THE SYNTHESIS OF SOME NOVEL PHOSPHORAMIDATES OF CHEMOTHERAPEUTIC INTEREST

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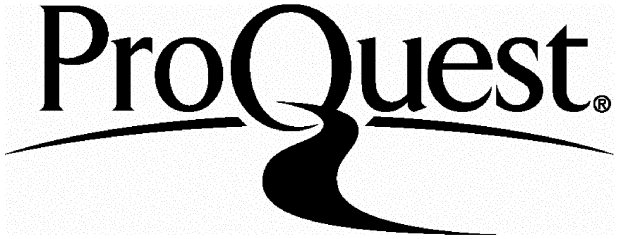

ABSTRACT

The human immunodeficiency virus (HIV) is known to be the causative agent of the acquired immunodeficiency syndrome (AIDS) and the most promising chemotherapeutic strategy used in combating this virus is to use inhibitors of HIV replication. At present nucleoside analogues are some of the most promising anti-HIV agents known. However, they suffer from a number of limitations. One new class of compounds which may play an important role in AIDS therapy is the alkylating agents. The design and synthesis of these agents as potential anti-HIV drugs is the subject of the first part of this thesis.

The synthesis of a variety of phosphoramidate derivatives of nitrogen mustard is described, along with the results of the biological evaluation of some of these agents against HIV. It is thought that these agents may act as inhibitors of HIV DNA and/or RNA replication. Their potential antineoplastic activity is also considered.

The synthesis of some alkyl and trihaloalkyl phosphoramidate derivatives of nitrogen mustard from N,N-bis(2-chloroethyl)amino (alkyl or trihaloalkyl) phosphorochloridate and various carboxy protected amino acids is described along with a discussion of the *in vitro* anti-HIV test results of some of these agents.

The next series of derivatives was designed with the aim of making it more lipophilic as it was thought that this property could be a contributory factor in the activity against the virus. Carboxy protected amino acids of varying degrees of lipophilicity were incorporated in 4-substituted phenyl phosphoramidate derivatives of nitrogen mustard. Some peptide derivatives were also synthesised and tested for potential anti-HIV activity.

The subject of the second part of this thesis was to investigate the synthesis of some analogues of cyclophosphamide, the most widely used alkylating agent in cancer

chemotherapy. Acrolein, a metabolite of cyclophosphamide is thought to be responsible for the cause of haemorrhagic cystitis which can be a fatal condition.

It was attempted to prepare 5-hydroxycyclophosphamide, a 6-membered ring derivative, in the hope that it would overcome the toxicity associated with the release of acrolein. However, it was possible to only isolate the alternative 5-membered ring analogue from reactions of dichlorophosphoramide with aminopropan-2,3-diol. The synthesis of 2-bis(2-chloroethyl)amino-1,3,2-oxaza, 2-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-dioxa and 2-bis(2-chloroethyl)amino-5-acetyloxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide to establish the nature of the isolated 5-membered ring analogue is described along with attempts to find alternative routes to 5-hydroxycyclophosphamide. Attempts were also made to prepare 5-O-allyl cyclophosphamide and some phosphite derivatives.

ACKNOWLEDGEMENTS

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Finally, and most importantly, I am very grateful to my parents, my brothers and sisters and Sayon, Maya and Kesha for their endless support and it is to them that I dedicate this thesis.

CONTENTS

ABBREVIATIONS

EC_{50} Median effective concentration. This is the minimum concentration of drug which reduces HIV antigen (Ag p24) by 50% in HIV infected cell culture.

ED_{50} Median effective dose. This is the minimum concentration of drug which reduces HIV antigen (Ag p24) by 50% in HIV infected cell culture.

IC_{50} Median inhibitory concentration. This is the minimum concentration of drug which causes 50% inhibition of viral proliferation.

TD_{50} Median toxic dose. This is the minimum concentration of drug which causes 50% inhibition of normal cell growth.

TI Therapeutic index. $\frac{TD_{50}}{ED_{50}}$ $\frac{\text{Median toxic dose}}{\text{Median effective dose}}$

INTRODUCTION

The first section of this thesis is devoted to anti-AIDS agents while the remaining section concentrates on anti-cancer agents.

The Acquired Immune Deficiency Syndrome (AIDS) apparently first appeared in 1979 and was brought to the attention of the medical community in 1981[1]. The first report of AIDS came from the Centre for Disease Control in America and since then there have been, up to October 1991, 418404 AIDS cases reported to the World Health Organisation[2]. The number of people who are HIV positive, throughout the world, is many times this figure .

AIDS is known to be caused by a virus, the Human Immunodeficiency Virus (HIV)[3] or the Human T-cell Lymphotropic Virus Type 3 (HTLV-III)[4,5]. The methods of transmission are known to be primarily intimate sexual contact, direct contamination of the blood (as when virus contaminated drug paraphernalia is shared) or the passage of virus from a mother to a foetus[6]. The body reacts against invasion of the virus by producing a specific antibody against the virus, (HIV antibody). In the early stages of infection it is possible, before antibodies are produced, to be antibody-negative but virus-positive. There is thus a wide spectrum of clinical expressions associated with HIV infection ranging from healthy antibody-negative virus-positive individuals to antibody-positive patients with fully developed AIDS. The majority of infected individuals are asymptomatic. The incubation period between infection and development of AIDS has been found to vary from 6 to 72 months with an average of around 28 months[7].

HIV causes a progressive derangement of immune function and AIDS is one late manifestation of that process. The outstanding clinical feature of AIDS is the occurrence of opportunistic infections and cancer in previously healthy individuals. Opportunistic infections are infections that occur because the immune system has

broken down. HIV is capable of destroying a specific type of white blood cell called T-helper lymphocytes which form an integral part of the immune system and this leaves the individual susceptible to organisms with which he had previously lived in relative harmony. The most important of these opportunistic infections include *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cryptococcosis and cytomegalovirus[8]. The breakdown in the immune system also predisposes the individual to the development of otherwise rare cancers such as Kaposi's sarcoma[9] (tumours of the skin and linings of internal organs) and lymphomas (cancers of lymphoid tissue). For some AIDS patients Kaposi's sarcoma is a rapidly progressive disease with the involvement of virtually every organ. Despite the aggressive nature of this cancer it is rarely fatal and most patients succumb first to severe opportunistic infections. Opportunistic infections and cancer are mortal illnesses for AIDS patients and the life expectancy for full blown AIDS is about 2-3 years.

To date there is no cure for AIDS, only treatment available for some of the complications of the disease. Effective therapy is available for some of the opportunistic infections but relapses are common[10]. Patients with PCP are known to respond to initial therapy with either co-trimoxazole or pentamidine. These drugs are not without problems since adverse reactions are common. *Toxoplasmosis gondii*-like pneumocystis, an opportunistic protozoan, is treated with sulphadiazine and pyrimethamine. Chemotherapy with cytotoxic agents or interferon is the treatment of choice for the more disseminated form of Kaposi's sarcoma[11,12]. A number of anti-cancer drugs have been employed including vinblastine, bleomycin, VP-16 and adriamycin. Although these drugs are very active in the treatment of Kaposi's sarcoma they are very toxic and not selective for the tumour. They may cause a further reduction in immune status since anti-cancer drugs usually attack actively dividing cells in the bone marrow and produce a fall in the production of white blood cells. In many cases the lowered immune status whilst on chemotherapy gives rise to increased

opportunistic infections. Interferon alfa has been used with some success to treat kaposi's sarcoma and has the advantage that it does not suppress the immune system. Interferons have also been shown to be anti-proliferative and so control the growth of some tumours.

An alternative therapeutic approach is to aim the treatment at the host rather than the tumour, that is, to correct the underlying immune deficiency. Biological response modifiers which have been utilised, in an effort to restore a balance in the immune system, include interferon gamma, interleukin II, thymic hormones, mixed bacterial vaccine and monoclonal antibodies[13]. The interferons are the only biological response modifiers which have shown any significant activity. Other treatments for AIDS include plasmapheresis, bone marrow transplantation and leukocyte transfusion[14].

The most promising chemotherapeutic strategy used in combating the AIDS virus is to use inhibitors of HIV replication[15]. Selected stages of HIV replication may be targets for therapeutic intervention. To understand how anti-HIV agents work, the structure and replicative cycle of the HIV virus must be considered.

HIV, the causative agent of AIDS and its related disorders, is a pathogenic retrovirus[16]. Retroviruses were so named because they reversed what seemed to be the normal flow of genetic information. In cells the genetic material is DNA and when genes are expressed the DNA is first transcribed into messenger RNA (mRNA) which then serves as the template for the production of proteins. The genes of a retrovirus are encoded in RNA and before they can be expressed the RNA must be converted into DNA. Only then are the viral genes transcribed and translated into proteins in the usual sequence.

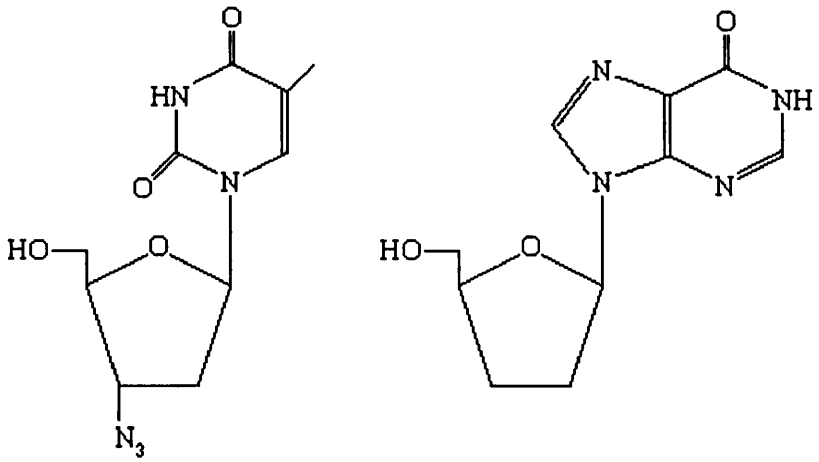
The replication cycle begins when an HIV particle binds to the outside of a cell. HIV has an envelope glycoprotein called gp120 which forms a strong bond with a glycoprotein called CD4 (or T4)[17]. CD4 is particularly abundant on the surface of a

class of white blood cells called helper T-cells. These cells are a prime target for HIV infection and their gradual depletion is a hallmark of AIDS[18]. There are several approaches to inhibiting the initial binding of HIV to a cell. Soluble CD4 or monoclonal antibodies specific for the viral envelope glycoprotein can be conjugated to toxins such as *Pseudomonas* exotoxin or ricin and this has been shown to inhibit viral infection *in vitro*[19,20]. Another approach involves creating free-floating, or soluble, form of CD4 that bind to CD4-binding sites on HIV[21]. Several large sulphated negatively charged molecules have also been shown to inhibit HIV replication[22]. One prototype is dextran sulphate and this has been shown to block viral binding and syncytia (giant cell) formation *in vitro*.

After HIV has bound to a cell, it fuses with the cell membrane and releases its contents into the cytoplasm. The inner protein coat is partially removed to expose the viral RNA. These steps are potential targets for therapy and antibodies which neutralize gp41, the envelope glycoprotein that mediates fusion, may prevent fusion from occurring. Antiviral drugs may be able to interfere with the uncoating process. The aromatic polycyclic dione compounds, hypericin and pseudohypericin have been found to be active against HIV[23]. It is thought that these compounds inhibit the uncoating process by conferring high stability on the viral capsid structure.

After viral entry and uncoating, viral RNA is used as a template for proviral DNA synthesis and this is catalysed by the viral DNA polymerase called reverse transcriptase[24]. The HIV reverse transcriptase is a heterodimer. The larger peptide contains a COOH-terminal sequence that has a ribonuclease H (RNase H) active site. RNase H specifically degrades the RNA template of an RNA-DNA hybrid to permit synthesis of a double-stranded viral DNA. The synthesis of the second DNA strand is also catalysed by the viral reverse transcriptase. This enzyme has proven to be a successful target for the development of anti-retroviral agents because it is unique to retroviruses.

It has been found that 2′,3′ dideoxynucleosides can inhibit the infectivity and replication of divergent HIV strains *in vitro*[25]. These nucleoside analogues are molecules that closely resemble the nucleosides that serve as building blocks in DNA and RNA: the pyrimidines (thymidine, uridine and cytidine) and the purines (adenosine and guanosine). One such compound, 3′-azido-2′,3′-dideoxythymidine, AZT **(1)** [Fig.1], was originally synthesised as a potential anticancer drug in 1964[26]. In 1985, this drug was found to be, at concentrations of between 0.25 and 1.25 μg/ml, an effective inhibitor of HIV in AIDS patients[27]. AZT and ddI **(2)**[28] are, to date, the only drugs licensed for clinical use. The mechanism of action of AZT involves its conversion to AZT triphosphate[29]. This process is called phosphorylation and the phosphate groups are added by cellular enzymes[30]. AZT triphosphate is an analogue of thymidine triphosphate, one of the building blocks of DNA. This analogue appears to inhibit production of viral DNA by at least two mechanisms, competitive inhibition and chain termination[31].



(1) **(2)**

[Fig. 1]

In competitive inhibition, AZT triphosphate binds to reverse transcriptase at a site that ordinarily binds to physiological nucleoside triphosphates. In chain termination, reverse transcriptase incorporates AZT triphosphate in a growing chain of viral DNA, in place of the normal thymidine triphosphate. The normal 5′ to 3′ phosphodiester linkage cannot be completed because AZT triphosphate lacks the hydroxyl group that is needed to form the chemical bond. The virus cannot repair this mistake and so viral DNA synthesis is terminated.

AZT can be toxic, particularly to bone marrow, so that patients on AZT often develop anaemia and in some cases low numbers of white blood cells[32]. This limits the amount of AZT that can be administered and bone marrow suppression is a major reason for failure of the drug. Other dideoxynucleosides (ddA, ddC, ddI and ddG) that are active against HIV also appear to work by the same mechanisms as AZT and each drug appears to have its own toxicity profile.

After a strand of DNA has been copied from viral RNA, the reverse transcription proceeds to the second stage: the synthesis of a second DNA copy of the first DNA strand. This stage is also subject to attack, and the enzymes that can be interfered with are RNase H and viral integrase. Several potent RNase H inhibitors, such as vanadyl sulphate or polyguanylic acid, are known[33]. Cellular toxicity is a significant issue with some of these agents.

The next target for therapy presents itself later in the cycle when the host cell is activated. The cell may begin to produce new proteins or receptors. This may also trigger the transcription and translation of viral DNA into viral proteins. The expression of retroviral genes may be blocked and this may involve the construction of negative strand (antisense) synthetic oligodeoxynucleotides[34]. A phosphorothioate analogue of a 28-nucleotide homo-oligodeoxycytidine (S-dC_{28}) has been found to be a potent inhibitor of HIV *in vitro*[35]. However, there are several complications with this approach. First, unmodified oligonucleotides are subject to rapid hydrolysis by host

nucleases. Second, certain chemically modified oligomers are nuclease-resistant but have poor solubility and require high concentrations for biological effects *in vitro*.

The late stages in the replication of HIV include secondary processing of viral proteins by an HIV protease[36]. This enzyme is unique to HIV and so represents a crucial target for therapy. Some peptide analogues, based on the transition-state mimetic concept, have been found to inhibit protease function and can therefore inhibit HIV replication[37].

In addition to the viral protease, cellular enzymes such as glycosylating or myristoylating enzymes, also function in the maturation of viral components. Several glycosidase inhibitors, including castanospermine (plant alkaloid) and N-butyldeoxynorjirimycin, have been found to block HIV-induced syncytium formation and interfere with HIV infectivity *in vitro*[38,39].

Finally, the viral proteins and RNA are transported to the cell membrane and there they are assembled into virus particles. These are then released by a process of viral budding. Interferon α (IFN-α) is thought to affect this process and is active against HIV *in vitro* during acute infection[40].

One class of compounds, which may play an important role in AIDS therapy, is the alkylating agents. The design and synthesis of alkylating agents as potential anti-HIV drugs is a relatively new field of study. To understand their potential role, one must understand the role that they already play in cancer chemotherapy. This is now discussed.

Second only to cardiovascular ailments in incidence, cancer is feared more than any other disease and accounts for one fifth of all deaths. Most multicellular organisms can be afflicted by cancer and cancerous lesions have been found in dinosaur bones. Only in the twentieth century, however, has there been much concern over the disease.

Cancer or neoplasm is a disease of cells characterised by a reduction or loss of effectiveness in normal cellular control and maturation mechanisms which regulate

multiplication[41]. Excessive cell proliferation usually results in the formation of a tumour. Tumours may be harmless or they may be cancerous and fatal, in which case they are termed as malignant. Malignant tumours duplicate continuously and rapidly without control. One of the unique properties of a malignant tumour is its ability to metastasise[42]. In the process of metastasis, there is an initial invasion of the malignant cells into surrounding tissues. Eventually, the normal tissue atrophies and dies. Fatality is a consequence of metastasis to vital organs. Following invasion, some of the malignant cells detach from the tumour and may enter the blood or lymph. This latter condition can lead to widespread metastasis. Those cells which survive in the blood or lymph may invade adjacent body tissues and establish secondary tumours. In all stages of metastasis the malignant cells resist the antitumour defences of the body.

It is now held that the major cause of human cancer is the exposure to a number of environmental chemicals, naturally occurring and synthetic[43]. It is also thought that cancer could be the consequence of an error in genetic programming[44]. This may be inherited but more frequently is believed to be due to DNA damage caused by carcinogens, radiation or even viruses. As a result, normal genes are converted to cancer causing genes or oncogenes.

According to their localization and shape, tumours receive different names: carcinoma (glandular tissue), sarcoma (connective tissue), lymphoma (lymphatic ganglia), leukaemia (blood cells; it can be myeloid leukaemia and lymphoid leukaemia)[45].

Treatment of cancer is normally a combination of surgery, radiotherapy and chemotherapy[41]. Surgical methods are generally used to remove the primary tumour and this may be of lasting benefit only if the tumour has not metastasised. Disseminated forms of cancer such as leukaemia cannot be attacked surgically. Radiation therapy is superior to surgery when it effectively destroys a tumour and at the same time causes minimal damage to the surrounding normal tissue. Radiation therapy is considered the

treatment of choice for many cancers. It may preserve the function of an organ or minimise the mutilating effect of a surgical procedure. As all forms of ionising radiation cause cancer under certain conditions, radiation therapy will always be subject to severe limitations. In contrast to surgery and radiotherapy, chemotherapy is not so much limited by metastasis as by the total mass of the tumour(s). Although antineoplastic drugs permeate the body, acting on clumps of cells that may have lodged in other organs, the drugs have great difficulty in destroying all cells in a large tumour.

Antineoplastic agents in clinical use are in general only palliative, especially in the case of leukaemia. Usually they do not cure cancer but only effect its temporary remission. Most antineoplastic agents are essentially antigrowth drugs, designed on the rationale that cancer cells multiply at a rate greater than all normal cells and therefore antineoplastic agents must somehow interfere with cellular mitosis[46]. However tumour cells do not undergo mitosis more rapidly than all cells. For instance, cells of the hemopoietic system, internal mucosa, oral mucosa, hair follicles and skin, divide faster than cancer cells. For this reason, drugs which act by destroying rapidly dividing cells mainly attack normal tissues and hence can be very toxic or even lethal to the patient. Common symptoms of intoxication with these drugs are leukopenia, thrombocytopenia, anorexia, nausea, alopecia, thrombophlebitis and cystitis. Nevertheless they are resorted to as adjuvants to surgery and radiotherapy, which remain the primary approaches to the cancer problem.

Antineoplastic agents can be divided into the following main classes; antimetabolites, antibiotics, plant products, enzymes, hormones and alkylating agents.

Antimetabolites

As cancer is characterised by an abnormal cellular metabolism and mitosis is controlled by the nucleic acids, most of the antimetabolites used as antineoplastic agents are structural analogues of the metabolites involved in the biosynthesis of nucleic

acids and the purine and pyrimidine containing cofactors. Antimetabolites most widely used are methotrexate[47], fluorouracil[48], dacarbazine[49], cytarabine[50], mercaptopurine[51] and thioguanine[52].

Antibiotics

Many antibiotics have antineoplastic activity and those that are most widely used are dactinomycin[53], mithramycin[54] and bleomycin[55]. Dactinomycin, isolated from *Streptomyces parvulus*, is the most active of a series of cyclic pentapeptides and is highly effective in Wilm's tumour. Their mechanism of action involves the inhibition of DNA and/or RNA synthesis.

Plant products

Many plant products have been shown to possess antineoplastic activity. The alkaloids, vinblastine and vincristine are two such compounds which have found an important role in cancer chemotherapy[56,57]. They are cell-cycle specific agents which block mitosis with metaphase arrest. Vincristine is included in the combinations against acute lymphocytic leukaemia, the lymphomas and childhood neoplasms. Vinblastine has similar capacities in lymphomas and choriocarcinoma.

Enzymes

Some lymphoid malignancies have been found to be dependent on an exogenous supply of asparagine. The enzyme L-asparaginase has been shown to be effective in the treatment of acute lymphoblastic leukaemia and the lymphomas[58]. Anaphylaxis and immunological problems are associated with this drug.

Hormones

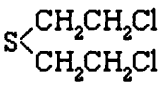
Some neoplasms have been found to be susceptible to hormone control; androgens, oestrogens and adrenocorticoids. Their main clinical indications are in the management of disseminated neoplasms. The most notable example is tamoxifen which, because of its low toxicity, is the agent of choice in the treatment of breast cancer[59]. Prednisone, a corticosteroid is an essential component of curative combinations used against acute lymphocytic leukaemia and Hodgkin's disease[60].

Alkylating agents

Alkylating agents with antitumour activity belong to a class of compounds that undergo chemical reactions leading to the addition, by covalent bonding, of organic carbon to biologically important molecules. The first study of the biological action of an alkylating agent was that of Ehrlich, who in 1898 investigated the effects of ethyleneimine[61]. However, the proposition that compounds of this type may have application in the control of cancer derived from much later work on potential war gases. It was during World War I that individuals heavily gassed with mustard gas, bis(2-chloroethyl)sulphide **(3)** [Fig.2], were found to suffer damage to bone marrow and lymphoid tissues. Studies on animals during World War II demonstrated that heavy exposure to the nitrogen mustards, bis(2-chloroethyl)amino compounds, destroyed lymphoid tissues[62]. It was decided to use these chemicals with caution to treat patients with cancers of the lymphoid tissues, such as lymphosarcoma and Hodgkin's disease[63]. The drugs were successful in reducing tumour masses but also damaged normal bone marrow. Since then many derivatives of the nitrogen mustards have been synthesised with various improvements.

Some members of this group of alkylating drugs are absorbed after oral administration but many require intravenous use. Their active moieties disappear rapidly from the blood, usually within 2 to 15 minutes. They are distributed to all

tissues except those of the central nervous system. All are toxic to bone marrow, cause immunodepression and are carcinogenic. Alkylating agents which are of interest as tumour inhibitors are now discussed.



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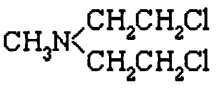

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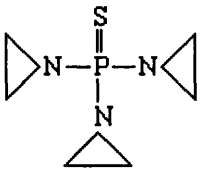

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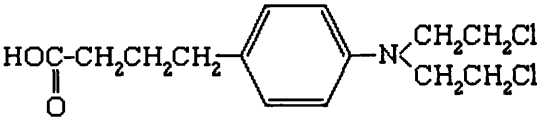

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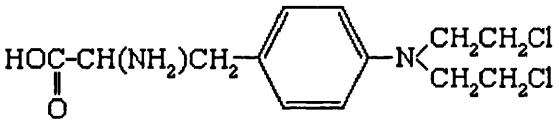

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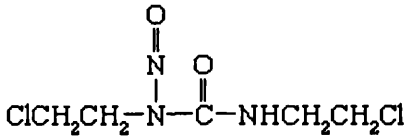

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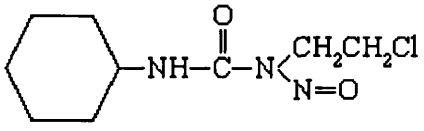

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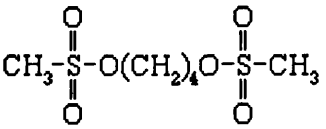

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[Fig. 2]

Mechlorethamine (4)[64]

The original agent, mechlorethamine, has largely been replaced with new congeners. It was first used as an antineoplastic agent in 1942. It is, like mustard gas, a strong vesicant and was usually injected into the tubing of a running infusion. The principle use of mechlorethamine is in combination chemotherapy of Hodgkin's disease and the non-Hodgkin's lymphomas. It has wide activity but more recent agents are safer and easier to use.

Thiotepa (5)[65]

Thiotepa, also called triethylenethiophosphoramide, has effects similar to those of mechlorethamine. It is now largely used in the treatment of bladder cancer.

Chlorambucil (6)[66]

The aromatic nitrogen mustards were introduced in the 1950s and are milder alkylating agents which may be administered orally. They reach their target sites before being dissipated extensively by side reactions. Chlorambucil is one of the slowest acting and least toxic of the nitrogen mustards. It is used chiefly in chronic lymphocytic leukaemia, malignant lymphomas and Hodgkin's disease. It is usually administered orally because of its favourable aqueous solubility as the sodium salt and rapid conversion to the free drug which is easily absorbed by the gastrointestinal tract.

Melphalan (7)[67]

This nitrogen mustard analogue was synthesised with the aim of attaching a nitrogen mustard group to an amino acid residue. It was anticipated that this combination would facilitate selective uptake by the tumour cell where rapid protein synthesis was taking place. The nitrogen mustard derivative of D-phenylalanine is less active than the natural L-form and so it has been suggested that melphalan is carried

into cells by the L-phenylalanine active transport mechanism. It is widely used in selected tumours such as multiple myeloma and ovarian carcinoma.

Carmustine (BCNU) (8) and Lomustine (CCNU) (9)[68]

These drugs are nitrosoureas with a broad spectrum of activity. The 2-chloroethyl-N-nitrosoureido group has been established as the structural unit for optimal activity. Advantages over other alkylating agents lie in their ability to cross the blood-brain barrier, making them drugs of choice in tumours involving the central nervous system. Alkylation of nucleic acids is thought to proceed via the generation of a chloroethyl carbonium ion whilst carbamoylation of amino groups is thought to occur via an alkyl isocyanate. BCNU and CCNU are also active against the lymphomas and gastrointestinal carcinomas.

Busulphan (10)[69]

This methanesulphonate ester has its greatest effect on granulocytes and is used in chronic myelocytic leukaemia. Its activity has been shown to be dependent on its ability to form a cyclic derivative by 1,4-bisalkylation of a nucleophilic group such as a cysteine residue in protein. It is thought that it may also act as a crosslinking agent.

Cyclophosphamide (11)[70]

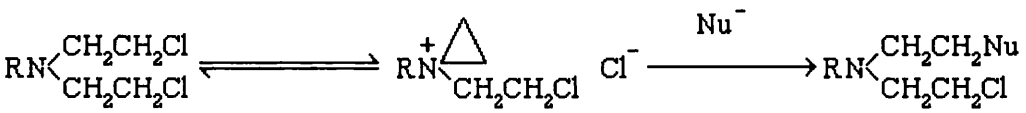
This is the most widely used alkylating agent in cancer chemotherapy and will be discussed in more detail further on in the thesis.

Mechanism of action of alkylating agents

Alkylating agents with antitumour activity share the property of being able to form covalent bonds with a variety of chemical groups on essential cellular molecules, under physiological conditions. The reaction proceeds through the formation of

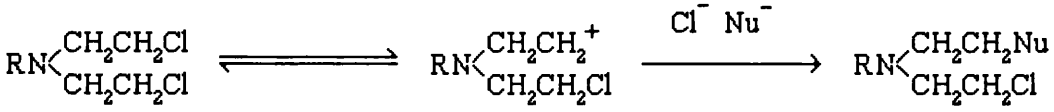
carbonium ion intermediates or of transition complexes with target molecules usually having phosphate, amino, sulphydryl, hydroxyl, carboxy, imidazole or other nucleophilic groups. The nucleophilic substitution step may follow second-order kinetics (S_N2), with the rate dependent on concentrations of both the alkylating agent and substrate or first-order kinetics (S_N1) where the rate-limiting step is solvent-assisted ionisation of the alkylating agent with subsequent rapid reaction of the resulting ion with substrate[71,72]. In the case of the basic aliphatic alkylating agents, alkylation proceeds through a second-order nucleophilic substitution. This has been shown to be preceded by a relatively fast unimolecular conversion to a cyclic aziridinium ion. This ion can then alkylate nucleophilic centres, [Fig.3].

The less basic aromatic nitrogen mustards and the sulphur mustards require longer periods of time to generate unstable active ions and so appear to follow first-order kinetics; being independent of the concentration of nucleophilic centres, [Fig.4]. These differences in mechanism may to some extent explain differences in biological effects between aliphatic and aromatic nitrogen mustards[73].



(R= alkyl)

[Fig. 3] Alkylation by a bimolecular (S_N2) mechanism



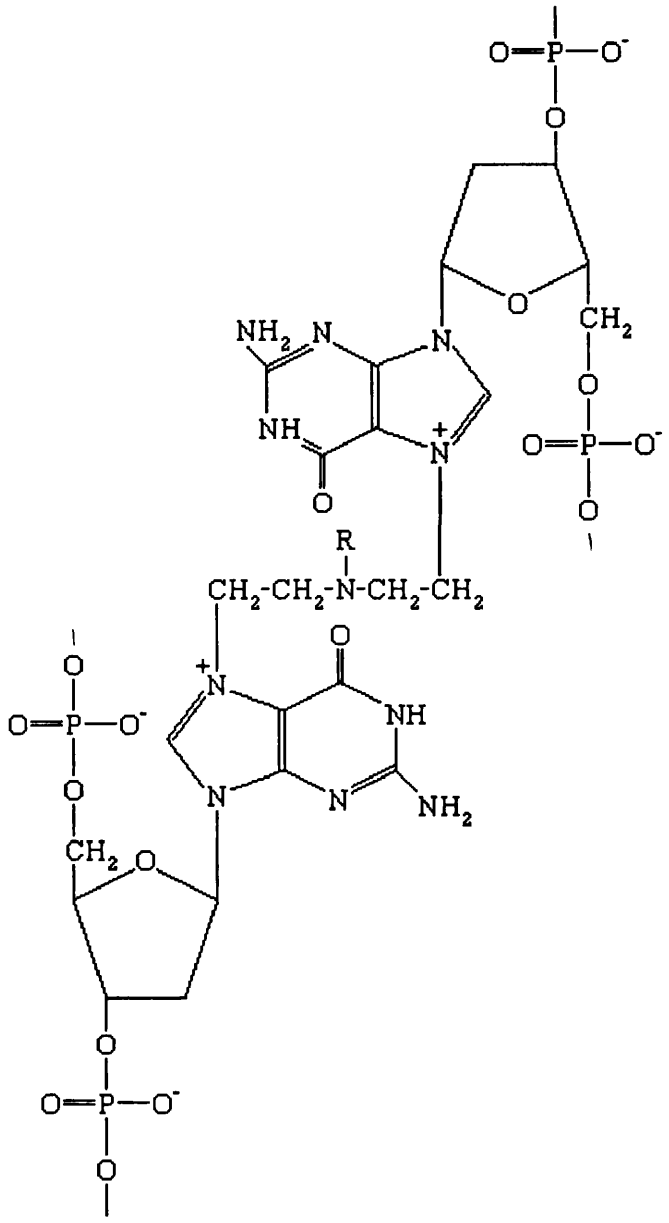
(R= aryl)

[Fig. 4] Alkylation by a S_N1 mechanism

The primary cytotoxic and mutagenic effects of these alkylating agents are probably the result of interaction with DNA[74], [Fig.5]. The favoured sites on DNA are the N-7 position of guanine, although other nucleophilic positions on guanine[75], adenine, cytosine and the sugar phosphate groups are reported to be liable to such attack[76]. Esterification of the backbone of DNA may also occur. It is not clear which of these sites of attack is most crucial in producing the end result. Alkylation of the N-7 position of guanine has a lesser effect on misreading of DNA and may result primarily in the loss of the purine base through scission of the purine-sugar bond (depurination). Alkylation of the N-3 of cytidine or the O-6 of guanine could also interfere with precise base pairing in DNA. The proximal effect of base alkylation includes misreading of the DNA codon and single-strand breakage of the DNA chain and the distal effect is mutation and/or cell death.

Most of the biologically active alkylating agents are at least bifunctional which means that there are at least two groups on the molecule capable of alkylation. Although monofunctional agents are capable of producing irreversible damage to DNA, agents that are bifunctional can cause greater damage by interstrand or intrastrand crosslinks[77]. Linking of a DNA strand with an adjacent protein is also possible.

Alkylating agents cause cytotoxic effects throughout the cell cycle but have pronounced activity against rapidly dividing cells. DNA alkylation and consequent single-strand breakage occur primarily through enzymatic processes of repair. The alkylated base is excised by an endonuclease enzyme and the gap is joined by a ligating enzyme. The repair process which acts at sites of base-alkylation or in regions that lack purine bases have less time to repair these damages in the rapidly dividing cell. Therefore the cell with damaged DNA is forced to enter the vulnerable DNA-synthetic phase of the cell cycle without repair and this makes it prone to mutation or cell death.



[Fig. 5] Cross-linking of DNA by a nitrogen mustard through two guanines

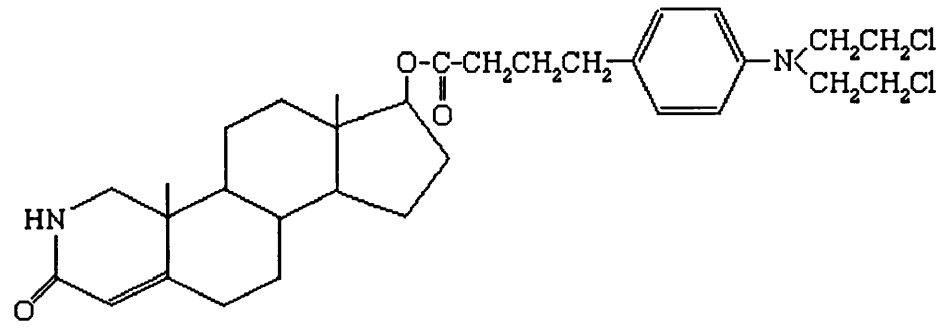
Although the alkylating agents as a class appear to have a common molecular mechanism of action, they differ substantially in their pharmacokinetic properties, lipid solubility and properties of transport across cell membranes.

Alkylating agents as anti-HIV agents

At present the most promising compounds which show activity against HIV are nucleoside analogues. However, in the future it seems certain that non-nucleoside type drugs will become more important in the experimental therapy of AIDS. Virtually every step in the replication of the HIV virus could serve as a target for therapeutic intervention. Alkylation of viral RNA and DNA, by alkylating agents such as the nitrogen mustards, could interfere with viral DNA replication. This could, in theory, suppress replication of the HIV virus.

An assessment of anti-HIV and antiproliferative activity of alkylating steroid derivatives was carried out by Pairas *et al* in 1990[78]. Their activity relationship studies of modified steroidal esters of carboxylic derivatives of N, N-bis(2-chloroethyl)aniline suggested that, in addition to an easily cleaved ester bond which is a basic requirement for antitumour activity, the lactam moiety is required for activity in L1210 leukaemia[79-82]. This prompted them to study the synthesis of *homo*-aza-steroidal esters of chlorambucil and the possible anti-HIV activity, [Fig.6]. All the alkylating steroid derivatives **(12)**, **(13)** and **(14)** were tested for both anti-HIV and antiproliferative activity in cultures of CEM-V or CEV-Z cells *in vitro*. Each agent was tested at varying concentrations ranging from 1.6×10^{-3} μg/ml to 5.0 μg/ml for **(12)** and **(14)** and from 3.0×10^{-3} to 9.4 μg/ml for **(13)**. These agents were tested for their ability to counteract the HIV-induced cytopathic effect on already HIV-infected CEM cells and to affect cell proliferation of uninfected CEM cells grown under similar culture conditions. The effect of each agent on cell growth of HIV, infected and uninfected cells was compared to that of uninfected cells incubated in the absence of drug. It was found that all the agents, **(12)**, **(13)** and **(14)** failed to counteract the cytopathic effects of HIV virus on CEM cells, since cell growth remained close to 10% of that of uninfected untreated cultures. Their results suggested that the three steroid derivatives lacked both anti-HIV

and antiproliferative activity at micromolar concentrations in culture despite the fact that they all carry mustargen bifunctional alkylating groups.



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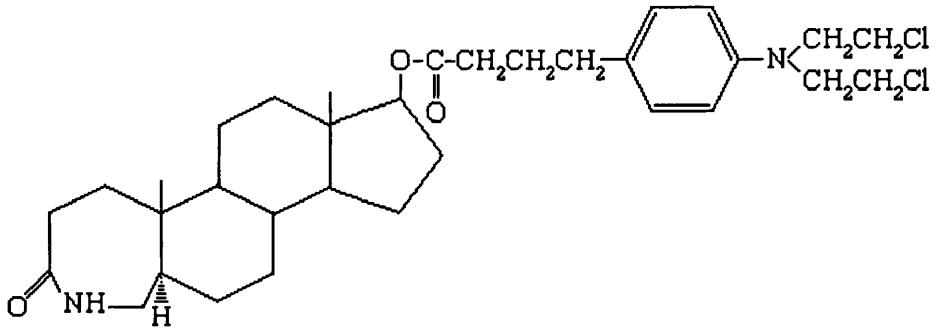

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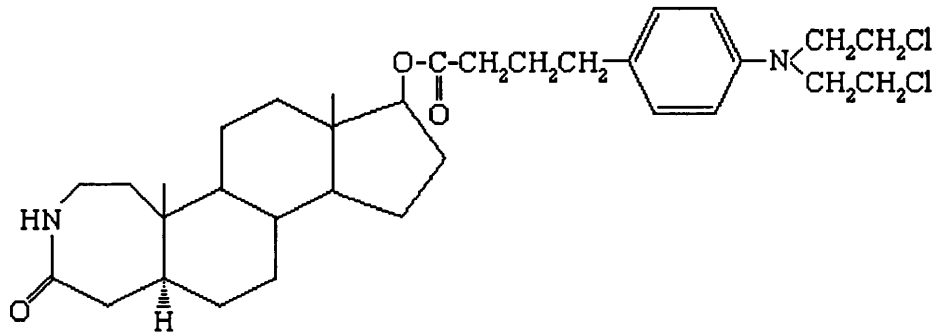
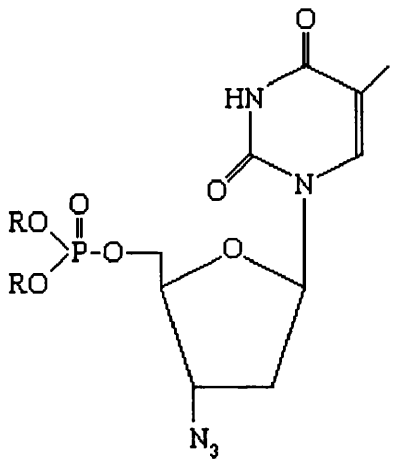


(14)

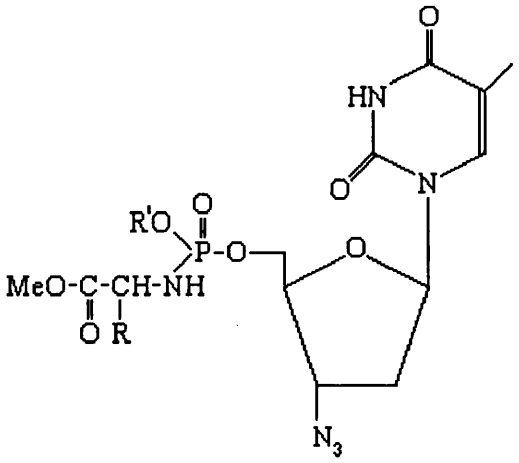
[Fig. 6] *Homo*-aza-steroidal esters of chlorambucil

A series of phosphate derivatives of the anti-HIV drug AZT (3′-azido-3′-deoxythymidine) has been prepared in this Department[83], [Fig.7]. The activity of AZT depends on its metabolic conversion to its 5′-triphosphate. The use of preformed 5′ monophosphate triesters[84-86] of the bioactive nucleoside analogue could enhance its activity, particularly if cellular phosphorylation is inefficient[87]. The major drawback of nucleoside analogues in current clinical use is their toxicity[88]. This approach may lead to a reduction in drug toxicity if the inactive prodrug form is hydrolysed to an active form selectively in the HIV-infected cell. The possibility of cleavage, by an HIV protease of an amino acid from the phosphate moiety of AZT phosphoramidate (P-N) triester was also investigated. HIV-1 protease is known to be an aspartic protease and has been identified as a homodimer[89-90]. It is responsible for the secondary processing of certain viral proteins in the last stages of replication.



R = Et, Pr, Bu

[Fig. 7] Dialkyl phosphate triesters of AZT



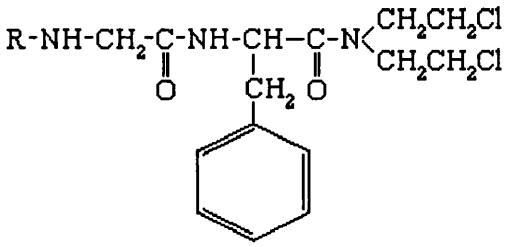
R	R'
Me	Et
iPr	Et
H	Et
CH_2iPr	Et
CH(Me)Et	Et
Bzl	Et
iPr	Me
iPr	nPr
iPr	nBu

[Fig. 8] Phosphoramidate derivatives of AZT

The three dialkyl phosphate triesters, [Fig.7], were found to be inactive at 100 μmol/l while the phosphoramidate derivatives, [Fig.8], gave EC_{50} values between 3 and 100 μmol/l, indicating the importance of the P-N link. Varying the amino acid groups was also found to confer changes in activity. These results were found to be consistent with a mode of action involving hydrolysis to a nucleotide derivative via P-N cleavage.

The observations made on the phosphoramidate derivatives of AZT generated interest in phosphoramidate derivatives of nitrogen mustard as potential anti-HIV agents.

Many amino acid derivatives of nitrogen mustard have been prepared in the hope that they would have enhanced activity as antineoplastic drugs. Gly-L-Phe **(15)**, Z-Gly-L-Phe **(16)**, Z-Gly-D-Phe **(17)** and Boc-Gly-L-Phe **(18)** derivatives of nitrogen mustard were prepared, by Raymond *et al*, as lysosomotropic agents[91], [Fig.9]. These agents were thought to enter cells by an active-transport mechanism and be hydrolysed, in lysosomes, to the bioactive amines.

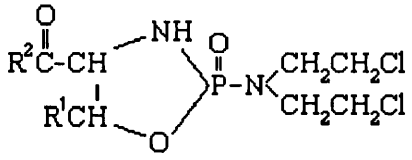


	R	Configuration
(15)	H	D-L
(16)	$PhCH_2OCO$	L
(17)	$PhCH_2OCO$	D
(18)	$(CH_3)_3COCO$	L

[Fig.9] Bioactive primary and secondary amines as lysosomotropic agents

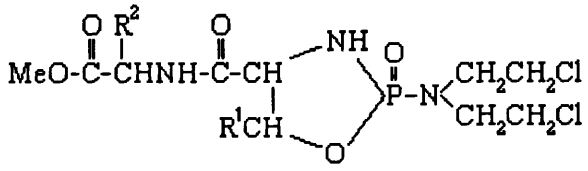
This combination of the 'latency and carrier' principles was also studied by Szekerke[92]. Ring systems incorporating the phosphoramide group were constructed

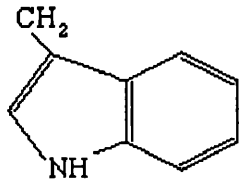
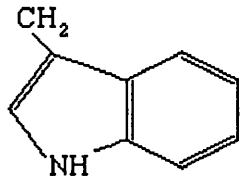
with the aid of amino acids which could function as selective transport moieties, [Fig.10]. The hydroxyamino acids or their esters used included serine **(19)-(21)**, α-phenyl-serine **(22)-(23)**, α-hydroxy-glutamic acid **(24)**, and α-(ρ-nitrophenyl)-serine **(25)**. In this report, peptide derivatives of these cyclic phosphoramide derivatives were also synthesised **(26)-(28)**, [Fig.11]. The results of antineoplastic tests indicated that the relative magnitude of antineoplastic activity and toxicity were influenced by varying the amino acid carrier, extending the chain to a dipeptide and using amino acids of different configuration.



	R^1	R^2	Configuration
(19)	H	OH	DL
(20)	H	OH	L
(21)	H	OH	D
(22)	Ph	OH	DL
(23)	Ph	OC_2H_5	DL
(24)	$CH_2COOC_2H_5$	OC_2H_5	DL
(25)	$PhNO_2$	OC_2H_5	DL

[Fig.10] Cyclic phosphoramide mustard derivatives



	R^1	R^2	Configuration
(26)	H		L-L
(27)	H		D-L
(28)	Ph	CH_2Ph	DL-L

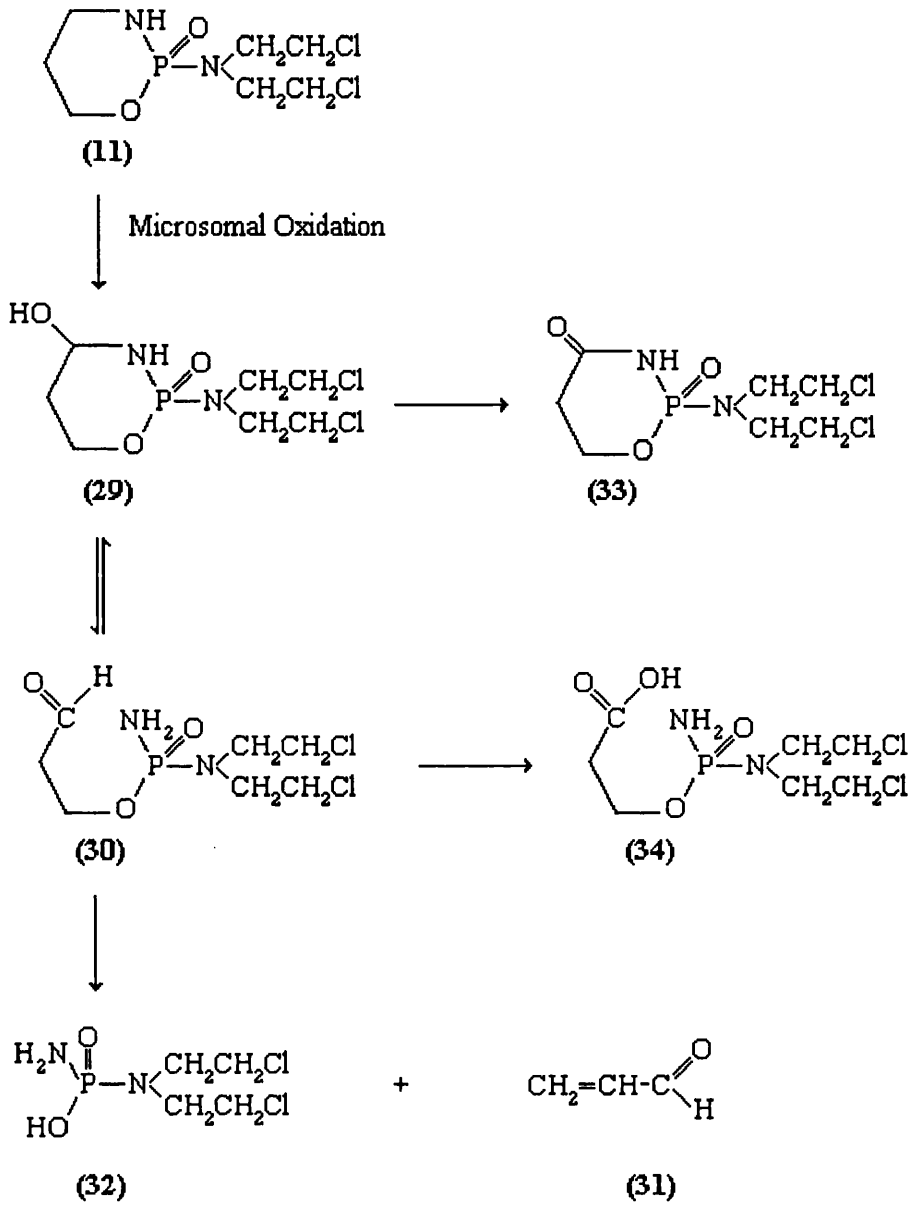
[Fig. 11] Peptide derivatives of cyclic phosphoramide mustards based on various hydroxy amino acids

The subject of the first part of the thesis was to prepare some phosphoramidate derivatives of nitrogen mustard as potential anti-HIV agents. The derivatives were to have attached to the phosphoryl group, a nitrogen mustard moiety and various alkyl, aryl and carboxy protected amino-linked amino acid groups. The electron withdrawing power of the phosphoryl group would be expected to have a deactivating effect on the

neighbouring nitrogen mustard group by reducing the availability of the lone pair of electrons on the nitrogen and thus slowing down the rate of formation of the cyclic aziridinium ion. This may aid the selectivity of the agent. The attachment of an agent to a carrier molecule is a common strategy in the design of drugs having increased selectivity. By using amino acids which are actively transported in the body by specific mechanisms, the distribution of biologically active compounds may be modulated. The phosphoramidate derivatives of nitrogen mustard were designed in the hope that cleavage of the amino acid moiety, via the P-N bond, by a specific HIV protease, would release the bisfunctional alkylating group selectively in HIV infected cells. It is also possible that cleavage is not a requirement for activity and that the agent may act as the intact molecule. These derivatives are also of interest in terms of their potential antineoplastic properties

As mentioned earlier on, cyclophosphamide **(11)** is the most widely used alkylating agent in cancer chemotherapy[93]. The reasons for this popularity are its demonstrated activity against a variety of tumours, a relatively high therapeutic index and the relative ease of administration. It may be administered orally or intravenously. It is frequently used in the treatment of chronic lymphocytic leukaemia, the lymphomas, carcinomas of the bronchus and ovary and various sarcomas.

Cyclophosphamide was synthesised[70] in the belief that it would be inactive in the body until its ring structure was broken down by an enzyme more common in cancer cells than in normal cells. It was thought that cyclophosphamide would be inert until it penetrated the cancer cell. It is now known that cyclophosphamide is metabolically activated in the liver[94]. The parent compound is not an alkylating or cytotoxic agent *in vitro* but is activated by hepatic microsomal enzymes to a variety of active chemicals that are responsible for its biological effects. The nature of the complex metabolism of the drug has been elucidated and the generally accepted metabolic scheme is shown in [Fig.12][95].

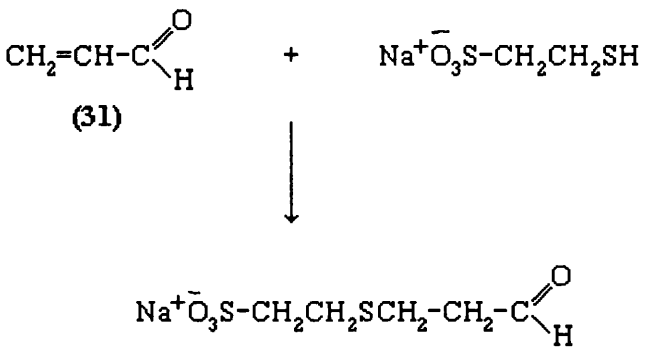


[Fig. 12] Schematic representation of the metabolism of cyclophosphamide (**11**)

The initial activation step is the oxidation of the heterocyclic ring to produce 4-hydroxycyclophosphamide (**29**). This compound is in spontaneous equilibrium with an open-chain aldehyde, aldophosphamide (**30**)[96]. Spontaneous or enzyme assisted elimination of acrolein (**31**)[97] from aldophosphamide yields phosphoramide mustard (**32**)[98]. This compound may enter the target cell either directly or as the non-alkylating

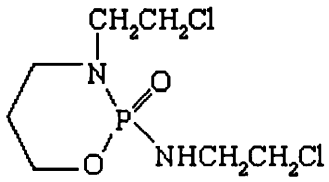
precursor, **(29)**. It is the reactive alkylating product, **(32)**, which is now generally regarded as the main source of the cytotoxicity of **(11)**[99]. Enzymic oxidation of **(29)** and **(30)** produces 4-ketocyclophosphamide **(33)** and carboxyphosphamide **(34)** respectively[100]. Compounds **(33)** and **(34)** have no antitumour activity and little toxicity and appear to be detoxification products. These two compounds appear to account for the majority of an administered dose of cyclophosphamide.

Two side-effects of cyclophosphamide that are uncommonly seen with other alkylating agents are alopecia and haemorrhagic cystitis. Alopecia is a frequent toxicity of other antitumour agents, including vincristine and adriamycin, but is usually not seen with alkylating agents. Phosphoramide mustard does not produce alopecia suggesting that this effect is mediated by lipophilic primary metabolites[101]. Fortunately, alopecia is not life-threatening and is reversible. Acrolein, a urinary metabolite of cyclophosphamide is thought to be responsible for the cause of haemorrhagic cystitis[102]. This serious complication can result in severe haemorrhage and can be fatal. Careful attention to hydration can reduce the frequency of this complication. This problem may be overcome in clinical practice by coadministering a sulphydryl compound, such as sodium 2-mercaptoethanesulphonate (MESNA), which undergoes a Michael-type addition reaction with acrolein to produce a non-toxic thioether[103], [Fig. 13].



[Fig. 13] A possible reaction between acrolein and MESNA

Despite the clinical availability of MESNA, a better solution to the problem would be to modify cyclophosphamide such that acrolein is not generated. Numerous analogues of cyclophosphamide have been prepared, with isophosphamide **(35)** being among the most promising analogues currently known[104], [Fig. 14].

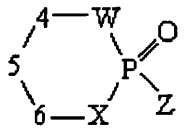


(35)

[Fig. 14] Isophosphamide

Investigations of cyclophosphamide analogues can be classified according to two possible approaches. Historically, the first of these approaches was to synthesise compounds having structural modifications which hopefully, would not preclude the essential features of cyclophosphamide metabolism. These are namely initial enzymatic oxidation and subsequent decomposition with release of a bis-alkylating agent similar to phosphoramide mustard. Structure **(36)** in [Fig.15] is a generalised representation of such compounds having at least one oxidizable carbon-hydrogen bond at either the 4 or 6 position and at least one abstractable proton at the 5 position. The structural elements W-Z are selected bearing in mind that they will also have an influence on the rate of release of the alkylating function. Although the vast majority of cyclophosphamide analogues have carbon atoms at positions 4-6 and 2-chloroethylamino ($ClCH_2CH_2N$) moieties as the potential alkylating functions, it is evident that a variety of alternative heterocycles and acyclic compounds are possible[95,105,106]. Preactivated compounds

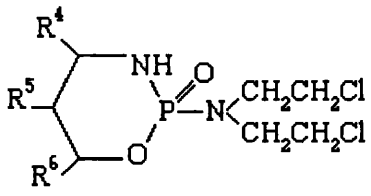
constitute the second general class of cyclophosphamide analogues and the majority bear a functional resemblance to 4-hydroxycyclophosphamide.

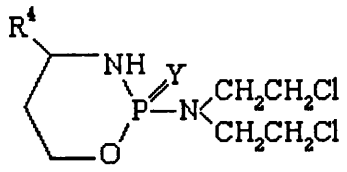


(36)

[Fig. 15] Generalised representation of class I cyclophosphamide analogues

In 1961, Arnold *et al* reported a list of compounds which fulfilled the structural criteria represented by formula **(36)** and the results of single-dose injections against Yoshida ascites sarcoma in rats[107]. Their data for **(37)**-**(42)** [Fig.16] suggested that therapeutic efficacy decreases as the degree of substitution (total number of carbon atoms) increases. It was also found that a relatively minor structural pertubation, such as monomethylation, **(37)**-**(39)** can result in a pronounced lowering of the therapeutic index (TI) value. Replacement of the phosphoryl oxygen with sulphur also caused a marked decrease in activity, as evidenced by comparisons of cyclophosphamide **(11)** (TI 20.0) with 2-thiocyclophosphamide **(43)** (TI 5.3) and 4-methylcyclophosphamide **(38)** (TI 12.5) with its 2-thio counterpart **(44)** (TI 1.5). These early studies also revealed the critical influence of elements W and X in formula **(36)**; for example, the 1,3-dioxy and 1,3-diaza analogues of cyclophosphamide were found to be therapeutically inactive.



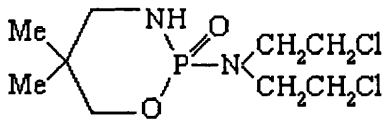


	R^4	R^5	R^6	TI
(11)	H	H	H	20.0
(37)	H	Me	H	14.4
(38)	Me	H	H	12.5
(39)	H	H	Me	12.0
(40)	H	Me	Et	6.0
(41)	Me	H	n-Pr	3.8
(42)	Me	H	$n\text{-}C_6H_{13}$	1.7

	Y	R^4	TI
(11)	O	H	20.0
(43)	S	H	5.3
(44)	S	Me	1.5

[Fig. 16] Adapted from data reported by Arnold *et al*[107]

The relative significance of metabolic pathways available to 4-hydroxycyclophosphamide and its methylated analogues was explored by Cox *et al* using racemates of 4-methyl **(38)**, 5,5-dimethyl **(45)** [Fig.17] and 6-methylcyclophosphamide **(39)**[108]. The virtually identical therapeutic indices found for cyclophosphamide (TI 93) and its 6-methyl derivative **(39)** (TI 92) against ADJ/PC6 plasma cell tumour in mice showed that methylation at this position does not alter drug efficacy. The lower anticancer activity found for the 4-methyl isomer was attributed to diminished oncostatic selectivity resulting from the absence of enzymatic detoxification pathways (further C-4 oxidation). The inactivity observed for **(45)** was consistent with its inability to release phosphoramide mustard.



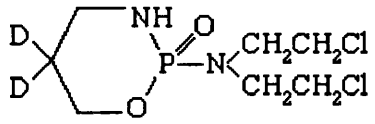
(45)

[Fig. 17]

The importance of phosphoramide mustard release and the nature of the accompanying α, β-unsaturated aldehyde has been probed by the use of various substituent effects. It has been found that during the first ten minutes of liver microsomal incubation of cyclophosphamide and its 5,5-D_2 analogue **(46)**, [Fig.18], the ratio of acrolein : acrolein-D was 5:3[109]. The 7-to-13-fold decrease in tumour toxicity found for the dideuterated drug is consistent with its isotopically retarded release of phosphoramide mustard *in vitro*. These results led to the suggestion that C-5 substituents which increase the efficiency of phosphoramide mustard release might therefore increase tumour toxicity and possibly lead to improvements in cancer treatment relative to **(11)**. The 5-trifluoromethyl derivative of cyclophosphamide **(47)** is of particular interest as the powerful electron-withdrawing effect of CF_3 would presumably lead to increased acidity of the proximate hydrogen and thus accelerate fragmentation to phosphoramide mustard.

Investigations of 5-bromocyclophosphamide **(48)** were motivated by a similar strategy, as inductive stabilization during nucleophilic additions to the expected α-bromoacrolein (CH_2=CBrCHO) fragment could result in enhanced toxicity[110]. In concert with this effect, the bromine substituent can facilitate fragmentation of the aldehydic metabolite by increasing hydrogen acidity within the CHBr moiety. Anticancer screening of a diastereomerically pure sample of 5-bromocyclophosphamide in ADJ/PC6 and L1210 test systems gave a TI of 25 and an increase in survival time of

46% respectively. However, these are less than the corresponding evaluation parameters for cyclophosphamide (91.8 and 250% respectively).



