Synthesis and Biological Evaluation of some Novel Phosphate Derivatives of the Anti-viral Drug AraA

by

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ABSTRACT

The nucleoside analogue 9-β-D-arabinofuranosyladenine (araA) has found widespread use as an anti-viral drug in the treatment of various herpesvirus infections. AraA suffers from a number of limitations, most importantly a low activity due to enzymatic deamination in the body by adenosine deaminase and, along with most nucleoside analogues, a dependence on enzymatic phosphorylation by cellular kinases to the active 5'-phosphate form(s). Other disadvantages include a low aqueous solubility, a low lipophilicity and a moderate toxicity.

The research presented in this thesis investigates the synthesis of some novel 5'-phosphate derivatives of araA as potential uncharged, membrane-soluble, deamination-resistant pro-drugs of ara-5'-monophosphate (araAMP). Generally, the method involved the preparation of the appropriate phosphorylating agent and its subsequent reaction with unprotected araA. The biological activity of these derivatives was evaluated by determining their ability to inhibit the synthesis of DNA in vitro using a tritiated thymidine incorporation assay employing mammalian epithelial cells.

Firstly, the 5'-bis(2-fluoroethyl) and 5'-bis(2-bromoethyl) phosphate triesters of araA were synthesized. Preparation of araA-5'-bis(2-iodoethyl) phosphate was subsequently attempted by the reaction of the 5'-bis(2-bromoethyl) derivative with potassium iodide in acetone.

The synthesis of a number of mixed, unsymmetrical 5'-phosphate derivatives of araA was investigated. A series of 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA were prepared by the method outlined above and additionally, in the case of araA-5'-butyl (2,2,2-trichloroethyl) phosphate, by a transesterification reaction. The synthesis of araA-5'-ethyl phenyl phosphate, araA-5'-(2,2,2-trichloroethyl) phenyl phosphate and some 5'-(2,2,2-trichloroethyl) para-substituted phenyl derivatives was carried out. A series of 5'-ethyl (benzyl-protected glycolyl) phosphate triesters of araA were also prepared. Hydrogenolysis of these derivatives gave the required deprotected products. AraA-5'-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate was synthesized and its hydrogenolysis was attempted. The results of in vitro biological testing of these
mixed, unsymmetrical derivatives are discussed.

The mechanism by which these 5'-phosphate triesters of araA exert their biological effects probably involves the hydrolysis of the phosphate moiety to yield either the 5'-monophosphate (araAMP) or the parent nucleoside (araA). The *in vitro* biological activity of two 5'-phosphate triesters of araA was compared with their analogous 5'-phosphinate esters to help elucidate the matter. The results of the assay were indicative of a mode of action largely involving the release of araAMP and also to a minor extent araA. Finally, the susceptibility to chemical hydrolysis of the 5'-bis(2,2,2-trifluoroethyl), 5'-bis(2,2,2-trichloroethyl) and 5'-butyl (2,2,2-trichloroethyl) phosphate triesters of araA was investigated and attempts to purify and characterize the resulting products were carried out.
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INTRODUCTION

The cells of animals, plants and bacteria are susceptible to viral diseases. These may vary in their severity from mild and transitory infections to life-threatening illnesses, e.g. polio, influenza, and acquired immune deficiency syndrome (AIDS) in man. Some viruses can also cause cancers in animals including man. Viruses are essentially infectious nucleic acid surrounded by a protective protein coat. They are obligate, intracellular parasites, capable of development only within the infected host cell since they lack the machinery required to generate metabolic energy and synthesize proteins. However, viral nucleic acid does contain the necessary genetic information to subvert the metabolism of the infected host cell for viral replication. Consequently, viruses are able to direct the activities of the infected host cell towards the synthesis of viral components and the assembly of these into identical progeny. Additionally, viruses are often able to induce the synthesis of their own specific enzymes, the viral coded enzymes.

The completed extracellular product of viral multiplication is called a virion (or virus particle). A virion is composed of a central core of nucleic acid and differs fundamentally from other cells in that it contains deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) but not both. The nucleic acid may be either double or single stranded and is surrounded by a protein coat, the capsid, which protects the nucleic acid from mechanical damage and enzymatic attack and also delivers it to a susceptible host. The capsid is composed of many often identical protein sub-units, the capsomers, the arrangement of which confers on the virion its external form. The latter generally assumes one of two principal arrangements, either a cylindrical structure with helical symmetry or a spherical structure with icosahedral symmetry. In some of the more complex animal viruses, the capsid is in turn surrounded by a membrane, the envelope, which is rich in both lipid and glycoprotein and derives from the infected host cell during replication. Between the capsid and the envelope of such viruses lies the tegument, a structure consisting of specific fibrous proteins. The structure of a virus, namely the herpes simplex virus (HSV), is illustrated on the following page (figure 1).
Figure 1: Structure of the herpes simplex virus

Figure 2: Schematic diagram of the virus replicative cycle showing the points of attack for anti-viral drugs
Viral replication is a unique process whereby virions proliferate within the infected host cell. The replicative cycle of a virus generally involves five main stages:

1. **Adsorption** - Viruses only recognize and adsorb to certain sites, the receptors, on the appropriate cell membranes of specific cells. The site of attachment may be a glycoprotein, a glycolipid, or contain functional sulphhydryl groups. The initial virion-host cell encounter is random, but as the two approach electrostatic interactions orientate the virion for binding with the host cell. A reversible phase of adsorption follows, before an increase in the fluidity of the cell membrane leading to permanent binding.

2. **Penetration and Uncoating** - The exact mechanism of penetration of the viral nucleic acid into the host cell varies from virus to virus. One common mechanism, termed the viropexis, involves the engulfment of the virion by a very fluid host cell membrane. Another method of penetration, whereby the viral envelope fuses with the host cell membrane, is also frequently observed. Removal of the capsid, i.e. uncoating, occurs either at this point or shortly after, to release the free nucleic acid.

3. **Synthesis of Viral Nucleic Acid and Protein** - The machinery of the infected host cell is now directed towards the synthesis of viral nucleic acid (DNA or RNA) and viral protein. The latter includes both structural protein (i.e. the capsomers) and enzymatic protein (i.e. the specific viral coded enzymes). DNA viruses recruit many host cell proteins in the replication and expression of their genome. However, in the case of RNA viruses, a problem arises because the host cells lack enzymes for synthesizing RNA from an RNA template. Consequently, RNA viruses must contain genetic information for the synthesis of an RNA-directed RNA polymerase (also called an RNA replicase or RNA synthetase) or an RNA-directed DNA polymerase (also called a reverse transcriptase).

4. **Assembly** - Following the synthesis phase, the viral components are assembled into new viral particles, the virions.

5. **Release** - Once complete, the infectious virions are released from the cell, a process often involving cell lysis. Alternatively, the virions may be dispersed into the extracellular medium.
without the immediate destruction of the host cell, by a process termed budding. This mechanism essentially involves the extrusion of a virion from the infected host cell, during which the virion may acquire an envelope derived from a portion of the host cell membrane. The latter is considerably modified by the addition of viral specific proteins before budding occurs. On their release, the virions are capable of infecting other suitable host cells and repeating the life cycle.

The prophylaxis and therapy of viral diseases was for a long time considered to be intractable to attack by chemotherapy due to the alliance between the replicative cycle of viruses and the normal metabolism of the cell. Consequently, it was assumed that agents capable of inhibiting or killing viruses might concomitantly damage the host cells that harbour them. However, it has become evident that viruses and virus-infected cells do exhibit some characteristics which differ quantitatively or qualitatively from those of uninfected cells\textsuperscript{45}. Many viruses clearly show such differences in their ability to induce a variety of specific viral coded enzymes, e.g DNA and RNA polymerases, endo- and exo-nucleases, ribonucleotide reductase and nucleoside kinases. These viral coded enzymes are either absent from the uninfected cell or fundamentally different from their normal cellular counterparts, and generally have a broader substrate specificity enabling them to accept compounds as substrates which would not be recognized by the cellular enzymes. Consequently, viral coded enzymes could serve as appropriate targets for the action of anti-viral drugs. Other possible targets for anti-viral chemotherapy include specific events that occur only in virus-infected cells. Indeed, most of the anti-viral drugs reported to date act at one or more stages in the replicative cycle of a virus, as depicted in the schematic diagram (figure 2)\textsuperscript{5}.

Firstly, the necessity for the binding of virions to the receptor sites of potential host cells provides a possible target for anti-viral strategies at the adsorption stage of viral replication. The initial step in the life cycle of the human immunodeficiency virus (HIV), the causative agent of AIDS\textsuperscript{6,7}, for example, involves the attachment of the HIV envelope gp120 glycoprotein to the CD4 glycoprotein on the cell surface of certain T-lymphocytes\textsuperscript{8}. Subsequently, the HIV envelope gp41 glycoprotein is thought to mediate the fusion of the viral and cellular membranes. The viral
envelope gp120 and gp41 glycoproteins may also be involved in inducing the fusion of healthy cells bearing CD4 receptors with HIV-infected cells to form giant multi-nucleated cells or syncytia. A number of approaches are available for preventing HIV infection at this early stage of the replicative cycle, in particular the development of agents capable of interfering with the binding process. A soluble form of the CD4 glycoprotein, consisting of the extracellular regions of CD4, has been reported to inhibit HIV replication and syncytia formation in vitro with low toxicity to healthy cells. Soluble CD4 is thought to saturate the HIV envelope gp120 glycoprotein with receptor sites, thereby neutralizing the infectivity of HIV. However, soluble CD4 suffers from a low plasma half-life and consequently related compounds with longer half-lives are also being investigated.

Dextran sulphate, a long-chain polymer of predominantly α-1,6 linked glucose molecules which may contain two sulphate groups per monosaccharide, also shows anti-viral activity at the adsorption stage of the HIV replicative cycle. Although dextran sulphate was originally developed as an anticoagulant in the 1950's, recent research has indicated its potent and selective inhibitory effect against HIV replication in vitro. This has been attributed to the ability of dextran sulphate to block viral binding to the target cell surface and inhibit syncytia formation, possibly due to its affinity for the gp120 and gp41 glycoproteins of the HIV envelope. Since dextran sulphate is inhibitory at a concentration below its anticoagulant threshold, this compound may be regarded as a potential drug for anti-viral chemotherapy due to its low toxicity.

Some oligopeptides have been reported to show anti-viral activity against paramyxoviruses and orthomyxoviruses at the virus-cell fusion and penetration stages of the viral replicative cycle. A number of compounds have been synthesized with amino acid sequences resembling the N-terminal regions of specific envelope glycoproteins of these viruses. Such oligopeptides may interfere with the binding of the N-termini of the viral glycoproteins at specific cell membrane sites and thereby prevent the initiation of the viral infection. Studies in vitro have indicated that carbobenzoxy-D-Phe-L-Phe-Gly-D-Ala-D-Val-D-Ile-Gly and carbobenzoxy-D-Phe-L-Phe-Gly (1) (figure 3) are potent inhibitors of the measles virus, while carbobenzoxy-Gly-L-Leu-L-Phe-Gly...
and carbobenzoxy-Gly-L-Phe-L-Phe-Gly are active against the influenza A virus\textsuperscript{16}. However, good \textit{in vivo} efficacy has not been reported for peptides of this type.

The anti-viral properties of derivatives of 1-adamantanamine (amantadine) (2) (\textit{figure 3}) are believed to arise from the ability of these compounds to inhibit viral entry into the host cell or viral uncoating. Amantadine hydrochloride (3) (\textit{figure 3}) selectively inhibits influenza A virus infections\textsuperscript{17}, probably by interfering with the uncoating process\textsuperscript{18,19}. Clinical trials have shown (3) to be effective in both the prophylaxis and early therapy of influenza A virus infections in man\textsuperscript{20}. Rimantadine hydrochloride (\textit{\alpha}-methyl-1-adamantanemethylamine hydrochloride) (4) (\textit{figure 3}) displays a similar activity to amantadine hydrochloride, but fewer side effects\textsuperscript{21}. The \textit{\beta}-diketone, arildone (4-(6-(2-chloro-4-methoxyphenoxy)hexyl)-3,5-heptanedione) (5) (\textit{figure 3}), also shows anti-viral activity at the uncoating stage of viral replication. This drug selectively inhibits a number of RNA and DNA viruses\textsuperscript{22} by interacting with the protein capsid of the virus and thus protecting against uncoating\textsuperscript{23}. However, the rapid metabolism of arildone in man makes this compound unlikely to be useful as a chemotherapeutic agent\textsuperscript{24}.

Most of the successful anti-viral drugs act at the stages of the viral replicative cycle involving transcription, translation and replication of nucleic acid. The majority of these compounds are analogues of naturally occurring nucleosides or their precursors, the heterocyclic nitrogenous purine or pyrimidine bases. Generally the targets of such chemotherapeutic agents include the viral nucleic acid polymerase and/or the viral nucleic acid itself. Such suppression of viral nucleic acid synthesis comprises the anti-viral activity of many clinical and experimental chemotherapeutic nucleoside analogues\textsuperscript{4,5}. These compounds, including for example the clinical anti-viral drugs 5-iodo-2'-deoxyuridine (idoxuridine, IDU) (6), 9-\textit{\beta}-D-arabinofuranosyladenine (araA) (7) and 9-(2-hydroxyethoxymethyl)guanine (acyclovir) (8) (\textit{figure 3}), often share a common requirement for kinase-mediated phosphorylation to their active nucleotide form(s) in order to exert their biological effects\textsuperscript{4,5}.

Idoxuridine (6) (\textit{figure 3}), originally prepared in 1959\textsuperscript{25}, might be regarded as the prototype for the further synthesis of nucleoside analogues as potential anti-viral drugs. Indeed, idoxuridine
was the first compound of this type demonstrated to possess activity against various DNA viruses \textit{in vitro}\textsuperscript{26}, and also to be indicated for the topical treatment of herpes keratitis in man\textsuperscript{27}. However, the toxicity of idoxuridine has precluded its systemic use as a chemotherapeutic agent\textsuperscript{28}. The antiviral activity of idoxuridine depends mainly on its incorporation in the 5'-'triphosphate form into newly synthesized viral DNA, instead of the natural nucleotide thymidine-5'-triphosphate, resulting in the formation of incorrect viral protein and hence poor assembly of the virions\textsuperscript{29,30}. Thus, it would appear that idoxuridine requires sequential phosphorylation through the 5'-monophosphate and then the 5'-diphosphate to the active 5'-triphosphate form in order to exert its biological effects. It has been reported that both viral and cellular kinases are capable of mediating the conversion of idoxuridine to its 5'-triphosphate and once formed the latter may be incorporated into viral and cellular DNA. Consequently, it appears to be uncertain as to where the anti-viral selectivity of idoxuridine arises. The clinical anti-herpetic drug araA (7) (figure 3) (discussed in detail later) is another example of a chemotherapeutic nucleoside analogue dependent on kinase-mediated phosphorylation for activation.

These "first generation" anti-viral drugs, e.g. idoxuridine and araA, are generally characterized by a non-selective inhibition of both viral and cellular DNA synthesis. Consequently, many of these compounds display a high degree of toxicity towards the host cells. More recent research has focused on the synthesis of chemotherapeutic agents which inhibit viral-specific processes and thus exert a low cytotoxicity. Such compounds have been termed "second generation" anti-viral drugs and include the nucleoside analogue acyclovir (8) (figure 3). The latter was first synthesized in the late 1970's and found to have a potent and selective activity against a number of herpesviruses in both cell culture and experimental animals\textsuperscript{31,32}. Acyclovir has since proved efficacious in the systemic and topical treatment and the systemic prophylaxis of various herpes simplex virus and varicella-zoster virus infections in man, including immunosuppressed patients\textsuperscript{33}. The selective anti-herpetic activity of acyclovir may be ascribed to its specific phosphorylation to the monophosphate by viral-encoded thymidine kinase and its subsequent conversion to the diphosphate and triphosphate by cellular kinases\textsuperscript{31,34}. Phosphorylation of acyclovir to the
monophosphate only occurs to a very limited extent in uninfected host cells and thus virus-infected cells are targeted by the drug. This accounts for the high selectivity and low cytotoxicity of acyclovir in its anti-viral activity. The active metabolite of acyclovir is the triphosphate, which selectively inhibits viral DNA polymerase and also acts as a chain terminator following its specific incorporation into viral DNA\textsuperscript{31,35}. The incorporated acyclovir nucleotide lacks a 3'-hydroxyl group to form a 3',5'-phosphodiester linkage with another deoxynucleoside-5'-triphosphate, thereby preventing further elongation of the viral DNA chain.

The pyrophosphate analogues, phosphonoacetic acid (PAA) (9) and phosphonoformic acid (foscarin, PFA) (10) (figure 3), are examples of non-nucleosidic compounds showing anti-viral activity at this stage of the viral replicative cycle. These compounds require no previous activation step, unlike the nucleoside analogues (7), (8) and (9), but selectively inhibit viral replication by directly interacting with the pyrophosphate binding sites of certain viral nucleic acid polymerases, thereby inhibiting elongation of the viral nucleic acid chain\textsuperscript{36}. PAA and PFA have been reported to selectively inhibit the DNA polymerases of various herpesviruses \textit{in vitro} and the replication of herpes simplex viruses \textit{in animals}\textsuperscript{37,38}. Additionally, PFA shows \textit{in vitro} anti-viral activity against the influenza virus RNA polymerase and the reverse transcriptases of several retroviruses at concentrations which are non-inhibitory towards host cells\textsuperscript{38,39}. (The reverse transcriptase enzyme is a unique viral DNA polymerase which essentially catalyses the synthesis of viral DNA from the viral genomic RNA template\textsuperscript{1}). More recently, PFA has been found to display an \textit{in vitro} inhibitory effect against the reverse transcriptase of HIV\textsuperscript{40,41}. The clinical usefulness of PAA is questionable since this compound causes skin irritation when applied topically\textsuperscript{42}. PFA, by contrast, is relatively free from this defect\textsuperscript{42} and may thus be regarded as a more promising chemotherapeutic agent than PAA. Indeed, recent clinical trials of PFA in man have proved this compound to be beneficial in the topical treatment of recurrent herpes genitalis and herpes labialis\textsuperscript{33}. However, initial results of evaluating the systemic use of PFA in man against cytomegalovirus infections and AIDS appear to be less encouraging, due to the need for constant infusion and the potential renal toxicity of the drug\textsuperscript{43,44}. 

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Other reverse transcriptase inhibitors include the clinical anti-viral drug 3'-azido-2',3'-dideoxythymidine (AZT) (figure 3), the only chemotherapeutic agent currently licensed for use in the treatment of AIDS. This 3'-modified-2',3'-dideoxynucleoside was originally prepared in 1964 and two decades later discovered to be a potent inhibitor of HIV in vitro. Clinical trials followed shortly after. Metabolic studies of AZT have indicated its dependence on enzymatic phosphorylation to the 5'-triphosphate for anti-viral activity. This activation of AZT is mediated by cellular kinases, since HIV does not appear to encode for the necessary enzymes in its own genome. The 5'-triphosphate is a potent and selective inhibitor of the HIV reverse transcriptase, competing with the natural nucleotide thymidine-5'-triphosphate for binding to this viral-encoded enzyme and also functioning as an alternative substrate. Once incorporated into the viral DNA chain, the AZT nucleotide acts as a chain terminator since it has no 3'-hydroxyl group to form a 3',5'-phosphodiester linkage necessary for chain elongation. The viral reverse transcriptase has a much greater affinity for AZT-5'-triphosphate as a substrate than the cellular DNA polymerases α and β, and this probably gives rise to the anti-viral selectivity of AZT. However, AZT-5'-triphosphate is a reasonable substrate for the cellular DNA polymerase γ, which may account for certain observed clinical toxicities of the drug.

A number of sites in the viral replicative cycle may be subject to the anti-viral activity of interferons, due to the abundance of biological effects these compounds are capable of generating. Interferons are essentially glycoproteins, unique to each animal species, which are synthesized and secreted by vertebrate cells in response to a number of stimuli, including the onset of a viral infection. Evidence has been provided that interferons can act at the stages of (i) virus uncoating, (ii) transcription of the viral genome, (iii) translation of the mRNA (mesenger RNA) and (iv) assembly of the virus particles. Predominant among these effects is an inhibition of viral protein synthesis, and this appears to be achieved by the activity of two enzymes, a protein kinase and an oligoadenylate synthetase. Interferons stimulate the production of these enzymes, which then become active in the presence of double stranded RNA. The protein kinase phosphorylates an initiation factor required for protein synthesis, thereby rendering it inactive. The
2',5'-oligoadenylate synthetase catalyzes the formation of oligoadenylates joined by 2',5'-rather than the usual 3',5'-phosphodiester bonds; these 2',5'-oligoadenylates then activate an endonuclease which degrades mRNA and rRNA (ribosomal RNA). Interferons have been reported to show anti-viral activity against a range of DNA and RNA virus infections, including those due to papilloma-, adeno-, herpes-, hepatitis B, influenza A, rhino- and corona-viruses. However, the low efficacy (on therapeutic use) and toxic side effects of interferons are a major problem in their application for the treatment of viral infections.

Finally, 2-deoxy-D-glucose and other glycosylation inhibitors show anti-viral activity at the assembly stage of the replicative cycle of a number of enveloped DNA and RNA viruses, e.g. the herpes-, togavirus-, rhabdovirus- and myxoviruses. The inhibitory effects of such compounds have generally been attributed to a deficient glycosylation and a loss of the proper function of the viral glycoproteins, resulting in either the inhibition of the viral assembly process or the formation of virions with decreased infectivity. Therapeutic effects have been observed against herpes keratitis in rabbits following the topical administration of 2-deoxy-D-glucose. Additionally, 2-deoxy-D-glucose has been advocated for the topical treatment of genital herpes simplex virus infections in man.

Therefore, a number of targets where various clinical and experimental anti-viral drugs exert their biological effects have been identified, and the reader is directed to two review articles by De Clercq for a more detailed discussion.

The anti-viral drug of particular interest to the research presented in this thesis is the nucleoside analogue 9-β-D-arabinofuranosyladenine (araA, vidarabine) (7). This compound (7) differs from the natural nucleoside adenosine (12) only in the configuration at the C2' of the sugar moiety (figure 4). AraA was first synthesized by Lee and co-workers in 1960 as a potential anti-cancer agent and later isolated by Parke-Davis and Company as a naturally occurring nucleoside antibiotic produced by Streptomyces antibioticus. The biological activity of araA was first demonstrated by Hubert-Habart and Cohen in studies with a purine deficient-strain of Str...
The final step from the 5'-diphosphate (14) to the 5'-triphosphate (15) involves catalysis by (deoxy)adenosine-diphosphate kinase.
*Escherichia coli,* where araA was found to inhibit DNA synthesis. Subsequently, araA was reported by De Garilhe and De Rudder\(^5^9\) to show significant *in vitro* anti-viral activity against herpes simplex and vaccinia viruses. Brink and LePage\(^6^0,6^1\) found araA to be effective against several transplantable tumours in mice, while Doering and co-workers\(^6^2\) indicated the activity of araA against mice fibroblasts (L cells) in cell culture.

More recently, araA has been shown to possess a broad spectrum of activity against DNA viruses in cultured cells and experimental animals but little, if any, activity against RNA viruses with the exception of some RNA tumour viruses and rhabdoviruses\(^6^3-6^5\). AraA has found widespread use as an anti-viral drug in the treatment of various herpesvirus infections\(^3^3,6^6\). Indeed, araA was the first nucleoside analogue approved for systemic use as an anti-herpetic agent. AraA shows a potent efficacy in the systemic therapy of herpes simplex encephalitis, providing it is administered sufficiently early in the course of the disease\(^6^7,6^8\). AraA can also be considered for the systemic treatment of varicella-zoster virus infections, e.g. disseminated herpes zoster and chickenpox, especially in immunosuppressed patients\(^6^9-7^1\). Systemic treatment with araA also brings about a significant reduction in the mortality rate of babies with neonatal herpes\(^7^2\). Additionally, araA has been advocated for clinical use in the topical treatment of herpes keratitis\(^7^3\).

The biologically active form of araA is the 5'-triphosphate (araATP) (15), which essentially inhibits or participates in various reactions associated with viral DNA synthesis. AraA requires sequential phosphorylation by cellular nucleoside and nucleotide kinases for activation. The nucleoside is firstly converted to the 5'-monophosphate (araAMP) (13), followed by further enzymatic phosphorylation to the 5'-diphosphate (araADP) (14) and then the 5'-triphosphate (araATP) (15), with these phosphorylation steps being catalyzed by (deoxy)adenosine kinase, (deoxy)adenylate kinase and (deoxy)adenosine-diphosphate kinase respectively (*equation 1*)\(^7^3,7^4\). This dependence on kinase-mediated phosphorylation to the active 5'-nucleotide form(s) is a problem common to most chemotherapeutic nucleoside analogues, e.g. the clinical anti-viral agents ioduxuridine (6)\(^2^9,3^0\), acyclovir (8)\(^3^1,3^5\) and AZT (11)\(^4^6,4^9\) (as previously discussed), and also to many other clinical and experimental drugs\(^4,5\).
The phosphorylation of araA in animal tissues was originally suggested by Brink and LePage\textsuperscript{50} after analyzing the acid-soluble fractions of tissues obtained from tumour-bearing mice treated with (8-\textsuperscript{14}C)araA. These results were subsequently confirmed by York and LePage\textsuperscript{75,76} in studies with cell-free tissue extracts and suspensions of intact TA3 ascites cells. More recently, Plunkett and Cohen\textsuperscript{77} have shown that cultured mouse fibroblasts phosphorylate a small, but significant, proportion of exogenously added (2-\textsuperscript{3}H)araA to arabinosyl nucleotides that attain inhibitory concentrations within the cell. Additionally, Rose and Brockman\textsuperscript{78}, using high pressure liquid chromatography to separate the araATP from ATP, have quantified araATP levels in mouse leukaemia L1210 cells under a variety of conditions.

There are several possible ways in which araA acts in the 5'-tri phosphate state (15) to inhibit viral DNA synthesis, but the inhibition causally related to the anti-viral activity has yet to be established. Primarily, araATP has been reported to inhibit viral DNA polymerase\textsuperscript{75,79,80}. This enzyme essentially catalyzes the formation of a phosphodiester bond between the 3'-hydroxyl group at the end of a growing DNA chain and the innermost phosphorus atom of an incoming deoxynucleoside-5'-triphosphate\textsuperscript{1}. Investigations of araATP inhibition of DNA synthesis in animal cells began with the observations that araA treatment of mice bearing TA3 and 6C3HED ascites tumours resulted in a marked reduction in DNA synthesis by the tumour cells\textsuperscript{50,61,81}. Subsequently, York and LePage\textsuperscript{75} studied the effect of araATP on the incorporation of (\textsuperscript{14}C)thymidine-5'-monophosphate into DNA by DNA polymerase in crude cell free extracts of TA3 ascites cells, and found araATP to be a non-competitive inhibitor of DNA polymerase. However, Furth and Cohen\textsuperscript{79,80} showed araATP inhibition of DNA polymerase from crude extracts of bovine lymphosarcoma and calf thymus to be competitive with deoxyadenosine-5'-monophosphate (dATP). It later became apparent that animal cells contain at least three separate species of DNA polymerase, termed \(\alpha\), \(\beta\), and \(\gamma\), which differ in their molecular weights, chromatographic properties and template requirements\textsuperscript{82}. Consequently, the use of crude preparations containing mixtures of the various species of DNA polymerase in these early studies of araATP inhibition of DNA polymerase\textsuperscript{75,79,80}, accounts for the conflicting results observed.
More recently, Muller and co-workers\textsuperscript{83,84} have investigated araATP inhibition of several purified preparations of DNA polymerases, including some cellular DNA $\alpha$- and $\beta$-polymerases and viral DNA polymerases. AraATP inhibition of each of the DNA polymerases was reported to be competitive with respect to dATP. However, araATP was found to inhibit the herpes-induced DNA polymerase to a significantly greater extent than the DNA $\alpha$- or $\beta$-polymerases of uninfected cells. Similarly, Shipman, Drach and co-workers\textsuperscript{85,86} observed that herpes DNA synthesis was considerably more sensitive to araA than cellular DNA synthesis. These results were of particular interest, given that the conversion of araA to araATP does not depend on viral coded enzymes and is essentially the same in uninfected and virus-infected cells. Consequently, araATP appears to preferentially inhibit viral DNA polymerase (of herpesvirus-infected cells) over cellular DNA polymerases, thereby providing a possible explanation for the selectivity of araA in its anti-viral action against herpesvirus infections.

Although the inhibition of DNA polymerase is the most readily apparent biochemical effect of araATP, the incorporation of araA nucleotides into viral and cellular DNA has also been reported. Early studies by Waqar and co-workers\textsuperscript{87}, involving the incorporation of $^{32}$P-labelled nucleotides into DNA synthesized by preparations of nuclei isolated from rat liver and thymus, raised the possibility that araATP may function as a DNA chain terminator. However, a number of subsequent investigations\textsuperscript{77,83,88} have demonstrated the incorporation of araA primarily into the internucleotide linkages of mouse fibroblast and lymphoma cell DNA, suggesting relatively low levels of chain-terminating activity. The incorporation of araA into viral-specific, as well as cellular, DNA has been observed in experiments in which herpesvirus-infected rabbit kidney cells were incubated with ($^{8}$-H)araA during the period of DNA synthesis\textsuperscript{84}. Although araA entered the internucleotide linkage in uninfected cells far more readily than it terminated DNA chains, the reverse was reported to be true for the viral DNA of herpes-infected cells\textsuperscript{89}. More recently, experiments involving the incorporation of araA into the DNA of leukaemia L1210 cells have indicated the presence of a greater proportion of araA residues at the 3'-terminus of DNA chains, suggesting that araA may provide a poor primer terminus for chain elongation\textsuperscript{90}.  

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AraA has also been found to inhibit the ribonucleotide reductase (or ribonucleoside diphosphate reductase) of animal cells\textsuperscript{5,91}, either in the 5′-triphosphate (15) or the 5′-diphosphate (14) forms. This enzyme essentially catalyzes the reduction of ribonucleoside-5′-diphosphates to their corresponding deoxyribonucleoside-5′-diphosphates\textsuperscript{1}. Consequently, an inhibition of ribonucleotide reductase by araA nucleotides may indirectly effect DNA synthesis by interfering with the formation of DNA precursors. AraATP, and possibly araADP, have been shown to inhibit the reduction of the four common ribonucleoside-5′-diphosphates, using a partially purified enzyme system from ascites tumour cells in rats\textsuperscript{91}. However, the araA nucleotides were found to be only one-tenth as active in this system as the natural allosteric inhibitor dATP. It has also been reported that the ribonucleotide reductase from herpes simplex virus-infected cells displays a greater sensitivity to inhibition by araATP than the enzyme from uninfected cells\textsuperscript{92}.

Although it is of clinical use, araA suffers from several other limitations besides a dependence on kinase-mediated phosphorylation for activation. These disadvantages include:

1. a low \textit{in vivo} activity due to the rapid conversion of araA by the enzyme adenosine deaminase to the much less efficacious metabolite arahypoxanthine (araHx) \textsuperscript{(16) (equation 2)}\textsuperscript{55,93} (discussed in detail below);

2. a low aqueous solubility (0.5 mg/ml at 25°C, 1.8 mg/ml at 37°C)\textsuperscript{50,94}, requiring the intravenous infusion of large volumes of fluid containing the dissolved drug for systemic use;

3. a low lipophilicity, which retards the diffusion of araA across biological membranes and its penetration into tissues and hence limits the use of the drug in the topical treatment of cutaneous herpesvirus infections\textsuperscript{65};

4. a moderate toxicity (at the large doses required), which may cause gastrointestinal disturbances (e.g. nausea, vomiting and diarrhoea), neurological disorders (e.g. hallucinations, tremors and extremity pains), and leucocyte chromosomal breakage\textsuperscript{33,65,85}.

The adenosine deaminase-mediated deactivation of araA within the body to arahypoxanthine (araHx) \textsuperscript{(16) (equation 2)}\textsuperscript{55,93} constitutes a major limitation in the use of araA as an anti-viral drug. The deamination of araA was observed in the early studies of this drug conducted by
Equation 2

\[
\text{adenosine deaminase}
\]

Figure 5

(17) \( R = \text{OH} \)
(18) \( R = \text{H} \)

(19)
Hubert-Habart and Cohen\textsuperscript{38}, where araA incubated for 2 hours with exponentially growing cultures of a purine-deficient strain of \textit{Escherichia coli} was almost completely converted to araHx. Similarly, Brink and LePage\textsuperscript{51} demonstrated that the cell-free extracts of various mouse tissues displayed a substantial capacity for araA deamination. It was also reported that on treating mice with (\textsuperscript{8-}\textsuperscript{14}C)araA, 35\% of the radioactivity administered was recovered as araHx in the urine within an hour\textsuperscript{61}. In man, intravenously administered araA was found to have a half-life in the bloodstream of 3.5 hours, with 50\% to 60\% of the drug excreted in the urine after 24 hours, primarily as araHx\textsuperscript{65}. A comparison of the araA deamination rates in several intact mammalian erythrocytes has also revealed substantial species-specific differences, with increasing levels of adenosine deaminase activity exhibited by rat, human, mouse and monkey erythrocytes\textsuperscript{93,96}. Additionally, significant tumour-specific variations in the rates of araA deamination have been observed. Studies conducted on a number of transplantable tumours in mice, for example, indicated that tumours with high adenosine deaminase activity were practically unresponsive to araA, while those with much lower adenosine deaminase activity exhibited the greatest sensitivity to the drug\textsuperscript{60,93,96}. Thus, it would appear that the extent of biological activity displayed by araA is inversely related to the levels of adenosine deaminase.

It might be possible to prevent or retard this mode of deactivation of araA by the co-administration of the drug with an inhibitor of adenosine deaminase. Indeed, several adenosine deaminase inhibitors have become available, the most potent of which include (R)-3-(\textbeta-D-erythropentafuranosyl)-3,6,7,8-tetrahydroimidazo(4,5-d)(1,3)diazapin-8-ol(coformycin) (17) and its 2\textsuperscript{-}deoxyribosyl homologue (deoxycoformycin, covidarabine) (18) (\textbf{figure 5}). Coformycin was originally isolated from culture filtrates of \textit{Nocardia interforma} and \textit{Streptomyces kaniharaensis}\textsuperscript{97,98} and later prepared by organic synthesis\textsuperscript{99}, while deoxycoformycin was originally isolated as a fermentation product of \textit{Streptomyces antibioticus}\textsuperscript{100,101}. Other slightly less potent inhibitors of adenosine deaminase include erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (19), synthesized by Schaeffer and Schwender\textsuperscript{102}, and 1,6-dihydro-6-hydroxymethyl purine ribonucleoside (20) (\textbf{figure 5}), prepared by Evans and Wolfenden\textsuperscript{103}. 

23
Deoxycoformycin (18) and EHNA (19) have been studied the most extensively as adenosine deaminase inhibitors that might enhance the therapeutic activity of araA. The inhibitory effect of EHNA on araA deamination was first reported by Plunkett and Cohen in studies involving homogenates of mouse fibroblasts and Ehrlich ascites tumour cells. Subsequently, LePage, Kimball and co-workers demonstrated that deoxycoformycin inhibited the deamination of araA in suspensions of intact mouse leukaemia L1210 cells. Additionally, both groups of investigators have observed that treatment with araA (or araA derivatives) in combination with EHNA or deoxycoformycin prolonged the survival time of tumour-bearing mice, as compared to therapy with araA alone. These adenosine deaminase inhibitors have also been found to potentiate the inhibitory effect of araA on the replication of several DNA viruses. A comparative study of the in vitro and in vivo anti-viral activity displayed by araA alone relative to araA when co-administered with deoxycoformycin, for example, was conducted by Sloan and co-workers. The combined drugs were found to possess a significantly greater (ten-fold) anti-viral activity against herpes and vaccinia viruses in tissue culture and subcutaneously against cranial herpes in mice. Similarly, North and Cohen have reported an increased inhibition of herpes simplex virus production in cell culture by araA in the presence of EHNA, over the anti-viral activity of araA alone.

Overall, it appears that a decrease in the rate of araA deamination has been achieved by the co-administration of araA with EHNA or deoxycoformycin, thereby allowing a greater accumulation of araA nucleotides in the cells. Indeed, Brockman, Rose and co-workers found significantly higher levels of araATP in mouse leukaemia L1210 cells and P388 cells when tumour-bearing mice were treated with araA in combination with EHNA or deoxycoformycin, as compared to therapy with araA alone. Similarly, Plunkett and Cohen observed significant increases in the cellular content of arabinose nucleotides when araA was co-administered with EHNA to mouse fibroblasts in cell culture. Studies conducted by Chang and Glazko concerning the effect of deoxycoformycin on araA metabolism in human tissues, revealed that deoxycoformycin not only protected against araA deamination in the blood, but also increased the
accumulation of araA nucleotides in the erythrocytes.

Although co-administration of araA with an adenosine deaminase inhibitor has proved successful in greatly increasing the plasma half-life of the anti-viral drug, the long term inhibition of crucial metabolic enzymes (e.g. adenosine deaminase) could have undesirable toxic side effects\textsuperscript{113}. Alternatively the problem of deamination might be overcome by synthesizing deamination-resistant forms of araA. The search for such compounds has largely focused on the preparation of 5'-modified derivatives of araA, since a major substrate requirement of adenosine deaminase is a free 5'-hydroxyl function\textsuperscript{114}. This approach essentially involves a biologically active drug being chemically modified to give an inactive precursor or pro-drug. A pro-drug may be defined as a pharmacologically inactive derivative of a parent drug that requires spontaneous or enzymatic transformation within the body to release the active drug. Thus, after administration or absorption of the pro-drug, the active drug might be released by catalyzed hydrolysis involving liver or intestinal enzymes or simply by direct chemical hydrolysis. The use of a pro-drug could lead to an enhanced activity over the parent drug itself, e.g. by a more efficient delivery of the pro-drug to the site of action than the parent drug.

There has been considerable interest in the synthesis of 5'-esters of araA as possible pro-drugs of the parent compound. Renis and co-workers\textsuperscript{115} in 1973, for example, synthesized the 5'-benzoyl (21) and 5'-palmitoyl (22) esters of araA (figure 6) as potential deamination-resistant forms of araA. Additionally, it was hoped that the lipophilicity of these derivatives might enhance their passive diffusion across the cell membrane, and once successful entry into the cell had been achieved the ester bond might be susceptible to intracellular hydrolysis to give the free nucleoside araA. AraA-5'-benzoate was found to possess a similar \textit{in vitro} anti-viral efficacy to araA, with both compounds showing activity against the DNA-containing herpesvirus but no activity against several RNA-containing viruses. However, araA-5'-palmitate displayed no \textit{in vitro} activity, probably due to the poor solubility of this compound. AraA was found to be effective in treating mice infected with herpesvirus, but the 5'-ester derivatives (21) and (22) were devoid of \textit{in vivo} activity. These results contrasted with the enhanced biological activity reported for some 5'-esters
of the anti-cancer drug 9-β-D-arabinofuranosylcytosine (araC), including the 5'-benzoate (23) and 5'-palmitate (24) derivatives (figure 6), over the parent drug^116,117. It has been suggested that the loss of in vivo activity following 5'-esterification of araA might arise from the esterases of the host being less effective in releasing araA than araC from their respective 5'-acylates^115.

Another potential deamination-resistant derivative of araA, namely araA-5'-formate (25) (figure 6), was synthesized by Repta and co-workers^44 in 1975. This compound was found to display a comparable activity to araA against experimental tumours in mice. Stability studies in various media indicated that the desired aim, involving the rapid conversion of (25) by chemical and enzymatic hydrolysis to the free nucleoside, had been achieved. Additionally, araA-5'-formate possessed the advantageous property of being more water soluble than araA. Further studies at this time included the synthesis of some O'-methylated derivatives of araA^118. The 5'-methyl (26) (figure 6) and 2',3'-dimethyl esters of araA have been reported to show complete resistance to enzymatic deamination, while the 2'-methyl and 3'-methyl esters of araA were much less susceptible to adenosine deaminase than the parent drug^118. Unfortunately, O'-methylation of araA resulted in a complete loss or considerable reduction of anti-viral activity against herpes simplex, vesicular stomatitis and vaccinia viruses in cell culture^119. This inactivity (or low activity) has been attributed to the lack of cleavage of these derivatives to release the free nucleoside. The 2'-methyl, 3'-methyl, and 2',3'-dimethyl esters of araA might also suffer from an unsuitability as substrates for the cellular kinases.

Overall, it is possible to conclude that 5'-esters of araA appear (in some cases) to be promising as deamination-resistant transport forms of the parent drug. However, such derivatives do not solve the other major limitation of araA, a dependence on enzymatic phosphorylation by cellular kinases for conversion to its 5'-nucleotide forms. AraAMP (13) might therefore be regarded as a potential deamination-resistant form of araA, with the added advantage that the presence of a 5'-phosphate group might obviate the dependence on nucleoside kinases for activation. Furthermore, the water solubility of araAMP (>100 mg/ml at 37°C)^120 is much greater than that of araA. However, a comparison of the anti-viral activities of araAMP and araA against
several DNA viruses in cell culture and herpes simplex virus infections in experimental animals, and more recently against varicella and disseminated zoster in immunosuppressed patients, revealed that the nucleotide showed no significant advantage over the nucleoside. Indeed, similar observations have been reported for the 5′-monophosphates of other chemotherapeutic nucleoside analogues when compared with their corresponding nucleosides. This feature is commonly attributed to the poor cellular membrane penetration of the nucleotide, presumably due to the presence of the negative charge on the 5′-monophosphate at physiological pH. Early experiments were indicative of the rapid extracellular cleavage of the nucleotide to the corresponding nucleoside, which then passed across the cell membrane and was rephosphorylated to the nucleotide within the cell. However, subsequent metabolic studies with doubly labelled (3H,32P)araAMP have shown that small amounts of araAMP slowly penetrated the cells without degradation, giving rise to intracellular araATP which could attain a potentially inhibitory concentration.

AraA cyclic 3′,5′-monophosphate (cyclic araAMP) (27) (figure 7), originally synthesized by Lee and co-workers in 1971 and later by Mian and co-workers in 1974, was found to exhibit a comparable in vitro and in vivo anti-viral activity to araA and araAMP. Studies conducted by LePage and Hersh have indicated the ability of cyclic araAMP to penetrate intact cells, with sufficient concentrations of this compound being attained to significantly inhibit the growth of ascites tumour cells in mice and the synthesis of DNA in cultured human lymphocytes. Additionally, it was demonstrated that homogenates of mouse leukaemia L1210 cells could convert (27) to the 5′-monophosphate, but no deamination or dephosphorylation of either cyclic araAMP or araAMP occurred. Hughes and Kimball have shown that cyclic araAMP inhibited the synthesis of DNA, but not RNA, and also the activity of DNA polymerase in L1210 tumour cells. This inhibition was probably due to the intracellular cleavage of the cyclic phosphodiester bond by cyclic phosphodiesterase and phosphorylation of the resulting araAMP to araATP. However, there was some indication that the cyclic nucleotide, itself, might also be inhibitory towards the DNA polymerase.
Revankar and co-workers\cite{Revankar1975} reported the first synthesis of a 5'-phosphate ester derivative of araA in 1975, with the preparation of araA-5'-methyl phosphate (28) (figure 7). This compound showed a similar activity to araA and araAMP against several DNA viruses in cell culture and also possessed the advantageous property of being more water soluble than araA.

More recently, a number of 5'-phosphate triester derivatives of araA have been synthesized. Farquhar and Smith\cite{Farquhar1980}, for example, have prepared the cyclic 5'-phosphate derivatives of araA, 9-(5'- (2-oxo-1,3,2-dioxaphosphorinan-2-yl)-β-D-arabinosyl)adenine (30) and 9-(5'- (2-oxo-1,3,2-oxazaphosphorinan-2-yl)-β-D-arabinosyl)adenine (31) (figure 8). It was hoped that (30) and (31) would undergo biotransformation \textit{in vivo} by hepatic cytochrome P-450 dependent mixed-function oxidases to give their respective 4-hydroxy analogues (32) and (33), in a similar manner to the mechanism by which the anti-cancer agent cyclophosphamide (29) (figure 8) exerts its biological effects\cite{Farquhar1983}. It was anticipated that these compounds (32) and (33) would enter the cells by passive diffusion and then undergo spontaneous ring opening to yield their corresponding acyclic tautomers (34) and (35), which would subsequently dissociate with elimination of acrolein (36) to give the 5'-monophosphate (13) and the 5'-phosphoramidate (37) respectively\cite{Farquhar1983} (figure 8). Conversion of (37) to (13) was envisaged to occur by chemical or enzymatic hydrolysis. However, studies of the \textit{in vivo} anti-tumour activity of (30) and (31) revealed that the former was only marginally effective at prolonging the life-span of mice bearing P-388 leukaemia, while the latter was inactive. The low activity of (30) and (31) was attributed to these compounds not undergoing significant bioactivation \textit{in vivo}. Indeed, on incubating (30) and (31) with mouse hepatic microsomal preparations in the presence of an NADPH-generating system (i.e. under conditions where cyclophosphamide was extensively degraded), these derivatives were only minimally biotransformed to their respective 4-hydroxy analogues (32) and (33). Farquhar and Smith concluded that the poor substrate properties of the cyclic 5'-phosphate derivatives (30) and (31) for the mixed-function oxidases were probably due to their low lipid solubility.

The synthesis of a series of simple 5'-dialkyl phosphate triesters of araA (38-41) (figure 9) has been reported in this Department\cite{Department1985}. It was hoped that these compounds would act as potential
Figure 8

\[
\begin{align*}
(29) & \quad X = \text{NH} \\
(30) & \quad X = \text{O}, Y = \text{H} \\
(31) & \quad X = \text{NH}, Y = \text{H} \\
(32) & \quad X = \text{O}, Y = \text{OH} \\
(33) & \quad X = \text{NH}, Y = \text{OH} \\
(34) & \quad X = \text{OH} \\
(35) & \quad X = \text{NH}_2 \\
(36) & \quad X = \text{NH} \\
(37) & \quad X = \text{F}
\end{align*}
\]

Figure 9

\[
\begin{align*}
(38) & \quad R = \text{Et} \\
(39) & \quad R = \text{Pr} \\
(40) & \quad R = \text{Bu} \\
(41) & \quad R = \text{Pen} \\
(42) & \quad X = \text{F} \\
(43) & \quad X = \text{Cl}
\end{align*}
\]
uncharged, membrane-soluble, deamination-resistant forms of the drug, which might also be preferentially hydrolyzed at the phosphorus-alkyl bond within the cell to release araAMP (13), thereby obviating nucleoside kinase dependence. These 5′-dialkyl phosphate derivatives showed complete resistance to deamination by the enzyme adenosine deaminase, and were also inhibitory towards DNA synthesis in vitro. Moreover, an increasing biological activity was observed for compounds (38-41) with increasing chain length of their constituent alkyl groups. This was attributed to the increasing lipophilicity of these compounds in the same series, suggesting membrane penetration by the intact 5′-phosphate triesters. Unfortunately, a decreasing water solubility was also reported for the 5′-dialkyl phosphate triesters of araA with increasing chain length of the alkyl moiety. Additionally, these derivatives were not susceptible to chemical or enzymatic hydrolysis at physiological pH and temperature.

AraA-5′-bis(2,2,2-trifluoroethyl) phosphate (42) and araA-5′-bis(2,2,2-trichloroethyl) phosphate (43) (figure 9) were subsequently prepared in this Department, with the aim of facilitating hydrolysis to araAMP. The biological activity of these 5′-bis(2,2,2-trihaloethyl) phosphate triesters of araA, when compared with their simple 5′-dialkyl analogues, was found to be significantly higher than one might expect on the basis of their lipophilicity alone. This was attributed to the electron-withdrawing halogen substituent labilizing the phosphorus-oxygen bond towards intracellular chemical cleavage to yield the 5′-monophosphate araAMP. Interestingly, araA-5′-bis(2,2,2-trichloroethyl) phosphate (43) showed a greater biological activity than its 5′-bis(2,2,2-trifluoroethyl) counterpart (42). Thus, the introduction of bis(2,2,2-trihaloethyl) substituents at the 5′-phosphate site appears to enhance lipophilicity, increase susceptibility to hydrolysis and increase biological activity, but the relative magnitude of these effects varies markedly with the nature of the halogen.

The research discussed above suggests the possibility of synthesizing other 5′-phosphate triesters of araA with alternative esterifying groups that might confer an enhanced biological activity on these compounds. Such derivatives might also act as intracellular sources of the free
nucleotides, thereby obviating nucleoside kinase dependence. Consequently, the research presented in this thesis involves an investigation into the synthesis and biological evaluation of some novel 5'-phosphate derivatives of araA as potential uncharged, membrane-soluble, deamination-resistant prodrugs of araAMP (13). It was hoped to obtain a derivative showing greater activity than the parent drug araA.

Firstly, an investigation into the synthesis of some 5'-bis(2-haloethyl) phosphate triesters of araA (44) (figure 10) was carried out. Subsequently, a number of mixed unsymmetrical 5'-alkyl (2,2,2-trichloroethyl) (45) and 5'-aryl (46) phosphate triesters of araA (figure 10) were synthesized. The preparation of some mixed, unsymmetrical 5'-(benzyl-protected glycolyl) phosphate triesters of araA (47) and the hydrogenolysis of these derivatives to their corresponding deprotected products (48) (figure 10) was also investigated. The biological activity of most of these derivatives was evaluated by determining their ability to inhibit the synthesis of DNA in vitro using a tritiated thymidine incorporation assay employing mammalian epithelial cells.

The mechanism by which these 5'-phosphate triesters of araA might exert their biological effects was unclear. It was hoped that the mode of action would involve the intracellular hydrolysis of the phosphate moiety via P-O-alkyl cleavage to the 5'-monophosphate (araAMP), thereby providing a "kinase by-pass". It is also possible that hydrolysis to the parent nucleoside (araA) might occur. The in vitro biological activity of two 5'-phosphate triesters of araA (49) were compared with their analogous 5'-phosphinate esters (50) (figure 11) to help elucidate the matter. Finally, the susceptibility of araA-5'-bis(2,2,2-trifluoroethyl) phosphate (42), araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) and a 5'-alkyl (2,2,2-trichloroethyl) phosphate triester of araA (45) to chemical hydrolysis was investigated and attempts to purify and characterize the products were carried out.
Figure 10

(44) $X = \text{halogen}$

(45) $R = \text{alkyl}$

(46) $\text{Ar} = \text{aryl}, R = \text{alkyl or 2,2,2-trihaloethyl}$

(47) $R = \text{alkyl or 2,2,2-trihaloethyl}, n = 1-3$

(48) $R = \text{alkyl or 2,2,2-trihaloethyl}, n = 1-3$

Figure 11

(49)

(50)
RESULTS AND DISCUSSION

Chapter 1: 5'-Bis(2-haloethyl) phosphate triesters of araA

The first aim of the research involved an investigation into the synthesis and biological evaluation of a series of 5'-bis(2-haloethyl) phosphate triesters of araA. It would be of interest to discover whether such compounds show an enhanced biological activity over their non-substituted counterparts as found for araA-5'-bis(2,2,2-trifluoroethyl) phosphate and araA-5'-bis(2,2,2-trichloroethyl) phosphate when compared with their simple 5'-dialkyl analogues\textsuperscript{135,136}. Additionally, it was hoped that the increasing biological activity observed for these 5'-bis(2,2,2-trihaloethyl) derivatives with increasing lipophilicity of the compound\textsuperscript{136}, would be similarly reflected in the 5'-bis(2-haloethyl) series.

