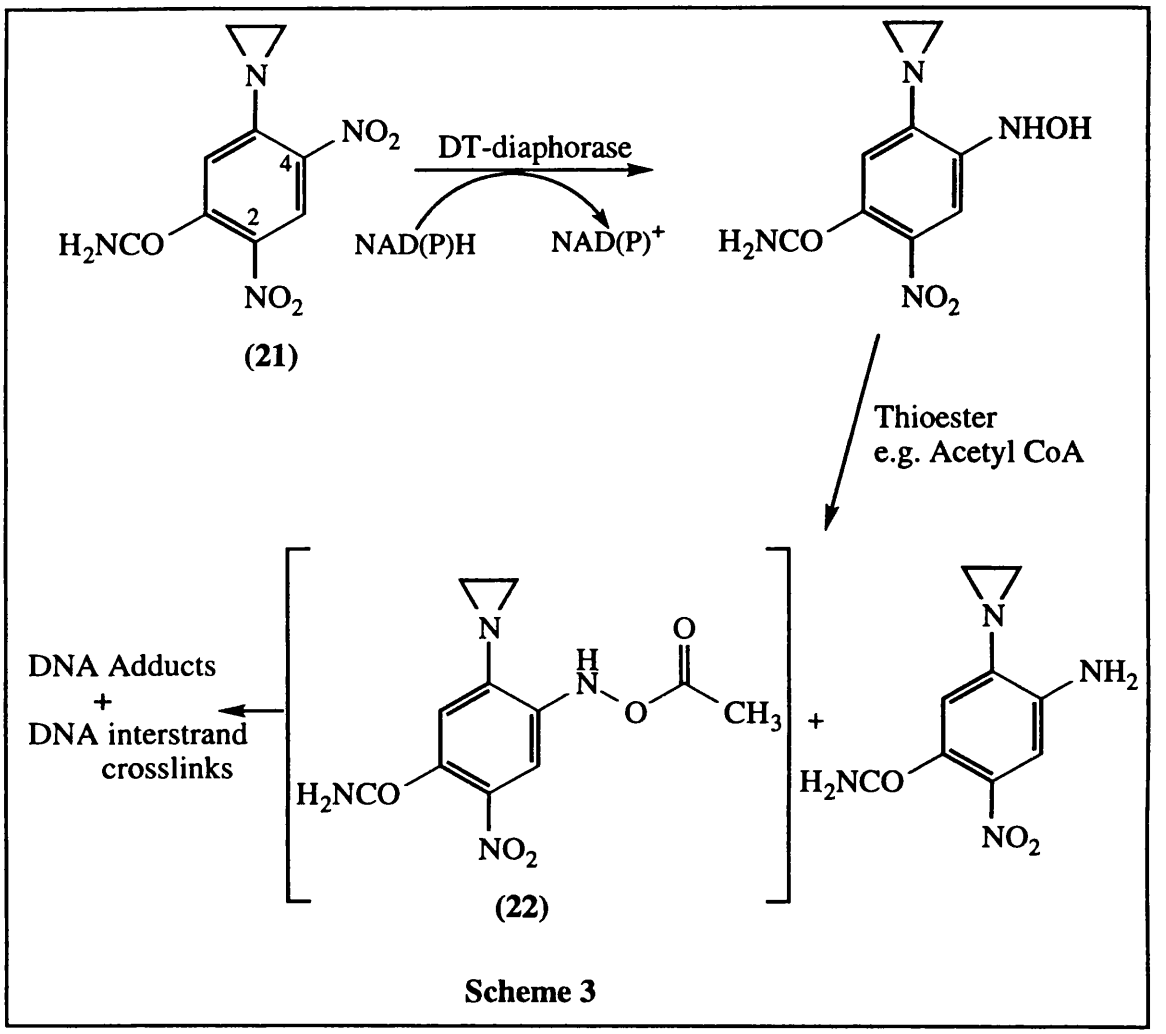


Scheme 2

The 2-nitroimidazole misonidazole (16) shows a hypoxic selectivity of *ca* 20-fold but has low cytotoxic potency (millimolar).[31] In comparison, RSU-1069 (19) not only has much higher cytotoxicity, but also shows enhanced selectivity for hypoxic cells (*ca* 50-100-fold).[16] While the predominant mechanism of cytotoxicity of this compound under aerobic conditions is DNA monoalkylation by the aziridine, reductive metabolism converts it into a much more toxic bifunctional alkylating agent capable of cross-linking DNA.[32] It has been demonstrated that the major sites for adduct formation upon the deoxyribosenucleotides are the phosphate groups and purine bases. These interactions are in competition with the slow hydrolysis of RSU-1069 to yield the corresponding aziridine ring-opened hydroxylated product which is inert to interactions with DNA.

CB 1954 (21)[33] is an agent which shows dramatic antitumour selectivity towards the rat Walker 256 tumour. The basis of the sensitivity is that CB 1954 forms DNA interstrand crosslinks in Walker cells but not in insensitive cells. Crosslink formation is due to the aerobic reduction of CB 1954 by the enzyme DT-diaphorase. The 4-hydroxylamine cannot crosslink DNA directly but requires further activation by a non-enzymatic reaction with a thioester (such as acetyl coenzyme A). The bioactivation of CB 1954 is shown in Scheme 3. The initial step is the reduction of CB 1954 by the enzyme DT-diaphorase to form the hydroxylamine product which can then react with thioesters to

produce DNA reactive species. It is postulated that this is the *N*-acetoxy derivative (22). The major product of this reaction is however, the reduced amino compound that does not react readily with DNA.

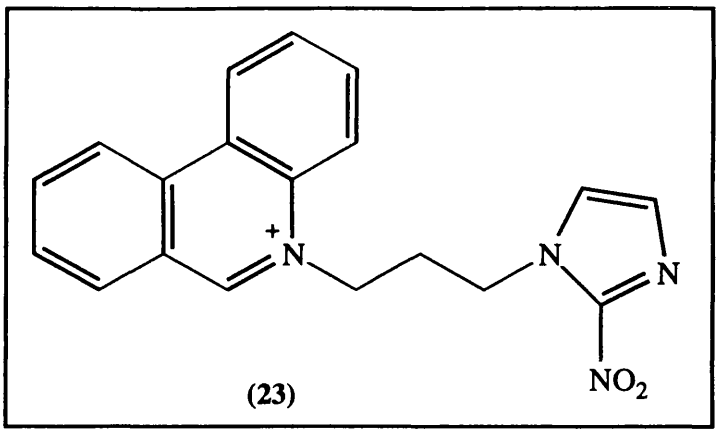


Scheme 3

The bioactivation of CB 1954 results in a vast increase in its cytotoxicity and the resulting dose modification can be up to 100,000-fold. Although this compound possesses the latent cytotoxicity required of a prodrug, this approach is thwarted by the intrinsic poor rate of reduction of CB 1954 by the human form of the enzyme DT-diaphorase.

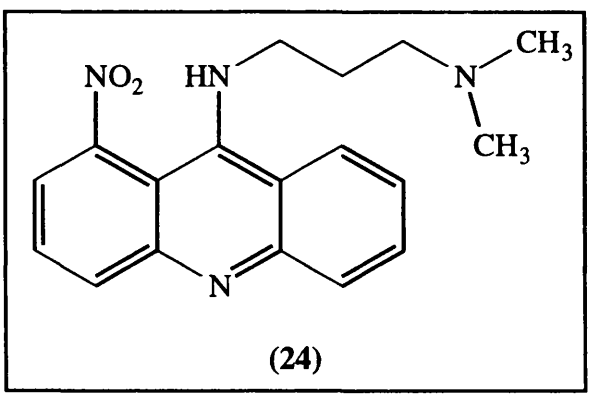
In attempts to improve the intrinsic cytotoxicity of the 2-nitroimidazoles, while preserving their selectivity, they have been attached to various types of reversible DNA-binding carriers. In such a manner, the phenanthridinium analogue, NLP-1 (23) shows

10-100-fold greater potency than misonidazole as a hypoxia-selective cytotoxin, even though the compound does not bind particularly well to DNA.[34]



(23)

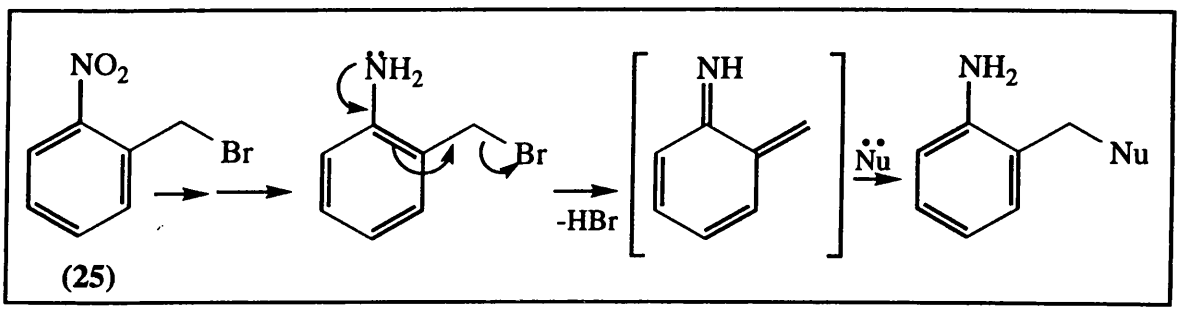
A related approach involves the use of compounds which exhibit both hypoxia selectivity and DNA affinity in the same chromophore. The best known examples are 1-nitroacridines, such as nitracrine (24). This compound shows very potent HSC activity against tumour cells in culture,[35] but is not active against hypoxic cells in solid tumours *in vivo* where it is suggested that it binds too tightly to DNA, resulting in slow extravascular diffusion.[36]



(24)

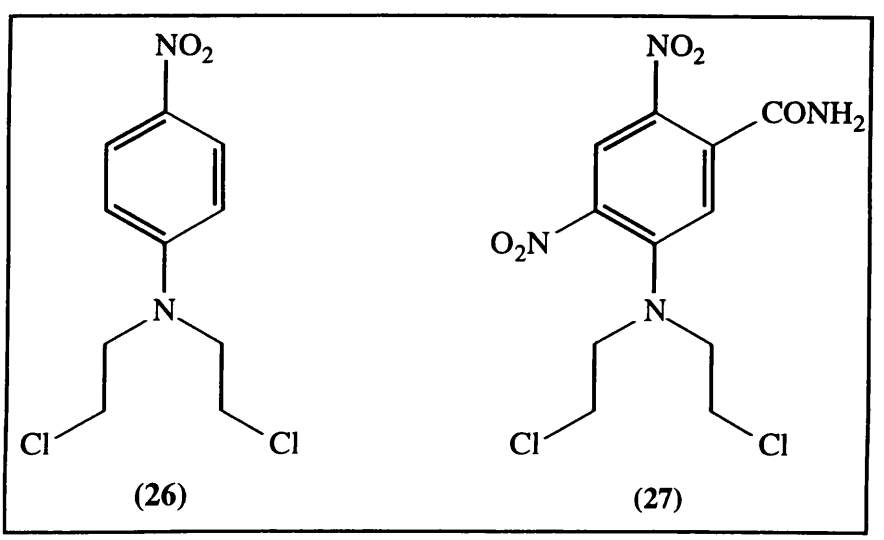
An alternative strategy for providing very cytotoxic species on reduction is the use of nitroaromatic compounds as prodrugs for the release of toxic moieties. In these compounds, a DNA-alkylating moiety is activated by cellular reduction of the nitro group. Since the stability and alkylating reactivity of aromatic nitrogen mustards is

determined almost entirely by the electron density on the nitrogen,[37] conversion of the powerfully electron-withdrawing nitro group into the electron-donating hydroxylamine or amino metabolites activates the mustard considerably.[38] One of the earliest attempts used nitroreduction as the trigger for methide generation.[39] *o*-Nitrobenzyl halides such as (25) showed moderate hypoxic selectivity *in vitro*, and were much more potent than nitroimidazoles of similar reduction potential.



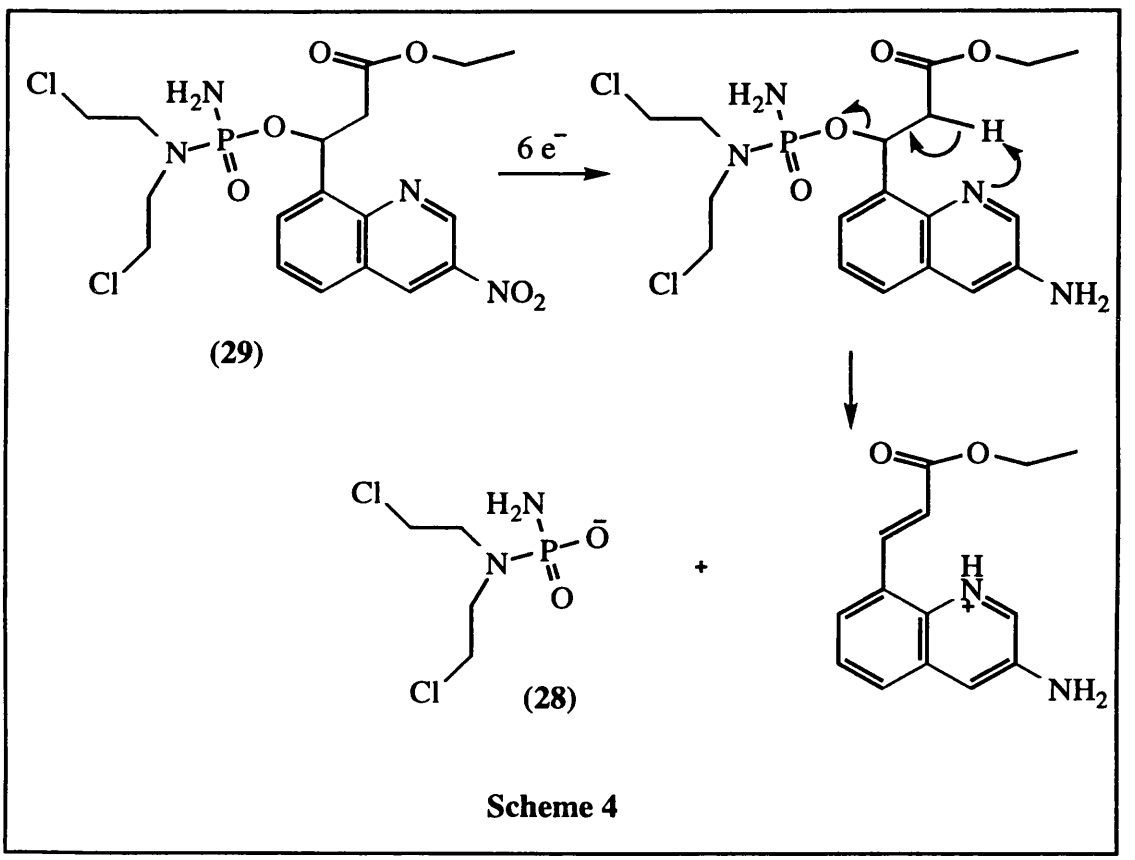
(25)

Mononitrophenyl mustards such as (26) have little hypoxic selectivity in cell culture, because the reduction potential (*ca.*-520 mV) is too low to allow appreciable reduction.[40] However, some dinitrocarboxamide analogues such as SN 23862 (27) with higher reduction potentials (*ca.*-450 mV) have more recently been shown to be highly selective for hypoxic cells (70-80-fold) in cell culture.[41]



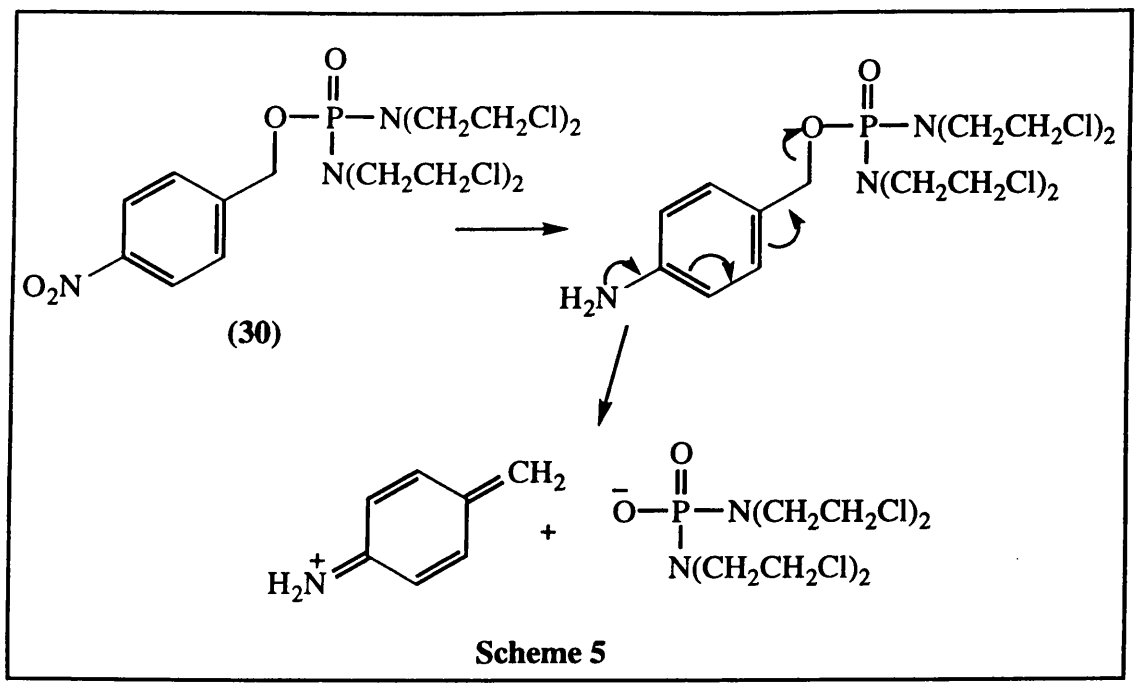
(26) (27)

The release of the much more toxic phosphoramide mustard (28) following reduction of a 3-nitroquinoline (29) has recently been cited.[42] The increased basicity on reduction to the electron-donating hydroxylamine or amine is proposed as triggering intramolecular base-catalysed elimination of the phosphoramide (Scheme 4). Compound (29) was shown to be 11-fold more toxic to hypoxic than aerobic HT-29 human colon tumour cells.



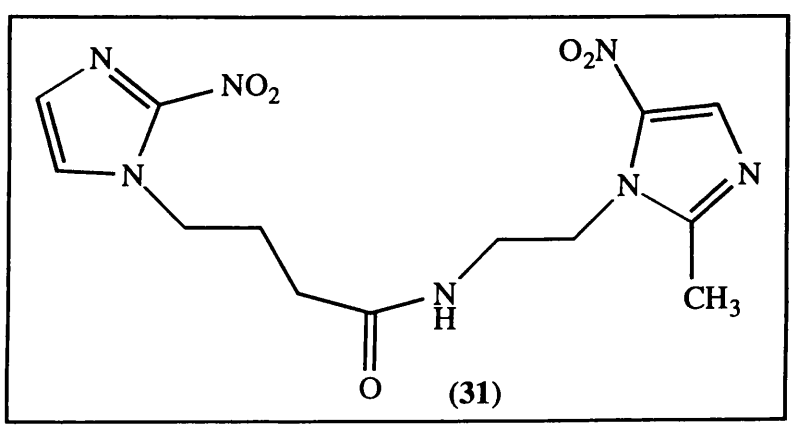
Scheme 4

A similar idea proposes that nitrobenzyltetrakis(chloroethyl)phosphorodiamidates (30) can be reduced to leave the corresponding aniline derivative which will then activate the alkylating moiety (Scheme 5).[43] Initial results have shown these compounds can be up to 90-fold selective towards hypoxic cells *via* alkylation of DNA.

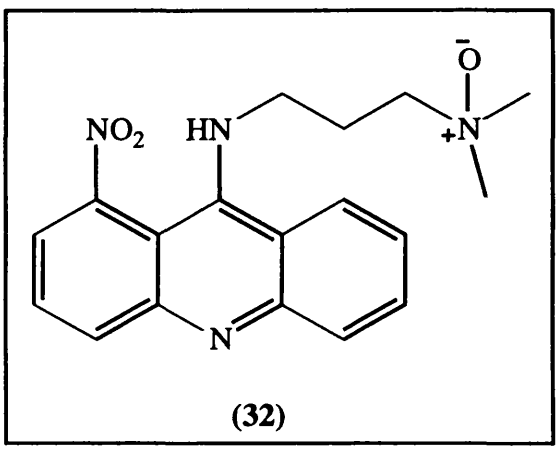


Scheme 5

A series of novel bis(nitroimidazolyl)alkanecarboxamides such as (31) have recently been prepared by Denny and Wilson[44] to act as bis-bioreducible agents possibly by generating DNA crosslinking agents when both nitro groups are reduced to reactive species. This compound (31) showed a high degree of toxicity with time under hypoxia which suggests that these compounds may be bis-bioreducible agents, with slow reduction at the redox centre of lower potential (5-nitroimidazole) generating a bis-reactive molecule under hypoxia.

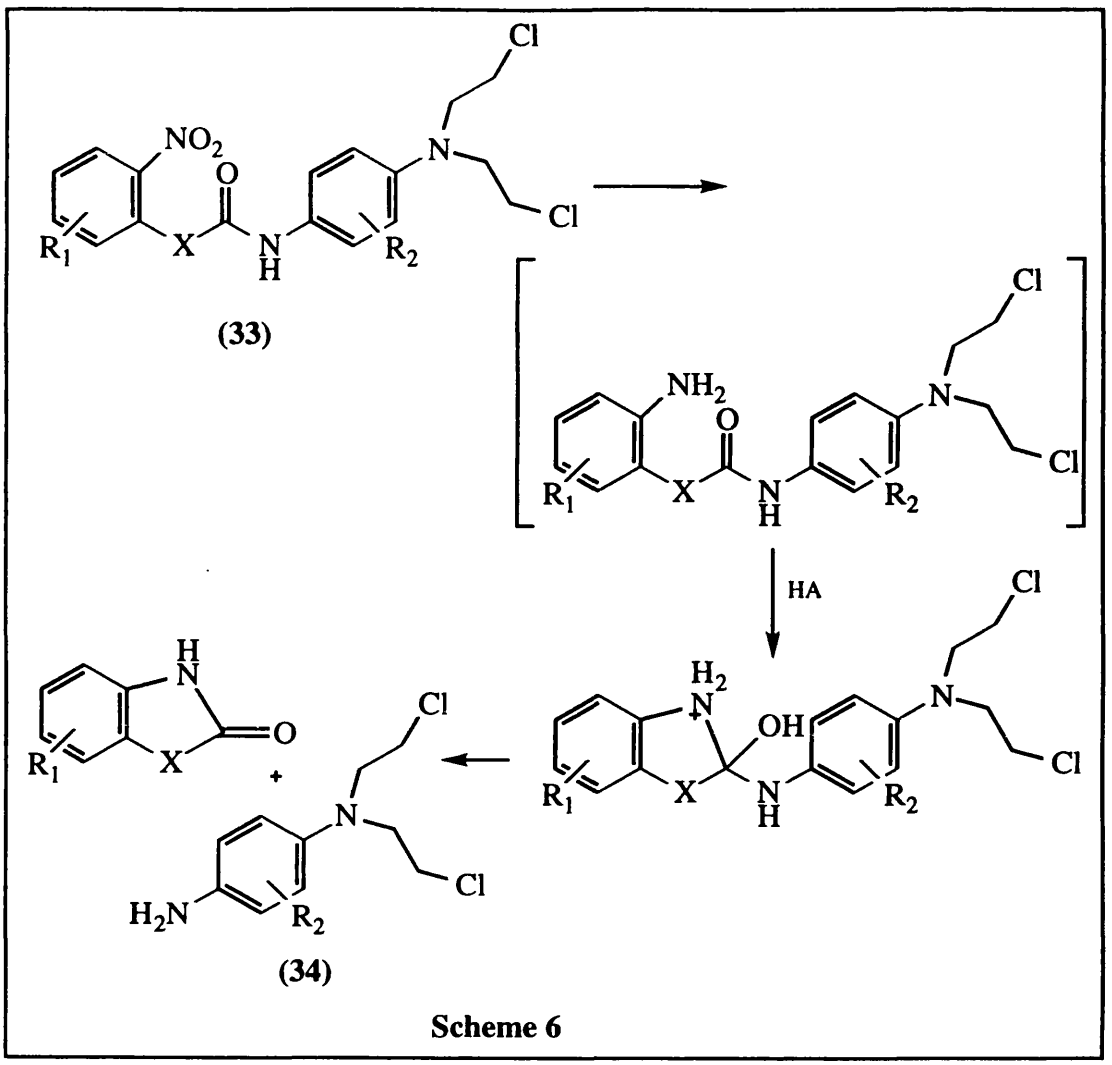


The same authors have also shown that another bis-bioreducible agent can achieve very high levels of hypoxic selectivity (> 1000-fold). The aliphatic *N*-oxide derivative (32) of the DNA intercalating bioreductive agent nitracrine[45] has been synthesised.[46] This compound is of particular interest since the formally neutral side chain might be expected to decrease DNA binding and, consequently, cytotoxicity. Full activation appears to require reduction of both the *N*-oxide (to increase physical DNA binding) and nitro groups (to generate reactive metabolites); both steps being inhibited by oxygen.



(32)

2-Nitroaryls of general structure (33) have been proposed as bioreducible drugs, capable of releasing cytotoxic aminoaniline mustards (34) on bioactivation.[47] This concept allows separate optimisation of the substituent effects influencing nitro-group reduction and mustard reactivity by electronically decoupling the nitro group and the mustard.



Scheme 6

In this design (Scheme 6), reduction of the nitro amide to the corresponding amine is followed by a spontaneous cyclisation to the tetrahedral intermediate. Breakdown of this results in formation of the lactam and release of the amine. The net conversion would expel an aromatic mustard bearing an amine substituent, which will be a much more reactive alkylating agent than the starting amide, due to an enhanced electron release.

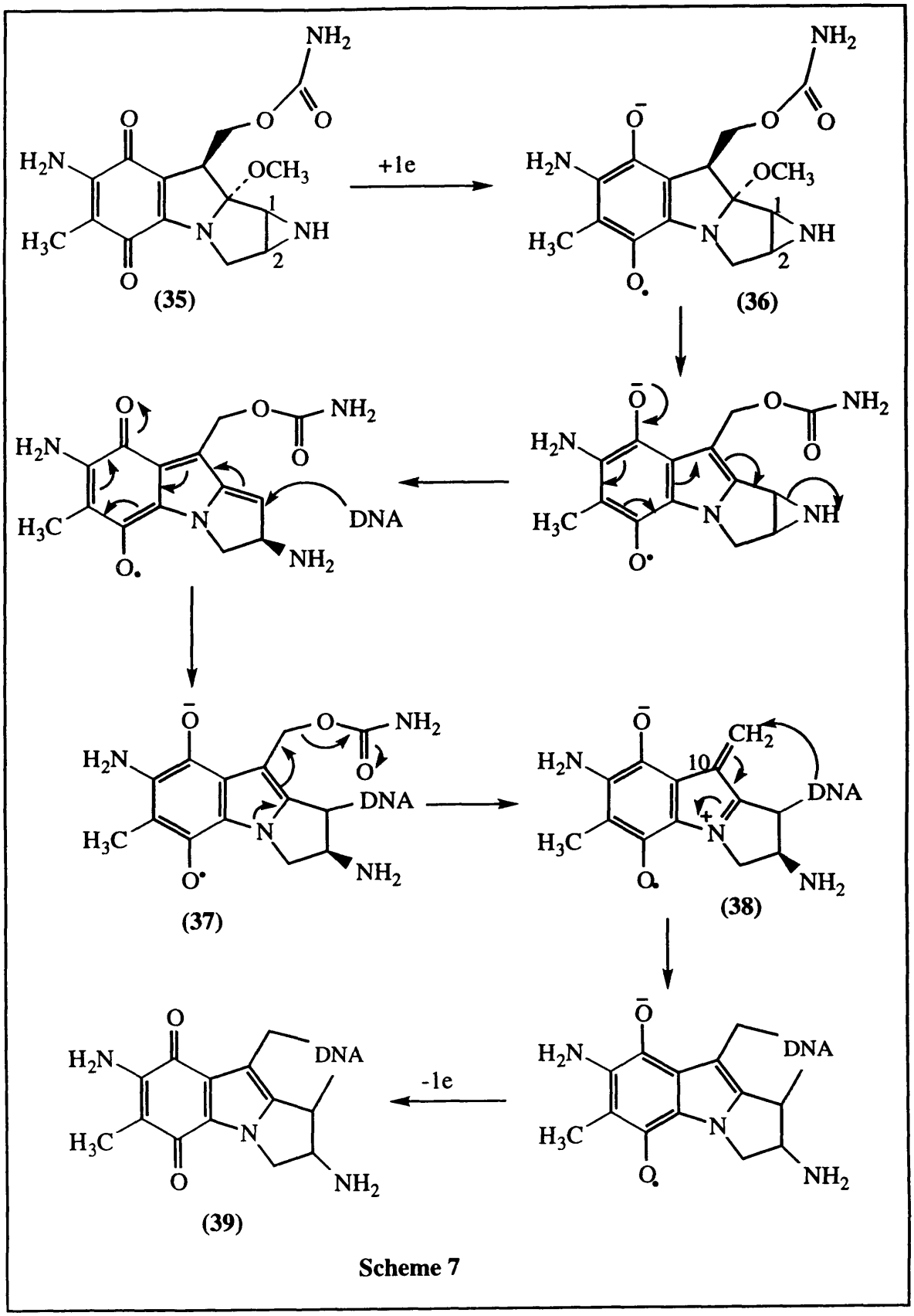
2.5 Quinones

Reduction of variously substituted 1,4-benzoquinones represents a second major biotransformation which can be inhibited by molecular oxygen. Like the nitroaromatics,

the quinones undergo 1-electron reduction, first to the semiquinone, and then another 1-electron reduction to the hydroquinone. The semiquinone radical is capable of being scavenged by oxygen in oxygenated normal tissues, resulting in a hypoxia-selective metabolism, although direct oxygen-irreversible two-electron reduction pathways (e.g. by the enzyme DT-diaphorase[13]) are also possible. The reduced quinones generally have low toxicity, so those in use usually contain potential alkylating functionality which is activated upon reduction.

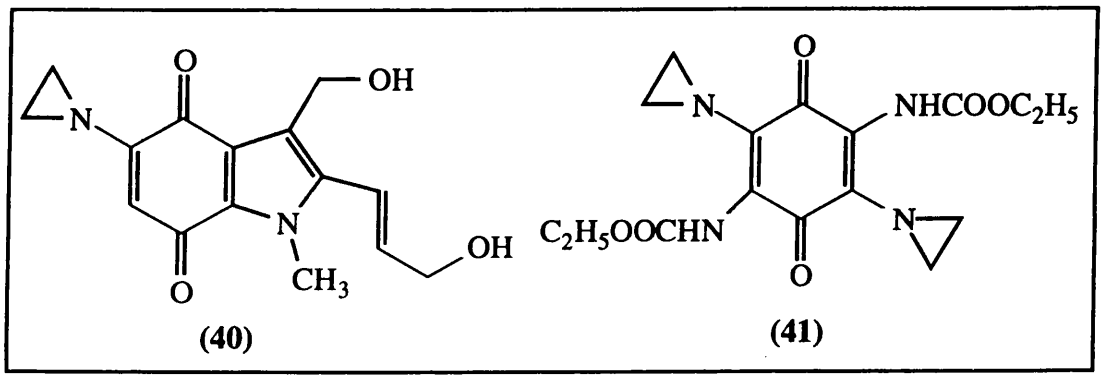
The quinone-containing antitumour antibiotic, mitomycin C (MMC) (35) was in clinical use as an anticancer agent well before the development of synthetic quinones as bioreducible alkylating agents, and can be considered as the prototype for this class since reduction to the semiquinone or dihydroquinone activates its alkylating activity.[48] The proposed mechanism for the activation of mitomycin C can be seen in Scheme 7.[49]

Following reduction to the mitomycin semiquinone radical anion (36), the C-1 position of the aziridine ring is activated and this results in alkylation at the N-2 position of guanine in DNA (37). Subsequent loss of the carbamate group leads to the formation of an additional reactive centre at C-10 (38), which then alkylates at a second neighbouring guanine N-2 forming a bis-adduct (39).[50] Both inter- and intra-strand links can be formed. Computer modelling shows that the bis-adduct fits snugly into the DNA minor grove with minimal distortion of the Watson-Crick double helix, possibly reducing the probability of damage recognition and repair. MMC is not as selectively toxic to hypoxic cells (*ca.* 2-5-fold) as the 2-nitroimidazoles and is markedly cell-line dependent.[51] It has been shown to provide improved survival as a supplement to radiotherapy of head and neck tumours[52], although it has not been established that its use in this context is a consequence of hypoxic cytotoxicity.



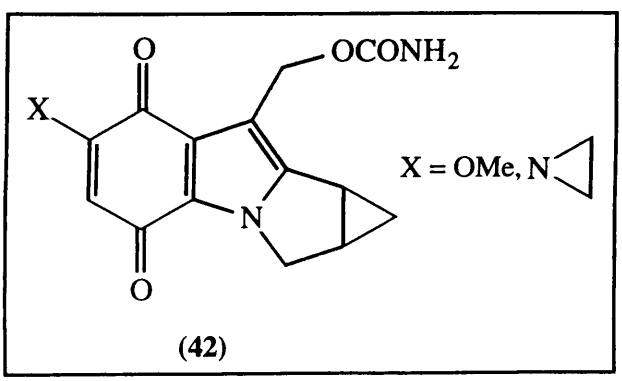
Scheme 7

The limited hypoxic differential of MMC has stimulated a search for more selective analogues. Synthetic aziridinyl quinones, such as the indoloquinone EO9 (40) synthesised by Oostveen and Speckamp[53] has hypoxic selectivity much superior to MMC *in vivo*.



Reduction of EO9 will activate the hydroxyl leaving groups and also the aziridine, giving a total of three reactive centres generated in the molecule as a result of bioreductive metabolism. The two aziridine moieties in diaziquone (41) are similarly activated on reduction, resulting in the formation of potentially lethal crosslinks,[54] but is similar to MMC in that it has only modest selectivity for hypoxic cells.[55]

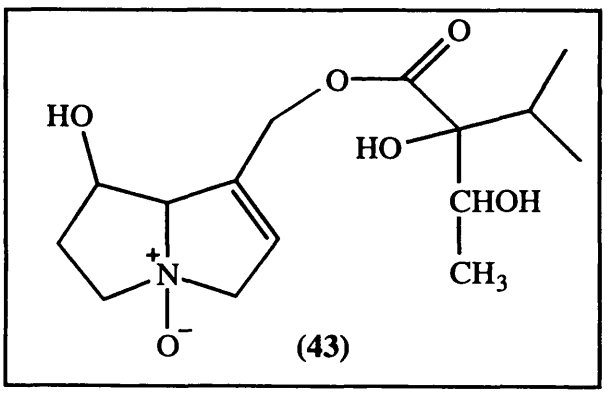
Another approach has been to investigate the role of C-10 in alkylation processes by preparing compounds in which the nucleophilicity at C-1 is reduced substantially by substituting a cyclopropane ring for the aziridine ring.[49] Such a compound (42), named a cyclopropamitosene, on reductive activation followed by elimination of the carbamate, could generate a powerful electrophile capable of alkylating DNA at C-10.



Under hypoxic conditions, the potency of the methoxy derivative is increased 34-fold. Both analogues acted as substrates for DT-diaphorase[56] and are better substrates than MMC at physiological pH, although they are inferior to indolequinone EO9 in this respect.

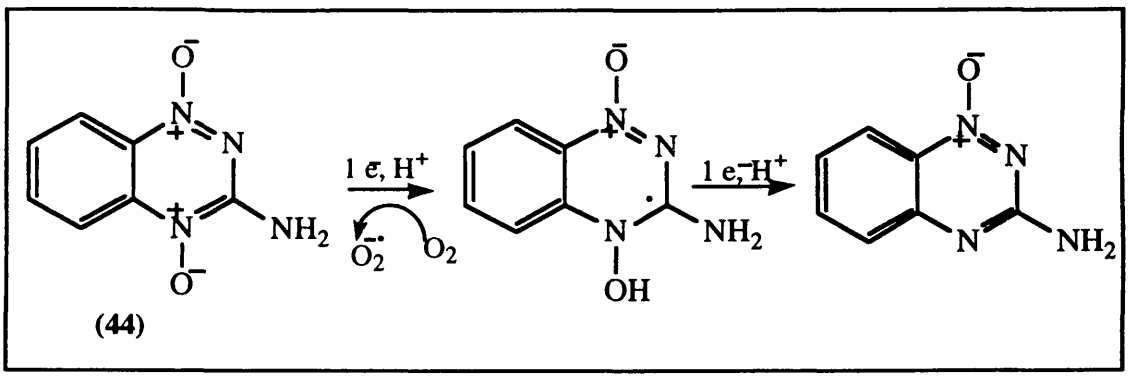
2.6 *N*-Oxides

Several *N*-oxides have been described as cytotoxic agents and some are believed to be intrinsically cytotoxic. These include the pyrrolizidine alkaloid *N*-oxides, as illustrated by indicine *N*-oxide (43).[57] Indicine *N*-oxide underwent intensive preclinical evaluation and was the subject of three Phase II clinical trials until evidence of severe hepatotoxicity halted its progress. The mechanism of action was thought to involve metabolic allylic oxidation and dehydration into alkylating species but only after its reduction to the indicine free base.



(43)

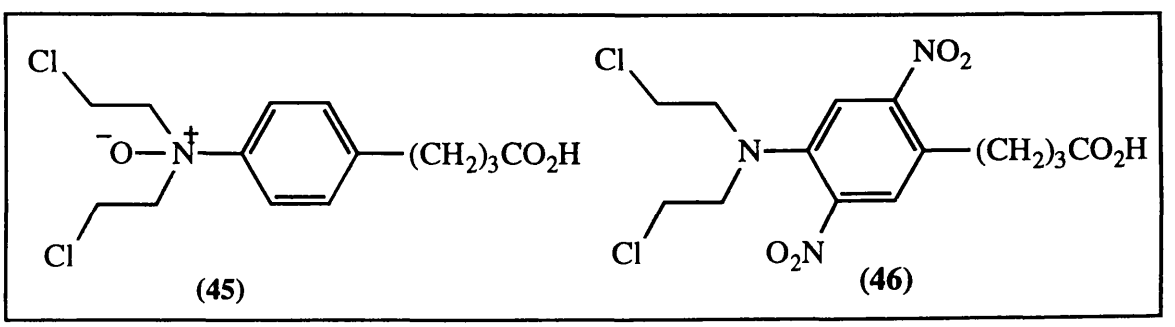
In the course of studies of non-nitro hypoxic cell radiosensitisers, the benzotriazine di-*N*-oxide SR4233 (44) was found to have a cytotoxicity 20-200-fold greater under hypoxic than under aerobic conditions in culture.[58] *In vivo* activity is excellent when it is combined with both single dose and fractionated radiotherapy, and the compound is now in clinical trials.



(44)

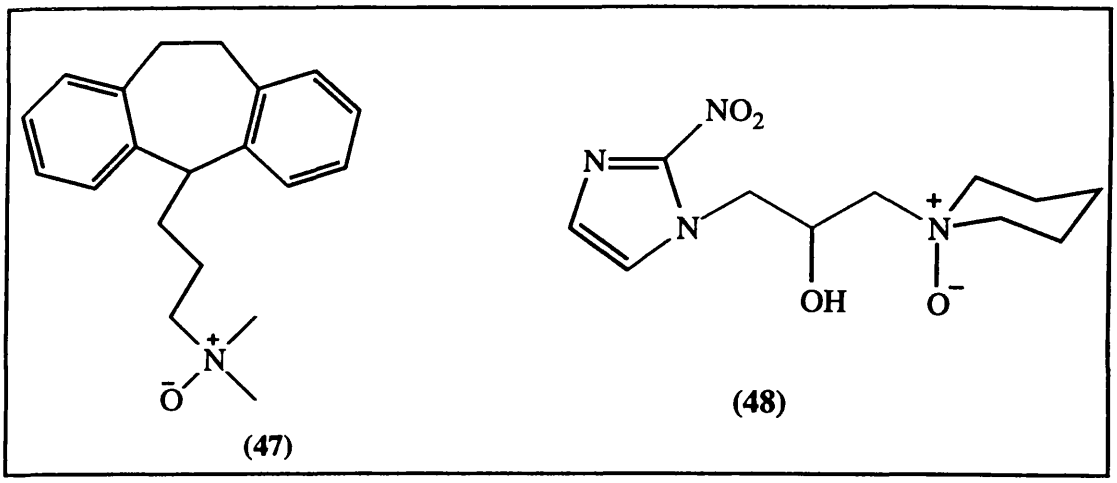
SR 4233 undergoes a 1 e^- reduction to a free radical,[59] which was difficult to detect because of its extreme instability. It causes double strand breaks to a much greater extent in hypoxic cells, but covalent binding to DNA and protein cannot be detected. The proposed mechanism requires the 1 e^- reduction product to act as an oxidising radical which abstracts hydrogen from bases in DNA.[59] The two and four electron reduction products are inactive, suggesting that the hypoxic selectivity of SR 4233 is due to the removal of the oxidising radical by this route in oxygenated cells.[60]

Synthesis of novel *N*- and *S*-mustards as potential prodrugs activated by bioreductive processes has been reported.[61] The synthesis of chlorambucil *N*-oxide (45) and the *S*-oxide analogue plus other derivatives, and initial biological activity were reported. Results showed that although all the compounds were cytotoxic, none had greater activity under hypoxic conditions, although a nitro derivative (46) showed greater activity than chlorambucil.



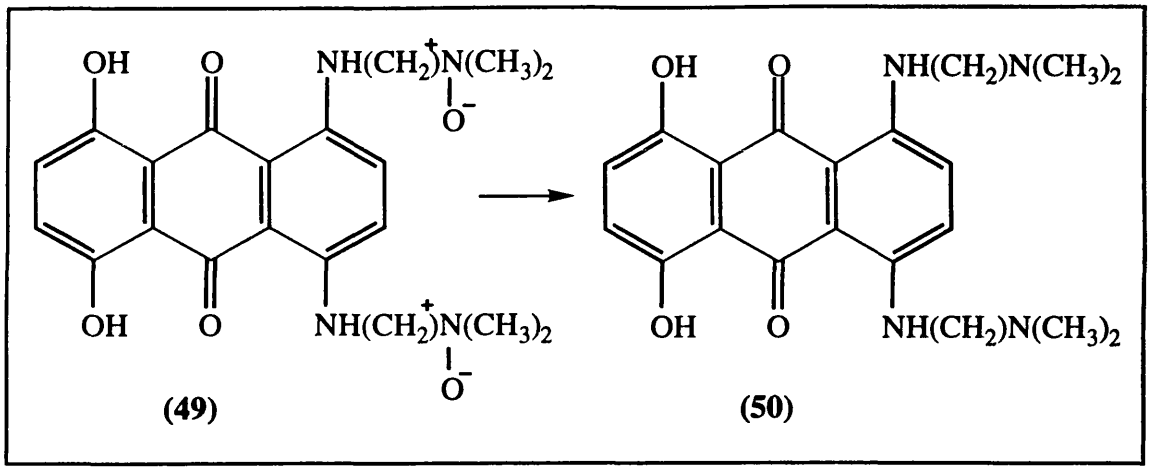
A variety of enzyme systems are known to catalyse the reduction of aliphatic amine *N*-oxides and to be inhibited by oxygen. Liver cytochrome P450 enzymes can

reduce[62] imipramine *N*-oxide (47), while extensive reduction of Ro 31-0313 (48), the piperidinyl-*N*-oxide metabolite of pimonidazole, has been observed in mice.[63]



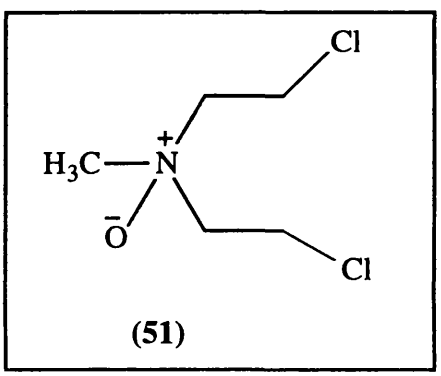
(47) (48)

While the reduction of aliphatic *N*-oxides is facile, there tends to be no toxic species generated as there is in the reduction of the other types. There is therefore a need to link reduction of the *N*-oxide to the enhancement of cytotoxic potency. This can be achieved through the use of a DNA intercalator as seen previously with nitracrine *N*-oxide and also with *N*-oxides of DNA binding topoisomerase II inhibitors. As conventional bioreducible agents are either intrinsically toxic and/or require chronic hypoxia to maintain their cytotoxicity, the use of *N*-oxides of DNA affinic agents as prodrugs that can be bioreduced to persistent, oxygen insensitive cytotoxic agents may avoid both these problems. This is illustrated with AQ4N (49), which can be reductively activated to a DNA affinic, potent cytotoxic agent AQ4 (50).[64]



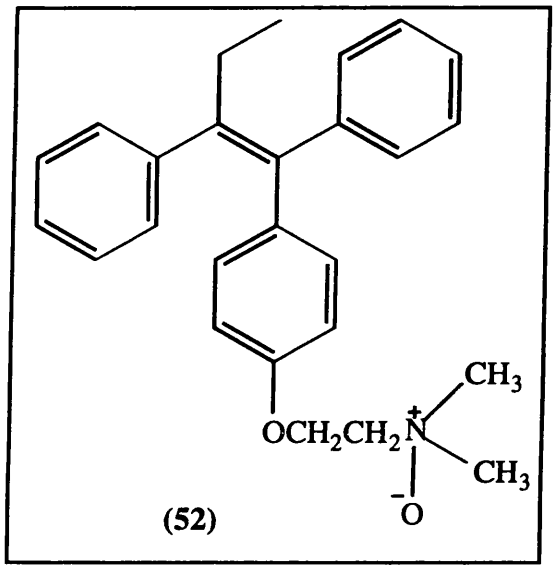
Results have shown that AQ4N has no intrinsic binding affinity to DNA and has low cytotoxicity. Under hypoxic conditions, AQ4N can be reduced *in vitro* to a DNA affinic agent with a 10-100-fold increase in cytotoxicity.

Another possibility is to use the *N*-oxide reduction to activate a nitrogen mustard. White *et al.*[65] have shown that the *N*-oxide (51) of mechlorethamine is reduced selectively under hypoxic conditions to form the more reactive des-*N*-oxide nitrogen mustard.



Nitromin (51) is selectively toxic in Walker rat tumour cell cultures under hypoxic conditions, but with a modest selectivity of only about 4-fold. This aspect appears to have resurrected interest in nitromin and a one electron reduction intermediate, the nitrogen anion radical has been proposed to explain the DNA strand breakage observed in addition to DNA crosslinking in cells treated with nitromin.[66]

Tamoxifen *N*-oxide (52) has been described but is no less toxic than tamoxifen.[67] This was taken to indicate, with other evidence, that the amine functionality of tamoxifen did not contribute to its anti-oestrogenicity or influence the intrinsic antitumour activity of this molecule.

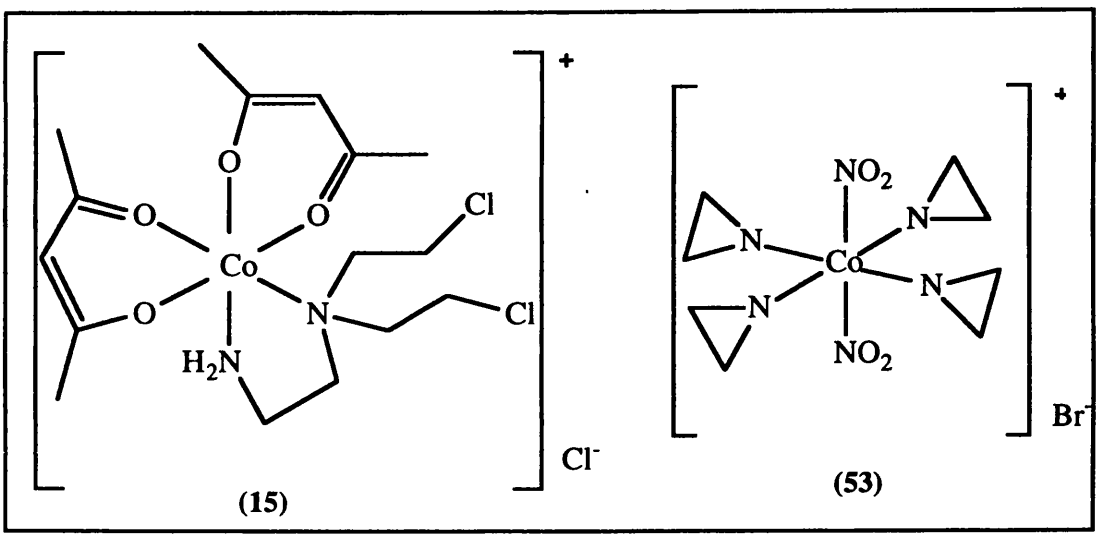


(52)

2.7 Transition Metal Complexes

Although metals are important redox centres in biological systems, little work has been done to explore their possible application in the context of hypoxia-selective cytotoxins. Pt(II) complexes with nitroimidazoles are known to be selectively toxic to hypoxic cells,[68] but the active redox centre is the nitroaromatic ligand rather than the metal itself. Various metals form substantially-inert co-ordination complexes with nitrogen ligands: for example $Co(NH_3)_6^{3+}$ has a displacement halflife[69] of 6 x 10^9 s. One electron reduction to a Co(II) complex results in enormous labilisation of such ligands.[70] Hypoxia-selective metabolism is therefore possible, with ligand displacement by water to form the very stable hexa-aquo Co(II) cation competing (in oxygenated cells) with reoxygenation of the Co(II) complex by molecular oxygen (futile metabolism). Such compounds are capable of hypoxia-selective cytotoxicity provided that the free ligands are much more cytotoxic than when metal bound. Recent studies[71] show this to be the case,

since complexes of aziridine with Co(III) (53) are greatly stabilised with respect to hydrolysis. Such compounds are not hypoxia selective, probably because the reduced Co(II) forms are too labile, but related complexes using chelating alkylating agents as ligands (15) do show limited hypoxia-selective cytotoxicity *in vitro*.[14]



2.8 Enzymology of Bioreduction

A number of enzymes are able to catalyse reduction of bioreducible agents.[72] The importance of understanding the enzymes involved in bioreduction is rapidly gaining widespread recognition[13] and it is becoming clear that many different enzymes are involved in the production of toxic bioreduction products. Enzymes known to be involved in bioreductive metabolism include cytochrome P-450, cytochrome P-450 reductase, xanthine oxidase, aldehyde oxidase, carbonyl reductase and DT-diaphorase, together with mitochondrial reductases and probably many others as well. In contrast to the other enzymes mentioned, DT-diaphorase is an obligate $2e^-$ donating enzyme.[73] Thus it reduces quinones directly to the hydroquinone, bypassing the semiquinone free radical. Cytochrome P-450 reductase is predominantly involved in the early stages of bioactivation of the benzotriazine, SR 4233, while cytochrome P-450 is involved in the later stages.[54] Cytochrome P-450 reductase also plays a critical role in the activation of mitomycin C,[28] whereas DT-diaphorase is much more important for EO9.[74]

The conventional approach to bioreducible drug development has involved screening for selective activity in hypoxic versus oxic cells *in vitro*. In general, hypoxic cell potency is predicted quite efficiently by one-electron redox potentials,[75] and this was a dominant consideration in the synthesis of new agents along with lipophilicity and charge. However, these factors cannot routinely predict for bioreducible cytotoxicity, particularly *in vivo*. The growing appreciation of the key role of bioreductive enzymes has led to the concept of enzyme-directed bioreductive drug development.[13] It is proposed that the antitumour selectivity of bioreducible agents can be enhanced by not only tumour hypoxia, but in addition by increased expression of key reductase enzymes in cancer cells. It may also be possible that further selectivity will be gained by tailor-making drugs to be activated specifically by particular reductases that are expressed at high levels in tumours. Initial results have shown that alterations of the chemical structure of mitomycin C and EO9 produce startling results in the reductive metabolism by DT-diaphorase.[76]

Since different enzymes participate to different extents with the various bioreducible agents, and even catalyse activation in one case and protection in another, it follows that the activity of a given drug might be enhanced by administrating it to patients with a particular tumour enzyme profile. So a tumour rich in DT-diaphorase might be an excellent target for EO9 but could be resistant to SR 4233 as this enzyme would miss out the toxic 1 electron reduction product. As DT-diaphorase has been reported to show a high level of expression in some human colon, breast, lung and liver tumours,[76] these may be good targets for EO9 therapy.

With further information of this type it seems possible that the bioreductive enzyme profile of human tumours could be used to guide the choice of bioreducible antitumour agent in individual patients.

2.9 Conclusions

The first bioreducible drugs, represented by misonidazole and mitomycin C, respectively lacked the potency and selectivity to be effective *in vivo*. The second-generation agents, represented by SR 4233, RB 6145 and EO9 have greater activity against hypoxic cells in mouse tumours and are now in clinical trials or preclinical development. Although these drugs may not be perfect, it is hoped that they will establish whether bioreducible drugs have a place in cancer chemotherapy today. This leaves exciting possibilities for rational design of new drugs capable of exploiting microenvironmental features in tumour cells.

It should be noted that bioreducible drugs may not necessarily exert significant activity as single agents. A 'clean' bioreducible drug would be expected to eradicate the hypoxic cells in the tumour but not the oxic cells. The general specificity of bioreducible agents for hypoxic cells means that *in vivo* testing should incorporate studies of the bioreducible agent in combination with treatments which will kill oxic cells. This might be radiation, conventional cytotoxics such as cyclophosphoramide, cisplatin and doxorubicin, or perhaps even endocrine therapy and targeted radiotherapy.

It is likely that interest in bioreductive drug development will continue to grow. This is due to a number of reasons.

a) The proposed basis of selectivity is well grounded in tumour biology, and can be readily modelled in tissue culture, unlike conventional anticancer agents, where there is little understanding of selectivity.

b) The mechanism of tumour selectivity (oxygen-inhibited bioactivation) is well understood in principle at the biochemical/chemical level and offers great scope to the medicinal chemist.

c) The conditions which make tumours sensitive to bioreducible agents (lack of oxygen and presence of activating enzymes) can be assessed so potential exists for developing assays which will help predict individual tumour sensitivity. Bioreducible agents are

unusual among prodrugs in that activation occurs locally in the tumour, making this approach advantageous.

d) If designed appropriately, bioreducible agents might provide diffusible cytotoxic products. As such, they have the potential to target local systems with delocalised effects, which will then overcome the fundamental problem of tumour heterogeniety.

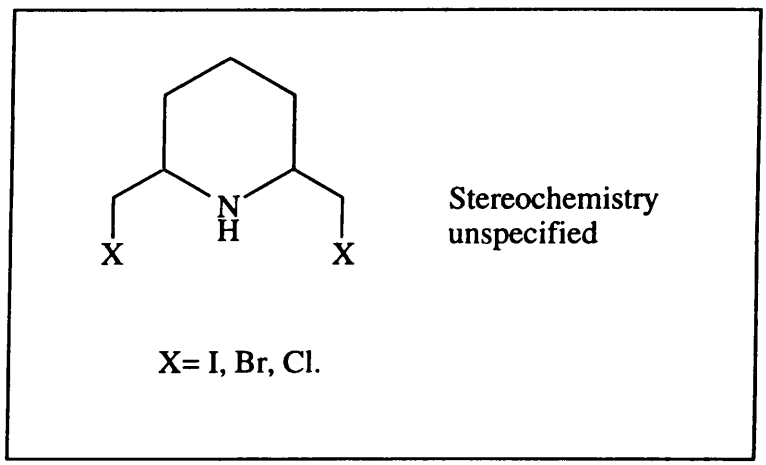
Of all the areas of anticancer drug development, the field of bioreducible drugs offers the most potential for rational design. Although dependent on reductase enzymes for activation, the target is primarily a physical condition (lack of oxygen). Strategies to maximise the extravascular diffusion, selectivity of activation and toxicity of the reduced produgs depend primarily on a knowledge of chemistry, and this class of compounds should be an attractive goal for the medicinal chemist.

Chapter 3

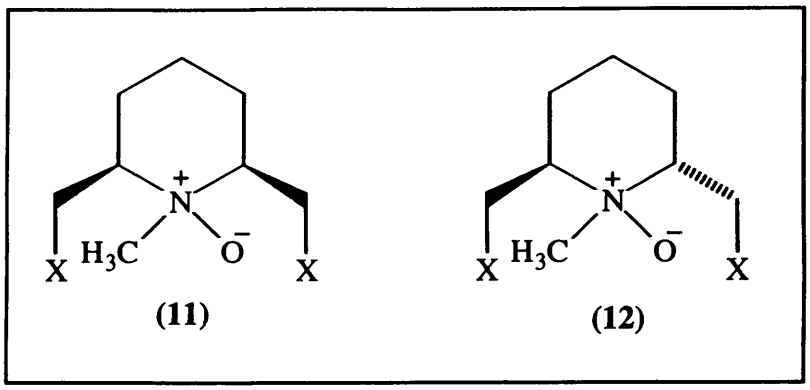
The Synthesis of *cis*-2,6-Disubstituted Piperidines

3.1 Introduction

An obscure publication in Japanese reported significant antitumour activity for 2,6-bis(halomethyl)piperidines and related compounds (stereochemistry unspecified).[77]



It seemed feasible that these toxic compounds could be incorporated into a prodrug which could release the toxic compounds after bioactivation. An *N*-oxide of an *N*-alkylated piperidine might be less toxic if the mode of action of these compounds is by aziridine formation followed by attack by nucleophilic biological molecules such as DNA. This *N*-oxide would then act as a prodrug if it was reduced in hypoxic microenvironments such as solid tumours, to produce a toxic intermediate, in this case a bifunctional alkylating agent via aziridine formation. To this end, *N*-methyl-*N*-oxides of 2,6-disubstituted piperidines with *cis*- (11) and *trans*- (12) stereochemistry with X= halogen or carbamate (novel compounds) have been prepared in this work for evaluation as bioreducible drugs.

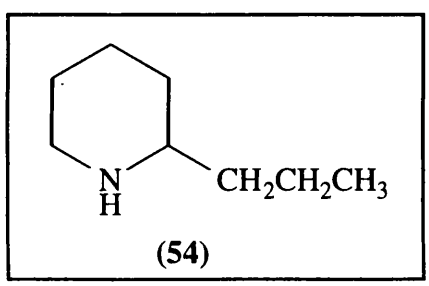


(11) (12)

3.2 Piperidine Alkaloids

One of the reasons for the interest in the synthesis and stereochemistry of piperidine derivatives is their wide occurrence in natural products. Some of these alkaloids have toxic properties and the possibility of making analogues which may be pharmacologically active is another reason for interest.

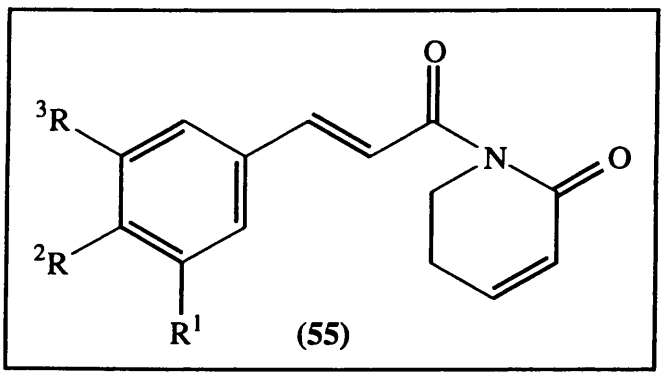
Six-membered rings containing nitrogen are common among alkaloids although biosynthetic studies have shown that they are formed by a number of different pathways.[78] In many of these alkaloids, (e.g. lobelia and lupin alkaloids), the amino-acid lysine serves as the precursor of the piperidine ring. In others, (e.g. hemlock group) the nitrogen appears to have been inserted into a straight carbon chain derived from acetate units. The toxic properties of the poison hemlock have been known since antiquity, and extracts were used in ancient Greece for the execution of criminals. One of the earliest contributors to hemlock literature was Plato, who described the death of Socrates from hemlock poisoning. Five alkaloids have been isolated from hemlock, of which the major one is coniine (54). This compound has neuromuscular blocking effects.



(54)

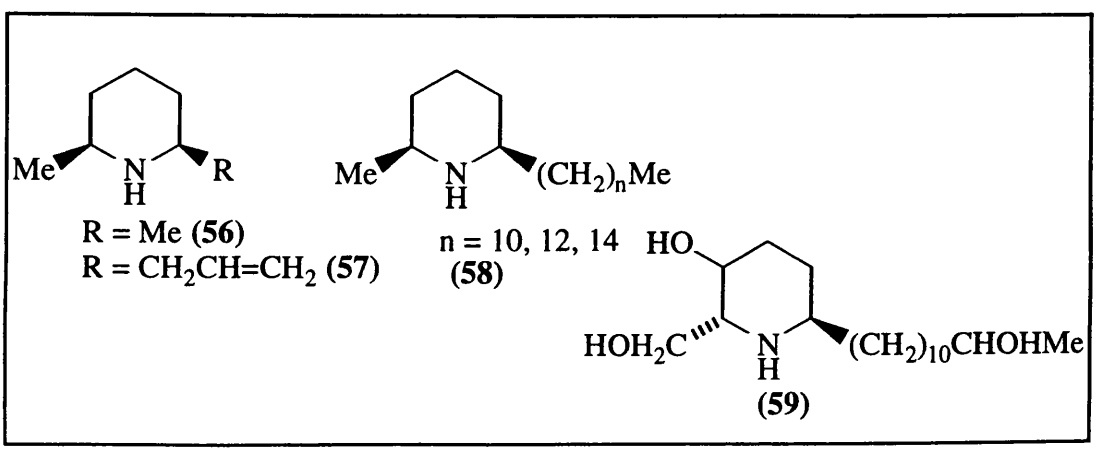
An excellent review on the chemistry and pharmacology of the piperidine alkaloids appears in 'Alkaloids', edited by Pelletier.[79]

The Taiwanese creeping shrub *Piper arborescens* has been found to contain three cytotoxic pyridone alkaloids of the general structure (55).[80]

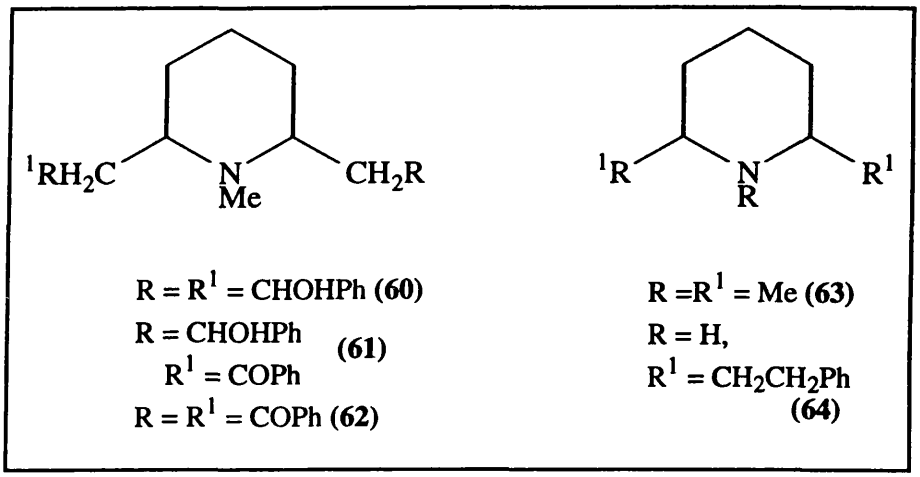


(55)

A minor subgroup of the piperidine alkaloids contains the 2-alkyl-6-methyl-piperidine skeleton.[81] Some representatives are 2,6-dimethylpiperidine (56), the pine alkaloid pinidine (57),[82] alkaloids of the fire-ant venom (58)[83] and hydroxylated alkaloids such as prosopine (59).[84] The venoms of the fire ants of the species *Sobnopsis xyloni, S. geminata, S. richteri* and *S. invicta* were found to contain a series of alkaloids, most of which are *trans*-2-methyl-6-alkylpiperidines with small amounts of the *cis*-isomer.[85] These are used to block neuromuscular transmission[86] and are also employed as antibacterial agents.[87]



Norlobelandinine has been isolated from *Lobelia polyphylla*.[88] Lobelanidine (60) reacts with iodine to give lobeline (61) and lobelanine (62).[89] The action of the latter two alkaloids, along with 1,2,6-trimethylpiperidine (63) and 2,6-diphenylethylpiperidine (64) on respiration and blood pressure has been examined.[90] The alkaloids affect both these properties while the other two piperidines have no effect on respiration but cause a decrease in blood pressure.



R = R^1 = CHOHPh **(60)**

R = CHOHPh
R^1 = COPh **(61)**

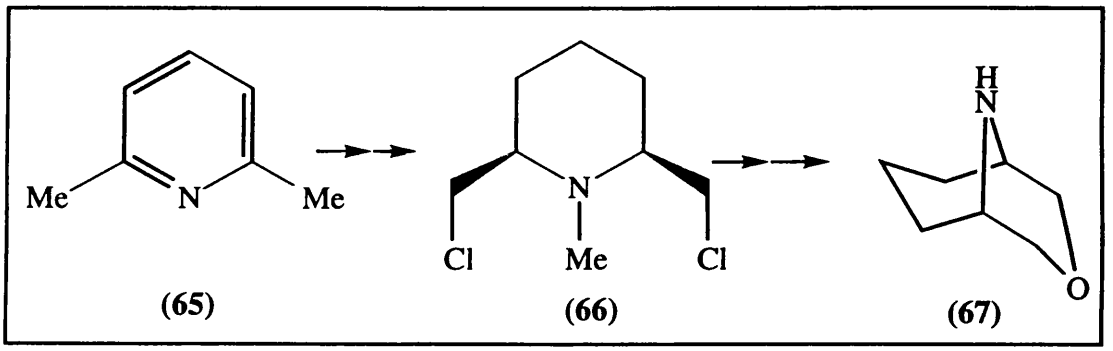
R = R^1 = COPh **(62)**

R =R^1 = Me **(63)**

R = H,
R^1 = CH_2CH_2Ph **(64)**

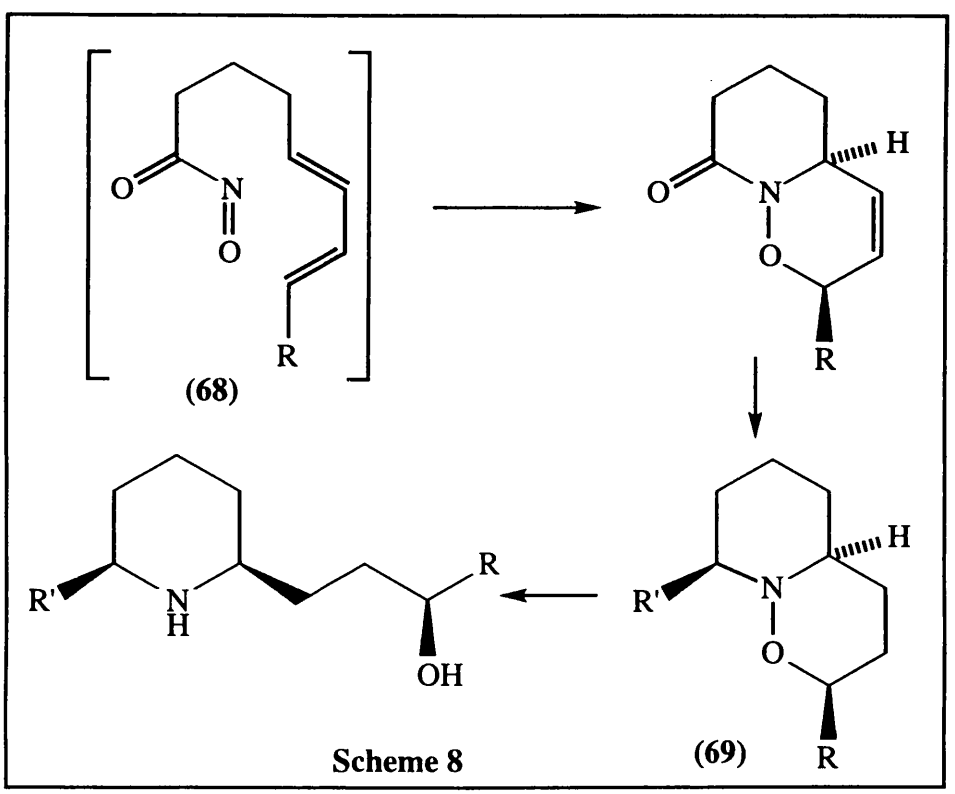
3.3 Methods of Synthesising *cis*-2,6-Disubstituted Piperidines

The most common way of achieving the *cis*-stereochemistry in *cis*-2,6-disubstituted piperidines is by catalytic hydrogenation of pyridine rings.[91] One such example is the synthesis of 1-methyl-*cis*-2,6-bis(chloromethyl)piperidine (66)[92] which was used as an intermediate towards the synthesis of bicyclic systems such as 3,9-oxazabicyclo[3.3.1]nonane (67). The synthesis started with the catalytic hydrogenation of 2,6-lutidine (65).