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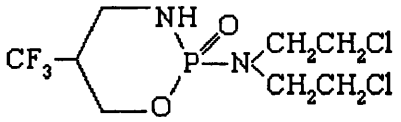

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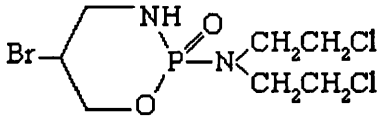
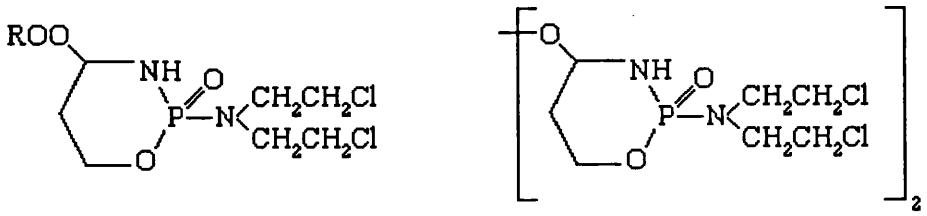


(48)

[Fig. 18]

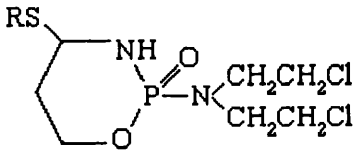
In the search for cyclophosphamide analogues which may have improved therapeutic efficacy against cancer, it is not surprising that 'preactivated' compounds of various types have received considerable attention. Activated (C-4 oxidized) derivatives such as 4-hydroperoxycyclophosphamide **(49)** and 4-peroxycyclophosphamide **(50)** can be obtained by chemical methods[111,112], [Fig.19]. The reactivity of these two compounds has been found to be similar to that of 4-hydroxycyclophosphamide[113,114]. 4-Sulphidocyclophosphamides such as **(51)** and **(52)**, [Fig.20], have been shown to have the same toxicity as 4-hydroxycyclophosphamide towards Yoshida ascites cells *in*

vitro, while analogues such as **(53)** and **(54)** have been found to be substantially less active[115].



(49) **(50)**

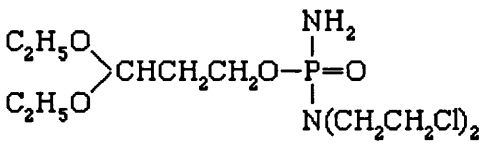
[Fig. 19] Activated (C-4 oxidized) derivatives of cyclophosphamide



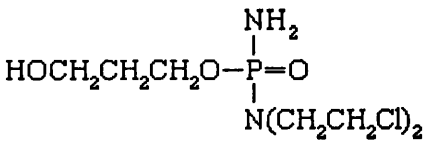
	R
(51)	CH_2CH_3
(52)	CH_2CH_2OH
(53)	$C(CH_3)_3$
(54)	CH_2Ph

[Fig. 20] 4-Sulphidocyclophosphamides

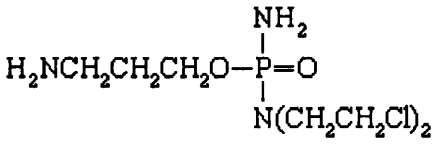
Acyclic compounds **(55)**-**(57)**, [Fig.21], are structurally related to aldophosphamide and could, in principle, serve as *in vivo* precursors to either this metabolite or functionally analogous compounds, that is, molecules capable of 1,2-elimination to give a phosphoramidic mustard[116]. Evaluation of prototypes **(55)**-**(57)** against L1210 leukaemia has indicated no significant increase in life-span.



(55)



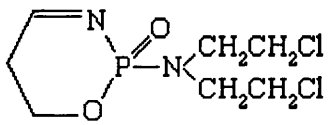
(56)



(57)

[Fig. 21] Some acyclic analogues of cyclophosphamide

Compared with the amount of exploratory synthetic work which has been devoted to 4-hydroxycyclophosphamide, aldophosphamide and their analogues, there have been only a few studies related to iminophosphamide (58), [Fig.22], a putative metabolite of cyclophosphamide[117]. The chemistry of this hypothetical compound and its use as a pro-drug form of the 4-hydroxy(sulphido)cyclophosphamides, has been investigated by Zon *et al*[117].



(58)

[Fig. 22] Iminophosphamide

Attachment of a cytotoxic agent to a carrier molecule is a commonly employed strategy in the design of potential anticancer drugs having increased selectivity. Foster and *et al* anticipated that steroid-cyclophosphamide hybrids might be more selective cytotoxic agents than cyclophosphamide, particularly in hormonally dependent neoplasms[118]. Estrone methyl ether, androstenolone and androstenedione were transformed into steroidal cyclophosphamides but the results of comparative screening tests were not published. Other applications of the carrier principle include the association of alkylating groups with nucleotides[119], proteins[120] and carbohydrates[121].

The subject of the second part of this thesis was to investigate the synthesis of some analogues of cyclophosphamide **(11)** which fulfilled the structural criteria represented by **(36)**. These compounds were to have structural modifications which hopefully would not preclude the essential features of cyclophosphamide metabolism, that is, initial enzymatic oxidation and subsequent decomposition with release of a bis-alkylating agent. The initial aim was to investigate the preparation of analogues with electronegative sustituents particularly at position 5 of **(11)**. It was anticipated that these analogues would be metabolised in an analogous manner to cyclophosphamide and give rise to, in addition to the active phosphoramide mustard, metabolites less toxic than acrolein **(31)**. By overcoming the toxicity associated with the release of acrolein, the need to administer sulphydryl compounds such as MESNA, may be eliminated. In concert with this effect, the electron withdrawing group effect of the electronegative substituents at the 5 position, may lead to increased acidity of the proximate hydrogen and thus accelerate fragmentation to phosphoramide mustard **(32)**. This combination of potent anticancer activity and low renal toxicity may generate analogues having improved chemotherapeutic value in the treatment of cancer. Some phosphite analogues of cyclophosphamide were also investigated.

In summary, it was proposed to prepare some phosphoramidate derivatives of nitrogen mustard with a view to these compounds being inhibitors of HIV and/or neoplasia.

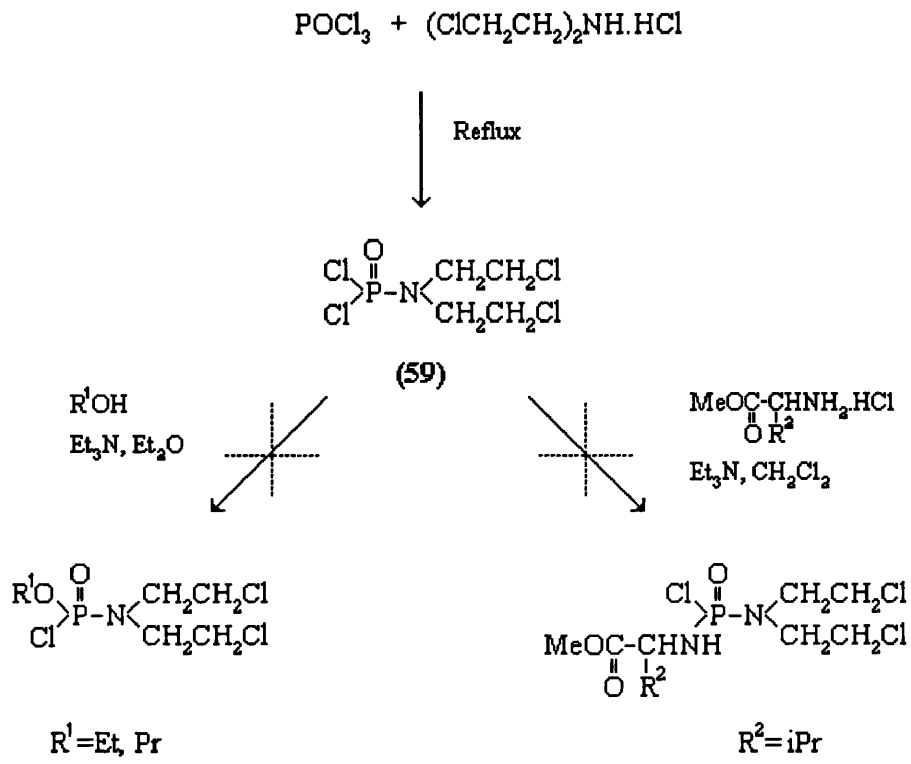
RESULTS AND DISCUSSION

1. Alkyl and aryl phosphoramidate analogues of nitrogen mustard

1.1. N,N-Bis(2-chloroethyl)amino alkyl phosphoramidates

Firstly, a route to alkyl and aryl phosphoramidate derivatives of nitrogen mustard was sought. It was proposed to follow a scheme which involved the preparation of the relevant N,N-bis(2-chloroethyl)amino alkyl phosphorochloridate and its reaction with the appropriate amino acid methyl ester hydrochloride. Initial attempts at the preparation of N,N-bis(2-chloroethyl)amino alkyl phosphorochloridates involved the reaction of dichlorophosphoramide **(59)** with the appropriate alcohol [Fig. 23]. Dichlorophosphoramide was obtained by refluxing bis(2-chloroethyl)amine hydrochloride in phosphoryl chloride and this reaction is discussed in more detail in chapter 2. It was found that the reactions of dichlorophosphoramide with ethanol and propanol, did not proceed to completion. In the former case, the reaction after 16 hours had proceeded to 18% completion while in the latter case it was 13% after 136 hours as judged by ^{31}P nmr spectroscopy. Therefore, increasing the reaction time appeared to have no beneficial effect as was found in the latter case. In both examples the reaction was conducted at room temperature, in ether and in the presence of the base triethylamine. No attempt was made to raise the reaction temperature for fear of generating the disubstituted products, the dialkyl phosphates.

An alternative route to N,N-bis(2-chloroethyl)amino alkyl phosphorochloridates incorporating the carboxy protected amino acid hydrochloride was then investigated. The reaction of dichlorophosphoramide with the carboxy protected amino acid hydrochloride, L-valine methyl ester hydrochloride, was carried out and found to be non-productive with the starting material dichlorophosphoramide being almost completely recovered [Fig. 23].



[Fig. 23]

The low yields and lack of products observed with these reactions of dichlorophosphoramide, were thought to be the results of the poor reactivity of the phosphorylating agent dichlorophosphoramide and the instability of the desired phosphorochloridate under these reaction conditions[122]. These results suggested that perhaps there was a preferred sequence for the addition of the nitrogen mustard, alkyloxy and amino acid methyl ester moieties. With this in mind an alternative route to N,N-bis(2-chloroethyl)amino alkyl phosphoramidates was pursued.

It was discovered that in general, alkyl phosphoramidate derivatives of nitrogen mustard could be prepared by adopting a common 3 step procedure. This involved the coupling of the appropriate N,N-bis(2-chloroethyl)amino alkyl phosphorochloridate with a carboxy protected amino acid hydrochloride. The alkyl phosphorochloridate

could be prepared by reacting the appropriate alkyl phosphorodichloridate with bis(2-chloroethyl)amine hydrochloride. The alkyl phosphorodichloridate could be obtained from the reaction of phosphoryl chloride with the relevant alcohol in the presence of the base triethylamine.

Ethyl phosphorodichloridate **(60)** was prepared by the reaction of ethanol with phosphoryl chloride in the presence of the base triethylamine [Fig. 24][123]. To minimise the formation of the by-products, the dialkyl phosphorochloridate and the trialkyl phosphate, the reaction was carried out at -78°C and in a large volume of solvent. On completion of addition, the reaction mixture was allowed to warm to ambient temperature and stirred for 20 hours. Filtration to remove the precipitated triethylamine hydrochloride and concentration of the filtrate, followed by a hexane extraction on the residue, gave the product as a colourless oil in 80% yield. The ^{31}P nmr spectrum of the product consisted of a single peak at δ5.03 which was close to its literature value and confirmed its identity and purity. In the ^{13}C nmr spectrum phosphorus coupling to both carbon atoms was observed.

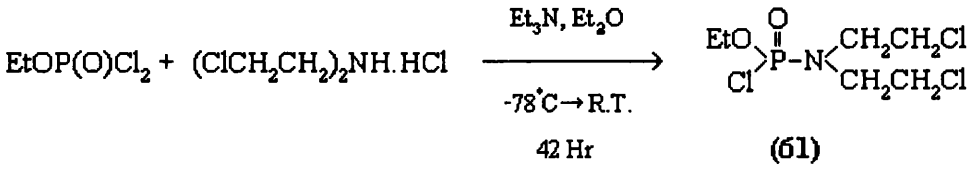
[Fig. 24]

$$POCl_3 + EtOH \xrightarrow[-78^{\circ}C \rightarrow R.T.,\ 20\ Hr]{Et_3N,\ Et_2O} EtOP(O)Cl_2 \quad \textbf{(60)}$$

The phosphorochloridate, N,N-bis(2-chloroethyl)amino ethyl phosphorochloridate **(61)**, was prepared from the reaction of N,N-bis(2-chloroethyl)amine hydrochloride with ethyl phosphorodichloridate with the reaction conditions being similar to those mentioned in the above method [Fig. 25]. An extra equivalent of the base triethylamine was used to release the free amine from the hydrochloride salt and the reaction was conducted at -78°C to minimise the formation

of bis[N,N-bis(2-chloroethyl)amino] ethyl phosphate. After stirring for 42 hours, the triethylamine hydrochloride was removed by filtration and the filtrate concentrated under reduced pressure. A hexane extraction on the residue gave **(61)** as a colourless oil in 86% yield.

[Fig. 25]



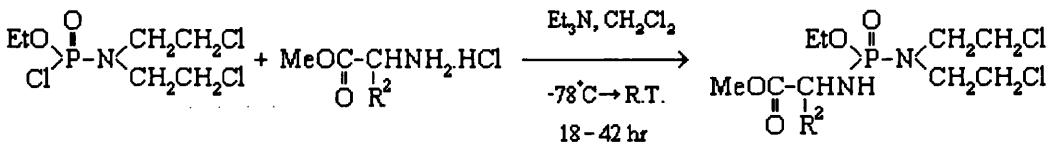
The ^{31}P nmr spectrum of **(61)** contained a single peak at δ13.44 which was in agreement with the value reported in the literature. Phosphorus coupling was observed in the ^{13}C nmr spectrum to the carbon atoms of the bis(2-chloroethyl) and ethyl groups. The 2-chloroethyl chains were observed as being equivalent with the carbons in each chain being recorded as two sets of doublets, the CH_2N at δ49.90 and the CH_2Cl at δ 41.53. It is also interesting to note that three bond phosphorus coupling is generally slightly larger than two bond coupling. For example, coupling constants of 6.0 Hz for $CH_3\underline{C}H_2OP$ and 8.0 Hz for $\underline{C}H_3CH_2OP$ were observed with the former atom giving the signal furthest downfield.

The $CH_3C\underline{H}_2OP$ in the ^1H nmr spectrum of **(61)** was seen as complex signals at δ4.27 and not a quartet as may have been expected. These signals are a consequence of phosphorus-proton coupling in addition to proton-proton coupling. Similar results were observed with the protons of the bis(2-chloroethyl) group with these being seen as two sets of multiplets at δ3.62 and δ3.46 representing CH_2N and CH_2Cl respectively. The methyl protons were seen at δ1.40 as a triplet which was duplicated as a result of phosphorus-proton coupling.

The instability of **(61)** precluded attempts to obtain a molecular ion peak by the technique of electron impact mass spectrometry (E.I.M.S). However, using fast atom bombardment mass spectrometry (F.A.B.M.S), the protonated molecular ion peak for both chlorine isotopes could be observed. Peaks at m/e 242 and m/e 240 indicated the loss of the ethyl group from the diprotonated molecular ion while those at m/e 144 and m/e 142 were assigned to $(ClCH_2CH_2)_2NH_2^+$. The base peak at m/e 102 suggested fragmentation to $P(O)N(CH{=}CH_2)CH_2^+$.

A series of short chain alkyl phosphoramidate derivatives of nitrogen mustard, depicted in [Fig. 26], was prepared by a common procedure. Firstly, N,N-bis(2-chloroethyl)amino ethyl phosphorochloridate **(61)** was stirred with glycine methyl ester hydrochloride in dichloromethane at -78°C to ambient temperature. An extra equivalent of the base triethylamine was used to release the free amino acid from its salt and the reaction was carried out for 42 hours. It was found that the required product could be isolated from the reaction mixture by successive ether extractions, followed by concentration of the combined filtered ether extracts. Thus **(62)** was obtained as a colourless oil in 82% yield without recourse to column chromatography.

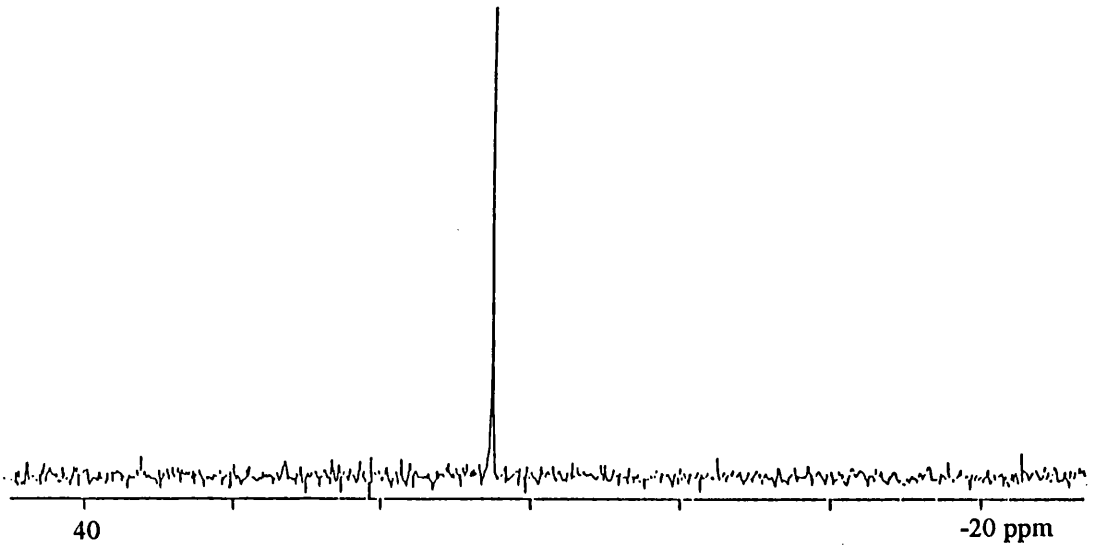
[Fig. 26]



(61)

(62) R^2=H
(63) R^2=$CH(CH_3)_2$
(64) R^2=CH_2Ph

The formation of **(62)** was confirmed by nmr spectroscopy. The ^{31}P nmr spectrum displayed a single resonance at δ12.55 which was in the region that one would expect for compounds of this type [Fig. 27][124]. The incorporation of the methoxyglycinyl moiety had produced an upfield shift of 0.89 ppm. In the ^{13}C nmr spectrum, the signals were entirely as predicted for this type of compound. The carbon atom of the carbonyl group and the CH_2NH were seen as doublets at 171.37 and 42.15 respectively with the former signal showing a larger phosphorus coupling of 8.0 Hz, 6 Hz greater than that seen with the latter signal. Again this is in accordance with the observation that three bond phosphorus coupling is slightly larger than two bond coupling. The methoxy carbon atom and the CH_2Cl were both recorded as singlets at 52.02 and 42.27 although it is usual for the latter signal to show phosphorus-carbon coupling. As observed in the ^{13}C nmr spectrum of **(61)**, both carbons of the ethyl group displayed phosphorus-carbon coupling. An upfield shift of 4 ppm was noted for $CH_3\underline{C}H_2OP$ whereas the chemical shift value for $\underline{C}H_3CH_2OP$ remained largely unchanged.



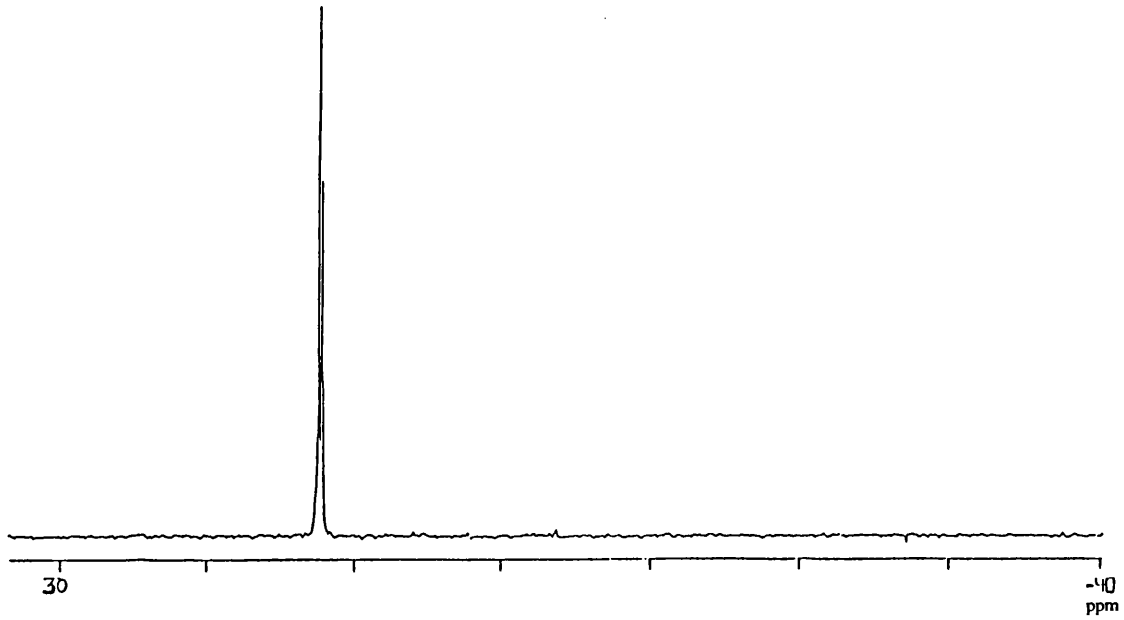
[Fig. 27]

The ^1H nmr spectrum of **(62)** was also consistent with the proposed structure. Additional signals to those seen in the spectrum of **(61)** included the methoxy protons which appeared as a singlet at δ3.74 and $C\underline{H}_2NH$ which increased the integration of the multiplet between δ3.37-3.50 by two protons. E.I. mass spectrometry supplied further evidence for the formation of **(62)** The mass spectrum contained the protonated molecular ion with an accurate mass measurement close to the calculated value. Also observed in the spectrum were peaks at m/e 271, m/e 180 and m/e 261 indicating the loss of CH_2Cl, $N(CH_2CH_2Cl)_2$ and CO_2Me respectively from the molecular ion. The expected chlorine isotope patterns were present in those peaks assigned to chlorine-containing fragments and confirmed the presence of two chlorine atoms in the compound. A peak at m/e 152 indicated the loss of both the ethyl and bis(2-chloroethyl) amino moieties from the protonated molecular ion. Satisfactory analytical data were obtained for this compound.

The next compound in the series, N,N-bis(2-chloroethyl)amino ethyl methoxyvalinyl phosphoramidate **(63)** was prepared by a similar method to that for **(62)**, differing only in the work-up and purification. Compound **(61)** was stirred with L-valine methyl ester hydrochloride in dichloromethane and again the addition of the base triethylamine was carried out at -78°C. Following a reaction time of 18 hours, the reaction mixture was concentrated, dissolved in chloroform and washed with sodium bicarbonate and water to remove any remaining L-valine methyl ester hydrochloride and phosphorochloridate. A yellow oil was obtained by concentrating the organic extract under reduced pressure. Characterisation of this material by ^{31}P nmr spectroscopy revealed minor signals at δ +3 and -7 ppm which were thought to represent products of hydrolysis. Repeated ether extractions failed to remove completely the unwanted material so it was found to be necessary to purify **(63)** by column chromatography. Two successive columns, using elution with chloroform followed by a mixture of methanol and chloroform, were needed to isolate the required

compound as a colourless oil in 65% yield. The absence of an ultra-violet chromophore in the compound meant that the components had to be visualised with an iodine and silica system. The compound being an oil rather than a solid suggested that a mixture of isomers had been isolated.

Evidence for a mixture of diastereoisomers being isolated was also found in the ^{31}P nmr spectrum which contained two distinct resonances at 12.51 and 12.28 [Fig. 28]. The isomerism is a result of the mixed stereochemistry at the phosphorus centre and was not observed in the spectrum of **(62)** due to the absence of a chiral centre in the glycinyl methyl ester moiety. In the case of **(63)**, the resonances were in the region expected for this type of alkylamino phosphate and were in a ratio of 2:1 with a separation of 0.2 ppm. An upfield shift of 1 ppm, similar to that noted for **(62)**, was produced upon the introduction of the methoxyvalinyl group.



[Fig. 28]

The presence of diastereoisomers was also evident from the ^{13}C nmr spectroscopic data. Most of the signals were split in a ratio which reflected the ratio of the diastereoisomers, that is 2:1. Further splitting was noted in the cases of resonances of carbon atoms within three bonds of the phosphorus, this being the result of phosphorus coupling. Examples of carbons showing both diastereomeric splitting and phosphorus coupling were $CH_3\underline{C}H_2OP$ and $\underline{C}H(CH_3)_2$ with both resonances appearing as clear doublets of doublets. The carbonyl carbon however, was observed as three signals at δ173.83. The carbons in each isomer contributing to these signals do not experience the same magnetic environment and so they resonate at slightly different chemical shifts. Each carbon couples to the phosphorus to give a doublet and these doublets overlap to give the appearance of a triplet. The signals assigned to $\underline{C}H_3CH_2OP$ were found to show the same effect. The largest diastereomeric splitting observed was 0.64 ppm for CHNH with this resonance being similar to those for CH_2N and CH_2Cl in that phosphorus coupling was absent. The non-equivalence of the valinyl methyl carbons was also apparent from the spectrum by virtue of their differing chemical shift values with one methyl carbon giving a diastereomeric splitting of 0.19 ppm and the other 0.02 ppm. It was thought that this may be an effect of the methyl carbons in an isomer experiencing different magnetic environments with the two isomers adopting dissimilar conformations.

The ^1H nmr data were also fully consistent with the structure of **(63)**. The non-equivalence of the valinyl methyl protons was also evident in the spectrum with the protons of one methyl group being seen as a clear doublet at δ0.98 while the other was a triplet at δ0.92. F.A.B. mass spectrometry also confirmed the isolation of **(63)**. In addition to the protonated molecular ion, the spectrum contained prominent peaks at m/e 222 (due to M^+-$N(CH_2CH_2Cl)_2$) and m/e 194 (due to M^+-$N(CH_2CH_2Cl)_2$-C_2H_5). Also observed were peaks at m/e 305 and m/e 303 which indicated the loss of the methoxy carbonyl group from the molecular ion for both chlorine isotopes.

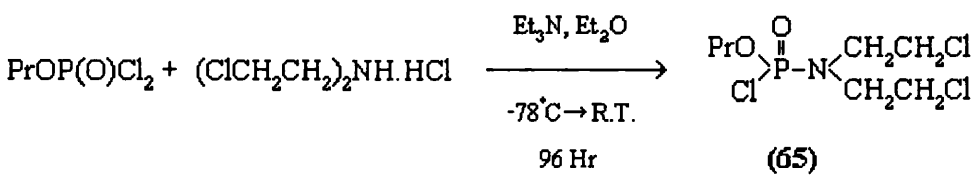
The next compound in the series, N,N bis(2-chloroethyl)amino ethyl methoxyphenylalaninyl phosphoramidate **(64)**, was prepared in an analogous manner to **(63)** except that the reaction was carried out for 42 hours and the step involving ether was excluded. The residue obtained from the organic-aqueous extraction was purified further by column chromatography with neat chloroform as the eluent. Analytical tlc had indicated the presence of a fast running component, possibly a product of hydrolysis which may have been generated during the organic-aqueous extraction. This impurity was successfully removed by chromatography to give **(64)** as a white solid in 77% yield. This material was fully characterised by spectroscopic and analytical methods.

Once again, the required compound was isolated as a mixture of diastereoisomers with these giving coincident resonances at δ11.40 in the ^{31}P nmr spectrum obtained at 82 MHz. This signal was resolved into two closely spaced resonances, in a ratio of 1:1 with a separation of 0.06 ppm, on a spectrometer operating at a higher frequency of 164 MHz. The presence of diastereoisomers was also evident from the ^{13}C nmr and ^{1}H nmr data. As in the case of **(63)**, the ^{13}C nmr spectrum contained signals which were split due to both diastereomeric splitting and phosphorus coupling. For example, both CH_2N and the methoxy carbon were observed as doublets of doublets at δ49 and δ52 respectively. The only signal showing neither effects was the CH_2Cl which was observed as a clear singlet at δ42.53. The only explanation for this is that the splittings were too small to be recorded. The spectrum also confirmed the presence of the phenylalaninyl group in the compound. The phenyl carbons were found to resonate in the expected region between δ127-136 with each carbon appearing as a doublet in a ratio of 1:1 due to diastereomeric splitting. The CH_2Ph, being three bonds away from the phosphorus atom and expected to show phosphorus coupling, appeared as a triplet at δ40.63 due to coincidence.

The ^1H nmr spectrum was also entirely consistent with the structure of **(64)**. All the protons in the compound were observed as multiplets with the exceptions of the methoxy protons and $C\underline{H}_3CH_2OP$. The former protons, in contrast to **(63)**, showed a diastereomeric splitting and were observed as two closely spaced signals at 3.70 and 3.69. The latter protons were found to resonate as a familiar triplet of doublets. F.A.B. mass spectrometry also confirmed the isolation of **(64)**. The spectrum displayed the protonated molecular ion and chlorine isotope effects in a ratio consistent with two chlorine atoms being present in the compound. Also observed were peaks at m/e 351, due to the loss of CO_2Me from the molecular ion, at m/e 285 (MH^+-2x CH_2CH_2Cl) and m/e 242 (MH^+-$N(CH_2CH_2Cl)_2$-C_2H_5). Also identified was a peak at m/e 319 due to the loss of CH_2Ph from the molecular ion.

The series of alkyl phosphoramidate derivatives of nitrogen mustard was continued with the preparation of a small number of compounds with the alkyl chain being lengthened from ethyl to propyl. It was thought to be of interest to investigate the effect this variation may have on the potential anti-HIV activity. Firstly, N,N-bis(2-chloroethyl)amino propyl phosphorochloridate **(65)** was prepared from propyl phosphorodichloridate [Fig. 29] by a similar method to that used for **(61)**. After an extended reaction time and the usual hexane work-up, **(65)** was obtained as a colourless oil in a yield of 63%. A single resonance at δ13.60 in the ^{31}P nmr spectrum supported the identity of the product. In the ^{13}C nmr spectrum all carbons within three bonds of the phosphorus were observed to show phosphorus coupling.

[Fig. 29]



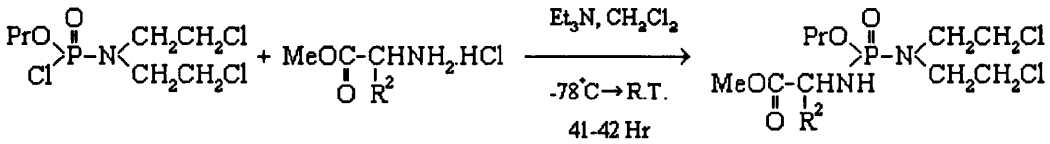
A similar ^{1}H nmr spectrum to **(61)** was obtained for **(65)** with the methyl and the additional adjacent methylene protons resonating as the expected triplet and sextet at δ0.98 and δ1.77 respectively. The F.A.B. mass spectrum displayed a peak at m/e 304 which corresponded to M^{+}+Na, this combination arising from the use of a sodium containing matrix to record the fragmentation of **(65)**. Again the isotope effects for the two chlorine atoms were observed. Other peaks in the spectrum included those at m/e 240 (MH_2^{+}-C_3H_7) and m/e 142 (MH^{+}-$N(CH_2CH_2Cl)_2$).

The synthesis of N,N-bis(2-chloroethyl)amino propyl methoxyglycinyl phosphoramidate **(66)** was the first to be undertaken in this short series [Fig. 30]. Thus **(65)** was treated with glycine methyl ester hydrochloride in dichloromethane, in the presence of triethylamine at -78°C to ambient temperature, for 42 hours. The normally employed organic-aqueous wash in the work-up was replaced with an ether extraction. It was felt that a cleaner mixture could be obtained prior to column chromatography if it was not exposed to an aqueous procedure. This indeed turned out to be the case when the crude product from the ether extraction was examined by ^{31}P nmr spectroscopy. There were, however, two minor signals on either side of the main signal which was at δ12.67. The minor signal at δ13.63 was thought to be unreacted phosphorochloridate **(65)** while the other minor signal at δ10.99 remained unidentified. Purification of this material was carried out by column chromatography with an eluent of neat chloroform and the components being visualised with iodine. This led to the isolation of the required compound **(66)** as a colourless oil in 57% yield.

The ^{31}P nmr spectrum of **(66)** displayed a single resonance at δ12.49 which was close to that observed for the analogous ethyl compound **(62)**. Replacing the ethyl group with a propyl group was noted to have produced an upfield shift of 0.1 ppm. The ^{13}C nmr data were also informative with all the signals except for CH_2Cl and $\underline{C}H_3CH_2CH_2OP$ appearing as doublets as a result of phosphorus coupling. CH_2Cl would be expected to couple to the phosphorus but as previously observed in the

spectra of the ethyl derivatives **(62)** and **(64)**, this signal has appeared as a singlet when the coupling has been too small to be resolved. Interestingly, in the ^{13}C nmr spectra of the phosphorochloridates **(61)** and **(65)**, CH_2Cl has been found to give a phosphorus-carbon coupling of 2 Hz. As in **(62)**, the absence of a chiral centre in the glycinyl moiety of **(66)** explained why there was no diastereomeric splitting.

[Fig. 30]



(66) R^2=H
(67) R^2=CH_2Ph

The ^1H nmr spectrum of **(66)** was assigned by analogy to the spectrum of **(62)**. The signals due to the protons of the bis(2-chloroethyl) moiety were observed as multiplets between δ3.40-3.70. The methoxy protons were also observed as a singlet at their characteristic position of δ3.72. The propyl protons were noted to have shifted upfield by 0.1-0.2 ppm in relation to **(65)**, with $CH_3CH_2C\underline{H}_2OP$ showing the largest shift. The F.A.B. mass spectrum further confirmed the successful isolation of **(66)**. The mono and diprotonated molecular ions were observed along with the familiar chlorine isotope patterns consistent with two chlorine atoms being present in the compound. The most prominent peak in the spectrum was that at m/e 194 assigned to M^+-$N(CH_2CH_2Cl)_2$. Other peaks observed were those at m/e 275 (due to M^+-OC_3H_7) and at m/e 247 due to loss of the glycinyl group (MeO_2CCH_2NH) from the protonated molecular ion.

The preparation of N,N-bis(2-chloroethyl)amino propyl methoxyphenylalaninyl phosphoramidate **(67)** was carried out by a similar method to **(66)**. The phosphorylating agent **(65)** was reacted with L-phenylalanine methyl ester hydrochloride in dichloromethane in the presence of the base triethylamine. Following a reaction time of 41 hours and successive ether and hexane extractions, the product was examined by ^{31}P nmr spectroscopy. The spectrum was found to consist of two closely spaced resonances at δ11.76 and δ11.65 which were in the region expected for this phosphoramidate. In contrast to the spectrum of the analogous ethyl compound **(64)**, the signal showed a small diastereomeric splitting of 0.1 ppm at 82 MHz. The data also suggested that the product had been isolated in the pure state. However, the ^{13}C nmr spectrum of this material was found to contain minor signals, all of which corresponded to unreacted L-phenylalanine methyl ester hydrochloride. An organic-aqueous extraction failed to remove the impurity and so it was found to be necessary to further purify the product by column chromatography. Using an eluent of a mixture of chloroform and petroleum spirit, the product was separated from the slower running impurity and was isolated as a white solid in 37% yield.

^{13}C nmr data confirmed the purity and identity of the isolated compound. The spectrum was assigned by analogy to the spectrum of **(64)** and again provided evidence for the presence of diastereoisomers in the ratio of 1:1. Most of the signals showed, in addition to diastereomeric splitting, the usual two and three bond phosphorus-carbon coupling and for $\underline{C}H_2Ph$, $CO_2\underline{Me}$ and $CH_3CH_2\underline{C}H_2OP$, these signals appeared as triplets due to the overlapping of two doublets. The largest diastereomeric splittings were observed for carbons of the methoxyphenylalaninyl group. For example, CHNH and $\underline{C}O_2Me$ were found to give splittings of 0.54 ppm and 0.28 ppm respectively. Again, the only signal showing neither diastereomeric splitting nor phosphorus coupling was that for CH_2Cl at δ42.52. This was in marked contrast to the adjacent CH_2N which was seen as a doublet of doublets at δ49.14 and δ49.03 showing a coupling of 4.6 Hz.

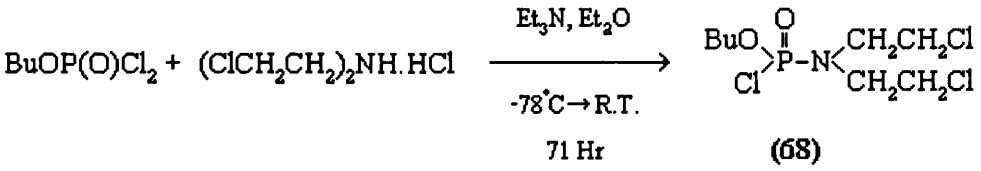
The signal due to $CH_3CH_2\underline{C}H_2OP$ at δ67.28, an upfield shift of 3 ppm relative to the spectrum of the starting **(65)**, appeared to be the only carbon in the propyl chain to be significantly affected by the introduction of the methoxyphenylalaninyl group.

The ^1H nmr spectrum was also informative and was assigned by analogy to the spectrum of **(64)**. The $CH_3CH_2C\underline{H}_2OP$ and $CH_3C\underline{H}_2CH_2OP$ signals were observed in their characteristic positions as a multiplet and sextet respectively, while $C\underline{H}_3CH_2CH_2OP$ was found to resonate as a triplet of doublets at δ0.89. The latter effect was presumed to be the result of diastereomeric splitting. Also exhibiting this type of splitting were the methoxy protons which appeared as two signals at δ3.7 with a fine splitting of 0.01 ppm. The phenyl protons, observed as a multiplet, were also in their characteristic position between δ7.13-7.30. The E.I. mass spectrum was also entirely consistent with the proposed structure of **(67)** and showed the protonated molecular ion peak at m/e 425 with a satisfactory accurate mass value. Also observed in the spectrum were peaks at m/e 365 and m/e 333 corresponding to the loss of OC_3H_7 and CH_2Ph respectively from the molecular ion. Other prominent peaks were those at m/e 284, arising from the loss of the bis(2-chloroethyl) group from the molecular ion, and at m/e 91 which was assigned to $PhCH_2^+$. The purity of **(67)** was confirmed by microanalysis and analytical hplc data.

The alkyl chain was gradually extended from ethyl to butyl with the preparation of some butyl phosphoramidate derivatives using the previously described methodology. N,N-Bis(2-chloroethyl)amino butyl phosphorochloridate **(68)** was obtained, from the reaction of butylphosphorodichloridate with bis(2-chloroethyl)amine hydrochloride, as an oil in 86% yield [Fig. 31]. The ^{31}P nmr spectrum contained a signal at δ13.62 which was consistent with the chemical shift values recorded for **(61)** and **(65)**. The ^{13}C nmr data were also in agreement with the structure of the monosubstituted phosphorylating agent. Phosphorus coupling was observed to the carbons of the bis(2-chloroethyl) group and to the two carbon atoms, in the alkyl chain,

closest to the phosphorus. A similar ^1H nmr spectrum to **(65)** was obtained for **(68)** with all protons, except those of the terminal methyl (triplet), being observed as multiplets.

[Fig. 31]



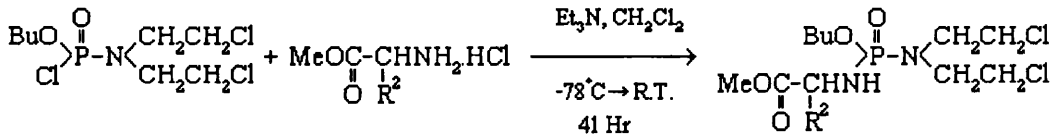
(68)

N,N-Bis(2-chloroethyl)amino butyl methoxyvalinyl phosphoramidate **(69)** was prepared by a using a similar method to that used for **(63)**. This involved the reaction of **(68)** with L-valine methyl ester hydrochloride in dichloromethane at -78°C to ambient temperature [Fig. 32]. Analytical tlc of the mixture, with iodine being used to visualise the components, indicated that there was a minor impurity having an R_f value identical to that of the starting material **(68)**. At this stage, after a reaction time of 41 hours, it was decided to remove the water-sensitive phosphorochloridate **(68)** by an organic-aqueous wash. As expected, ^{31}P nmr spectroscopy of the resulting pale yellow oil indicated that **(68)** had been successfully removed by the organic-aqueous wash but at the expense of generating some minor products (<5%). Therefore the material was further purified by column chromatography and this procedure led to the isolation of a colourless oil in 69% yield. The same system of iodine and silica was used to monitor the separation of the components.

The ^{31}P nmr spectrum of this oil was composed of two closely spaced resonances at δ12.54 and δ12.30. As observed with other members of this series, these resonances in a ratio of 1:1 were due to the presence of diastereoisomers. The ^{13}C nmr spectrum also confirmed the identity and purity of **(69)** with most signals displaying

diastereomeric splitting and where appropriate, two and three bond phosphorus-carbon coupling. Interestingly, CH_2Cl which is generally observed as a singlet, was found to display a diastereomeric splitting of 0.05 ppm which was comparable with that noted for compound **(63)**. The chemical shift values of the butyl carbons remained relatively unchanged except for $CH_3CH_2CH_2\underline{C}H_2OP$ which was noted to have shifted upfield by 3 ppm upon the introduction of a second P-N bond. Both $CH_3CH_2\underline{C}H_2CH_2OP$ and $\underline{C}H(CH_3)_2$ were found to appear as a multiplet between δ32.14-32.47 as a result of similar chemical shift values. The ^1H nmr spectrum was also in good agreement with the structure of **(69)**. Most signals were observed as multiplets due to phosphorus-proton and proton-proton couplings and possibly diastereomeric splitting. The latter effect was visible with the methoxy protons which were observed as two very closely spaced signals at their characteristic position of δ3.7. However, at a frequency of 400 MHz this splitting was too small to be quantified.

[Fig. 32]



(69) $R^2=CH(CH_3)_2$
(70) $R^2=CH_2Ph$

The F.A.B. mass spectrum provided further evidence for the successful isolation of **(69)**. In addition to the protonated molecular ion at m/e 391, the spectrum contained peaks at m/e 331, (due to M^+-CO_2Me) and at m/e 250 (due to M^+-$N(CH_2CH_2Cl)_2$). Also observed was a peak at m/e 275 which corresponded to the fragmentation of both the methoxy carbonyl and butyl groups from the protonated

molecular ion. Both microanalysis and analytical hplc data also confirmed the identity and purity of **(69)**.

Compound **(70)** was similarly prepared from **(68)** and L-phenylalanine methyl ester hydrochloride and was isolated as a colourless oil in a reasonable yield of 56%. This compound was also successfully characterised by spectroscopic and analytical methods. The diastereoisomers of the product were observed to give resonances which were coincident, appearing at δ11.69 in the ^{31}P nmr spectrum obtained at 82 MHz. It was found that this signal could not be resolved when a higher frequency of 164 MHz was used. The ^{13}C nmr spectrum of **(70)** was assigned by analogy to the spectra of **(64)** and **(67)** and was also entirely consistent with the proposed structure. Again, many signals were found to exhibit both diastereomeric splitting and phosphorus-carbon coupling. These included the carbonyl carbon which was observed as a doublet of doublets at δ173.53 and δ173.24 with one signal showing a phosphorus coupling of 3.2 Hz while the other showed a larger coupling of 5.8 Hz. All the phenyl carbons, resonating in their characteristic region between δ127-136, displayed diastereomeric splittings ranging between 0.07 ppm and 0.11 ppm. The largest splitting was that of 0.55 ppm noted for CHNH and this has been the case in some of the previously described analogues. It was also of interest to note that the magnitude of the diastereomeric splittings, in the carbons of the butyl chain, decreased as one moved further away from the phosphorus centre so that it was completely absent in the terminal methyl.

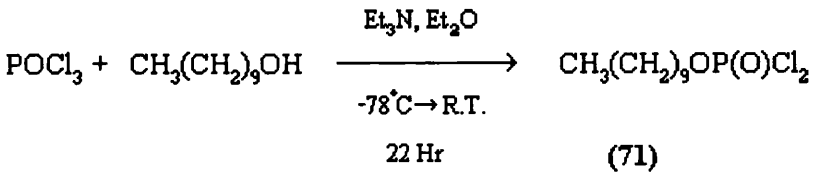
The ^{1}H nmr data also supported the structure of **(70)**. The spectrum contained a number of signals between δ2.94-3.53 which were assigned to the bis(2-chloroethyl)amino and alaninyl protons. The methoxy protons in each isomer were found to give separate signals showing a diastereomeric splitting of 0.012 ppm. The phenyl protons were found to resonate as complex signals downfield between δ7.15-7.32 with the complexity being attributed to both proton-proton and phosphorus-proton

couplings and diastereomeric splitting. The terminal methyl protons of the butyl chain were observed as a triplet of doublets at δ0.93 while the adjacent methylene protons were resolved into a sextet of doublets at δ1.34 ppm, both effects being duplicated due to diastereomeric splitting. The remaining protons were observed as multiplets with $CH_3CH_2CH_2C\underline{H}_2OP$ at δ3.87, an upfield shift of 0.4 ppm relative to **(68)**. Further evidence for the successful isolation of **(70)** was provided by the F.A.B. mass spectrum which contained the protonated molecular ion at m/e 439. A peak at m/e 441 and its splitting was consistent with the presence of two chlorine atoms. The fragmentation of this compound was found to be similar to that for analogous compounds. Microanalysis and analytical hplc data confirmed the purity of **(70)**.

In view of the success of the strategy developed for the synthesis of compounds previously described, the synthesis of a number of long chain alkyl phosphoramidate derivatives of nitrogen mustard was undertaken. The presence of long alkyl groups such as decyl, octadecyl and oleyl in the compound would inevitably increase the lipophilicity of the compound. Therefore, it would be of interest to see what advantage the increased lipophilicity would have on the activity of the compound.

The synthetic route employed for the decyl phosphoramidates **(73)** and **(74)** shown in [Fig. 35] was similar to that for the short chain alkyl compounds and involved the reaction of the appropriate phosphorochloridate with a variety of carboxy protected amino acid hydrochlorides. Firstly, the synthesis of decyl phosphorodichloridate **(71)** was carried out in an analogous manner to **(60)** [Fig. 33]. This method involved the low temperature reaction of phosphoryl chloride with 1-decanol in ether in the presence of triethylamine. A hexane extraction on the concentrated reaction mixture gave **(71)** as a colourless oil in a yield of 98%.

[Fig. 33]

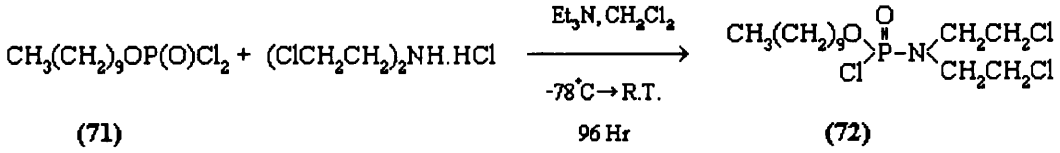


The ^{31}P nmr spectrum consisted of a major signal at δ5.12 corresponding to **(71)** and a minor signal at δ2.95 with the former chemical shift being consistent with this type of alkyl phosphorodichloridate[124]. The ^{13}C nmr spectrum displayed some interesting features and was assigned by reference to model compounds such as decanol. The multiplet of signals appearing between δ28.92-29.63 was assigned to the methylene carbon atoms (C-4→C-8) towards the centre of the decyl chain. C-10 was found to resonate at δ72.43 and showed a phosphorus-carbon coupling of 9.9 Hz while the remaining carbons were observed as singlets. The ^{1}H nmr spectrum also confirmed the isolation of **(71)**. The $CH_2C\underline{H}_2OP$ and $C\underline{H}_2CH_2OP$ signals were observed as multiplets at δ4.31 and δ1.78 respectively, this pattern arising as a result of additional coupling to the adjacent phosphorus. The broad singlet at δ1.25 integrating for 14 protons was assigned to the remaining methylene protons.

The phosphorodichloridate **(71)** was then treated with bis(2-chloroethyl)amine hydrochloride, in dichloromethane in the presence of two equivalents of triethylamine, by a slightly different procedure to that for **(61)**, **(65)** and **(68)** from their corresponding phosphorodichloridates [Fig. 34]. An extended reaction time of 96 hours and the use of two equivalents rather than one of bis(2-chloroethyl)amine hydrochloride was required to take into account the expected lack of reactivity of **(71)** due to its steric bulk. Dichloromethane was chosen as the solvent suitable for the reaction as previous attempts in ether had generated a mixture of products comprising of the required product and the starting material. It was found that by concentrating the reaction

mixture under reduced pressure and carrying out an ether extraction on the residue in the usual way, **(72)** could be obtained as an oil. In this case the isolated product was again a mixture of components with the required phosphorylating agent being the dominant component resonating at δ13.38 in the ^{31}P nmr spectrum.

[Fig. 34]



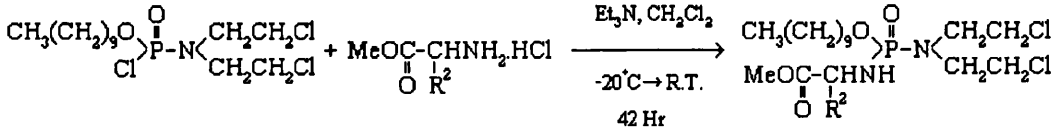
(71) **(72)**

The ^{13}C nmr spectrum showed similar signals to **(71)** along with some minor impurities. Additional doublets were observed at δ49.92 and δ41.53 arising from the bis(2-chloroethyl)amino carbons. Compared to the spectrum of **(71)**, there was an upfield shift of 4 ppm for C-10, the decyl carbon closest to the phosphorus. Again, the ^{1}H nmr spectrum of **(72)** showed similar resonances to **(71)** with the multiplets at δ3.64 and δ3.47 integrating for 4 protons each and being assigned to $N(CH_2CH_2Cl)_2$. It was decided to use the crude product directly, in subsequent reactions.

The decyl phosphoramidate derivatives, **(73)** and **(74)**, were the first compounds to be prepared in the series of long chain alkyl derivatives [Fig. 35]. The preparation of **(73)**, the least lipophilic of the two compounds, involved reacting the phosphorylating agent **(72)** with L-alanine methyl ester hydrochloride in dichloromethane in the presence of triethylamine. It was predicted that the reaction would not proceed to completion if it was conducted at the usual low temperature of -78°C. Therefore, to take into account the predicted low reactivity of **(72)**, the addition of the base triethylamine was carried out at -20°C. On completion of addition, the reaction was allowed to warm to ambient temperature and stirred for 42 hours. It was

attempted to follow the course of the reaction by analytical tlc using iodine to visualise the components and this suggested that 42 hours was adequate. This process of analysis also revealed the presence of some minor impurities which were more lipophilic than the required product (R_f 0.48). The next step involved concentrating the reaction mixture and extracting the crude product with ether. In this way, a yellow oil was isolated and this was purified further by column chromatography. Due to the closeness on tlc, of the minor impurities to the required product, an eluent consisting of a mixture of chloroform and petroleum spirit was used. This slow procedure resulted in the isolation of **(73)** as a colourless oil in 60% yield.

[Fig. 35]



(73) $R^2=CH_3$
(74) $R^2=CH(CH_3)_2$

The isolated product was characterised by spectroscopic and analytical techniques. Two resonances at δ11.75 and δ11.38 in the ^{31}P nmr spectrum indicated that once again an equal mixture of isomers had been isolated. The ^{13}C nmr data further confirmed the structure of **(73)** with the effects of diastereoisomerism and phosphorus-carbon coupling being observed with the relevant carbons. In some cases the expected doublet of doublets was found to merge to give the appearance of a triplet. For example, the carbonyl carbon and C-10 gave triplets at δ174.56 and δ65.71 respectively. An upfield shift of 3 ppm, relative to **(72)**, was noted in the latter case. A doublet of doublets at δ21.39 and δ21.05 representing the alaninyl $CH\underline{C}H_3$, supported

the presence of the alaninyl group. In all other respects the ^{13}C nmr data were similar to those obtained for **(72)**.

The ^{1}H nmr data were also in agreement with the structure of **(73)**. Evidence for the presence of a decyl group were a multiplet and a quintet at δ3.90 and δ1.59, a triplet at δ0.83 and a broad singlet at δ1.22, which were assigned to $CH_2C\underline{H}_2OP$ and $C\underline{H}_2CH_2OP$, the terminal methyl and the remaining methylene protons respectively. Evidence for the presence of the methoxyalaninyl group were a fine singlet at δ3.70, a triplet at δ1.37 and a small multiplet at δ3.20 which were assigned to the methoxy, alaninyl methyl and amino protons respectively. The increased integration, by an additional proton, of the multiplet between δ3.51-3.65 arising from CH_2N was due to the presence of $C\underline{H}NH$ of the alaninyl moiety. Peaks at m/e 451, m/e 449 and m/e 447 in the F.A.B. mass spectrum corresponded to the expected protonated molecular ion cluster of this compound. Also observed was a peak at m/e 344 indicating fragmentation with the loss of the methoxyalaninyl group from the protonated molecular ion and peaks at m/e 306 (due to M^{+}-$N(CH_2CH_2Cl)_2$) and m/e 166 (due to MH^{+}-$N(CH_2CH_2Cl)_2$-$(CH_2)_9CH_3$).

Compound **(74)** was similarly obtained from the coupling of **(72)** with L-valine methyl ester hydrochloride. After an identical reaction time of 42 hours and the usual ether work-up, it was found by analytical tlc that the isolated yellow oil contained several components. Therefore, this material was further purified by the technique of column chromatography with the use of a mixture of chloroform and petroleum spirit as the eluent. This procedure led to the isolation of **(74)** as a colourless oil in 48% yield. The unexpected low yield of the product was attributed to the use of a crude phosphorylating agent. Evidence for the diastereoisomers being isolated in a ratio of 2:1 was provided by the ^{31}P nmr spectrum which contained two resonances at 14.97 and 14.71. As an inspection of the ^{31}P nmr spectrum was not carried out, prior to the column, it

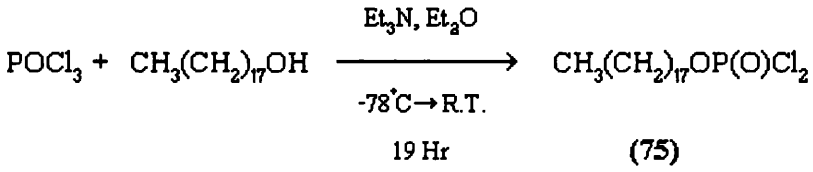
was not known whether or not the formation of one isomer had been preferred over the other.

The ^{13}C nmr data confirmed the identity of the isolated product with the ratio of the diastereoisomers in the spectrum being consistent with that shown in the ^{31}P nmr spectrum. The spectrum was assigned by analogy to **(73)**, with many similar features being observed. Surprisingly, only one carbon (C-10), exhibited both phosphorus-carbon coupling and diastereomeric splitting. The methylene carbons of the decyl chain were found to resonate as singlets between δ22.63-31.83 with several showing diastereomeric splitting ranging between 0.02-0.03 ppm. As noted with previously described methoxyvalinyl compounds, diastereomeric splitting was observed for only one of the two valinyl methyl carbons.

The ^{1}H nmr data were also in agreement with the proposed structure and displayed signals which could be correctly assigned to all three groups attached to the phosphoryl centre. All protons, with the exception of $CH_3(C\underline{H}_2)_7$ and all methyl protons, were observed as multiplets in their characteristic positions. The methylene protons were found to resonate as the usual broad singlet at δ1.23 while the methoxy protons were observed as a fine singlet at δ3.70. One of the valinyl methyl protons was resolved into a clear doublet of doublets at δ0.94, a consequence of an expected doublet showing a diastereomeric splitting The other valinyl methyl protons resonated slightly further upfield at a similar chemical shift to the terminal methyl of the decyl chain and so appeared as a multiplet. Structural confirmation was provided by the F.A.B. mass spectrum which displayed the protonated molecular ion at m/e 475 and m/e 477. The pattern of peaks assigned to chlorine containing fragments was entirely consistent with two chlorine atoms being present in the compound. The most noticeable peaks were those at m/e 334, (due to MH^+-$(CH_2)_9CH_3$), m/e 275 (due to MH^+-$(CH_2)_9CH_3$-CO_2Me) and m/e 194 (due to MH^+-$(CH_2)_9CH_3$-$N(CH_2CH_2Cl)_2$). Satisfactory microanalysis and analytical data were obtained for this compound.

The series of long chain alkyl phosphoramidate derivatives of nitrogen mustard was elaborated with the synthesis of compounds (77) and (78) where the alkyl chain was extended to octadecyl [Fig. 38]. The synthetic route to these compounds required the preparation of the appropriate alkyl phosphorodichloridate. Thus, octadecyl phosphorodichloridate (75) was duly synthesised from 1-octadecanol and phosphoryl chloride by the routine method for preparing alkyl phosphorodichloridates [Fig. 36].

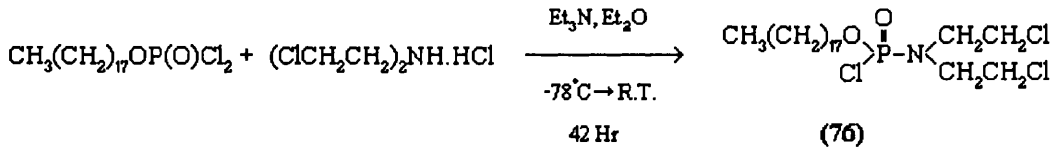
[Fig. 36]



The required product (75), obtained in the form of a powdery white solid in 89% yield, was characterised by ^{31}P nmr spectroscopy. The spectrum consisted of a single resonance at δ5.26 which was close to the value recorded for decyl phosphorodichloridate (71). The ^{13}C nmr spectrum contained similar features to those found in the corresponding spectrum of (71) and was also dominated by a multiplet between δ29.37-29.69 representing the methylene carbons C-4-C-15. The signal due to C-18 at δ72.30 was noted to be a doublet with a large phosphorus-carbon coupling constant of 10.1 Hz. The ^{1}H nmr spectrum obtained was also very similar to the spectrum of (71) with the only noticeable difference being in the broad singlet at δ1.23 which integrated for 30 protons. Further confirmation of (75) came from its F.A.B. mass spectrum which contained peaks at m/e 409 and m/e 411, corresponding to (M^++Na) and showing the chlorine isotope effect for the two chlorine atoms in the product.

N,N-Bis(2-chloroethyl)amino octadecyl phosphorochloridate (**76**) was obtained from the reaction of (**75**) with bis(2-chloroethyl)amine hydrochloride in ether at a low temperature, followed by a routine filtration and concentration of the mixture [Fig. 37]. This procedure gave the required phosphorochloridate as a white solid in 89% yield. The ^{31}P nmr spectrum displayed a major signal at δ13.57 which indicated a downfield shift of 8 ppm upon the introduction of the bis(2-chloroethyl)amino group. The ^{13}C nmr spectrum of (**76**) contained similar signals to (**75**) with additional resonances at δ49.96 and δ41.69 arising from the carbons of the bis(2-chloroethyl)amino group. Also noted was an upfield shift of 3.6 ppm for the octadecyl carbon (C-18) closest to the phosphorus.

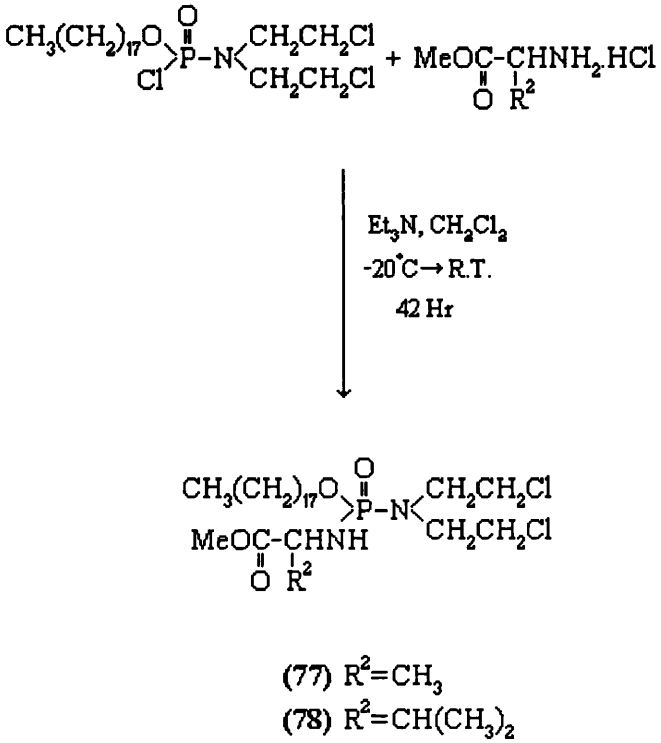
[Fig. 37]



Again, the ^{1}H nmr spectrum of (**76**) showed similar resonances to (**75**) with the multiplet between δ3.35-3.56 integrating for 8 protons, being assigned to $N(CH_2CH_2Cl)_2$. The structure of (**76**) was confirmed by its F.A.B. mass spectrum which contained the molecular ion peak at m/e 491 and additional peaks corresponding to the fragmentation of the octadecyl chain. For example a peak at m/e 222 indicated the loss of $O(CH_2)_{17}CH_3$ from the molecular ion and another peak at m/e 240 indicated the loss of $(CH_2)_{17}CH_3$ from the diprotonated molecular ion.