The proposed route for the synthesis of some 5'-bis(2-haloethyl) phosphate triesters of araA involved a two-step procedure, this methodology having been successfully used in the preparation of a series of 5'-dialkyl phosphate triesters of araA\textsuperscript{135}, and subsequently in the synthesis of the 5'-bis(2,2,2-trihaloethyl) phosphate triesters of araA\textsuperscript{136}. The first step involved the preparation of the appropriate phosphorylating agent, in this case a series of bis(2-haloethyl) phosphorochloridates. Several alternative methods are available for the synthesis of phosphorochloridates\textsuperscript{137}, some examples include:

(1) The reaction of tertiary or secondary phosphites with (i) chlorine\textsuperscript{138,139}, (ii) sulphuryl chloride\textsuperscript{140,142} and (iii) thionyl chloride\textsuperscript{141,143}.

\begin{align*}
(\text{RO})_3\text{P} + \text{Cl}_2 & \rightarrow (\text{RO})_2\text{POCl} + \text{RCl} \\
(\text{RO})_2\text{PHO} + \text{Cl}_2 & \rightarrow (\text{RO})_2\text{POCl} + \text{HCl}
\end{align*}
(ii) \((\text{RO})_3\text{P} + \text{SO}_2\text{Cl}_2 \rightarrow (\text{RO})_2\text{POCl} + \text{SO}_2 + \text{RCl}\)

\((\text{RO})_2\text{PHO} + \text{SO}_2\text{Cl}_2 \rightarrow (\text{RO})_2\text{POCl} + \text{SO}_2 + \text{HCl}\)

(iii) \((\text{RO})_3\text{P} + \text{SOCl}_2 \rightarrow (\text{RO})_2\text{POCl} + \text{SO} + \text{RCl}\)

\((\text{RO})_2\text{PHO} + \text{SOCl}_2 \rightarrow (\text{RO})_2\text{POCl} + \text{SO} + \text{HCl}\)

(2) The reaction of phosphoryl chloride with two molar equivalents of alcohol in the absence or presence of a tertiary organic base, usually in an inert solvent at or below ambient temperature\(^{144,145}\).

\(\text{POCl}_3 + 2\text{ROH} \rightarrow (\text{RO})_2\text{POCl} + 2\text{HCl}\)

This last example was the method chosen for the preparation of the bis(2-haloethyl) phosphorochloridates. Thus, phosphoryl chloride was reacted with two molar equivalents of the appropriate 2-haloethyl alcohol in diethyl ether solvent at reduced temperature (equation 3). Triethylamine was present in the reaction mixture to remove the hydrogen chloride by-product by precipitation as triethylamine hydrochloride. Although pyridine is commonly used as a base in this type of reaction, triethylamine was chosen instead, because phosphorochloridates can react with pyridine or pyridine hydrochloride on heating to give alkyl halides and pyridine-phosphorus compounds\(^{146,147}\) and this may cause problems if the product is to be purified by vacuum distillation. Elimination of the hydrogen halide by-product may also be accomplished by carrying out the reaction under reduced pressure or by bubbling an inert gas, e.g. nitrogen or carbon dioxide, through the reaction mixture\(^{145}\). The above procedure, involving the use of a tertiary organic base, was favoured from the range of methods available since the reaction is relatively easy to control under these conditions.
Equation 3

\[
\text{POCl}_3 + 2\text{XCH}_2\text{CH}_2\text{OH} + 2\text{Et}_3\text{N} \rightarrow (\text{XCH}_2\text{CH}_2\text{O})_2\text{POCl} + 2\text{Et}_3\text{NHCl}
\]

\(X = \text{halogen}\)

\(-78^\circ\text{C} \text{ to } \text{RT}\)

Equation 4

\[
(X\text{CH}_2\text{CH}_2\text{O})_2\text{POCl} + \text{HO} \xrightarrow{\text{pyridine}} X\text{CH}_2\text{CH}_2\text{O} - \text{P} - \text{O} - \text{XCH}_2\text{CH}_2\text{O}
\]

\(X = \text{halogen}\)

Equation 5

\[
\text{HO} \xleftrightarrow{\text{(Me)}_2\text{CO}} \text{HO}
\]

\[
\xrightarrow{\text{H}^+ / \text{H}_2\text{O}} \text{HO}
\]

(12)  \(\rightarrow\)  (51)
The second step involved the subsequent reaction of the appropriate bis(2-haloethyl) phosphorochloridate with unprotected araA in basic solvent at reduced temperature to give a series of 5′-bis(2-haloethyl) phosphate triesters of araA (equation 4). Pyridine is commonly used as the solvent and base in reactions of this type, due to the poor solubility of most nucleosides in other common solvents and the basic nature of pyridine facilitating the removal of the hydrogen halide by-product as pyridine hydrochloride. However, araA is only sparingly soluble in pyridine at ambient temperature, but complete dissolution can be achieved by heating a suspension of the nucleoside in pyridine to reflux then cooling to ambient temperature or below to give a super-saturated solution, before the addition of the appropriate bis(2-haloethyl) phosphorochloridate.

The use of pyridine may cause difficulties if purification of the resulting product involves column chromatography, since small amounts of this solvent can bind to silica and consequently elute in a broad band with the product. It was hoped that in the work-up of reactions using pyridine, after the removal of the bulk of this solvent under reduced pressure, final traces would be removed by trituration with diethyl ether and co-evaporation with toluene under reduced pressure in an attempt to overcome this problem. Several other polar solvents, namely 1,4-dioxan, ethyl acetate, triethylamine and acetone, have been investigated as alternatives, but none proved successful\(^{148}\). Another possibility would be the reaction of the appropriate bis(2-haloethyl) phosphorochloridate with unprotected araA in anhydrous tetrahydrofuran containing an excess of N-methylimidazole. A similar reaction has proved successful in the synthesis of some phosphoramidate and aryl phosphate derivatives of the anti-HIV drug AZT\(^{149-152}\). However, reaction of araA was found to be poor under these conditions, probably due to the sparing solubility of this nucleoside in tetrahydrofuran even at reflux. Pyridine was therefore used as both solvent and base in all syntheses discussed in this thesis involving reaction of the appropriate phosphorylating agent with araA.

The main disadvantage of the proposed route for the synthesis of 5′-bis(2-haloethyl) phosphate triesters of araA is the use of an unprotected nucleoside. Reaction of the
phosphorochloridate may occur at the 2'-hydroxyl group, the 3'-hydroxyl group, and/or the amino group in addition to the required 5'-hydroxyl group. However, preferential reaction at the amino group is unlikely due to its low reactivity relative to the hydroxyl groups, and of these the sterically less hindered primary 5'-hydroxyl group is a more reactive site than the secondary 2'- and 3'-hydroxyl groups. The 5'-selectivity of the reaction can be ensured by protecting the 2'- and 3'-hydroxyl groups, and less importantly the amino group, providing that the product is stable under the conditions required for deprotection. Protection at the 2'- and 3'-hydroxyl sites of ribonucleosides, e.g. adenosine (12), is achieved relatively easily by reaction with propanone to give the isopropylidene acetal derivative (51), with the deprotection step occurring under dilute acid conditions\textsuperscript{153,154} (equation 5). However, the trans stereochemistry of the 2' - and 3'-hydroxyl groups of arabinonucleosides, e.g. araA, means protection of these sites is more complicated. Firstly 5'-protection is required, followed by 2'- and 3'-protection, and finally 5'-deprotection, before reaction with the appropriate phosphorochloridate. Consequently, since optimization of yields was not of primary importance in the research, it was decided to carry out all reactions on unprotected nucleosides. This strategy has proved successful in similar reactions with unprotected araA\textsuperscript{135,136}, where only minor formation of compounds other then the required 5'-phosphorylated product was observed and the latter was readily separated from such impurities on purification.

The target compounds, araA-5'-bis(2-fluoroethyl) phosphate (53) and araA-5'-bis(2-bromoethyl) phosphate (55), were synthesized by the two-step procedure outlined above, involving preparation of the appropriate bis(2-haloethyl) phosphorochloridate and its subsequent reaction with unprotected araA. Bis(2-fluoroethyl) phosphorochloridate (52) has been previously prepared by the reaction of phosphoryl chloride with two molar equivalents of 2-fluoroethanol, while passing carbon dioxide through the reaction mixture to remove the hydrogen chloride by-product\textsuperscript{155}. Bis(2-bromoethyl) phosphorochloridate (54) is not reported in the literature.

The synthesis of the bis(2-haloethyl) phosphorochloridates (52) and (54) involved the separate and simultaneous addition of two molar equivalents of both the appropriate 2-haloethyl alcohol
and triethylamine to phosphoryl chloride at -78°C, using diethyl ether as the solvent (equation 6). Possible impurities resulting from these reactions include 2-haloethyl phosphorodichloridate, tris(2-haloethyl) phosphate and hydrolyzed material. It was hoped to minimize the formation of these by-products by the scrupulous drying of the reagents prior to the reaction, the slow addition of the reagents at reduced temperature, and using an excess of solvent to dilute the reaction mixture.

Firstly, bis(2-fluoroethyl) phosphorochloridate (52) was prepared by the reaction of phosphoryl chloride with two molar equivalents of both 2-fluoroethanol and triethylamine in diethyl ether solvent at -78°C (equation 6). After allowing the reaction mixture to warm to ambient temperature with stirring overnight, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. The $^{31}$P n.m.r. spectrum of the crude phosphorylating agent showed a major peak due to the required product (52) and also two minor peaks probably due to 2-fluoroethyl phosphorodichloridate and tris(2-fluoroethyl) phosphate. These peaks were assigned by comparison with the values for diethyl phosphorochloridate, ethyl phosphorodichloridate and triethyl phosphate respectively. The crude phosphorylating agent was stirred under reduced pressure for several hours, after which the $^{31}$P n.m.r. spectrum of the resulting product revealed that the impurity of 2-fluoroethyl phosphorodichloridate had been successfully removed. This is consistent with the fact that 2-fluoroethyl phosphorochloridate is known to have a much lower boiling point than bis(2-fluoroethyl) phosphorochloridate and tris(2-fluoroethyl) phosphate. The $^{31}$P n.m.r. spectrum of (52) now showed a major peak at $\delta$ 3.38 ppm due to bis(2-fluoroethyl) phosphorochloridate and a minor peak at $\delta$ -3.36 ppm probably due to tris(2-fluoroethyl) phosphate. An E.I. mass spectrum was also obtained on (52), further confirming the identity of this phosphorylating agent. The spectrum showed peaks due to the protonated molecular ion with an isotope pattern for this, and other chlorine containing fragments, characteristic of one chlorine atom in the intact molecule. The base peak was observed at m/e 47 due to FCH$_2$CH$_2^+$. 

Bis(2-bromoethyl) phosphorochloridate (54) was prepared in an entirely analogous way to (52) (equation 6). The $^{31}$P n.m.r. spectrum of the crude phosphorylating agent showed a major peak
Equation 6

\[
\text{POCl}_3 + 2\text{XCH}_2\text{CH}_2\text{OH} + 2\text{Et}_3\text{N} \rightarrow \text{Et}_2\text{O} \rightarrow (\text{XCH}_2\text{CH}_2\text{O})_2\text{POCl} + 2\text{Et}_3\text{NHCl}
\]

-78°C to RT

(52) X = F
(54) X = Br

Equation 7

\[
(\text{XCH}_2\text{CH}_2\text{O})\text{POCl} + \text{HO} \rightarrow \text{pyridine} \rightarrow \text{XCH}_2\text{CH}_2\text{O} \rightarrow \text{XCH}_2\text{CH}_2\text{O}
\]

0°C to RT

(52) X = F
(54) X = Br

(53) X = F
(55) X = Br

Figure 12: $^{31}\text{P}$ n.m.r. spectrum of (53)
due to the required product (54) and two minor peaks probably due to 2-bromoethyl phosphorodichloridate and tris(2-bromoethyl) phosphate. These peaks were assigned by comparison with the values for diethyl phosphorochloridate, ethyl phosphorodichloridate, and triethyl phosphate respectively\textsuperscript{156-158}. The crude phosphorylating agent was stirred under reduced pressure for several hours, after which the $^{31}\text{P}$ n.m.r. spectrum of the resulting product revealed that the impurity of 2-bromoethyl phosphorodichloridate had been successfully removed. The $^{31}\text{P}$ n.m.r. spectrum of (54) now showed a major peak at $\delta$ 1.72 ppm due to bis(2-bromoethyl) phosphorochloridate, slightly upfield of the value for (52) due to the lower electronegativity of bromine relative to fluorine. A minor peak was also observed at $\delta$ -5.35 ppm probably due to tris(2-bromoethyl) phosphate. The heterocoupled $^{31}\text{P}$ n.m.r. spectrum of (54) showed a quintet for the major peak, as expected for the required product.

Although not entirely pure, additional spectra were obtained on (54), confirming the structural assignment of this compound. The $^{13}\text{C}$ n.m.r. spectrum, assigned by comparison with the spectrum of 2-bromoethanol\textsuperscript{160}, showed phosphorus-coupled doublets at $\delta$ 68.23 ppm (the CH$_2$OP resonance) and at $\delta$ 28.22 ppm (the BrCH$_2$ resonance). The three-bond coupling of 9.0 Hz for the latter was slightly larger than the two-bond coupling of 6.6 Hz for the former, probably due to the angular dependence of coupling constants\textsuperscript{161}. The $^1\text{H}$ n.m.r spectrum, assigned by comparison with the spectrum of 2-bromoethanol\textsuperscript{162}, consisted of a multiplet at $\delta$ 4.50 ppm (the CH$_2$OP resonance) and at $\delta$ 3.60 ppm (the BrCH$_2$ resonance) rather than an expected triplet for each, due to phosphorus-proton coupling being observed for these signals in addition to proton-proton coupling. The F.A.B. mass spectrum of (54) showed peaks due to the protonated molecular ion with an isotope pattern for this, and other halogen containing fragments, characteristic of one chlorine and two bromine atoms in the intact molecule. The base peak was observed at m/e 107 due to $^{79}\text{BrCH}_2\text{CH}_2^+$, with another peak of marginally lower intensity at m/e 109 due to $^{81}\text{BrCH}_2\text{CH}_2^+$.

Further purification of bis(2-fluoroethyl) phosphorochloridate (52) and bis(2-bromoethyl) phosphorochloridate (54) by vacuum distillation was not attempted since previous attempts in this Department to purify the corresponding chlorine derivative using this technique had proved
unsuccessful, probably due to the boiling point of this compound being so high that it decomposed before it could be distilled. The presence of acidic β-hydrogens in the bis(2-haloethyl) phosphorochloridates causes these compounds to be less stable to heat than their corresponding bis(2,2,2-trihaloethyl) analogues, so the latter have been purified relatively easily in this Department by vacuum distillation. The only impurities present in the phosphorylating agents (52) and (54) were probably due to traces of the appropriate tris(2-haloethyl) phosphate, which were not expected to be reactive towards nucleosides. Consequently, it was possible to use these bis(2-haloethyl) phosphorochloridates in the next stage of the synthesis.

The synthesis of araA-5'-bis(2-fluoroethyl) phosphate (53) and araA-5'-bis(2-bromoethyl) phosphate (55) involved the subsequent reaction of 1.5 molar equivalents of the appropriate bis(2-haloethyl) phosphorochloridate with unprotected araA in pyridine at 0°C (equation 7). The reaction employed 1.5 molar equivalents of phosphorochloridate, since a similar amount of phosphorylating agent was required to drive some analogous reactions to completion. It was hoped that carrying out the reaction at reduced temperature (i.e. 0°C) would increase the probability of reaction at the primary 5'-hydroxyl site rather than the more sterically hindered secondary 2'- and 3'-hydroxyl sites.

Firstly, 1.5 molar equivalents of (52) were added to araA in pyridine at 0°C (equation 7) and the course of the reaction was followed by t.l.c. After two hours stirring at 0°C, t.l.c. indicated that the reaction had proceeded to completion with all of the araA having reacted to give a more lipophilic major component, assumed to be due to the required product araA-5'-bis(2-fluoroethyl) phosphate (53). Two even more lipophilic minor components and a baseline component were also observed. The reaction mixture was quenched with deionized water to react with any excess phosphorylating agent. Pyridine was removed and the resulting residue purified by column chromatography to give a yellow oil, shown to be impure by 31P n.m.r spectroscopy. Addition of chloroform to the crude product, followed by cooling of the resulting suspension to 0°C for an hour, caused a precipitate to collect. After warming to ambient temperature and removal of solvent, the product (53) was isolated as a white solid in 41% yield.
The $^{31}$P n.m.r. spectrum of (53) (figure 12) showed a single peak at $\delta$ -0.84 ppm which compares with the value of $\delta$ 1.10 ppm for araA-5'-'-diethyl phosphate. The $^1$H n.m.r. spectrum, assigned by comparison with the spectra of araA and 2-fluorethanol, was consistent with the structure of the product. The base protons appeared as single peaks at $\delta$ 8.32 ppm (H2) and $\delta$ 8.19 ppm (H8). A doublet was observed for H1' due to coupling with H2', with a coupling constant of 3.96 Hz. The FCH$_2$ resonance appeared as two multiplets at $\delta$ 4.65 ppm and $\delta$ 4.53 ppm, the multiplicity within each signal being due to proton-proton and phosphorus-proton coupling. Two such multiplets appeared due to fluorine-proton coupling, with the coupling constant of 47.67 Hz lying in the expected range for this type of coupling. The signals due to H2', H3', H4', H5', and CH$_2$OP comprised an unresolved multiplet at 4.10-4.50 ppm. The F.A.B. mass spectrum of (53) further confirmed its structural assignment, showing a peak due to the protonated molecular ion at m/e 440 and a minor peak at m/e 462 due to the molecular ion plus sodium from the matrix. The base peak at m/e 136 was due to protonated adenine, while peaks at m/e 115, 99, 97, and 81 were probably due to ionized sugar fragments and were assigned to C$_7$H$_{12}$O$_7$', C$_7$H$_{12}$O$_8^+$, C$_5$H$_9$O$_3^+$, and C$_5$H$_9$O$^+$ respectively. Finally, reverse phase analytical H.P.L.C. revealed that the product (53) had been isolated in a pure state, with no contaminating araA.

AraA-5'-bis(2-bromoethyl) phosphate (55) was prepared in an entirely analogous way to (53) (equation 7), except that the reaction mixture was stirred for 2.5 hours and the product was isolated as a white solid in 47% yield following column chromatography and trituration of the resulting gum with diethyl ether. The $^{31}$P n.m.r. spectrum of (55) showed a single peak at $\delta$ -1.68 ppm, slightly upfield of the value for (53) due to the lower electronegativity of bromine relative to fluorine. The $^{13}$C n.m.r. spectrum of (55), assigned by comparison with the spectra of araA (figure 13) and 2-bromoethanol, was consistent with the structure of the required product. This spectrum was expected to provide evidence that (55) was the product of 5'-'-phosphorylation and not 2'-'- or 3'-'-phosphorylation by the appearance of a doublet for C5', but a multiplet was observed for this resonance due to its overlap with the resonance for the CH$_2$OP moiety. However, the shift of this multiplet at $\delta$ 68.06-68.12 ppm was significantly downfield compared with the
Figure 13: $^{13}$C n.m.r. chemical shifts of araA

\[
\begin{align*}
\text{NH}_2 & \quad 155.9 \text{ (C6)}, 152.5 \text{ (C2)}, 149.5 \text{ (C4)}, 140.5 \text{ (C8)}, 118.4 \text{ (C5)} \\
\text{C6} & \quad 84.1 \text{ (C1')}, 83.8 \text{ (C4')}, 75.9, 75.1 \text{ (C2', C3')*}, 61.0 \text{ (C5')} \\
\end{align*}
\]

* chemical shifts may be assigned to either carbon

Figure 14: Thermospray mass spectrum of (55)
value of $\delta$ 61.0 ppm for C5' of araA$^{155}$ and is consequently still indicative of 5'-phosphorylation. A doublet was observed for C4' due to phosphorus-carbon coupling, with the three-bond coupling constant of 7.6 Hz lying in the expected range for this type of coupling$^{166}$. Phosphorus-carbon coupling was also observed for the BrCH$_2$ resonance, doublets appearing at $\delta$ 29.84 ppm and at $\delta$ 29.82 ppm with identical coupling constants of 7.8 Hz. The presence of two doublets, one for each of the BrCH$_2$ moieties present in (55), has been observed for other 5'-phosphate triesters of araA$^{155}$ and corresponds to the diastereotopic nature of the two (2-haloalkyl) chains attached to the prochiral phosphorus atom.

The $^1$H n.m.r. spectrum of (55) was similar to that of (53), single peaks being observed for the base protons, a doublet for H1', and an unresolved multiplet for H2', H3', H4', H5', and CH$_2$OP. This multiplet, appearing at $\delta$ 4.14-4.36 ppm, was downfield of the multiplet assigned to BrCH$_2$. The analogous multiplet in the spectrum of (53), by contrast, appeared upfield to the multiplets assigned to FCH$_2$ due to the greater electronegativity of fluorine relative to bromine. The use of d$_6$-DMSO as nmr solvent in this spectrum, rather than CD$_3$OD as in the spectrum of (53), enabled a broad singlet to be observed for NH$_2$, and a doublet for both 2'-OH and 3'-OH due to coupling with H2' and H3' respectively. Confirmation of the identity of these signals was provided by their absence from the $^1$H n.m.r. spectrum on D$_2$O exchange.

The mass spectrum of (55) (figure 14) was obtained using the thermospray technique. The latter, in common with F.A.B. mass spectrometry, is characterized by the production of a relatively high concentration of molecular ions and little fragmentation of the compound, as opposed to E.I. mass spectrometry where the molecular ion is often not observed and extensive fragmentation is common. The thermospray mass spectrum of (55) further confirmed the structural assignment of this compound, showing a cluster of peaks due to the molecular ion. The isotopic pattern for these peaks and other bromine containing fragments was characteristic of two bromine atoms in the intact molecule. The base peak was observed at m/e 231 due to (BrCH$_2$CH$_2$O)(HO)PO$_2$CH$_2$CH$_2^+$, with another peak of marginally lower intensity at m/e 233 due to the corresponding $^{81}$Br fragment. A prominent peak appeared at m/e 250 due to (MH$^+$ - (BrCH$_2$CH$_2$O)$_2$PO$_2$H), while minor peaks
included those at m/e 136 and m/e 115 assigned to protonated adenine and the ionized sugar fragment C₉H₇O₅⁺ respectively. Finally, microanalysis data were consistent with a hydrated form of (55) and reverse phase analytical H.P.L.C. revealed that the product had been isolated in a pure state, with no contaminating araA.

The synthesis of araA-5′-bis(2-iodoethyl) phosphate (57) (figure 15) was attempted by an alternative route to that employed in the preparation of the other 5′-bis(2-haloethyl) phosphate triesters of araA. It was hoped that araA-5′-bis(2-bromoethyl) phosphate (55) would undergo a nucleophilic substitution reaction with potassium iodide to yield (57). Thus, ten molar equivalents of potassium iodide were added to a solution of (55) in acetone, dissolution having been achieved by heating (55) in acetone to reflux and cooling to ambient temperature prior to the addition. After 48 hours stirring at reflux, t.l.c. indicated that the reaction had proceeded to completion with all of the starting material (55) having reacted to give a marginally more lipophilic product. The reaction mixture was filtered, solvent removed from the filtrate and the resulting residue purified by column chromatography.

The 3¹P n.m.r. spectrum showed a major peak at δ -2.08 ppm and a minor peak at δ -1.86 ppm. The major peak may have been due to the required product (57) and the minor peak due to unreacted starting material (55), but the shift for the latter in this spectrum differs slightly from the value of δ -1.65 ppm observed earlier in the spectrum of (55). Alternatively, these peaks may have been due to the two possible diastereoisomers of araA-5′-(2-bromoethyl) (2-iodoethyl) phosphate (56), one being formed more favorably than the other probably due to the steric hindrance associated with the large iodine group. This is a more likely explanation since it is possible that introduction of the first iodine group into araA-5′-bis(2-bromoethyl) phosphate (55) to give the mixed, unsymmetrical araA-5′-(2-bromoethyl) (2-iodoethyl) phosphate (56) proceeds relatively quickly, but the introduction of the second iodine group to give (57) does not take place at all due to the greater steric hindrance to the incoming iodine group from the iodine group now present in the molecule (equation 8).
Figure 15

\[
\text{ICH}_2\text{CH}_2\text{O} - \text{P} - \text{O} \\
\text{ICH}_2\text{CH}_2\text{O} - \text{HO} - \text{OH}
\]  

(57)

Equation 8

\[
\begin{align*}
\text{BrCH}_2\text{CH}_2\text{O} - \text{P} - \text{O} \\
\text{BrCH}_2\text{CH}_2\text{O} - \text{HO} - \text{OH}
\end{align*}
\]

(55)

\[
\begin{align*}
\text{KI (excess)} / \text{acetone} & \rightarrow \\
\text{BrCH}_2\text{CH}_2\text{O} - \text{P} - \text{O} & \rightarrow \\
\text{ICH}_2\text{CH}_2\text{O} - \text{P} - \text{O} \\
\text{ICH}_2\text{CH}_2\text{O} - \text{HO} - \text{OH}
\end{align*}
\]

(56)

(57)
The $^{13}$C n.m.r. spectrum, assigned by comparison with the spectra of (55) and 2-iodoethanol\textsuperscript{160}, provided further evidence that (56) was the identity of the product. A doublet was observed for C4' and C5' due to phosphorus-carbon coupling, indicating the retention of the 5'-phosphate. The CH$_2$OP and BrCH$_2$ resonances both appeared as doublets with phosphorus-carbon coupling constants of 5.6 Hz and 6.8 Hz respectively, in contrast to the spectrum of (55) where a multiplet was assigned to C4' and CH$_2$OP and two closely spaced doublets were observed for the two diastereotopic BrCH$_2$ moieties. It is of interest to note that the three-bond couplings of 6.4 Hz for C4' and 6.8 Hz for BrCH$_2$ are greater than the two-bond couplings of 5.5 Hz for C5' and 5.8 Hz for CH$_2$OP. A doublet was also observed at $\delta$ 24.20 ppm, which was not present in the analogous spectrum of (55). This signal was assigned to ICH$_2$, although it appeared further downfield than expected when compared with the shift for the corresponding group of 2-iodoethanol\textsuperscript{160}. Finally, no diastereomeric splitting was seen in the spectrum, suggesting that only one of the two possible diastereoisomers of (56) had been formed, unless the other diastereoisomer was present in such a small quantity that the appropriate signals were too weak to be observed in the spectrum. Alternatively, but less likely, all the signals due to the two diastereoisomers may have been coincident. The $^1$H n.m.r. spectrum of the product, although not integrated, was instructive in showing a doublet at $\delta$ 3.17 ppm which was not present in the analogous spectrum of (55). This signal was in the expected region for the ICH$_2$ resonance\textsuperscript{162}.

The F.A.B. mass spectrum showed no peaks due to the protonated molecular ion or the molecular ion of (56) or (57), and extensive fragmentation was observed. However, evidence supporting (56) as the identity of the product was provided by the appearance of spectral peaks at m/e 528 due to (M$^+$ - Br), and at m/e 395 and 397 due to (BrCH$_2$CH$_2$O)(ICH$_2$CH$_2$O)PO$_2$\textsuperscript{+} and the corresponding $^{81}$Br fragment respectively. Other peaks of interest included those due to ICH$_2$CH$_2$O$, BrCH$_2$CH$_2$O$, adenine, and the ionized sugar fragment C$_2$H$_7$O$_3$$. The peaks due to bromine containing fragments displayed an isotopic pattern characteristic of one bromine atom in the intact molecule. Reverse phase analytical H.P.L.C. showed the presence of a major peak with a retention time of 5.93 mins, which compares with a retention time of 8.16 mins for the starting...
material (55). Although no diastereomeric splitting was observed in the H.P.L.C. spectrum, this
is not necessarily suggestive of only one of the two possible diastereoisomers of (56) being
formed, since the two peaks expected may have been unresolved under the conditions employed.
A number of minor peaks were also observed but these did not include one due to the starting
material (55).

It was hoped to evaluate the biological activity of the 5’-bis(2-haloethyl) phosphate triesters
of araA (53) and (55), and also the previously prepared araA-5’-bis(2-chloroethyl) phosphate, by
determining the ability of these compounds to inhibit the synthesis of DNA in vitro using a
tritiated thymidine incorporation assay employing mammalian epithelial cells. Unfortunately
some time elapsed between the synthesis of these compounds and their submission for biological
testing, during which they appeared to have undergone some decomposition, as indicated by 31P
n.m.r. spectroscopy and analytical H.P.L.C. Consequently, it was not possible to proceed with the
biological testing. Time did not allow for these compounds to be resynthesized and submitted for
biological testing immediately after their preparation. However, the rapid decomposition of these
5’-bis(2-haloethyl) phosphate triesters of araA, even when stored at low temperature, suggests that
their therapeutic potential would be rather limited.
Chapter 2: Investigating the mechanism of action of 5'-phosphate triesters of araA

The biological evaluation of some simple 5'-dialkyl phosphate triesters of araA\textsuperscript{135} and their 5'-bis(2,2,2-trifluoroethyl) and 5'-bis(2,2,2-trichloroethyl) counterparts\textsuperscript{136} revealed that these compounds were potent inhibitors of the synthesis of DNA \textit{in vitro}, as determined by an assay developed by Riley and co-workers\textsuperscript{168} measuring the incorporation of tritiated thymidine into the DNA of mammalian epithelial cells. Moreover, a correlation was observed between increasing biological activity and increasing lipophilicity for the 5'-dialkyl derivatives, suggesting membrane penetration by the intact 5'-phosphate triesters of araA as opposed to their extracellular cleavage prior to entering the cell. The 5'-bis(2,2,2-trihaloethyl) phosphate triesters of araA, when compared with their simple 5'-dialkyl analogues, were found to have a significantly higher activity than might be expected on the basis of their lipophilicity alone. This was attributed to the electron-withdrawing halogen substituent labilizing the phosphorus-oxygen bond towards chemical cleavage to yield the 5'-monophosphate araAMP. Similar observations have been made for the analogous 5'-phosphate triesters of the anti-cancer drug araC\textsuperscript{136,169}.

However, the mechanism by which these 5'-phosphate triesters of araA were exerting their biological effects was unclear. It was hoped that the mode of action of the 5'-phosphate triesters of araA would involve intracellular hydrolysis via P-O-alkyl cleavage to the 5'-monophosphate araAMP (equation 9). If this were the case, these compounds would be acting as intracellular sources of araAMP, thereby obviating the dependence of araA on nucleoside kinase-mediated phosphorylation to the 5'-monophosphate. AraAMP could then be sequentially phosphorylated to the 5'-diphosphate and then further to the active 5'-triphosphate form. Another possible, but clearly less desirable, route would be the intracellular hydrolysis of the 5'-phosphate triesters via P-O-nucleoside cleavage to the parent nucleoside araA (equation 10), resulting in these compounds merely acting as depot forms of araA. The aim of establishing a "kinase by-pass" would not have been achieved, but the intracellular release of the nucleoside might still be of some chemotherapeutic benefit\textsuperscript{64}. 
Equation 9

\[
\begin{align*}
\text{(49)} & \quad \text{RO—P—O—RO} \\
\text{hydrolysis} & \quad \text{NH}_2
\end{align*}
\]

Equation 10

\[
\begin{align*}
\text{(49)} & \quad \text{RO—P—O—RO} \\
\text{hydrolysis} & \quad \text{NH}_2
\end{align*}
\]

Figure 16

\[
\begin{align*}
\text{(49)} & \quad \text{RO—P—O—RO} \\
\text{(50)} & \quad \text{RCH}_2—P—O—RCH_2
\end{align*}
\]

Figure 17

\[
\begin{align*}
\text{(58)} & \quad \text{HO—O—C(}^{3}\text{H})_3
\end{align*}
\]

\[
\begin{align*}
\text{(59)} & \quad \text{HO—PO—PO—PO—PO—O—RO}
\end{align*}
\]
It was hoped to elucidate the mechanism by which 5'-phosphate triesters of araA exert their biological effects by comparing the biological activity of two 5'-phosphate triesters of araA (49) with their analogous 5'-phosphinate esters (50) (figure 16), in the aforementioned in vitro tritiated thymidine incorporation assay. The assay essentially involves extracellular thymidine passing across the cell membrane and undergoing sequential phosphorylation by thymidine kinase and thymidylate kinases to thymidine-5'-triphosphate (59), which is then incorporated into the DNA of mammalian epithelial cells (figure 17). Measurement of the thymidine uptake requires extracting the DNA from the cells and recording the radioactivity caused by β-emission from the tritium by scintillation counting in a suitable medium. Thymidine incorporation into cellular DNA may be regarded as a measure of DNA synthesis and hence it is possible to evaluate the ability of 5'-phosphate derivatives of araA to inhibit DNA synthesis in vitro.

The oxygen atoms in the esterifying groups of 5'-phosphate triesters of araA (49) are replaced by methylene groups in their analogous 5'-phosphinate esters (50). These moieties are not too dissimilar in size, the Van der Waals radius of 140 ppm for oxygen comparing with a value of 200 ppm for the methyl group. Consequently, compounds of type (49) and (50) with identical R groups should be comparable. However, phosphorus-carbon bonds are much more chemically and enzymatically stable than phosphorus-oxygen bonds. It follows that if 5'-phosphate triesters of araA exert their biological effects by hydrolysis via P-O-alkyl cleavage to the 5'-monophosphate araAMP (equation 9), the 5'-phosphinate esters of araA would be expected to show a lower biological activity in the in vitro tritiated thymidine incorporation assay than their 5'-phosphate triester counterparts. Alternatively, if the mode of action involves hydrolysis via P-O-nucleoside cleavage to the nucleoside araA (equation 10), the 5'-phosphinate esters would be expected to show a similar biological activity to their analogous 5'-phosphate triesters.

Two pairs of the general compounds (49) and (50) were biologically evaluated in vitro, namely araA-5'-dipropyl phosphate (39) and araA-5'-dibutyl phosphinate (60) in the alkyl series, and araA-5'-diphenyl phosphate (61) and araA-5'-dibenzyl phosphinate (62) in the aryl series (figure 18). These four compounds had been previously prepared in this Department. The
Figure 18

Equation 11

Equation 12
synthesis of the 5'-phosphate triesters of araA (39) and (61) involved the reaction of unprotected araA with two molar equivalents of the appropriate dialkyl and diaryl phosphorochloridate respectively, in pyridine at ambient temperature^{151,172} (equation 11). Dipropyl phosphorochloridate (63) was prepared by reacting 2 molar equivalents of both n-propanol and triethylamine with phosphoryl chloride in ether solvent at ambient temperature, while commercial diphenyl phosphorochloridate (64) was used.

A convenient route to the 5'-phosphinate esters of araA (60) and (62) similarly involved the reaction of unprotected araA with two molar equivalents of the appropriate dialkyl and diaryl phosphinic chloride respectively, in pyridine at ambient temperature^{172} (equation 12). Dibutyl phosphinic chloride (66) was synthesized by a two-step procedure. Firstly, dibutyl phosphinic acid (65) was prepared by the method of Kosolapoff and Watson^{173}, involving the reaction of diethyl phosphite with the Grignard reagent from 1-bromobutane, followed by hydrolysis and oxidation (equation 13). Dibutyl phosphinic acid (65) was subsequently chlorinated with thionyl chloride^{174}, yielding dibutyl phosphinic chloride (66) (equation 14). Likewise, dibenzyl phosphinic chloride (68) was synthesized by a two-step procedure. Firstly, dibenzyl phosphinic acid (67) was prepared by the method of Sauvage^{175}, involving the reaction of phosphoryl chloride with benzyl magnesium chloride, followed by acid hydrolysis (equation 15). Dibenzyl phosphinic acid (67) was subsequently chlorinated with thionyl chloride^{174} as above, yielding dibenzyl phosphinic chloride (68) (equation 16).

The in vitro biological activities of these 5'-phosphate triesters of araA (39) and (61), and their respective 5'-phosphinate ester analogues (60) and (62) were duly evaluated and compared^{172}. The in vitro tritiated thymidine incorporation assay employed cells for the testing of these compounds from a mammalian epithelial cell line, CNCM I.221. The cells were routinely subcultured at weekly intervals and all experiments were carried out only on cells of passage number 21 to 30, which were screened for Mycoplasma contamination by autoradiography. The use of a small range of passage numbers minimizes the possibility of the cells undergoing transformations over long periods of time involving many subcultures. The cells were screened
Equation 13

\[(\text{EtO})_2\text{PHO} + 2\text{BuMgBr} \rightarrow \text{Bu}_2\text{PHO} \rightarrow \text{Bu}_2\text{P(O)OH}\]

(i) 0°C, N\(_2\), 1 h / Et\(_2\)O
(ii) reflux, N\(_2\), 2 h
(iii) H\(^+\) / H\(_2\)O

Equation 14

\[\text{Bu}_2\text{P(O)OH} \rightarrow \text{Bu}_2\text{POCl}\]

(i) SOCl\(_2\), 0°C, N\(_2\) / toluene
(ii) reflux, 1 h

Equation 15

\[\text{POCl}_3 + 2\text{BzlMgCl} \rightarrow \text{Bzl}_2\text{P(O)OH}\]

(i) 0°C, 1.5 h / Et\(_2\)O
(ii) 40°C, 1 h
(iii) HCl / H\(_2\)O

Equation 16

\[\text{Bzl}_2\text{P(O)OH} \rightarrow \text{Bzl}_2\text{POCl}\]

(i) SOCl\(_2\), 0°C, N\(_2\) / toluene
(ii) reflux, 2 h
for the presence of *Mycoplasma* since contamination by these micro-organisms can affect the uptake of thymidine. *Mycoplasma* is a genus of the family *Mycoplasmataceae* characterized by minute, polymorphic, gram-negative, nonmotile micro-organisms without cell walls, which are intermediate in some respects between viruses and bacteria and are usually parasitic in mammals\(^7\). The procedure of testing for *Mycoplasma* contamination involved growing cells in growth medium on a microscope slide and labelling them with tritiated thymidine for a period of time. The slide was then washed carefully to remove any unincorporated label, fixed, washed again, and dried. The slide was coated with a thin layer of photographic emulsion and left for a week, before it was developed and fixed. Giemsa staining of the slide enabled the radiolabel to be seen as dark spots under the microscope. Normal incorporation of thymidine into cellular DNA appears as nuclear clusters of label, while the presence of cytoplasmic label or large clumps of extracellular label suggest infection by *Mycoplasma* and bacteria respectively. All the cells involved in the biological evaluation of 5'-phosphate derivatives of araA were found to be free from contamination by *Mycoplasma* and bacteria.

Cells were grown to confluence in multiwell trays over a period of 48 hours in a 37°C incubator with a 2% CO\(_2\) atmosphere. Only the inner eight wells of each tray were seeded with cells at a density of 5x10\(^5\) cells/ml in growth medium (1 ml/well), while the outer 16 wells were filled with an equal volume of serum-free medium (SFM). Solutions of araA, (39), (60), (61) and (62) in non-sterile distilled water were prepared, filter sterilized, and diluted to the desired concentrations. These solutions were freshly prepared prior to use in the first week of testing, then immediately stored at 4°C until required for use in a subsequent week of testing. On completion of the testing, t.l.c. and H.P.L.C. analysis of the solutions indicated that no decomposition of the compounds had taken place. Aliquots (100 µl) of the sterile distilled water control and each of the solutions of araA, (39), (60), (61) and (62) at concentrations of 0.3 mM and 0.03 mM were added to four replicate wells (giving final concentrations/well of 0.03 mM and 0.003 mM respectively). The cultures were incubated at 37°C in a 2% CO\(_2\) atmosphere for 30 minutes. An aliquot of tritiated thymidine in phosphate buffered saline solution was then added to each well and the
cultures incubated for a further 30 minutes. The cultures were carefully washed several times with phosphate buffered saline solution to remove any unincorporated label (since this would affect the scintillation counts if allowed to remain), fixed with dilute 2,2,2-trichloroacetic acid, washed again, and carefully dried. The cells were digested overnight at 37°C with sodium hydroxide solution to dissolve the acid insoluble cell contents. An aliquot of digest was then removed from each cell, mixed with hydrochloric acid and Ecosint A, and the radioactivity caused by β-emission from the tritium was measured using a scintillation counter. The remainder of the digests from each set of four replicate wells was pooled and the absorbance at λ=280 nm was measured as an estimate of the total cellular protein. This gives some indication of the regularity of cell seeding and whether cells have been lost during the washings. If the absorbance values are very variable, a correction can be made by dividing the scintillation count for each well by the absorbance for that particular set of four replicate wells. It was found that such a correction was unnecessary in the experiments described above.

The scintillation counts are proportional to the amount of tritiated thymidine incorporated into the DNA of the cells. Thus, thymidine incorporation into cellular DNA may be regarded as a measure of DNA synthesis. Consequently, the results of the in vitro assay may be expressed as a % inhibition of DNA synthesis, relative to the distilled water control, caused by a particular compound at a given concentration. Each experiment was carried out at least twice on cells of different passage number and the mean % inhibition and standard error of the mean (SEM) were calculated for each set of % inhibition values (table 1, biological testing section). A two-tail student’s t-test was used to determine the degree of significant difference between the mean % inhibition for araA and each of the compounds (39), (60), (61) and (62), and also between the mean % inhibition for the 5′-phosphate triesters of araA and their 5′-phosphinate esters analogues, i.e. (39) vs. (60) and (61) vs. (62), at a given concentration. This data was subsequently used to determine the probability of the difference between the mean % inhibition values of two compounds at a given concentration being statistically significant (table 2, biological testing section). A commonly used threshold for the probability is a value of 5%, i.e. (P < 5%) indicates
that this difference is statistically significant, while \( P > 5\% \) suggests that this difference is not statistically significant (marked (NS) in table 2).

The results of the assay are presented as a bar chart (figure 19), in which the mean % inhibition of DNA synthesis (relative to the distilled water control) is plotted against compound at final concentrations of 0.03 mM and 0.003 mM. AraA caused 78% inhibition of cellular DNA synthesis at 0.03 mM, while araA-5’-dipropyl phosphate (39) and araA-5’-dibutyl phosphinate (60) displayed 45% and 24% inhibition respectively. However in the aryl series, araA-5’-diphenyl phosphate (61) was equi-active with araA, whereas araA-5’-dibenzyl phosphinate (62) showed 52% inhibition. Considering the lower concentration of 0.003 mM, araA caused 31% inhibition of cellular DNA synthesis, with araA-5’-dipropyl phosphate (39) and araA-5’-dibutyl phosphinate (60) displaying 23% and 6% inhibition respectively. The corresponding inhibition values for araA-5’-diphenyl phosphate (61) and araA-5’-dibenzyl phosphinate (62) were 21% and 16% respectively.

Two main observations are evident from the results of the assay. Firstly, in both the alkyl and aryl series at both concentrations studied, the 5’-phosphinate esters of araA (60) and (62) retain an inhibitory effect on DNA synthesis. Thus, the stabilizing of the phosphorus to alkyl link in the 5’-phosphinate esters does not remove the biological activity of these compounds relative to their 5’-phosphate triester analogues. This is indicative of the 5’-phosphate triesters of araA not acting solely by a mechanism involving hydrolysis via P-O-alkyl cleavage to the 5’-monophosphate araAMP (equation 9). Secondly, in both the alkyl and aryl series at both concentrations studied, the 5’-phosphinate esters of araA (60) and (62) show a lower biological activity than their respective 5’-phosphate triester counterparts (39) and (61). Indeed, a two-tail student’s t-test indicates that these differences are statistically significant (i.e. \( P < 5\% \)) in the alkyl series for (39) vs. (60) at 0.03 mM and 0.003 mM, and in the aryl series for (61) vs. (62) only at the higher concentration. This suggests that the labilizing of the phosphorus to alkyl link in the 5’-phosphate triesters of araA enhances the biological activity of these compounds relative to their analogous 5’-phosphinate esters, which is consistent with a mode of action for the 5’-phosphate triesters.
Figure 19: % Inhibition of DNA synthesis against compound

% Inhibition of DNA synthesis

- 0.003 mM
- 0.03 mM

-compound araA (39) (60) (61) (62)
involving hydrolysis via P-O-alkyl cleavage to araAMP (equation 9).

Overall it is possible to conclude that 5'-phosphinate esters of araA show a biological activity that is reduced, but not abolished, relative to their 5'-phosphate triester counterparts. Therefore, 5'-phosphate triesters of araA would appear to be exerting their biological effects by a mechanism largely involving the intracellular release of the free nucleotide araAMP (equation 9), and also to a minor extent the free nucleoside araA (equation 10).

The possibility that the 5'-phosphate triesters of araA might be acting by a mechanism involving extracellular hydrolysis of these compounds was investigated by incubating araA, (39), (60), (61) and (62) under assay conditions, but excluding the cellular material. Hydrolysis was not observed for any of these compounds either by t.l.c. analysis over 24 hours at 37°C or by H.P.L.C. analysis over a week at 37°C. Therefore, extracellular hydrolysis is unlikely to be involved in the mechanism by which these 5'-phosphate triesters of araA exert their biological effects. Further confirmation of this would be provided by preparing a 5'-phosphate triester of araA with a radiolabelled 32P atom and assaying this compound in the cell culture experiment (in the absence of tritiated thymidine). The radiolabelled 5'-phosphate triester of araA would be synthesized by the reaction of araA with the appropriate phosphorochloridate, prepared from 32P labelled phosphoryl chloride. Scintillation counting of the cell digests would then determine whether the labelled phosphorus had passed across the cell membrane. If this were the case, the labelled compound would presumably have entered the cell intact and evaded extracellular hydrolysis, since it is unlikely that charged fragments would enter the cell by passive diffusion.

It remains uncertain whether the 5'-phosphate triesters of araA undergo intracellular hydrolysis or act directly without hydrolysis. However, the latter has been tentatively ruled out on the basis of some experiments carried out by Dr. I. Weller of University College and Middlesex School of Medicine, University of London on cells infected with the Hepatitis B virus. The Hepatitis B virus only contains one enzyme, DNA polymerase. This enzyme catalyzes the formation of a phosphodiester bond between the 3'-hydroxyl group at the end of a growing DNA chain and the innermost phosphorus atom of an incoming deoxynucleoside-5'-triphosphate. It follows that only
those compounds which in their original form inhibit viral DNA polymerase will show any activity against this virus. AraA and its 5'-dialkyl and 5'-bis(2,2,2-trihaloethyl) phosphate triester derivatives were found to show no activity in this investigation, suggesting that these 5'-phosphate triesters of araA are unlikely to exert their biological effects in their original forms. It is also possible that the 5'-phosphate triesters of araA might be interfering with the transport of the exogenously added thymidine across the cell membrane, rather than actually exerting an effect on DNA synthesis. This could be investigated by growing cultures of cells in the absence and presence of these compounds and recording cell counts over a period of several cell cycles. If the 5'-phosphate triesters of araA were interfering with the replication process of the cell, this would be observed in the relative growth rates.

Finally, another possibility for investigating the mechanism by which 5'-phosphate triesters of araA exert their biological effects would involve a similar approach to the study discussed above\cite{172}, but this time the \textit{in vitro} biological activity of 5'-phosphate triesters of araA (49) would be compared with their analogous 5'-deoxy-5'-phosphinate esters (69) (figure 20), rather than their 5'-phosphinate ester counterparts (50). This study would be expected to provide corroborating evidence for the mechanism of action of 5'-phosphate triesters of araA largely involving the hydrolysis of these compounds via P-O-alkyl cleavage to araAMP (equation 9). Unfortunately, there was insufficient time to synthesize the required 5'-deoxy-5'-phosphinate ester of araA and compare the biological activity of this compound with its 5'-phosphate triester analogue.

Returning to the synthesis of araA-5'-dibutyl phosphinate (60) carried out previously in this Department\cite{172}, the required phosphorylating agent dibutyl phosphinic chloride (66) was prepared via dibutyl phosphinic acid (65)\cite{173} and its subsequent chlorination\cite{174} (equations 13, 14). Initially, a novel route to (66) had been proposed, involving the reaction of phosphoryl chloride with two molar equivalents of the Grignard reagent from 1-chlorobutane and subsequent heating of the resulting Grignard salt (70) to cause the latter to decompose to the phosphinic chloride (66)\cite{148}.
Equation 17

\[
\text{POCl}_3 + 2\text{BuMgCl} \rightarrow \begin{align*}
\text{OMgBu} \quad \text{Bu} & \quad \text{P} \quad \text{Cl} \\
\text{Bu} & \quad \text{P} \quad \text{Cl} 
\end{align*}
\text{heat to ca. 180°C} \quad 0.05 \text{ mm Hg} \rightarrow \text{Bu}_2\text{POCl}
\]

Equation 18

\[
\text{POCl}_3 + \text{BuMgBr} \rightarrow \begin{align*}
\text{OMgBu} \quad \text{Bu} & \quad \text{P} \quad \text{Cl} \\
\text{Cl} & \quad \text{P} \quad \text{Cl} 
\end{align*}
\text{heat to ca. 170°C} \quad 0.3 \text{ mm Hg} \rightarrow \text{BuPOCl}_2
\]
(equation 17). However, this method proved unsuccessful resulting in the formation of butyl phosphinic dichloride (71), rather than (66), in low yield. Recently, difficulties have also been encountered in attempts to modify this route (equation 17) for the preparation of butyl phosphinic dichloride (71) in improved yield, by employing one molar equivalent in the reaction scheme. Use of the more reactive butyl magnesium bromide was initially considered to be preferable to butyl magnesium chloride as the required Grignard reagent.

The first attempt to synthesize butyl phosphinic dichloride (71) employed butyl magnesium bromide as the Grignard reagent, the latter being prepared by the standard method involving the reaction of 1-bromobutane and a slight excess of magnesium in diethyl ether. The Grignard reagent was filtered to remove any unreacted magnesium, then added dropwise to a solution of phosphoryl chloride in diethyl ether at -78°C under an atmosphere of nitrogen. This order of addition was used to promote the formation of the required product (71). Solvent was removed from the reaction mixture to give a white solid, presumably the Grignard salt (72), to which a little silicone oil was added (to aid the distillation process) prior to vacuum distillation. Strong heating was required before a yellow oil began to collect (equation 18).