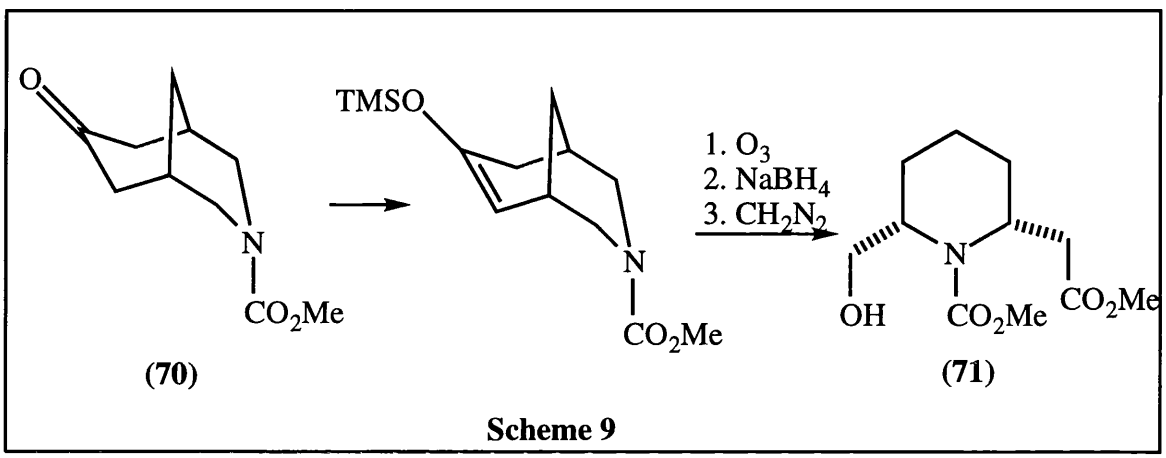
(65) (66) (67)

A general approach to *cis*-2,6-dialkyl piperidines has been obtained via intramolecular acyl nitroso Diels-Alder cycloadditions.[93] This reaction is highly regio- and stereo-selective and could have great potential for alkaloid synthesis. Key features involved in this approach are an intramolecular Diels-Alder cycloaddition of the acyl nitroso compound (68) and a stereoselective introduction of an alkyl side chain (R') into the C-8 position of the oxazinolactam ring (69) followed by reductive cleavage of the N-O bond (Scheme 8).



Scheme 8 (69)

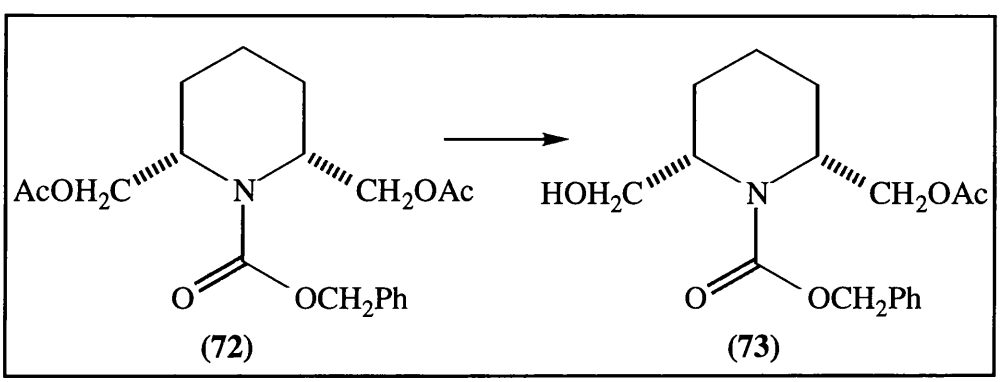
An enantioselective route to *cis*-2,6-bis(hydroxymethyl)piperidine in a seven step synthesis has been published.[94] This involved an asymmetric cleavage of the 'fork-head' ketone of 9-azabicyclo[3.3.1]nonan-3-one (70) to give a *cis*-2,6-disubstituted piperidine derivative (71) in 93% ee (Scheme 9). The obtainable diol may be an important chiral building block for the synthesis of many naturally occurring piperidine alkaloids.



(70) **(71)**

Scheme 9

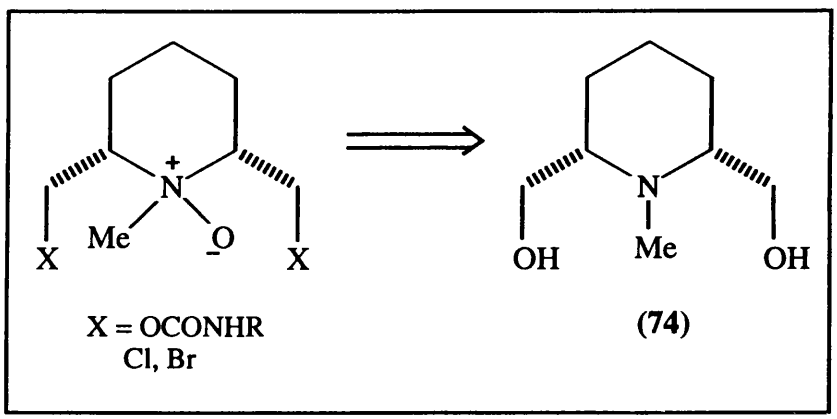
The first enzymatic asymmetrisation of a piperidine system was published by Chenevert and Dickman.[95] Hydrolysis of *N*-benzyloxycarbonyl-*cis*-2,6-bis(acetoxymethyl)piperidine (72) in the presence of *Aspergillus niger* lipase gave the corresponding (2*R*, 6*S*)-mono-acetate (73) in good chemical yield and very high optical purity (ee > 98%).



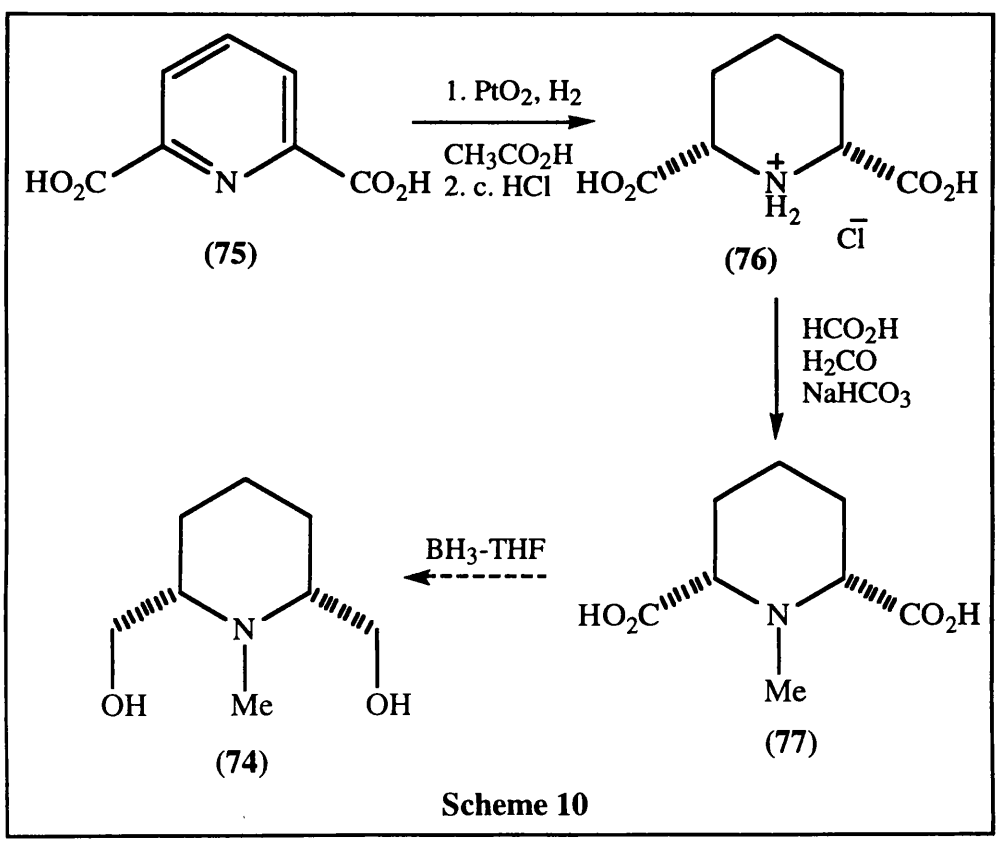
(72) **(73)**

3.4 Synthesis of *N*-Methyl-*cis*-2,6-(hydroxymethyl)piperidine

This compound (74) was considered a key intermediate in the formation of the target compounds (11) and (12).

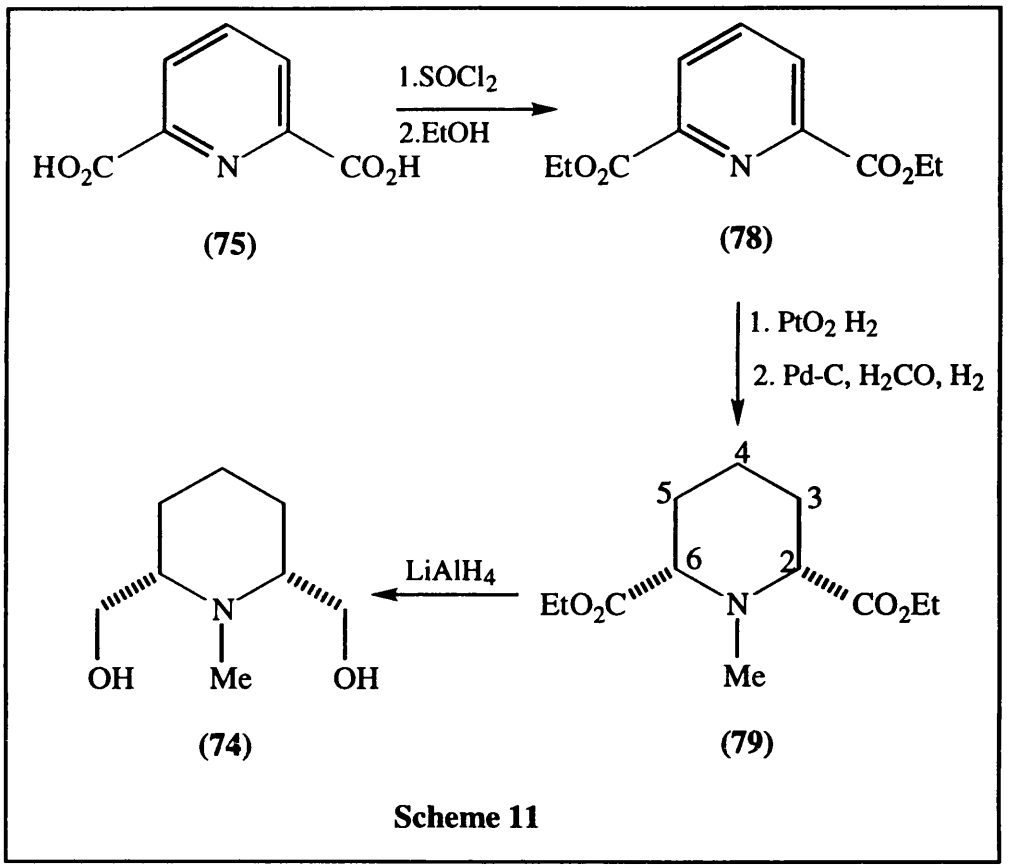


A number of methods have been reported in the literature in which the common starting material is dipicolinic acid. The first attempt to synthesise the hydroxyl compound can be seen in Scheme 10.



Scheme 10

Anderson and Soine[96] reported the synthesis of *cis*-2,6-piperidinedicarboxylic acid hydrochloride (76) by hydrogenation of dipicolinic acid (75) in acetic acid using Adams' catalyst, followed by acidification. This method was repeated by us to give the *cis*-product in 90% yield. *N*-Methylation was carried out by heating at reflux a mixture of the *cis*-piperidine, formic acid, formaldehyde and sodium bicarbonate. The first step in the reaction is the conversion of the hydrochloride salt into the free base using sodium bicarbonate. The nitrogen lone pair can then attack the formaldehyde and this is followed by dehydration to give the immonium ion. Formic acid acts as a hydrogen donor and reduces the immonium group to give the required *N*-methyl derivative (77). The next step involved reduction of the acid groups by diborane to give the desired diol (74). However, this reduction did not occur due to the extreme insolubility of the starting diacid in THF. Attempts to solubilise it by making the diester were then tried. The diacid (77) was dissolved in methanol and HCl gas was bubbled through for 30 minutes, but no reaction took place, as was the case when thionyl chloride and methanol were tried, probably due to the same reason. Esterification using 2,2-dimethoxypropane and conc. HCl did appear to work over the course of a few days, but significant isomerisation to the *trans*-product had also occurred. It was therefore decided to esterify at an earlier stage in the synthesis, as reported by Barnes and Fales.[97] To this end, dipicolinic acid (75) was heated at reflux in thionyl chloride for 48 hours before the acid chloride was reacted with ethanol in quantitative yield to produce the diester (78) (Scheme 11). In the IR spectrum, a carbonyl stretching band at 1745 cm^{-1} and aromatic vibrations at 1576, 1532 and 1481 cm^{-1} were evident. The ^1H NMR peaks were as expected for the compound with the usual substitution pattern for the pyridine as a doublet of doublets at δ 8.07 and a doublet at δ 8.30. The main peak in the mass spectrum was for *m/z* 150 due to loss of one ester group, and a small molecular ion was observed.



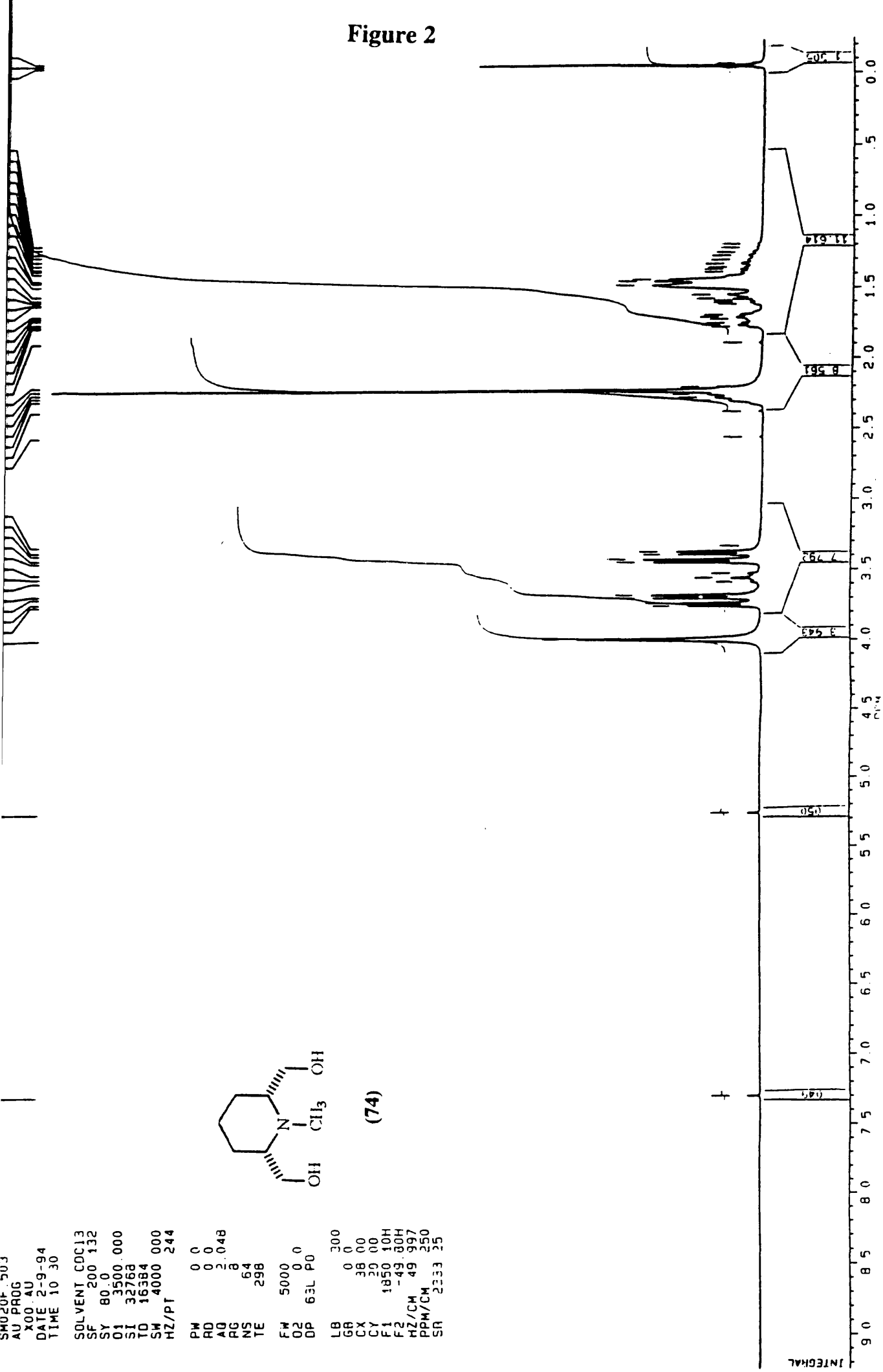
Scheme 11

The next step was to hydrogenate the diester (78) and *N*-methylate the product as before. We chose to follow the method of Feldkamp *et al.*[98] where 2-, 3-, and 4-(hydroxymethyl)piperidines were synthesised in a programme to find new antispasmodic agents. In their procedure, hydrogenation of the pyridine ring was carried out in aqueous acetic acid with Adams' catalyst. Isolation of this unmethylated piperidine derivative proved unnecessary because *N*-methylation could proceed in the same solution by using a reductive methylation procedure. This meant exchanging the platinum catalyst for palladium-on-charcoal and adding a slight excess of aqueous formaldehyde. This solution was then hydrogenated to afford the methylated product. This latter step was advantageous as the authors had reported difficulties in their attempts to methylate using formic acid and formaldehyde. Thus we isolated diethyl *N*-methyl-*cis*-2,6-piperidine dicarboxylate (79) in a yield of 50% as white crystals. In the IR spectrum the characteristic carbonyl stretch was found at 1730 cm^{-1} whilst new bands had appeared at 2861 cm^{-1} indicating CH stretch and 1376 cm^{-1} corresponding to CH_3 symmetrical

deformation. The multiplet in the ^1H NMR spectrum at δ 1.52 - 1.68 was due to the ring CH_2 protons. The equivalent protons at C-2 and C-6 appeared as a doublet of doublets at δ 2.69. In the ^{13}C NMR spectrum, the two methylene signals at δ 22.7 and δ 29.1 were in a ratio of 1 : 2, and correspond respectively to C-4 and C-3 plus C-5. The methine signal came at δ 67.9 while the *N*-methyl was at δ 41.8. One quaternary signal was seen at δ 172.5 which was attributed to both carbonyl carbons. In the mass spectrum, the parent ion was observed at *m/z* 243, whilst the largest ion was at *m/z* 170 due to loss of an ester group.

This diester (79) could now be readily reduced in THF with lithium aluminium hydride to the diol (74). The reduction of the unmethylated *cis*- piperidine with $LiAlH_4$ to the diol has been reported in the literature[99] without any isomerisation to the *trans*-isomer. This reduction was successfully carried out in a yield of 97% to afford *N*-methyl-cis-2,6-bis(hydroxymethyl)piperidine (74) as a clear oil. A broad band at 3374 cm^{-1} indicated OH stretch and the carbonyl band had disappeared in the IR spectrum. The ^1H NMR spectrum of the diol (74) can be seen in Figure 2. The *N*-methyl peak had shifted slightly to δ 2.21, as had the methine protons, to a new position of δ 2.26. The two diastereotopic CH_2 protons next to the OH appeared as two doublet of doublets at δ 3.39 and δ 3.70 due to strong vicinal coupling and weak geminal coupling. The ^{13}C NMR spectrum showed similar shifts for the *N*-methyl peak, now at δ 34.9, while the equivalent carbons at C-2 and C-6 were located at δ 64.1. The new methylene signal at δ 63.5 belonged to the carbons next to the hydroxyl groups.

Figure 2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AU PROG
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DATE 2-9-94
TIME 10 30
SOLVENT CDCl3
SF 200 132
SY 80.0
O1 3500.000
SI 32768
TD 16384
SW 4000.000
HZ/PT 244
PW 0.0
RD 0.0
AQ 2.048
RG 8
NS 64
TE 298
FW 5000
O2 0.0
DP 63L P0
LB 300
GB 0.0
CX 38.00
CY 20.00
F1 1850.10H
F2 -49.80H
HZ/CM 49.997
PPM/CM 250
SR 2233.25
OH
CH3
N
OH
(74)
INTEGRAL
11.614
8.561
7.792
3.543
1.307
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0

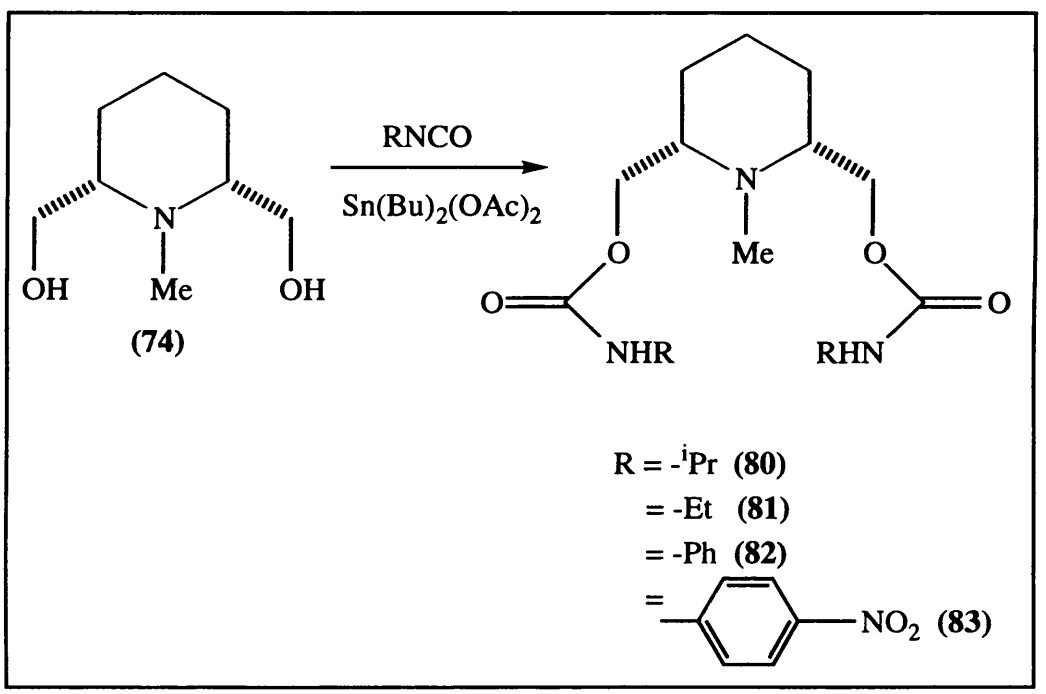
3.5 Synthesis of *N*-Methyl-*cis*-2,6-bis(aminocarbonyloxymethyl)-piperidines

Now that we had prepared our key intermediate in a satisfactory overall yield of 47%, we could concentrate on putting on appropriate leaving groups. We had decided on making carbamates because:

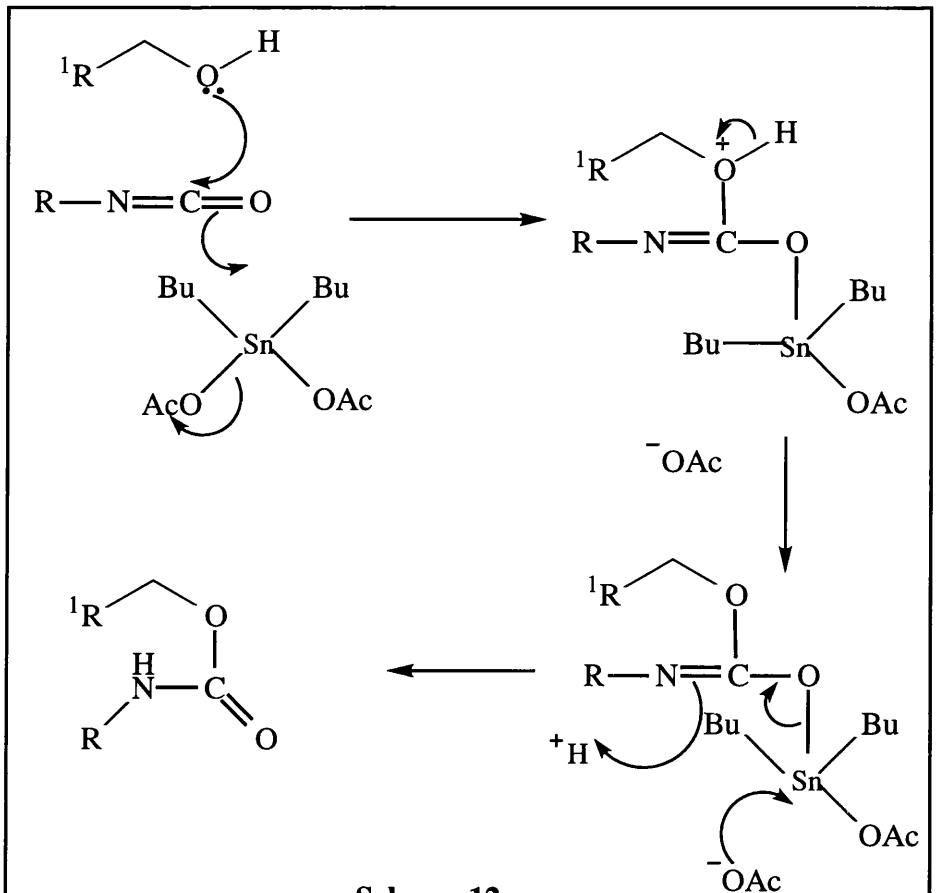
a) their reasonable stability allowed easy handling although they could be displaced in physiological conditions;

b) their ease of formation; and

c) carbamates are usually crystalline and therefore can be purified and characterised easily.



The bis-carbamates were made using an appropriate isocyanate, the diol and a catalytic amount of dibutyl tin diacetate.[100] This reaction goes quickly and cleanly at room temperature in dichloromethane to produce high yields of the corresponding bis-carbamate. The ethyl, isopropyl, phenyl and *p*-nitrophenyl bis-carbamates were synthesised. The mechanism of reaction is assumed to take place as in Scheme 12.



Scheme 12

N-Methyl-*cis*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine (80) was prepared in good yield as white crystals. In the IR spectrum, the carbonyl absorption was at 1690 cm^{-1} and a band at 1370 cm^{-1} was indicative of an isopropyl group. The ^1H NMR spectrum showed a doublet at δ 1.14 for the isopropyl methyl protons, while the CH was found at δ 3.81 as a multiplet. The *N*-methyl signal came at δ 2.33 as a singlet while the two protons at C-2 and C-6 were a multiplet at δ 2.28. The methylene protons next to the carbamate had shifted downfield to a new position of δ 4.10 and appeared as a multiplet. In the ^{13}C NMR spectrum two signals were observed for the isopropyl methyl groups at δ 23.0 and δ 23.5. This may be due the π-bonding between the nitrogen atom and the carbonyl carbon atom slowing the rotation about this bond, thus leaving two environments for the methyl groups. A similar effect is seen with dimethylformamide but with greater effect as the two methyl groups are joined directly to the nitrogen. The *N*-methyl signal and the methine signal of C-2 and C-6 were similar to that found in the diol

and were observed at δ 37.7 and δ 63.3 respectively. The methylene carbons next to the carbamate had shifted slightly from their position in the diol to δ 66.9 while the new carbonyl carbon signal was observed at δ 155.7. No molecular ion was present in the mass spectrum, the heaviest ion found was at *m/z* 314 which corresponded to loss of the *N*-methyl. The largest ion found was at *m/z* 213 which was due to the loss of two $NH^{i}Pr$ groups.

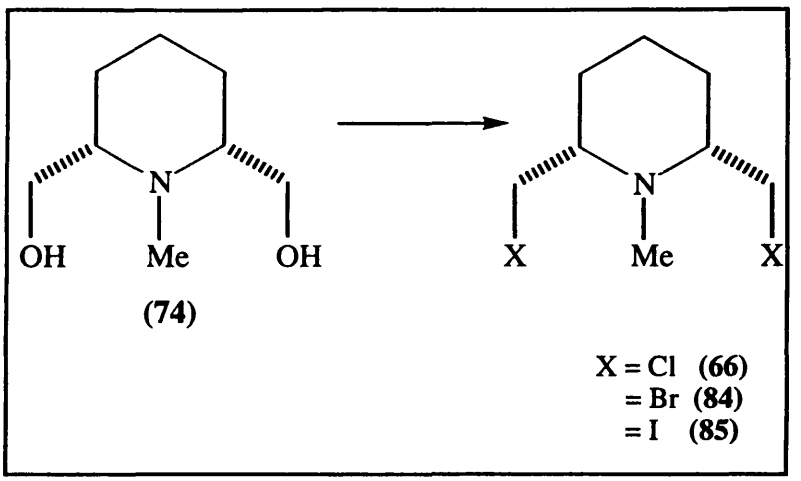
Preparation of the bis(ethyl carbamate) (81) was achieved in a similar manner, although this compound could not be crystallised as only an oily solid was obtained after a week in the freezer. The spectra of this compound were very similar to that of the isopropyl carbamate although this time a molecular ion was observed in the mass spectrum.

N-Methyl-*cis*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine (82) was obtained as white crystals after column chromatography. The IR spectrum showed a slight carbonyl shift to 1700 cm^{-1} and the characteristic aromatic bands at 1600 cm^{-1} and 1525 cm^{-1}. The monosubstitution pattern of the aromatic ring was obvious with strong bands at 750 cm^{-1} and 690 cm^{-1}. In the ^{1}H NMR spectrum, the peaks were all shifted slightly downfield compared to the aliphatic carbamates. Thus the *N*-methyl singlet came at δ 2.43 while the two protons at C-2 and C-6 were observed as a multiplet at δ 2.55. The methylene protons adjacent to the two carbamate groups were found at the chemical shift of δ 4.26 as a multiplet. In the aromatic region, the *para*-protons were observed as a triplet at δ 7.07, but the *ortho*- and *meta*-protons overlapped with each other between δ 7.27 and 7.37 to give a multiplet. The ^{13}C NMR spectrum again showed similarities to the aliphatic carbamates with the *N*-methyl signal observed at δ 36.4. Signals at δ 118.6, 123.3 and 128.9 are typical of a phenyl ring and correspond respectively to the *ortho*-, *para*- and *meta*-carbons. The two quaternary signals at δ 137.8 and δ 153.3 were attributed to the aromatic quaternary carbon and carbonyl carbon respectively. Again, no molecular ion was observed in the mass spectrum, but the usual fragmentation products at *m/z* 247, 213 and 110 did appear.

Synthesis of the bis(nitrophenyl carbamate) (83) posed more of a problem. This was due to two main reasons, the first being the impurity of *p*-nitrophenyl isocyanate and the second due to the poor solubility of the product. Purification was obtained using a gravity alumina column, eluting with toluene : ether 2:1 to leave bright yellow crystals, but the yield was poor (58%). The IR spectrum showed the carbonyl stretch to have shifted to 1725 cm^{-1}. The aromatic bands appeared at 1600 cm^{-1} and 1505 cm^{-1} with a strong band at 850 cm^{-1} indicating *para*-disubstitution. The two strong bands at 1545 and 1330 cm^{-1} are due to asymmetrical and symmetrical stretching of the NO bonds. The ^{1}H NMR spectrum, taken in d_6-acetone showed the *N*-methyl singlet at δ 2.35 with the protons at C-2 and C-6 at δ 2.40. In the aromatic region, signals at δ 7.75 and δ 8.17 are typical of an AA'BB' system. In the ^{13}C NMR spectrum, the *N*-methyl signal and the C-2 and C-6 carbons appeared at δ 38.0 and 63.4 respectively while the methylene carbons next to each carbamate had shifted slightly downfield to δ 68.3. The aromatic methine signals came at δ 118.6 and 125.7, corresponding to the *ortho*- and *meta*-protons respectively. Three quaternary carbons were present, the CNH signal appearing at δ 143.2, the CNO_2 signal at δ 146.4 and the carbonyl signal at δ 154.0. The parent ion did not survive in the mass spectrum, but the fragmentation pattern of the molecule was consistant with the structure.

3.6 Synthesis of *N*-Methyl-*cis*-2,6-bis(halomethyl)piperidines

Compounds with halogen leaving groups were also synthetic targets due to the reported activity of the unmethylated disubstituted piperidine system.[77] Halogens are also present in a number of conventional nitrogen mustards, whose pharmacology is well documented.[101]



N-Methyl-*cis*-2,6-bis(chloromethyl)piperidine (66) was synthesised by dissolving the diol (74) in dichloromethane and adding it dropwise at 0 °C to an excess of thionyl chloride. This reaction went in good yield and the product was isolated as the free base which is quite stable if kept at 0 °C (no noticeable change after 6 months). Although this compound has been made before,[92] no characterisation had been reported. The IR spectrum showed the usual CH stretching peaks at 2939 cm^{-1} with the NCH_2 bands at 2860 and 2787 cm^{-1}. A medium intensity band at 674 cm^{-1} was assigned to the C-Cl stretch. The ^1H NMR spectrum of (66) was very similar to that of the diol, with the *N*-methyl peak at δ 2.32 and the methylene protons next to the chlorines showing two doublet of doublets at δ 3.53 and δ 3.63. Again the ^{13}C NMR spectrum showed the usual *N*-methyl at δ 37.0, and the C-2 and C-6 methine carbons at δ 64.2 while a new signal at δ 47.3 was attributed to the methylenes adjacent to the chlorines. The largest fragment in the mass spectrum was obtained at *m/z* 160 which corresponded to loss of one chlorine atom.

The corresponding dibromo compound (84) was made from the diol (74) using phosphorus tribromide in a similar fashion to that described above. However, the yield was disappointing at only 23%, possibly due to quick formation of the amine salt, rendering the compound insoluble in the solution and thus unable to react, even with heating at reflux over long periods of time (72 hours). Again, the spectra were similar to that of the diol. The IR showed the C-Br band at 570 cm^{-1}, while in the ^1H NMR

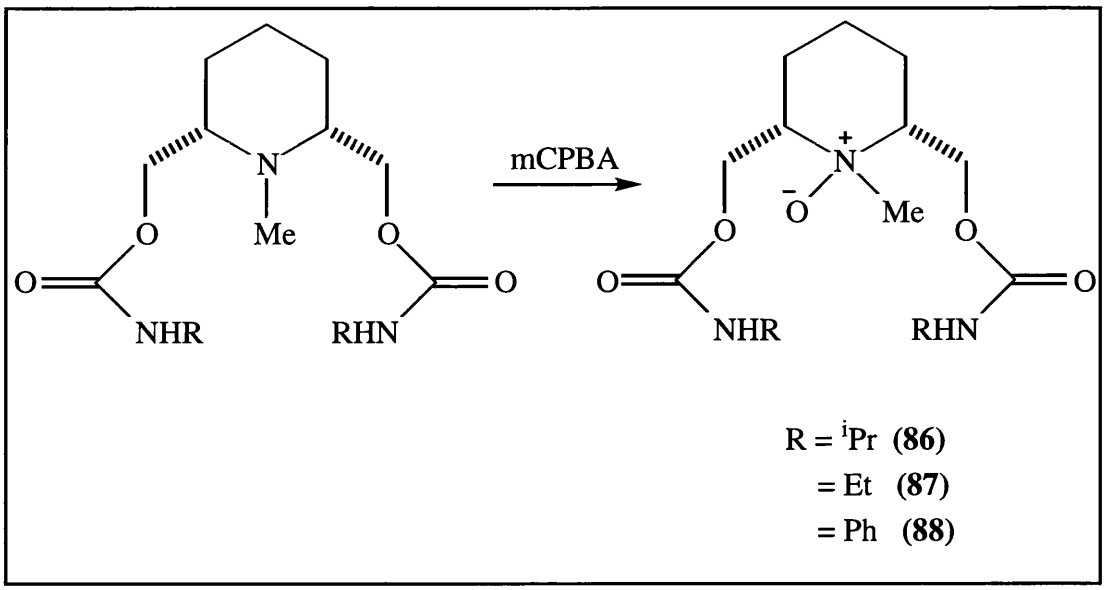
spectrum, the methylene protons adjacent to the bromines appeared at δ 3.41 and δ 3.50 as two doublet of doublets. The respective carbon signal was found at δ 36.5 in the ^{13}C NMR spectrum. In the mass spectrum, the parent ion was observed at *m/z* 284, with the main fragment being loss of CH_2Br.

Attempts to make the corresponding diiodo compound (85) from the dibromo compound (84) by Finkelsteins method[102] were futile as the product, which appeared to form due to observation of the precipitation of sodium bromide, could not be isolated.

3.7 Synthesis of *N*-Methyl-*cis*-2,6-bis(aminocarbonyloxymethyl)-piperidine *N*-Oxides

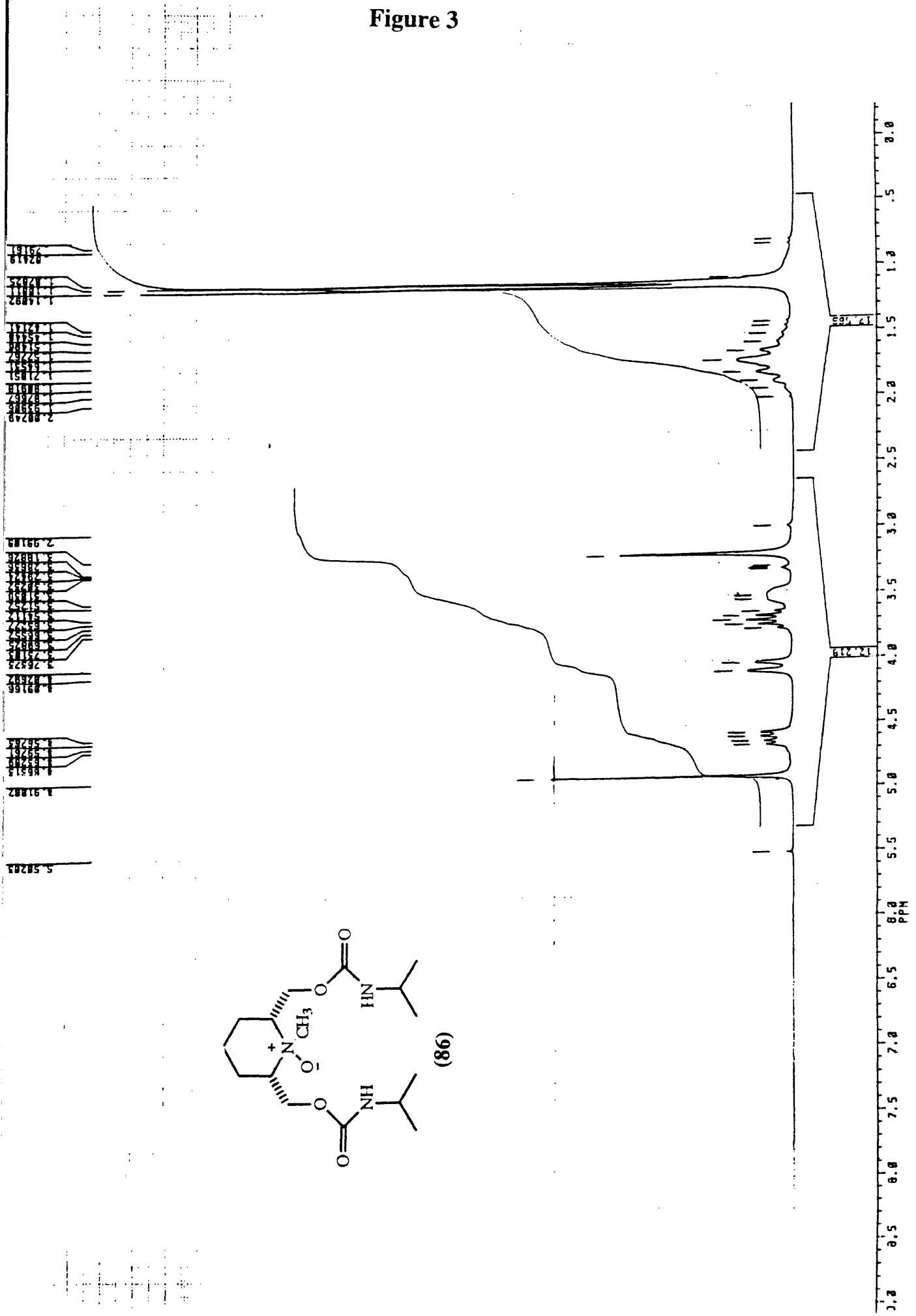
Tertiary amines and other basic nitrogen compounds react readily with hydrogen peroxide or peracids to give the *N*-oxides. The reaction depends on the nucleophilicity of the amine and the electrophilicity of the peroxide. Pyridine and other less basic nucleophilic nitrogen heterocycles require a peracid such as trifluoroperacetic acid. For our purposes, *m*-chloroperoxybenzoic acid (mCPBA) was the most suitable to use due to ease of handling.

The *N*-oxides were obtained simply by mixing the disubstituted piperidine with the peracid in dry acetone. After careful removal of the solvent, the residue was chromatographed on an alumina column to remove the acid residues. In general, good yields were obtained with the exception of the bis(nitrophenyl carbamate). In this case, the lack of solubility of the starting bis-carbamate prevented reaction.



N-Methyl-*cis*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine *N*-oxide (86) was obtained as white crystals. The IR spectrum was similar to that of the free base, with the carbonyl stretch present at 1680 cm^{-1}, and the new band at 920 cm^{-1} was assigned to the *N*-oxide. The ^{1}H NMR spectrum can be seen in Figure 3. The *N*-methyl singlet had shifted considerably downfield to δ 3.18 as expected, due to the influence of the positive charge on the nitrogen. Accordingly, the signal attributed to the methine protons at C-2 and C-6 appeared at δ 3.52 as a multiplet. The methylene protons at C-7 and C-8 had also changed considerably, occurring as a doublet at δ 4.05 and a doublet of doublets at δ 4.61. The ^{13}C NMR spectrum showed that all the ring methylene carbons had grouped together to become one signal at δ 25.4, as had the isopropyl methyl groups at δ 22.9. The *N*-methyl signal had shifted to δ 55.8, and the methine signal for C-2 and C-6 appeared at δ 76.2. The quaternary carbonyl carbon was present in a similar position at δ 157.3. As there was only one set of carbon signals in this spectra, this indicated that although two diastereoisomers could be formed, the reaction only yielded one isomer. The mass spectrum showed no parent ion.

Figure 3



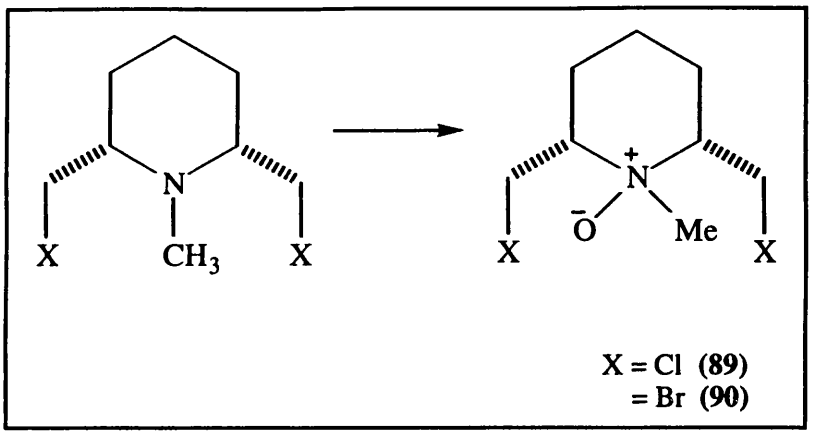
(86)

The bis(ethyl carbamate) *N*-oxide (87), unlike its free base, was obtained as white crystals. This compound had similar spectra to the isopropyl derivative, with the carbonyl stretch at 1730 cm^{-1} and N-O stretch at 920 cm^{-1} in the IR spectrum. The ^1H NMR spectrum showed the shifting of the *N*-methyl to δ 2.96, where it became part of a multiplet with the methylene protons of the bis(ethyl carbamate). Again, the doublet at δ 3.89 and the doublet of doublets at δ 4.30 were attributed to the methylenes at C-7 and C-8. The ^{13}C NMR spectrum showed a similar story with the *N*-methyl moved to δ 55.4 and the C-2 and C-6 carbons at δ 75.6. Unexpectedly, a parent ion was found at *m/z* 317 in the mass spectrum along with the expected fragmentation pattern.

N-Methyl-*cis*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine *N*-oxide (88) was isolated as white crystals. The IR spectrum was almost identical to the free base with the addition of a band at 920 cm^{-1} which was attributed to the N-O stretch. This time, the *N*-methyl had not shifted quite so far downfield in the ^1H NMR spectrum and appeared at δ 2.87, as was the case with the protons at C-2 and C-6 which had their multiplet positioned at δ 3.13. The familiar pattern of the C-7 and C-8 methylene groups was in evidence at δ 4.00 and 4.52. Multiplets at δ 6.82, 7.02 and 7.29 in the ratio of 1:2:2 corresponded to the *para*-, *meta*- and *ortho*-aromatic protons. The ^{13}C NMR spectrum showed one diastereoisomer present with the *N*-methyl signal at δ 55.9 and the C-2 and C-6 methine carbons at δ 76.2. The mass spectrum showed no parent ion, but the usual fragmentation pattern.

3.8 Synthesis of *N*-Methyl-*cis*-2,6-bis(halomethyl)piperidine *N*-Oxides

As before, oxidation of the amine was achieved using mCPBA. This tended to give slightly discoloured oils which could not be purified further due to the small amounts being used and uncertainty of the stability of the compounds at higher temperatures.



N-Methyl-*cis*-2,6-bis(chloromethyl)piperidine *N*-oxide (89) was obtained as a yellow oil which required storage in the freezer to prevent further discolourisation. In the IR spectrum, an additional band at 910 cm^{-1} was attributed to the N-O stretch. The ^{1}H NMR spectrum showed shifting of the *N*-methyl as expected to δ 2.80 as was the same with C-2 and C-6 protons which appeared at δ 3.21. Again, the two doublet of doublets at δ 3.88 and 4.17 were due to the C-7 and C-8 methylene protons. The ^{13}C NMR spectrum showed one diasteroeisomer and was similarly affected with the *N*-methyl signal at δ 44.6 and the signal for C-2 and C-6 at δ 80.9. A large (9.4%) parent ion was found at *m/z* 211 in the mass spectrum.

The dibromo derivative (90) was prepared in a similar way to the dichloro compound and was also obtained as a yellow oil which was unstable. The compound yielded similar spectra to that of the dichloro derivative.

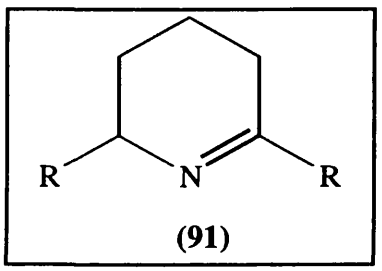
Chapter 4

Synthesis of *trans*-2,6-Disubstituted Piperidines.

4.1 Methods of Synthesising *trans*-2,6-Disubstituted Piperidines.

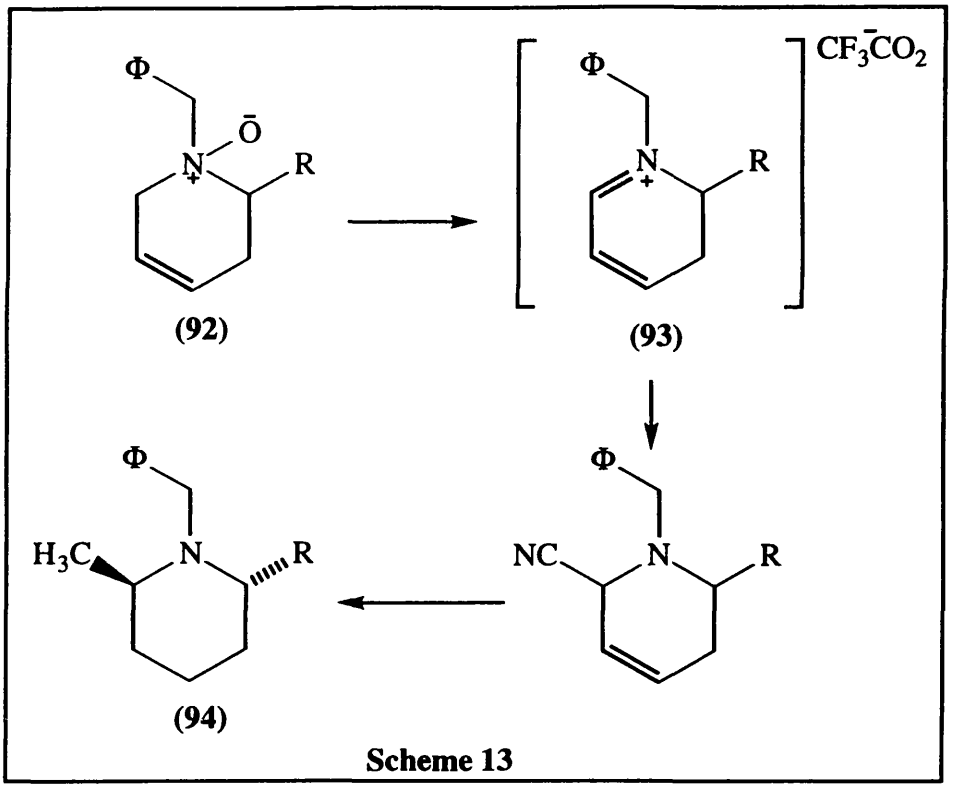
The family of *trans*-2,6-disubstituted piperidine alkaloids includes a number of products exhibiting notable biological activity.[91] Despite interest in the pharmacological properties of these compounds, there are few general, stereoselective methods for their synthesis. The reduction of substituted pyridine derivatives[103] or various intramolecular cyclisations[104] have generally yielded product mixtures of the *cis*- and *trans*-isomers. However, in the past ten years, a number of approaches have been made to combat the problem.

Hydride reduction of cyclic imines (91)[105] can be manipulated to give either *cis*- or *trans*-stereochemistry, depending on the reagent and conditions.



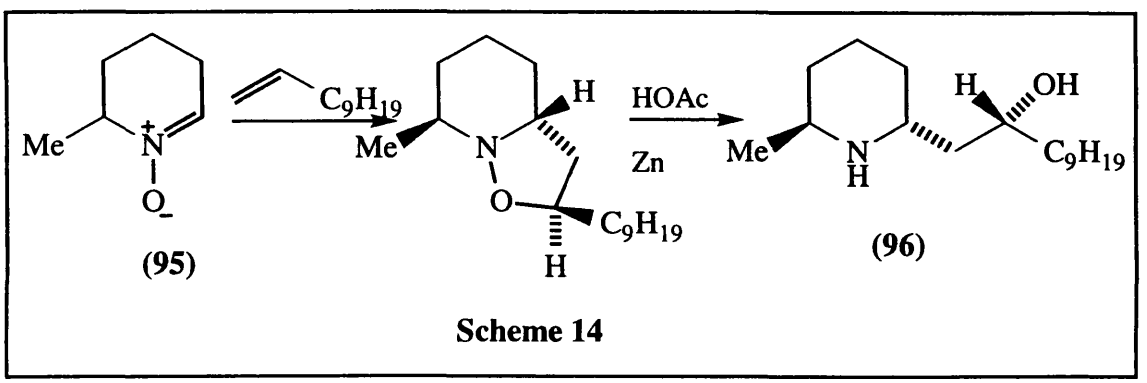
(91)

The reaction of tetrahydropyridine *N*-oxides (92) with trifluoroacetic anhydride in dichloromethane at 0 °C (Polonovski-Potier reaction)[106] afforded 5,6-dihydropyridinium salts (93).[107] Treatment with potassium cyanide, followed by reduction gave a series of *trans*-2,6-dialkyl-piperidines (94) (Scheme 13).



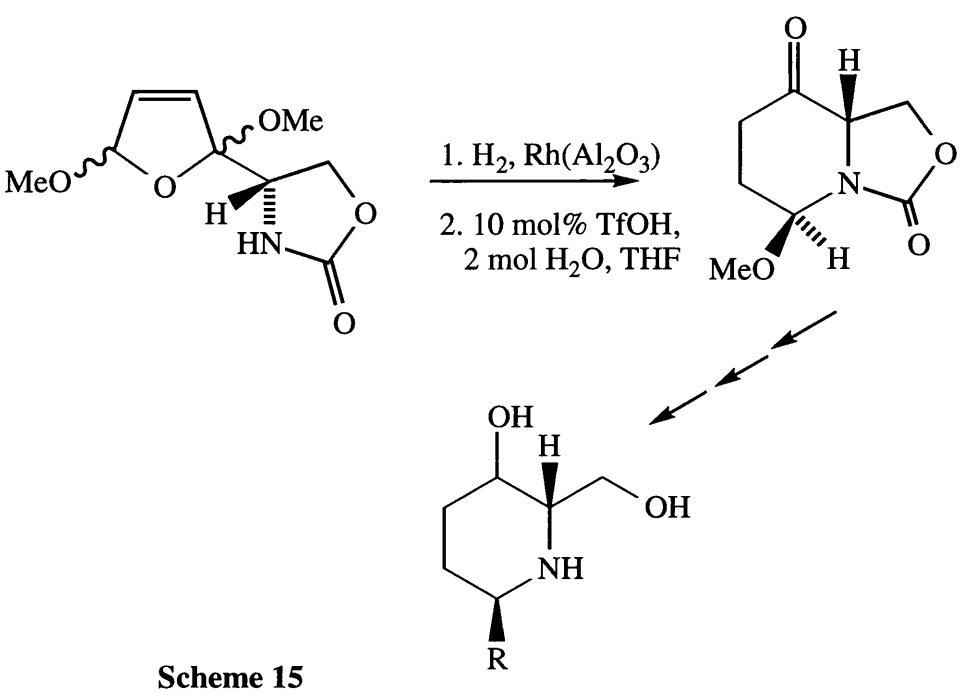
Scheme 13

Cycloadditions of alkenes with tetrahydropyridine *N*-oxides (95) followed by reductive cleavage has furnished *trans*-2,6-dialkylpiperidines (96) (Scheme 14).[108] The cycloaddition of an alkene to an *N*-oxide takes place preferentially by orthogonal approach of the alkene to the nitrone in a conformation in which the 2-alkyl substituent is equatorial, to give an isoxazolidine which will furnish a *trans*-2,6-dialkylpiperidine by reductive cleavage of the N-O bond.



Scheme 14

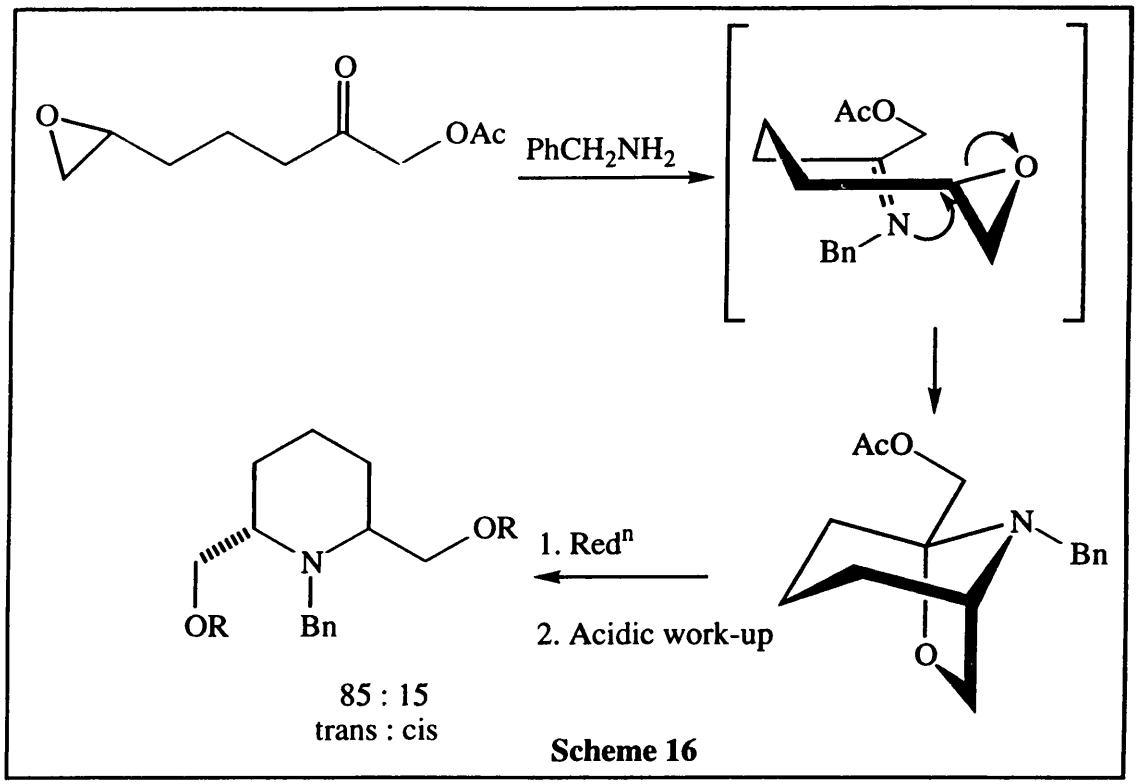
Alkylation and reduction of bicyclic carbamates, via the aza-Achmatowicz reaction[109] using chemoenzymatic methods, also gives the *trans*-isomer in a stereospecific reaction (Scheme 15).[110]



Scheme 15

Wasserman *et al.* have shown that an imine-epoxide rearrangement followed by hydride reduction proves a mild, efficient method for the formation of *trans*-2,6-disubstituted piperidines[111] and have used that procedure for the synthesis of the fire-ant venom alkaloid, solenopsin A[112] in which the *cis* : *trans* ratio is 1:99.

Using the same methodology, the same group has also synthesised *trans*-piperidine-2,6-dicarboxylic acid,[113] commonly known as teneraic acid, which can be isolated from the red alga *Porphyra tenera* [114] in which the *cis:trans* ratio is 15:85 (Scheme 16).

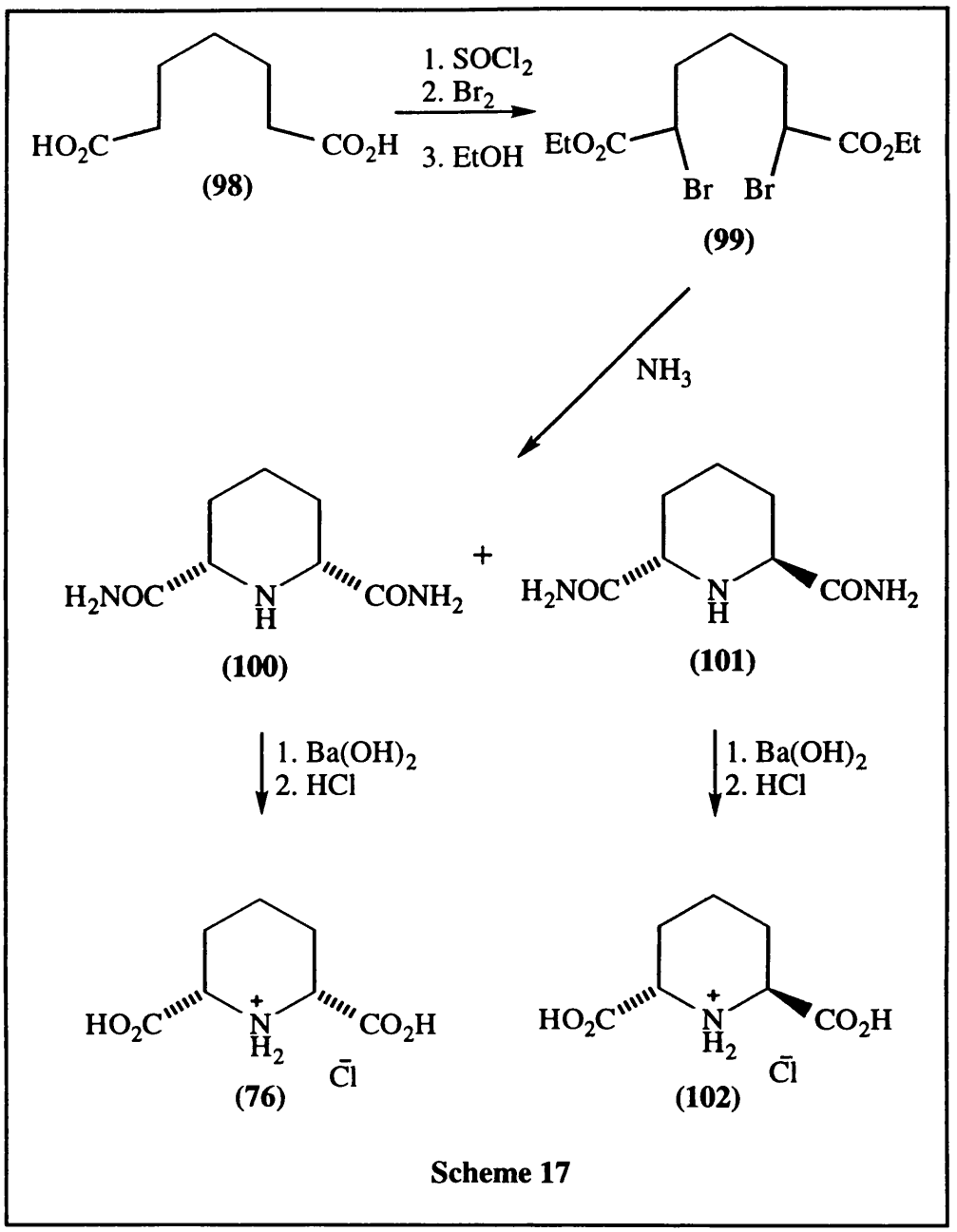


Scheme 16

4.2 Synthesis of *N*-Methyl-*trans*-2,6-bis(hydroxymethyl)piperidine

By analogy with the *cis*-series of compounds, *N*-methyl-*trans*-2,6-bis(hydroxymethyl)-piperidine (97) was seen as a key intermediate. Steps were therefore taken to synthesise this compound.

A route had been devised to *trans*-2,6-piperidinedicarboxylic acid in work to synthesise enzyme inhibitors of the diaminopimelate pathway to L-lysine.[115] We repeated this work (Scheme 17) and so pimelic acid (98) was converted into the diacid chloride by warming with thionyl chloride.[116] The α-positions were then brominated using bromine, with iodine as a catalyst. This is the Hell-Volhard-Zelinsky reaction where only the carbon α to an acid chloride is halogenated. Addition of ethanol at the end of the reaction converted the diacid chloride into the diethyl ester (99).

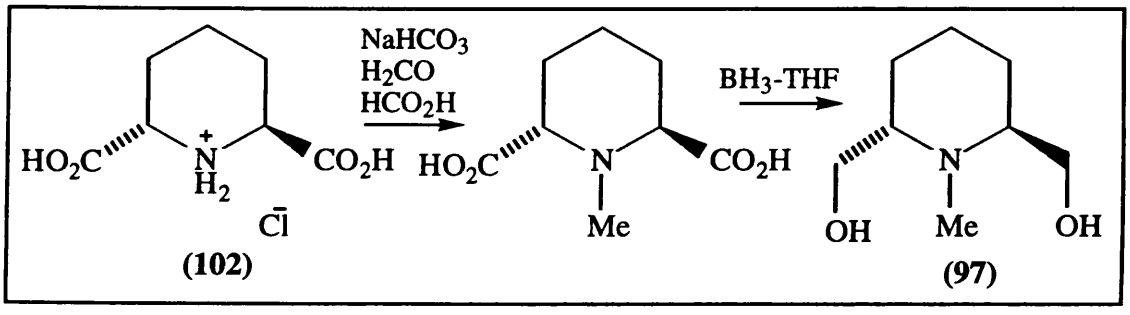


Scheme 17

Cyclisation of diethyl α,α'-dibromopimelate was carried out in a sealed tube with liquid ammonia. After three days, the tube was opened and the ammonia was allowed to evaporate off leaving 2,6-piperidinedicarboxamide as a mixture of isomers. Separation of the isomers was achieved by careful crystallisation. On addition of iced water, the remaining solid was filtered off and dried. This is the *cis*-isomer (100) which was identified by its melting point (lit.[117] m.p. 226-228 °C) and by subsequent hydrolysis of the diamide to the diacid which showed that the hydrolysis product was indeed the *cis*-

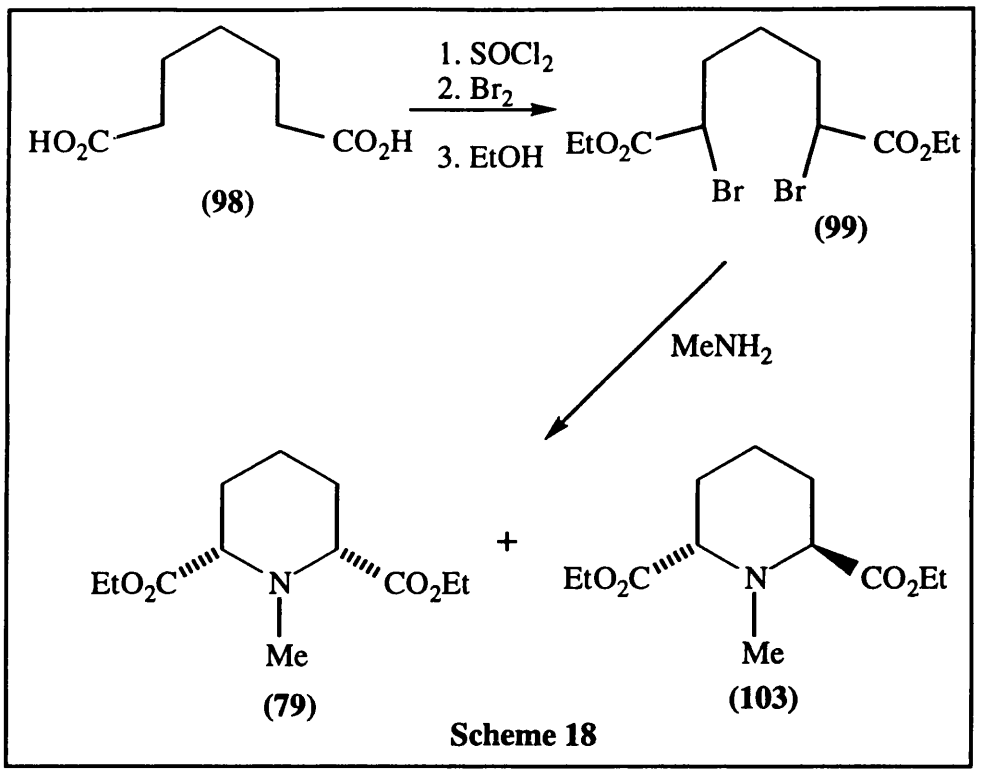
2,6-piperidine dicarboxylic acid. Concentration of the filtrate obtained above gave the *trans*-diamide (101) as white crystals. This isomer was also identified by its melting point (lit.[117] m.p. 265-269 °C). The separated *cis*-and *trans*-2,6-piperidinedicarboxamides were hydrolysed to the corresponding diacids by heating at reflux in 10% barium hydroxide solution followed by acidification. The isomers were identified by their different melting points[117,96] and the *cis*-isomer (76) was identical to the product of the hydrogenation of dipicolinic acid, which is known to give solely the *cis*-product.

The intention next was to *N*-methylate the *trans*-diacid (102) using formaldehyde and formic acid, followed by reduction of the diacid using borane-THF. However, it was decided at this point to try to find a new route to the *trans*-isomer (97) because of the poor yields obtained in the above method (25% for the diamide and 45% after hydrolysis).



4.3 An Alternative Synthesis of *N*-Methyl-*trans*-2,6-bis(hydroxymethyl)-piperidine

After a search through the literature, it was discovered that esters of *N*-substituted piperidines had been synthesised as part of a search for improved local anaesthetics.[118] In a similar procedure to that described above, the reaction of a dihalogenated ester with three molar proportions of the appropriate primary amine gave a piperidine derivative. Although not reported here, methylamine has been used before in this reaction,[119] but yields of product were poor.