The final step in the route to N,N-bis(2-chloroethyl)amino octadecyl methoxyalaninyl phosphoramidate (**77**) involved the low temperature reaction of (**76**) with L-alanine methyl ester hydrochloride [Fig. 38].

[Fig. 38]



(77) $R^2=CH_3$
(78) $R^2=CH(CH_3)_2$

Several purification steps were required before compound (77) could be obtained in the pure state. Following a reaction time of 42 hours, the resulting reaction mixture was subjected to a routine ether extraction. Analytical tlc on the concentrated residue indicated the presence of two slow running components in addition to the required product. It was interesting to note that on tlc, the diastereoisomers of (77) were represented by two coalescing spots. It was attempted to purify this material by silica-gel column chromatography, with neat chloroform as the eluent and iodine to monitor the separation. Pooling and evaporation of the fast fractions and characterisation of the isolated white solid, by ^{31}P nmr spectroscopy, revealed the presence of minor impurities at δ5 and δ-2. These minor signals were present in addition to the two main signals at δ12 corresponding to the diastereoisomers of (77). A slow second column with elution by petroleum spirit and ether was successful in

removing the polar impurities but this appeared to be at the expense of generating a minor fast running impurity. A second ^{31}P nmr spectrum of the isolated white solid revealed that this minor impurity was a phosphorus containing component and that it resonated at a chemical shift not too dissimilar to the desired product. Therefore, it was decided to employ the technique of preparative hplc to further purify this material. This was achieved with the use of ethylacetate (100%) and normal phase conditions involving a refractive index detector. Also achieved by this method was the complete separation of the isomers **(77A)** and **(77B)** as white solids in 16% and 11% yields respectively. It is quite likely that the separate isomers of a compound may possess different biological activity. Therefore, each isomer of **(77)** was characterised separately by nmr spectroscopy and analytical methods.

The ^{31}P nmr spectra of **(77A)** and **(77B)** contained single peaks at δ12.70 and δ 13.07 respectively with the more lipophilic isomer **(77A)** resonating at the lower chemical shift. ^{13}C nmr spectroscopy fully supported the identity and purity of the isolated products. Not surprisingly, the ^{13}C nmr spectrum of each isomer showed very similar features with all the relevant carbons in each of the three moieties giving rise to doublets due to two and three bond phosphorus-carbon coupling. The only exception to this rule was CH_2Cl which was observed as the usual singlet at δ43. The ^{1}H nmr data were also consistent with the proposed structure of **(77)** and again were very similar for the two isomers. For example, the methoxy protons and $CHC\underline{H}_3$ of the alaninyl group were observed as a singlet at δ3.72 and a doublet between δ1.38-1.40 respectively in each spectrum.

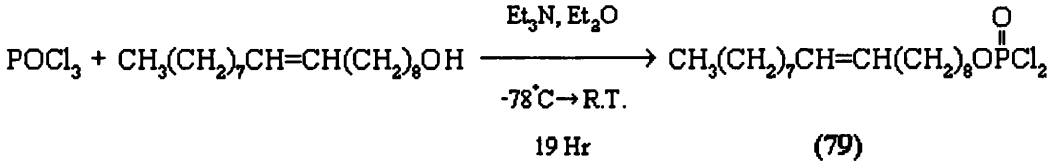
Both isomers were also found to give peaks corresponding to M^+ and MH_3^+ in their F.A.B. mass spectra. Peaks observed at m/e 417, m/e 289 and m/e 499 in both spectra, indicated the loss of $(CH_2)_9CH_3$, $O(CH_2)_{17}CH_3$ and CO_2Me respectively from the molecular ion. Also observed was the base peak at m/e 166 which was due to MH^+-$N(CH_2CH_2Cl)_2$-$(CH_2)_{17}CH_3$. The loss of $N(CH_2CH_2Cl)_2$ from the molecular ion,

represented by a prominent peak at m/e 418, was noted only in the spectrum of **(77A)**. Satisfactory microanalysis and analytical hplc data were obtained for each isomer.

In a similar manner, **(76)** was treated with L-valine methyl ester hydrochloride to generate N,N-bis(2-chloroethyl)amino octadecyl methoxyvalinyl phosphoramidate **(78)**. The required compound was obtained as a colourless oil in 61% yield after purification by silica-gel column chromatography. The diastereoisomers of the isolated product were found to resonate as two distinct signals at δ12.04 and δ11.80 in the generally observed ratio of 1:1. Also in agreement with the proposed structure were the ^{13}C nmr and ^{1}H nmr data with diastereomeric splitting and phosphorus coupling being observed where they were expected. Both spectra were assigned by analogy to the corresponding spectra of **(76)**. The introduction of the methoxyvalinyl group was supported by additional resonances appearing at chemical shifts characteristic of this particular group. For example, $CH(\underline{C}H_3)_2$ was observed between δ17.56-18.90 and was also noted to display diastereomeric splitting for just one of the methyl carbons. Peaks at m/e 587 and m/e 589 in the F.A.B. mass spectrum corresponded to the expected protonated molecular ion cluster of this compound. Satisfactory microanalytical data were also collected for this compound.

The analogous oleyl phosphoramidates **(81)** and **(82)** were made the next targets for synthesis [Fig. 41]. Oleyl phosphorodichloridate **(79)** was routinely prepared from oleyl alcohol and phosphoryl chloride in the presence of one equivalent of triethylamine [Fig. 39]. The required phosphorylating agent, isolated as an oil in a high yield of 99%, was characterised by ^{31}P nmr spectroscopy. The spectrum obtained displayed a resonance at δ5.19 which was in agreement with the chemical shift values noted for the analogous decyl and octadecyl phosphorodichloridates.

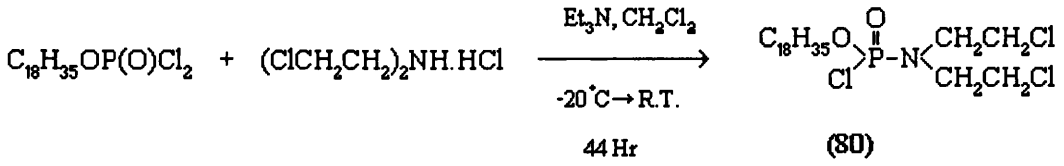
[Fig. 39]



Compound **(79)** was also characterised by its ^{13}C nmr spectrum which displayed very similar characteristics to that of **(75)** with additional singlets at δ130.00 and δ129.65 corresponding to the alkenyl carbons (CH=CH). Again, similar resonances to those in the spectrum of **(75)** were observed in the ^{1}H nmr spectrum of **(79)**. Additional multiplets between δ5.30-5.35 and δ1.98-2.00 were assigned to the alkenyl protons (CH=CH) and the adjacent protons ($C\underline{H}_2CH=CHC\underline{H}_2$) respectively. Satisfactory microanalytical data were obtained for **(79)**.

In the next step, the phosphorodichloridate **(79)** was reacted with bis(2-chloroethyl)amine hydrochloride in a manner similar to **(76)** from **(75)**, to generate the corresponding phosphorochloridate **(80)** as a pale yellow oil in 91% yield [Fig. 40]. This material was found to resonate at δ13.59 in its ^{31}P nmr spectrum, a downfield shift of 8 ppm compared to the starting phosphorodichloridate **(79)**. Also present in the spectrum were some minor impurities on either side of the main peak. Previous attempts at this reaction had produced **(80)** in a less pure state. ^{13}C nmr and ^{1}H nmr spectra were also obtained and given assignments analogous to **(79)** with additional signals being recorded, at 49.91 and 41.51 in the former spectrum and at 3.62 and 3.44 in the latter, confirming the presence of the bis(2-chloroethyl)amino group. Both spectra also contained minor signals corresponding to impurities.

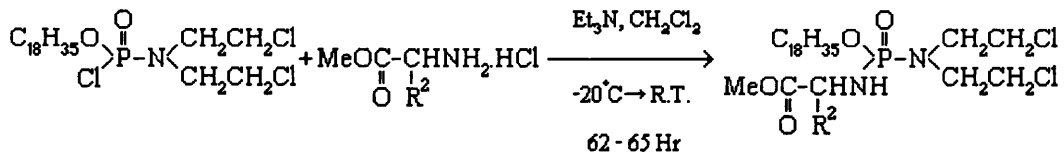
[Fig. 40]



(80)

The final step in the scheme required the reaction of the phosphorochloridate **(80)** with the appropriate amino acid methyl ester hydrochloride [Fig. 41]. Thus, compound **(81)** was prepared from **(80)** and L-alanine methyl ester hydrochloride in dichloromethane with the addition of triethylamine being carried out at -20°C. After a reaction time of 65 hours, a hexane extraction was carried out on the concentrated reaction mixture. The resulting material was then subjected to two successive columns with elution by varying mixtures of chloroform and petroleum spirit. Pooling and evaporation of the appropriate fractions afforded **(81)** as a colourless oil in a respectable yield of 51%.

[Fig. 41]



(81) $R^2=CH_3$
(82) $R^2=CH_2Ph$

The isolated compound **(81)** was found to resonate as two peaks at δ11.84 and δ11.48 in its ^{31}P nmr spectrum. ^{13}C nmr and ^{1}H nmr spectra were also obtained and these displayed characteristics resembling those observed in the phosphorochloridate **(80)**.

For instance, a multitude of signals appearing between δ28.95-30.41 in the ^{13}C nmr spectrum was due to the familiar overlapping of the methylene carbons towards the centre of the oleyl chain. Signals exhibiting both diastereomeric splitting and phosphorus-carbon coupling included those at δ52.46 and δ52.33 (J=3.7-4.9 Hz) and at δ49.75 and δ49.52 (J=3.6-11.4 Hz) corresponding to the alaninyl $CO_2\underline{Me}$ and CHNH respectively. It is interesting to note that the former carbon displayed phosphorus coupling despite being more than three bonds away from the phosphorus centre. Further confirmation of the formation of **(81)** was provided by its F.A.B. mass spectrum which displayed the protonated molecular ion peak. Noticeable peaks were those at m/e 247 and m/e 185 arising from MH^+-$C_{18}C_{35}$-CO_2Me and MH_2^+-$C_{18}C_{35}$-CO_2Me-CH_2CH_2Cl respectively.

N,N-Bis(2-chloroethyl)amino oleyl phosphoramidate **(82)** was similarly prepared from the reaction of **(80)** with L-phenylalanine methyl ester hydrochloride. Again, two columns were needed to purify the desired compound which was eventually isolated as a colourless oil in a respectable yield. The isomers of the product were found to give coincident shifts at δ12.56 in the ^{31}P nmr spectrum which was consistent with previously described phenyl-containing phosphoramidates. The ^{13}C nmr and ^1H nmr spectra supported the isolation of **(82)** and were assigned by analogy to the spectra of **(80)** and **(81)**. The presence of the phenylalaninyl moiety in the compound was supported by the appearance in the ^{13}C nmr spectrum, of characteristic signals between δ127.05-136.00 corresponding to the phenyl carbons. The splittings observed with these signals confirmed the isolation of diastereoisomers in a ratio of 1:1. The presence of the oleyl and bis(2-chloroethyl)amino groups was also supported by familiar signals in their characteristic positions. The introduction of a second P-N link was found to have induced an upfield shift of approximately 3 ppm for C-18 ($CH_2\underline{C}H_2OP$). The remaining carbons of the oleyl group resonated at chemical shifts similar to those recorded for **(80)**. Signals due to $CH_2C\underline{H}_2OP$ in the ^1H nmr spectrum were also noted

to have shifted upfield (by 0.3 ppm) in relation to the spectrum of **(80)**. The structure of **(82)** was established by its F.A.B. mass spectrum which displayed the protonated molecular ion and diprotonated molecular ion clusters predicted for this chlorine containing compound. Also observed was a prominent peak at m/e 242 (due to MH^+-$N(CH_2CH_2Cl)_2$-$C_{18}H_{35}$) and the base peak at m/e 120 which was assigned to $(PhCH_2CHNH_2)^+$.

Some of the phosphoramidate derivatives, whose syntheses were described in this chapter, were evaluated for anti-HIV activity by various test centres. The short chain alkyl phosphoramidates **(62)-(64)**, were tested against HIV by T.J. O'Connor and D. Kinchington of the Department of Virology at St. Mary's Hospital Medical School London. The compounds were tested for anti-HIV and toxicity on human C8166 T-lymphoblastoid cells which were incubated with $10TCID_{50}$ HIV-1 (HTLV III, RF strain). The IC_{50} of all three compounds was less than 100μM. The compounds were also found to be non-toxic at the concentrations tested. Under the conditions of the assay, AZT, the control, reduced viral proliferation by 50% at a concentration as low as 0.03 μM. An interesting observation made with these results was that the degree of viral inhibition appeared to be affected by the lipophilicity of the compound. For example, both **(62)** and **(63)** produced less than 10% inhibition with **(62)** showing the least inhibition, while **(64)** containing a phenyl group produced 40% inhibition.

Compounds **(69)** and **(70)** were tested at two independent test centres, one at Mill Hill London and the other at Cambridge. However, both compounds were found to be inactive in the tests against the virus. Both compounds were also toxic with **(69)** showing selective toxicity at low concentrations in those tests conducted at Cambridge. The toxicity profile was reversed in the tests carried out at Mill Hill.

1.2. N,N-Bis(2-chloroethyl)amino trihaloalkyl phosphoramidates

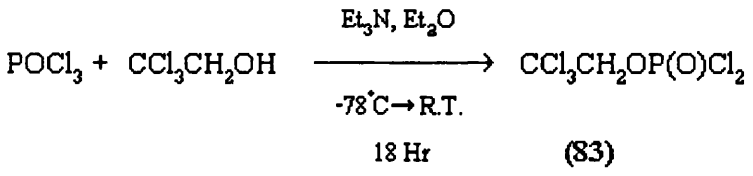
The initial rationale behind the synthesis of phosphoramidates was the idea that HIV aspartate proteinase[89] might specifically hydrolyse these compounds to release the bisfunctional alkylating moiety. The replacement of the alkyl groups in dialkyl phosphate triesters of AZT by trihaloalkyl groups such as trifluoroethyl, has been found to convert inactive compounds into compounds with notable anti-HIV activity[125-126]. It has also been demonstrated that the introduction of these electron withdrawing bis(trihaloethyl) groups at the phosphate site of phosphate triesters of certain nucleosides can enhance lipophilicity and increase lability towards hydrolysis and that the relative magnitude of these effects varies markedly with the nature of the halogen substituent[127]. Phosphate triester derivatives of AZT where the phosphorus centre carries a trichloro or trifluoro group and a carboxyl-protected, amino-linked amino acid have also been found to display potent anti-HIV activity but the introduction of the haloalkyl moiety does not appear to increase the activity of the compound[128]. A trichloroethyl methoxy alaninyl compound was found to be the one exception. The activity of this compound was enhanced 50-fold by the introduction of the trichloroethyl group. In these examples, electron-withdrawing halogen atoms were contained at the β-carbon of the phosphate-esterifying group. These results suggested that the combination of a haloethyl and an amino acid moiety on the phosphorus attached to a nitrogen mustard group may yield compounds which are active against HIV.

In order to probe the effect of replacing alkyl groups with trihaloalkyl ones the synthesis of a small series of 2,2,2-trichloroethyl and 2,2,2-trifluoroethyl alkyloxy phosphoramidate derivatives of nitrogen mustard was undertaken. The synthetic route employed to phosphoramidate derivatives of AZT involved the reaction of the appropriate amino acid methyl ester with the haloalkyl phosphorodichloridate[129]. These agents were then allowed to react with AZT in THF containing N-methylimidazole[130].

The methodology used to prepare the trihaloalkyl phosphoramidate derivatives of nitrogen mustard, **(85)**, **(86)**, **(90)** and **(91)**, was one used to prepare previously described analogues. That is, the reaction of the relevant phosphorochloridate with the appropriate amino acid methyl ester hydrochloride. Alternative routes involving the reaction of dichlorophosphoramide with the relevant alcohol or the methyl ester of the appropriate amino acid have been tried and found to be unsuccessful as explained in the previous chapter.

Firstly, the phosphorochloridates **(84)** and **(89)** were prepared from their corresponding phosphorodichloridates **(83)** and **(88)**. The phosphorylating agent **(83)** was obtained from the reaction of trichloroethanol with phosphoryl chloride in ether [Fig. 42]. After a reaction time of 18 hours and a hexane extraction on the concentrated reaction mixture, a clear oil with a hint of yellow was isolated in 98% yield. This oil gave a single peak at δ6.42, in its ^{31}P nmr spectrum, which compared to the previously prepared short and long chain alkyl phosphorodichloridates, was downfield by about 1 ppm.

[Fig. 42]



The ^{13}C nmr spectrum contained two doublets at δ93.05 and δ78.28 which were assigned to $\underline{C}Cl_3CH_2OP$ and $CCl_3\underline{C}H_2OP$ respectively. This assignment was based on the peak heights. CCl_3 may have a longer relaxation time and therefore would be expected to give the smaller resonance under these conditions. Also the magnitude of the phosphorus-carbon coupling constant observed with the former signal (13.5 Hz)

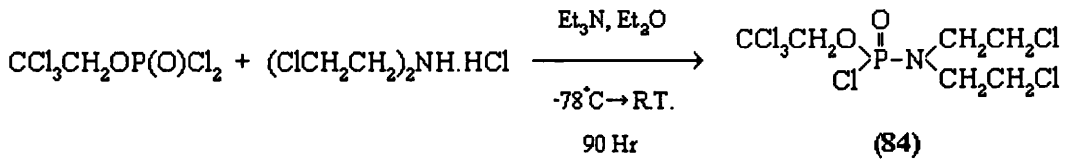
was almost twice that noted for $CCl_3\underline{C}H_2OP$ (7.5 Hz) which was in accordance with the observation that three bond coupling is generally greater than two bond coupling in such systems. This magnitude of 13.5 Hz was also much greater than that noted for $\underline{C}H_3CH_2OP$ (2.6 Hz) in the ^{13}C nmr spectrum of the analogous ethyl compound **(60)**, this presumably being due to the introduction of the three chlorine atoms in the ethyl group. The ^1H nmr spectrum of **(83)** contained a simple doublet at δ4.76 arising from phosphorus-proton coupling. The F.A.B. mass spectrum contained peaks at m/e 264, 266 aand 268 corresponding to the molecular ion and its isotope effects due to the presence of chlorine atoms. The pattern of the cluster observed was consistent with five chlorine atoms being present in the phosphorylating agent and provided further evidence for a selective reaction having taken place.

Three attempts were required to couple successfully **(83)** with bis(2-chloroethyl)amine hydrochloride. Previous attempts using just one equivalent of bis(2-chloroethyl)amine hydrochloride and a reaction time of 66 hours failed to generate the phosphorochloridate **(84)** in a sufficiently pure state, as indicated by ^{31}P nmr spectroscopy. A substantial amount of the starting material was found to be the main impurity. Therefore, 1.5 equivalents of bis(2-chloroethyl)amine hydrochloride were used and the reaction time increased to 90 hours [Fig. 43] and this combination appeared to give the desired results. Filtration of the reaction mixture and concentration of the filtrate under reduced pressure led to the isolation of a pale yellow solid in a high yield of 96%. A single resonance at δ12.56 in the ^{31}P nmr spectrum confirmed the identity of the product. This chemical shift was noted to be upfield by about 1 ppm when compared to bis(2-chloroethyl) amino ethyl phosphorochloridate **(61)**.

The ^{13}C nmr spectrum showed a similar $\underline{C}Cl_3CH_2OP$ resonance to **(83)** at δ93.94 with a phosphorus coupling constant of 14.6 Hz. The resonance due to $CCl_3\underline{C}H_2OP$ was obscured by the signals arising from deuterochloroform. The use of an alternative solvent such as d-methanol would have overcome this slight problem.

The ^1H nmr spectrum was in marked contrast to that obtained for **(83)** and contained two very complex multiplets between δ3.50-3.72 and downfield at δ4.65 which were assigned to CH_2CH_2Cl and CCl_3CH_2OP respectively by analogy to **(61)**.

[Fig. 43]



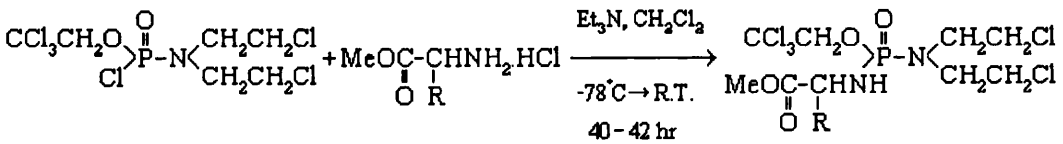
(84)

The F.A.B. mass spectrum was also in agreement with the structure of **(84)** and like **(83)** displayed some interesting features. The protonated molecular ion was observed at m/e 370, 372, 374, 376 and 378 and the isotopic abundance ratios for this ion were entirely consistent with there being six chlorine atoms in this phosphorylating agent. Also of interest were peaks at m/e 240 and 142 which were assigned to the loss of CH_2CCl_3 from MH_2^+ and the chlorine containing fragment $(ClCH_2CH_2)_2NH_2^+$ respectively.

An unusually high antiviral activity in the AZT series from a trichloroethyl methoxy alanine compound has been noted in this Department[128] and it was of interest to see whether this combination would produce a similar effect in the nitrogen mustard series. Therefore the first compound to be synthesised in this series was N,N-bis(2-chloroethyl)amino trichloroethyl methoxyalaninyl phosphoramidate **(85)** from the reaction of **(84)** with L-alanine methyl ester hydrochloride [Fig. 44]. Ether and hexane extractions on the concentrated reaction mixture gave a colourless solid. Characterisation of this material by ^{31}P nmr spectroscopy revealed the presence of some very minor impurities in addition to the two resonances downfield between δ11-12 ppm representing the diastereoisomers of the product. This oil was further purified by silica-

gel column chromatography (20% chloroform in petroleum spirit) and this led to the isolation of **(85)** as an isomer and a mixture of isomers. A white solid was isolated in each case and was characterised separately by spectroscopic and analytical techniques. As mentioned with compound **(77)** in the previous chapter the separated isomers of a product or a mixture of the isomers in various ratios may give varying biological results which may arise from differences in behaviour in a biological environment. A tenfold difference in activity between the two isomers of 3′-azido-3′-deoxythymidine-5′-(ethyl methoxyvalinyl) phosphate has been observed with the most lipophilic being the most active[131]. This reinforces the idea that the precise environment at the phosphorus-nitrogen bond may be crucial for the antiviral properties of these phosphoramidates which is consistent with the suggestion of P-N cleavage as a possible mode of action.

[Fig. 44]



(85) R =Me
(86) R =CH_2Ph

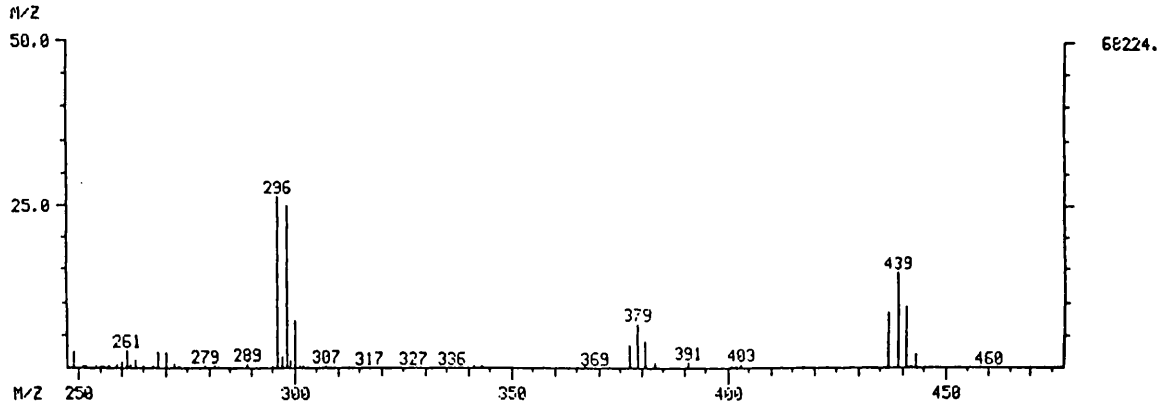
In the case of **(85)** chromatographic procedure and the difficulty encountered with the use of iodine to visualise the separated components were thought to have influenced the degree of separation of the isomers. The ^{31}P nmr spectrum of the isomer **(85A)** which was collected first, the more lipophilic component, consisted of a single signal at δ11.87. The mixture of isomers **(85B)** was found to resonate at δ11.26 and δ11.94 in a ratio of 1:2.5, in its ^{31}P nmr spectrum. The ratio of the isomers in the material prior to column chromatography was 1:1.5. It is of interest to note that in the

case of diastereoisomers the component with the higher column mobility, that is the more lipophilic component, generally resonates at the more downfield shift.

The ^{13}C nmr data confirmed the isolation of **(85A)** and **(85B)** with the spectrum of the former compound showing similar features to the latter with the exception of diastereomeric splitting. The $\underline{C}Cl_3CH_2OP$ signals of both **(85A)** and **(85B)** were observed as a doublet and a doublet of doublets respectively at δ96 with a downfield shift of about 2 ppm when compared to the spectrum of **(84)**. The introduction of the methoxyalaninyl group appeared to have reduced considerably (by more than 7 Hz) the coupling of $\underline{C}Cl_3CH_2OP$ to the phosphorus. The $CCl_3\underline{C}H_2OP$ was also coupled to the phosphorus and showed a coupling constant of 3.3 Hz. The CHNH in the spectrum of **(85A)** resonated as a doublet at δ49.57 due to phosphorus coupling while $CO_2\underline{Me}$ appeared as a singlet at δ52.64. In contrast, these carbons in the spectrum of **(85B)** were observed as doublets at δ49.94 and δ49.56 due to diastereomeric splitting with phosphorus couping being absent and a doublet of doublets at δ52.64 and δ52.53, due to both diastereomeric splitting and phosphorus coupling. The ^1H nmr data gathered on both **(85A)** and **(85B)** also supported the ^{13}C nmr data. The effect of diastereoisomerism in **(85B)** was most noticeable in those resonances arising from CCl_3CH_2OP and CO_2Me with the former protons being observed as a doublet of doublets due to additional coupling to the phosphorus and the latter a signal with a small splitting of 0.005 ppm.

The protonated molecular ion was present in the F.A.B. mass spectra of both **(85A)** and **(85B)** and not surprisingly, a very similar fragmentation pattern was observed [Fig. 45]. Other prominent peaks were those at m/e 377 and m/e 296 indicating the loss of CO_2Me and $N(CH_2CH_2Cl)_2$ respectively from the molecular ion. Satisfactory analytical hplc and microanalysis data were also collected for both samples.

[Fig. 45]



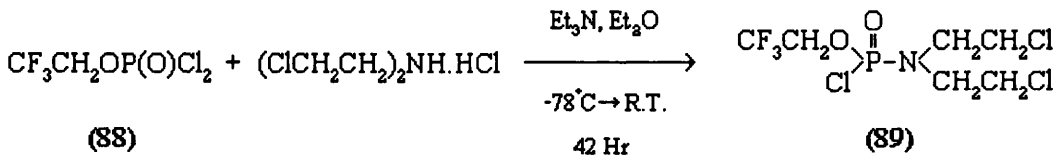
The reaction of **(84)** with L-phenylalanine methyl ester hydrochloride was then carried out and after column chromatography gave a colourless oil, which was characterised as the product **(86)**, in 68% yield. The ^{31}P nmr spectrum of this material contained two closely spaced signals at δ11.52 and δ11.25 (1.5:1) representing the isomers of the product. Signals between δ127.29 and δ135.77 in the ^{13}C nmr spectrum confirmed the presence of a phenyl group in the compound. All of these signals were observed as doublets due to the expected diastereomeric splitting with the exception of the carbon in the *para* position which was a singlet possibly due to coincident signals. The remaining carbons in each isomer also resonated at different chemical shifts with phosphorus-carbon coupling being observed where expected. The CHNH and CH_2Cl signals were both usual exceptions to this rule. As noticed with the previously described compound **(85B)**, $\underline{C}Cl_3CH_2OP$ and $CCl_3\underline{C}H_2OP$ each gave two sets of doublets with the former signal appearing downfield by about 2 ppm relative to the spectrum of **(84)**. Again, the ^{31}P coupling constant noted for $\underline{C}Cl_3CH_2OP$ was about twice the magnitude of that noted for the adjacent carbon.

The ^{1}H nmr spectrum also confirmed the isolation of **(86)** with all protons resonating as multiplets with the exception of the methoxy protons which were

observed as two very closely spaced signals. The F.A.B. mass spectrum confirmed the identity of **(86)** by displaying a cluster of peaks corresponding to the protonated molecular ion and an isotopic abundance pattern which was similar to that observed in the spectra of **(85)**.

The series was then continued with the preparation of a small number of analogues containing a trifluoroethyl substituted alkyl group. N,N-Bis(2-chloroethyl)amino trifluoroethyl phosphorochloridate **(89)** was obtained from commercial **(88)** by a similar method to that used for the preparation of **(84)** from **(83)**. There were slight modifications in the procedure with respect to the number of equivalents of bis(2-chloroethyl)amine hydrochloride (1 equivalent) and the reaction time (42 hours) [Fig. 46]. A hexane extraction on the concentrated reaction mixture led to the isolation of **(89)** as a colourless oil in 67% yield. A ^{31}P nmr spectrum of this oil consisted of a single resonance at δ14.18 which indicated that the replacement of a trichloroethyl group with the more electron withdrawing trifluoroethyl group had produced a downfield shift of 1.6 ppm.

[Fig. 46]



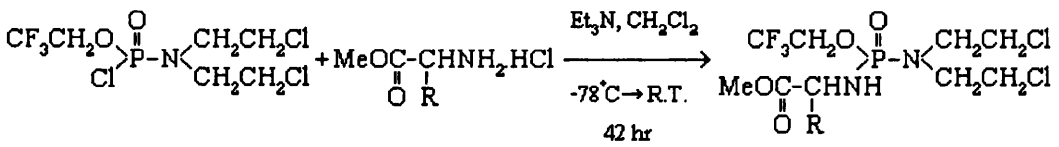
(88) **(89)**

The ^{13}C nmr spectrum contained, in addition to the bis(2-chloroethyl)amino carbons at their characteristic positions, two sets of multiplets at 118.70 ppm and 124.78 ppm (J_{C-F}=306 Hz) corresponding to $\underline{C}F_3CH_2OP$ and a quartet of doublets at 63.19 ppm (J_{C-F}=37 Hz) corresponding to $CF_3\underline{C}H_2OP$. The trifluoromethyl signal was

noted to have shifted downfield by more than 100 ppm relative to the methyl signal in the spectrum of **(61)**. The pattern observed with each carbon of the trifluoroethyl chain is a result of coupling to the three fluorine atoms in addition to the phosphorus. The ^1H nmr spectrum was similar to that of **(84)** and consisted of two sets of multiplets corresponding to CF_3CH_2OP and the bis(2-chloroethyl)amino protons. Again the former signals were shifted downfield relative to the methylene signals in **(61)** by the deshielding effect of the fluorine atoms. The F.A.B. mass spectrum was entirely consistent with the structure of **(89)** and contained the protonated molecular ion peaks at m/e 322, 324 and 326. The pattern of this cluster was also consistent with three chlorine atoms being present in the compound. The base peak at m/e 142, as in **(84)**, was due to $(ClCH_2CH_2)_2NH_2^+$.

The 2,2,2-trifluoroethyl compounds **(90)** and **(91)** [Fig. 47] were prepared by a similar method to that used for the analogous trichloroethyl compounds. Purification by silica-gel column chromatography gave **(90)** as a white solid in 63% yield. The ^{31}P nmr spectrum displayed the commonly observed two signals at δ13.01 and δ12.30 with a splitting of 0.71 ppm. The signals were in the ratio of 1:1.5 and in the expected region although they were shifted slightly upfield in relation to **(89)** upon introduction of the methoxyalaninyl group. The chemical shift was also noted to be greater than that observed for the analogous trichloroethyl compound which was as expected.

[Fig. 47]



(90) R =Me
(91) R =CH_2Ph

^{13}C nmr spectroscopy also supported the successful isolation of **(90)** with the spectrum containing some interesting features due to the presence of the fluorine atoms. The most complex resonance was that due to $CF_3\underline{C}H_2OP$ which was resolved into two sets of quartets of doublets at δ62.29 and δ62.16. Coupling of this carbon to the three adjacent equivalent fluorine atoms gives rise to a quartet which also couples to the neighbouring phosphorus. Additional splitting due to the diastereomeric mixture also accounts for the complexity of the resonance. The larger coupling constant of 36.8 Hz was attributed to carbon-fluorine coupling and the smaller constant of 3.6 Hz, to 2 bond phosphorus-carbon coupling. The signal due to $\underline{C}F_3CH_2OP$ was also expected to show two sets of quartets of doublets but this effect was obscured by the level of noise contained in the spectrum and the low intensity of the signal. However it was possible to identify two sets of doublets from which carbon-fluorine and phosphorus-carbon coupling constants of 318 Hz and 2 Hz were obtained. An unusual feature contained in this particular spectrum was the resonance of the CHNH which was coupled to the phosphorus atom in only one of the two isomers.

The ^1H nmr spectrum also featured some complex resonances. For example, CF_3CH_2OP was observed as a multiplet at δ4.34 which was more complex than the one observed in the spectrum of **(89)** due to the combination of diastereomeric splitting, proton-phosphorus and proton-fluorine couplings. The F.A.B. mass spectrum displayed the protonated molecular ion in addition to prominent peaks at m/e 329 and m/e 248 corresponding to the cleavage of the methoxy carbonyl and the bis(2-chloroethyl)amino groups respectively from the parent ion. Microanalysis and analytical hplc data confirmed the purity of this compound.

Compound **(91)** was prepared from **(89)** and L-phenylalanine methyl ester hydrochloride in an entirely analogous manner to **(90)**. The technique of column chromatography was also used in this case and gave the product as a white solid in 67% yield. ^{31}P nmr spectroscopy revealed two signals in the ratio of 1:1.5

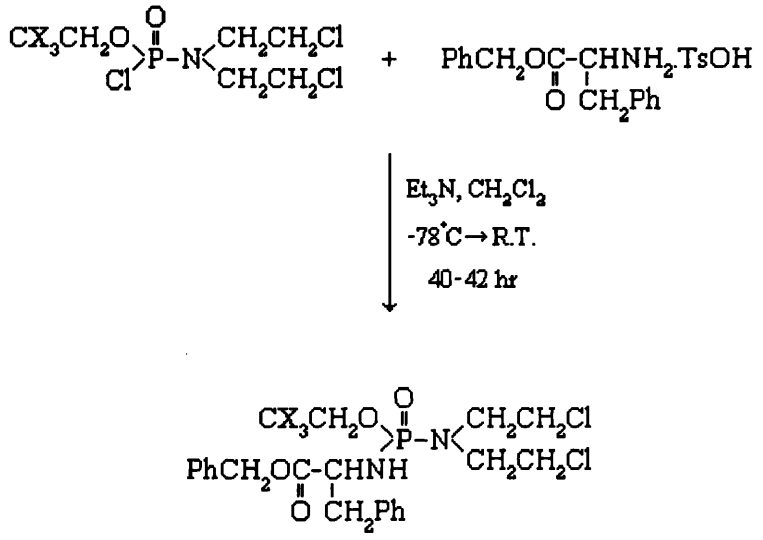
corresponding to the expected isomers of **(91)**. The ^{13}C nmr spectrum was informative with most signals showing diastereomeric splitting in a ratio consistent with that displayed in the ^{31}P nmr spectrum. The presence of a phenyl group was supported by resonances in their characteristic positions between δ127.24 and δ135.78. The $CF_3\underline{C}H_2OP$ resonance was noted to be a multiplet and not two sets of quartets of doublets due to poor resolution. The ^{1}H nmr spectrum was equally informative and was assigned by analogy to **(86)**. All signals were multiplets with the exclusion of those corresponding to the methoxy protons. These were observed as two signals at δ3.75 and δ3.73 in a ratio consistent with that shown in the ^{31}P nmr spectrum. The F.A.B. mass spectrum displayed the protonated molecular ion at m/e 465, m/e 467 and m/e 469 (due to the isotope ^{37}Cl). The fragmentation of this compound was not too dissimilar to **(90)** with the most prominent peaks being those at m/e 405 and m/e 324 corresponding to the loss of the methoxy carbonyl and the bis(2-chloroethyl)amino groups respectively from the molecular ion. Microanalysis and analytical hplc data confirmed the purity of this compound.

It was also thought to be of interest to investigate the effect of structural variation arising from the replacement of the usual methyl ester protecting group with a benzyl ester protecting group. The presence of a benzyl group rather than a methyl one may be expected to modify biological properties as a result of differences in steric and electronic properties. Thus the benzyl-containing trichloroethyl **(87)** and trifluoroethyl **(92)** analogues depicted in [Fig. 48] were prepared.

The synthetic strategy used for the preparation of **(87)** and **(92)** involved the use of an amino acid benzyl ester and was very similar to the preparations of previously described methyl ester containing derivatives. The reaction of **(84)** with L-phenylalanine benzyl ester p-toluenesulphonate was carried out in dichloromethane in the presence of triethylamine and was followed by the usual work-up. L-Phenylalanine

benzyl ester hydrochloride could also have been used and would be expected to give similar results.

[Fig. 48]



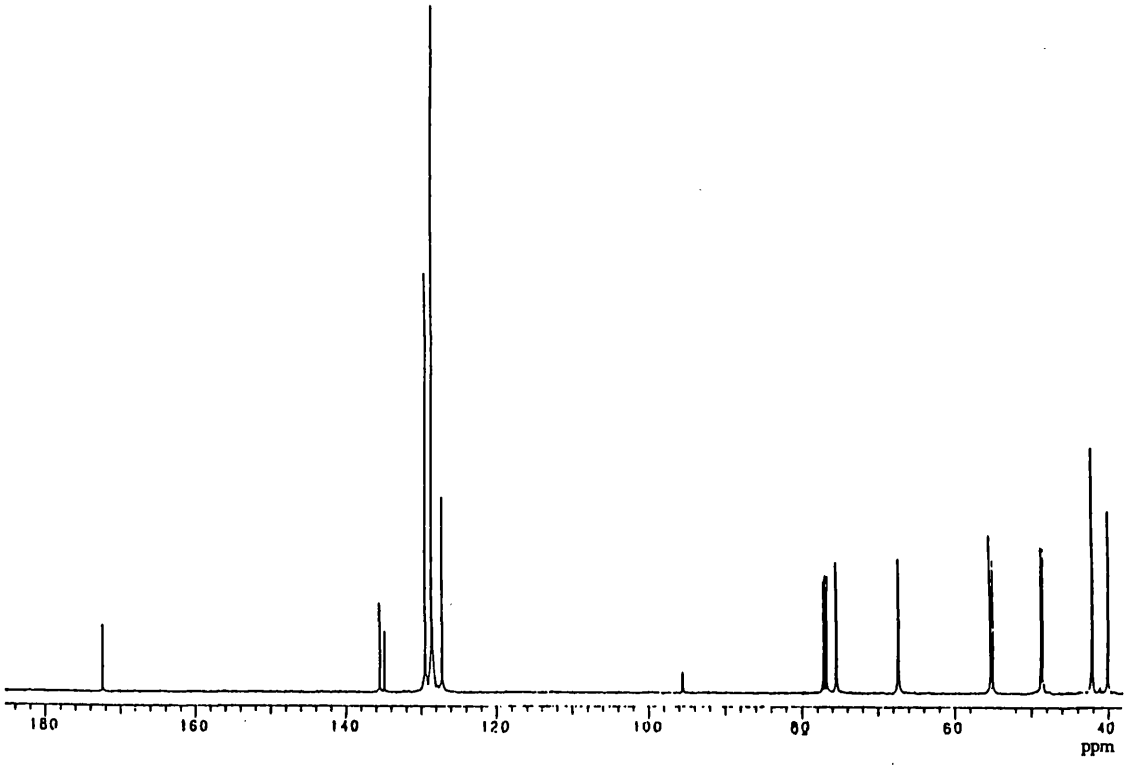
(87) X=Cl

(92) X=F

Purification by column chromatography with a mixture of petroleum spirit and chloroform as the eluent and pooling and evaporation of the appropriate fractions gave rise to **(87)** as a white solid in 50% yield. The ^{31}P nmr spectrum of this material displayed two signals at δ11.62 and δ11.27 which was very close to values reported for the trichloroethyl methoxyphenylalaninyl compound **(86)**. The ^{13}C nmr spectrum [Fig. 49] was fully consistent with the structure of **(87)** with most signals showing diastereomeric splitting and the relevant carbons coupling to phosphorus. Resonances between δ127.18 and δ135.60 confirmed the presence of two phenyl groups in the compound. Any splitting observed in this particular region was due solely to

diastereoisomerism. The remainder of the spectrum was similar to that of **(86)** with an additional signal (multiplet) between δ67.23-67.45 being identified as $Ph\underline{C}H_2OCO$.

[Fig. 49]



The ^1H nmr spectrum obtained was also similar to that of **(86)** with the signals due to the methoxy protons being replaced by a multiplet downfield at δ5.14 arising from $PhC\underline{H}_2OCO$. F.A.B. mass spectral data were also consistent with the structure of the compound. The spectrum displayed the protonated molecular ion at m/e 589 and a peak at m/e 591 (due to the presence of the ^{37}Cl isotope). The pattern of the peaks observed in this region did not confirm the presence of five chlorine atoms in the compound. However, the expected isotope effect was demonstrated in peaks at m/e

453, 455 and 457 arising from the loss of the benzyloxycarbonyl group from the molecular ion.

The synthesis of the analogous trifluoroethyl compound **(92)** was accomplished by similar means from **(89)** and L-phenylalanine benzyl ester p-toluenesulphonate. Purification of the reaction mixture by silica-gel column chromatography gave rise to the product as a white solid in 61% yield. This was successfully characterised by ^{31}P, ^{13}C and ^{1}H nmr spectroscopy. Each isomer of the isolated product gave a separate signal in the ^{31}P nmr spectrum. The ^{13}C nmr spectrum displayed similar features to the spectrum of the analogous methyl ester compound and was entirely consistent with the presence of two phenyl groups in the compound. The *ipso* carbons of the benzyl and phenyl groups both appeared as distinct doublets at δ136 and δ135 due to diastereomeric splitting, with the resonances appearing most downfield being assigned to the benzyl group. Due to poor resolution, both $\underline{C}F_3CH_2OP$ and $CF_3\underline{C}H_2OP$ were observed as multiplets rather than the expected two sets of quartets of doublets.

The ^{1}H nmr spectrum also supported the ^{13}C nmr data with all protons giving complex signals at their characteristic positions. For example, the CF_3CH_2OP resonances were observed as a multiplet at δ4.17 due to diastereomeric splitting, proton-phosphorus and proton-fluorine couplings. The F.A.B. mass spectrum confirmed the isolation of **(92)** by displaying the protonated molecular ion peak at m/e 541 and m/e 543 in a ratio characteristic of the presence of two chlorine atoms. Other prominent peaks were those at m/e 405 and m/e 372 corresponding to the loss of $PhCH_2OCO$ and both CH_2Ph and Ph from the molecular ion. The base peak at m/e 91 was assigned to $PhCH_2^+$. Analytical hplc and microanalysis data confirmed the purity of this compound.

The haloalkyl phosphoramidates **(85A)**, **(85B)**, **(87)**, **(90)** and **(92)** were evaluated, by methods previously described, for their ability to inhibit the proliferation of HIV-1 in vitro; the results are summarised in table 2. The trichloroethyl compounds

(85) and **(87)**, tested at the Mill Hill centre, were found to be inactive at concentrations up to 200 μM. This suggested that there was no enhancement of activity upon introduction of this haloalkyl moiety. The same lack of enhancement was noted for the analogous trifluoroethyl compounds. Similar data were obtained, from Cambridge, for compounds **(87)** and **(92)** with **(90)** being toxic to uninfected cells. However the trichloroethyl compound **(85)**, also tested at Cambridge, was found to produce very different results to those seen at Mill Hill. This compound was tested as one of its isomers **(85A)** and as a mixture of isomers in a ratio of 1:2.5 **(85B)**. The former sample was found to be selectively active at 5 μM while the latter sample was rather less active. The reasons for the differences in behaviour are not entirely known but the results indicate that the introduction of the trichloroethyl moiety may have increased the activity of the ethyl phosphoramidate. The unsubstituted ethyl phosphoramidates **(62)**-**(64)**, which were tested at St. Bartholomews, were all noted to have been inactive at concentrations up to 200 μM.

Table 2. Anti-HIV activities from 3 test centres. Data are given as ED_{50} (TD_{50}) in μM

	St. Bartholomews	Mill Hill	Cambridge
85A	—	>200	5(>200)
85B	—	>200	50(>200)
87	15(10)	>200	>200
90	>200	>200	toxic
92	70(60)	>200	>200

Compounds **(87)**, **(90)** and **(92)** were also tested at St. Bartholomews. The trifluoroethyl methoxyalaninyl compound **(90)** was found to be inactive at the concentrations studied whereas both the trichloroethyl and trifluoroethyl-

benzylphenylalaninyl compounds were noted to be toxic. Compound **(87)** was toxic at 10 μM while **(92)** was toxic at a higher concentration of 60 μM. The potential anti-HIV activity of the analogous trichloroethyl and trifluoroethyl methoxyphenylalaninyl compounds **(86)** and **(91)** are currently being investigated and this may shed some light on the previous results.

In conclusion, the results obtained from two different test centres suggest that phosphoramidate derivatives of nitrogen mustard bearing amino acid moieties and trihaloethyl groups may have anti-HIV activity. The trichloroethyl and trifluoroethyl benzylphenylalaninyl compounds were found to be the most active compounds in this series of trihaloalkyl derivatives with the former compound showing the greatest activity. At present the exact mechanism of action of these compounds is uncertain. One explanation could be of membrane penetration followed by intracellular cleavage of the labile substituted alkyl to phosphorus bond resulting in the release of the amino acid containing bisfunctional alkylating moiety. However, many of the compounds exert toxicity at, or near, their antiviral concentrations. This may limit the development of these leads.

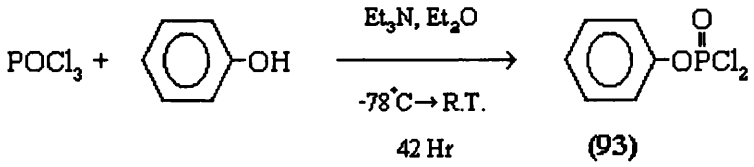
1.3. N,N-Bis(2-chloroethyl)amino aryl phosphoramidates

The results of anti-HIV testing of alkyl and trihaloalkyl phosphoramidate derivatives of nitrogen mustard suggested that there may be a correlation between biological activity and lipophilicity and that studies should be directed towards the development of compounds with a more lipophilic character. Thus it was of interest to prepare compounds in which the aliphatic alkyl or substituted alkyl group was replaced with an aryl group to see what effect this variation would have on the activity of the compound. The presence of an aryl group would be expected to promote lipid solubilty which may be a property that is required for the activity of these phosphoramidates. Thus the synthesis of some phenyl and 4-substituted phenyl phosphoramidates of nitrogen mustard was undertaken in the hope of providing compounds which may serve as chemically stable, lipophilic prodrugs. The halogens, fluorine, chlorine and bromine, were the substituents chosen for this study of structure-activity relationship to determine whether incorporating electronegative substituents in the aryl group would produce results which were as promising as those obtained with the trihaloalkyl derivatives described in the previous chapter. The synthetic strategy chosen was one that was applied to the synthesis of previously described similar derivatives and involved phosphorochloridate chemistry. Alternative routes which may involve the sequential reaction of dichlorophosphoramide with the relevant 4-halosubstituted phenol and protected amino acid were not studied as it was predicted that these reactions would proceed at a very slow rate due to the steric bulk of the aryl group. Also, past experience with studies of alternative routes discouraged any further attempts. Again, a variety of methyl ester and benzyl ester protected amino acids was employed.

The synthesis of the phenyl derivatives **(95)**and **(96)** depicted in [Fig. 52] was the first to be approached. The route to these compounds began with the preparation of

the phosphorylating agent **(93)** from the reaction of phenol with phosphoryl chloride by the routine method [Fig. 50]. This procedure gave the agent as a colourless oil in 80% yield.

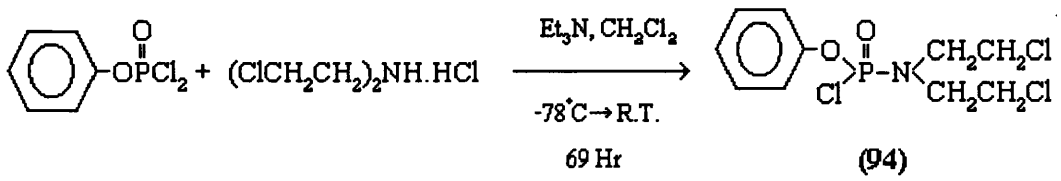
[Fig. 50]



The ^{31}P nmr and ^{13}C nmr data confirmed the identity and purity of the agent with the former spectrum containing a single resonance upfield at 1.56 which compares well with the spectrum of an authentic commercial sample. The *ipso* and *ortho* carbons were observed as doublets, due to 2 and 3 bond phosphorus coupling, at 149.70 and 120.58 respectively in the ^{13}C nmr spectrum. Singlets at 130.33 and 127.21 were assigned to the *meta* and *para* carbons respectively[132]. The phenyl protons were observed as a multiplet in the expected region between δ7.25-7.47 in the ^{1}H nmr spectrum. The F.A.B. mass spectrum was also entirely consistent with the structure of **(93)** and displayed the molecular ion peak at m/e 210 and also at m/e 212 due to the existence of the isotope ^{37}Cl.

The phosphorylating agent **(94)** was obtained from the reaction of **(93)** with two equivalents of bis(2-chloroethyl)amine hydrochloride in dichloromethane rather than in ether [Fig. 51]. Previous attempts at this reaction using one equivalent of bis(2-chloroethyl)amine hydrochloride in ether had resulted in an incomplete reaction. The lack of solubility of the hydrochloride in ether may have contributed to this problem.

[Fig. 51]

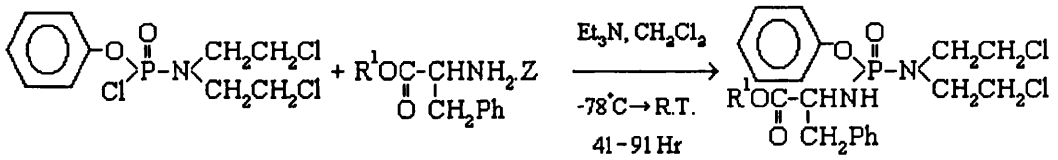


A hexane extraction on the concentrated reaction mixture gave a colourless oil, the ^{31}P nmr spectrum of which revealed the presence of some unreacted starting material. This oil was then heated to 65-70°C in a high vacuum (*ca.* 0.5 mmHg) for 6 hours in order to remove the unreacted material. After this period of time, the colourless oil was examined again by ^{31}P nmr spectroscopy and this indicated the presence of a single peak at δ8.98 corresponding to the desired material. Structural confirmation was further provided by ^{13}C nmr and ^{1}H nmr spectra which contained similar resonances to **(93)** with additional ones arising from the bis(2-chloroethyl)amino group. The E.I. mass spectrum contained the molecular ion peak on which it was possible to obtain an accurate mass measurement close to the calculated one.

N,N-Bis(2-chloroethyl)amino phenyl methoxyphenylalaninyl phosphoramidate **(95)** was obtained from the coupling reaction of **(94)** with L-phenylalanine methyl ester hydrochloride [Fig. 52]. Purification by silica-gel column chromatography gave the product as a white solid in 21% yield. The ^{31}P nmr spectrum of this material consisted of signals at δ8.18 and δ8.05 in a ratio of 3:1 which suggested that perhaps the formation of one of the isomers had been preferred over the other. These signals were found to be upfield by more than 3 ppm when compared to the resonances given by analogous alkyloxy derivatives. The ^{13}C nmr data also indicated that the isomers of the product had been isolated in a ratio of 3:1. Signals appearing between δ120.06-135.85 supported the presence of two phenyl groups in the compound with those signals

appearing the most downfield in each set of resonances, being assigned to the carbons of the phenyl group closest to the phosphorus. The ^{1}H nmr data were found to be consistent with the ^{13}C nmr data. Structural confirmation was provided by F.A.B. mass spectrometry which showed peaks at m/e 459 and m/e 461 corresponding to the protonated molecular ion. Also observed were peaks at m/e 399 and m/e 318 indicating the loss of CO_2Me and $N(CH_2CH_2Cl)_2$ respectively from the molecular ion. Other prominent peaks included one at m/e 367 due to the loss of CH_2Ph and another at m/e 290 due to the loss of both CH_2Ph and Ph from the molecular ion.

[Fig. 52]



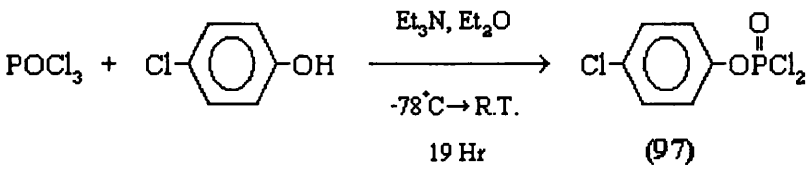
(95) R^1=Me, Z=HCl
(96) R^1=CH_2Ph, Z=$SO_3C_7H_8$

N,N-Bis(2-chloroethyl)amino phenyl benzylphenylalaninyl phosphoramidate **(96)** was prepared in a similar manner to **(95)** with the exception of the reaction time which was increased to 91 hours. Purification of the reaction mixture by column chromatography led to the isolation of an isomer **(96A)** and a mixture of isomers **(96B)** as white solids. Both samples gave single resonances at approximately δ8 in their ^{31}P nmr spectra. The isomer **(96A)** isolated was the least lipophilic of the two isomers and gave the resonance that appeared the most downfield. The ^{13}C nmr spectra of both samples were consistent with the structure of the product, with the isomer **(96A)** giving signals showing phosphorus coupling and not diastereomeric splitting as expected.

The F.A.B. mass spectrum displayed peaks for the protonated molecular ion in a ratio that was consistent with two chlorine atoms being present in the compound. Also observed were peaks at m/e 399 and m/e 366 which indicated the loss of $PhCH_2OCO$ and both CH_2Ph and Ph respectively from the molecular ion. Microanalysis and analytical hplc data confirmed the purity of only sample **(96A)** and so it was this sample which was evaluated for anti-HIV activity. The minor impurity present in the sample of mixed isomers was thought to be the starting material **(94)** which probably could have been removed by a slower chromatographic procedure if time had allowed.

The first structural modification to be investigated was the incorporation of a chlorine atom at the *para* position of the phenoxy group as halogenation of this group may modify the biological properties of the phosphoramidate. The synthetic methodology which was developed for the preparation of the alkyl derivatives was employed again. This involved the preparation of 4-chlorophenyl phosphorodichloridate **(97)** by the routine method used for preparing phosphorodichloridates [Fig. 53]. Thus phosphoryl chloride was reacted with 4-chlorophenol in ether and this was followed by filtration and concentration of the reaction mixture. This procedure gave a pale yellow oil in 83% yield. This material was found to give, in its ^{31}P nmr spectrum, a single peak at $\delta 2.17$ which was consistent with the structure of this type of phosphorylating agent[133].

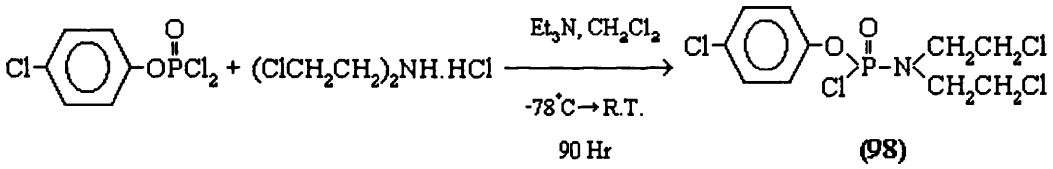
[Fig. 53]



The introduction of a chlorine atom at the *para* position of the phenyl group was noted to have induced a downfield shift of 0.6 ppm. In the ^{13}C nmr spectrum, all the phenyl carbons resonated as doublets due to phosphorus coupling with the *ipso* carbon at δ148.00 showing the largest coupling of 11.6 Hz. The ^{1}H nmr spectrum contained the phenyl protons at their characteristic position between δ7.18-7.42. The F.A.B. mass spectrum displayed peaks between m/e 244-249 corresponding to the expected molecular ion cluster of this compound.

The subsequent reaction of **(97)** with bis(2-chloroethyl)amine hydrochloride in ether [Fig. 54], followed by a hexane extraction on the concentrated reaction mixture, produced the phosphorylating agent N,N-bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate **(98)** as a colourless oil in 74% yield.

[Fig. 54]



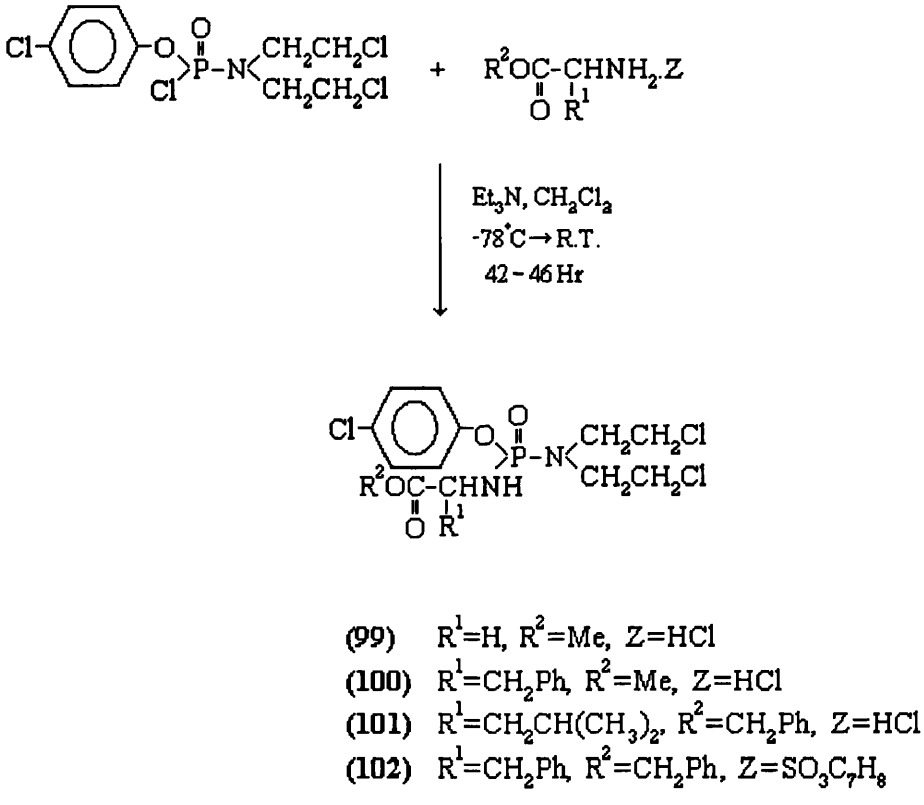
(98)

A single resonance was observed at δ9.23 in the ^{31}P nmr spectrum and this was noted to show a downfield shift of 7.0 ppm upon the introduction of the P-N link. Again, doublets were observed in the ^{13}C nmr spectrum but only for the *ipso* (δ148.11) and *ortho* (δ121.83) phenyl carbons and for the 2-chloroethyl carbons. The ^{1}H nmr data were similar to those obtained for **(97)** with additional resonances between δ3.52-3.74 due to CH_2CH_2Cl. F.A.B. mass spectrometry provided structural confirmation by displaying the expected molecular ion cluster.

The synthesis of N,N-bis(2-chloroethyl)amino 4-chlorophenyl methoxyglycinyl phosphoramidate **(99)** was next to be approached and this compound was obtained

from the reaction of **(98)** with glycine methyl ester hydrochloride in dichloromethane [Fig. 55]. An ether extraction on the concentrated reaction mixture followed by purification by silica-gel column chromatography gave rise to the required product as a colourless oil in 64% yield. This oil gave a familiar single peak ^{31}P nmr spectrum with the signal appearing at δ9.12.