The 31P n.m.r. spectrum of the distillate showed a major peak at δ 49.75 ppm due to butyl phosphinic dichloride (71). This value lies in the region where compounds of type RPGCl are expected to resonate. A minor peak was also observed at δ 35.77 ppm. It would appear that the use of butyl magnesium bromide as the Grignard reagent has interfered with the synthesis of (71) by the proposed route (equation 18) due to possible halide exchange reactions taking place. The presence of a bromine containing compound in the distillate would account for its yellow colouration, contrasting with the colourless oil identified as butyl phosphinic dichloride in previous work. Consequently, further attempts to synthesize butyl phosphinic dichloride (71) employed butyl magnesium chloride as the Grignard reagent.

The second attempt to synthesize butyl phosphinic dichloride (71) involved an analogous method to the first attempt, except for the use of butyl magnesium chloride as the Grignard reagent. The latter was prepared by the standard method, whereby 1-chlorobutane was reacted
with a slight excess of magnesium in diethyl ether. Strong heating was again required during the vacuum distillation of the white solid, presumably the Grignard salt (73), before the product (71) began to collect as a colourless oil in 15% yield (equation 19a). The $^{31}$P n.m.r. spectrum of the distillate showed a single peak at $\delta$ 49.70 ppm due to butyl phosphinic dichloride. Carbon and proton n.m.r. spectra of (71) were consistent with the structure of the product, as compared with those reported in previous work\textsuperscript{148}.

Although butyl phosphinic dichloride (71) had been prepared in a pure state by the proposed route (equation 19a), the yield of the product was rather low. This factor, coupled with the observation that a significant amount of phosphoryl chloride (identified by $^{31}$P n.m.r. spectroscopy) was collected during the distillation, suggested that the reaction between phosphoryl chloride and butyl magnesium chloride had not proceeded to completion. Consequently, a further two attempts to prepare (71) by the proposed route involved increasing the temperature at which the addition and subsequent reaction of phosphoryl chloride and butyl magnesium chloride occurred (equations 19b, 19c). Firstly, the addition of the reagents was carried out at 0°C followed by warming to ambient temperature overnight, contrasting with the subsequent experiment involving addition at ambient temperature followed by refluxing overnight. However, this appeared to have little affect on the reaction. The $^{31}$P n.m.r. spectra of the distillate from both experiments showed a single peak due to butyl phosphinic dichloride, but a slight decrease in yield from 9% to 5% was observed with increasing temperature of the reaction.

A final attempt to synthesize butyl phosphinic dichloride (71) involved the addition of butyl magnesium chloride to phosphoryl chloride, initially at ambient temperature and then cooled to -10°C due to the vigorous nature of the reaction. The use of a Kugelruhr flask (180 ml) as the reaction vessel in this experiment, rather than a round bottomed flask (200 ml), to avoid the difficult transfer of the Grignard salt to a distillation vessel encountered in previous attempts (equation 19, methods 1-3) where distillation did not involve the use of a Kugelruhr, may have contributed to the rather vigorous nature of the reaction in this attempt. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature over 2 hours then
**Equation 19**

\[
\text{POCl}_3 + \text{BuMgCl} \quad \xrightarrow{\text{Et}_2\text{O}} \quad \begin{bmatrix} \text{OMgCl} \\ \text{Bu} \quad \text{P} \\ \text{Cl} \quad \text{Cl} \quad \text{Cl} \end{bmatrix} \quad \xrightarrow{\text{heat to ca. 150°C/0.05 mm Hg}} \quad \text{BuPOCl}_2
\]

(73) (71)

Conditions:

(a) method 1 \(-78^\circ\text{C} (2 \text{ h}) \text{ to } \text{RT} (16 \text{ h})\) \quad \text{heat to ca. } 150^\circ\text{C}/0.05 \text{ mm Hg}

(b) method 2 \(0^\circ\text{C} (1 \text{ h}) \text{ to } \text{RT} (20 \text{ h})\) \quad \text{heat to ca. } 160^\circ\text{C}/0.05 \text{ mm Hg}

(c) method 3 \(\text{RT} (1 \text{ h}), \text{ reflux} (20 \text{ h})\) \quad \text{heat to ca. } 200^\circ\text{C}/0.02 \text{ mm Hg}

**Equation 20**

\[
\text{POCl}_3 + \text{BuMgCl} \quad \xrightarrow{\text{Et}_2\text{O}} \quad \begin{bmatrix} \text{OMgBr} \\ \text{Bu} \quad \text{P} \\ \text{Cl} \quad \text{Cl} \quad \text{Cl} \end{bmatrix} \quad \xrightarrow{\text{heat to ca. 250°C} \quad /0.2 \text{ mm Hg}} \quad \text{BuPOCl}_2 + \text{Bu}_2\text{POCl}
\]

(73) (71) (70) (66)
refluxed for a further 42 hours. The white solid, presumably the Grignard salt (73), obtained on removal of solvent from the reaction mixture, was subjected to distillation under reduced pressure using a Kugelruhr (employed due to its ability to attain higher temperatures than an oil bath). Strong heating was required to cause a small volume of a colourless oil to collect, but more distillate could only be encouraged to collect by further stronger heating.

The $^{31}$P n.m.r. spectrum of the distillate showed a major peak at $\delta$ 70.37 ppm due to dibutyl phosphinic chloride (66), comparing well with the literature value of $\delta$ 70.7 ppm$^{179}$ for this compound. A minor peak was also observed at $\delta$ 49.64 ppm due to the required product butyl phosphinic dichloride (71). A heterocoupled $^{31}$P n.m.r. spectrum further confirmed the structural assignments of these peaks, with a quintet and triplet observed for (66) and (71) respectively. A significant amount of phosphoryl chloride (identified by $^{31}$P n.m.r. spectroscopy) was again collected during the distillation.

It would appear that this reaction is not very controllable and a mixture of products are formed (equation 20). Raising the temperature at which reaction between phosphoryl chloride and butyl magnesium chloride occurred, rather than encouraging the reaction to proceed to completion, seemed to favour the formation of dibutyl phosphinic chloride (66). The formation of (66) in this final attempt to synthesize butyl phosphinic dichloride (71) had probably not been observed in the previous attempts due to the decomposition temperature of the dibutyl Grignard salt (70) being higher than that of the mono-butyl Grignard salt (73). Only by use of a Kugelruhr, with the ability to attain higher temperatures during the distillation, was it possible to cause decomposition of (70) to yield a significant amount of (66) relative to (71). This would account for the low yields of butyl phosphinic dichloride even when reacting phosphoryl chloride and butyl magnesium chloride at -78°C and the significant amount of phosphoryl chloride collected during the distillations of the Grignard salts. Overall, it is possible to conclude that the proposed novel route to butyl phosphinic dichloride (71) was far from ideal.

The butyl phosphinic dichloride (71), obtained in a pure state from the above experiments, was subsequently used to synthesize butyl (2,2,2-trichloroethoxy) phosphinic chloride (74) with the aim
of preparing the mixed, unsymmetrical 5′-butyl (2,2,2-trichloroethoxy) phosphinate ester of araA (75). It would be of interest to compare the biological activity of 5′-alkyl (2,2,2-trichloroethoxy) phosphinate esters of araA (76) with their analogous 5′-alkyl (2,2,2-trichloroethyl) phosphate triesters (45) (figure 21), following the synthesis and biological evaluation of a series of the latter compounds earlier in the course of this research (as discussed in chapter 3). The proposed route to araA-5′-butyl (2,2,2-trichloroethoxy) phosphinate (75) involved a two-step procedure similar to the one previously employed in the preparation of a number of mixed, unsymmetrical 5′-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (chapter 3). Generally, the method involved the synthesis of the appropriate phosphorylating agent and its subsequent reaction with unprotected araA.

Firstly, butyl (2,2,2-trichloroethoxy) phosphinic chloride (74) was prepared by reacting one molar equivalent of both butyl phosphinic dichloride (71) and triethylamine with phosphoryl chloride in diethyl ether solvent at -78°C (equation 21). After allowing the reaction mixture to warm to ambient temperature with stirring overnight, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. The $^3\text{P}$ n.m.r. spectrum of the product, obtained after extraction with hexane, showed a number of peaks including a major peak at δ 32.53 ppm. The latter was assumed to be due to the required product (74) by comparison with a value of δ 45.0 ppm for ethyl (ethoxy) phosphinic chloride (i.e. (Et)(EtO)POCl))$^{157,158}$. The product (74) was isolated by vacuum distillation in 43% yield as a colourless oil, which solidified on standing at ambient temperature.

The $^{13}\text{C}$ n.m.r. and $^1\text{H}$ n.m.r. spectra of (74) were consistent with the structure of the product, showing signals due to both the BuP and CCl$_3$CH$_2$OP moieties. The $^{13}\text{C}$ n.m.r. spectrum showed a cluster of peaks composed of three sets of interspersed phosphorus-coupled doublets for the CH$_3$P, CH$_2$CHP and CH$_3$CH$_2$ resonances. The magnitude of the coupling constants for these doublets were instructive in their assignments, by comparison with those reported earlier for the starting material (71). Coupling constants of 2.5 Hz, 55.9 Hz, and 18.9 Hz were observed for the doublets at δ 23.99 ppm, at δ 23.81 ppm and at δ 23.80 ppm respectively, corresponding to a
Figure 21

Equation 21

BuPOCl₂ + CCl₃CH₂OH + Et₃N $\xrightarrow{\text{Et₂O}}$ (Bu)(CCl₃CH₂O)POCl + Et₃NHCl

(71)

Equation 22

(Bu)(CCl₃CH₂O)POCl + HO $\xrightarrow{\text{pyridine}}$ BuPOCl₂ + CCl₃CH₂OH

(74)
small two-bond coupling for the CH₂CH₂P resonance, a large one-bond coupling for the CH₃P resonance and a three-bond coupling, larger than the two-bond coupling, for the CH₂CH₂ resonance respectively. Phosphorus-carbon coupling was also observed at δ 95.22 ppm (the CCl₃ resonance) and at δ 75.46 ppm (the CH₂OP resonance), these doublets appearing further downfield than the other signals in the spectrum due to the deshielding effect of the chlorine atoms. The former doublet was also of lower intensity than the other peaks, presumably due to the longer relaxation time of the CCl₃ moiety. A single peak was also observed in the spectrum for the methyl group. The ¹H n.m.r. spectrum of (74) consisted of multiplets for the CH₂OP, CH₃P and CH₂CH₂P resonances, due to phosphorus-proton coupling being observed for these signals in addition to proton-proton coupling. The resonances for the methylene (i.e. CH₂CH₂) and methyl groups showed multiplicities due only to proton-proton coupling, with the appearance of a sextet and triplet respectively.

The F.A.B. mass spectrum of (74) further confirmed its structural assignment. A cluster of peaks were observed due to the molecular ion plus sodium from the matrix. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of four chlorine atoms in the intact molecule. Prominent peaks appeared at m/e 139 and m/e 141 due to BuPOCl⁺ and the corresponding ⁷Cl fragment respectively, while minor peaks included those due to (M⁺ - CH₃), (M⁺ - Cl) and (M⁺ - CCl₃). Finally, microanalysis data indicated that the product (74) had been isolated in a pure state.

The second step in the preparation of araA-5’-butyl (2,2,2-trichloroethoxy) phosphinate (75) would have involved the subsequent reaction of butyl (2,2,2-trichloroethoxy) phosphinic chloride (74) with unprotected araA in pyridine at reduced temperature (equation 22). Unfortunately, there was insufficient of this phosphorylating agent (74) to carry out the reaction with araA and time did not allow for the resynthesis of this compound.
Chapter 3: 5'-Alkyl (2,2,2-trichloroethyl) phosphate triesters of araA

The comparative biological evaluation of some 5'-phosphate triesters of araA with their analogous 5'-phosphinate esters\textsuperscript{172}, suggested that the mechanism of action of the 5'-phosphate triesters of araA largely involved the intracellular hydrolysis of these compounds via P-O-alkyl cleavage to the 5'-monophosphate araAMP. Thus, the aim of obviating the dependence of araA on nucleoside kinase-mediated phosphorylation to the 5'-monophosphate would appear to have been achieved. This leads to the possibility of synthesizing other 5'-phosphate triesters of araA as potential prodrugs of araAMP, with alternative esterifying groups that might enhance the biological activity of these compounds.

The aim of the present chapter involved an investigation into the synthesis and biological evaluation of a series of mixed, unsymmetrical 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (45) (figure 22). It was hoped that the presence of the 2,2,2-trichloroethyl moiety would increase the susceptibility to chemical hydrolysis and hence the biological activity of these compounds over their non-substituted counterparts, as found for araA-5'-bis(2,2,2-trifluoroethyl) phosphate and araA-5'-bis(2,2,2-trichloroethyl) phosphate when compared with their simple 5'-dialkyl analogues\textsuperscript{133,136}. If the 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (45) were to penetrate the cell membrane and undergo chemical hydrolysis of the 2,2,2-trichloroethyl moiety to give the 5'-alkyl phosphate diesters of araA (77), the latter might be subject to phosphodiesterase induced cleavage of the alkyl moiety to yield the 5'-monophosphate, thereby combining chemical and enzymatic cleavage (equation 23). Additionally, it would be of interest to discover whether the increasing biological activity observed for the 5'-dialkyl phosphate triesters of araA with the increasing chain length of their constituent alkyl groups\textsuperscript{135}, would be similarly reflected in the 5'-alkyl (2,2,2-trichloroethyl) series.

The preparation of a series of mixed, unsymmetrical 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA was attempted via two different routes. The first route generally involved the synthesis of the appropriate alkyl phosphorodichloridate and its subsequent reaction with
Figure 22

\[
\text{NH}_2\quad\text{NH}\quad\text{NH}\quad\text{NH}
\]
\[
\text{RO} - \text{P} - \text{O} \\
\text{CCl}_3\text{CH}_2\text{O} \\
\text{OH}
\]

(45) \( R = \text{alkyl} \)

Equation 23

\[
\text{NH}_2\quad\text{NH}_2\quad\text{NH}_2
\]
\[
\text{RO} - \text{P} - \text{O} \\
\text{OH}
\]

chemical hydrolysis

\[
\text{RO} - \text{P} - \text{O} \\
\text{OH}
\]

(77)

(13)

phosphodiesterase
2,2,2-trichloroethanol to give the required alkyl 2,2,2-trichloroethyl phosphorochloridate, which was then reacted with unprotected araA. Several alternative methods are available for the preparation of alkyl phosphorodichloridates\textsuperscript{137}, some examples include:

1. The reaction of pyrophosphoryl chloride with one molar equivalent of a primary alcohol in the absence of solvent at reduced temperature, followed by vacuum distillation to isolate the alkyl phosphorodichloridate\textsuperscript{180,181}.

\[
\text{Cl}_2\text{P}(	ext{O})\text{OP(O)}\text{Cl}_2 + \text{ROH} \rightarrow \text{Cl}_2\text{PO}_2\text{H} + \text{ROPOCl}_2
\]

2. The reaction of phosphoryl chloride with one molar equivalent of aliphatic alkyl acetate in the presence of a catalyst of phosphoric acid at reflux, followed by vacuum distillation to isolate the alkyl phosphorodichloridate\textsuperscript{182}.

\[
\text{POCl}_3 + \text{ROCOCH}_3 \rightarrow \text{ROPOCl}_2 + \text{CH}_3\text{COCl}
\]

3. The reaction of phosphoryl chloride with one molar equivalent of alcohol in the absence or presence of an organic tertiary base, usually in an inert solvent at or below ambient temperature\textsuperscript{144,145}.

\[
\text{POCl}_3 + \text{ROH} \rightarrow \text{ROPOCl}_2 + \text{HCl}
\]

This last example was the method chosen for the preparation of a series of alkyl phosphorodichloridates. Thus, phosphoryl chloride was reacted with one molar equivalent of both the appropriate alcohol and triethylamine in diethyl ether solvent at reduced temperature (equation 24), in an analogous manner to the procedure involved in the previous synthesis of a series of dialkyl phosphorochloridates\textsuperscript{135}, except for the use of different proportions of reagents.
Equation 24

\[
\text{POCl}_3 + \text{ROH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}} \text{ROPOCl}_2 + \text{Et}_3\text{NHCl}
\]

-78°C to RT

(R = alkyl)

Equation 25

\[
\text{ROPOCl}_2 + \text{CCl}_3\text{CH}_2\text{OH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}} (\text{RO})(\text{CCl}_3\text{CH}_2\text{O})\text{POCl} + \text{Et}_3\text{NHCl}
\]

-78°C to RT

(R = alkyl)

Equation 26

\[
(\text{RO})(\text{CCl}_3\text{CH}_2\text{O})\text{POCl} + \text{HO} \xrightarrow{\text{pyridine}} \text{RO-P-O} \text{CCl}_3\text{CH}_2\text{O}
\]

0°C to RT

(R = alkyl)
The appropriate alkyl phosphorodichloridate was subsequently reacted with one molar equivalent of both 2,2,2-trichloroethanol and triethylamine in diethyl ether solvent at reduced temperature to give the required alkyl 2,2,2-trichloroethyl phosphorochloridate (equation 25). This reaction of a primary phosphorodichloridate with one molar equivalent of alcohol to give a secondary phosphorochloridate may take place in the absence or presence of a base. The use of a tertiary organic base for the elimination of the hydrogen halide by-product was employed in the above two reactions (equations 24, 25), rather than carrying out the reaction under reduced pressure or the bubbling of an inert gas through the reaction mixture, since the reaction is more controllable under the former conditions. The appropriate alkyl 2,2,2-trichloroethyl phosphorochloridate was then reacted with unprotected araA in pyridine at reduced temperature to give a series of 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (equation 26).

The target compounds, araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80), araA-5'-propyl (2,2,2-trichloroethyl) phosphate (83) and araA-5'-ethyl (2,2,2-trichloroethyl) phosphate (86), were prepared by the route outlined above. Firstly, the synthesis of the required alkyl phosphorodichloridates, butyl phosphorodichloridate (78), propyl phosphorodichloridate (81) and ethyl phosphorodichloridate (84), involved the addition of one molar equivalent of both the appropriate alcohol and triethylamine to phosphoryl chloride at -78°C, using diethyl ether as the solvent (equation 27). Possible impurities resulting from these reactions include unreacted phosphoryl chloride, dialkyl phosphorochloridate, and hydrolyzed material. It was hoped to minimize the formation of these by-products by the scrupulous drying of the reagents prior to the reaction, the slow addition of the reagents at reduced temperature under an atmosphere of nitrogen, and using an excess of solvent to dilute the reaction mixture.

After allowing the reaction mixture to warm to ambient temperature with stirring overnight, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. Butyl phosphorodichloridate (78), propyl phosphorodichloridate (81) and ethyl phosphorodichloridate (84) were isolated as colourless liquids in yields of 87% to 99%.

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Equation 27

\[ \text{POCl}_3 + \text{ROH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}} \text{ROPOCl}_2 + \text{Et}_3\text{NHCl} \]

\(-78^\circ \text{C to RT}\)

(78) \(R = \text{Bu}\)  
(81) \(R = \text{Pr}\)  
(84) \(R = \text{Et}\)

Equation 28

\[ \text{ROPOCl}_2 + \text{CCl}_3\text{CH}_2\text{OH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}} (\text{RO})(\text{CCl}_3\text{CH}_2\text{O})\text{POCl} + \text{Et}_3\text{NHCl} \]

\(-78^\circ \text{C to RT}\)

(78) \(R = \text{Bu}\)  
(81) \(R = \text{Pr}\)  
(84) \(R = \text{Et}\)

Equation 29

\[ (\text{RO})(\text{CCl}_3\text{CH}_2\text{O})\text{POCl} + \text{HO} \xrightarrow{\text{pyridine}} \text{RO} - \text{P} - \text{O} \text{CCl}_3\text{CH}_2\text{O} \]

\(0^\circ \text{C to RT}\)

(79) \(R = \text{Bu}\)  
(82) \(R = \text{Pr}\)  
(85) \(R = \text{Et}\)

Figure 23: Diastereoisomers of (80)
The $^3$P n.m.r. spectra of all three phosphorodichloridates showed single peaks with chemical shifts lying in the region where compounds of type ROPOCl$_2$ are expected to resonate$^{184}$. The heterocoupled $^3$P n.m.r. spectrum of (78) showed a triplet, as expected for the required product. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (78), (81) and (84), assigned by comparison with the corresponding spectra of n-butanol, n-propanol and ethanol respectively$^{162,166}$, were consistent with the structures of these phosphorylating agents. Phosphorus-carbon coupling was observed in the $^{13}$C n.m.r. spectra of all three alkyl phosphorodichloridates, but only for the two nearest carbon atoms to the phosphorus atom. The $^1$H n.m.r. spectra of (78), (81) and (84) showed multiplets for all protons within three bonds of the phosphorus atom, due to phosphorus-proton coupling being observed for these signals in addition to proton-proton coupling. The E.I. mass spectra of (78), (81) and (84) further confirmed the structural assignments of these compounds. Although the spectra of (78) and (81) showed no peaks due to the molecular ion, peaks were identified due to the sequential loss of carbon fragments from the alkyl chains with those of highest m/e value in both spectra being assigned to (M$^+$ - Et). The spectrum of (84), by contrast, showed a cluster of peaks due to (M$^+$ - H), while a similar fragmentation pattern was observed to those in the spectra of (78) and (81). The peaks due to chlorine containing fragments in all three spectra displayed an isotopic pattern characteristic of two chlorine atoms in the intact molecule. Satisfactory microanalysis data were also obtained on all three alkyl phosphorodichloridates. It is therefore evident that each of the phosphorylating agents (78), (81) and (84) was isolated in a pure state, without the need for vacuum distillation.

The alkyl 2,2,2-trichloroethyl phosphorochloridates, butyl 2,2,2-trichloroethyl phosphorochloridate (79), propyl 2,2,2-trichloroethyl phosphorochloridate (82) and ethyl 2,2,2-trichloroethyl phosphorochloridate (85), were subsequently prepared by the reaction of the appropriate alkyl phosphorodichloridate with one molar equivalent of both 2,2,2-trichloroethanol and triethylamine in diethyl ether solvent at -78°C (equation 28). Possible impurities resulting from these reactions include unreacted alkyl phosphorodichloridate, alkyl bis(2,2,2-trichloroethyl) phosphate, and hydrolyzed material. It was hoped to minimize the formation of these by-products
by the usual procedure, involving the scrupulous drying of the reagents prior to the reaction, the slow addition of the reagents at reduced temperature under an atmosphere of nitrogen, and using an excess of solvent to dilute the reaction mixture.

Firstly, butyl 2,2,2-trichloroethyl phosphorochloridate (79) was prepared by the addition of one molar equivalent of both 2,2,2-trichloroethanol and triethylamine to butyl phosphorodichloridate (78) at -78°, using diethyl ether as solvent (equation 28). After allowing the reaction mixture to warm to ambient temperature with stirring overnight, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. Extraction of the resulting cloudy oil with hexane gave a pale yellow oil, shown to be slightly impure by $^{31}$P n.m.r. spectroscopy. The crude phosphorylating agent was stirred under reduced pressure at 50°C for several hours to remove a minor impurity of unreacted starting material (78). Further purification by vacuum distillation enabled the product (79) to be isolated as a colourless oil in 28% yield. The $^{31}$P n.m.r spectrum of the distillate showed a single peak at $\delta$ 1.90 ppm due to butyl 2,2,2-trichloroethyl phosphorochloridate, comparing with the values of $\delta$ 3.4 ppm for dibutyl phosphorochloridate and $\delta$ 1.30 ppm for bis(2,2,2-trichloroethyl) phosphorochloridate.

The n.m.r. and $^1$H n.m.r. spectra of (79) further confirmed the identity of this phosphorylating agent, showing signals due to both the BuOP and CCl$_3$CH$_2$OP moieties. The $^{13}$C n.m.r. spectrum showed phosphorus-coupled doublets for all carbon atoms within three bonds of the phosphorus atom. It is of interest to note that the three-bond coupling constant for the C$_3$CH$_2$OP resonance is of similar magnitude to the two bond coupling constant for the CH$_2$CH$_2$OP resonance, while the three-bond coupling constant for the CCl$_3$ resonance is larger than the two-bond coupling constant for the CCl$_3$CH$_2$OP resonance. The doublet due to the CCl$_3$ moiety appeared further downfield than the other signals in the spectrum due to the deshielding effect of the chlorine atoms. This doublet was also of lower intensity than the other peaks, presumably due to the longer relaxation time of the CCl$_3$ moiety. Single peaks were also observed in the spectrum for the methylene (i.e. CH$_2$CH$_2$) and methyl groups. The $^1$H n.m.r. spectrum of (79) consisted of multiplets for the CCl$_3$CH$_2$OP, CH$_2$CH$_2$OP and CH$_3$CH$_2$OP resonances due to phosphorus-proton
coupling being observed for these signals in addition to proton-proton coupling. The signals for protons further than three bonds away from the phosphorus atom showed multiplicities due only to proton-proton coupling, with a sextet and a triplet observed for the methylene (i.e. CH\textsubscript{2}CH\textsubscript{2}) and methyl groups respectively.

Propyl 2,2,2-trichloroethyl phosphorochloridate (82) was prepared in an entirely analogous manner to (79), except that the purification of the crude phosphorylating agent did not involve vacuum distillation. An impurity of unreacted starting material (81), shown to be present by \textsuperscript{31}P n.m.r. spectroscopy, was successfully removed by stirring the crude product under reduced pressure at 30°C for several hours to give the product (82) as a colourless oil in 81% yield. The \textsuperscript{31}P n.m.r. spectrum of (82) now showed a single peak at δ 1.99 ppm due to propyl 2,2,2-trichloroethyl phosphorochloridate, comparing with the values of δ 2.61 ppm for dipropyl phosphorochloridate\textsuperscript{148} and δ 1.30 ppm for bis(2,2,2-trichloroethyl) phosphorochloridate\textsuperscript{186}. The \textsuperscript{13}C n.m.r. and \textsuperscript{1}H n.m.r. spectra of (82) were consistent with the structure of the product, showing signals due to both the PrOP and CCl\textsubscript{3}CH\textsubscript{2}OP moieties. These spectra were similar to the corresponding spectra of (79), with phosphorus-coupling only observed for those atoms within three bonds of the phosphorus atom. The E.I. mass spectrum of (82) further confirmed the structural assignment of this phosphorylating agent, showing a cluster of minor peaks due to (M\textsuperscript{+} - H). The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 117 due to (HO)\textsubscript{3}PCI\textsuperscript{+}, with another peak of lower intensity at m/e 119 due to the corresponding \textsuperscript{37}Cl fragment. A prominent peak also appeared at m/e 43 due to C\textsubscript{3}H\textsubscript{7}\textsuperscript{+}.

Ethyl 2,2,2-trichloroethyl phosphorochloridate (85) was prepared by a similar method to (82), except that the reaction mixture was stirred for a further 56 hours after allowing it to warm to ambient temperature with stirring overnight. A longer reaction time was employed in the synthesis of (85) since the presence of unreacted starting material in the crude products obtained in the preparation of (79) and (82), may have been due to the reactions involved not having proceeded to completion. However, the \textsuperscript{31}P n.m.r spectrum of the crude product resulting from the present
reaction showed a major peak due to the required product (85) and two minor peaks including one due to unreacted starting material (84). The crude phosphorylating agent was stirred under reduced pressure at 30°C for several hours, after which the $^{31}$P n.m.r spectrum of the resulting product revealed that the impurity of ethyl phosphorodichloridate (84) had been successfully removed. The $^{31}$P n.m.r. spectrum now showed a major peak at $\delta$ 1.22 ppm due to ethyl 2,2,2-trichloroethyl phosphorochloridate, comparing with the values of $\delta$ 2.8 ppm for diethyl phosphorochloridate$^{156-158}$ and $\delta$ 1.30 ppm for bis(2,2,2-trichloroethyl) phosphorochloridate$^{185}$. A minor peak was also observed at $\delta$ -6.79 ppm, probably due to ethyl bis(2,2,2-trichloroethyl) phosphate and comparing with a value of $\delta$ -2.3 ppm for diethyl 2,2,2-trichloroethyl phosphate$^{187}$. Further purification of (85) was not attempted since the only impurity present was probably due to a trace of ethyl bis(2,2,2-trichloroethyl) phosphate, which was not expected to be reactive towards nucleosides.

Although not entirely pure, additional spectra were obtained on (85), confirming the identity of this phosphorylating agent. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (85) were consistent with the structure of the product and similar to the corresponding spectra of (79) and (82). The $^{13}$C n.m.r. spectrum of (85) consisted of phosphorus-coupled doublets for all resonances in the spectrum. The $^1$H n.m.r spectrum of (85) showed multiplets for the CCl$_3$CH$_2$OP and CH$_3$CH$_2$OP moieties and a triplet of doublets for the methyl group, due to phosphorus-proton coupling being observed for these signals in addition to proton-proton coupling. The E.I. mass spectrum of (85) showed no peaks due to the molecular ion, but the cluster of peaks of highest m/e value were assigned to (M$^+$ - Et). The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 129 due to (CH$_2$O)(HO)POCl$^+$, with another peak of lower intensity at m/e 131 due to the corresponding $^{37}$Cl fragment.

The synthesis of araA-5′-butyl (2,2,2-trichloroethyl) phosphate (80), araA-5′-propyl (2,2,2-trichloroethyl) phosphate (83) and araA-5′-ethyl (2,2,2-trichloroethyl) phosphate (86) involved the subsequent reaction of the appropriate alkyl 2,2,2-trichloroethyl phosphorochloridate with unprotected araA in pyridine at 0°C (equation 29). Firstly, 1.5 molar equivalents of butyl
2,2,2-trichloroethyl phosphorochloridate (79) were added to araA in pyridine at 0°C and the course of the reaction was followed by t.l.c. The reaction employed 1.5 molar equivalents of phosphorochloridate, since a similar amount of phosphorylating agent had been required to drive some analogous reactions to completion\(^{135,136}\). After 21 hours stirring at 0°C, t.l.c. indicated that the reaction had not yet proceeded to completion with some araA remaining. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 35 hours. T.l.c. now indicated that the reaction had proceeded further to completion with most of the araA having reacted to give a more lipophilic major component assumed to be due to the required product araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80). Two even more lipophilic minor components were also observed. The addition of more phosphorylating agent (79) would probably have enabled the reaction to proceed to completion and the latter to have been achieved in a shorter period of time. However, this was not possible since further butyl 2,2,2-trichloroethyl phosphorochloridate was not available.

The reaction mixture was quenched with deionized water to remove any unreacted phosphorylating agent and solvent removed. Attempted purification of the resulting residue by column chromatography, using a methanol-chloroform eluent, proved unsuccessful as indicated by t.l.c. analysis. Further purification of the crude product by column chromatography, employing a slower methanol-ethyl acetate eluent, gave a white solid. The latter, although appearing pure by t.l.c. analysis, was shown to be slightly impure by \(^{31}\)P n.m.r. spectroscopy. The product (80) was finally isolated as a white solid in 29% yield, following recrystallization from ethyl acetate. The \(^{31}\)P n.m.r. spectrum of the product (run at 82 MHz) consisted of a single peak at \(\delta -2.31\) ppm, which compares with the values of \(\delta -0.54\) ppm for araA-5'-dibutyl phosphate\(^{135}\) and \(\delta -3.83\) ppm for araA-5'-bis(2,2,2-trichloroethyl) phosphate\(^{136}\). However, a second \(^{31}\)P n.m.r. spectrum of (80) (run at 164 MHz) showed two closely spaced signals of similar intensity, indicating the presence of an approximately equal mixture of the two possible diastereoisomers of (80) (figure 23) in the product. This isomerism arises from the asymmetry at the chiral phosphorus centre. It was believed that the first spectrum (run at 82 MHz) had only shown a singlet due to the coincidence of the two
peaks in this spectrum.

The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (80), assigned by comparison with the corresponding spectra of araA-5'-dibutyl phosphate$^{135}$ and araA-5'-bis(2,2,2-trichloroethyl) phosphate$^{136}$, were consistent with the structure of the product and further confirmed its diastereomeric nature. The latter was evident in the $^{13}$C n.m.r. spectrum of (80) by the duplication of many signals in the same ratio (i.e. 1:1) as observed for the peaks in the $^{31}$P n.m.r. spectrum (run at 164 MHz). Coincidence of peaks also occurred in some cases. Diastereomeric splitting was observed in the presence of two distinct peaks for all the nucleosidic carbon atoms except for C6, C2', C3' and C4'. The spectrum showed single peaks for C6, C2' and C3', while a doublet appeared for C4' due to phosphorus-carbon coupling. Diastereomeric splitting was observed in addition to phosphorus-carbon coupling for the CH$_2$CH$_2$OP and C5' resonances, the former appearing as a "triplet" due to the overlap of these splittings while two doublets were clearly visible for the latter. Only phosphorus-carbon coupling was observed in the doublets for the CCl$_3$, CCl$_2$CH$_2$OP and CH$_3$CH$_2$OP moieties, contrasting with the resonances for the methylene (i.e. CH$_3$CH$_2$) and methyl groups where only diastereomeric splitting was present. Evidence that (80) was the product of 5'-phosphorylation and not 2'- or 3'-phosphorylation was provided by the appearance of phosphorus-coupled doublets for C5' at $\delta$ 70.14 ppm and $\delta$ 70.08 ppm, showing a significant downfield shift compared with the value of $\delta$ 61.0 ppm for C5' of araA$^{135}$. It is of interest to note that the three-bond coupling constants for the C4', CCl$_3$ and CH$_3$CH$_2$OP resonances were greater than the two-bond coupling constants for the C5', CCl$_3$CH$_2$OP and CH$_3$CH$_2$OP resonances respectively, probably due to the angular dependence of coupling constants$^{161}$.

The $^1$H n.m.r. spectrum of (80) also showed diastereomeric splitting for a number of peaks in the spectrum, e.g. two distinct signals were observed for the base proton H2. The H1' resonance displayed diastereomeric splitting in addition to proton-proton coupling, with the appearance of a "triplet" due to the overlap of these splittings. The spectrum featured unresolved multiplets for H2', and CCl$_3$CH$_2$OP, and for H4' and CH$_3$CH$_2$OP, while separate multiplets were observed for H3', H5', CH$_3$CH$_2$OP, CH$_3$CH$_2$ and CH$_3$. The multiplicity of these signals was attributed to
proton-proton coupling and diastereomeric splitting, with phosphorus-proton coupling also present for those signals within three bonds of the phosphorus atom.

The F.A.B. mass spectrum of (80) further confirmed the structural assignment of this compound, showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of three chlorine atoms in the intact molecule. The base peak was observed at m/e 136 due to protonated adenine, while a prominent peak appeared at m/e 99 due to the ionized sugar fragment C$_2$H$_7$O$_2^+$. Finally, microanalysis data and reverse phase analytical H.P.L.C. indicated that the product (80) had been isolated in a pure state. The latter technique displayed one signal rather than the two expected for the diastereomeric product (80), presumably due to the coincidence of these peaks under the conditions employed, and also indicated the absence of contaminating araA.

AraA-5'-propyl (2,2,2-trichloroethyl) phosphate (83) was prepared by a similar procedure to (80), except for the use of more of the appropriate phosphorylating agent (82) (initially 2.0 molar equivalents with a further 1.0 molar equivalents added during the course of the reaction) and a longer reaction time, to enable the reaction to proceed to completion. The product (83) was isolated as a white solid in 25% yield, following column chromatography and recrystallization from ethyl acetate. The $^{31}$P n.m.r. spectrum of the product (run at 82 MHz) featured a single peak at $\delta$ -2.29 ppm, comparing with the values of $\delta$ -0.54 ppm for araA-5'-dipropyl phosphate$^{135}$ and $\delta$ -3.83 ppm for araA-5'-bis(2,2,2-trichloroethyl) phosphate$^{136}$. However, a second $^{31}$P n.m.r. spectrum of (83) (run at 164 MHz) showed two closely spaced signals of similar intensity, indicating that the product was actually composed of an approximately equal mixture of the two possible diastereoisomers. A similar phenomenon was found in the corresponding $^{31}$P n.m.r spectra of (80), where the two peaks expected for the diastereomeric product were coincident in the spectrum run at 82 MHz, but clearly visible in the spectrum run at 164 MHz.

The $^{13}$C n.m.r and $^1$H n.m.r spectra of (83) were consistent with the structure of the product, showing signals due to both the PrOP and CCl$_3$CH$_2$OP moieties in addition to the nucleosidic resonances. These spectra further confirmed the diastereomeric nature of the product with many
of the signals split in the same ratio (i.e. 1:1) as observed for the peaks in the $^{31}\text{P}$ n.m.r. spectrum (run at 164 MHz). A comparison of the $^{13}\text{C}$ n.m.r. spectrum of (83) with that of (80) revealed very few differences. Some features of the former spectrum which contrasted with the latter spectrum included the appearance of diastereomeric splitting for the C2' and C3' resonances, a phosphorus-coupled doublet for the methylene (i.e. CH\text{CH}_2) group, and a single peak for the methyl group. The $^1\text{H}$ n.m.r spectrum of (83) was similar to that of (80) except that two interspersed triplets were clearly visible for the methyl group, the presence of two such signals being due to diastereomeric splitting.

The F.A.B. mass spectrum of (83) showed a cluster of peaks due to the protonated molecular ion with an isotopic pattern for this, and other chlorine containing fragments, characteristic of three chlorine atoms in the intact molecule. The base peak was observed at m/e 136 due to protonated adenine. Minor peaks of interest included those assigned to (PrO)(CCl\text{CH}_2O)P(OH)\text{H}^+ and the ionized sugar fragments C\text{H}_2O_5\text{H}^+, C\text{H}_3O_4\text{H}^+, C\text{H}_4O_3\text{H}^+ and C\text{H}_5O. Finally, reverse phase analytical H.P.L.C. revealed that the product (83) had been isolated in a pure state, with no contaminating araA. It is of interest to note that the H.P.L.C. spectrum of (83) showed coincident peaks for the diastereomeric product, as similarly observed for (80).

AraA-5'-ethyl (2,2,2-trichloroethyl) phosphate (86) was prepared in an analogous manner to (80), except for the use of 2.5 molar equivalents of the appropriate phosphorylating agent (85) and carrying out the reaction at ambient temperature (after an initial hour at 0°C). These conditions enabled the reaction to proceed to completion and the latter to be achieved in a slightly shorter period of time. The product (86) was isolated as a white solid in 44% yield, following column chromatography. The $^{31}\text{P}$ n.m.r. spectrum of the product (figure 24) showed a singlet at $\delta$ -2.09 ppm, which compares with the values of $\delta$ -0.70 ppm for araA-5'-diethyl phosphate\textsuperscript{135} and $\delta$ -3.83 ppm for araA-5'-bis(2,2,2-trichloroethyl) phosphate\textsuperscript{136}. Only one signal was observed in this spectrum due to the coincidence of the two peaks expected for the two possible diastereoisomers of (86), as similarly observed in the $^{31}\text{P}$ n.m.r. spectra of (80) and (83) (run at 82 MHz). Diastereomeric splitting was observed in the $^{13}\text{C}$ n.m.r and $^1\text{H}$ n.m.r. spectra of (86) with the
Figure 24: $^{31}$P n.m.r. spectrum of (86)

Figure 25: F.A.B. mass spectrum of (86)
duplication of a number of the peaks in a 1:1 ratio, confirming that the product was composed of an approximately equal mixture of the two possible diastereoisomers.

The $^{13}$C n.m.r. and $^1$H n.m.r spectra of (86) were consistent with the structure of the product and similar to the corresponding spectra of (80), with the main exception being the appearance of phosphorus coupling in the resonances for the methyl group. The $^{13}$C n.m.r spectrum of (86) showed a doublet for the methyl group due to phosphorus-carbon coupling, rather than the two signals observed for this resonance in the spectrum of (80) due to diastereomeric splitting. The $^1$H n.m.r. spectrum of (86) displayed two sets of interspersed triplets of doublets for the methyl group. The multiplicity within each signal was due to proton-proton coupling and phosphorus-proton coupling, while two such signals occurred due to diastereomeric splitting. This contrasted with the spectrum of (80) where a multiplet was observed for the methyl group, due to the overlap of proton-proton coupling and diastereomeric splitting.

The F.A.B. mass spectrum of (86) (figure 25) confirmed the structural assignment of the product, showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks was characteristic of three chlorine atoms in the intact molecule. The base peak appeared at m/e 136 due to protonated adenine, while minor peaks of interest included those due to the ionized sugar fragments $C_7H_7O_2^+$, $C_7H_5O_2^+$ and $C_7H_3O^+$. Finally, microanalysis data and reverse phase analytical H.P.L.C. revealed that the product (86) had been isolated in a pure state. The latter technique again showed no resolution of the two peaks expected for the diastereomeric product, and also indicated the absence of contaminating araA.

An alternative route investigated for the synthesis of the mixed, unsymmetrical 5'-alkyl (2,2,2-trichloroethyl) phosphates of araA involved a transesterification reaction, proceeding via the displacement of one of the 2,2,2-trichloroethoxy moieties of araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) by an alkoxy moiety of the appropriate alcohol in the presence of an excess of caesium fluoride (equation 30). Ogilvie and co-workers have employed this type of reaction for the synthesis of a number of mixed trialkyl phosphates with the general formula $(CCl_3CH_2)PO(OR)(OR')$. 

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Equation 30

\[
\begin{align*}
&\text{CCl}_3\text{CH}_2\text{O}osphate \\
&\text{CsF (excess)/ROH} \\
&\text{RT} \\
&\rightarrow
\end{align*}
\]

Equation 31

\[
\text{POCl}_3 + 2\text{CCl}_3\text{CH}_2\text{OH} + 2\text{Et}_3\text{N} \rightarrow (\text{CCl}_3\text{CH}_2\text{O})_2\text{POCl} + 2\text{Et}_3\text{NHCl}
\]

\(-78^\circ \text{C to RT} \quad (87)\)

Equation 32

\[
\text{(CCl}_3\text{CH}_2\text{O})_2\text{POCl} + \text{HO} \rightarrow \text{CCl}_3\text{CH}_2\text{O}osphate
\]

\(0^\circ \text{C to RT} \quad (87)\)

Equation 33

\[
\begin{align*}
&\text{CCl}_3\text{CH}_2\text{O}osphate \\
&\text{CsF (excess)/ROH} \\
&\text{RT} \\
&\rightarrow
\end{align*}
\]

\( (80) \text{ R = Bu} \\
(88) \text{ R = Me} \)
The appropriate alcohol (R'OH) acted as both reactant and solvent in the preparation of these compounds from alkyl bis(2,2,2-trichloroethyl) phosphates ((CCl₃CH₂O)₂PO(OR)) in the presence of an excess of caesium fluoride. It has been suggested that the mechanism of the reaction occurs via the initial attack of the fluoride ion on the phosphorus atom, followed by the rapid reaction of the resulting phosphorofluoridate intermediate ((CCl₃CH₂O)PO(OR)F) with the alcohol¹⁸⁸,¹⁸⁹. A similar reaction has proved successful in the synthesis of some mixed, unsymmetrical 5'-alkyl (2,2,2-trichloroethyl) phosphates of the anti-cancer drug araC¹⁹⁰.

A prerequisite for the synthesis of some 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA by the above route was the preparation of the appropriate starting material, araA-5'-bis(2,2,2-trichloroethyl) phosphate (43)¹³⁶, requiring the prior synthesis of the phosphorylating agent bis(2,2,2-trichloroethyl) phosphorochloridate (87)¹⁹¹. The latter was prepared by an analogous procedure to that previously employed in the synthesis of (87) in this Department¹⁹⁰. Thus, two molar equivalents of both 2,2,2-trichloroethanol and triethylamine were reacted with phosphoryl chloride at -78°C, using diethyl ether as the solvent (equation 31).

After allowing the reaction mixture to warm to ambient temperature with stirring overnight, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. The product (87) was isolated as a white solid in 64% yield, following distillation using a Kugelruhr. The ³¹P n.m.r. spectrum of the distillate showed a single peak at δ 1.55 ppm, comparing well with the literature value of δ 1.30 ppm for (87)¹⁸⁶. The ¹³C n.m.r and ¹H n.m.r. spectra of (87) were consistent with the structure of the product, as compared with those reported in previous work¹⁹⁰. The F.A.B. mass spectrum of (87) further confirmed the identity of this phosphorylating agent, showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of seven chlorine atoms in the intact molecule.

AraA-5'-bis(2,2,2-trichloroethyl) phosphate (43) was subsequently prepared by the reaction of bis(2,2,2-trichloroethyl) phosphorochloridate (87) with araA in pyridine at 0°C (equation 32). The method involved was similar to that previously reported for the synthesis of (43)¹³⁶ except that
the reaction was carried out at 0°C, rather than at ambient temperature, to ensure a more controlled reaction. The phosphorylating agent (87) was also added "neat", rather than dissolving it in pyridine prior to its addition to araA, since the latter procedure had proved unsuccessful earlier in the course of this research in an initial small scale attempt to prepare araA-5'-bis(2-bromoethyl) phosphate (55).

The product (43) was isolated as a white solid in 53% yield, following column chromatography. The $^{31}$P n.m.r. spectrum of the product showed a single peak at $\delta$ -3.89 ppm, comparing well with the value of $\delta$ -3.83 ppm for this compound. The $^{13}$C n.m.r and $^1$H n.m.r spectra of (43) displayed signals due to the CCl$_3$CH$_2$OP moiety in addition to the nucleosidic resonances. These spectra were consistent with the structure of the product, as compared with those reported in previous work. The F.A.B. mass spectrum of (43) showed a cluster of peaks due to the protonated molecular ion with an isotopic pattern for this, and other chlorine containing fragments, characteristic of six chlorine atoms in the intact molecule. Other peaks of interest included those due to protonated adenine, adenine and the ionized sugar fragments C$_3$H$_7$O$_3$+, C$_5$H$_7$O$_2$+ and C$_5$H$_5$O$_2$+. Finally, microanalysis data and reverse phase analytical H.P.L.C. revealed that the product (43) had been isolated in a pure state. The latter technique also indicated the absence of contaminating araA.

The synthesis of araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80) and araA-5'-methyl (2,2,2-trichloroethyl) phosphate (88) was subsequently investigated via the "Ogilvie-type" transesterification reaction employing araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) as the starting material, as outlined earlier. These reactions were initially carried out on a small scale to ensure the benefit of pursuing them on a larger scale for the complete isolation and characterization of the products (80) and (88). Firstly, araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) was reacted with n-butanol in the presence of an excess of caesium fluoride at ambient temperature (equation 33). The course of the reaction was followed by t.l.c. After 232 hours stirring at ambient temperature, t.l.c. indicated that the reaction had proceeded to completion with all of the starting material (43) having reacted to give a marginally less lipophilic major
component assumed to be due to the required product (80). An even less lipophilic minor component was also observed. Solvent was removed and the resulting residue purified by column chromatography to give a white solid, shown to be slightly impure by $^{31}$P n.m.r. spectroscopy. The $^{31}$P n.m.r. spectrum showed a major peak at $\delta$ -2.45 ppm, comparing well with the value of $\delta$ -2.31 ppm for (80) prepared via the first route investigated for the synthesis of some 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA.

The synthesis of araA-5'-methyl (2,2,2-trichloroethyl) phosphate (88) from araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) via a transesterification reaction was subsequently investigated on a small scale. Thus, (43) was reacted with methanol in the presence of an excess of caesium fluoride at ambient temperature (equation 33) in an analogous manner to the synthesis of (80) by this route, except that a shorter period of time (131 hours) was required for the reaction to proceed to completion. Methanol was removed and the resulting residue was purified by column chromatography to give a cream solid, shown to be slightly impure by $^{31}$P n.m.r. spectroscopy. The $^{31}$P n.m.r spectrum of the product showed a major peak consisting of two closely spaced signals of approximately equal intensity, probably due to the two possible diastereoisomers of the required product (43). The shifts of these peaks at $\delta$ 1.66 ppm and $\delta$ 1.62 ppm compare well with the value of $\delta$ 1.67 ppm for one of the two possible diastereoisomers of the corresponding 5'-methyl (2,2,2-trichloroethyl) phosphate triester of the anti-cancer drug araC. A minor peak was also observed at $\delta$ -3.33 ppm, assumed to be due to unreacted starting material (43) by comparison with the value of $\delta$ -3.83 ppm reported for this compound. The $^1$H n.m.r. spectrum of the product, assigned by comparison with the spectra of (43) and methanol, provided further evidence that the product consisted of an approximately equal mixture of the two possible diastereoisomers of (88). This spectrum was particularly instructive in showing two sets of doublets at $\delta$ 3.77 ppm and $\delta$ 3.76 ppm, which were not present in the corresponding spectrum of (43). These signals appeared in the expected region for the CH$_2$O resonance, and their multiplicity was due to diastereomeric splitting being observed in addition to phosphorus-proton coupling. Minor peaks also appeared in the spectrum due to a nucleosidic impurity, probably unreacted starting material (43).
The results of these small scale reactions suggest that the preparation of araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80) and araA-5'-methyl (2,2,2-trichloroethyl) phosphate (88) from araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) via a transesterification reaction was a feasible route to these compounds. Unfortunately, there was only sufficient time to repeat the synthesis of (80) (equation 33) on a larger scale for complete isolation and characterization of the product. The method involved an analogous procedure to the one employed in the small scale reaction, except that a longer period of time (332 hours) was required for the reaction to proceed to completion.

Purification of the product by column chromatography gave a white solid, shown to be impure by $^{31}\text{P}$ n.m.r. spectroscopy. The $^{31}\text{P}$ n.m.r. spectrum showed several peaks including one at $\delta$ -2.30 ppm, assumed to be due to the required product by comparison with the values of $\delta$ -2.45 ppm for the major product of the small scale transesterification reaction and $\delta$ -2.31 ppm for (80) prepared by the first route investigated for the synthesis of some 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA. The product (80) was isolated as a white solid in 25% yield, following further purification by reverse phase preparative H.P.L.C. The $^{31}\text{P}$ n.m.r. spectrum now showed a single peak at $\delta$ -4.92 ppm. The disagreement in shifts between the spectra run before and after preparative H.P.L.C. was believed to be due to the former spectrum being run at 82 MHz with referencing to external phosphoric acid, while the latter spectrum was run at 164 MHz with no referencing. Only one signal was observed in these spectra due to the coincidence of the two peaks expected for the two possible diastereoisomers of (80). The $^{13}\text{C}$ n.m.r. and $^1\text{H}$ n.m.r spectra of (80) confirmed the diastereomeric nature of the product, with many peaks split in a 1:1 ratio due to the presence of the two possible diastereoisomers of (80) in approximately equal proportions. It would appear that the synthesis of (80) by either of the two routes investigated does not result in the preferential formation of one of the two possible diastereoisomers of (80) over the other, but gives a product composed of an approximately equal mixture of the two.