Scheme 18

Diethyl α,α'-dibromopimelate (99) was prepared in good yield and obtained as a colourless oil. Cyclisation with methylamine was carried out at reflux for 24 hours. After an acid-base work up and column chromatography, a pale yellow oil was obtained.

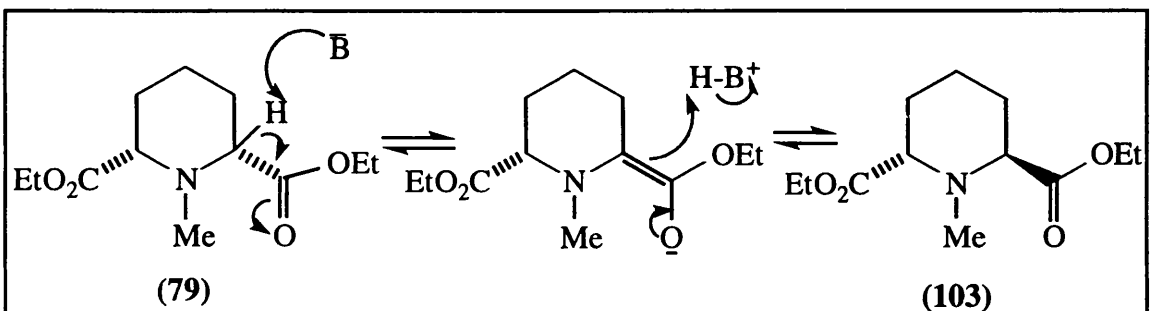
Questions of stereochemistry now arise. If the diethyl α,α'-dibromopimelate is the *meso*-isomer, the resulting piperidine diethyl esters should have the *meso-cis* (79) configuration. If the dibromo diester is a racemic mixture, the products should be the racemic *trans*-piperidine esters (103). It was quite obvious from the NMR data that the product was a mixture of the isomers in a 1 : 1 ratio. The ^{1}H NMR spectrum showed the usual complex multiplet for the ring methylenes between δ 1.48 and 1.95. Two distinct singlets of equal intensity were observed for the *N*-methyl at δ 2.21 and 2.40 one of which corresponds to the *cis*-isomer and the other to the *trans*-isomer. The protons α to the nitrogen also came as two multiplets, one at δ 2.80 and the other at δ 3.78. This large difference in chemical shift is consistent with literature reports,[120] as the protons are more shielded in the *cis*-isomer (δ 2.80) than in the *trans*-isomer (δ 3.78). This is reported to be due to the presence of $n\sigma\sigma^*$-type hyperconjugative interaction in the *cis*-isomer,[121] where, in the most populated conformation, *cis* (eee), the two CH bonds are

anti-coplanar with the nitrogen lone pair. The ^{13}C NMR spectrum showed two signals for each carbon, further supporting the presence of two isomers. The *N*-methyl carbons appeared at δ 40.6 and 42.1, while the C-2 and C-6 methine carbons had a greater difference, consistent with the ^{1}H NMR spectrum, with signals at δ 61.6 and 68.2. In the IR spectrum, the carbonyl stretch band appeared at 1731 cm^{-1}, whilst a small amount of the parent ion *m/z* 243 was found in the mass spectrum.

There now remained two alternatives. Either to separate the isomers by column chromatography, or to epimerise one centre in the *cis*-isomer to give wholly the *trans*-isomer.

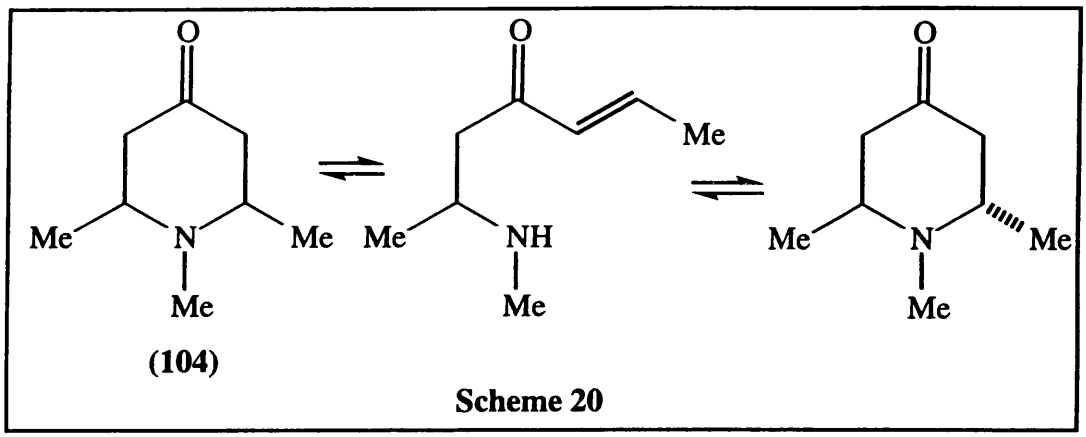
Conformational Analysis of Piperidine Systems

There have been several reports in the literature on epimerisations in 2,6-disubstituted piperidine rings. In basic conditions, it is possible to convert, through repetition, the *cis*-isomer into the *trans*. Barnes and Fales[97] noticed that their sample of dimethyl *N*-methyl-*cis*-2,6-piperidine dicarboxylate (79) isomerised to the *trans*-isomer during attempted acyloin condensation in alkaline conditions (Scheme 19). They also observed that even on standing, the diester was gradually isomerised and they concluded that the tertiary nitrogen was a strong enough base to cause some enolisation.



Scheme 19

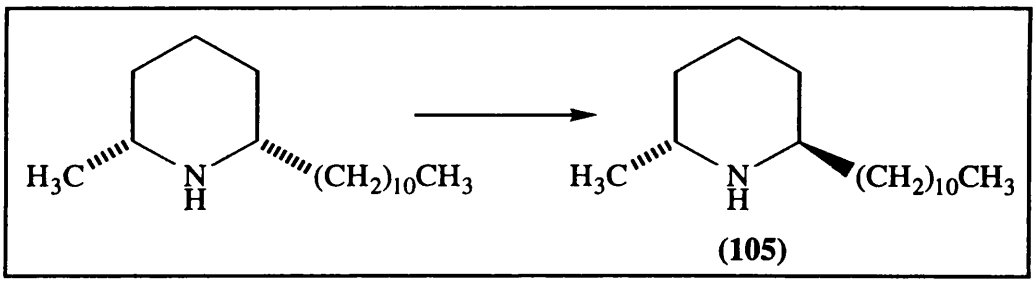
Another example quotes epimerisation of C-2 in 1,2,6-trimethyl-4-piperidone (104)[122] using Bu^tNH_2-H_2O at 50 °C. This is believed to occur via a ring opened species.



(104)

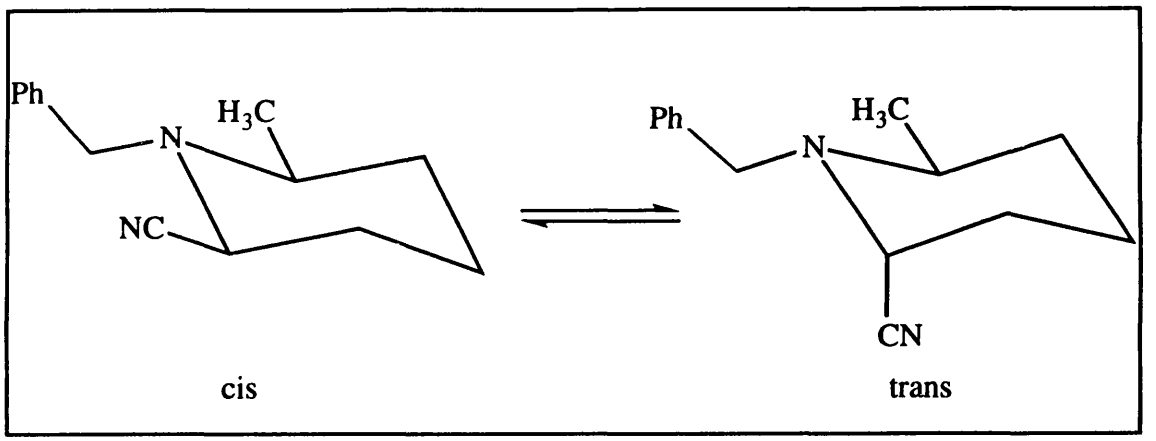
Scheme 20

Using similar conditions, the alkaloid, solenopsin A (105) which has haemolytic, insecticidal and antibiotic activities, has been made through epimerisation of the *cis*-compound using K^tOBu.[123]

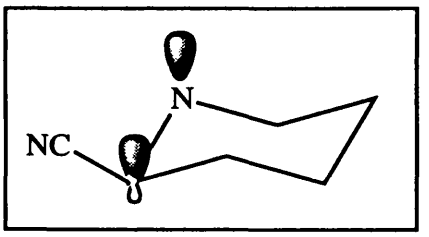


(105)

In the preparation of *N*-benzyl-2-cyano-6-methyl-piperidine, Husson *et al.*[124] noted that the molecule preferred a conformation where the cyano group is pseudoaxial. There appeared to be a gain in stabilisation energy when the cyano group is pseudoaxial, which was sufficiently large to prevent ring inversion to the alternate and *a priori* more stable diequatorial conformer.



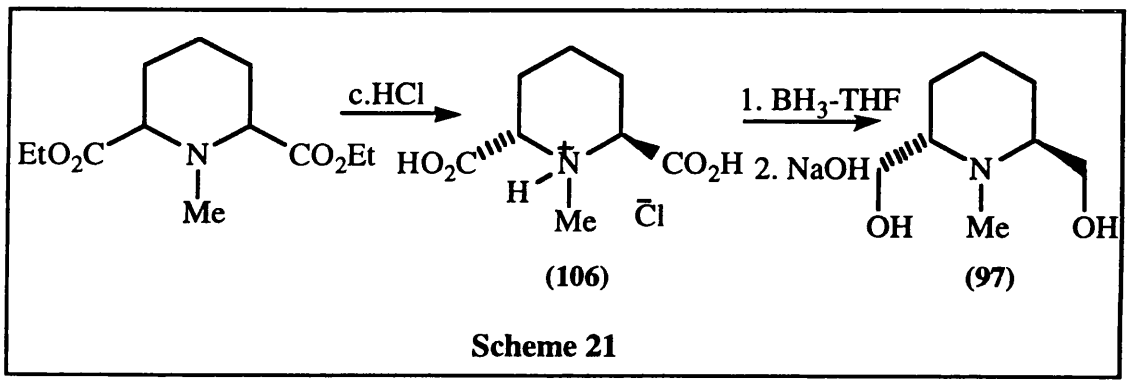
This preferred orientation of the nitrile group has been likened to the 'anomeric effect' observed in pyranose sugars.[125] When a polar group is located on a carbon α to a heteroatom instead of being in the equatorial position, it prefers the axial position. An explanation of this is that the lone pairs of the polar group connected to the carbon can be stabilised by overlapping with an anti-bonding orbital of the bond between the carbon and the heteroatom, i.e. there is a higher coefficient on the electropositive α-carbon.



A review on the conformation equilibria of nitrogen-containing saturated heterocycles has been published by Crabb and Katritzky.[126]

It seemed feasible that complete conversion of the mixture of *cis*- and *trans*-diesters to the single *trans*-isomer would be possible. Following literature precedence, the mixture of isomers was dissolved in xylene and small pieces of fresh sodium were added. The mixture was then heated at reflux for 24 hours after which it was discovered that 30% of the *cis*-isomer remained. Unsatisfied with this result, we then attempted acidic epimerisation, by heating the mixture of isomers in conc. HCl in a sealed tube at 100 °C for 18 hours (Scheme 21). After the reaction had cooled and the pressure was

released, the resulting hydrochloride salt precipitate (106) was removed by filtration and analysed. The ^1H NMR spectrum showed a singlet at δ 2.75 corresponding to three protons and a multiplet at δ 3.77 for two protons. In the ^{13}C NMR spectrum, only one set of signals could be seen, with the *N*-methyl signal coming at δ 42.7 and the α protons resonating at δ 67.9. Definitive proof that we had obtained 100% pure *N*-methyl-*trans*-2,6-piperidinedicarboxylic acid hydrochloride (106) was obtained from the melting point which was not identical to that of an authentic sample of the *cis* isomer which had been made by hydrogenation. In the IR spectrum, a carbonyl stretching band at 1745 cm^{-1} and a hydroxyl band at 3440 cm^{-1} from the carboxylic acids were evident. The mass spectrum of the diacid showed a very small molecular ion at *m/z* 187. The largest ion, *m/z* 142 was due to loss of one carboxyl group.

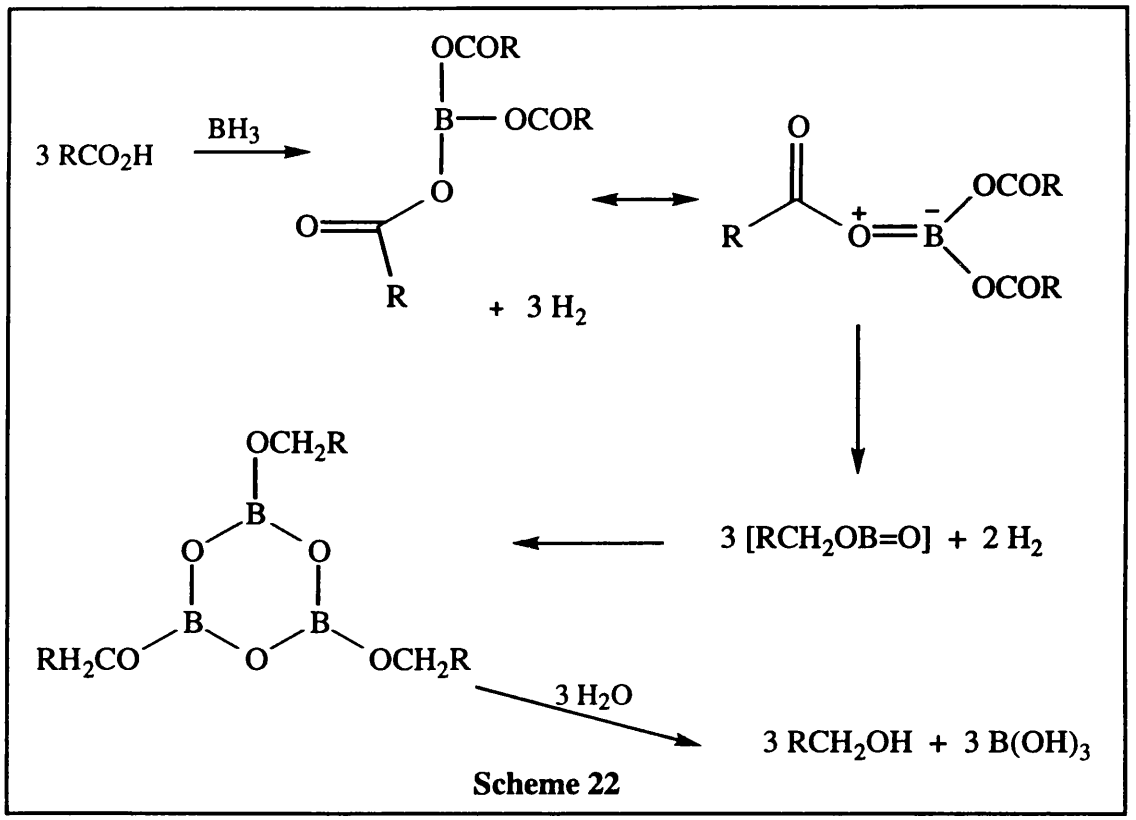


Scheme 21

There now remained only the problem of the reduction of the acid groups to the corresponding diol (97). Reduction of acids is normally facile using diborane. However, in this case, the starting acid is highly insoluble, either as the amino acid or as its hydrochloride salt.

Diborane is a dimer although it acts as if it were the monomer, BH_3. In THF, however, the reagent exists as the monomer, in the form of an acid-base complex with the solvent. Unlike complex hydride anions, which are nucleophilic, boranes are electrophilic and combine with the lone pair of the oxygen. The mechanism (Scheme 22) for the reduction of carboxylic acids using diborane was first established by Brown and

Heim.[127] Each acid group requires three active hydride equivalents for reduction to the alcohol.



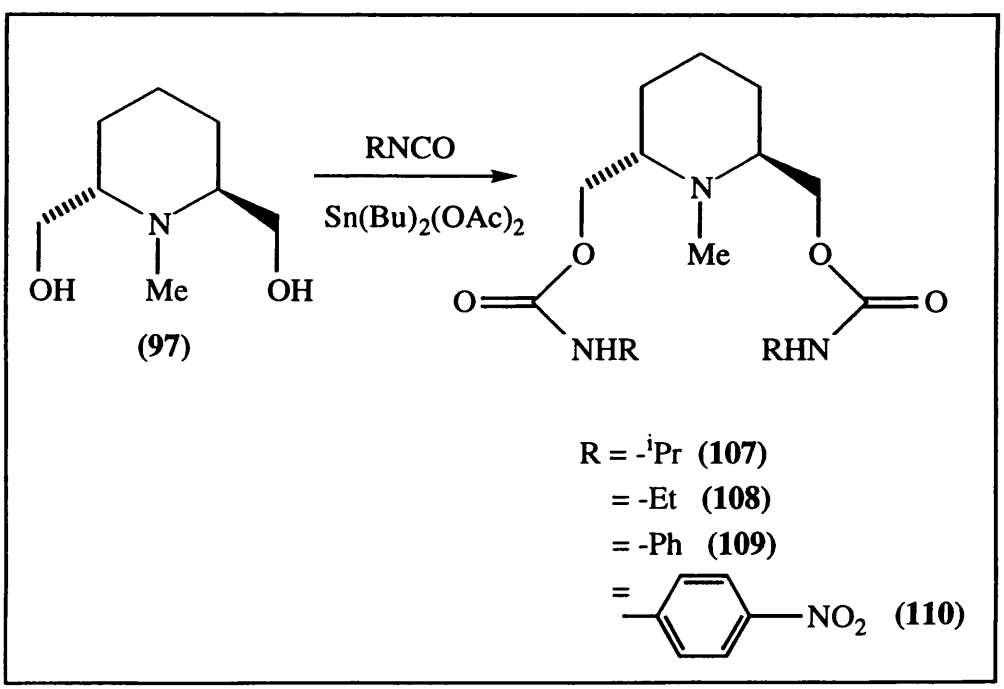
Scheme 22

Fortunately, in a large excess of borane-THF, kept at reflux for 48 hours, reduction of the hydrochloride salt to the diol (97) occured in good yield. The IR and mass spectra were of a similar pattern to the *cis*-isomer, although there were subtle differences in the NMR spectra. The *N*-methyl peak came at δ 2.32 (*cf. cis*,δ 2.21) and the C-2 and C-6 protons were at δ 2.43 (*cf. cis*, δ 2.26) as a multiplet in the ^{1}H NMR spectrum. The methylenes adjacent to the hydroxyl groups were again slightly different, appearing as a doublet of doublets at δ 3.45 and 3.91. The ^{13}C NMR spectrum was almost identical to that of the *cis*-isomer.

4.4 Synthesis of *N*-Methyl-*trans*-2,6-bis(aminocarbonyloxymethyl)-piperidines

The bis-carbamate derivatives of the *trans*-diol were prepared in an analogous manner to those of the *cis*-series in similar yields.

N-Methyl-*trans*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine (107) was obtained as white crystals after purification. The IR and mass spectra were almost identical to those of the *cis*-compound. In the NMR spectra, only slight differences in chemical shift were observed, such as the ring methylene protons which had resolved into two separate multiplets, found at δ 1.40 and δ 1.68 in the ratio 1 : 2, corresponding to C-4 : C-3 and C-5. The ^{13}C NMR spectrum showed similar patterns, where the C-4 methylene carbon had shifted to δ 25.6, compared to δ 23.7 in the *cis*-compound. Only one methyl signal was observed for the isopropyl groups. The largest difference in properties was observed for the melting points of the two compounds in which the *trans*-isomer melted at a temperature of 40 °C higher than the *cis*-isomer.



The bis(ethyl carbamate) (108) derivative also had identical spectra to that of its *cis*-isomer, except for the ^{1}H NMR spectrum in which the ring methylenes had been resolved as in the previous compound. Also, the protons on C-2 and C-6 had shifted downfield and were now part of a complex multiplet involving the methylenes of the bis(ethyl carbamate) at δ 3.09-3.27. Again, the largest difference came in the melting points, where this time the *trans*-isomer would not even solidify, remaining a colourless oil.

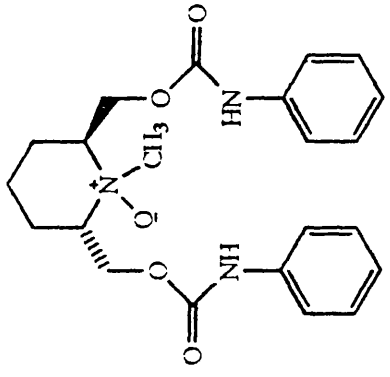
N-Methyl-*trans*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine (109), acquired as white crystals had small differences from the *cis*-isomer in the NMR spectra, but this could be due to the different deuteriated solvent used, in this case, d_6-DMSO. The ^{1}H NMR spectrum can be seen in Figure 4. For example, the *N*-methyl singlet appeared at δ 2.32, compared to δ 2.43 in the *cis*-isomer, and the C-2 and C-6 methine protons came as a multiplet at δ 2.35, in comparison to the *cis*-compound where they appeared at δ 2.54. The melting points of the *cis*- and *trans*-isomers differed by 15 °C with the *trans*-isomer melting at the lower temperature of 130 - 132 °C.

The bis(*p*-nitrophenyl carbamate) (110) caused the same problems as before with purity but yellow crystals were obtained after column chromatography. Again, no noticeable difference in the spectra was apparent, but the melting points of the isomers differed by 30 °C, with the *trans*-isomer melting at 49 °C and the *cis*-compound melting at 78-80 °C.

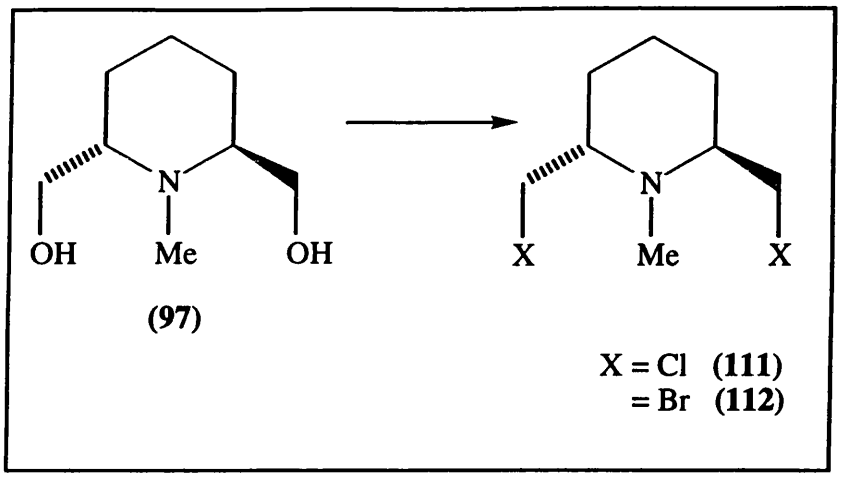
4.5 Synthesis of *N*-Methyl-*trans*-2,6-bis(halomethyl)piperidines

These compounds were prepared in the same manner as for the *cis*-isomers using thionyl chloride and phosphorus tribromide in equivalent yields to give oils as the products. Both the chloro- (111) and bromo- (112) compounds had similar spectra to their corresponding *cis*-analogues, and gave correct accurate mass spectra

Figure 4

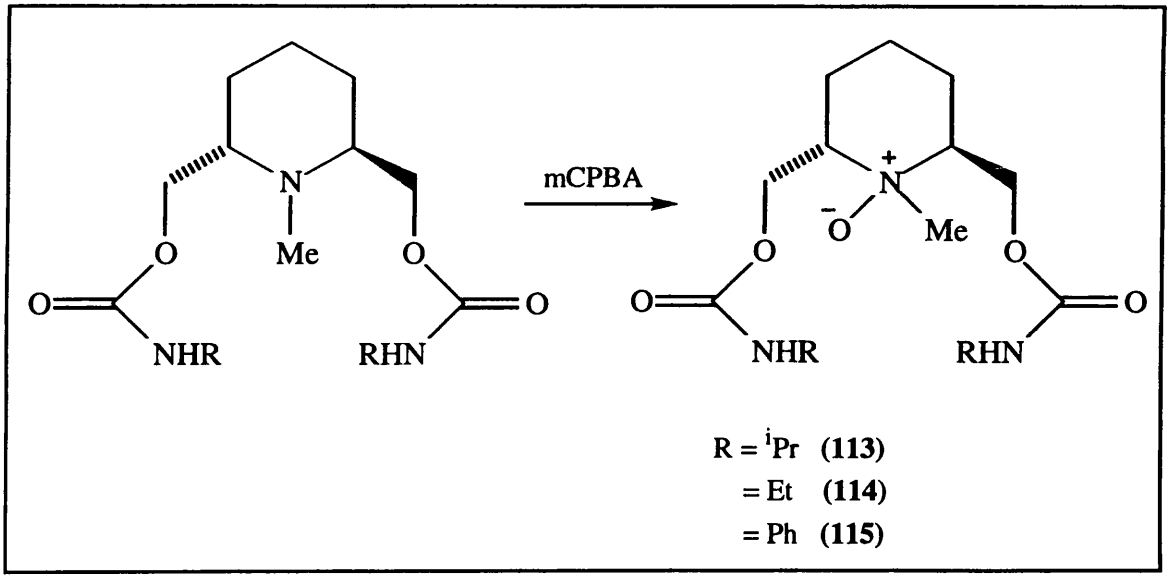


(115)



4.6 Synthesis of *N*-Methyl-*trans*-2,6-bis(aminocarbonyloxymethyl)-piperidine *N*-Oxides

mCPBA was again used as the oxidising agent to form the *N*-oxides in analogous conditions to those of the *cis*-series. Again, the *N*-oxide of the *p*-nitrophenyl derivative could not be made.



N-Methyl-*trans*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine *N*-oxide (113) was prepared in reasonable yield as white crystals. Similar IR and mass spectra to the *cis*-series were not a surprise and again only small differences in the NMR spectra

emerged. The most notable difference in the ^{1}H NMR spectrum was the shift of the methine protons at C-2 and C-6, from δ 3.52 in the *cis*-isomer to δ 3.30 in the *trans*-isomer. This time, even the melting points were quite similar, with the *trans*-isomer melting at 177 °C and the *cis*-isomer melting at 180 °C.

In the bis(ethyl carbamate) derivative (114), the ^{1}H NMR spectrum showed the same difference as found above. The protons α to the nitrogen were shifted to δ 3.38 in the *trans*-isomer, compared to δ 3.61 in the *cis*-isomer. No difference in the ^{13}C NMR spectrum was apparent for these two methine carbons. The melting points of the two *N*-oxides could be compared, unlike their free bases and it was obvious that the two compounds were different, with the *trans*-compound melting at 178 °C and the *cis*-isomer melting at 158 °C.

N-Methyl-*trans*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine *N*-oxide (115) was obtained as white crystals and had similar spectra to its *cis*-counterpart. The ^{1}H NMR spectrum can be seen in Figure 5. The *N*-methyl and α protons did resonate at different chemical shifts to those in the *cis*-compound (δ 3.15 and 3.59 respectively compared to the *cis*-isomer δ 2.87 and 3.43) but whether this is due to the different deuteriated solvents employed (d_6 DMSO and d_4 MeOD) or some stereoelectronic effect is unknown. Their two melting points were quite similar at 178 °C for the *trans*-isomer and 170 °C for the *cis*-isomer.

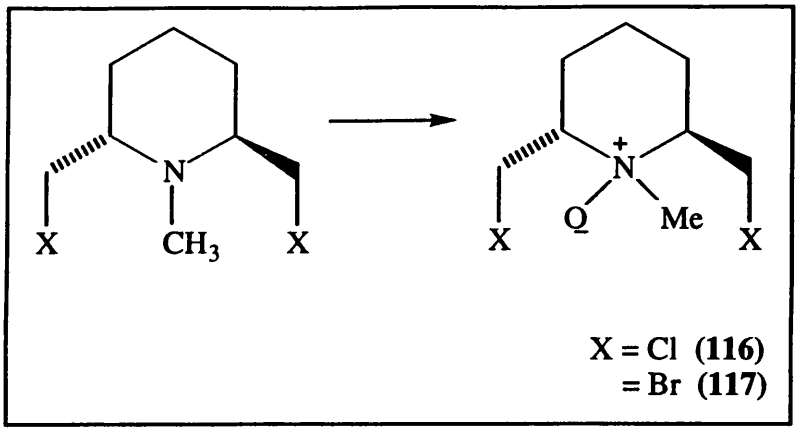
4.7 Synthesis of *N*-Methyl-*trans*-2,6-bis(halomethyl)piperidine *N*-Oxides

The same procedure used in the *cis*-series was followed to make these *N*-oxides. The yields were poor due to difficulties in purifying the products. Although the products were coloured, it seemed that in each case, it was only a very small impurity that was present.

Again, similar spectra to the *cis*-series were obtained.

Figure 5


(109)
NH
HN
CH3
N
O
O
O
O
SOLVENT DGDMSO
SF 200.133
SY 80.0
O1 4450.000
SI 32768
TD 16384
SW 4000.000
HZ/PT .244
PW 0.0
RD 0.0
AG 2.048
AG 16
RG 64
NS 298
TE
FW 5000 0.0
O2 0.0
DP 63L PO
LB .300
GB 0.0
CX 38.00
CY 20.00
F1 1850.10H
F2 -49.80H
HZ/CM 49.997
PPM/CM .250
SR 3315.97
INTEGRAL
PPM



4.8 Conclusions

A range of novel compounds have been prepared and characterised by a synthetically useful route. The biological testing data of these compounds can be seen in Chapter 8. It was now decided to investigate similar molecules that could incorporate two *N*-oxides which would then have different reduction potentials. It is crucial in the development of these compounds that the reduction potentials lie in the correct range in order to be reduced in the hypoxic cells. Two different types of molecule were chosen to synthesise due to the availability of starting materials and their similarity in structure to those compounds already made, so rational comparisons could be made regarding their biological activity. This work is presented in the next two chapters.

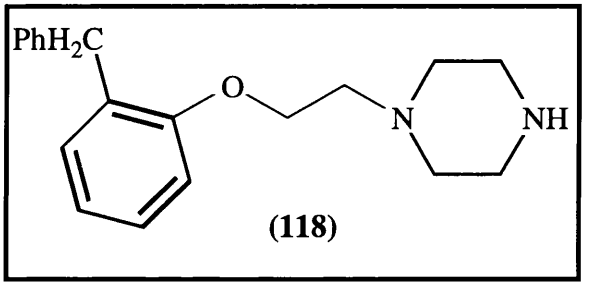
Chapter 5

Synthesis of 1,4-Disubstituted Piperazines

5.1 Introduction

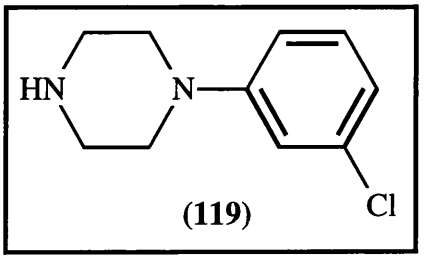
The piperazine structure is present in many pharmacologically active compounds and there has been much interest in the pharmaceutical and herbicidal[128] application of such compounds. Some selected examples will now be briefly mentioned.

New *N*-substituted piperazine derivatives (118) have been prepared as drugs for improving the functional disorder of the brain by inhibiting calcium uptake of the cranial nerve.[129]



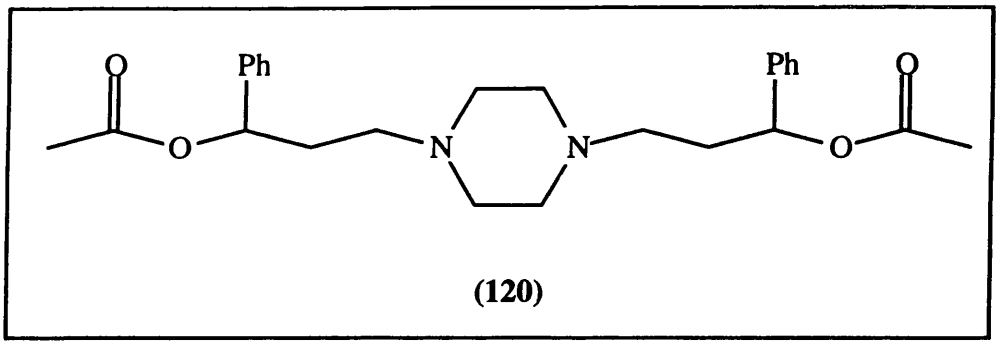
(118)

A similar type of compound (119) is a $GABA_A$ receptor blocker.[130] The authors suggest that the antidepressant and anti-psychotic effects as well as the convulsions, anxiety, panic attacks and insommnia caused by this compound may be due to $GABA_A$ receptor blockade.



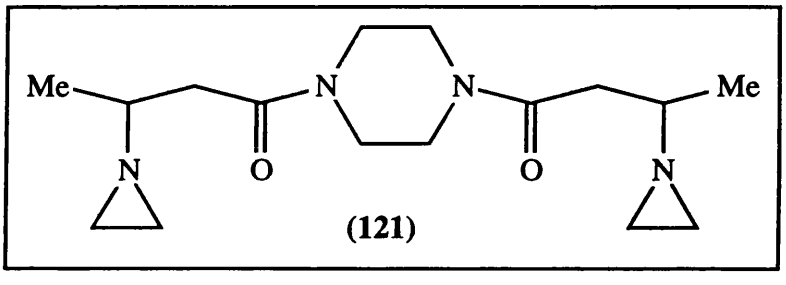
(119)

The synthesis and pharmacological screening of a group of piperazine derivatives (120) showing analgesic activity have recently been published.[131]



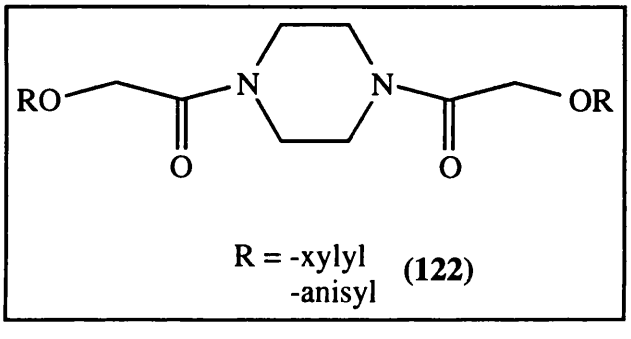
(120)

Other 1,4-disubstituted piperazines (121) have shown immunotropic activity, with marked suppressive effect.[132]



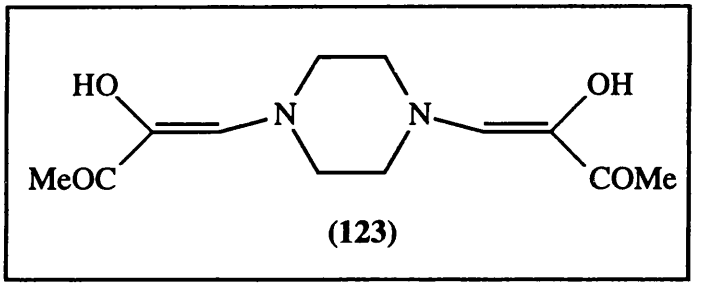
(121)

Similarly, compounds of type (122) have undergone pharmacological and physical tests.[133]



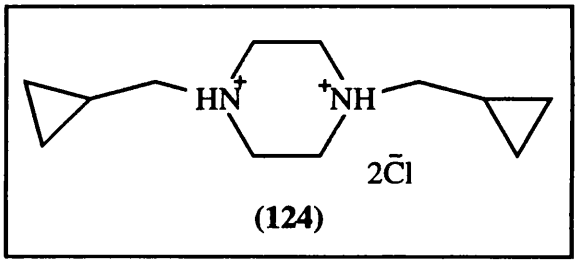
R = -xylyl
-anisyl **(122)**

Several types of derivatives of 1,4-disubstituted piperazines have been shown to have anticancer activity. Compounds such as (123) inhibit Lewis' pulmonary carcinoma amongst others.[134]



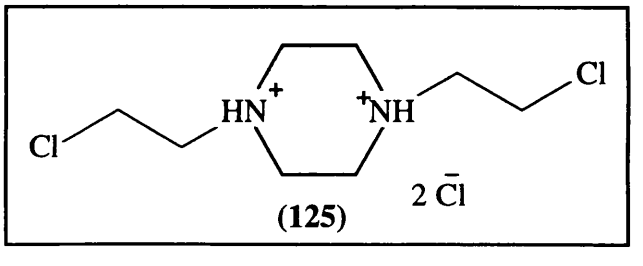
(123)

N,N'-Bis(cyclopropylmethyl)piperazine dihydrochloride (124) is a novel inotropic compound which shows potassium channel blocker activity and has antimuscarinic activity.[135]



(124)

1,4-Bis(2-chloroethyl)piperazine (125) has been prepared before,[136] along with 20 other β-chloroethylamine derivatives of mono-, bi- and polyfunctionality where the authors were making *N*-oxides in order to decrease the toxicity of nitrogen mustard compounds.



(125)

Their findings included the following.

a) The necessity of two or more β-chloroethylamine groups in the molecule in order to be effective in cancer retardation. This polyfunctionality was also true in the case of the *N*-oxides.

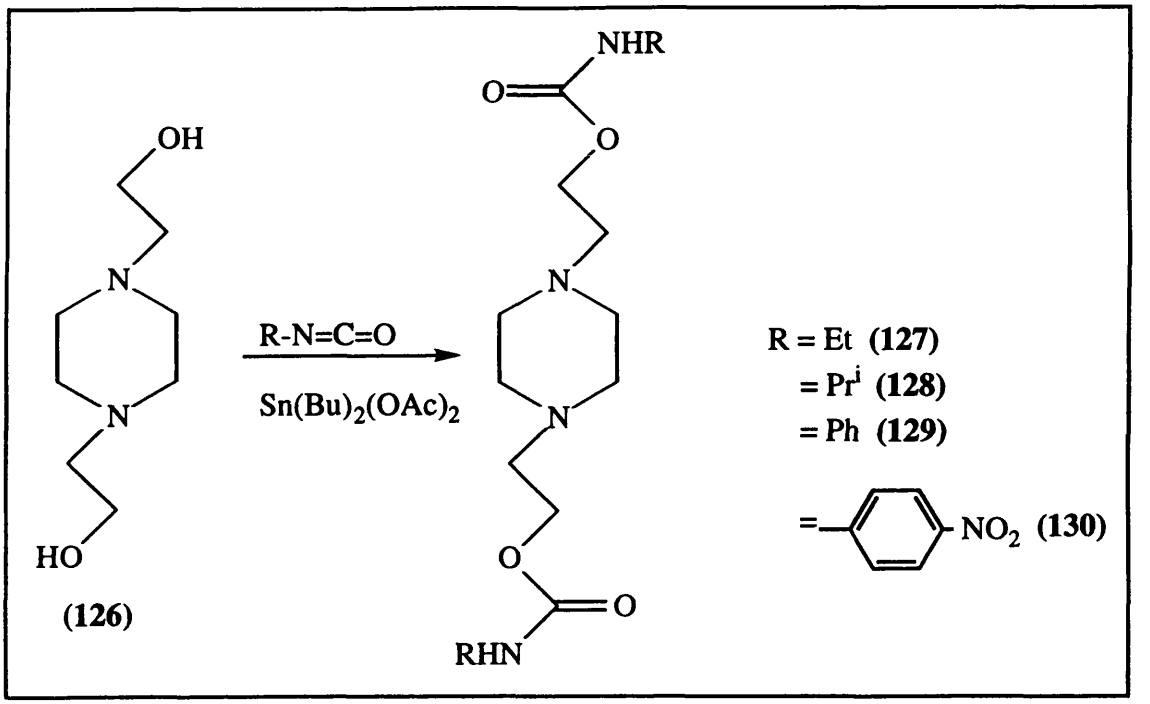
b) A decrease in toxicity was generally far more dominant than a decrease in effectiveness when the tertiary amines were changed into their *N*-oxides. Accordingly, the chemotherapeutic index was improved with *N*-oxide formation.

They reported that both the free base and the bis-*N*-oxide of the piperazine derivative were active against tumour cells, but not as effective as nitromin (Chapter 2), which they took forward to clinical trials.

5.2 Synthesis of 1,4-Disubstituted Piperazines

The piperazine series of derivatives was chosen so that two *N*-oxides could be incorporated into each molecule, thereby altering the reduction potential of the compound, which is important in the activation of such compounds. Another factor in choosing these compounds was the ease of formation from convenient starting materials. The same set of derivatives as before were chosen as targets so a rational comparison of the different systems could occur.

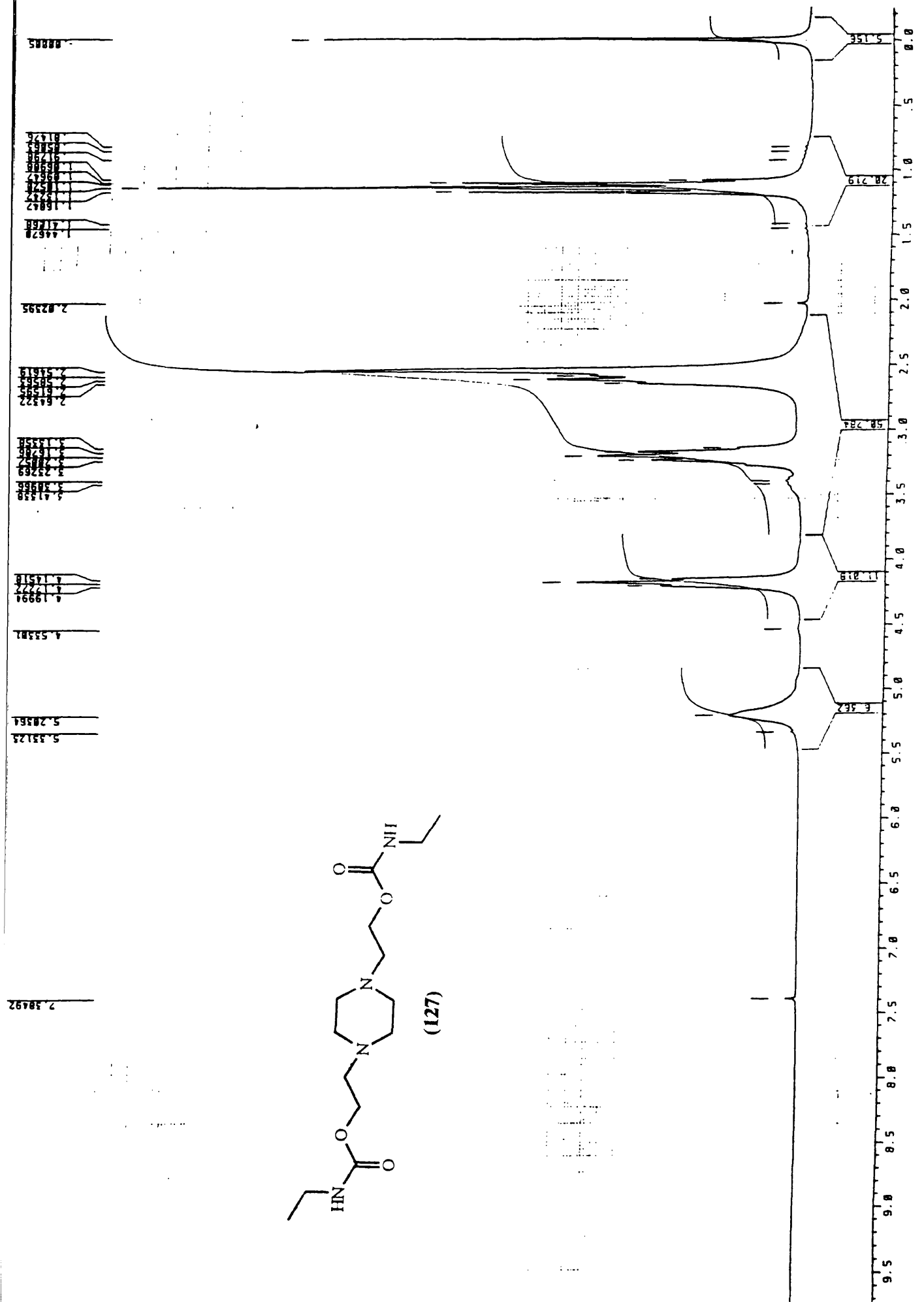
We started with the readily available 1,4-bis(2-hydroxyethyl)piperazine (126) and, in the same manner as before, reacted it with a number of isocyanates to produce a range of bis-carbamates in excellent yield. Although the starting diol is sparingly soluble in dichloromethane, as the reaction proceeded, the very soluble bis-carbamate was produced. In this manner the reaction was known to be complete when a clear solution was obtained.



1,4-Bis(2-isopropylaminocarbonyloxyethyl)piperazine (128) was obtained as white crystals. The IR spectrum showed bands at 2780 and 2810 cm^{-1} which relate to the *N*-CH_2 stretch, and a carbonyl band at 1690 cm^{-1}. The isopropyl group was evident due to a strong signal at 1365 cm^{-1}, corresponding to $C(CH_3)_2$. In the ^1H NMR spectrum, a multiplet appeared at δ 2.60 which had an integral of 12 H. The methylene group next to the carbamate came further downfield at δ 4.16 as expected. A broad singlet at δ 4.83 was attributed to the NH of the carbamate. The ^{13}C NMR spectrum showed three methylene signals, at δ 53.1, 57.1 and 61.5, with intensities of 2:1:1. Therefore, the ring methylenes were assigned the signal at δ 53.1. The signal at δ 61.5 was similar to that found for the methylene next to the carbamate in previous examples, so the signal at δ 57.1 was attributed to the exocyclic *N*-CH_2. A parent ion *m/z* 344 was found as a small percentage in the mass spectrum, whilst the largest fragment at *m/z* 125 corresponded to loss of both carbamate groups plus a methylene group.

The bis(ethylcarbamate) (127) behaved in a similar fashion to that of the *iso*-propyl carbamate and was obtained as white crystals. The spectra were consistent with the structure and full analytical data were obtained.The ^1H NMR spectrum of this compound can be seen in Figure 6.

Figure 6



(127)

1,4-Bis(2-phenylaminocarbonyloxyethyl)piperazine (129) was made as above, but the resulting bis-carbamate was also insoluble in dichloromethane, and a heavy white precipitate formed during the reaction. However, as all the diol had reacted, it was easy to purify the product by recrystallisation with methanol. The expected bands were apparent in the IR spectrum, with the carbonyl stretch occurring at 1700 cm^{-1}. The ^1H NMR spectrum was run in d_6-DMSO and signals were obtained at δ 2.52 (singlet) and δ 2.63 (triplet) in a ratio of 2 : 1. The triplet was obviously the exocyclic *N*-CH_2 which was also coupled to the triplet at δ 4.22 which corresponded to the adjacent methylene group. In the ^{13}C NMR spectrum, the methylenes appeared at the same shifts and same ratios as in the previous examples. No parent ion was found in the mass spectrum, just the expected fragmentation pattern.

The bis(*p*-nitrophenylcarbamate) derivative (130) behaved similarly to the bis(phenylcarbamate) in that it precipitated as it was being formed. This presented problems when purification was attempted due to the extreme insolubility of the compound. Crystallisation was therefore very difficult as solubility was only obtained with DMSO, from which the compound was unrecoverable. However, repeated washing of the material with hot methanol rendered a solid pure enough for good analytical data to be obtained.

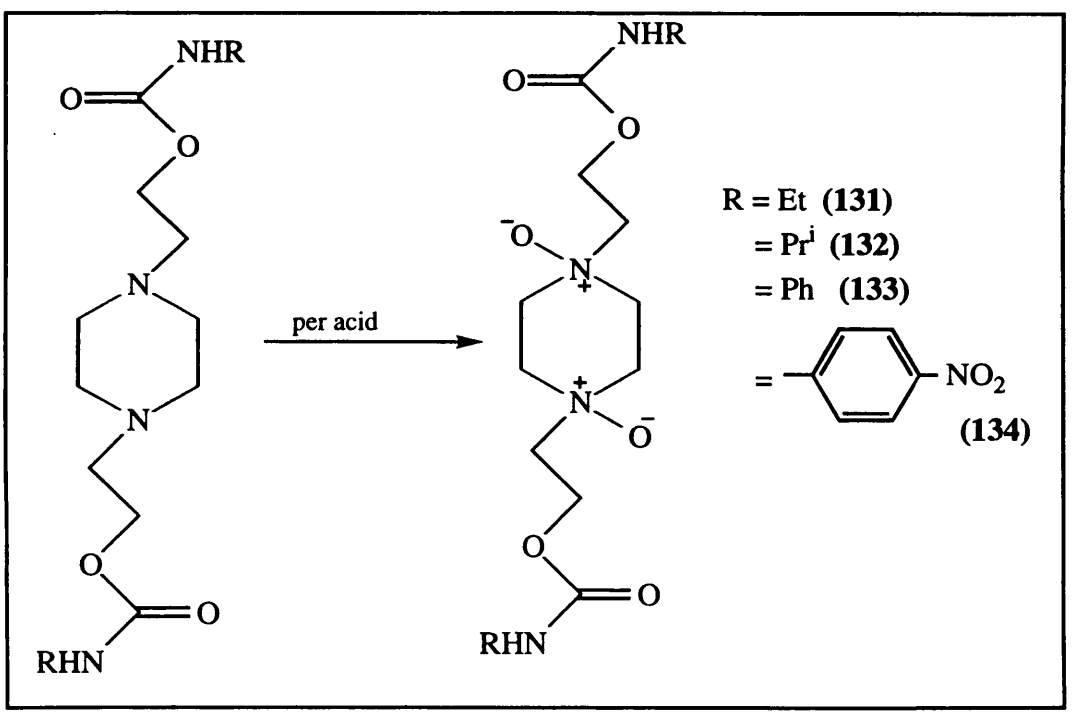
The production of the bis(2-haloethyl)piperazines was also attempted. 1,4-Bis(2-chloroethyl)piperazine hydrochloride (125) was prepared using thionyl chloride. The product was kept as the dihydrochloride salt to stop any decomposition, which would occur if the nitrogens were liberated to the free base. All spectra obtained were consistent with the structure. The ^1H NMR spectrum was similar to that of the bis(phenyl carbamate), where the ring *N*-CH_2 groups were distinguishable from the exocyclic *N*-CH_2, and appeared as a singlet at δ 3.77. The exocyclic methylenes gave signals at δ 3.70 and 3.94 and were coupled to each other.

Attempts to make the dibromo compound were numerous, but futile. Phosphorus tribromide was used initially, but the insolubility of the starting diol prevented any reaction taking place as the starting material just clumped together on addition of the

reagent. Heating for long periods of time did not improve the situation. Then a mixture of triphenylphosphine and bromine was tried, but no product could be isolated. Carbon tetrabromide with triphenylphosphine was also used, but only starting material was isolated. In both the last two cases, bromination probably happened, but attempts to isolate the free base failed probably because of aziridinium formation and hydrolysis back to the alcohol. Problems with removal of triphenylphosphine and its oxide were also encountered. At this stage it was decided not to pursue this synthesis.

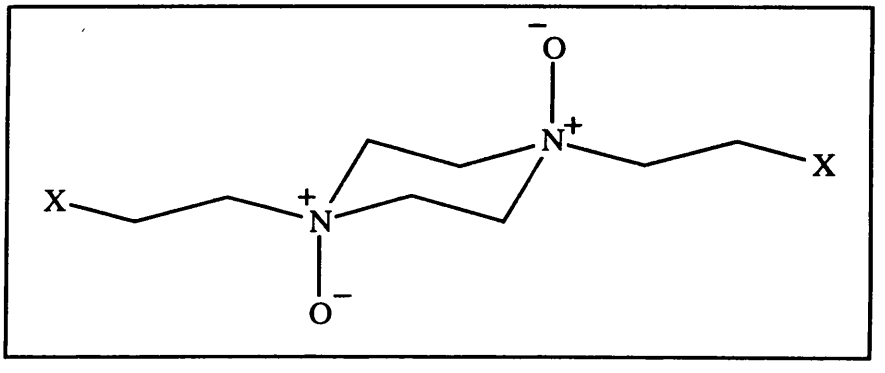
5.3 Synthesis of 1,4-Disubstituted Piperazine bis-*N*-Oxides

The majority of the piperazine derivatives were oxidised in the same manner as before using mCPBA. As all the products were insoluble in the solvent used (acetone), it was easy to follow the progress of each reaction by the formation of a white precipitate.



1,4-Bis(2-isopropylaminocarbonyloxyethyl)piperazine bis-*N*-oxide (132) was prepared in reasonable yield and obtained as white crystals. In the IR spectrum, a new band at 980 cm^{-1} indicated the formation of the *N*-oxide. The solubility of the compound

had decreased substantially and NMR spectra were only obtainable in d_6-DMSO at 333K. The ^1H NMR spectrum showed a multiplet at δ 3.63 which corresponded to the ring *N*-CH_2 protons. Two broad singlets at δ 3.98 and 4.58 corresponded to the exocyclic methylenes, with the protons next to the carbamate occurring the furthest downfield. From the ^{13}C NMR spectrum, it appeared that only one diastereoisomer wa present and the three methylene signals appeared at δ 57.3, 58.6 and 67.8, this time in the ratio 1:2:1. Evidently, the ring methylene carbons are more affected by the presence of the oxygens than the acyclic ones. This can be seen in the diagram below, where more effect from the axial oxygens can be envisaged with the ring carbons than the equatorial methylene groups.



No molecular ion was found in the mass spectum; indeed, the compound seemed to fall apart very easily into small, recognisable fragments.

The bis(ethylcarbamate)-bis-*N*-oxide (131) was more soluble than the bis(isopropylcarbamate) and a ^1H NMR spectrum was obtained in D_2O which can be seen in Figure 7. This shows the methylene protons as four different types of proton in a AA'BB' system.

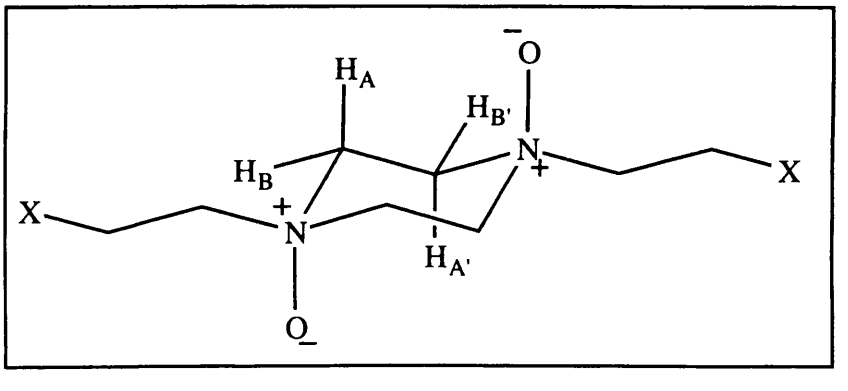
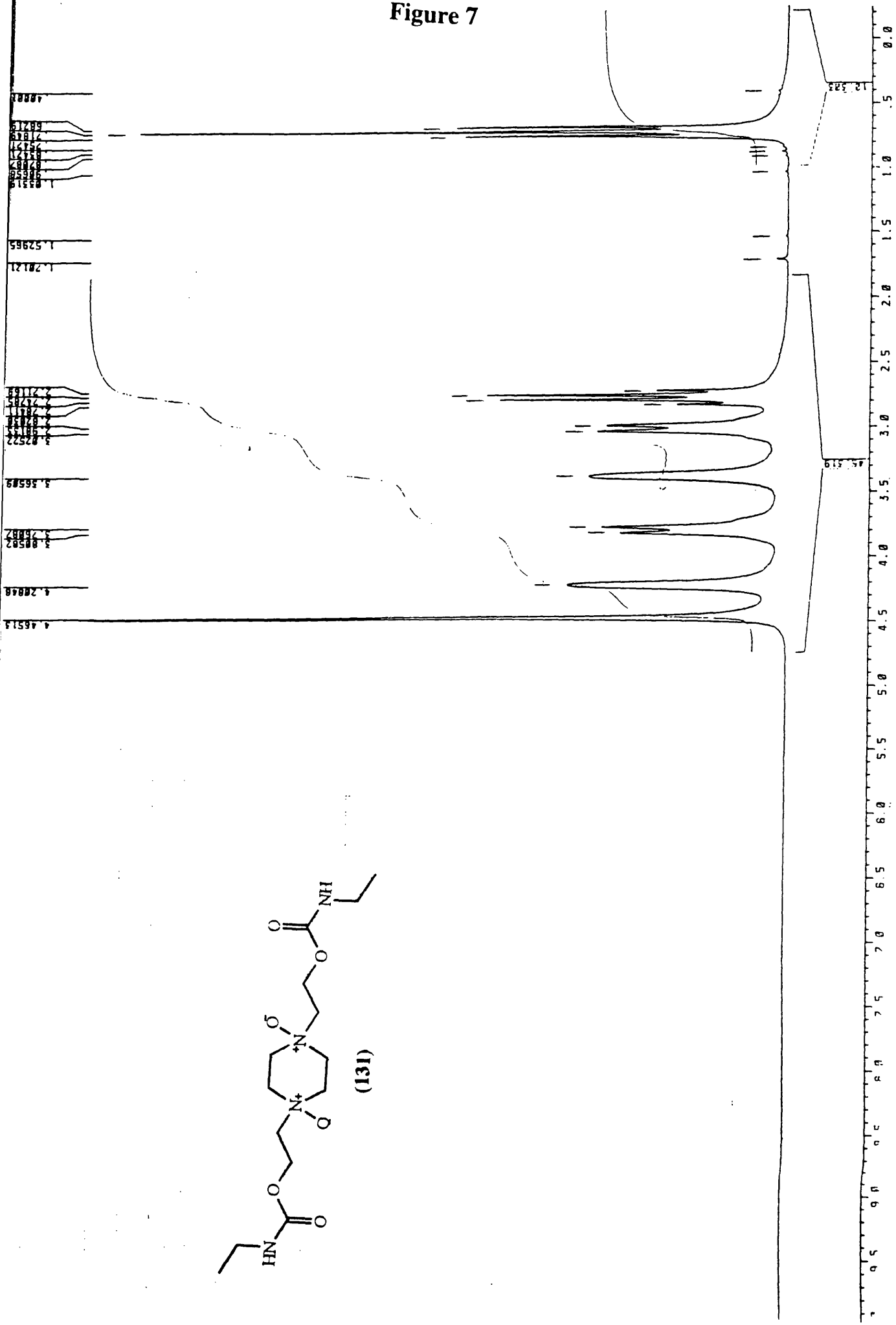


Figure 7



(131)

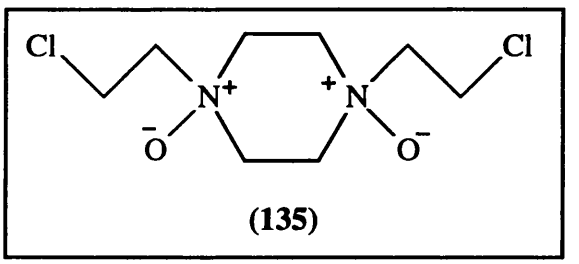
There also appeared in the ^1H NMR spectrum two doublets at δ 3.24 and 4.12 with two broad singlets at δ 3.50 and 4.44, all of equal intensity. In assigning the signals, it was assumed that as the methylene protons next to the carbamate had always appeared the furthest downfield, they would do so again, so those protons were assigned the singlet at δ 4.44. Since the methylene protons next to that carbon would be coupled to it, it seemed logical that the singlet at δ 3.50 would belong to those protons. The two doublets remaining were more difficult to assign. From the diagram it appears that H_B and $H_{B'}$ are more affected by the oxygen than the H_A and $H_{A'}$, and so would have a signal further downfield. Thus H_A was assigned δ 3.24 and H_B assigned δ 4.12. Although the difference could be seen in the ^1H NMR spectrum, there was still only the three methylene signals in the ^{13}C NMR spectrum as before, indicating the presence of one diastereoisomer.

The same pattern appeared in the ^1H NMR spectrum for bis(phenylcarbamate)-bis-*N*-oxide (133) at approximately the same shifts. This bis-*N*-oxide is highly insoluble, barely dissolving in DMSO unless it is heated.

1,4-Bis(2-*p*-nitrophenylaminocarbonyloxyethyl)piperazine bis-*N*-oxide (134) could not be prepared using mCPBA with acetone as solvent due to the insolubility of the starting material. Therefore another method was required. Trifluoroacetic acid is well known as an excellent solvent, so it seemed obvious to use this in combination with hydrogen peroxide to produce trifluoroperacetic acid *in situ*. Fortunately, the bis(nitrophenyl carbamate) did dissolve in the acid and the bis-*N*-oxide was produced in this manner. Like the bis(phenyl carbamate), this compound is highly insoluble, dissolving only in DMSO. The *N*-oxide stretch band appeared at 920 cm^{-1} in the IR spectrum along with the expected bands for the aromatic region. The ^1H NMR spectrum was run in d_6-DMSO, and all the *N*-CH_2 methylene protons appeared in a multiplet ranging from δ 4.40 to 4.52. In this case these protons appeared further downfield than the methylenes next to the carbamate, which appeared at their normal shift of δ 4.10. Solvent effects due to the 'wet' d_6-DMSO are a possible explanation for this large shift of

ca 1 ppm. No such effects were seen in the ^{13}C NMR spectrum, with the signals appearing at comparable shifts as before.

The dichloro bis-*N*-oxide (135) was made as usual, but care was taken to form the free base in completely dry conditions, using distilled acetone and distilled triethylamine, before the addition of mCPBA. This gave the product as white crystals which were highly soluble in most organic solvents.



(135)

The IR spectrum showed bands at 950 cm^{-1} for the *N*-oxide stretch and 670 cm^{-1} for the C-Cl stretch. The ^1H NMR spectrum was different from the spectrum of the dihydrochloride salt, with the ring protons appearing between δ 2.73 and 3.36. The acyclic methylene groups appeared as two triplets at δ 3.64 and 3.90, corresponding to the *N*-CH_2 and the Cl-CH_2 respectively. The ^{13}C NMR spectrum indicated the presence of one diastereoisomer. A suprisingly large molecular ion *m/z* 244 was found in the mass spectrum, with the main peak at *m/z* 163, corresponding to loss of an oxygen and a chloroethyl group.

5.4 Conclusions

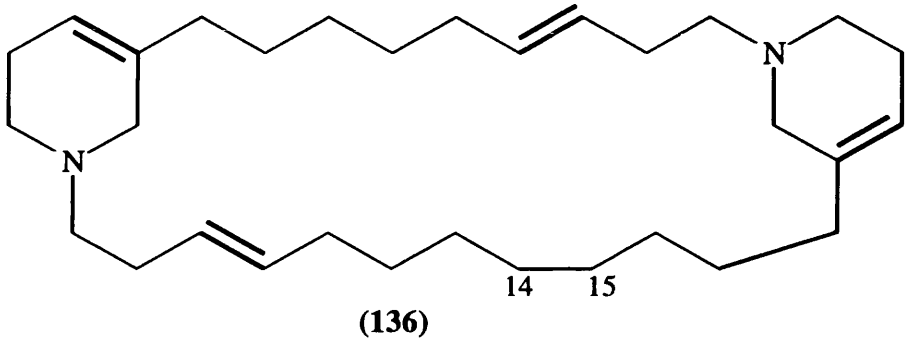
Crystalline solids of a range of 1,4-disubstituted piperazines and their corresponding bis-*N*-oxides have been synthesised and fully characterised and the biological testing results can be found in Chapter 8.

Chapter 6

Synthesis of N,N'-bridged bis(2-substituted piperidines)

6.1 Bis-piperidine Alkaloids

This type of structure with two piperidines joined by an alkyl chain, is relatively uncommon in nature, and natural compounds joined through both nitrogens are rare. Of those that do exist, many have toxic properties. Haliclamines A and B (136) are two cytotoxic alkaloids occurring in an unnamed marine sponge of the genus *Haliclona*. Their structures and configurations are based on extensive NMR spectroscopy.[137]



(136)

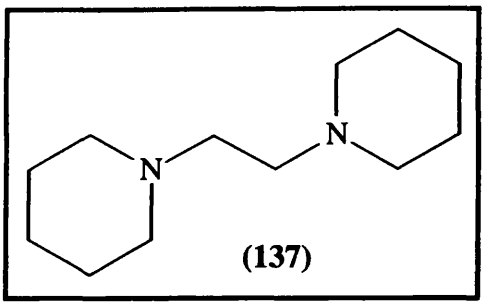
Haliclamine A
Haliclamine B(Δ^{14}, *cis*)

Other alkaloids with a two carbon chain linking the two nitrogens include thermopsidine,[138] convolvidine,[139] and salsamine.[140]

6.2 N,N'-Alkylbispiperidines

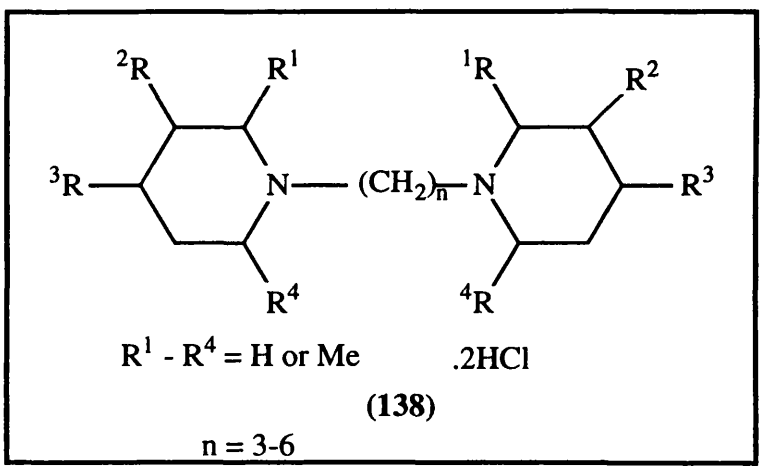
Neurotoxicity has been reported[141] to exist in the simplest backbone structure of *N,N'*-1,2-ethanediyl-bis-piperidine (137). This is not a cumulative poison and

neurotoxicity is absent in derivatives with different ring systems or with different connecting chains.



(137)

N,N'-αω-Dialkylbis-piperidine dihydrochlorides (138) have been prepared by alkylation of pyridine derivatives with αω-dihalo-C_{3-6}-alkanes followed by hydrogenation of the resulting bis-pyridinoalkanes in the presence of Adams' catalyst in acetic acid.[142] These compounds showed high radioprotectant activities when R = H, while the Me-substituted salts were more toxic and displayed no radioprotectant activity.

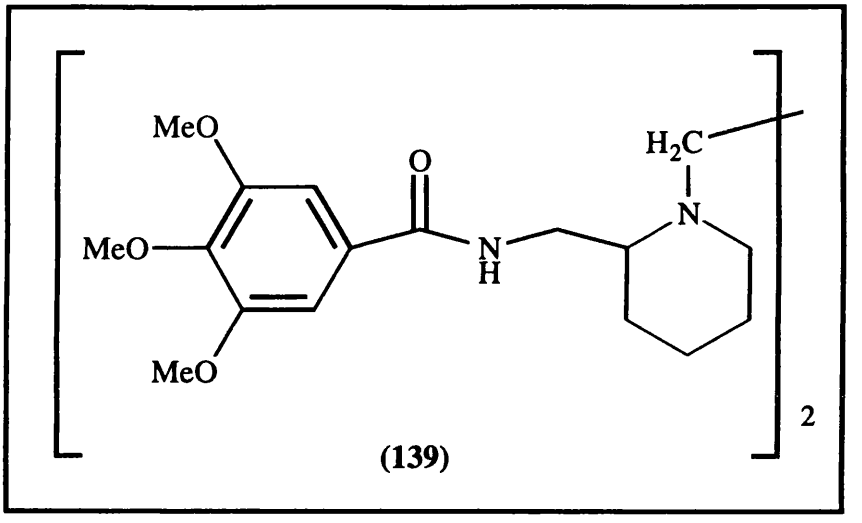


R^1 - R^4 = H or Me .2HCl

(138)

n = 3-6

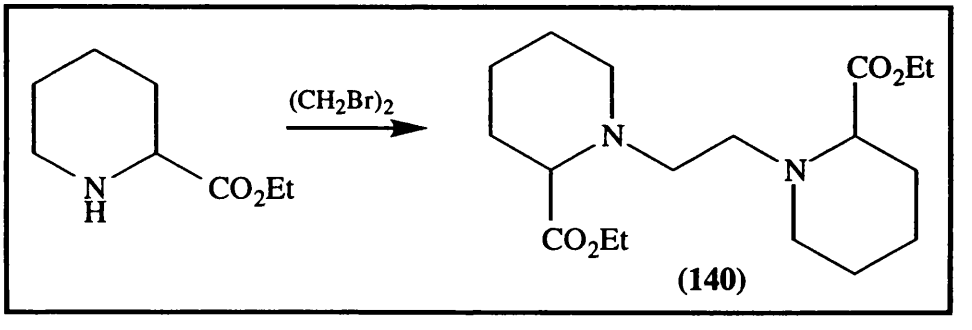
Substituted amides of (139) have been found to possess cardiovascular activity, in particular calcium antagonistic activity, which indicates that they have potential use in the treatment of angina.[143] This type of compound is well absorbed and shows high metabolic stability with no toxic effects.