[Fig. 55]



(99) R^1=H, R^2=Me, Z=HCl
(100) R^1=CH_2Ph, R^2=Me, Z=HCl
(101) R^1=$CH_2CH(CH_3)_2$, R^2=CH_2Ph, Z=HCl
(102) R^1=CH_2Ph, R^2=CH_2Ph, Z=$SO_3C_7H_8$

The ^{13}C nmr data confirmed the successful isolation of **(99)** and were found to be similar to those acquired for **(98)** with additional resonances in the expected region supporting the presence of the glycinyl moiety. For example, doublets at δ171.12 and δ52.48 were assigned to the methoxy carbonyl and methoxy carbon respectively. A phosphorus-carbon coupling constant of 12.2 Hz was noted for the latter carbon even

though this particular carbon is more than three bonds away from the phosphorus centre. The resonances arising from $CH_2\underline{C}H_2Cl$ and the glycinyl CH_2NH were found to have similar chemical shift values and appeared as a multiplet between δ42.15-42.51. The absence of a chiral centre in the glycinyl moiety explained the absence of diastereomeric splitting in this spectrum. All protons were observed as multiplets in the ^1H nmr spectrum with the exception of the methoxy protons which appeared as a singlet at δ3.72. The $C\underline{H}_2NH$ resonances at δ3.76 were resolved into a doublet of doublets arising from both proton-proton and proton-phosphorus coupling. F.A.B. mass spectrometry also confirmed the proposed structure of the isolated product. The spectrum displayed the protonated molecular ion and related peaks arising from the isotope of chlorine, ^{37}Cl. Also observed was a familiar fragmentation pattern showing the loss of CO_2Me (m/e 343) and $N(CH_2CH_2Cl)_2$ (m/e 262) from the molecular ion. Microanalysis and analytical hplc data confirmed the purity of this compound.

The series was then continued with the preparation of the phenylalaninyl analogue **(100)** to investigate the effect of varying the amino acid moiety within this group of 4-chlorophenyl analogues. Compound **(100)** was obtained, in an analogous manner to **(99)**, as a white solid in 56% yield. This material was fully characterised by spectroscopic and analytical techniques with the data gathered being fully supportive of its structure. For example, in the ^{31}P nmr spectrum at δ8.19, a single peak was observed which is generally the case with these phenyl-containing phosphoramidates. The ^{13}C nmr and ^1H nmr data confirmed the presence of two phenyl groups in the molecule.

The series was extended with the preparation of **(101)** to investigate the effect of varying the protecting group in the amino acid moiety. A benzyl ester amino acid was chosen to replace the methyl ester one as previous results, with trihaloalkyl derivatives containing this protecting group, were found to be promising. An additional aryl group would be expected to increase the lipophilicity of the phosphoramidate and it

would be interesting to compare the results of this compound with those obtained for the unsubstituted phenyl compounds.

Thus **(101)** was prepared in an entirely analogous manner to **(99)** and was isolated as a white solid in 63% yield. Two closely spaced signals at δ8.21 and δ8.14 and in an approximate ratio of 1:1 were observed in the ^{31}P nmr spectrum obtained at 164 MHz. The ^{13}C nmr data were consistent with the structure of **(101)** and also supported the isolation of diastereoisomers. The presence of two phenyl groups in the compound was confirmed by two sets of resonances in the expected region. The *ipso* carbon at δ149.34 and δ149.25 appeared to be the only phenyl carbon to display both diastereomeric splitting and phosphorus coupling with the others mostly showing only the former. The methyl groups of the leucinyl moiety of each isomer were found to give separate signals, due to non-equivalence, at similar chemical shifts. The resulting two sets of doublets were observed between δ21.80-δ22.65. A complex set of signals between δ7.06-7.38 in the ^{1}H nmr spectrum was assigned to the protons of the two phenyl groups. Two sets of signals, appearing below δ1 and representing the leucinyl methyl protons, were resolved into a doublet due to proton-proton coupling and a doublet of doublets due to additional long range proton-phosphorus coupling or diastereomeric splitting. The remaining signals were observed as multiplets with $PhC\underline{H}_2O$ at δ5.16 being the least complex. The F.A.B. mass spectrum contained peaks at m/e 534, 536 and 538 corresponding to the molecular ion with the ratio of the peaks being consistent with the compound possessing three chlorine atoms.

The introduction of an additional aryl group would be expected to increase further the lipophilicity of the compound. This was the basis for the preparation of the benzylphenylalaninyl analogue **(102)** which was prepared in a similar manner to **(99)** with slight modifications in the procedure. The routine ether extraction was followed by a hexane extraction in an attempt to remove some of the impurities present in the isolated pale yellow oil. These slow running impurities, which were more polar than the

required product, were revealed by the process of tlc. The purification procedure was only partially successful and so it was decided to further purify the oil by silica-gel column chromatography using a mixture of chloroform and petroleum spirit as the eluent. This led to the isolation of a cream coloured solid in a yield of 64%. This material was then characterised by standard techniques.

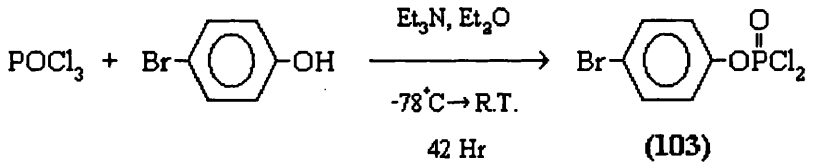
A single peak arising from coincident signals was observed at δ8.17 in the ^{31}P nmr spectrum. The ^{13}C nmr spectrum provided evidence for the existence of diastereoisomers in equal proportions. The spectrum was dominated by a mass of resonances appearing between δ121.44 and δ149.27 arising from the three phenyl groups. These resonances were assigned by analogy to the spectra of **(99)**, **(100)** and **(101)** and were in agreement with the structure of the isolated compound. The phenyl carbons mostly appeared as doublets due to diastereomeric splitting with the exception of the *ipso* carbon of the 4-chlorophenyl group, resonating at δ149.27 and δ149.18, which showed both phosphorus coupling (7 Hz) and diastereomeric splitting. It was of interest to note that a phosphorus-carbon coupling of 2.6 Hz was given by CHNH of the amino acid ester moiety in only one of the two isomers. The other isomer was observed as a singlet at a similar chemical shift.

The ^{1}H nmr data supported the ^{13}C nmr data with all protons appearing as multiplets, due to proton-proton coupling and phosphorus-proton coupling where relevant, at their characteristic positions. The F.A.B. mass spectrum also confirmed the successful isolation of **(102)** and contained the protonated molecular ion at m/e 569. Also evident was a peak at m/e 571 corresponding to the ^{37}Cl-containing protonated molecular ion. Fragmentation of the parent ion with the loss of the benzyloxycarbonyl group and both CH_2Ph and Ph were indicated by peaks at m/e 433 and m/e 400 respectively.

The series of 4-substituted phenyl derivatives of nitrogen mustard was elaborated with the preparation of a small number of 4-bromophenyl derivatives which

are depicted in [Fig. 58]. It was thought to be of interest to see what effect on biological activity the replacement of the chlorine atom, with the less electronegative bromine, would produce. The set of amino acids used was identical to that chosen for the phenyl and 4-chlorophenyl compounds so that any variations in biological activity within the whole series of phenyl and 4-substituted phenyl compounds would largely be a consequence of the nature of the *para* substituent. The synthetic methodology used was identical to that used for the previously described phenyl and 4-chlorophenyl derivatives and involved the preparation of 4-bromophenyl phosphorodichloridate **(103)** [Fig. 56]. The reaction of 4-bromophenol with phosphoryl chloride led to the isolation of this phosphorylating agent as a colourless oil in 88% yield. This was found to give a single resonance, in the ^{31}P nmr spectrum, at δ1.93 which was close to the chemical shift values reported for the analogous phenyl and 4-chlorophenyl agents.

[Fig. 56]



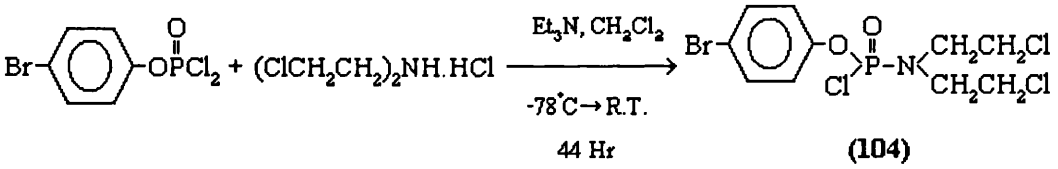
(103)

The ^{13}C nmr data indicated that the presence of the bromine atom at the 4 position had caused an upfield shift of 6ppm, for the *para*-carbon, with respect to the spectrum of **(93)**. The other phenyl carbons were also noted to have shifted upfield by 1-3 ppm. All of these carbons appeared as doublets due to phosphorus coupling with the *ipso*-carbon showing the largest coupling of 11.4 Hz. The ^{1}H nmr spectrum was also in agreement with the ^{13}C nmr data and showed some interesting features. Two sets of doublets of doublets were observed at δ7.17 and δ7.50 and these were assigned to the *ortho* and *meta* protons respectively. A combination of proton-proton and

phosphorus-proton couplings accounted for the observed splittings. The F.A.B. mass spectrum of **(103)** displayed, in the molecular ion region, the expected isotope cluster for a compound containing one bromine and two chlorine atoms.

The preparation of the phosphorylating agent **(104)** [Fig. 57] was carried out in an analogous manner to **(94)** and **(98)** and this led to the isolation of a colourless oil in 54% yield. This material was characterised by ^{31}P nmr spectroscopy which revealed the presence of a single resonance at δ9.00 which again was very close to the values reported for the analogous phenyl and 4-chlorophenyl phosphorochloridates. ^{13}C nmr and ^{1}H nmr data collected on **(104)** were similar to those on **(103)** with additional resonances arising from the bis(2-chloroethyl)amino moiety being observed. In the ^{1}H nmr spectrum, the proton resonances of the phenyl group were again resolved into two sets of doublets of doublets.

[Fig. 57]

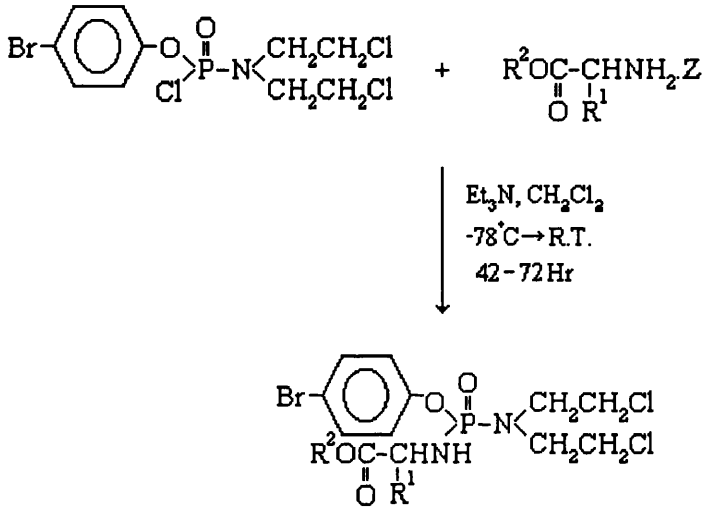


(104)

The first compound to be synthesised, in this series of 4-bromophenyl derivatives, was N,N-bis(2-chloroethyl)amino 4-bromophenyl methoxyphenylalaninyl phosphoramidate **(105)** which was obtained from the reaction of **(104)** with the appropriate amino acid methyl ester hydrochloride. Following a reaction time of 44 hours and the routine ether work-up, the product was purified by column chromatography using an eluent of chloroform and petroleum spirit. Pooling and evaporation of the appropriate fractions gave the product as a white solid in a yield of 66%. This solid was found to give a one peak ^{31}P nmr spectrum, the chemical shift

being δ8.00 which compared well with the shifts noted for the analogous phenyl **(95)** and 4-chlorophenyl **(100)** compounds.

[Fig. 58]



(105) R^1=CH_2Ph, R^2=Me, Z=HCl
(106) R^1=$CH_2CH(CH_3)_2$, R^2=CH_2Ph, Z=HCl
(107) R^1=CH_2Ph, R^2=CH_2Ph, Z=$SO_3C_7H_8$

The ^{13}C nmr spectrum supported the identity of the isolated product and consisted of signals displaying diastereomeric splitting and where applicable, phosphorus-carbon coupling. The presence of two phenyl groups was supported by peaks between δ117.58-149.87, with the *ipso*-carbon of the 4-bromophenyl group having the higher chemical shift (downfield) and the *para*-carbon accomodating the bromine atom, having the lower shift in this region. The ^1H nmr spectrum was also consistent with the structure of the product with all protons giving complex signals excluding the methoxy protons which appeared as two separate but closely spaced

signals, at δ3.70 and 3.65, of approximately equal intensities. The F.A.B. mass spectrum contained peaks at m/e 537, 539 and 541 corresponding to the expected molecular ion cluster of this compound. Notable peaks at m/e 477 and m/e 445 were assigned to the loss of CO_2Me and CH_2Ph respectively from the molecular ion. The loss of OPhBr was represented by peaks at m/e 365 and m/e 367 due to the presence of ^{37}Cl or ^{81}Br in these fragments.

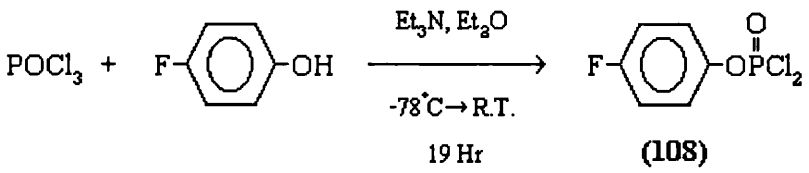
Similarly prepared was the benzylleucinyl analogue **(106)**, as a white solid in 61% yield, from the reaction of **(105)** with L-leucine benzyl ester hydrochloride. This product was found to give a single resonance, due to the isomers having coincident chemical shift values, at δ7.93 in its ^{31}P nmr spectrum. Both the ^{13}C nmr and ^{1}H nmr spectra were entirely consistent with the structure of the isolated product and were assigned by analogy to the corresponding spectra of **(101)**. It was of interest to note that in the ^{1}H nmr spectrum of **(106)**, as in the spectrum of **(101)**, the leucinyl methyl protons were observed as a doublet for one methyl group and a doublet of doublets for the other. The doublet of doublets was thought to be a consequence of additional long range phosphorus-proton coupling or diastereomeric splitting. F.A.B. mass spectrometry provided structural confirmation by displaying the molecular ion and protonated molecular ion peaks.

The preparation of **(107)** was the next to be carried out and concluded this short series of 4-bromophenyl analogues. The regular method of purification using the technique of silica-gel column chromatography, with a mixture of chloroform and petroleum spirit as the eluent, was used in this case and found to have partially separated the isomers of the product. ^{31}P nmr spectroscopy revealed that one isomer had been isolated in a pure state; the other fraction being a mixture of isomers. Both samples of the product were recrystallised as white solids from petroleum spirit (b.p. 40-60°C) in order to remove some impurities which were present in trace amounts. The isomer **(107A)** which was obtained in 23% yield was found to give a single resonance

at δ10.19 in its ^{31}P nmr spectrum while the mixture of isomers **(107B)** obtained in 30% yield was found to give two closely spaced signals of equal intensities at 10.19 and 10.14. As expected the ^{13}C nmr and ^{1}H nmr spectra for both samples were very similar with additional signals due to diastereomeric splitting being observed in the spectra of the mixture of isomers. F.A.B. mass spectrometry confirmed the structure of the isolated compounds and displayed the protonated molecular ion in each case. The fragmentation pattern was also very similar for each sample. Further confirmation of identity came from analytical hplc and microanalysis data.

The next *para* substituent to be studied was the fluorine one to see whether it would increase the biological activity of the compound to which it was introduced. The analogous 4-fluorophenyl derivatives which were synthesised are shown in [Fig. 61]. The synthetic strategy used was similar to that described for the other analogues in this series and required the preparation of 4-fluorophenyl phosphorodichloridate **(108)** and N,N-bis(2-chloroethyl)amino 4-fluorophenyl phosphorochloridate **(109)** by established methods. Thus, the former phosphorylating agent was obtained from the reaction of phosphoryl chloride and 4-fluorophenol [Fig. 59] as a pale yellow oil in 82% yield. The ^{31}P nmr spectrum of this material consisted of a peak at δ2.30 which was similar in chemical shift value to the analogous 4-chlorophenyl compound **(97)** and slightly upfield in comparison to the analogous 4-bromophenyl compound **(103)**.

[Fig. 59]

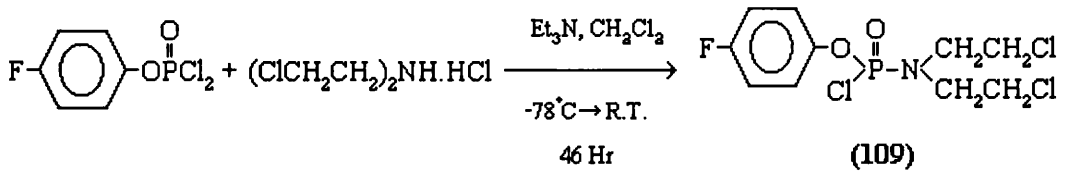


(108)

The ^{13}C nmr spectrum confirmed the identity of the isolated product and displayed some interesting features. The presence of the fluorine atom at the *para* position was found to have caused a large downfield shift of 34 ppm with respect to the *para* carbon of **(93)** while the the remaining phenyl carbons were found to have shifted upfield by 4-8 ppm. All signals were observed as doublets of doublets due to phosphorus-carbon and fluorine-carbon couplings with the latter type giving the larger coupling constant. For example, the *para* carbon appeared as two distinct doublets showing phosphorus-carbon and fluorine-carbon couplings with coupling constants of 3 Hz and 247 Hz respectively. In the case of the *ortho* carbons the constant for fluorine-carbon coupling was also noted to be larger than that for phosphorus-carbon coupling. There was a less apparent difference in these coupling constants for the *meta* carbons. In the ^{1}H nmr spectrum, the phenyl protons were found to give resonances which were more complex than those usually observed for analogous compounds. The F.A.B. mass spectrum contained peaks corresponding to the molecular ion and peaks at m/e 111 and m/e 95 which were assigned to the fluorine containing ions $FPhO^{+}$ and FPh^{+} respectively.

N,N-Bis(2-chloroethyl)amino 4-fluorophenyl phosphorochloridate **(109)** was obtained from **(108)** and bis(2-chloroethyl)amine hydrochloride [Fig. 60] in an analogous manner to **(98)**. The colourless oil which was isolated in 63% yield was successfully characterised by nmr spectroscopy.

[Fig. 60]



(109)

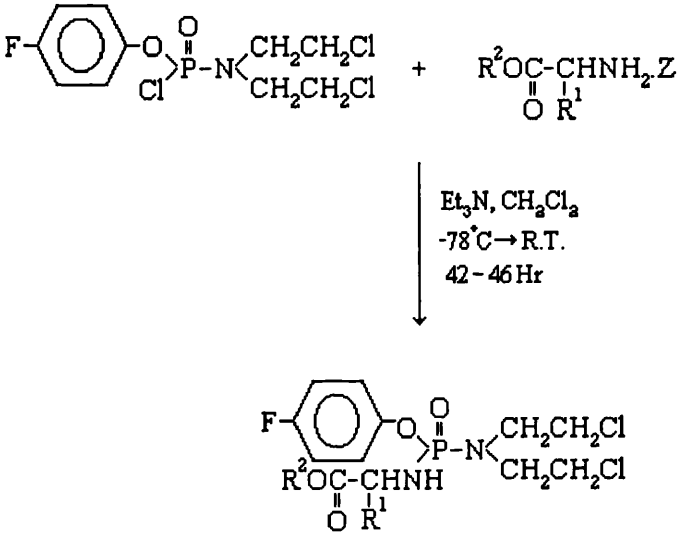
The ^{31}P nmr spectrum consisted of a single peak at δ9.45 which indicated a downfield shift of 0.5 ppm with respect to the unsubstituted phenyl phosphorochloridate **(93)**. ^{13}C nmr and ^{1}H nmr data were similar to those collected for **(108)** with additional resonances being present due to the bis(2-chloroethyl)amino group. The incorporation of this group appeared to affect only the *ipso* carbon which showed a larger phosphorus-carbon coupling constant and a smaller carbon-fluorine coupling constant than the corresponding ones in the spectrum of **(108)**. F.A.B. mass spectrometry confirmed the identity of the isolated oil by displaying the protonated molecular ion peak. The loss of OPhF and CH_2Cl were represented by peaks at m/e 222 and m/e 284 respectively.

N,N-Bis(2-chloroethyl)amino 4-fluorophenyl methoxyphenylalaninyl phosphoramidate **(110)** was the next compound chosen to be synthesised and evaluated for anti-HIV activity. This involved the reaction of **(109)** with L-phenylalanine methyl ester hydrochloride in dichloromethane followed by purification by column chromatography. This led to the isolation of a product in 69% yield.

This material was characterised by ^{31}P nmr spectroscopy which revealed a single peak, due to coincident resonances, at δ8.13. The ^{13}C nmr spectrum consisted of resonances showing both diastereomeric splitting and phosphorus-carbon coupling and confirmed the isolation of diastereoisomers. It was apparent from the heights of the signals that the ratio of the isomers in the product was approximately 1:1. Also apparent were certain signals showing additional splitting due to the coupling of carbon to the fluorine atom. For example, the *para*-FPh carbon was observed as a doublet of doublets downfield, between δ159.45 and δ159.50 and showing a large carbon-fluorine coupling of 243.5 Hz. The *meta* carbon of the 4-fluorophenyl group was observed as a complex signal between δ121.38-121.54 due to the combination of diastereomeric splitting, and phosphorus-carbon and carbon-fluorine couplings. The *ipso* carbon was also observed as a complex signal (δ146.44-146.64) which is probably the result of

long range coupling, between this carbon and fluorine, being in effect. The ^{1}H nmr data supported the ^{13}C nmr data with all protons appearing as multiplets with the exception of the methoxy protons which appeared as separate signals at δ3.70 and 3.65. Some protons were found to give signals more complex than usually observed and this was due to the presence of the fluorine atom. Further confirmation of the structure came from the F.A.B. mass spectrum which contained the protonated and diprotonated molecular ions.

[Fig. 61]



(110) $R^1=CH_2Ph$, $R^2=Me$, $Z=HCl$
(111) $R^1=CH_2CH(CH_3)_2$, $R^2=CH_2Ph$, $Z=HCl$
(112) $R^1=CH_2Ph$, $R^2=CH_2Ph$, $Z=SO_3C_7H_8$

The benzyl ester-containing analogues **(111)** and **(112)** were prepared in a similar manner to the analogous chlorophenyl and bromophenyl analogues. Both compounds were purified by silica-gel column chromatography and isolated as white solids in 66-67% yield. Each displayed, in its respective ^{31}P nmr spectrum, a single

resonance at δ8 which was entirely consistent with its proposed identity. The ^{13}C nmr spectrum in each case indicated that a mixture of isomers had been isolated and as noted with other fluorine-containing compounds, extensive coupling to the fluorine atom was evident. For example, a carbon-fluorine coupling constant of 244 Hz was observed for the *para*-F in each compound. Also observed were the familiar diastereomeric splitting and phosphorus-carbon coupling. The ^{1}H nmr data for **(111)** and **(112)** were also in agreement with their ^{13}C nmr data with all protons resonating at their characteristic positions. With the exception of the leucinyl methyl protons of **(111)**, all protons were observed as multiplets. It was noticed that the leucinyl methyl protons were non-equivalent, as previously noted with the bromo analogue **(106)**, with one group appearing as a distinct doublet and the other a doublet of doublets. F.A.B. mass spectrometry and analytical data also confirmed the identity and purity of the isolated compounds.

The series of 4-halogenosubstituted phenyl derivatives was completed with a small number of dipeptide-containing phosphoramidate derivatives of nitrogen mustard. It was hoped that by synthesising these compounds information on the influence of the peptide chain length on the biological activity of the compound could be obtained. Peptides containing two amino acids can be obtained by protecting the amino group of the first amino acid and then activating the carboxyl group before allowing it to react with the second protected amino acid. The final step can be carried out at ambient temperature and under conditions which are neither acidic nor basic. The carboxyl group can be activated by converting it into an anhydride or acid chloride[134]. Protecting groups which are generally used for protecting the amino group and converting it into one of low nucleophilicity, are the benzyloxycarbonyl and tert-butyloxycarbonyl groups as both can be removed under conditions that do not affect amide linkages[135]. However, dipeptides are commercially available and so there was no need to carry out these particular steps. The dipeptide chosen for this study was phenylalaninyl-phenylalanine

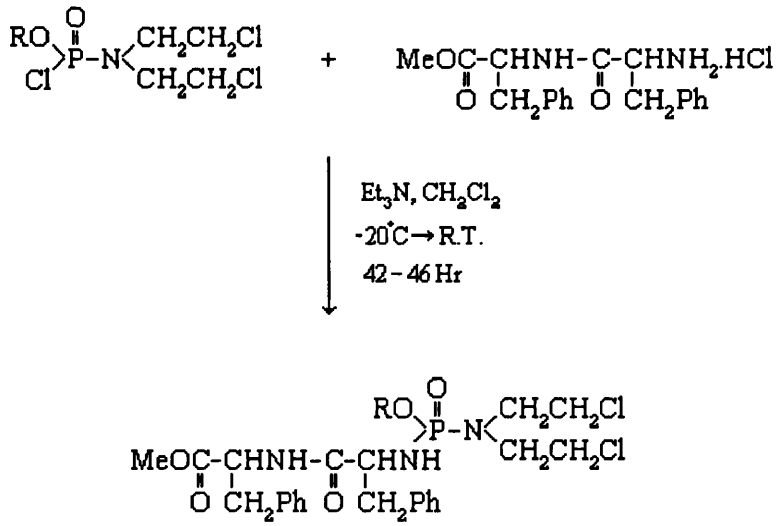
and this was protected at the carboxyl terminus with a methoxy group, by standard procedures involving refluxing in methanol and thionyl chloride[136]. This step was essential to enable P-N bond formation to take place at the terminal amino group. Thus, compounds composed of a nitrogen mustard, methoxyphenylalaninyl-phenylalaninyl and an alkyl or 4-substituted aryl moiety attached to the phosphorus were prepared.

The synthesis of propyl methoxyphenylalaninyl-phenylalaninyl phosphoramidate **(113)** was the first to be carried out and necessitated the reaction of bis(2-chloroethyl)amino propyl phosphorochloridate **(65)** with L-phenylalaninyl-phenylalanine methyl ester hydrochloride. The reaction was conducted in dichloromethane in the presence of the base triethylamine and at -20°C rather than -78°C to take into account the predicted low reactivity of the bulky protected amino acid [Fig. 62]. Concentration of the reaction mixture and an ether extraction gave rise to a yellow oil which, by analytical tlc, was found to contain minor impurities. The ^{31}P nmr spectrum, of the material obtained after purification by silica-gel column chromatography, revealed the presence of impurities which had not previously been observed. It was thought that these impurities had probably been generated during the attempt at purifying the compound by column chromatography. Therefore, it was found to be necessary to further purify this material by preparative HPLC. This technique allowed the desired compound to be isolated as a white solid in 30% yield. The product was characterised by ^{31}P nmr spectroscopy which indicated that a mixture of diastereoisomers had been successfully isolated. No attempt was made to resolve these isomers which were found to resonate as closely spaced signals at δ14.49 and δ13.96 in a ratio of 1:1.

The ^{13}C nmr spectrum confirmed the isolation of **(113)** and contained signals showing diastereomeric splitting and where relevant phosphorus coupling. Both of the carbonyl carbons displayed diastereomeric splitting but only the carbon nearest to the phosphorus showed phosphorus-carbon coupling which would be expected as the

terminal carbonyl carbon is positioned more than three bonds away from the phosphorus centre. The presence of the phenylalaninyl groups in the compound was supported by two sets of aryl signals appearing between δ127.12 and δ136.60. The ^1H nmr data were also consistent with the structure of **(113)** with all signals being observed as multiplets with the exclusion of the methyl protons of the propyl group which were observed as a doublet of triplets and the methoxy methyl protons which were observed as two signals. The protonated molecular ion peak was observed at m/e 572 and m/e 574 (due to ^{37}Cl) in the F.A.B. mass spectrum. The base peak at m/e 120 was assigned to the phenyl-containing ion $(PhCH_2CHNH_2)^+$.

[Fig. 62]



(113) R = n-C_3H_7
(114) R = p-ClPh
(115) R = p-BrPh
(116) R = p-FPh

The 4-chlorophenyl methoxyphenylalaninyl-phenylalaninyl analogue **(114)** was the next target compound to be prepared. This preparation involved the reaction of

N,N-bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate **(98)** with L-phenylalaninyl-phenylalanine methyl ester hydrochloride under similar conditions to those described for **(113)**. This included the addition of the base triethylamine at -20°C and a reaction period of 42 hours. An ether work-up on the concentrated reaction mixture, followed by column chromatography, was found to give a colourless oil. A ^{31}P nmr spectrum of this material consisted of two closely spaced signals, in an approximate ratio of 1:1, with no evidence of any phosphorus-containing impurities being present.

The 4-chlorophenyl compounds described earlier on were generally found to exist in the solid state and so this suggested that the product **(114)** should be a solid rather than an oil. Therefore, it was attempted to crystallise this material from petroleum spirit and this led to the isolation of three samples of the product containing the isomers in various ratios. The partial separation of the two isomers was evident from the changing ratios of the two ^{31}P nmr signals. All three mixtures were obtained as white solids and were characterised separately by spectroscopic and analytical techniques as it is possible that the two diastereoisomers of a given product may show notable differences in biological activities.

^{31}P nmr spectroscopy indicated that the isomers resonated between 11 and δ12 and that they had been isolated in the ratios of 8:2 **(114A)**, 3:7 **(114B)** and 1:9 **(114C)**. The isomer with the higher chemical shift value was found to be the most lipophilic and so the last mixture to crystallise from the petroleum spirit was the one dominated by this isomer, that is **(114A)**. Not unexpectedly, the mixtures of the product gave very similar signals in their respective ^{13}C nmr spectra with the ratios of the signals being consistent with the ratios observed in the corresponding ^{31}P nmr spectra. The spectrum of **(114A)** confirmed the presence of the phenylalaninyl and the 4-chlorophenyl groups by displaying three sets of phenyl resonances in the characteristic positions. For example, the *ipso* carbon of the chlorophenyl group in each isomer resonated as two

singlets at δ149. The signals at δ136.23 and δ136.02 were assigned to the *ipso* carbon of the phenylalaninyl group furthest away from the phosphorus centre while those nearby at δ135.77 and δ135.63 were assigned to the remaining *ipso* carbon. As expected the amide carbonyl carbon gave doublets downfield at δ171.17 and at δ171.03 with phosphorus-carbon couplings of 5.1 Hz and 4.5 Hz respectively. The methoxy carbonyl carbon appeared as two resonances at higher chemical shifts of δ171.41 and δ171.34 with diastereoisomerism alone accounting for the observed splitting.

The ^{1}H nmr spectrum of **(114A)** was entirely consistent with the structure of the isolated product and again confirmed the ratio of the isomers in the mixture. The protons of the three phenyl groups were observed as a multitude of signals between δ6.80-7.33. The remaining protons, with the exception of the methoxy protons, were also observed as multiplets. The F.A.B. mass spectrum contained both the molecular ion and the protonated molecular ion for **(114A)**. Very similar spectroscopic data were obtained for compounds **(114B)** and **(114C)** with the only notable variation being in the ratios of the signals observed. The purity of each sample was confirmed in each case by microanalysis and analytical hplc data.

To continue this series of lipophilic compounds, N,N-bis(2-chloroethyl)amino 4-bromophenyl methoxyphenylalaninyl-phenylalaninyl phosphoramidate **(115)** was duly prepared. The method used was analogous to that for **(114)** and involved similar purification steps. Crystallisation of the material partially purified by column chromatography, resulted in the isolation of two samples of the product, **(115A)** (32%) and **(115B)** (24%), with the ratio of the isomers varying in each case. As in the example of **(114)**, the isolated solids were characterised separately by spectroscopic and analytical techniques.

^{31}P nmr spectroscopy indicated that the isomers had been isolated in ratios of 8:1 **(115A)** and 1:3 **(115B)** and that the isomer with the higher chemical shift was the

one that was the least lipophilic, which was in contrast to **(114)**. The ^{13}C nmr spectra of both samples were very similar with the exception of the ratios of the isomers which were consistent with those displayed in the corresponding ^{31}P nmr spectra. The ^{13}C nmr spectrum of **(115A)** was assigned by analogy to the spectrum of the 4-chlorophenyl compound **(114)**. The spectrum showed the methoxy carbonyl carbon as two closely spaced singlets at δ171.41 and δ171.42 while the amide carbonyl carbon of the prominent isomer was resolved into a doublet appearing slightly upfield at δ171.04 and showing a phosphorus-carbon coupling of 4.5 Hz. The presence of the phenylalaninyl and 4-bromophenyl groups was supported by resonances appearing between δ117.72 and δ149.65 which were assigned to the phenyl carbons. In general, these carbons were observed to show diastereomeric splitting alone with the *ortho* and *ipso* carbons of the 4-bromophenyl group also showing phosphorus-carbon couplings of 5-6 Hz. The $\underline{C}H_2Ph$ carbons were found to resonate as doublets close to each other at δ39 and δ38 with the former set of signals also showing a coupling of 5.5 Hz and being assigned to the carbon closest to the phosphorus centre.

The ^{1}H nmr spectrum of **(115A)** supported the ^{13}C nmr data with all protons resonating as multiplets excluding the methoxy protons which resonated as singlets in a ratio of 8:1 at δ3.67 and δ3.64. The F.A.B. mass spectrum contained peaks in the molecular ion region which were in agreement with the presence of one bromine and two chlorine atoms, and which confirmed the identity of **(115A)**. Also observed were peaks at m/e 543 and m/e 512 due to the loss of $N(CH_2CH_2Cl)_2$ and OPhBr respectively from the molecular ion. Satisfactory microanalysis and analytical data were also obtained for this sample. Very similar spectroscopic and analytical data were collected for the second sample **(115B)** and these also confirmed its identity and purity.

Similarly prepared was the 4-fluorophenyl analogue, N,N-bis(2-chloroethyl)amino 4-fluorophenyl methoxyphenylalaninyl-phenylalaninyl phosphoramidate **(116)** from N,N-bis(2-chloroethyl)amino 4-fluorophenyl phosphorochloridate **(109)**, and phenylalaninyl-

phenylalanine methyl ester hydrochloride. Again, the technique of silica-gel column chromatography was needed to purify the product. This was isolated as a white solid in 62% and found to resonate, in the ^{31}P nmr spectrum, as two signals at δ12.16 and δ 11.75 in an approximate ratio of 1:1. This mixture of isomers was not resolved or partially separated and was tested as such. Further characterisation was by ^{13}C nmr spectroscopy and this was also entirely consistent with the successful isolation of **(116)** as a mixture of diastereoisomers in a ratio of 1:1. As noted with the spectra of similar fluorine containing compounds, this spectrum contained signals exhibiting diastereomeric splitting and phosphorus-carbon and carbon-fluorine couplings. For example, the *para*-fluorophenyl and *meta*-fluorophenyl carbons were observed as complex signals with the former showing a carbon-fluorine coupling of 242.5 Hz and a diastereomeric splitting of 0.1 ppm. The presence of the phenylalaninyl and bis(2-chloroethyl)amino groups was supported by resonances in their characteristic positions.

The ^{1}H nmr spectrum was also consistent with the structure of **(116)** with all protons, excluding the methoxy group, resonating as multiplets. Structural confirmation was provided by the F.A.B. mass spectrum which contained the protonated molecular ion peak and showed peaks at m/e 512 and m/e 483 corresponding to the loss of OPhF and $N(CH_2CH_2Cl)_2$ respectively from the molecular ion. The base peak at m/e 417 was assigned to the loss of CH_3, OPhF and $H_2NP(O)N(CH_2CH_2Cl)_2$ from the molecular ion.

Some of the compounds whose synthesis have been described in this chapter were evaluated for anti-HIV activity by methods previously described. The unsubstituted phenyl analogue **(95)** and the 4-chlorophenyl analogues **(100)-(102)** and **(114A-B)** shown in table 3 were all found to show slight activity at concentrations greater than 200 μM in tests conducted at Mill Hill. In this system, AZT is active at a concentration of 0.03 μM. These results suggested that replacing the alkyl group of these phosphoramidates with an aryl or a 4-chloro-substituted aryl group had produced

no obvious beneficial effect. Incorporating a dipeptide in the molecule also appeared to be ineffective. In marked contrast, compounds **(95)** and **(100)-(102)** were found to be active at much lower concentrations in similar tests carried out at Cambridge. The unsubstituted phenyl compound **(95)** was active at 20 μM but equitoxic whereas the 4-chlorophenyl methoxyphenylalaninyl **(100)**, benzylleucinyl **(101)** and benzylphenylalaninyl **(102)** compounds were found to be selectively active and non-toxic.

Table 3. Anti-HIV activities from 2 test centres. Data are given as ED_{50} (TD_{50}) in μM

	Mill Hill	Cambridge
(95)	>200	20(20)
(100)	>200	30(>200)
(101)	>200	10(>200)
(102)	>200	2(>200)
(114A)	>200	>200
(114B)	>200	>200
(114C)	>200	>200

The results obtained from Cambridge suggested that increasing the lipophilic nature of the molecule and/or incorporating *para* substituted halogens in the aryl group had produced an activating effect. Given the high activity of AZT in the biological assay used it was found to be necessary to confirm, by analytical hplc, that these particular compounds were entirely free of this contaminant. These compounds are much less active than the anti-HIV drug AZT but are perhaps less toxic and are of novel structure.

The promising results obtained with some of these compounds suggest that perhaps further studies should be carried out on this particular group of analogues. The

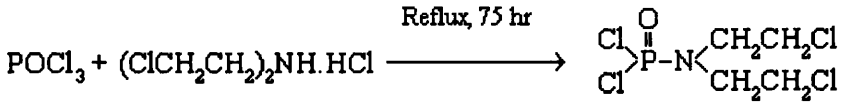
remainder of the compounds in this particular series of phenyl and 4-halogenosubstituted phenyl derivatives of nitrogen mustard are currently undergoing tests for potential anti-HIV activity.

2. Analogues of cyclophosphamide

Cyclophosphamide **(11)**, an alkylating agent prepared over 20 years ago by Arnold[70], has become widely used in cancer chemotherapy. Along with its structural isomer isophosphamide[104], it is effective against a wide range of human tumours. These two drugs share a rather unique toxicity profile amongst anti-cancer drugs, in particular of haemorrhagic cystitis. This is thought to be caused by the non-chemotherapeutic metabolite acrolein which is produced in the bio-activation pathway of cyclophosphamide and its isomer[102]. Current clinical practice is to co-administer a thiol such as 2-mercaptoethanesulphonate (MESNA) to trap the liberated acrolein[103]. However, a better solution to the problem might be to chemically modify cyclophosphamide such that acrolein is not released. With this in mind, the synthesis of 5-substituted cyclophosphamides was investigated, in particular the 5-hydroxy compound **(117)**.

This particular analogue of cyclophosphamide has been prepared by Eibl, but by a long route requiring numerous protection and deprotection steps[137]. Therefore, it was wondered if this compound could be prepared by the simple reaction of unprotected aminopropan-2,3-diol with dichlorophosphoramide **(59)**. This latter reagent has been employed in the synthesis of cyclophosphamide and is prepared from the reaction of phosphoryl chloride with bis(2-chloroethyl)amine hydrochloride[138]. This reaction is carried out at an elevated temperature, in the absence of a base and the crude solid product is vacuum distilled. However, in this report a procedure has been found which obviates the necessity for troublesome vacuum distillation and provides **(59)** in a quantitative yield. This involves refluxing bis(2-chloroethyl)amine hydrochloride in phosphoryl chloride [Fig. 63] and then removing the solvent by evaporation under reduced pressure to give dichlorophosphoramide as a solid in the pure state as judged by ^{31}P nmr spectroscopy.

[Fig. 63]

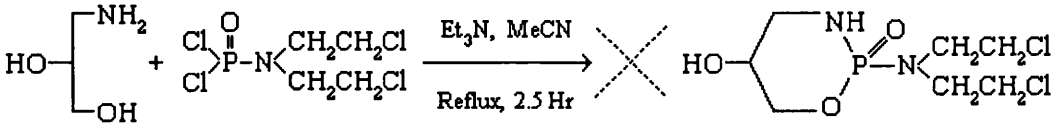


(59)

The chemical shift value of δ15.80 obtained for the isolated material was noted to be close to that reported for the model compound dipropylamino phosphorodichloridate (δ15.3)[124]. The ^{13}C nmr data also confirmed the structure of the product, with two doublets being noted at chemical shifts comparable to those reported for the bis(2-chloroethyl)amino moiety of cyclophosphamide[139]. The ^{1}H nmr and microanalysis data also confirmed the identity and purity of the isolated product.

Thus, the reaction of dichlorophosphoramide with aminopropan-2,3-diol was investigated [Fig. 64]. This reaction was conducted in anhydrous acetonitrile and at reflux with the product being isolated following evaporation of the solvent and a tetrahydrofuran extraction.

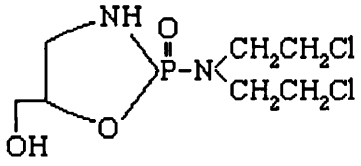
[Fig. 64]



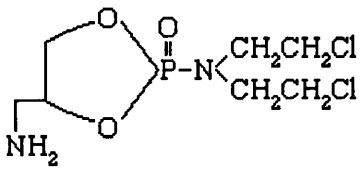
(117)

The ^{31}P nmr spectrum of the isolated material consisted of two closely spaced signals at δ28.57 and 27.61 in an approximate ratio of 1:1. The close spacing of these two signals suggested that diastereoisomers of the product had been isolated. However,

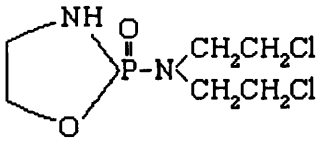
the chemical shift was noted to be rather different to that reported for cyclophosphamide which is known to resonate at δ13[140]. This difference was thought to be larger than might be expected simply upon the introduction of a 5-hydroxy substituent. Thus, the possibility that the product was a structural isomer **(118)** or **(119)** of the intended product was considered.



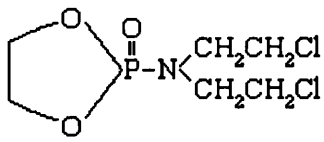
(118)



(119)



(120)



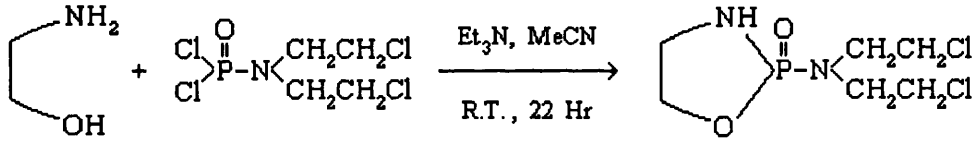
(121)

Phosphorus nmr chemical shift values have not been reported for these compounds or for the un-branched compounds **(120)**[141,107] and **(121)**[107]. However, it is known that 5-membered ring phosphorus heterocycles generally resonate approximately 20 ppm downfield of their 6-membered ring analogues in the ^{31}P nmr spectra. For instance, 2-ethyl-1,3,2-dioxaphosphacyclopentane 2-oxide and its cyclohexane analogue are noted to resonate at δ+17 and δ-7 respectively in the ^{31}P nmr spectra[142]. Also, nucleotide derivatives bearing 1,3,2-oxazaphosphacyclopentane 2-oxide heterocycles have been noted to appear at δ+21[143] while the cyclohexane derivatives appear at δ-4[144]. These observations suggested that the product isolated from the reaction in question had the structure **(118)** or **(119)**. Infra-red data collected on the isolated product supported the structure **(118)** in favour of **(119)** or **(117)**.

Primary hydroxyl bands were noted at 3273 and 1056 cm^{-1} with no primary amino bands being observed.

Further experiments were then conducted in order to establish the identity of the product in question and to probe the effect of structure on the ^{31}P nmr chemical shift. Thus, the reaction of dichlorophosphoramide with ethanolamine was carried out in a similar manner to that for the preparation of **(117)** with the exception that the reaction was conducted at ambient temperature rather than at reflux [Fig. 65].

[Fig. 65]

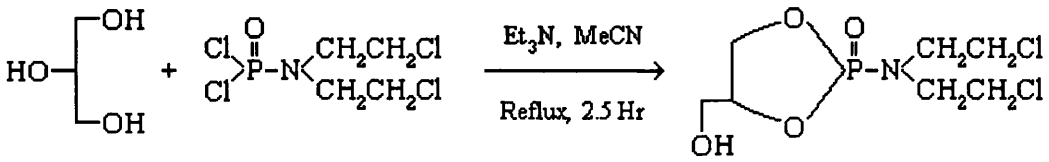


(120)

Following a reaction time of 22 hours and a tetrahydrofuran extraction, the product **(120)** was isolated as a white solid in a quantitative yield. The ^{13}C nmr data were found to correspond closely with those reported for cyclophosphamide with both $\underline{C}H_2CH_2Cl$ and CH_2OP carbons displaying phosphorus-carbon coupling (2.7-4.9 Hz). Most importantly, the product was observed to resonate as a single signal at δ30.09 in its ^{31}P nmr spectrum. This chemical shift value was very close to that noted for the product in question and suggested that it had the structure **(118)**. Also, the bands assigned to the primary hydroxyl group in **(118)** were absent in the infra red spectrum of **(120)**. FAB mass spectrometry and microanalysis data also confirmed the identity of this compound.

To provide further evidence for the structure of **(118)**, the reaction of dichlorophosphoramide with glycerol was carried out in a similar manner to that for the preparation of **(120)** [Fig. 66].

[Fig. 66]



(122)

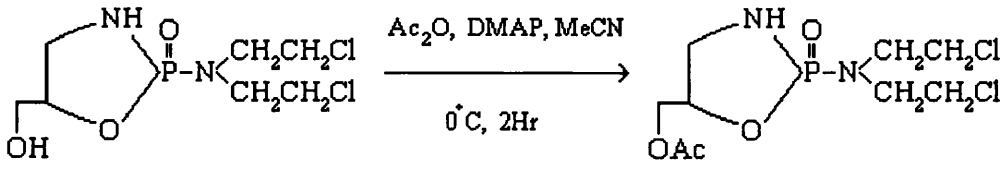
Thus, the reaction mixture was refluxed for 2.5 hours and then the product extracted with diethyl ether. This procedure led to the isolation of a clear colourless oil in a quantitative yield and this was found to give two closely spaced signals at δ24.61 and 23.89 (1:1) in the ^{31}P nmr spectrum. These resonances were assigned to the diastereoisomers of the 5-membered ring compound **(122)** and not the alternative 6-membered ring analogue. This assignment was strongly supported by the ^{13}C nmr data which would have identified the latter compound on the basis of its symmetry. The spectrum was found to contain three distinct sets of signals in addition to those arising from the bis(2-chloroethyl)amino group. Interestingly, the CHOP signal showed coupling to phosphorus in only one of its isomers, a feature which was also noted in the spectrum of **(118)**. This reaction is particularly informative for several reasons. Firstly, in the reaction of dichlorophosphoramide with glycerol, a five membered ring product is clearly preferred over the 6-membered ring alternative. None of the latter product was detected in the spectra of **(122)**. Secondly, the ^{31}P nmr chemical shift of **(122)** is approximately 4 ppm upfield of that observed for the product in question. The

replacement of a hydroxymethyl group in **(122)** with an aminomethyl group in **(119)** would not be expected to produce such a large shift. However the presence of an oxazaphosphaheterocycle **(118)** rather than a dioxaphosphaheterocycle **(122)** would be expected to induce such a shift. A downfield shift of 4-5 ppm has been noted in the literature for similar replacements[142,143].

Therefore, the ^{31}P nmr and ^{13}C nmr data gathered on model compounds confirmed that the product derived from the reaction of dichlorophosphoramide with aminopropan-2,3-diol was indeed N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide **(118)**. The ^{13}C nmr and ^{1}H nmr data fully supported the assigned structure. The former spectrum contained signals exhibiting diastereomeric splitting and where expected, phosphorus-carbon coupling. The EI mass spectrum also supported the isolation of **(118)** and displayed the protonated molecular ion peak at m/e 277. Also observed were peaks at m/e 279, corresponding to the ^{37}Cl-containing protonated molecular ion, and at m/e 136 corresponding to the loss of $N(CH_2CH_2Cl)_2$ from the molecular ion.

The hydroxy product **(118)** was then derivatised in order to further confirm its structure. Thus, the product was stirred with acetic anhydride in acetonitrile, in the presence of dimethylaminopyridine (DMAP) [Fig. 67][145].

[Fig. 67]

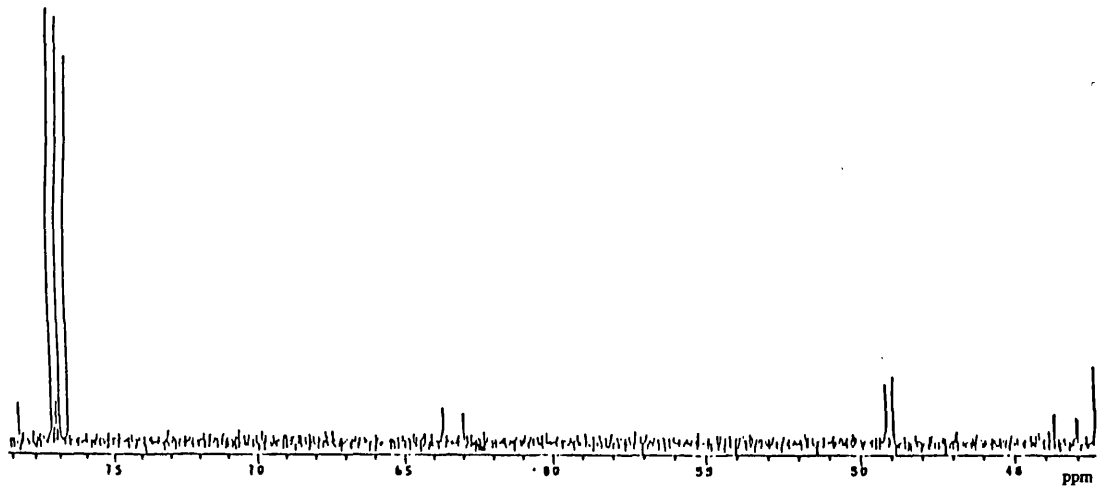


(118) **(123)**

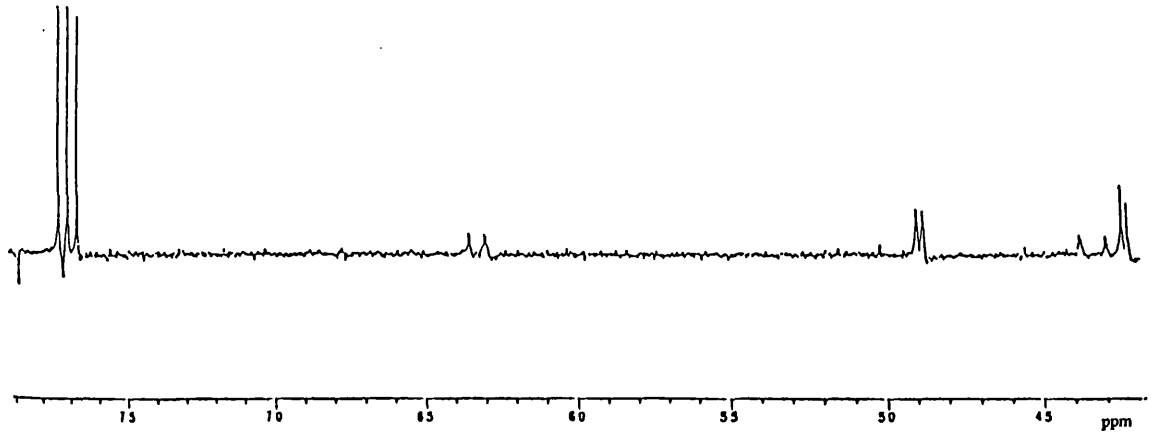
An organic-aqueous extraction on the concentrated reaction mixture gave rise to a mono-acetylated product **(123)** in a high yield. The ^{31}P nmr and ^{13}C nmr data confirmed the structure of this product and provided further evidence for the structure of **(118)**. In the ^{13}C nmr spectrum of **(118)** [Fig. 68], the most downfield signal was assigned to CHOP and appeared as a multiplet due to the combination of diastereomeric splitting and phosphorus-carbon coupling. This resonance which appeared as a singlet and a doublet (J=2.4 Hz) was assigned to CHOP, the only methine in the compound, on the basis of its behaviour in an Attached Proton Test (APT) [Fig. 69][146]. This pulse sequence is able to distinguish between methine and methyl carbons from methylenes by inverting those signals arising from the former. Acetylation of this compound was found to shift the resonance from this particular carbon upfield by approximately 3 ppm while the resonance from the exocyclic hydroxymethyl carbon was noted to have moved downfield by approximately 1 ppm. It has been found in the literature that in general carbon atoms bearing hydroxyl groups move downfield upon acetylation whilst β carbons move upfield[147]. This observation confirmed that the CH in **(118)** was β to the hydroxyl group undergoing acetylation whilst the CH_2 was adjacent to the hydroxyl group, further confirming the structure of **(118)**.

The 5-membered ring analogue, N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide **(118)** was also of interest in terms of its potential anti-HIV activity, in addition to its potential anti-cancer properties, and was tested against HIV by methods previously discussed. However, this compound was found to be inactive and non-toxic at the concentrations tested.

It was then attempted to oxidize the 5-membered ring compound **(118)** in order to provide the corresponding aldehyde [Fig. 70]. The oxidation was attempted several times with the first attempt employing the use of silver carbonate precipitated on celite which was prepared by the method of Fetizon, Balogh and Golfier[148]. This has proved



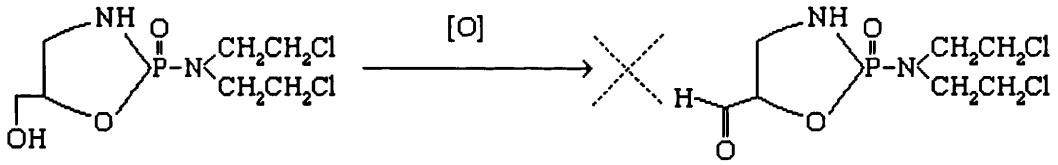
[Fig. 68]



[Fig. 69]

to be an efficient reagent for the oxidation of primary and secondary alcohols to the corresponding aldehydes or ketones in high yields and under mild conditions. These oxidations are usually carried out in boiling benzene with an excess amount of freshly prepared reagent and the course of the reaction is generally followed by analytical tlc. In the case of **(118)**, the attempted oxidations were carried out in anhydrous tetrahydrofuran and at ambient temperature for 70 hours to 13 days or at reflux for 21 hours. In each case the oxidation was found to be unsuccessful with the starting material being recovered. The reason for the failure of the reaction was thought to involve the reaction of the silver carbonate on celite reagent with the chlorides of the bis(2-chloroethyl) amino group resulting in the liberation of silver chloride.

[Fig. 70]



(118)

The oxidation was then attempted using an alternative oxidising agent, dinitrogen tetroxide[149]. This agent has been found to be a valuable reagent for converting triesters of phosphorus acid to the corresponding phosphates in methylene chloride solution at 0°C[149]. The oxidant is reduced to a mixture of dinitrogen oxide and nitrogen. This reaction was duly attempted at 0°C for a period of 1 hour and was also found to be non-productive with the starting material being almost completely recovered. It was felt that perhaps this reagent was too mild for this particular oxidation.

Pyridinium chlorochromate (PCC) is a stable reagent used in the oxidation of primary and secondary alcohols to carbonyl compounds and this is known to proceed with high efficiency[150]. This reagent was prepared by the method of Corey and Suggs and involved the addition of pyridine to a solution of chromium trioxide in hydrochloric acid (6M) at 0°C followed by filtration to obtain the air-stable oxidising agent. The oxidation of **(118)** was attempted twice in the presence of sodium acetate to take into account the slightly acidic nature of the oxidising reagent. In the first attempt the course of the reaction was followed by tlc, using an iodine and silica visualising system, and this suggested that the reaction was complete after 2 hours. The reaction mixture was noted to have turned from orange to black in colour which also suggested that an oxidation had taken place. A chloroform extraction on the reaction mixture produced a black oil and its ^{31}P nmr spectrum contained two sets of signals at δ30 and δ26 with relative intensities of 2:2 and 1:1 respectively. The former set of signals was assigned to the starting material whilst the latter set of signals was thought to represent diastereoisomers of the intended product. The low yield of isolated material prevented any further purification. This reaction was then repeated with the reaction time being extended to 3 hours after a tlc analysis of the reaction mixture. In this case various extractions including chloroform, tetrahydrofuran and ether failed to produce a phosphorus-containing material.

Despite the efficient oxidation of a wide range of alcohols to carbonyl compounds, the mildly acidic nature of pyridinium chlorochromate was thought to have caused P-N bond and possibly ring cleavages in the case of the acid-sensitive **(118)**. Therefore, the oxidation was attempted with pyridinium dichromate (PDC) which is milder than PCC and which is known to be a very useful and versatile oxidant with widespread application including in the oxidation of alcohols[151]. This reagent was prepared by the method of Corey and Schmidt and was collected as an orange solid from the addition of pyridine to chromium trioxide in water. The attempted oxidation of

(118) was carried out in anhydrous dichloromethane, as the use of this oxidant in this particular solvent is known to oxidise primary alcohols to the corresponding aldehydes and no further regardless of the nature of the substrate[151]. The reaction was conducted at ambient temperature for 23 hours and then it was attempted, without success, to isolate the required product from the black reaction mixture. As found in the second attempt with pyridinium chlorochromate, the work-up involving numerous extractions failed to produce a phosphorus-containing material.

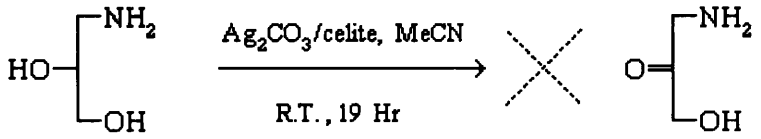
The pursuit of an alternative and convenient route to 5-hydroxycyclophosphamide **(117)** was continued. The reaction of dichlorophosphoramide with aminopropan-2,3-diol at various temperatures was then investigated in order to determine whether an alteration in temperature would encourage the formation of the desired 6-membered ring product. The reactions were conducted in acetonitrile, in the presence of triethylamine and the temperatures ranged between -78°C to +70°C. In each case the 5-membered ring product was formed exclusively.

Alternative routes to 5-hydroxycyclophosphamide involving selectively protected aminopropan-2,3-diol were then sought. The selective oxidation of the secondary hydroxyl group was one of the first reactions to be investigated. It was hoped that this oxidation would give 2-ketoaminopropan-3-ol which if coupled with dichlorophosphoramide would lead to the production of 5-ketocyclophosphamide. The latter compound is of interest in terms of its potential anti-cancer properties. It may then be possible to reduce this compound, in the presence of a suitable reducing agent, to 5-hydroxycyclophosphamide. Thus, the oxidation of aminopropan-2,3-diol was attempted with silver carbonate on celite[148], in acetonitrile rather than in benzene due to solubility problems and at ambient temperature rather than at reflux to aid selectivity [Fig. 71].

The green reaction mixture was observed to turn black which suggested that an oxidation had taken place and the ^1H nmr spectrum of the isolated material, although unclear, indicated that a number of products had been formed and that oxidation may

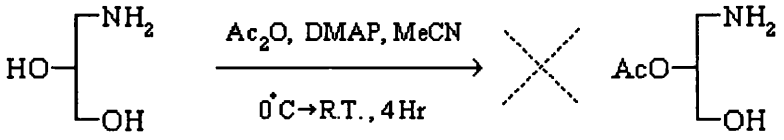
have occurred at more than one site. This was not unexpected considering the nature of the starting material and the close proximity of the two potential oxidation sites.