The $^{13}\text{C}$ n.m.r. and $^1\text{H}$ n.m.r. spectra of (80) were consistent with the structure of the product, as compared with the corresponding spectra of this compound prepared earlier in the research.
Diastereomeric splitting was again observed in the $^{13}$C n.m.r. spectrum of (80) for all nucleosidic carbons apart from C6, C2’ and C3’, for the methylene (i.e. CH$_2$CH$_2$) and methyl groups, and in addition to phosphorus coupling for CH$_2$CH$_2$OP. The resonances for the CCl$_3$ and CCl$_2$CH$_2$OP moieties again appeared as doublets due only to phosphorus-carbon coupling. The $^1$H n.m.r. spectrum of (80) again consisted of two closely spaced singlets for H2 due to diastereomeric splitting, a single peak for H8, and multiplets for H2’, H3’, H4’, H5’ and the protons of the esterifying groups. These multiplets showed diastereomeric splitting in addition to proton-proton coupling, while phosphorus-proton coupling was only observed for those protons within three bonds of the phosphorus atom. The F.A.B. mass spectrum of (80) further confirmed the structural assignment of this compound, showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of three chlorine atoms in the intact molecule. Finally, analytical H.P.L.C. indicated that the product (80) had been isolated in a pure state, with no contaminating araA.

A comparison of the two alternative pathways investigated for the synthesis of the 5’-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA, reveals that each route may be advantageous under different circumstances. The first route studied for the preparation of these derivatives, involving the standard procedure of synthesizing the appropriate phosphorylating agent and subsequently reacting it with araA, would be preferable if only one particular 5’-alkyl (2,2,2-trichloroethyl) phosphate triester of araA was required. This is apparent from the reaction of araA with butyl 2,2,2-trichloroethyl phosphorochloridate (79) proceeding at a faster rate (46 h vs. 382 h) and in slightly greater yield (29% vs. 25%) than the analogous conversion of araA-5’bis(2,2,2-trichloroethyl) phosphate (43) to araA-5’-butyl (2,2,2-trichloroethyl) phosphate (80) via a transesterification reaction. However, the latter route would offer a greater flexibility for preparing a series of 5’-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA once a supply of araA-5’-bis(2,2,2-trichloroethyl) phosphate (43) had been synthesized, by avoiding the prior need to prepare a series of alkyl 2,2,2-trichloroethyl phosphorochloridates for subsequent reaction with araA.
A final aim of this chapter of the research involved an attempt to isolate and characterize the products resulting from the chemical hydrolysis of araA-5′-bis(2,2,2-trifluoroethyl) phosphate (42), araA-5′-bis(2,2,2-trichloroethyl) phosphate (43) and araA-5′-butyl (2,2,2-trichloroethyl) phosphate (80). Previous studies have investigated the stability of simple 5′-dialkyl phosphate triesters of araA\(^{135,172}\) and their 5′-bis(2,2,2-trihaloethyl) counterparts\(^{136}\) in water at 37°C. These experiments were carried out in order to determine whether the presence of the 2,2,2-trihaloethyl moieties in (42) and (43) had successfully increased the susceptibility of these compounds to chemical hydrolysis over their non-substituted 5′-dialkyl analogues. Under such conditions, the 5′-dialkyl phosphate triesters of araA were found to be resistant to hydrolysis over several months\(^{135,172}\), araA-5′-bis(2,2,2-trichloroethyl) phosphate (43) showed no change over a week\(^{136}\), and araA-5′-bis(2,2,2-trifluoroethyl) phosphate (42) was completely converted to one much less lipophilic product after a week\(^{136}\). The \(^{31}\)P n.m.r. spectrum of the hydrolysis product showed a single peak at \(\delta -0.75\) ppm, downfield of the value of \(\delta -2.0\) ppm for (42) (with both spectra run at 82 MHz with referencing to external phosphoric acid and using \(D_2O\) as solvent). Analysis by \(^{31}\)P n.m.r. spectroscopy and t.l.c. revealed that the hydrolysis product was neither araA nor araAMP. Consequently, the diester araA-5′-(2,2,2-trifluoroethyl) phosphate (89) (equation 34) was suggested as the identity of the hydrolysis product. However, the latter was not fully isolated or characterized.

There is particular interest in 5′-phosphate diesters of araA as these compounds would be expected to be more promising prodrugs of araAMP than the 5′-phosphate triester derivatives. This is because the former should be suitable substrates for the phosphodiesterase-mediated hydrolysis to araAMP, while the latter would probably be dependent on chemical hydrolysis for activation. However, there are also several disadvantages associated with these 5′-phosphate diesters of araA as potential prodrugs of araAMP. Firstly, the presence of the negative charge on the hydroxyl group of the 5′-phosphate diesters at physiological pH might retard the passive diffusion of these compounds across the cell membrane relative to the 5′-phosphate triesters. It is also possible that the 5′-phosphate diesters might undergo extracellular cleavage, induced by
Equation 34

\[
\begin{align*}
&\text{NH}_2 \quad \text{H}_2\text{O}, 37^\circ\text{C} \quad \text{7 days} \\
\text{CF}_3\text{CH}_2\text{O} - \text{PO} - \text{O} \\
\text{CF}_3\text{CH}_2\text{O} &\rightarrow \\
&\text{CF}_3\text{CH}_2\text{O} - \text{PO} - \text{O} \\
\end{align*}
\]

Equation 35

\[
\begin{align*}
\text{POCl}_3 + 2\text{CF}_3\text{CH}_2\text{OH} + 2\text{Et}_3\text{N} &\rightarrow (\text{CF}_3\text{CH}_2\text{O})_2\text{POCl} + 2\text{Et}_3\text{NHCl} \\
\text{Et}_2\text{O} &\rightarrow \\
-40^\circ\text{C} \text{ to RT} &\rightarrow
\end{align*}
\]

Equation 36

\[
\begin{align*}
&(\text{CF}_3\text{CH}_2\text{O})_2\text{POCl} + \text{HO} \quad \text{pyridine} \quad 0^\circ\text{C} \text{ to RT} \\
&\text{CF}_3\text{CH}_2\text{O} - \text{PO} - \text{O} \\
&\text{CF}_3\text{CH}_2\text{O} &\rightarrow \\
\end{align*}
\]
the enzyme phosphodiesterase. Additionally, the 5'-phosphate diesters are likely to be more suitable substrates than the 5'-phosphate triesters for the enzyme alkaline phosphatase, which mediates the conversion to the free nucleoside araA. If this were the case, the aim of obviating the dependence of araA on nucleoside kinase-mediated phosphorylation to the 5'-monophosphate would not have been achieved and the possibility of araA undergoing rapid deactivation by the enzyme adenosine deaminase might arise.

The chemical hydrolysis of araA-5'-bis(2,2,2-trifluoroethyl) phosphate (42) was duly investigated with the aim of isolating and characterizing the hydrolysis product. A prerequisite for this reaction was the synthesis of (42), requiring the prior preparation of the phosphorylating agent bis(2,2,2-trifluoroethyl) phosphorochloridate (90).

The synthesis of bis(2,2,2-trifluoroethyl) phosphorochloridate (90) involved an analogous procedure to the one previously employed for the preparation of (90) in this Department. Thus, two molar equivalents of both 2,2,2-trifluoroethanol and triethylamine were reacted with phosphoryl chloride at -40°C, using diethyl ether as solvent (equation 35). The product (90) was isolated as a colourless oil in 65% yield, following vacuum distillation. The 31P n.m.r. spectrum of the distillate showed a single peak at δ 5.67 ppm, which agrees well with the value of δ 5.61 ppm for this compound. The n.m.r and n.m.r. spectra of (90) were consistent with the structure of the product, as compared with those reported in previous work. It is of interest to note that all the resonances in the 13C n.m.r. and 1H n.m.r. spectra of (90) showed fluorine coupling. The F.A.B. mass spectrum of (90) further confirmed the identity of this phosphorylating agent, showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of one chlorine atom in the intact molecule.

AraA-5'-bis(2,2,2-trifluoroethyl) phosphate (42) was subsequently prepared by the reaction of bis(2,2,2-trifluoroethyl) phosphorochloridate (90) with araA in pyridine at 0°C (equation 36). The method involved was analogous to the one previously reported for the synthesis of (42), except that the reaction was carried out at 0°C, rather than at ambient temperature, to ensure a
more controlled reaction. The product (42) was isolated as a white solid in 46% yield, following column chromatography and repeated trituration with diethyl ether. The $^{31}$P n.m.r. spectrum of (42) showed a single peak at $\delta$(D$_2$O) -2.02 ppm, agreeing well with the value of $\delta$ (D$_2$O) -2.00 ppm for this compound$^{148}$. The $^1$H n.m.r spectrum of (42) was consistent with the structure of the product, as compared with previously reported values$^{136}$, and further confirmed the identity of this compound.

Following the successful preparation of araA-5′-bis(2,2,2-trifluoroethyl) phosphate (42), this compound was subjected to chemical hydrolysis under aqueous conditions. Thus, (42) was dissolved in deionized water and the resulting solution initially stirred at ambient temperature. The course of the reaction was followed by t.l.c. After 310 hours stirring at ambient temperature, t.l.c. indicated that approximately half of the starting material (42) had been converted to a much less lipophilic product. The reaction was allowed to warm to 37°C to encourage it to proceed to completion, the latter being achieved after a further 271 hours stirring at this temperature. An aqueous/organic extraction was carried out to remove any impurities of the by-product 2,2,2-trifluoroethanol. The hydrolysis product (89) was isolated as a white solid in 99% yield, following lyophilization of the aqueous layer. The $^{31}$P n.m.r. spectrum of the hydrolysis product showed a single peak at $\delta$ -0.74 ppm, agreeing well with the value of $\delta$ -0.75 ppm for (89) reported in previous work$^{148}$ and appearing downfield of the value of $\delta$ -2.02 ppm for the starting material (42) (with all the spectra being run at 82 MHz with referencing to external phosphoric acid and using D$_2$O as solvent). A comparison of the t.l.c. properties of the hydrolysis product with those of araA (more lipophilic) and adenosine-5′-monophosphate (AMP) (less lipophilic), appear to rule out araA and araAMP as possible identities of this compound. The most likely explanation is that araA-5′-bis(2,2,2-trifluoroethyl) phosphate (42) undergoes chemical hydrolysis of only one of the 2,2,2-trifluorothioxy moieties to give the diester, araA-5′-(2,2,2-trifluoroethyl) phosphate (89) (equation 37).

The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of the hydrolysis product provided further evidence for the diester (89) as the identity of this compound. The $^{13}$C n.m.r. spectrum was assigned by
Equation 37

\[
\text{CF}_3\text{CH}_2\text{O} - \text{P} - \text{O} \xrightarrow{\text{H}_2\text{O}} \text{HO} - \text{P} - \text{O} \quad \text{RT to 37°C}
\]

(42) \rightarrow (89)

Equation 38

\[
\text{CCl}_3\text{CH}_2\text{O} - \text{P} - \text{O} \xrightarrow{\text{H}_2\text{O}} \text{HO} - \text{P} - \text{O} \quad \text{reflux}
\]

(43) \rightarrow (91)

Equation 39

\[
\text{BuO} - \text{P} - \text{O} \xrightarrow{\text{H}_2\text{O, reflux}} \text{HO} - \text{P} - \text{O}
\]

(80) \rightarrow (91)

(91)
comparison with the spectra of araA$^{165}$ and 2,2,2-trifluoroethanol$^{193}$, rather than the corresponding spectrum of (42), since the latter had unfortunately not been recorded. Doublets were observed for C4' and C5' due to phosphorus coupling, indicating the retention of the 5'-phosphate in the hydrolysis product. It is of interest to note that the shift of $\delta$ 61.13 ppm for C5' is comparable with that of $\delta$ 61.0 ppm for C5' of araA$^{165}$, rather than appearing significantly downfield of this value as found for some 5'-phosphate triesters of araA$^{135,136}$. The spectrum of (89) displayed a downfield shift for the C4' resonance relative to the C1' resonance, contrasting with the former being observed upfield of the latter as reported for some 5'-phosphate triesters of araA$^{135,136}$. The CF$_3$CH$_2$OP and CF$_3$CH$_2$OP resonances both appeared as a quartet of doublets, due to the coupling of each carbon atom with three equivalent fluorine atoms (giving a quartet) and a phosphorus atom (splitting each signal of the quartet into a doublet). The CF$_3$CH$_2$OP resonance showed a large one bond fluorine-carbon coupling constant of 277 Hz, while a smaller two bond coupling constant of 36.4 Hz was observed for the CF$_3$CH$_2$OP resonance. These coupling constants were of the expected magnitude for their respective types of coupling$^{166}$. It is also of interest to note that the three bond phosphorus-carbon coupling constant of 9.8 Hz for the CF$_3$CH$_2$OP resonance was larger than the two bond coupling constant of 4.7 Hz for the CF$_3$CH$_2$OP resonance.

The $^1$H n.m.r. spectrum of the hydrolysis product was similar to that of the starting material (42), showing single peaks for the base protons H2 and H8, and a doublet for H1' due to coupling with H2'$. However, the unresolved multiplet assigned to the H2', H3', H4', H5' and CH$_2$OP resonances integrated for seven protons in the spectrum of the hydrolysis product, as opposed to nine protons for the corresponding signal in the spectrum of (42). This was consistent with (89) as the identity of the hydrolysis product. The F.A.B. mass spectrum of the hydrolysis product further confirmed the structural assignment of this compound, with a peak observed for the molecular ion of (89) at m/e 429. Prominent peaks appeared at m/e 250 and m/e 136 due to (MH$^+$ - (CF$_3$CH$_2$O)(HO)PO$_2$H) and protonated adenine respectively, while minor peaks of interest included those assigned to CF$_3$CH$_2^+$ and CF$_3^+$. Finally, reverse phase analytical H.P.L.C. revealed that the product (89) had been isolated in a pure state, with no contaminating araA.
The chemical hydrolysis of araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) was subsequently investigated under more vigorous conditions than those employed in the analogous reaction involving (42), due to the previously reported stability of (43) in water at 37°C over a week. Thus, (43) was dissolved in deionized water, stirred at reflux, and the course of the reaction followed by t.l.c. After 93 hours stirring at reflux, t.l.c. indicated that the reaction had proceeded to completion with all of the araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) having been converted to a much less lipophilic major component. A minor component, less lipophilic than (43), was also observed and identified as araA. An aqueous/organic extraction was carried out to remove any impurities of the by-product 2,2,2-trichloroethanol, and solvent was removed from the aqueous layer by lyophilization to give a white solid. The $^3\text{P}$ n.m.r. spectrum of the hydrolysis product showed a single peak at $\delta$ -2.24 ppm, appearing downfield of the value of $\delta$ -3.83 ppm for the starting material (43) (with both spectra being run at 82 MHz with referencing to external phosphoric acid and using CH$_3$OD as solvent). A comparison of the t.l.c. properties of the hydrolysis product with those of araA (more lipophilic) and AMP (less lipophilic), appear to rule out araA and araAMP as possible identities of this compound. The most likely explanation is that araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) undergoes chemical hydrolysis of only one of the 2,2,2-trichloroethoxy moieties to give the diester, araA-5'-(2,2,2-trichloroethyl) phosphate (91) (equation 38).

Recrystallization of a sample of the crude hydrolysis product was ineffective in removing the trace of araA shown to be present by t.l.c. analysis. Reverse phase preparative H.P.L.C. also proved unsuccessful in purifying a second sample of the crude hydrolysis product, with the latter appearing to be unstable under the eluent conditions employed. Unfortunately, time did not allow for the resynthesis of araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) and a further attempt to isolate and characterize the product resulting from the subsequent chemical hydrolysis of this compound under aqueous conditions at reflux.

An appropriate conclusion to this series of studies was an investigation into the chemical hydrolysis of araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80). Thus, (80) was subjected to
chemical hydrolysis in an analogous manner to (43), except that the reaction mixture was stirred at reflux for a longer period of time. After 118 hours stirring at reflux, t.l.c. indicated that the reaction had proceeded to completion with all of the araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80) having been converted to a much less lipophilic major component. A minor component, less lipophilic than (80), was also observed and identified as araA. Following an aqueous/organic extraction, solvent was removed from the aqueous layer by lyophilization to give a white solid. The $^3$P n.m.r. spectrum of the hydrolysis product showed a major peak at $\delta$ -0.074 ppm and a minor peak at -0.723 ppm, both appearing downfield of the value of $\delta$ -1.99 ppm for the starting material (80) (with both spectra being run at 82 MHz with referencing to external phosphoric acid and using D$_2$O as solvent). A comparison of the t.l.c. properties of the hydrolysis product with those of araA (more lipophilic) and AMP (less lipophilic), appear to rule out araA and araAMP as possible identities of this compound. A consideration of the resistance to chemical hydrolysis previously noted for 5'-dialkyl phosphates of araA in water at 37°C over several months$^{335}$ and the present investigations involving araA-5'-bis(2,2,2-trichloroethyl) phosphate (43), suggest that (80) probably undergoes chemical hydrolysis of only the more labile 2,2,2-trichloroethoxy moiety to give the diester, araA-5'-butyl phosphate (92) (equation 39a).

Attempted purification of a sample of the crude hydrolysis product by recrystallization proved ineffective in removing the trace of araA shown to be present by t.l.c. analysis. However, a second sample of the crude hydrolysis product was successfully purified by reverse phase preparative H.P.L.C., yielding a white solid. The $^3$P n.m.r. spectra of the hydrolysis product now showed a single peak at $\delta$ -0.649 ppm. A comparison of this spectrum (run at 164 MHz) with that of the crude hydrolysis product (run at 82 MHz) suggested that the minor product might have been isolated rather than the major product. However, this was believed to be inconclusive due to the referencing of only the spectrum of the crude hydrolysis product to external phosphoric acid and the use of different spectrometers. Furthermore, analytical H.P.L.C. data identified the isolated product as the major component of the crude product, the former showing a comparable retention time to the major peak of the latter under the same conditions.
The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of the hydrolysis product were not consistent with (92) as the proposed structure for this compound. The $^{13}$C n.m.r. spectrum of the hydrolysis product unexpectedly revealed the presence of phosphorus-coupled doublets for the CCl$_3$ and CCl$_3$CH$_2$OP resonances in addition to peaks for the nucleosidic carbon atoms, while no resonances were observed for the BuOP moiety. Phosphorus-coupled doublets were observed for C5' and C4', indicating the retention of the 5'-phosphate. The shift of the C5' doublet at $\delta$ 66.88 ppm was significantly downfield of the shift of $\delta$ 61.0 ppm for the C5' resonance of araA$^{165}$, also suggesting the presence of 5'-phosphorylation. The $^1$H n.m.r spectrum of the hydrolysis product similarly showed peaks due to the CCl$_3$CH$_2$OP moiety in addition to the nucleosidic protons, and the absence of peaks for the BuOP moiety. These data suggest that the identity of the compound isolated from the crude product obtained on chemical hydrolysis of araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80) was the diester, araA-5'-(2,2,2-trichloroethyl) phosphate (91) (equation 39b).

If (91) was indeed the identity of the hydrolysis product, the $^{31}$P n.m.r. spectrum of this compound would be expected to show a comparable shift to that of the crude product resulting from the chemical hydrolysis of araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) and believed to be the diester (91). However, the shift of $\delta$(D$_2$O) -0.64 ppm (run at 164 MHz) of the product resulting from the hydrolysis of (80) is significantly downfield of the shift of $\delta$(CH$_3$OD) -2.24 ppm (run at 82 MHz) of the product resulting from the chemical hydrolysis of (43). This disagreement in shifts was believed to be due to the use of different n.m.r. solvents and spectrometers for recording the two spectra and the referencing of only the latter spectrum (run at 82 MHz) to external phosphoric acid, so it would be inappropriate to draw any conclusions from such a comparison.

Further evidence for the diester (91) as the identity of the hydrolysis product was provided by the F.A.B. mass spectrum of this compound, with a cluster of minor peaks being observed for the molecular ion of (91). The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of three chlorine atoms in the intact molecule. A prominent peak appeared at
m/e 136 due to protonated adenine, while minor peaks of interest included those assigned to 
CCl₃CH₂OPO₂H⁺, CCl₃CH₂OPOH⁺ and CCl₃CH⁺. Finally, reverse phase analytical H.P.L.C. 
revealed that the hydrolysis product had been isolated in a pure state, with no contaminating araA. 
Additionally, the hydrolysis product was found to have an identical retention time to the major 
component of the crude product resulting from the chemical hydrolysis of araA-5'-bis(2,2,2-
trichloroethyl) phosphate (43) and believed to be the diester (91). It was clearly unexpected that 
araA-5'-(2,2,2-trichloroethyl) phosphate (91) should be identified as the product obtained on 
chemical hydrolysis of araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80) under aqueous 
conditions at reflux. Unfortunately, time did not allow for a repeat of the experiment to fully 
establish the course of this reaction.

The above experiments have concentrated on the isolation and characterization of the products 
resulting from the chemical hydrolysis of araA-5'-bis(2,2,2-trifluoroethyl) phosphate (42), araA-
5'-bis(2,2,2-trichloroethyl) phosphate (43), and araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80). 
However, it would also have been of interest to carry out some kinetic studies investigating the 
stability of compounds (42), (43) and (80) under conditions of varying pH (using buffer solutions) 
and in human plasma. Additionally, it would have been worthwhile to determine the stability of 
the 5'-phosphate diesters of araA in human plasma and the susceptibility of these derivatives to 
the enzyme phosphodiesterase. Unfortunately, their was insufficient time to carry out these 
investigations but they may lead to scope for future research.

The biological activities of the 5'-alkyl (2,2,2-trichloroethyl) phosphates of araA (80), (83) 
and (86), and the diester araA-5'-(2,2,2-trichloroethyl) phosphate (91) were evaluated by 
determining the ability of these compounds to inhibit the synthesis of cellular DNA 
using the in vitro tritiated thymidine incorporation assay developed by Riley and co-workers¹⁶⁸, 
in an entirely analogous manner to the procedure employed for the testing of some 5'-phosphate 
triesters of araA and their 5'-phosphinate ester counterparts¹⁷² (discussed earlier in chapter 2). 
Each experiment was carried out at least twice on cells of different passage number and
the mean % inhibition of DNA synthesis (relative to the distilled water control) and standard error of the mean (SEM) were calculated for each set of % inhibition values (tables 3, 5, biological testing section). A two-tail student’s t-test was used to determine the degree of significant difference between the mean % inhibition values of two compounds at a given concentration. This data was subsequently used to determine the probability of this difference being statistically significant (tables 4, 6, biological testing section).

Firstly, the in vitro biological activities of the 5'-alkyl (2,2,2-trichloroethyl) phosphates of araA, araA-5'-ethyl (2,2,2-trichloroethyl) phosphate (86), araA-5'-propyl (2,2,2-trichloroethyl) phosphate (83) and araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80), were evaluated along with their respective 5'-dialkyl analogues, araA-5'-diethyl phosphate (38), araA-5'-dipropyl phosphate (39) and araA-5'-dibutyl phosphate (40). The latter three compounds have been previously synthesized and biologically evaluated in this Department\(^\text{135}\). However, it was felt that the inclusion of (38), (39) and (40) in the same experiment as (86), (83) and (80) would allow for a fairer comparison between the biological activities of these two sets of compounds.

The results of the assay are presented as a bar chart (figure 26), in which the mean % inhibition of DNA synthesis (relative to the distilled water control) is plotted against compound at final concentrations of 0.03 mM and 0.003 mM. This graph clearly illustrates the relationship between the compounds and their biological activity. Each of the compounds tested displays an inhibitory effect on DNA synthesis in vitro, and in every case a dose-response is evident. Considering the higher concentration of 0.03 mM, the 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (86), (83) and (80) show an increasing biological activity with increasing chain length of their constituent alkyl group, as observed in previous work\(^\text{135}\) (as well as in the present study) for the 5'-dialkyl phosphate triesters of araA. However, a comparison of the biological activities of the 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (86), (83) and (80) with their respective 5'-dialkyl counterparts (38), (39) and (40), reveals that replacing an alkyl chain by a 2,2,2-trichloroethyl moiety enhances the biological activity of the former over the latter. Indeed, a two-tail student’s t-test (table 4, biological testing section) indicates that these
Figure 26: % Inhibition of DNA synthesis against compound

% Inhibition of DNA synthesis

Compound

araA (86) (83) (80) (38) (39) (40)

0.003 mM  0.03 mM
differences are significant (i.e. P < 5%) for (86) vs. (38), (83) vs. (39), and (80) vs. (40). It is also of interest to note that compounds (86) and (83) display biological activities which do not differ significantly from that of araA, while compound (80) shows a biological activity significantly greater than araA. However at the lower concentration of 0.003 mM, the 5’-alkyl (2,2,2-trichloroethyl) phosphates of araA (86), (83) and (80) display comparable biological activities to their respective 5’-dialkyl analogues (38), (39) and (40), probably reflecting the weaker effects at lower concentrations of compound. The two-tail student’s t-test indicates that these differences in biological activities are not significant (i.e. P > 5%). Additionally, compounds (86) and (83) show biological activities significantly lower than araA, while compound (80) displays a biological activity which does not differ significantly from araA.

The biological activity of the diester araA-5’-(2,2,2-trichloroethyl) phosphate (91) was subsequently evaluated along with araA-5’-butyl (2,2,2-trichloroethyl) phosphate (80) (from which it was derived) and araA-5’-bis(2,2,2-trichloroethyl) phosphate (43). It would be of interest to discover whether (extracellular) hydrolysis adversely or beneficially affected the activity of the parent compound (80) and the related compound (43). The results of the assay are presented in a bar chart (figure 27), in which the mean % inhibition of DNA synthesis (relative to the distilled water control) is plotted against compound at final concentrations of 0.03 mM and 0.003 mM. Considering the higher concentration of 0.03 mM, compounds (91) and (80) are equi-active. The 5’-diester (91) also displays a slightly greater biological activity than araA and a greater biological activity than (43), with the inhibition value of the latter being lower than the corresponding value for araA. A two-tail student’s t-test (table 6, biological testing section) indicates that these differences in biological activities are significant (i.e. P < 5%). However at the lower concentration of 0.003 mM, comparable biological activities are observed for araA, (91), (80) and (43), probably reflecting the weaker effects at lower concentration of compound. These results contrast with those reported in previous work\cite{35}, where the diester araA-5’-(2,2,2-trifluoroethyl) phosphate (89) resulting from the chemical hydrolysis of araA-5’-bis(2,2,2-trifluoroethyl) phosphate (42) (but not isolated or fully characterized prior to its inclusion in the biological assay) showed a much lower
Figure 27: % Inhibition of DNA synthesis against compound

% Inhibition of DNA synthesis

- 0.003 mM
- 0.03 mM

<table>
<thead>
<tr>
<th>Compound</th>
<th>0.003 mM</th>
<th>0.03 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>(91)</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>(80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(43)</td>
<td></td>
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</tbody>
</table>
biological activity than (42). A possible explanation for this may lie in the greater lipophilicity of
the chlorine atoms relative to the fluorine atoms in the 2,2,2-trihaloethyl moieties of these diesters,
(91) and (89) respectively.

Thus, some interesting relationships were observed between the structures of the compounds
tested and their ability to inhibit the synthesis of DNA *in vitro*. These may lead to scope for
future research and development. The effect of introducing a 2,2,2-trichloroethyl moiety into a
5'-phosphate derivative of araA is particularly noteworthy, and in the case of araA-5'-butyl
(2,2,2-trichloroethyl) phosphate (80) appears to cause a slight but significant enhancement in the
biological activity of the parent drug araA.
Chapter 4: 5'-Aryl phosphate triesters of araA

Recent research (chapter 3) has revealed that mixed, unsymmetrical 5'-phosphate triesters of araA containing only one 2,2,2-trichloroethyl group at the 5'-phosphate site, i.e. the series of 5'-alkyl (2,2,2-trichloroethyl) derivatives, show an enhanced biological activity over their simple 5'-dialkyl counterparts. It would be of interest to extend this study to include 5'-aryl phosphate triesters of araA, especially in view of the fact that araA-5'-diphenyl phosphate (61) was reported to be equi-active with araA, when evaluating the biological activity of some 5'-phosphate triesters of araA in comparison with their analogous 5'-phosphinate esters to elucidate the mechanism of action of the former type of compound\textsuperscript{172}. Thus, an investigation was carried out into the synthesis and biological evaluation of the mixed, unsymmetrical 5'-phosphate triester of araA containing one simple aryl group and one simple alkyl group, namely araA-5'-ethyl phenyl phosphate (94), and subsequently the corresponding derivative containing one simple aryl group and one substituted alkyl group, namely araA-5'-(2,2,2-trichloroethyl) phenyl phosphate (97) (figure 28).

Figure 28

![Chemical structures](image)

Additionally, it would be of interest to discover the effect of changing the para-substituent of the aryl group of some 5'-aryl (2,2,2-trichloroethyl) phosphate triesters of araA on the biological activity of these compounds.
The preparation of araA-5'-ethyl phenyl phosphate and some 5'-aryl (2,2,2-trichloroethyl) phosphate triesters of araA generally involved the synthesis of the appropriate phosphorylating agent, in this case ethyl phenyl phosphorochloridate and a number of aryl 2,2,2-trichloroethyl phosphorochloridates, and its subsequent reaction with unprotected araA. Two alternative routes were employed for the synthesis of the required phosphorochloridates. Firstly, ethyl phenyl phosphorochloridate and the majority of the aryl 2,2,2-trichloroethyl phosphorochloridates were prepared via ethyl phosphorodichloridate and 2,2,2-trichloroethyl phosphorodichloridate respectively, and their subsequent reaction with the required aryl alcohol. Several alternative methods are available for the synthesis of phosphorodichloridates (as discussed in chapter 3). The method of choice involved the reaction of phosphoryl chloride with one molar equivalent of both the appropriate alcohol and triethylamine in diethyl ether solvent at reduced temperature (equation 40). This procedure was analogous to the one employed in the earlier preparation of a series of alkyl phosphorodichloridates (chapter 3). The tertiary base triethylamine was again present in the reaction mixture to remove the hydrogen halide by-product by precipitation as triethylamine hydrochloride.

Ethyl phosphorodichloridate and 2,2,2-trichloroethyl phosphorodichloridate were subsequently reacted with one molar equivalent of both the appropriate aryl alcohol and triethylamine at reduced temperature, using diethyl ether as the solvent, to give ethyl phenyl phosphorochloridate and the required aryl 2,2,2-trichloroethyl phosphorochloridates respectively (equation 41). This reaction of a primary phosphorodichloridate with an alcohol to give a secondary phosphorochloridate may take place in the absence or presence of base. The use of the tertiary organic base triethylamine was again favoured for the removal of the hydrogen chloride by-product, rather than carrying out the reaction under reduced pressure or the bubbling of an inert gas through the reaction mixture since the reaction is more controllably under the former conditions.

An alternative route to a few of the aryl 2,2,2-trichloroethyl phosphorochloridates was employed in cases where the required commercial aryl phosphorodichloridate was available. The method then involved the reaction of the appropriate aryl phosphorodichloridate with one molar
Equation 40

\[
\begin{align*}
\text{POCl}_3 + \text{ROH} + \text{Et}_3\text{N} & \xrightarrow{\text{Et}_2\text{O}} \text{ROPOCl}_2 + \text{Et}_3\text{NCl} \\
-78^\circ\text{C} \text{ to RT} &
\end{align*}
\]

\(R = \text{alkyl or 2,2,2-trihaloethyl}\)

Equation 41

\[
\begin{align*}
\text{ROPOCl}_2 + \text{ArOH} + \text{Et}_3\text{N} & \xrightarrow{\text{Et}_2\text{O}} (\text{ArO})(\text{RO})\text{POCl} + \text{Et}_3\text{NCl} \\
-78^\circ\text{C} \text{ to RT} &
\end{align*}
\]

\(\text{Ar} = \text{aryl,}
\text{R} = \text{alkyl or 2,2,2-trihaloethyl}\)

Equation 42

\[
\begin{align*}
\text{ArOPOCl}_2 + \text{CCl}_3\text{CH}_2\text{OH} + \text{Et}_3\text{N} & \xrightarrow{\text{Et}_2\text{O}} (\text{ArO})(\text{CCl}_3\text{CH}_2\text{O})\text{POCl} + \text{Et}_3\text{NCl} \\
-78^\circ\text{C} \text{ to RT} &
\end{align*}
\]

\(\text{Ar} = \text{aryl}\)

Equation 43

\[
\begin{align*}
(\text{ArO})(\text{RO})\text{POCl} + \text{HO} & \xrightarrow{\text{pyridine}} \text{ArO} - \overset{\text{O}}{\overset{\text{P}}{\text{O}}} - \overset{\text{RO}}{\overset{\text{O}}{\text{H}}} \\
0^\circ\text{C} \text{ to RT} &
\end{align*}
\]

\(\text{Ar} = \text{aryl,}
\text{R} = \text{alkyl or 2,2,2-trihaloethyl}\)
equivalent of both 2,2,2-trichloroethanol and triethylamine in diethyl ether solvent at reduced temperature (equation 42), in an analogous manner to the procedure involved in the earlier synthesis of a series of alkyl 2,2,2-trichloroethyl phosphorochloridates (chapter 3), except for the use of an aryl phosphorodichloridate rather than an alkyl phosphorodichloridate.

These mixed, unsymmetrical aryl phosphorochloridates, obtained by one of the two routes outlined above (equations 40 and 41, or equation 42), were subsequently reacted with unprotected araA in pyridine at reduced temperature to give araA-5'-ethyl phenyl phosphate and the required 5'-aryl (2,2,2-trichloroethyl) phosphate triesters of araA (equation 43). This methodology had been successfully employed in the earlier synthesis of a series of mixed, unsymmetrical 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (chapter 3).

The synthesis of the first target compound, araA-5'-ethyl phenyl phosphate (94), proceeded via the route outlined above involving the preparation of the required alkyl aryl phosphorochloridate from ethyl phosphorodichloridate (84). Ethyl phenyl phosphorochloridate (93) has been previously synthesized by several alternative methods\textsuperscript{196}, some examples include:

1. The reaction of phenyl phosphorodichloridate with one molar equivalent of ethanol, while removing the hydrogen chloride by-product by passing a stream of carbon dioxide through the reaction mixture and then under reduced pressure\textsuperscript{197}.

   \[
   \text{PhOPOCl}_2 + \text{EtOH} \rightarrow (\text{PhO})(\text{EtO})\text{POCl} + \text{HCl}
   \]

2. The reaction of ethyl phenyl phosphite (prepared from phenyl dichlorophosphite and ethanol) with sulphuryl chloride in carbon tetrachloride solvent, while removing the volatile by-products and solvent by passing dry air through the reaction mixture under reduced pressure\textsuperscript{198}.

   \[
   (\text{PhO})(\text{EtO})\text{PHO} + \text{SO}_2\text{Cl}_2 \rightarrow (\text{PhO})(\text{EtO})\text{POCl} + \text{SO}_2 + \text{HCl}
   \]
The present synthesis of ethyl phenyl phosphorochloridate (93) involved the reaction of ethyl phosphorodichloridate (84), prepared earlier (as discussed in chapter 3), with one molar equivalent of both phenol and triethylamine in diethyl ether solvent at -78°C (equation 44). Possible impurities resulting from this reaction include unreacted ethyl phosphorodichloridate, ethyl diphenyl phosphate, and hydrolyzed material. It was hoped to minimize the formation of these by-products by the scrupulous drying of the reagents prior to the reaction, the slow addition of the reagents at reduced temperature under an atmosphere of nitrogen, and using an excess of solvent to dilute the reaction mixture.

After allowing the reaction mixture to warm to ambient temperature with stirring overnight, followed by a further 50 hours stirring at this temperature, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. The resulting cloudy oil was extracted with hexane to give the product (93) as a colourless oil in 100% yield. The $^{31}\text{P}$ n.m.r. spectrum of (93) showed a single peak at $\delta$ -2.34 ppm, comparing with the values of $\delta$ 2.8 ppm for diethyl phosphorochloridate$^{156-158}$ and $\delta$ -6.2 ppm for diphenyl phosphorochloridate$^{158}$. The $^{13}\text{C}$ n.m.r. and $^1\text{H}$ n.m.r. spectra of (93) were consistent with the structure of the product, showing signals due to both the EtOP and PhOP moieties. These spectra were assigned by comparison with the corresponding spectra of phenol$^{162,165}$ and ethanol$^{162,166}$. The $^{13}\text{C}$ n.m.r. spectrum showed phosphorus-coupled doublets for the ipso-Ph, ortho-Ph, methylene and methyl resonances. Other signals in the spectrum, due to carbon atoms further than three bonds away from the phosphorus atom, appeared as singlets with no phosphorus coupling. The $^1\text{H}$ n.m.r. spectrum consisted of multiplets at $\delta$ 7.19 - 7.40 ppm (the PhOP resonance), $\delta$ 4.37 ppm (the CH$_2$OP resonance) and $\delta$ 1.43 ppm (the CH$_3$ resonance). The E.I. mass spectrum of (93) (figure 29) further confirmed the structural assignment of this phosphorylating agent, showing a cluster of peaks due to the molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of one chlorine atom in the intact molecule. The base peak was observed at m/e 94 due to C$_3$H$_7$O$^+$ and other prominent peaks included those assigned to (MH$^+$ - Et) and C$_6$H$_5^+$. Satisfactory microanalysis data were also obtained on (93), indicating the purity of the product.
Equation 44

\[
\text{EtOPOCl}_2 + \text{PhOH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}} \text{(PhO)(EtO)POCl} + \text{Et}_3\text{NHCl}
\]

(84)

-78°C to RT

(93)

Figure 29: E.I. mass spectrum of (93)

Equation 45

\[
\text{(PhO)(EtO)POCl} + \text{HO} \xrightarrow{\text{pyridine}} \text{PhO} \xrightarrow{\text{Py}} \text{EtO} \xrightarrow{\text{OH}} \text{HO}
\]

(93)

0°C to RT

(94)
The synthesis of araA-5′-ethyl phenyl phosphate (94) involved the subsequent reaction of two molar equivalents of ethyl phenyl phosphorochloridate (93) with araA in pyridine at 0°C (equation 45). The reaction employed two molar equivalents of phosphorochloridate since this amount of phosphorylating agent had been required to drive some analogous reactions to completion in the earlier synthesis of a series of 5′-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA (chapter 3). After stirring initially at 0°C for an hour, the reaction mixture was allowed to warm to ambient temperature and stirred at this temperature for a further 21 hours. T.l.c. indicated that the reaction had proceeded to completion, with all of the araA having reacted to give a more lipophilic major component assumed to be due to the required product araA-5′-ethyl phenyl phosphate (94). Two even more lipophilic minor components were also observed. The reaction mixture was quenched with deionized water to remove any excess phosphorylating agent. Pyridine was removed and the resulting residue was purified by column chromatography using a methanol-chloroform eluent. The product, although appearing pure by t.l.c. analysis, was shown to be slightly impure by $^3$P n.m.r. spectroscopy. After further purification by column chromatography, employing a slower methanol-ethyl acetate eluent, the product (94) was isolated as a white solid in 40% yield.

The $^3$P n.m.r. spectrum of (94) (figure 30) showed two closely spaced singlets of similar intensity at $\delta$ -5.89 ppm and $\delta$ -6.01 ppm, corresponding to the presence of an approximately equal mixture of the two possible diastereoisomers of (94) (figure 31) in the product. This isomerism arises from the asymmetry at the chiral phosphorus centre. The shift of these peaks compares with the values of $\delta$ -0.70 ppm for araA-5′-diethyl phosphate$^{135}$ and $\delta$ -11.25 ppm for araA-5′-diphenyl phosphate$^{172}$. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (94), assigned by comparison with the corresponding spectra of araA-5′-diethyl phosphate$^{135}$ and araA-5′-diphenyl phosphate$^{172}$, further confirmed the identity of the product and its diastereomeric nature. The latter was evident in both spectra by the duplication of many of the peaks in the same ratio (i.e. 1:1) as observed in the $^3$P n.m.r. spectrum. The multiplicity of these signals indicates the non-equivalence of the carbon atoms and protons in the two diastereoisomers of the product. The $^{13}$C n.m.r. spectrum, for
Figure 30: $^{31}$P n.m.r. spectrum of (94)

Figure 31: Diastereoisomers of (94)
example, showed diastereomeric splitting for all nucleosidic carbon atoms except for C6, C5, and C2′ or C3′. Further splitting due to phosphorus-carbon coupling was observed for the resonances of some of the carbon atoms within three bonds of the phosphorus atom, with the appearance of two doublets for C4′, and multiplets for C5′ and CH₂OP due to the overlap of these splittings. Only phosphorus coupling was observed for the doublets assigned to the ipso-Ph and methyl groups. Evidence that (94) was the product of 5′-phosphorylation and not 2′- or 3′-phosphorylation was provided by the multiplet for C5′ at δ 69.03 ppm appearing significantly downfield compared with the value of 61.0 ppm for C5′ of araA. The ¹H n.m.r. spectrum also showed diastereomeric splitting for a number of peaks in the spectrum, e.g. two distinct signals were observed for the base proton H2. The H1′ resonance appeared as two doublets due to both proton-proton coupling and diastereomeric splitting. The spectrum also featured separate multiplets for the PhOP, H2′, H3′ and methyl resonances, while the signals due to H4′, H5′ and CH₂OP comprised an unresolved multiplet.

The F.A.B. mass spectrum of (94) further confirmed the structural assignment of this compound, showing a prominent peak due to the protonated molecular ion and a minor peak due to the molecular ion plus sodium from the matrix. The base peak was observed at m/e 136 due to protonated adenine. A minor peak at m/e 203 was assigned to (PhO)(EtO)P(OH)_3⁺, while other peaks indicative of a phenyl moiety included those at m/e 175 and m/e 94 due to PhOP(OH)₃⁺ and C₆H₅OH⁺ respectively. Finally, microanalysis data and reverse phase analytical H.P.L.C. revealed that the product (94) had been isolated in a pure state. The latter technique displayed only one signal rather than the two expected for the diastereomeric product (94), presumably due to the coincidence of these peaks under the conditions employed, and also indicated the absence of contaminating araA.

An investigation into the synthesis of araA-5′-(2,2,2-trichloroethyl) phenyl phosphate (97) and araA-5′-(2,2,2-trichloroethyl) p-nitrophenyl phosphate (100) was subsequently carried out. The proposed route to these compounds, by contrast to that employed in the synthesis of
araA-5′-ethyl phenyl phosphate (94), involved the preparation of the required aryl 2,2,2-
trichloroethyl phosphorochloridate from the appropriate commercial aryl phosphorodichloridate. 
Thus, 2,2,2-trichloroethyl phenyl phosphorochloridate (96) and 2,2,2-trichloroethyl p-nitrophenyl 
phosphorochloridate (99) were prepared by the reaction of the appropriate aryl 
phosphorodichloridate with one molar equivalent of both 2,2,2-trichloroethanol and triethylamine 
in diethyl ether solvent at -78°C (equation 46). Possible impurities resulting from these reactions 
include unreacted aryl phosphorodichloridate, aryl bis(2,2,2-trichloroethyl) phosphate, and 
hydrolyzed material. It was hoped to minimize the formation of these by-products by the usual 
procedure involving the scrupulous drying of the reagents prior to the reaction, the slow addition 
of the reagents at reduced temperature under an atmosphere of nitrogen, and using an excess of 
solvent to dilute the reaction mixture.

Firstly, 2,2,2-trichloroethyl phenyl phosphorochloridate (96) was prepared by the addition of 
one molar equivalent of both 2,2,2-trichloroethanol and triethylamine to commercial phenyl 
phosphorodichloridate (95) at -78°C, using diethyl ether as the solvent (equation 46). After 
allowing the reaction mixture to warm to ambient temperature with stirring overnight, followed 
by a further 30 hours stirring at this temperature, the precipitated triethylamine hydrochloride was 
filtered off and solvent removed under reduced pressure. The $^{31}$P n.m.r. spectrum of the product, 
obtained after extraction with hexane, showed a major peak at $\delta$ -2.55 ppm due to 2,2,2-
trichloroethyl phenyl phosphorochloridate. The chemical shift of this peak compares with the value 
of $\delta$ -2.34 ppm for (93), and is approximately intermediate between the values of $\delta$ 1.30 ppm for 
bis(2,2,2-trichloroethyl) phosphorochloridate$^{186}$ and $\delta$ -6.2 ppm for diphenyl phosphorochloridate$^{158}$. 
A minor peak was also observed at $\delta$ -11.17 ppm, probably due to phenyl bis(2,2,2-trichloroethyl) 
phosphate and comparing with a value of $\delta$ -12.0 ppm for ethyl diphenyl phosphate$^{199}$. 

Although not entirely pure, additional spectra were obtained on (96), further confirming the 
structural assignment of this phosphorylating agent. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra, assigned 
by comparison with the corresponding spectra of 2,2,2-trichloroethanol and phenol$^{102,105}$, showed 
signals due to both the CCl$_3$CH$_2$OP and PhOP moieties. The $^{13}$C n.m.r. spectrum of (96) was
Equation 46

\[
\begin{align*}
\text{ArOPOCl}_2 + \text{CCl}_3\text{CH}_2\text{OH} + \text{Et}_3\text{N} & \quad \xrightarrow{\text{Et}_2\text{O}} \quad (\text{ArO})(\text{CCl}_3\text{CH}_2\text{O})\text{POCl} + \text{Et}_3\text{NHCl} \\
(95) \ R = \text{Ph} & \quad \quad (96) \ R = \text{Ph} \\
(98) \ R = p-\text{NO}_2\text{Ph} & \quad \quad (99) \ R = p-\text{NO}_2\text{Ph}
\end{align*}
\]

-78°C to RT

Figure 32: E.I. mass spectrum of (96)

Equation 47

\[
\text{(ArO)(CCl}_3\text{CH}_2\text{O)}\text{POCl} + \text{HO} \quad \xrightarrow{\text{pyridine}} \quad \text{ArO-P-O} \\
\text{CCl}_3\text{CH}_2\text{O} \\
0°C to RT
\]

(96) \ R = \text{Ph} \\
(99) \ R = p-\text{NO}_2\text{Ph} \\
(97) \ R = \text{Ph} \\
(100) \ R = p-\text{NO}_2\text{Ph}
similar to the spectrum of (93), with phosphorus-coupled doublets observed for all the carbon atoms within three bonds of the phosphorus atom. However, the doublet assigned to the CCl$_3$CH$_2$OP moiety of (96) appeared further downfield than the corresponding resonance for the CH$_3$CH$_2$OP moiety of (93), probably due to the deshielding effect of the chlorine atoms. The $^1$H n.m.r. spectrum of (96) consisted of multiplets at $\delta$ 7.23-7.43 ppm (the PhOP resonance) and $\delta$ 4.74 ppm (the CCl$_3$CH$_2$OP resonance). An E.I. mass spectrum was also obtained on (96) (figure 32), showing a cluster of peaks for the molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 94 due to C$_6$H$_4$OH$^+$ and other prominent peaks included those due to (MH$^+$ - CCl$_3$CH$_2$) and C$_2$H$_4^+$.

2,2,2-Trichloroethyl $p$-nitrophenyl phosphorochloridate (99) was prepared in an entirely analogous manner to (96). A colourless oil was obtained, following work-up of the reaction mixture and extraction of the resulting cloudy oil with hexane. The $^{31}$P n.m.r. spectrum of the product showed a major peak at $\delta$ -3.16 ppm due to 2,2,2-trichloroethyl $p$-nitrophenyl phosphorochloridate, comparing with the value of $\delta$ -2.55 ppm for (96). A minor peak was also observed at $\delta$ 11.87 ppm, probably due to bis(2,2,2-trichloroethyl) $p$-nitrophenyl phosphate and comparing with the value of -12.0 ppm for ethyl diphenyl phosphate$^{199}$. Despite the presence of a minor impurity, additional spectra were obtained on (99), further confirming the identity of this phosphorylating agent. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (99) were consistent with the structure of the product, showing signals due to both the CCl$_3$CH$_2$OP and $p$-NO$_2$C$_6$H$_4$OP moieties. These spectra were assigned by comparison with the corresponding spectra of (96) and $p$-nitrophenol$^{162,200}$. The $^{13}$C n.m.r. spectrum of (99) was similar to the spectrum of (96), except for the influence of the nitro substituent on the chemical shift of the resonances for the phenyl carbon atoms. The $^1$H n.m.r. spectrum of (99) consisted of two distinct signals for the phenyl protons with a doublet at $\delta$ 8.31 ppm (the meta-Ph resonance) and a multiplet at $\delta$ 7.48 ppm (the ortho-Ph resonance), in contrast to the spectrum of (96) where an unresolved multiplet was observed for the phenyl protons. A multiplet was again observed for the CCl$_3$CH$_2$OP group. The
E.I. mass spectrum of (99) showed a cluster of peaks due to the molecular ion with an isotopic pattern for this, and other chlorine containing fragments, characteristic of four chlorine atoms in the intact molecule. A similar fragmentation pattern was observed in this spectrum to that in the spectrum of (96), with peaks of moderate intensity associated with the loss of the CCl₃ and CCl₃CH₂ moieties from the parent ion. Peaks indicative of the presence of an aryl moiety in the intact molecule included those assigned to C₆H₄OH⁺ and C₆H₅⁺.

Further purification of the 2,2,2-trichloroethyl phenyl phosphorochloridate (96) and 2,2,2-trichloroethyl p-nitrophenyl phosphorochloridate (99) by vacuum distillation was not attempted since a consideration of the boiling point of the related phosphorylating agent, ethyl phenyl phosphorochloridate (93), suggested that the boiling points of (96) and (99) might be so high that decomposition of these compounds would occur before they could be distilled. The only impurities present in compounds (96) and (99) were probably due to traces of the appropriate aryl bis(2,2,2-trichloroethyl) phosphate, which were not expected to be reactive towards nucleosides. Consequently, it was possible to use these aryl 2,2,2-trichloroethyl phosphorochloridates in the next stage of the synthesis.

The synthesis of araA-5′-(2,2,2-trichloroethyl) phenyl phosphate (97) and attempted preparation of araA-5′-(2,2,2-trichloroethyl) p-nitrophenyl phosphate (100) involved the subsequent reaction of the appropriate aryl 2,2,2-trichloroethyl phosphorochloridate with unprotected araA in pyridine at 0°C (equation 47). Firstly, araA-5′-(2,2,2-trichloroethyl) phenyl phosphate (97) was prepared in a similar manner to (94), except for the use of more of the appropriate phosphorylating agent (96) (initially 2.0 molar equivalents with a further 1.0 molar equivalents added during the course of the reaction) and a longer reaction time. These conditions were employed to enable the present reaction to proceed to completion. Purification of the crude product (97) differed slightly to the procedure employed for (94). Following column chromatography, using a methanol-ethyl acetate eluent, a white solid was obtained which was shown to be impure by t.l.c. analysis and ³¹P n.m.r. spectroscopy. Attempted recrystallizations of the product from ethyl acetate and ethanol/hexane proved ineffective in removing the impurities.
The product was finally purified by reverse phase preparative H.P.L.C., allowing (97) to be isolated as a white solid in 25% yield. The $^{31}$P n.m.r. spectrum of the product now showed two closely spaced singlets at $\delta$ -7.63 ppm and $\delta$ -7.76 ppm in a 1:1 ratio, indicating that (97) had been isolated as an approximately equal mixture of the two possible diastereoisomers. This isomerism again results from the mixed stereochemistry at the phosphorus centre. The chemical shifts of these peaks compare with the values of $\delta$ -5.89 ppm and -6.01 ppm for (94), and the values of $\delta$ -6.99 ppm and $\delta$ -7.11 ppm for the corresponding 5'-[(2,2,2-trichloroethyl) phenyl phosphate triester of the anti-HIV drug AZT\textsuperscript{152}, and are also approximately intermediate between the values of $\delta$ -3.83 ppm for araA-5'-bis(2,2,2-trichloroethyl) phosphate\textsuperscript{136} and $\delta$ -11.25 ppm for araA-5'-diphenyl phosphate\textsuperscript{172}.