(139)

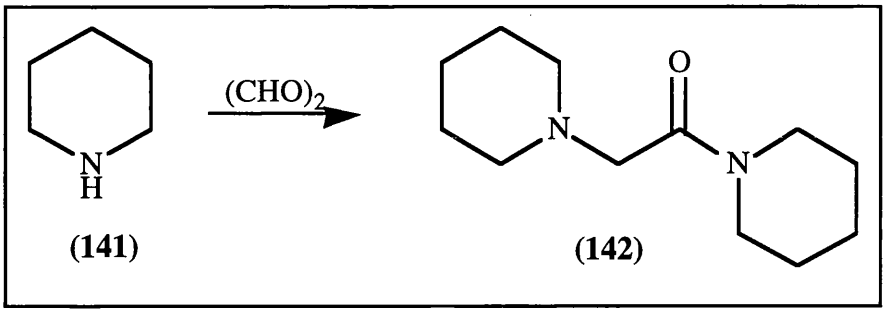
6.3 Synthesis of N,N'-(1,2-Bisethanediyl)bis(2-hydroxymethyl-piperidine)

Literature precedence[144] had suggested that pipecolinic acid derivatives (140) could be linked with 1,2-dibromoethane. However, when we repeated this reaction, mostly starting material was isolated after using a variety of conditions.



(140)

We then tried to use the reductive alkylation procedure which had worked well in the *N*-methylation of *cis*-piperidine systems. It had been reported[145] that aqueous glyoxal reacts with piperidine (141) to give a bis-piperidine system (142).



(141) **(142)**

We considered that it should be possible to condense two pipecolinic acids with glyoxal and reduce the resultant product to the aliphatic two carbon chain *in situ* with hydrogen and palladium-carbon, as done previously (Chapter 3). However, this was unsuccessful and only starting material was isolated.

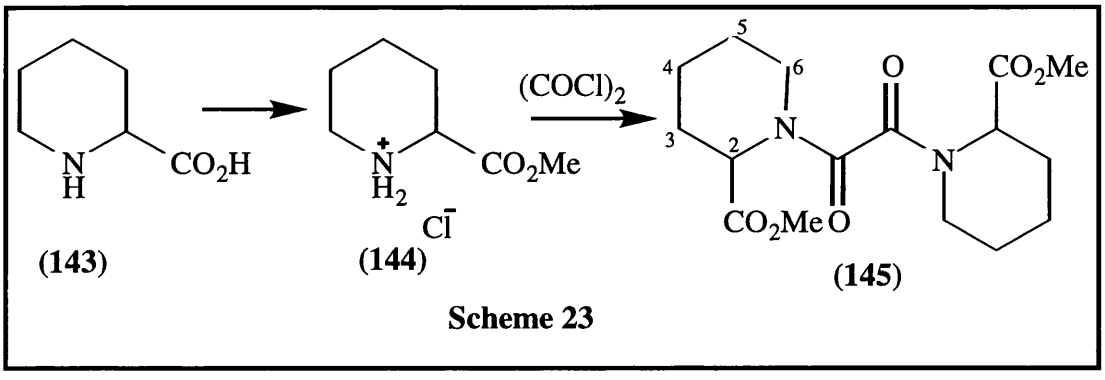
Vicinal diamination of ethene using a palladium promoted sequence[146] followed by oxidation was considered but not attempted due to the complexity of the process.

We chose oxalyl chloride as our coupling agent for ease of use and ready availability of the reagent, even if it did add a couple of steps on to our route (Scheme 23).

Methyl DL-pipecolinate hydrochloride (144) was prepared from pipecolinic acid (143) using 2,2-dimethoxypropane and conc. hydrochloric acid. This is an excellent method of esterification when the starting acid is highly insoluble. Because the molecule is chiral, the protons are diastereotopic and the ^{1}H NMR spectrum was quite complex. Five protons appeared as a broad multiplet at δ 1.42-1.77 which corresponds to C-4 and C-5 methylenes and one proton of C-3. The other proton of C-3 appeared as a multiplet at δ 2.16, as these protons are diastereotopic. The methylene protons of C-6 were split into two multiplets, one at δ 2.91 and the other at δ 3.31. The methine signal appeared further downfield at a shift of δ 3.88 as a doublet of doublets. The ^{13}C NMR spectrum showed methylene signals at δ 22.0 and 22.1 corresponding to C-4 and C-5, while C-3 appeared at δ 26.5. The other methylene carbon, C-6 had its signal at δ 44.9, quite far downfield due to the positive charge on the adjacent nitrogen. The methyl carbon of the ester was at δ 54.4, close to the methine C-6 carbon at δ 57.6. The one quaternary carbon of the ester appeared at δ 171.0. A molecular ion of *m/z* 143 was observed in the mass spectrum,

with the largest fragment belonging to *m/z* 84 which corresponds to loss of the ester group.

The key reaction was now to couple two of these molecules together using oxalyl chloride. This was achieved in chloroform with two equivalents of triethylamine as a base to form the piperidine base *in situ* and to mop up the hydrochloric acid formed in the reaction to minimise ester hydrolysis.



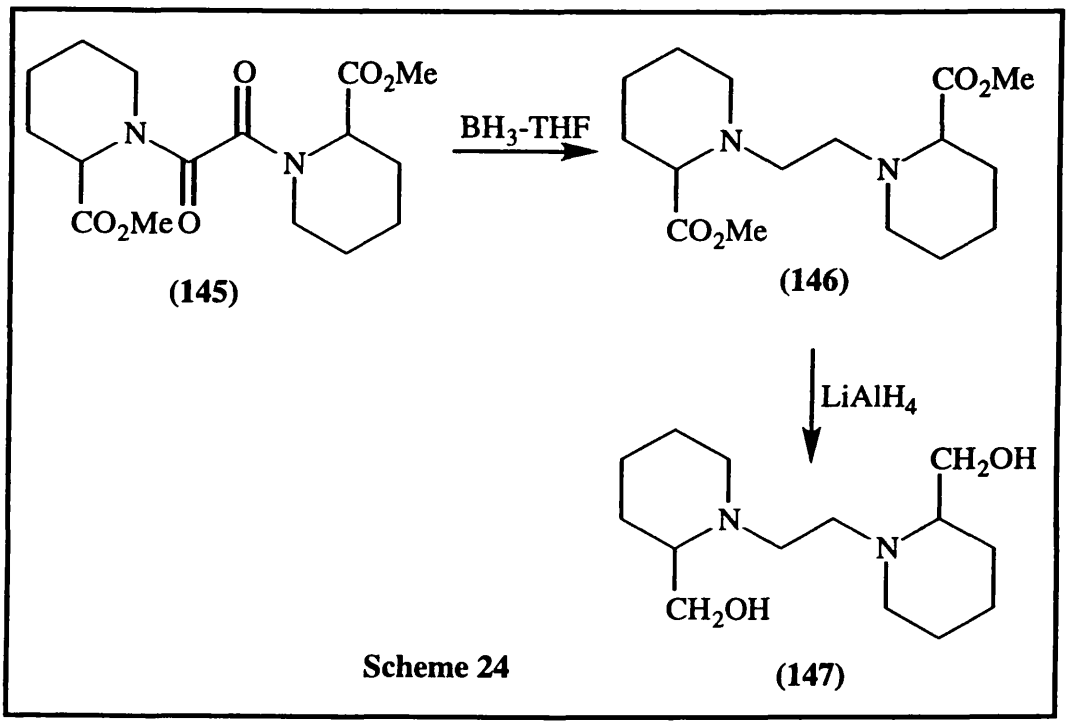
(143) **(144)** **(145)**

Scheme 23

The dimer (145) was obtained as a clear oil after purification. The IR spectrum showed two carbonyl stretch bands, one at 1739 cm^{-1} for the ester carbonyl, and one at 1659 cm^{-1} for the amide carbonyl. The ^1H NMR spectrum revealed a broad multiplet between δ 1.20 and 1.82 with an intensity of 10 H, corresponding to the methylene protons in the rings not attached to nitrogen. A multiplet at δ 2.25 with intensity 2H was attributed to one proton of the methylene (C3) adjacent to the chiral centre as in methyl DL-pipecolinate. There were two different signals for the axial and equatorial protons next to the nitrogen (C6), one multiplet at δ 2.90 and the other at δ 3.30. The methyl protons of the esters occurred as a singlet at δ 3.77. A multiplet at δ 5.26 was assigned to the methine protons. This compound is a mixture of a racemate and a meso form, and two sets of signals were seen in the ^{13}C NMR spectrum. Four methylene carbons were observed in duplicate at *ca.* δ 21, 25, 26 and 44. These were assigned to C-4, C-5, C-3 and C-6 respectively. There was only one signal at δ 52.3 due to the methine proton at C-2. Two quaternary carbons in duplicate at δ 164.9 and 170.8 correspond to the amide carbonyl carbon and the ester carbonyl carbon respectively. In the mass

spectrum, a small molecular ion was observed at *m/z* 340. The largest ion was at *m/z* 142 which is due to cleavage of an amide bond to leave the pipecolinate ester fragment.

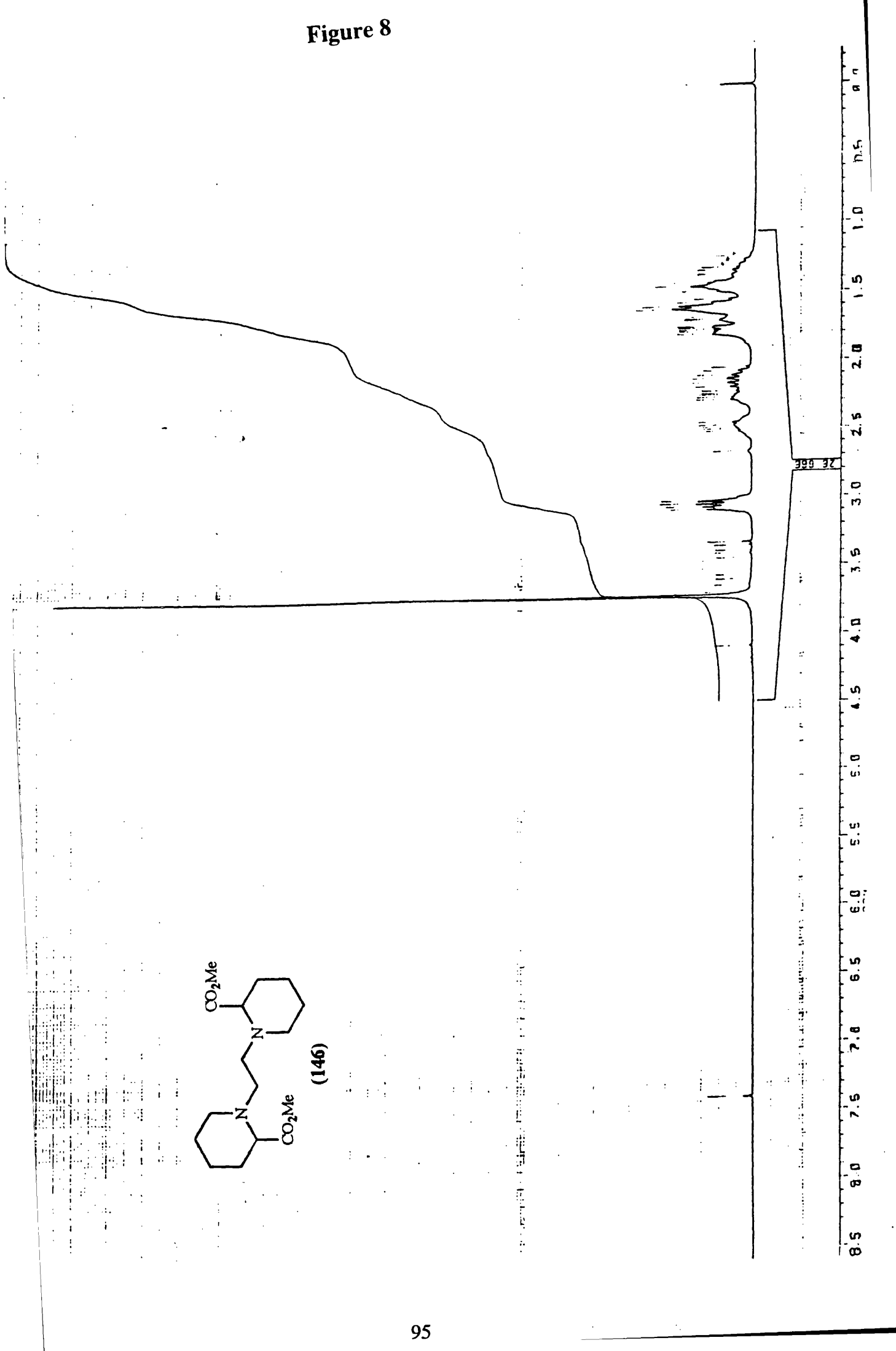
Reduction was now required to produce the desired diol intermediate. It was envisaged that we might be able to reduce both the amide and ester in one step with $LiAlH_4$. When this was attempted, instead of reducing the amide to the amine, the amide bond was cleaved to produce the original starting material. This happens with some secondary amides depending on the reaction conditions.[147] The solution was to reduce each functional group in turn, starting with the amide, which could be reduced easily with borane, followed by reduction of the ester using $LiAlH_4$.



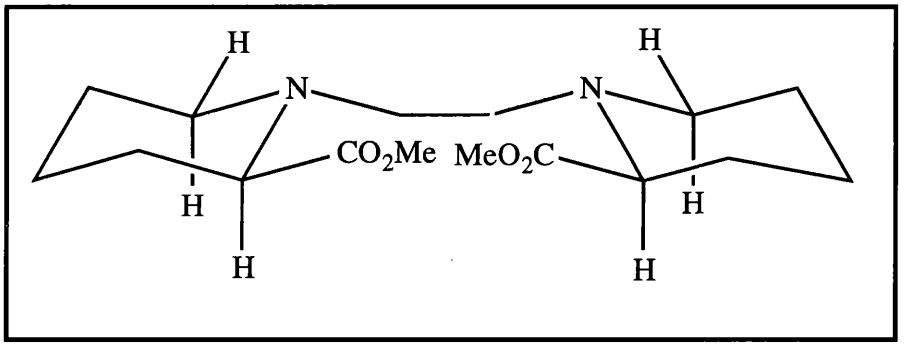
Scheme 24

Reduction of the diamide went smoothly and the free base (146) was obtained in good yield as a clear oil. As expected, there was only one carbonyl band in the IR spectrum at 1737 cm^{-1} which corresponded to the ester group. The ^1H NMR spectrum can be seen in Figure 8. The two methine protons appeared together as one multiplet at δ 2.16. Multiplets at δ 2.42 and 2.57 of equal intensity was attributed to the axial and equatorial protons of C-6.The slightly less complex signal at δ 3.00 was assigned to the

Figure 8



linear bridging methylenes protons. Assignment was consistent with literature precedence.[167]

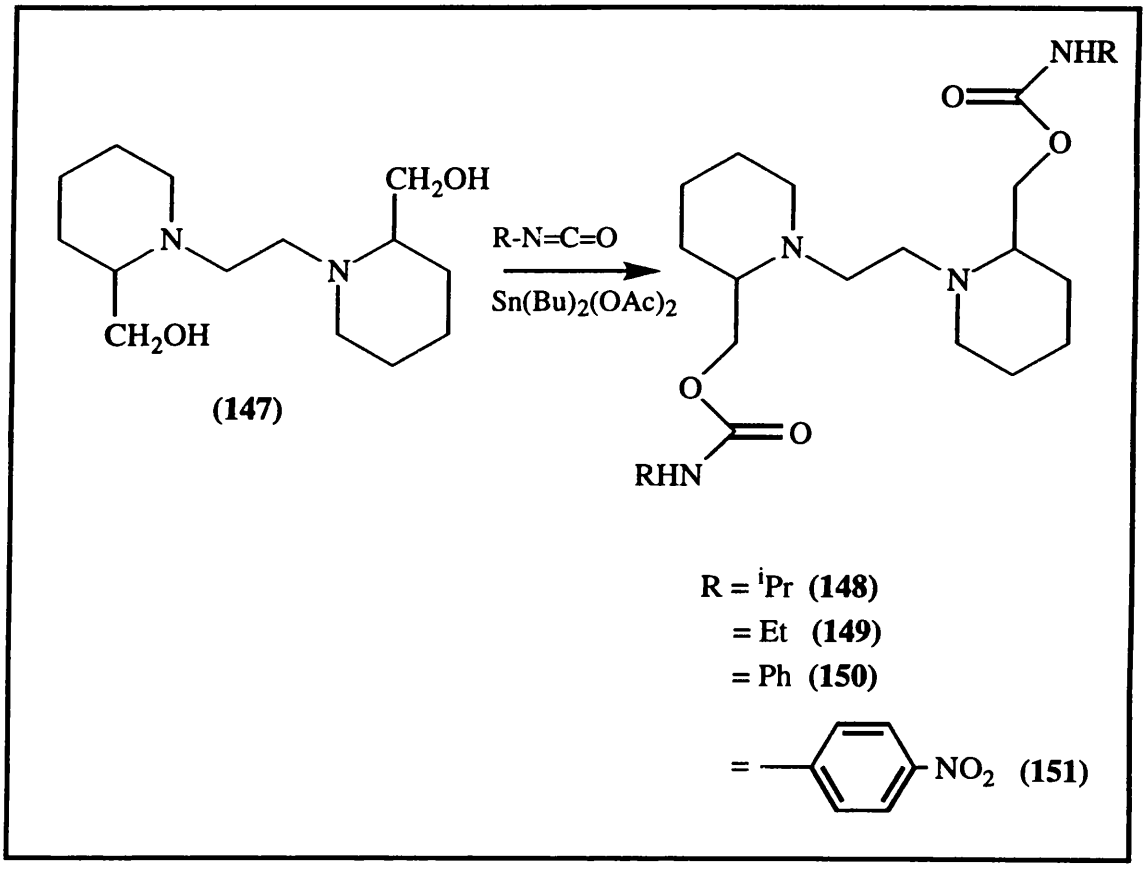


The ^{13}C NMR spectrum was similar to that of the diamide (145) with the signals in duplicate. Again, the *N*-methylene signals were distinguishable, with one set apparent at δ 50.7 and 50.9 while the other set was at δ 53.8 and 54.0. These were assigned for the same reasons as above to the cyclic and linear carbons respectively. The methine signals had shifted 10 ppm downfield to new positions of δ 65.1 and 65.4, whilst the single quaternary carbon appeared at δ 174.1 and 174.2. The parent ion *m/z* 312 was found as a small percentage with the major ion being that of exactly half the molecule with *m/z* of 156.

The second reduction, this time with $LiAlH_4$ went in high yield to give the diol (147) as a thick clear oil. There were no bands in the carbonyl region of the IR spectrum; instead, new bands at 3329 and 1284 cm^{-1} corresponding to OH stretch and OH bend had appeared. The major difference in the ^1H NMR spectrum was the emergence of the hydroxymethylene protons, which appeared as two doublet of doublets at δ 3.17 and 3.85. A broad singlet at δ 4.26 was attributed to the OH proton. The ^{13}C NMR spectrum showed no duplication of signals. However the rest of the spectrum was similar to that above with the exception of the quaternary carbon of the ester and the addition of the hydroxymethylene signal which appeared at δ 63.1. The molecular ion minus one hydrogen was found at *m/z* 255, but again, the major ion was that of half the molecule at *m/z* 128.

6.4 Synthesis of *N,N'*-(1,2-Ethanediyl)bis(aminocarbonyloxymethyl-piperidines)

After synthesising our key intermediate, we prepared a series of bis-carbamates and a dihalogen derivative.



The bis(isopropylcarbamate) (148) was made in the standard way and was obtained as white crystals. Bands at 1684 and 1364 cm^{-1} were evident in the IR spectrum and were due to the carbonyl and isopropyl methyl groups respectively. In the ^1H NMR spectrum the ring methylenes C-3, C-4 and C-5 were grouped together in a complex multiplet at δ 1.36-1.74, whilst the methine protons and the C-6 protons were also found together as a broad multiplet between δ 2.12 and 2.72. The methylene protons between the rings appeared at δ 2.89 as a multiplet and the remaining methylene protons next to the carbamate groups came at δ 4.15, also as a multiplet. The peaks corresponding to the

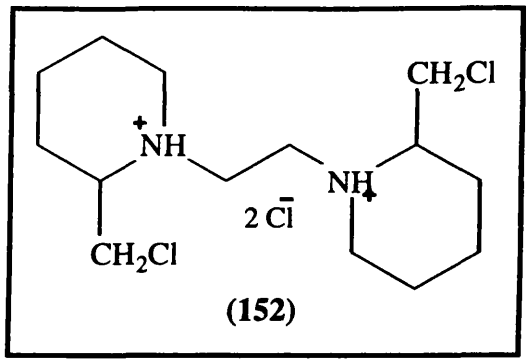
isopropyl component were found at δ 1.15 and 3.80 as a doublet and multiplet respectively. In the mass spectrum, the molecular ion was found at *m/z* 426 as a small percentage with the major ion being the peak at *m/z* 213 corresponding to half of the molecule.

Preparation of the bis(ethylcarbamate) (149) yielded the product as a thick clear oil which would not crystallise. Similar spectra to that described for the bis(isopropyl carbamate) were obtained, and full characterisation was achieved.

In preparing the bis(phenylcarbamate) (150) problems were encountered with the crude phenyl isocyanate and this required distillation before use. This derivative was insoluble in dichloromethane and came out of solution during the reaction as a white precipitate. After purification, white crystals of the product were obtained. The IR spectrum showed the carbonyl stretch to be at 1730 cm^{-1} with the predictable aromatic bands appearing at 1600 and 1538 cm^{-1}. The ^{1}H NMR spectrum, run in d_6-acetone, was very complex, showing complex multiplets for each group of signals. The ^{13}C NMR spectrum was as expected with methylene signals at δ 23.7, 26.0 and 29.4 corresponding to the ring methylenes C-3, C-4 and C-5. The methylene carbons next to the nitrogens came at δ 52.1 and 53.1 and were assigned to the endocyclic and exocyclic methylenes respectively. The signal for the methine protons was obvious at δ 60.3 and the remaining methylene carbons next to the carbamates came at δ 66.2. The aromatic methines and quaternary carbons appeared at the shifts expected for these groups. No parent ion was found in the mass spectrum.

The bis(*p*-nitrophenyl carbamate) (151) was obtained as yellow crystals after column chromatography. The spectra followed a similar pattern to those described for the bis(phenylcarbamate).

The dichloro derivative (152) was also synthesised. This compound has been made before[143] as a precursor to the corresponding primary diamine.

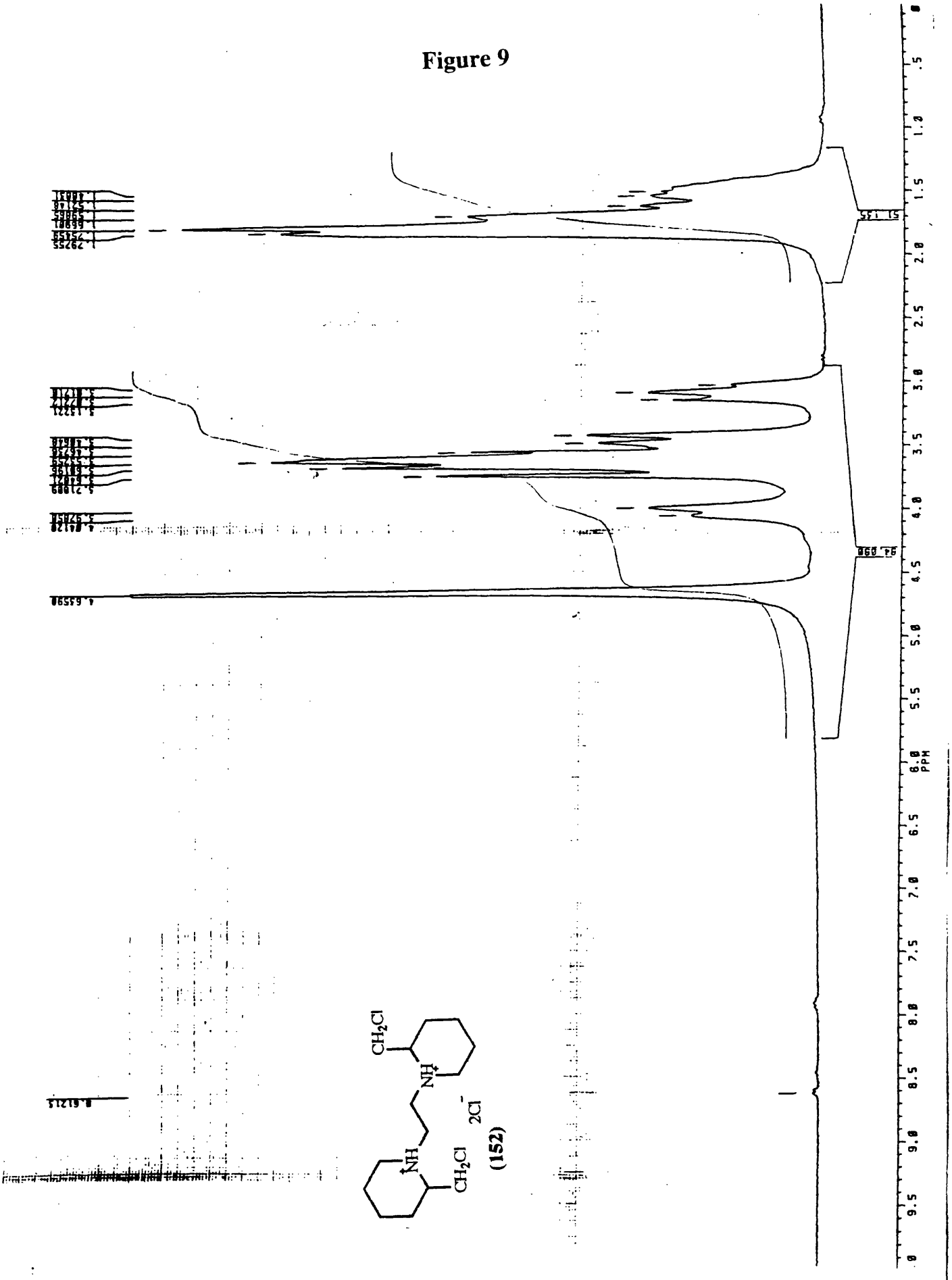


(152)

The compound was made in the usual manner from the diol with thionyl chloride and kept as the dihydrochloride salt for analysis. The IR spectrum showed a band at 746 cm^{-1} which was attributed to the C-Cl stretch. The NMR spectra were run in D_2O and the ^1H NMR spectrum is shown in Figure 9. It shows the usual broad multiplet at δ 1.58-1.90 for the ring protons. The methylene and methine protons adjacent to the nitrogen also appear as a complex multiplet between δ 3.51-3.81. To either side of this multiplet, there are two more smaller multiplets corresponding to the chloromethylene protons appearing as they had in the diol. This time, however, it was not possible to distinguish the doublets. The ^{13}C NMR spectrum showed a new methylene signal at δ 43.8 belonging to the carbon adjacent to the chlorine. The highest ion found in the mass spectrum was due to loss of one chlorine at *m/z* 257, while the largest fragment of *m/z* 146 corresponded to half the molecule.

The dibromo-derivative was not made because of the problems encountered in previous syntheses and the generally poor yielding reactions.

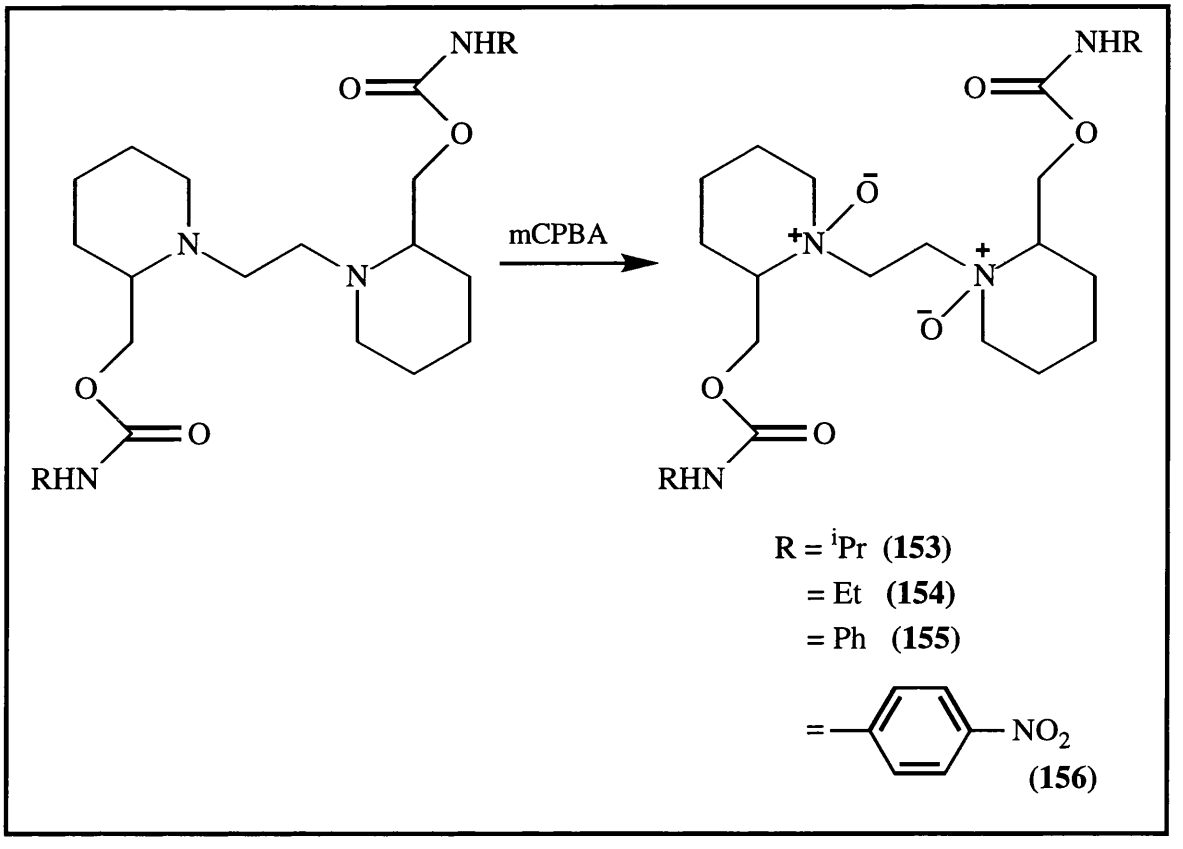
Figure 9



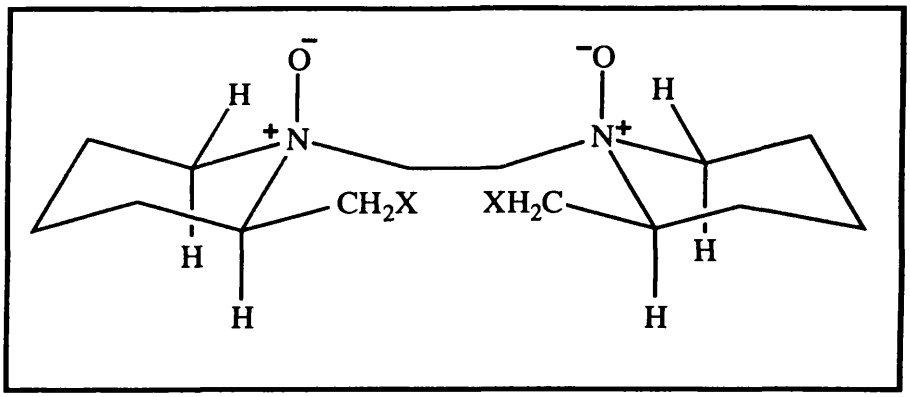
(152)

6.5 Synthesis of *N,N'*-(1,2-Ethanediyl)bis(aminocarbonyloxymethyl-piperidines) bis-*N*-Oxides

The synthesis of the bis-*N*-oxides was undertaken as before.



The bis(isopropylcarbamate) bis-*N*-oxide (153) was produced in this manner to afford white crystals after purification. The characteristic band of the *N*-oxide was at 960 cm^{-1} in the IR spectrum. The ^1H NMR spectrum showed that the signal for the methine protons had moved downfield to δ 3.06 as was also true of the methylenes adjacent to the nitrogens. Multiplets at δ 3.4 and 3.75 were assigned to the endocyclic and exocyclic methylene protons respectively.



The ^{13}C NMR spectrum also revealed the downfield shift of the methylene groups to δ 61.4 and 63.2, with the signal for the methine protons at a shift of δ 75.1. No parent ion was found in the mass spectrum.

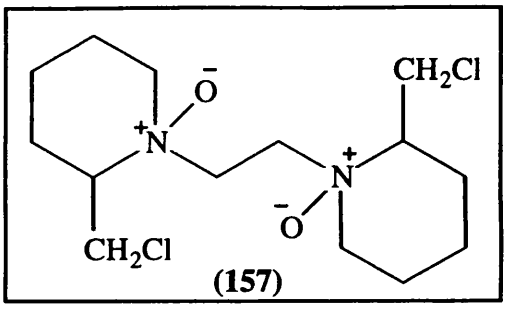
The bis(ethylcarbamate) bis-*N*-oxide (154) was obtained as white crystals. The IR spectrum showed the N-O bond at 964 cm^{-1} along with the other expected bands. The NMR spectra was similar to that of the bis(isopropyl carbamate). Again, no parent ion was found in the mass spectrum.

Both of these compounds take water into their lattices when crystallising and this is a feature which has been reported before[148] for this type of compound.

The bis(2-phenylcarbamate) bis-*N*-oxide (155) was also obtained as white crystals after purification. A band at 1070 cm^{-1} in the IR spectrum was attributed to the N-O bond. The expected chemical shifts were found in the NMR spectra, following the same pattern as before and no parent ion was found in the mass spectrum.

The bis(*p*-nitrophenylcabamate) bis-*N*-oxide (156) was recovered as a pale yellow solid which could not be crystallised. In the IR spectrum, a band at 1064 cm^{-1} was assigned to the *N*-oxide bond. The NMR data were also consistent with the structure.

The bis(chloro) bis-*N*-oxide (157) was prepared in a similar manner and obtained as a yellow oil.

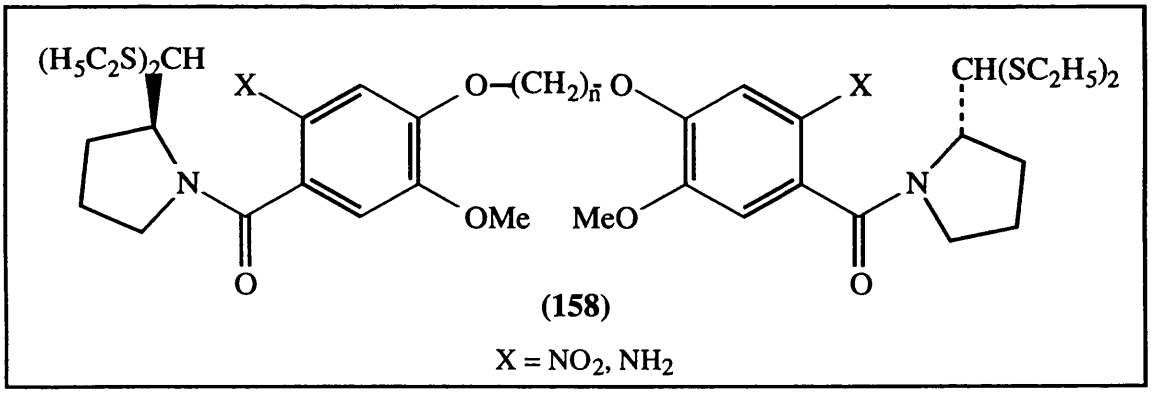


(157)

The IR spectrum showed the *N*-O band at 932 cm^{-1} while the C-Cl band was at 732 cm^{-1}. In the ^1H NMR spectrum, the endocyclic methylenes adjacent to the nitrogen appeared as a multiplet at δ 3.11 while the exocyclic methylenes, methine protons and chloro methylene protons appeared together as one large complex multiplet between δ 3.28 and δ 4.16. A small molecular ion was found at *m/z* 324 in the mass spectrum.

6.6 Homologous Analogues

It has been reported that varying the linker length between pyrrolobenzodiazepine dimers (158) affects DNA binding affinity, cross-linking efficiency and cytotoxicity.[149]



(158)

X = NO_2, NH_2

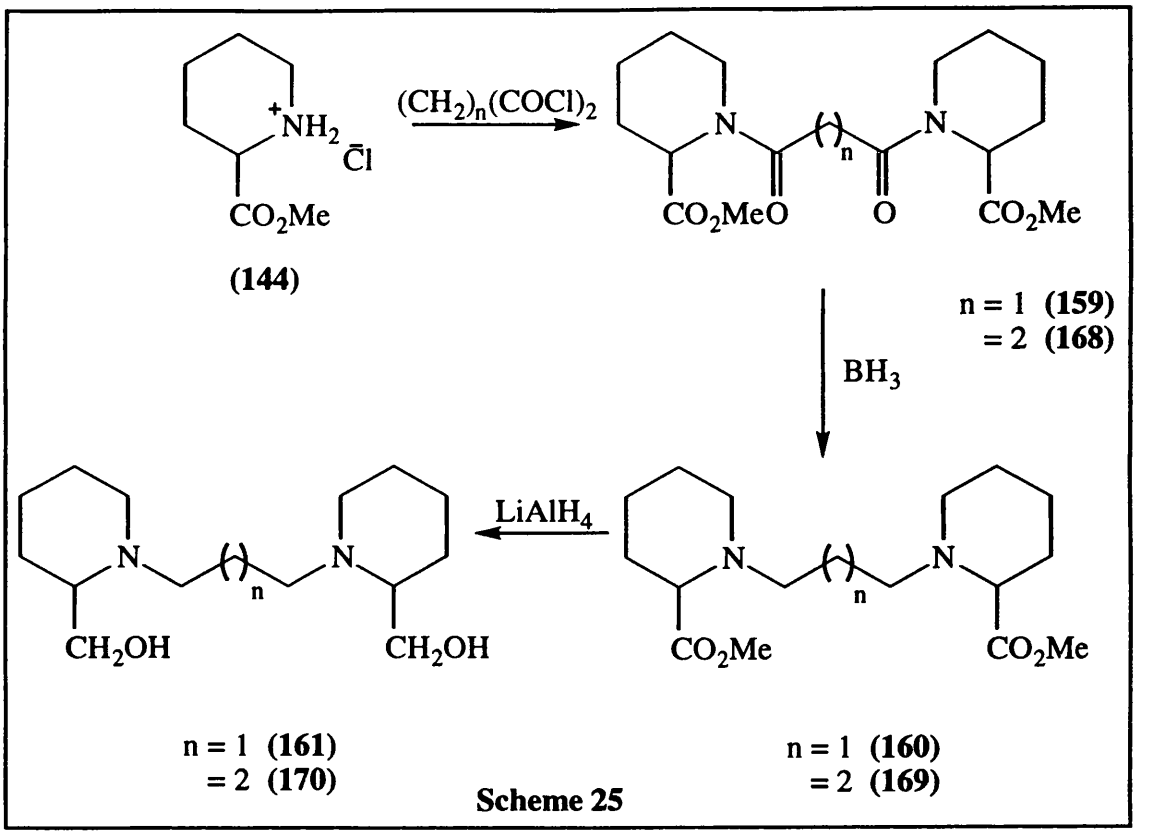
Compounds with an odd number of methylenes (n = 3 or 5) in the linker showed a higher affinity for DNA, enhanced cross-linking efficiency and were more cytotoxic compared with compounds with either n = 4 or 6. These data were consistent with the differences in ability of the compounds to stabilise DNA helix-coil transitions.

This led us to investigate the effect of toxicity of the carbon chain length of our bis-piperidine derivatives and it was decided to synthesise compounds with 3 and 4 carbons between the piperidines. This could be easily achieved using malonyl chloride and succinyl chloride respectively in the same manner as before (Scheme 25).

6.7 Synthesis of *N,N'*-(1,3-Propanediyl)bispiperidines

Starting with methyl DL-pipecolinate hydrochloride (144), the diamide (159) was prepared using malonyl dichloride to leave the product as a thick clear oil after column chromatography. Again, two carbonyl bands were found in the IR spectrum at 1746 cm^{-1} and 1645 cm^{-1}. The NMR spectra were similar to the analogue with a two carbon bridge, with the extra methylene group appearing at δ 3.50 and 3.54 as two singlets in the ^{1}H NMR spectrum. This is due to the presence of a racemate and a mseo form as discussed previously. One signal was seen at δ 41.2 in the ^{13}C NMR spectrum. The molecular ion *m/z* 354 was present in the mass spectrum as a small percentage.

This diamide was reduced in turn with borane to give the tertiary amine (160) which was isolated as a clear oil. The ^{1}H NMR spectrum showed the axial and equatorial protons of C-6 at δ 2.34-2.73 as a broad multiplet, with the exocyclic methylene protons next to the nitrogen appearing between δ 2.90 and 3.10. As before there were two sets of signals in the ^{13}C NMR spectrum corresponding to the presence of the racemate and meso forms. Reduction of the diester with $LiAlH_4$ produced the diol (161) as a clear oil. The hydroxymethylene protons were apparent at δ 3.70 in the ^{1}H NMR spectrum, and the corresponding signal in the ^{13}C NMR spectrum appeared at δ 63.0.



Scheme 25

This time, only two bis-carbamates, the phenyl and *p*-nitrophenyl were synthesised as it was felt that only two were necessary to compare toxicities. The bis(phenyl carbamate) (162) was isolated as a white solid and had bands in the IR spectrum at 1600, 1538, 753 and 693 cm^{-1} which are all indicative of a phenyl ring. A signal at δ 4.22 in the ^1H NMR spectrum corresponded to the methylene protons adjacent to the newly formed carbamate. Similar spectra was observed for the bis(*p*-nitrophenyl carbamate) (163) compound. Signals at δ 118.4 and 125.5 in the ^{13}C NMR spectrum corresponded to the aromatic methine carbons. Quaternary signals at δ 143.1, 146.3 and 154.0 were attributed to CNH, CNO_2 and C=O respectively. The dichloro derivative (164) was also made and was obtained as a clear oil. The spectra of this compound were similar to those of the diol (161), with the exception of the ^{13}C NMR spectrum in which the chloromethylene carbon appeared at δ 43.3.

Formation of the bis-*N*-oxides proceeded in an analogous manner as before using mCPBA. The bis(phenyl carbamate) bis-*N*-oxide (165) was obtained as a white solid. The IR spectrum showed the N-O stretch band at 694 cm^{-1} and the signals corresponding

to the methylenes next to the *N*-oxides were all shifted downfield as expected. Formation of the bis(*p*-nitrophenyl carbamate) bis-*N*-oxide (166) went smoothly in reasonable yield. The ^{1}H NMR spectrum was rather complex consisting of large multiplets. The ^{13}C NMR spectrum was clearer and showed signals at δ 64.9 and 65.2 for the methylene carbons adjacent to the *N*-oxides. The bis-chloro bis-*N*-oxide (167) was isolated as a yellow oil and showed a signal at δ 41.1 for the chloromethylene carbon. The methine carbons appeared at δ 72.5 which is consistent with the series of compounds.

6.8 Synthesis of *N,N'*-(1,4-Butanediyl)bispiperidines

Succinyl chloride was reacted with methyl DL-picolinate to produce the system with a four carbon bridge. This diamide (168) was isolated as white crystals after purification and full characterisation was obtained. The exocyclic methylenes occurred as a broad multiplet at δ 2.55-2.90 in the ^{1}H NMR spectrum, while in the ^{13}C NMR spectrum the corresponding signal came at a higher shift than before at δ 28.2 which is consistent with the theoretical calculated value.

Reduction of the diamide with borane furnished the diamine (169). Unlike the corresponding C_2 and C_3 bridged compounds there was only one set of signals in the ^{13}C NMR spectrum. Reduction of this amine diester with $LiAlH_4$ furnished the diol (170) as a white solid. Characteristic OH bands were found in the IR spectrum at 3126 and 1374 cm^{-1}. The ^{1}H NMR spectrum showed a large multiplet at δ 1.07-1.56 corresponding to the alkyl methylenes of the piperidine rings and the central protons in the bridge. The endocyclic *N*-CH_2 protons and methine protons came together as a multiplet between δ 2.02 and 2.32, whereas the exocyclic *N*-CH_2 protons appeared further downfield as a multiplet at δ 2.65. The hydroxymethyl protons were also a multiplet and appeared at δ 3.27-3.53. A broad singlet at δ 3.67 was attributed to the OH signal. Signals at δ 23.3, 23.5, 24.6 and 27.8 in the ^{13}C NMR spectrum corresponding to the methylenes not connected to nitrogen were not assigned specifically.

Again, the two aromatic bis-carbamates and the dichloro compound were the only derivatives to be synthesised. The bis(phenylcarbamate) (171) was obtained as a white solid which had all the expected bands in its IR spectrum, such as the carbonyl stretch at 1702 cm^{-1} and the strong mono-substituted aromatic bands at 751 and 688 cm^{-1}. The ^1H NMR spectrum was again quite complex with a multiplet between δ 1.25 and 1.66 corresponding to the methylene protons not attached to nitrogen. The methine protons appeared at δ 2.20 with the endocyclic *N*-CH_2 groups at δ 2.45 and the exocyclic *N*-CH_2 protons at δ 2.80, all of which were complex multiplets. The aromatic protons were distinguishable from each other, with the *para*-protons appearing at δ 7.06, the *ortho*-protons at δ 7.27 and the *meta*-protons at δ 7.38, again, all of which were multiplets. The ^{13}C NMR spectrum is shown in Figure 10 and shows methylene carbons at δ 52.1 and 53.6 corresponding to the endocyclic and exocyclic *N*-CH_2 carbons respectively. The methine signal appears at δ 59.4 and the methylenes next to the carbamates occur at δ 65.9. The two quaternary carbons at δ 137.9 and 153.7 correspond to the phenyl carbons and the carbamate carbons respectively. The mass spectrum showed no parent ion but many fragments.

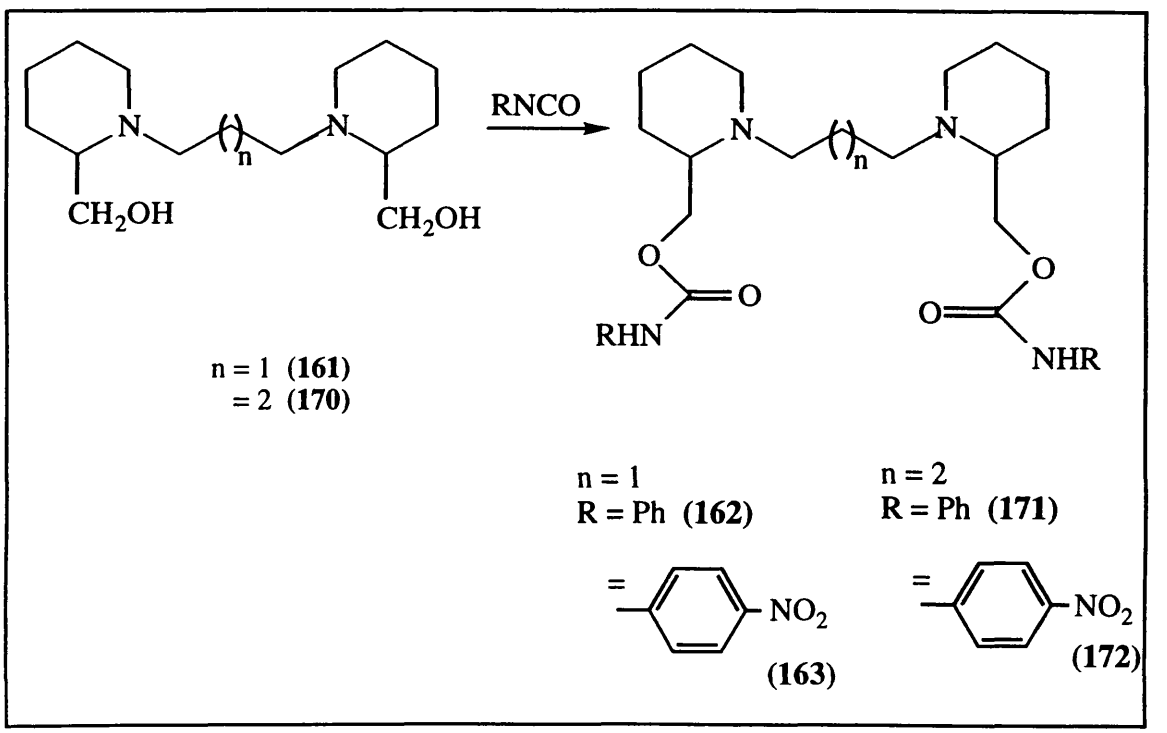
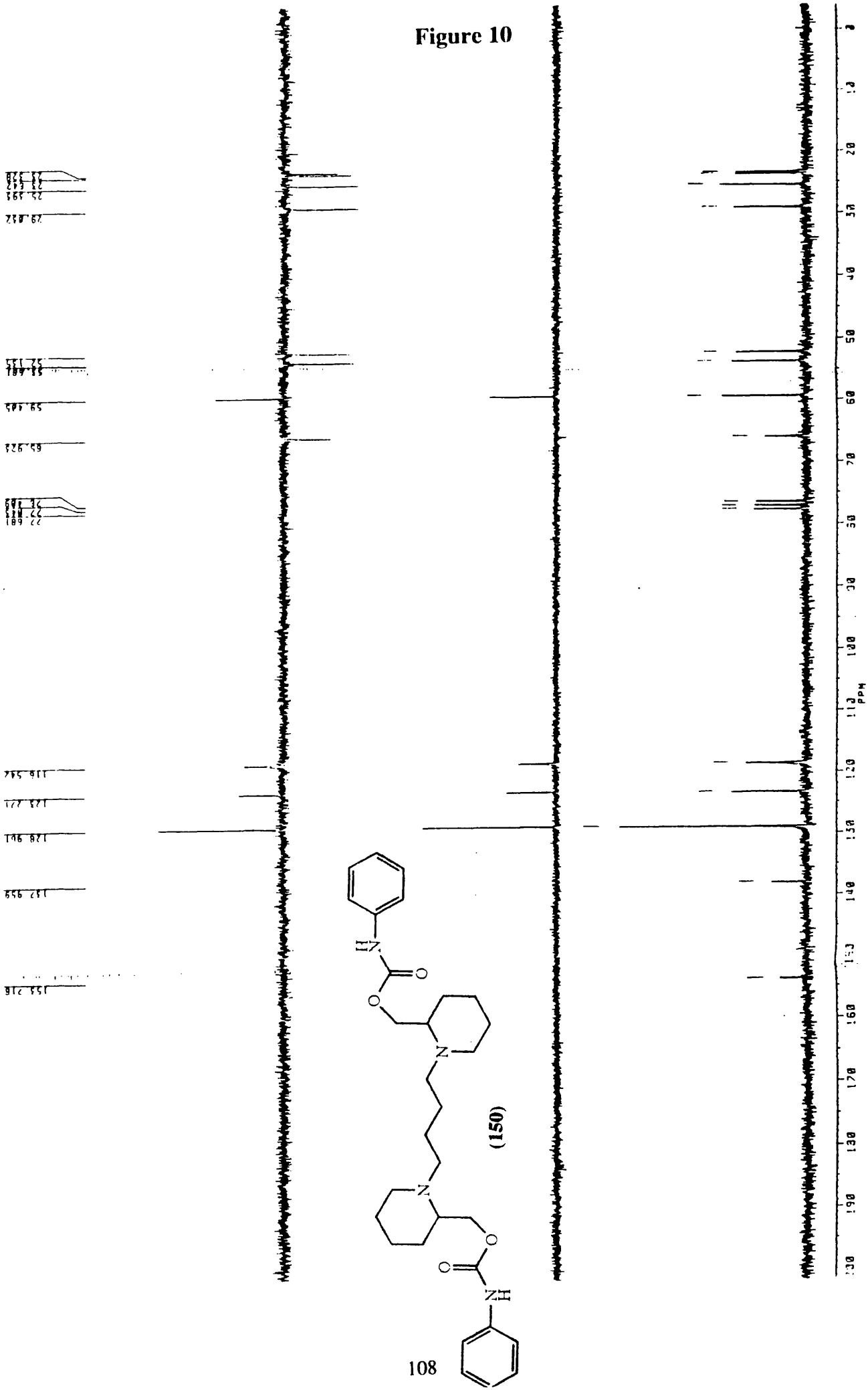


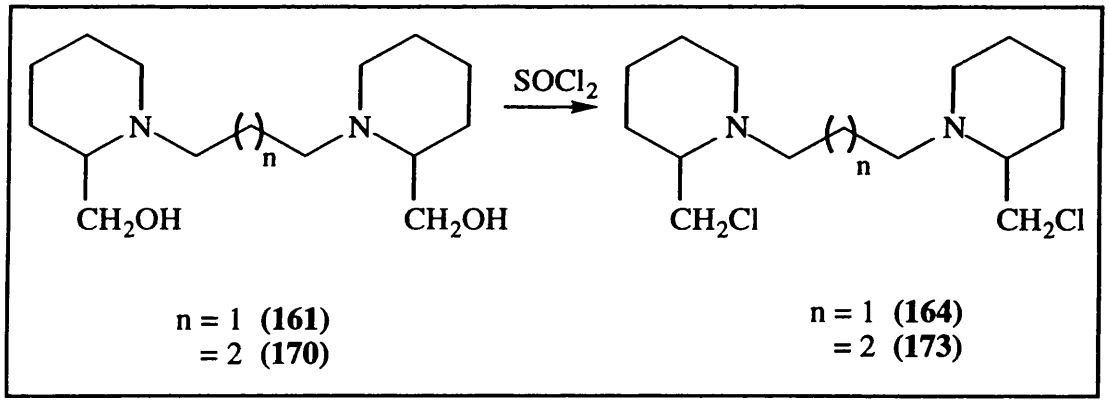
Figure 10



(150)

The bis(*p*-nitrophenylcarbamate) (172) was obtained as a yellow solid and gave spectra similar to that of the phenyl derivative. Bands at 1737, 1552, 1509 and 852 cm^{-1} in the IR spectrum were all indicative of the compound. Once more, a large mutiplet in the ^1H NMR spectrum at δ 1.17-1.46 contained the signals of the methylenes not attached to the nitrogens. This spectrum is shown in Figure 11. The endo- and exo-cyclic *N*-methylene protons appeared at δ 2.40 and 2.75 respectively. The ^{13}C NMR spectrum showed the methine signal at δ 60.0 and the methylene next to the carbamate at δ 66.6. The aromatic methine carbons came at δ 118.4 and 125.6 while the three quaternary carbons had signals at δ 143.1, 146.4 and 154.1, belonging to CNH, CNO_2 and C=O respectively.

The dichloro derivative (173) was made using thionyl chloride and was obtained as a clear oil. The spectra obtained were all consistent with the structure, with the C-Cl stretch appearing at 738 cm^{-1} in the IR spectrum and the methylenes adjacent to the chlorines appearing at δ 45.2 in the ^{13}C NMR spectrum.

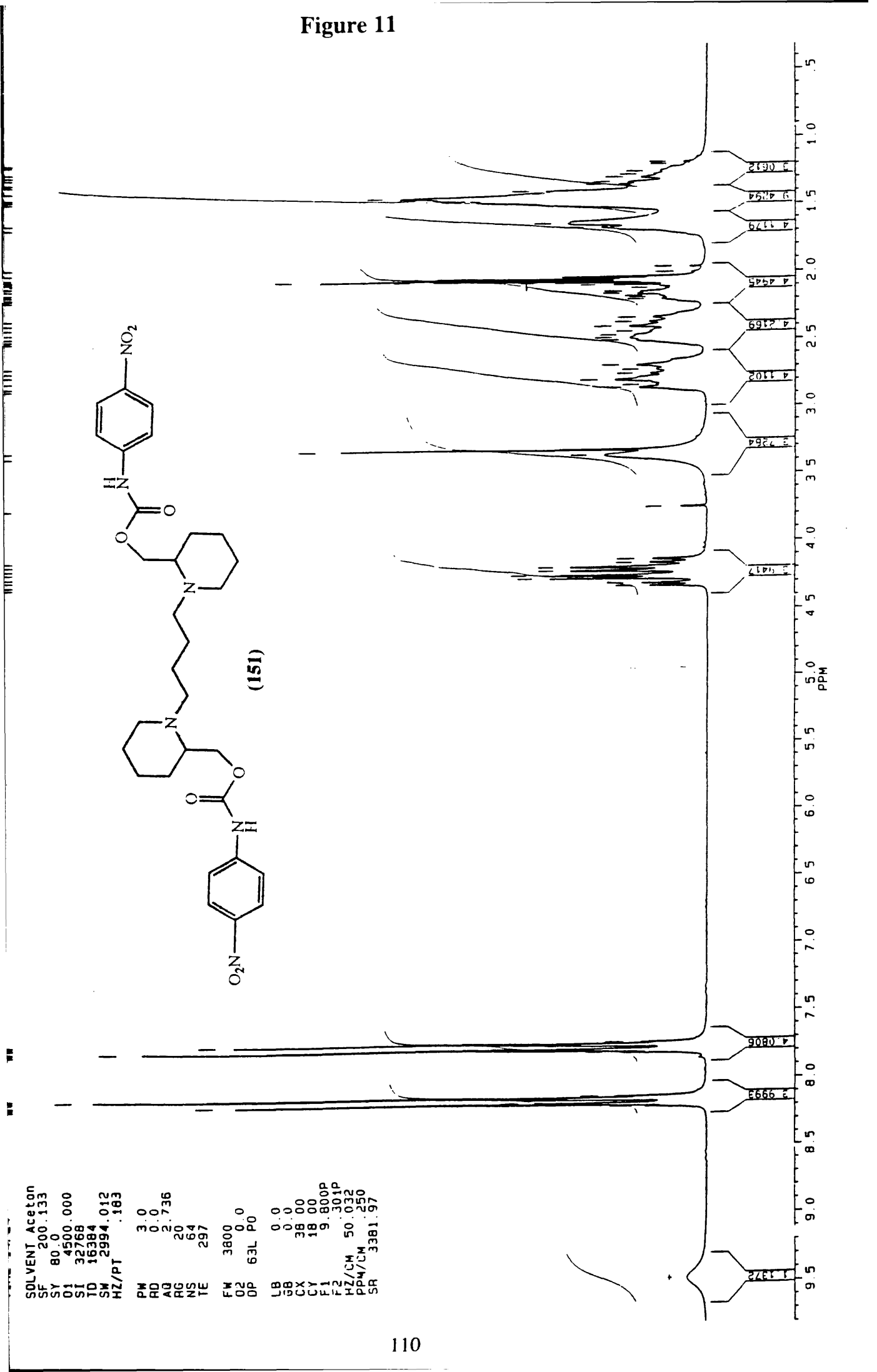


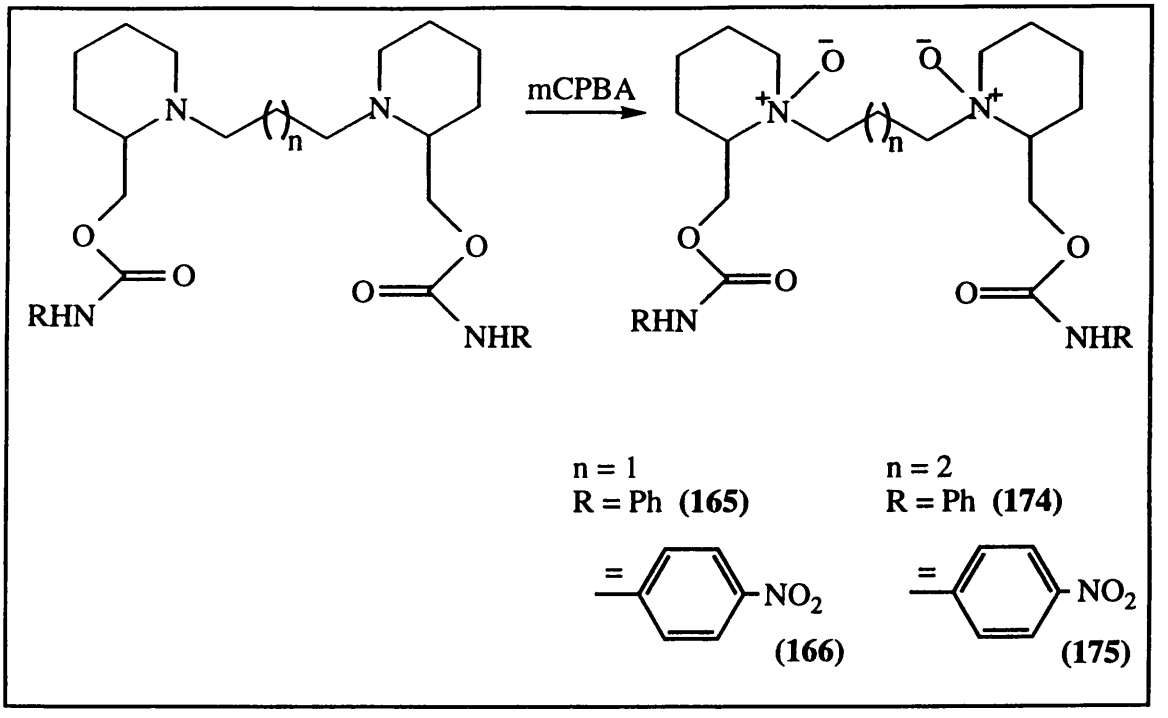
n = 1 **(161)**
= 2 **(170)**

n = 1 **(164)**
= 2 **(173)**

Bis-*N*-oxide formation was achieved in the same manner as before and full characterisation was obtained for each of the compounds.

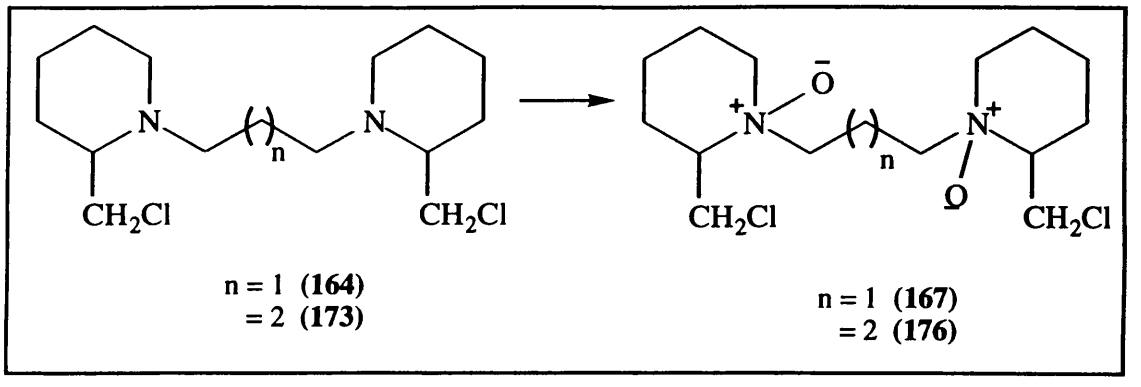
Figure 11





The bis(*p*-nitrophenyl carbamate) bis-*N*-oxide (175) was obtained as a pale yellow solid. In the IR spectrum, the band at 1070 cm^{-1} was attributed to the *N*-oxide. The ^{1}H NMR spectrum showed the expected shifts of the methylenes adjacent to the nitrogen and these appeared together as a broad multiplet at δ 3.42-3.85. The methine protons had shifted downfield to δ 4.15 as a complex multiplet, with the methylene protons next to the carbamate appearing at δ 4.60, also as a complex multiplet. Signals in the ^{13}C NMR spectrum at δ 63.8 and 64.8 were assigned to the endo- and exo-cyclic methylenes adjacent to the nitrogen respectively. The methine signal had also shifted to δ 72.3 as expected.

The bis-chloro bis-*N*-oxide (176) was obtained as a yellow oil after chromatography.



The IR spectrum showed bands at 977 and 718 cm^{-1} which were attributed to the *N*-O and C-Cl stretch. In the ^1H NMR spectrum, a large multiplet from δ 1.63 to 2.10 corresponded to the exocyclic and endocyclic protons not attached to nitrogen. A multiplet at δ 2.71 of intensity integrating for two protons corresponded to the methine protons adjacent to the *N*-oxides. The methylene groups next to the positive nitrogens appeared at δ 3.74 to 4.20 as a broad multiplet, with the methylenes next to the chlorines occurring at δ 4.43-4.84, also as a multiplet. The ^{13}C NMR followed the same pattern as seen before. M^+ (*m/z* 353), was not found in the mass spectrum, the heaviest fragment being loss of a chloromethylene group and an oxygen.

6.9 Conclusions

A new series of disubstituted bis-piperidine derivatives with corresponding bis-*N*-oxides have been prepared and characterised from a general route. These compounds are a 1:1 mixture of a racemate and meso form as shown by the ^{13}C NMR spectra. The biological testing results for these compounds are presented in Chapter 8.

Chapter 7

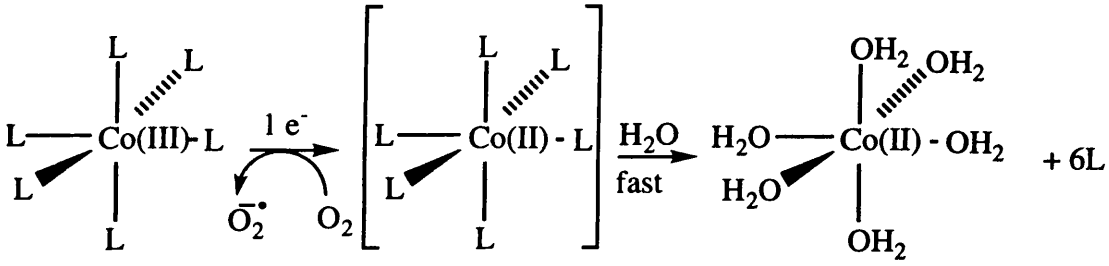
Synthesis of Cobalt (III) Complexes containing Amines

7.1 Introduction

Complexes of many transition metals have demonstrated cytotoxicity in cell culture and/or antitumour activity in tumour-bearing animals.[150] Only complexes of platinum, however, are currently in routine clinical use.[151] A wide variety of metal complexes have also been shown to be radiation-sensitisers of mammalian and/or bacterial cells. These include complexes of Ag(I), Cu(I), Cu(II), Zn(II), Rh(II), Pt(II), Co(III) and Fe(III).[152]

Co(III) forms kinetically inert octahedral coordination complexes. This inertness is due to the d^6 low spin electron configuration of trivalent cobalt.[153] Kinetically inert transition metal complexes (e.g. Rh(III) and Cr(III)) undergo the water exchange reaction relatively slowly, with half-lives of about 1 day.[154] By contrast, complexes of kinetically labile metal ions (e.g. Mn(II), Cr(II)) undergo this reaction with half-lives of less than 1 second.[154] The biological consequence of kinetic inertness is that many Co(III) complexes will remain intact when added to a culture medium or injected into animals and should arrive at their cellular targets with their ligand configuration intact.

Cobalt is known to form stable octahedral Co(III) complexes with a range of electron-donating ligands (L), including both aromatic and aliphatic amines. The reduction potentials of the Co(III)/Co(II) couple in these amine complexes fall within an appropriate range (-650 to +280 mV)[155] so that one-electron bioreduction to the Co(II) species $Co(II)L_6$ is possible. This reduction labilises the ligands enormously, and in aqueous conditions they are normally rapidly lost by water displacement to give the $Co(II)(H_2O)_6$ ion (Scheme 26).