[Fig. 71]



The acetylation of aminopropan-2,3-diol was next to be attempted in the hope that either one of the two hydroxyl groups would be acetylated selectively. However, it was predicted that a mixture of O-acetylated products would be generated and that this could be purified by silica-gel column chromatography. If the product containing a protected secondary hydroxyl group was isolated, then its subsequent reaction with dichlorophosphoramide could lead to the production of the 6-membered ring derivative, 5-O-acetylcyclophosphamide. The first attempt at this reaction involved stirring aminopropan-2,3-diol with acetic anhydride in anhydrous acetonitrile at 0°C and in the presence of the catalyst dimethylaminopyridine [Fig. 72][145].

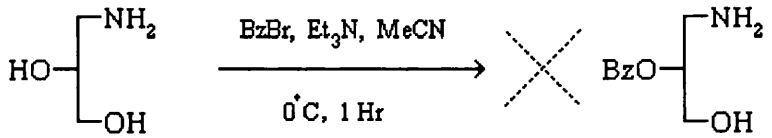
[Fig. 72]



Following an organic-aqueous extraction on the concentrated reaction mixture, an oil was isolated and characterised by ^1H nmr spectroscopy. The presence of two methyl signals at δ2.04 and δ2.06 and the proton-proton couplings observed suggested that acetylation of two sites in the same product had occurred, and that it was O-acetylation in each case. Further attempts involved the use of a large volume of solvent, the addition of a solution of acetic anhydride and dimethylaminopyridine to aminopropan-2,3-diol rather than in the reverse order and a slow rate of addition of reactants. All of these attempts were found to produce mixtures of products containing diacetylated starting material.

The reaction of aminopropan-2,3-diol with benzyl bromide was also expected to give rise to products protected at the primary or secondary hydroxyl groups. Protection of the secondary hydroxyl group would be ideal as this would provide a direct route to a 5-substituted analogue of cyclophosphamide. The product with a protected primary hydroxyl group would require further steps involving protection and deprotection at the remaining reactive sites. The first attempt at the selective benzylation involved the reaction of benzyl bromide with aminopropan-2,3-diol in the presence of one equivalent of the base triethylamine [Fig. 73].

[Fig. 73]



The addition was carried out at 0°C and the progress of the reaction followed by analytical tlc. After a period of one hour, it appeared that the reaction had proceeded to completion. The major component of the reaction was isolated by silica-gel column

chromatography and characterised by nmr spectroscopy. The ^{13}C nmr spectrum of this material contained a signal at δ53.94 which was assigned to the benzylic carbon $\underline{C}H_2Ph$. The chemical shift of this signal suggested that benzylation had occurred at the amino site. It has been found that in general, N-$\underline{C}H_2Ph$ carbons resonate between δ41 and δ56 whilst O-$\underline{C}H_2Ph$ carbons give signals appearing between δ73 and δ76. The ^1H nmr data strongly suggested that monobenzylation had occurred. The spectrum of this material in dimethylsulphoxide contained a broad singlet at δ5.36 which integrated for one proton and was assigned to NH. A singlet at δ4.13 representing $C\underline{H}_2Ph$ was noted to integrate for two protons and also supported monobenzylation of the amino group. Infra-red and mass spectrometry confirmed this with the former showing the NH stretch and the latter displaying the molecular ion peak for a monobenzylated product.

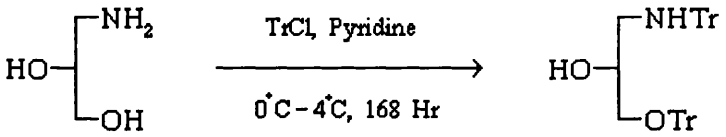
The second attempt at this reaction, with the reaction time extended to 26 hours to encourage benzylation of the hydroxyl groups, was found to have resulted in dibenzylation at the amino site. An intense fast running component and two very close running components were evident from a tlc of the reaction mixture. One of these slow running components was thought to be a monobenzylated product. It was attempted without much success to separate the slow running components by silica-gel column chromatography. However the use of this technique did lead to the isolation of the fast running component as a white solid and the ^{13}C nmr of this material revealed it to be N,N-dibenzylaminopropan-2,3-diol. The resonance at δ58.76 was noted to be in the expected region for N-benzylation and appeared to be twice as intense as the signal observed in the corresponding spectrum of the product isolated in the previous attempt. The ^1H nmr and mass spectral data were also consistent with dibenzylation at nitrogen.

Other attempts at the preparation of selectively protected aminopropan-2,3-diol included reactions with trityl chloride. Again, it was hoped that one of the hydroxyl groups would be selectively protected and that if a mixture of monotritylated products was formed, it would be possible to separate them and couple the secondary hydroxyl

protected product with dichlorophosphoramide. This procedure may result in the generation of 5-O-tritylcyclophosphamide which on deprotection may give rise to 5-hydroxycyclophosphamide.

Thus, the tritylation was conducted in the presence of trityl chloride in pyridine between 0°C-4°C [Fig. 74][152]. The course of the reaction was monitored by tlc which indicated the presence of a fast running component. After a reaction period of 7 days the reaction mixture was slowly added to a mixture of ice and water and the precipitated white solid collected by filtration.

[Fig. 74]



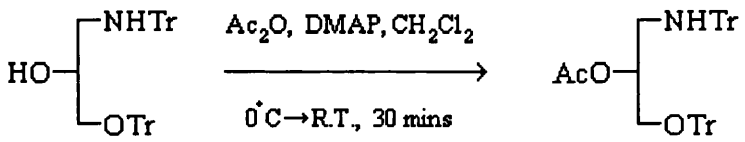
(124)

The isolated product was examined by ^{1}H nmr spectroscopy and this suggested that tritylation had occurred at two sites. The multiplet appearing between δ7.12-7.53 and integrating for 30 protons indicated that there were two trityl groups in the molecule. The ^{13}C nmr spectrum of **(124)** also supported the ^{1}H nmr data and contained singlets at δ65.63 and δ46.62 corresponding to $\underline{C}H_2OTr$ and $\underline{C}H_2NHTr$ respectively indicating that tritylation had taken place at the primary hydroxyl and amino groups.

It was then decided to further protect the secondary hydroxyl group of this ditritylated compound with the aim of eventually removing the protecting groups from the two primary sites and then coupling the resulting product with dichlorophosphoramide. The acetyl group was chosen for the protection and this

reaction was carried out with acetic anhydride and dimethylaminopyridine in dichloromethane at 0°C [Fig. 75][145].

[Fig. 75]

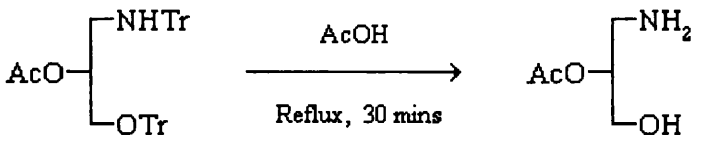


(125)

The course of the reaction was followed by tlc and after 30 minutes, this indicated the presence of a single component which was more lipophilic than the starting material. The reaction mixture was then concentrated under reduced pressure and after an organic-aqueous extraction a product was isolated as a white solid. This material was successfully characterised as the required acetylated compound **(125)** by nmr spectroscopy.

The next step in the scheme was to isolate 2-O-acetylaminopropan-3-ol by removing the two trityl protecting groups. This deprotection was attempted twice with the first attempt requiring the use of acetic acid [Fig. 76][153].

[Fig. 76]



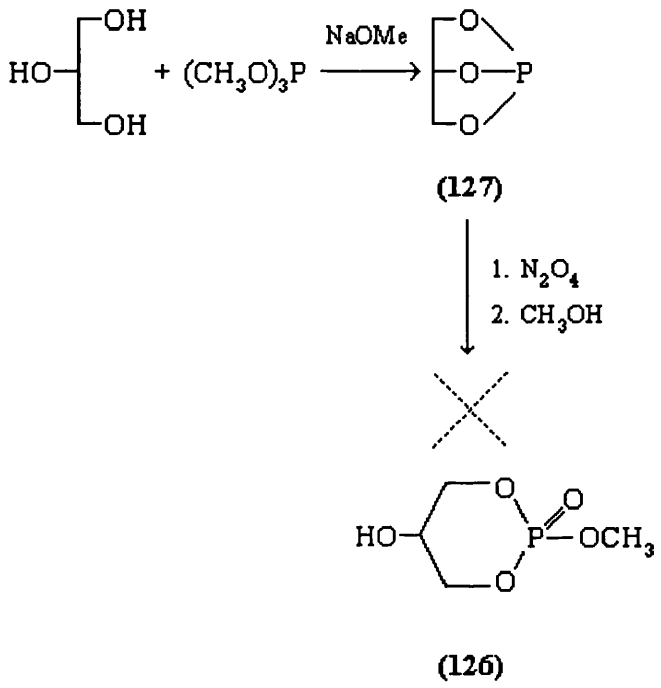
The reaction mixture was refluxed for 30 minutes and this was followed by the addition of a mixture of ice and water. The precipitated white solid was removed by filtration and the resulting solution concentrated to give a white solid. The ^{1}H nmr spectrum of this material, although inconclusive, suggested that the detritylation had been successful. This was supported by the absence of trityl group signals in this spectrum and the corresponding ^{13}C nmr spectrum. However, the low yield of product obtained (10 mg) prevented a subsequent reaction with dichlorophosphoramide. These results suggested that perhaps the deprotection should be conducted in conditions more acidic than those employed in the first attempt. This may encourage the reaction to proceed much further than it did in this first attempt and give a better yield of product. Therefore, the second attempt involved refluxing **(125)** with hydrochloric acid for 3 hours[154] and again the course of the reaction was followed by tlc. It was evident that a single component, which was less lipophilic than the starting material, was present in the reaction mixture. Attempts to isolate this by silica-gel column chromatography were unsuccessful with insufficient material being available for characterisation by nmr spectroscopy. If time had permitted, this particular route to 5-O-acetylcyclophosphamide would have undergone further investigations.

In seeking an alternative route to 5-hydroxycyclophosphamide, the method of Denney and Varga for the preparation of compound **(126)**[155] was followed in the hope that it would provide a model route for its preparation. The first step leading to the bicyclic phosphite **(127)** was accomplished by the reaction of trimethyl phosphite with glycerol and the subsequent treatment with catalytic amounts of sodium methoxide [Fig. 77].

This procedure gave rise to a colourless oil of **(127)** in a low yield. The structural assignment was based on its method of preparation and its nmr data. The ^{31}P nmr spectrum contained two signals with the most intense being at δ103.10 and this chemical shift value was found to agree well with that reported in the literature

(+105.4 ±0.1 ppm)[156]. An attempt at the oxidation of this material, with dinitrogen tetroxide[149], to the corresponding bicyclic phosphate was found to be unsuccessful owing to the instability of the starting material. The oxidised product is also known to be susceptible to hydrolysis.

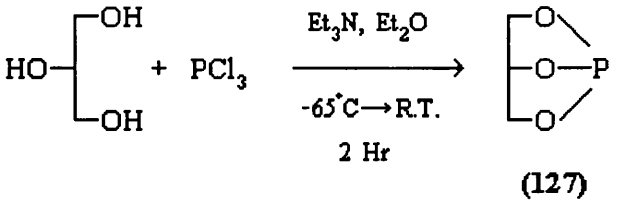
[Fig. 77]



It was then attempted to prepare the bicyclic phosphite **(127)** by a route more convenient than the one proposed by Denney and Varga. This involved the reaction of glycerol with phosphorus trichloride between -65 to -70°C and in the presence of triethylamine [Fig. 78].

This reaction gave rise to an oil which, in its ^{31}P nmr spectrum, resonated at δ103.33 which was found to compare well with the literature value. The spectrum also contained a minor signal which was thought to represent an intermediate in this reaction.

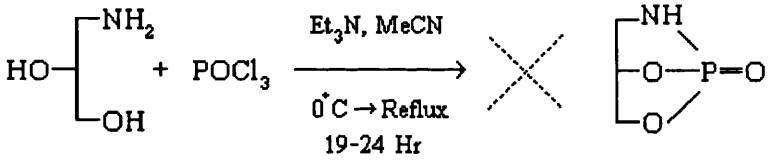
[Fig. 78]



(127)

The apparent success of the above reaction prompted an attempt at the reaction of aminopropan-2,3-diol with phosphoryl chloride [Fig. 79] which may yield the bicyclic phosphate **(128)**. The coupling of this compound with dichlorophosphoramide may provide an alternative route to 5-hydroxycyclophosphamide.

[Fig. 79]



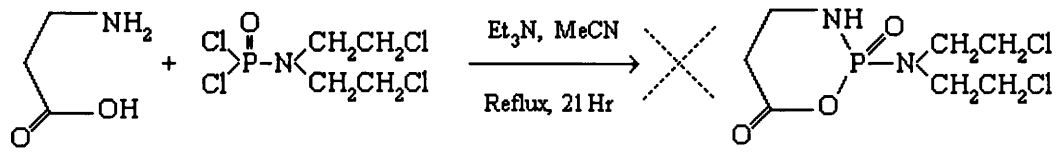
(128)

This reaction was attempted several times and was generally conducted in acetonitrile at 0°C and then at reflux for 19-24 hours. Extraction with tetrahydrofuran or ether was found to give a material which resonated as a single peak at δ-6 in the ^{31}P nmr spectrum. This compound was noted to resonate in the expected region which was found from structurally related compounds[157,158]. The results of further characterisations by nmr spectroscopy were inconclusive. In one case, the ^{1}H nmr spectrum contained a multiplet at δ5.16 which was assigned to the bridgehead hydrogen. Two sets of triplets at δ3.50 and δ3.94 were assigned to the remaining

methylene hydrogens. The spectrum also contained signals arising from impurities. The EI mass spectrum displayed the molecular ion peak at m/e 135 and the infra-red spectrum showed absorptions at 3240 cm^{-1} (NH) and 1340 cm^{-1} (P=O) which were consistent with the structure **(128)**. However, the ^{13}C nmr data suggested that the isolated product had a structure other than the one proposed as signals in the expected regions and phosphorus-carbon couplings were not observed. This scheme was not investigated any further as the character of the material isolated from each attempt was questionable.

Analogues of cyclophosphamide, other than 5-hydroxycyclophosphamide, were made the next targets for synthesis. The first such compound to be investigated was the 6-membered ring analogue of cyclophosphamide, 6-ketocyclophosphamide **(129)** which is of interest in terms of its potential anti-cancer properties. It was thought that this derivative would be metabolically activated in an analogous manner to cyclophosphamide with the release of a metabolite which may be less toxic than acrolein. Several attempts were made to prepare this derivative with each one involving the addition of dichlorophosphoramide to a suspension of β-alanine in a large volume of acetonitrile [Fig. 80].

[Fig. 80]



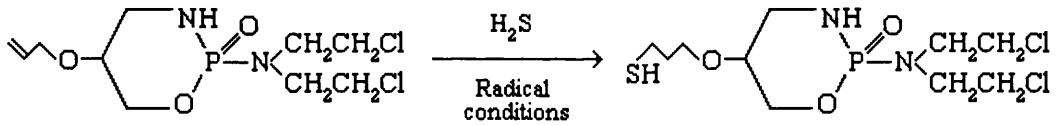
(129)

The reactions were conducted either at ambient temperature or at temperatures ranging between 40-80°C and were found to be unsuccessful. However, in some cases, the ^{31}P nmr spectra contained minor resonances in the region that the required product

would be expected to resonate. One of the main problems of this particular reaction was thought to be the high reactivity of the carbonyl group of the amino acid, which may have led to the formation of metaphosphates represented by signals in the negative region of the spectrum. An alternative route to the desired product would be to protect the carbonyl group, react the protected amino acid with dichlorophosphoramide and then remove the protecting group in situ and under mild aprotic conditions. However, this type of reaction has been carried out and was found to be non-productive as discussed in a previous chapter.

The preparation of 5-O-allylcyclophosphamide **(130)** was next to be attempted. The intention was to react this novel derivative of 5-hydroxycyclophosphamide with hydrogen sulphide to give the thiol-containing compound **(131)** [Fig. 81].

[Fig. 81]



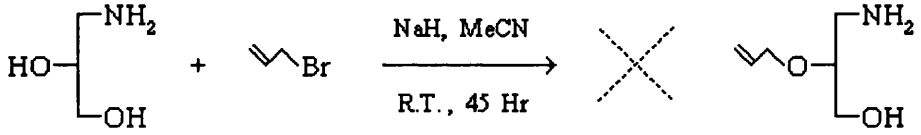
(130) **(131)**

The rationale for preparing a thiol-containing compound was that its metabolism may generate, in addition to the active phosphoramide mustard, a metabolite which may be less toxic in comparison to acrolein. The latter is a urinary metabolite of cyclophosphamide and is thought to be the cause of haemorrhagic cystitis[102]. This serious complication may be overcome by administering sulphydryl compounds such as mercaptoethanesulphonic acid (MESNA)[103]. This compound is known to undergo a Michael-type addition with acrolein to produce a non-toxic adduct. A better solution to this problem may be to incorporate a thiol-containing group in the

parent drug. Metabolism of this compound may then generate a thiol-containing metabolite which may undergo an intramolecular Michael addition reaction giving rise to a non-toxic metabolite.

Firstly, the preparation of the allyl derivative of aminopropan-2,3-diol, 2-O-allylaminopropan-3-ol **(132)** was attempted by reacting aminopropan-2,3-diol with allyl bromide in the presence of the base sodium hydride [Fig. 82]. It was predicted that this base would remove the hydrogen of the secondary hydroxyl group as this was thought to be the most acidic hydrogen in the molecule.

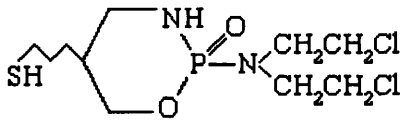
[Fig. 82]



(132)

The attempted allylation involved the addition of aminopropan-2,3-diol to a slurry of the base in acetonitrile. The tlc of the reaction mixture indicated the presence of 80-90% of a main component. Two successive columns were needed to purify this material which was characterised as a diallylated compound by nmr spectroscopy. A resonance at δ56.96 in the ^{13}C nmr spectrum indicated that the allylation had taken place at the amino group and was assigned accordingly to $N(\underline{C}H_2CH{=}CH_2)_2$. The resonance arising from $O\underline{C}H_2CH{=}CH_2$ generally appears at δ70[132]. The mass spectrum of this material confirmed that it was a diallylated product. Other attempts at this reaction were found to produce similar results. The use of n-butyl lithium as an alternative base was also found to be non-productive.

N,N-Bis(2-chloroethyl)amino-5-(3-thiopropyl)tetrahydro-2H-1,3,2-oxaza-phosphorine-2-oxide **(133)** was made the next target for synthesis.



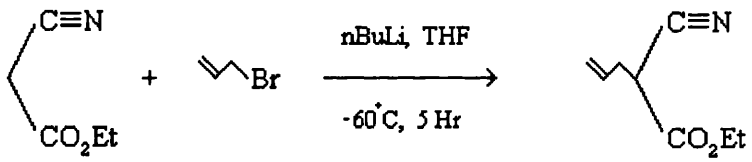
(133)

The rationale for its preparation was similar to that for the previously discussed thiol-containing compound **(131)**. The first step in the scheme involves the preparation of monoallylethylcyanoacetate **(134)**. A reported procedure by Normant and Grugny requires the addition of allyl bromide to a mixture of sodium hydride and ethylcyanoacetate in HMPT at 0°C[159]. The reagents present in equimolar amounts are then refluxed at 70°C for 2 hours. Distillation of the mixture separates the required product, monoallylethylcyanoacetate (b.p. 108/17 mmHg) from the diallylethylcyanoacetate (b.p. 122/17 mmHg). Thus, the preparation of **(134)** was carried out by following a similar method to the one reported [Fig. 83]. The reaction conditions were modified slightly with n-butyl lithium replacing sodium hydride as the base for generating the carboanion and tetrahydrofuran being the preferred solvent for the reaction.

It was found that the optimum reaction conditions, for the preparation of **(134)** as the exclusive product, included having allyl bromide, ethylcyanoacetate and n-butyl lithium in a ratio of 1:3:1 respectively. Ratios deviating from this were found to produce side products such as diallylethylcyanoacetate. Thus, allyl bromide was added to n-butyl lithium and ethylcyanoacetate at -60°C. After a reaction time of 6 hours and an ether and sodium bicarbonate extraction, the residue was purified by column

chromatography to give the required product as a clear yellow oil in 26% yield. The ^{13}C nmr and ^{1}H nmr spectra were entirely consistent with the structure **(134)** with the latter spectrum also indicating the presence of a minor impurity (<10%).

[Fig. 83]



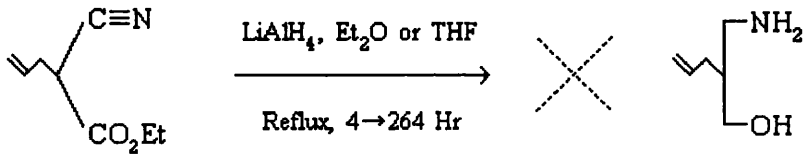
(134)

Successive attempts at the reduction of **(134)** to the amino alcohol **(135)** appeared to have been futile. A general reduction method consists of the use of lithium aluminium hydride in ether[160]. This method has been used in the reduction of simple nitriles to amines as well as for the reduction of esters to alcohols. In the former case, the absence of secondary amines in the product is a highly advantageous feature of this method. The first attempt at the hydride reaction was carried out following a procedure by McMurray[161].

The reaction involved the slow addition of **(134)** to a slurry of lithium aluminium hydride in ether [Fig. 84]. The reaction mixture was refluxed gently and then cooled in an ice bath. Quenching of excess reagent and hydrolysis of aluminate salts was effected by the cautious slow addition of water and aqueous sodium hydroxide. The ether layer was filtered from the granular aluminium salts and washed with brine. The dried organic extract was then concentrated to produce a yellow oil. The tlc of this material indicated the presence of a slow running component, in addition to a fast running one, which appeared as an intense spot with an iodine and silica visualising system. The required amino alcohol **(135)** was expected to have a much

smaller R_f value than the one obtained for the intense spot which was thought to be an intermediate in the reduction. This suggested that the reduction had been unsuccessful and that the reaction time needed to be extended.

[Fig. 84]



(135)

The second attempt at the reduction was carried out in an analogous manner to the first attempt with the exception of the reaction time which was extended to 16 hours. This reaction was found to produce results which were similar to those obtained in the previous attempt. In this case, it was attempted without success to purify the major product by column chromatography. The ^1H nmr spectrum of the isolated material was unclear but suggested that the reduction of the ester group was more difficult than that of the nitrile group.

The reduction was then repeated in conditions more vigorous than those employed in previous attempts. Tetrahydrofuran replaced ether as the preferred solvent for the reaction, allowing it to be refluxed at a temperature of 60°C rather than 30°C. An excess of lithium aluminium hydride was used and the reaction time extended to 11 days to encourage the reduction to proceed to completion. The progress of the reaction was followed by tlc which, after a reaction time of 8 days, indicated the presence of three major products and one minor one in addition to some unreacted monoallylethylcyanoacetate. At this point, an additional equivalent of the reducing agent was added to the mixture and refluxing continued for a further three days. The ^1H nmr

data of the water soluble minor component showed promise but the exceptionally low yield of product prevented any further characterisation.

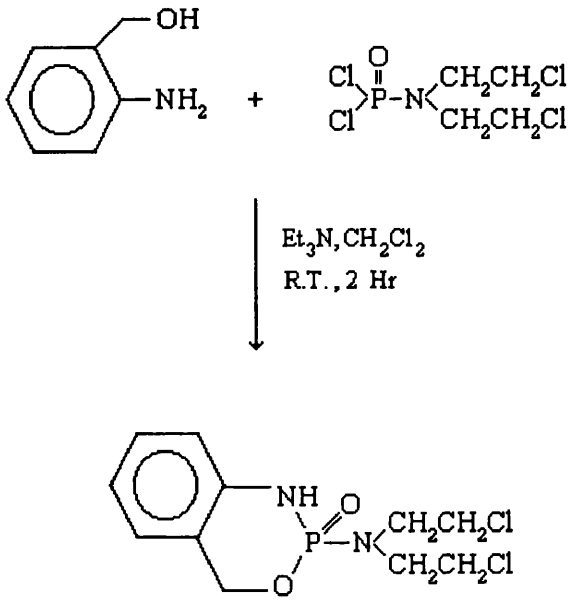
The major products of the reaction were found to be ether soluble and attempts to isolate them by column chromatography were unsuccessful. It was hoped that if a useful intermediate was isolated it could be reduced further with lithium aluminium hydride. Spectral data on the mixture of products again suggested that the reduction of the ester group was more difficult than that of the nitrile group. The absence of the $(CH_2{=}CHC\underline{H}_2)C\underline{H}$ protons in the ^1H nmr spectrum also suggested that the hydride reduction was not as selective as expected. This was thought to be the result of active hydrogens being present in monoallylethylcyanoacetate, leading to other reduction products such as diamines and secondary amines. The action of lithium aluminium hydride on nitriles containing active hydrogens has been modified by Nystrom[162]. This procedure involves the addition of a lewis acid such as aluminium chloride to the reaction mixture. The use of this 'acidic' hydride has been found to improve the yields of the desired products. However, the use of this method in the reduction of **(134)** may lead to further problems such as an increase in the chelation of the reduced product, the amino alcohol, with the aluminium metal ion.

The benzocyclophosphamide **(136)** was made the next target for synthesis primarily for the purpose of determining whether it was active against HIV. This compound was expected to be more lipophilic than the 5-membered ring analogue **(118)** due to the presence of a benzenoid nucleus. A method for its preparation has been reported[163] and this was modified with respect to the reaction time, solvent and the method of purification. Thus, O-aminobenzyl alcohol was reacted with dichlorophosphoramide in dichloromethane, at ambient temperature and in the presence of triethylamine [Fig. 85].

Following purification by column chromatography and recrystallisation from petroleum spirit, the product was isolated as white needles in 51% and successfully

characterised by nmr spectroscopy. The purity of **(136)** was also confirmed by analytical hplc. This compound was submitted for anti-HIV tests and these indicated that it was inactive and non-toxic at the concentrations tested.

[Fig. 85]



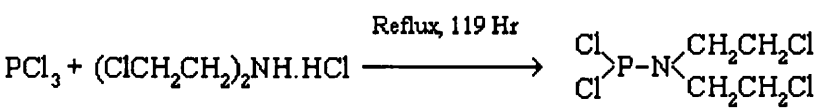
(136)

It was also thought to be of interest to prepare some phosphite analogues of cyclophosphamide by adopting the procedure used for the preparation of analogous phosphates. The preparation of N,N-bis(2-chloroethyl)aminophosphorus dichloride **(137)** and its separate reactions with aminopropan-2,3-diol and β-alanine may provide novel routes to phosphite analogues of 5-hydroxycyclophosphamide and 6-ketocyclophosphamide respectively. The synthesis of the phosphite analogues of cyclophosphamide, isophosphamide and triphosphamide have been reported and are known to polymerise easily at room temperature[164]. The method of Okruszek and

Verkade was followed in the initial attempts at the preparation of **(137)**[164]. This involved the addition of the base triethylamine to a suspension of bis(2-chloroethyl)amine hydrochloride in phosphorus trichloride and benzene in anhydrous conditions. The mixture was then refluxed for 3-5 hours and the required product extracted with benzene as a yellow/brown solid in yields ranging between 39-41%. The ^{31}P nmr spectrum of this material consisted of a single peak at δ160.68 which was in good agreement with the literature value of δ162.2. The literature method for the preparation of **(137)** states that the product is collected by distillation at a reduced pressure (106°C/0.5 mmHg). However, it was found that purification of the product by distillation was unnecessary as it was shown to be pure by various spectroscopic techniques prior to this step. The material obtained from these reactions was noted to be yellow/brown in colour and it was found that this colouring could be removed by distillation at a reduced pressure. The colourless solid which was obtained by this procedure was successfully characterised as the required phosphite.

It was then attempted to prepare the phosphite **(137)** by a convenient method which was analogous to that for the preparation of dichlorophosphoramide **(59)**. The latter reagent is obtained in a pure state and in a quantitative yield by refluxing bis(2-chloroethyl)amine hydrochloride in phosphoryl chloride as described earlier on in this chapter. Thus, the analogous reaction with phosphorus trichloride was carried out for 119 hours and in the absence of a base [Fig. 86]. The removal of the excess solvent under a reduced pressure gave rise to an off-white solid in a quantitative yield.

[Fig. 86]

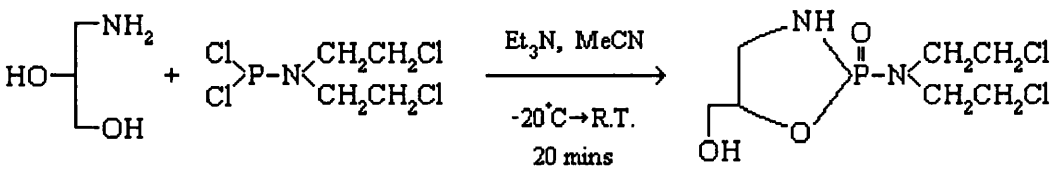


(137)

The ^{31}P nmr spectrum contained a single resonance at δ160.68. Again, the chemical shift of the isolated compound compared well with the literature value. The ^{13}C nmr spectrum was consistent with the ^{31}P nmr data and contained two sets of doublets at δ41.69 (CH_2Cl) and δ50.06 (CH_2N) with phosphorus-carbon coupling constants of 3.3 Hz and 20.3 Hz respectively. The ^{1}H nmr spectrum was also consistent with the ^{31}P nmr data. These data suggested that the phosphite **(137)** could be prepared in a pure state without the use of a base or the need to distil.

It was thought to be of interest to identify the product from the reaction of the phosphite **(137)** with aminopropan-2,3-diol and to compare it with the product generated from the analogous phosphate reaction. In the latter case, it was found that the 5-membered ring phosphate with a hydroxymethyl side chain, compound **(118)**, was the exclusive product. The reaction of **(137)** with aminopropan-2,3-diol was generally carried out in tetrahydrofuran and at -20°C with extreme precautions being taken to prevent the entry of water to the system [Fig. 87]. The reaction mixture was then stirred for a time ranging between 15 minutes and 1 hour and this was followed by filtration and an ether extraction to give a pale yellow oil. In each case, the ^{31}P nmr spectrum consisted of two closely spaced signals of equal intensities between δ27-28 and these chemical shifts appeared to correspond with those obtained for the 5-membered ring phosphate **(118)**, (δ28.57/27.61).

[Fig. 87]

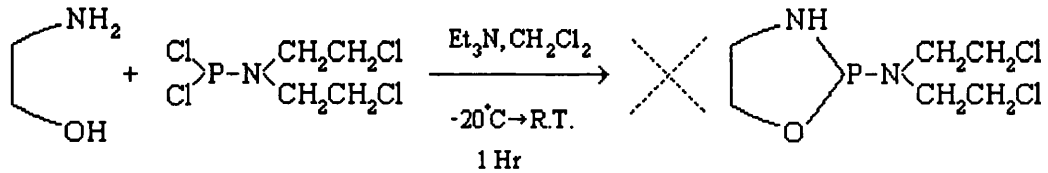


(118)

These results suggested that the required phosphite may have been oxidised to the corresponding phosphate at some point in the reaction although the nature of the oxidation remains unclear. Similar results were obtained when the ether extraction was replaced with a tetrahydrofuran one and when the solvent for the reaction was altered to acetonitrile. Examination by ^{31}P nmr spectroscopy, of the crude material from a reaction conducted in acetonitrile, revealed the presence of two major signals at δ145.01 and δ15.34. The resonance appearing at the most downfield shift was noted to be in the expected region for a 5-membered ring phosphite[124] which suggested that as in the case of the phosphate, a 5-membered ring product was preferred over the 6-membered ring alternative.

The reaction of **(137)** with ethanolamine [Fig. 88] was carried out in a similar manner to the previously described attempts with aminopropan-2,3-diol. This experiment was conducted in an attempt to establish the nature of the product giving rise to the resonance appearing at δ145.01 in the ^{31}P nmr spectrum obtained in the previous reaction. The reaction conducted at -20°C was found to produce a yellow polymeric gum and successive attempts to extract the product were found to be futile.

[Fig. 88]

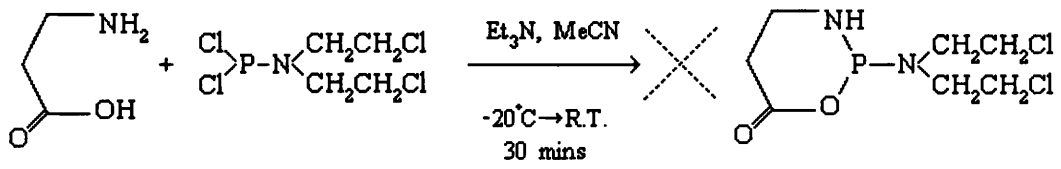


(137)

The reaction of the phosphite **(137)** with β-alanine [Fig. 89] was considered to be of interest as it may provide a novel route to the phosphite analogue of 6-ketocyclophosphamide. It was thought that problems such as the formation of

metaphosphates and other side reactions encountered with the analogous phosphate reaction may be overcome with the use of an agent possessing a phosphorus centre which is more reactive than the one found in dichlorophosphoramide.

[Fig. 89]



The reaction was observed to produce a yellow solid which, in its ^{31}P nmr spectrum, gave rise to major signals of equal intensities at δ21.47 and δ10.52. Neither of these chemical shifts were consistent with the structure of the phosphite analogue of 6-ketocyclophosphamide. Further attempts at purifying this material were found to be futile. The lack of products obtained in these reactions involving phosphites was attributed to the instability of the desired phosphites. This instability may arise from intra and/or intermolecular nucleophilic attack by the phosphorus lone pair on a chlorine-bearing carbon.

SUMMARY AND CONCLUSIONS

The synthesis of a variety of amino acid linked nitrogen mustard derivatives has been described in this thesis. It was thought that these compounds would have potential as anti-HIV and/or antineoplastic agents.

Firstly, some alkyl and trihaloalkyl phosphoramidate derivatives of nitrogen mustard were prepared from reactions of N,N-bis(2-chloroethyl)amino(alkyl or trihaloalkyl) phosphorochloridate with various carboxy protected amino acids. Some of these agents were tested for anti-HIV activity *in vitro* and it was found that the trichloroethyl and trifluoroethyl benzylphenylalaninyl compounds were the most active, in this series, at concentrations less than 200 μM. However, these compounds were noted to exert toxicity at, or near, their antiviral concentrations.

The next series of derivatives was designed bearing in mind that the lipophilicity of the compound may have an influence on its activity. Carboxy protected amino acids of varying degrees of lipophilicity were incorporated in phenyl and 4-substituted phenyl phosphoramidate derivatives of nitrogen mustard and some of these agents were tested for biological activity. Some peptide derivatives were also synthesised. The phenyl methoxyphenylalaninyl and 4-chlorophenyl compounds were found to inhibit the proliferation of HIV *in vitro* at concentrations less than 200 μM. Again, these compounds were noted to exert toxicity at, or near, their antiviral concentrations which may limit the development of these leads.

The synthesis of some cyclic analogues of cyclophosphamide was also investigated. Acrolein, a metabolite of cyclophosphamide, is one of its major sources of toxicity. Attempts were made to prepare 5-hydroxycyclophosphamide in the hope that this agent would overcome the toxicity associated with the release of acrolein. However, it was possible to only isolate the alternative 5-membered ring analogue from reactions of dichlorophosphoramide with aminopropan-2,3-diol. The synthesis of 2-bis(2-

chloroethyl)amino-1,3,2-oxaza, 2-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-dioxa and 2-bis(2-chloroethyl)amino-5-acetyloxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide to establish the nature of the isolated 5-membered ring analogue was described along with attempts to find alternative routes to 5-hydroxycyclophosphamide.

EXPERIMENTAL

General methods

Commercially available Merck Kieselgel 60 F_{254} plates were used for analytical thin layer chromatography and components visualised by iodine or ultra-violet light. Column chromatography was carried out using Woelm silica (32-63 μm) as the stationary phase. The ratio of silica: compound varied between 50:1 and 100:1 (w/w). Melting points were determined on a Riechert hot stage melting point apparatus and are uncorrected. Infra-red spectra were recorded on a Perkin Elmer 983 infra-red spectrophotometer. Electron impact mass spectra (E.I.M.S.) and Fast atom bombardment mass spectra (F.A.B.M.S.) were carried out by Dr. M. Mruzek on a VG 7070H mass spectrometer fitted with a Finnigan Incos II data system and a VG Zab1F spectrometer respectively. HPLC was carried out by Mr. S. Corker on a Gilson Binary Gradient HPLC system, fitted with a Gilson 115 uv detector and Rheodyne injector. Microanalyses were carried out by the University College London microanalysis service.

Phosphorus nuclear magnetic resonance (^{31}P nmr) spectra were recorded on a Varian XL-200 spectrometer operating at 82 MHz or on a VXR-400 spectrometer operating at 164 MHz and are reported in units of δ values relative to 85% phosphoric acid as external reference, positive shifts are downfield. Carbon nuclear magnetic resonance (^{13}C nmr) spectra were recorded on a Varian XL-200 spectrometer operating at 50 MHz or on a VXR-400 spectrometer operating at 100 MHz and are reported in units of δ values relative to tetramethylsilane (TMS) as internal standard. Unless otherwise stated, both ^{31}P and ^{13}C nmr spectra were proton noise decoupled and all signals were singlets. Proton nuclear magnetic resonance (^{1}H nmr) spectra were recorded on either a Varian XL-200 spectrometer operating at 200 MHz or on a VXR-400 spectrometer operating at 400 MHz and are reported in units δ values relative to TMS as internal standard. The following abbreviations are used in the assignment of nmr signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and b (broad).

All experiments involving water sensitive reagents were carried out under scrupulously dry conditions. Where needed, anhydrous solvents and reagents were obtained in the following ways: benzene, dichloromethane and diethyl ether were heated to reflux over calcium hydride for several hours, distilled and stored over activated 4 Å molecular sieves. Tetrahydrofuran was heated to reflux over lithium aluminium hydride for several hours, distilled and stored over activated 4 Å molecular sieves. Acetonitrile was distilled from phosphorus pentoxide twice, onto activated 3 Å molecular sieves. Triethylamine was heated to reflux over calcium hydride for several hours and distilled immediately prior to use. Anhydrous methanol was obtained by heating with magnesium activated iodine, followed by distillation and storage over activated molecular sieves. Acetic anhydride, phosphoryl chloride, phosphorus trichloride and thionyl chloride were distilled prior to use. Decanol, ethanol, propan-1,2-diol, propanol and 2,2,2-trichloroethanol were stored over activated molecular sieves for at least 24 hours before use.

Ethyl phosphorodichloridate (60)

A solution of triethylamine (7.48 ml, 53.64 mmol) and ethanol (3.15 ml, 53.64 mmol) in ether (100 ml) was added dropwise to a solution of phosphoryl chloride (5.00 ml, 53.64 mmol) in ether (100 ml). The addition was carried out at -78°C in an atmosphere of nitrogen. The white reaction mixture was then allowed to warm to ambient temperature overnight and stirring was continued for a total period of 20 hr. Filtration of the reaction mixture and concentration of the filtrate under reduced pressure produced a colourless oil, (6.99 g, 80%).

^{31}P nmr $\delta(CDCl_3)$ 5.03.

^{13}C nmr $\delta(CDCl_3)$ 68.52 (d, $CH_3\underline{C}H_2OP$, J=9.0 Hz), 15.39 (d, $\underline{C}H_3CH_2OP$, J=2.6 Hz).

N,N-Bis(2-chloroethyl)amino ethyl phosphorochloridate (61)

Bis(2-chloroethyl)amine hydrochloride (2.19 g, 12.27 mmol) was suspended in a solution of ethyl phosphorodichloridate (2.00 g, 12.27 mmol) in ether (49 ml). A solution of triethylamine (3.42 ml, 24.55 mmol) in ether (49 ml) was added dropwise, with stirring, to this mixture at -78°C. The reaction mixture was stirred at ambient temperature for 42 hr and then filtered and concentrated under reduced pressure. Hexane (150 ml) was added to the residue and the solution filtered and concentrated under reduced pressure to give the product as a colourless oil, (2.84 g, 86%).

^{31}P nmr $\delta(CDCl_3)$ 13.44.

^{13}C nmr $\delta(CDCl_3)$ 65.02 (d, $CH_3\underline{C}H_2OP$, J=6.0 Hz), 49.90 (d, 2x CH_2N, J=4.3 Hz), 41.53 (d, 2x CH_2Cl, J=2.5 Hz), 15.79 (d, $\underline{C}H_3CH_2OP$, J=8.0 Hz).

^{1}H nmr $\delta(CDCl_3)$ 4.27 (m, 2H, $CH_3C\underline{H}_2OP$), 3.62 (m, 4H, 2x CH_2N), 3.46 (m, 4H, 2x CH_2Cl), 1.40 (2xt, 3H, $C\underline{H}_3CH_2OP$).

FABMS m/e 270 (MH^+, ^{37}Cl, 3.06%), 268 (MH^+, 4.24%), 242 (MH_2^+-C_2H_5, ^{37}Cl, 0.82%), 240 (MH_2^+-C_2H_5, 1.25%), 144 ($(ClCH_2CH_2)_2NH_2^+$, ^{37}Cl, 13.67%), 142 ($(ClCH_2CH_2)_2NH_2^+$, 26.31%), 102 ($P(O)N(CH{=}CH_2)CH_2^+$, 100%), 65 ($ClCH_2CH_2^+$, ^{37}Cl, 0.96%), 63 ($ClCH_2CH_2^+$, 6.62%).

N,N-Bis(2-chloroethyl)amino ethyl methoxyglycinyl phosphoramidate (62)

A solution of triethylamine (0.21 ml, 1.49 mmol) in dichloromethane (6 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino ethyl phosphorochloridate (0.20 g, 0.74 mmol) and glycine methyl ester hydrochloride (94 mg, 0.74 mmol) in dichloromethane (12 ml). The addition was carried out at -78°C in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature overnight and stirred for a total period of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 150 ml). The ether extracts were filtered, combined and concentrated under reduced pressure to produce a colourless oil, (0.20 g, 82%).

^{31}P nmr δ($CDCl_3$) 12.55.

^{13}C nmr δ($CDCl_3$) 171.37 (d, $\underline{C}O_2Me$, J=8.0 Hz), 61.56 (d, $CH_3\underline{C}H_2OP$, J=5.1 Hz), 52.02 ($CO_2\underline{Me}$), 48.97 (d, 2x $CH_2\underline{C}H_2N$, J=4.7 Hz), 42.27 (2x CH_2Cl), 42.15 (d, CH_2NH, J=2.0 Hz), 15.99 (d, $\underline{C}H_3CH_2OP$, J=7.1 Hz).

^{1}H nmr δ($CDCl_3$) 4.05 (m, 2H, $CH_3C\underline{H}_2OP$), 3.74 (s, 3H, CO_2Me), 3.66 (m, 4H, 2x CH_2N), 3.37-3.50 (m, 6H, 2x CH_2Cl, $C\underline{H}_2NH$), 3.12 (m, 1H, $CH_2N\underline{H}$), 1.29 (t, 3H, $C\underline{H}_3CH_2OP$).

EIMS m/e 323 (MH^+, ^{37}Cl, 0.03%), 321.0544 (MH^+, 0.02%, calcd for $C_9H_{20}Cl_2N_2O_4P$ 321.0538), 273 (M^+-CH_2Cl, ^{37}Cl, 5.16%), 271 (M^+-CH_2Cl, 16.33%), 263 (M^+-CO_2Me, ^{37}Cl, 9.00%), 261 (M^+-CO_2Me, 14.27%), 180 (M^+-$N(CH_2CH_2Cl)_2$, 100%), 152 (MH^+-$N(CH_2CH_2Cl)_2$-C_2H_5, 90.24%).

Analytical hplc stationary phase 50+250 mm x 4.6 mm kromasil C18 5 μM column, mobile phase methanol-water-triethylamine (70:29.99:0.01), flow rate 1 ml/min. Retention time 5.63 min.

Analysis $C_9H_{19}Cl_2N_2O_4P$ requires: C 33.66 %, H 5.96, N 8.72. Found: C 33.87, H 6.08, N 8.37.

N,N-Bis(2-chloroethyl)amino ethyl methoxyvalinyl phosphoramidate (63)

A solution of triethylamine (0.75 ml, 5.36 mmol) in dichloromethane (22 ml) was added dropwise with stirring to a solution of N,N-bis(2-chloroethyl)amino ethyl phosphorochloridate (0.72 g, 2.68 mmol) and L-valine methyl ester hydrochloride (0.45 g, 2.68 mmol) in dichloromethane (56 ml). The addition was carried out at -78°C in an atmosphere of nitrogen. The reaction mixture was then stirred at ambient temperature for a total of 18 hr. The solvent was concentrated to 1-2 ml by rotary evaporation under reduced pressure and the concentrate dissolved in chloroform (20 ml). This was washed with a saturated solution of sodium bicarbonate (20 ml) and then water (3x 10 ml). The organic extract was dried over anhydrous magnesium sulphate, filtered and the filtrate concentrated under reduced pressure to produce a yellow oil. This was dissolved in ether (100 ml) which was then filtered and concentrated to a pale yellow oil. This procedure was repeated and the material then purified by two successive columns with elution by methanol and chloroform (2.5:97.5) and chloroform. Pooling and evaporation of the appropriate fractions gave the product as a colourless oil, (0.63 g, 65%).

^{31}P nmr $\delta(CDCl_3)$ 12.51, 12.28 (2:1).

^{13}C nmr $\delta(CDCl_3)$ 173.83 (t, $\underline{C}O_2Me$, J=3.6 Hz), 61.97, 61.81 (2xd, $CH_3\underline{C}H_2OP$, J=5.3 Hz, J=5.0 Hz), 59.70, 59.06 (CHNH), 52.12, 52.04 ($CO_2\underline{Me}$), 49.25, 49.20 (2x CH_2N), 42.63, 42.57 (2x CH_2Cl), 32.35, 32.19 (2xd, $\underline{C}H(CH_3)_2$, J=5.7 Hz, J=6.3 Hz), 18.97, 18.95 ($CH\underline{C}H_3$), 17.79, 17.60 ($CH\underline{C}H_3$), 16.28 (t, $\underline{C}H_3CH_2OP$, J=6.0 Hz).

^1H nmr $\delta(CDCl_3)$ 4.06 (m, 2H, $CH_3C\underline{H}_2OP$), 3.75 (s, 3H, CO_2Me), 3.60-3.74 (m, 5H, 2x CH_2N, $C\underline{H}NH$), 3.42 (m, 4H, 2x CH_2Cl), 3.22 (2xt, 1H, $CHN\underline{H}$), 2.06 (m, 1H, $C\underline{H}(CH_3)_2$), 1.30 (2xt, 3H, $C\underline{H}_3CH_2OP$), 0.98 (d, 3H, $CHC\underline{H}_3$), 0.92 (t, 3H, $CHC\underline{H}_3$).

FABMS m/e 365 (MH^+, ^{37}Cl, 6.14%), 363 (MH^+, 11.89%), 305 (M^+-CO_2Me, ^{37}Cl, 6.04%), 303 (M^+-CO_2Me, 11.47%), 291 (MH^+-OMe-$CH(CH_3)_2$, ^{37}Cl, 0.06%), 289 (MH^+-OMe-$CH(CH_3)_2$, 0.56%), 277 (MH^+-OC_2H_5-$CH(CH_3)_2$, ^{37}Cl, 0.01%), 275 (MH^+-OC_2H_5-$CH(CH_3)_2$, 0.13%), 222 (M^+-$N(CH_2CH_2Cl)_2$, 100%), 194 (MH^+-C_2H_5-$N(CH_2CH_2Cl)_2$, 34.68%), 178 (MH^+-$N(CH_2CH_2Cl)_2$-OC_2H_5, 1.18%).

Analytical hplc stationary phase 250 mm x 4 mm lichrosorb 7 μM column, refractometer range 20, mobile phase methanol-water-triethylamine (80:20:0.1), flow rate 1 ml/min. Retention time 3.67 min.

Analysis $C_{12}H_{25}Cl_2N_2O_4P(H_2O)_{0.5}$ requires: C 38.72 %, H 7.31, N 7.53. Found C 38.63, H 7.05, N 7.47.

N,N-Bis(2-chloroethyl)amino ethyl methoxyphenylalaninyl phosphoramidate (64)

A solution of triethylamine (0.21 ml, 1.49 mmol) in dichloromethane (6 ml), was added dropwise with stirring, to a solution of N,N-bis(2-chloroethyl)amino ethyl phosphorochloridate (0.20 g, 0.74 mmol) and L-phenylalanine methyl ester hydrochloride (0.16 g, 0.74 mmol) in dichloromethane (20 ml). The addition was

carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was stirred at ambient temperature for 42 hr after which time the solvent was removed by rotary evaporation under reduced pressure and the remaining material dissolved in chloroform (20 ml). This was washed with aqueous sodium bicarbonate (20 ml) and then water (3 x 10 ml). The organic extract was dried over anhydrous magnesium sulphate and filtered. The filtrate was concentrated under reduced pressure to give a pale yellow oil which was purified by column chromatography. Elution with chloroform followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.24 g, 77%).

^{31}P nmr (82 MHz), δ($CDCl_3$) 11.40.

^{31}P nmr (164 MHz), δ($CDCl_3$) 14.13, 14.07 (1:1).

^{13}C nmr δ($CDCl_3$) 173.47, 173.19 (2xd, $\underline{C}O_2Me$, J=4.1 Hz, J=5.4 Hz), 136.07, 135.99 (*ipso*-Ph), 129.55, 129.45 (*ortho*-Ph), 128.62, 128.50 (*meta*-Ph), 127.16, 127.10 (*para*-Ph), 61.82 (bs, $CH_3\underline{C}H_2OP$), 55.48, 54.97 ($\underline{C}HNH$), 52.30, 52.24 (2xd, $CO_2\underline{Me}$, J=2.8 Hz, J=3.6 Hz), 49.16, 49.07 (2xd, 2x CH_2N, J=4.4 Hz, J=4.7 Hz), 42.53 (2x CH_2Cl), 40.63 (t, CH_2Ph, J=6.0 Hz), 16.24 (t, $\underline{C}H_3CH_2OP$, J=3.8 Hz).

^{1}H nmr δ($CDCl_3$) 7.15-7.30 (m, 5H, Ph), 4.14 (m, 1H, C$\underline{H}$NH), 3.95 (m, 2H, $CH_3C\underline{H}_2OP$), 3.70, 3.69 (2xs, 3H, CO_2Me), 3.51 (m, 4H, 2x CH_2N), 3.24 (m, 4H, 2x CH_2Cl), 2.98-3.07 (m, 3H, CHN$\underline{H}$, CH_2Ph), 1.23 (2xt, 3H, $C\underline{H}_3CH_2OP$).

FABMS m/e 413 (MH^+, ^{37}Cl, 8.65%), 411 (MH^+, 15.32%), 353 (M^+-CO_2Me, ^{37}Cl, 2.80%), 351 (M^+-CO_2Me, 6.70%), 319 (M^+-CH_2Ph, 0.49%), 285 (MH^+-2x

CH_2CH_2Cl, 0.02%), 270 (M^+-$N(CH_2CH_2Cl)_2$, 58.16%), 242 (MH^+-$N(CH_2CH_2Cl)_2$-C_2H_5, 29.28%).

Analytical hplc stationary phase 50+250 mm x 4.6 mm techsphere OD52 5 μM column, mobile phase A=water B=acetonitrile, gradient conditions 15% B 0 min, 15% B 12 min, 80% B 30 min, flow rate 1 ml/min. Retention times 30.84 min, 31.00 min.

Analysis $C_{16}H_{25}Cl_2N_2O_4P$ requires: C 46.73 %, H 6.13, N 6.81, P 7.53. Found C 47.01, H 5.85, N 6.67, P 7.25.

N,N-Bis(2-chloroethyl)amino propyl phosphorochloridate (65)

Bis(2-chloroethyl)amine hydrochloride (2.00 g, 11.20 mmol) was suspended in a solution of propyl phosphorodichloridate (1.98 g, 11.20 mmol) in ether (45 ml). A solution of triethylamine (3.12 ml, 22.41 mmol) in ether (45 ml) was added dropwise with stirring to this mixture at -78°C. The reaction mixture was then allowed to warm to ambient temperature overnight and stirred for a total period of 96 hr. Filtration of this mixture and concentration of the filtrate under reduced pressure produced a cloudy oil. Hexane (2x 100 ml) was added and the solution filtered and concentrated under reduced pressure to give a colourless oil, (2.00 g, 63%).

^{31}P nmr $\delta(CDCl_3)$ 13.60.

^{13}C nmr $\delta(CDCl_3)$ 70.26 (d, $CH_3CH_2\underline{C}H_2OP$, J=6.7 Hz), 49.94 (d, 2x CH_2N, J=4.4 Hz), 41.56 (d, 2x CH_2Cl, J=2.4 Hz), 23.26 (d, $CH_3\underline{C}H_2CH_2OP$, J=8.3 Hz), 10.00 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 4.16 (m, 2H, $CH_3CH_2C\underline{H}_2OP$), 3.67 (m, 4H, 2x CH_2N), 3.48 (m, 4H, 2x CH_2Cl), 1.77 (sextet, 2H, $CH_3C\underline{H}_2CH_2OP$), 0.98 (t, 3H, $C\underline{H}_3CH_2CH_2OP$).

FABMS m/e 308 (M^++Na, 2x ^{37}Cl, 4.01%), 306 (M^++Na, ^{37}Cl, 17.71%), 304 (M^++Na, 16.62%), 242 (MH_2^+-C_3H_7, ^{37}Cl, 1.51%), 240 (MH_2^+-C_3H_7, 2.23%), 176 (MH^+-CH_2CH_2Cl-C_3H_7, 5.26%), 144 (MH^+-$N(CH_2CH_2Cl)_2$, ^{37}Cl, 3.12%), 142 (MH^+-$N(CH_2CH_2Cl)_2$, 8.68%), 65 ($ClCH_2CH_2^+$, ^{37}Cl, 11.48%), 63 ($ClCH_2CH_2^+$, 46.77%).

N,N-Bis(2-chloroethyl)amino propyl methoxyglycinyl phosphoramidate (66)

A solution of triethylamine (0.20 ml, 1.42 mmol) in dichloromethane (6 ml) was added dropwise with stirring to a solution of N,N-bis(2-chloroethyl)amino propyl phosphorochloridate (0.20 g, 0.71 mmol) and glycine methyl ester hydrochloride (89 mg, 0.71 mmol) in dichloromethane (11 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The cloudy white reaction mixture was then allowed to warm to ambient temperature overnight and stirred for a total period of 42 hr. The solvent was removed by rotary evaporation under reduced pressure and ether (2x 100 ml) added to the residue. Filtration of the combined ether extracts and concentration under reduced pressure produced an oil which was purified by column chromatography. Elution with chloroform followed by pooling and evaporation of the appropriate fractions gave a colourless oil, (0.14 g, 57%).

^{31}P nmr δ($CDCl_3$) 12.49.

^{13}C nmr δ($CDCl_3$) 171.50 (d, $\underline{C}O_2Me$, J=8.5 Hz), 67.25 (d, $CH_3CH_2\underline{C}H_2OP$, J=5.4 Hz), 52.27 (d, $CO_2\underline{Me}$, J=6.6 Hz), 49.18 (d, 2x CH_2N, J=4.5 Hz), 42.50 (2x CH_2Cl), 42.34 (d, CH_2NH, J=2.2 Hz), 23.65 (d, $CH_3\underline{C}H_2CH_2OP$, J=7.2 Hz), 10.08 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 3.92 (m, 2H, $CH_3CH_2C\underline{H}_2OP$), 3.72 (s, 3H, CO_2Me), 3.56-3.70 (m, 6H, 2x CH_2N, $C\underline{H}_2NH$), 3.40 (m, 4H, 2x CH_2Cl), 3.16 (m, 1H, $CH_2N\underline{H}$), 1.64 (sextet, 2H, $CH_3C\underline{H}_2CH_2OP$), 0.91 (t, 3H, $C\underline{H}_3CH_2CH_2OP$).

FABMS m/e 339 (MH^+, 2x ^{37}Cl, 3.54%), 338 (MH_2^+, ^{37}Cl, 2.34%), 337 (MH^+, ^{37}Cl, 51.02%), 336 (MH_2^+, 7.50%), 335 (MH^+, 82.88%), 299 (M^+-Cl, 1.01%), 285 (M^+-CH_2Cl, 0.20%), 277 (M^+-OC_3H_7, ^{37}Cl, 0.25%), 275 (M^+-OC_3H_7, 1.07%), 249 (MH^+-MeO_2CCH_2NH, ^{37}Cl, 1.21%), 247 (MH^+-MeO_2CCH_2NH, 2.79%), 194 (M^+-$N(CH_2CH_2Cl)_2$, 100%), 166 (MH^+-$N(CH_2CH_2Cl)_2$-C_2H_5, 23.43%), 152 (MH^+-$N(CH_2CH_2Cl)_2$-C_3H_7, 56.44%), 136 (MH^+-$N(CH_2CH_2Cl)_2$-OC_3H_7, 10.13%).

Analytical hplc stationary phase 250 mm x 4.6 mm kromasil C18 5 μM column, refractive index 2x, mobile phase methanol-water-triethylamine (70:30:0.01), flow rate 1 ml/min. Retention time 5.65 min.

Analysis $C_{10}H_{21}Cl_2N_2O_4P$ requires: C 35.84 %, H 6.32, N 8.36. Found C 35.74, H 6.14, N 7.94.

N,N-Bis(2-chloroethyl)amino propyl methoxyphenylalaninyl phosphoramidate (67)

A solution of triethylamine (0.30 ml, 2.12 mmol) in dichloromethane (9 ml) was added dropwise with stirring to a solution of N,N-bis(2-chloroethyl)amino propyl phosphorochloridate (0.30 g, 1.06 mmol) and L-phenylalanine methyl ester hydrochloride (0.23 g, 1.06 mmol) in dichloromethane (29 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and after stirring for 41 hr, the solvent was removed by rotary evaporation under reduced pressure. Ether (2x 150 ml) was added

to the residue and the solution filtered and concentrated under reduced pressure. Successive hexane (4x 50 ml) extractions and purification by column chromatography with elution by 20% chloroform in petroleum spirit (b.p. 60-80°C) afforded a white solid, (0.06g, 37%).

^{31}P nmr δ($CDCl_3$) 11.76, 11.65 (1:1).

^{31}C nmr δ($CDCl_3$) 173.54, 173.26 (2xd, $\underline{C}O_2Me$, J=4.1 Hz, J=5.5 Hz), 136.02, 135.96 (*ipso*-Ph), 129.54, 129.43 (*ortho*-Ph), 128.61, 128.50 (*meta*-Ph), 127.16, 127.10 (*para*-Ph), 67.28 (t, $CH_3CH_2\underline{C}H_2OP$, J=5.2 Hz), 55.48, 54.94 (CHNH), 52.25 (t, $CO_2\underline{Me}$, J=6.2 Hz), 49.14, 49.03 (2xd, 2x CH_2N, J=4.6 Hz), 42.52 (2x CH_2Cl), 40.62 (t, $\underline{C}H_2Ph$, J=6.2 Hz), 23.71 (d, $CH_3\underline{C}H_2CH_2OP$, J=7.4 Hz), 10.16, 10.13 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 7.13-7.30 (m, 5H, Ph), 4.14 (m, 1H, C$\underline{H}$NH), 3.82 (m, 2H, $CH_3CH_2C\underline{H}_2OP$), 3.70, 3.69 (2xs, 3H, CO_2Me), 3.51 (m, 4H, 2x CH_2N), 3.25 (m, 4H, 2x CH_2Cl), 2.92-3.20 (m, 3H, CHN$\underline{H}$, C$\underline{H}_2$Ph), 1.60 (m, 2H, $CH_3C\underline{H}_2CH_2OP$), 0.89 (2xt, 3H, $C\underline{H}_3CH_2CH_2OP$).

EIMS m/e 425.1147 (MH^+, 0.12%, calcd. for $C_{17}H_{28}Cl_2N_2O_4P$ 425.1164), 367 (M^+-OC_3H_7, ^{37}Cl, 10.67%), 365 (M^+-OC_3H_7, 15.93%), 335 (M^+-CH_2Ph, ^{37}Cl, 33.07%), 333 (M^+-CH_2Ph, 49.17%), 284 (M^+-$N(CH_2CH_2Cl)_2$, 26.09%), 91 ($PhCH_2^+$, 100%).

Analytical hplc stationary phase 50+250 mm x 4.6 mm kromasil C18 5 μM column, mobile phase methanol-water-triethylamine (80:20:0.1), flow rate 1 ml/min. Retention time 8.23 min.

Analysis $C_{17}H_{27}Cl_2N_2O_4P(H_2O)_{0.5}$ requires: C 47.02 %, H 6.27, N 6.45. Found C 46.80, H 6.03, N 6.78.