The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (97), assigned by comparison with the spectra of araA-5'-bis(2,2,2-trichloroethyl) phosphate\textsuperscript{136} and araA-5'-diphenyl phosphate\textsuperscript{172}, were consistent with the structure of the product. Signals were observed for both the CCl$_3$CH$_2$OP and PhOP moieties in addition to the nucleosidic resonances. These spectra further confirmed the diastereomeric nature of the product, with many signals split in the same ratio (i.e. 1:1) as observed for the peaks in the $^{31}$P n.m.r. spectrum. A comparison of the $^{13}$C n.m.r. spectrum of (97) with the spectrum of (94) revealed few differences between these spectra, apart from those associated with the presence of a CCl$_3$CH$_2$OP moiety rather than a CH$_3$CH$_2$OP moiety. Phosphorus coupling was again observed for all carbon atoms within three bonds of the phosphorus atom. The $^1$H n.m.r. spectrum of (97) was similar to the spectrum of (94), with the main exception being the slight downfield shift observed for the CH$_2$OP resonance in the former due to the deshielding effect of the chlorine atoms.

The F.A.B. mass spectrum of (97) showed a cluster of peaks due to the protonated molecular ion with an isotopic pattern characteristic of three chlorine atoms in the intact molecule. The base peak was observed at m/e 136 due to protonated adenine. A prominent peak appeared at m/e 77 due to C$_6$H$_5^+$, while minor peaks of interest included those assigned to C$_6$H$_4$OH$^+$ and the ionized sugar fragments C$_2$H$_7$O$_2^+$, C$_3$H$_5$O$_2^+$ and C$_3$H$_7$O$^+$. Finally, reverse phase analytical H.P.L.C. revealed
that the product (97) had been isolated in a pure state, with no contaminating araA. It is of interest to note that the H.P.L.C. spectrum of (97) showed two distinct signals for the diastereomeric product in a 1:1 ratio, contrasting with that for (94) where coincidence of the two expected peaks occurred.

The synthesis of araA-5′-(2,2,2-trichloroethyl) p-nitrophenyl phosphate (100) was attempted by an analogous procedure to the one employed in the preparation of (94), except that the reaction was stirred at ambient temperature for a longer period of time to encourage it to proceed to completion. After 65 hours stirring at ambient temperature (following an initial hour at 0°C), t.l.c. indicated that the all of the araA had reacted to give two more lipophilic major components of approximately equal intensity and two even more lipophilic minor components. The two major components were close running and assumed to due to the two possible diastereoisomers of (100), although the diastereomeric nature of (94) and (97) had not been similarly visualized by t.l.c. under ultra violet (uv) light.

The 31P n.m.r. spectrum of the crude product, following work-up of the reaction mixture, showed a major peak composed of two closely spaced singlets of approximately equal intensity at δ -7.47 ppm and δ -7.71 ppm. These peaks were assumed to be due to the two possible diastereoisomers of the required product (100), by comparison with the values of δ -7.63 ppm and δ -7.76 ppm for (97). Several minor peaks were also observed in the spectrum. Attempted purification of the crude product by column chromatography, using a methanol-chloroform eluent, proved unsuccessful. T.l.c. analysis and 31P n.m.r. spectroscopy of fractions collected from the column indicated that decomposition of the product had occurred. Unfortunately, time did not allow for the resynthesis of araA-5′-(2,2,2-trichloroethyl) p-nitrophenyl phosphate (100).

An investigation into the synthesis of araA-5′-(2,2,2-trichloroethyl) p-cyanophenyl phosphate (103), araA-5′-(2,2,2-trichloroethyl) p-isopropylphenyl phosphate (105), araA-5′-biphenyl (2,2,2-trichloroethyl) phosphate (107), araA-5′-(2,2,2-trichloroethyl) p-chlorophenyl phosphate (109) and araA-5′-(2,2,2-trichloroethyl) p-methoxyphenyl phosphate (111) was subsequently carried out. The
proposed route to these compounds, in contrast to that employed in the synthesis of araA-5'-(2,2,2-trichloroethyl) phenyl phosphate (97), involved the preparation of the required aryl 2,2,2-trichloroethyl phosphorochloridates from 2,2,2-trichloroethyl phosphorodichloridate. A prerequisite for these reactions was the synthesis of the phosphorylating agent 2,2,2-trichloroethyl phosphorodichloridate\(^1\) (101).

2,2,2-Trichloroethyl phosphorodichloridate (101) was prepared by an analogous method to that employed in the earlier synthesis of a series of alkyl phosphorodichloridates (chapter 3). Thus, one molar equivalent of both 2,2,2-trichloroethanol and triethylamine were added to phosphoryl chloride at -78°C, using diethyl ether as the solvent (equation 48). After allowing the reaction mixture to warm to ambient temperature with stirring overnight, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. The product (101) was isolated as a colourless oil in 74% yield, following vacuum distillation. The \(^{31}\)P n.m.r. spectrum of the distillate showed a single peak at \(\delta 6.43\) ppm, which is in the region where compounds of type \((RO)POCl_2\) are expected to resonate\(^1\). Additional spectra were obtained on (101), further confirming the identity and purity of this phosphorylating agent. The \(^{13}\)C n.m.r. spectrum showed phosphorus-coupled doublets for the \(CCl_3\) and \(CH_2OP\) moieties, with the three bond coupling constant for the former greater than the two bond coupling constant for the latter probably due to the angular dependence of coupling constants\(^1\). The \(^{1}\)H n.m.r. spectrum consisted of a phosphorus-coupled doublet for the methylene group. The F.A.B. mass spectrum of (101) showed no peaks for the protonated molecular ion, but the cluster of peaks of highest \(m/e\) value were assigned to \((M^+ - Cl)\). Other peaks of interest included those due to the loss of the \(CCl_3\) and \(CCl_3CH_2O\) moieties from the parent compound. Finally, microanalysis data indicated that the product (101) had been isolated in a pure state.

Several aryl 2,2,2-trichloroethyl phosphorochloridates were subsequently prepared by the reaction of 2,2,2-trichloroethyl phosphorodichloridate (101) with one molar equivalent of both the appropriate aryl alcohol and triethylamine in diethyl ether solvent at -78°C (equation 49). Possible impurities resulting from these reactions include unreacted 2,2,2-trichloroethyl
Equation 48

\[
\text{POCl}_3 + \text{CCl}_3\text{CH}_2\text{OH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}} \text{CCl}_3\text{CH}_2\text{OPoCl}_2 + \text{Et}_3\text{NHCl}
\]

\(-78^\circ\text{C to RT}\)

(101)

Equation 49

\[
\text{CCl}_3\text{CH}_2\text{OPoCl}_2 + \text{ArOH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}} (\text{ArO})(\text{CCl}_3\text{CH}_2\text{O})\text{PoCl} + \text{Et}_3\text{NHCl}
\]

\(-78^\circ\text{C to RT}\)

(101)

(102) \(\text{Ar} = p\text{-CNPh}\)
(104) \(\text{Ar} = p\text{-iPrPh}\)
(106) \(\text{Ar} = p\text{-PhPh}\)
(108) \(\text{Ar} = p\text{-ClPh}\)
(110) \(\text{Ar} = p\text{-MeOPh}\)

Equation 50

\[
(\text{ArO})(\text{CCl}_3\text{CH}_2\text{O})\text{POCl} + \text{HO} \xrightarrow{\text{pyridine}} \text{ArO} = \text{P} \xrightarrow{\text{0}^\circ\text{C to RT}} \text{CCl}_3\text{CH}_2\text{O}
\]

(102) \(\text{Ar} = p\text{-CNPh}\)
(104) \(\text{Ar} = p\text{-iPrPh}\)
(106) \(\text{Ar} = p\text{-PhPh}\)
(108) \(\text{Ar} = p\text{-ClPh}\)
(110) \(\text{Ar} = p\text{-MeOPh}\)

(103) \(\text{Ar} = p\text{-CNPh}\)
(105) \(\text{Ar} = p\text{-iPrPh}\)
(107) \(\text{Ar} = p\text{-PhPh}\)
(109) \(\text{Ar} = p\text{-ClPh}\)
(111) \(\text{Ar} = p\text{-MeOPh}\)
phosphorodichloridate, diaryl 2,2,2-trichloroethyl phosphate, and hydrolyzed material. It was hoped to minimize the formation of these by-products by the usual procedure involving the scrupulous drying of the reagents prior to the reaction, the slow addition of the reagents at reduced temperature under an atmosphere of nitrogen, and using an excess of solvent to dilute the reaction mixture.

Firstly, 2,2,2-trichloroethyl p-cyanophenyl phosphorochloridate (102) was synthesized from 2,2,2-trichloroethyl phosphorodichloridate (101) by the reaction of the latter with one molar equivalent of p-cyanophenol in the presence of one molar equivalent of triethylamine at -78°C, using diethyl ether as the solvent (equation 49). After allowing the reaction mixture to warm to ambient temperature with stirring overnight, followed by a further 30 hours stirring at this temperature, the precipitated triethylamine hydrochloride was filtered off. Solvent was removed under reduced pressure to give the product (102) as a colourless oil in 100% yield. The $^{31}$P n.m.r. spectrum of (102) showed a single peak at $\delta$ -3.04 ppm, which compares with the value of $\delta$ -2.55 ppm for 2,2,2-trichloroethyl phenyl phosphorochloridate (96). The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (102) were consistent with the structure of the product, further confirming the identity of this phosphorylating agent. These spectra were assigned by comparison with the corresponding spectra of (96), and also the $^{13}$C n.m.r. spectra of benzonitrile and phenol$^{105}$ and the $^1$H n.m.r. spectrum of p-cyanophenol$^{102}$. The $^{13}$C n.m.r. spectrum of (102) was similar to that of (96), with phosphorus-coupled doublets for carbon atoms within three bonds of the phosphorus atom. However, an additional signal was observed in the spectrum of (102) due to the cyano substituent, and the influence of the latter on the chemical shift of the phenyl carbon atoms was apparent. The $^1$H n.m.r. spectrum of (102) consisted of multiplets at $\delta$ 7.38-7.74 ppm (the C$_{6}$H$_{4}$OP resonance) and $\delta$ 4.76 ppm (the CH$_{2}$OP resonance). The E.I. mass spectrum of (102) showed a cluster of peaks due to the molecular ion with an isotopic pattern for these peaks, and other chlorine containing fragments, characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 119 due to CNC$_{5}$H$_{4}$OH$^+$. Satisfactory microanalysis data were also obtained on (102), indicating the purity of the product.

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2,2,2-Trichloroethyl p-isopropylphenyl phosphorochloridate (104) was prepared in an entirely analogous manner to (102), except that purification of the crude phosphorylating agent was required. An impurity of unreacted starting material (101), shown to be present by $^{31}$P n.m.r. spectroscopy, was successfully removed by stirring the crude product under reduced pressure at 60°C for several hours to give the product (104) as a pale yellow oil in 86% yield. The $^{31}$P n.m.r. spectrum now showed a single peak at δ -2.27 ppm due to 2,2,2-trichloroethyl p-isopropylphenyl phosphorochloridate (104). The $^{13}$C n.m.r. and $^1$H n.m.r. of (104) were consistent with the structure of the product, showing peaks due to both the CCl$_3$CH$_2$OP and p-iPrC$_6$H$_4$OP moieties. These spectra were assigned by comparison with the corresponding spectra of (96) and also the $^{13}$C n.m.r. spectra of p-isopropylbenzene and phenol$^{165}$ and the $^1$H n.m.r. spectrum of p-isopropylphenol$^{162}$. The $^{13}$C n.m.r. spectrum of (104) was similar to that of (96), with the main exception being the appearance of two singlets for the isopropyl substituent and the influence of the latter on the chemical shifts of the phenyl carbon atoms in the spectrum of (104). The $^1$H n.m.r. spectrum of (104) showed an unresolved multiplet for the phenyl protons, as similarly observed in the spectrum of (96). The resonances for the isopropyl substituent appeared as a septet and a doublet for the CH(CH$_3$)$_2$ and CH(CH$_2$)$_2$ moieties respectively, due to proton-proton coupling. An E.I. mass spectrum was also obtained on (104), showing a cluster of peaks due to the molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 121 due to CH$_3$CHC$_6$H$_4$OH$^+$ and to a minor extent (CCl$_3^+$, 2x$^{37}$Cl). Other prominent peaks included those assigned to (M$^+$ - CH$_3$), C$_7$H$_7^+$ and C$_6$H$_4^+$. Finally, microanalysis data revealed that the product (104) had been isolated in a pure state.

The synthesis of biphenyl 2,2,2-trichloroethyl phosphorochloridate (106) was carried out by a similar method to (102), except that the reaction mixture was stirred at ambient temperature for a longer period of time (100 hours). The latter was employed to encourage this reaction, involving the more sterically hindered aryl alcohol p-phenylphenol, to proceed to completion. However, the $^{31}$P n.m.r. spectrum of the crude phosphorylating agent revealed the presence of a minor impurity
of unreacted starting material (101). The product was isolated as a white solid in 75% yield, following extraction of the 2,2,2-trichloroethyl phosphorodichloridate with hexane. The $^{31}$P n.m.r. spectrum of (106) now showed a single peak at $\delta$ -2.28 ppm, which compares with the value of $\delta$ -2.55 ppm for 2,2,2-trichloroethyl phenyl phosphorochloridate (96). Additional spectra were obtained on the product (106), further confirming the identity of this phosphorylating agent. The $^{13}$C n.m.r. spectrum, assigned by comparison with the spectra of (96), biphenyl$^{201}$ and phenol$^{162}$ and also by use of a correlation table to predict the chemical shift of phenyl carbon atoms$^{161}$, was consistent with the structure of the product. Phosphorus-coupled doublets were observed for carbon atoms within three bonds of the phosphorus atom. The $^1$H n.m.r. spectrum consisted of multiplets at $\delta$ 7.21-7.63 ppm (the $\text{C}_6\text{H}_5\text{C}_6\text{H}_4\text{OP}$ resonance) and $\delta$ 4.79 ppm (the $\text{CH}_2\text{OP}$ resonance). The E.I. mass spectrum of (106) showed a cluster of peaks due to the molecular ion with the isotopic pattern for this, and other chlorine containing fragments, characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 170 due to $\text{C}_7\text{H}_5\text{C}_6\text{H}_4\text{OH}^+$. Other prominent peaks included those attributed to $\text{C}_6\text{H}_5\text{C}_6\text{H}_4\text{OPO(OH)}\text{Cl}^+$, $\text{C}_6\text{H}_5\text{C}_6\text{H}_4\text{O}^+$, $\text{C}_11\text{H}_7^+$ and $\text{C}_9\text{H}_9^+$. Satisfactory microanalysis data were also obtained (106), indicating the purity of the product.

2,2,2-Trichloroethyl p-chlorophenyl phosphorochloridate (108) was subsequently prepared. Although this phosphorylating agent (108) has not been reported in the literature, it is of interest to note that the related 2,2,2-trichloroethyl o-chlorophenyl phosphorochloridate has been previously synthesized by a similar method to (96), but involving the addition of triethylamine to a solution of o-chlorophenyl phosphorodichloridate and 2,2,2-trichloroethanol in diethyl ether$^{202}$. The present synthesis of 2,2,2-trichloroethyl p-chlorophenyl phosphorochloridate (108) was carried out in an analogous manner to (102), except that the reaction was stirred for a longer period of time at ambient temperature (74 hours) to allow it to proceed to completion. The product (108) was isolated as a colourless oil in 97% yield. The $^{31}$P n.m.r. spectrum of (108) showed a single peak at $\delta$ -2.53 ppm, which compares with the value of $\delta$ -2.55 ppm for 2,2,2-trichloroethyl phenyl phosphorochloridate (96). The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (108) were consistent with the
structure of the product, showing signals due to both the CCl₂CH₂OP and p-ClC₆H₄OP moieties. These spectra were assigned by comparison with the corresponding spectra of (96) and p-chlorophenol¹⁶²,²⁰⁰. The ¹³C n.m.r. spectra of (108) was similar to the spectrum of (96), with the main exception being the influence of the chlorine substituent on the chemical shifts of the resonances for the phenyl carbon atoms in the spectrum of (108). The ¹H n.m.r. spectrum consisted of multiplets for the p-ClC₆H₄OP and CCl₂CH₂OP moieties. The E.I. mass spectrum of (108) further confirmed the structural assignment of this phosphorylating agent, showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of five chlorine atoms in the intact molecule. The base peak was observed at m/e 230 due to CCl₂CH₂OPO(OH)Cl⁺, with a second prominent peak at m/e 232 and a third peak of lower intensity at m/e 234 due to the corresponding ³⁷Cl and 2x³⁷Cl fragments respectively. Finally, microanalysis data revealed that the product (108) had been isolated in a pure state.

The synthesis of 2,2,2-trichloroethyl p-methoxyphenyl phosphorochloridate (110) was performed by an identical method to (108). Following work-up of the reaction mixture, the product (110) was isolated as a colourless oil in 100% yield. The ³¹P n.m.r. spectrum showed a single peak at δ -1.81 ppm due to 2,2,2-trichloroethyl p-methoxyphenyl phosphorochloridate (110). The chemical shift of this peak lies in the region where compounds of type (RO)₂POCl are expected to resonate¹⁸⁵. The ¹³C n.m.r. and ¹H n.m.r. spectra were consistent with the structure of the product, further confirming the identity of this phosphorylating agent. These spectra were assigned by comparison with the corresponding spectra of (96) and p-methoxyphenol¹⁶²,²⁰³. The ¹³C n.m.r. spectrum of (110) was similar to that of (96), with the main difference being the appearance of a singlet for the methoxy substituent and the influence of the latter on the chemical shifts of the phenyl carbon atoms in the spectrum of (110). The ¹H n.m.r. spectrum of (110) consisted of two distinct signals for the phenyl protons with a multiplet at δ 7.19 ppm (the ortho-Ph resonance) and a doublet at δ 6.87 ppm (the meta-Ph resonance), contrasting with the unresolved multiplet for the phenyl protons in the spectrum of (96). An additional singlet also appeared in the spectrum of
(110) due to the methoxy group. The E.I. mass spectrum of (110) showed a cluster of peaks due to the molecular ion with an isotopic pattern for this, and other chlorine containing fragments, characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 222 due to CH$_3$OC$_2$H$_4$OP(O)(OH)Cl$^+$, with another peak of lower intensity at m/e 224 due to the corresponding $^{37}$Cl fragment. Other prominent peaks included those at m/e 123 and m/e 124 due to CH$_3$OC$_2$H$_4$OH$^+$ and CH$_3$OC$_2$H$_4$O$^+$ respectively. Satisfactory microanalysis data were also obtained on the product (110).

The aryl 2,2,2-trichloroethyl phosphorochloridates (102), (104), (106), (108) and (110), successfully prepared above, were subsequently reacted with unprotected araA in pyridine at 0°C to give the appropriate 5′-aryl (2,2,2-trichloroethyl) phosphate triesters of araA (equation 50). Firstly, the synthesis of araA-5′-(2,2,2-trichloroethyl) p-cyanophenyl phosphate (103) was attempted in an analogous manner to the preparation of araA-5′-ethyl phenyl phosphate (94). However, difficulties were encountered in the present experiment in isolating the required product (103). Attempted purification of the crude product by column chromatography using a methanol-chloroform eluent, followed by recrystallization from ethyl acetate, and then column chromatography using a methanol-ethyl acetate eluent, gave a slightly impure white solid. The $^{31}$P n.m.r. spectrum (run at 82 MHz) showed two closely spaced singlets of similar intensity at δ -7.58 ppm and δ -7.70 ppm, these peaks being attributed to the two possible diastereoisomers of araA-5′-(2,2,2-trichloroethyl) p-cyanophenyl phosphate (103) by comparison with the values of δ -7.63 ppm and -7.76 ppm for araA-5′-(2,2,2-trichloroethyl) phenyl phosphate (97). However, t.l.c. analysis revealed the presence of two minor components in addition to the major component assumed to be due to (103). These minor impurities, if they were indeed phosphorus containing, may have been masked in the above spectrum by the noisy baseline encountered due to the n.m.r. sample being rather weak. A second $^{31}$P n.m.r. spectrum of the product (run at 164 MHz) showed several minor peaks besides the major peak, the latter being composed of two closely spaced singlets of approximately equal intensity at δ -10.75 ppm and δ -10.85 ppm. The difference in the chemical shift of the major peak, attributed to the required product (103), in the two spectra was
believed to be due to only the first spectrum (run at 82 MHz) being referenced to external phosphoric acid and the use of different spectrometers. A final attempt to purify the product by reverse phase preparative H.P.L.C. proved unsuccessful. Reverse phase analytical H.P.L.C. of the appropriate fraction, immediately after its collection from the preparative column, indicated that the purified product (103) was decomposing as it was eluted off the preparative column. Unfortunately, time did not allow for a further attempt to synthesize araA-5'-{(2,2,2-trichloroethyl)} p-cyanophenyl phosphate (103).

AraA-5'-{2,2,2-trichloroethyl} p-isopropylphenyl phosphate (105) was prepared by a similar procedure to (94), except that a longer reaction time (49 hours at ambient temperature, following an initial hour at 0°C) was required for the reaction to proceed to completion. Purification of the crude product also differed slightly from the procedure employed for (94), with (105) being isolated as a white solid in 21% yield following column chromatography and recrystallization from ethyl acetate. The $^{31}$P n.m.r. spectrum of the product (105) showed two closely spaced peaks of approximately equal intensity at δ -7.22 ppm and δ -7.35 ppm. The multiplicity of these signals corresponds to the presence of the two possible diastereoisomers of (105) in the product, with the ratio of the isomers 1:1, and arises from the mixed stereochemistry at the chiral phosphorus centre. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (105) were consistent with the structure of the product, showing signals due to both the CCl$_2$CH$_2$OP and p-iPrC$_6$H$_4$OP moieties in addition to the nucleosidic resonances. These spectra further confirmed the diastereomeric nature of the product, with many signals split in the same ratio (i.e. 1:1) as observed for the peaks in the $^{31}$P n.m.r. spectrum. A comparison of the $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (105) with the corresponding spectra of (97) revealed that the main differences between these spectra were those associated with the presence of the isopropyl substituent and its influence (in particular) on the chemical shift of the phenyl carbon atoms in the spectrum of (105). Phosphorus-coupling, in cases where it could be resolved, was observed for carbon atoms and protons within three bonds of the phosphorus atom in the $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (105) respectively. The F.A.B. mass spectrum of (105) showed a cluster of peaks for the protonated molecular ion with an isotopic pattern for this,
and other chlorine containing fragments, characteristic of three chlorine atoms in the intact molecule. The base peak was observed at m/e 44 due to C₆H₅⁺. Minor peaks indicative of an aryl moiety in the intact molecule included those assigned to C₆H₃⁺, C₇H₇⁺ and C₈H₅⁺. Other minor peaks of interest appeared at m/e 136 due to protonated adenine or iPrC₅H₅OH⁺ and m/e 81 due to the ionized sugar fragment C₃H₅O⁺. Finally, microanalysis data and reverse phase H.P.L.C. revealed that the product (105) had been isolated in a pure state. The latter technique displayed only one signal due to the coincidence of the two peaks expected for the diastereomeric product, and also indicated the absence of contaminating araA.

AraA-5'-biphenyl (2,2,2-trichloroethyl) phosphate (107) was prepared in an entirely analogous manner to (94), except that the product (107) was isolated as a white solid in 19% yield following column chromatography and recrystallization from ethyl acetate. The ³¹P n.m.r. spectrum of (107) showed two closely spaced singlets of similar intensity at δ -6.94 ppm and δ -7.06 ppm, indicating that the product had been isolated as a mixture of the two possible diastereoisomers. The diastereomeric nature of the product (107) was also evident by the duplication of many signals in the ¹³C n.m.r. and ¹H n.m.r. spectra. These spectra further confirmed the structural assignment of the product, displaying signals due to both the CCl₃CH₂OP and p-C₆H₅C₆H₄OP moieties in addition to the nucleosidic resonances. A F.A.B. mass spectrum was also obtained on (107), showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of three chlorine atoms in the intact molecule. Prominent peaks appeared at m/e 91 and m/e 76 due to C₇H₇⁺ and C₈H₅⁺ respectively, while minor peaks included those attributed to C₆H₅C₆H₄O⁺, protonated adenine and the ionized sugar fragment C₃H₅O₂⁺. Finally, microanalysis data and reverse phase analytical H.P.L.C. revealed that the product (107) had been isolated in a pure state. The latter technique showed coincident peaks for the diastereomeric product, as similarly observed for (94) and (105), and also indicated the absence of contaminating araA.

The synthesis of araA-5'-bis(2,2,2-trichloroethyl) p-chlorophenyl phosphate (109) was carried out by a similar method to (94), except that the reaction mixture was stirred at ambient
temperature for 27 hours after an initial hour at 0°C. Following work-up of the reaction mixture and purification by column chromatography, the product (109) was isolated as a white solid in 23% yield. The $^{31}$P n.m.r. spectrum of (109) showed two closely spaced singlets of approximately equal intensity at $\delta$ -7.25 ppm and $\delta$ -7.37 ppm, corresponding to the presence of the two possible diastereoisomers of (109) in the product. This isomerism again arises from the asymmetry at the chiral phosphorus centre. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (109) further confirmed the identity of the product and its diastereomeric nature. The latter was evident by the presence of many peaks split in the same ratio (i.e. 1:1) as observed for the peaks in the $^{31}$P n.m.r. spectrum, reflecting the non-equivalence of the carbon atoms and protons in the two isomers. The F.A.B. mass spectrum of (109) showed a cluster of peaks due to the protonated molecular ion with an isotopic pattern for this, and other chlorine containing fragments, characteristic of four chlorines in the intact molecule. The base peak appeared at m/e 136 due to protonated adenine, as similarly observed in the spectra of (94) and (97). Minor peaks of interest included those assigned to C$_8$H$_4^+$ and the ionized sugar fragments C$_7$H$_2$O$_5^+$ and C$_3$H$_5$O$_2^+$. Finally, microanalysis data and reverse phase analytical H.P.L.C. revealed that the product (109) had been isolated in a pure state. The latter technique again showed no resolution of the two peaks expected for the diastereomeric product, and also indicated the absence of contaminating araA.

AraA-5'-((2,2,2-trichloroethyl)-p-methoxyphenyl phosphate (111) was prepared in an analogous manner to (94). Thus, the product (111) was isolated as a white solid in 34% yield following column chromatography. The $^{31}$P n.m.r. spectrum of (111) showed two closely spaced signals of similar intensity at $\delta$ -6.63 ppm and $\delta$ -6.79 ppm, indicating that the product was composed of an approximately equal mixture of the two possible diastereoisomers. The diastereomeric nature of the product was also evident by the duplication of many peaks in the $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (111). These spectra were consistent with the structure of the product, showing signals due to both the CCl$_3$CH$_2$OP and $p$-CH$_3$OC$_6$H$_4$OP moieties in addition to the nucleosidic resonances. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (111) were similar to the corresponding spectra of (97), with the main exception being the appearance of resonances for the methoxy group.
Additionally, the influence of the methoxy substituent on the chemical shift of the phenyl carbon atoms and protons was apparent in the spectra of (111). The $^1$H n.m.r. spectrum of (111), for example, showed multiplets at δ 7.15 ppm (the ortho-Ph resonance) and δ 6.84 ppm (the meta-Ph resonance) which contrasted with the unresolved multiplet observed for the PhOP moiety in the spectrum of (97). The F.A.B. mass spectrum of (111) further confirmed the structural assignment of the product, with a cluster of peaks observed for (M - H)Na$^+$. The isotopic pattern for these peaks was characteristic of three chlorine atoms in the intact molecule. Prominent peaks appeared in the spectrum at m/e 136 and m/e 77 due to protonated adenine and C$_7$H$_5$Na$^+$ respectively. Minor peaks of interest included those attributed to CH$_3$OC$_6$H$_4$OH$^+$ and CH$_3$OC$_6$H$_4$O$^+$. Finally, microanalysis data and reverse phase analytical H.P.L.C. revealed that the product (111) had been isolated in a pure state. It is of interest to note that the H.P.L.C. spectrum of (111) displayed two distinct signals for the diastereomeric product in a 1:1 ratio, as similarly observed for (97), and also indicated the absence of contaminating araA.

The biological activities of araA-5'-ethyl phenyl phosphate (94) and the 5'-aryl (2,2,2-trichloroethyl) phosphate triesters of araA (97), (105), (109) and (111) were evaluated by determining the ability of these compounds to inhibit the synthesis of cellular DNA using the in vitro tritiated thymidine incorporation assay developed by Riley and co-workers$^{168}$, in an entirely analogous manner to the procedure employed earlier for the testing of some 5'-phosphate derivatives of araA (chapters 2 and 3). AraA-5'-diphenyl phosphate (61), previously prepared and biologically evaluated in this Department$^{172}$, was also tested along with the above compounds. It was felt that the inclusion of (61) in the same experiment as (94), (97), (105), (109) and (111) would allow for a fairer comparison between the biological activities of these compounds. Each experiment was carried out at least twice on cells of different passage number and the mean % inhibition of DNA synthesis (relative to the distilled water control) and standard error of the mean (SEM) were calculated for each set of % inhibition values (table 7, biological testing section). A two-tail student’s t-test was used to determine the degree of significant difference between the
mean % inhibition values of two compounds at a given concentration. This data was subsequently used to determine the probability of this difference being statistically significant (table 8, biological testing section).

The results of the assay are presented as a bar chart (figure 33) in which the mean % inhibition of DNA synthesis (relative to the distilled water control) is plotted against compound at final concentrations of 0.03 mM and 0.003 mM. This graph clearly illustrates that all of the compounds tested display an inhibitory effect on DNA synthesis in vitro, and these effects are dose dependent. Considering the higher concentration of 0.03 mM, araA-5’-ethyl phenyl phosphate (94) and araA-5’-(2,2,2-trichloroethyl) p-isopropylphenyl phosphate (105) are equi-active with araA, while the 5’-aryl (2,2,2-trichloroethyl) phosphate triesters of araA (97), (109) and (111) display a greater biological activity than araA. A two-tail student’s t-test reveals these differences in biological activities to be significant (i.e. P < 5%) for all these compounds when compared with araA, except for (94) vs. araA, and (105) vs. araA. A comparison of the biological activity of araA-5’-(2,2,2-trichloroethyl) phenyl phosphate (97) with its 5’-ethyl phenyl analogue (94) reveals that replacing a simple alkyl chain by a 2,2,2-trichloroethyl moiety enhances biological activity, as similarly observed in recent research comparing the biological activities of some 5’-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA with their 5’-dialkyl counterparts (chapter 3). It is also of interest to note that (94) is less active than araA-5’-diphenyl phosphate (61), while (97) shows a comparable biological activity to (61). Indeed a two-tail student’s t-test indicates that these differences in biological activities are significant (i.e. P < 5%) only for (94) vs. (97), and (94) vs. (61). It would appear, in a simply additive sense, that the phenyl and 2,2,2-trichloroethyl moieties are equally efficacious in their inhibitory effect on DNA synthesis in vitro, relative to the simple alkyl groups. Similar observations have been made for the 5’-diphenyl and 5’-(2,2,2-trichloroethyl) phenyl phosphate triesters of the anti-HIV drug AZT\textsuperscript{152}. Additionally, (109) and (111) show biological activities which do not differ significantly from (97), while (105) displays a biological activity significantly lower than (97). This suggests that changing the para-substituent of the aryl group in these 5’-aryl (2,2,2-trichloroethyl) phosphate triesters of araA appears to have
Figure 33: % Inhibition of DNA synthesis against compound

% Inhibition of DNA synthesis

- 0.003 mM
- 0.03 mM

Compound

araA (61) (94) (97) (105) (109) (111)
little effect on the biological activity of these compounds relative to the parent compound (97), with the exception of araA-5'-\((2,2,2\text{-trichloroethyl})\) \(p\)-isopropylphenyl phosphate (105). A possible explanation for the latter may be due to the greater steric hindrance, associated with a large isopropyl substituent, interfering with the mechanism of action of this compound (105). It is also of interest to note that the above trends observed for the assay results at 0.03 mM are less apparent at the lower concentration of 0.003 mM, probably reflecting the weaker effects at lower concentrations of the compound.

Finally, since araA shows in vitro activity against a number of DNA viruses, including the herpes simplex-1 virus (HSV-1), it would be of interest to evaluate the anti-HSV-1 activities of the 5'-aryl phosphate triesters of araA and compare them with that of araA. The testing of these compounds, i.e. (97), (105), (109) and (111), against HSV-1 was carried out by Dr. N. Mahmood at the Medical Research Council Collaborative Centre, Mill Hill, London. The assay essentially involves measuring levels of viral antigen production in HSV-1 infected Vero cells in the presence of a test compound at various concentrations204. The levels of antigen recorded are indicative of the extent of viral infection of the host cells, and therefore give a measure of the anti-viral activity of the test compound under consideration. Several other 5'-phosphate triesters of araA, namely araA-5'-diphenyl phosphate (61), araA-5'-bis(2,2,2-trichloroethyl) phosphate (43), and the mixed, unsymmetrical 5'-ethyl \((2,2,2\text{-trichloroethyl})\) (86), 5'-butyl \((2,2,2\text{-trichloroethyl})\) (80) and 5'-(2,2,2-trichloroethyl) \((8\text{-benzyloxy-3,6-dioxaoctyl})\) (128) phosphate triesters of araA, were also similarly evaluated in a separate experiment. The results of the assay are tabulated (tables 11 and 12) and discussed in the appendix.
Chapter 5: 5'-Glycolyl phosphate derivatives of araA

The biological evaluation of some simple 5'-dialkyl phosphate triesters of araA revealed an increasing activity of these compounds with increasing chain length of their constituent alkyl groups\textsuperscript{135}. This correlation was attributed to the increasing lipophilicity and hence improved membrane penetration of these compounds in the same series. However, a decreasing water solubility was also apparent on passing from the 5'-diethyl to the 5'-dipentyl derivative\textsuperscript{148}. Indeed, araA-5'-dipentyl phosphate was reported to be equi-active with araA but less soluble in water. This suggests that little benefit would be gained from the preparation of higher alkyl analogues, especially in view of the low aqueous solubility of araA\textsuperscript{30,94} being an inherent problem in the clinical use of this drug. A solution may lie in the incorporation of oxygen atoms into the alkyl chain, with the possibility of increasing lipophilicity without loss of water solubility. The presence of oxygen atoms may also enhance the leaving group propensity of the alkyl chain and thereby increase the susceptibility to chemical hydrolysis. Consequently, the present chapter involves an investigation into the synthesis and biological evaluation of a series of mixed, unsymmetrical 5'-ethyl glycolyl phosphate derivatives of araA (118), (122) and (126) (figure 34). It would also be of interest to discover whether the enhanced biological activity observed for the 5'-bis(2,2,2-trihaloethyl) phosphate triesters of araA when compared with their simple 5'-dialkyl analogues\textsuperscript{135,136}, would be similarly reflected by 5'-(2,2,2-trichloroethyl) glycolyl phosphate derivatives of araA over their 5'-ethyl glycolyl counterparts.

The preparation of a number of mixed, unsymmetrical 5'-ethyl glycolyl and 5'-((2,2,2-trichloroethyl) glycolyl phosphate derivatives of araA consisted of a multi-step procedure. The proposed route generally involved the synthesis of the appropriate ethyl (benzyl-protected glycolyl) and 2,2,2-trichloroethyl (benzyl-protected glycoyl) phosphorochloridates, followed by their subsequent reaction with unprotected araA. The resulting 5'-ethyl (benzyl-protected glycolyl) and 5'-(2,2,2-trichloroethyl) (benzyl-protected glycolyl) phosphate triesters of araA were then subjected to hydrogenolysis to yield the corresponding deprotected products.
Figure 34

\[
\begin{align*}
\text{NH}_2 & \\
\text{H(OCH}_2\text{CH}_2)_n\text{O} & \text{P} \quad \text{O} \\
\text{RO} & \\
\end{align*}
\]

(118) \( R = \text{Et}, n = 1 \)
(122) \( R = \text{Et}, n = 2 \)
(126) \( R = \text{Et}, n = 3 \)

Figure 35

\[
\begin{align*}
\text{POCl}_3 & \\
\text{OCH}_2\text{CH}_2\text{O} & \text{POCl}_3 \\
\end{align*}
\]

(112) (113)

Equation 51

\[
\begin{align*}
\text{POCl}_3 + \text{ROH} & \xrightarrow{\text{Et}_3\text{N/Et}_2\text{O}, 0^\circ\text{C}, \text{N}_2} \text{ROPOCl}_2 \\
\text{ROPOCl}_2 & \xrightarrow{\text{HOCH}_2\text{CH}_2\text{OH, 2Et}_3\text{N/ Et}_2\text{O}, 0^\circ\text{C to RT}} \text{ROPOPOPO} \\
\end{align*}
\]

(114)

Equation 52

\[
\begin{align*}
\text{H(OCH}_2\text{CH}_2)_n\text{OH} & \xrightarrow{\text{(i) 50\% aq. NaOH (excess), reflux, 0.5 h}} \text{R(OCH}_2\text{CH}_2)_n\text{OH} \\
\text{R(OCH}_2\text{CH}_2)_n\text{OH} & \xrightarrow{\text{(ii) RX (5 to 10 molar equivs.), reflux, 24 h}} \text{R(OCH}_2\text{CH}_2)_n\text{OH} \\
\end{align*}
\]

(R = alky, X = halogen)
Firstly, the ethyl (benzyl-protected glycolyl) and 2,2,2-trichloroethyl (benzyl-protected glycolyl) phosphorochloridates were synthesized via the reaction of ethyl phosphorodichloridate and 2,2,2-trichloroethyl phosphorodichloridate respectively with the appropriate benzyl-protected glycol. Several alternative methods are available for the preparation of phosphorodichloridates (as discussed in chapter 3). The method of choice for the earlier synthesis of ethyl phosphorodichloridate (84) (chapter 3) and 2,2,2-trichloroethyl phosphorodichloridate (101) (chapter 4), from which supplies of these phosphorylating agents were readily available for use in the present reactions, involved the addition of one molar equivalent of both the appropriate alcohol and triethylamine to phosphoryl chloride in diethyl ether solvent at -78°C.

A series of benzyl-protected glycols were subsequently prepared. The requirement for the glycols to be protected at one of their hydroxyl functions by a benzyl group was proposed to avoid the possibility of ring formation in the subsequent reaction of these compounds with the appropriate phosphorodichloridate. Indeed, the reactions of monoethylene glycol with phosphoryl chloride are complex and appear to involve cyclization to give products of type (112) and (113) (figure 35). Magolda and Johnson have also reported the formation of a cyclic phosphate (114) on reacting a phosphorodichloridate (prepared from phosphoryl chloride and alcohol in the presence of triethylamine) with one molar equivalent of monoethylene glycol and two molar equivalents of triethylamine (equation 51). The required benzyl-protected glycols were prepared by a method similar to the one of Gibson for the synthesis of some alkyl ethylene glycols, whereby a primary alkyl halide was reacted with an excess of 50% aqueous sodium hydroxide and 5 to 10 molar equivalents of glycol at 100°C for 24 hours to give the monoalkylation product in 70% to 90% yield (equation 52). This procedure has been successfully used, e.g. by Coudert and co-workers, for the preparation of some monobenzyl ethylene glycols using a ratio of benzyl chloride, glycol and sodium hydroxide of (1:4:4) (equation 53).

Following the synthesis of the appropriate benzyl-protected glycols, these compounds were reacted with ethyl phosphorodichloridate or 2,2,2-trichloroethyl phosphorodichloridate in the presence of triethylamine at reduced temperature, using diethyl ether as the solvent, to give the
Equation 53

\[ \text{H(OCH}_2\text{CH}_2)_n\text{OH} \xrightarrow{(i) 50\% \text{ aq. NaOH, 100°C, N}_2, 0.5 \text{ h}} \text{Bzl(OCH}_2\text{CH}_2)_n\text{OH} \]
\[ \xrightarrow{(ii) \text{ BzlCl (0.25 molar equivs.), 100°C, N}_2, 24 \text{ h}} \]

(n = 1-3)

Equation 54

\[ \text{ROPOCl}_2 + \text{Bzl(OCH}_2\text{CH}_2)_n\text{OH} + \text{Et}_3\text{N} \xrightarrow{\text{Et}_2\text{O}, -78°C \text{ to RT}} \text{Bzl(OCH}_2\text{CH}_2)_n\text{O} - \text{P} - \text{Cl} + \text{Et}_3\text{NHCl} \]

(R = alkyl or 2,2,2-trihaloethyl, n = 1-3)

Equation 55

(R = alkyl or 2,2,2-trihaloethyl, n = 1-3)

Equation 56

(R = alkyl or 2,2,2-trihaloethyl, n = 1-3)
required ethyl (benzyl-protected glycolyl) and 2,2,2-trichloroethyl (benzyl-protected glycolyl) phosphorochloridates respectively (equation 54). The procedure involved was similar to the one of the two methods employed in the earlier synthesis of some aryl 2,2,2-trichloroethyl phosphorochloridates (chapter 4). Elimination of the hydrogen halide by-product was again accomplished by use of the tertiary organic base triethylamine, rather than carrying out the reaction under reduced pressure or the bubbling of an inert gas through the reaction mixture\textsuperscript{145}, since the reaction is more controllable under these conditions.

The appropriate ethyl (benzyl-protected glycolyl) and 2,2,2-trichloroethyl (benzyl-protected glycolyl) phosphorochloridates were then reacted with unprotected araA in pyridine at reduced temperature to give the required 5'-ethyl (benzyl-protected glycolyl) and 5'-(2,2,2-trichloroethyl) (benzyl-protected glycolyl) phosphate triesters of araA respectively (equation 55), in an analogous manner to the procedure employed in the earlier synthesis of several other mixed, unsymmetrical 5'-phosphate triesters of araA (chapters 3 and 4). Finally, these 5'-ethyl (benzyl-protected glycolyl) and 5'-(2,2,2-trichloroethyl) (benzyl-protected glycolyl) phosphate triesters of araA were converted to the required deprotected products by hydrogenolysis, involving the treatment of a methanolic solution of these compounds with hydrogen in the presence of a 10% palladium on charcoal catalyst at ambient temperature (equation 56).

The target compounds, araA-5'-ethyl (2-hydroxyethyl) phosphate (118), araA-5'-ethyl (5-hydroxy-3-oxapentyl) phosphate (122) and araA-5'-ethyl (8-hydroxy-3,6-dioxaoctyl) phosphate (126), were synthesized by the procedure outlined above. Firstly, the preparation of the required benzyl-protected glycols involved heating a stirred mixture of benzyl chloride and four molar equivalents of both the appropriate glycol and 50% aqueous sodium hydroxide solution at 100°C for 24 hours under an atmosphere of nitrogen (equation 57). Following work-up of the reaction mixture, the resulting oil was purified by vacuum distillation. Thus, 2-O-benzylethanol (115) was isolated as a colourless oil in 49% yield. The other benzyl-protected glycols, 2-(2-O-benzylethoxy) ethanol (119) and 2-(2-(2-O-benzylethoxy)ethoxy) ethanol (123), had been previously synthesized.
Equation 57

H(OCH₂CH₂)nOH → Bzl(OCH₂CH₂)nOH

(i) 50% aq. NaOH, 100°C, N₂, 0.5 h

(ii) BzCl (0.25 molar equivs.), 100°C, N₂, 24 h

(115) n = 1
(119) n = 2
(123) n = 3

Equation 58

EtOPOCl₂ + Bzl(OCH₂CH₂)nOH + Et₃N → Bzl(OCH₂CH₂)nO—P—Cl + Et₃NHCl

Et₂O

-78°C to RT

(84) (115) n = 1
(119) n = 2
(123) n = 3

Equation 59

Bzl(OC₂H₄)nO—P—Cl + HO—P—O—Et

pyridine

0°C to RT

(116) n = 1
(120) n = 2
(124) n = 3

(117) n = 1
(121) n = 2
(125) n = 3
by an identical method in this Department\textsuperscript{148}, and sufficient quantities of these compounds were still available in a pure state (as indicated by \textsuperscript{13}C n.m.r. and \textsuperscript{1}H n.m.r. spectroscopy) for use in the present research.

Spectral data were obtained on 2-\textsubscript{O}-benzylethanol (\textbf{115}), confirming the identity of this compound. The \textsuperscript{13}C n.m.r. and \textsuperscript{1}H n.m.r. spectra of (\textbf{115}) were consistent with the structure of the product, as compared with literature values\textsuperscript{208}. The \textsuperscript{13}C n.m.r. spectrum showed singlets for the phenyl carbon atoms between $\delta$ 127 ppm and $\delta$ 138 ppm, while the PhCH\textsubscript{2} resonance appeared as a singlet further upfield at $\delta$ 73.26 ppm. Single peaks were also observed for the methylene groups at $\delta$ 71.49 ppm (the CH$_2$CH$_2$OH resonance) and $\delta$ 61.78 ppm (the CH$_2$OH resonance). The \textsuperscript{1}H n.m.r. spectrum consisted of singlets for the phenyl protons and the PhCH$_2$ resonance, multiplets for the methylene protons and a triplet for the hydroxyl proton. The E.I. mass spectrum of (\textbf{115}) showed a prominent peak at m/e 152 due to the molecular ion and another of much lower intensity at m/e 153 due to the protonated molecular ion. The base peak was observed at m/e 91 due to C$_6$H$_7^+$ and other prominent peaks appeared at m/e 107 and m/e 92 due to C$_6$H$_5$CH$_2$O$^+$ and C$_7$H$_6^+$ respectively.

A series of ethyl (benzyl-protected glycolyl) phosphorochloridates were subsequently prepared by the reaction of ethyl phosphorochloridate (\textbf{84}), synthesized earlier (as discussed in chapter 3), with one molar equivalent of both the appropriate benzyl-protected glycol and triethylamine in diethyl ether solvent at -78°C (\textbf{equation 58}). Possible impurities resulting from these reactions include unreacted ethyl phosphorochloridate, ethyl bis(benzyl-protected glycolyl) phosphate, and hydrolyzed material. It was hoped to minimize the formation of these by-products by the scrupulous drying of the reagents prior to the reaction, the slow addition of the reagents at reduced temperature under an atmosphere of nitrogen, and using an excess of solvent to dilute the reaction mixture.

Firstly, ethyl (2-benzyloxyethyl) phosphorochloridate (\textbf{116}) was prepared by the addition of one molar equivalent of both 2-\textsubscript{O}-benzylethanol (\textbf{115}) and triethylamine to ethyl phosphorodichloridate (\textbf{84}) at -78°C, using diethyl ether as the solvent (\textbf{equation 58}). After
allowing the reaction mixture to warm to ambient temperature with stirring overnight, followed by a further 50 hours stirring at this temperature, the precipitated triethylamine hydrochloride was filtered off and solvent removed under reduced pressure. The resulting cloudy oil was extracted with hexane to give the product (116) as a colourless oil in 88% yield. The 31P n.m.r. spectrum of the product showed a single peak at δ 3.07 ppm, which compares with the values of δ 2.8 ppm for diethyl phosphorochloridate155-158 and δ 4.7 ppm for dibenzyloxy phosphorochloridate209.

The 13C n.m.r. and 1H n.m.r. spectra of (116) were consistent with the structure of the product, showing signals due to both the ethyl and 2-benzyloxyethyl moieties. These spectra were assigned by comparison with the corresponding spectra of the starting materials (84) and (115). The 13C n.m.r. spectrum of (116) showed phosphorus coupling only for those carbon atoms within three bonds of the phosphorus atom, with an unresolved multiplet observed at δ 67.99-68.25 ppm (the CH2CH2OP resonance) and doublets at δ 66.00 ppm (the CH2CH2OP resonance) and δ 15.71 ppm (the CH2CH2OP resonance). The three-bond coupling constant of 8.0 Hz for the methyl doublet was slightly greater than the two-bond coupling constant of 7.2 Hz for the methylene doublet, probably due to the angular dependence of coupling constants161. Other signals in the spectrum due to the benzylic carbon atoms appeared as singlets. The 1H n.m.r. spectrum consisted of singlets for the phenyl and PhCH3 resonances, a separate multiplet for the CH2CH2OP moiety, and an unresolved multiplet for the remaining methylene protons (i.e. CH2CH2OP and CH3CH2OP). The presence of phosphorus-proton coupling in addition to proton-proton coupling for the signals due to protons within three bonds of the phosphorus atom was particularly evident in the appearance of a triplet of doublets for the methyl resonance.

The E.I. mass spectrum of (116) further confirmed the structural assignment of this phosphorylating agent, showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of one chlorine atom in the intact molecule. The base peak was observed at m/e 91 due to C7H7+ and other prominent peaks included those assigned to C8H12O+, C7H5CHO+, C7H4CO+ and C7H8+. The spectrum also showed minor peaks associated with other fragments of the 2-benzyloxyethyl...
moiety and the loss of these from the parent compound. Satisfactory microanalysis data were also obtained on (116), indicating the purity of the product.

Ethyl (5-benzyl-3-oxapentyl) phosphorochloridate (120) was prepared by a similar method to (116), except that purification of the crude phosphorylating agent was required. An impurity of unreacted starting material (84), shown to be present by $^{31}$P n.m.r. spectroscopy, was successfully removed by stirring the crude product under reduced pressure at 30° for several hours. This is consistent with the known boiling point of ethyl phosphorodichloridate$^{180,181}$. Hence, the product (120) was isolated as a colourless oil in 63% yield. The $^{31}$P n.m.r. spectrum now showed a single peak at $\delta$ 3.02 ppm, which compares well with the value of $\delta$ 3.07 ppm for (116). The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (120), assigned by comparison with the corresponding spectra of the starting materials (84) and (119), were consistent with the structure of the product. The $^{13}$C n.m.r. spectrum of (120) was similar to that of (116), with the main exception being the appearance of a phosphorus-coupled doublet for each of the carbon atoms within three bonds of the phosphorus atom. Additional peaks were also observed in the spectrum of (120) due to the PhCH$_2$OCH$_2$ and PhCH$_2$OCH$_2$CH$_2$ resonances. The $^1$H n.m.r. spectrum of (120) showed singlets for the phenyl and PhCH$_3$ resonances, separate multiplets for the CH$_3$CH$_2$OP and CH$_3$CH$_2$OP moieties, and an unresolved multiplet for the remaining glycol methylene protons. The E.I. mass spectrum of (120) further confirmed the identity of this phosphorylating agent, showing a cluster of peaks due to the protonated molecular ion. These peaks and other chlorine containing fragments displayed an isotopic pattern characteristic of two chlorine atoms in the intact molecule. The base peak was observed at m/e 91 due to C$_7$H$_4^+$ and other prominent peaks included those due to C$_6$H$_3$CH$_2$O$, C$_6$H$_3$CHO$, C$_6$H$_3$CO$^+$ and C$_7$H$_4^+$, as similarly found in the spectrum of (116). The spectrum of (120) also featured peaks attributed to other fragments of the 5-benzyloxy-3-oxapentyl moiety and the loss of these from the parent compound. Finally, microanalysis data revealed that the product (120) had been isolated in a pure state.