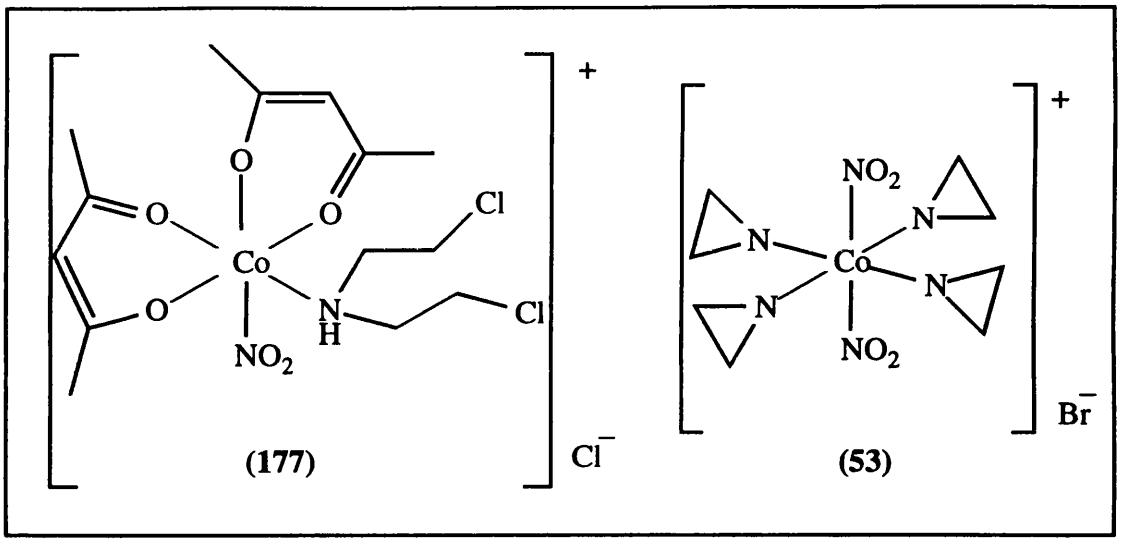
Scheme 26

While this overall process is essentially irreversible, kinetically rapid oxidation of the transient Co(II)L_6 intermediate by molecular oxygen can occur[156] to regenerate the parent Co(III) complex.

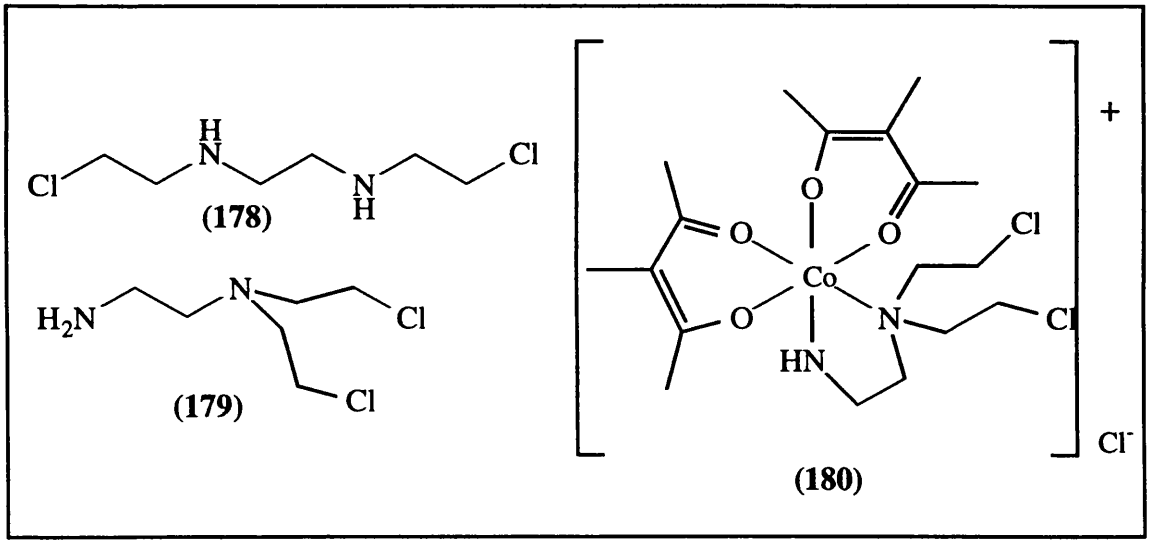
One-electron reduction of Co(III) complexes could therefore, in principle, be used to trigger the oxygen-inhibited release of attached amine ligands. If these ligands were aliphatic nitrogen mustards, the resulting complexes would be potential bioreducible drugs. Donation of the nitrogen lone pair to the metal on complexation greatly lowers the nucleophilic character, and so stabilises the mustard. Following one electron reduction, the potent aliphatic nitrogen mustards would be released.

A number of Co(III) complexes of the monodentate monoalkylating agent aziridine are known, containing up to four aziridines.[71] The strained, three-membered heterocycle is a highly reactive molecule that readily undergoes ring-opening reactions, particularly under acid conditions. However, once coordinated to an inert metal such as Co(III), the reactivity of aziridine toward nucleophiles is much diminished and the heterocycle is stabilised with respect to ring-opening reactions. Free aziridine is a potent alkylating agent and is active towards substrates such as DNA and aziridine groups are present in a number of anticancer agents.[157] A Co(III) complex of the monodentate bisalkylating agent bis(2-chloroethyl)amine (177) has also recently been prepared but, like the aziridine complexes (53), is not selective for hypoxic cells as a cytotoxin.[158] The reason for this is probably that the corresponding Co(II) complexes are not sufficiently stable to allow reoxidation by molecular oxygen to compete with ligand release. This

means that the toxicity towards normal cells is increased and so selectivity between oxic and hypoxic cells is decreased.



Since the kinetic stability of metal complexes is greatly increased if chelating ligands are used,[159] a series of Co(III) complexes containing the bidentate bisalkylating nitrogen mustards (178) and (179) have been prepared.[14]



Complexes of the unsymmetrical mustard (180) have reduction potentials in the range of -300 to -450 mV and show hypoxic selectivity in a range of cell lines. The complex (180) has 20- to 30-fold selectivity for hypoxic cells and is currently undergoing

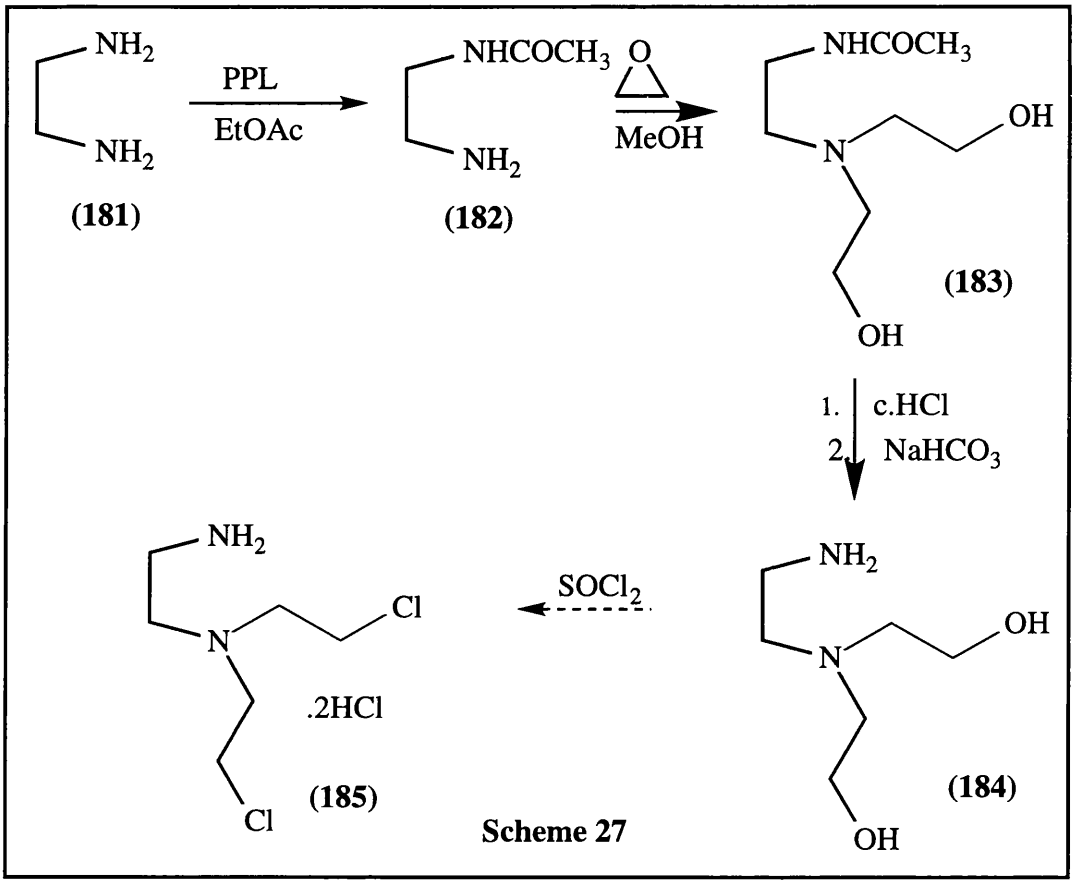
investigation *in vivo* as the lead compound of this class. The reduction potentials of metal complexes are modulated by the nature of the ligands; thus complexes of the symmetrical mustard have potentials *ca.* 125 mV lower than the corresponding complexes of the unsymmetrical mustard. The use of 3-alkylpentane-2,4-dionato auxiliary ligands also allows some variation in reduction potentials.[160]

The work done to date suggests that use of deactivated mustards in a cobalt complex is a viable design for bioreducible drugs. Advantages include the fact that reductive activation is necessarily a one-electron process and that the pharmacology of the released nitrogen mustard is well understood. It was therefore decided that a range of diamines, triamines and tetraamines would be synthesised and their complexation to Co(III) would be attempted.

7.2 Synthesis of *N,N*-Bis(2-chloroethyl)ethane-1,2-diamine Dihydrochloride (185)

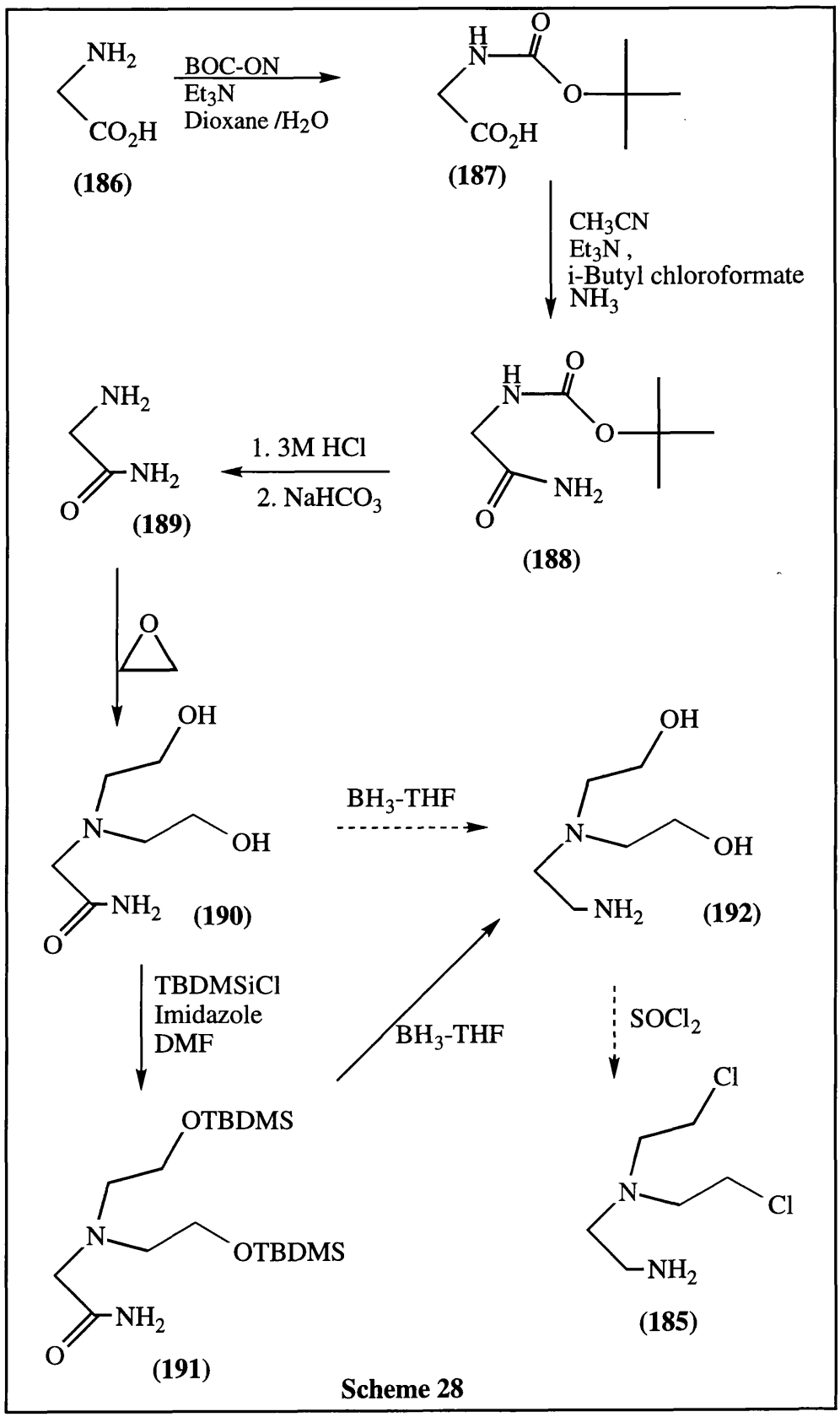
In order to provide a reference compound for our own complexes, it was decided to remake the cobalt complex which Ware *et al.*[160] reported had significant hypoxic selectivity. This ligand has been reported before[161] and the synthesis can be seen in Scheme 27. *N*-Acetylethylenediamine (182) was prepared by selective monoacetylation using pig pancreatic lipase, ethylenediamine (181) and ethyl acetate in a shaking bath at 37 °C for three days. This oil was then treated with excess ethylene oxide in distilled methanol to produce the diol (183)[162] as a thick oil. This was deacetylated with concentrated HCl after five hours at reflux to give the salt (184). However, this compound was isolated as a dark brown oil which could not be crystallised or purified. Even after making the free base using sodium bicarbonate, it was impossible to rid the compound of its colour. Activated charcoal had little effect and column chromatography was impossible due to the polar nature of the compound. As the NMR spectra indicated that it was the right compound, it was decided to try the next step of chlorination but the free base would not dissolve in thionyl chloride so no reaction could take place.

Deacetylation was tried using sodium hydroxide to see if a cleaner diamine could be obtained, but even after 72 hours heating at reflux, the reaction had not gone to completion. This method was therefore abandoned. What was needed was a different type of protecting group which could be removed easily and cleanly.



Scheme 27

It was decided to use a BOC protecting group as these usually give crystalline solids and are easily removed under mild acidic conditions. The second synthesis can be seen in Scheme 28. We chose to use [2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile] (commonly referred to as BOC-ON) as this is specially meant for use with amino acids, since our starting material was to be glycine (186). The BOC protection went easily in high yield and the product (187) was obtained as white crystals. The amide (188) was prepared by means of a mixed anhydride using *i*-butyl chloroformate followed by excess ammonia gas.

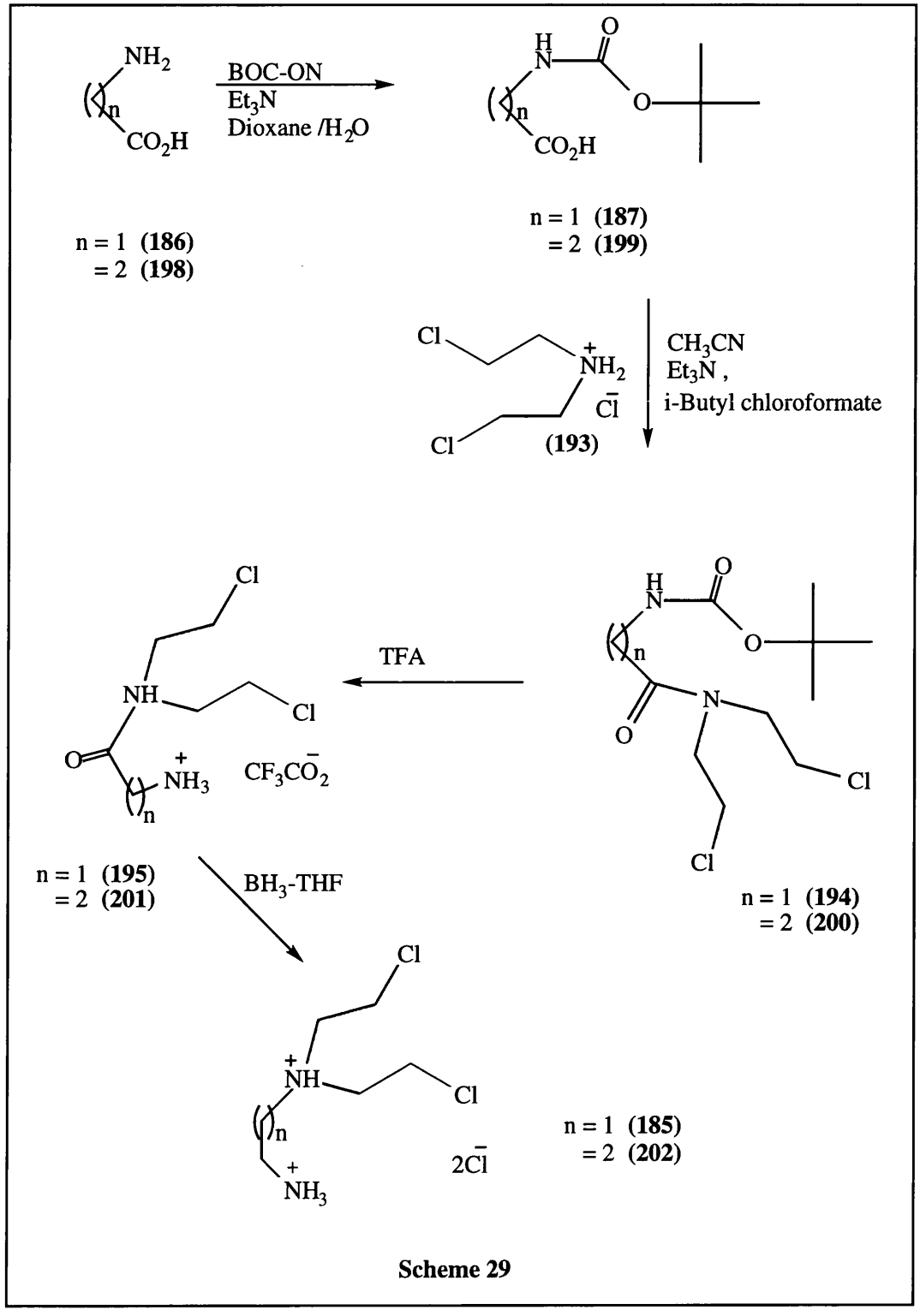

BOC-ON
Et3N
Dioxane /H2O
(186)
(187)
CH3CN
Et3N ,
i-Butyl chloroformate
NH3
(188)
1. 3M HCl
2. NaHCO3
(189)
(190)
BH3-THF
(192)
TBDMSiCl
Imidazole
DMF
BH3-THF
SOCl2
(191)
(185)

Scheme 28

Deprotection of the amine using aqueous acid went in high yield but it was difficult to obtain the free base as the polarity of this compound made it impossible to extract into organic solvents. Eventually, addition of sodium bicarbonate followed by extracting repeatedly with methanol furnished the amine (189) as a clear oil. Treatment of this with ethylene oxide as before gave the diol (190) as a very thick oil, which, again, was totally insoluble in all organic solvents except methanol. This led to problems in the next step which was supposed to be reduction of the amide with borane, as the oil would not dissolve in THF. An extra protection step was necessary in order to render the compound more soluble in THF. A TBDMS group was chosen to decrease the polarity caused by the hydroxyl groups, as these were the main problem with solubility. TBDMS was also a good choice as it could be removed in the acid work-up of the borane reduction. This protected diol (191) was obtained in reasonable yield and only the final step was required. However, as before, the diol (192) was obtained as a thick oil which would not dissolve in thionyl chloride even with heat over long periods of time. It was apparent that replacement of the hydroxyls with chlorines in order to improve the polarity was of vital importance in making these compounds. As the above route was getting rather long and arduous with its own share of problems, a third route was sought. The one we eventually chose involved avoiding the diol completely. Based on the above method, it involved simply using the nitrogen mustard (193) itself in the mixed anhydride reaction instead of ammonia. This meant we had shortened the route considerably and removed purification problems encountered with the polar diols. The synthesis can be seen in Scheme 29.

The BOC-protected amino acid (187) had characteristic acid bands at 1749 and 1669 cm^{-1} with the amide band at 1540 cm^{-1}. ^1H NMR spectroscopy showed a large singlet at δ 1.45 corresponding to the tertiary butyl methyl protons. The methylene protons appeared as a doublet at δ 3.91 with the amide proton as a triplet at δ 5.40. A broad singlet at δ 11.08 was attributed to the carboxylic acid proton. The ^{13}C NMR spectrum had the methyl carbons at δ 28.2 and the methylene carbon at δ 42.1. The three quaternary carbons were spread out, with the tertiary butyl carbon appearing at δ 80.3,

the carbamate carbonyl carbon at δ 156.1 and the acid carbonyl carbon at δ 173.9. No molecular ion was found in the mass spectrum, as the tertiary butyl group did not survive the bombardment.

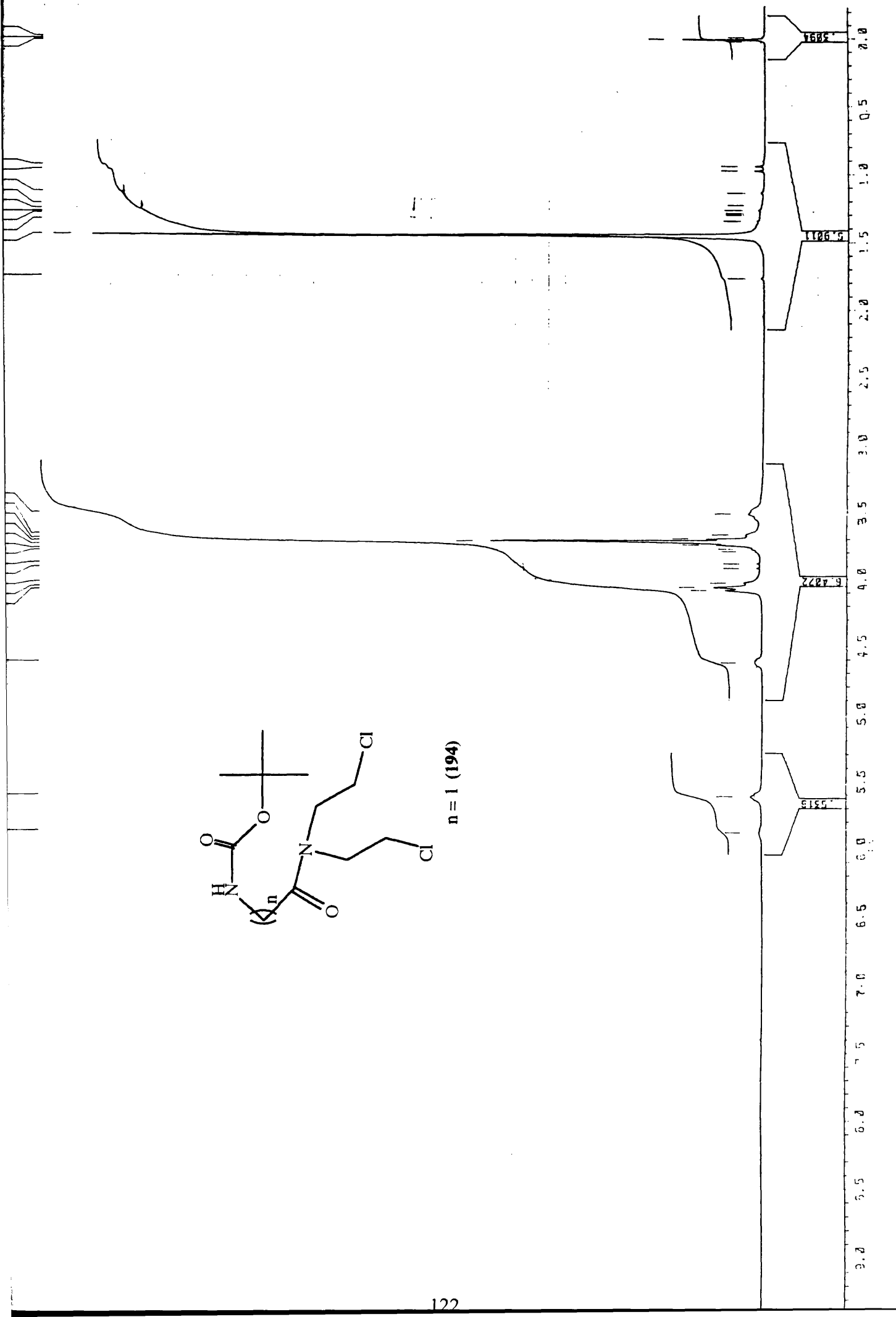


Scheme 29

The reaction between the mixed anhydride and bis(2-chloroethyl)amine hydrochloride (193) was an unpredictable one as it was necessary to ensure that all the chloroformate had reacted with the acid. If the amine was added too quickly to the mixture, the chloroformate would react with that instead. Alternatively, if the chloroformate was left to react for too long, it would give a brown mess. The addition of triethylamine was also found to be important for the success of the reactions. Two equivalents are required, one for the removal of the acid proton and the other to form the free base of the amine. It was found that if all the base was added initially to the acid (187) before addition of the chloroformate, the chloroformate did not react as well with the acid, presumably due to the competing reaction of the tertiary amine. So addition of one equivalent before addition of the chlorofomate and addition of the other after the amine hydrochloride so that the free base could react immediately with the mixed anhydride led to suppression of the side products and the amide (194) could be isolated in a pure state as a thick clear oil in good yield. Carbamate carbonyl stretch for the amide (194) was apparent in the IR spectrum at 1751 cm^{-1} and the amide carbonyl band came at 1702 cm^{-1}. A medium band at 700 cm^{-1} was attributed to the C-Cl stretch. In the ^{1}H NMR spectrum (Figure 12) the methylenes of the chloroethyl mustard all appeared together as a triplet at δ 3.69 with the glycine methylene coming at δ 4.05. The carbamate proton was apparent as a triplet at δ 5.61. The ^{13}C NMR spectrum had three methylene carbon signals, at δ 41.1, 43.6 and 49.5, which were attributed to CH_2-Cl, $NRCH_2$ and $COCH_2$ respectively. The newly formed amide carbonyl signal appeared at δ 169.3. The molecular ion minus one chlorine was found at *m/z* 263 in the mass spectrum.

Deprotection of the BOC-protected amide (194) was attempted using aqueous acid. This went easily within an hour but difficulties were encountered in isolating the free base. So, alternatively, trifluoroacetic acid in dichloromethane was used to remove the BOC group and the product was obtained as the salt after triturating with diethyl ether. This gave the trifluroacetate salt (195) as white crystals. The amide carbonyl stretch band appeared at 1685 cm^{-1} with a signal at 722 cm^{-1} attributed to the C-Cl bond. The ^{1}H NMR spectrum showed three multiplets of intensity 4 : 4 : 2 at δ 3.39, 3.74 and 4.44.

Figure 12

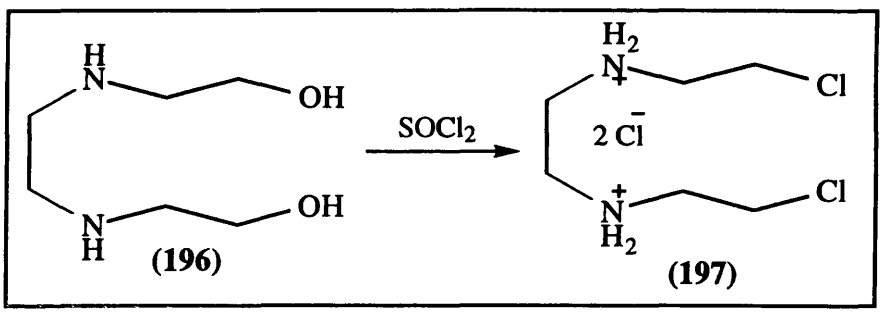


These signals were assigned to $RNCH_2$, CH_2Cl and $COCH_2$ respectively. The ^{13}C NMR spectrum showed signals for each carbon present, two signals at δ 39.9 and 40.9 for the methylenes adjacent to the chlorine and two signals at δ 46.8 and 49.8 for the methylenes next to the amide. It was assumed that this was because the π bonding between the nitrogen atom and the carbonyl carbon slows the rotation about this bond, as is seen in spectra of dimethylformamide (discussed previously in Chapter 3). The molecular ion was found at *m/z* 198 in the mass spectrum.

The final step involved reduction of the amide bond with borane. The trifluoroacetate salt (195) was used directly so excess borane was required as the acetate group would be reduced in preference to the amide group. This was indeed the case as the salt could be seen to dissolve in the THF minutes after addition of the borane. After acidic work-up, *N,N*-bis(2-chloroethyl)ethane-1,2-diamine (185) was obtained as the dihydrochloride salt in a crystalline form. The ^1H NMR spectrum again showed three multiplets, this time with intensities 2 : 6 : 4. This corresponded to the methylene next to the primary amine at δ 3.40, the three methylenes adjacent to the tertiary nitrogen at δ 3.62 and the remaining methylenes next to the chlorines appearing at δ 3.88. This is consistent with the values reported previously.[160] The ^{13}C NMR spectrum had four signals in it, one at δ 36.0 corresponding to the methylene adjacent to the primary amine and one at δ 39.7 corresponding to the chloromethylenes. The signal at δ 52.4 was assigned to the single methylene CH_2NR_2 whilst the one at δ 57.4 was attributed to the remaining two methylenes of the tertiary nitrogen, as indicated by the intensities of the signals. No molecular ion was found in the mass spectrum, but the largest fragment at *m/z* 154 belonged to an ion with loss of CH_2NH_2.

7.3 Synthesis of *N,N'*-bis(2-chloroethyl)ethane-1,2-diamine Dihydrochloride (197)

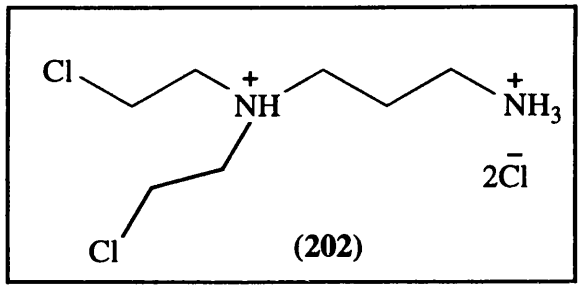
This ligand had also been reported by Ware *et al.* and was made by us for comparison. This was very easily made[163] by direct chlorination of the commercially available *N,N'*-bis(2-hydroxyethyl)ethane-1,2-diamine) (196) using thionyl chloride.



The IR spectrum of the dihydrochloride (197) showed bands at 2924 and 2770 cm^{-1} corresponding to CH stretch and N-CH stretch. A band at 773 cm^{-1} was attributed to the C-Cl stretch. In the ^1H NMR spectrum, a singlet at δ 3.49 was surrounded by two multiplets at δ 3.23 and 3.80, all of equal intensity. The chloromethylenes were assigned the shift at δ 3.80 and the β-chloromethylenes the signal at δ 3.23 as it was obvious that these signals were coupled to each other. This left the singlet at δ 3.49 to which the remaining methylenes were assigned. The ^{13}C NMR spectrum showed three signals. The signal at δ 43.9 was assigned to the chloromethylene carbons, with the signal at δ 50.6 corresponding to the CH_2NHR methylenes. The remaining signal at δ 57.4 was attributed to the β-chloromethylene carbons. The highest peak in the mass spectrum at *m/z* 149 was due to loss of a chlorine atom from the parent compound.

7.4 Synthesis of *N,N*-Bis(2-chloroethyl)propane-1,3-diamine Dihydrochloride (202)

This higher homologue was also made in an attempt to see if a five or six membered ring would be more stable in the complex with cobalt, which would then have implications for the release of the ligand and therefore selectivity.



(202)

This ligand was made in exactly the same way as before (Scheme 29), but starting with β-alanine (198). BOC protection to give (199) was achieved as before and this was followed by amide (200) formation *via* the mixed anhydride. Deprotection using TFA afforded the salt (201) and this time the free base was isolated as a clear oil. The amide carbonyl band appeared at 1682 cm^{-1} with the C-Cl band appearing at 722 cm^{-1}. The ^{1}H NMR spectrum showed a large multiplet at δ 3.70 which corresponded to all the methylenes except for the methylene adjacent to the carbonyl, which appeared as a triplet at δ 4.37. The ^{13}C NMR spectrum showed signals at δ 41.7, 45.2 and 45.9 which corresponded to the chloromethylenes, the methylene next to the primary amine and the methylenes adjacent to the tertiary amine, respectively. Another methylene signal at δ 62.0 was assigned to the carbon next to the carbonyl and the quaternary carbon of the carbonyl itself appeared at δ 158.3. No parent ion was found in the mass spectrum, but there was a signal at *m/z* 178 which corresponded to loss of one chlorine atom.

Reduction of the amide with borane gave the diamine (202) as the dihydrochloride salt. The ^{1}H NMR spectrum had a multiplet at δ 1.80 which corresponded to the methylene protons not attached to either nitrogen. Four multiplets at δ 3.07, 3.25, 3.53 and 3.67 corresponded to those methylenes which were attached to the nitrogens. It was

assumed that the protons β to the chlorines would appear further downfield than those further away from their influence. The chloromethylenes appeared at δ 3.93, also as a multiplet.

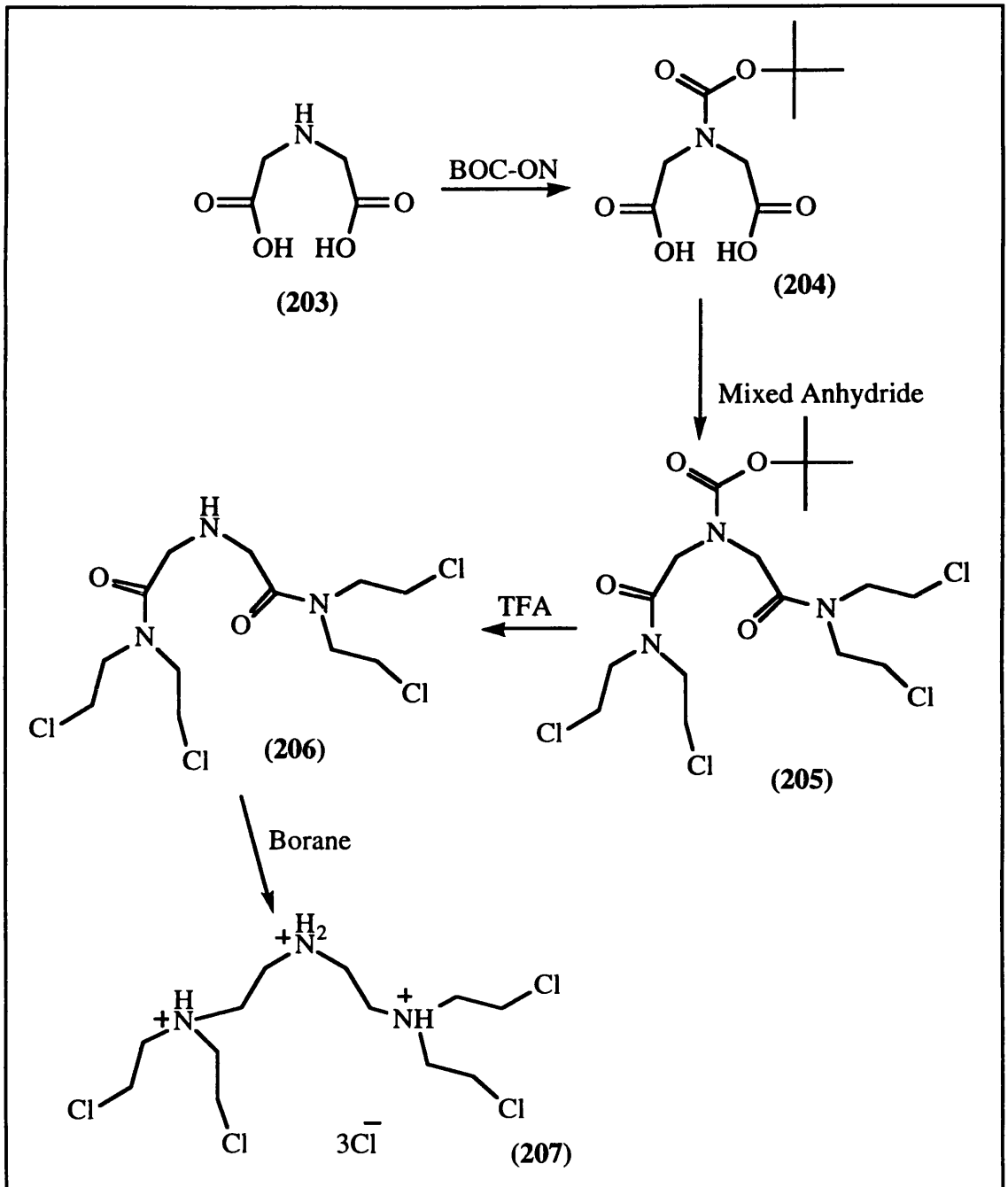
7.5 Synthesis of a Triamine Ligand

It seemed reasonable to expect that a triamine would have a different binding ability in comparison to a diamine and as this is of fundamental importance in achieving selectivity, a triamine was chosen to test this hypothesis.

The synthesis is shown in Scheme 30. We started with the commercially available iminodiacetic acid (203) and protected the amine with BOC-ON. The yield of this reaction was very poor for a BOC protection, because isolation of the product was difficult due to the poor solubility of this compound (204) in organic solvents.

Reaction with the mixed anhydride gave the diamide (205) as a thick clear oil. Bands at 1755 and 1698 cm^{-1} in the IR spectrum corresponded to the carbamate carbonyl and the amide carbonyl respectively, with the signal at 733 cm^{-1} attributed to the C-Cl stretch. In the ^{1}H NMR spectrum, the singlet at δ 1.36 corresponded to the tertiary butyl protons. Multiplets at δ 3.43 and 3.83 of equal intensity were assigned to the β chloromethylene protons and the chloromethylene protons respectively. The methylenes adjacent to the carbonyls appeared at δ 4.23 as a singlet.

Treatment with TFA followed by basification yielded the diamide (206) as a thick clear oil. The ^{1}H NMR spectrum simply had a multiplet for the mustard methylene protons at δ 3.72 and the methylenes adjacent to the carbonyls appeared as a doublet at δ 4.34. A broad singlet at δ 9.87 was attributed to the secondary amine proton. In the ^{13}C NMR spectrum, signals due to the chloromethylene carbons came at δ 41.2 and 41.4. There were four signals due to the methylenes β to the chlorines at shifts of δ 48.8, 48.9, 49.8 and 49.9. The methylene carbon adjacent to the secondary amine was further downfield at δ 72.3. Two signals were seen for the carbonyl carbons, one at δ 169.0 and the other at δ 169.5.



Scheme 30

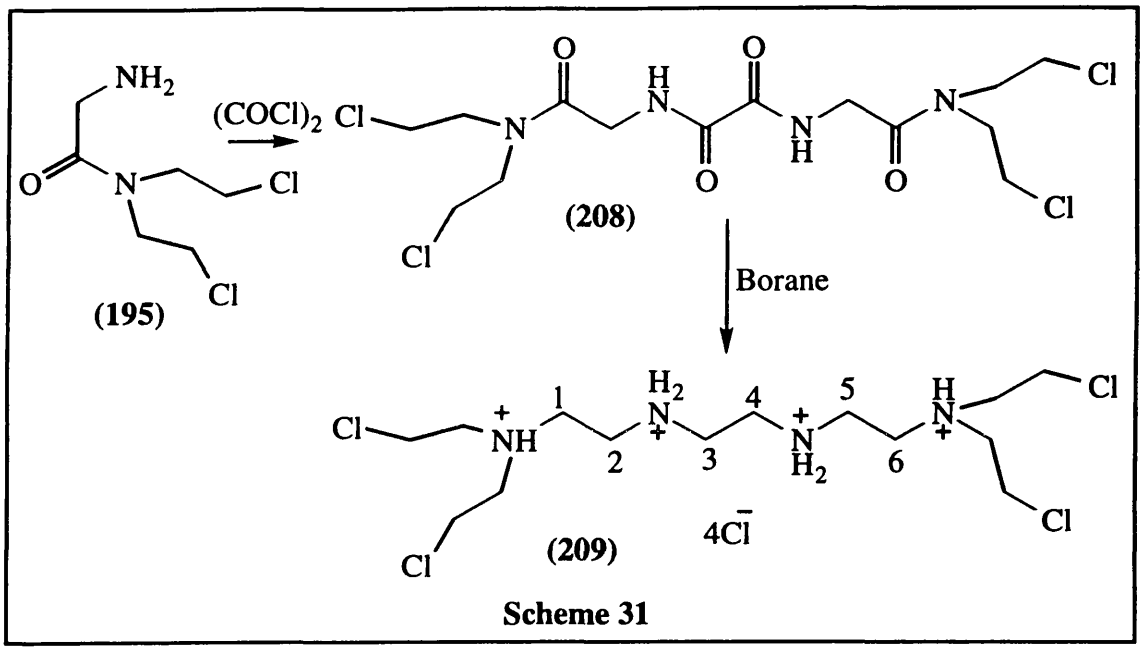
Reduction of the diamide (206) to the triamine (207) using borane went smoothly to leave the trihydrochloride salt as white crystals. The ^1H NMR spectrum consisted of three multiplets of relative intensity 1 : 3 : 2. This corresponded to the methylenes adjacent to the secondary amine at δ 3.05, with the methylene protons next to the tertiary

amines coming in a large multiplet at δ 3.33-3.54. The chloromethylene protons appeared at a shift of δ 3.68. The ^{13}C NMR spectrum was similar to those of the diamines. A molecular ion was observed at *m/z* 354 but the compound was too involatile for an accurate mass measurement to be obtained.

7.6 Synthesis of a Tetraamine Ligand

Polyamines have been used to target anticancer agents to DNA[164] because of two special features:[165] (i) the nature of their interaction with DNA, and (ii) the existence of an active uptake system for polyamines in a variety of cell types. Conjugation of a drug to a polyammonium cation will confer a high DNA affinity with mobility to allow drugs with high specificity to locate appropriate sites on DNA. If a polyamine could be combined with a cytotoxic part, then these compounds would be expected to target DNA. If these compounds could be further complexed to cobalt, then more selectivity could be achieved. It has been reported that the tetraamine, spermine has been complexed successfully to cobalt,[166] so we considered it desirable to make a tetraamine with nitrogen mustards incorporated that could be complexed to cobalt.

The most simple tetraamine to synthesise was a dimer of the diamine. Two of these molecules can be coupled together using oxalyl chloride and the amide (195) to give a symmetrical tetraamide (208), with each nitrogen linked by a two carbon chain. It was decided to use the trifluoroacetate of (195) as the precursor as then only one reduction of the compound (208) would be necessary (Scheme 31).



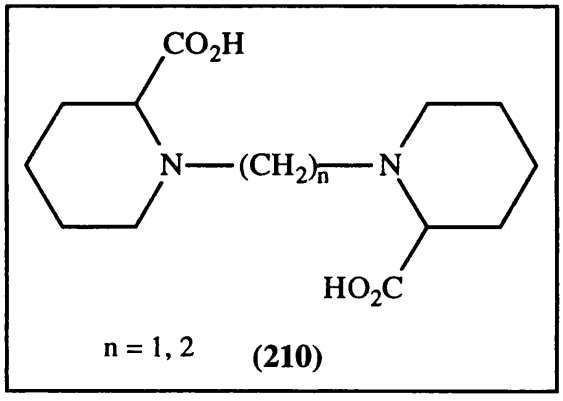
Scheme 31

The tetraamide (208) was made from the trifluoroacetate salt (195) and the free base was made *in situ* with triethylamine. Oxalyl chloride was added to form the tetraamide which was isolated as a clear oil. Three carbonyl stretches were observed in the IR spectrum, at 1732 cm^{-1} for the tertiary amides and at 1682 and 1514 cm^{-1} for the secondary amides. The ^{1}H NMR spectrum was relatively simple with a large multiplet at δ 3.76 for the mustard methylene protons and a smaller multiplet at δ 4.27 for the methylenes next to the secondary amines. A broad singlet was observed at δ 7.69 which was assigned to the NH protons. Duplicate signals were seen in the ^{13}C NMR spectrum for the methylene carbons adjacent to the chlorines with signals at δ 41.0, 41.1, 41.2 and 41.4. Only two signals were seen for the corresponding β-methylenes but with twice the intensity at δ 48.5 and 49.5. The methylene carbons adjacent to the carbonyls appeared as one signal at δ 63.4. Again, four signals were seen for the quaternary carbons, two at δ 156.6 and 159.7 which were assigned to the tertiary amides, and two at δ 167.4 and 167.9 which were attributed to the secondary amides. A large fragment corresponding to half the molecule at *m/z* 227 was found in the mass spectrum but there was no peak at *m/z* 454.

This tetraamide (208) was then subjected to reduction by borane which after acidic work up yielded the tetrahydrochloride salt (209). In the ^1H NMR spectrum, multiplets were observed for each set of signals. The methylene protons at C-3 and C-4 appeared at δ 3.04, while the protons on C-2 and C-5 were observed at δ 3.27. The methylene protons adjacent to the tertiary nitrogens all came together in a multiplet at δ 3.65 and the chloromethylenes were furthest downfield at a shift of δ 3.86. No parent ion was found in the mass spectrum, but a signal at *m/z* 322 corresponded to loss of two chlorine atoms.

7.7 Miscellaneous Diamines

It has been reported[167] that *N,N'*-linked pipecolinic acids (210) can be chelated to nickel(II) to give stable complexes.

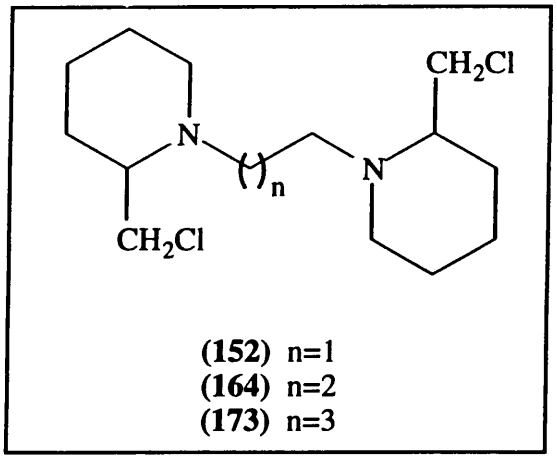


(210)

These ligands contain four potential coordinating centres. The system with a C_2 bridge produces a highly stable complex. This is attributed to its ability to form a planar chelate with three fused rings. Extension of the *N,N'*-bridge by one methylene group causes a sharp decrease in the ability to form a chelate, presumably reflecting the lower stability of a six-membered chelate ring. However, the longer the methylene bridge, the more tolerable the steric interference.

This led us to think it might be possible to complex our bis-piperidine systems to cobalt(III). The dichloro derivative was chosen to minimise steric interference and

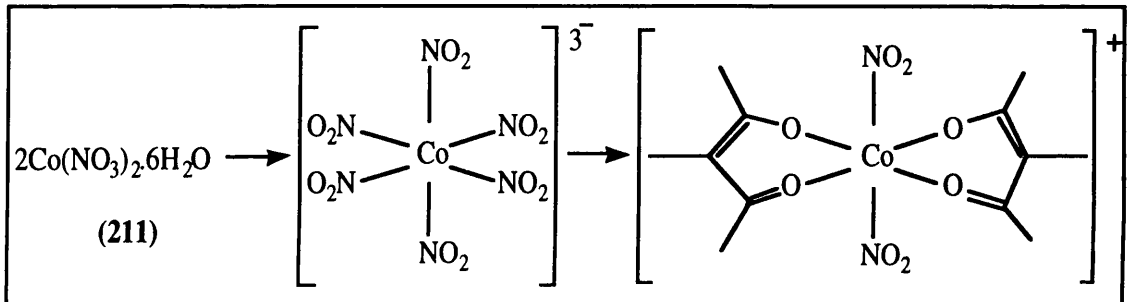
attempts were made to prepare all three cobalt complexes containing (152), (164) and (173).



(152) n=1
(164) n=2
(173) n=3

7.8 Synthesis of the Cobalt(III) Precursor Complex

Most Co(III) complexes undergo very slow substitution at the inert metal centre, rendering the synthesis of complexes containing very reactive ligands difficult. The preparation of the Co(III) complexes of the bidentate mustards required a cobalt-ligand system which undergoes relatively rapid substitution at Co(III), since the nitrogen mustards are unstable when in the required free base form. A suitable cobalt(III) precursor is *trans*-$Na[Co(3\text{-}Meacac)_2(NO_2)_2]$ (213) (acac = pentane-2,4-dionato anion) which can be prepared by treatment of $Na_3[Co(NO_2)_6]$ (212) with Na[Meacac] (Scheme 32).



Scheme 32

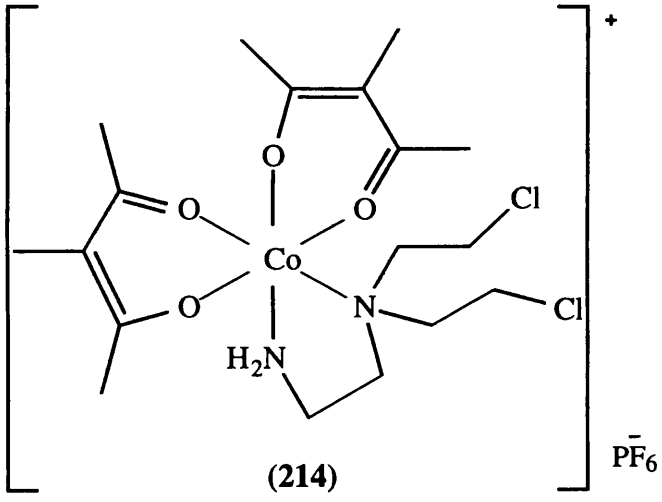
Sodium hexanitrocobaltate (III) (212) was made from cobalt nitrate (211) by a literature preparation,[168] by reacting cobalt(III) nitrate with sodium nitrite in aqueous acidic conditions with a stream of air passing through the solution. This compound was obtained as a yellow powder in good yield. This was converted into sodium dinitro-bis(3-methyl-acetylacetonato) cobaltate (213) by treating with 3-methylacetylacetone in base following a general procedure by Boucher and Bailar.[169] This compound was obtained as fine red-brown crystals which are slightly paramagnetic and are soluble in water and methanol. In the IR spectrum, signals at 1359 and 1298 cm^{-1} indicated the presence of nitro groups, whilst the carbonyl stretch band appeared at 1562 cm^{-1}. The signals in the ^1H NMR spectrum were present as singlets at δ 1.90 and 2.22 corresponding to the β methyl group and α methyl groups respectively. In the ^{13}C NMR spectrum, the signals appeared at δ 15.4 and 26.3 and were attributed to CCH_3 and $COCH_3$ as before. Quaternary signals at δ 100.0 and 187.4 corresponded to the CCH_3 and $COCH_3$ carbons and this is in accordance with literature precedence.[160]

7.9 Complexation of the Ligands to Cobalt

Procedures for the synthesis of the known complexes were taken from the literature.[160] The basic method involves dissolving the cobalt precursor in a 1:1 mixture of water and methanol, followed by addition of activated charcoal. An equimolar amount of diamine salt was then added with the careful addition of a slight excess of base next. The mixture was stirred for one hour before isolation of the complex by addition of ammonium hexafluorophosphate was permitted. In this manner, isolation of a number of complexes was accomplished although the yields were very poor after recrystallisation. The best yield came from the symmetrical mustard (197) and this is thought to be due to the greater stability of this ligand in comparison to the unsymmetrical mustards.[160]

Bis(3-methyl-2,4-pentanedionato)[*N*,*N*-bis(2-chloroethyl)ethane-1,2-diamine]-cobalt(III) hexafluorophosphate (214) was isolated as green crystals in poor yield. Bands at 2926 and 1569 cm^{-1} indicated the presence of aliphatic chains and carbonyl groups

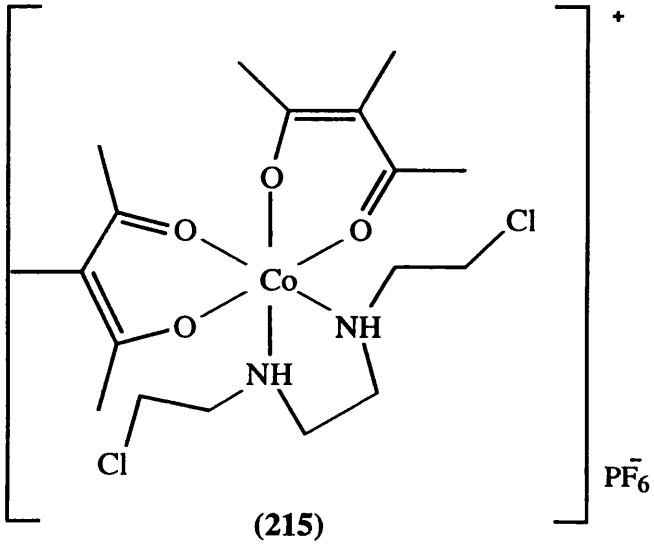
respectively. The band at 843 cm^{-1} was assigned to P-F stretch and the band at 737 cm^{-1} to C-Cl stretch. In the ^1H NMR spectrum, there were four singlets present for the four CH_3CO methyl groups at δ 1.86, 1.92, 1.96 and 2.07. The remaining CCH_3 methyl groups appeared as two singlets slightly further downfield at δ 2.23 and 2.32. The multiplet at δ 2.70 of intensity two was assigned to the CH_2NR_2 methylene protons, whilst the adjacent CH_2NH_2 protons appeared at δ 3.84. This is in line with literature reports.[160] The remaining β-chloromethylene protons appeared at δ 2.97 with the chloromethylene protons themselves showing at δ 3.62. A broad singlet at δ 4.06 was assigned to the amino protons. The ^{13}C NMR spectrum had two signals for the CCH_3 methyl carbons at δ 14.6 and 14.9. For the $COCH_3$ methyl carbons there were again four signals ranging from δ 25.6 to 26.4. Two signals were also apparent for the chloromethylene carbons, appearing at δ 36.0 and 38.2, as was true of the β-chloromethylene carbons which came at δ 53.5 and 55.7. One signal was present for the CH_2NH_2 carbon at δ 41.7 and one for the CH_2NR_2 which appeared at δ 61.0. Signals at δ 102.1 and 103.9 were assigned to the quaternary carbon CCH_3. Four signals from δ 187.7 to 189.5 corresponded to the remaining carbonyl carbons.



(214)

Bis(3-methyl-2,4-pentanedionato)[*RS-N,N'*-bis(2-chloroethyl)ethane-1,2-diamine]-cobalt-(III) hexafluorophosphate (215) was prepared in a similar manner and the product was obtained as purple needles after slow recrystallisation. It is possible to

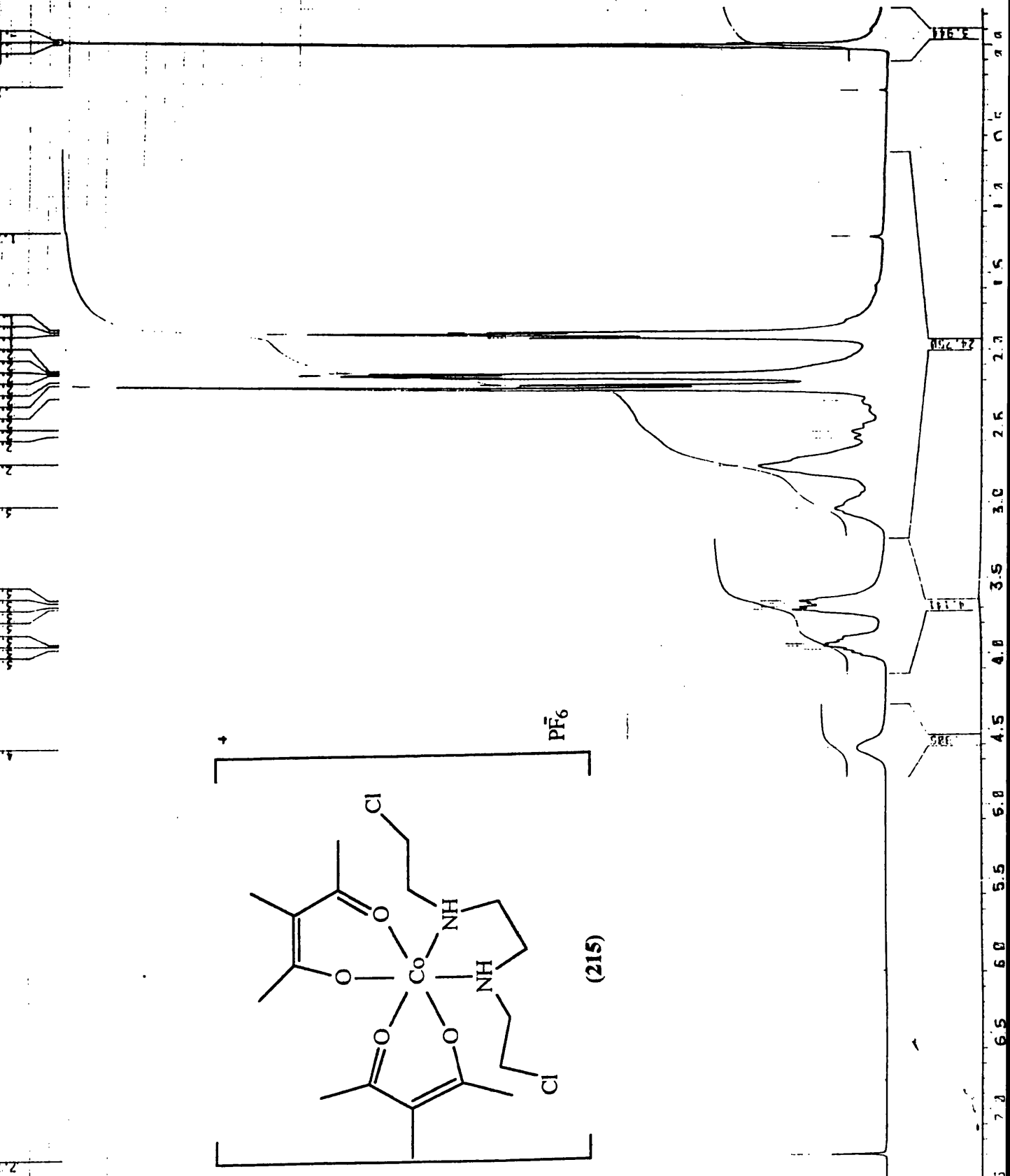
form three diastereoisomers when the ligand is complexed to cobalt as on coordination, each nitrogen atom becomes chiral. This can produce the *RR, SS* racemate and *meso-RS* compounds but the more stable form (*RS*) was enriched during crystallisation. This is more clearly seen in the NMR spectra. In the ^{1}H NMR spectrum (Figure 13), the Meacac signals were similar to that of the above compound (214). The β-chloromethylene protons appeared as a multiplet between δ 2.53 and 2.90 with the chloromethylene protons appearing as a multiplet at δ 3.66. The CH_2NH protons were split and appeared as multiplets at δ 3.03 and 3.94, each of intensity 2. A broad singlet at δ 4.61 accounted for the NH protons. The ^{13}C NMR spectrum was similar to the previous complex, except only one signal was apparent for each of the methyl carbon and the quaternary carbon of CCH_3. The IR spectrum was almost identical to that of the previous compound (214).



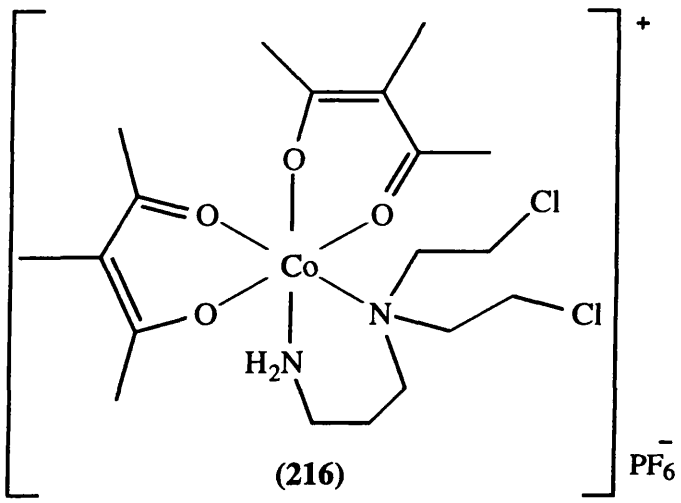
(215)

Bis(3-methyl-2,4-pentanedionato)[*N,N*-bis(2-chloroethyl)propane-1,3-diamine]-cobalt(III) hexafluorophosphate (216) was prepared in poor yield in the same fashion to afford the product as red-brown crystals. The NMR spectra were similar to that of the analogous compound as expected with the extra methylene group appearing as a multiplet at δ 1.19 in the ^{1}H NMR spectrum and at δ 31.3 in the ^{13}C NMR spectrum.

Figure 13

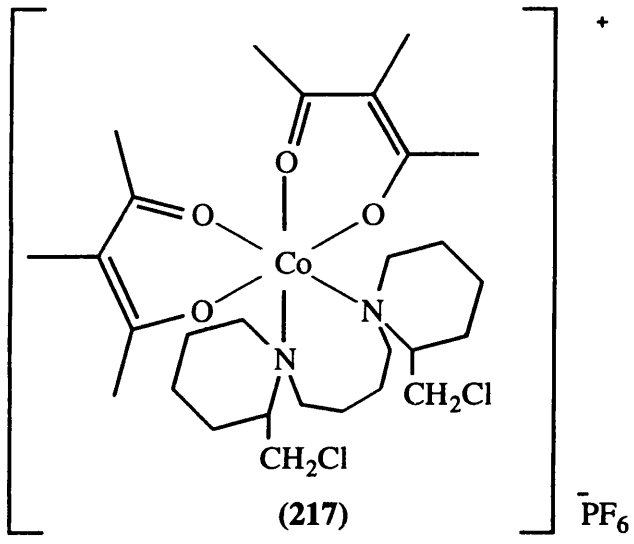


(215)



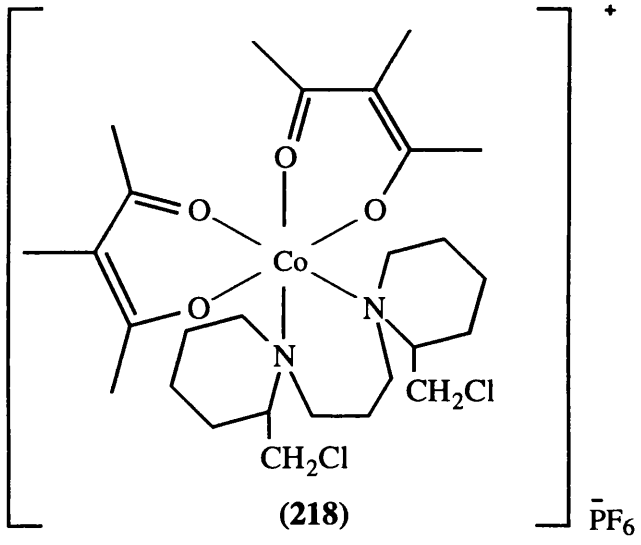
(216)

Bis(3-methyl-2,4-pentanedionato)[*N,N'*-(1,4-butanediyl)bis(2-chloromethyl-piperidine)] cobalt(III) hexafluorophosphate (217) was made by direct reaction between the free base of the diamine and the cobalt precursor complex. This resulted in the formation of red-brown crystals after slow recrystalliation. The ^1H NMR spectrum showed a broad multiplet between δ 1.55 and 2.05 corresponding to all the methylenes not attached to nitrogen. Four singlets at δ 1.86, 1.92, 2.12 and 2.16 were assigned to the CH_3CO methyl protons while the two singlets at δ 2.21 and 2.26 corresponded to the CCH_3 protons. A multiplet at δ 2.90-3.10 with intensity 8 was attributed to both the exo- and endo-cyclic methylenes adjacent to the nitrogens. Another mutiplet at δ 3.38 of intensity 4 was assigned to the chloromethylene protons with a small multiplet at δ 3.64 attributed to the CHN methine protons. No ^{13}C NMR spectrum could be obtained due to poor resolution with the small amount of material available.



(217)

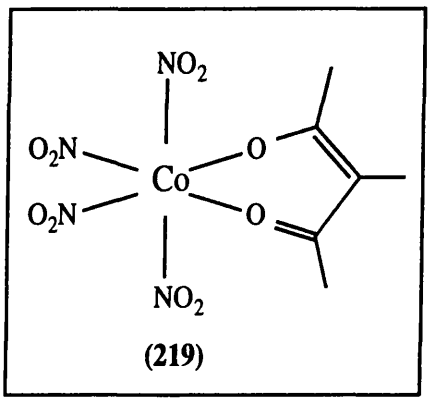
Bis(3-methyl-2,4-pentanedionato)[*N,N'*-(1,3-propanediyl)bis(2-chloromethyl-piperidine)]cobalt(III) hexafluorophosphate (218) was prepared in a similar manner to that above and was obtained as red-brown crystals, although in poor yield. The ^{1}H NMR spectrum was almost identical to that of its higher homologue, and as before, no ^{13}C NMR spectrum could be obtained.



(218)

Although complexation of the N-C_2-N bis(chloromethylpiperidine) compound (152) to cobalt was attempted repeatedly, no product could be isolated. This could be due to the steric hindrance caused by the side chains making it difficult for the nitrogens to complex to the cobalt.

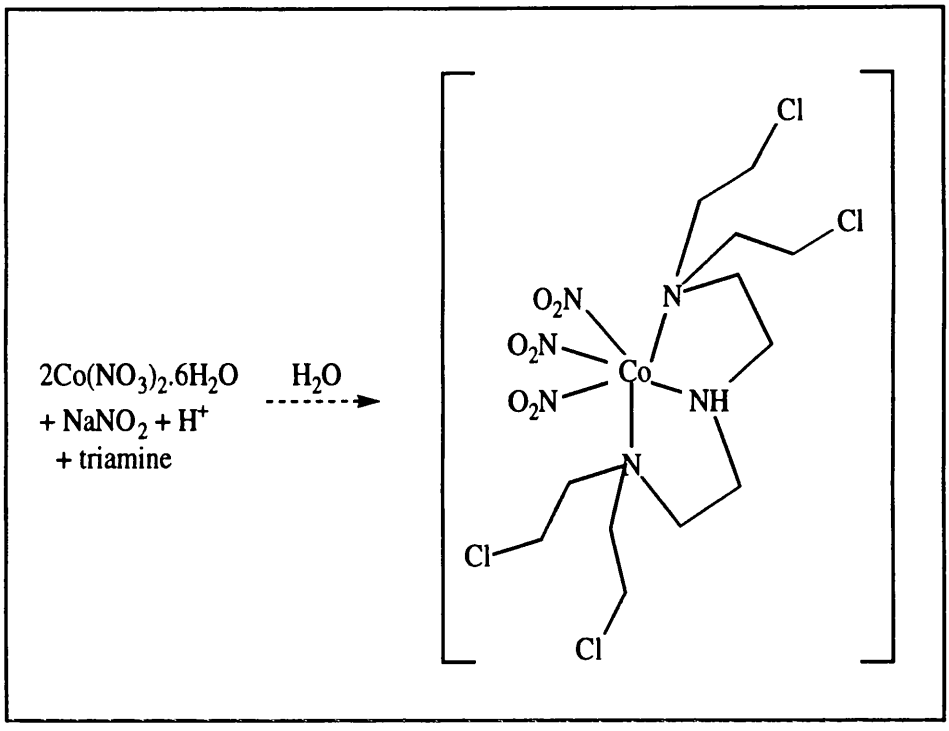
Attempts to complex the triamine (207) and tetraamine (209) to cobalt were hampered by the lack of a suitable precursor. Ideally, we would like a complex (219) with one Meacac and four nitro groups for both the triamine and tetraamine, so we could displace three or four nitro groups and still have the Meacac group in place as this group seems to be important for achieving the right reduction potential.



(219)

A search of the literature was unsuccessful in finding such a compound so it was decided to complex each polyamine to a cobalt precursor to obtain a complex containing nitro groups.

A method was found in the literature to complex a triamine to cobaltous nitrate hexahydrate.[170] This involved a method similar to that used for sodium hexanitrocobaltate in which a mixture of cobaltous nitrate, sodium nitrite and the triamine were aerated in acidic aqueous solution for one hour.