N,N-Bis(2-chloroethyl)amino butyl phosphorochloridate (68)

Bis(2-chloroethyl)amine hydrochloride (0.65 g, 3.64 mmol) was suspended in a solution of butyl phosphorodichloridate (0.70 g, 3.64 mmol) in ether (14 ml). A solution of triethylamine (1.02 ml, 7.28 mmol) in ether (14 ml) was added dropwise to this mixture at -78°C and in an atmosphere of nitrogen. The reaction mixture was then allowed to warm to ambient temperature and stirred for 71 hr. Filtration of the mixture and concentration under reduced pressure produced a pale yellow oil, (0.93 g, 86%).

^{31}P nmr $\delta(CDCl_3)$ 13.62 .

^{13}C nmr $\delta(CDCl_3)$ 68.55 (d, $CH_3CH_2CH_2\underline{C}H_2OP$, J=3.9 Hz), 49.92 (d, 2x CH_2N, J=4.4 Hz), 41.55 (d, 2x CH_2Cl, J=2.1 Hz), 31.76 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=8.0 Hz), 18.66 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.48 ($\underline{C}H_3CH_2CH_2CH_2OP$).

^{1}H nmr $\delta(CDCl_3)$ 4.22 (m, 2H, $CH_3CH_2CH_2C\underline{H}_2OP$), 3.31-3.68 (m, 8H, 2x CH_2CH_2Cl), 1.69 (m, 2H, $CH_3CH_2C\underline{H}_2CH_2OP$), 1.38 (m, 2H, $CH_3C\underline{H}_2CH_2CH_2OP$), 0.93 (t, 3H, $C\underline{H}_3CH_2CH_2CH_2OP$).

N,N-Bis(2-chloroethyl)amino butyl methoxyvalinyl phosphoramidate (69)

A solution of triethylamine (0.47 ml, 3.37 mmol) in dichloromethane (14 ml) was added dropwise with stirring to a solution of N,N-bis(2-chloroethyl)amino butyl phosphorochloridate (0.50 g, 1.69 mmol) and L-valine methyl ester hydrochloride (0.28 g, 1.69 mmol) in dichloromethane (35 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was then allowed to warm to ambient

temperature and stirred for 41 hr. The solvent was removed by evaporation under reduced pressure and the residue dissolved in chloroform (40 ml). This was washed with aqueous sodium bicarbonate (40 ml) and then water (3x 15 ml). The organic extract was dried over magnesium sulphate, filtered and concentrated under reduced pressure to produce a yellow oil. Purification of this material by column chromatography with elution by chloroform gave a white solid, (0.45 g, 69%).

^{31}P nmr δ($CDCl_3$) 12.54, 12.30 (1:1).

^{13}C nmr ($CDCl_3$) 173.80 (t, $\underline{C}O_2Me$, J=3.0 Hz), 65.73, 65.54 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=5.4 Hz, J=5.5 Hz), 59.64, 59.02 (CHNH), 52.12, 52.01 ($CO_2\underline{Me}$), 49.18, 49.14 (2x CH_2N), 42.62, 42.57 (2x CH_2Cl), 32.14-32.47 (m, $CH_3CH_2\underline{C}H_2CH_2OP$, $\underline{C}H(CH_3)_2$), 18.82-18.89 (m, $CH_3\underline{C}H_2CH_2CH_2OP$, $CHC\underline{H}_3$), 17.73, 17.55 ($CHC\underline{H}_3$), 13.65 ($\underline{C}H_3CH_2CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 3.95 (m, 2H, $CH_3CH_2CH_2C\underline{H}_2OP$), 3.73 (s, 3H, CO_2Me), 3.58-3.72 (m, 5H, $C\underline{H}NH$, 2x CH_2N), 3.38 (m, 4H, 2x CH_2Cl), 3.11 (m, 1H, $CHN\underline{H}$), 2.03 (m, 1H, $C\underline{H}(CH_3)_2$), 1.60 (m, 2H, $CH_3CH_2C\underline{H}_2CH_2OP$), 1.37 (m, 2H, $CH_3C\underline{H}_2CH_2CH_2OP$), 0.88-0.98 (m, 9H, $C\underline{H}_3CH_2CH_2CH_2OP$, $CH(C\underline{H}_3)_2$).

FABMS m/e 393 (MH^+, ^{37}Cl, 14.45%), 391 (MH^+, 23.71%), 333 (M^+-CO_2Me, ^{37}Cl, 5.11%), 331 (M^+-CO_2Me, 10.08%), 291 (MH^+-CO_2Me-$CH(CH_3)_2$, ^{37}Cl, 1.20%), 289 (MH^+-CO_2Me-$CH(CH_3)_2$, 3.26%), 277 (MH^+-CO_2Me-C_4H_9, ^{37}Cl, 6.61%), 275 (MH^+-CO_2Me-C_4H_9, 11.86%), 250 (M^+-$N(CH_2CH_2Cl)_2$, 100%).

Analytical hplc stationary phase 50+250 mm x 4.6 mm kromasil C18 μM column, mobile phase methanol-water-triethylamine (80:20:0.01), flow rate 1 ml/min. Retention times 9.03 min, 9.60 min.

Analysis $C_{14}H_{29}Cl_2N_2O_4P$ requires: C 42.98 %, H 7.47, N 7.16. Found C 42.99, H 7.23, N 6.66.

N,N-Bis(2-chloroethyl)amino butyl methoxyphenylalaninyl phosphoramidate (70)

A solution of triethylamine (0.28 ml, 2.02 mmol) in dichloromethane (8 ml) was added dropwise with stirring to a solution of N,N-bis(2-chloroethyl)amino butyl phosphorochloridate (0.30 g, 1.01 mmol) and L-phenylalanine methyl ester hydrochloride (0.22 g, 1.01 mmol) in dichloromethane (27 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. After stirring the reaction mixture for 41 hr at ambient temperature, the solvent was removed by rotary evaporation under reduced pressure. The residue was dissolved in chloroform (24 ml), washed with aqueous sodium bicarbonate (24 ml) and then water (3x 12 ml). The organic extract was dried over magnesium sulphate, filtered and concentrated under reduced pressure to produce an oil. Purification by column chromatography with elution by chloroform gave a white solid, (0.25 g, 56%).

^{31}P nmr $\delta(CDCl_3)$ 11.69.

^{13}C nmr $\delta(CDCl_3)$ 173.53, 173.24 (2xd, $\underline{C}O_2Me$, J=3.2 Hz, J=5.8 Hz), 136.01, 135.94 (*ipso*-Ph), 129.52, 129.42 (*ortho*-Ph), 128.59, 128.48 (*meta*-Ph), 127.14, 127.08 (*para*-Ph), 65.54 (t, $CH_3CH_2CH_2\underline{C}H_2OP$, J=5.4 Hz), 55.46, 54.91 (CHNH), 52.28, 52.20 ($CO_2\underline{Me}$), 49.13, 49.02 (2xd, 2x CH_2N, J=4.6 Hz, J=4.7 Hz), 42.52 (2x CH_2Cl),

40.61 (t, $\underline{C}H_2Ph$, J=6.0 Hz), 32.39 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=7.3 Hz), 18.84, 18.82 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.66 ($\underline{C}H_3CH_2CH_2CH_2OP$).

^1H nmr $\delta(CDCl_3)$ 7.15-7.32 (m, 5H, Ph), 4.16 (m, 1H, C$\underline{H}$NH), 3.87 (m, 2H, $CH_3CH_2CH_2C\underline{H}_2OP$), 3.72, 3.70 (2xs, 3H, CO_2Me), 3.53 (m, 4H, 2x CH_2N), 3.27 (m, 4H, 2x CH_2Cl), 2.94-3.12 (m, 3H, CHN$\underline{H}$, $C\underline{H}_2Ph$), 1.58 (m, 2H, $CH_3CH_2C\underline{H}_2CH_2OP$), 1.34 (2x sextet, 2H, $CH_3C\underline{H}_2CH_2CH_2OP$), 0.93 (2xt, 3H, $C\underline{H}_3CH_2CH_2CH_2OP$).

FABMS m/e 441 (MH^+, ^{37}Cl, 15.68%), 439 (MH^+, 30.42%), 379 (M^+-CO_2Me, 0.17%), 323 (MH^+-CO_2Me-C_4H_9, 0.45%), 298 (M^+-$N(CH_2CH_2Cl)_2$, 58.54%), 270 (MH^+-$N(CH_2CH_2Cl)_2$-C_2H_5, 6.13%), 120 (($PhCH_2CHNH_2)^+$, 100%), 91 ($PhCH_2^+$, 43.09%).

Analytical hplc stationary phase 50+250 mm x 4.6 mm kromasil C18 5 μM column, mobile phase methanol-water-triethylamine (80:20:0.01), flow rate 1 ml/min. Retention times 10.46 min, 10.88 min.

Analysis $C_{18}H_{29}Cl_2N_2O_4P$ requires: C 49.21 %, H 6.65, N 6.38. Found C 49.03, H 6.64, N 6.33.

L-Alanine methyl ester hydrochloride

Thionyl chloride (25.22 ml, 0.35 mol) was added dropwise to methanol (56.00 ml, 1.38 mol) at -10°C over 45 min. L-alanine (15.40 g, 0.17 mol) was then added slowly over 1 hr at -10°C. The mixture was allowed to warm to ambient temperature and refluxed at 40°C for 23 hr. The solvent was removed by rotary evaporation under reduced pressure to produce a colourless oil. This was concentrated further in a high vacuum (*ca.* 0.2

mmHg). Ether (200 ml) was then added to the oil and this was left at 0°C for 14 hr. The solvent was then removed by rotary evaporation under reduced pressure and the remaining material triturated with toluene (3x 100 ml). The solid collected was dried under a high vacuum and then dissolved in a minimum of warm methanol (20 ml). Ether (200 ml) was then added to precipitate the product. This was collected by filtration and dried at 50°C/*ca.* 0.2 mmHg to give a white solid, (15.40 g, 64%).

^{1}H nmr δ($CDCl_3$) 8.61 (bs, 3H, NH_3^+), 4.26 (bs, 1H, C<u>H</u>CH_3), 3.78 (s, 3H, CO_2Me), 1.68 (d, 3H, $CHC\underline{H}_3$).

Decyl phosphorodichloridate (71)

A solution of triethylamine (7.48 ml, 53.64 mmol) and 1-decanol (10.24 ml, 53.64 mmol) in ether (100 ml) was added dropwise to a solution of phosphoryl chloride (5.00 ml, 53.64 mmol) in ether (100 ml) at -78°C over a period of 4 hr. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 22 hr. Filtration and concentration of the filtrate under reduced pressure produced a cloudy oil to which was added hexane (2x 125 ml). The solution was then decanted and concentrated under reduced pressure to give a colourless oil, (14.51 g, 98%).

^{31}P nmr δ($CDCl_3$) 5.12 (major), 2.95 (minor).

^{13}C nmr δ($CDCl_3$) 72.43 (d, C-10, J=9.9 Hz), 31.84 (C-3), 28.92-29.63 (m, C-4-C-8), 25.19 (C-9), 22.65 (C-2), 14.09 (C-1).

^{1}H nmr δ($CDCl_3$) 4.31 (m, 2H, $CH_2C\underline{H}_2OP$), 1.78 (m, 2H, $C\underline{H}_2CH_2OP$), 1.25 (bs, 14H, 7x CH_2), 0.86 (t, 3H, CH_3).

Analysis $C_{10}H_{21}Cl_2O_2P$ requires: Cl 25.77 %. Found Cl 25.24.

N,N-Bis(2-chloroethyl)amino decyl phosphorochloridate (72)

A solution of triethylamine (2.53 ml, 18.17 mmol) in dichloromethane (38 ml) was added to bis(2-chloroethyl)amine hydrochloride (3.24 g, 18.17 mmol) and decyl phosphorodichloridate (2.50 g, 9.09 mmol) in dichloromethane (72 ml) at -78°C. The mixture was allowed to warm to ambient temperature and stirred for a total of 96 hr. The mixture was then filtered and concentrated under reduced pressure and ether (2x 100 ml) added to the residue. Filtration and concentration of the ether extracts under reduced pressure gave a pale yellow oil, (2.87 g, 83%).

^{31}P nmr δ($CDCl_3$) 13.38 (major), 6.88 (minor).

^{13}C nmr δ($CDCl_3$) 68.85 (d, C-10, J=6.5 Hz), 49.92 (d, 2x CH_2N, J=4.3 Hz), 41.53 (d, 2x CH_2Cl, J=2.5 Hz), 31.85 (C-3), 28.98-30.19 (m, C-4-C8), 25.38 (C-9), 22.66 (C-2), 14.10 (C-1).

^{1}H nmr δ($CDCl_3$) 4.21 (m, 2H, $CH_2C\underline{H}_2OP$), 3.64 (m, 4H, 2x CH_2N), 3.47 (m, 4H, 2x CH_2Cl), 1.68 (m, 2H, $C\underline{H}_2CH_2OP$), 1.24 (bs, 14H, 7x CH_2), 0.85 (t, 3H, CH_3).

N,N-Bis(2-chloroethyl)amino decyl methoxyalaninyl phosphoramidate (73)

A solution of triethylamine (0.37 ml, 2.63 mmol) in dichloromethane (11 ml) was added to a solution of L-alanine methyl ester hydrochloride (0.18 g, 1.31 mmol) and N,N-bis(2-chloroethyl)amino decyl phosphorochloridate (0.50 g, 1.31 mmol) in dichloromethane (39 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. Following the addition, the reaction mixture was stirred at ambient temperature for 42 hr. The solvent was then removed by rotary evaporation under

reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated under reduced pressure and the residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave a colourless oil, (0.35 g, 60%).

^{31}P nmr δ($CDCl_3$) 11.75, 11.38 (1:1).

^{13}C nmr δ($CDCl_3$) 174.56 (t, $\underline{C}O_2Me$, J=7.29 Hz), 65.71 (t, C-10, J=4.3 Hz), 52.36 (t, $CO_2\underline{Me}$, J=2.5 Hz), 49.73, 49.44 (CHNH), 49.28, 49.23 (2x CH_2N), 42.49 (2x CH_2Cl), 31.80 (C-3), 29.11-30.37 (m, C-4-C-8), 25.57 (C-9), 22.60 (C-2), 21.39, 21.05 (2xd, $CH\underline{C}H_3$, J=4.2 Hz, J=5.2 Hz), 14.04 (C-1).

^{1}H nmr δ($CDCl_3$) 3.90 (m, 2H, $CH_2C\underline{H}_2OP$), 3.70 (s, 3H, CO_2Me), 3.51-3.65 (m, 5H, 2x CH_2N, $C\underline{H}NH$), 3.35 (m, 4H, 2x CH_2Cl), 3.20 (m, 1H, $CHN\underline{H}$), 1.59 (quintet, 2H, $C\underline{H}_2CH_2OP$), 1.37 (t, 3H, $CHC\underline{H}_3$), 1.22 (bs, 14H, 7x CH_2), 0.83 (t, 3H, $C\underline{H}_3CH_2$).

FABMS m/e 451 (MH^+, 2x ^{37}Cl, 4.42%), 449 (MH^+, ^{37}Cl, 26.99%), 447 (MH^+, 36.07%), 389 (M^+-CO_2Me, ^{37}Cl, 2.41%), 387 (M^+-CO_2Me, 4.08%), 346 (M^+-$NHCH(CH_3)CO_2Me$, ^{37}Cl, 0.32%), 344 (M^+-$NHCH(CH_3)CO_2Me$, 2.60%), 306 (M^+-$N(CH_2CH_2Cl)_2$, 58.66%), 249 (MH^+-$NHCH(CH_3)CO_2Me$-2x CH_2Cl, ^{37}Cl, 8.94%), 247 (MH^+-$NHCH(CH_3)CO_2Me$-2x CH_2Cl, 15.95%), 166 (MH^+-$N(CH_2CH_2Cl)_2$-$(CH_2)_9CH_3$, 100%).

Analysis $C_{18}H_{37}Cl_2N_2O_4P$ requires: C 48.32 %, H 8.34, N 6.26, P 6.92. Found C 49.03, H 8.88, N 5.67, P 6.71.

N,N-Bis(2-chloroethyl)amino decyl methoxyvalinyl phosphoramidate (74)

A solution of triethylamine (0.33 ml, 2.39 mmol) in dichloromethane (10 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino decyl phosphorochloridate (0.45 g, 1.93 mmol) and L-valine methyl ester hydrochloride (0.20 g, 1.19 mmol) in dichloromethane (43 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for 42 hr. The solvent was then removed by evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave a colourless oil, (0.28 g, 48%).

^{31}P nmr δ($CDCl_3$) 14.97, 14.71 (2:1).

^{13}C nmr δ($CDCl_3$) 173.77, 173.74 ($\underline{C}O_2Me$), 66.00, 65.81 (2xd, C-10, J=5.3 Hz, J=5.4 Hz), 59.63, 58.99 (CHNH), 51.97-52.10 (m, $CO_2\underline{Me}$), 49.21, 49.17 (2x CH_2N), 42.58, 42.54 (2x CH_2Cl), 32.32, 32.16 (2xd, $\underline{C}H(CH_3)_2$, J=5.6 Hz, J=6.1 Hz), 31.83 (C-3), 30.42, 30.40 (C-8), 30.35, 30.32 (C-7), 29.47 (C-6), 29.25 (C-5), 29.14 (C-4), 25.60, 25.57 (C-9), 22.63 (C-2), 18.88 ($CH\underline{C}H_3$), 17.71, 17.54 ($CH\underline{C}H_3$), 14.08 (C-1).

^{1}H nmr δ($CDCl_3$) 3.92 (m, 2H, $CH_2C\underline{H}_2OP$), 3.70 (s, 3H, CO_2Me), 3.57-3.70 (m, 5H, $C\underline{H}NH$, 2x CH_2N), 3.36 (m, 4H, 2x CH_2Cl), 3.10 (m, 1H, $CHN\underline{H}$), 1.98 (m, 1H, $C\underline{H}(CH_3)_2$), 1.60 (m, 2H, $C\underline{H}_2CH_2OP$), 1.23 (bs, 14H, 7x CH_2), 0.94 (2xd, 3H, $CHC\underline{H}_3$), 0.82-0.89 (m, 6H, $CHC\underline{H}_3$, $C\underline{H}_3CH_2$).

FABMS m/e 477 (MH^+, ^{37}Cl, 7.82%), 475 (MH^+, 16.97%), 417 (M^+-CO_2Me, ^{37}Cl, 1.17%), 415 (M^+-CO_2Me, 3.61%), 334 (MH^+-$(CH_2)_9CH_3$, 70.17%), 277 (MH^+-CO_2Me-$(CH_2)_9CH_3$, ^{37}Cl, 10.92%), 275 (MH^+-CO_2Me-$(CH_2)_9CH_3$, 23.41%), 194 (MH^+-$(CH_2)_9CH_3$-$N(CH_2CH_2Cl)_2$, 78.18%).

Analytical hplc stationary phase 250 mm x 4.6 mm Kromasil C18 7 μM column, refractometer range 10. Mobile phase acetonitrile-water (95:5), flow rate 1 ml/min. Retention times 6.50 min, 7.00 min.

Analysis $C_{20}H_{41}Cl_2N_2O_4P$ requires: C 50.53 %, H 8.69, N 5.89. Found C 50.50, H 8.78, N 5.65.

Octadecyl phosphorodichloridate (75)

A solution of triethylamine (2.06 ml, 14.79 mmol) and 1-octadecanol (4.00 g, 14.79 mmol) in ether (162 ml) was added dropwise to a solution of phosphoryl chloride (1.38 ml, 14.79 mmol) in ether (28 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was then warmed to ambient temperature and stirred for 19 hr. Filtration of the mixture and concentration of the filtrate under reduced pressure produced a white solid, (5.11 g, 89%).

^{31}P nmr δ($CDCl_3$) 5.26.

^{13}C nmr δ($CDCl_3$) 72.30 (d, C-18, J=10.1 Hz), 31.93 (C-3), 29.37-29.69 (m, C-4-C-15), 28.94 (C-17), 25.21 (C-16), 22.70 (C-2), 14.13 (C-1).

^1H nmr δ($CDCl_3$) 4.31 (m, 2H, $CH_2C\underline{H}_2OP$), 1.76 (m, 2H, $C\underline{H}_2CH_2OP$), 1.23 (bs, 30H, 15x CH_2), 1.14 (t, 3H, $C\underline{H}_3CH_2$).

FABMS m/e 411 ($(M+Na)^+$, ^{37}Cl, 7.27%), 409 ($(M+Na)^+$, 11.57%), 199 ($(MH+Na)^+$-$(CH_2)_{14}CH_3$, 0.73%), 176 (MH^+-$(CH_2)_{14}CH_3$, 18.25%), 157 ($(MH+Na)^+$-$(CH_2)_{17}CH_3$, 9.83%).

N,N-Bis(2-chloroethyl)amino octadecyl phosphorochloridate (76)

A solution of triethylamine (1.78 ml, 12.91 mmol) in ether (61 ml) was added dropwise to a suspension of octadecyl phosphorodichloridate (2.50 g, 6.45 mmol) and bis(2-chloroethyl)amine hydrochloride (1.15g, 6.45 mmol) in ether (61 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was then allowed to warm to ambient temperature and stirred for a total of 42 hr. Filtration of the mixture and concentration of the filtrate under reduced pressure produced a white solid, (2.82 g, 89%).

^{31}P nmr δ($CDCl_3$) 13.57 (major), 5.27 (minor).

^{13}C nmr δ($CDCl_3$) 68.70 (d, C-18, J=10.1 Hz), 49.96 (d, 2x CH_2N, J=4.2 Hz), 41.69 (d, 2x CH_2Cl, J=2.2 Hz), 31.92 (C-3), 29.16-30.54 (m, C-4-C-15), 29.01 (C-17), 25.40 (C-16), 22.70 (C-2), 14.13 (C-1).

^1H nmr δ($CDCl_3$) 3.64 (m, 2H, $CH_2C\underline{H}_2OP$), 3.35-3.56 (m, 8H, 2x CH_2CH_2Cl), 1.70 (m, 2H, $C\underline{H}_2CH_2OP$), 1.24 (bs, 30H, 15x CH_2), 0.84 (t, 3H, $C\underline{H}_3CH_2$).

FABMS m/e 491 (M^+, 0.09%), 427 (M^+-H-CH_2CH_2Cl, 0.03%), 244 (MH_2^+-$(CH_2)_{17}CH_3$, 2x ^{37}Cl, 5.48%), 242 (MH_2^+-$(CH_2)_{17}CH_3$, ^{37}Cl, 21.33%), 240 (MH_2^+-$(CH_2)_{17}CH_3$, 24.67%), 224 (M^+-$O(CH_2)_{17}CH_3$, ^{37}Cl, 2.06%), 222 (M^+-$O(CH_2)_{17}CH_3$, 5.48%), 43 ($CH_3CH_2CH_2^+$, 100%).

N,N-Bis(2-chloroethyl)amino octadecyl methoxyalaninyl phosphoramidate (77)

A solution of triethylamine (0.14 ml, 1.01 mmol) in dichloromethane (17 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino octadecyl phosphorochloridate (0.25 g, 0.51 mmol) and L-alanine methyl ester hydrochloride (71mg, 0.51 mmol) in dichloromethane (17 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 150 ml). The extracts were filtered, combined and concentrated to give a residue, 90% of which was purified by two successive columns with elution by chloroform and 30% chloroform in petroleum spirit (b.p. 60-80°C). Further purification of this material was achieved by preparative hplc. Stationary phase 250 mm x 4.6 mm Nucleosil 100 5 μM silica gel column, mobile phase ethylacetate. The diastereoisomers of the product were isolated as white solids, **77A** (44 mg, 16%) and **77B** (32 mg, 11%).

77A

^{31}P nmr δ($CDCl_3$) 12.70.

^{13}C nmr δ($CDCl_3$) 174.58 (d, $\underline{C}O_2Me$, J=7.3 Hz), 65.82 (d, C-18, J=5.1 Hz), 52.45 (d, $CO_2\underline{Me}$, J=4.5 Hz), 49.47 (CHNH), 49.29 (d, 2x CH_2N, J=4.7 Hz), 42.55 (2x CH_2Cl), 31.90 (C-3), 29.35-30.43 (C-4-C-15), 29.18 (C-17), 25.62 (C-16), 22.68 (C-2), 21.48 (d, $CH\underline{C}H_3$, J=4.2 Hz), 14.12 (C-1).

^{1}H nmr δ($CDCl_3$) 3.90-3.95 (m, 3H, $CH_2C\underline{H}_2OP$, $C\underline{H}NH$), 3.72 (s, 3H, CO_2Me), 3.58 (m, 4H, 2x CH_2N), 3.37 (m, 4H, 2x CH_2Cl), 3.18 (t, 1H, $CHN\underline{H}$), 1.61 (quintet, 2H, $C\underline{H}_2CH_2OP$), 1.40 (d, 3H, $CHC\underline{H}_3$), 1.22 (bs, 30H, 15x CH_2), 0.83 (t, 3H, $C\underline{H}_3CH_2$).

FABMS m/e 561 (MH_3^+, 15.83%), 558 (M^+, 18.28%), 499 (M^+-CO_2Me, 2.14%), 418 (M^+-$N(CH_2CH_2Cl)_2$, 23.75%), 417 (M^+-$(CH_2)_9CH_3$, 9.49%), 289 (M^+-$O(CH_2)_{17}CH_3$, 0.33%), 249 (MH^+-CO_2Me-$(CH_2)_{17}CH_3$, ^{37}Cl, 9.96%), 247 (MH^+-CO_2Me-$(CH_2)_{17}CH_3$, 15.51%), 213 ($CH_3(CH_2)_{14}^+$, ^{37}Cl, 1.72%), 211 ($CH_3(CH_2)_{14}^+$, 3.90%), 197 ($CH_3(CH_2)_{13}^+$, 1.87%), 166 (MH^+-$N(CH_2CH_2Cl)_2$-$(CH_2)_{17}CH_3$ 100%).

Analytical hplc stationary phase 250 mm x 4.6 mm Nucleosil 100 5 μM silica gel column, 2x Refractive Index. Mobile phase ethylacetate-hexane (70:30), flow rate 1 ml/min. Retention time 7.34 min.

Analysis $C_{26}H_{53}Cl_2N_2O_4P(H_2O)_{0.5}$ requires: C 54.92 %, H 9.40, N 4.93, P 5.45. Found C 54.60, H 9.64, N 4.70, P 5.71.

77B

^{31}P nmr δ($CDCl_3$) 13.07.

^{13}C nmr δ($CDCl_3$) 174.65 (d, $\underline{C}O_2Me$, J=6.8 Hz), 65.78 (d, C-18, J=5.2 Hz), 52.39 (d, $CO_2\underline{Me}$, J=3.4 Hz), 49.76 (CHNH), 49.24 (d, 2x CH_2N, J=4.6 Hz), 42.58 (2x CH_2Cl), 31.90 (C-3), 29.34-30.42 (C-4-C-15), 29.18 (C-17), 25.60 (C-16), 22.67 (C-2), 21.12 (d, $CH\underline{C}H_3$, J=5.2 Hz), 14.12 (C-1).

^1H nmr δ($CDCl_3$) 3.90-3.96 (m, 3H, $CH_2C\underline{H}_2OP$, $C\underline{H}NH$), 3.72 (s, 3H, CO_2Me), 3.60 (m, 4H, 2x CH_2N), 3.38 (m, 4H, 2x CH_2Cl), 3.14 (t, 1H, $CHN\underline{H}$), 1.61 (quintet, 2H, $C\underline{H}_2CH_2OP$), 1.38 (d, 3H, $CHC\underline{H}_3$), 1.22 (bs, 30H, 15x CH_2), 0.85 (t, 3H, $C\underline{H}_3CH_2$).

FABMS m/e 561 (MH_3^+, 19.20%), 558 (M^+, 20.40%), 499 (M^+-CO_2Me, 1.59%), 417 (M^+-$(CH_2)_9CH_3$, 29.10%), 289 (M^+-$O(CH_2)_{17}CH_3$, 0.54%), 249 (MH^+-CO_2Me-

$(CH_2)_{17}CH_3$, ^{37}Cl, 10.44%), 247 (MH^+-CO_2Me-$(CH_2)_{17}CH_3$, 16.15%), 213 ($CH_3(CH_2)_{14}^+$, ^{37}Cl, 1.72%), 211 ($CH_3(CH_2)_{14}^+$, 3.82%), 197 ($CH_3(CH_2)_{13}^+$, 2.02%), 166 (MH^+-$N(CH_2CH_2Cl)_2$-$(CH_2)_{17}CH_3$, 100%).

Analytical hplc stationary phase 250 mm x 4.6 mm Nucleosil 100 5 μM silica gel column, 2x Refractive Index. Mobile phase ethylacetate-hexane (70:30), flow rate 1 ml/min. Retention time 8.62 min.

Analysis $C_{26}H_{53}Cl_2N_2O_4P(H_2O)_{0.5}$ requires: C 54.92 %, H 9.40, N 4.93. Found C 55.11, H 9.59, N 4.67.

N,N-Bis(2-chloroethyl)amino octadecyl methoxyvalinyl phosphoramidate (78)

A solution of triethylamine (0.25 ml, 1.79 mmol) in dichloromethane (30 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino octadecyl phosphorochloridate (0.44 g, 0.90 mmol) and L-valine methyl ester hydrochloride (0.15 g, 0.90 mmol) in dichloromethane (30 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for 42 hr. The solvent was then removed by evaporation under reduced pressure and the product extracted with ether (2x 150 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 20% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave a colourless oil, (0.32 g, 61%).

^{31}P nmr $\delta(CDCl_3)$ 12.04, 11.80 (1:1).

^{13}C nmr δ($CDCl_3$) 173.80 (t, $\underline{C}O_2Me$, J=3.3 Hz), 66.03, 65.85 (2xd, C-18, J=5.4 Hz, J=5.5 Hz), 59.65, 59.02 (CHNH), 52.12, 52.02 ($CO_2\underline{Me}$), 49.22, 49.18 (2x CH_2N), 42.62, 42.57 (2x CH_2Cl), 32.36, 32.19 (2xd, $\underline{C}H(CH_3)_2$, J=5.7 Hz, J=6.1 Hz), 31.91 (C-3), 29.35-30.45 (C-4-C-15), 29.20 (C-17), 25.63, 25.61 (C-16), 22.68 (C-2), 18.90 ($CH\underline{C}H_3$), 17.74, 17.56 ($CH\underline{C}H_3$), 14.12 (C-1).

^1H nmr δ($CDCl_3$) 4.30 (t, 1H, $C\underline{H}NH$), 3.95 (m, 2H, $CH_2C\underline{H}_2OP$), 3.74 (s, 3H, CO_2Me), 3.54-3.73 (m, 5H, $C\underline{H}NH$, 2x CH_2N), 3.38 (m, 4H, 2x CH_2Cl), 3.10 (m, 1H, $CHN\underline{H}$), 2.04 (m, 1H, $C\underline{H}(CH_3)_2$), 1.67 (m, 2H, $C\underline{H}_2CH_2OP$), 1.25 (bs, 30H, 15x CH_2), 0.97 (2xd, 3H, $CHC\underline{H}_3$), 0.80-0.96 (m, 6H, $CHC\underline{H}_3$, $C\underline{H}_3CH_2$).

FABMS m/e 589 (MH^+, ^{37}Cl, 0.65%), 587 (MH^+, 0.83%), 528 (MH^+-CO_2Me, 1.39%), 446 (M^+-$N(CH_2CH_2Cl)_2$, 8.61%), 277 (MH^+-CO_2Me-$(CH_2)_{17}CH_3$, ^{37}Cl, 11.05%), 275 (MH^+-CO_2Me-$(CH_2)_{17}CH_3$, 11.67%), 194 (MH^+-$N(CH_2CH_2Cl)_2$-$(CH_2)_{17}CH_3$, 100%).

Analysis $C_{28}H_{57}Cl_2N_2O_4P(H_2O)_{0.2}$ requires: C 56.88 %, H 9.72, N 4.74. Found C 56.55, H 9.78, N 3.55.

Oleyl phosphorodichloridate (79)

A solution of triethylamine (2.20 ml, 15.81 mmol) and oleyl alcohol (5.00 ml, 15.81 mmol) in ether (172 ml) was added dropwise to a solution of phosphoryl chloride (1.47 ml, 15.81 mmol) in ether (30 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was then warmed to ambient temperature and stirred for a total period of 19 hr. Filtration of the mixture and concentration of the filtrate under reduced pressure produced a colourless oil, (6.06 g, 99%).

^{31}P nmr $\delta(CDCl_3)$ 5.19.

^{13}C nmr $\delta(CDCl_3)$ 130.00 ($CH=\underline{C}H(CH_2)_8OP$), 129.65 ($\underline{C}H=CH(CH_2)_8OP$), 72.39 (d, C-18, J=9.4 Hz), 31.91 (C-3), 27.21-29.67 (m, C-4-C-8, C-11-C-15), 27.21 (C-17), 25.21 (C-16), 22.69 (C-2), 14.11 (C-1).

^{1}H nmr $\delta(CDCl_3)$ 5.30-5.35 (m, 2H, CH=CH), 4.31 (2xt, 2H, $CH_2C\underline{H}_2OP$), 1.98-2.00 (m, 4H, $C\underline{H}_2CH=CHC\underline{H}_2$), 1.76 (quintet, 2H, $C\underline{H}_2CH_2OP$), 1.24 (bs, 22H, 11x CH_2), 0.86 (t, 3H, $C\underline{H}_3CH_2$).

FABMS m/e 410 ($(M+Na)^+$-H, 2x ^{37}Cl, 0.20%), 408 ($(M+Na)^+$-H, ^{37}Cl, 0.93%), 406 ($(M+Na)^+$-H, 1.56%), 218 (MH^+-$C_{12}H_{23}$, 0.05%).

Analysis $C_{18}H_{35}Cl_2O_2P$ requires: C 56.10 %, H 9.16, P 8.04. Found C 58.73, H 9.95, P 8.46.

N,N-Bis(2-chloroethyl)amino oleyl phosphorochloridate (80)

A solution of triethylamine (2.71 ml, 19.46 mmol) in dichloromethane (39 ml) was added dropwise to a suspension of oleyl phosphorodichloridate (2.50 g, 6.49 mmol) and bis(2-chloroethyl)amine hydrochloride (5.79 g, 32.44 mmol) in dichloromethane (61 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 44 hr. The solvent was then removed under reduced pressure and the product extracted with hexane (2x 100 ml). Filtration of the combined extracts and concentration of the filtrate under reduced pressure produced a pale yellow oil, (2.91 g, 91%).

^{31}P nmr $\delta(CDCl_3)$ 14.65 (minor), 13.59 (major), 7.70 (minor).

^{13}C nmr δ($CDCl_3$) 129.94 (CH=$\underline{C}$H(CH_2)$_8$OP), 129.68 ($\underline{C}$H=CH(CH_2)$_8$OP), 68.82 (d, C-18, J=6.6 Hz), 49.91 (d, 2x CH_2N, J=4.4 Hz), 41.51 (d, 2x CH_2Cl, J=2.2 Hz), 31.88 (C-3), 28.96-30.40 (m, C-4-C-8, C-11-C-15), 27.17 (C-17), 25.36 (C-16), 22.66 (C-2), 14.10 (C-1).

^{1}H nmr δ($CDCl_3$) 5.28-5.33 (m, 2H, CH=CH), 4.15 (m, 2H, $CH_2C\underline{H}_2OP$), 3.62 (m, 4H, 2x CH_2N), 3.44 (m, 4H, 2x CH_2Cl), 1.96-1.98 (m, 4H, $C\underline{H}_2CH$=CH$C\underline{H}_2$), 1.67 (m, 2H, $C\underline{H}_2CH_2OP$), 1.22 (bs, 22H, 11x CH_2), 0.84 (t, 3H, $C\underline{H}_3CH_2$).

N,N-Bis(2-chloroethyl)amino oleyl methoxyalaninyl phosphoramidate (81)

A solution of triethylamine (0.23 ml, 1.63 mmol) in dichloromethane (7 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino oleyl phosphorochloridate (0.40 g, 0.82 mmol) and L-alanine methyl ester hydrochloride (0.11g, 0.82 mmol) in dichloromethane (24 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 65 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with hexane (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by two successive columns with elution by 30% chloroform in petroleum spirit (b.p. 60-80°C) and 25% chloroform in petroleum spirit (b.p. 60-80°C). Pooling and evaporation of the appropriate fractions gave the product as a colourless oil, (0.23 g, 51 %).

^{31}P nmr δ($CDCl_3$) 11.84, 11.48 (1:1).

^{13}C nmr δ($CDCl_3$) 174.59 (t, $\underline{C}O_2Me$, J=6.6 Hz), 129.61-129.97 (m, CH=CH), 65.74 (t, C-18, J=7.7 Hz), 52.46, 52.33 (2xd, $CO_2\underline{Me}$, J=4.9 Hz, J=3.7 Hz), 49.75, 49.52 (2xd, CHNH, J=11.4 Hz, J=3.6 Hz), 49.26, 49.22 (2x CH_2N,), 42.56, 42.53 (2x

CH_2Cl), 31.87 (C-3), 28.95-30.41 (C-4-C-8, C-11-C-15), 27.17 (C-17), 25.59 (C-16), 22.65 (C-2), 21.44, 21.08 ($CH\underline{C}H_3$), 14.09 (C-1).

^1H nmr $\delta(CDCl_3)$ 5.26-5.40 (m, 2H, CH=CH), 3.92, (m, 2H, $CH_2C\underline{H}_2OP$), 3.74, 3.73 (2xs, 3H, CO_2Me), 3.02-3.64 (m, 10H, 2x CH_2CH_2Cl, CHNH), 1.98 (m, 2H, $C\underline{H}_2CH_2OP$), 1.80 (2H, $CH_2CH{=}CHC\underline{H}_2(CH_2)_7OP$), 1.15-1.40 (bs, 24H, $C\underline{H}_2CH{=}CHCH_2(CH_2)_7OP$, 11x CH_2), 0.78-0.88 (m, 6H, $CHC\underline{H}_3$, $C\underline{H}_3CH_2$).

FABMS m/e 559 (MH^+, ^{37}Cl, 2.25%), 557 (MH^+, 2.95%), 416 (M^+-$N(CH_2CH_2Cl)_2$, 1.73%), 249 (MH^+-$C_{18}H_{35}$-CO_2Me, ^{37}Cl, 9.83%), 247 (MH^+-$C_{18}H_{35}$-CO_2Me, 12.72%), 185 (MH_2^+-$C_{18}H_{35}$-CO_2Me-CH_2CH_2Cl, 46.24%).

Analysis $C_{26}H_{51}Cl_2N_2O_4P(H_2O)_{0.4}$ requires: C 55.29 %, H 9.10, N 4.96, P 5.48. Found C 54.90, H 9.35, N 5.14, P 5.80.

N,N-Bis(2-chloroethyl)amino oleyl methoxyphenylalaninyl phosphoramidate (82)

A solution of triethylamine (0.34 ml, 2.44 mmol) in dichloromethane (10 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino oleyl phosphorochloridate (0.60 g, 1.22 mmol) and L-phenylalanine methyl ester hydrochloride (0.26 g, 1.22 mmol) in dichloromethane (33 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 62 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by two successive columns. Elution with 50% chloroform in petroleum spirit (b.p. 60-80°C) and 25% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and

evaporation of the appropriate fractions gave the product as a colourless oil, (0.39 g, 50%).

^{31}P nmr δ($CDCl_3$) 12.56.

^{13}C nmr δ($CDCl_3$) 173.47, 173.21 (2xd, $\underline{C}O_2Me$, J=4.3 Hz, J=5.6 Hz), 136.00, 135.93 (*ipso*-Ph), 129.59-130.09 (m, CH=CH), 129.49, 129.38 (*ortho*-Ph), 128.56, 128.44 (*meta*-Ph), 127.10, 127.05 (*para*-Ph), 65.79 (t, C-18, J=4.3 Hz), 55.46, 54.91 (CHNH), 52.25, 52.15 (2xd, $CO_2\underline{Me}$, J=6.9 Hz, J=6.7 Hz), 49.09 (2x CH_2N), 49.01 (2x CH_2N', J=4.9 Hz), 42.47 (2x CH_2Cl,), 40.57 (t, CH_2Ph, J=6.1 Hz), 31.86 (C-3), 29.16-30.38 (C-4-C-8, C-11-C-15), 27.16 (C-17), 25.57 (C-16), 22.64 (C-2), 14.09 (C-1).

^{1}H nmr δ($CDCl_3$) 7.14-7.31 (m, 5H, Ph), 5.32-5.37 (m, 2H, CH=CH), 4.14 (m, 1H, C$\underline{H}$NH), 3.86 (m, 2H, $CH_2C\underline{H}_2OP$), 3.71, 3.70 (2xs, 3H, CO_2Me), 3.50 (m, 4H, 2x CH_2N), 3.30 (m, 4H, 2x CH_2Cl), 3.01-3.06 (m, 3H, CH_2Ph, CHN$\underline{H}$), 2.00 (m, 2H, $C\underline{H}_2CH_2OP$), 1.56-1.61 (m, 4H, $C\underline{H}_2CH$=CHC$\underline{H}_2$), 1.16-1.27 (m, 22H, 11x CH_2), 0.87 (t, 3H, $C\underline{H}_3CH_2$).

FABMS m/e 637 (MH^+, 2x ^{37}Cl, 0.81%), 636 (MH_2^+, ^{37}Cl, 0.93%), 635 (MH^+, ^{37}Cl, 2.56%), 634 (MH_2^+, 1.34%), 633 (MH^+, 3.43%), 575 (M^+-CO_2Me, ^{37}Cl, 0.81%), 573 (M^+-CO_2Me, 0.93%), 492 (M^+-$N(CH_2CH_2Cl)_2$, 2.50%), 385 (MH_2^+-$C_{18}H_{35}$, ^{37}Cl, 0.52%), 383 (MH_2^+-$C_{18}H_{35}$, 0.84%), 242 (MH^+-$N(CH_2CH_2Cl)_2$-$C_{18}H_{35}$, 31.98%), 120 ($(PhCH_2CHNH_2)^+$, 100%).

Analysis $C_{32}H_{55}Cl_2N_2O_4P(H_2O)_{0.5}$ requires: C 59.97 %, H 8.65, N 4.37. Found C 59.80, H 8.63, N 4.36.

2,2,2-Trichloroethyl phosphorodichloridate (83)

A solution of triethylamine (14.95 ml, 0.11 mmol) and trichloroethanol (10.29 ml, 0.11 mmol) in ether (200 ml) was added dropwise to a solution of phosphoryl chloride (10.00 ml, 0.11 mmol) in ether (200 ml) at -78°C over a period of 2 hr. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 18 hr. Filtration and concentration of the filtrate under reduced pressure produced a cloudy oil to which was added hexane (2x 125 ml). The solution was then decanted and concentrated under reduced pressure to give an oil with a yellow tint, (28.11 g, 98%).

^{31}P nmr δ($CDCl_3$) 6.42.

^{13}C nmr δ($CDCl_3$) 93.05 (d, $\underline{C}Cl_3CH_2OP$, J=13.5 Hz), 78.28 (d, $CCl_3\underline{C}H_2OP$, J=7.5 Hz).

^{1}H nmr δ($CDCl_3$) 4.76 (d, 2H, CCl_3CH_2OP, J=9.17 Hz).

FABMS m/e 289 ($(M+Na)^+$, ^{37}Cl, 0.02%), 287 ($(M+Na)^+$, 0.32%), 268 (M^+, 2x ^{37}Cl, 0.38%), 266 (M^+, ^{37}Cl, 1.48%), 264 (M^+, 0.84%), 231 (M^+-Cl, ^{37}Cl, 6.74%), 229 (M^+-Cl, 7.43%), 117 (M^+-OCH_2CCl_3, 17.47%).

Analysis $C_2H_2Cl_5O_2P$ requires: C 9.02 %, H 0.76, P 11.63. Found C 8.95, H 0.76, P 12.10.

N,N-Bis(2-chloroethyl)amino trichloroethyl phosphorochloridate (84)

A solution of triethylamine (2.62 ml, 18.78 mmol) in ether (38 ml) was added to bis(2-chloroethyl)amine hydrochloride (2.51 g, 14.08 mmol) and trichloroethyl phosphorodichloridate (2.50 g, 9.39 mmol) in ether (56 ml). The addition was carried

out dropwise at -78°C and in an atmosphere of nitrogen. The mixture was then allowed to warm to ambient temperature and stirred for a total of 90 hr. Filtration of the reaction mixture and concentration of the filtrate under reduced pressure produced a pale yellow solid, (3.34 g, 96%).

^{31}P nmr δ($CDCl_3$) 12.56.

^{13}C nmr δ($CDCl_3$) 93.94 (d, $\underline{C}Cl_3CH_2OP$, J=14.6 Hz), 49.81 (d, 2x CH_2N, J=4.5 Hz), 41.41 (d, 2x CH_2Cl, J=1.6 Hz). The signals representing $CCl_3\underline{C}H_2OP$ were masked by those representing deuterochloroform.

^{1}H nmr δ($CDCl_3$) 4.65 (m, 2H, CCl_3CH_2OP), 3.50-3.72 (m, 8H, 2x CH_2CH_2Cl).

FABMS m/e 378 (MH^+, 4x ^{37}Cl, 0.35%), 376 (MH^+, 3x ^{37}Cl, 4.60%), 374 (MH^+, 2x ^{37}Cl, 12.95%), 372 (MH^+, ^{37}Cl, 18.74%), 370 (MH^+, 11.80%), 338 (M^+-Cl, 2x ^{37}Cl, 3.89%), 336 (M^+-Cl, ^{37}Cl, 7.07%), 334 (M^+-Cl, 4.28%), 240 (MH_2^+-CH_2CCl_3, 24.62%), 224 (MH_2^+-OCH_2CCl_3, ^{37}Cl, 2.63%), 222 (MH_2^+-OCH_2CCl_3, 4.58%), 144 ($(ClCH_2CH_2)_2NH_2^+$, ^{37}Cl, 57.51%), 142 ($(ClCH_2CH_2)_2NH_2^+$, 100%).

Analysis $C_6H_{10}Cl_6NO_2P$ requires: C 19.38 %, H 2.71, N 3.77. Found C 19.99, H 2.87, N 3.82.

N,N-Bis(2-chloroethyl)amino trichloroethyl methoxyalaninyl phosphoramidate (85)

A solution of triethylamine (0.22 ml, 1.61 mmol) in dichloromethane (7 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino trichloroethyl phosphorochloridate (0.30 g, 0.81 mmol) and L-alanine methyl ester hydrochloride

(0.11g, 0.81 mmol) in dichloromethane (23 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 40 hr. The solvent was then removed by rotary evaporation under reduced pressure and ether (2x 150 ml) added to the residue. The extracts were filtered, combined and concentrated under reduced pressure. This procedure was repeated with hexane. The resulting residue was then purified by column chromatography with elution by 20% chloroform in petroleum spirit (b.p. 60-80°C) to give the product (white solid) as an isomer, 85A (39 mg, 11%) and a mixture of isomers 85B (141 mg, 40%).

85A

^{31}P nmr δ($CDCl_3$) 11.87.

^{13}C nmr δ($CDCl_3$) 174.22 ($\underline{C}O_2Me$), 95.62 (d, $\underline{C}Cl_3CH_2OP$, J=3.6 Hz), 75.83 (d, $CCl_3\underline{C}H_2OP$, J=3.3 Hz), 52.64 ($CO_2\underline{Me}$), 49.57 (d, CHNH, J=3.3 Hz), 48.98 (d, 2x CH_2N, J=4.8 Hz), 42.31 (2x CH_2Cl), 21.31 (d, $CH\underline{C}H_3$, J=4.5 Hz).

^{1}H nmr δ($CDCl_3$) 4.53 (d, 2H, CCl_3CH_2OP), 4.02 (quintet, 1H, C$\underline{H}$NH), 3.74 (s, 3H, CO_2Me), 3.64 (m, 4H, 2x CH_2N), 3.44-3.58 (m, 5H, 2x CH_2Cl, CHN$\underline{H}$), 1.44 (d, 3H, CHC$\underline{H}_3$).

FABMS m/e 443 (MH^+, 3x ^{37}Cl, 1.99%), 441 (MH^+, 2x ^{37}Cl, 9.29%), 439 (MH^+, ^{37}Cl, 14.59%), 437 (MH^+, 8.56%), 383 (M^+-CO_2Me, 3x ^{37}Cl, 0.75%), 381 (M^+-CO_2Me, 2x ^{37}Cl, 4.02%), 379 (M^+-CO_2Me, ^{37}Cl, 6.57%), 377 (M^+-CO_2Me, 3.58%), 300 (M^+-$N(CH_2CH_2Cl)_2$, 2x ^{37}Cl, 7.31%), 298 (M^+-$N(CH_2CH_2Cl)_2$, ^{37}Cl, 24.86%), 296 (M^+-$N(CH_2CH_2Cl)_2$, 26.59%), 289 (M^+-OCH_2CCl_3, 0.71%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column, refractometer range 50. Mobile phase methanol-water-triethylamine (70:30:0.01), flow rate 1 ml/min. Retention time 13.76 min.

Analysis $C_{10}H_{18}Cl_5N_2O_4P$ requires: C 27.39 %, H 4.14, N 6.39. Found C 27.35, H 4.09, N 6.44.

85B

^{31}P nmr δ($CDCl_3$) 11.94, 11.26 (2.5:1).

^{13}C nmr δ($CDCl_3$) 174.35, 174.26 (2xd, $\underline{C}O_2Me$, J=6.6 Hz, J=7.6 Hz), 95.63, 95.52 (2xd, $\underline{C}Cl_3CH_2OP$, J=3.6 Hz), 75.85 (t, $CCl_3\underline{C}H_2OP$, J=3.3 Hz), 52.64, 52.53 (2xd, $CO_2\underline{Me}$, J=2.8 Hz), 49.94, 49.56 (CHNH), 48.99, 48.87 (2xd, 2x CH_2N, J=4.8 Hz), 42.40, 42.33 (2x CH_2Cl), 21.34, 20.98 (2xd, $CH\underline{C}H_3$, J=4.5 Hz, J=5.5 Hz).

^1H nmr δ($CDCl_3$) 4.52 (2xd, 2H, CCl_3CH_2OP), 4.03 (quintet, 1H, C$\underline{H}$NH), 3.742, 3.737 (2xs, 3H, CO_2Me), 3.67 (m, 4H, 2x CH_2N), 3.40-3.60 (m, 5H, 2x CH_2Cl, CHN$\underline{H}$), 1.43 (d, 3H, $CHC\underline{H}_3$).

FABMS m/e 443 (MH^+, 3x ^{37}Cl, 6.95%), 441 (MH^+, 2x ^{37}Cl, 25.35%), 439 (MH^+, ^{37}Cl, 39.73%), 437 (MH^+, 23.58%), 383 (M^+-CO_2Me, 3x ^{37}Cl, 2.88%), 381 (M^+-CO_2Me, 2x ^{37}Cl, 11.33%), 379 (M^+-CO_2Me, ^{37}Cl, 17.92%), 377 (M^+-CO_2Me, 10.82%), 300 (M^+-$N(CH_2CH_2Cl)_2$, 2x ^{37}Cl, 20.95%), 298 (M^+-$N(CH_2CH_2Cl)_2$, ^{37}Cl, 67.52%), 296 (M^+-$N(CH_2CH_2Cl)_2$, 72.83%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column, refractometer range 50. Mobile phase methanol-water-triethylamine (70:30:0.01), flow rate 1 ml/min. Retention times 12.51 min, 13.61 min.

Analysis $C_{10}H_{18}Cl_5N_2O_4P$ requires: C 27.39 %, H 4.14, N 6.39. Found C 27.50, H 4.06, N 6.27.

N,N-Bis(2-chloroethyl)amino trichloroethyl methoxyphenylalaninyl phosphoramidate (86)

A solution of triethylamine (0.19 ml, 1.34 mmol) in dichloromethane (12 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino trichloroethyl phosphorochloridate (0.25 g, 0.67 mmol) and L-phenylalanine methyl ester hydrochloride (0.14g, 0.67 mmol) in dichloromethane (38 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography with elution by 30% chloroform in petroleum spirit (b.p. 60-80°C). Pooling and evaporation of the appropriate fractions gave the product as a colourless oil, (0.24 g, 68%).

^{31}P nmr δ($CDCl_3$) 11.52, 11.25 (1.5:1).

^{13}C nmr δ($CDCl_3$) 173.16, 173.09 (2xd, $\underline{C}O_2Me$, J=4.2 Hz, J=5.2 Hz), 135.77, 135.66 (*ipso*-Ph), 129.48, 129.41 (*ortho*-Ph), 128.70, 128.63 (*meta*-Ph), 127.29 (*para*-Ph), 95.61, 95.49 (2xd, $\underline{C}Cl_3CH_2OP$, J=7.3 Hz, J=8.0 Hz), 75.67, 75.48 (2xd, $CCl_3\underline{C}H_2OP$, J=3.5 Hz, J=3.3 Hz), 55.46, 55.04 (CHNH), 52.49, 52.36 (2xd, $CO_2\underline{Me}$, J=2.5 Hz),

48.87, 48.66 (2xd, CH_2N, J=4.7 Hz, J=4.8 Hz), 42.25 (2x CH_2Cl), 40.25 (t, CH_2Ph, J=7.0 Hz).

^1H nmr $\delta(CDCl_3)$ 7.16-7.33 (m, 5H, Ph), 4.16-4.48 (m, 3H, CCl_3CH_2OP, C<u>H</u>NH), 3.75, 3.73 (2xs, 3H, CO_2Me), 3.64 (m, 4H, 2x CH_2N), 3.32 (m, 4H, 2x CH_2Cl), 2.94-3.20 (m, 3H, CHN<u>H</u>, C<u>H</u>$_2$Ph).

FABMS m/e 519 (MH^+, 3x ^{37}Cl, 6.09%), 517 (MH^+, 2x ^{37}Cl, 26.66%), 515 (MH^+, ^{37}Cl, 44.02%), 513 (MH^+, 23.22%), 459 (M^+-CO_2Me, 3x ^{37}Cl, 0.86%), 457 (M^+-CO_2Me, 2x ^{37}Cl, 9.28%), 455 (M^+-CO_2Me, ^{37}Cl, 17.79%), 453 (M^+-CO_2Me, 10.25%), 376 (M^+-$N(CH_2CH_2Cl)_2$, 2x ^{37}Cl, 22.42%) 374 (M^+-$N(CH_2CH_2Cl)_2$, ^{37}Cl, 72.94%), 372 (M^+-$N(CH_2CH_2Cl)_2$, 74.15%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 8.60 min, 9.26 min.

Analysis $C_{16}H_{22}Cl_5N_2O_4P$ requires: C 37.34 %, H 4.31, N 5.44. Found C 37.51, H 4.31, N 5.16.

N,N-Bis(2-chloroethyl)amino trichloroethyl benzylphenylalaninyl phosphoramidate (87)

A solution of triethylamine (0.15 ml, 1.08 mmol) in dichloromethane (4 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino trichloroethyl phosphorochloridate (0.20 g, 0.54 mmol) and L-phenylalanine benzyl ester p-toluenesulphonate (0.23g, 0.54 mmol) in dichloromethane (29 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was

allowed to warm to ambient temperature and stirred for a total of 40 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 150 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 30% petroleum spirit (b.p. 60-80°C) in ether followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.16 g, 50%).

^{31}P nmr δ($CDCl_3$) 11.62, 11.27 (1.5:1).

^{13}C nmr δ($CDCl_3$) 172.44 (s, $PhCH_2O\underline{C}O$), 172.40 (d, $PhCH_2O\underline{C}O'$, J=1.9 Hz), 135.60, 135.47 (*ipso*-Bz), 134.92, 134.88 (*ipso*-Ph), 129.50, 129.43 (*ortho*-Bz), 128.54-128.59 (m, *ortho*-Ph , *meta*-Ph, *meta*-Bz, *para*-Bz), 127.21, 127.18 (*para*-Ph), 95.57, 95.46 (2xd, $\underline{C}Cl_3CH_2OP$, J=6.1 Hz, J=6.5 Hz), 75.58, 75.43 (2xd, $CCl_3\underline{C}H_2OP$, J=3.4 Hz, J=2.8 Hz), 67.23-67.45 (m, $Ph\underline{C}H_2OCO$), 55.40, 54.99 (CHNH), 48.81, 48.49 (2xd, 2x CH_2N, J=4.5 Hz, J=4.4 Hz), 42.22, 42.18 (2x CH_2Cl), 40.14 (t, $Ph\underline{C}H_2CH$, J=6.0 Hz).

^{1}H nmr δ($CDCl_3$) 7.08-7.38 (m, 10H, 2x Ph), 5.14 (m, 2H, $PhC\underline{H}_2OCO$), 4.20-4.42 (m, 3H, CCl_3CH_2OP, C<u>H</u>NH), 3.52 (m, 4H, 2x CH_2N), 3.32 (m, 4H, 2x CH_2Cl), 2.99-3.22 (m, 3H, $PhC\underline{H}_2CH$, CHN<u>H</u>).

FABMS m/e 591 (MH^+, ^{37}Cl, 0.18%), 589 (MH^+, 0.34%), 459 (M^+-$PhCH_2OCO$, 3x ^{37}Cl, 0.04%), 457 (M^+-$PhCH_2OCO$, 2x ^{37}Cl, 0.31%), 455 (M^+-$PhCH_2OCO$, ^{37}Cl, 0.65%), 453 (M^+-$PhCH_2OCO$, 0.27%), 314 (MH^+-$N(CH_2CH_2Cl)_2$-$PhCH_2OCO$, 0.02%), 91 ($PhCH_2^+$, 100%).

Analytical hplc Stationary phase 50+250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water-triethylamine (80:20:0.01), flow rate 1 ml/min. Retention times 20.83 min, 22.56 min.

Analysis $C_{22}H_{26}Cl_5N_2O_4P.H_20$ requires: C 43.41 %, H 4.30, N 4.60. Found C 43.04, H 3.59, N 4.20.

N,N-Bis(2-chloroethyl)amino trifluoroethyl phosphorochloridate (89)

A solution of triethylamine (3.21 ml, 23.05 mmol) in ether (46 ml) was added dropwise to bis(2-chloroethyl)amine hydrochloride (2.06 g, 11.52 mmol) and trifluoroethyl phosphorodichloridate (2.50 g, 11.52 mmol) in ether (46 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The reaction mixture was then filtered and concentrated under reduced pressure. A hexane extraction (2x 100 ml) on the residue produced a colourless oil, (2.48 g, 67%).

^{31}P nmr δ($CDCl_3$) 14.18.

^{13}C nmr δ($CDCl_3$) 124.78, 118.70 (2xm, $\underline{C}F_3CH_2OP$, J_{C-F}=306.4 Hz), 63.19 (qd, $CF_3\underline{C}H_2OP$, J_{C-F}=37.1 Hz, J_{C-P}=2.9 Hz), 49.52 (d, 2x CH_2N, J=3.6 Hz), 41.16 (2x CH_2Cl).

^{1}H nmr δ($CDCl_3$) 4.42 (m, 2H, CF_3CH_2OP), 3.44-3.69 (m, 8H, 2x CH_2CH_2Cl).

FABMS m/e 326 (MH^+, 2x ^{37}Cl, 0.01%), 324 (MH^+, ^{37}Cl, 1.35%), 322 (MH^+, 1.94%), 288 (M^+-Cl, ^{37}Cl, 0.18%), 286 (M^+-Cl, 0.44%), 274 (M^+-CH_2Cl, ^{37}Cl, 0.53%), 272 (M^+-CH_2Cl, 0.84%), 224 (MH^+-2x CH_2Cl, 0.58%), 181 (M^+-$N(CH_2CH_2Cl)_2$, 2.11%),

146 ($(ClCH_2CH_2)_2NH_2^+$, 2x ^{37}Cl, 7.91%), 144 ($(ClCH_2CH_2)_2NH_2^+$, ^{37}Cl, 61.75%), 142 ($(ClCH_2CH_2)_2NH_2^+$, 100%).

N,N-Bis(2-chloroethyl)amino trifluoroethyl methoxyalaninyl phosphoramidate (90)

A solution of triethylamine (0.22 ml, 1.55 mmol) in dichloromethane (7 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino trifluoroethyl phosphorochloridate (0.25 g, 0.78 mmol) and L-alanine methyl ester hydrochloride (0.11g, 0.78 mmol) in dichloromethane (23 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 20% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.19 g, 63%).