Ethyl (8-benzyloxy-3,6-dioxaoctyl) phosphorochloridate (124) was prepared in an entirely analogous manner to (116). Thus, the product (124) was isolated as a colourless oil in 85% yield.
The \(^{31}\text{P}\) n.m.r. spectrum of (124) showed a single peak at \(\delta 3.01\) ppm, which compares well with the values of \(\delta 3.07\) ppm and \(\delta 3.01\) ppm for (116) and (120) respectively. Additional spectra were obtained on (124), confirming the structure of this phosphorylating agent. The \(^{13}\text{C}\) n.m.r. and \(^{1}\text{H}\) n.m.r. spectra, assigned by comparison with the corresponding spectra of the starting materials (84) and (123), were consistent with the structure of the product. The \(^{13}\text{C}\) n.m.r. spectrum of (124) was similar to the spectra of (116) and (120) with phosphorus-coupled doublets, where they could be resolved, for carbon atoms within three bonds of the phosphorus atom. The \(^{1}\text{H}\) n.m.r. spectrum of (124) was analogous to the spectrum of (120), with singlets observed for the phenyl and PhCH\(_2\) resonances, a multiplet for the CH\(_2\)CH\(_2\)OP group, and the remaining protons of the glycolyl moiety comprising an unresolved multiplet. The E.I. mass spectrum of (124) showed a cluster of peaks due to the protonated molecular ion with the isotopic pattern for this, and other chlorine containing fragments, characteristic of one chlorine atom in the intact molecule. The base peak was observed at m/e 91 due to C\(_6\)H\(_7\)\(^+\), as similarly found in the spectra of (116) and (120). Prominent peaks again included those assigned to C\(_5\)H\(_2\)CH\(_2\)O\(^+\), C\(_6\)H\(_5\)CHO\(^+\), C\(_5\)H\(_4\)CO\(^+\) and C\(_7\)H\(_8\)\(^+\). The spectrum also consisted of peaks due to other fragments of the 8-benzyloxy-3,6-dioxaoctyl moiety and the loss of these from the parent compound. Microanalysis data revealed that the product (124) had been isolated in a pure state.

The ethyl (benzyl-protected glycolyl) phosphorochloridates (116), (120) and (124), successfully prepared above, were subsequently reacted with unprotected araA in pyridine at 0°C to give the appropriate 5'-ethyl (benzyl-protected glycolyl) phosphate triesters of araA (equation 59). Firstly, araA-5'-ethyl (2-benzyloxyethyl) phosphate (117) was synthesized by the addition of two molar equivalents of (116) to araA in pyridine at 0°C. The reaction employed two molar equivalents of phosphorochloridate since this amount of phosphorylating agent had been required to drive some analogous reactions to completion in the earlier synthesis of some 5'-aryl phosphate triesters of araA (chapter 4). The course of the reaction was followed by t.l.c. After stirring initially at 0°C for an hour, followed by a further 22 hours at ambient temperature, t.l.c. indicated that the reaction had proceeded to completion with all of the araA having reacted to give a more lipophilic major
component assumed to be due to the required product (117). Two even more lipophilic minor components were also observed. The reaction mixture was quenched with deionized water to remove any unreacted phosphorylating agent and solvent was removed. Purification of the resulting residue by column chromatography, employing a methanol-chloroform eluent, gave a slightly impure product (as indicated by t.l.c. analysis and $^{31}$P n.m.r. spectroscopy). After further purification by column chromatography, using a slower methanol-ethyl acetate eluent, the product (117) was isolated as a white solid in 59% yield.

The $^{31}$P n.m.r. spectrum of (117) (figure 36) showed a single peak at $\delta$ -0.74 ppm, which compares with the values of $\delta$ -0.70 ppm for araA-5'-diethyl phosphate$^{135}$ and $\delta$ -1.4 ppm for (EtO)$_2$(BzlO)PO$^{210}$. The spectrum displayed a single peak, rather than the two distinct resonances expected for the presence of the two possible diastereoisomers of (117) (figure 37) in the product, presumably due to the coincidence of these peaks. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (117), assigned by comparison with the corresponding spectra of araA-5'-diethyl phosphate$^{135}$ and (116), were consistent with the structure of the product and confirmed its diastereomeric nature. The latter was apparent in both spectra by the presence of many signals split in a 1:1 ratio. The multiplicity of these signals indicates the non-equivalence of the carbon atoms and protons in the two diastereoisomers of the product, while their ratio reflects the presence of the two isomers in approximately equal proportions. The $^{13}$C n.m.r. spectrum, for example, showed diastereomeric splitting for the nucleosidic carbon atoms C2 and either C2' or C3', and also for all the benzylic carbon atoms. Diastereomeric splitting was observed in addition to phosphorus coupling for the resonances of some of the carbon atoms within three bonds of the phosphorus atom, with the appearance of two doublets for C4' and CH$_2$CH$_2$OP, and a "triplet" for CH$_2$CH$_2$OP due to the overlap of these splittings. Only phosphorus coupling was observed in the doublets assigned to the methyl group. Evidence that (121) was the product of 5'-phosphorylation and not 2'- or 3'-phosphorylation was provided by the multiplet for C5' at $\delta$ 68.25-68.50 ppm showing a significant downfield shift compared with the value of $\delta$ 61.0 ppm for C5' of araA$^{155}$. The multiplicity of the C5' signal in the spectrum of (117) was due to the overlap of this resonance with that for the
Figure 36: $^{31}$P n.m.r. spectrum of (117)

Figure 37: Diastereoisomers of (117)

- BzlOCH$_2$CH$_2$O
- EtO
- NH$_2$
- OH
- BzlOCH$_2$CH$_2$O
The \( ^1H \) n.m.r. spectrum of (117) also showed diastereomeric splitting for a number of peaks in the spectrum, e.g. two distinct signals were observed for the H2 and PhCH\(_3\) resonances. The methyl group appeared as two sets of interspersed triplets of doublets, with the multiplicity within each signal due to proton-proton and phosphorus-proton coupling and two such signals occurring due to diastereomeric splitting. The spectrum also featured a singlet for H8 and multiplets for the phenyl protons and the CH\(_2\)CH\(_2\)OP moiety, while the signals for H2', H3', H4', H5', CH\(_2\)CH\(_2\)OP, and CH\(_3\)CH\(_2\)OP comprised an unresolved multiplet.

The F.A.B. mass spectrum of (117) further confirmed the structural assignment of the product, showing peaks at m/e 510 due to the protonated molecular ion and m/e 532 due to the molecular ion plus sodium from the matrix. The base peak was observed at m/e 136 due to protonated adenine, and a prominent peak was observed at m/e 107 due to \( C_6H_3CH_2O^+ \). Minor peaks included those associated with fragments of the 2-benzylolxyethyl moiety, and the ionized sugar fragments \( C_7H_5O_4^+ \), \( C_7H_7O_3^+ \) and \( C_7H_7O_4^+ \). Finally, microanalysis data were consistent with a hydrated form of (117) and reverse phase analytical H.P.L.C. revealed that the product had been isolated in a pure state. The latter technique displayed coincident peaks for the diastereomeric product, and also indicated the absence of contaminating araA.

The synthesis of araA-5'-ethyl (5-benzyloxy-3-oxapentyl) phosphate (121) was carried out in an entirely analogous manner to (117). Thus, the product (121) was isolated as a white solid in 63% yield following column chromatography. The \( ^31P \) n.m.r. spectrum of (121) showed a single peak at \( \delta = -3.30 \text{ ppm} \), contrasting with the value of \( \delta = -0.74 \text{ ppm} \) for (117). This difference in chemical shift was probably due to the use of CDCl\(_3\) as the n.m.r. solvent in the spectrum of (121), rather than CD\(_3\)OD as in the spectrum of (117). However, the chemical shift for (121) is comparable with the value of \( \delta = -3.17 \text{ ppm} \) for araA-5'-bis(8-benzyloxy-3,6-dioxapentyl) phosphate\(^{148} \) (with both spectra employing CDCl\(_3\) as the n.m.r. solvent). The spectrum of (121) showed no resolution of the two peaks expected for the two possible diastereoisomers of the product, as similarly observed in the spectrum of (117). However, diastereomeric splitting was evident in the \( ^13C \) n.m.r. and \( ^1H \) n.m.r. spectra of (121) with the duplication of a number of signals.
in a 1:1 ratio, confirming that the product was composed of an approximately equal mixture of the two possible diastereoisomers of (121). This isomerism again arises from the asymmetry at the chiral phosphorus centre.

The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (121) were consistent with the structure of the product, showing signals due to both the ethyl and 5-benzyloxy-3-oxapentyl moieties in addition to the nucleosidic resonances. The $^{13}$C n.m.r. spectrum of (121) was similar to that of (117), with diastereomeric splitting observed for all the benzylic carbon atoms, and in addition to phosphorus coupling for the $\text{CH}_2\text{CH}_2\text{OP}$ and $\text{CH}_3\text{CH}_2\text{OP}$ moieties. The methyl resonance showed both diastereomeric splitting and phosphorus coupling, with the appearance of a "triplet" due to the overlap of the two doublets expected for these splittings. This contrasted with the spectrum of (117) where a phosphorus-coupled doublet was observed for the methyl group. The $^{13}$C n.m.r. spectrum of (121) also differed from that of (117) in the appearance of broader signals, especially for some of the nucleosidic carbon atoms. This feature may have been due to the use of CDCl$_3$ as the n.m.r. solvent in the spectrum of (121), rather than CD$_3$OD as in the spectrum of (117), and may have resulted in the multiplicity of some signals in the spectrum of (121) being unresolved. The spectrum of (121), for example, displayed a broad singlet for C$_4'$, contrasting with the two doublets observed for the corresponding resonance in the spectrum of (117) due to phosphorus coupling and diastereomeric splitting. The $^1$H n.m.r. spectrum of (121) similarly showed a broadening of some of its signals when compared with the corresponding resonances in the spectrum of (117). This was particularly evident in the appearance of broad singlets for H2 and H1' in the spectrum of (121), as opposed to the respective diastereomeric splitting and phosphorus coupling observed for these resonances in the spectrum of (117). However the use of CDCl$_3$ as the n.m.r. solvent in the spectrum of (121), enabled the resonances for the NH$_2$, 2'-OH and 3'-OH groups to be observed.

The F.A.B. mass spectrum of (121) further confirmed the identity of this compound, showing a peak at m/e 554 due to the protonated molecular ion. The base peak was observed at m/e 136 due to protonated adenine and a prominent peak appeared at m/e 107 due to C$_8$H$_7$CH$_2$O$, as
similarly found in the spectrum of (117). A minor peak at m/e 305 was attributed to (EtO)(BzlOCH₂CH₃OCH₂CH₃O)P(OH)₂⁺. Other minor peaks of interest included those due to fragments of the 5-benzyloxy-3-oxapentyl moiety, and the ionized sugar fragments C₅H₇O₃⁺, C₅H₇O₂⁺ and C₅H₅O₂⁺. Finally, microanalysis data were consistent with a hydrated form of (121) and reverse phase analytical H.P.L.C. revealed that the product had been isolated in a pure state with no contaminating araA. The H.P.L.C. spectrum of (121) displayed two distinct signals for the diastereomeric product in a 1:1 ratio, contrasting with the spectrum of (117) where coincidence of the two expected peaks occurred.

AraA-5'-ethyl (8-benzyl-3,6-dioxaoctyl) phosphate (125) was prepared by a similar procedure to (117), except for the use of more of the appropriate phosphorylating agent (124) (initially 2.0 molar equivalents with a further 1.0 molar equivalent added during the course of the reaction) and a longer reaction time. These conditions were employed to enable the present reaction to proceed to completion. The product (125) was isolated as a white solid in 57% yield, following column chromatography. The ³¹P n.m.r. spectrum of (125) showed a single peak at δ -3.36 ppm, which compares well with the value of δ -3.30 ppm for (121) (with both spectra employing CDCl₃ as the n.m.r. solvent). Only one signal was observed in the spectrum of (125) due to the coincidence of the two peaks expected for the two possible diastereoisomers of the product, as similarly found in the spectra of (117) and (121).

The ¹³C n.m.r. and ¹H n.m.r. spectra of (125) confirmed the identity of the product and its diastereomeric nature. The latter was evident by the presence of a number of the peaks in these spectra split in a 1:1 ratio, indicating that the product had been isolated as an approximately equal mixture of the two possible diastereoisomers of (125). These spectra were consistent with the structure of the product, showing signals due to both the ethyl and 8-benzyloxy-3,6-dioxaoctyl moieties, and were also similar to the corresponding spectra of (121) (with all these spectra employing CDCl₃ as the n.m.r. solvent). A F.A.B. mass spectrum of (125) further confirmed the structural assignment of the product, with a peak at m/e 598 due to the protonated molecular ion. The base peak was observed at m/e 136 due to protonated adenine and a prominent peak appeared
at m/e 107 due to \( \text{C}_3\text{H}_7\text{O}^+ \), as similarly found in the spectra of (117) and (121). Minor peaks of interest included those attributed to \( (\text{EtO})(\text{BzlOCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{O})\text{P(OH)}_2^+ \), fragments of the 8-benzxyloxy-3,6-dioxaoctyl moiety, and the ionized sugar fragments \( \text{C}_3\text{H}_7\text{O}_2^+ \), \( \text{C}_3\text{H}_7\text{O}_{12}^+ \) and \( \text{C}_3\text{H}_7\text{O}_{15}^+ \). Finally, microanalysis data were consistent with a hydrated form of (125) and reverse phase analytical H.P.L.C. revealed that the product had been isolated in a pure state. It is of interest to note that the H.P.L.C. spectrum of (125) displayed coincident peaks for the diastereomeric product, as similarly observed in the spectrum of (117), and also indicated the absence of contaminating araA.

Following the successful preparation of the 5'-ethyl (benzyl-protected glycolyl) phosphate triesters of araA (117), (121) and (125), these compounds were subsequently converted to their respective 5'-ethyl glycolyl counterparts (118), (122) and (126) by hydrogenolysis. The method involved the treatment of a methanolic solution of the former compounds with hydrogen in the presence of a 10% palladium on charcoal catalyst at ambient temperature, to give the required deprotected products (equation 60). Firstly, araA-5'-ethyl (2-hydroxyethyl) phosphate (118) was synthesized from (117) by the procedure outlined above. The course of the reaction was followed by t.l.c. analysis and fresh hydrogen was added to the reaction mixture at intervals. After 23 hours stirring at ambient temperature, more catalyst was added to encourage the reaction to proceed to completion. The latter was achieved after stirring the reaction mixture for a further 19 hours at ambient temperature. T.l.c. now indicated that all of the araA-5'-ethyl (2-benzxyloxyethyl) phosphate (117) had reacted to give a much less lipophilic major component, assumed to be due to the required product (118), and also a less lipophilic minor component.

The reaction mixture was filtered, washing the catalyst with methanol, and solvent removed from the filtrate. Purification of the resulting oil by column chromatography, employing a methanol-chloroform eluent, gave a slightly impure product (as indicated by both t.l.c. analysis and \( ^{31}\text{P} \) n.m.r. spectroscopy). After further purification by column chromatography, using a methanol-ethyl acetate eluent, the product (118) was isolated as a white gum in 17% yield. The \( ^{31}\text{P} \) n.m.r. spectrum of (118) (figure 38) showed a single peak at \( \delta -0.30 \) ppm, which compares
Equation 60

\[
\text{Bzl(OCH}_2\text{CH}_2\text{)}_n\text{O} - \text{P} - \text{EtO} \xrightarrow{\text{H}_2, \text{Pd/C (10\%)} / \text{MeOH}} \text{H(OCH}_2\text{CH}_2\text{)}_n\text{O} - \text{P} - \text{EtO}
\]

Figure 38: \textsuperscript{31}P n.m.r. spectrum of (118)
with the value of δ -0.74 ppm for araA-5'-ethyl (2-benzyloxyethyl) phosphate (117). Only one signal was observed in the spectrum of (118), presumably due to the coincidence of the two peaks expected for the two possible diastereoisomers of the product.

The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (118) were consistent with the structure of the product, showing peaks due to both the ethyl and 2-hydroxyethyl moieties in addition to the nucleosidic resonances. A comparison of the $^{13}$C n.m.r. spectrum of (118) with the corresponding spectrum of (117) revealed these spectra to differ mainly in the absence of the resonances for the benzylic carbon atoms of the starting material (117) in the spectrum of the product (118), confirming the successful removal of the benzyl protecting group by hydrogenolysis. Additionally, the $^{13}$C n.m.r. spectrum of (118) showed a significant upfield shift for the HOCH$_2$CH$_2$OP resonance (at ca. δ 62 ppm) when compared with the value for the corresponding BzIOCH$_2$CH$_2$OP resonance (at ca. δ 70 ppm) in the spectrum of (117). This is consistent with the value of δ 63.4 ppm for the CH$_2$OH resonance of monoethylene glycol$^{156}$ displaying an upfield shift relative to the value of δ 71.4 ppm for the BzIOCH$_2$ resonance of benzyl 2-hydroxyethyl ether$^{210}$. Evidence for the retention of the 5'-phosphate in the product (118) was provided by the doublets for C5' at δ 68.31 ppm and δ 68.26 ppm appearing significantly downfield when compared with the value of δ 61.0 ppm for C5' of araA$^{155}$. The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (118) also confirmed the diastereomeric nature of the product, with many signals split in a 1:1 ratio due to the presence of an approximately equal mixture of the two possible diastereoisomers of (118) in the product. The $^{13}$C n.m.r. spectrum, for example, showed diastereomeric splitting for the nucleosidic carbon atoms C2, C8, and either C2' or C3'. Diastereomeric splitting was observed in addition to phosphorus coupling for the resonances of some carbon atoms within three bond of the phosphorus atom, with the methyl resonance consisting of two doublets and "triplets" appearing for C5' and CH$_2$CH$_2$OP due to the overlap of the two doublets expected for these splittings. Only phosphorus coupling was displayed in the doublets assigned to C4', CH$_2$CH$_2$OP and CH$_2$CH$_2$OP. It is of interest to note that all of the three bond phosphorus-carbon coupling constants were slightly greater than the two bond coupling constants, probably due to the angular
dependence of coupling constants. The $^1$H n.m.r. spectrum of (118) also showed no resonances for the benzylic protons present in the corresponding spectrum of the starting material (117). However, in other respects, the spectrum of (118) was similar to the spectrum of (117), with a singlet being observed for H8, a doublet for H1, and a multiplet for CH$_2$CH$_2$OP, and the signals due to H2, H3, H4, H5 and remaining methylene protons (i.e. CH$_2$CH$_2$OP and CH$_2$CH$_2$OP) comprising an unresolved multiplet.

The F.A.B. mass spectrum of (118) further confirmed the structural assignment of the product, showing a peak at m/e 420 due to the protonated molecular ion and another of much lower intensity at m/e 442 due to the molecular ion plus sodium from the matrix. The base peak was observed at m/e 136 due to protonated adenine, while minor peaks of interest included those assigned to (EtO)(HOCH$_2$CH$_2$O)P(OH)$_2$ and the ionized sugar fragment C$_9$H$_7$O$_3$. Finally, microanalysis data were consistent with a hydrated form of (118) and reverse phase analytical H.P.L.C. revealed that the product had been isolated in a pure state with no contaminating araA. It is of interest to note that the H.P.L.C. spectrum of (118) displayed two distinct signals for the diastereomeric product in a 1:1 ratio.

AraA-5′-ethyl (5-hydroxy-3-oxapentyl) phosphate (122) was prepared by hydrogenolysis of (121) in a similar manner to the synthesis of (118) from (117), except for the initial use of more catalyst which enabled the reaction to proceed to completion in a shorter period of time.

Purification of the crude product (122) also differed slightly from the method employed for (118). Only one chromatographic column, using a methanol-ethyl acetate eluent, was required to isolate the product (122) as a white gum in 60% yield. The $^{31}$P n.m.r. spectrum of (122) showed a single peak at δ -0.34 ppm, which compares well with the value of δ -0.30 ppm for (118). The presence of only one signal in the spectrum of (122), rather than the two expected for a diastereoisomeric product, was presumably due to the coincidence of these peaks. This feature was similarly observed in the spectrum of (118).

The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (122) confirmed the identity of the product and its diastereomeric nature. The latter was evident by the duplication of many peaks in these spectra,
with the ratio of these peaks (1:1) reflecting the approximately equal mixture of the two possible diastereoisomers of (122) in the product. These spectra were consistent with the structure of the product, showing signals due to both the ethyl and 5-hydroxy-3-oxapentyl moieties in addition to the nucleosidic resonances, and also similar to the corresponding spectra of (118) except for the resonances associated with the presence of the extra glycolyl methylene groups in (122). The resonances for the benzyl group in the $^{13}$C n.m.r. and $^1$H n.m.r. spectra of the starting material (121) were not similarly observed in the corresponding spectra of (122), confirming that the benzyl protecting group had been successfully removed. The $^1$H n.m.r. spectrum of the product (122) also differed from that of the starting material (121) in the absence of resonances for the NH$_2$, 2'-OH and 3'-OH groups. This was due to the use of CD$_3$OD as the n.m.r. solvent in the spectrum of (122) rather than CDCl$_3$ as in the spectrum of (121).

A F.A.B. mass spectrum was also obtained on (122), showing a peak at m/e 464 due to the protonated molecular ion and another of much lower intensity at m/e 486 due to the molecular ion plus sodium from the matrix. The base peak was observed at m/e 136 due to protonated adenine. Minor peaks of interest included those attributed to (EtO)(HOCH$_2$CH$_2$OCH$_2$CH$_2$O)P(OH)$_3$ and the ionized sugar fragment C$_2$H$_2$O$^+$. Finally, microanalysis data were consistent with a hydrated form of (122) and reverse phase analytical H.P.L.C. revealed that the product had been isolated in a pure state with no contaminating araA. The H.P.L.C. spectrum of (122) displayed only one signal due to the coincidence of the two peaks expected for the diastereomeric product, contrasting with the spectrum of (118) where two distinct signals were observed.

AraA-5'-ethyl (8-hydroxy-3,6-dioxaocetyl) phosphate (126) was prepared by hydrogenolysis of (125) in an analogous manner to the synthesis of (122) from (121), except that a longer period of time (50 hours) was required for the reaction to proceed to completion. The product (126) was isolated as a white gum in 79% yield, following column chromatography. The $^{31}$P n.m.r. spectrum of (126) showed a single peak at $\delta$ -0.36 ppm, which compares with the values of $\delta$ -0.30 ppm for (118) and $\delta$ -0.35 ppm for (122). The spectrum of (126) displayed no resolution of the two peaks expected for two possible diastereoisomers of the product, as similarly found in the spectra
of (118) and (122). However, many signals in the \(^{13}\text{C}\) n.m.r. and \(^{1}\text{H}\) n.m.r. spectra of (126) were split in a 1:1 ratio, indicating that the product was composed of an approximately equal mixture of the two possible diastereoisomers of (126). The \(^{13}\text{C}\) n.m.r. and \(^{1}\text{H}\) n.m.r. spectra of (126) were consistent with the structure of the product, showing signals due to both the ethyl and 8-hydroxy-3,6-dioxaoctyl moieties in addition to the nucleosidic resonances, and also the absence of signals for the benzyl group of (125). These spectra were similar to the corresponding spectra of (118) and (122), with the main exception being the presence of additional peaks associated with the extra glycolyl methylene groups in (126).

The F.A.B. mass spectrum of (126) further confirmed the identity of the product, with a peak at m/e 508 due to the protonated molecular ion and another of lower intensity at m/e 530 due to the molecular ion plus sodium from the matrix. The base peak was observed at m/e 136 due to protonated adenine. A minor peak of interest at m/e 259 was attributed to the fragment (EtO)(HOCH\(_2\)CH\(_2\)OCH\(_2\)CH\(_2\)OCH\(_2\)CH\(_2\)O)P(OH)\(_2\)). Finally, microanalysis data were consistent with a hydrated form of (126) and reverse phase analytical H.P.L.C. revealed that the product had been isolated in a pure state with no contaminating araA. The latter technique displayed two distinct signals for the diastereomeric product in a 1:1 ratio, as similarly found in the spectrum of (118).

Following the successful preparation of the 5'-ethyl glycolyl phosphate derivatives of araA (118), (122) and (126), the synthesis of araA-5'-((2,2,2-trichloroethyl) (8-hydroxy-3,6-dioxaoctyl) phosphate (129) was investigated by an analogous multi-step procedure. The proposed route generally involved the preparation of the appropriate 2,2,2-trichloroethyl (benzyl-protected glycolyl) phosphorochloridate and its subsequent reaction with araA, followed by hydrogenolysis to give the required deprotected product.

Firstly, (2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphorochloridate (127) was synthesized by the reaction of 2,2,2-trichloroethyl phosphorodichloridate (101), prepared earlier (as discussed in chapter 4), with one molar equivalent of both 2-(2-(2-\(O\)-benzyloxy)ethoxy)ethanol (123) and triethylamine in diethyl ether solvent at -78°C (equation 61). The method
Equation 61

\[
\text{CCl}_3\text{CH}_2\text{OPCl}_2 + \text{Bzl}[(\text{OC}_2\text{H}_4)_3\text{OH}] + \text{Et}_3\text{N} \rightarrow \text{Bzl}[(\text{OC}_2\text{H}_4)_3\text{O}]\text{P} \rightarrow \text{Cl} + \text{Et}_3\text{NHCl}
\]

-78°C to RT \( \text{CCl}_3\text{CH}_2\text{O} \)

(101) (123) (127)

Equation 62

\[
\text{Bzl}[(\text{OC}_2\text{H}_4)_3\text{O}]\text{P} \rightarrow \text{Cl} + \text{HO} \rightarrow \text{Bzl}[(\text{OC}_2\text{H}_4)_3\text{O}]\text{P} \rightarrow \text{O} \rightarrow \text{CCI}_3\text{CH}_2\text{O}
\]

0°C to RT \( \text{CCI}_3\text{CH}_2\text{O} \)

(127) (128)

Equation 63

\[
\text{Bzl}[(\text{OC}_2\text{H}_4)_3\text{O}]\text{P} \rightarrow \text{O} \rightarrow \text{HO} \rightarrow \text{H}[(\text{OC}_2\text{H}_4)_3\text{O}]\text{P} \rightarrow \text{O} \rightarrow \text{CCI}_3\text{CH}_2\text{O}
\]

RT \( \text{CCI}_3\text{CH}_2\text{O} \)

(128) (129)
involved was similar to the one employed in the synthesis of ethyl (8-benzyloxy-3,6-dioxaoctyl) phosphorochloridate (124) from ethyl phosphorodichloridate (84), except that the reaction mixture was stirred at ambient temperature for a longer period of time (71 hours). The latter was employed to encourage the present reaction, involving the more sterically hindered 2,2,2-trichloroethyl phosphorodichloridate, to proceed to completion. Following work-up of the reaction mixture, the product (127) was isolated as a colourless oil in 100% yield. The $^{31}$P n.m.r. spectrum of (127) showed a single peak at $\delta$ 2.41 ppm, which compares with the values of $\delta$ 3.08 ppm for (124), $\delta$ 1.30 ppm for bis(2,2,2-trichloroethyl) phosphorochloridate$^{186}$ and $\delta$ 4.70 ppm for dibenzyloxy phosphorochloridate$^{189}$.

The $^{13}$C n.m.r. and $^1$H n.m.r. spectra of (127) were consistent with the structure of the product, showing signals due to both the 2,2,2-trichloroethyl and 8-benzyloxy-3,6-dioxaoctyl moieties. These spectra were assigned by comparison with the corresponding spectra of the starting materials (101) and (123). The $^{13}$C n.m.r. spectrum of (127) was similar to that of (124), with singlets observed for all the benzylic carbon atoms and phosphorus-coupled doublets, in cases where they could be resolved, for carbon atoms within three bonds of the phosphorus atom. However, the doublet assigned to the CCl$_3$CH$_2$OP moiety of (127) displayed a significant downfield shift when compared with the corresponding doublet for the CH$_3$CH$_2$OP moiety of (124), probably due to the deshielding effect of the chlorine atoms. It is of interest to note that the doublet for the CCl$_3$ resonance was of lower intensity than the other peaks in the spectrum of (127), presumably due to the longer relaxation time of the CCl$_3$ group. The $^1$H n.m.r. spectrum of (127) was similar to that of (124), with the main exception being the appearance of resonances associated with a CCl$_3$CH$_2$OP moiety rather than a CH$_3$CH$_2$OP moiety. An E.I. mass spectrum was also obtained on the product (127), showing a cluster of peaks due to the molecular ion. The isotopic pattern for these peaks and other chlorine containing fragments was characteristic of four chlorine atoms in the intact molecule. The base peak was observed at m/e 91 due to C$_7$H$_7^+$ and other prominent peaks included those assigned to C$_8$H$_5$CH$_2$O$^+$, C$_6$H$_5$CHO$^+$ and C$_6$H$_5$CO$^+$. The spectrum also featured minor peaks associated with other fragments of the 8-benzyloxy-3,6-dioxaoctyl moiety.
and the loss of these from the parent compound. Finally, microanalysis data revealed that the product (127) had been isolated in a pure state.

AraA-5′-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate (128) was subsequently prepared by the reaction of two molar equivalents of 2,2,2-trichloroethyl (8-benzyloxy-3,6-dioxaoctyl) phosphorochloridate (127) with unprotected araA in pyridine at 0°C (equation 62). The method involved was similar to the one employed in the synthesis of (117) and (121), except that a longer period of time stirring at ambient temperature (46 hours) was required for the reaction to proceed to completion. Thus, the product (128) was isolated as a white solid in 52% yield, following column chromatography. The 31P n.m.r. spectrum of (128) showed two closely spaced singlets of similar intensity at δ -2.18 ppm and δ -2.36 ppm. The multiplicity of these signals arises from the asymmetry at the chiral phosphorus centre and corresponds to the presence of an approximately equal mixture of the two possible diastereoisomers of (128) in the product. This contrasts with the 31P n.m.r. spectra of the 5′-ethyl (benzyl-protected glycolyl) phosphate triesters of araA (117), (121) and (125), where coincidence of the two expected peaks occurred. The chemical shift of the peaks in the spectrum of (128) displayed a slight downfield shift when compared with the value of δ -3.36 ppm for araA-5′-ethyl (8-benzyloxy-3,6-dioxaoctyl) phosphate (125) due to the deshielding effect of the chlorine atoms.

The 13C n.m.r. and 1H n.m.r. spectra of (128) confirmed the identity of the product and its diastereomeric nature. These spectra were consistent with the structure of the product, showing signals due to both the 2,2,2-trichloroethyl and 8-benzyloxy-3,6-dioxaoctyl moieties in addition to the nucleosidic resonances. A comparison of the 13C n.m.r. spectrum of (128) with that of (125) revealed these spectra to be similar. However, the spectrum of (128) showed fewer signals duplicated due to diastereomeric splitting than the spectrum of (125). All the resonances for the phenyl carbon atoms in the spectrum of (128), for example, appeared as singlets. This contrasted with the spectrum of (125) where each of the signals for the phenyl carbon atoms consisted of two peaks in a 1:1 ratio. Additionally, the phosphorus-coupled doublet observed for the CCl3CH2OP resonance of (128) appeared further downfield than the corresponding signal for the CH3CH2OP
resonance of (125), probably due to the deshielding effect of the chlorine atoms. The \( ^1H \) n.m.r.
spectrum of (128) was similar to that of (125), with the main exception being the presence of
signals for a CCl\(_3\)CH\(_2\)OP moiety rather than a CH\(_3\)CH\(_2\)OP moiety.

The F.A.B. mass spectrum of (128) further confirmed the structural assignment of the product,
showing a cluster of peaks due to the protonated molecular ion. The isotopic pattern for these
peaks and other chlorine containing fragments was characteristic of three chlorine atoms in the
intact molecule. The base peak was observed at m/e 136 due to protonated adenine and a
prominent peak appeared at m/e 91 due to C\(_7\)H\(_8\)\(^+\). Minor peaks of interest included those due to
(CCl\(_3\)CH\(_2\)O)(BzI\(_2\)OCH\(_2\)OCH\(_2\)OCH\(_2\)CH\(_2\)O)PO\(_2\)^+ fragments of the 8-benzyloxy-3,6-dioxaoctyl
moiety, and the ionized sugar fragments C\(_3\)H\(_7\)O\(_3\)^+ , C\(_3\)H\(_7\)O\(_2\)^+ and C\(_3\)H\(_5\)O\(_2\)^+. Finally, microanalysis
data were consistent with a hydrated form of (128) and reverse phase analytical H.P.L.C. revealed
that the product had been isolated in a pure state with no contaminating araA. It is of interest to
note that the H.P.L.C. spectrum of (128) displayed two distinct signals for the diastereomeric
product in a 1:1 ratio, contrasting with the spectrum of (125) where coincidence of the two
expected peaks occurred.

The synthesis of araA-5'--(2,2,2-trichloroethyl) (8-hydroxy-3,6-dioxaoctyl) phosphate (129) was
attempted by the subsequent hydrogenolysis of araA-5'--(2,2,2-trichloroethyl) (8-benzyloxy-3,6-
dioxaoctyl) phosphate (128), involving the treatment of a methanolic solution of (128) with
hydrogen in the presence of a 10% palladium on charcoal catalyst at ambient temperature
(equation 63). The method involved was similar to the one employed in the earlier preparation
of araA-5'-ethyl (8-hydroxy-3,6-dioxaoctyl) phosphate (126) from araA-5'-ethyl (8-benzyloxy-3,6-
dioxaoctyl) phosphate (125), except that a shorter period of time was required for the present
reaction to proceed to completion. After 26 hours stirring at ambient temperature, t.l.c. indicated
that all of the araA-5'--(2,2,-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate (128) had been
converted to a major baseline component, which was significantly less lipophilic than the starting
material (128) and even araA. This contrasted with the synthesis of (126) from (125), where the
major component of the reaction mixture (isolated and characterized as the required product (126))
was less lipophilic than the starting material (125) but more lipophilic than araA under the same conditions of t.l.c. analysis. Furthermore, (129) might be expected to show similar or perhaps marginally more lipophilic properties than (126) under identical t.l.c. conditions. A possible explanation may lie in the loss of both the benzyl protecting group and the 2,2,2-trichloroethyl moiety from the starting material (128) under the hydrogenolysis conditions, but the feasibility of this suggestion could not be confirmed due to the circumstances detailed below.

The reaction mixture was filtered, washing the catalyst with methanol, and solvent removed from the filtrate under reduced pressure. Purification of the crude product was attempted by column chromatography, using a methanol-ethyl acetate eluent. Unfortunately, the major component of the reaction mixture appeared to have been lost during the work-up of the reaction mixture and subsequent purification by column chromatography. However, the more lipophilic minor component of the reaction mixture was isolated as a white solid from the chromatographic column. Although the identity of this compound was not fully ascertained, spectral data did rule out the possibility of it being the required product (129) and suggested a decomposition product. Time did not allow for the resynthesis of araA-5'-((2,2,2-trichloroethyl) (8-benzyl-3,6-dioxaocetyl) phosphate (128) and a further investigation into the conversion of this compound to the required deprotected product, araA-5'-((2,2,2-trichloroethyl) (8-hydroxy-3,6-dioxaocetyl) phosphate (129).

The biological activities of the 5'-ethyl (benzyl-protected glycolyl) phosphate triesters of araA (117), (121) and (125), araA-5'-((2,2,2-trichloroethyl) (8-benzyl-3,6-dioxaocetyl) phosphate (128), and the 5'-ethyl glycolyl phosphate derivatives of araA (118), (122) and (126) were evaluated by determining the ability of these compounds to inhibit the synthesis of cellular DNA using the in vitro tritiated thymidne incorporation assay developed by Riley and co-workers, in an entirely analogous manner to the procedure employed earlier for the testing of some 5'-phosphate derivatives of araA (chapters 2, 3 and 4). AraA-5'-diethyl phosphate (38), previously synthesized and biologically evaluated in this Department, was also tested along with the above derivatives.
to allow for a fairer comparison between the biological activities of these compounds. Each experiment was carried out at least twice on cells of different passage number and the mean % inhibition of DNA synthesis (relative to the distilled water control) and standard error of the mean (SEM) were calculated for each set of % inhibition values (table 9, biological testing section). A two-tail student's t-test was used to determine the degree of significant difference between the mean % inhibition values of two compounds at a given concentration. This data was subsequently used to determine the probability of this difference being statistically significant (table 10, biological testing section).

The results of the assay are presented as a bar chart (figure 39) in which the mean % inhibition of DNA synthesis (relative to the distilled water control) is plotted against compound at a final concentration of 0.03 mM. This graph clearly illustrates the relationship between the compounds and their biological activity, in particular each of the compounds tested displays an inhibitory effect on DNA synthesis in vitro. Several other interesting features are evident from the results of the assay. Considering the 5'-ethyl (benzyl-protected glycolyl) phosphate triesters of araA (117), (121) and (125), these compounds show a decreasing activity with increasing length of the glycolyl chain. A similar correlation is also apparent for the 5'-ethyl glycolyl phosphate derivatives of araA (118), (122) and (126). These trends are the reverse of those expected on the basis of the increasing biological activity observed for the 5'-dialkyl phosphate triesters of araA with increasing chain length of their constituent alkyl chains, which was attributed to the increasing lipophilicity and hence improved membrane penetration of these compounds in the same series. This suggests that either membrane penetration is not a limiting factor for these 5'-ethyl (benzyl-protected glycolyl) phosphate triesters of araA and their 5'-ethyl glycolyl analogues, or that other detrimental effects overwhelm any such advantage. However, all the 5'-ethyl (benzyl-protected glycolyl) phosphate triesters of araA and the 5'-ethyl glycolyl phosphate derivatives display a greater biological activity than araA-5'-diethyl phosphate (38), except for (126) which is equi-active with (38). Indeed, a two-tail student's t-test reveals these differences in biological activities to be significant (i.e. P < 5%) for all these compounds when compared with (38), apart
Figure 39: % Inhibition of DNA synthesis against compound

% Inhibition of DNA synthesis

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from (126) vs. (38). Thus, it would appear that replacing an ethyl moiety by a benzyl-protected glycolyl or a glycolyl moiety enhances biological activity, but this effect decreases with increasing length of the glycolyl chain.

A comparison of the biological activities of the 5′-ethyl (benzyl-protected glycolyl) phosphate triesters of araA (117), (121) and (125) with their respective 5′-ethyl glycolyl analogues (118), (122) and (126) reveals that, in every case, the former show a biological activity significantly greater than the latter (as indicated by the two-tail student’s t-test). A possible explanation for this may lie in the fact that compounds (117), (121) and (125) are 5′-phosphate triesters of araA, while compounds (118), (122) and (126) are 5′-phosphate diesters of araA. The presence of the negative charge on the hydroxyl group of the glycolyl moiety of the 5′-ethyl glycolyl phosphate derivatives of araA at physiological pH may retard the passive diffusion of these compound across the cell membrane relative to the 5′-ethyl (benzyl-protected glycolyl) phosphate triesters of araA, and hence be reflected in the differing biological activities of these two sets of compounds. It is also of interest to note that araA-5′-ethyl (2-benzyloxyethyl) phosphate (117) is equi-active with araA, while compounds (121), (125), (118), (122) and (126) all show biological activities significantly lower than araA.

Finally, a comparison of the biological activity of araA-5′-ethyl (8-benzyloxy-3,6-dioxaoctyl) phosphate (125) with araA-5′-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate (128), reveals that replacing an ethyl moiety by a 2,2,2-trichloroethyl moiety enhances biological activity. Similar observations have been previously made for the 5′-bis(2,2,2-trihaloethyl) phosphate triesters of araA when compared with their simple 5′-dialkyl counterparts, and also in recent research (chapters 3 and 4). AraA-5′-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate (128) also displays a greater biological activity than araA. The two-tail student’s t-test indicates that these differences in biological activities are significant (i.e. P < 5%) for (128) vs. (125), and (128) vs. araA.

Thus some interesting relationships were observed between the structures of the compounds tested and their ability to inhibit the synthesis of DNA in vitro. These may lead to scope for future
research and development. The presence of a 2,2,2-trichloroethyl moiety in a 5'-phosphate derivative of araA is again particularly noteworthy (as observed earlier in chapters 3 and 4), with araA-5'-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate (128) appearing to improve the biological activity of the parent drug araA.
SUMMARY AND CONCLUSION

The research presented in this thesis has investigated the synthesis and biological evaluation of some novel 5'-phosphate derivatives of the anti-viral drug 9-β-D-arabinofuranosyladenine (araA). It was hoped that these compounds would act as potential uncharged, membrane-soluble, deamination-resistant forms of the drug, which might also undergo preferential hydrolysis within the cell to release araA-5'-monophosphate (araAMP), thereby obviating nucleoside kinase dependence. Generally, the method involved the preparation of the appropriate phosphorylating agent, from phosphoryl chloride, and its subsequent reaction with unprotected araA in pyridine. The biological activity of these derivatives was evaluated by determining their ability to inhibit the synthesis of DNA in vitro using a tritiated thymidine incorporation assay employing mammalian epithelial cells.

Firstly, the 5'-bis(2-fluoroethyl) and 5'-bis(2-bromoethyl) phosphate triesters of araA were synthesized. Preparation of araA-5'-bis(2-iodoethyl) phosphate was subsequently attempted by the reaction of the 5'-bis(2-bromoethyl) derivative with potassium iodide in acetone.

The synthesis of a series of mixed, unsymmetrical 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA was carried out. AraA-5'-ethyl (2,2,2-trichloroethyl) phosphate, araA-5'-propyl (2,2,2-trichloroethyl) phosphate and araA-5'-butyl (2,2,2-trichloroethyl) phosphate were prepared by the general method outlined above. The latter compound was also synthesized from araA-5'-bis(2,2,2-trichloroethyl) phosphate by a transesterification reaction involving the appropriate alcohol as solvent in the presence of an excess of caesium fluoride.

Several mixed, unsymmetrical 5'-aryl phosphate triesters of araA were prepared, initially araA-5'-ethyl phenyl phosphate and its 5'-((2,2,2-trichloroethyl) phenyl analogue. The synthesis of a number of 5'-(2,2,2-trichloroethyl) para-substituted phenyl phosphate triesters of araA, with para-substituents consisting of isopropyl, phenyl, chloro and methoxy groups, was subsequently carried out. Preparation of araA-5'-(2,2,2-trichloroethyl) p-cyanophenyl phosphate and araA-5'-(2,2,2-trichloroethyl) p-nitrophenyl phosphate was also attempted.
Some mixed, unsymmetrical 5′-ethyl (benzyl-protected glycolyl) phosphate triesters of araA were synthesized. AraA-5′-ethyl (2-benzylxyethyl) phosphate, araA-5′-ethyl (5-benzylxy-3-oxapentyl) phosphate and araA-5′-ethyl (8-benzylxy-3,6-dioxaoctyl) phosphate were prepared. Hydrogenolysis of these derivatives, employing a palladium on charcoal catalyst in methanol solvent, gave the required deprotected products. AraA-5′-(2,2,2-trichloroethyl) (8-benzylxy-3,6-dioxaoctyl) phosphate was synthesized and its hydrogenolysis was attempted.

The in vitro biological activity of these mixed, unsymmetrical 5′-phosphate derivatives of araA was evaluated and the results are discussed. Each of the compounds tested displayed an inhibitory effect on the synthesis of DNA by mammalian epithelial cells and some interesting structure-activity relationships were observed. The replacement of a simple alkyl chain by a 2,2,2-trichloroethyl moiety in a 5′-phosphate derivative of araA was particularly noteworthy, appearing to enhance biological activity.

Research has also been carried out to establish the mechanism by which 5′-phosphate triesters of araA exert their biological effects. This probably involves the intracellular hydrolysis of the phosphate moiety to yield either the 5′-monophosphate (araAMP) or the parent nucleoside (araA). The in vitro biological activity of two 5′-phosphate triesters of araA was compared with their analogous 5′-phosphinate esters to help elucidate the matter. The results of the assay were indicative of a mode of action largely involving the intracellular release of araAMP and also to a minor extent araA.

Finally, the susceptibility of the 5′-bis(2,2,2-trifluoroethyl), 5′-bis(2,2,2-trichloroethyl) and 5′-butyl (2,2,2-trichloroethyl) phosphate triesters of araA to chemical hydrolysis was investigated and attempts to purify and characterize the resulting products were carried out. Hydrolysis of the 5′-bis(2,2,2-trihaloethyl) derivatives appeared to proceed with the loss of only one of the 2,2,2-trihaloethyl moieties to give the appropriate diesters. The product isolated from the chemical hydrolysis of araA-5′-butyl (2,2,2-trichloroethyl) phosphate was unexpectedly identified as araA-5′-(2,2,2-trichloroethyl) phosphate. This diester was subsequently biologically evaluated and found to possess an inhibitory effect on the synthesis of DNA in vitro.
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General Methods

All experiments involving water sensitive reagents were carried out under scrupulously dry conditions. Solvents and reagents were dried, where appropriate, in the following ways. Hexane, pyridine and toluene were heated to reflux over calcium hydride for several hours, distilled and stored over activated molecular sieves. Diethyl ether was heated to reflux over calcium hydride for several hours, distilled and either used fresh or after storage over activated molecular sieves. Triethylamine was heated to reflux over calcium hydride for several hours and freshly distilled prior to use. Phosphoryl chloride was distilled prior to use. Methanol was heated to reflux with magnesium activated with iodine, distilled and stored over activated molecular sieves. Ethanol, 2-fluoroethanol, 2-bromoethanol, 2,2,2-trifluoroethanol, 2,2,2-trichloroethanol, benzyl chloride, glycols and benzyl protected glycols were stored over activated molecular sieves for at least 24 hours prior to use. Nucleosides and phenols were dried at ambient temperature under reduced pressure (0.1 mm Hg) for at least 24 hours prior to use. Caesium fluoride was dried by heating to ca. 120°C under reduced pressure (0.1 mm Hg) for 6 hours. Molecular sieves were activated by heating to ca. 160°C under reduced pressure (0.1 mm Hg) for at least 6 hours.

Commercially available Merck Kieselgel 60 F254 pre-coated silica plates were used for analytical t.l.c. and visualized by ultra violet (uv) light. Column chromatography was carried using Woelm silica (32-64 μM) as the stationary phase, the ratio of silica:compound varying from 75:1 to 150:1 (w/w). Electron impact mass spectra (E.I.M.S.) were recorded by Dr. M. Mruzek on a VG7070H mass spectrometer fitted with a Finnigan Incos II data system. Fast atom bombardment mass spectra (F.A.B.M.S.) were recorded either by Dr. M. Mruzek on the aforementioned spectrometer or by the University of London mass spectrometry service on a VG Zab1F spectrometer, both using m-nitrobenzyl alcohol as matrix unless otherwise stated. Microanalyses were carried out by the microanalytical section of the Chemistry Department, University College London; nucleoside derivatives were noted to be hygroscopic and the analytical data are represented accordingly. Analytical and preparative H.P.L.C. were carried out by Mr. S. Corker on a Gilson Binary Gradient H.P.L.C. system fitted with a Gilson 115 uv detector (detection at
254 nm) and a Rheodyne injection valve.

Phosphorus nuclear magnetic resonance spectra ($^{31}$P n.m.r.) were recorded on a Varian XL-200 spectrometer operating at 82 MHz and are reported in units of $\delta$ relative to 85% phosphoric acid (H$_3$PO$_4$) as external standard. In a few cases where the n.m.r. spectra were recorded on a Varian VXR-400 spectrometer operating at 164 MHz or a Jeol-500 spectrometer operating at 205 MHz, or not referenced to phosphoric acid, these conditions are indicated. The n.m.r. spectra of some nucleoside derivatives were recorded in a non-deuterated solvent of methanol or chloroform, by using an internal tube of deuterium oxide (D$_2$O) for the lock. Carbon nuclear magnetic resonance ($^{13}$C n.m.r.) spectra were recorded on a Varian XL-200 operating at 50 MHz for all compounds except for nucleoside derivatives, which were recorded on a Varian VXR-400 spectrometer operating at 100 MHz. The n.m.r. spectra are reported in units of $\delta$ relative to the solvent employed, or relative to sodium 3-(trimethylsilyl)-1-propane sulphonic acid (TSP) in deuterium dioxide (D$_2$O) as external standard when using D$_2$O as solvent, or relative to tetramethylsilane (TMS) as internal standard, as indicated. Phosphorus and carbon n.m.r. spectra were heterodecoupled, unless otherwise stated. Proton nuclear magnetic resonance ($^1$H n.m.r.) spectra were recorded on a Varian XL-200 spectrometer operating at 200 MHz for all compounds except for nucleoside derivatives, which were recorded on a Varian VXR-400 spectrometer operating at 400 MHz unless otherwise stated. The n.m.r. spectra are reported in units of $\delta$ relative to the solvent employed, or relative to sodium 3-(trimethyl)-1-propane sulphonic acid (TSP) in deuterium dioxide (D$_2$O) as external standard when using D$_2$O as solvent, or relative to tetramethylsilane (TMS) as internal standard, as indicated. All peaks reported in the n.m.r. spectra were singlets, unless otherwise stated. The following abbreviations were used in the assignment of n.m.r. signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad), dm (doublet of multiplets), td (triplet of doublets) and qd (quartet of doublets).
Bis(2-fluoroethyl) phosphorochloridate (52)

Phosphoryl chloride (0.79 ml, 1.30 g, 8.5 mmol) was dissolved in diethyl ether (25 ml) and cooled to -78°C. Separately and simultaneously, 2-fluoroethanol (1 ml, 1.09 g, 0.017 mol) dissolved in diethyl ether (25 ml) and triethylamine (2.34 ml, 1.72 g, 0.017 mol) dissolved in diethyl ether (25 ml) were added dropwise over 0.75 h with vigorous stirring to the cooled solution. On completion of the addition, the reaction mixture was stirred at -78°C for a further hour then allowed to warm to ambient temperature overnight (16 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure to give a pale yellow oil (1.74 g). A minor impurity of 2-fluoroethyl phosphorodichloridate was removed by stirring the crude product under reduced pressure (0.1 mm Hg) at ambient temperature for 7 h, yielding a pale yellow oil (52) (1.44 g, ca. 81%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 3.383 (major, (52)), -3.363 (minor)

E.I.M.S. m/e 211 (MH$^+$, 0.03%), 209 (MH$^+$, 0.16%), 208 (M$^+$, 0.02%), 174 (MH$^+$ - Cl, 0.27%), 173 (M$^+$ - Cl, 3.81%), 165 (FCH$_2$CH$_2$OP(OH)$_2^{37}$Cl$^+$, 1.99%), 163 (FCH$_2$CH$_2$OP(OH)$_2$Cl$^+$, 13.26%), 131 ((CH$_2$O)(HO)PO$^{37}$Cl$^+$, 2.91%), 129 ((CH$_2$O)(HO)POCl$^+$, 9.61%) 127 (FCH$_2$CH$_2$OP(OH)$_2$H$^+$, 54.95%), 111 (FCH$_2$CH$_2$OPOH$^+$, 21.30%), 94 ((CH$_2$O)(HO)PO$^+$, 58.77%), 81 (OP(OH)$_2$H$^+$, 62.86%), 63 (FCH$_2$CH$_2$O$^+$, 22.08%), 47 (FCH$_2$CH$_2$$^+$, 100%), 33 (FCH$_2$$^+$, 55.65%)

9-β-D-Arabinofuranosyladenine-5′-bis(2-fluoroethyl) phosphate (53)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and bis(2-fluoroethyl) phosphorochloridate (52) (0.29 g, 1.39 mmol, 1.5 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for a further 2 h. After warming to ambient temperature, the reaction mixture was quenched with deionized water (25 μl, 1.39 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were
removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml)
under reduced pressure. The resulting yellow oil was purified by column chromatography on silica,
eluting with 15% methanol in chloroform. Pooling and evaporation of appropriate fractions yielded
a yellow oil (0.42 g). Chloroform (50 ml) was added to the impure product and the resulting
suspension was left at 0°C for 1 h, causing a precipitate to collect. After warming to ambient
temperature, solvent was decanted off and final traces were removed under reduced pressure,
yielding a white solid (53) (0.170 g, 41%).