Unfortunately, no complex could be isolated from the reaction mixture and it was not certain that the triamine had complexed at all. Material isolated from the solution had a ^{1}H NMR spectrum which was consistent with the structure of the triamine However, it could not be ascertained whether the triamine was part of a cobalt complex or was in the salt form due to lack of other protons present in the desired complex. No further analysis was undertaken.

Complexation of the tetraamine was carried out following a general method[171] in a similar manner. A mixture of cobalt chloride, lithium hydroxide, sodium nitrite and the tetraamine in water was aerated for a period of eight hours before the counter ion of hexafluorophosphate was added. Unfortunately, any complex formed could not be identified due to the same reasons as above.

7.10 Conclusions

Synthetically useful routes have been devised to diamine ligands which could themselves be used as precursors to make polyamine ligands. The two cobalt complexes

which have been made previously were successfully prepared and all spectral data were consistent with those reported before. Three new complexes have been isolated and characterised and it should be possible to improve the yields achieved. A suitable cobalt precursor needs to be made before complexation of the polyamines can be accomplished. This may be a promising area to enter as there is plenty of scope for design of complexes which may be useful in the area of bioreducible drugs.

Chapter 8

Biological Testing Results

8.1 Introduction

The compounds were tested in the Department of Medical Oncology, University of Glasgow. The *cis*- and *trans*-2,6-disubstituted piperidine derivatives were tested by Dr. Jane Plumb while the rest of the compounds were tested by myself under her supervision.

Aerobic toxicity was assessed using a microtitration assay called the MTT based cytotoxicity assay, from which IC_{50} values were calculated after drug exposure for 24 hours. The selective toxicity of the compounds toward hypoxic cells was determined by comparing the toxicity in aerobic and hypoxic conditions after drug exposure for 3 hours. The prodrug (*N*-oxide or cobalt complex) requires a reduction potential within the normal range of cellular reductants (about -0.20 to -0.40 V *vs* normal hydrogen electrode) in order to be reduced to the toxic free base.

8.2 Cell Lines

The role of the obligate two-electron-reducing enzyme DT-diaphorase in the bioactivation of mitomycin C, EO9 and other bioreducible agents has received a lot of attention in recent years.[76] This enzyme is of significant interest because DT-diaphorase is often found in high concentrations in tumours compared with normal tissue.[76] Two human colon carcinoma cell lines were employed in these studies which could determine the role of DT-diaphorase in the bioreductive activation of our prodrugs. HT 29 cells produce high levels of DT-diaphorase and so the mechanism of activation for prodrugs may proceed through a $2e^-$ reduction pathway. It should be noted that reduction by DT-diaphorase is dependent on the compound and some prodrugs are activated better than

others. For example quinones and CB 1954 are especially good substrates for DT-diaphorase. BE cells contain a mutation in the NQ1 gene and express a mutant form with essentially no enzyme activity[172] and so a $1e^-$ reduction pathway may be expected to predominate. The combined results from these two cell lines should provide evidence for the mode of action for individual compounds and it should be clear if any compound has a preference for activation by DT-diaphorase

8.3 MTT Based Cytotoxicity Assay

Cytotoxicity tests are widely used to evaluate the effects of anticancer drugs on cells. Clonogenic assays use plating efficiency, either on plastic or in agar, as an end point such that the number of colonies relative to the control indicates the number of cells that survived the treatment. Furthermore, the size of the colony can indicate whether the treatment was cytotoxic, cytostatic or mitogenic. These assays are slow and the development of microtitration assays has been an enormous benefit to those who screen new compounds for cytotoxic activity. The end point of a microtitration assay is an estimate of cell number. This can be achieved directly by cell counts or by indirect methods such as isotope incorporation. Use of MTT as a cell viability measure[173] can be chosen as the optimal end point.[174] MTT is a yellow water soluble tetrazolium dye that is reduced by live, but not dead cells, to a purple formazan product that is insoluble in aqueous solutions. It should be noted that a number of factors can influence the reduction of MTT.[175] However, in the assay used, the same result has been achieved as with a standard clonogenic assay.[176]

Cells in exponential phase of growth were exposed to the cytotoxic drug of interest. The duration of exposure is usually determined as the time required for maximal damage to occur but is also influenced by the stability of the drug. After removal of the drug the cells were allowed to proliferate for two to three doubling times in order to distinguish between cells which remain viable and are capable of proliferation and those which remain viable but cannot proliferate. Surviving cell numbers were then determined

indirectly by MTT dye reduction. The amount of MTT-formazan produced can be determined spectrophotometrically once solubilised in a suitable solvent. It is important to note that the following conditions are met.

1) MTT colour is shown to be in the linear range of dependence on the cell number.

2) The colour produced is proportional to the time of MTT exposure.

8.4 Hypoxic Cytotoxicity

Plastic multiwell plates are not suitable for carrying out cytotoxicity experiments with drugs that are activated under hypoxic conditions. This is because oxygen is soluble in tissue culture plastic and is released slowly into the surrounding environment when external oxygen tension decreases.[177] To overcome this problem, glass petri dishes were used for the drug exposure under nitrogen. The exposure was limited to three hours as cells will die without drug exposure if the absence of oxygen is prolonged beyond this time. The medium containing the drug was then removed and replaced with fresh medium. After two days, surviving cell numbers were then counted directly using a Coulter counter, and this number was taken as a percentage of the controls.

8.5 *cis*- and *trans*-Disubstituted Piperidines

The results of the testing of these compounds can be seen in Tables 1 and 2. The compounds in the shaded areas are the corresponding *N*-oxides which are required to be non-toxic in oxic conditions but toxic in hypoxic conditions.

The *N*-oxides were inactive, indicating that using the lone pair of the nitrogen to form a bond reduces the toxicity substantially. This is consistent with the mechanism of aziridine formation of conventional nitrogen mustards. There appears to be little difference in the oxic and hypoxic toxicities for individual *N*-oxides, so we can assume that the reduction potentials of these types of compound are not in the correct range for cellular reductants.

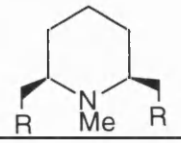
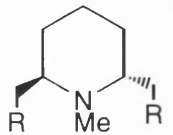
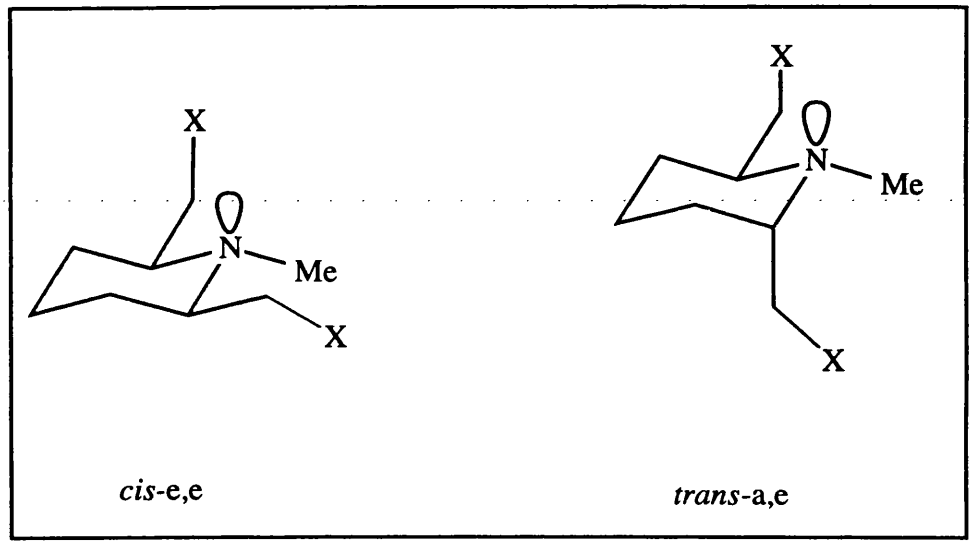
Structure	Survival as % of control at 1mM after 3h; IC_{50} values are after 24 h exposure					
	HT29 Cells			BE Cells		
R Equals	Oxic	Hypoxic	IC_{50} (μM)	Oxic	Hypoxic	IC_{50} (μM)
OCONHEt (**81**)	80	60	-	0	0	-
OCONHPr^{i} (**80**)	0	0	250	0	0	220
OCONHPh (**82**)	0	0	41	0	0	77
$CO_2NHPhNO_2$ (**83**)	0	0	38	0	0	28
OCONHEt (**87**)	100	100	-	100	100	-
OCONHPr^{i} (**86**)	100	100	-	100	100	-
OCONHPh (**88**)	100	100	-	100	100	-
Cl (**66**)	100	100	-	87	100	-
Br (**84**)	85	62	8.5	51	42	11
Cl (**89**)	98	84	-	85	96	-
Br (**90**)	95	93	-	92	100	-

Table 1

Table 2

Structure	Survival as % of control at 1mM after 3h; IC_{50} values are after 24 h exposure					
	HT29 Cells			BE Cells		
R Equals	Oxic	Hypoxic	IC_{50} (μM)	Oxic	Hypoxic	IC_{50} (μM)
OCONHEt (**108**)	100	100	-	83	100	-
OCONHPr^{i} (**107**)	0	0	350	0	0	400
OCONHPh (**109**)	0	0	130	0	0	130
$CO_2NHPhNO_2$(**110**)	76	53	0.25	44	100	0.22
OCONHEt (114)	100	100	-	100	100	-
OCONHPr^{i} (113)	100	100	-	100	100	-
OCONHPh (115)	100	100	-	100	100	-
Cl (**111**)	100	100	-	100	100	-
Br (**112**)	17	14	8.2	2	4	6.5
Cl (116)	100	100	-	100	100	-
Br (117)	91	82	-	78	100	-

In general, there is little difference between the two cell lines and what difference there is can probably be attributed to experimental error. We can therefore conclude that none of the compounds are activated appreciably by reductase enzymes including DT-diaphorase. Both *cis*- and *trans*-dibromo-compounds are more toxic than the dichloro-compounds and this is consistent with the theory that the incorporation of better leaving groups increases alkylating potency. It is interesting to note that the *trans*- aromatic carbamates are up to 100-fold more toxic than the *cis*-series, indicating that spatial arrangements are important for toxicity. This was unexpected as it was assumed that the equatorial groups would be more susceptible to attack by the nitrogen lone pair.

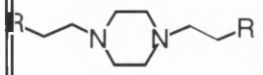


cis-e,e *trans*-a,e

8.6 Piperazine Derivatives

The results of the testing of the piperazine derivatives are shown in Table 3. Again we see little hypoxic selectivity from the *N*-oxides, but a dramatic decrease in toxicity is observed in comparison to the free bases, where the *N*-oxides are basically non-toxic. This would appear to mean that the lone pair on the nitrogens is essential for toxic activity, as for the 2,6-disubstituted piperidines and is consistent with aziridine formation and attack by biological nucleophiles.

Table 3

Structure	Survival as % at 1mM after 3 h; IC_{50} values are after 24 h exposure					
	HT29 Cells			BE Cells		
R Equals	Oxic	Hypoxic	IC_{50} (μM)	Oxic	Hypoxic	IC_{50} (μM)
OCONHPr^{i} (**128**)	0	0	220	0	0	260
OCONHEt (**127**)	0	0	190	0	0	100
OCONHPh (**129**)	0	0	7.2	0	0	13
OCONHPhNO_2 (**130**)	0	0	9.4	0	0	1.7
Cl (**125**)	0	0	650	0	0	300
OCONHEt (**131**)	95	85	>1000	100	100	>1000
OCONHPr^{i} (**132**)	90	100	>1000	60	51	650
OCONHPh (**133**)	90	65	>1000	100	100	600
OCONHPhNO_2 (**134**)	84	100	>1000	100	100	500
Cl (**135**)	18	12	>1000	25	42	260

The toxicities of the free bases are similar to those of the piperidine derivatives, with the aromatic carbamates being the most active.

There is a significant difference in the toxicities of the *N*-oxides towards the two cell lines. It appears in this case that most of the compounds are selectively more toxic towards the BE cells than the HT 29 cells.Compound 131 does not show this selectivity as it seems to be completley non-toxic. On the other extreme, compound 135 has an increase of around 10-fold in toxicity. This is probably due to straight 2e- reduction. However, it may mean that instead of activating our *N*-oxides, DT-diaphorase may be acting as a protective enzyme which is detoxifying a reactive intermediate in our compounds. This is the case for SR 4233 where a 2e- reduction protects against toxicity. We could therefore expect greater toxicity in BE cells if this is the case for this type of *N*-oxide. This is an interesting result which may have implications for enzyme profiling, if a reductase enzyme can be found which does activate these prodrugs.

8.7 Bis-piperidine Derivatives

Tables 4, 5 and 6 show the results of testing the bis-piperidine series with the 2, 3 and 4 bridging carbons, respectively. All three sets of compounds show a greater toxicity for the free bases in comparison to their *N*-oxides. Again, the *N*-oxides appear to have little selectivity for cells under hypoxic conditions in preference to those under oxic conditions.

Compounds with two carbons between the nitrogens (Table 4) show greatest toxicity in the aromatic carbamates. There appears to be little difference between the two cell lines except for the dichloro (152) and bis(phenyl carbamate) (150) derivatives. These compounds in the free base form are *ca.* 10-fold more toxic to HT 29 cells than BE cells. It may be that these derivatives are genuinely being activated in some way by the high levels of DT-diaphorase present in this cell line.

Compounds with three carbons between the nitrogens (Table 5) seem generally to show the same magnitude of toxicity as the N-C_2-N set with the possible exception of the

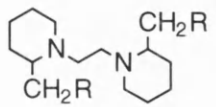
Structure	Survival as % at 1mM after 3 h; IC_{50} values are after 24 h exposure					
	HT29 Cells			BE Cells		
R Equals	Oxic	Hypoxic	IC_{50} (μM)	Oxic	Hypoxic	IC_{50} (μM)
OCONHEt (**149**)	0	0	78	0	0	45
$OCONHPr^i$ (**148**)	0	0	450	0	0	295
OCONHPh (**150**)	0	0	2.9	0	0	16
$OCONHPhNO_2$(**151**)	0	0	0.95	0	0	0.75
Cl (**152**)	10	23	144	50	35	1300
OCONHEt (**154**)	88	57	1200	75	100	<1000
$OCONHPr^i$ (**153**)	93	93	250	50	100	150
OCONHPh (**155**)	45	78	320	37	73	190
$OCONHPhNO_2$(**156**)	23	81	275	68	60	215
Cl (**157**)	100	100	275	87	68	165

Table 4

Table 5

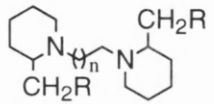
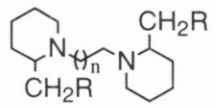
Structure	Survival as % at 1mM after 24 h					
	HT29 Cells			BE Cells		
R Equals	Oxic	Hypoxic	IC_{50} (μM)	Oxic	Hypoxic	IC_{50} (μM)
n=2						
OCONHPh (**162**)	0	0	2.9	0	0	1.2
OCONHPhNO_2 (**163**)	0	0	0.3	0	0	2.5
Cl (**164**)	0	0	66	0	0	20
OCONHPh (**165**)	18	40	320	23	43	110
OCONHPhNO_2 (**166**)	12	37	470	26	43	670
Cl (**167**)	0	14	120	25	37	640

Table 6

Structure	Survival as % at 1mM after 24 h					
	HT29 Cells			BE Cells		
R Equals	Oxic	Hypoxic	IC_{50} (μM)	Oxic	Hypoxic	IC_{50} (μM)
n=3						
OCONHPh (**171**)	0	0	0.083	0	0	1.2
$OCONHPhNO_2$ (**172**)	0	0	0.012	0	0	0.013
Cl (**173**)	0	0	80	0	0	20
OCONHPh (**174**)	0	7	430	0	14	270
$OCONHPhNO_2$ (**175**)	16	46	660	10	34	500
Cl (**176**)	25	5	780	0	14	210

dichloro derivative (164) which has a slightly greater toxicity, especially for the BE cells, where it shows a 65-fold increase in toxicity over the corresponding N-C_2-N compound.

It does appear that when the bridge length is increased to four carbons (Table 6), the toxicity of the carbamates is increased substantially, by around 100-fold for the HT 29 cells. This is an interesting observation and it may be that the optimum chain length for toxicity has still to be reached. It would be desirable to synthesise some aromatic bis-carbamates with a bridging length of greater than four to see if this is the case. The bis(*p*-nitrophenyl carbamate) (172) is the most toxic compound made in this project with IC_{50} value of 12 nanomolar against both cell lines. Surprisingly, the dichloro derivative does not show the same increase in toxicity, with values comparable to those of the N-C_3-N series.

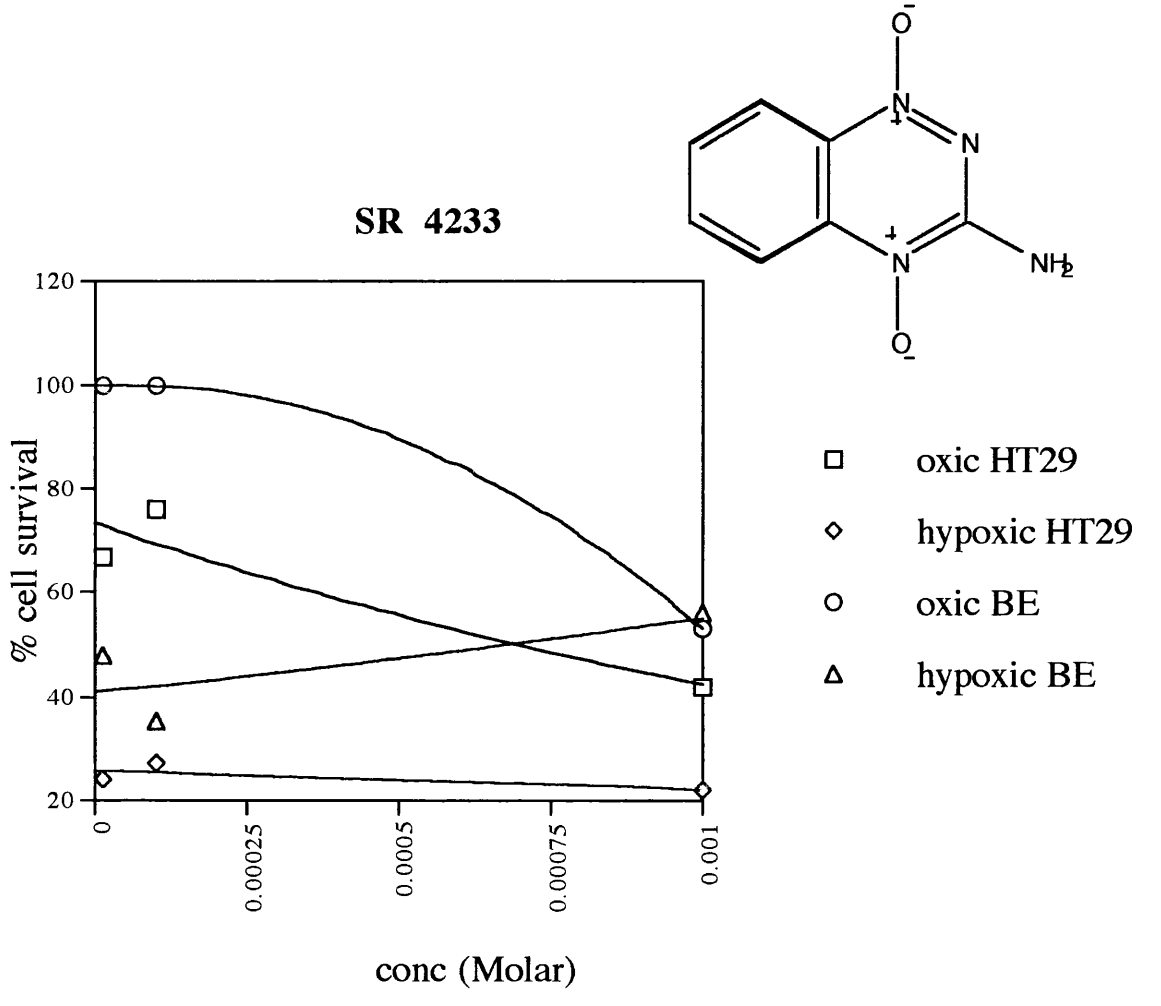
8.8 Cobalt Complexes

This set of compounds was tested for oxic/hypoxic selectivity, with IC_{50} values measured for the mustard hydrochloride salts. Table 7 shows IC_{50} values for the polyamine salts and some of the cobalt complexes, along with oxic/hypoxic selectivity at the lower concentration of 0.1 mmol. The IC_{50} values cover a wide range. The salts seem to show a preferential toxicity towards BE cells but, surprisingly, the triamine (207) and the tetraamine (209) do not show the higher toxicity that was expected because of the four potential alkylating chloroethyl groups. It may be that this is due to steric effects of the extra chloroethyl groups. The two diamines (185) and (197) that have been made before[160] do not show as high toxicity as reported but this is probably due to the different cell lines used. The cobalt complexes show varying toxicities. The values of (214) and (215) (which had been made before) correlate very well with those reported,[160] with the unsymmetrical diamine complex (214) showing the highest toxicity of those made. Of the two bis-piperidine complexes, the compound with N-C_4-N (217) shows less toxicity than the free diamine indicating that the complex is quite stable in these conditions. On the other hand, the complex containing N-C_3-N (218) is 10-fold more toxic than the N-C_4-N

Structure	Survival as % at 0.1mM after 3 h; IC_{50} values are after 24 h exposure					
	HT29 Cells			BE Cells		
	Oxic	Hypoxic	IC_{50} (μM)	Oxic	Hypoxic	IC_{50} (μM)
(185)	-	-	310	-	-	120
(197)	-	-	1250	-	-	290
(207)	-	-	880	-	-	830
(209)	-	-	230	-	-	94
(202)	-	-	23	-	-	12
(214)	50	25	4.7	53	71	72
(215)	100	100	1000	100	100	1300
(216)	82	54	-	33	47	-
(217)	88	100	510	100	100	860
(218)	100	100	52	100	60	110

Table 7

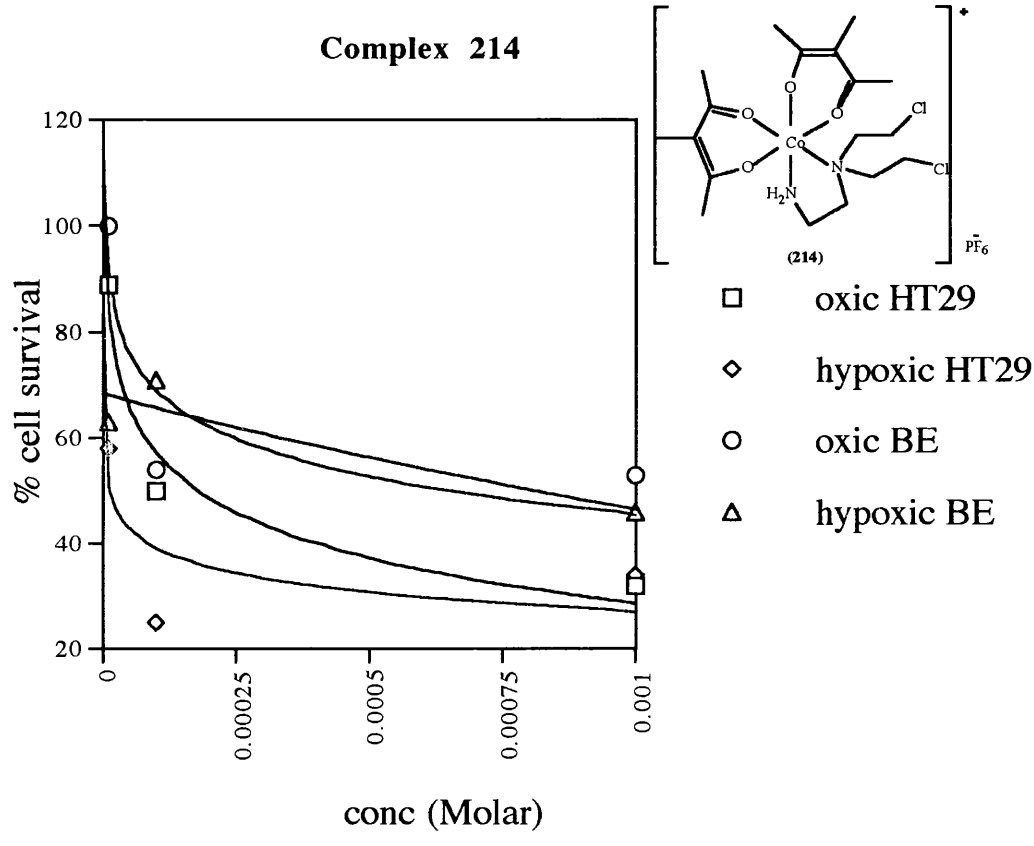
complex, and its IC_{50} values correlate quite well with the free base values (Table 5). This would indicate that this complex is less stable and quickly breaks down to give the free diamine. There is a slightly higher toxicity towards the HT 29 cells than the BE cell line, but not much is known about the enzymology of cobalt complexes. We therefore felt it was worth conducting a more detailed oxic/hypoxic screen on the cobalt complexes. These results are shown in graph form.



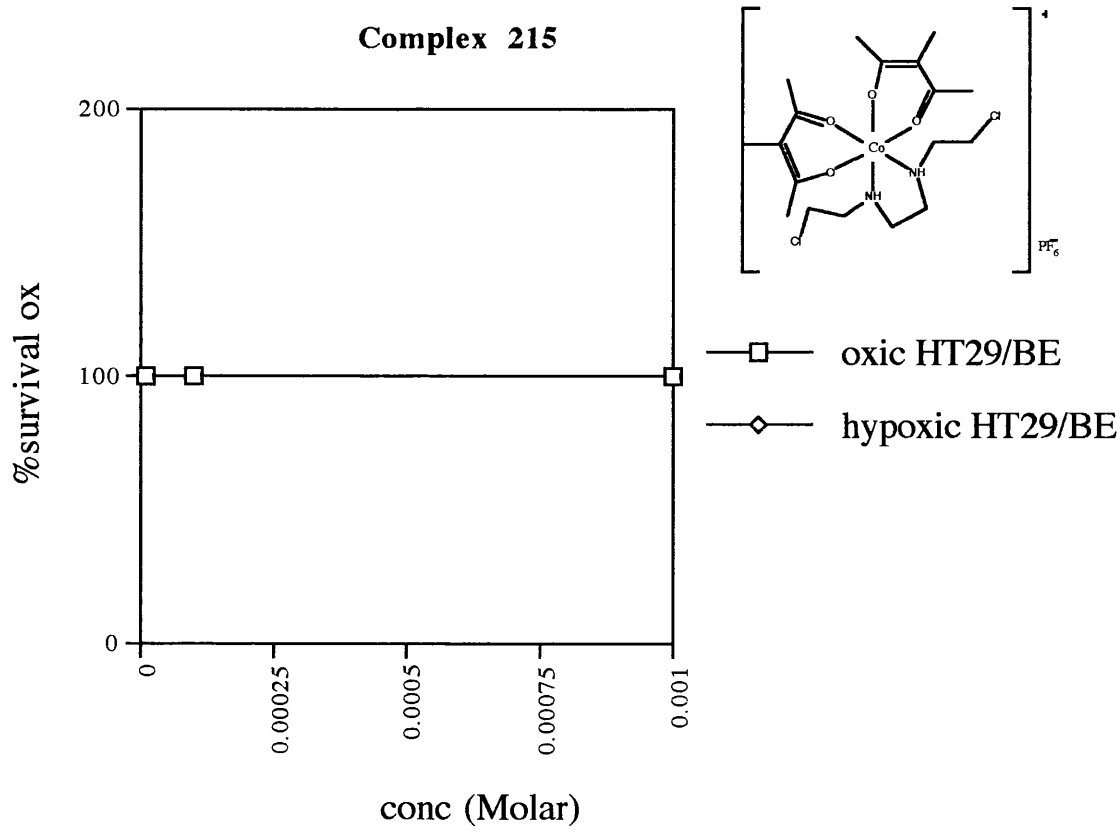
SR 4233 (44) was also tested as a standard to see if the test system was working in a satisfactory manner. The complexes were tested at three different concentrations, starting at 1 mmol with a five-fold dilution each time, under oxic and hypoxic conditions with a three hour drug exposure.

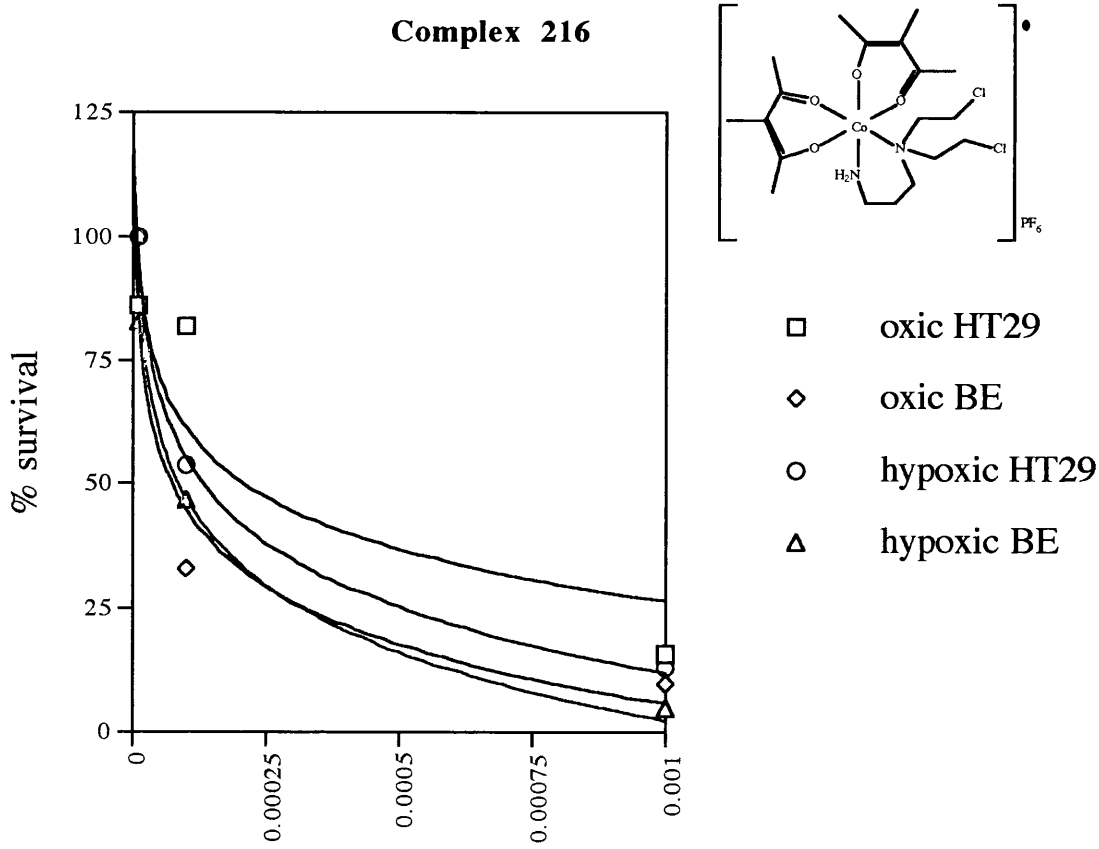
SR 4233 (44) can be seen to be more toxic under hypoxic than oxic conditions for both cell lines at the concentration of 40 mM. This is consistent with results obtained by others using the same system.[28]

Of all the cobalt complexes tested, the greatest selectivity was seen with that complex (214) which had been proven before to be selective.[160] It had been reported to have 20-fold selectivity for hypoxic cells but our own results showed a selectivity of *ca.* 6-fold. This result can easily be explained by the different cell lines used.

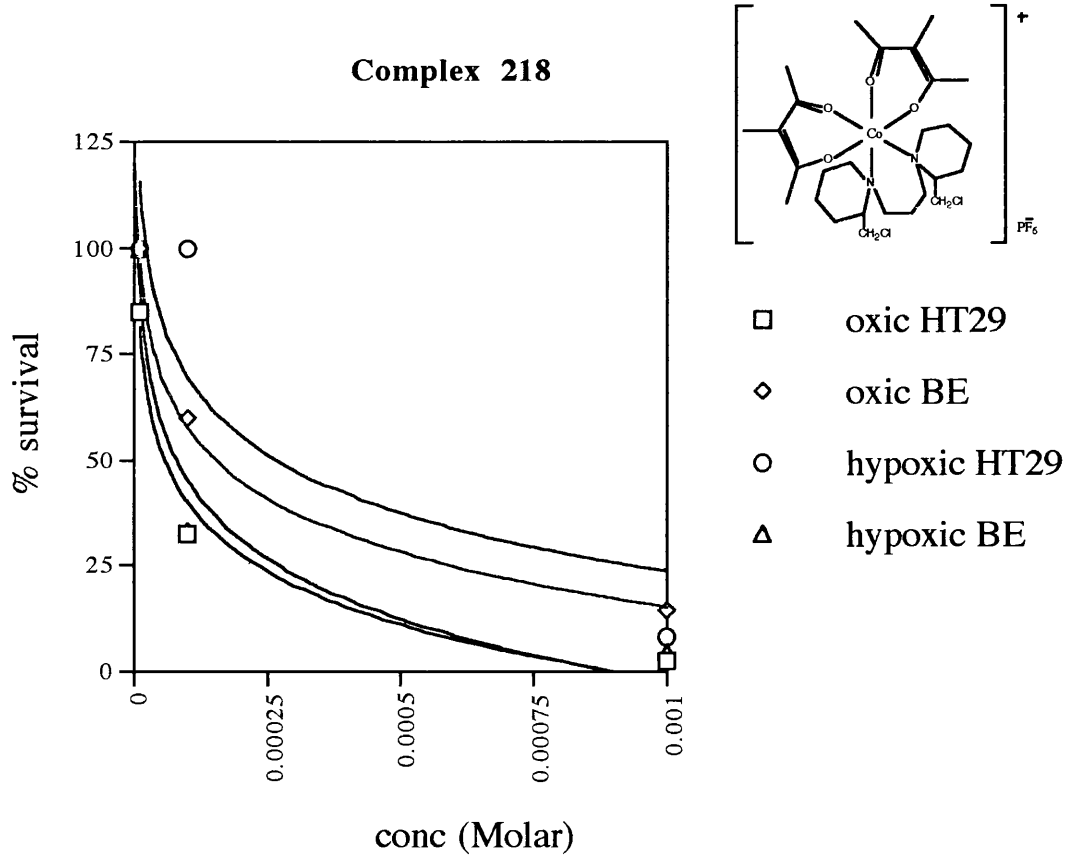


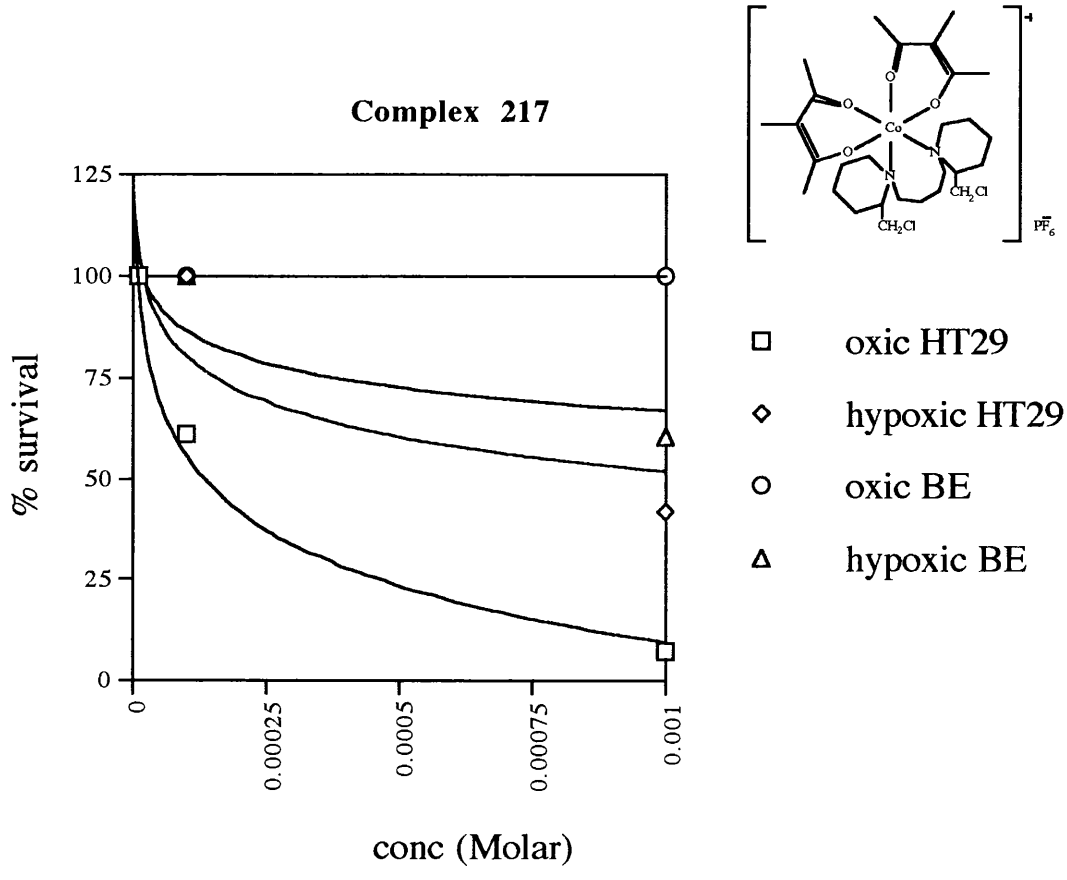
The diamine complex (215) did not show any activity at all towards either cell line and this is in line with the results of Ware *et al.*[160] The higher homologue (216) did not show any selectivity at all with the BE cells, with very similar toxicity under both oxic and hypoxic conditions, but some selectivity was observed with the HT 29 cells at a concentration of 200 mM.


Complex 215
%survival ox
200
100
0
0
0.00025
0.0005
0.00075
0.001
conc (Molar)
oxic HT29/BE
hypoxic HT29/BE


Complex 216
% survival
125
100
75
50
25
0
0
0.00025
0.0005
0.00075
0.001
oxic HT29
oxic BE
hypoxic HT29
hypoxic BE

The bis-piperidine complexes showed very similar behaviour. The N-C_3-N derivative (218) had some selectivity, with the compound being more toxic towards BE cells under hypoxic conditions at a concentration of 200 mM. The compound was actually more toxic towards oxic HT 29 cells than hypoxic HT 29 cells at all concentrations. The derivative with N-C_4-N (217) showed similar results except that it was more toxic to BE cells under hypoxic conditions at concentration of 1 mmol only. Again, greater toxicity was observed for the HT 29 cells under oxic conditions.




Complex 217
% survival
125
100
75
50
25
0
0
0.00025
0.0005
0.00075
0.001
conc (Molar)
oxic HT29
hypoxic HT29
oxic BE
hypoxic BE
Co
O
N
CH2Cl
PF6

8.9 Conclusions

It would appear that under the conditions used, none of the *N*-oxides are suitable for use as bioreducible drugs. This is either due to these compounds not having appropriate reduction potentials or due to lack of activation by the reductase enzymes present in the HT 29 cell line. Many of the free bases are highly toxic species. These are probably acting as alkylating agents by forming aziridinium rings which can then be attacked by DNA. It is probable that on release from the molecule the aromatic carbamates are exerting their own toxic actions which are contributing to the high toxicity of these compounds. It would be desirable if more detailed testing on a selection of these compounds could establish the nature of their toxic actions. More work is required on the cobalt complexes to see if these compounds could be counted as a viable class of bioreducible drug. There are some encouraging results presented here which indicate that this could be worthwhile.

Experimental

General

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. Infra red spectra were obtained on a Perkin Elmer 580 spectrophotometer in the form of KBr discs for solids and thin films for oils and liquids unless otherwise stated. Nuclear magnetic resonance spectra were recorded with a Bruker WP200-SY spectrometer operating at 200 MHz (δ_H) and 50 MHz (δ_C). The multiplicities of the ^{13}C NMR spectra were determined using DEPT spectra with pulse angles of $\phi = 90°$ and $\phi = 135°$. Spectra were recorded for solutions in deuteriochloroform unless otherwise stated, with tetramethylsilane as internal standard. Mass spectra were obtained with A.E.I. MS 12 or 902 spectrometers. Elemental analyses were obtained with a Carlo-Erba 1106 elemental analyser.

TLC was carried out on neutral aluminium oxide 60 F_{254} plates of 0.2 mm thickness obtained from Merck and were detected using UV light, Dragendorff reagent[178] (tertiary amines) and vanillin (carbonyl compounds).

Tetrahydrofuran was dried by distillation from sodium-benzophenone under nitrogen prior to use. Dichloromethane was dried by distillation from calcium hydride. Methanol was dried by distillation from clean, dry magnesium turnings and iodine. Acetone was dried with anhydrous potassium carbonate followed by distillation. DMF was dried by distillation from magnesium sulfate. Solvents were evaporated off under reduced pressure below 50 °C.

General Procedures

General Procedure A

A mixture of the diol, isocyanate (quantities are given under individual compounds) and 3 drops of dibutyl tin diacetate were stirred together at room temperature in dichloromethane (20 ml) until TLC indicated that the reaction was complete. The solution was then concentrated to leave a residue which was purified as described.

General Procedure B

mCPBA was dissolved in dry acetone and the amine (quantities are given under individual compounds) in acetone (5 ml) was added dropwise. After TLC had indicated the reaction had gone to completion, the solution was carefully concentrated. Flash chromatography on alumina with methanol (50 ml) removed the acid residues and evaporation of the methanol left a residue which was purified as described.

Experimental to Chapter 3

cis-2,6-Piperidinedicarboxylic Acid Hydrochloride[96] (76)

Dipicolinic acid (1.50 g, 8.7 mmol) in glacial acetic acid (50 ml) was hydrogenated at atmospheric pressure and room temperature for 30 h using platinum (IV) oxide (0.15 g) as catalyst. The catalyst was removed by filtration through Celite and the filtrate was acidified by conc. HCl to precipitate the hydrochloride as a white solid. Crystallisation from water gave *cis*-2,6-piperidinedicarboxylic acid hydrochloride (1.4 g, 89%), m.p. 284-286 °C, ν_{max} 3400, 3000 and 1750 cm^{-1}; δ_H (90 MHz) (D_2O) 1.5-2.2 (6H, m, ring CH_2) and 4.35 (2H, m, CHN); *m/z* 173 (M^+ 0.1 %), 128 and 82 (100%).

N-Methyl-*cis*-2,6-Piperidinedicarboxylic Acid[115] (77)

A mixture of *cis*-2,6-piperidinedicarboxylic acid hydrochloride (1.00 g, 5.8 mmol), 37 % formaldehyde solution (0.96 g), 90 % formic acid (1.2 ml) and sodium bicarbonate (0.5 g, 5.8 mmol) was heated at reflux for 19 h. Precipitated sodium chloride was filtered off and the filtrate concentrated to an oil. Crystallisation from methanol gave *N*-methyl-*cis*-2,6-piperidinedicarboxylic acid as white crystals (0.68 g, 68%), m.p. 212-216 °C, δ_H (90 MHz) (D_2O) 1.5-2.3 (m, 6H, ring CH_2), 2.83 (3H, s, NCH_3) and 3.9 (2H, m, CHN); *m/z* 187 (M^+ 0.2 %), 142, 114, 96, 82 and 70.

Diethyl 2,6-pyridinedicarboxylate[97] (78)

Dipicolinic acid (5.0 g, 30 mmol) was refluxed for 42 h with thionyl chloride (15 ml). The excess thionyl chloride was removed *in vacuo* and the residue was taken up in CCl_4 and treated with ethanol at 50-60 °C. The crude diester precipitated on cooling and was recrystallised with methanol to leave white crystals (5.65 g, 97%), R_F 0.58 (EtOAc) m.p. 38 °C, ν_{max} 1745, 1576, 1532, 1482, 1385, 1242 and 764 cm^{-1}; δ_H 1.46 (6H, t, *J* 7 Hz,

CH_3), 4.49 (4H, q, *J* 7 Hz, CH_2), 8.07 (1H, dd *J* 8 Hz, and *J* 8 Hz, *CH*CHN), and 8.30 (2H, d, *J* 8 Hz, *CH*N); δ_C 14.1 (q), 62.2 (t), 127.7 (d, *C*HC), 138.1 (d, *CH*CHC), 148.4 (s, *C*N) and 164.4 (s, *C*=O); *m/z* 223 (M^+, 0.3%), 178 (9%) and 150 (19%) (Found: C, 59.33; H, 6.02; N, 6.21. $C_{11}H_{13}NO_4$ requires C, 59.19; H, 5.83; N, 6.28%).

Diethyl *N*-Methyl *cis*-2,6-piperidinedicarboxylate (79)

Diethyl 2,6-pyridinedicarboxylate (2.0 g, 8.9 mmol) was suspended in glacial acetic acid (50 ml) and hydrogenated at atmospheric pressure and room temperature for 30 h using PtO_2 (0.20 g) as catalyst. The catalyst was removed by filtration through Celite and the filtrate was returned to the hydrogenator with 36% formaldehyde solution and 10% palladium on charcoal catalyst (0.20 g). Absorption of H_2 proceeded in 18 h at atmospheric pressure and room temperature. The catalyst was removed by filtration through Celite and the clear filtrate was covered with ether and cooled to 5 °C. The solution was made basic slowly with 35% NaOH solution. The ether layer containing the liberated ester was separated and, after the aqueous layer was completely extracted with the same solvent, the combined extracts were dried over magnesium sulfate, filtered and concentrated to leave diethyl *N*-methyl *cis*-2,6-piperidinedicarboxylate as white crystals (1.09 g, 50%), m.p. 85-86 °C, R_F 0.55 (EtOAc: pet ether, 1:1), ν_{max} (2940, 2861, 1730 and 1376 cm^{-1}; δ_H 1.21 (6H, t, *J* 7 Hz, CH_2CH_3), 1.52-1.68 (6H, m, ring CH_2), 2.13 (3H, s, NCH_3), 2.65-2.72 (2H, dd, *J* 2 Hz and *J* 11 Hz, N*CH*) and 4.13 (4H, q, *J* 7 Hz, CH_2CH_3); δ_C 13.9 (q, CH_2CH_3), 22.7 (t, CH_2CH_2CH), 29.1 (t, CH_2CH), 41.8 (q, NCH_3), 60.4 (t, CH_2O), 67.9 (d, *C*HN) and 172.5 (s, *C*=O); *m/z* 243 (M^+ 0.6%), 170 (100%), 156, 142 and 96 (Found: M^+, 243.1470. $C_{12}H_{21}NO_4$ requires 243.1458).

N-Methyl-*cis*-2,6-bis(hydroxymethyl)piperidine (74)

Diethyl *N*-methyl-*cis*-2,6-piperidinedicarboxylate (2.0 g, 8.2 mmol) in dry THF (20 ml) was added to $LiAlH_4$ (16 ml, 16 mmol) under nitrogen and heated at reflux for 3 h. Water was added at 0 °C to decompose the $LiAlH_4$ and the resulting slurry was concentrated to leave a white residue. This was washed with pyridine and filtered. The filtrate was concentrated to leave *N*-methyl *cis*-2,6-bis(hydroxymethyl)piperidine as a clear oil (1.45 g, 97%), R_F 0.35 (EtOAc), ν_{max} 3373, 2934, 2860, 2793, 1235, 1064 and 1025 cm^{-1}; δ_H 1.37-1.77 (6H, m, ring CH_2), 2.21 (3H, s, NCH_3), 2.26 (2H, m, NC*H*), 3.39 (2H, dd, *J* 4 Hz and *J* 11 Hz, CH_2O), 3.70 (2H, dd, *J* 4 Hz, *J* 11 Hz, CH_2O) and 3.98 (2H, bs, O*H*); δ_C 23.7 (t, CH_2CH_2CHN), 26.7 (t, CH_2CHN), 34.9 (q, NCH_3), 63.5 (t, CH_2O) and 64.1 (d, *C*HN); *m/z* 159 (M^+ 0.1%), 142, 125, 128 (100%), 110, 96 and 82 (Found: M^+ 159.1241. $C_8H_{17}NO_2$ requires M^+ 159.1259).

N-Methyl-*cis*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine (80)

N-Methyl-*cis*-2,6-bis(hydroxymethyl)piperidine (0.46 g, 2.9 mmol) and isopropyl isocyanate (0.74 g, 8.7 mmol) were reacted according to procedure A for 24 h. The product was obtained as white crystals after recrystallisation from EtOAc/hexane (0.82 g, 86%), m.p. 95-97 °C, R_F 0.46 (EtOAc: hexane, 1:1), ν_{max} 3400, 2990, 2800, 1690 and 1370 cm^{-1}; δ_H 1.14 (12H, d, *J* 7 Hz, CH_3CH), 1.35-1.81 (6H, m, ring CH_2), 2.28 (2H, m, $CHNCH_3$), 2.33 (3H, s, NCH_3), 3.81 (2H, m, $CHCH_3$), 4.10 (4H, m, CH_2O) and 4.75 (2H, bs, N*H*); δ_C 23.0 and 23.5 (q, CH_3CH), 23.7 (t, CH_2CH_2CH), 28.66 (t, CH_2CH), 37.7 (q, NCH_3), 43.1 (d, *C*HNH), 63.3 (d, $CHNCH_3$), 66.9 (t, CH_2O) and 155.7 (s, *C*=O); *m/z* 314 , 227, 213 (100%), 128, 110 and 96 (Found: C, 58.17; H, 9.77; N; 12.59. $C_{16}H_{31}N_3O_4$ requires C, 58.36; H, 9.42; N, 12.77%).

N-Methyl-*cis*-2,6-bis(ethylaminocarbonyloxymethyl)piperidine (81)

N-Methyl-*cis*-2,6-bis(hydroxymethyl)piperidine (0.46 g, 2.9 mmol) and ethyl isocyanate (0.62 g, 8.7 mmol) were reacted according to procedure A for 24 h. The product was obtained as a clear oil after flash chromatography on alumina with ethyl acetate as eluant. The oil solidified after a week in the freezer but could not be crystallised (0.83 g, 95%), m.p. 58-60 °C, R_F 0.33 (EtOAc:hexane, 1:1), ν_{max} 3400, 2800 and 1690 cm^{-1}; δ_H 1.14 (6H, t, *J* 7 Hz, CH_3CH_2), 1.36-1.81 (6H, m, ring CH_2), 2.28 (2H, m, $CHNCH_3$), 2.34 (3H, s, NCH_3), 3.20 (4H, q, *J* 7 Hz, CH_2CH_3), 4.13 (4H, m, CH_2O) and 5.24 (2H, bs, NH); δ_C 15.3 (q, CH_3CH_2), 23.6 (t, CH_2CH_2CH), 28.3 (t, CH_2CHN), 35.2 (t, CH_2CH_3), 37.4 (q, NCH_3), 63.1 (d, CHN), 66.7 (t, CH_2O) and 156.4 (s, C=O); *m/z* 302 (M^+, 0.1%), 287, 213, 199 (100%), 128, 110 and 96 (Found: M^+, 302.2076. $C_{14}H_{28}N_3O_4$ requires 302.2080).

N-Methyl-*cis*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine (82)

N-Methyl-*cis*-2,6-bis(hydroxymethyl)piperidine (0.46 g, 2.9 mmol) and phenyl isocyanate (1.03 g, 8.7 mmol) were reacted according to procedure A for 24 h. The product was obtained as a yellow oil and was purified by flash chromatography on alumina with ethyl acetate. After concentration the residue was recrystallised with EtOAc/hexane to leave white crystals (0.82 g, 71%), m.p. 145-147 °C, R_F 0.7 (EtOAc:hexane, 1:1), ν_{max} 3380, 2800, 1700, 1600, 1525, 750 and 690 cm^{-1}; δ_H 1.27-1.91 (6H, m, ring CH_2), 2.43 (3H, s, NCH_3), 2.54 (2H, m, $CHNCH_3$), 4.26 (4H, m, CH_2O), 7.07 (2H, m, *p*-CH), 7.27-7.37 (8H, m, *m*- and *o*-CH) and 9.35 (2H, s, NH); δ_C 23.4 (t, CH_2CH_2CHN), 27.5 (t, CH_2CHN), 36.4 (q, NCH_3), 63.2 (d, $CHNCH_3$), 66.2 (t, CH_2O), 118.6 (d, *o*-CH), 123.3 (d, *p*-CH), 128.9 (d, *m*-CH), 137.8 (s, CNH) and 153.3 (s, C=O); *m/z* 247, 213, 128, 119 (100%) and 110.

_N_-Methyl-_cis_-2,6-bis(_p_-nitrophenylaminocarbonyloxymethyl)piperidine (83)

_N_-Methyl-_cis_-2,6-bis(hydroxymethyl)piperidine (0.46 g, 2.9 mmol) and _p_-nitrophenyl isocyanate (1.43 g, 8.7 mmol) were reacted according to procedure A for 24 h. After removal of the solvent, a yellow powder was left which was purified using a gravity alumina column, eluting with toluene:ether (2:1) to produce yellow crystals (0.82 g, 58%), m.p. 78-80 °C, R_F 0.40, (EtOAc:hexane, 1:4), ν_{max} 3420, 2920, 2760, 1725, 1600, 1545, 1505, 1330 and 850 cm^{-1}; δ_H (d_6-acetone) 1.29-1.78 (6H, m, ring CH_2), 2.35 (3H, s, NCH_3), 2.40 (2H, m, $CHNCH_3$), 4.21 (4H, m, CH_2O), 7.75 and 8.17 (8H, d, _J_ ca. 9 Hz, AA'BB' system) and 9.40 (2H, s, NH); δ_C 24.1 (t, CH_2CH_2CHN), 28.9 (t, CH_2CHN), 38.0 (q, NCH_3), 63.42 (d, $CHNCH_3$), 68.3 (t, CH_2O), 118.6 (d, CHCNH), 125.7 (d, $CHCNO_2$), 143.3 (s, CNH), 146.4 (s, CNO_2) and 154.0 (s, C=O); _m/z_ 334, 292 (19%), 260 and 110 (24%) (Found: C, 54.06; H, 5.17; N, 14.40. $C_{22}H_{25}N_5O_8$ requires C, 54.21; H, 5.13; N, 14.37%).

_N_-Methyl _cis_-2,6-bis(chloromethyl)piperidine[92] (66)

_N_-Methyl-_cis_-2,6-bis(hydroxymethyl)piperidine (0.46 g, 3.2 mmol) was dissolved in dichloromethane and added slowly to thionyl chloride at 0 °C. The mixture was heated at reflux for 3 h, then water was added carefully and the solution was saturated with sodium bicarbonate. Separation of the two phases was followed by washing of the organic phase with $NaHCO_3$ solution (2 x 20 ml), drying, filtration and evaporation to yield _N_-methyl-_cis_-2,6-bis(chloromethyl)piperidine as a brown oil. Flash chromatography on alumina using ethyl acetate as eluant gave the product as a clear oil (0.46 g, 80%), R_F 0.66 (EtOAc:hexane, 1:8), ν_{max} 2939, 2860, 2737 and 674 cm^{-1}; δ_H 1.38-1.83 (6H, m, ring CH_2), 2.32 (3H, s, NCH_3), 2.42 (2H, m, NCH), 3.53 (2H, dd, _J_ 6 Hz and _J_ 11 Hz, CH_2Cl) and 3.63 (2H, dd, _J_ 6 Hz and _J_ 11 Hz, CH_2Cl); δ_C 22.9 (t, CH_2CH_2CHN),

28.6 (t, CH_2CHN), 37.0 (q, NCH_3), 47.3 (t, CH_2Cl) and 64.2 (d, *C*HN); *m/z* 160, 110 and 96 (Found: M^+ (-Cl) 160.0906. $C_8H_{15}NCl$ requires 160.0893).

N-Methyl-*cis*-2,6-bis(bromomethyl)piperidine (84)

N-Methyl *cis*-2,6-bis(hydroxymethyl)piperidine (0.46 g, 3.2 mmol) was dissolved in dichloromethane and added slowly to phosphorus tribromide (1.92 g, 7.1 mmol) at 0 °C. The mixture was heated at reflux for 3 h, then water was added carefully and the solution was saturated with sodium bicarbonate. Separation of the two phases was followed by washing of the organic phase with $NaHCO_3$ solution (2 x 20ml), drying, filtration and evaporation to yield *N*-methyl-*cis*-2,6-bis(bromomethyl)piperidine as a clear oil (0.2 g, 23%) R_F 0.66 (EtOAc:hexane, 1:8), ν_{max} 2937, 2860, 2781 1301and 570 cm^{-1}; δ_H 1.42-1.82 (6H, m, ring CH_2), 2.31 (3H, s, NCH_3), 2.42 (2H, m, NC*H*), 3.41 (2H, dd, *J* 6 Hz and *J* 11 Hz, CH_2Br) and 3.50 (2H, dd, *J* 6 Hz and *J* 11 Hz, CH_2Br); δ_C 22.6 (t, CH_2CH_2CHN), 29.1 (t, CH_2CHN), 36.5 (t, CH_2Br), 36.7 (q, NCH_3) and 63.5 (d, *C*HN); *m/z* 285 (M^+ 1.2%), 204, 190 (100%), 110 and 96 (Found M^+ 284.9552. $C_8H_{15}NBr_2$ requires M^+ 284.9552).

N-Methyl-*cis*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine *N*-oxide (86)

N-Methyl-*cis*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine (0.2 g, 0.6 mmol) and mCPBA (0.14 g, 0.64 mmol) were reacted according to procedure B for 24 h. The remaining white powder was recrystallised from ethyl acetate to leave the product, (0.18 g, 87%), m.p. 180 °C, R_F 0.68 (EtOAc), ν_{max} 3420, 2990, 1680 and 920 cm^{-1}; δ_H (CD_3OD) 1.12 (12H, d, *J* 7 Hz, CH_3CH), 1.45-1.93 (6H, m, ring CH_2), 3.18 (3H, s, N^+CH_3), 3.52 (2H, m, C*H*N$^+$), 3.70 (2H, m, $CHCH_3$), 4.05 (2H, d, *J* 13 Hz, CH_2O) and 4.61 (2H, dd, *J* 6 Hz and *J* 13 Hz, CH_2O); δ_C 22.9 (q, CH_3CH), 25.4 (t, ring CH_2), 44.1 (d, $CHCH_3$), 55.8 (q, N^+CH_3), 64.3 (t, CH_2O), 76.2 (d, CHN^+) and

157.3 (s, *C*=O); *m/z* , 314, 213, 126, 110 (65%) and 96 (Found: C, 55.95; H, 9.01; N, 11.91. $C_{16}H_{31}N_3O_5$ requires C, 55.65; H, 8.99; N, 12.17%).

_N_-Methyl-*cis*-2,6-bis(ethylaminocarbonyloxymethyl)piperidine _N_-oxide (87)

_N_-Methyl-*cis*-2,6-bis(ethylaminocarbonyloxymethyl)piperidine (0.20 g, 0.6 mmol) and mCPBA (0.14 g, 0.64 mmol) were reacted according to procedure B for 24 h. The remaining white powder was recrystallised from ethyl acetate to give the product (0.17 g, 81%), m.p. 158-159 °C, R_F 0.60 (MeOH), ν_{max} 3420, 2990, 1730 and 920 cm^{-1}; δ_H (D_2O) 0.99 (6H, t, *J* 7 Hz, CH_3CH_2), 1.51-1.83 (6H, m, ring CH_2), 2.94-3.09 (7H, m, CH_2CH_3 and N^+CH_3), 3.75 (2H, d, *J* 13 Hz, CHN^+) and 4.45 (4H, m, CH_2O); δ_C 14.9 (q, CH_3CH_2), 22.1 (t, $CH_2CH_2CHN^+$), 24.8 (t, CH_2CHN^+), 36.3 (t, CH_2CH_3), 55.4 (q, N^+CH_3), 64.4 (t, CH_2O), 75.6 (d, CHN^+) and 158.5 (s, C=O); *m/z* 317 (0.2%), 199, 144, 128 (100%), 126, 110 (100%) and 96 (Found: M^+ 317.2005. $C_{14}H_{27}N_3O_5$ requires 317.1950).

_N_-Methyl-*cis*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine _N_-oxide (88)

_N_-Methyl-*cis*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine (0.15 g, 0.4 mmol) and mCPBA (0.10 g, 0.45 mmol) were reacted according to procedure B for 18 h. The remaining pale yellow powder was recrystallised from methanol to give the product as a white solid (0.10 g, 67%), m.p. 170-172 °C, R_F 0.50 (EtOAc), ν_{max} 3400, 2950, 1700, 1600, 1530, 920, 760 and 690 cm^{-1}; δ_H (CD_3OD) 1.50-1.94 (6H, m, ring CH_2), 2.87 (3H, s, N^+CH_3), 3.43 (2H, m, CHN^+), 4.00 and 4.52 (4H, m, CH_2O), 6.82 (2H, m, *p*-CH), 7.02 (4H, m, *m*-CH) and 7.29 (4H, m, *o*-CH); δ_C 25.5 (t, $CH_2CH_2CHN^+$), 26.4 (t, CH_2CHN^+), 55.9 (q, CH_3N^+), 62.0 and 64.5 (t, CH_2O), 76.2 and 79.7 (d, CHN^+), 119.8 (d, *o*-CH), 124.0 (d, *p*-CH), 129.8 (d, *m*-CH), 140.0 (s, CNH) and

155.1 (s, *C*=O); *m/z* 247, 213, 144, 126, 110 and 96 (Found: C, 64.27; H, 6.55; N, 10.17. $C_{22}H_{27}N_3O_5$ requires C, 63.92; H, 6.53; N, 10.17%).

_N_-Methyl-*cis*-2,6-bis(chloromethyl)piperidine _N_-oxide (89).

N-Methyl-*cis*-2,6-bis(chloromethyl)piperidine (0.10 g, 0.5 mmol) and mCPBA (0.13 g, 60 mmol) were reacted according to procedure B for 24 h. This left *N*-methyl *cis*-2,6-bis(chloromethyl)piperidine *N*-oxide as a yellow oil (0.04 g, 44%), R_F 0.47 (MeOH), δ_H (CD_3OD) 1.53-1.88 (4H, m, C*H*$_2$CHN$^+$), 1.98-2.16 (2H, m, C*H*$_2$R), 2.80 (3H, s, C*H*$_3$N$^+$), 3.21 (2H, m, C*H*N$^+$), 3.88 (2H, dd, *J* 4 Hz and 11 Hz, C*H*$_2$Cl) and 4.17 (2H, dd, *J* 4 Hz and 11 Hz, C*H*$_2$Cl); δ_C 23.1 (t, *C*H$_2$R), 27.2 (t, *C*H$_2$CHN$^+$), 42.0 (t, *C*H$_2$Cl), 44.6 (q, *C*H$_3$N$^+$) and 80.9 (d, *C*HN$^+$); *m/z* 211 (*M*$^+$ 9.4%), 196, 176, 162, 146 (100%), 110 and 94 (Found: *M*$^+$ 211.0525. $C_8H_{15}NOCl_2$ requires *M*$^+$ 211.1530).

_N_-Methyl-*cis*-2,6-bis(bromomethyl)piperidine _N_-oxide (90).

N-Methyl-*cis*-2,6-bis(bromomethyl)piperidine (0.1 g, 0.37 mmol) and mCPBA (0.08 g, 45 mmol) were reacted according to procedure B for 24 h. This left *N*-methyl-*cis*-2,6-bis(bromomethyl)piperidine *N*-oxide as a yellow oil (0.04 g, 41%), R_F 0.55 (MeOH), ν_{max} 2943, 2829, 916 and 541 cm^{-1}; δ_H (CD_3OD) 1.33-1.69 (4H, m, C*H*$_2$CHN$^+$), 2.04 (2H, m, C*H*$_2$R), 2.87 (3H, s, C*H*$_3$N$^+$), 3.18 (2H, m, C*H*N$^+$), 3.66 (2H, m, C*H*$_2$Br) and 4.02 (2H, m, C*H*$_2$Br); δ_C 22.1 (t, *C*H$_2$R), 28.6 (t, *C*H$_2$CHN$^+$), 36.0 (t, *C*H$_2$Br), 45.8 (q, *C*H$_3$N$^+$) and 75.2 (d, *C*HN$^+$); *m/z* 285, 204, 190 (100%), and 96.

Experimental to Chapter 4

Diethyl α,α'-Dibromopimelate[116] (99)

Pimelic acid (10.80 g, 68 mmol) in thionyl chloride (12.2 ml) was heated at 40 °C for 18 h. Iodine (0.2 g) and bromine (8.04 ml, 150 mmol) were added and the mixture was heated at 80 °C for 6 h. After cooling, the solution was added to ethanol (60 ml). Water (150 ml) was added and the aqueous solution was extracted with diethyl ether (3 x 25 ml). The combined ether extracts were washed with 10% sodium thiosulfate solution (5 x 20 ml). The ether layer was dried, filtered and concentrated to leave a pale yellow oil. Vacuum distillation gave the product as a clear oil (19.50 g, 77%), R_F 0.68 (hexane), b.p. 165-170 (4 mmHg), ν_{max} 2980, 1740, 1425, 1230 and 1100 cm^{-1}; δ_H 1.30 (6H, t, *J* 7 Hz, CH_3CH_2), 1.62 (2H, m, CH_2CH_2CHBr), 2.10 (4H, m, CH_2CHBr), 4.00 (2H, m, CHBr) and 4.20 (4H, q, *J* 7 Hz, CH_2CH_3); δ_C 13.9 (q, CH_3CH_2), 25.0 (t, CH_2CH_2CHBr), 33.9 (t, CH_2CHBr), 45.3 (d, CHBr), 62.1 (t, CH_3CH_2O) and 169.6 (s, C=O); *m/z* 249, 222, 166 and 73 (100%).

cis- and *trans*-2,6-Piperidinedicarboxamide[117] (100) and (101)

Diethyl αα'-dibromopimelate (13.1 g, 36 mmol) in liquid ammonia in a sealed tube was left standing at room temperature for 3 d. The tube was opened at -78 °C and allowed to warm to room temperature. Ice water (10 ml) was added to the solid residue. The remaining solid residue was filtered off and dried to give *cis*-piperidinedicarboxamide (2.50 g, 41 %), m.p. 226-228 °C, ν_{max} 3300, 2980, 1680, 1630 and 1400 cm^{-1}; δ_H (90MHz) (D_2O) 1.3-2.15 (6H, m, ring CH_2) and 3.45 (2H, m, CHN); *m/z* 172 (M^+ 0.3 %), 127 and 82 (100%). Concentration of the filtrate gave crystals of *trans*-piperidinedicarboxamide (2.10 g, 34%), m.p. 264-268 °C, ν_{max} 3300, 2980, 1680, 1630 and 1400 cm^{-1}; δ_H (90 MHz) (D_2O) 1.5-2.35 (6H, m, ring CH_2) and 4.4 (2H, m, CHN); *m/z* 172 (M^+ 0.3%), 127 and 82 (100%).

cis-2,6-Piperidinedicarboxylic Acid[96] (76)

cis-2,6-Piperidinedicarboxamide (1.58 g, 9.1 mmol) in 10% barium hydroxide solution was heated at reflux for 2 h. Carbon dioxide was added to the solution and the solid barium carbonate was filtered off. The filtrate was acidified with conc. HCl and concentrated to leave *cis*-piperidinedicarboxylic acid hydrochloride as a white solid (0.79 g, 50%), m.p. 286-288 °C, ν_{max} 3400, 3000 and 1750; δ_H (90 MHz) (D_2O) 1.5-2.25 (6H, m, ring C*H*$_2$) and 3.85 (2H, m, C*H*N); *m/z* 173 (M^+ 0.1 %), 128 and 82 (100%).

trans-2,6-Piperidinedicarboxylic Acid[117] (102)

trans-2,6-Piperidinedicarboxamide (1.58 g, 9.1 mmol) in 10% barium hydroxide solution was heated at reflux for 2 h. Carbon dioxide was added to the solution and the solid barium carbonate was filtered off. The filtrate was acidified with conc. HCl and concentrated to leave *cis*-piperidinedicarboxylic acid hydrochloride as white crystals (0.65 g, 41%), m.p. 264-266 °C, δ_H (90 MHz) (D_2O) 1.5-2.15 (6H, m, ring C*H*$_2$) and 4.25 (2H, m, C*H*N); *m/z* 173 (M^+ 1%), 139, 111, 95 and 82.