^{31}P nmr δ($CDCl_3$) 13.01, 12.30 (1:1.5).

^{13}C nmr δ($CDCl_3$) 174.30, 174.15 (2xd, $\underline{C}O_2Me$, J=6.6 Hz, J=8.0 Hz), 122.99 (2xd, $\underline{C}F_3CH_2OP$, J_{C-F}=317.7 Hz, J_{C-P}=2.0 Hz), 62.29, 62.16 (2xqd, $CF_3\underline{C}H_2OP$, J_{C-F}=36.8 Hz, J_{C-P}=3.6 Hz), 52.63, 52.51 ($CO_2\underline{Me}$), 49.88 (s, CHNH), 49.51 (d, CHNH′, J=2.3 Hz), 48.80, 48.64 (2xd, 2x CH_2N, J=4.7 Hz, J=4.9 Hz), 42.24, 42.19 (2x CH_2Cl), 21.16, 20.85 (2xd, $CH\underline{C}H_3$, J=4.3 Hz, J=5.7 Hz).

^1H nmr δ($CDCl_3$) 4.34 (m, 2H, CF_3CH_2OP), 3.96 (m, 1H, C<u>H</u>NH), 3.76, 3.75 (2xs, 3H, CO_2Me), 3.64 (m, 4H, 2x CH_2N), 3.35-3.50 (m, 5H, 2x CH_2Cl, CHN<u>H</u>), 1.43 (dd, 3H, $CHC\underline{H}_3$).

FABMS m/e 393 (MH^+, 2x ^{37}Cl, 2.03%), 391 (MH^+, ^{37}Cl, 15.22%), 389 (MH^+, 21.96%), 333 (M^+-CO_2Me, 2x ^{37}Cl, 1.25%), 331 (M^+-CO_2Me, ^{37}Cl, 9.82%), 329 (M^+-CO_2Me, 15.56%), 248 (M^+-$N(CH_2CH_2Cl)_2$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column, refractive index 2x. Mobile phase methanol-water-triethylamine (70:30:0.01), flow rate 1 ml/min. Retention times 11.51 min, 12.42 min.

Analysis $C_{10}H_{18}Cl_2F_3N_2O_4P$ requires: C 30.87 %, H 4.66, N 7.20. Found C 30.95, H 4.71, N 7.00.

N,N-Bis(2-chloroethyl)amino trifluoroethyl methoxyphenylalaninyl phosphoramidate (91)

A solution of triethylamine (0.30 ml, 2.17 mmol) in dichloromethane (15 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino trifluoroethyl phosphorochloridate (0.35 g, 1.08 mmol) and L-phenylalanine methyl ester hydrochloride (0.23g, 1.08 mmol) in dichloromethane (48 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.34 g, 67%).

^{31}P nmr $\delta(CDCl_3)$ 14.46, 14.20 (1:1.5).

^{13}C nmr $\delta(CDCl_3)$ 173.14, 173.04 (2xd, $\underline{C}O_2Me$, J=4.4 Hz, J=5.0 Hz), 135.78, 135.65 (*ipso*-Ph), 129.47, 129.40 (*ortho*-Ph), 128.82, 128.71 (*meta*-Ph), 128.71, 128.61 (*meta*-Ph), 127.30, 127.24 (*para*-Ph), 123.10 (2xm, $\underline{C}F_3CH_2OP$, J_{C-F}=302.4 Hz), 61.72-62.40 (m, $CF_3\underline{C}H_2OP$), 55.42, 55.06 (CHNH), 52.51, 52.37 ($CO_2\underline{Me}$), 48.69, 48.44 (2xd, CH_2N, J=4.7 Hz, J=4.8 Hz), 42.15 (2x CH_2Cl), 40.29, 40.19 (2xd, $\underline{C}H_2Ph$, J=5.6 Hz, J=6.6 Hz).

^1H nmr $\delta(CDCl_3)$ 7.15-7.36 (m, 5H, Ph), 4.20 (m, 2H, $CF_3\underline{C}H_2OP$), 3.99 (m, 1H, C$\underline{H}$NH), 3.75, 3.73 (2xs, 3H, CO_2Me), 3.54 (m, 4H, 2x CH_2N), 3.35 (m, 4H, 2x CH_2Cl), 3.14 (m, 2H, C$\underline{H}_2$Ph), 2.98 (m, 1H, CHN$\underline{H}$).

FABMS m/e 469 (MH^+, 2x ^{37}Cl, 0.33%), 468 (MH_2^+, ^{37}Cl, 0.47%), 467 (MH^+, ^{37}Cl, 8.36%), 466 (MH_2^+, 2.50%), 465 (MH^+, 18.18%), 407 (M^+-CO_2Me, ^{37}Cl, 13.20%), 405 (M^+-CO_2Me, 24.48%), 373 (M^+-CH_2Ph, 0.35%), 324 (M^+-$N(CH_2CH_2Cl)_2$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention time 6.63 min.

Analysis $C_{16}H_{22}Cl_2F_3N_2O_4P$ requires: C 41.31 %, H 4.77, N 6.02, P 6.66. Found C 41.82, H 4.70, N 5.78, P 6.72.

N,N-Bis(2-chloroethyl)amino trifluoroethyl benzylphenylalaninyl phosphoramidate (92)

A solution of triethylamine (0.13 ml, 0.94 mmol) in dichloromethane (4 ml) was added to a solution of N,N-bis(2-chloroethyl)amino trifluoroethyl phosphorochloridate (0.15 g, 0.47 mmol) and L-phenylalanine benzyl ester p-toluenesulphonate (0.20 g, 0.47 mmol)

in dichloromethane (25 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.16 g, 61%).

^{31}P nmr δ($CDCl_3$) 15.12, 14.76 (1:1).

^{13}C nmr δ($CDCl_3$) 172.39 (t, $\underline{C}O_2Me$, J=4.8 Hz), 135.59, 135.47 (*ipso*-Bz), 135.00, 134.94 (*ipso*-Ph), 129.51 (*ortho*-Bz), 128.41-128.98 (m, *ortho*-Ph, *meta*-Ph, *meta*-Bz, *para*-Bz), 127.31, 127.25 (*para*-Ph), 124.34, 121.57 (2xm, $\underline{C}F_3CH_2OP$, $J_{C\text{-}F}$=279.2 Hz), 67.52, 67.41 ($Ph\underline{C}H_2OCO$), 61.17-62.90 (m, $CF_3\underline{C}H_2OP$), 55.43, 55.02 (2xd, CHNH, J=8.5 Hz, J=7.0 Hz), 48.71 (d, 2x CH_2N, J=4.7 Hz), 48.35 (s, 2x CH_2N'), 42.18, 42.15 (2x CH_2Cl), 40.28, 40.18 (2xd, $Ph\underline{C}H_2CH$, J=5.5 Hz, J=6.8 Hz).

^{1}H nmr δ($CDCl_3$) 7.05-7.36 (m, 10H, 2x Ph), 5.12 (m, 2H, $PhC\underline{H}_2OCO$), 4.17 (m, 2H, CF_3CH_2OP), 3.94 (m, 1H, $C\underline{H}NH$), 3.48 (m, 4H, 2x CH_2N), 3.25 (m, 4H, 2x CH_2Cl), 2.93-3.15 (m, 3H, $PhC\underline{H}_2CH$, $CHN\underline{H}$).

FABMS m/e 543 (MH^+, ^{37}Cl, 1.60%), 541 (MH^+, 2.49%), 407 (M^+-$PhCH_2OCO$, ^{37}Cl, 2.24%), 405 (M^+-$PhCH_2OCO$, 3.67%), 372 (M^+-CH_2Ph-Ph, 6.59%), 91 ($PhCH_2^+$, 100%).

Analytical hplc Stationary phase 50+250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention time 10.13 min.

Analysis $C_{22}H_{26}Cl_2F_3N_2O_4P$ requires: C 48.81 %, H 4.84, N 5.18. Found C 48.91, H 5.03, N 5.32.

Phenyl phosphorodichloridate (93)

A solution of triethylamine (7.40 ml, 0.11 mmol) and phenol (5.00 g, 0.05 mmol) in ether (100 ml) was added dropwise to a solution of phosphoryl chloride (4.95 ml, 0.05 mmol) in ether (100 ml) at -78°C over a period of 2 hr. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. Filtration and concentration of the filtrate under reduced pressure produced a colourless oil, (8.92 g, 80%).

^{31}P nmr $\delta(CDCl_3)$ 1.56.

^{13}C nmr $\delta(CDCl_3)$ 149.70 (d, *ipso*-Ph, J=11.7 Hz), 130.33 (*meta*-Ph), 127.21 (*para*-Ph), 120.58 (d, *ortho*-Ph, J=5.4 Hz).

^{1}H nmr $\delta(CDCl_3)$ 7.25-7.47 (m, 5H, Ph).

FABMS m/e 213 (MH^+, ^{37}Cl, 0.79%), 212 (M^+, ^{37}Cl, 14.84%), 211 (MH^+, 3.26%), 210 (M^+, 30.22%), 174 (M^+-HCl, 5.22%), 77 (Ph^+, 49.25%).

Analysis $C_6H_5Cl_2O_2P$ requires: C 34.16 %, H 2.39. Found C 35.13, H 2.54.

N,N-Bis(2-chloroethyl)amino phenyl phosphorochloridate (94)

A solution of triethylamine (6.61 ml, 47.40 mmol) in dichloromethane (142 ml) was added to a suspension of bis(2-chloroethyl)amine hydrochloride (8.46 g, 47.40 mmol) and phenyl phosphorodichloridate (5.00 g, 23.70 mmol) in dichloromethane (189 ml). The addition was carried out dropwise at -78°C and in an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature and stirred for a total of 69 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with hexane (2x 100 ml). The hexane extracts were filtered and concentrated under reduced pressure and the residue then heated to 65-70°C in a high vacuum (*ca.* 0.5 mmHg) for 6 hr to give a colourless oil, (6.03 g, 80%).

^{31}P nmr δ($CDCl_3$) 8.98.

^{13}C nmr δ($CDCl_3$) 149.47 (*ipso*-Ph), 130.05 (*meta*-Ph), 126.22 (*para*-Ph), 120.43 (d, *ortho*-Ph, J=4.1 Hz), 49.72 (2x CH_2N), 41.43 (2x CH_2Cl).

^{1}H nmr δ($CDCl_3$) 7.21-7.42 (m, 5H, Ph), 3.45-3.72 (m, 8H, 2x CH_2CH_2Cl).

EIMS m/e 319 (M^+, 2x ^{37}Cl, 0.29%), 318 (MH^+, ^{37}Cl, 0.08%), 317 (M^+, ^{37}Cl, 1.64%), 316 (MH^+, 0.12%), 314.9777 (M^+, 2.10%, calcd. for $C_{10}H_{13}Cl_3NO_2P$ 314.9749), 270 (M^+-CH_2Cl, 2x ^{37}Cl, 18.72%), 269 (MH^+-CH_2Cl, ^{37}Cl, 11.44%), 268 (M^+-CH_2Cl, ^{37}Cl, 87.30%), 267 (MH^+-CH_2Cl, 18.39%), 266 (M^+-CH_2Cl, 100%).

Analysis $C_{10}H_{13}Cl_3NO_2P$ requires: C 37.94 %, H 4.14, N 4.42. Found C 38.04, H 3.86, N 3.65.

N,N-Bis(2-chloroethyl)amino phenyl methoxyphenylalaninyl phosphoramidate (95)

A solution of triethylamine (0.35 ml, 2.53 mmol) in dichloromethane (10 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino phenyl phosphorochloridate (0.40 g, 1.26 mmol) and L-phenylalanine methyl ester hydrochloride (0.27g, 1.26 mmol) in dichloromethane (34 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 41 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 150 ml). The filtered extracts were combined and concentrated under reduced pressure and the resulting residue purified by column chromatography. Elution with 30% ether in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.12 g, 21%).

^{31}P nmr δ($CDCl_3$) 8.18, 8.05 (3:1).

^{13}C nmr δ($CDCl_3$) 173.08, 173.03 ($\underline{C}O_2Me$), 150.80 (*ipso*-PhO), 135.85, 135.76 (*ipso*-CH_2Ph), 129.81, 129.74 (*meta*-PhO), 129.52, 129.46 (*meta*-CH_2Ph), 128.62 (*ortho*-CH_2Ph), 127.22 (*para*-CH_2Ph), 124.76 (*para*-PhO), 120.15, 120.06 (*ortho*-PhO, J=6.1 Hz, J=5.1 Hz), 55.41, 55.04 (CHNH), 52.30, 52.24 (2xd, $CO_2\underline{Me}$, J=2.6 Hz, J=3.3 Hz), 48.98, 48.88 (2x CH_2N, J=4.6 Hz), 42.24 (2x CH_2Cl), 40.71, 40.62 (CH_2Ph, J=6.2 Hz, J=6.7 Hz).

^{1}H nmr δ($CDCl_3$) 7.14-7.31 (m, 10H, 2x Ph), 4.25 (m, 1H, C$\underline{H}$NH), 3.65 (s, 3H, CO_2Me), 3.32-3.50 (m, 9H, 2x CH_2CH_2Cl, CHN$\underline{H}$), 3.27 (t, 2H, C$\underline{H}_2$Ph).

FABMS m/e 461 (MH^+, ^{37}Cl, 9.08%), 459 (MH^+, 17.75%), 401 (M^+-CO_2Me, ^{37}Cl, 2.55%), 399 (M^+-CO_2Me, 5.69%), 369 (M^+-CH_2Ph, ^{37}Cl, 0.97%), 367 (M^+-CH_2Ph, 2.91%), 318 (M^+-$N(CH_2CH_2Cl)_2$, 46.30%), 290 (M^+-CH_2Ph-Ph, 16.65%).

Analytical hplc Stationary phase 250 mm x 4.6 mm lichrosorb RP Select B 7 μM column. Mobile phase methanol-water-triethylamine (70:30:0.01), flow rate 1 ml/min. Retention time 8.99 min.

Analysis $C_{20}H_{25}Cl_2N_2O_4P$ requires: C 52.30 %, H 5.49, N 6.10. Found C 53.06, H 5.97, N 4.93.

N,N-Bis(2-chloroethyl)amino phenyl benzylphenylalaninyl phosphoramidate (96)

A solution of triethylamine (0.31 ml, 2.21 mmol) in dichloromethane (9 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino phenyl phosphorochloridate (0.35 g, 1.11 mmol) and L-phenylalanine benzyl ester p-toluenesulphonate (0.47 g, 1.11 mmol) in dichloromethane (59 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 91 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 25% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave one isomer of the product as a white solid, (0.18 g, 30%).

^{31}P nmr $\delta(CDCl_3)$ 7.67.

^{13}C nmr δ($CDCl_3$) 172.40 (CO_2Me, J=4.6 Hz), 150.64 (*ipso*-PhO), 135.55 (*ipso*-Bz), 135.06 (*ipso*-$PhCH_2$), 129.73 (*meta*-PhO) 129.52 (*meta*-Bz), 128.58-128.60 (m, *meta*-$PhCH_2$, *ortho*-Bz, *ortho*-$PhCH_2$), 128.48 (*para*-Bz), 127.19 (*para*-$PhCH_2$), 124.76 (*para*-PhO), 120.08 (*ortho*-PhO), 67.20 ($PhCH_2OCO$), 55.41 (CHNH), 48.84 (d, 2x CH_2N, J=4.7 Hz), 42.25 (2x CH_2Cl), 40.57 (d, $CHCH_2Ph$, J=6.4 Hz).

^1H nmr δ($CDCl_3$) 7.08-7.35 (m, 15H, 3x Ph), 6.15 (2H, $PhCH_2OCO$), 5.08 (m, 1H, CHNH), 3.27-3.50 (m, 9H, 2x CH_2CH_2Cl, CHNH), 3.03 (d, 2H, $PhCH_2CH$).

FABMS m/e 537 (MH^+, ^{37}Cl, 2.83%), 535 (MH^+, 3.86%), 443 (M^+-CH_2Ph, 0.19%), 401 (M^+-$PhCH_2OCO$, ^{37}Cl, 1.73%), 399 (M^+-$PhCH_2OCO$, 2.98%), 366 (M^+-CH_2Ph-Ph, 16.56%), 91 ($PhCH_2^+$, 100%).

Analytical hplc Stationary phase 50+250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention time 13.68 min.

Analysis $C_{26}H_{29}Cl_2N_2O_4P(H_2O)_2$ requires: C 54.65 %, H 5.12, N 4.90, P 5.42. Found C 54.25, H 5.12, N 4.78, P 5.59.

4-Chlorophenyl phosphorodichloridate (97)

A solution of triethylamine (5.42 ml, 38.89 mmol) and 4-chlorophenol (5.00 g, 38.89 mmol) in ether (44 ml) was added dropwise to a solution of phosphoryl chloride (3.62 ml, 38.89 mmol) in ether (44 ml) at -78°C over a period of 2 hr. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 19 hr. Filtration and concentration of the filtrate under reduced pressure produced a pale yellow oil, (7.93 g, 83%).

^{31}P nmr $\delta(CDCl_3)$ 2.17.

^{13}C nmr $\delta(CDCl_3)$ 148.00 (d, *ipso*-Ph, J=11.6 Hz), 132.84 (d, *para*-Ph, J=2.8 Hz), 130.36 (d, *meta*-Ph, J=1.5 Hz), 121.97 (d, *ortho*-Ph, J=5.6 Hz).

^{1}H nmr $\delta(CDCl_3)$ 7.18-7.42 (m, 4H, Ph).

FABMS m/e 249 (MH^+, 2x ^{37}Cl, 10.32%), 248 (M^+, 2x ^{37}Cl, 29.42%), 247 (MH^+, ^{37}Cl, 43.92%), 246 (M^+, ^{37}Cl, 100%), 245 (MH^+, 45.99%), 244 (M^+, 98.34%), 130 (MH^+-$POCl_2$, ^{37}Cl, 6.57%), 129 (M^+-$POCl_2$, ^{37}Cl, 21.20%), 128 (MH^+-$POCl_2$, 33.49%), 127 (M^+-$POCl_2$, 72.38%), 113 ($ClPh^+$, ^{37}Cl, 10.76%), 111 ($ClPh^+$, 40.68%).

Analysis $C_6H_4Cl_3O_2P$ requires: C 29.36 %, H 1.64, P 12.62. Found C 29.95, H 1.64, P 12.65.

N,N-Bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate (98)

A solution of triethylamine (4.26 ml, 30.56 mmol) in ether (92 ml) was added to a suspension of bis(2-chloroethyl)amine hydrochloride (4.09 g, 22.92 mmol) and 4-chlorophenyl phosphorodichloridate (3.75 g, 15.28 mmol) in ether (92 ml). The addition was carried out dropwise at -78°C and in an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature and stirred for a total of 90 hr. The solvent was then removed under reduced pressure and the product extracted with hexane (2x 100 ml). The combined hexane extracts were filtered and concentrated under reduced pressure to give a colourless oil, (3.98 g, 74%).

^{31}P nmr $\delta(CDCl_3)$ 9.23.

^{13}C nmr δ($CDCl_3$) 148.11 (d, *ipso*-Ph, J=11.6 Hz), 131.70 (*para*-Ph), 130.06 (*meta*-Ph), 121.83 (d, *ortho*-Ph, J=5.4 Hz), 49.66 (d, 2x CH_2N, J=4.3 Hz), 41.36 (d, 2x CH_2Cl, J=2.0 Hz).

^1H nmr δ($CDCl_3$) 7.16-7.36 (m, 4H, Ph), 3.52-3.74 (m, 8H, 2x CH_2CH_2Cl).

FABMS m/e 353 (M^+, 2x ^{37}Cl, 5.45%), 351 (M^+, ^{37}Cl, 13.82%), 349 (M^+, 12.11%), 314 (M^+-Cl, 0.14%), 238 (M^+-ClPh, 0.52%), 146 ($(ClCH_2CH_2)_2NH_2^+$, 2x ^{37}Cl, 8.22%), 144 ($(ClCH_2CH_2)_2NH_2^+$, ^{37}Cl, 62.25%), 142 ($(ClCH_2CH_2)_2NH_2^+$, 100%).

Analysis $C_{10}H_{12}Cl_4NO_2P$ requires: Cl 40.40 %, P 8.82. Found Cl 39.25, P 9.83.

N,N-Bis(2-chloroethyl)amino 4-chlorophenyl methoxyglycinyl phosphoramidate (99)

A solution of triethylamine (0.33 ml, 2.39 mmol) in dichloromethane (10 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate (0.42 g, 1.20 mmol) and glycine methyl ester hydrochloride (0.15g, 1.20 mmol) in dichloromethane (19 ml). The addition was carried out dropwise at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions produced a colourless oil, (0.31 g, 64%).

^{31}P nmr δ($CDCl_3$) 9.12.

^{13}C nmr $\delta(CDCl_3)$ 171.12 (d, $\underline{C}O_2Me$, J=9.0 Hz), 149.11 (d, *ipso*-Ph, J=6.3 Hz), 130.11 (*para*-Ph), 129.73 (*meta*-Ph), 121.45 (d, *ortho*-Ph, J=4.8 Hz), 52.48 (d, $CO_2\underline{Me}$, J=12.2 Hz), 49.03 (d, 2x CH_2N, J=4.7 Hz), 42.15-42.51 (m, CH_2NH, 2x CH_2Cl).

^1H nmr $\delta(CDCl_3)$ 7.12-7.15 (m, 2H, *meta*-Ph), 7.24-7.27(m, 2H, *ortho*-Ph), 3.76 (2xd, 2H, $C\underline{H}_2NH$), 3.72 (s, 3H, CO_2Me), 3.58 (m, 4H, 2x CH_2N), 3.44-3.50 (m, 5H, 2x CH_2Cl, $CH_2N\underline{H}$).

FABMS m/e 407 (MH^+, 2x ^{37}Cl, 16.28%), 405 (MH^+, ^{37}Cl, 66.64%), 403 (MH^+, 64.45%), 345 (M^+-CO_2Me, ^{37}Cl, 8.11%), 343 (M^+-CO_2Me, 8.52%), 277 (M^+-OPhCl, ^{37}Cl, 8.77%), 275 (M^+-OPhCl, 16.48%), 264 (M^+-$N(CH_2CH_2Cl)_2$, ^{37}Cl, 25.90%), 262 (M^+-$N(CH_2CH_2Cl)_2$, 90.15%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column, refractive index 2x. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention time 9.77 min.

Analysis $C_{13}H_{18}Cl_3N_2O_4P$ requires: C 38.68 %, H 4.50, N 6.94. Found C 39.69, H 4.78, N 6.71.

N,N-Bis(2-chloroethyl)amino 4-chlorophenyl methoxyphenylalaninyl phosphoramidate (100)

A solution of triethylamine (0.16 ml, 1.14 mmol) in dichloromethane (5 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate (0.20 g, 0.57 mmol) and L-phenylalanine methyl ester hydrochloride (0.12 g, 0.57 mmol) in dichloromethane (15 ml). The addition was

carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 46 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (3x 50 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 20% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.16 g, 56%).

^{31}P nmr δ($CDCl_3$) 8.19.

^{13}C nmr δ($CDCl_3$) 172.96, 172.73 (2xd, $\underline{C}O_2Me$, J=4.4 Hz, J=6.5 Hz), 149.25 (t, *ipso*-ClPh, J=6.6 Hz), 135.68, 135.38 (*ipso*-CH_2Ph), 130.11, 130.02 (*para*-ClPh), 129.74, 129.68 (*meta*-ClPh), 129.48, 129.44 (*meta*-CH_2Ph), 128.64, 128.51 (*ortho*-CH_2Ph), 127.27, 127.14 (*para*-CH_2Ph), 121.46 (t, *ortho*-ClPh, J=4.5 Hz), 55.40, 54.72 (CHNH), 52.42, 52.34 (2xd, $CO_2\underline{Me}$, J=4.1 Hz, J=1.7 Hz), 48.90, 48.80 (2xd, 2x CH_2N, J=4.7 Hz, J=3.9 Hz), 42.25 (2x CH_2Cl), 40.58, 40.36 (2xd, CH_2Ph, J=7.0 Hz, J=4.4 Hz).

^{1}H nmr δ($CDCl_3$) 6.99-7.30 (m, 9H, ClPh, $CH_2\underline{Ph}$), 4.26 (m, 1H, C$\underline{H}$NH), 3.71, 3.66 (2xs, 3H, CO_2Me), 3.26-3.57 (m, 9H, 2x CH_2CH_2Cl, CHN$\underline{H}$), 3.03 (m, 2H, $C\underline{H}_2Ph$).

FABMS m/e 497 (MH^+, 2x^{37}Cl, 9.83%), 495 (MH^+, ^{37}Cl, 35.33%), 493 (MH^+, 32.15%), 437 (M^+-CO_2Me, 2x ^{37}Cl, 3.96%), 435 (M^+-CO_2Me, ^{37}Cl, 15.68%), 433 (M^+-CO_2Me, 15.47%), 405 (M^+-CH_2Ph, 2x^{37}Cl, 0.89%), 403 (M^+-CH_2Ph, ^{37}Cl, 5.52%), 401 (M^+-CH_2Ph, 6.00%), 367 (M^+-OPhCl, ^{37}Cl, 3.62%), 365 (M^+-OPhCl, 6.08%), 354 (M^+-$N(CH_2CH_2Cl)_2$, ^{37}Cl, 31.58%), 352 (M^+-$N(CH_2CH_2Cl)_2$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water-triethylamine (80:20:0.01), flow rate 1 ml/min. Retention time 9.42 min.

Analysis $C_{20}H_{24}Cl_3N_2O_4P$ requires: C 48.65 %, H 4.90, N 5.67, P 6.27. Found C 49.30, H 4.91, N 5.38, P 6.24.

N,N-Bis(2-chloroethyl)amino 4-chlorophenyl benzylleucinyl phosphoramidate (101)

A solution of triethylamine (0.20 ml, 1.42 mmol) in dichloromethane (6 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate (0.25 g, 0.71 mmol) and L-leucine benzyl ester hydrochloride (0.18 g, 0.71 mmol) in dichloromethane (23 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 20% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.24 g, 63%).

^{31}P nmr δ($CDCl_3$) 8.21, 8.14 (1:1).

^{13}C nmr δ($CDCl_3$) 173.80, 173.67 (2xd, $\underline{C}O_2Me$, J=4.6 Hz, J=3.5 Hz), 149.34, 149.25 (2xd, *ipso*-ClPh, J=6.4 Hz, J=6.5 Hz), 135.25, 135.16 (*ipso*-Bz), 130.00, 129.92 (*para*-ClPh), 129.65 (m, *meta*-ClPh), 128.62, 128.59 (*meta*-Bz), 128.53 (*para*-Bz), 128.49, 128.42 (*ortho*-Bz), 121.38 (d, *ortho*-ClPh, J=4.9 Hz), 67.24, 67.05 (Ph$\underline{C}H_2OCO$), 53.01, 52.55 (CHNH), 49.04, 48.93 (2xd, 2x CH_2N, J=4.5 Hz, J=4.6

Hz), 43.84 (t, $\underline{C}H_2CH(CH_3)_2$, J=6.1 Hz), 42.39, 42.24 (2x CH_2Cl), 24.56, 24.34 ($CH_2\underline{C}H(CH_3)_2$), 22.65, 22.57 ($CH_2CH\underline{C}H_3$), 21.99, 21.80 ($CH_2CH\underline{C}H_3$).

^1H nmr $\delta(CDCl_3)$ 7.06-7.38 (m, 9H, ClPh, $\underline{Ph}CH_2OCO$), 5.16 (m, 2H, $PhC\underline{H}_2OCO$), 4.00 (m, 1H, $C\underline{H}NH$), 3.28-3.61 (m, 9H, 2x CH_2CH_2Cl, $CHN\underline{H}$), 1.74 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.56 (m, 2H, $C\underline{H}_2CH(CH_3)_2$), 0.93, 0.91 (2xs, 3H, $CH_2CHC\underline{H}_3$), 0.87, 0.86 (2xd, 3H, $CH_2CHC\underline{H}_3$).

FABMS m/e 538 (M^+, 2x ^{37}Cl, 2.68%), 536 (M^+, ^{37}Cl, 13.32%), 534 (M^+, 7.78%), 403 (M^+-$PhCH_2OCO$, 2x ^{37}Cl, 7.56%), 401 (M^+-$PhCH_2OCO$, ^{37}Cl, 32.95%), 399 (M^+-$PhCH_2OCO$, 30.76%), 394 (M^+-$N(CH_2CH_2Cl)_2$, 1.00%), 368 (M^+-$CH_2CH(CH_3)_2$-ClPh, ^{37}Cl, 31.27%), 366 (M^+-$CH_2CH(CH_3)_2$-ClPh, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water-triethylamine (80:20:0.01), flow rate 1 ml/min. Retention time 17.45 min.

Analysis $C_{23}H_{30}Cl_3N_2O_4P$ requires: C 51.56 %, H 5.64, N 5.23. Found C 51.54, H 5.73, N 5.24.

N,N-Bis(2-chloroethyl)amino 4-chlorophenyl benzylphenylalaninyl phosphoramidate (102)

A solution of triethylamine (0.20 ml, 1.40 mmol) in dichloromethane (6 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate (0.25 g, 0.70 mmol) and L-phenylalanine benzyl ester p-toluenesulphonate (0.30 g, 0.70 mmol) in dichloromethane (38 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was

allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was removed by rotary evaporation under reduced pressure and then ether (3x 50 ml) was added to the residue. The filtered extracts were combined and concentrated under reduced pressure. This procedure was repeated using hexane (3x 50 ml). The resulting residue was then purified by column chromatography with an eluent of 20% chloroform in petroleum spirit (b.p. 60-80°C). Pooling and evaporation of the appropriate fractions gave the product as a cream coloured solid, (0.25 g, 64%).

^{31}P nmr δ($CDCl_3$) 8.17.

^{13}C nmr δ($CDCl_3$) 172.33, 172.15 (2xd, $\underline{C}O_2Me$, J=4.3 Hz, J=6.6 Hz), 149.27, 149.18 (2xd, *ipso*-ClPh, J=6.8 Hz, J=7.1 Hz), 135.46, 135.20 (*ipso*-Bz), 134.99, 134.91 (*ipso*-CH_2Ph), 130.07, 129.96 (*para*-ClPh), 129.73, 129.66 (*meta*-ClPh), 129.53, 129.49 (*meta*-CH_2Ph), 128.46-128.77 (m, *meta*-Bz, *para*-Bz, *ortho*-Bz, *ortho*-CH_2Ph), 127.22, 127.08 (*para*-CH_2Ph), 121.44 (t, *ortho*-ClPh, J=5.0 Hz), 67.44, 67.25 (Ph$\underline{C}H_2OCO$), 55.39 (CHNH), 54.67 (d, CHNH′, J=2.6 Hz), 48.89, 48.73 (2xd, 2x CH_2N, J=4.5 Hz, J=4.6 Hz), 42.25, 42.22 (2x CH_2Cl), 40.51, 40.28 (2xd, CH$\underline{C}H_2Ph$, J=6.7 Hz, J=4.5 Hz).

^{1}H nmr δ($CDCl_3$) 6.87-7.36 (m, 14H, ClPh, $\underline{Ph}CH_2OCO$, $\underline{Ph}CH_2CH$), 5.09 (m, 2H, PhC$\underline{H}_2$OCO), 4.29 (m, 1H, C$\underline{H}$NH), 3.23-3.53 (m, 9H, 2x CH_2CH_2Cl, CHN$\underline{H}$), 3.02 (m, 2H, PhC$\underline{H}_2$CH).

FABMS m/e 571 (MH^+, ^{37}Cl, 4.73%), 569 (MH^+, 13.61%), 433 (M^+-$PhCH_2OCO$, 20.71%), 400 (M^+-$PhCH_2$-Ph, 78.70%), 91 ($PhCH_2^+$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water-triethylamine (85:15:0.01), flow rate 1 ml/min. Retention times 9.35 min, 10.13 min.

Analysis $C_{26}H_{28}Cl_3N_2O_4P$ requires: C 54.80 %, H 4.95, N 4.92, P 5.44. Found C 55.74, H 5.06, N 4.54, P 5.30.

4-Bromophenyl phosphorodichloridate (103)

A solution of triethylamine (4.03 ml, 28.90 mmol) and 4-bromophenol (5.00 g, 28.90 mmol) in ether (54 ml) was added dropwise to a solution of phosphoryl chloride (2.69 ml, 28.90 mmol) in ether (54 ml) at -78°C over a period of 2 hr. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. Filtration and concentration of the filtrate under reduced pressure produced a colourless oil, (7.35 g, 88%).

^{31}P nmr $\delta(CDCl_3)$ 1.93.

^{13}C nmr $\delta(CDCl_3)$ 148.57 (d, *ipso*-Ph, J=11.4 Hz), 133.36 (d, *meta*-Ph, J=1.8 Hz), 122.35 (d, *ortho*-Ph, J=5.5 Hz), 120.53 (d, *para*-Ph, J=3.4 Hz).

^{1}H nmr $\delta(CDCl_3)$ 7.50 (2xd, 2H, *meta*-Ph), 7.17 (2xd, 2H, *ortho*-Ph).

FABMS m/e 293 (MH^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 14.19%), 292 (M^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 8.08%), 291 (MH^+, ^{81}Br or ^{37}Cl, 38.64%), 290 (M^+, ^{81}Br or ^{37}Cl, 20.08%), 289 (MH^+, 27.48%), 288 (M^+, 8.81%).

Analysis $C_6H_4BrCl_2O_2P$ requires: C 24.86 %, H 1.39, P 10.68. Found C 25.38, H 1.54, P 11.43.

N,N-Bis(2-chloroethyl)amino 4-bromophenyl phosphorochloridate (104)

A solution of triethylamine (3.85 ml, 27.60 mmol) in dichloromethane (83 ml) was added to a suspension of bis(2-chloroethyl)amine hydrochloride (6.16 g, 34.50 mmol) and 4-bromophenyl phosphorodichloridate (4.00 g, 13.80 mmol) in dichloromethane (138 ml). The addition was carried out dropwise at -78°C and in an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature and stirred for a total of 44 hr. The solvent was then removed under reduced pressure and the product extracted with hexane (2x 125 ml). The hexane extract was filtered and concentrated under reduced pressure to give a colourless oil, (2.95 g, 54%).

^{31}P nmr $\delta(CDCl_3)$ 9.00.

^{13}C nmr $\delta(CDCl_3)$ 148.57 (d, *ipso*-Ph, J=7.5 Hz), 133.06 (*meta*-Ph), 122.23 (d, *ortho*-Ph, J=5.3 Hz), 119.38 (d, *para*-Ph, J=2.2 Hz), 49.70 (d, 2x CH_2N, J=4.2 Hz), 41.36 (d, 2x CH_2Cl, J=2.0 Hz).

^{1}H nmr $\delta(CDCl_3)$ 7.40 (2xd, 2H, *meta*-Ph), 7.14 (2xd, 2H, *ortho*-Ph), 3.52-3.74 (m, 8H, 2x CH_2CH_2Cl).

FABMS m/e 400 (MH^+, ^{81}Br + 2x ^{37}Cl or 3x ^{37}Cl, 9.04%), 399 (M^+, ^{81}Br + 2x ^{37}Cl or 3x ^{37}Cl, 3.98%), 3.98 (MH^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 51.97%), 397 (M^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 12.11%), 396 (MH^+, ^{81}Br or ^{37}Cl, 88.38%), 395 (M^+, ^{81}Br or ^{37}Cl, 11.07%), 394 (MH^+, 51.33%), 393 (M^+, 0.29%), 362 (M^+-Cl, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 2.79%), 360 (M^+-Cl, ^{81}Br or ^{37}Cl, 11.10%), 358 (M^+-Cl, 5.50%), 348 (M^+-CH_2Cl, ^{81}Br + ^{37}Cl

or 2x ^{37}Cl, 4.97%), 346 (M^+-CH_2Cl, ^{81}Br or ^{37}Cl, 17.11%), 344 (M^+-CH_2Cl, 9.21%), 65 ($ClCH_2CH_2^+$, ^{81}Br or ^{37}Cl, 34.01%), 63 ($ClCH_2CH_2^+$, 100%).

Analysis $C_{10}H_{12}BrCl_3NO_2P$ requires: C 30.37 %, H 3.06, N 3.54. Found C 30.13, H 2.94, N 3.34.

N,N-Bis(2-chloroethyl)amino 4-bromophenyl methoxyphenylalaninyl phosphoramidate (105)

A solution of triethylamine (0.35 ml, 2.53 mmol) in dichloromethane (10 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-bromophenyl phosphorochloridate (0.50 g, 1.26 mmol) and L-phenylalanine methyl ester hydrochloride (0.27 g, 1.26 mmol) in dichloromethane (68 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 44 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 20% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.45 g, 66%).

^{31}P nmr $\delta(CDCl_3)$ 8.00.

^{13}C nmr $\delta(CDCl_3)$ 173.00, 172.75 (2xd, $\underline{C}O_2Me$, J=4.4 Hz, J=6.6 Hz), 149.87, 149.80 (*ipso*-BrPh), 135.67, 135.36 (*ipso*-CH_2Ph), 132.74, 132.69 (*meta*-BrPh), 129.47, 129.44 (*meta*-CH_2Ph), 128.64, 128.50 (*ortho*-CH_2Ph), 127.26, 127.14 (*para*-CH_2Ph), 121.88 (t, *ortho*-BrPh, J=4.4 Hz), 117.67, 117.58 (*para*-BrPh), 55.39, 54.69 (CHNH),

52.37 (t, $CO_2\underline{Me}$, J=9.4 Hz), 48.89, 48.79 (2xd, 2x CH_2N, J=4.8 Hz, J=4.6 Hz), 42.24 (2x CH_2Cl), 40.57, 40.34 (2xd, CH_2Ph, J=6.4 Hz, J=4.5 Hz).

^1H nmr δ($CDCl_3$) 6.99-7.44 (m, 9H, BrPh, $CH_2\underline{Ph}$), 4.28 (m, 1H, C$\underline{H}$NH), 3.70, 3.65 (2xs, 3H, CO_2Me), 3.25-3.57 (m, 9H, 2x CH_2CH_2Cl, CHN$\underline{H}$), 3.02 (m, 2H, C$\underline{H_2}$Ph).

FABMS m/e 541 (MH^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 9.89%), 539 (MH^+, ^{81}Br or ^{37}Cl, 28.05%), 537 (MH^+, 14.86%), 481 (M^+-CO_2Me, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 3.99%), 479 (M^+-CO_2Me, ^{81}Br or ^{37}Cl, 10.41%), 477 (M^+-CO_2Me, 5.25%), 449 (M^+-CH_2Ph, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 0.89%), 447 (M^+-CH_2Ph, ^{81}Br or ^{37}Cl, 3.68%), 445 (M^+-CH_2Ph, 1.81%), 398 (M^+-$N(CH_2CH_2Cl)_2$, ^{81}Br or ^{37}Cl, 31.94%), 396 (M^+-$N(CH_2CH_2Cl)_2$, 28.43%), 367 (M^+-OPhBr, ^{81}Br or ^{37}Cl, 3.49%), 365 (M^+-OPhBr, 7.15%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 9.94 min, 10.97 min.

Analysis $C_{20}H_{24}BrCl_2N_2O_4P$ requires: C 44.63 %, H 4.50, N 5.20, P 5.76. Found C 44.52, H 4.45, N 5.09, P 6.01.

N,N-Bis(2-chloroethyl)amino 4-bromophenyl benzylleucinyl phosphoramidate (106)

A solution of triethylamine (0.28 ml, 2.02 mmol) in dichloromethane (8 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-bromophenyl phosphorochloridate (0.40 g, 1.01 mmol) and L-leucine benzyl ester hydrochloride (0.26g, 1.01 mmol) in dichloromethane (78 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was removed by rotary

evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 20% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.36 g, 61%).

^{31}P nmr δ($CDCl_3$) 7.93.

^{13}C nmr δ($CDCl_3$) 173.80, 173.67 (2xd, $\underline{C}O_2Me$, J=4.6 Hz, J=3.6 Hz), 149.89, 149.81 (2xd, *ipso*-BrPh, J=6.2 Hz, J=7.0 Hz), 135.24, 135.16 (*ipso*-Bz), 132.65 (*meta*-BrPh), 128.49-128.60 (m, *meta*-Bz, *para*-Bz, *ortho*-Bz), 121.85, 121.80 (*ortho*-BrPh), 117.58, 117.52 (*para*-BrPh), 66.97-67.34 (m, $Ph\underline{C}H_2OCO$), 53.01, 52.56 (2xd, CHNH, J=5.3 Hz, J=4.0 Hz), 49.03, 48.93 (2xd, 2x CH_2N, J=4.6 Hz, J=4.9 Hz), 43.83 (t, $C\underline{H}_2CH(CH_3)_2$, J=6.2 Hz), 42.39, 42.24 (2x CH_2Cl), 24.56, 24.34 ($CH_2\underline{C}H(CH_3)_2$), 22.66, 22.58 ($CH_2CHC\underline{H}_3$), 21.99, 21.80 ($CH_2CHC\underline{H}_3$).

^{1}H nmr δ($CDCl_3$) 6.99-7.41 (m, 9H, BrPh, $\underline{Ph}CH_2OCO$), 5.14 (m, 2H, $PhC\underline{H}_2OCO$), 3.98 (m, 1H, $C\underline{H}NH$), 3.28-3.57 (m, 9H, 2x CH_2CH_2Cl, $CHN\underline{H}$), 1.72 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.50 (m, 2H, $C\underline{H}_2CH(CH_3)_2$), 0.91, 0.89 (2xs, 3H, $CH_2CHC\underline{H}_3$), 0.85, 0.84 (2xd, 3H, $CH_2CHC\underline{H}_3$).

FABMS m/e 585 (MH^+, ^{81}Br + 2x ^{37}Cl, 4.65%), 584 (M^+, ^{81}Br + 2x ^{37}Cl, 6.98%), 583 (MH^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 26.16%), 582 (M^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 14.53%), 581 (MH^+, ^{81}Br or ^{37}Cl, 52.91%), 580 (M^+, ^{81}Br or ^{37}Cl, 10.46%), 579 (MH^+, 33.72%), 578 (M^+, 1.45%), 447 (M^+-$PhCH_2OCO$, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 27.91%), 445 (M^+-$PhCH_2OCO$, ^{81}Br or ^{37}Cl, 56.40%), 443 (M^+-$PhCH_2OCO$, 35.46%), 332 (M^+-$PhCH_2$-BrPh, 2.33%), 91 ($PhCH_2^+$, 100%).

Analysis $C_{23}H_{30}BrCl_2N_2O_4P$ requires: C 47.61 %, H 5.21, N 4.83, P 5.34. Found C 47.70, H 5.34, N 4.45, P 5.47.

N,N-Bis(2-chloroethyl)amino 4-bromophenyl benzylphenylalaninyl phosphoramidate (107)

A solution of triethylamine (0.14 ml, 1.01 mmol) in dichloromethane (4 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-bromophenyl phosphorochloridate (0.20 g, 0.51 mmol) and L-phenylalanine benzyl ester p-toluenesulphonate (0.22 g, 0.51 mmol) in dichloromethane (27 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 72 hr. The solvent was then removed by rotary evaporation under reduced pressure and ether (2x 100 ml) added to the residue. The filtered extracts were combined and concentrated under reduced pressure and the resulting residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid. This was recrystallized to give an isomer, **(107A)** (71 mg, 23%), and a mixture of isomers, **(107B)** (92 mg, 30%).

(107A)

^{31}P nmr $\delta(CDCl_3)$ 10.19.

^{13}C nmr $\delta(CDCl_3)$ 172.32 (d, $\underline{C}O_2Me$, J=4.1 Hz), 149.74 (d, *ipso*-BrPh, J=6.6 Hz), 135.46 (*ipso*-Bz), 134.98 (*ipso*-CH_2Ph), 132.63 (*meta*-BrPh), 129.48 (*meta*-Bz, *meta*-CH_2Ph), 128.60 (*ortho*-Bz, *ortho*-CH_2Ph), 128.53 (*para*-Bz), 127.22 (*para*-CH_2Ph), 121.85 (d, *ortho*-BrPh, J=5.1 Hz), 117.54 (*para*-BrPh), 67.25 (t, Ph$\underline{C}H_2OCO$, J=7.9

Hz), 55.38 (CHNH), 48.72 (d, 2x CH_2N, J=4.4 Hz), 42.24 (2x CH_2Cl), 40.50 (d, $CH\underline{C}H_2Ph$, J=6.7 Hz).

^1H nmr δ($CDCl_3$) 6.99-7.39 (m, 14H, BrPh, $\underline{Ph}CH_2OCO$, $\underline{Ph}CH_2CH$), 5.08, 5.07 (2xs, 2H, $PhC\underline{H}_2OCO$), 4.18-4.36 (m, 1H, $C\underline{H}NH$), 3.26-3.47 (m, 9H, 2x CH_2CH_2Cl, $CHN\underline{H}$), 2.99-3.05 (m, 2H, $PhC\underline{H}_2CH$).

FABMS m/e 615 (MH^+, ^{81}Br or ^{37}Cl, 2.02%), 613 (MH^+, 0.73%), 483 (MH_2^+-$PhCH_2OCO$, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 0.32%), 481 (MH_2^+-$PhCH_2OCO$, ^{81}Br or ^{37}Cl, 1.69%), 479 (MH_2^+-$PhCH_2OCO$, 0.14%), 448 (MH_2^+-CH_2Ph-Ph, ^{81}Br or ^{37}Cl, 2.44%), 446 (MH_2^+-CH_2Ph-Ph, 2.54%), 393 (M^+-$N(CH_2CH_2Cl)_2$-Br, 0.09%), 91 ($PhCH_2^+$, 100%).

Analysis $C_{26}H_{28}BrCl_2N_2O_4P$ requires: C 50.84 %, H 4.59, N 4.56. Found C 50.77, H 4.53, N 4.46.

(107B)

^{31}P nmr δ($CDCl_3$) 10.19, 10.14 (1:1).

^{13}C nmr δ($CDCl_3$) 172.35, 172.17 (2xd, $\underline{C}O_2Me$, J=4.1 Hz, J=6.4 Hz), 149.84, 149.75 (2xd, *ipso*-BrPh, J=6.7 Hz, J=6.1 Hz), 135.47, 135.20 (*ipso*-Bz), 135.00, 134.92 (*ipso*-CH_2Ph), 132.72, 132.65 (*meta*-BrPh), 129.54 (*meta*-Bz), 129.50 (*meta*-CH_2Ph), 128.54-128.78 (m, *para*-Bz, *ortho*-Bz), 128.48 (*ortho*-CH_2Ph), 127.24, 127.09 (*para*-CH_2Ph), 121.89 (t, *ortho*-BrPh, J=5.4 Hz), 117.69, 117.58 (*para*-BrPh), 67.27-67.53 (m, $Ph\underline{C}H_2OCO$), 55.40 (CHNH), 54.69 (d, CHNH′, J=2.6 Hz), 48.89, 48.74 (2xd, 2x CH_2N, J=4.4 Hz, J=4.2 Hz), 42.25, 42.22 (2x CH_2Cl), 40.52, 40.29 (2xd, $CH\underline{C}H_2Ph$, J=6.5 Hz, J=4.6 Hz).

^{1}H nmr δ($CDCl_3$) 6.86-7.41 (m, 14H, BrPh, $\underline{Ph}CH_2OCO$, $\underline{Ph}CH_2CH$), 5.03-5.15 (m, 2H, $PhC\underline{H}_2OCO$), 4.24-4.32 (m, 1H, $C\underline{H}NH$), 3.24-3.53 (m, 9H, 2x CH_2CH_2Cl, $CHN\underline{H}$), 2.97-3.07 (m, 2H, $PhC\underline{H}_2CH$).

FABMS m/e 617 (MH^+, 2x ^{81}Br or 2x ^{37}Cl, 1.40%), 615 (MH^+, ^{81}Br or ^{37}Cl, 0.30%), 613 (MH^+, 0.18%), 91 ($PhCH_2^+$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 19.82 min, 22.14 min.

Analysis $C_{26}H_{28}BrCl_2N_2O_4P$ requires: C 50.84 %, H 4.59, N 4.56. Found C 50.62, H 4.43, N 4.44.

4-Fluorophenyl phosphorodichloridate (108)

A solution of triethylamine (6.22 ml, 44.60 mmol) and 4-fluorophenol (5.00 g, 44.60 mmol) in ether (51 ml) was added dropwise to a solution of phosphoryl chloride (4.16 ml, 44.60 mmol) in ether (51 ml) at -78°C over a period of 2 hr. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 19 hr. Filtration and concentration of the filtrate under reduced pressure produced a pale yellow oil, (8.37, 82%).

^{31}P nmr δ($CDCl_3$) 2.30.

^{13}C nmr δ($CDCl_3$) 160.70 (2xd, *para*-Ph, J_{C-P}=2.6 Hz, J_{C-F}=247.0 Hz), 145.38 (2xd, *ipso*-Ph, J_{C-P}=1.3 Hz, J_{C-F}=11.6 Hz), 122.22 (2xd, *meta*-Ph, J_{C-P}=5.4 Hz, J_{C-F}=8.3 Hz), 117.01 (2xd, *ortho*-Ph, J_{C-P}=1.9 Hz, J_{C-F}=23.8 Hz).

^{1}H nmr $\delta(CDCl_3)$ 7.00-7.11 (m, 2H, *meta*-Ph), 7.20-7.28 (m, 2H, *ortho*-Ph).

FABMS m/e 230 (M^+, ^{37}Cl, 21.38%), 228 (M^+, 42.59%), 192 (M^+-HCl, 1.97%), 117 (M^+-OPhF, 4.49%), 111 ($FPhO^+$, 69.35%), 95 (FPh^+, 25.00%), 83 (MH^+-OPhF-Cl, 100%).

Analysis $C_6H_4Cl_2FO_2P$ requires: C 31.47 %, H 1.76, P 13.53. Found C 32.90, H 1.78, P 11.74.

N,N-Bis(2-chloroethyl)amino 4-fluorophenyl phosphorochloridate (109)

A solution of triethylamine (3.04 ml, 21.84 mmol) in dichloromethane (65 ml) was added to a suspension of bis(2-chloroethyl)amine hydrochloride (4.87 g, 27.30 mmol) and 4-fluorophenyl phosphorodichloridate (2.50 g, 10.92 mmol) in dichloromethane (109 ml). The addition was carried out dropwise at -78°C and in an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature and stirred for a total of 46 hr. The solvent was then removed under reduced pressure and the product extracted with hexane (2x 100 ml). The hexane extract was filtered and concentrated under reduced pressure to give a colourless oil, (2.30 g, 63%).

^{31}P nmr $\delta(CDCl_3)$ 9.45.

^{13}C nmr $\delta(CDCl_3)$ 160.34 (2xd, *para*-Ph, J_{C-P}=1.8 Hz, J_{C-F}=245.6 Hz), 145.34 (2xd, *ipso*-Ph, J_{C-P}=3.0 Hz, J_{C-F}=5.8 Hz), 121.98 (2xd, *meta*-Ph, J_{C-P}=5.3 Hz, J_{C-F}=8.3 Hz), 116.69 (d, *ortho*-Ph, J_{C-F}=24.1 Hz), 49.67 (d, 2x CH_2N, J=4.3 Hz), 41.36 (d, 2x CH_2Cl, J=1.8 Hz).

^{1}H nmr δ($CDCl_3$) 7.01-7.09 (m, 2H, *meta*-Ph), 7.18-7.26 (m, 2H, *ortho*-Ph), 3.52-3.73 (m, 8H, 2x CH_2CH_2Cl).

FABMS m/e 338 (MH^+, 2x ^{37}Cl, 23.55%), 337 (M^+, 2x ^{37}Cl, 8.82%), 336 (MH^+, ^{37}Cl, 88.04%), 335 (M^+, ^{37}Cl, 15.32%), 334 (MH^+, 100%), 333 (M^+, 4.45%), 300 (M^+-Cl, ^{37}Cl, 10.11%), 298 (M^+-Cl, 19.81%), 286 (M^+-CH_2Cl, ^{37}Cl, 17.11%), 284 (M^+-CH_2Cl, 28.32%), 224 (M^+-OPhF, ^{37}Cl, 2.10%), 222 (M^+-OPhF, 9.90%).

Analysis $C_{10}H_{12}Cl_3FNO_2P$ requires: C 35.90 %, H 3.62, N 4.19, Cl 31.79. Found C 36.75, H 3.45, N 4.00, Cl 30.00.

N,N-Bis(2-chloroethyl)amino 4-fluorophenyl methoxyphenylalaninyl phosphoramidate (110)

A solution of triethylamine (0.29 ml, 2.09 mmol) in dichloromethane (10 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-fluorophenyl phosphorochloridate (0.35 g, 1.05 mmol) and L-phenylalanine methyl ester hydrochloride (0.23g, 1.05 mmol) in dichloromethane (29 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 45 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 25% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.35 g, 69%).

^{31}P nmr δ($CDCl_3$) 8.13.

^{13}C nmr δ($CDCl_3$) 173.04, 172.78 (2xd, $\underline{C}O_2Me$, J=4.5 Hz, J=6.6 Hz), 159.50, 159.45 (2xd, *para*-FPh, J_{C-F}=243.5 Hz), 146.44-146.64 (m, *ipso*-FPh), 135.70, 135.42 (*ipso*-CH_2Ph), 129.48, 129.44 (*meta*-CH_2Ph), 128.63, 128.48 (*ortho*-CH_2Ph), 127.25, 127.12 (*para*-CH_2Ph), 121.38-121.54 (m, *meta*-FPh), 116.29, 116.23 (2xd, *ortho*-FPh, J_{C-F}=23.4 Hz), 55.40, (CHNH), 54.71 (d, CHNH′, J=2.3 Hz), 52.25-52.44 (m, $CO_2\underline{Me}$), 48.90, 48.81 (2xd, 2x CH_2N, J=4.9 Hz, J=4.6 Hz), 42.25 (2x CH_2Cl), 40.59, 40.36 (2xd, CH_2Ph, J=6.5 Hz, J=4.3 Hz).

^1H nmr δ($CDCl_3$) 6.95-7.31 (m, 9H, FPh, $CH_2\underline{Ph}$), 4.25 (m, 1H, C$\underline{H}$NH), 3.70, 3.65 (2xs, 3H, CO_2Me), 3.24-3.64 (m, 9H, 2x CH_2CH_2Cl, CHN$\underline{H}$), 3.02 (m, 2H, C$\underline{H_2}$Ph).

FABMS m/e 480 (MH_2^+, ^{37}Cl, 0.53%), 479 (MH^+, ^{37}Cl, 5.58%), 478 (MH_2^+, 2.65%), 477 (MH^+, 12.01%), 419 (M^+-CO_2Me, ^{37}Cl, 4.17%), 417 (M^+-CO_2Me, 6.77%), 387 (M^+-CH_2Ph, ^{37}Cl, 0.49%), 385 (M^+-CH_2Ph, 0.90%), 367 (M^+-OPhF, ^{37}Cl, 0.38%), 365 (M^+-OPhF, 0.40%), 336 (M^+-$N(CH_2CH_2Cl)_2$, 30.27%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water: (80:20), flow rate 1 ml/min. Retention times 7.69 min, 11.28 min.

Analysis $C_{20}H_{24}Cl_2FN_2O_4P$ requires: C 50.33 %, H 5.07, N 5.87, P 6.49. Found C 49.89, H 5.00, N 5.60, P 6.65.

N,N-Bis(2-chloroethyl)amino 4-fluorophenyl benzylleucinyl phosphoramidate (111)

A solution of triethylamine (0.33 ml, 2.39 mmol) in dichloromethane (11 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-fluorophenyl phosphorochloridate (0.40 g, 1.20 mmol) and L-leucine benzyl ester hydrochloride

(0.31 g, 1.20 mmol) in dichloromethane (40 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 18% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.41 g, 66%).

^{31}P nmr δ($CDCl_3$) 8.32.

^{13}C nmr δ($CDCl_3$) 173.84, 173.70 (2xd, $\underline{C}O_2Me$, J=4.6 Hz, J=3.6 Hz), 159.46, 159.41 (2xd, *para*-FPh, J_{C-F}=243.5 Hz), 146.48-146.68 (m, *ipso*-FPh), 135.26, 135.17 (*ipso*-Bz), 128.40-128.61 (m, *meta*-Bz, *ortho*-Bz, *para*-Bz), 121.33-121.46 (*meta*-FPh), 116.32, 116.08 (*ortho*-FPh), 67.22, 67.02 ($Ph\underline{C}H_2OCO$), 53.01 (CHNH), 52.57 (d, CHNH′, J=2.0 Hz), 49.05, 48.92 (2xd, 2x CH_2N, J=4.6 Hz, J=4.7 Hz), 43.88, 43.82 (2xd, $\underline{C}H_2CH(CH_3)_2$, J=2.7 Hz, J=4.1 Hz), 42.39, 42.26 (2x CH_2Cl), 24.55, 24.32 ($CH_2\underline{C}H(CH_3)_2$), 22.65, 22.56 ($CH_2CH\underline{C}H_3$), 22.00, 21.78 ($CH_2CH\underline{C}H_3$).

^{1}H nmr δ($CDCl_3$) 6.94-7.38 (m, 9H, $\underline{Ph}CH_2OCO$, FPh), 5.16 (m, 2H, $PhC\underline{H}_2OCO$), 4.00 (m, 1H, $C\underline{H}NH$), 3.24-3.60 (m, 9H, 2x CH_2CH_2Cl, $CHN\underline{H}$), 1.74 (m, 1H, $CH_2C\underline{H}(CH_3)_2$), 1.56 (m, 2H, $C\underline{H}_2CH(CH_3)_2$), 0.93, 0.92 (2xs, 3H, $CH_2CHC\underline{H}_3$), 0.87, 0.85 (2xd, 3H, $CH_2CHC\underline{H}_3$).

FABMS m/e 520 (M^+, ^{37}Cl, 2.55%), 518 (M^+, 3.83%), 407 (M^+-OPhF, 0.39%), 385 (M^+-$PhCH_2OCO$, ^{37}Cl, 4.80%), 383 (M^+-$PhCH_2OCO$, 7.23%), 350 (MH^+-2x CH_2CH_2Cl-$CH(CH_3)_2$, 20.93%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention time 14.22 min.

Analysis $C_{23}H_{30}Cl_2FN_2O_4P$ requires: C 53.19 %, H 5.82, N 5.39. Found C 52.88, H 5.71, N 5.22.

N,N-Bis(2-chloroethyl)amino 4-fluorophenyl benzylphenylalaninyl phosphoramidate (112)

A solution of triethylamine (0.33 ml, 2.39 mmol) in dichloromethane (11 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-fluorophenyl phosphorochloridate (0.40 g, 1.20 mmol) and L-phenylalanine benzyl ester p-toluenesulphonate (0.51 g, 1.20 mmol) in dichloromethane (63 ml). The addition was carried out at -78°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated under reduced pressure and the resulting residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.44 g, 67%).

^{31}P nmr $\delta(CDCl_3)$ 8.19.

^{13}C nmr $\delta(CDCl_3)$ 172.36, 172.17 (2xd, $\underline{C}O_2Me$, J=4.3 Hz, J=6.5 Hz), 159.48, 159.41 (2xd, *para*-FPh, $J_{C\text{-}F}$=243.6 Hz), 146.42-146.62 (m, *ipso*-FPh), 135.48, 135.23 (*ipso*-Bz), 134.99, 134.92 (*ipso*-CH_2Ph), 129.52, 129.48 (*meta*-Bz), 128.43-128.75 (*ortho*-

Bz, *meta*-CH_2Ph, *para*-Bz, *ortho*-CH_2Ph), 127.20, 127.04 (*para*-CH_2Ph), 121.36-121.53 (m, *meta*-FPh), 116.27, 116.20 (2xd, *ortho*-FPh, J_{C-F}=23.4 Hz), 67.40, 67.20 ($PhCH_2OCO$), 55.39 (CHNH), 54.68 (d, CHNH', J=1.9 Hz), 48.88, 48.72 (2xd, 2x CH_2N, J=4.6 Hz), 42.24, 42.21 (2x CH_2Cl), 40.52, 40.27 (2xd, $CHCH_2Ph$, J=6.5 Hz, J=4.5 Hz).

^1H nmr δ($CDCl_3$) 6.90-7.39 (m, 14H, $PhCH_2CH$, $PhCH_2OCO$, FPh), 5.11 (m, 2H, $PhCH_2OCO$), 4.31 (m, 1H, CHNH), 3.27-3.58 (m, 9H, 2x CH_2CH_2Cl, CHNH), 3.04 (m, 2H, $PhCH_2CH$).