$^{31}$P n.m.r. $\delta$(CH$_2$OD) - 0.837

$^1$H n.m.r. $\delta$(CD$_2$OD) 8.324 (1H, s, H2), 8.192 (1H, s, H8), 6.455 (1H, d, H1', J=3.96 Hz), 4.649,
4.530 (4H, dm, FCH$_2$, J=47.67 Hz), 4.096-4.497 (9H, m, H2', H3', H4', H5', CH$_2$OP)

F.A.B.M.S. (NO$_2$C$_6$H$_5$CH$_2$OH/Nal) m/e 462 (MNa+, 0.92%), 440 (MH+, 18.23%), 305 (MH+ -
adenine, 0.31%), 250 (MH+ - (FCH$_2$CH$_2$O)$_2$PO$_2$H, 0.33%), 203 ((FCH$_2$CH$_2$O)$_2$PO$_2$CH$_3$+, 2.54%),
191 ((FCH$_2$CH$_2$O)$_2$P(OH)$_2$+, 2.82%) 164 (adenineCHO+, 4.14%) 145 ((FCH$_2$CH$_2$O)P(OH)$_3$+, 1.85%),
136 (adenineH+, 100%), 135 (adenine+, 9.66%), 128 ((FCH$_2$CH$_2$O)P(OH)$_2$+, 2.01%) 115
(C$_3$H$_7$O$_3$+, 4.86%), 111 (FCH$_2$CH$_2$OPOH+, 3.81%) 99 (C$_3$H$_7$O$_2$+, 1.55%), 97 (C$_3$H$_7$O$_2$+, 7.51%), 81
(C$_3$H$_5$O+, 17.30%), 63 (FCH$_2$CH$_2$O+, 17.80%).

H.P.L.C. (analytical)
Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column
Mobile phase: methanol/0.01% triethylamine in water (20/80), isocratic conditions
(53): 32.24 mins

Bis(2-bromoethyl) phosphoryl chloride (54)
Phosphoryl chloride (5 ml, 8.23 g, 0.054 mol) was dissolved in diethyl ether (25 ml) and cooled
to -78°C. Separately and simultaneously, 2-bromoethanol (7.75 ml, 11.05 g, 0.109 mol) dissolved in diethyl ether (25 ml) and triethylamine (15.2 ml, 11.04 g, 0.109 mol) dissolved in diethyl ether (25 ml) were added dropwise over 0.5 h with vigorous stirring to the cooled solution. On completion of the addition, the reaction mixture was stirred at -78°C for a further 0.5 h then allowed to warm to ambient temperature overnight (16 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure to give a yellow/brown oil (17.53 g). A minor impurity of 2-bromoethyl phosphorochloridate was removed by stirring the crude product under reduced pressure (0.1 mm Hg) at ambient temperature for 6 h, yielding a paler yellow/brown oil (54) (14.91 g, ca. 84%).

\(^{31}\text{P n.m.r.}\) δ(CDCl₃) 1.715 (major, (54), quintet in heterocoupled spectrum, J=9.3 Hz), -5.346 (minor)

\(^{13}\text{C n.m.r.}\) δ(CDCl₃) 68.232 (d, CH₂OP, J=6.6 Hz), 28.224 (d, BrCH₂, J=9.0 Hz)

\(^{1}\text{H n.m.r.}\) δ(CDCl₃/TMS) 4.495 (4H, m, CH₂OP), 3.602 (4H, m, BrCH₂)

F.A.B.M.S. (glycerol/thioglycerol/2,2,2-trifluoroacetic acid) m/e 335 (MH⁺, 2x\(^{85}\)Br 1x\(^{37}\)Cl, 2.17%), 333 (MH⁺, 2x\(^{81}\)Br and 1x\(^{85}\)Br 1x\(^{37}\)Cl, 7.61%), 331 (MH⁺, 1x\(^{85}\)Br and 1x\(^{37}\)Cl, 11.41%), 329 (MH⁺, 5.98%), 297 (M⁺ - Cl, 2x\(^{85}\)Br, 2.99%), 295 (M⁺ - Cl, 1x\(^{85}\)Br, 4.89%), 293 (M⁺ - Cl, 9.24%), 109 (\(^{81}\)BrCH₂CH₂⁺, 98.37%), 107 (BrCH₂CH₂⁺, 100%)

9-\(\beta\)-D-Arabinofuranosyladenine-5′-bis(2-bromoethyl) phosphate (55)

AraA (1.00 g, 3.74 mmol) was dissolved in pyridine (60 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and bis(2-bromoethyl) phosphorochloridate (54) (1.85 g, 5.61 mmol, 1.5 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for a further 2.5 h.
After warming to ambient temperature, the reaction mixture was quenched with deionized water (100 µl, 5.61 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 25 ml) and then co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting green oil was purified by column chromatography on silica, eluting with 20% methanol in chloroform. Pooling and evaporation of appropriate fractions gave a pale green gum, which on repeated trituration with diethyl ether yielded a white solid (55) (0.98 g, 47%).

$^{31}$P n.m.r. $\delta$(CH$_3$OD) -1.675

$^{13}$C n.m.r. $\delta$(CH$_3$OD) 156.101 (C6), 152.622 (C2), 149.575 (C4), 141.859 (C8), 118.695 (C5), 85.611 (C1'), 83.130 (d, C4', J=7.6 Hz), 76.454 (C2' or C3'), 76.011 (C2' or C3'), 68.063-68.115 (m, C5', CH$_2$OP), 29.843, 29.820 (2xd, BrCH$_2$, J=7.8 Hz, 7.8 Hz)

$^1$H n.m.r. (d$_2$-DMSO/TMS) 8.172 (1H, s, H2), 8.139 (1H, s, H8), 7.220 (2H, bs, NH$_2$, absent on D$_2$O exchange), 6.329 (1H, d, H1', J=4.31 Hz), 5.786 (1H, d, OH, J=3.82 Hz, absent on D$_2$O exchange), 5.727 (1H, d, OH, J=3.82 Hz, absent on D$_2$O exchange), 4.139-4.355 (9H, m, H2', H3', H4', H5', CH$_2$OP), 3.666 (4H, m, BrCH$_2$)

Thermospray Mass Spectrum m/e 563 (M$^+$, 2x$^{73}$Br, 6.69%), 561 (M$^+$, $^{73}$Br, 12.79%), 559 (M$^+$, 6.98%), 483 (MH$^+$ - Br, $^{73}$Br, 2.91%), 481 (MH$^+$ - Br, 2.62%), 427 (MH$^+$ - adenine, 2x$^{73}$Br, 0.58%), 425 (MH$^+$ - adenine, $^{73}$Br, 1.16%), 423 (MH$^+$ - adenine, 0.58%), 374 (MH$^+$ - 2xBrCH$_2$CH$_2$ 5.81%), 250 (MH$^+$ - (BrCH$_2$CH$_2$O)$_2$PO$_2$H, 51.16%), 249 (M$^+$ - (BrCH$_2$CH$_2$O)$_2$PO$_2$H, 30.81%), 233 ($^{73}$BrCH$_2$CH$_2$O)(HO)PO$_2$CH$_2$CH$_2^+$, 93.60%), 231 ((BrCH$_2$CH$_2$O)(HO)PO$_2$CH$_2$CH$_2^+$, 100%), 136 (adenineH$^+$, 11.63%), 115 (C$_2$H$_7$O$_5^+$, 1.16%), 109 ($^{73}$BrCH$_2$CH$_2^+$, 1.45%), 107 (BrCH$_2$CH$_2^+$, 1.74%)
Microanalysis

Found: C 29.08%; H 3.51%; N 11.46%

C_{14}H_{20}Br_{2}N_{5}O_{7}P(H_{2}O)_{1.0} requires: C 29.04%; H 3.83%; N 12.09%

H.P.L.C. (analytical)

Stationary phase: 4 + 250 x 4.0 mm Lichrosorb rp select b column

Mobile phase: methanol/water/acetic acid (50/50/0.1), isocratic conditions

(55): retention time 8.16 mins

Reaction of 9-β-D-arabinofuranosyladenine-5′-bis(2-bromoethyl) phosphate (55) with potassium iodide

9-β-D-Arabinofuranosyladenine-5′-bis(2-bromoethyl) phosphate (55) (0.18 g, 0.321 mmol) was dissolved in acetone (25 ml) by heating the stirred suspension to reflux at 70°C. Potassium iodide (0.532 g, 3.21 mmol, 10 molar equivs.) was added to the resulting solution. After 1 h stirring at reflux, the potassium iodide had not completely dissolved so more acetone (20 ml) was added and the reaction mixture was stirred at reflux for a further 47 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting dark brown solid was purified by column chromatography on silica, eluting with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions yielded a yellow/brown solid (0.170 g).

\(^{31}\text{P n.m.r.}\) δ(CH₃OD) -1.862 (minor), -2.084 (major)

\(^{13}\text{C n.m.r.}\) δ(CD₃OD) 156.542 (C6), 152.948 (C2), 150.394 (C4), 142.931 (C8), 119.490 (C5), 86.316 (C1′), 83.897 (d, C4′, J=6.4 Hz), 77.160 (C2′ or C3′), 76.740 (C2′ or C3′), 69.625 (d, CH₂OP, J=5.6 Hz), 69.010 (d, C5′, J=5.5 Hz), 30.868 (d, BrCH₂, J=6.8 Hz), 24.185 (d, ICH₂, J=3.4 Hz)

177
$^1$H n.m.r. $\delta$ (d$_e$-DMSO/TMS, 200 MHz) includes 8.205 (s, H2), 8.196 (s, H8), 7.277 (bs, NH$_2$), 6.341 (d, H1', J = 4.48 Hz), 5.845 (d, OH, J = 3.43 Hz), 5.785 (d, OH, J = 3.35 Hz), 3.642-3.394 (m, H2', H3', H4', H5', BrCH$_2$CH$_2$OP, Ich$_2$CH$_2$OP), 3.170 (d, ICH$_2$)

F.A.B.M.S. m/e 528 (M$^+$ - Br, 1.16%), 359 ($^{11}$BrCH$_2$CH$_2$O)(ICH$_2$CH$_2$O)PO$_2$$^+$, 1.45%), 357 ((BrCH$_2$CH$_2$O)(ICH$_2$CH$_2$O)PO$_2$$^+$, 1.74%), 250 (MH$^+$ - (BrCH$_2$CH$_2$O)(ICH$_2$CH$_2$O)PO$_2$H, 1.31%), 171 (ICH$_2$CH$_2$O$^+$, 2.03%), 135 (adenine$^+$, 16.28%), 125 ($^{11}$BrCH$_2$CH$_2$O$^+$, 11.33%), 123 (BrCH$_2$CH$_2$O$^+$, 12.50%), 115 (C$_3$H$_7$O$_3$$^+$, 0.58%)

H.P.L.C. (analytical)
Stationary phase: 4 + 250 x 4.0 mm Lichrosorb rp select b column
Mobile phase: methanol/water/acetic acid (50/50/0.01), isocratic conditions
Major peak: retention time 5.93 min (42%)

Investigating the synthesis of butyl phosphinic dichloride (71) from butyl magnesium bromide and phosphoryl chloride

The Grignard reagent from 1-bromobutane (11.6 ml, 14.8 g, 108 mmol) and magnesium (2.87 g, 118 mmol, 1.1 molar equivs.) in diethyl ether (35 ml) was filtered and added dropwise over 1 h with vigorous stirring to a solution of phosphoryl chloride (10.07 ml, 16.56 g, 108 mmol) in diethyl ether (125 ml) at -78°C under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (15 h). Solvent was removed from the reaction mixture under reduced pressure giving a white solid, to which silicone oil (45 ml) was added before subjecting it to vacuum distillation. Heating the solid to ca. 170°C was required before a yellow oil (2.83 g) started to collect (bp 36-38°C, 0.3 mm Hg).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 49.749 (major, (71)), 35.769 (minor)
Investigating the synthesis of butyl phosphinic dichloride (71) from butyl magnesium chloride and phosphoryl chloride

Method 1

The Grignard reagent from 1-chlorobutane (11.29 ml, 10 g, 108 mmol) and magnesium (2.87 g, 118 mmol, 1.1 molar equivs.) in diethyl ether (35 ml) was filtered and added dropwise over 1 h with vigorous stirring to a solution of phosphoryl chloride (10.07 ml, 16.56 g, 108 mmol) in diethyl ether (125 ml) at -78°C under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was stirred at -78°C for a further hour then allowed to warm to ambient temperature overnight (16 h). Solvent was removed from the reaction mixture under reduced pressure giving a white solid, to which silicone oil (45 ml) was added before subjecting it to vacuum distillation. Heating the solid to ca. 150°C was required before a colourless oil (71) (2.901 g, 15%) started to collect (bp 41-42°, 0.05 mm Hg).

\(^{31}\text{P n.m.r.} \delta (\text{CDCl}_3) \ 49.702

\(^{13}\text{C n.m.r.} \delta (\text{CDCl}_3) \ 42.460 \ (d, \text{CH}_2\text{P}, J=96.5 \text{ Hz}), \ 24.668 \ (d, \text{CH}_3\text{CH}_2\text{P}, J=6.8 \text{ Hz}), \ 22.743 \ (d, \text{CH}_3\text{CH}_2, J=22.2 \text{ Hz}), \ 13.374 \ (\text{CH}_3)

\(^{1}\text{H n.m.r.} \delta (\text{CDCl}_3) \ 2.511 \ (2H, m, \text{CH}_2\text{P}), \ 1.717 \ (2H, m, \text{CH}_3\text{CH}_2\text{P}), \ 1.432 \ (2H, \text{sextet}, \text{CH}_3\text{CH}_2, J=7.65 \text{ Hz}), \ 0.889 \ (3H, t, \text{CH}_3, J=7.27 \text{ Hz})

Method 2

The Grignard reagent from 1-chlorobutane (11.29 ml, 10 g, 108 mmol) and magnesium (2.87 g, 118 mmol, 1.1 molar equivs.) in diethyl ether (35 ml) was filtered and added dropwise over 1 h with vigorous stirring to a solution of phosphoryl chloride (10.07 ml, 16.56 g, 108 mmol) in diethyl ether (125 ml) at 0°C under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (20 h). Solvent was
removed from the reaction mixture under reduced pressure giving a white solid, to which silicone oil (45 ml) was added before subjecting it to vacuum distillation. Heating the solid to ca. 160°C was required before a colourless oil (71) (1.67 g, 9%) began to collect (bp 44-46°C, 0.05 mm Hg).

\[^{31}\text{P n.m.r. } \delta(\text{CDCl}_3) 49.715\]

**Method 3**

The Grignard reagent from 1-chlorobutane (11.29 ml, 10 g, 108 mmol) and magnesium (2.87 g, 118 mmol, 1.1 molar equivs.) in diethyl ether (35 ml) was filtered and added dropwise over 1 h with vigorous stirring to a solution of phosphoryl chloride (10.07 ml, 16.56 g, 108 mmol) in diethyl ether (125 ml) at ambient temperature under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was stirred at reflux overnight (20 h) then allowed to cool to ambient temperature. Solvent was removed under reduced pressure giving a white solid. Silicone oil (45 ml) was added to the latter before subjecting it to vacuum distillation. Heating the solid to ca. 200°C was required before a colourless oil (71) (0.955 g, 5%) started to collect (bp 40°C, 0.02 mm Hg).

\[^{31}\text{P n.m.r. } \delta(\text{CDCl}_3) 49.722\]

**Method 4**

The Grignard reagent from 1-chlorobutane (11.29 ml, 10 g, 108 mmol) and magnesium (2.87 g, 118 mmol, 1.1 molar equivs.) in diethyl ether (35 ml) was filtered and added dropwise over 1 h with vigorous stirring to a solution of phosphoryl chloride (16.56 g, 108 mmol) in diethyl ether (125 ml) in a Kugelruhr distillation flask, initially at ambient temperature and then at -10°C due to the vigorous nature of the reaction. Filtering and addition of the Grignard reagent were both carried out under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature over 2 h then stirred at reflux for a further 42 h. The
reaction mixture was allowed to cool to ambient temperature before removing solvent under reduced pressure. The white solid obtained was subjected to distillation under reduced pressure (0.02 mm Hg) using a Kugelruhr. Heating the solid to ca. 200°C was initially required to cause a small volume of colourless oil to collect, but only by heating to ca. 250°C was it possible to encourage more distillate (3.083 g) to collect.

$^{31}$P n.m.r. \( \delta (\text{CDCl}_3) \) 70.369 (major, (66), quintet in heterocoupled spectrum, \( J = 9.7 \) Hz), 49.642 (minor, (71), triplet in heterocoupled spectrum, \( J = 16.2 \) Hz)

**Butyl (2,2,2-trichloroethoxy) phosphinic chloride (74)**

Butyl phosphinic dichloride (71) (1.5 g, 8.57 mmol) was dissolved in diethyl ether (25 ml) and cooled to -78°C. A solution of 2,2,2-trichloroethanol (0.82 ml, 1.28 g, 8.57 mmol) and triethylamine (1.20 ml, 0.87 g, 8.60 mmol) dissolved in diethyl ether (25 ml) was added dropwise over 1 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was stirred at -78°C for an further hour then allowed to warm to ambient temperature overnight (18 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy yellow solution was extracted with hexane, to give a colourless oil (1.937 g). The crude product was purified by vacuum distillation (bp 88-90°C, 0.5 mm Hg), yielding a colourless oil (74) (1.061 g, 43%) which solidified on standing at ambient temperature.

$^{31}$P n.m.r. \( \delta (\text{CDCl}_3) \) 32.572

$^{13}$C n.m.r. \( \delta (\text{CDCl}_3) \) 95.222 (d, CCl\(_3\), \( J = 9.0 \) Hz), 75.458 (d, CH\(_2\)OP, \( J = 5.6 \) Hz), 23.988 (d, CH\(_3\)CH\(_2\)P, \( J = 2.5 \) Hz), 23.810 (d, CH\(_2\)P, \( J = 55.9 \) Hz), 23.800 (d, CH\(_3\)CH\(_2\), \( J = 18.9 \) Hz), 13.467 (CH\(_3\))
$^1$H n.m.r. $\delta$(CDCl$_3$) 4.601 (2H, m, CH$_2$OP), 1.990 (2H, m, CH$_2$P), 1.696 (2H, CH$_2$CH$_2$P), 1.444
(2H, sextet, CH$_3$CH$_2$, J=7.06 Hz), 0.914 (3H, t, CH$_3$, J=7.25 Hz)

F.A.B.M.S. (tetraglyme) m/e 315 (M$^{+}$, 3x$^{35}$Cl, 4.07%), 313 (M$^{+}$, 2x$^{35}$Cl, 19.77%), 311
(M$^{+}$, $^{37}$Cl, 57.56%), 309 (M$^{+}$, 62.79%), 277 (M$^{+}$ - CH$_3$, 3x$^{35}$Cl, 4.07%), 275 (M$^{+}$ - CH$_3$,
2x$^{35}$Cl, 9.30%), 273 (M$^{+}$ - CH$_3$, $^{37}$Cl, 13.95%), 271 (M$^{+}$ - CH$_3$, 12.79%), 255 (M$^{+}$ - Cl, 2x$^{35}$Cl,
4.07%), 253 (M$^{+}$ - Cl, $^{37}$Cl, 8.14%), 251 (M$^{+}$ - Cl, 8.14%), 171 (M$^{+}$ - CCl$_3$, $^{37}$Cl, 2.33%), 169
(M$^{+}$ - CCl$_3$, 5.23%), 141 (BuPO$^{35}$Cl$^*$, 12.91%), 139 (BuPOCl$^*$, 56.98%)

Microanalysis

Found: C 26.42%; H 4.10%; Cl 48.96%

C$_6$H$_{11}$Cl$_4$O$_2$P requires: C 25.03%; H 3.85%; Cl 49.25%

Butyl phosphorodichloridate (78)
Phosphoryl chloride (8.20 ml, 13.49 g, 0.088 mol) was dissolved in diethyl ether (200 ml) and
cooled to -78°C. A solution of n-butanol (8.05 ml, 6.52 g, 0.088 mol) and triethylamine (12.26
ml, 8.9 g, 0.088 mol) dissolved in diethyl ether (200 ml) was added dropwise over 2 h with
vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the
addition, the reaction mixture was allowed to warm to ambient temperature overnight (19 h). The
reaction mixture was filtered and solvent removed under reduced pressure, yielding a colourless
oil (78) (16.134 g, 96%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 4.917 (triplet in heterocoupled spectrum, J=8.6 Hz)

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 72.062 (d, CH$_2$OP, J=9.7 Hz), 31.333 (d, CH$_2$CH$_2$OP, J=8.6 Hz), 18.350
(CH$_2$CH$_2$), 13.374 (CH$_3$)
\[ ^1H\text{ n.m.r. } \delta(\text{CDCl}_3)\] 4.313 (2H, m, CH\text{OP}), 1.744 (2H, m, CH\text{OP}), 1.436 (2H, m, CH\text{H}_2), 0.935 (3H, t, CH_3, J=7.18 Hz)

**E.I.M.S.** m/e 163 (M\(^+\) - Et, \text{Cl}^\text{37}, 0.08\%), 161 (M\(^+\) - Et, 0.26\%), 152 (MH\(^+\) - Pr, 2x\text{Cl}^\text{37}, 0.03\%), 151 (M\(^+\) - Pr, 2x\text{Cl}^\text{37}, 0.05\%), 150 (MH\(^+\) - Pr, \text{Cl}^\text{37}, 1.52\%), 149 (M\(^+\) - Pr, \text{Cl}^\text{37}, 1.54\%), 148 (MH\(^+\) - Pr, 3.05\%), 147 (M\(^+\) - Pr, 2.41\%), 139 ((\text{HO})_2\text{PCl}_2^\text{+}, 2x\text{Cl}^\text{37}, 3.70\%), 137 ((\text{HO})_2\text{PCl}_2^\text{+}, \text{Cl}^\text{37}, 29.31\%), 135 ((\text{HO})_2\text{PCl}_2^\text{+}, 41.55\%), 122 (\text{HOPCl}_2^\text{+}, 2x\text{Cl}^\text{37}, 0.24\%), 121 (\text{OPCl}_2^\text{+}, 2x\text{Cl}^\text{37}, 0.56\%), 120 (\text{HOPCl}_2^\text{+}, \text{Cl}^\text{37}, 3.72\%), 119 (\text{OPCl}_2^\text{+}, \text{Cl}^\text{37}, 5.69\%), 118 (\text{HOPCl}_2^\text{+}, 7.08\%), 117 (\text{OPCl}_2^\text{+}, 8.55\%), 101 (\text{OP(OH)}\text{Cl}^\text{37}, 1.54\%), 99 (\text{OP(OH)}\text{Cl}^\text{37}, 4.38\%), 73 (\text{C}_4\text{H}_9\text{O}^\text{+}, 1.94\%), 57 (\text{C}_4\text{H}_9^\text{+}, 17.56\%), 56 (\text{C}_4\text{H}_9^\text{+}, 100\%), 55 (\text{C}_4\text{H}_9^\text{+}, 16.7\%)

**Microanalysis**

Found: C 25.28\%; H 4.80\%; P 16.97\%

\[ \text{C}_4\text{H}_9\text{Cl}_2\text{O}_2\text{P} \text{ requires: C 25.25\%, H 4.75\%; P 16.22\%} \]

**Butyl 2,2,2-trichloroethyl phosphorochloridate (79)**

Butyl phosphorodichloridate (78) (4.01 g, 0.021 mols) was dissolved in diethyl ether (200 ml) and cooled to -78\(^\circ\)C. A solution of 2,2,2-trichloroethanol (2.01 ml, 3.13 g, 0.021 mol) and triethylamine (2.93 ml, 2.13 g, 0.021 mol) dissolved in diethyl ether (50 ml) were added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was stirred at -78\(^\circ\)C for a further 2 h then allowed to warm to ambient temperature overnight (16 h). The reaction mixture was filtered and solvent was removed under reduced pressure. The resulting cloudy oil was extracted with hexane to give a clear pale yellow oil (5.484 g). A minor impurity of unreacted starting material (78) was removed by stirring the crude product under reduced pressure (0.06 mm Hg) at ca. 50\(^\circ\)C for 8 h. The resulting pale yellow oil (3.543 g) was further purified by vacuum distillation (bp 98\(^\circ\)C, 0.06 mm Hg), yielding a colourless oil (79) (1.794 g, 28\%).

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$^{31}$P n.m.r. $\delta$(CDCl$_3$) 1.903

$^{13}$C n.m.r $\delta$(CDCl$_3$) 93.806 (d, CCl$_3$, $J$=12.4 Hz), 77.025 (d, CCl$_3$CH$_2$OP, $J$=7.3 Hz), 70.587 (d, CH$_2$CH$_2$OP, $J$=8.0 Hz), 31.520 (d, CH$_2$CH$_2$OP, $J$=7.9 Hz), 18.345 (CH$_3$), 13.292 (CH$_3$)

$^1$H n.m.r. $\delta$(CDCl$_3$) 4.613 (2H, m, CCl$_3$CH$_2$OP), 4.248 (2H, m, CH$_3$CH$_2$OP), 1.696 (2H, m, CH$_3$CH$_2$OP), 1.394 (2H, sextet, CH$_3$CH$_2$, $J$=7.66 Hz), 0.899 (3H, t, CH$_3$, $J$=7.36 Hz)

9-β-D-Arabinofuranosyladenine-5′-butyl (2,2,2-trichloroethyl) phosphate (80)

AraA (0.5 g, 1.87 mmol) was dissolved in pyridine (35 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and butyl 2,2,2-trichloroethyl phosphorochloridate (79) (0.85 g, 2.8 mmol, 1.5 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 21 h, allowed to warm to ambient temperature and stirred for a further 35 h. The reaction mixture was quenched with deionized water (50 µl, 2.76 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting solid was purified by successive column chromatography on silica, eluting with 15% methanol in chloroform and 10% methanol in ethyl acetate respectively. Pooling and evaporation of the appropriate fractions gave a white solid (0.727 g) which was further purified by recrystallization from ethyl acetate, yielding a white solid (80) (0.286 g, 29%).

$^{31}$P n.m.r. $\delta$(CD$_3$OD) -2.311

$^{31}$P n.m.r. $\delta$(CD$_3$OD, 164 MHz, not referenced to H$_3$PO$_4$) 0.246, 0.221 (1:1)
$^{13}$C n.m.r. $\delta$(CD$_3$OD) 157.210 (C6), 153.807, 153.776 (2xs, C2), 150.547, 150.500 (2xs, C4),
142.667, 142.641 (2xs, C8), 119.593, 119.560 (2xs, C5), 96.286 (d, CCl$_3$, $J$=12.0 Hz), 86.418,
86.286 (2xs, C1'), 83.940 (d, C4', $J$=6.6 Hz), 78.097 (d, CCl$_3$CH$_2$OP, $J$=4.4 Hz), 77.352 (C2' or C3'),
76.913 (C2' or C3'), 70.142, 70.082 (2xd, CH$_2$CH$_2$OP, $J$=6.1 Hz, 6.0 Hz), 69.193,
69.162 (2xd, C5', $J$=6.0 Hz, 5.9 Hz), 33.177 (d, CH$_2$CH$_2$OP, $J$=6.6 Hz), 19.629, 19.609 (2xs, 
CH$_3$CH$_2$), 13.833, 13.819 (2xs, CH$_3$)

$^1$H n.m.r. $\delta$(CD$_3$OD) 8.346, 8.342 (1H, 2xs, H2), 8.193 (1H, s, H8), 6.462 (1H, t, H1', $J$=3.33 Hz),
4.596-4.669 (3H, m, H2', CCl$_3$CH$_2$OP), 4.474 (1H, m, H3'), 4.289 (2H, m, H5'),
4.128-4.178 (3H, m, H4', CH$_2$CH$_2$OP), 1.645 (2H, m, CH$_2$CH$_2$OP), 1.406 (2H, m, CH$_3$CH$_2$), 0.907
(3H, m, CH$_3$)

F.A.B.M.S. m/e 536 (MH$^+$, $^{37}$Cl, 7.18%), 534 (MH$^+$, 6.58%), 250 (MH$^+$ - (BuO)(CCl$_3$CH$_2$O)PO$_2$H, 
1.48%), 231 ((CCl$_3$CH$_2$O)POH$^+$, $^{37}$Cl, 0.20%), 229 ((CCl$_3$CH$_2$O)POH$^+$, 0.23%), 155 
((BuO)POH$^+$, 0.20%), 136 (adenineH$^+$, 100%), 135 (adenine$^+$, 3.85%), 115 (C$_3$H$_7$O$_3$$^+$, 1.71%),
99 (C$_3$H$_2$O$_2$$^+$, 16.85%), 81 (C$_2$H$_2$O$^+$, 1.30%)

Microanalysis

Found: C 35.82%; H 4.40%; N 11.76%; P 5.73%

C$_{16}$H$_{23}$Cl$_3$N$_5$O$_7$P requires: C 35.94%; H 4.34%; N 13.10%; P 5.79%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Spherisorb ODS2 5 $\mu$M column

Mobile phase: water (A)/0.01% triethylamine in acetonitrile (B), gradient conditions 0 min 20% 
(B), 30 min 80% (B), 40 min 80% (B)

(80): retention time 16.88 min
Propyl phosphorodichloridate (81)

Phosphoryl chloride (10 ml, 16.46 g, 0.107 mol) was dissolved in diethyl ether (200 ml) and cooled to -78°C. A solution of n-propanol (8 ml, 6.43 g, 0.107 mol) and triethylamine (14.92 ml, 10.83 g, 0.107 mol) dissolved in diethyl ether (200 ml) was added dropwise over 3.5 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (17 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure, yielding a colourless oil (81) (18.69 g, 99%).

$^{31}$P n.m.r.  $\delta$(CDCl$_3$) 4.951

$^{12}$C n.m.r.  $\delta$(CDCl$_3$) 73.766 (d, CH$_2$OP, $J$=9.7 Hz), 22.936 (d, CH$_3$CH$_2$, $J$=8.9 Hz), 9.731 (CH$_3$)

$^1$H n.m.r.  $\delta$(CDCl$_3$) 4.263 (2H, m, CH$_2$OP), 1.799 (2H, m, CH$_3$CH$_2$), 0.988 (3H, t, CH$_3$, $J$=7.41 Hz)

E.I.M.S. m/e 151 (M$^+$ - Et, $2^3$Cl, 0.43%), 149 (M$^+$ - Et, $^{37}$Cl, 9.52%), 147 (M$^+$ - Et, 14.59%), 139 ((HO)$_2$PCl$_2$+, $2^3$Cl, 5.52%), 137 ((HO)$_2$PCl$_2$+, $^{37}$Cl, 52.90%), 135 ((HO)$_2$PCl$_2$+, 85.77%), 122 (HOPCl$_2$+, $2^3$Cl, 0.06%), 121 (OPCl$_2$+, $2^3$Cl, 0.34%), 120 (HOPCl$_2$+, $^{37}$Cl, 6.16%), 119 (OPCl$_2$+, $^{37}$Cl, 13.91%), 118 (HOPCl$_2$+, 11.99%), 117 (OPCl$_2$+, 29.08%), 101 (OP(OH)$_2$Cl$^+$, 2.11%), 99 (OP(OH)Cl$^+$, 9.69%), 59 (C$_3$H$_7$O$^+$, 15.52%), 43 (C$_3$H$_7$+, 67.02%), 42 (C$_3$H$_8$+, 100%), 41 (C$_3$H$_9$+, 50.11%)

Microanalysis

Found: C 20.55%; H 4.21%; P 17.20%

C$_3$H$_7$Cl$_2$O$_2$P requires: C 20.36%; H 3.99%; P 17.50%
Propyl 2,2,2-trichloroethyl phosphorochloridate (82)

Propyl phosphorodichloridate (81) (3 g, 0.017 mmol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2,2,2-trichloroethanol (1.63 ml, 2.54 g, 0.017 mmol) and triethylamine (2.37 ml, 1.72 g, 0.017 mmol) dissolved in diethyl ether (100 ml) was added dropwise over 1 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (16 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy solution was extracted with hexane to give a colourless oil (4.085 g). A minor impurity of unreacted starting material (81) was removed by stirring the crude product under reduced pressure (0.04 mm Hg) at ca. 30°C for 6 h, yielding a colourless oil (82) (3.981 g, 81%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 1.990

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 93.875 (d, CCl$_3$, J=12.8 Hz), 77.136 (d, CCl$_3$CH$_2$OP, J=5.2 Hz), 72.376 (d, CH$_2$CH$_2$OP, J=7.8 Hz), 23.123 (d, CH$_3$CH$_2$, J=7.6 Hz), 9.798 (CH$_3$)

$^1$H n.m.r. $\delta$(CDCl$_3$) 4.619 (2H, m, CCl$_3$CH$_2$OP), 4.172 (2H, m, CH$_2$CH$_2$OP), 1.743 (2H, m, CH$_3$CH$_2$), 0.958 (3H, t, CH$_3$, J=7.34 Hz)

E.I.M.S. m/e 289 (M$^+$ - H, $^{35}$Cl, 0.04%), 287 (M$^+$ - H, 0.02%), 253 (MH$_2^+$ - Pr, 3x$^{37}$Cl, 0.26%), 251 (MH$_2^+$ - Pr, 2x$^{37}$Cl, 2.24%), 249 (MH$_2^+$ - Pr, $^{37}$Cl, 5.19%), 247 (MH$_2^+$ - Pr, 4.04%), 215 (MH$^+$ - Pr - Cl, 2x$^{37}$Cl, 7.86%) 213 (MH$^+$ - Pr - Cl, $^{37}$Cl, 23.64%), 211 (MH$^+$ - Pr - Cl, 24.85%), 173 (M$^+$ - CCl$_3$, $^{37}$Cl, 5.39%), 171 (M$^+$ - CCl$_3$, 17.07%), 131 ((CH$_2$O)(HO)PO$_{37}$Cl$^+$, 40.89%), 129 ((CH$_2$O)(HO)POCl$^+$, 63.89%), 119 ((HO)$_2$P$^{37}$Cl$^+$, 71.24%), 117 ((HO)$_2$PCl$^+$, 100%), 59 (C$_3$H$_7$O$^+$, 9.20%), 43 (C$_3$H$_7^+$, 73.69%)
9-β-D-Arabinofuranosyladenine-5′-propyl (2,2,2-trichloroethyl) phosphate (83)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and propyl 2,2,2-trichloroethyl phosphorochloridate (82) (0.54 g, 1.86 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 18 h, allowed to warm to ambient temperature and stirred for a further 7 h. More phosphorylating agent (82) (0.27 g, 0.93 mmol, 1 molar equiv.) was added dropwise at 0°C with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 49 h. The reaction mixture was quenched with deionized water (50 μl, 2.78 mmol) and solvent removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting pink solid was purified by successive column chromatography on silica, eluting with 5% methanol in chloroform and 10% methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions gave a white solid (0.286 g) which was further purified by recrystallization from ethyl acetate, yielding a white solid (83) (0.120 g, 25%).

$^31$P n.m.r. $\delta$(CH$_3$OD) -2.291

$^31$P n.m.r. $\delta$(CD$_3$OD, 164 MHz, not referenced to H$_3$PO$_4$) 1.089, 1.044 (1:1)

$^{13}$C n.m.r. $\delta$(CD$_3$OD) 157.215 (C6), 153.858, 153.770 (2xs, C2), 150.539, 150.492 (2xs, C4), 142.626 (m, C8), 119.596, 119.558 (2xs, C5), 96.218 (d, CCl$_3$, J=11.0 Hz), 86.374 (m, C1′), 83.925 (m, C4′), 78.113 (d, CCl$_3$CH$_2$OP J=4.3 Hz) 77.417, 77.303 (2xs, C2′ or C3′), 76.974, 76.862 (2xs, C3′ or C2′), 71.932, 71.810 (2xd, CH$_3$CH$_2$OP, J=6.0 Hz, 6.1 Hz), 69.194, 69.114 (2xd, C5′, J=6.1 Hz, 5.9 Hz), 24.552 (d, CH$_3$CH$_3$, J=6.7 Hz), 10.250 (CH$_3$)
H n.m.r. δ(CD$_3$OD) 8.343, 8.331 (1H, 2x, H2), 8.191 (1H, s, H8), 6.459 (1H, t, 1H', J=3.87 Hz), 4.537-4.642 (3H, m, H2', CCl$_3$CH$_2$OP), 4.391 (1H, m, H3'), 4.288 (2H, m, H5'), 4.168 (1H, m, H4'), 4.112 (2H, q, CH$_3$CH$_2$OP, J=6.83 Hz), 1.711 (2H, m, CH$_3$CH$_2$), 0.953, 0.929 (3H, 2xt, CH$_3$, J=4.1 Hz, 4.1 Hz)

F.A.B.M.S m/e 524 (MH$^+$, 2x$^{35}$Cl, 4.06%), 522 (MH$^+$, $^{37}$Cl, 14.61%), 520 (MH$^+$, 13.06%), 387 (MH$^+$ - adenine, $^{37}$Cl, 0.35%), 385 (MH$^+$ - adenine, 0.36%), 273 ((PrO)(CCl$_3$CH$_2$O)P(OH)$_2$)$^+$, $^{37}$Cl, 0.21%), 271 ((PrO)(CCl$_3$CH$_2$O)P(OH)$_2$)$^+$, 0.27%), 250 (MH$^+$ - (PrO)(CCl$_3$CH$_2$O)PO$_2$H, 2.48%), 233 (CCl$_3$CH$_2$OP(OH)$_3$)$^+$, 2x$^{35}$Cl, 0.15%), 231 (CCl$_3$CH$_2$OP(OH)$_3$)$^+$, $^{37}$Cl, 0.88%), 229 (CCl$_3$CH$_2$OP(OH)$_3$)$^+$, 0.89%), 164 (adenineCHO$^+$, 11.56%), 141 (PrOP(OH)$_3$)$^+$, 1.14%), 136 (adenineH$^+$, 100%), 135 (adenine$^+$, 8.42%), 115 (C$_3$H$_7$O$_3$)$^+$, 3.90%), 99 (C$_3$H$_7$O$_2$)$^+$, 13.58%), 97 (C$_3$H$_5$O$_2$)$^+$, 5.86%), 81 (C$_3$H$_5$O$^+$, 6.29%)

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column

Mobile phase: methanol/water/2,2,2-trifluoroacetic acid (50/50/0.1), isocratic conditions

(83): retention time 12.75 min

Ethyl phosphorodichloridate (84)

Phosphoryl chloride (15 ml, 24.675 g, 0.161 mol) was dissolved in diethyl ether (200 ml) and cooled to -78°C. A solution of ethanol (9.445 ml, 7.414 g, 0.161 mol) and triethylamine (22.43 ml, 16.284 g, 0.161 mol) dissolved in diethyl ether (200 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (17 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure, yielding a colourless oil (84) (22.936 g, 87%).
$^{31}$P n.m.r. δ($\text{CDCl}_3$) 4.837

$^{13}$C n.m.r. δ($\text{CDCl}_3$) 68.535 (d, CH$_2$OP, J=9.0 Hz), 15.377 (d, CH$_3$, J=8.9 Hz)

$^1$H n.m.r. δ($\text{CDCl}_3$) 4.353 (2H, m, CH$_2$OP), 1.418 (3H, td, CH$_3$, J=7.16 Hz, 1.30 Hz)

E.I.M.S. m/e 163 (M* - H, $^{37}$Cl, 0.65%), 160.9323 (VP - H, C$_2$H$_4$Cl$_2$O$_2$P requires 160.9326, 1.26%), 151 (M* - CH$_3$, 2x$^{37}$Cl, 1.89%), 149 (M* - CH$_3$, $^{37}$Cl, 15.65%), 147 (M* - CH$_3$, 25.12%), 139 ((HO)$_2$PCl$_2$*, 2x$^{37}$Cl, 9.63%), 137 ((HO)$_2$PCl$_2$*, $^{37}$Cl, 63.96%), 135 ((HO)$_2$PCl$_2$*, 100%), 129 (M* - Cl, $^{37}$Cl, 3.20%), 127 (M* - Cl, 10.05%), 122 (HOPCl$^+$, 2x$^{37}$Cl, 0.39%), 121 (OPCl$^+$, 2x$^{37}$Cl, 3.18%), 120 (HOPCl$^+$, $^{37}$Cl, 5.69%), 119 (OPCl$^+$, $^{37}$Cl, 19.54%), 118 (HOPCl$^+$, 9.36%), 117 (OPCl$^+$, 28.94%), 101 (OP(OH)$^{37}$Cl$^+$, 11.69%), 99 (OP(OH)Cl$^+$, 34.78%), 45 (C$_2$H$_4$O$^*$, 45.86%)

Microanalysis

Found: C 14.99%; H 3.31%; Cl 43.32%
C$_2$H$_4$Cl$_2$O$_2$P requires: C 14.74%; H 3.09%; Cl 43.52%

Ethyl 2,2,2-trichloroethyl phosphorochloridate (85)

Ethyl phosphorodichloridate (84) (4.50 g, 0.028 mol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2,2,2-trichloroethanol (2.65 ml, 4.13g, 0.028 mol) and triethylamine (3.85 ml, 2.80 g, 0.028 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (16 h) and stirred for a further 56 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure to give a pale yellow oil (7.055 g). A minor impurity of unreacted starting material (84) was removed by stirring the crude product under reduced pressure (0.07 mm Hg) at
ca. 30°C for 8 h, yielding a pale yellow oil (85) (5.966 g, ca. 78%).

\[ ^{31}P \text{n.m.r.} \delta(\text{CDCl}_3) 1.219 \text{ (major, (85))}, -6.786 \text{ (minor)} \]

\[ ^{13}C \text{n.m.r.} \delta(\text{CDCl}_3) 93.813 \text{ (d, CCl}_3, J=12.8 \text{ Hz),} 77.108 \text{ (d, CCl}_3\text{CH}_2\text{OP,} J=5.4 \text{ Hz),} 67.076 \text{ (d, CCl}_3\text{H}_2\text{OP,} J=7.4 \text{ Hz),} 15.589 \text{ (d, CH}_3, J=7.8 \text{ Hz)} \]

\[ ^{1}H \text{n.m.r.} \delta(\text{CDCl}_3) 4.622 \text{ (2H, m, CCl}_3\text{CH}_2\text{OP),} 4.303 \text{ (2H, m, CH}_3\text{CH}_2\text{OP),} 1.391 \text{ (3H, td, CH}_3, J=7.18 \text{ Hz,} 1.13 \text{ Hz)} \]

E.I.M.S. m/e 251 (M⁺ - Et, 3x^37Cl, 0.70%), 249 (M⁺ - Et, 2x^37Cl, 5.87%), 247 (M⁺ - Et, ^37Cl, 12.88%), 245 (M⁺ - Et, 9.56%), 214 (M⁺ - Et - Cl, 2x^37Cl, 9.03%), 212 (M⁺ - Et - Cl, ^37Cl, 29.69%), 210 (M⁺ - Et - Cl, 32.31%), 158 (M⁺ - H - CCl₃, 2x^37Cl, 11.59%), 156 (M⁺ - H - CCl₃, ^37Cl, 31.34%), 131 ((CH₂O)(HO)PO^37Cl⁺, 14.25%), 129 ((CH₂O(HO)POCl⁺, 100%), 119 ((HO)₂P^37Cl⁺, 25.09%), 117 ((HO)₂PCl⁺, 66.67%), 101 (OP(OH)^37Cl⁺, 8.32%), 99 (OP(OH)Cl⁺, 22.33%), 45 (C₂H₂O⁺, 17.75%)

9-β-D-Arabinofuranosyladenine-5’-ethyl (2,2,2-trichloroethyl) phosphate (86)
AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and ethyl 2,2,2-trichloroethyl phosphorochloridate (85) (0.65 g, 2.36 mmol, 2.5 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 47 h. The reaction mixture was quenched with deionized water (45 μl, 2.5 mmol) and solvent removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting orange/brown oil was purified by successive column chromatography on silica, eluting with 5% methanol in chloroform and 5%...
methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions yielded a white solid (86) (0.207 g, 44%).

\[ ^{31}P \text{n.m.r.} \delta(CH_3OH/D_2O) \delta = -2.090 \]

\[ ^{13}C \text{n.m.r.} \delta(CD_2OD) 157.204 (C6), 153.807, 153.782 (2xs, C2), 150.534, 150.483 (2xs, C4), 142.630, 142.554 (2xs, C8), 119.606, 119.563 (2xs, C5), 96.211 (d, CCl_3, J=11.6 Hz), 86.434, 86.303 (2xs, C1'), 83.884 (bs, C4'), 78.104 (d, CCl_3CH_2OP, J=4.2 Hz), 77.347 (C2' or C3'), 76.922 (C2' or C3'), 69.151, 69.042 (2xd, C5', J=5.7 Hz, 5.8 Hz), 66.606, 66.540 (2xd, CH_2CH_2OP, J=6.5 Hz, 6.6 Hz), 16.342 (d, CH_3, J=6.4 Hz)

\[ ^{1}H \text{n.m.r.} \delta(CD_2OD) 8.340, 8.324 (1H, 2xs, H2), 8.159 (1H, s, H8), 6.458 (1H, t, H1', J=4.14 Hz) 4.565-4.637 (3H, m, H2', CCl_3CH_2OP), 4.399 (1H, m, H3'), 4.293 (2H, m, H5'), 4.177-4.243 (3H, m, H4', CH_2CH_2OP), 1.348, 1.340 (3H, 2xtd, CH_3, J=7.06 Hz, 1.02 Hz, J=7.10 Hz, 0.98 Hz)

\[ \text{F.A.B.M.S. m/e 510 (MH', 2x}^{35}\text{Cl, 1.90%), 509 (M', 2x}^{37}\text{Cl, 0.14%), 508 (MH', }^{37}\text{Cl, 11.41%), 507 (M', }^{37}\text{Cl, 1.04%), 506 (MH', 12.40%), 505 (M', 1.53%), 250 (MH' - (EtO)(CCl_3CH_2O)PO_2H, 0.23%), 164 (adenineCHO', 3.17%), 136 (adenineH', 100%), 135 (adenine', 49.75%), 99 (C_5H_7O_2', 0.28%), 97 (C_5H_7O_2', 0.23%), 81 (C_5H_7O_2', 0.53%) \]

**Microanalysis**

Found: C 33.48%; H 3.53%; N 13.55%; P 6.62%

C_{14}H_{19}Cl_3N_7O_{7}P requires: C 33.19%; H 3.78%; N 13.82%; P 6.11%
H.P.L.C. (analytical)
Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column
Mobile phase: methanol/water (50/50), isocratic conditions
(86): retention time 13.93 min

**Bis(2,2,2-trichloroethyl) phosphorochloridate (87)**

Phosphoryl chloride (9.7 ml, 15.96 g, 0.104 mol) was dissolved in diethyl ether (75 ml) and cooled to -78°C. A solution of 2,2,2-trichloroethanol (20 ml, 31.14 g, 0.208 mol) and triethylamine (29 ml, 21.05 g, 0.208 mol) dissolved in diethyl ether (75 ml) was added dropwise over 2.5 h with vigorous stirring to the cooled solution. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (24 h). The reaction mixture was filtered and solvent removed under reduced pressure to give a yellow oil (35.501 g). The crude product was purified by distillation under reduced pressure (0.5 mm Hg) using a Kugelruhr, yielding a white solid (87) (25.339 g, 64%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 1.554

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 93.455 (d, CCl$_3$, J=11.8 Hz), 77.669 (d, CH$_2$OP, J=3.9 Hz)

$^1$H n.m.r. $\delta$(CDCl$_3$) 4.715 (4H, d, CH$_2$OP, J=7.86 Hz)

F.A.B.M.S. m/e 383 (MH$^+$, $3x^{37}$Cl, 0.31%), 381 (MH$^+$, $2x^{37}$Cl, 1.15%), 379 (MH$^+$, $^{37}$Cl, 1.18%), 377 (MH$^+$, 0.15%), 215 (CCl$_3$CH$_2$OPO$_2$H$^+$, $2x^{37}$Cl, 0.58%), 213 (CCl$_3$CH$_2$OPO$_2$H$^+$, $^{37}$Cl, 4.40%), 211 (CCl$_3$CH$_2$OPO$_2$H$^+$, 4.52%), 135 (CCl$_3$CH$_2^+$, $2x^{37}$Cl, 6.00%), 133 (CCl$_3$CH$_2^+$, $^{37}$Cl, 20.02%) 131 (CCl$_3$CH$_2^+$, 21.21%), 119 ((HO)$_2$P$^{37}$Cl$^+$, 11.28%), 117 ((HO)$_2$PCl$^+$, 25.45%)
9-β-D-Arabinofuranosyladenine-5'-bis(2,2,2-trichloroethyl) phosphate (43)

AraA (0.75 g, 2.81 mmol) was dissolved in pyridine (100 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and bis(2,2,2-trichloroethyl) phosphorochloridate (87) (2.13 g, 5.62 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for a further 3 h. After warming to ambient temperature, the reaction mixture was quenched with deionized water (150 µl, 8.33 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 50 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting pale yellow solid was purified by column chromatography on silica, eluting with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions yielded a white solid (43) (0.907 g, 53%).