Diethyl *N*-methyl 2,6-piperidinedicarboxylate[119] (79) and (103)

Methylamine (33% in methylated spirits, 10.23 g, 111 mmol) was added carefully to diethyl α,α'-dibromopimelate (13.30 g, 36 mmol) at 0 °C and was left standing for 30 min. Potassium iodide (0.20 g) was added and the solution was heated at 75 °C for 24 h. After cooling, the solution was added to water (30 ml) and extracted with diethyl ether (3 x 30 ml). The ether extracts were then shaken with 3M H_2SO_4 (5 x 10 ml) and the combined aqueous extracts were made slightly alkaline with 5M NaOH at 0 °C. The resulting free base was extracted from the aqueous layer with diethyl ether (4 x 25 ml). The combined ether extracts were then dried, filtered and concentrated to leave a pale yellow oil. This was purified using flash chromatography, eluting with ethyl acetate to

give a 1:1 mixture of the *cis*- and *trans*-isomers (6.58 g, 76%), R_F (0.60) (EtOAc:hexane, 1:3), ν_{max} 2942, 2864 1731cm^{-1}; δ_H 1.29 (6H, t, *J* 7 Hz, CH_3CH_2), 1.48-1.95 (6H, m, ring CH_2), 2.21 and 2.40 (3H, s, NCH_3), 2.80 (1H, m, *CH*N), 3.78 (1H, m, *CH*N) and 4.19 (4H, q, *J* 7 Hz, CH_2CH_3); δ_C 14.2 and 14.3 (q, CH_3CH_2), 18.9 and 23.0 (t, CH_2CH_2CHN), 28.8 and 29.7 (t, CH_2CHN), 40.6 and 42.1 (q, CH_3N), 60.3 and 60.7 (t, CH_2O), 61.6 and 68.2 (d, *C*HN) and 172.8 and 173.4 (s, *C*=O); *m/z* 243 (M^+ 0.5%), 170, 142 (100%) and 96 (Found: M^+ 243.1470. $C_{12}H_{21}NO_4$ requires M^+ 243.1474).

N-Methyl-*trans*-2,6-piperidinedicarboxylic Acid Hydrochloride (106)

Diethyl *N*-methyl-2,6-piperidinedicarboxylate (4.0 g, 16 mmol) was dissolved in 10 M HCl (20 ml) and left for 2 h at room temperature. The solution was then heated at 100 °C for 24 h in a sealed tube, then cooled and evaporated to dryness. The residue was washed with acetone and filtered to leave the hydrochloride salt as white crystals (3.4 g, 94 %), m.p. 228-230 °C, ν_{max} 3440, 2945, 1745, 1459, 1369 and 718 cm^{-1}; δ_H (D_2O) 1.41-1.77 and 2.03 (6H, m, ring CH_2), 2.75 (3H, s, NCH_3) and 3.77 (2H, d, *J* 9 Hz, *CH*N); δ_C (D_2O) 21.8 (t, CH_2CH_2CH), 28.0 (t, CH_2CHN), 42.7 (q, NCH_3), 67.9 (d, *C*HN) and 171.1 (s, *C*=O); *m/z* 187 (M^+ 0.1%), 142 (100%), 96 and 70 (Found: M^+, 187.0845; C, 42.75; H, 6.05; N, 6.10. $C_8H_{13}NO_4$ requires M^+187.0804; C, 42.95; H, 5.82; N, 6.26%).

N-Methyl-*trans*-2,6-bis(hydroxymethyl)piperidine (97)

N-Methyl-*trans*-2,6-piperidinedicarboxylic acid hydrochloride (2.0 g, 9 mmol) was placed in a dry flask under nitrogen. Dry THF (10 ml) was added to form a slurry and the mixture was cooled to 0 °C. 1 M Borane-THF solution (60 ml, 60 mmol) was added over a period of 10 min after which the solution was heated at reflux for 48 h. The solution was cooled to 0 °C and the reaction was quenched with THF:water (1:1). The THF was

then removed *in vacuo* to leave the aqueous solution which was basified with 5M NaOH. This was then extracted with dichloromethane (3 x 30 ml) and the organic extracts were dried, filtered and concentrated to give the product as a clear oil (1.2 g, 84%), R_F 0.69 (EtOAc), ν_{max} 3741, 2874 and 1308 cm^{-1}; δ_H 1.15-1.85 (6H, m, ring CH_2), 2.32 (3H, s, NCH_3), 2.43 (2H, m, CHN), 3.45 and 3.91 (4H, dd, *J* 4 Hz and *J* 11 Hz, CH_2O) and 3.56 (2H, bs, OH); δ_C 23.5 (t, CH_2CH_2CHN), 26.5 (t, CH_2CHN), 34.5 (q, CH_3N), 63.3 (t, CH_2O) and 64.0 (d, CHN); *m/z* 158 (M^+ (-1H) 0.5%), 142, 128 (100%), 126, 110, 96 and 82 (Found: M^+ -H 158.1181. $C_8H_{16}NO_2$ requires M^+-H 158.1181).

N-Methyl-*trans*2,6-bis(isopropylaminocarbonyloxymethyl)piperidine (107)

N-Methyl-*trans*-2,6-bis(hydroxymethyl)piperidine (0.46 g, 2.9 mmol) and isopropyl isocyanate (0.74 g, 8.7 mmol) were reacted according to procedure A for 24 h. The product was obtained as an oil which was subjected to flash chromatography on alumina with ethyl acetate. After evaporation, the remaining white powder was crystallised from dichloromethane/hexane to leave the product as flakey white crystals (0.82 g, 86%), R_F 0.67 (EtOAc), m.p. 138-140 °C, ν_{max} 3400, 2980, 2800, 1690 and 1370 cm^{-1}; δ_H 1.15 (12H, d, *J* 7 Hz, CH_3CH), 1.40 (2H, m, CH_2CH_2CHN), 1.68 (4H, m, CH_2CHN), 2.30 (2H, m, $CHNCH_3$), 2.33 (3H, s, NCH_3), 3.81 (2H, m, $CHCH_3$), 4.10 (4H, m, CH_2O) and 4.71 (2H, bs, NH); δ_C 23.0 (q, CH_3CH), 25.6 (t, CH_2CH_2CH), 28.5 (t, CH_2CH), 37.5 (q, NCH_3), 42.9 (d, CHNH), 63.2 (d, $CHNCH_3$), 66.7 (t, CH_2O) and 155.8 (s, C=O); *m/z* 314 , 227, 213, 227,128, 110 (100%) and 96 (Found: C, 58.11; H, 9.49; N; 12.98. $C_{16}H_{31}N_3O_4$ requires C, 58.36; H, 9.42; N, 12.77%).

_N_-Methyl-*trans*-2,6-bis(ethylaminocarbonyloxymethyl)piperidine (108)

_N_-Methyl-*trans*-2,6-bis(hydroxymethyl)piperidine (0.46 g, 2.9 mmol) and ethyl isocyanate (0.62 g, 8.7 mmol) were reacted according to procedure A for 24 h. The product was obtained as a thick clear oil after flash chromatography on alumina using ethyl acetate as eluant (0.83 g, 95%), R_F 0.43 (EtOAc:hexane, 1:1), ν_{max} 3460, 2980, 2800 and 1700 cm^{-1}; δ_H 1.13 (6H, t, _J_ 7 Hz, CH_3CH_2), 1.40 (2H, m, CH_2CH_2CHN), 1.81 (4H, m, CH_2CHN), 2.31 (3H, s, NCH_3), 3.09-3.27 (6H, m, CH_2CH_3 & CHN), 4.10 (4H, m, CH_2O), 5.30 (2H, bs, NH); δ_C 14.9 (q, CH_3CH_2), 23.5 (t, CH_2CH_2CH), 28.2 (t, CH_2CHN), 35.7 (t, CH_2CH_3), 37.1 (q, NCH_3), 63.1 (d, CHN), 66.5 (t, CH_2O), 156.6 (s, C=O); _m/z_ 302 (M^+, 0.2%), 286, 257, 213, 199 (100%), 128, 110 (100%), 96 and 82 (Found: M^+, 302.2072. $C_{14}H_{28}N_3O_4$ requires M^+302.2079).

_N_-Methyl-*trans*-2,6-bis(phenylaminocarbonyloxymethyl)piperidine (109)

_N_-Methyl-*trans*-2,6-bis(hydroxymethyl)piperidine (0.50 g, 3.1 mmol) and phenyl isocyanate (1.12 g, 9.3 mmol) were reacted according to procedure A for 24 h. The product was obtained as an oil which was purified using a flash chromatography column on alumina with ethyl acetate as eluant. The resulting white powder after evaporation was crystallised from dichloromethane/hexane (1.10 g, 88%), m.p. 130-132 °C, R_F 0.58 (EtOAc:hexane, 1:1), ν_{max} 3390, 2800, 1700, 1590, 1525, 750 and 690 cm^{-1}; δ_H (d_6-DMSO) 1.20-1.93 (6H, m, ring CH_2), 2.32 (3H, s, NCH_3), 2.35 (2H, m, $CHNCH_3$), 4.11 (4H, m, CH_2O), 6.99 (2H,m, _p_-CH), 7.28 (4H, m, _m_-CH) 7.53 (4H, m, _o_-CH) and 9.55 (2H, s, NH); δ_C 23.3 (t, CH_2CH_2CHN), 27.5 (t, CH_2CHN), 36.8 (q, NCH_3), 63.4 (d, $CHNCH_3$), 66.4 (t, CH_2O), 118.3 (d, _o_-CH), 122.4 (d, _p_-CH), 128.8 (d, _m_-CH), 139.3 (s, CNH), 153.6 (s, C=O); _m/z_ 247, 213, 128, 119, 110, and 93 (100%) (Found: C, 66.32; H, 6.99; N, 10.29. $C_{22}H_{27}N_3O_4$ requires C, 66.50; H, 6.80; N, 10.57%).

_N_-Methyl-_trans_-2,6-bis(_p_-nitrophenylaminocarbonyloxymethyl)piperidine (110)

_N_-Methyl-_trans_-2,6-bis(hydroxymethyl)piperidine (0.50 g, 3.1 mmol) and _p_-nitrophenyl isocyanate (1.55 g, 9.4 mmol) were reacted according to procedure A for 24 h. After removal of the solvent, a yellow powder was left which was purified using a gravity alumina column, eluting with toluene:ether (2:1) to produce yellow crystals (0.65 g, 42%), R_F 0.40 (toluene:ether, 2:1) m.p. 49-50 °C, ν_{max} 3360, 2920, 2800, 1720, 1600, 1550, 1500, 1330 and 850 cm^{-1}; δ_H (CD_3OD) 1.34-1.90 (6H, m, ring CH_2), 2.40 (3H, s, NCH_3), 2.42 (2H, m, $CHNCH_3$), 4.17 (4H, m, CH_2O), 7.52 and 8.04 (8H, d, _J_ 9 Hz, AA'BB' aromatic system); δ_C 24.4 (t, CH_2CH_2CHN), 29.0 (t, CH_2CHN), 38.1 (q, NCH_3), 64.6 (d, $CHNCH_3$), 67.9 (t, CH_2O), 118.8 (d, CHCNH), 125.8 (d, $CHCNO_2$), 143.8 (s, CNH), 146.6 (s, CNO_2) and 154.9 (s, C=O); _m/z_ 350, 322, 242, 110 and 96 (Found: C, 54.58; H, 5.53; N, 14.42. $C_{22}H_{25}N_5O_8$ requires C, 54.21; H, 5.13; N, 14.37%).

_N_-Methyl-_trans_-2,6-bis(chloromethyl)piperidine (111)

_N_-Methyl-_trans_-2,6-bis(hydroxymethyl)piperidine (0.46 g, 3.2 mmol) was dissolved in dichloromethane and added slowly to thionyl chloride at 0 °C. The mixture was heated at reflux for 3 h, then water was added carefully and the solution was saturated with sodium bicarbonate. Separation of the two phases was followed by washing of the organic phase with $NaHCO_3$ solution (2 x 20 ml), drying, filtration and evaporation to yield _N_-methyl-_trans_-2,6-bis(chloromethyl)piperidine as a brown oil. Flash chromatography using ethyl acetate as eluant gave the product as a clear oil (0.45 g, 72%), R_F 0.70 (EtOAc:hexane, 1:8), ν_{max} 2939, 2860, 2786 and 674 cm^{-1}; δ_H 1.61-1.91 (6H, m, ring CH_2), 2.32 (3H, s, NCH_3), 2.40 (2H, m, NCH) and 3.58 (4H, m, CH_2Cl); δ_C 23.0 (t, CH_2CH_2CHN), 27.3 (t, CH_2CHN), 36.0 (q, NCH_3), 47.5 (t, CH_2Cl) and 63.6 (d, CHN); _m/z_ 197 (M^+ 0.7%), 160, 110 and 96 (Found: M^+ 197.0561. $C_8H_{15}NCl_2$ requires M^+197.0552).

N-Methyl-*trans*-2,6-bis(bromomethyl)piperidine (112)

N-Methyl-*trans*-2,6-bis(hydroxymethyl)piperidine (0.46 g, 3.2 mmol) was dissolved in dichloromethane and added slowly to phosphorus tribromide (1.92 g, 7.1 mmol) at 0 °C. The mixture was heated at reflux for 3 h, then water was added carefully and the solution was saturated with sodium bicarbonate. Separation of the two phases was followed by washing of the organic phase with $NaHCO_3$ solution (2 x 20 ml), drying, filtration and evaporation to yield *N*-methyl-*trans*-2,6-bis(bromomethyl)piperidine as a clear oil (0.31 g, 34%), R_F 0.70 (EtOAc:hexane, 1:8), ν_{max} 2945, 1313 and 568 cm^{-1}; δ_H 1.41-1.85 (6H, m, ring CH_2), 2.32 (3H, s, NCH_3), 2.44 (2H, m, NC*H*), 3.42 (2H, dd, *J* 6 Hz and *J* 11 Hz, CH_2Br) and 3.56 (2H, dd, *J* 6 Hz and *J* 11 Hz, CH_2Br); δ_C 22.5 (t, CH_2CH_2CHN), 28.9 (t, CH_2CHN), 36.4 (t, CH_2Br), 36.6 (q, NCH_3) and 63.5 (d, *C*HN); *m/z* 285 (M^+ 1.2%), 204, 190 (100%), 110 and 96 (Found M^+ 284.9542. $C_8H_{15}NBr_2$ requires M^+ 284.9553).

N-Methyl-*trans*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine *N*-oxide. (113)

N-Methyl-*trans*-2,6-bis(isopropylaminocarbonyloxymethyl)piperidine (0.27 g, 0.8 mmol) and mCPBA (0.20 g, 0.9 mmol) were reacted according to procedure B for 18 h. This left a white powder which was recrystallised from ethyl acetate to give the product (0.14 g, 42%), m.p. 177-178 °C, R_F 0.70 (MeOH), ν_{max} 3330, 2970, 1690 and 920 cm^{-1}; δ_H 1.16 (12H, d, *J* 7 Hz, CH_3CH), 1.53-2.07 (6H, m, ring CH_2), 3.15 (3H, s, N^+CH_3), 3.30 (2H, m, CHN^+), 3.77 (2H, m, $CHCH_3$), 4.07 (2H, d, *J* 13 Hz, CH_2O), 4.77 (2H, dd, *J* 6 Hz and *J* 13 Hz, CH_2O) and 5.93 (2H, bd, *J* 7 Hz, N*H*); δ_C 22.1 (t, $CH_2CH_2CHN^+$), 22.8 (q, CH_3CH), 24.2 (t, CH_2CHN^+), 43.1 (d, *C*HNH), 55.6 (q, N^+CH_3), 63.6 (t, CH_2O), 75.2 (d, CHN^+) and 155.5 (s, *C*=O); *m/z* 327, 314, 213, 158, 126, 110 (100%) and 96 (Found: C, 55.41; H, 8.76; N, 11.81. $C_{16}H_{31}N_3O_5$ requires C, 55.65; H, 8.99; N, 12.17%).

_N_-Methyl-_trans_-2,6-bis(ethylaminocarbonyloxymethyl)piperidine _N_-oxide. (114)

_N_-Methyl-_trans_-2,6-bis(ethylaminocarbonyloxymethyl)piperidine (0.50 g, 1.6 mmol) and mCPBA (0.36 g, 1.68 mmol) were reacted according to procedure B for 4 h. This left a yellow oil which was crystallised from acetone to leave the product as a white solid (0.26 g, 52%), m.p. 178-179 °C, R_F 0.63 (MeOH), ν_{max} 3420, 2990, 1720 and 920 cm^{-1}; δ_H (D_2O) 0.98 (6H, t, _J_ 7 Hz, CH_3CH_2), 1.44-1.80 (6H, m, ring CH_2), 2.95-3.12 (7H, m, CH_3N^+ and CH_2CH_3), 3.38 (2H, m, CHN^+), 3.93 (2H, d, _J_ 13 Hz, CH_2O) and 4.44 (2H dd, _J_ 6 Hz and _J_ 13 Hz, CH_2O); δ_C 14.9 (q, CH_3CH), 22.1 (t, CH_2CH_2CHN), 24.8 (t, CH_2CHN), 36.3 (t, CH_2CH_3), 55.4 (q, CH_3N), 64.4 (t, CH_2O), 75.6 (d, CHNO) and 158.5 (s, C=O); _m/z_ 317 (M^+ 0.5%), 246, 215 199, 126, 110 and 96 (Found: M^+ 317.1973. $C_{14}H_{27}N_3O_5$ requires M^+317. 1951).

_N_-Methyl-_trans_-2,6-bis(phenylaminocarbonyloxymethyl)piperidine _N_-oxide. (115)

_N_-Methyl-_trans_-2,6-bis(phenylaminocarbonyloxymethyl)piperidine (0.30 g, 0.75 mmol) and mCPBA (0.20 g, 0.9 mmol) were reacted according to procedure B for 4 h. This left a white powder which was recrystallised with methanol and the mother liquors were crystallised from ethyl acetate (0.14 g, 45%), m.p. 178-180 °C, R_F 0.67 (EtOAc), ν_{max} 3400, 2990, 1700, 1600, 1525, 920, 760 and 690 cm^{-1}; δ_H (d_6-DMSO) 1.56-1.88 (6H, m, ring CH_2), 3.15 (3H, s, N^+CH_3), 3.59 (2H, m, CHN^+), 4.07 (2H, d, _J_ 11 Hz, CH_2O), 4.76 (4H, dd, _J_ 6 Hz and _J_ 13 Hz, CH_2O), 7.02 (2H, t, _J_ 8 Hz, _p_-CH), 7.31 (4H, t, _J_ 8 Hz, _m_-CH) and 7.53 (4H, d, _J_ 8 Hz, _o_-CH); δ_C 21.5 (t, $CH_2CH_2CHN^+$), 24.2 (t, CH_2CHN^+), 55.2 (q, CH_3N^+), 63.4 (t, CH_2O), 73.0 (d, CHN^+), 118.3 (d, _o_-CH), 122.5 (d, _p_-CH), 128.7 (d, _m_-CH), 139.1 (s, CNH) and 153.3 (s, C=O); _m/z_ 247, 213, 144, 126, 110 and 96 (Found: C, 60.42; H, 5.89; N, 9.10. $C_{22}H_{27}N_3O_5.1.5H_2O$ requires C, 60.00; H, 6.13; N, 9.54%).

_N_-Methyl-*trans*-2,6-bis(chloromethyl)piperidine _N_-oxide. (116)

_N_-Methyl-*trans*-2,6-bis(chloromethyl)piperidine (0.10 g, 0.5 mmol) and mCPBA (0.14 g, 64 mmol) were reacted according to procedure B for 18 h. This left _N_-methyl-*trans*-2,6-bis(chloromethyl)piperidine _N_-oxide as a yellow oil (0.06 g, 58%), R_F 0.40 (MeOH), ν_{max} 2947, 915 and 667 cm^{-1}; δ_H (CD_3OD) 1.60-1.91 (4H, m, CH_2CHN^+), 2.09-2.16 (2H, m, CH_2R), 2.81 (3H, s, CH_3N^+), 3.18 (2H, m, CHN^+), 3.87 (2H, dd, _J_ 4 Hz and 11 Hz, CH_2Cl) and 4.18 (2H, dd, _J_ 3 Hz and 11 Hz, CH_2Cl); δ_C 23.1 (t, CH_2R), 27.2 (t, CH_2CHN^+), 41.0 (t, CH_2Cl), 44.4 (q, CH_3N^+) and 80.7 (d, CHN^+); _m/z_ 211 (M^+ 4.7%), 196, 176, 146 (100%), 110 and 94 (Found: C, 45.10; H, 7.24; N, 6.56. $C_8H_{15}NOCl_2$ requires C, 45.28; H, 7.08; N, 6.60%).

_N_-Methyl-*trans*-2,6-bis(bromomethyl)piperidine _N_-oxide. (117)

_N_-Methyl-*trans*-2,6-bis(bromomethyl)piperidine (0.10 g, 0.4 mmol) and mCPBA (0.08 g, 45 mmol) were reacted according to procedure B for 18 h. This left _N_-methyl-*trans*-2,6-bis(bromomethyl)piperidine _N_-oxide as a yellow oil (0.06 g, 50%), ν_{max} 2948, 2870, 923 and 574 cm^{-1}; δ_H (CD_3OD) 1.37-1.76 (4H, m, CH_2CHN^+), 2.12 (2H, m, CH_2R), 2.94 (3H, s, CH_3N^+), 3.13 (2H, m, CHN^+), 3.64 (2H, m, CH_2Br) and 3.95 (2H, m, CH_2Br); δ_C 21.7 (t, CH_2R), 28.3 (t, CH_2CHN^+), 36.6 (t, CH_2Br), 46.4 (q, CH_3N^+) and 75.1 (d, CHN^+); _m/z_ 285, 204, 190 (100%), and 96.

Experimental to Chapter 5

1,4-Bis(2-isopropylaminocarbonyloxyethyl)piperazine (128)

1,4-Bis(2-hydroxyethyl)piperazine (1.0 g , 5.75 mmol) and isopropyl isocyanate (1.47 g, 17.2 mmol) were reacted according to procedure A for 3 h until a clear solution was obtained. The solvent was then removed *in vacuo* to leave a white powder. This was recrystallised from dichloromethane/hexane to produce a white solid (1.91 g, 100%), m.p. 127-128 °C, R_F 0.70 (EtOAc), ν_{max} 3330, 2780, 2810, 1690, 1365 cm^{-1}; δ_H 1.15 (12H, d, *J* 7 Hz, CH_3CH), 2.55-2.64 (12H, m, NCH_2), 3.78 (2H, m, $CHCH_3$), 4.16 (4H, t, *J* 6 Hz, CH_2O) and 4.83 (2H, bs,N*H*); δ_C 23.4 (q, CH_3CH), 43.0 (d, $CHCH_3$), 53.1 (t, ring CH_2), 57.1 (t, NCH_2), 61.5 (t, CH_2O), and 155 7 (s, C=O); *m/z* 334 (M^+, 0.1%), 242 (8.6%), 228 (35.2%), 156 (82.0%), 125 (100%) (Found: M^+, 344.2423, C, 55.87; H, 9.51; N, 16.47. $C_{16}H_{32}N_4O_4$ requires M^+ 344.2407, C, 55.80; H, 9.30; N, 16.27%).

1,4-Bis(2-ethylaminocarbonyloxyethyl)piperazine (127)

1,4-Bis(2-hydroxyethyl)piperazine (1.0 g, 5.75 mmol) and ethyl isocyanate (1.25 g, 17.2 mmol) were reacted according to procedure A for 3 h until a clear solution was obtained. The solvent was then removed *in vacuo* to leave an oil. This was then crystallised from dichloromethane/hexane to produce a white solid (1.73 g, 95%), m.p. 102 °C, R_F 0.70 (EtOAc); ν_{max} 3330, 2830, 1710 cm^{-1}; δ_H 1.13 (6H, t, *J* 7 Hz, CH_3CH_2), 2.54-2.64 (12H, m, NCH_2), 3.20 (4H, q, *J* 7 Hz, CH_2CH_3), 4.17 (4H, t, *J* 5.5 Hz, CH_2O) 5.20 (2H, bs, N*H*); δ_C 15.1 (q, CH_3CH), 35 6 (t, CH_2CH_3), 53.1 (t, ring CH_2), 57.0 (t, NCH_2), 61.6 (t, CH_2O), and 156.3 (s, C=O); *m/z* 316 (M^+, 0.1%) 227 (14.4%), 214 (36.3%), 156 (32.5%), and 125 (100%); (Found: M^+, 316.2110, C, 52.80; H, 8.64; N, 18.08. $C_{14}H_{28}N_4O_4$ requires M^+ 316.2108, C, 53.16; H, 8.86; N, 17.72%).

1,4-Bis(2-phenylaminocarbonyloxyethyl)piperazine (129)

1,4-Bis(2-hydroxyethyl)piperazine (1.0 g, 5.75 mmol) and phenyl isocyanate (2.05 g, 17.2 mmol) were reacted according to procedure A for 6 h and a white precipitate was obtained. The solvent was then removed *in vacuo* to leave a white powder. This was recrystallised from methanol to produce a white solid (1.91 g, 100%), m.p. 158-159 °C, R_F 0.75 (EtOAc); ν_{max} 3330, 2810, 2780, 1700, 1600, 1530, 750, 690 cm^{-1}; δ_H (d_6-DMSO) 2.52 (8H, s, ring CH_2), 2.63 (4H, t, *J* 6 Hz, NCH_2), 4.22 (4H, t, *J* 6 Hz, CH_2O), 6.96 (2H, t, *J* 7 Hz, *p*-C*H*), 7.25 (4H, t, *J* 7 Hz, *m*-C*H*), and 7.46 (4H, d, *J* 7 Hz, *o*-C*H*); δ_C (d_6-DMSO) 53.1 (t, ring CH_2), 56.7 (t, NCH_2), 61.5 (t, CH_2O), 118.4 (d, *o*-CH), 122.3 (d, *p*-CH), 128.5 (d, *m*-CH), 139.0 (s, CNH) and 153.7 (s, C=O); *m/z* 262 (20.6%), 156 (40.0%) and 125 (65.5%) (Found: C, 64.37; H, 6.60; N, 13.52. $C_{22}H_{28}N_4O_4$ requires C, 64.10; H, 6.97; N, 13.59%).

1,4-Bis(2-*p*-nitrophenylaminocarbonyloxyethyl)piperazine (130)

1,4-Bis(2-hydroxyethyl) piperazine (1.0 g , 5.75 mmol) and *p*-nitrophenyl isocyanate (2.83 g, 17.2 mmol) were reacted according to procedure A for 18 h. Undissolved starting material was filtered off and the solvent was removed *in vacuo* to leave a yellow powder. This was then washed repeatedly with methanol to produce a yellow solid (1.62 g, 56%), m.p. 215-217 °C, ν_{max} 3340, 2820, 1730, 1600, 1550, 1500, 1330 and 850 cm^{-1}; δ_H (d_6-DMSO) 2.39-2.55 (8H, m, ring CH_2N), 2.62 (4H, t, *J* 5 Hz, NCH_2), 4.27 (4H, t, *J* 5 Hz, CH_2O), 7.74 and 8.30 (8H, d, *J ca.* 9 Hz, AA'BB' system) and 10.46 (2H, bs, N*H*); δ_C 52.9 (t, ring CH_2), 56.5 (t, CH_2N), 62.0 (t, CH_2O), 117.8 (d, CHCNH), 125.2 (d, $CHCNO_2$), 141.7 (s, CNH), 145.9 (s, CNO_2) and 153.4 (C=O); *m/z* 307, 164, 143, 125 and 70 (Found: C, 52.41; H, 5.14; N, 16.80. $C_{22}H_{26}N_6O_8$ requires C, 52.59; H, 5.18; N, 16.73%).

1,4-bis(2-chloroethyl)piperazine dihydrochloride[136] (125)

1,4-Bis(2-hydroxyethyl)piperazine (1.0 g, 5.74 mmol) was dissolved in dichloromethane and added slowly to thionyl chloride (10 ml) at 0 °C. The reaction was left to stir for 8 h at room temperature after which the solvent was removed to leave a yellow solid. This was recrystallised from methanol to leave white crystals (1.20 g, 74%), m.p. 215-216 °C, ν_{max} 2820, 2790 and 670 cm^{-1}; δ_H (D_2O) 3.70 (4H, t, *J* 4 Hz, CH_2N^+), 3.77 (8H, s, ring CH_2) and 3.94 (4H, t, *J* 4 Hz, CH_2Cl); δ_C 37.6 (t, CH_2Cl), 49.2 (t, ring CH_2) and 58.4 (t, CH_2N^+); *m/z* 212 (M^+ 2.1%), 177, 174, 161 (100%), 125, 118 and 70 (100%) (Found: M^+ 212.0662. $C_8H_{16}N_2Cl_2$ requires M^+212.0664).

1,4-Bis(2-isopropylaminocarbonyloxyethyl)piperazine *N*-oxide (132)

mCPBA (0.43 g, 2 mmol) and 1,4-bis(2-isopropylaminocarbonyloxyethyl)piperazine (0.30 g, 0.90 mmol) were reacted according to procedure B for 3 h. This left a white powder which was recrystallised from methanol to leave the product (0.22 g, 64%), R_F 0.50 (EtOAc) m.p. 157-158 °C, ν_{max} 3430, 2870, 1690, 1466, 1367, 1257 and 960 cm^{-1}; δ_H (d_6-DMSO) 1.10 (12H, d, *J* 7 Hz, CH_3CH), 3.63 (6H, m, ring $CH_2N^+_{ax}$ and $CHCH_3$), 3.98 (4H, bs, CH_2N^+), 4.33 (4H, d, *J* 9 Hz, ring $CH_2N^+_{eq}$) and 4.58 (4H, bs, CH_2O); δ_C 22.6 (q, CH_3CH), 42.6 (d, $CHCH_3$), 57.3 (t, CH_2N^+), 58.6 (t, ring CH_2), 67.8 (t, CH_2O) and 154.7 (s, C=O); *m/z* 172, 156, 143, 126, 115 and 70 (100%) (Found: C, 44.47; H, 8.53; N, 12.95. $C_{16}H_{32}N_4O_6.3H_2O$ requires C, 44.65; H, 8.83: N, 13.02%).

1,4-Bis(2-ethylaminocarbonyloxyethyl)piperazine *N*-oxide (131)

mCPBA (0.57 g, 2.65 mmol) and 1,4-bis(2-ethylaminocarbonyloxyethyl)piperazine (0.40 g, 1.27 mmol) were reacted according to procedure B for 4 h. This left a white powder which was recrystallised from methanol to give the product (0.35 g, 79%), m.p. 205-207 °C, R_F 0.56 (MeOH), ν_{max} 3330, 1690 and 960 cm^{-1}; δ_H (D_2O) 0.95 (6H, t, *J* 7 Hz, CH_3CH_2), 2.96 (4H, q, *J* 7 Hz, CH_2CH_3), 3.24 (4H, d, *J* 9 Hz, ring $CH_2N^+_{ax}$), 3.50 (4H, bs, CH_2N^+), 4.12 (4H, d, *J* 9 Hz, ring $CH_2N^+_{eq}$) and 4.44 (4H, bs CH_2O); δ_C 14.9 (q, CH_3), 36.3 (t, CH_2CH_3), 58.9 (t, CH_2N^+), 59.9 (t, ring CH_2), 69.8 (t, CH_2O) and 158.1 (s, C=O); *m/z* 243, 188, 175, 157, 143 and 70 (Found: C, 43.53; H, 8.06; N, 14.35. $C_{14}H_{28}N_4O_6.2H_2O$ requires C, 43.75; H, 8.33; N, 14.58).

1,4-Bis(2-phenylaminocarbonyloxyethyl)piperazine *N*-oxide (133)

mCPBA (0.45 g, 2.1 mmol) and 1,4-bis(2-phenylaminocarbonyloxyethyl)piperazine (0.40 g, 0.97 mmol) were reacted according to procedure B for 4 h. This left a white powder which was recrystallised from methanol (0.36 g, 84%), m.p. 151-152 °C, R_F 0.70 (EtOAc), ν_{max} 3400, 1720, 1074 cm^{-1}; δ_H (d_6-DMSO) 3.82 (4H d, *J* 9 Hz, ring $CH_2N^+_{ax}$), 4.15 (4H, bs, CH_2N^+), 4.40 (4H, d, *J* 9 Hz, ring $CH_2N^+_{eq}$), 4.76 (4H, bs, CH_2O), 7.02 (2H, t, *J* 7 Hz, *p*-CH), 7.31 (4H, t, *J* 7 Hz, *m*-CH), 7.48 (4H, d, *J* 7 Hz, *o*-CH) and 9.82 (2H, bs, NH); δ_C 57.9 (t, CH_2N), 58.5 (t, ring CH_2N), 67.2 (t, CH_2O), 118.5 (d, *o*-CH), 122.7 (d, *p*-CH), 128.8 (d, *m*-CH), 139.0 (s, NHCH) and 152.9 (s, C=O); *m/z* 262, 188, 175, 157, 143, 119 (100%) and 70 (Found: C, 54.79; H, 6.71; N, 11.63. $C_{22}H_{28}N_4O_6.2H_2O$ requires C, 55.00; H, 6.66; N, 11.66%).

1,4-Bis(2-*p*-nitrophenylaminocarbonyloxyethyl)piperazine *N*-oxide (134)

1,4-Bis(2-*p*-nitrophenylaminocarbonyloxyethyl)piperazine (0.30 g, 0.60 mmol) was dissolved in TFA (15 ml). H_2O_2 (0.27 g, 2.4 mmol) was added and the solution was stirred for 18 h at room temperature. The solution was then filtered to remove undissolved starting material and the filtrate was added to ether upon which a precipitate appeared. This was filtered to leave a pale yellow solid which was washed with methanol (0.27 g, 84%), m.p. 173-175 °C (dec), ν_{max} 3340, 1740, 1600, 1550, 1500, 1330, 920 and 850 cm^{-1}; δ_H (d_6-DMSO) 4.05-4.22 (4H, m, C*H*$_2$O), 4.40-4.52 (12 H, m, C*H*$_2$N$^+$), 7.75 and 8.26 (8H, d, *J* ca. 9 Hz, AA'BB' system) and 10.59 (2H, bs, N*H*); δ_C 57.2 (t, ring *C*H$_2$), 57.6 (t, *C*H$_2$N), 66.6 (t, *C*H$_2$O), 117.9 (d, *C*HCNH), 125.2 (d, *C*HCNO$_2$), 141.9 (s, *C*NH), 145.3 (s, *C*NO$_2$) and 152.4 (s, *C*=O); *m/z* 534, 234, 208, 188, 172, 164 (84%), 157, 90 (100%) and 70; (Found: C, 46.74; H, 5.88; N, 14.77. $C_{22}H_{26}N_6O_{10}.2H_2O$ requires C, 46.32; H, 5.26; N, 14.74%).

1,4-bis(2-chloroethyl)piperazine *N*-oxide[136] (135)

1,4-bis(2-chloroethyl)piperazine hydrochloride (0.2 g, 0.71 mmol) and triethylamine (0.11 g, 1.48 mmol) were dissolved in acetone and stirred for 4 h to form the free base. mCPBA (0.32 g, 1.5 mmol) was then added and the solution was stirred for another 2.5 h. The solvent was removed *in vacuo* and the residue taken up in methanol. This solution was then subjected to alumina flash column chromatography with methanol after which the solvent was removed to leave a white solid which was recrystallised from dichloromethane/hexane (0.13 g, 76%), R_F 0.70 (EtOAc:hexane, 1:3), m.p. 62-64 °C, ν_{max} 670 and 950 cm^{-1}; δ_H 2.73-3.36 (8H, m, ring C*H*$_2$), 3.64 (4H, t, *J* 6 Hz, C*H*$_2$N$^+$) and 3.90 (4H, t, *J* 6 Hz, C*H*$_2$O); δ_C 42.1 (t, ring *C*H$_2$), 54.4 (t, *C*H$_2$N$^+$) and 71.6 (t, *C*H$_2$Cl); *m/z* 244 (*M*$^+$ 8.1%), 165, 163 (100%), 147 and 84 (Found: M$^+$ 244.0558; C, 39.58; H, 6.28; N, 11.28. $C_8H_{16}N_2O_2Cl_2$ requires M$^+$ 244.0557; C, 39.51; H, 6.58; N, 11.52%).

Experimental to Chapter 6

Methyl DL-Pipecolinate Hydrochloride (144)

A solution of DL-pipecolinic acid (10.0 g, 77.5 mmol) in distilled 2,2-dimethoxypropane (160 ml) with conc. HCl (88 ml) was heated at reflux for 30 min then stirred at room temperature for 18 h. The solution was concentrated to leave a red solid. This was washed with isopropanol to remove the red colour and leave a white powder (12.6 g, 91%), m.p. 205-207 °C, ν_{max} 2990, 1750, 1250 and 1100 cm^{-1}; δ_H (D_2O) 1.42-1.77 and 2.16 (6H, m, ring CH_2), 2.91 and 3.31 (1H, m, NCH_2), 3.68 (3H, s, CO_2CH_3) and 3.88 (1H, m, CHN); δ_C 22.0 and 22.1 (t, ring CH_2), 26.5 (t, $CH_2CH_2CH_2N$), 44.9 (t, CH_2N), 54.4 (q, CO_2CH_3), 57.6 (d, CHN) and 171.0 (s, C=O); *m/z* 143 (M^+ 8.4 %), 128, 84 (100%) and 82 (Found: M^+ 143.0946. $C_7H_{13}NO_2$ requires 143.0944).

N,N'-(1,2-Dioxo-1,2-ethanediyl)bis(2-methoxycarbonylpiperidine) (145)

To a solution of chloroform (30 ml) was added methyl pipecolinate hydrochloride (5.0 g, 27.8 mmol) and triethylamine (6.80 g, 66.7 mmol) under nitrogen. The solution was stirred for 10 min before being cooled to -5 °C. Oxalyl chloride (1.65 g, 13.0 mmol) was then added dropwise over a period of 10 min. The flask was then allowed to return to room temperature and the components were stirred for 2 h. Removal of the solvent *in vacuo* left a brown residue which was taken up in water and extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were dried, filtered and evaporated to leave a yellow oil. Flash chromatography on alumina using EtOAc followed by concentration produced a clear oil (3.60 g, 82%), R_F 0.70 (EtOAc), ν_{max} 2948, 2861, 2712, 1739 and 1659 cm^{-1}; δ_H 1.20-1.82 (10H, m, ring CH_2), 2.18-2.35 (2H, m, ring CH_2CH_2N), 2.85 (1H, m, CH_2N), 3.31 (1H, m, CH_2N), 3.77 (6H, s, CH_3) and 5.26 (2H, m, CHN); δ_C 20.9, 21.1, 24.6 25.0, 26.0 and 26.2 (t, ring CH_2), 43.4 and 43.9 (t, CH_2N), 51.0 (q, $COCH_3$), 52.3 (d, CHN), 164.3 and 164.9 (s, NC=O) and 170.6 and

170.8 (s, $COCH_3$); *m/z* 340 (M^+ 5%), 281, 197, 170, 142 (100%) and 111 (Found: M^+ 340.1634. $C_{16}H_{24}N_2O_6$ requires M^+ 340.1635).

N,N'-(1,2-Ethanediyl)bis(2-methoxycarbonylpiperidine) (146)

N,N'-(1,2-Dioxo-1,2-ethanediyl)bis(2-methoxycarbonylpiperidine) (2.0 g, 5.9 mmol) in dry THF (20 ml) was added to a solution of 1M borane-THF (30 ml, 30 mmol) under nitrogen at 0 °C. The solution was then heated at reflux for 2 h. 6M HCl (5 ml) was added dropwise at 0 °C with the formation of hydrogen, followed by the addition of water (10 ml). The THF was then removed by distillation to leave an aqueous solution which was concentrated *in vacuo*. Methanol (20 ml) and conc. HCl (2 drops) were added to the residue and the mixture was concentrated *in vacuo* to remove the methyl borate formed. This procedure was repeated twice, after which no more weight loss was recorded. The remaining thick clear oil was taken up in chloroform (25 ml) and triethylamine (5 ml) was added. The resulting solution was stirred for 30 min before being concentrated to leave a white residue. This was taken up in water and extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were dried, filtered and evaporated to leave a clear oil (1.46 g, 73%), R_F 0.60 (EtOAc), ν_{max} 2935, 2855 and 1737 cm^{-1}; δ_H 1.32-1.74 (12H, m, ring CH_2), 2.16 (2H, m, CHN), 2.42 and 2.57 (4H, m, ring CH_2N), 3.05 (4H, m, NCH_2CH_2N) and 3.65 (6H, s, CO_2CH_3); δ_C 22.5, 22.6, 25.3, 29.6 and 29.7 (t, ring CH_2), 50.7 and 50.9 (t, ring CH_2N), 51.6 (q, CO_2CH_3), 53.8 and 54.0 (t, NCH_2CH_2N), 65.1 and 65.4 (d, CHN) and 174.1 and 174.2 (s, CO_2CH_3); *m/z* 312 (M^+ 8%), 253, 170, 156 (100%) and 112 (Found: M^+ 312.2049. $C_{16}H_{28}N_2O_4$ requires M^+ 312.2028).

N,N'-(1,2-Ethanediyl)bis(2-hydroxymethylpiperidine) (147)

N,N'-(1,2-Ethanediyl)bis(2-methoxycarbonylpiperidine) (2.0 g, 6.4 mmol) in dry THF (20 ml) was added dropwise to a solution of 1M $LiAlH_4$ (15 ml, 15 mmol) at 0 °C under nitrogen. The solution was warmed to room temperature and stirred for 4 h. The flask was then cooled to 0 °C and water : THF (1 : 1, 20 ml) was added dropwise. Both solvents were evaporated *in vacuo* which left a white powder. This was stirred with dichloromethane (30 ml) for 30 min then the solution was filtered and the filtrate was concentrated *in vacuo* to leave a clear oil (1.56 g, 95%), R_F 0.34 (MeOH), ν_{max} 3329, 2926, 2853 and 1284 cm^{-1}; δ_H 1.34-1.71 (12H, m, ring CH_2), 1.99 (2H, d, *J* 11 Hz, CHN), 2.17 (4H, m, ring CH_2N), 2.84 (4H, d, *J* 11 Hz, NCH_2CH_2N), 3.18 (2H, dd, *J* 1.5 Hz and *J* 12 Hz, CH_2OH), 3.85 (2H, dd, *J* 3.5 Hz and *J* 12 Hz, CH_2OH) and 4.26 (2H, bs, OH); δ_C 24.2, 25.0 and 28.7 (t, ring CH_2), 49.3 (t, ring CH_2N), 54.4 (t, NCH_2CH_2N), 62.1 (d, CHN) and 63.0 (t, CH_2OH); *m/z* 256, 255, 238, 225, 142, 128 (100%), 110, 98 and 84 (Found: M^+ -H, 255.2072. $C_{14}H_{28}N_2O_2$ requires M^+ -H 255.2069).

N,N'-(1,2-Ethanediyl)-bis(2-isopropylaminocarbonyloxymethyl-piperidine) (148)

N,N'-(1,2-Ethanediyl)bis(2-hydroxymethylpiperidine) (0.30 g, 1.2 mmol) and isopropyl isocyanate (0.31 g, 3.6 mmol) were reacted according to procedure A for 18 h. This gave the product as a yellow oil which was purified by flash chromatography on alumina using ethyl acetate as eluant. After concentration the remaining white powder was recrystallised using dichloromethane/hexane (0.45 g, 88%), R_F 0.60 (EtOAc), m.p. 162-164 °C, ν_{max} 3331, 2928, 2853, 2795, 1684, 1364 and 1259 cm^{-1}; δ_H 1.15 (12H, d, *J* 11 Hz, CH_3CH), 1.36-1.74 (12H m, ring CH_2), 2.12-2.72 (4H, m, CHN and ring CH_2N), 2.89 (4H, m, NCH_2CH_2N), 3.80 (2H, m, $CHCH_3$), 4.15 (4H, m, CH_2O) and 5.17 (2H, bs, NH); δ_C 22.8 (q, CH_3CH), 25.3, 28.8 and 29.4 (t, ring CH_2), 42.8 (d,

*C*HCH$_3$), 50.72 (t, ring *C*H$_2$N), 52.7 (t, NCH$_2$*C*H$_2$N), 59.5 (d, *C*HN), 65.7 (t, *C*H$_2$O) and 155.7 (s, *C*=O); *m/z* 426 (M^+ 0.3%), 310, 227, 213 (100%), 128 and 110 (Found: M^+ 426.3230. $C_{22}H_{42}N_4O_4$ requires M^+ 426.3180).

N,N'-(1,2-Ethanediyl)-bis(2-ethylaminocarbonyloxymethylpiperidine) (149)

N,N'-(1,2-Ethanediyl)bis(2-hydroxymethylpiperidine) (0.30 g, 1.2 mmol) and ethyl isocyanate (0.25 g, 3.6 mmol) were reacted according to procedure A for 18 h. This gave the product as a clear oil after flash chromatography on alumina eluting with ethyl acetate (0.40 g, 82%), R_F 0.40 (EtOAc), ν_{max} 3414, 2982, 2806, 1716, 1450 and 1265 cm^{-1}; δ_H 1.14 (6H, t, *J* 7 Hz, *C*H$_3$CH$_2$), 1.21-1.80 (12H, m, ring C*H*$_2$), 2.23 (2H, m, C*H*N), 2.52 (4H, m, C*H*$_2$N), 2.88 (4H, m, NC*H*$_2$C*H*$_2$N), 3.20 (4H, q, *J* 7 Hz, C*H*$_2$CH$_3$), 4.13 (4H, m, C*H*$_2$O) and 5.31 (2H, bs, N*H*); δ_C 15.2 (q, *C*H$_3$CH$_2$), 23.5, 25.4 and 29.1 (t, ring *C*H$_2$), 35.8 (t, *C*H$_2$CH$_3$), 50.7 (t, *C*H$_2$N), 52.9 (t, NCH$_2$*C*H$_2$N), 59.7 (d, *C*HN), 66.1 (t, *C*H$_2$O) and 156.7 (s, *C*=O); *m/z* 398 (M^+ 0.1%) 296, 220, 213, 199 (100%), 142,128 and 110 (Found: M^+ 398.2892. $C_{20}H_{38}N_4O_4$ requires M^+ 398.2885).

N,N'-(1,2-Ethanediyl)-bis(2-phenylaminocarbonyloxymethylpiperidine) (150)

N,N'-(1,2-Ethanediyl)bis(2-hydroxymethylpiperidine) (0.30 g, 1.2 mmol) and phenyl isocyanate (0.43 g, 3.6 mmol) were reacted according to procedure A for 24 h. This gave the product as an oil which was purified on an alumina flash chromatography column eluting with ethyl acetate. The resulting white powder was then recrystallised from dichloromethane (0.45 g, 77%), R_F 0.75 (EtOAc), m.p. 142-143 °C, ν_{max} 3323, 2856, 1730, 1600, 1538, 754 and 693 cm^{-1}; δ_H (d_6-acetone) 1.26-1.71 (12H, m, ring C*H*$_2$), 2.31 (2H, m, C*H*N), 2.56 (4H, m, C*H*$_2$N), 2.89 (4H, m, NC*H*$_2$C*H*$_2$N), 4.23 (4H, m,

CH_2O), 6.90 (2H, m, *p*-C*H*), 7.26 (4H, m, *m*-C*H*), 7.57 (4H, m, *o*-C*H*) and 8.85 (2H, bs, N*H*); δ_C 23.7 26.0 and 29,4 (t, ring CH_2), 52.1 (t, CH_2N), 53.1 (t, NCH_2CH_2N), 60.3 (d, *C*HN), 66.2 (t, CH_2O), 119.2 (d, *o*-*C*H), 123.2 (d, *p*-*C*H), 129.4 (d, *m*-*C*H), 140.0 (s, *C*NH) and 154.4 (s, *C*=O); *m/z* 212, 194, 119 and 93 (100%) (Found: C, 67.75; H, 7.75; N, 11.46. $C_{28}H_{38}N_4O_4$ requires C, 68.02, H, 8.04, N 11.37%).

N,N'-(1,2-Ethanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethyl-piperidine) (151)

N,N'-(1,2-Ethanediyl)bis(2-hydroxymethylpiperidine) (0.48 g, 1.87 mmol) and *p*-nitrophenyl isocyanate (0.62 g, 3.8 mmol) were reacted in dichloromethane (25 ml) with 3 drops of dibutyl tin diacetate for 20 h. The solution was then filtered and the filtrate was concentrated to leave a yellow residue. This was purified on a gravity column on alumina using ethyl acetate : pet ether 40-60 °C which left the product as yellow crystals after concentration (0.53 g, 48%), R_F 0.55 (EtOAc:pet ether, 1:2), m.p. 92-93 °C, ν_{max} 3406, 2932, 2810, 1736, 1599, 1554, 1508, 1332, 1219 and 852 cm^{-1}; δ_H 1.42-2.00 (12H, m, ring CH_2), 2.54 (2H, m, C*H*N), 2.89 (4H, m, CH_2N), 3.18-3.40(4H, m, NCH_2CH_2N), 4.23 (4H, m, CH_2O), 7.58 and 8.13 (8H, m, aromatic C*H*) and 9.35 (2H, bs, N*H*); δ_C 22.6, 24.0 and 27.8 (t, ring CH_2), 50.7 (t, CH_2N), 53.2 (t, NCH_2CH_2N), 61.1 (d, *C*HN), 65.4 (t, CH_2O), 117.8 (d, *C*HCNH), 125.0 (d, *C*HCNO$_2$), 142.7 (s, *C*NH), 144.6 (s, *C*NO$_2$) and 152.8 (s, *C*=O); *m/z* 343, 267, 238, 225, 223, 164, 142, 128 (100%) and 110.

N,N'-(1,2-Ethanediyl)bis(2-chloromethylpiperidine) Dihydrochloride[143] (152)

N,N'-(1,2-Ethanediyl)bis(2-hydroxymethylpiperidine) (0.40 g, 1.56 mmol) in dichloromethane was added slowly to thionyl chloride (15 ml) at 0°C. The solution was stirred at room temperature for 24 h, after which the solvent was removed to leave a

white residue. This was dissolved in methanol and concentrated again, this time to leave white crystals (0.51 g, 89%), m.p. 231-233 °C, ν_{max} 2948, 2870 and 746 cm^{-1}; δ_H (D_2O) 1.58-1.90 (12H, m, ring CH_2), 3.17 (2H, m, CHN), 3.51-3.81 (8H, m, NCH_2CH_2N and CH_2N) and 4.07 (4H, m, CH_2Cl); δ_C 21.6, 23.2 and 28.0 (t, ring CH_2), 43.8 (t, CH_2Cl), 46.9 (t, CH_2N), 54.3 (t, NCH_2CH_2N) and 65.5 (d, CHN); *m/z* 257, 243, 222, 192, 160, 146 (100%), 110 and 97 (Found M^+ -Cl 257.1797. $C_{14}H_{26}N_2Cl$ requires M^+ -Cl 257.1791).

_N,N'_-(1,2-Ethanediyl)-bis(2-isopropylaminocarbonyloxymethyl-piperidine) bis-_N_-Oxide (153)

_N,N'_-(1,2-Ethanediyl)-bis(2-isopropylaminocarbonyloxymethylpiperidine) (0.30 g, 0.7 mmol) and mCPBA (0.33 g, 1.55 mmol) were reacted according to procedure B for 18 h. This gave the product as white crystals after recrystallisation from acetone (0.20 g, 62%), m.p. 152-154 °C, R_F 0.66 (MeOH), ν_{max} 3343, 2946, 1686, 1364, 1250 and 960 cm^{-1}; δ_H 1.14 (12H, d, *J* 7 Hz, CH_3CH), 1.37-2.24 (12H m, ring CH_2), 3.06 (2H, m, CHN^+), 3.44 (4H, m, CH_2N^+), 3.76 (4H, m, $N^+CH_2CH_2N^+$), 3.97 (2H, m, $CHCH_3$), 4.13 (4H, m, CH_2O) and 4.67 (2H, bs, NH); δ_C 23.6 (q, CH_3CH), 19.9, 22.8 and 24.8 (t, ring CH_2), 43.1 (d, $CHCH_3$), 61.4 (t, CH_2N^+), 63.2 (t, $N^+CH_2CH_2N^+$), 65.2 (t, CH_2O), 75.1 (d, CHN^+) and 155.2 (s, C=O); *m/z* 245, 158, 144, 142 and 102 (Found: C, 53.82; H, 8.91; N, 11.60. $C_{22}H_{42}N_4O_6.2H_2O$ requires C, 53.44; H, 9.31; N, 11.34%).

_N,N'_-(1,2-Ethanediyl)-bis(2-ethylaminocarbonyloxymethylpiperidine) bis-_N_-Oxide (154)

_N,N'_-(1,2-Ethanediyl)-bis(2-ethylaminocarbonyloxymethylpiperidine) (0.30 g, 0.75 mmol) and mCPBA (0.36 g, 1.66 mmol) were reacted according to procedure B for 18 h. This gave the product as a clear oil which was crystallised from acetone and

recrystallised from dichloromethane (0.24 g, 74 %), m.p. 132-134 °C, R_F 0.66 (MeOH), ν_{max} 3396, 2972, 1730, 1248 and 964 cm^{-1}; δ_H 1.10 (6H, t, CH_3CH_2), 1.31-2.16 (12H, m, ring CH_2), 3.15 (6H, m, CHN^+ and CH_3CH_2), 3.38 (4H, $N^+CH_2CH_2N^+$), 3.68 (4H, m, CH_2N^+), 4.52 (4H, m, CH_2O) and 5.31 (2H, m, NH); δ_C 15.0 (q, CH_3CH_2), 19.9, 22.1 and 24.4 (t, ring CH_2), 35.7 (t, CH_2CH_3), 61.3 (t, $N^+CH_2CH_2N^+$), 62.9 (t, CH_2N^+), 65.8 (t, CH_2O), 74.7 (d, CHN^+) and 156.1 (s, C=O); *m/z* 228, 202, 140, 126, 113, 110 and 100 (100%) (Found: C, 47.69; H, 8.94; N, 10.92. $C_{20}H_{38}N_4O_6.4H_2O$ requires C, 47.81; H, 9.16; N, 11.15%).

N,N'-(1,2-Ethanediyl)-bis(2-phenylaminocarbonyloxymethylpiperidine) bis-*N*-Oxide (155)

N,N'-(1,2-Ethanediyl)-bis(2-phenylaminocarbonyloxymethylpiperidine) (0.30 g, 0.6 mmol) and mCPBA (0.29 g, 1.3 mmol) were reacted according to procedure B for 18 h. This gave the product as white crystals after recrystallisation from acetone (0.20 g, 63%), R_F 0.72 (MeOH), ν_{max} 3431, 1726, 1601, 1500, 1230, 1070, 752 and 694 cm^{-1}; δ_H (d_6-Acetone) 1.35-2.21 (12H, m, ring CH_2), 3.37 (2H, m, CHN^+), 3.66 (4H, m, CH_2N^+), 3.89 (4H, m, $N^+CH_2CH_2N^+$), 4.65 (4H, m, CH_2O), 6.94 (2H, m, *p*-CH), 7.25 (4H, *m*-CH) and 7.43 (4H, m, *o*-CH); δ_C 20.6, 22.7 and 25.1 (t, ring CH_2), 61.6 (t, CH_2N^+), 63.8 (t, $N^+CH_2CH_2N^+$), 66.0 (t, CH_2O), 119.1 (d, *o*-CH), 123.4 (d, *p*-CH), 129.5 (d, *m*-CH), 140.6 (s, CNH) and 154.0 (s, C=O); *m/z* 276, 248, 212, 140, 126, 119 (100%), 113, 110 and 98 (Found: C, 64.03; H, 6.32; N, 10.60. $C_{28}H_{38}N_4O_6$ requires C, 63.88; H, 7.22; N, 10.66%).

N,N'-(1,2-Ethanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethyl-piperidine) bis-*N*-Oxide (156)

N,N'-(1,2-Ethanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethylpiperidine) (0.20 g, 0.34 mmol) and mCPBA (0.16 g, 0.75 mmol) were reacted together in acetone for 18 h

at room temperature. After careful concentration of the solution, the residue was purified using flash chromatography on alumina, eluting first with ethyl acetate to remove any impurities, and then with methanol to remove the product. This gave the product as yellow crystals (0.11 g, 51%), m.p. 176-177 °C, ν_{max} 3426, 1736, 1599, 1560, 1508, 1332, 1224, 1064 and 854 cm^{-1}; δ_H 1.25-2.20 (12H, m, ring CH_2), 3.15 (2H, m, CHN^+), 3.50 (4H, m, CH_2N^+), 3.82 (4H, m, $N^+CH_2CH_2N^+$), 4.70 (4H, m, CH_2O), 7.58 and 8.13 (8H, AA'BB' system); δ_C 19.7, 21.7 and 24.1 (t, ring CH_2), 61.7 (t, CH_2N^+), 63.4 (t, $N^+CH_2CH_2N^+$), 65.4 (t, CH_2O), 71.6 (d, CHN^+), 117.7 (d, CHCNH), 124.9 (d, $CHCNO_2$), 142.5 (s, CNH), 144.8 (s, CNO_2) and 153.0 (s, C=O); *m/z* 292, 261, 196, 142, 138 (100%), 128, 113, 112 and 100.

_N,N'_-(1,2-Ethanediyl)bis(2-chloromethylpiperidine) bis-_N_-Oxide (157)

_N,N'_-(1,2-Ethanediyl)bis(2-chloromethylpiperidine) (0.2 g, 0.68 mmol) and mCPBA (0.32 g, 1.5 mmol) were reacted according to procedure B for 18 h. This gave the product as a yellow oil (0.14 g, 65%), R_F 0.61(MeOH), ν_{max} 2947, 2869, 932 and 732 cm^{-1}; δ_H (D_2O) 1.52-2.14 (12H, m, ring CH_2), 3.11 (4H, m, CH_2N^+) and 3.28-4.16 (10H, m, $N^+CH_2CH_2N^+$, CHN^+ and CH_2Cl); *m/z* 324, 221, 162, 148, 126, 112, 100 and 84 (100%) (Found M^+ 324.1369. $C_{14}H_{26}N_2O_2Cl_2$ requires M^+ 324.1353).

_N,N'_-(1,3-Dioxo-1,3-propanediyl)bis(2-methoxycarbonylpiperidine) (159)

To a solution of chloroform (30 ml) was added methyl pipecolinate hydrochloride (5.0 g, 27.8 mmol) and triethylamine (6.80 g, 66.7 mmol) under nitrogen. The solution was stirred for 10 min before being cooled to -5 °C. Malonyl dichloride (1.27 ml, 13.0 mmol) was then added dropwise over a period of 10 min. The flask was then allowed to warm to room temperature and the mixture was stirred for 2 h. Removal of the solvent *in vacuo* left a brown residue which was taken up in water and extracted with ethyl acetate (3 x 30

ml). The combined organic extracts were dried, filtered and concentrated to leave a yellow oil. Flash chromatography on alumina eluting with EtOAc followed by concentration produced a clear oil (3.40 g, 74%), R_F 0.67 (EtOAc), ν_{max} 2951, 2864, 1746, 1645 and 1278 cm^{-1}; δ_H 1.29-1.72 (10H, m, ring CH_2), 2.28 (2H, m, CH_2CH_2N) 3.25-3.44 (4H, m, CH_2N), 3.50 and 3.54 (2H, s, $NCOCH_2CON$), 3.73 (6H, s, CH_3) and 5.38 (2H, m, CHN); δ_C 20.8, 24.8 and 26.1 (t, ring CH_2), 43.9 (t, CH_2N), 51.9 (q, $COCH_3$), 53.8 (d, CHN), 61.2 (t, $NCOCH_2CON$), 167.2 (s, NC=O) and 170.9 (s, $COCH_3$); *m/z* 354 (M^+ 5%), 323, 295, 212, 170, 142 and 84 (100%) (Found: M^+ 354.1796. $C_{17}H_{26}N_2O_6$ requires M^+ 354.1779).

N,N'-(1,3-Propanediyl)bis(2-methoxycarbonylpiperidine) (160)

N,N'-(1,3-Dioxo-1,3-propanediyl)bis(2-methoxycarbonylpiperidine) (3.50 g, 9.8 mmol) in dry THF (20 ml) was added to a solution of 1M borane-THF (77 ml, 77 mmol) under nitrogen at 0 °C. The solution was then heated at reflux for 2 h. 6M HCl (5 ml) was added dropwise at 0 °C with the formation of hydrogen, followed by the addition of water (10 ml). The THF was removed by distillation to leave an aqueous solution which was concentrated *in vacuo*. Methanol (20 ml) and conc. HCl (2 drops) were added to the residue and the mixture was evaporated *in vacuo* to remove the methyl borate ester formed. This procedure was repeated twice, after which no more weight loss was recorded. The remaining thick clear oil was taken up in chloroform (25 ml) and triethylamine (5 ml) was added. The resulting solution was stirred for 30 min before being evaporated to leave a white residue. This was taken up in water and extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were then dried, filtered and concentrated to leave a clear oil (2.40 g, 75%), R_F 0.55 (EtOAc), ν_{max} 2936, 2855, 1737 and 1261 cm^{-1}; δ_H 1.12-1.35 (2H, m, $NCH_2CH_2CH_2N$), 1.47-1.85 (12H, m, ring CH_2), 1.90-2.22 (2H, m, CHN), 2.34-2.73 (4H, m, CH_2N), 2.90-3.10 (4H, m, $NCH_2CH_2CH_2N$) and 3.62 (6H, s, CO_2CH_3); δ_C 22.4 (t, $NCH_2CH_2CH_2N$), 24.0, 24.1, 25.0, 25.3, 29.3 and 29.4 (t, ring CH_2), 50.1 and 50.2 (t, CH_2N), 51.3 (q,

CO_2CH_3), 54.1 and 54.3 (t, $NCH_2CH_2CH_2N$), 64.8 and 65.1 (d, *C*HN) and 173.9 (s, CO_2CH_3); *m/z* 326 (M^+ 17%), 267, 184, 170, 156, 142, 126, 124 and 98 (100%) (Found: M^+ 326.2205. $C_{17}H_{30}N_2O_4$ requires 326.2197).

N,N'-(1,3-Propanediyl)bis(2-hydroxypiperidine) (161)

N,N'-(1,3-Propanediyl)bis(2-methoxycarbonylpiperidine) (2.5 g, 7.67 mmol) in dry THF (20 ml) was added dropwise to a solution of 1M $LiAlH_4$ in THF (32.2 ml, 32.2 mmol) at 0 °C under nitrogen. The solution was warmed to room temperature and stirred for 4 h. The flask was then cooled to 0 °C and water : THF (1 : 1, 20 ml) was added dropwise. Both solvents were evaporated *in vacuo* which left a white powder. This was stirred with dichloromethane (30 ml) for 30 min after which the solution was filtered and the fitrate concentrated *in vacuo* to leave a clear oil (1.81 g, 87%), R_F 0.65 (MeOH), ν_{max} 3348, 2934 and 1383 cm^{-1}; δ_H 1.19-1.77 (14H, m, ring CH_2 and $NCH_2CH_2CH_2N$), 2.05-2.38 (4H, m, CH_2N) 2.45 (2H, m, C*H*N), 2.90-3.22 (4H, m, $NCH_2CH_2CH_2N$), 3.55-3.87 (4H, m, CH_2OH) and 4.33 (2H, bs, O*H*); δ_C 22.6 (t, $NCH_2CH_2CH_2N$), 24.7, 27.8 and 29.6 (t, ring CH_2), 51.9 (t, CH_2N), 56.7 (t, $NCH_2CH_2CH_2N$), 62.4 (d, *C*HN) and 63.0 (t, CH_2OH); *m/z* 222, 156, 142, 128, 110, 98 and 84 (100%) (Found: M^+ ($-H_2O$), 252.2180. $C_{15}H_{28}N_2O$ requires 252.2178).

N,N'-(1,3-Propanediyl)-bis(2-phenylaminocarbonyloxymethylpiperidine) (162)

N,N'-(1,3-Propanediyl)bis(2-hydroxypiperidine) (0.40 g, 1.5 mmol) and phenyl isocyanate (0.35 g, 2.9 mmol) were reacted according to procedure A for 18 h. Flash chromatography on alumina with ethyl acetate produced a white powder which was crystallised with dichloromethane. This gave the product as white crystals (0.35 g, 46%), R_F 0.70 (EtOAc), 128-130 °C, ν_{max} 3302, 2857, 1710, 1600, 1538,1224, 753 and 693 cm^{-1}; δ_H 1.15-1.93 (14H, m, ring CH_2 and $NCH_2CH_2CH_2N$), 2.45 (2H, m, C*H*N),

2.66-3.08 (8H, m, $NCH_2CH_2CH_2N$ and CH_2N), 4.22 (4H, m, CH_2O), 6.92 (2H, m, *p*-C*H*), 7.23 (4H, m, *m*-C*H*), 7.46 (4H, m, *o*-C*H*) and 8.78 (2H, bs, N*H*); δ_C 20.2 (t, $NCH_2CH_2CH_2N$), 22.4 23.6 and 27.6 (t, ring CH_2), 50.9 (t, CH_2N), 51.9 (t, $NCH_2CH_2CH_2N$), 57.9 (d, CHN), 64.2 (t, CH_2O), 118.8 (d, *o*-CH), 122.7 (d, *p*-CH), 128.8 (d, *m*-CH), 138.8 (s, CNH) and 153.6 (s, C=O); *m/z* 261, 247, 234, 212, 142, 128, 119, 98 and 84 (100%) (Found: C, 68.75; H, 8.08, N, 10.95. $C_{29}H_{40}N_4O_4$ requires C, 68.50; H, 7.87; N, 11.02%).

N,N'-(1,3-Propanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethyl-piperidine) (163)

N,N'-(1,3-Propanediyl)bis(2-hydroxypiperidine) (0.20 g, 0.74 mmol) and *p*-nitrophenyl isocyanate (0.28 g, 1.7 mmol) were reacted according to procedure A for 18 h. The residue was dissolved in methanol and was purified by flash column chromatography on alumina, eluting first with ethyl acetate to remove starting material impurities and then with methanol to remove the product. The methanol was removed to leave the product as yellow crystals (0.24 g, 54%) R_F 0.55 (EtOAc:), m.p. 109-111 °C, ν_{max} 3432, 2937, 2858, 1735, 1598, 1553, 1508, 1332, 1218 and 852 cm^{-1}; δ_H (d_6-acetone) 1.20-1.90 (14H, m, ring CH_2 and $NCH_2CH_2CH_2N$), 2.18 (2H, m, CHN), 2.47 (4H, m, CH_2N), 2.84 (4H, m, $NCH_2CH_2CH_2N$), 4.24 (4H, m, CH_2O), 7.75 and 8.12 (8H, m, aromatic CH) and 9.40 (2H, bs, NH); δ_C 23.8 (t, $NCH_2CH_2CH_2N$), 26.1, 26.5 and 29.4 (t, ring CH_2), 50.6 (t, CH_2N), 52.2 (t, $NCH_2CH_2CH_2N$), 59.9 (d, CHN), 66.8 (t, CH_2O), 118.4 (d, CHCNH), 125.5 (d, $CHCNO_2$), 143.1 (s, CNH), 146.3 (s, CNO_2) and 154.0 (s, C=O); *m/z* 292, 261, 142, 138 (100%), 124, 111 and 98.

N,N'-(1,3-Propanediyl)bis(2-chloropiperidine) (164)

N,N'-(1,3-Propanediyl)bis(2-hydroxypiperidine) (0.40 g, 1.48 mmol) was dissolved in dichloromethane (5 ml) and was slowly added to thionyl chloride (15 ml) at 0 °C. The

solution was stirred at room temperature for 24 h, after which the solvent was removed. The residue was dissolved in dichloromethane (20 ml) and basified with sodium bicarbonate solution. The organic layer was removed and the aqueous layer was extracted with dichloromethane (2 x 20 ml). The combined extracts were dried, filtered and concentrated to leave a brown oil. Flash chromatography on alumina with ethyl acetate yielded after evaporation, a clear oil (0.38 g, 83%), R_F 0.60 (EtOAc:hexane, 1:6), ν_{max} 2936, 2857, 2797 and 733 cm^{-1}; δ_H 1.36-1.69 (14H, m, ring CH_2 and $NCH_2CH_2CH_2N$), 1.92-2.25 (2H, m, CHN), 2.54 (4H, m, CH_2N), 2.81 (4H, m, NCH_2RCH_2N) and 3.61 (4H, m, CH_2Cl); δ_C 22.7 (t, $NCH_2CH_2CH_2N$), 25.3, 28.8 and 29.4 (t, ring CH_2), 43.3 (t, CH_2Cl), 45.3 (t, CH_2N), 51.5 (t, $NCH_2CH_2CH_2N$) and 60.4 (d, CHN); *m/z* 273, 235, 174, 162, 160, 146, 138 (100%), 124, 111 and 98 (Found M^+ -Cl 273.1893. $C_{15}H_{28}N_2Cl$ requires M^+ 273.1901).