FABMS m/e 557 (MH^+, 2x ^{37}Cl, 0.81%), 556 (MH_2^+, ^{37}Cl, 1.05%), 555 (MH^+, ^{37}Cl, 3.37%), 554 (MH_2^+, 1.51%), 553 (MH^+, 4.77%), 465 (MH_2^+-CH_2Ph, ^{37}Cl, 1.28%), 463 (MH_2^+-CH_2Ph, 1.98%), 421 (M^+-$PhCH_2OCO$, 2x ^{37}Cl, 1.63%), 419 (M^+-$PhCH_2OCO$, ^{37}Cl, 5.70%), 417 (M^+-$PhCH_2OCO$, 7.56%), 384 (M^+-$PhCH_2$-Ph, 20.00%), 91 ($PhCH_2^+$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 13.48 min, 14.70 min.

Analysis $C_{26}H_{28}Cl_2FN_2O_4P$ requires: C 56.43 %, H 5.10, N 5.06. Found C 55.69, H 4.78, N 4.78.

N,N-Bis(2-chloroethyl)amino propyl methoxyphenylalaninyl-phenylalaninyl phosphoramidate (113)

A solution of triethylamine (0.12 ml, 0.85 mmol) in dichloromethane (13 ml) was added dropwise with stirring to a solution of bis(2-chloroethyl)amino propyl

phosphorochloridate (0.12 g, 0.42 mmol) and di-L-phenylalanine methyl ester hydrochloride (0.15 g, 0.42 mmol) in dichloromethane (22 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 150 ml). The extracts were filtered, combined and concentrated to give a residue which was partially purified by column chromatography (30% chloroform in petroleum spirit (b.p. 60-80°C). Further purification of this material was achieved by preparative HPLC. Stationary phase 250 mm x 4.6 mm Kromasil 5 μM silica gel column, mobile phase methanol and water. The product was isolated as a white solid, (72 mg, 30%).

^{31}P nmr δ($CDCl_3$) 14.49, 13.96 (1:1).

^{31}C nmr δ($CDCl_3$) 171.85, 171.80 ($\underline{C}O_2Me$), 171.61, 171.43 (2xd, NHCO, J=4.4 Hz, J=3.5 Hz), 136.60, 136.42 (*ipso*-Ph2), 135.90, 135.81 (*ipso*-Ph1), 129.86, 129.64 (*meta*-Ph2), 129.15, 129.11 (*meta*-Ph1), 128.73, 128.52 (*ortho*-Ph2), 128.43, 128.35 (*ortho*-Ph1), 126.86-127.12 (m, *para*-Ph2, *para*-Ph1), 67.48, 67.36 (2xd, $CH_3CH_2CH_2OP$, J=5.4 Hz, J=5.3 Hz), 56.15, 55.89 ($MeO_2C\underline{C}HNH$), 53.15, 53.22 (2xd, $NHCO\underline{C}HNH$, J=6.2 Hz, J=6.5 Hz), 52.23, 52.17 (2xd, $CO_2\underline{Me}$, J=3.5 Hz, J=3.6 Hz), 48.65, 48.52 (2xd, 2x CH_2N, J=4.6 Hz, J=4.7 Hz), 42.56 (2x CH_2Cl), 39.31, 38.96 (2xd, $\underline{C}H_2$Ph-Ph-2, J=5.4 Hz, J=5.5 Hz), 37.82, 37.74 ($\underline{C}H_2$Ph-Ph1), 23.64, 23.57 (2xd, $CH_3\underline{C}H_2CH_2OP$, J=3.9 Hz), 10.10, 10.02 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 7.05-7.50 (m, 10H, 2x Ph), 4.98 (m, 2H, $CH_3CH_2C\underline{H}_2OP$), 4.14 (m, 1H, $MeO_2CC\underline{H}NH$), 4.00 (m, 1H, $NHCOC\underline{H}NH$), 3.86, 3.85 (2xs, 3H, $CO_2\underline{Me}$),

3.60-3.69 (m, 4H, 2x CH_2N), 3.05-3.43 (m, 10H, 2x CH_2Cl, N<u>H</u>COCHN<u>H</u>, 2x C<u>H</u>$_2$Ph), 1.78 (m, 2H, $CH_3C\underline{H}_2CH_2OP$), 1.07 (2xt, 3H, $C\underline{H}_3CH_2CH_2OP$).

FABMS m/e 574 (MH^+, ^{37}Cl, 0.30%), 572 (MH^+, 0.64%), 430 (M^+-H-$N(CH_2CH_2Cl)_2$, 90.41%), 366 (MH_2^+-OC_3H_7-CO_2Me-CH_2Ph, ^{37}Cl, 8.22%), 364 (MH_2^+-C_3H_7O-CO_2Me-CH_2Ph, 15.07%), 120 ($(PhCH_2CHNH_2)^+$, 100%).

Analytical hplc Stationary phase 50+250 mm x 4.6 mm kromasil C18 5 μM column, mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 8.62 min, 9.13 min.

Analysis $C_{26}H_{36}Cl_2N_3O_5P$ requires: C 54.55 %, H 6.34, N 7.34. Found C 54.16, H 6.54, N 7.10.

N,N-Bis(2-chloroethyl)amino 4-chlorophenyl methoxyphenylalaninyl-phenylalaninyl phosphoramidate (114)

A solution of triethylamine (0.15 ml, 1.10 mmol) in dichloromethane (16 ml) was added dropwise to a suspension of N,N-bis(2-chloroethyl)amino 4-chlorophenyl phosphorochloridate (0.19 g, 0.55 mmol) and di-L-phenylalanine methyl ester hydrochloride (0.20g, 0.55 mmol) in dichloromethane (29 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 10% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave a colourless oil. This was recrystallized from petroleum

spirit (b.p. 40-60°C) to give the product (white solid) as mixtures of isomers, **(114A)** (44 mg, 12%), **(114B)** (77 mg, 22%) and **(114C)** (34 mg, 10%).

(114A)

^{31}P nmr δ($CDCl_3$) 11.80, 11.40 (8:2).

^{13}C nmr δ($CDCl_3$) 171.41, 171.34 ($\underline{C}O_2Me$), 171.17, 171.03 (2xd, NHCO, J=5.1 Hz, J=4.5 Hz), 149.14, 149.08 (2xd, *ipso*-ClPh, J=3.7 Hz, J=4.0 Hz), 136.23, 136.02 (*ipso*-Ph2), 135.77, 135.63 (*ipso*-Ph1), 130.20, 130.13 (*para*-ClPh), 129.90, 129.78 (*meta*-ClPh), 129.73 (*meta*-Ph2), 129.20, 129.15 (*meta*-Ph1), 128.88, 128.64 (*ortho*-Ph2), 128.52, 128.46 (*ortho*-Ph1), 127.35, 127.10 (*para*-Ph2), 127.05, 127.00 (*para*-Ph1), 121.46, 121.37 (2xd, *ortho*-ClPh, J=5.1 Hz), 56.10, 55.90 ($MeO_2C\underline{C}HNH$), 53.47 (d, NHCO$\underline{C}$HNH, J=2.5 Hz), 53.20 (NHCO$\underline{C}$HNH'), 52.34, 52.28 (2xd, $CO_2\underline{Me}$, J=4.7 Hz, J=4.0 Hz), 48.56, 48.42 (2xd, 2x CH_2N, J=4.9 Hz, J=4.6 Hz), 42.33 (2x CH_2Cl), 39.45, 39.20 (2xd, $\underline{C}H_2$Ph-Ph2, J=4.6 Hz, J=5.5 Hz), 37.88, 37.79 ($\underline{C}H_2$Ph-Ph1).

^{1}H nmr δ($CDCl_3$) 6.80-7.33 (m, 14H, 2x Ph, ClPh), 4.81 (m, 1H, $MeO_2CC\underline{H}NH$), 4.09 (m, 1H, NHCOC$\underline{H}$NH), 3.68, 3.65 (2xs, 3H, CO_2Me), 3.42 (m, 4H, 2x CH_2N), 3.12-3.30 (m, 6H, 2x CH_2Cl, N$\underline{H}$COCHN$\underline{H}$), 2.94-3.10 (m, 4H, 2x C$\underline{H}_2$Ph).

FABMS m/e 640 (MH^+, 9.34%), 639 (M^+, 6.24%), 498 (M^+-H-N($CH_2CH_2Cl)_2$, 1.91%), 413 (M^+-H-ClPhO-2x CH_2Cl, 6.41%), 326 (MH^+-ClPhOP(O)N($CH_2CH_2Cl)_2$, 13.46%), 176 (MH^+-CO_2Me-CH_2Ph-ClPhOP(O)N($CH_2CH_2Cl)_2$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 14.60 min, 16.64 min.

Analysis $C_{29}H_{33}Cl_3N_3O_5P$ requires: C 54.35 %, H 5.19, N 6.56, P 4.83. Found C 53.99, H 5.28, N 6.16, P 4.96.

(114B)

^{31}P nmr δ($CDCl_3$) 12.06, 11.66 (3:7).

^{13}C nmr δ($CDCl_3$) 171.42, 171.40 ($\underline{C}O_2Me$), 171.17, 171.02 (NHCO, J=5.3 Hz, J=4.5 Hz), 149.05-149.16 (m, *ipso*-ClPh), 136.22, 136.01 (*ipso*-Ph2), 135.76, 135.61 (*ipso*-Ph1), 130.20, 130.12 (*para*-ClPh), 129.89, 129.77 (*meta*-ClPh), 129.71 (*meta*-Ph2), 129.19, 129.14 (*meta*-Ph1), 128.87, 128.63 (*ortho*-Ph2), 128.52, 128.45 (*ortho*-Ph1), 127.34, 127.09 (*para*-Ph2), 127.04, 126.99 (*para*-Ph1), 121.45, 121.36 (2xd, *ortho*-ClPh, J=4.9 Hz, J=5.2 Hz), 56.09, 55.88 ($MeO_2C\underline{C}HNH$), 53.47, 53.17 ($NHCO\underline{C}HNH$), 52.35, 52.28 ($CO_2\underline{Me}$), 48.54, 48.40 (2xd, 2x CH_2N, J=4.4 Hz, J=4.6 Hz), 42.33 (2x CH_2Cl), 39.43, 39.19 (2xd, $\underline{C}H_2Ph$-Ph2, J=4.8 Hz, J=5.5 Hz), 37.87, 37.78 ($\underline{C}H_2Ph$-Ph1).

^{1}H nmr δ($CDCl_3$) 6.82-7.35 (m, 14H, 2x Ph, ClPh), 4.78 (m, 1H, $MeO_2CC\underline{H}NH$), 4.09 (m, 1H, $NHCOC\underline{H}NH$), 3.69, 3.66 (2xs, 3H, CO_2Me), 2.99-3.53 (m, 14H, 2x CH_2CH_2Cl, $N\underline{H}COCHN\underline{H}$, 2x $C\underline{H}_2Ph$).

FABMS m/e 644 (MH^+, 2x ^{37}Cl, 3.18%), 642 (MH^+, ^{37}Cl, 8.88%), 640 (MH^+, 9.17%), 499 (M^+-$N(CH_2CH_2Cl)_2$, 100%), 327 (MH_2^+-$ClPhOP(O)N(CH_2CH_2Cl)_2$, 32.51%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 12.48 min, 14.08 min.

Analysis $C_{29}H_{33}Cl_3N_3O_5P$ requires: C 54.35 %, H 5.19, N 6.56. Found C 53.49, H 5.14, N 6.14.

(114C)

^{31}P nmr δ($CDCl_3$) 12.22, 11.79 (1:9).

^{13}C nmr δ($CDCl_3$) 171.49 ($\underline{C}O_2Me$), 171.25 (d, NHCO, J=5.1 Hz), 149.10 (d, *ipso*-ClPh, J=6.7 Hz), 136.26 (*ipso*-Ph2), 135.78 (*ipso*-Ph1), 130.23, 130.18 (*para*-ClPh), 129.82, 129.75 (*meta*-ClPh), 129.70 (*meta*-Ph2), 129.15 (*meta*-Ph1), 128.86, 128.62 (*ortho*-Ph2), 128.51, 128.44 (*ortho*-Ph1), 127.07 (*para*-Ph2), 127.03 (*para*-Ph1), 121.45 (d, *ortho*-ClPh, J=4.9 Hz), 56.17 ($MeO_2C\underline{C}HNH$), 53.48 ($NHCO\underline{C}HNH$), 52.35 (d, $CO_2\underline{Me}$, J=3.3 Hz), 48.53 (2x CH_2N, J=4.4 Hz), 42.31 (2x CH_2Cl), 39.45 (d, $\underline{C}H_2Ph$-Ph2, J=5.1 Hz), 37.84, 37.76 ($\underline{C}H_2Ph$-Ph1).

^1H nmr δ($CDCl_3$) 6.90-7.22 (m, 14H, 2x Ph, ClPh), 4.72 (m, 1H, $MeO_2CC\underline{H}NH$), 4.03 (m, 1H, $NHCOC\underline{H}NH$), 3.63, 3.60 (2xs, 3H, CO_2Me), 3.39 (m, 4H, 2x CH_2N), 3.14-3.29 (m, 6H, 2x CH_2Cl, $N\underline{H}COCHN\underline{H}$), 2.94-3.07 (m, 4H, 2x $C\underline{H}_2Ph$).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 13.50 min, 24.02 min.

Analysis $C_{29}H_{33}Cl_3N_3O_5P$ requires: C 54.35 %, H 5.19, N 6.56. Found C 57.56, H 6.07, N 5.69.

N,N-Bis(2-chloroethyl)amino-4-bromophenyl methoxyphenylalaninyl-phenylalaninyl phosphoramidate (115)

A solution of triethylamine (0.19 ml, 1.36 mmol) in dichloromethane (20 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-bromophenyl phosphorochloridate (0.27 g, 0.68 mmol) and di-L-phenylalanine methyl ester hydrochloride (0.25g, 0.68 mmol) in dichloromethane (36 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 20% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid. This was recrystallized from petroleum spirit (b.p. 60-80°C) to give the product as mixtures of isomers, **(115A)** (0.15 g, 32%) and **(115B)** (0.11 g, 24%).

(115A)

^{31}P nmr δ($CDCl_3$) 11.66, 11.25 (8:1).

^{13}C nmr δ($CDCl_3$) 171.41, 171.42 ($\underline{C}O_2Me$), 171.04 (d, NHCO, J=4.5 Hz), 149.65 (d, *ipso*-BrPh, J=6.2 Hz), 136.20, 136.03 (*ipso*-Ph2), 135.76, 135.63 (*ipso*-Ph1), 132.75, 132.71 (*meta*-BrPh), 129.87, 129.71 (*meta*-Ph2), 129.20, 129.15 (*meta*-Ph1), 128.87, 128.64 (*ortho*-Ph2), 128.52, 128.46 (*ortho*-Ph1), 127.34, 127.09 (*para*-Ph2), 127.05, 127.00 (*para*-Ph1), 121.89, 121.80 (2xd, *ortho*-BrPh, J=5.0 Hz, J=5.1 Hz), 117.72

(*para*-BrPh), 56.13, 55.91 ($MeO_2C\underline{C}HNH$), 53.50, 53.20 ($NHCO\underline{C}HNH$), 52.36, 52.28 (2xd, $CO_2\underline{Me}$, J=3.4 Hz, J=3.2 Hz), 49.08, 48.44 (2xd, 2x CH_2N, J=4.4 Hz, J=4.6 Hz), 42.32 (2x CH_2Cl), 39.44, 39.22 (2xd, $\underline{C}H_2Ph$-Ph2, J=5.5 Hz), 37.87, 37.78 ($\underline{C}H_2Ph$-Ph1).

^1H nmr δ($CDCl_3$) 6.81-7.42 (m, 14H, 2x $CH_2\underline{Ph}$, BrPh), 4.80 (m, 1H, $MeO_2CC\underline{H}NH$), 4.08 (m, 1H, $NHCOC\underline{H}NH$), 3.67, 3.64 (2xs, 3H, CO_2Me), 3.39 (m, 4H, 2x CH_2N), 3.09-3.29 (m, 6H, 2x CH_2Cl, $N\underline{H}COCHN\underline{H}$), 2.94-3.07 (m, 4H, 2x $C\underline{H}_2Ph$).

FABMS m/e 687 (M^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 12.79%), 685 (M^+, ^{81}Br or ^{37}Cl, 25.58%), 683 (M^+, 15.12%), 606 (M^+-Ph, 5.81%), 545 (M^+-$N(CH_2CH_2Cl)_2$, ^{81}Br, 95.35%), 543 (M^+-$N(CH_2CH_2Cl)_2$, 100%), 512 (M^+-OPhBr, 3.49%), 465 (MH^+-$N(CH_2CH_2Cl)_2$-Br, 45.84%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 14.60 min, 16.55 min.

Analysis $C_{29}H_{33}BrCl_2N_3O_5P$ requires: C 50.82 %, H 4.85, N 6.13. Found C 51.13, H 4.78, N 5.84.

(115B)

^{31}P nmr ($CDCl_3$) 9.15, 8.60 (1:3)

^{13}C nmr δ($CDCl_3$) 171.44 ($\underline{C}O_2Me$), 171.23, 171.07 (2xd, NHCO, J=5.1 Hz, J=6.0 Hz), 149.68 (d, *ipso*-BrPh, J=6.4 Hz), 136.23, 136.04 (*ipso*-Ph2), 135.77, 135.61 (*ipso*-Ph1), 132.70, 132.59 (*meta*-BrPh), 129.84, 129.67 (*meta*-Ph2), 129.13, 129.12

(*meta*-Ph1), 128.81, 128.57 (*ortho*-Ph2), 128.47, 128.41 (*ortho*-Ph1), 127.27, 127.02 (*para*-Ph2), 126.99, 126.95 (*para*-Ph1), 121.86, 121.77 (2xd, *ortho*-BrPh, J=4.8 Hz, J=5.0 Hz), 117.72 (*para*-BrPh), 56.11, 55.91 ($MeO_2C\underline{C}HNH$), 53.45, 53.19 ($NHCO\underline{C}HNH$), 52.30, 52.26 (d, $CO_2\underline{Me}$, J=3.7 Hz), 48.56, 48.41 (2xd, 2x CH_2N, J=4.4 Hz, J=4.7 Hz), 42.26 (2x CH_2Cl), 39.47, 39.20 (2xd, $\underline{C}H_2Ph$-Ph2, J=4.7 Hz, J=5.1 Hz), 37.82, 37.74 ($\underline{C}H_2Ph$-Ph1).

^1H nmr ($CDCl_3$) 6.95-7.41 (m, 14H, 2x $CH_2\underline{Ph}$, BrPh), 4.78 (m, 1H, $MeO_2CC\underline{H}NH$), 4.07 (m, 1H, $NHCOC\underline{H}NH$), 3.66, 3.64 (2xs, 3H, CO_2Me), 3.42 (m, 4H, 2x CH_2N), 3.22-3.30 (m, 6H, 2x CH_2Cl, $N\underline{H}COCHN\underline{H}$), 2.81-3.09 (m, 4H, 2x $C\underline{H}_2Ph$).

FABMS m/e 688 (MH^+, ^{81}Br + ^{37}Cl or 2x ^{37}Cl, 3.49%), 686 (MH^+, ^{81}Br or ^{37}Cl, 6.39%), 684 (MH^+, 4.07%), 606 (M^+-Ph, 2.33%), 545 (M^+-$N(CH_2CH_2Cl)_2$, ^{81}Br, 40.12%), 543 (M^+-$N(CH_2CH_2Cl)_2$, 39.54%), 512 (M^+-OPhBr, 5.23%), 465 (MH^+-$N(CH_2CH_2Cl)_2$-Br, 29.65%), 120 ($(PhCH_2CHNH_2)^+$, 91.28%), 91 ($PhCH_2^+$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 14.59 min, 16.62 min.

Analysis $C_{29}H_{33}BrCl_2N_3O_5P$ requires: C 50.82 %, H 4.85, N 6.13, P 4.52. Found C 51.47, H 4.88, N 6.06, P 4.47.

N,N-Bis(2-chloroethyl)amino 4-fluorophenyl methoxyphenylalaninyl-phenylalaninyl phosphoramidate (116)

A solution of triethylamine (0.17 ml, 1.20 mmol) in dichloromethane (18 ml) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino 4-fluorophenyl

phosphorochloridate (0.20 g, 0.60 mmol) and di-L-phenylalanine methyl ester hydrochloride (0.22g, 0.60 mmol) in dichloromethane (32 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for a total of 42 hr. The solvent was then removed by rotary evaporation under reduced pressure and the product extracted with ether (2x 100 ml). The filtered extracts were combined and concentrated and the resulting residue purified by column chromatography. Elution with 30% chloroform in petroleum spirit (b.p. 60-80°C) followed by pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.23 g, 62%).

^{31}P nmr δ($CDCl_3$) 12.16, 11.75 (1.5:1).

^{13}C nmr δ($CDCl_3$) 171.42 ($\underline{C}O_2Me$), 171.21, 171.07 (2xd, NHCO, J=5.2 Hz, J=4.4 Hz), 159.58, 159.50 (2xd, *para*-FPh, J_{C-F}=242.5 Hz), 146.40 (m, *ipso*-FPh), 136.26, 136.03 (*ipso*-Ph2), 135.78, 135.64 (*ipso*-Ph1), 129.92, 129.74 (*meta*-Ph2), 129.21, 129.17 (*meta*-Ph1), 128.89, 128.65 (*ortho*-Ph2), 128.53, 128.46 (*ortho*-Ph1), 127.36, 127.19 (*para*-Ph2), 127.10, 127.05 (*para*-Ph1), 121.34-121.57 (m, *meta*-FPh), 116.36, 116.31 (*ortho*-FPh, J_{C-F}=23.6 Hz), 56.13, 55.89 ($CO_2\underline{Me}$), 53.48, 53.20 (2xd, $CO_2MeC\underline{H}NH$, J=2.3 Hz, J=2.3 Hz), 52.27-52.37 (m, NHCOC$\underline{H}$NH), 48.54, 48.41 (2xd, 2x CH_2N, J=4.7 Hz, J=4.6 Hz), 42.36 (2x CH_2Cl), 39.40, 39.18 (2xd, Ph2-$\underline{C}H_2Ph$, J=4.6 Hz, J=5.4 Hz), 37.89, 37.80 (Ph1-$\underline{C}H_2Ph$).

^{1}H nmr δ($CDCl_3$) 6.82-7.30 (m, 14H, 2x $CH_2\underline{Ph}$, FPh), 4.79 (m, 1H, $MeO_2CC\underline{H}NH$), 4.07 (m, 1H, NHCOC$\underline{H}$NH), 3.69, 3.66 (2xs, 3H, CO_2Me), 3.18-3.47 (m, 10H, 2x CH_2CH_2Cl, N$\underline{H}$COCHN$\underline{H}$), 2.99-3.17 (m, 4H, 2x $C\underline{H}_2Ph$).

FABMS m/e 626 (MH^+, ^{37}Cl, 1.74%), 624 (MH^+, 2.33%), 512 (M^+-OPhF, 1.16%), 485 (M^+-$N(CH_2CH_2Cl)_2$, ^{37}Cl, 13.66%), 483 (M^+-$N(CH_2CH_2Cl)_2$, 45.64%), 419 (M^+-M^+-CO_2Me-$PhCH_2CHNHCO$, ^{37}Cl, 6.98%), 417 (M^+-CO_2Me-$PhCH_2CHNHCO$, 9.88%), 294 (M^+-CH_3-OPhF-$H_2NP(O)N(CH_2CH_2Cl)_2$, 100%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water (80:20), flow rate 1 ml/min. Retention times 10.87 min, 11.90 min.

Analysis $C_{29}H_{33}Cl_2FN_3O_5P(H_2O)_{0.5}$ requires: C 54.98 %, H 5.25, N 6.63. Found C 54.82, H 5.34, N 5.92.

Dichlorophosphoramide (59)

Bis(2-chloroethyl)amine hydrochloride (10.00 g, 56.34 mmol) was mixed with phosphoryl chloride (26.34 ml, 0.28 mol) and the mixture heated under reflux at 120-140°C for 75 hr. Excess phosphoryl chloride was then removed under reduced pressure to yield the product as off-white crystals, (14.48 g, 100%), m.p. 51°C.

^{31}P nmr ($CDCl_3$) 15.80.

^{13}C nmr ($CDCl_3$) 49.29 (d, 2x CH_2N, J=4.1 Hz), 40.79 (d, 2x CH_2Cl, J=2.7 Hz).

^{1}H nmr ($CDCl_3$) 3.55-3.75 (m, 8H, 2x CH_2CH_2Cl).

FABMS m/e 542 (M_2HNa^+, 2x ^{37}Cl, 4.01%), 540 (M_2HNa^+, ^{37}Cl, 9.32%), 538 (M_2HNa^+, 6.41%), 286 (MNa^+, 3x ^{37}Cl, 5.51%), 284 (MNa^+, 2x ^{37}Cl, 44.06%), 282 (MNa^+, ^{37}Cl, 100%), 280 (MNa^+, 76.34%), 264 (MH^+, 3x ^{37}Cl, 1.05%), 262 (MH^+, 2x

^{37}Cl, 13.70%), 260 (MH^+, ^{37}Cl, 36.79%), 258 (MH^+, 32.78%), 224 (M^+-Cl, ^{37}Cl, 4.03%), 222 (M^+-Cl, 4.47%), 208 (M^+-CH_2Cl, 3.10%), 194 (M^+-CH_2CH_2Cl, 2.03%).

Analysis $C_4H_8Cl_4NOP$ requires: C 18.56 %, H 3.12, N 5.41, Cl 54.77. Found C 18.55, H 3.02, N 5.29, Cl 54.70.

N,N-Bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (118)

A solution of aminopropan-1,2-diol (0.36 g, 3.95 mmol) and triethylamine (1.10 ml, 7.90 mmol) in acetonitrile (50 ml) was added dropwise with vigorous stirring to a solution of dichlorophosphoramide (1.17 g, 4.50 mmol) in acetonitrile (50 ml) at ambient temperature. The mixture was refluxed for 2.5 hr and then the solvent was removed by rotary evaporation under reduced pressure. The residue was extracted with tetrahydrofuran (4 x 50 ml) and the combined extracts concentrated under reduced pressure to yield the product as a colourless oil, (1.10 g, 100%), m.p. 102-103°C.

^{31}P nmr ($CDCl_3$) 28.57, 27.61 (1:1).

^{13}C nmr ($CDCl_3$) 78.80 (CHOP), 77.24 (d, CHOP′, J=2.4 Hz), 63.67, 63.00 (2xd, CH_2OH, J=2.9 Hz, J=7.6 Hz), 49.12, 48.91 (2xd, 2x CH_2N, J=5.0 Hz), 43.69, 42.97 (2xd, CH_2NH, J=7.6 Hz, J=8.5 Hz), 42.39, 42.23 (2x CH_2Cl).

^1H nmr ($CDCl_3$) 4.63, 4.49 (2xm, 1H, CHOP), 3.54-3.87 (m, 6H, $C\underline{H}_2OH$, 2x CH_2N), 3.28-3.49 (m, 7H, 2x CH_2Cl, $C\underline{H}_2N\underline{H}$), 271 (t, 1H, $CH_2O\underline{H}$).

EIMS m/e 279 (MH^+, ^{37}Cl, 0.63%), 277 (MH^+, 1.32%), 241 (M^+-Cl, 0.76%), 229 (M^+-CH_2Cl, ^{37}Cl, 25.16%), 227 (M^+-CH_2Cl, 72.47%), 136 (M^+-$N(CH_2CH_2Cl)_2$,

17.61%), 94 ($ClCH_2CH_2N(H)CH_2^+$, ^{37}Cl, 47.96%), 92 ($ClCH_2CH_2N(H)CH_2^+$, 100%), 65 (CH_2CH_2Cl, ^{37}Cl, 9.78%), 63 (CH_2CH_2Cl, 26.96%).

I.R. 3273 (OH), 2957, 2873, 2707, 1451, 1400, 1350, 1305, 1219, 1151, 1132, 1091, 1056 (OH), 986, 928, 854, 798, 753, 736 cm^{-1}.

Analysis $C_7H_{15}Cl_2N_2O_3P$ requires: C 30.34 %, H 5.46, N 10.11. Found C 30.23, H 5.29, N 9.77.

N,N-Bis(2-chloroethyl)amino-1,3,2-oxazaphosphacyclopentane 2-oxide (120)

A solution of ethanolamine (0.12 g, 2.00 mmol) and triethylamine (0.51 ml, 3.66 mmol) in acetonitrile (35 ml) was added dropwise with vigorous stirring to a volume of acetonitrile (15 ml) at ambient temperature. Separately but simultaneously, a solution of dichlorophosphoramide (0.52 g, 2.00 mmol) in acetonitrile (15 ml) was added dropwise. The mixture was stirred at ambient temperature for 22 hr and then the solvent was removed under reduced pressure. The residue was extracted with tetrahydofuran (2x 50 ml) and the combined extracts concentrated under reduced pressure to yield the product as a white solid, (0.49 g, 100%), m.p. 94-95°C.

^{31}P nmr ($CDCl_3$) 30.09.

^{13}C nmr ($CDCl_3$) 66.27 (d, CH_2O, J=2.7 Hz), 48.96 (d, 2x CH_2N, J=4.9 Hz), 42.24 (CH_2NH), 42.16 (2x CH_2Cl).

^{1}H nmr ($CDCl_3$) 4.36 (m, 2H, CH_2O), 3.35-3.71 (m, 11H, CH_2NH, 2x CH_2CH_2Cl).

EIMS m/e 250 (M^+, 2x ^{37}Cl, 0.05%), 248 (M^+, ^{37}Cl, 0.24%), 246 (M^+, 0.35%), 213 (M^+-Cl, ^{37}Cl, 2.33%), 211 (M^+-Cl, 0.87%), 199 (M^+-CH_2Cl, ^{37}Cl, 32.56%), 197 (M^+-CH_2Cl, 100%).

I.R. 2919, 2851, 1454. 1376, 1263, 1215, 1197, 1128, 1091, 1015, 988, 934, 823, 752 cm^{-1}.

Analysis $C_6H_{13}Cl_2N_2O_2P$ requires: C 29.17 %, H 5.30, N 11.34. Found C 29.49, H 5.12, N 10.91.

N,N-Bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-dioxazaphosphacyclopentane 2-oxide (122)

A solution of glycerol (0.18 g, 1.96 mmol) and triethylamine (0.64 ml, 4.59 mmol) in acetonitrile (35 ml) was added dropwise with vigorous stirring to a volume of acetonitrile (15 ml) at ambient temperature. Separately and simultaneously, a solution of dichlorophosphoramide (0.58 g, 2.25 mmol) in acetonitrile (25 ml) was added dropwise. The mixture was refluxed for 2.5 hr and then the solvent was removed under reduced pressure. The residue was extracted with diethyl ether (4x 50 ml) and the combined extracts concentrated under reduced pressure to yield the product as an oil, (0.56 g, 100%).

^{31}P nmr ($CDCl_3$) 24.61, 23.89 (1:1).

^{13}C nmr ($CDCl_3$) 78.53 (CHOP), 76.40 (d, CHOP', J=2.3 Hz), 66.89, 66.68 (CH_2NH), 62.41, 61.40 (2xd, CH_2OH, J=2.8 Hz, J=7.8 Hz), 49.41, 49.29 (2xd, 2x CH_2N, J=4.9 Hz), 42.00, 41.78 (2x CH_2Cl).

^{1}H nmr ($CDCl_3$) 4.70 (m, 1H, CHOP), 4.32 (m, 2H, CH_2OP), 3.29-3.95 (m, 11H, CH_2OH, 2x CH_2CH_2Cl).

FABMS m/e 302 (MNa^+, ^{37}Cl, 3.12%), 300 (MNa^+, 7.77%), 280 (MH^+, ^{37}Cl, 0.76%), 278 (MH^+, 1.04%), 228 (M^+-CH_2Cl, 0.15%), 137 (M^+-$N(CH_2CH_2Cl)_2$, 1.61%).

I.R. 3370, 2960, 2921, 2855, 1260, 1093, 1021, 931, 899, 866, 801 cm^{-1}.

Analysis $C_7H_{14}Cl_2NO_4P$ requires: C 30.24 %, H 5.08, N 5.04. Found C 30.88, H 5.71, N 4.66.

N,N-Bis(2-chloroethyl)amino-5-acetyloxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (123)

Acetic anhydride (37 mg, mmol) and dimethylaminopyridine (ca. 4 mg) were added to a solution of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphospha cyclopentane 2-oxide (0.10 g, 0.36 mmol) in acetonitrile (10 ml) at 0°C. The solution was stirred for 2 hr and then the solvent was removed by rotary evaporation under reduced pressure. The residue was dissolved in chloroform (100 ml) and extracted with water (2x 10 ml). The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give the product (0.11 g, 96%), m.p. 90-92°C.

^{31}P nmr ($CDCl_3$) 29.31, 28.95 (1:1).

^{13}C nmr ($CDCl_3$) 170.54, 170.46 ($MeCO_2$), 75.39 (CHOP), 74.28 (d, CHOP′, J=4.2 Hz), 64.62, 63.77 (2xd, CH_2OAc, J=3.9 Hz, J=8.4 Hz), 49.11, 48.99 (2xd, 2x CH_2N, J=5.0 Hz), 43.79 (t, CH_2NH, J=7.9 Hz), 42.14, 42.02 (2x CH_2Cl), 20.67, 20.65 ($MeCO_2$).

^{1}H nmr ($CDCl_3$) 4.60 (m, 1H, CHOP), 4.21 (m, 2H, CH_2OAc), 3.19-3.62 (m, 11H, CH_2NH, 2x CH_2CH_2Cl), 2.09, 2.07 (2xs, 3H, OAc).

EIMS m/e 321 (MH^+, ^{37}Cl, 0.97%), 319.0403 (MH^+ calcd. for $C_9H_{18}Cl_2N_2O_4P$ 319.0381, 1.40%), 271 (M^+-CH_2Cl, ^{37}Cl, 6.12%), 269 (M^+-CH_2Cl, 19.27%), 178 (M^+-$N(CH_2CH_2Cl)_2$, 91.30%), 136 (M^+-$N(CH_2CH_2Cl)_2$-Ac), 94 ($ClCH_2CH_2N(H)CH_2^+$, ^{37}Cl, 18.81%), 92 ($ClCH_2CH_2N(H)CH_2^+$, 56.38%), 65 (CH_2CH_2Cl, ^{37}Cl, 8.02%), 63 (CH_2CH_2Cl, 20.56%), 43 ($MeCO^+$, 100%).

I.R. 3253, 2956, 2893, 1741 (C=O), 1663, 1646, 1558, 1450, 1369, 1232, 1151, 1132, 1086, 1037, 987, 965, 941, 873, 801, 754 cm^{-1}.

Analysis $C_9H_{17}Cl_2N_2O_4P$ requires: C 33.87 %, H 5.37, N 8.78%. Found C 33.45, H 5.45, N 7.69.

Attempted oxidation of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (118) with silver carbonate on celite

Silver carbonate on celite (1.86 g, 1.62 mmol) was added to a solution of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (0.10 g, 0.36 mmol) in tetrahydrofuran (13 ml). The reaction mixture was stirred at ambient temperature for 70 hr. The mixture was then filtered and the solid residue washed with tetrahydrofuran (50 ml). Concentration of the combined extracts under reduced pressure produced an oil, (0.31 g).

^{31}P nmr ($CDCl_3$) 28.20, 27.30 (1:1), 21.90.

Attempted oxidation of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (118) with dinitrogen tetroxide

Dinitrogen tetroxide (1.76 ml, 0.20 mmol) was added dropwise to a solution of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (0.20 g, 0.72 mmol) in dichloromethane (3 ml) at 0°C and in an atmosphere of nitrogen. The solution was stirred at ambient temperature for 1 hr and then the solvent was evaporated under reduced pressure to produce a pale yellow oil, (0.80 g).

^{31}P nmr ($CDCl_3$) 28.82, 27.62 (1:1).

Attempted oxidation of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (118) with pyridinium chlorochromate

N,N-Bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (0.25 g, 0.90 mmol) in dichloromethane (3 ml) was rapidly added to a suspension of pyridinium chlorochromate (0.29 g, 1.34 mmol) and sodium acetate (0.55 g, 6.70 mmol) in dichloromethane (3 ml) at ambient temperature. The orange reaction mixture was stirred for 2 hr during which time it turned black. This mixture was washed with chloroform (4x 50 ml) and the organic extracts filtered through purified celite. Concentration of the filtrate under reduced pressure produced a black oil, (0.28 g).

^{31}P nmr ($CDCl_3$) 32.26, 30.20 (2:2), 26.28, 25.33 (1:1).

Attempted oxidation of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (118) with pyridinium dichromate

Pyridinium dichromate (0.61 g, 1.62 mmol) was added to a solution of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane 2-oxide (0.30 g, 1.08 mmol) in dichloromethane (10 ml). This suspension was stirred vigorously at

ambient temperature for 23 hr. The black reaction mixture was then diluted with ether (100 ml) and the solvent decanted. Filtration and concentration of the filtrate under reduced pressure produced no material. This procedure was repeated with dichloromethane and produced a brown/yellow oil which contained no phosphorus by ^{31}P nmr spectroscopy.

Attempted preparation of 2-ketoaminopropan-3-ol

Freshly prepared silver carbonate on celite reagent (1.56 g, 1.88 mmol) was added to a solution of aminopropan-2,3-diol (0.46 g, 5.05 mmol) in acetonitrile (60 ml). The reaction mixture was stirred vigorously for 19 hr at ambient temperature during which time the green oxidant had turned black. Filtration and concentration of the filtrate under reduced pressure produced a yellow oil, (0.14 g). The ^{1}H nmr spectrum of this material contained many signals.

Attempted preparation of 2-O-acetylaminopropan-3-ol

Aminopropan-2,3-diol (0.30 g, 3.29 mmol) in acetonitrile (10 ml) was added to acetic anhydride (0.37 g, 3.62 mmol) and dimethylaminopyridine (40 mg, 0.33 mmol) at 0°C. The reaction mixture was stirred at ambient temperature for 4 hr and then the solvent was removed by evaporation under reduced pressure. The residue was dissolved in chloroform (100 ml) and washed with water (10 ml). The organic solution was dried over magnesium sulphate and concentrated under reduced pressure to yield a yellow oil, (0.26 g).

^{1}H nmr ($CDCl_3$) 6.04 (bs, 1H, NH), 5.14 (q, 1H, C<u>H</u>OAc), 4.15 (m, 2H, C<u>H</u>$_2$OAc), 3.46 (m, 2H, C<u>H</u>$_2$NH$_2$), 2.06 (s, 3H, Me), 2.04 (s, 3H, Me).

2-O-Benzyl aminopropan-3-ol (1st attempt)

A solution of benzyl bromide (0.23 g, 1.34 mmol) in acetonitrile (5 ml) and a solution of triethylamine (0.19 ml, 1.34 mmol) in acetonitrile (3 ml) were added dropwise, separately but simultaneously, to a solution of aminopropan-2,3-diol (0.12 g, 1.34 mmol) in acetonitrile (50 ml) at 0°C. The reaction mixture was stirred at 0°C for 1 hr and then allowed to warm to ambient temperature. The solvent was removed by evaporation under reduced pressure and the residue purified by column chromatography. Elution with 27% methanol in chloroform followed by pooling and evaporation of the appropriate fractions produced a white solid (0.12 g).

^{13}C nmr (D_2O) 133.60 (*ipso*-Ph), 132.57 (*ortho*-Ph), 132.36 (*para*-Ph), 132.00 (*meta*-Ph), 70.00 (CHOH), 66.06 (CH_2OH), 53.94 ($NHCH_2Ph$), 51.72 ($CH(OH)\underline{C}H_2NH$).

^1H nmr (DMSO) 7.41-7.56 (m, 5H, Ph), 5.36 (bs, 1H, NH), 4.13 (s, 2H, $C\underline{H}_2Ph$). Signals arising from the remaining protons were masked by those from DMSO.

EIMS m/e 182 (MH^+, 2.34%), 181 (M^+, 2.06%), 150 (M^+-CH_2OH, 6.37%), 120 ($PhCH_2NHCH_2^+$, 81.27%), 106 ($PhCH_2NH^+$, 8.85%).

2-O-Benzyl aminopropan-3-ol (2nd attempt)

A solution of benzyl bromide (1.88 g, 10.98 mmol) in acetonitrile (40 ml) and a solution of triethylamine (1.53 ml, 10.98 mmol) in acetonitrile (20 ml) were added dropwise, separately but simultaneously, to a solution of aminopropan-2,3-diol (1.00 g, 10.98 mmol) in acetonitrile (400 ml) at 0°C. The reaction mixture was stirred at 0°C for 26 hr and then allowed to warm to ambient temperature. The solvent was removed by evaporation under reduced pressure and the residue subjected to column

chromatography. Elution with 10% methanol in dichloromethane followed by pooling and evaporation of the appropriate fractions gave a white solid (0.60 g).

^{13}C nmr ($CDCl_3$) 138.26 (2x *ipso*-Ph), 129.10 (2x *ortho*-Ph), 128.51 (2x *meta*-Ph), 127.42 (2x *para*-Ph), 67.62 (CHOH), 64.66 (CH_2OH), 58.76 ($N(\underline{C}H_2Ph)_2$), 55.69 ($CH(OH)\underline{C}H_2NH$).

^1H nmr ($CDCl_3$) 7.32 (s, 10H, 2x Ph), 2.44-3.83 (m, 11H, 2x $N(C\underline{H}_2Ph)_2$, $CH(OH)CH_2N$, CH_2OH).

EIMS m/e 271 (M^+, 0.03%), 240 (M^+-CH_2OH, 1.30%), 210 ($(PhCH_2)_2NCH_2^+$, 77.70%), 196 ($(PhCH_2)_2N^+$, 0.38%), 91 ($PhCH_2^+$, 100%).

Analysis $C_{17}H_{21}NO_2$ requires: C 75.25, H 7.80, N 5.90. Found C 74.56, H 8.01, N 4.94.

Preparation of 1-tritylamino-3-trityloxypropan-2-ol (124)

A solution of trityl chloride (1.53 g, 5.49 mmol) and aminopropan-2,3-diol (0.50 g, 5.49 mmol) in pyridine (10 ml) was stirred at 0°C-4°C for 168 hr. The reaction mixture was then slowly added to crushed ice (400 g) and the precipitated white solid collected by filtration. This was dried in vacuo at 40°C to give white crystals, (1.40 g, 44%).

^{13}C nmr ($CDCl_3$) 126.30-129.32 (m, 2x Tr), 70.12-71.26 (m, 2x $\underline{C}$-Ph_3), 66.76 (CHOH), 65.63 ($\underline{C}H_2OTr$), 46.62 ($\underline{C}H_2NHTr$).

^1H nmr ($CDCl_3$) 7.12-7.53 (m, 30H, 2x Tr), 3.92 (m, 1H, $C\underline{H}OH$), 3.22 (m, 2H, CH_2O), 2.41 (m, 2H, CH_2N).

Preparation of 1-tritylamino-2-0-acetyl-3-trityloxypropane (125)

1-Tritylamino-3-trityloxypropan-2-ol (0.30 g, 0.52 mmol) in dichloromethane (30 ml) was added to acetic anhydride (53 mg, 0.52 mmol) and dimethylaminopyridine (6 mg, 0.05 mmol) at 0°C. The reaction mixture was stirred at ambient temperature for 30 mins and then the solvent was removed by evaporation under reduced pressure. The residue was dissolved in chloroform (100 ml) and washed with water (30 ml). The organic solution was dried over magnesium sulphate and concentrated under reduced pressure to yield a white solid, (0.25 g, 78%).

^{13}C nmr ($CDCl_3$) 170.51 (COMe), 86.42 (CHOAc), 72.84 ($CH_2O\underline{C}Ph_3$), 70.53 ($CH_2NH\underline{C}Ph_3$), 63.05 ($\underline{C}H_2OTr$), 44.08 (CH_2NH), 21.21 (CO$\underline{Me}$).

^1H nmr ($CDCl_3$) 7.15-7.43 (m, 30H, 2x Tr), 5.23 (m, 1H, C$\underline{H}$OAc), 3.28 (d, 2H, $C\underline{H}_2OTr$), 2.46 (t, 1H, NH), 2.08 (s, 3H, CH_3).

Preparation of 2-O-acetylaminopropan-3-ol (1st attempt)

A mixture of 1-tritylamino-2-O-acetyl-3-trityloxypropane (0.25 g, 0.40 mmol) in acetic acid (1 ml) was refluxed at 100°C for 30 mins. A mixture of ice and water was then added to the resulting solution and the precipitated white solid removed by filtration. The solution was then concentrated under reduced pressure to give a white solid.

^{13}C nmr ($CDCl_3$) 72.69 (CHOAc), 65.72 (CH_2OH), 43.98 (CH_2NH_2), 22.65 (CH_3). The spectrum also contained signals arising from impurities.

^1H nmr ($CDCl_3$) 4.07 (m, 1H, C$\underline{H}$OAc), 3.55 (m, 2H, $C\underline{H}_2OH$), 3.10 (m, 2H, $C\underline{H}_2NH_2$), 2.02 (s, 3H, CH_3).

The spectrum also contained signals arising from impurities.

Preparation of 2-O-acetylaminopropan-3-ol (2nd attempt)

A mixture of 1-tritylamino-2-0-acetyl-3-trityloxypropane (0.25 g, 0.40 mmol) and hydrochloric acid (0.1 ml), in acetone (4 ml) was stirred at ambient temperature for 3 hr. The solvent was then removed by rotary evaporation under reduced pressure and the resulting residue subjected to column chromatography with elution by 5% methanol in chloroform. Following pooling and evaporation of the appropriate fractions it was found that there was insufficient material available for characterisation by nmr spectroscopy.

2,6,7-Trioxa-1-phosphabicyclo[2,2,1]heptane (127)

Glycerol (11.51 g, 0.12 mol) and trimethylphosphite (15.04 ml, 0.13 mol) in silicone oil (13 ml) were heated with vigorous stirring in an atmosphere of nitrogen. When the distillation of methanol had ceased, a solution of sodium methoxide (0.25 g, 4.63 mmol) was added to the reaction mixture at ambient temperature. The product was distilled from the mixture under reduced pressure (40-50°C/3.0 mmHg) and collected as a colourless oil, (5.50 g, 37%).

^{31}P nmr ($CDCl_3$) 103.10 (major), 31.27.

2,6,7-Trioxa-1-phosphabicyclo[2,2,1]heptane (127)

A solution of glycerol (5.76 g, 62.55 mmol) in ether (25 ml), a solution of phosphorus trichloride (5.46 ml, 62.55 mmol) in ether (10 ml) and a solution of triethylamine (26.48 ml, 0.19 mol) in ether (10 ml) were added dropwise, separately but simultaneously, to a volume of ether (25 ml). The addition was carried out between -65 to -70°C and in an atmosphere of nitrogen. The reaction mixture was then stirred for 2 hours at ambient temperature. The solvent was removed by evaporation under reduced pressure and the residue extracted with ether (2x 100 ml). Filtration and concentration of the filtrate under reduced pressure produced a white solid (0.36 g).

^{31}P nmr ($CDCl_3$) 136.00, 103.33 (major).

Dioxoamino-1-phosphabicyclo[2,2,1]heptane (128)

A solution of aminopropan-2,3-diol (1.00 g, 10.98 mmol) and triethylamine (4.59 ml, 32.94 mmol) in acetonitrile (80 ml) and a solution of phosphoryl chloride (1.07 ml, 10.98 mmol) in acetonitrile (10 ml) were added dropwise, separately but simultaneously, to a volume of acetonitrile (10 ml) at 0°C. The reaction mixture was allowed to warm to ambient temperature and then refluxed for 19 hr.The solvent was removed by evaporation under reduced pressure and the residue extracted with tetrahydrofuran (2x 50 ml). The extracts were combined and concentrated under reduced pressure to yield a colourless oil, (0.20 g, 14%).

^{31}P nmr ($CDCl_3$) -6.97.

^{1}H nmr ($CDCl_3$) 5.16 (m, 1H, CH), 3.94 (t, 2H, CH_2O), 3.50 (t, 2H, CH_2NH).
The spectrum also contained signals arising from impurities.

EIMS m/e 135 (M^+, 1.11%), 117 (M^+-H_2O, 4.44%).

Attempted preparation of 6-ketocyclophosphamide (129)

A solution of dichlorophosphoramide (0.52 g, 2.00 mmol) in acetonitrile (25 ml) was added dropwise to triethylamine (0.56 ml, 4.00 mmol) and a suspension of β-alanine (89 mg, 1.00 mmol) in acetonitrile (75 ml). The mixture was then refluxed for 21 hr and the solvent removed by evaporation under reduced pressure. Extraction of the residue with tetrahydrofuran (2x 50 ml) produced a brown solid (0.13g).

^{31}P nmr ($CDCl_3$) 10.56. (Also present were signals between δ-14.34 to -25.93).

Attempted preparation of 2-O-allylaminopropan-3-ol (132)

A solution of aminopropan-2,3-diol (0.40 g, 4.39 mmol) in acetonitrile (67 ml) was added dropwise to a slurry of sodium hydride (96 mg, 2.99 mmol) in acetonitrile (13 ml). The addition was carried out at ambient temperature over a period of 1 hr. When the release of hydrogen had ceased, a solution of allyl bromide (0.34 ml, 3.99 mmol) in acetonitrile (1 ml) was added dropwise. The reaction mixture was then stirred at ambient temperature for 45 hr. The solution was decanted and concentrated at reduced pressure and the resulting residue subjected to two successive columns with elution by 10% methanol in chloroform and 5% methanol in chloroform respectively. This gave rise to a colourless oil, (0.21 g).

^{13}C nmr ($CDCl_3$) 134.12 ($CH_2\underline{C}H=CH_2$), 118.86 ($CH_2CH=\underline{C}H_2$), 67.65 (CHOH), 64.93 (CH_2OH), 56.96 ($N(\underline{C}H_2CH=CH_2)_2$), 55.47 ($CH(OH)\underline{C}H_2N$).

^1H nmr ($CDCl_3$) 5.81 (m, 2H, $N(CH_2C\underline{H}=CH_2)_2$), 5.18 (m, 4H, $N(CH_2CH=C\underline{H}_2)_2$), 3.75 (m, 2H, $C\underline{H}_2OH$), 3.46 (m, 1H, $C\underline{H}OH$), 3.16 (m, 2H, $CH(OH)C\underline{H}_2N$), 2.56 (m, 4H, $N(C\underline{H}_2CH=CH_2)_2$).

EIMS m/e 171 (M^+, 0.92%), 110 ($((CH_2=CHCH_2)_2NCH_2^+$, 100%).

2-Cyanopent-4-ene acetate (134)

N-Butyl lithium (11.05 ml, 22.10 mmol) was added to a solution of ethylcyanoacetate (7.06 ml, 66.30 mmol) in tetrahydrofuran (150 ml) at -60°C and in an atmosphere of nitrogen. A solution of allyl bromide (1.91 ml, 22.10 mmol) in tetrahydrofuran (20 ml) was then added dropwise with stirring over 1 hr. The mixture was stirred at -60°C for a further 5 hr and then the solvent was removed under reduced pressure. The residue was dissolved in diethyl ether (600 ml) and washed with sodium bicarbonate solution (600

ml). The aqueous solution was then extracted with diethyl ether (2x 600 ml) and the organic solution dried over magnesium sulphate. Filtration and concentration of the filtrate under reduced pressure produced an oil which was purified by column chromatography with elution by 10% ether in petroleum spirit (b.p. 60-80°C). The product was isolated as , (0.88 g, 26%).

^{13}C nmr ($CDCl_3$) 165.73 ($\underline{C}O_2Et$), 131.39 ($CH(CH_2\underline{C}H=CH_2)$), 120.05 ($CH(CH_2CH=\underline{C}H_2)$), 116.09 (C≡N), 62.89 ($CO_2\underline{C}H_2CH_3$), 37.50 ($\underline{C}H(CH_2CH=CH_2)$), 33.85 ($CH(\underline{C}H_2CH=CH_2)$), 14.03 ($CO_2CH_2\underline{C}H_3$).

^1H nmr ($CDCl_3$) 5.81 (m, 1H, $CH(CH_2\underline{C}H=CH_2)$), 5.28 (m, 2H, $CH(CH_2CH=C\underline{H}_2)$), 4.26 (q, 2H, $CO_2C\underline{H}_2CH_3$), 3.58 (t, 1H, $C\underline{H}(CH_2CH=CH_2)$), 2.70 (t, 2H, $CH(C\underline{H}_2CH=CH_2)$), 1.33 (t, 3H, $CO_2CH_2C\underline{H}_3$).

EIMS m/e 154 (MH^+, 24.14%), 111 (M^+-H-$CH_2CH=CH_2$, 3.43%), 109 (MH^+-OEt, 9.22%).

Attempted preparation of 2-allylaminopropan-3-ol (135)

Monoallylethylcyanoacetate (1.00 g, 6.53 mmol) in tetrahydrofuran (20 ml) was added dropwise, over 1 hr at ambient temperature, to a slurry of lithium aluminium hydride (0.74 g, 19.50 mmol) in tetrahydrofuran (45 ml). The mixture was refluxed at 60°C for 192 hr and then a further equivalent of lithium aluminium hydride (0.25 g, 6.53 mmol) was added. The mixture was then refluxed for a further 72 hr. Quenching of excess reagent and hydrolysis of aluminate salts was effected by the cautious slow addition of water (10 ml) followed by 10% sodium hydroxide solution (15 ml) and then water (15 ml). The solution was decanted from the granular aluminium salts, concentrated under reduced pressure and the residue washed with diethyl ether (2x 100 ml). The aqueous

solution was then concentrated under reduced pressure and the residue extracted with dichloromethane (2x 100 ml). The organic extract was dried over magnesium sulphate, filtered and concentrated under reduced pressure to produce an oil. It was attempted to purify this material by column chromatography with elution by 2.5% methanol in chloroform and this gave rise to a white solid (5 mg). The ^{1}H nmr spectrum contained many signals and was inconclusive.

N,N-Bis(2-chloroethyl)amino-1,4-dihydro-2H-3,1,2-benzoxazaphosphorin 2-oxide (136)

A solution of dichlorophosphoramide (3.15 g, 12.18 mmol) in dichloromethane (15 ml) was added dropwise to a solution of O-aminobenzylalcohol (1.50 g, 12.18 mmol) and triethylamine (3.40 ml, 24.36 mmol) in dichloromethane (19 ml). The reaction was carried out at ambient temperature for 24 hr and then the solvent was removed by evaporation under reduced pressure. Ether (2x 100 ml) was added to the residue and the extracts concentrated under reduced pressure. The concentrate was purified by column chromatography with elution by 40% chloroform in petroleum spirit (b.p. 60-80 °C). This gave a yellow solid which was recrystallised from petroleum spirit (b.p. 40-60°C) to afford white needles, (1.92 g, 51%).

^{31}P nmr ($CDCl_3$) 5.61.

^{13}C nmr ($CDCl_3$) 116.86-140.07 (m, Ph), 67.58, 67.47 (2xd, CH_2O, J=5.4 Hz, J=4.6 Hz), 48.71, 48.66 (2x CH_2N), 42.20 (2x CH_2Cl).

^{1}H nmr ($CDCl_3$) 6.47-7.24 (m, 4H, Ph), 5.17 (m, 2H, CH_2O), 3.56 (m, 4H, 2x CH_2N), 3.34 (m, 4H, 2x CH_2Cl).

FABMS m/e 313 (MH^+, 2x ^{37}Cl, 5.69%), 311 (MH^+, ^{37}Cl, 42.41%), 309 (MH^+, 66.94%), 261 (M^+-CH_2Cl, ^{37}Cl, 5.26%), 259 (M^+-CH_2Cl, 17.11%), 168 (M^+-$N(CH_2CH_2Cl)_2$, 9.08%).

Analytical hplc Stationary phase 250 mm x 4.6 mm Kromasil C18 5 μM column. Mobile phase methanol-water-triethylamine (60:40:0.01), flow rate 1 ml/min. Retention time 10.72 min.

Analysis $C_{11}H_{15}Cl_2N_2O_2P$ requires: C 42.74 %, H 4.89, N 9.06. Found C 41.83, H 4.89, N 8.38.

N,N-Bis(2-chloroethyl)amino phosphorus dichloride (137)

Bis(2-chloroethyl)amine hydrochloride (1.50 g, 8.40 mmol) was added to a solution of phosphorus trichloride (0.73 ml, 8.40 mmol) in benzene (8 ml) at ambient temperature and in an atmosphere of nitrogen. Triethylamine (2.34 ml, 17 mmol) was then added dropwise and the reaction mixture stirred under reflux for 3 hr. After cooling to ambient temperature, the precipitated triethylamine hydrochloride was removed by filtration and washed with benzene (3x 50 ml). The combined extracts were concentrated under reduced pressure to produce a yellow/brown solid, (0.79 g, 39%).

^{31}P nmr ($CDCl_3$) 160.68.

^{13}C nmr ($CDCl_3$) 50.05 (d, 2x CH_2N, J=20.4 Hz), 41.63 (d, 2x CH_2Cl, J=3.7 Hz).

^{1}H nmr ($CDCl_3$) 3.61-3.67 (m, 8H, 2x CH_2CH_2Cl).

EIMS m/e 245 (MH_2^+, ^{37}Cl, 0.27%), 243 (MH_2^+, 1.20%), 94 ($ClCH_2CH_2NHCH_2^+$, ^{37}Cl, 69.06%), 92 ($ClCH_2CH_2NHCH_2^+$, 100%), 65 ($ClCH_2CH_2^+$,^{37}Cl, 68.99%), 63 ($ClCH_2CH_2^+$, 78.77%).

N,N-Bis(2-chloroethyl)amino phosphorus dichloride (137)

A mixture of bis(2-chloroethyl)amine hydrochloride (5.00 g, 28.01 mmol) in phosphorus trichloride (12.22 ml, 0.14 mol) was stirred under reflux for 119 hr. Evaporation of the solvent under reduced pressure produced an off-white solid, (6.80 g, 100%).

^{31}P nmr ($CDCl_3$) 160.68.

^{13}C nmr ($CDCl_3$) 50.06 (d, 2x CH_2N, J=20.3 Hz), 41.69 (d, 2x CH_2Cl, J=3.3 Hz).

^{1}H nmr ($CDCl_3$) 3.64-3.69 (m, 8H, 2x CH_2CH_2Cl).

Attempted preparation of N,N-bis(2-chloroethyl)amino-5-hydroxymethyl-1,3,2-oxazaphosphacyclopentane

A solution of N,N-bis(2-chloroethyl)amino phosphorus dichloride (0.30 g, 1.21 mmol) in acetonitrile (2 ml) was added dropwise to a solution of aminopropan-2,3-diol (0.11 g, 1.21 mmol) and triethylamine (0.34 ml, 2.41 mmol) in acetonitrile (49 ml) at -20°C and in an atmosphere of nitrogen. The reaction mixture was allowed to warm to ambient temperature and stirred for 20 mins. The mixture was concentrated under reduced pressure and the residue examined by ^{31}P nmr spectroscopy.

^{31}P nmr ($CDCl_3$) 145.07 (major), 15.34.

Attempted preparation of N,N-bis(2-chloroethyl)amino-1,3,2-oxazaphospha-cyclopentane

A solution of N,N-bis(2-chloroethyl)amino phosphorus dichloride (0.20 g, 0.82 mmol) in dichloromethane (1 ml) was added to a solution of ethanolamine (50 mg, 0.82 mmol) and triethylamine (0.23 ml, 1.64 mmol) in dichloromethane (33 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was stirred at ambient temperature for 1 hr and then the solvent was removed under reduced pressure. The residue was extracted with diethyl ether (130 ml) and the extract concentrated under reduced pressure but no material was isolated. This was repeated with hexane (130 ml) and gave the same result. The reaction mixture at this stage was a yellow/white polymeric gum.

Attempted preparation of N,N-bis(2-chloroethyl)amino-6-keto-1,3,2-oxazaphospha-cyclohexane

A solution of N,N-bis(2-chloroethyl)amino phosphorus dichloride (0.30 g, 1.24 mmol) in acetonitrile (2 ml) was added dropwise to triethylamine (0.34 ml, 2.48 mmol) and a suspension of β-alanine (0.11 g, 1.24 mmol) in dichloromethane (100 ml). The addition was carried out at -20°C and in an atmosphere of nitrogen. The reaction mixture was stirred at ambient temperature for 30 mins and then the solvent was removed under reduced pressure. The residue was extracted with tetrahydrofuran (2x 100 ml) and the extract concentrated under reduced pressure to give a yellow oil, (0.16 g).

^{31}P nmr ($CDCl_3$) 21.47, -10.52 (1:1).

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