_N,N'_-(1,3-Propanediyl)-bis(2-phenylaminocarbonyloxymethylpiperidine) bis-_N_-Oxide (165)

_N,N'_-(1,3-Propanediyl)-bis(2-phenylaminocarbonyloxymethylpiperidine) (0.17 g, 0.33 mmol) and mCPBA (0.14 g, 0.83 mmol) were reacted according to procedure B for 24 h. This gave the product as white crystals after recrystallisation from acetone (0.11 g, 63%), R_F 0.73 (MeOH), ν_{max} 3334, 1729, 1601, 1504, 1231, 1070, 758 and 694 cm^{-1}; δ_H 1.25-2.06 (14H, m, ring CH_2 and $N^+CH_2CH_2CH_2N^+$), 3.10 (2H, m, CHN^+), 3.22-3.64 (4H, m, CH_2N^+), 4.07-4.32 (4H, m, $N^+CH_2RCH_2N^+$) 4.02 (2H, m, CHN^+) 4.41-4.76 (4H, m, CH_2O), 7.01 (2H, m, *p*-CH), 7.26 (4H, m, *m*-CH), 7.52 (4H, m, *o*-CH); δ_C 20.0 (t, $N^+CH_2CH_2CH_2N^+$), 21.5 23.5 and 24.6 (t, ring CH_2), 53.8 (t, CH_2N^+), 55.1 (t, $N^+CH_2CH_2CH_2N^+$), 63.1 (t, CH_2O), 74.5 (d, CHN^+), 118.6 (d, *o*-CH), 123.1 (d, *p*-CH), 128.9 (d, *m*-CH), 138.5 (s, CNH) and 153.0 (s, C=O); *m/z* 322, 250, 216, 127, 119 and 84 (100%).

N,N'-(1,3-Propanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethyl-piperidine) bis-*N*-Oxide (166)

N,N'-(1,3-Propanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethylpiperidine) (0.20 g, 0.33 mmol) and mCPBA (0.18 g, 0.83 mmol) were reacted according to procedure B for 24 h. This gave the product as yellow crystals after recrystallisation from acetone, (0.100 g, 48%), m.p. 142-144 °C, ν_{max} 1729, 1596, 1569, 1505, 1332, 1224, 1062 and 856 cm^{-1}; δ_H (CD_3OD) 1.67-2.16 (14H, m, ring CH_2 and $N^+CH_2CH_2CH_2N^+$), 3.22-3.62 (8H, m, $N^+CH_2CH_2CH_2N^+$ and N^+CH_2), 4.17 (2H, m, CHN^+), 4.69 (4H, m, CH_2O), 7.65 (4H, m, CH) and 8.13 (4H, m, CH); δ_C 21.7 (t, $N^+CH_2CH_2CH_2N^+$), 22.9, 25.6 and 26.5 (t, ring CH_2), 64.9 (t, CH_2N^+), 65.2 (t, $N^+CH_2RCH_2N^+$), 70.4 (t, CH_2O), 72.6 (d, CHN^+), 118.9 (d, CHCNH), 125.9 (d, $CHCNO_2$), 143.9 (s, CNH), 146.5 (s, CNO_2) and 154.5 (s, C=O); *m/z* 301, 295, 261, 258, 251, 244, 164, 156, 139 and 100 (100%).

N,N'-(1,3-Propanediyl)bis(2-chloropiperidine) bis-*N*-Oxide (167)

N,N'-(1,3-Propanediyl)bis(2-chloropiperidine) (0.25 g, 0.8 mmol) and mCPBA (0.45 g, 2.4 mmol) were reacted according to procedure B for 24 h. This gave the product as a yellow oil (0.16 g, 62%), R_F 0.53 (MeOH), ν_{max} 2951, 1448, 978 and 687 cm^{-1}; δ_H 1.54-1.80 (14H, m, ring CH_2 and $N^+CH_2CH_2CH_2N^+$), 2.75 (2H, m, CHN^+), 4.15 (4H, m, CH_2Cl) and 4.38-5.03 (8H, m, N^+CH_2); δ_C 20.1 (t, $N^+CH_2CH_2CH_2N^+$), 21.5 25.96 and 28.7 (t, ring CH_2), 41.1 (t, CH_2Cl), 65.9 (t,CH_2N^+), 72.1 (t, $N^+CH_2CH_2CH_2N^+$), 72.5 (d, CHN^+); *m/z* 190, 176, 154, 146, 140 and 98.

N,N'-(1,4-Dioxo-1,4-butanediyl)bis(2-methoxycarbonylpiperidine) (168)

To a solution of chloroform (30 ml) was added methyl pipecolinate hydrochloride (5.0 g, 27.8 mmol) and triethylamine (6.80 g, 66.7 mmol) under nitrogen. The solution was

stirred for 10 min before being cooled to -5 °C. Succinyl dichloride (1.44 ml, 13.0 mmol) was then added dropwise over a period of 10 min. The flask was then allowed to warm to room temperature and the mixture was stirred for 2 h. Removal of the solvent *in vacuo* left a brown residue which was taken up in water and extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were dried, filtered and concentrated to leave a yellow oil. Flash chromatography on alumina eluting with EtOAc followed by concentration produced white crystals (4.0 g, 84%), m.p. 92 °C, R_F 0.44 (EtOAc), ν_{max} 2951, 2858, 1736 and1634 cm^{-1}; δ_H 1.22-1.74 (10H, m, ring CH_2), 2.24 (2H, m, ring CH_2CH_2N), 2.55-2.90 (4H, m, $COCH_2CH_2CO$), 3.22 (2H, m, CH_2N) 3.72 (6H, s, CH_3), 3.88 (2H, m, CH_2N) and 5.37 (2H, m, CHN); δ_C 20.8, 25.0 and 27.0 (t, ring CH_2), 42.9 (t, CH_2N), 52.0 (q, $COCH_3$), 53.8 (d, CHN), 167.2 (s, NC=O) and 171.9 (s, $COCH_3$); *m/z* 368 (M^+ 1.3%), 337, 209, 226 (100%), 198, 142 and 84 (Found: C, 58.85; N, 7.76; N, 7.66; M^+ 368.1947. $C_{17}H_{26}N_2O_6$ requires C, 58.69; H, 7.61; N, 7.61%; M^+ 368.1937).

N,N'-(1,4-Butanediyl)bis(2-methoxycarbonylpiperidine) (169)

N,N'-(1,4-Dioxo-1,4-butanediyl)bis(2-methoxycarbonylpiperidine) (4.40 g, 12 mmol) in dry THF (20 ml) was added to a solution of 1M borane-THF (94 ml, 94 mmol) under nitrogen at 0 °C. The solution was heated at reflux for 2 h. 6M HCl (5 ml) was added dropwise at 0 °C with the formation of hydrogen, followed by the addition of water (10 ml). The THF was then removed by distillation to leave an aqueous solution which was then concentrated *in vacuo*. Methanol (20 ml) and conc. HCl (2 drops) were added to the residue and concentration *in vacuo* removed the methyl borate ester formed. This procedure was repeated twice, after which no more weight loss was recorded. The remaining thick clear oil was taken up in chloroform (25 ml) and triethylamine (5 ml) was added. The resulting solution was stirred for 30 min before being concentrated to leave a white residue. This was taken up in water and extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were then dried, filtered and evaporated to leave a clear oil

(2.80 g, 72%), R_F 0.50 (EtOAc), ν_{max} 2937, 2857, 1737 and 1260 cm^{-1}; δ_H 1.24-1.80 (16H, m, ring CH_2 and $CH_2CH_2CH_2CH_2$), 2.04-2.20 (2H, m, CHN), 2.25 (2H, m, CH_2N), 2.48 (2H, m, CH_2N), 3.06 (4H, m, $NCH_2CH_2CH_2CH_2N$) and 3.71 (6H, s, CO_2CH_3); δ_C 22.5 (t, $NCH_2CH_2CH_2CH_2N$), 24.4, 25.1 and 29.5 (t, ring CH_2), 50.4 (t, CH_2N), 51.4 (q, CO_2CH_3), 56.2 (t, NCH_2RCH_2N), 65.2 (d, CHN) and 174.1 (s, CO_2CH_3); *m/z* 340 (M^+ 19%), 281, 198 (100%), 182, 170, 156, 111 and 98 (Found: M^+ 340.2361. $C_{18}H_{32}N_2O_4$ requires 340.2372).

N,N'-(1,4-Butanediyl)bis(2-hydroxypiperidine) (170)

N,N'-(1,4-Butanediyl)bis(2-methoxycarbonylpiperidine) (2.8 g, 8.1 mmol) in dry THF (20 ml) was added dropwise to a solution of 1M $LiAlH_4$ (36 ml, 36 mmol) at 0 °C under nitrogen. The solution was warmed to room temperature and stirred for 4 h. The flask was then cooled to 0 °C and water : THF (1 : 1, 20 ml) was added dropwise. Both solvents were evaporated *in vacuo* which left a white powder. This was stirred with dichloromethane (30 ml) for 30 min after which the solution was filtered and the filtrate concentrated *in vacuo* to leave a white solid (1.90 g, 83%), m.p.117-119 °C, R_F 0.51 (MeOH), ν_{max} 3126, 2934, 2855, 1374 and 1231 cm^{-1}; δ_H 1.07-1.56 (16H, m, ring CH_2 and $NCH_2CH_2CH_2CH_2N$), 2.02-2.32 (6H, m, CHN and CH_2N), 2.52-2.78 (4H, m, NCH_2RCH_2N), 3.27-3.53 (4H, m, CH_2OH) and 3.67 (2H, bs, OH); δ_C 23.3 (t, $NCH_2CH_2CH_2CH_2N$), 23.5, 24.5 and 27.8 (t, ring CH_2), 51.3 (t, CH_2N), 53.1 (t, NCH_2RCH_2N), 61.0 (d, CHN) and 62.4 (t, CH_2OH); *m/z* 267, 253, 236, 224, 170 (100%), 156, 140 (100%), 128, 112 and 98 (Found: M^+ -4H, 281.2201. $C_{16}H_{28}N_2O_2$ requires M^+ -4H 281.2193)

N,N'-(1,4-Butanediyl)-bis(2-phenylaminocarbonyloxymethyl-piperidine) (171)

N,N'-(1,4-Butanediyl)bis(2-hydroxypiperidine) (0.40 g, 1.4 mmol) and phenyl isocyanate (0.42 g, 3.5 mmol) were reacted according to procedure A for 18 h. Flash chromatography on alumina with ethyl acetate left a white powder which was recrystallised from dichloromethane to afford the product as white crystals (0.63 g, 86%), m.p. 138-140 °C, R_F 0.63 (EtOAc:pet ether, 1:1), ν_{max} 3338, 2852, 1702, 1602, 1540, 1242, 751 and 688 cm^{-1}; δ_H 1.25-1.66 (16H, m, ring CH_2 and $NCH_2CH_2CH_2CH_2N$), 2.20 (2H, m, CHN), 2.45 (4H, m, CH_2N), 2.80 (4H, NCH_2RCH_2N), 4.23 (4H, m, CH_2O), 7.06 (2H, m, *p*-CH), 7.27 (4H, m, *m*-CH) and 7.38 (4H, m, *o*-CH) ; δ_C 23.3 (t, $NCH_2CH_2CH_2CH_2N$), 23.6, 25.4 and 29.0 (t, ring CH_2), 52.1 (t, CH_2N), 53.7 (t, NCH_2RCH_2N), 59.4 (d, CHN), 65.9 (t, CH_2O), 116.5 (d, *o*-CH), 123.3 (d, *p*-CH), 128.9 (d, *m*-CH), 138.0 (s, CNH) and 153.7 (s, C=O); *m/z* 372, 282, 266, 253, 236, 224, 170, 140, 119 (100%) and 98.

_N,N'_-(1,4-Butanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethyl-piperidine) (172)

N,N'-(1,4-Butanediyl)bis(2-hydroxypiperidine) (0.40 g, 1.41 mmol) and *p*-nitrophenyl isocyanate (0.58 g, 3.5 mmol) were reacted according to procedure A for 18 h. Flash chromatography was carried out on alumina, first eluting with ethyl acetate to remove starting material and then with methanol to remove the product.This gave the product as yellow crystals (0.54 g, 63%) R_F 0.56 (EtOAc), m.p. 91-92 °C, ν_{max} 3355, 2936, 2808, 1737, 1600, 1552, 1509, 1332, 1216 and 852 cm^{-1}; δ_H (d_6-acetone) 1.17-1.46 (16H, m, ring CH_2 and $NCH_2CH_2CH_2CH_2N$), 2.15 (2H, m, CHN), 2.40 (4H, m, CH_2N), 2.75 (4H, m, NCH_2RCH_2N), 4.21 (4H, dd, *J* 5 Hz and *J* 11 Hz, CH_2O), 7.77 and 8.17 (8H, d, *J ca.* 9 Hz, AA'BB' system) and 9.45 (2H, bs, NH); δ_C 23.8 (t, $NCH_2CH_2CH_2CH_2N$), 24.6, 26.0 and 29.8 (t, ring CH_2), 52.2 (t, CH_2N), 54.4 (t, NCH_2RCH_2N), 59.9 (d, CHN), 66.6 (t, CH_2O), 118.4 (d, $CHCNH$), 125.5 (d, $CHCNO_2$), 143.1 (s, CNH), 146.4 (s, CNO_2) and 154.1 (s, C=O); *m/z* 261, 253, 236, 196, 170, 164, 140, 138 (100%) and 98.

N,N'-(1,4-Butanediyl)bis(2-chloropiperidine) (173)

N,N'-(1,4-Butanediyl)bis(2-hydroxypiperidine) (0.60 g, 2.11 mmol) was dissolved in dichloromethane (5 ml) and was slowly added to thionyl chloride (15 ml) at 0 °C. The solution was stirred at room temperature for 24 h, after which the solvent was removed. The residue was dissolved in dichloromethane (20 ml) and basified with sodium bicarbonate solution. The organic layer was removed and the aqueous layer was extracted with dichloromethane (2 x 20 ml). The combined extracts were dried, filtered and concentrated to leave a brown oil. Flash chromatography on alumina eluting with ethyl acetate left after evaporation a clear oil (0.52 g, 77%), R_F 0.60 (hexane:EtOAc, 6:1), ν_{max} 2934, 2856, 2792, 1462, 1445 and 738 cm^{-1}; δ_H 0.88-1.55 (16H, m, ring C*H*$_2$ and NCH$_2$C*H*$_2$C*H*$_2$CH$_2$N), 2.20 (2H, m, C*H*N), 2.48-2.72 (8H, m, NC*H*$_2$RC*H*$_2$N and CH$_2$N) and 3.58 (4H, m, C*H*$_2$Cl); δ_C 22.7 (t, NCH$_2$*C*H$_2$*C*H$_2$CH$_2$N), 23.1, 25.1 and 28.7 (t, ring *C*H$_2$), 45.2 (t, *C*H$_2$Cl), 51.1 (t, *C*H$_2$N), 53.1 (t, N*C*H$_2$R*C*H$_2$N) and 60.5 (d, *C*HN); *m/z* 285, 271, 250, 235, 188, 159, 152, 146, 140, 138 and 112 (100%) (Found M^+ -Cl 285.2095. $C_{16}H_{30}N_2Cl$ requires M^+ -Cl 285.2080).

N,N'-(1,4-Butanediyl)-bis(2-phenylaminocarbonyloxymethyl-piperidine) bis-*N*-Oxide (174)

N,N'-(1,4-Butanediyl)-bis(2-phenylaminocarbonyloxymethylpiperidine) (0.38 g, 0.73 mmol) and mCPBA (0.30 g, 1.74 mmol) were reacted according to procedure B for 24 h. This gave the product as white crystals after recrystallisation from acetone (0.30 g, 73%), m.p. 66-68 °C, R_F 0.44 (MeOH), δ_H 1.26-2.16 (16H, m, ring C*H*$_2$ and N$^+$CH$_2$C*H*$_2$C*H*$_2$CH$_2$N$^+$), 2.20 (2H, m, C*H*N$^+$), 3.43-3.67 (8H, m, N$^+$C*H*$_2$RC*H*$_2$N$^+$ and C*H*$_2$N$^+$), 4.02 (2H, m, C*H*N$^+$) 4.21-4.53 (4H, m, C*H*$_2$O), 7.01 (2H, m, *p*-C*H*), 7.21 (4H, m, *m*-C*H*), 7.60 (4H, m, *o*-C*H*) and 9.75 (2H, bs, N*H*); δ_C 21.0 (t,

$N^+CH_2CH_2CH_2CH_2N^+$), 22.5, 25.4 and 29.6 (t, ring CH_2), 55.3 (t, CH_2N^+), 62.0 (t, $N^+CH_2RCH_2N^+$), 64.3 (t, CH_2O), 79.4 (d, CHN^+), 119.8 (d, *o*-CH), 124.3 (d, *p*-CH), 129.8 (d, *m*-CH), 139.6 (s, CNH) and 154.7 (s, C=O); *m/z* 328, 247, 216, 127, 119 and 93 (100%) (Found: C, 61.21; H, 7.20; N, 10.10. $C_{30}H_{42}N_4O_6.2H_2O$ requires C, 61.02; H, 7.79; N, 9.49%).

N,N'-(1,4-butanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethyl-piperidine) bis-*N*-oxide (175)

N,N'-(1,4-Propanediyl)-bis(2-*p*-nitrophenylaminocarbonyloxymethylpiperidine) (0.30 g, 0.49 mmol) and mCPBA (0.20 g, 1.18 mmol) were reacted according to procedure B for 24 h. This gave the product as yellow crystals after recrystallisation from acetone (0.16 g, 55%), m.p. 117-119 °C, ν_{max} 3422, 2950, 1734, 1597, 1560, 1508, 1332, 1222, 1070 and 853 cm^{-1}; δ_H (CD_3OD) 1.48-2.21 (16H, m, ring CH_2 and $N^+CH_2CH_2CH_2CH_2N^+$), 3.42-3.85 (8H, m, N^+CH_2), 4.15 (2H, m, CHN^+), 4.67 (4H, m, CH_2O), 7.64 (4H, m, CH) and 8.11 (4H, m, CH); δ_C 20.9 (t, $N^+CH_2CH_2CH_2CH_2N^+$), 22.0, 23.6 and 25.7 (t, ring CH_2), 63.8 (t, CH_2N^+), 64.8 (t, $N^+CH_2RCH_2N^+$), 69.6 (t, CH_2O), 72.3 (d, CHN), 118.9 (d, CHCNH), 125.8 (d, $CHCNO_2$), 143.8 (s, CNH), 146.5 (s, CNO_2) and 154.1 (s, C=O); *m/z* 315, 278, 252, 186, 156, 164, 138, 114 and 90 (100%) (Found: C, 56.20; H, 6.81; N, 12.97. $C_{30}H_{40}N_6O_{10}$ requires C, 55.90; H, 6.21; N, 13.04%).

N,N'-(1,4-Butanediyl)bis(2-chloropiperidine) bis-*N*-oxide (176)

N,N'-(1,4-Butanediyl)bis(2-chloropiperidine) (0.23 g, 0.74 mmol) and mCPBA (0.43 g, 1.84 mmol) were reacted according to procedure B for 24 h. This gave the product as a yellow oil (0.20g, 76%), R_F 0.63 (MeOH), ν_{max} 2955, 1448, 978 and 718 cm^{-1}; δ_H (CD_3OD) 1.63-2.10 (16H, m, ring CH_2 and $N^+CH_2CH_2CH_2CH_2N^+$), 2.71 (2H, m, CHN^+), 3.74-4.20 (8H, m, N^+CH_2) and 4.43-4.84 (4H, m, CH_2Cl); δ_C 20.7 (t,

$N^+CH_2CH_2CH_2CH_2N^+$), 22.7 27.0 and 28.7 (t, ring CH_2), 41.1 (t, CH_2Cl), 66.2 (t, CH_2N^+), 73.3 (t, $N^+CH_2RCH_2N^+$), 75.0 (d, CHN^+); *m/z* 337, 237, 235, 221, 196, 187, 156, 138, 128 and 100 (100%) (Found: M^+ -2Cl, $-CH_2$ and -O 251.2079. $C_{15}H_{26}N_2O$ requires 251.2085).

Experimental to Chapter 7

N-Acetylethane-1,2-diamine (182)

Ethylenediamine (5.0 g, 83 mmol) was dissolved in ethyl acetate (50 ml) and PPL (5.0 g) was added. The flask was shaken at 100 rpm in a temperature controlled water bath at 25 °C. Portions were taken out at intervals to follow the reaction by TLC. After 3 d, the reaction solvent was decanted from the flask and retained. Chloroform:methanol (9:1) (2 ml) was added to the enzyme which was stirred for 1 h at room temperature. This broke up the enzyme (which had clumped together) which was filtered through Celite and the combined extracts and solvent was dried over sodium sulfate, filtered and concentrated to yield an oil (5.8 g, 68%), δ_H (90 MHz) 2.0 (3H, s, CH_3), 2.75 (2H, m, CH_2NH_2) and 3.2 (2H, m, CH_2NH).

N'-Acetyl-*N*,*N*-bis(2-hydroxyethyl)ethane-1,2-diamine[162] (183)

Ethylene oxide (2.3 ml, 49 mmol) was collected in a measuring cylinder at -30 °C. This was added to a flask containing *N*-acetylethane-1,2-diamine (2.0 g, 19.6 mmol) in methanol (25 ml) at -30 °C. This solution was stirred for 18 h. at room temperature after which the solution was heated at 70 °C for 6 h. The solvent was then removed to leave a thick oil which was distilled to leave the product as a clear oil (2.86 g, 72%), δ_H (90 MHz) (CD_3OD) 1.80 (3H, s, CH_3), 2.5 (6H, m, NCH_2), 3.1 (2H, m, CH_2NH) and 3.5 (4H, m, CH_2OH); *m/z* 190 (M^+ 0.4%), 174, 159, 147, 130, 118 (94%) and 74.

N,*N*-Bis(2-hydroxyethyl)ethane-1,2-diamine (184)

N'-Acetyl-*N*,*N*-bis(2-hydroxyethyl)ethane-1,2-diamine (2.8 g, 15 mmol) and conc. HCl (5 ml) in water (2 ml) were heated together at reflux for 6 h. This solution was added to an ethanolic solution of NaOH (0.6 g, 15 mmol). After 30 min. stirring, the solution was

diluted with acetone to precipitate sodium chloride, filtered and concentrated to leave a thick, dark oil (1.76 g, 80%), δ_H (D_2O) 2.50 (8H, m, CH_2N) and 3.45 (4H, m, CH_2OH); δ_C 38.5 (t, CH_2NH_2), 56.4 (t, CH_2N) and 59.7 (t, CH_2OH).

t-Butoxycarbonylaminoethanoic acid (187)

To a stirred solution of glycine (5.0 g, 66 mmol), in distilled triethylamine (8.0 g, 80 mmol), was added a mixture of BOC-ON (19.7 g, 80 mmol) in 1,4-dioxane (75 ml) and water (75 ml). The solution quickly became homogeneous and was left to stir for 2 h at room temperature. The solution was then washed with ethyl acetate (5 x 40 ml) followed by acidification with 5% citric acid. The aqueous layer was then extracted with ethyl acetate (3 x 30 ml) and the organic extracts were dried, filtered and concentrated to leave the product as white crystals (11.5 g, 100%), m.p. 86 °C (lit, 87-88 °C), R_F 0.25 (EtOAc), ν_{max} 3408, 940, 2618, 2537, 1749, 1669, 1540, 1450 and 1368 cm^{-1}; δ_H 1.45 (9H, s, $C(CH_3)_3$), 3.91 (2H, dd, *J* 5 Hz and *J* 13 Hz, $NHCH_2$), 5.40 (1H, t, NH) and 11.08 (1H, bs, OH); δ_C 28.2 (q, $C(CH_3)_3$), 42.1 and 43.3 (t, $NHCH_2$), 80.3 and 81.8 (s, $C(CH_3)_3$), 156.1 and 157.4 (s, NHCO), 173.9 and 174.4 (s, CO_2H); *m/z* 170, 120, 101, 76, 59 and 57 (100%) (Found: C, 48.02; H, 7.64; N, 8.12. $C_7H_{13}NO_4$ requires C, 48.00; H, 7.43; N, 8.00%).

N'-t-Butoxycarbonylaminoethanamide (188)

t-Butoxycarbonylaminoethanoic acid (5.0 g, 28.6 mmol) and distilled triethylamine (3.46 g, 34.3 mmol) were cooled to -5 °C in distilled acetonitrile (100 ml). Added dropwise to this was *i*-butylchloroformate (4.70 g, 34.3 mmol) whilst stirring. Ammonia gas was bubbled through the solution for 5 min in which time the precipitate turned from yellow to white. The solution was then stirred for 2 h at room temperature. The solution was then concentrated to leave a solid yellow residue which was taken up in water (30 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were then dried, filtered

and concentrated to leave a white solid which was crystallised from ethyl acetate (4.22 g, 84%), δ_H 1.44 (9H, s, C(CH_3)$_3$), 3.79 (2H, d, *J* 5 Hz, CH_2NH), 5.90 (1H, bs, N*H*) and 6.72 and 6.84 (2H, bs, NH_2); δ_C 28.2 (q, C(CH_3)$_3$), 43.6 (t, CH_2NH), 79.9 (s, C(CH$_3$)$_3$), 156.2, (s, O*C*ONH) and 173.1 (s, N*C*OCH$_2$); *m/z* 130, 101, 59 and 57 (100%).

Aminoethanamide Hydrochloride (189)

t-Butoxycarbonylaminoethanamide (4.0 g, 23 mmol) was dissolved in 3M HCl/EtOAc (50 ml). After 1 h the solution was concentrated to leave a solid residue. This was washed with ethanol and filtered to leave white crystals of the hydrochloride salt (2.40 g, 95%), m.p. 180 °C, δ_H 3.75 (CH_2); δ_C 41.1 (t, CH_2) and 170.3 (s, *C*=O); *m/z* 74 (M^+ 100%) and 57 (Found: M^+ 74.0465, C, 21.79; H, 6.73; N, 25.16. $C_2H_6N_2O$.HCl requires M^+ 74.0479, C, 21.72; H, 6.33; N, 25.34).

***N',N'*-bis(2-hydroxyethyl)aminoethanamide (190)**

Aminoethanamide hydrochloride (1.35 g, 12.2 mmol) was dissolved in methanol (10 ml) and sodium bicarbonate was added. The solution was stirred for 30 min before the solution was filtered. The filtrate was concentrated to leave a solid residue which was extracted with ethanol. This solution was filtered and the filtrate was cooled to -30 °C. Ethylene oxide (5 ml) was added to the filtrate and the resultant mixture was stirred for 24 h at room temperature. The solution was concentrated to leave a very thick clear oil (1.62 g, 82%), δ_H (CD_3OD) 2.85 (4H, m, NCH_2CH$_2$OH), 3.40 (2H, m, CH_2CO) and 3.75 (4H, m, CH_2OH); δ_C 42.8 (t, CH_2CO), 59.3 (t, CH_2N), 61.5 (t, CH_2OH) and 174.6 (s, *C*=O).

N',N'-bis(2-*t*-Butyldimethylsilyloxyethyl)aminoethanamide (191)

A solution of the diol (1.8 g, 11 mmol), imidazole (3.32 g, 48 mmol) and TBDMSiCl (4.2 g, 27.7 mmol) in dry DMF (5 ml) was stirred at room temperature for 18 h under nitrogen. Water (20 ml) was added and the aqueous solution was extracted with ether (3 x 25 ml). The combined organic extracts were washed with brine (6 x 25 ml), dried, filtered and evaporated to leave the product as a clear oil (4.2 g, 97%), δ_H (90 MHz) 0.0 (12 H, s, $SiCH_3$), 1.60 (18H, s, $C(CH_3)_3$), 2.6 (4H, m, CH_2N), 3.0 (2H, m, CH_2CO) and 3.5 (4H, m, CH_2O).

N',N'-Bis(2-hydroxyethyl)ethane-1,2-diamine Dihydrochloride (192)

N',N'-bis(2-*t*-Butyldimethylsilyloxyethyl)aminoethanamide (4.2 g, 10.7 mmol) was placed in a dry flask under nitrogen. Dry THF (10 ml) was added to form a slurry which was then cooled to 0 °C. 1 M Borane-THF (21 ml, 21 mmol) was added dropwise over 10 min in which time the solution became clear. The solution was then heated at reflux for 2 h. The reaction was then quenched with 6M HCl (5 ml) followed by water (10 ml). The THF was removed by distillation and the remaining solution was concentrated to leave a white residue. This was dissolved in methanol (10 ml) and conc HCl (2 drops) which was subsequently evaporated *in vacuo* to remove the methyl borate formed. This procedure was repeated twice until no more weight loss was recorded. The residue was then dissolved in ethanol to which sodium bicarbonate was added. This solution was filtered and the filtrate was concentrated to give the product as a thick clear oil (1.0 g, 64%), δ_H 2.6 (8H, m, CH_2N), and 3.5 (4H, m, CH_2O); δ_C 38.5 (t, CH_2NH_2), 56.4 (t, CH_2N) and 59.8 (t, CH_2OH).

N'-t-Butoxycarbonylamino*N,N*-bis(2-chloroethyl)ethanamide (194)

t-Butoxycarbonylaminoethanoic acid (2.0 g, 11.4 mmol) and distilled triethylamine (1.70 g, 17.1 mmol) were cooled to -5 °C in distilled acetonitrile. Added dropwise to this was *i*-butylchloroformate (2.06 g, 15 mmol) whilst stirring. After a white precipitate formed, bis-(2chloroethyl)amine hydrochloride (2.0 g, 11.4 mmol) was added. Triethylamine (1.7 g, 17.1 mmol) was then added dropwise and the solution was stirred for 2 h at room temperature. The solution was then concentrated to leave a solid yellow residue which was taken up in water (30 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were then dried, filtered and concentrated to leave a yellow oil. After flash chromatography on alumina using ethyl acetate the product was left as a clear oil (2.6 g, 76%), R_F 0.55 (EtOAc:hexane 1:1), ν_{max} 3399, 2966, 1751, 1702, 1368 and 700 cm^{-1}; δ_H 1.45 (9H, s, $C(CH_3)_3$), 3.69 (8H, t, *J* 5 Hz, NCH_2CH_2Cl), 4.05 (2H, d, *J* 5 Hz, $NHCH_2$) and 5.61 (1H, t, NH); δ_C 28.2 (q, $C(CH_3)_3$), 41.1 (t, CH_2Cl), 43.6 (t, NCH_2CH_2Cl), 49.5 ($NHCH_2$), 79.5 (s, $C(CH_3)_3$), 155.6 (s, OCONH) and 169.3 (s, $NCOCH_2$); *m/z* 263, 229, 207, 175, 171, 146, 133, 105 and 92 (100%) (Found: M^+ -Cl 263.1155. $C_{11}H_{20}N_2O_3Cl$ requires 263.1162).

2-Amino-*N,N*-Bis(2-chloroethyl)ethanamide Trifluoroacetate (195)

N'-t-Butoxycarbonylamino*N,N*-bis(2-chloroethyl)ethanamide (2.0 g, 6.7 mmol) was dissolved in dry dichloromethane (25 ml) under nitrogen and trifluoroacetic acid (5 ml) was added dropwise at 0 °C. The solution was allowed to return to room temperature and left to stir for 1 h. The solution was concentrated to a thick oil and triturated with diethyl ether. White crystals precipitated immediately and were filtered and air dried (1.98 g, 95%), m.p. 117-118 °C, ν_{max} 3155, 1685, 1626, 1527 and 722 cm^{-1}; δ_H (D_2O) 3.39 (4H, m, NCH_2CH_2Cl), 3.74 (4H, m, CH_2Cl) and 4.44 (2H, m, $COCH_2$); δ_C 39.9 and 40.9 (t, CH_2Cl), 46.8 and 49.8 (t, NCH_2CH_2Cl), 62.1 (t, $COCH_2$) and 166.5 (s, C=O);

m/z 198 (M^+ 0.3%), 165,163, 147, 126, 106 and 92 (100%) (Found: M^+ 198.0340. $C_6H_{12}N_2OCl_2$ requires M^+ 198.0327).

N,N-Bis(2-chloroethyl)ethane-1,2-diamine Dihydrochloride[160] (185)

2-Amino-*N,N*-Bis(2-chloroethyl)ethanamide trifluoroacetate (1.5 g, 4.8 mmol) was placed in a dry flask under nitrogen. Dry THF (10 ml) was added to form a slurry which was then cooled to 0 °C. 1 M Borane-THF (20 ml) was added dropwise over 10 min in which time the solution became clear. The solution was then heated at reflux for 2 h. The reaction was quenched with 6M HCl (5 ml) followed by water (10 ml). The THF was removed by distillation and the remaining solution was concentrated to leave a white residue. This was dissolved in methanol (10 ml) and conc HCl (2 drops) which was subsequently evaporated *in vacuo* to remove the methyl borate ester formed. This procedure was repeated twice until no more weight loss was recorded. The residue was then crystallised from ethanol to give the product (0.9 g, 90%), m.p. 135-137 °C, ν_{max} 2954, 2514, 1638, 1450 and 763 cm^{-1}; δ_H 3.40 (2H, m, CH_2NH_2), 3.62 (6H, m, CH_2N) and 3.88 (4H, m, CH_2Cl); δ_C 36.0 (t, CH_2NH_2), 39.7 (t, CH_2Cl), 52.4 (t, CH_2NR_2) and 57.4 (t, NCH_2CH_2Cl); *m/z* 158, 154 (100%), 142, 120, 112, 106 and 92 (Found: C, 27.73; H, 5.27; N, 10.59. $C_6H_{14}N_2Cl_2.2HCl$ requires C, 27.90; H, 5.42; N, 10.85%).

N,N'-Bis(2-chloroethyl)ethane-1,2-diamine Dihydrochloride[163] (197)

N,N'-Bis(2-hydroxyethyl)ethane-1,2-diamine (0.5 g, 3.4 mmol) was added slowly to thionyl chloride (10 ml) at 0 °C. The resulting slurry was left to stir for 2 h at room temperature. Excess thionyl chloride was removed *in vacuo* and the residue was washed with methanol to obtain white crystals of the dihydrochloride salt (0.65 g, 74%), m.p. 138-139 °C, ν_{max} 2924, 2478, 1638, 1446 and 773 cm^{-1}; δ_H 3.24 (4H, m, NCH_2CH_2Cl), 3.49 (4H, s, CH_2NHR) and 3.81 (4H, m, CH_2Cl); δ_C 43.9 (t, CH_2Cl),

50.7 (t, CH_2NHR), and 57.4 (t, NCH_2CH_2Cl); *m/z* 159, 149, 128, 117, 104 and 74 (100%) (Found: C, 27.81; H, 5.52; N, 10.66. $C_6H_{14}N_2Cl_2.2HCl$ requires C, 27.90; H, 5.42; N, 10.85%).

t-Butoxycarbonylaminopropanoic acid (199)

To a stirred solution of β-alanine (4.50 g, 50 mmol), in distilled triethylamine (8.0g, 80 mmol), was added a mixture of BOC-ON (13.7 g, 54 mmol) in 1,4-dioxan (75 ml) and water (75 ml). The solution quickly became homogeneous and was left to stir for 2 h at room temperature. The solution was then washed with ethyl acetate (5 x 40 ml) followed by acidification with 5% citric acid. The aqueous layer was then extracted with ethyl acetate (3 x 30 ml) and the organic extracts were dried, filtered and concentrated to leave the product as white crystals (8.0 g, 90%), m.p. 72-74 °C, R_F 0.28 (EtOAc), ν_{max} 3443, 2940, 2633, 2575, 1709, 1649, 1560, 1458 and 1365 cm^{-1}; δ_H 1.44 (9H, s, $C(CH_3)_3$), 2.57 (2H, t, *J* 5.5 Hz, CH_2CO_2H), 3.40 (2H, q, *J* 5.5 Hz, $NHCH_2$), 5.28 (1H, t, NH) and 10.44 (1H, bs, OH); δ_C 28.2 (q, $C(CH_3)_3$), 34.3 and 35.8 (t, $NHCH_2CH_2$), 79.6 (s, $C(CH_3)_3$), 156.0 (s, NHCO), 177.1 (s, CO_2H); *m/z* 190, 133, 116, 98, 90, 70 and 57 (Found: C, 50.58; H, 7.92; N, 7.27. $C_8H_{15}NO_4$ requires C, 50.79; H, 7.94; N, 7.41%).

N'-t-Butoxycarbonylamino*N,N*-bis(2-chloroethyl)propanamide (200)

t-Butoxycarbonylaminopropanoic acid (5.0 g, 26.6 mmol) and distilled triethylamine (3.05 g, 30 mmol) were cooled to -5 °C in distilled acetonitrile. Added dropwise to this was *i*-butylchloroformate (4.12 g, 30 mmol) whilst stirring. After a white precipitate formed, bis-(2-chloroethyl)amine hydrochloride (4.75 g, 26.6 mmol) was added. Triethylamine (3.0 g, 30 mmol) was then added dropwise and the solution was stirred for 2 h at room temperature. The solution was then concentrated to leave a solid yellow residue which was taken up in water (30 ml) and extracted with ethyl acetate (3 x 30 ml).

The combined extracts were then dried, filtered and concentrated to leave a yellow oil. After flash chromatography on alumina using ethyl acetate the product was left as white crystals (6.2 g, 74%), m.p. 100-102 °C, R_F 0.70 (EtOAc:pet. ether, 1:1), ν_{max} 3370, 2977, 1740, 1690, 1367 and 670 cm^{-1}; δ_H 1.42 (9H, s, $C(CH_3)_3$), 2.63 (2H, t, $NHCH_2$) 3.48 (8H, t, NCH_2CH_2Cl), 4.04 (2H, d, $NCOCH_2$) and 5.45 (1H, t, NH); δ_C 28.4 (q, $C(CH_3)_3$), 35.0 (t, $NHCH_2$) 36.3 (t, CH_2Cl), 38.8 (t, NCH_2CH_2Cl), 49.0 (t, $NCOCH_2$), 79.5 (s, $C(CH_3)_3$), 156.2 (s, OCONH) and 171.9 (s, $NCOCH_2$); *m/z* 221, 185, 131, 105 92 (100%) and 88 (Found: M^+ -(-CH_3)$_3$C and -Cl 221.0676. $C_8H_{13}N_2O_3Cl$ requires 221.0686).

2-Amino-*N,N*-Bis(2-chloroethyl)propanamide (201)

N'-t-Butoxycarbonylamino*N,N*-bis(2-chloroethyl)propanamide (2.0 g, 6.4 mmol) was dissolved in dry dichloromethane (25 ml) under nitrogen and trifluoroacetic acid (5 ml) was added dropwise at 0 °C. The solution was allowed to return to room temperature and left to stir for 1 h. The solution was concentrated to a thick oil and was dissolved in dichloromethane (20 ml). Sodium bicarbonate solution was added untill there was no more effervescing. The organic layer was removed and the aqueous layer was extracted twice more with dichloromethane (20 ml). The combined organic extracts were dried, filtered and concentrated to leave a clear oil (1.30 g, 95%), R_F 0.56 (EtOAc), ν_{max} 3430, 2972, 1682, 1434 and 722 cm^{-1}; δ_H 3.70 (10H, m, NCH_2CH_2Cl and CH_2NH_2) and 4.37 (2H, t, *J* 8 Hz, CH_2CON); δ_C 41.7 (t, CH_2Cl), 45.2 (t, CH_2NH_2), 45.9 (t, NCH_2CH_2Cl), 62.0 (t, CH_2CON) and 158.3 (s, C=O); *m/z* 178, 170, 168, 142, 141, 114, 86 and 72 (Found: M^+ -Cl 178.0795. $C_7H_{14}N_2OCl$ requires 178.0798).

***N,N*-Bis(2-chloroethyl)propane-1,3-diamine Dihydrochloride (202)**

2-Amino-*N,N*-Bis(2-chloroethyl)propanamide (1.0 g, 4.7 mmol) was placed in a dry flask under nitrogen. Dry THF (10 ml) was added to form a slurry which was then

cooled to 0 °C. 1 M Borane-THF (15 ml, 15 mmol) was added dropwise over 10 min in which time the solution became clear. The solution was then heated at reflux for 2 h. The reaction was then quenched with 6M HCl (5 ml) followed by water (10 ml). The THF was removed by distillation and the remaining solution was concentrated to leave a white residue. This was dissolved in methanol (10 ml) and conc. HCl (2 drops) which was subsequently evaporated *in vacuo* to remove the methyl borate ester formed. This procedure was repeated twice until no more weight loss was recorded. The residue was then crystallised from ethanol to give the product (1.0 g, 80%), m.p. 152-153 °C, ν_{max} 2953, 2515, 1643, 1454 and 674 cm^{-1}; δ_H 1.80 (2H, $NH_2CH_2C\mathit{H}_2CH_2N$), 3.07, 3.25 (2H, m, $C\mathit{H}_2NH_2$), 3.53 and 3.67 (8H, m, $NC\mathit{H}_2CH_2Cl$) and 3.93 (4H, m, $C\mathit{H}_2Cl$); δ_C 29.9 (t, $NCH_2\mathit{C}H_2CH_2N$), 37.9 (t, $\mathit{C}H_2NH_2$), 40.1 (t, $\mathit{C}H_2Cl$), 49.4 (t, $\mathit{C}H_2NR_2$) and 57.3 ($N\mathit{C}H_2CH_2Cl$); *m/z* 183, 170, 127, 113, 100, 88 (100%) and 86.

_t_-Butoxycarbonylaminodiacetic acid (204)

To a stirred solution of iminodiacetic acid (2.0 g, 15 mmol), in distilled triethylamine (4.0 g, 40 mmol), was added a mixture of BOC-ON (4.93 g, 20 mmol) in 1,4-dioxane (75 ml) and water (75 ml). The solution became homogeneous and was left to stir for 18 h at room temperature. The solution was then washed with ethyl acetate (5 x 40 ml) followed by acidification with 5% citric acid. The aqueous layer was then extracted with ethyl acetate (6 x 30 ml) and the organic extracts were dried, filtered and concentrated to leave a residue which was crystallised from dichloromethane/hexane to leave white crystals (2.1 g, 60%), m.p. 132-134 °C, ν_{max} 3413, 2947, 2657, 1731, 1661, 1465, 1369 and 1248 cm^{-1}; δ_H (D_2O) 1.20 (9H, s, $C\mathit{H}_3C$) and 3.86 (4H, d, *J* 3 Hz, $C\mathit{H}_2N$); δ_C 28.2 (q, $\mathit{C}H_3C$), 50.7 (t, $\mathit{C}H_2N$), 83.8 (s, $CH_3\mathit{C}$), 157.5 (s, $N\mathit{C}O$) and 174.7 ($\mathit{C}OOH$); *m/z* 177 (1.2%), 172, 161, 144, 133, 116 and 89(100%) (Found: M^+ $-C(CH_3)_3$ 177.0273. $C_5H_6NO_6$ requires M^+ 177.0273).

_N'_-bis(2-chloroethyl)-N''-bis(2-chloroethyl)-_t_-butoxycarbonylamino-diethanamide (205)

_t_-Butoxycarbonylaminodiacetic acid (1.75 g, 7.5 mmol) and distilled triethylamine (1.80 g, 18 mmol) were cooled to -5 °C in distilled acetonitrile. Added dropwise to this was _i_-butylchloroformate (2.46 g, 18 mmol) whilst stirring. After a white precipitate formed, bis(2-chloroethyl)amine hydrochloride (2.8 g, 15 mmol) was added. Triethylamine (1.80 g, 18 mmol) was then added dropwise and the solution was stirred for 2 h at room temperature. The solution was then concentrated to leave a solid yellow residue which was taken up in water (30 ml) and extracted with ethyl acetate (3 x 30 ml). The combined extracts were then dried, filtered and concentrated to leave a yellow oil. After flash chromatography on alumina using ethyl acetate the product was left as white crystals (1.87 g, 52%), R_F 0.43 (EtOAc), ν_{max} 3390, 2968, 1755, 1698, 1368, 1253 and 733 cm^{-1}; δ_H 1.36 (9H, s, CH_3C), 3.43 (8H, m, NCH_2CH_2Cl), 3.62-3.83 (8H, m, CH_2Cl) and 4.23 (4H, s, NCH_2CO); δ_C 28.1 and 28.2 (q, CH_3C), 38.1, 38.2 and 38.5 (CH_2Cl), 41.4 (t, NCH_2CO), 46.3, 48.4, 48.9 and 49.9 (t, NCH_2CH_2Cl), 81.2 (s, $(CH_3)_3C$), 155.2 and 155.3 (s, NCOO) and 169.5 and 169.9 (s, $NCOCH_2$);

_N'_-bis(2-chloroethyl)-N''-bis(2-chloroethyl)diethanamide (206)

_N'_-bis(2-chloroethyl)-N''-bis(2-chloroethyl)-_t_-butoxycarbonylaminodiethanamide (2.0 g, 4.2 mmol) was dissolved in dry dichloromethane (25 ml) under nitrogen and trifluoroacetic acid (5 ml) was added dropwise at 0 °C. The solution was allowed to return to room temperature and was left to stir for 1 h. The solution was concentrated to a thick oil and basified with $NaHCO_3$ solution. The aqueous layer was then extracted with ethyl acetate (3 x 30 ml) and the combined extracts were dried, filtered and concentrated to leave an oil (1.15 g, 72%), R_F 0.52 (EtOAc : hexane, 1:1), ν_{max} 3395, 2967, 1677 and 722 cm^{-1}; δ_H 3.72 (16H, m, NCH_2CH_2Cl), 4.34 (4H, d, _J_ 3 Hz, CH_2NH) and 9.87 (1H, bs, NH); δ_C 41.2 and 41.4 (t, CH_2Cl), 48.8, 48.9, 49.8 and 49.9 (t,

NCH_2CH_2Cl), 72.3 (t, CH_2NH) and 169.0 and 169.5 (s, C=O); *m/z* 216, 215, 194, 192, 136 and 92 (100%).

N-Bis(2-chloroethyl)aminoethyl-*N'*-bis(2-chloroethyl)(ethane-1,2-diamine Trihydrochloride (207)

N'-bis(2-chloroethyl)-N''-bis(2-chloroethyl)diethanamide (0.5 g, 1.3 mmol) was placed in a dry flask under nitrogen. Dry THF (10 ml) was added to form a slurry which was then cooled to 0 °C. 1 M Borane-THF (10 ml, 10 mmol) was added dropwise over 10 min in which time the solution became clear. The solution was then heated at reflux for 2 h. The reaction was then quenched with 6M HCl (5 ml) followed by water (10 ml). The THF was removed by distillation and the remaining solution was concentrated to leave a white residue. This was dissolved in methanol (10 ml) and conc. HCl (2 drops) which was subsequently evaporated *in vacuo* to remove the methyl borate ester formed. This procedure was repeated twice until no more weight loss was recorded. The residue was then crystallised from ethanol to give the product (0.48 g, 80%), m.p.> 300 °C, ν_{max} 2955, 1643, 1462 and 668 cm^{-1}; δ_H (D_2O) 3.05 (4H, m, CH_2NH), 3.33 and 3.54 (12H, m, CH_2N) and 3.68 (8H, m, CH_2Cl); δ_C 37.6 and 38.0 (t, CH_2Cl), 39.3 and 40.0 (t, CH_2NH), 49.1 and 49.3 ($NHCH_2CH_2NR$) and 55.7 and 55.8 (t, NCH_2CH_2Cl); *m/z* 356 (M^+ 3.8%), 354, 283, 255, 248, 226, 212, 199, 184 and 169 (Too involatile for high res. MS).

N,N'-[Bis(2-chloroethyl)aminocarbonylmethyl]oxalamide (208)

2-Amino-*N,N*-bis(2-chloroethyl)ethanamide trifluoroacetate (1.0 g, 3.2 mmol) was dissolved in chloroform (20 ml) and distilled triethylamine (0.7 g, 7 mmol) was added. The solution was then cooled to -5 °C and oxalyl chloride (0.14 ml, 1.6 mmol) was added dropwise. The solution was left to stir at room temperature for 4 h before the solvent was removed *in vacuo* . The residue was dissolved in water and was extracted

with ethyl acetate (3 x 30 ml). The combined extracts were dried, filtered and concentrated to leave a clear oil (0.65 g, 90%), R_F 0.60 (EtOAc), ν_{max} 3378, 2976, 2874, 1732, 1682, 1514 and 1454 cm^{-1}; δ_H 3.76 (16H, m, NCH_2CH_2Cl), 4.27 (4H, m, $NHCH_2$) and 7.69 (2H, bs, NH); δ_C 41.0, 41.1, 41.2 and 41.4 (t, CH_2Cl), 48.48 and 49.5 (t, NCH_2CH_2Cl), 63.4 (t, $NHCH_2CO$), 156.6, 159.7 (s, NHCOCONH) and 167.4 and 167.9 (s, CH_2CON); *m/z* 336, 325, 263, 227, 225, 168, 142, 126, 106, 92 and 63 (100%) (Found: M^+ (1/2 molecule), 227.0642. $C_7H_{11}N_2O_2Cl_2$ requires 227.0640).

Bis[*N,N'*-bis(2-chloroethyl)aminoethyl]ethane-1,2-diamine Tetrahydrochloride (209)

N,N'-[Bis(2-chloroethyl)aminocarbonylmethyl]oxalamide (2.12 g, 4.7 mmol) was placed in a dry flask under nitrogen. Dry THF (10 ml) was added to form a slurry which was then cooled to 0 °C. 1 M Borane-THF (40 ml, 40 mmol) was added dropwise over 10 min in which time the solution became clear. The solution was then heated at reflux for 2 h. The reaction was then quenched with 6M HCl (5 ml) followed by water (10 ml). The THF was removed by distillation and the remaining solution was concentrated to leave a white residue. This was dissolved in methanol (10 ml) and conc. HCl (2 drops) which was subsequently evaporated *in vacuo* to remove the methyl borate ester formed. This procedure was repeated twice until no more weight loss was recorded. The residue was then crystallised from methanol to give the product (2.20 g, 86%), m.p.192-194 °C, ν_{max} 2968, 2450 and 680 cm^{-1}; δ_H (D_2O) 3.04 (4H, m, $NHCH_2CH_2NH$), 3.27 (4H, m, $NHCH_2CH_2NR$), 3.65 (12H, m, CH_2N) and 3.86 (8H, m, CH_2Cl); δ_C 37.9 (t, CH_2Cl), 42.4 and 44.3 (NCH_2CH_2N), 49.3 and 49.5 (t, CH_2NH), 50.3 and 51.7 (t, CH_2N) and 55.8 (t, NCH_2CH_2Cl); *m/z* 322, 204, 163, 161, 154, 143, 127 and 118 (Found: C, 28.07; H, 6.67; N, 8.81. $C_{14}H_{26}N_4Cl_4.4HCl.4H_2O$ requires C, 27.36; H, 6.84; N, 9.12%. M^+ (-2Cl) 322.1650. $C_{14}H_{26}N_4Cl_2$ requires 322.1661,).

Sodium Hexanitrocobaltate(III)[168] (212)

A solution of sodium nitrite (15 g) in water (15 ml) was cooled to 50-60 °C and cobalt nitrate hexahydrate (5 g) was added, followed by 50% acetic acid (5 ml) in small portions with stirring. A fast stream of air was then bubbled through the solution for 30 min. After standing for 2 h the brown precipitate was filtered off to leave a clear solution. The precipitate was then stirred with water (5 ml) at 70-80 °C and filtered again. The two filtrates were combined and treated with 96% methanol (25 ml). After 2 h, the resulting precipitate was filtered, washed with methanol (4 x 25 ml) then diethyl ether (2 x 20 ml) and air dried to leave a yellow powder (6.0 g, 86%), m.p. 215 °C (dec), ν_{max} 3423, 1423, 1396, 1333, 1116, 847 and 832 cm^{-1}; *m/z* 269, 239, 149, 105, 97, 81, 71 and 46.

Sodium Bis(3-methyl-2,4-pentanedionato)dinitrocobaltate(III) Hydrate[160] (213)

Sodium hexanitrocobaltate(III) (3.27 g, 8.1 mmol) was dissolved in water (11 ml) and added to a mixture of NaOH (0.7 g, 17.5 mmol) and 3-methyl-2,4-pentanedione (2.0 g, 17.5 mmol) in water (11 ml) which had been cooled in an ice bath. Rapid formation of red-brown crystals occurred in 10 min, and after cooling at 5 °C for 12 h these were collected by filtration and washed with acetone (20 ml) and diethyl ether (20 ml) (2.90 g, 75%). This was recrystallised by dissolving 1 g in water (35 ml) and filtering into sodium nitrite solution (5 g in 15 ml water). The resulting crystalline product was washed with EtOH/acetone (2:1) and dried in air, m.p. 175 °C (dec), ν_{max} 3457, 1562, 1443, 1359, 1328, 1298 and 1181 cm^{-1}; δ_H (D_2O) 1.90 (6H, s, CH_3C) and 2.23 (12H, s, CH_3CO); δ_C 15.40 (q, CH_3C), 26.3 (q, CH_3CO), 100.3 (s, CCH_3) and 187.4 (C=O); *m/z* 285, 270, 242, 172, 143, 114, 99 and 72.

Bis(3-methyl-2,4-pentanedionato)(*N,N*-bis(2-chloroethyl)ethane-1,2-diamine)cobalt(III) Hexafluorophosphate[160] (214)

Sodium bis(3-methyl-2,4-pentanedionato)dinitrocobaltate(III) hydrate (0.37 g, 0.87 mmol) was dissolved in a mixture of methanol (6 ml) and water (6 ml). Activated charcoal (0.1 g) was added to the solution followed by *N,N*-bis(2-chloroethyl)ethane-1,2-diamine dihydrochloride (0.25 g, 1 mmol) in methanol (2 ml) and water (2 ml). A solution of NaOH (0.08 g, 2 mmol) in water (4 ml) was added dropwise to the cobalt solution and the mixture was stirred for 1 h. The charcoal was filtered off through Celite and washed with methanol which was added to the filtrate. Ammonium hexafluorophosphate (0.3 g) was added to the filtrate which was then extracted with chloroform (3 x 25 ml). The combined extracts were concentrated to leave a green residue which was taken up in water (6 ml). Methanol (6 ml) was then added and the solution was left open to the air for slow evapouration of the methanol. After 1 week, the resulting green crystals were collected by filtration and washed with 20% methanol/water, water and ether, m.p. 82-83 °C, ν_{max} 2926, 1569, 1426, 1339, 1299, 843 and 737 cm^{-1}; δ_H 1.86, 1.92, 1.96 and 2.07 (12H, s, CH_3CO), 2.23 and 2.32 (6H, s, CH_3C), 2.70 (2H, m, CH_2NR_2), 2.97 (4H, m, NCH_2CH_2Cl), 3.62 (4H, m, CH_2Cl), 3.84 (2H, m, CH_2NH_2) and 4.06 (2H, bs, NH_2); δ_C 14.6 and 14.9 (q, CH_3C), 25.6, 26.2, 26.3 and 26.4 (q, CH_3CO), 36.0 and 38.2 (t, CH_2Cl), 41.7 (t, CH_2NH_2), 53.5 and 55.7 (t, CH_2CH_2Cl), 61.0 (t, CH_2NR_2), 102.1 and 103.9 (s, CH_3C) and 187.7, 188.9, 189.2 and 189.5 (s, C=O); *m/z* 371, 343, 341, 329, 285, 172, 151, 123 and 107 (100%) (Found C, 35.13; H, 5.42; N, 4.31. $C_{18}H_{32}N_2O_4Cl_2CoPF_6$ requires C, 35.12; H, 5.20; N, 4.55%).

Bis(3-methyl-2,4-pentanedionato)(*RS*-*N,N'*-bis(2-chloroethyl)ethane-1,2-diamine)cobalt(III) Hexafluorophosphate[160] (215)

Sodium bis(3-methyl-2,4-pentanedionato)dinitrocobaltate(III) hydrate (0.50 g, 1.2 mmol) was dissolved in a mixture of methanol (6 ml) and water (6 ml). Activated charcoal (0.1 g) was added to the solution followed by *N,N'*-bis(2-chloroethyl)ethane-1,2-diamine dihydrochloride (0.34 g, 1.3 mmol) in methanol (2 ml) and water (2 ml). A solution of NaOH (0.08 g, 2 mmol) in water (4 ml) was added dropwise to the cobalt solution and the mixture was stirred for 1 h. The charcoal was filtered off through Celite and washed with methanol which was added to the filtrate. Ammonium hexafluorophosphate (0.3 g) was added to the filtrate which was then extracted with chloroform (3 x 25 ml). The combined extracts were concentrated to leave a purple residue which was taken up in water (6 ml). Methanol (6 ml) was then added and the solution was left open to the air for slow evaporation of the methanol. After 48 h, the resulting purple needles were filtered and washed with water (10 ml) and ether (10 ml) and finally dried in air, m.p. 141-142 °C, ν_{max} 2926, 1569, 1426, 1339, 1299, 842 and 737 cm^{-1}; δ_H 1.88 and 1.98 (6H, s, CH_3C), 2.15, 2.17, 2.22 and 2.24 (12H, s, CH_3CO), 2.53-2.90 (4H, m, CH_2CH_2Cl), 3.03 (2H, m, CH_2NH), 3.66 (4H, m, CH_2Cl), 3.94 (2H, m, CH_2NH) and 4.61 (2H, bs, N*H*); δ_C 14.9 (q, CH_3C), 26.1, 26.2, 26.4 and 26.5 (q, CH_3CO), 48.9, 49.4, 50.0 and 50.9 (t, CH_2CH_2Cl and CH_2NHR), 51.4 and 56.9 (t, CH_2CH_2Cl), 102.2 (s, CH_3C) and 188.1 and 188.9 (s, CH_3CO); *m/z* 285, 172, 151, 123, 107 and 85 (100%) (Found C, 36.69; H, 5.30; N, 4.70. $C_{18}H_{32}N_2O_4Cl_2CoPF_6$ requires C, 35.12; H, 5.20; N, 4.55%).

Bis(3-methyl-2,4-pentanedionato)(*N,N*-bis(2-chloroethyl)propane-1,3-diamine)cobalt(III) Hexafluorophosphate (216)

Sodium bis(3-methyl-2,4-pentanedionato)dinitrocobaltate(III) hydrate (0.31 g, 0.74 mmol) was dissolved in a mixture of methanol (6 ml) and water (6 ml). Activated

charcoal (0.1 g) was added to the solution followed by *N,N*-bis(2-chloroethyl)propane-1,3-diamine (0.20 g, 0.74 mmol) in methanol (2 ml) and water (2 ml). A solution of NaOH (0.06 g, 1.48 mmol) in water (4 ml) was added dropwise to the cobalt solution and the mixture was stirred for 1 h. The charcoal was filtered off through Celite and washed with methanol which was added to the filtrate. Ammonium hexafluorophosphate (0.3 g) was added to the filtrate which was then extracted with chloroform (3 x 25 ml). The combined extracts were concentrated to leave a brown residue which was taken up in water (6 ml) . Methanol (6 ml) was then added and the solution left open to the air for slow evaporation of the methanol. After 1 week, the resulting dark crystals were collected by filtration and washed with 20% methanol/water, water and ether (0.08 g, 17%), m.p. 136-137 °C, ν_{max} 2930, 1568, 1424, 1339, 1299, 845 and 737 cm^{-1}; δ_H (CD_3OD) 1.24 (2H, m, $NCH_2CH_2CH_2N$), 1.44, 1.46, 1.49 and 1.53 (12H, s, CH_3CO), 1.55 and 1.66 (6H, s, CH_3C), 2.41 (2H, m, CH_2NR_2), 2.86 (8H, m, NCH_2CH_2Cl and CH_2Cl) and 3.16 (2H, m, CH_2NH_2); δ_C 13.5 (q, CH_3C), 27.0 (q, CH_3CO), 31.3 (t, $NCH_2CH_2CH_2N$) 38.8 (t, CH_2Cl), 40.6 (t, CH_2NH_2), 43.0 (t, CH_2CH_2Cl), 58.9 (t, CH_2NR_2), 100.8 (s, CH_3C) and 187.9 (s, C=O); *m/z* 285, 242, 143, 123 99 and 72 (Found C, 37.78; H, 5.18; N, 4.00. $C_{19}H_{34}N_2O_4Cl_2CoPF_6$ requires C, 36.25; H, 5.41; N, 4.45%).

Bis(3-methyl-2,4-pentanedionato)(*N,N'*-(1,4-Propanediyl)bis(2-chloropiperidine)-cobalt(III) Hexafluorophosphate (218).

Sodium bis(3-methyl-2,4-pentanedionato)dinitrocobaltate(III) hydrate (0.31 g, 0.74 mmol) was dissolved in a mixture of methanol (6 ml) and water (6 ml). Activated charcoal (0.1 g) was added to the solution. *N,N'*-(1,3-propanediyl)bis(2-chloropiperidine) (0.22 g, 0.74 mmol) in methanol (4 ml) was added dropwise to the cobalt solution and the mixture was stirred for 1 h. The charcoal was filtered off through Celite and washed with methanol which was added to the filtrate. Ammonium hexafluorophosphate (0.3 g) was added to the filtrate which was then extracted with

chloroform (3 x 25 ml). The combined extracts were concentrated to leave a red residue which was taken up in water (6 ml). Methanol (6 ml) was then added and the solution was left open to the air for slow evapouration of the methanol. After 1 week, the resulting red-brown crystals were collected by filtration and washed with 20% methanol/water, water and ether, (0.05 g, 9%), m.p. 144-145 °C, ν_{max} 2932, 1575, 1426, 1339, 1299, 843 and 735 cm^{-1}; δ_H 1.48-1.98 (14H, m, ring CH_2 and $NCH_2CH_2CH_2N$), 1.78, 1.86, 2.02, 2.10 (12H, s, CH_3CO), 2.18, 2.21 (6H, CH_3C), 2.86-3.07 (8H, m, CH_2N), 3.29 (4H, m, CH_2Cl) and 3.58 (2H, m, CHN); *m/z* 415, 271, 257, 242, 158 (100%), 143, 115 and 100.

Bis(3-methyl-2,4-pentanedionato)[*N,N'*-(1,4-butanediyl)bis(2-chloro-piperidine)]cobalt(III) Hexafluorophosphate (217)

Sodium bis(3-methyl-2,4-pentanedionato)dinitrocobaltate(III) hydrate (0.15 g, 0.87 mmol) was dissolved in a mixture of methanol (6 ml) and water (6 ml). Activated charcoal (0.1 g) was added to the solution. *N,N'*-(1,4-butanediyl)bis(2-chloropiperidine) (0.10 g, 0.34 mmol) in methanol (4 ml) was added dropwise to the cobalt solution and the mixture was stirred for 1 h. The charcoal was filtered off through Celite and washed with methanol which was added to the filtrate. Ammonium hexafluorophosphate (0.3 g) was added to the filtrate which was then extracted with chloroform (3 x 25 ml). The combined extracts were concentrated to leave a red residue which was taken up in water (6 ml) . Methanol (6 ml) was then added and the solution was left open to the air for slow evaporation of the methanol. After 1 week, the resulting red-brown crystals were collected by filtration and washed with 20% methanol/water, water and ether, (0.04 g, 16%), m.p. 145-147 °C, ν_{max} 2924, 1564, 1428, 1339, 1299, 850 and 737 cm^{-1}; δ_H 1.55-2.05 (16H, m, ring CH_2 and $NCH_2CH_2CH_2CH_2N$), 1.86, 1.92, 2.12, 2.16 (12H, s, CH_3CO), 2.21, 2.26 (6H, CH_3C), 2.90-3.10 (8H, m, CH_2N), 3.38 (4H, m, CH_2Cl) and 3.64 (2H, m, CHN); *m/z* 415, 271, 257, 242, 158 (100%), 143, 115 and 100.

Experimental to Chapter 8

Biological Testing

Materials

Microtitre plates (Linbro, ICN flow)

Multichannel pipette (Costar)

Tip box, autoclavable (ICN flow)

Pipette tips (ICN flow)

Petri dishes, 5 cm and 10 cm (Sterilin)

Universal containers, 30 ml and 100 ml (Sterilin)

Plastic lunch box

Growth medium

Trypsin (0.25% + EDTA 1 mmol in PBS)

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma)

Sorensen's glycine buffer (0.1 M glycine, 0.1 M NaCl adjusted to pH 10.5 with 1 M NaOH)

Dimethylsulphoxide (DMSO)

DMSO dispenser (Well-fill, Denley)

ELIZA plate reader-Emax from Molecular Devices-Mento Park, California with Softmax® software.

Cell lines

Two human colon-carcinoma cell lines were used: HT 29 was obtained from the ATCC (Rockville, MD) and BE from Dr. N. Gibson (School of Pharmacy, University of Southern California, Los Angeles, CA). Both were grown as monolayer cultures in a mixture of Ham's F10 and Dulbecco's modified Eagle's medium (50:50, Life

Technologies, Paisley, UK) supplemented with glutamine (2 mM) and FCS (10%). Both cell lines had a doubling time of about 24 h.

Cytotoxicity assay : Protocol

1) Plating out cells

Growth medium was removed from cell culture and trypsin (2 ml) was added. After a couple of minutes, the trypsin was removed and the cells were seen to be sliding down the plate indicating that they were loosened. The cells were then collected in growth medium (10 ml) containing serum and counted using the Coulter counter. Dilution of the cells to the required concentration (which depends on the cell line) with medium, allowing 20 ml per microtitre plate was then carried out. The resulting cell suspension was transfered to a 10 cm petri dish from which 200 µl was added to each well of the central 10 columns of a flat bottomed 96 well plate (80 wells per plate) using a multichannel pipette, starting with column 2 and ending with column 11 (6×10^2 cells/well for HT 29 and 1×10^3 cells/well for BE). 200 µl of growth medium was added to the eight wells in column 1 and column 12 as blanks. The plates were placed in a plastic lunch box and incubated in a humidified atmosphere at 37 °C and 2% CO_2 for two days so that the cells would be in the exponential phase of growth for drug addition.

2) Drug addition

A serial 5-fold dilution of the cytotoxic drug was prepared in growth medium to give 4 concentrations, starting at 1 mmol. Two plates was used for each drug to give duplicate determinations within one experiment.

The medium was then removed from the wells in columns 2 to 11. This was achieved using a hypodermic needle attatched to a suction line. The cells in the eight wells in columns 2 and 11 were fed with 200 µl of fresh growth medium and these cells were

used as controls. The cytotoxic drugs were then added to the cells in columns 3 to 10. Only four wells were needed for each drug concentration so in this way, four compounds were screened in one plate, each drug with 16 wells. The drug solutions were transferred to 5 cm petri dishes and 200 µl added to the wells. The plates were returned to the lunch box and incubated for 24 h.

3) Growth period.

At the end of the drug exposure period, the medium was removed from all the wells containing cells and the cells fed were with 200 µl of fresh medium, and incubated for 4 days.

4) Estimation of surviving cell numbers

The plates were fed with 200 µl of fresh medium and 50 µl of MTT (5 mg/ml in PBS and filter sterilised) was added to all wells in columns 1 to 11. The plates were then wrapped in aluminium foil and incubated for 4 h in a humidified atmosphere at 37 °C. This is a minimum incubation time and plates can be left for up to 8 h. The medium and MTT were then removed from the wells and the MTT-formazan crystals were dissolved by addition of 200 µl of DMSO to all wells in columns 1 to 11. Glycine buffer was added (25 µl per well, 0.1M, pH 10.5) to all wells containing DMSO. Absorbance was recorded at 570 nm immediately since the product is unstable. The wells in column 1, which contained medium, MTT but no cells was used to blank the plate reader.

5) Analysis of results

A graph was plotted of absorbance (y axis) against drug concentration (x axis). The mean absorbance reading from the wells in columns 2 and 11 were used as the control absorbance and the IC_{50} concentration was determined as the drug concentration required

to reduce the absorbance to half that of the control. The absorbance values in columns 2 and 11 should be the same, but on occasion they were not and this was taken to indicate uneven plating of cells across the plate.

Hypoxic cytotoxicity assay

For estimation of the oxic:hypoxic cytotoxicity ratio, cells were grown on glass petri dishes for drug exposure under hypoxic conditions and on plastic petri dishes for oxic conditions. Cells were plated out at a density of 1 x 10^5 cells per 5 cm petri dish for the HT 29 cells and a density of 2 x 10^5 cells per 5 cm petri dish for the BE cells. The cells were allowed to attach and grow for 2 d. The medium was removed from the dishes and replaced with 2 ml of fresh medium containing the drug. Then the dishes were exposed to the drug for 3 h in a humidified atmosphere in oxic (2% CO_2 in air) or hypoxic (2% CO_2 in N_2) conditions. The medium was removed and the dishes were fed with fresh medium and incubated for 36 h. After removal of the medium, cells were detached with trypsin (1 ml per dish). After 5-10 min, medium was added (1 ml) and the number of live cells counted using the Coulter Counter.

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