$^{31}$P n.m.r. δ(CH$_3$OD) -3.892

$^{13}$C n.m.r δ(CD$_3$OD) 157.244 (C6), 153.854 (C2), 150.614 (C4), 142.700 (C8), 119.649 (C5), 97.715, 95.927 (2xd, CCl$_3$, J=12.6 Hz, 11.9 Hz), 86.385 (Cl'), 83.859 (d, C4', J=6.1 Hz), 78.451, 78.081 (2xd, CH$_2$OP, J=3.6 Hz, 3.3 Hz), 77.523 (C2' or C3'), 76.985 (C2' or C3'), 70.104 (d, C5', J=5.6 Hz)

$^1$H n.m.r. δ(CD$_3$OD) 8.370 (1H, s, H2), 8.192 (1H, s, H8), 6.473 (1H, d, H1', J=3.52 Hz), 4.708 (4H, m, CH$_2$OP), 4.474-4.539 (2H, m, H2', H3'), 4.296 (2H, m, H5'), 4.211 (1H, m, H4')

F.A.B.M.S. (tetraglyme) m/e 616 (MH$^+$, 4x$^{37}$Cl, 0.58%), 615 (MH$_2^+$, 3x$^{37}$Cl, 0.58%), 614 (MH$^+$, 3x$^{37}$Cl, 1.74%), 613 (MH$_2^+$, 2x$^{37}$Cl, 1.16%), 612 (MH$^+$, 2x$^{37}$Cl, 4.07%), 611 (MH$_3^+$, $^{37}$Cl, 1.74%), 610 (MH$^+$, $^{37}$Cl, 4.94%), 609 (MH$_2^+$, 1.16%), 608 (MH$^+$, 2.62%), 250 (MH$^+$ - (CCl$_3$CH$_2$O)$_2$PO$_2$H, 4.65%), 164 (adenineCHO$^+$, 4.65%), 136 (adenineH$^+$, 11.05%), 135 (adenine$^+$, 4.36%), 115 (C$_3$H$_7$O$_3^+$, 6.10%), 99 (C$_3$H$_4$O$_2^+$, 5.81%), 97 (C$_3$H$_2$O$_2^+$, 1.45%)
Microanalysis

Found: C 26.02%; H 2.54%; N 9.93%

C_{14}H_{16}Cl_{6}N_{2}O_{3}P(H_{2}O)_{2.0} requires: C 26.03%; H 3.12%; N 10.84%

**H.P.L.C. (analytical)**

Stationary phase: 50 + 250 x 4.6 mm Spherisorb ODS2 5 μM column

Mobile phase: methanol/water/triethylamine (60/40/0.01), isocratic conditions

(43): retention time 15.64 min

Attempted synthesis of 9-β-D-arabinofuranosyladenine-5′-butyl (2,2,2-trichloroethyl) phosphate (80) via a transesterification reaction

A suspension of 9-β-D-arabinofuranosyladenine-5′-bis(2,2,2-trichloroethyl) phosphate (43) (0.03 g, 0.049 mmol) and caesium fluoride (0.27 g, 1.78 mmol, 36 molar equivs.) in n-butanol (3 ml) was stirred in a dry atmosphere at ambient temperature for 232 h. Solvent was removed from the reaction mixture under reduced pressure to give a white solid which was purified by column chromatography on silica, eluting with 10% methanol in ethyl acetate. Pooling and evaporation of appropriate fractions yielded a slightly impure white solid (80) (0.015 g).

$^{31}$P n.m.r. δ(CH$_3$OD, 164 MHz) -1.658 (minor), -2.452 (major, (80))

Synthesis of 9-β-D-arabinofuranosyladenine-5′-butyl (2,2,2-trichloroethyl) phosphate (80) via a transesterification reaction

A suspension of 9-β-D-arabinofuranosyladenine-5′-bis(2,2,2-trichloroethyl) phosphate (43) (0.26 g, 0.426 mmol) and caesium fluoride (1.94 g, 12.77 mmol, 30 molar equivs.) in n-butanol (26 ml) was stirred in a dry atmosphere at ambient temperature for 382 h. Solvent was removed from the reaction mixture under reduced pressure to give a white solid which was purified by column chromatography on silica, eluting with 10% methanol in ethyl acetate. Pooling and evaporation of appropriate fractions yielded an impure white solid (0.150 g).
The product was further purified by reverse phase preparative H.P.L.C. (stationary phase: 250 x 10 mm Kromasil C18 7 µM column; mobile phase: methanol/water/2,2,2-trifluoroacetic acid (55/45/0.1), isocratic conditions; (80): retention time 7.81 min). Pooling and evaporation of appropriate fractions yielded a white solid (80) (0.058 g, 25%).

\[ ^{31}P \text{ n.m.r.} \delta(\text{CH}_3\text{OD}) \quad -0.489 \text{ (major), -2.298 (major, (80)), -4.274 (bs, minor)} \]

\[ ^{13}C \text{ n.m.r.} \delta(\text{CD}_3\text{OD}) \quad 157.239 \text{ (C6), 153.689, 153.618 (2xs, C2), 150.504, 150.433 (2xs, C4), 142.581, 142.556 (2xs, C8), 119.639, 119.589 (2xs, C5), 96.739 (d, CCl}_3, J=12.0 \text{ Hz}, 86.981, 86.853 (2xs, C1'), 84.312 (d, C4', J=7.0 \text{ Hz}), 78.154 (d, CCl}_3\text{CH}_2\text{OP, J=4.5 Hz}, 77.154 \text{ (C2' or C3'), 76.968 (C2' or C3'), 70.139 (m, C5'), 69.127 (d, CH}_2\text{CH}_2\text{OP, J=5.5 Hz), 33.222 (d, CH}_2\text{CH}_2\text{OP, J=6.7 Hz), 19.660, 19.638 (2xs, CH}_2\text{CH}_2, 13.857, 13.831 (2xs, CH}_3 \]

\[ ^{1}H \text{ n.m.r.} \delta(\text{CD}_3\text{OD}) \quad 8.489, 8.484 (1H, 2xs, H2), 8.349 (1H, s, H8), 6.510, 6.505 (1H, 2xd, H1'), J=4.43 \text{ Hz, 4.34 Hz), 4.524-4.666 (3H, m, H2', CCl}_3\text{CH}_2\text{OP), 4.409 (1H, m, H3'), 4.295 (2H, m, H5'), 4.143-4.211 (3H, m, H4', CH}_2\text{CH}_2\text{OP), 1.681 (2H, m, CH}_2\text{CH}_2\text{OP), 1.404 (2H, m, CH}_3\text{CH}_2), 0.923 (3H, m, CH}_3 \]

F.A.B.M.S. m/e 537 (M\(^{+}\), 2x\(^{37}\text{Cl}, 3.19\%), 535 (M\(^{+}\), \(^{37}\text{Cl}, 11.02\%), 533 (M\(^{+}\), 10.37\%), 250 (MH\(^{+}\) -(BuO)(CCl}_3\text{CH}_2\text{OP)OH}, 2.76\%), 233 (CCl}_3\text{CH}_2\text{OP(OH)}_2, 2x\(^{37}\text{Cl}, 0.02\%), 231 (CCl}_3\text{CH}_2\text{OP(OH)}_2, \(^{37}\text{Cl}, 0.78\%), 229 (CCl}_3\text{CH}_2\text{OP(OH)}_2, 0.72\%), 164 (adenineCHO\(^{+}\), 10.17\%), 155 (BuOP(OH))\(^{+}\), 2.29\%), 136 (adenineH\(^{+}, 100\%), 135 (adenine\(^{+}, 6.47\%), 115 (\text{C}_4\text{H}_2\text{O}_3, 4.15\%), 99 (\text{C}_4\text{H}_2\text{O}_3, 18.82\%), 97 (\text{C}_4\text{H}_2\text{O}_3, 5.56\%), 81 (\text{C}_4\text{H}_2\text{O}_3, 6.06\%), 57 (\text{C}_4\text{H}_2, 17.12\%), 55 (\text{C}_4\text{H}_2, 22.02\%) \]
H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column

Mobile phase: methanol/water/2,2,2-trifluoroacetic acid (60/40/0.1), isocratic conditions

(80): retention time 8.01 min

Attempted synthesis of 9-β-D-arabinofuranosyladenine-5′-methyl (2,2,2-trichloroethyl) phosphate (88) via a transesterification reaction

A solution of 9-β-D-arabinofuranosyladenine-5′-bis(2,2,2-trichloroethyl) phosphate (43) (0.03 g, 0.049 mmol) and caesium fluoride (0.23 g, 1.51 mmol, 30.8 molar equivs.) in anhydrous methanol (3 ml) was stirred in a dry atmosphere at ambient temperature for 161 h. Solvent was removed under reduced pressure and the resulting cream solid was purified by column chromatography on silica, eluting with 10% methanol in ethyl acetate. Pooling and evaporation of the appropriate fractions yielded an impure cream solid (88) (0.020 g).

$^{31}$P n.m.r. δ(CD$_3$OD, 164 MHz) 1.657, 1.623 (1:1, major, (88)), -3.327 (minor, (43))

$^1$H n.m.r. δ(CD$_3$OD) 8.301 (1H, s, H2), 8.193 (1H, s, H8), 6.450 (1H, d, H1′, J=4.25 Hz), 4.128-4.489 (7H, m, CH$_2$OP, H2′, H3′, H4′, H5′), 3.770, 3.764 (3H, 2xd, CH$_3$, J=11.17 Hz, 11.17 Hz)

Minor peaks were also observed in the proton n.m.r. spectrum due to a nucleosidic impurity.

Bis(2,2,2-trifluoroethyl) phosphorochloridate (90)

Phosphoryl chloride (2.52 ml, 5.15 g, 0.027 mol) was dissolved in diethyl ether (30 ml) and cooled to -40°C. A solution of 2,2,2-trifluoroethanol (3.925 ml, 5.39 g, 0.054 mol) and triethylamine (7.5 ml, 5.45 g, 0.054 mol) dissolved in diethyl ether (30 ml) was added dropwise over 1 h with vigorous stirring to the cooled solution. On completion of the addition, the reaction mixture was stirred at -40°C for a further hour then allowed to warm to ambient temperature
overnight (15 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure to give a colourless oil (6.63 g). The crude product was purified by vacuum distillation (bp 28-30°C, 0.125 mm Hg), yielding a colourless oil (90) (4.89 g, 65%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 5.668

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 121.739 (qd, CF$_3$, $J$=277.6 Hz, 11.2 Hz), 64.63 (qd, CH$_2$OP, $J$=39.1Hz, 5.3Hz)

$^1$H n.m.r. $\delta$(CDCl$_3$) 4.460 (4H, m, CH$_2$OP)

F.A.B.M.S. (tetraglyme) m/e 283 (MH$^+$, $^{37}$Cl, 1.45%), 281 (MH$^+$, 6.98%), 263 (MH$^+$ - HF, $^{37}$Cl, 2.91%), 261 (MH$^+$ - HF, 6.69%), 245 (M$^+$ - Cl, 19.19%), 225 (M$^+$ - HF - Cl, 5.23%), 183 (M$^+$ - CF$_3$CH$_2$O, $^{37}$Cl, 0.29%), 181 (M$^+$ - CF$_3$CH$_2$O, 1.16%), 99 (CF$_3$CH$_2$O$^+$, 6.40%)

9-β-D-Arabinofuranosyladenine-5'-bis(2,2,2-trifluoroethyl) phosphate (42)

AraA (0.5 g, 1.87 mmol) was dissolved in pyridine (60 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and bis(2,2,2-trifluoroethyl) phosphorochloridate (90) (1.05 g, 3.74 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for a further 3.5 h. After warming to ambient temperature, the reaction mixture was quenched with deionized water (70 μl, 3.89 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 25 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting pale orange oil was purified by column chromatography on silica, eluting with 10% methanol in chloroform. Pooling and evaporation of appropriate fractions gave a yellow gum, which on repeated trituration with diethyl ether yielded a white solid (42) (0.438 g, 46%).
$^3$P n.m.r. $\delta$(D$_2$O) -2.023

$^1$H n.m.r. $\delta$(D$_2$O, 200 MHz) 8.155 (1H, s, H2), 8.096 (1H, s, H8), 6.274 (1H, d, H1', $J$=5.4 Hz), 4.024-4.746 (9H, m, H$_2'$, H$_3'$, H$_4'$, H$_5'$, CH$_{OP}$)

9-β-D-Arabinofuranosyladenine-5'-bis(2,2,2-trifluoroethyl) phosphate (89)

A solution of 9-β-D-arabinofuranosyladenine-5'-bis(2,2,2-trifluoroethyl) phosphate (42) (0.205 g, 40.1 mmol) in deionized water (100 ml) was stirred at ambient temperature for 310 h and analyzed at intervals by t.l.c. The reaction mixture was allowed to warm to 37°C and stirred for a further 271 h. An aqueous/organic extraction was carried out, adding diethyl ether (100 ml) to the reaction mixture. Solvent was removed from the aqueous layer by lyophilization under reduced pressure, yielding a white solid (89) (0.107 g, 99%).

$^3$P n.m.r. $\delta$(D$_2$O) -0.744

$^{13}$C n.m.r. $\delta$(D$_2$O) 159.236 (C6), 151.840 (C2), 143.721 (C4), 142.431 (C8), 125.824 (qd, CF$_3$, $J$=277.3 Hz, 9.8 Hz), 122.407 (C5'), 92.349 (d, C4', $J$= 2.3 Hz), 85.844 (C1'), 81.533 (C2' or C3'), 77.340 (C2' or C3'). 65.204 (qd, CH$_{OP}$, $J$=36.5 Hz, 4.7 Hz), 61.131 (d, C5', $J$=5.0 Hz)

$^1$H n.m.r. $\delta$(D$_2$O, 200 MHz) 8.450 (1H, s, H2), 8.122 (1H, s, H8), 6.308 (1H, d, H1', $J$= 5.01 Hz), 3.915-4.868 (7H, m, H$_2'$, H$_3'$, H$_4'$, H$_5'$, CH$_{OP}$)

F.A.B.M.S. m/e 429 (M$, 0.27\%$), 250 (M$^+$ - (CF$_3$CH$_2$O)(HO)PO$_2^+$, 90.40$\%$), 181 (CF$_3$CH$_2$OP(OH)$_2^+$, 0.73$\%$), 164 (adenineCHO$^+$ and CF$_3$CH$_2$OP(OH)$_2^+$, 1.85$\%$), 136 (adenineH$^+$, 94.44$\%$), 135 (adenine$, 9.43\%$), 115 (C$_3$H$_5$O$_5^+$, 5.01$\%$), 99 (C$_3$H$_5$O$_2^+$ and CF$_3$CH$_2$O$^+$, 0.55$\%$), 97 (C$_3$H$_5$O$_2^+$, 4.54$\%$), 83 (CF$_3$CH$_2^+$, 8.47$\%$), 81 (C$_3$H$_5$O$^+$, 12.01$\%$), 69 (CF$_3^+$, 24.19$\%$)
Hydrolysis of 9-β-D-arabinofuranosyladenine-5'-bis(2,2,2-trichloroethyl) phosphate (43)

A solution of 9-β-D-arabinofuranosyladenine-5'-bis(2,2,2-trichloroethyl) phosphate (43) (0.199 g, 0.326 mmol) in deionized water (80 ml) was stirred at reflux for 93 h and analyzed at intervals by t.l.c.. An aqueous/organic extraction was carried out, adding diethyl ether (80 ml) to the reaction mixture. Solvent was removed from the aqueous layer by lyophilization under reduced pressure, yielding a slightly impure white solid (91) (0.161 g).

$^{31}$P n.m.r. δ(CH$_3$OD) -2.238

Analysis of the crude product by t.l.c. revealed the presence of araA. Recrystallization of a sample of the crude product from ethyl acetate/methanol (4:1 by volume) proved unsuccessful. Attempted purification of a sample of the crude product by reverse phase preparative H.P.L.C. (stationary phase: 250 x 10 mm Spherisorb ODS2 5 μM column; mobile phase: water (A)/acetonitrile (B) (both contain 0.1% 2,2,2-trifluoroacetic acid), gradient conditions 0 min 0% (B), 18 min 18% (B), 20 min 80% (B), 30 min 80% (B), 40 min 80% (B)) also proved unsuccessful.

Hydrolysis of 9-β-D-arabinofuranosyladenine-5'-butyl (2,2,2-trichloroethyl) phosphate (80) and isolation of araA-5'-2,2,2-trichloroethyl phosphate (91)

A solution of 9-β-D-arabinofuranosyladenine-5'-butyl (2,2,2-trichloroethyl) phosphate (80) (0.106 g, 0.198 mmol) in deionized water (49 ml) was stirred at reflux for 118 h and analyzed at intervals by t.l.c. An aqueous/organic extraction was carried out, adding diethyl ether (49 ml) to
the reaction mixture. Solvent was removed from the aqueous layer by lyophilization under reduced pressure, yielding a white solid (0.108 g)

$^31$P n.m.r. $\delta$(D$_2$O) -0.074 (major), -0.723 (minor)

Attempted purification of a sample of the crude product by recrystallization from ethyl acetate/methanol (2:1 by volume) proved unsuccessful. A sample of the crude product was purified by reverse phase preparative H.P.L.C. (stationary phase: 250 x 10 mm Spherisorb ODS2 5 µM column; mobile phase: water (containing 0.05% triethylamine + 2,2,2-trifluoroacetic acid until pH 6.8) (A)/acetonitrile (B), gradient conditions 0 min 0% (B), 20 min 20% (B), 30 min 20% (B), 40 min 20% (B); (91) retention time 19.31 min). Pooling and evaporation of appropriate fractions yielded a white solid (91) (0.006 g from 0.033 g).

$^31$P n.m.r. $\delta$(D$_2$O, 164 MHz, not referenced to H$_3$PO$_4$) -0.649

$^{13}$C n.m.r. $\delta$(D$_2$O) 157.923 (C6), 154.956 (C2), 151.575 (C4), 144.001 (C8), 120.754 (C5), 97.871 (d, CCl$_3$, J=12.6 Hz), 85.383 (C1'), 83.230 (d, C4', J=8.7 Hz), 78.606 (d, CH$_2$OP, J=3.9 Hz), 77.976 (C2' or C3'), 75.953 (C2' or C3'), 66.883 (d, C5', J=5.4 Hz)

$^1$H n.m.r. $\delta$(D$_2$O) 8.423 (1H, s, H2), 8.266 (1H, s, H8), 6.450 (1H, d, J=6.17 Hz), 4.149-4.719 (7H, m, H2', H3', H4', H5', CH$_2$OP)

F.A.B.M.S. m/e 479 (M$^+$, 37Cl, < 0.01%), 477 (M$^+$, < 0.01%), 459 (M$^+$ - H$_2$O, 0.03%), 213 (CCl$_3$CH$_2$OPO$_2$H$^+$, 37Cl, 0.07%), 211 (CCl$_3$CH$_2$OPO$_2$H$^+$, 0.05%), 197 (CCl$_3$CH$_2$OPOH$^+$, 37Cl, 0.08%), 195 (CCl$_3$CH$_2$OPOH$^+$, 0.09%), 164 (adenineCHO$^+$, 1.09%), 136 (adenineH$^+$, 83.99%), 135 (adenine$^+$, 6.49%), 133 (CCl$_3$CH$_2$+$^+$, 37Cl, 0.35%), 131 (CCl$_3$CH$_2$+$^+$, 0.40%), 115 (C$_3$H$_5$O$^+$, 4.43%), 99 (C$_3$H$_7$O$_2$+$^+$, 0.20%), 97 (C$_3$H$_5$O$_2$+$^+$, 0.48%), 81 (C$_3$H$_4$O$^+$, 3.14%)
H.P.L.C. (analytical)
Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column
Mobile phase: water (contains 0.1% 2,2,2-trifluoroacetic acid + 2% methanol), isocratic conditions
(91): retention time 7.26 min

Ethyl phenyl phosphorochloridate (93)
Ethyl phosphorodichloridate (84) (2.5 g, 0.015 mol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of phenol (1.44 g, 0.015 mol) and triethylamine (2.14 ml, 1.55 g, 0.015 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (16 h) and stirred for a further 50 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy pale yellow oil was extracted with hexane, yielding a colourless oil (93) (3.41 g, 100%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) -2.338

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 149.659 (d, ipso-Ph, J=8.7 Hz), 129.839 (meta-Ph), 126.053 (para-Ph), 120.203 (d, ortho-Ph, J=5.3 Hz), 66.657 (d, CH$_2$OP, J=7.5 Hz), 15.609 (d, CH$_3$, J=7.7 Hz)

$^1$H n.m.r. $\delta$(CDCl$_3$) 7.187-7.396 (5H, m, Ph), 4.368 (2H, m, CH$_2$OP), 1.430 (3H, m, CH$_3$)

E.I.M.S. m/e 223 (MH$^+$, $^{35}$Cl, 0.20%), 222 (M$^+$, $^{37}$Cl, 6.31%), 221 (MH$^+$, 1.69%), 220.0060 (M$^+$, C$_8$H$_{10}$ClO$_3$P requires 220.0056, 20.86%), 207 (M$^+$ - Me, $^{37}$Cl, 0.31%), 205 (M$^+$ - Me, 1.61%), 194 (MH$^+$ - Et, $^{37}$Cl, 9.5%), 192 (MH$^+$ - Et, 29.38%), 185 (M$^+$ - Cl, 0.66%), 94 (C$_6$H$_5$OH$^+$, 100%), 77 (C$_6$H$_5^+$, 17.53%)
Microanalysis

Found: C 44.71%; H 4.78%; P 14.42%

C₄H₁₀ClO₃P requires: C 43.56%; H 4.57%; P 14.01%

9-β-D-Arabinofuranosyladenine-5′-ethyl phenyl phosphate (94)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and ethyl phenyl phosphorochloridate (93) (0.42 g, 1.90 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 21 h. The reaction mixture was quenched with deionized water (35 µl, 1.94 mmol) and solvent removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting white solid was purified by successive column chromatography on silica, eluting with 5% methanol in chloroform and 5% methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions gave a white solid (94) (0.167 g, 40%).

³¹P n.m.r. δ(CH₂OD) -5.889, -6.009 (1:1)

¹³C n.m.r. δ(CD₂OD) 157.183 (C6), 153.773, 153.749 (2xs, C2), 151.840 (d, ipso-Ph, J=7.1 Hz), 150.460 (C4), 142.525, 142.484 (2xs, C8), 130.926, 130.882 (2xs, meta-Ph), 126.550, 126.525 (2xs, para-Ph), 121.129 (m, ortho-Ph), 119.571 (C5), 86.506, 86.399 (2xs, C1’), 84.001, 83.872 (2xd, C4’, J=7.4 Hz, 7.7 Hz), 77.317, 77.272 (2xs, C2’ or C3’), 76.909 (C2’ or C3’), 69.030 (m, C5’), 66.539 (m, CH₂OP), 16.338 (d, CH₃, J=6.8 Hz)

¹H n.m.r. δ(CD₂OD) 8.288, 8.243 (1H, 2xs, H2), 8.182 (1H, s, H8), 7.155-7.351 (5H, m, Ph), 6.446, 6.431 (1H, 2xd, H1’, J=4.09 Hz, 4.15 Hz), 4.533 (1H, m, H2’), 4.420 (1H, m, H3’), 4.144-4.293 (5H, m, H4’, H5’, CH₂OP), 1.277-1.328 (3H, m, CH₃)
F.A.B.M.S. (NO₂C₆H₅CH₂OH/NaI) m/e 474 (MNa⁺, 0.34%), 452 (MH⁺, 34.56%), 317 (MH⁺ - adenine, 4.64%), 250 (MH⁺ - (PhO)(EtO)PO₂H, 3.00%), 203 ((PhO)(EtO)P(OH)₃⁺, 7.13%), 175 (PhOP(OH)₂⁺, 23.18%), 164 (adenineCHO⁺, 10.86%), 136 (adenineH⁺, 100%), 135 (adenine⁺, 5.95%), 127 (EtOP(OH)₂⁺, 0.14%), 115 (C₄H₇O₃⁺, 2.88%), 99 (C₄H₆O₂⁺, 0.29%), 97 (C₄H₆O₂⁺, 3.68%), 94 (C₆H₄OH⁺, 4.09%)

Microanalysis

Found: C 47.46%; H 4.92%; N 14.51%; P 6.48%

C₁₅H₂₂N₃O₃P(H₂O)₀.₅ requires: C 46.96%; H 5.04%; N 15.21%; P 6.73%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column

Mobile phase: methanol/water/triethylamine (50/50/0.01), isocratic conditions

(94): retention time 8.88 min

2,2,2-Trichloroethyl phenyl phosphorochloridate (96)

Phenyl phosphorodichloridate (1.79 ml, 2.53 g, 0.012 mol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2,2,2-trichloroethanol (1.15 ml, 1.79 g, 0.012 mol) and triethylamine (1.67 ml, 1.21 g, 0.012 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (16 h) and stirred for a further 30 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy white oil was extracted with hexane to yield a colourless oil (96) (4.116 g, 100%).

³¹P n.m.r. δ(CDCl₃) -2.546 (major, (96)), -11.168 (minor)
\(^{13}\text{C n.m.r.}\) δ(CDC\textsubscript{3}) 149.360 (d, ipso-Ph, J=9.0 Hz), 129.998 (meta-Ph), 126.535 (para-Ph), 120.230 (d, ortho-Ph, J=5.3 Hz), 93.605 (d, CCl\textsubscript{3}, J=12.7 Hz), 77.651 (CH\textsubscript{2}OP, J=5.7 Hz)

\(^{1}\text{H n.m.r.}\) δ(CDC\textsubscript{3}) 7.228-7.430 (5H, m, Ph), 4.736 (2H, m, CH\textsubscript{2}OP)

E.I.M.S. m/e 328 (M\(^{+}\), 3xCl, 1.91%), 326 (M\(^{+}\), 2xCl\textsubscript{3}, 10.40%), 324 (M\(^{+}\), Cl\textsubscript{3}, 20.96%), 321.8859 (M\(^{+}\), C\textsubscript{6}H\textsubscript{5}Cl\textsubscript{2}O\textsubscript{3}P requires 321.8877, 17.37%), 291 (M\(^{+}\) - Cl, 2xCl\textsubscript{3}, 3.96%), 289 (M\(^{+}\) - Cl, Cl\textsubscript{3}, 13.26%), 287 (M\(^{+}\) - Cl, 13.70%), 207 (M\(^{+}\) - CCl\textsubscript{3}, Cl\textsubscript{3}, 9.85%), 205 (M\(^{+}\) - CCl\textsubscript{3}, 31.15%), 194 (MH\(^{+}\) - CCl\textsubscript{3}CH\textsubscript{2}, Cl\textsubscript{3}, 37.02%), 193 (M\(^{+}\) - CCl\textsubscript{3}CH\textsubscript{2}, Cl\textsubscript{3}, 3.71%), 192 (MH\(^{+}\) - CCl\textsubscript{3}CH\textsubscript{2}, 89.94%), 191 (M\(^{+}\) - CCl\textsubscript{3}CH\textsubscript{2}, 9.40%), 137 (CCl\textsubscript{3}CH\textsubscript{2}+, 3xCl, 0.93%), 135 (CCl\textsubscript{3}CH\textsubscript{2}+, 2xCl\textsubscript{3}, 3.66%), 133 (CCl\textsubscript{3}CH\textsubscript{2}+, Cl\textsubscript{3}, 7.23%), 131 (CCl\textsubscript{3}CH\textsubscript{2}+, 7.60%), 121 (CCl\textsubscript{3}+, 2xCl\textsubscript{3}, 1.29%), 119 (CCl\textsubscript{3}+, Cl\textsubscript{3}, 5.87%), 117 (CCl\textsubscript{3}+, 6.78%), 94 (C\textsubscript{6}H\textsubscript{5}OH\(^{+}\), 100%), 77 (C\textsubscript{6}H\textsubscript{5}+, 85.30%)

9-β-D-Arabinofuranosyladenine-5′-(2,2,2-trichloroethyl) phenyl phosphate (97)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and 2,2,2-trichloroethyl phenyl phosphorochloridate (96) (0.61 g, 1.88 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for an hour, allowed to warm to ambient temperature and stirred for a further 16 h. More phosphorylating agent (96) (0.30 g, 0.926 mmol, 1 molar equiv.) was added dropwise at 0°C with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 72 h. The reaction mixture was quenched with deionized water (50 μl, 2.78 mmol) and solvent removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting orange solid was purified by column chromatography on silica, eluting with 5% methanol in ethyl acetate. Pooling and evaporation of appropriate fractions gave a white solid (0.339 g). Further attempts to purify the product by recrystallizations from ethyl
acetate and ethanol/hexane proved unsuccessful. The product was finally purified by reverse phase preparative H.P.L.C. (stationary phase: 250 x 10 mm Spherisorb ODS2 5 μM column; mobile phase: water (A)/methanol (B) (both contain 0.1% 2,2,2-trifluoroacetic acid), gradient conditions 0 min 0% (B), 20 min 100% (B), 40 min 100% (B); (97) retention time 19.5 min). Pooling and evaporation of appropriate fractions yielded a white solid (97) (0.130 g, 25%).

$^{31}$P n.m.r. $\delta$(CD$_3$OD, 205 MHz) -7.634, -7.763 (1:1)

$^{13}$C n.m.r. $\delta$(CD$_3$OD) 157.191 (C6), 153.833, 153.757 (2xs, C2), 151.530 (d, ipso-Ph, J=8.6 Hz), 150.469, 150.405 (2xs, C4), 142.743, 142.699 (2xs, C8), 131.057, 130.975 (2xs, meta-Ph), 126.984, 126.904 (2xs, para-Ph), 121.273, 121.246 (2xd, ortho-Ph, J=4.6 Hz, 4.7 Hz), 119.558 (C5), 95.396 (d, CCl$_3$, J=11.5 Hz), 87.102, 86.984 (2xs, C1'), 84.314, 84.277 (2xd, C4', J=5.9 Hz, 6.0 Hz), 78.590 (d, CH$_2$OP, J=4.4 Hz), 77.096 (C2' or C3'), 76.950 (C2' or C3'), 69.888 (m, C5')

$^1$H n.m.r. $\delta$(CD$_3$OD) 8.506, 8.464 (1H, 2xs, H2), 8.370 (1H, s, H8), 7.171-7.393 (5H, m, Ph), 6.521, 6.496 (1H, 2xd, H1', J=4.30 Hz, 4.27 Hz), 4.636-4.771 (3H, m, H2', CH$_2$OP), 4.527 (1H, m, H3'), 4.304 (2H, m, H5'), 4.222 (1H, m, H4')

F.A.B.M.S. m/e 558 (MH$^+$, 2x$^{35}$Cl, 1.11%), 556 (MH$^+$, $^{37}$Cl, 6.83%), 554 (MH$^+$, 5.71%), 250 (MH$^+$ - (PhO)(CCl$_3$CH$_2$O)PO$_2$H, 1.05%), 175 (PhOP(OH)$_3$)$^+$, 5.86%), 164 (adenineCHO$^+$, 5.96%), 136 (adenineH$^+$, 100%), 135 (adenine$, 7.47%)$, 115 (C$_5$H$_7$O$_2$)$^+$, 3.95%), 97 (C$_5$H$_7$O$_2$)$^+$, 3.06%), 94 (C$_6$H$_5$OH$^+$, 3.44%), 81 (C$_3$H$_7$O$^+$, 6.95%), 77 (C$_6$H$_5^+$, 31.71%)
H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column

Mobile phase: methanol/water/2,2,2-trifluoroacetic acid (50/50/0.1), isocratic conditions

(97): retention time 18.83 min, 20.36 min (1:1)

2,2,2-Trichloroethyl p-nitrophenyl phosphorochloridate (99)

p-Nitrophenyl phosphorodichloridate (3.51 g, 0.014 mmol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2,2,2-trichloroethanol (1.32 ml, 2.06 g, 0.014 mmol) and triethylamine (1.91 ml, 1.39 g, 0.014 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (16 h) and stirred for a further 30 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy yellow oil was extracted with hexane to yield a colourless oil (99) (3.737 g, 79%).

$^{31}P$ n.m.r. $\delta$(CDCl$_3$) -3.155 (major, (99)), -11.871 (minor)

$^{13}C$ n.m.r. $\delta$(CDCl$_3$) 153.570 (d, ipso-Ph, J=8.3 Hz), 145.831 (para-Ph), 125.965 (meta-Ph), 121.269 (d, ortho-Ph J=5.4 Hz), 93.404 (d, CCl$_3$, J=13.4 Hz), 77.865 (d, CH$_2$OP, J=5.2 Hz)

$^1$H n.m.r. $\delta$(CDCl$_3$) 8.293 (2H, d, ortho-Ph, J=9.13 Hz), 7.477 (2H, m, meta-Ph), 4.771 (2H, m, CH$_2$OP)

E.I.M.S. m/e 373 (M$^+$, 3x$^{37}$Cl, 0.91%), 371 (M$^+$, 2x$^{37}$Cl, 8.32%), 369 (M$^+$, $^{37}$Cl, 20.58%), 366.8783 (M$^+$, C$_4$H$_8$Cl$_4$NO$_3$P requires 366.8734, 15.34%), 338 (M$^+$ - Cl, 3x$^{37}$Cl, 0.15%), 336 (M$^+$ - Cl, 2x$^{37}$Cl, 5.64%), 334 (M$^+$ - Cl, $^{37}$Cl, 20.58%), 332 (M$^+$ - Cl, 22.95%), 251 (MH$^+$ - CCl$_3$, $^{37}$Cl, 5.77%), 249 (MH$^+$ - CCl$_3$, 36.21%), 238 (M$^+$ - CCl$_3$CH$_2$, $^{37}$Cl, 11.29%), 236 (M$^+$ - CCl$_3$CH$_2$, 207
Attempted synthesis of 9-β-D-arabinofuranosyladenine-5′-(2,2,2-trichloroethyl) p-nitrophenyl phosphate (100)

AraA (0.175 g, 0.655 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and 2,2,2-trichloroethyl p-nitrophenyl phosphorochloridate (99) (0.48 g, 1.30 mmol, 2 molar equivs.) was added dropwise at 0°C with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 65 h. The reaction mixture was quenched with deionized water (25 µl, 1.39 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure, yielding an orange/brown solid.

³¹P n.m.r. δ(CH₃OH/D₂O) -2.217 (minor), -3.370 (minor), -4.656 (minor), -5.795 (minor), -6.780 (minor), -7.470, -7.711 (1:1, major, (100)), -9.406 (minor)

Attempted purification of the crude product by column chromatography on silica, eluting with 5% methanol in chloroform proved unsuccessful.

2,2,2-Trichloroethyl phosphorodichloridate (101)

Phosphoryl chloride (5 ml, 8.23g, 0.054 mol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2,2,2-trichloroethanol (5.15 ml, 8.02 g, 0.054 mol) and triethylamine (7.48 ml, 5.43 g, 0.054 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (15 h). The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure to give a yellow oil (14.283 g). The crude product was purified by vacuum distillation (bp 48-50°C,
0.4 mm Hg), yielding a colourless oil (101) (10.537 g, 74%) which solidified on standing at ambient temperature.

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 6.431

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 92.889 (d, CCl$_3$, J=13.5 Hz), 78.123 (d, CH$_2$OP, J=7.5 Hz)

$^1$H n.m.r. $\delta$(CDCl$_3$) 4.759 (2H, d, CH$_2$OP, J=9.17 Hz)

F.A.B.M.S. m/e 235 (M$^+$ - Cl, 4x$^{37}$Cl, 0.04%), 233 (M$^+$ - Cl, 3x$^{37}$Cl, 3.02%), 231 (M$^+$ - Cl, 2x$^{37}$Cl, 9.39%), 229 (M$^+$ - Cl, 6.74%), 151 (M$^+$ - CCl$_3$, 2x$^{37}$Cl, 0.60%), 149 (M$^+$ - CCl$_3$, 37Cl, 13.84%), 147 (M$^+$ - CCl$_3$, 21.56%), 121 (OPCl$_2^+$, 2x$^{37}$Cl, 2.45%), 119 (OPCl$_2^+$, $^{37}$Cl, 22.62%), 117 (OPCl$_2^+$, 34.18%)

Microanalysis

Found: C 8.97%; H 0.81%; P 11.17%

C$_2$H$_2$Cl$_2$O$_2$P requires: C 9.02%; H 0.76%; P 11.63%

2,2,2-Trichloroethyl $p$-cyanophenyl phosphorochloridate (102)

2,2,2-Trichloroethyl phosphorodichloridate (101) (2.5 g, 9.39 mmol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of $p$-cyanophenol (1.12 g, 9.40 mmol) and triethylamine (1.31 ml, 0.95 g, 9.40 mmol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (15 h) and stirred for a further 30 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure, yielding a colourless oil (102) (4.27 g, 100%).
\[^{31}\text{P n.m.r.} \delta(\text{CDCl}_3) \text{ } -3.035\]

\[^{13}\text{C n.m.r.} \delta(\text{CDCl}_3) \text{ } 152.250 \text{ (d, ipso-Ph, } J=8.3 \text{ Hz), 134.399 \text{ (meta-Ph), 121.492 \text{ (d, ortho-Ph, } J=5.4 \text{ Hz), 117.573 \text{ (CN), 110.849 \text{ (para-Ph), 93.424 \text{ (d, } C\text{Cl}_3, J=12.5 \text{ Hz), 77.853 \text{ (d, CH}_2\text{OP, } J=5.5 \text{ Hz)}}\text{)}}\]

\[^{1}H \text{n.m.r.} \delta(\text{CDCl}_3) \text{ } 7.347-7.740 \text{ (4H, m, Ph), 4.755 \text{ (2H, m, CH}_2\text{OP)}}\]

\text{E.I.M.S. } m/e 553 (M\(^+\), 3x\(^{37}\text{Cl}, \text{ 0.97\%}), 551 \text{ (M}\(^+\), 2x\(^{37}\text{Cl}, \text{ 5.52\%}), 549 \text{ (M}\(^+\), \text{ } 11.69\%), 546.8844 \text{ (M}\(^+\), C\text{Cl}_6\text{H}_4\text{NO}_3\text{P requires } 546.8839, \text{ 8.88\%}), 316 \text{ (M}\(^+\) - Cl, 2x\(^{37}\text{Cl}, \text{ 3.29\%}), 314 \text{ (M}\(^+\) - Cl, 10.60\%), 312 \text{ (M}\(^+\) - Cl, 10.96\%), 235 \text{ (M}\(^+\) - CNC\text{Cl}_2\text{H}_4\text{O}, 3x\(^{37}\text{Cl}, \text{ 1.80\%}), 233 \text{ (M}\(^+\) - CNC\text{Cl}_2\text{H}_4\text{O, } 2x\(^{37}\text{Cl}, 10.01\%)\text{, 232 (M}\(^+\) - CCI}_3, \text{ 8.41\%), 231 \text{ (M}\(^+\) - CNC\text{Cl}_2\text{H}_4\text{O, } 3^{37}\text{Cl, 21.37\%), 230 (M}\(^+\) - CCI}_3, \text{ 25.38\%), 229 (M}\(^+\) - CNC\text{Cl}_2\text{H}_4\text{O, } 14.85\%)\text{, 219 (MH}\(^+\) - CCI}_3\text{CH}_2, \text{ 37^\text{Cl, 20.18\%), 217 (MH}\(^+\) - CCI}_3\text{CH}_2, \text{ 61.52\%), 151 (CCl}_3\text{CH}_2\text{O}^+, 2x^{37}\text{Cl, 5.98\%}, 149 (CCl}_3\text{CH}_2\text{O}^+, \text{ 37^\text{Cl, 38.53\%), 147 (CCl}_3\text{CH}_2\text{O}^+, 59.09\%), 119 (CNC\text{Cl}_2\text{H}_4\text{OH}^+, \text{ 100\%), 102 (CNC\text{Cl}_2\text{H}_4^+, \text{ 48.22\%), 76 (C}_4\text{H}_4^+, \text{ 18.10\%}}\text{)}}\]

\text{Microanalysis}

Found: C 32.23\%; H 1.84\%; N 3.96\%; P 9.35\%

C\text{Cl}_6\text{H}_4\text{NO}_3\text{P requires: C 30.98\%; H 1.73\%; N 4.01\%; P 8.88\%}

\text{Attempted synthesis of 9-\beta-D-arabinofuranosyladenine-5'-\text{(2,2,2-trichloroethyl) p-cyanophenyl phosphate (103)}}\]

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140\(^\circ\text{C}\). The resulting solution was cooled to 0\(^\circ\text{C}\) and 2,2,2-trichloroethyl p-cyanophenyl phosphorochloridate (102) (0.65 g, 1.87 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred at 0\(^\circ\text{C}\) in a dry atmosphere for 1 h, allowed to warm to ambient temperature and stirred for a further 23 h. The reaction mixture
was quenched with deionized water (35 µl, 1.94 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting pale orange solid was purified by column chromatography on silica, eluting with 0-10% methanol in chloroform. Pooling and evaporation of appropriate fractions yielded an impure white solid (0.325 g). Recrystallization of the product from ethyl acetate proved unsuccessful. The product was further purified by column chromatography on silica, eluting with 5% methanol in ethyl acetate. Pooling and evaporation of appropriate fractions yielded a slightly impure white solid (103) (0.067 g).

$^{31}$P n.m.r. $\delta$(CH$_3$OH/D$_2$O) -7.584, -7.697 (1:1)

$^{31}$P n.m.r. $\delta$(CH$_3$OD, 164 MHz, not referenced to H$_3$PO$_4$) 0.532 to -1.106 (m, minor), -3.910 (minor), -5.477 (minor), -9.838 (minor), -10.144 (minor), -10.749, -10.852 (1:1, major, (103))

A final attempt to purify the product by reverse phase preparative H.P.L.C. (stationary phase: 250 x 10 mm Kromasil C18 5 µM column; mobile phase: acetonitrile/0.01% triethylamine in water (30/70), isocratic conditions; (103): retention time 17.80 min, 20.15 min (1:1)) proved unsuccessful. Analytical H.P.L.C. of the appropriate fraction immediately after its collection from the preparative column indicated that the purified product (103) was decomposing as it was eluted off the preparative column.

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column

Mobile phase: acetonitrile/0.01% triethylamine in water (30/70), isocratic conditions (103): retention time 17.80 min (16.8%), 20.15 min (16.3%)
2,2,2-Trichloroethyl \( p \)-isopropylphenyl phosphorochloridate (104)

2,2,2-Trichloroethyl phosphorodichloridate (101) (2.5 g, 9.39 mmol) was dissolved in diethyl ether (100 ml) and cooled to \(-78^\circ\text{C}\). A solution of \( p \)-isopropylphenol (1.28 g, 9.40 mmol) and triethylamine (1.31 ml, 0.95 g, 9.40 mmol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (15 h) and stirred for a further 30 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure to give a yellow oil (3.449 g). A minor impurity of unreacted starting material (101) was removed by stirring the crude product under reduced pressure (0.03 mm Hg) at ca. 60ºC for 6 h, yielding a pale yellow oil (104) (2.955 g, 86%).

\(^{31}\text{P}\) n.m.r. \(\delta(\text{CDCl}_3)\) -2.271

\(^{13}\text{C}\) n.m.r. \(\delta(\text{CDCl}_3)\) 147.410 (d, \(ipso\)-Ph, \(J=7.6\) Hz), 147.298 (\(para\)-Ph), 127.392 (\(meta\)-Ph), 120.506 (d, \(ortho\)-Ph, \(J=5.0\) Hz), 93.726 (d, \(\text{CCl}_3\), \(J=12.7\) Hz), 77.635 (d, \(\text{CH}_2\)OP, \(J=4.6\) Hz), 33.542 (\(\text{CH(CH}_3)_2\)), 23.946 (\(\text{CH(CH}_3)_2\))

\(^1\text{H}\) n.m.r. \(\delta(\text{CDCl}_3)\) 7.171-7.261 (4H, m, Ph), 4.782 (2H, m, \(\text{CH}_2\)OP), 2.913 (1H, septet, \(\text{CH(CH}_3)_2\)), \(J=7.00\) Hz), 1.235 (6H, d, \(\text{CH(CH}_3)_2\)), \(J=6.91\) Hz)

E.I.M.S. m/e 370 (M\(^+\), 3x\(^{37}\text{Cl}, 0.85\%)\), 368 (M\(^+\), 2x\(^{37}\text{Cl}, 5.52\%)\), 366 (M\(^+\), \(^{37}\text{Cl}, 11.93\%)\), 363.9377 (M\(^+\), \(\text{C}_11\text{H}_{13}\text{Cl}_3\text{O}_3\)P requires 363.9357, 9.12\%), 355 (M\(^+\) - \(\text{CH}_3\), 3x\(^{37}\text{Cl}, 6.95\%)\), 353 (M\(^+\) - \(\text{CH}_3\), 2x\(^{37}\text{Cl}, 33.27\%)\), 351 (M\(^+\) - \(\text{CH}_3\), \(^{37}\text{Cl}, 67.27\%)\), 349 (M\(^+\) - \(\text{CH}_3\), 53.33\%), 333 (M\(^+\) - Cl, 2x\(^{37}\text{Cl}, 0.44\%)\), 331 (M\(^+\) - Cl, \(^{37}\text{Cl}, 1.99\%)\), 329 (M\(^+\) - Cl, 2.07\%), 235 (M\(^+\) - \(\text{CCl}_3\)\(\text{CH}_2\), \(^{37}\text{Cl}, 2.84\%)\), 233 (M\(^+\) - \(\text{CCl}_3\)\(\text{CH}_2\), 11.65\%), 221 (MH\(^+\) - \(\text{CH}_3\) - \(\text{CCl}_3\)\(\text{CH}_2\), \(^{37}\text{Cl}, 20.73\%)\), 219 (MH\(^+\) - \(\text{CH}_3\) - \(\text{CCl}_3\)\(\text{CH}_2\), 65.03\%), 183 (\(\text{CH}_3\)\(\text{CH}_2\)\(\text{H}_2\)\(\text{PO}_2\)\(^+\), 10.34\%), 151 (\(\text{CCl}_3\)\(\text{CH}_2\)O\(^+\), 2x\(^{37}\text{Cl}, 3.12\%)\), 149 (\(\text{CCl}_3\)\(\text{CH}_2\)O\(^+\), \(^{37}\text{Cl}, 21.58\%)\), 147 (\(\text{CCl}_3\)\(\text{CH}_2\)O\(^+\), 32.34\%), 136 ((\(\text{CH}_3\)\(\text{CH}_2\)\(\text{CH}_2\)OH\(^+\), 17.81\%), 135
9-β-D-Arabinofuranosyladenine-5′-(2,2,2-trichloroethyl) p-isopropylphenyl phosphate (105)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and 2,2,2-trichloroethyl p-isopropylphenyl phosphorochloridate (104) (0.69 g, 1.89 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 49 h. The reaction mixture was quenched with deionized water (35 µl, 1.94 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting cream solid was purified by successive column chromatography on silica, eluting with 5% methanol in ethyl acetate and 2.5% methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions gave a cream solid (0.145 g) which was further purified by recrystallization from ethyl acetate, yielding a white solid (105) (0.116 g, 21%).

$^{31}$P n.m.r. $\delta$(CH$_3$OD) -7.222, -7.349 (1:1)

$^{13}$C n.m.r. $\delta$(CD$_3$OD) 157.196 (C6), 153.812, 153.773 (2xd, C2, J=3.9 Hz, 3.9 Hz), 150.532, 150.466 (2xs, C4), 149.466 (d, ipso-Ph, J=7.4 Hz), 147.887, 147.848 (2xs, para-Ph), 142.751,
142.719 (2xs, C8), 128.799, 128.730 (2xs, meta-Ph), 121.060 (d, ortho-Ph, J=4.4 Hz), 119.567 (C5), 96.068 (d, CCl3, J=13.1 Hz), 86.378 (m, Cl''), 83.941, 83.815 (2xd, C4', J=7.1 Hz, 7.4 Hz), 78.455 (d, CH2OP, J=4.4 Hz), 77.331 (C2'' or C3''), 76.894, 76.851 (2xs, C2'' or C3''), 70.013, 69.867 (2xd, C5'', J=6.3 Hz, 6.4 Hz), 34.793, 34.756 (2xs, CH(CH3)3), 24.408, 24.374 (2xs, CH(CH3)3)

1H n.m.r. &CD3OD) 8.345, 8.284 (1H, 2xs, H2), 8.188 (1H, s, H8), 7.138-7.217 (4H, m, Ph), 6.470, 6.436 (1H, 2xd, H1', J=3.8 Hz, 3.5 Hz), 4.613-4.729 (3H, m, H2'', CH2OP), 4.496 (1H, m, H3''), 4.272 (2H, m, H5''), 4.188 (1H, m, H4''), 2.853 (1H, septet, CH(CH3)2, J=7.08 Hz), 1.203, 1.169 (6H, 2xd, CH(CH3)2, J=6.89 Hz, 6.89 Hz)

F.A.B.M.S. m/e 598 (MH+, 37Cl, 1.22%), 596 (MH+, 1.07%), 208 (C6H10OP+, 2.43%), 182 (CCl3CH2OP+, 2x37Cl, 0.13%), 180 (CCl3CH2OP+, 37Cl, 0.41%), 178 (CCl3CH2OP+, 0.49%), 166 ((CH3)2CHC6H4OP+, 0.29%), 164 (adenineCHO+, 0.52%), 136 (adenineH+ or (CH3)2CHC6H4OH+, 3.42%), 135 (adenine+ or (CH3)2CHC6H4O+, 0.99%), 121 (CH3CHC6H4OH+, 1.84%), 120 (CH3CHC6H4O+, 1.97%), 99 (C6H2O5+, 0.03%), 97 (C6H2O5+, 0.17%), 92 (C6H4+, 16.36%), 91 (C6H6+, 9.55%), 81 (C6H2O+, 14.09%), 77 (C6H6+, 18.38%), 44 (C3H8+, 100%)

Microanalysis
Found: C 42.30%; H 4.14%; N 10.74%; Cl 17.84%; P 5.21%
C21H25Cl3N2O3P requires: C 42.26%; H 4.22%; N 11.73%; Cl 17.82%; P 5.19%

H.P.L.C. (analytical)
Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column
Mobile phase: methanol/water/2,2,2-trifluoroacetic acid (65/35/0.1), isocratic conditions
(105): retention time 11.98 min
Biphenyl 2,2,2-trichloroethyl phosphorochloridate (106)

2,2,2-Trichloroethyl phosphorodichloridate (101) (2.5 g, 9.39 mmol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of p-phenylphenol (1.6 g, 9.40 mmol) and triethylamine (1.31 ml, 0.95 g, 9.40 mmol) dissolved in diethyl ether (100 ml) was added dropwise over 1.5 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (17 h) and stirred for a further 100 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure to give a white solid (3.752 g), from which a minor impurity of starting material (101) was removed by a subsequent hexane extraction. Final traces of solvent were removed from the hexane insoluble residue under reduced pressure, yielding a white solid (106) (2.822 g, 75%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) -2.278

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 148.781 (d, ipso-Ph, J=9.1 Hz), 139.844 (ipso'-Ph), 139.607 (para-Ph), 128.851 (meta-Ph), 128.711 (meta-Ph), 127.655 (para'-Ph), 127.027 (ortho-Ph), 120.615 (d, ortho-Ph, J=5.1 Hz), 93.649 (d, CCl$_3$, J=12.7 Hz), 77.688 (d, CH$_2$OP, J=5.3 Hz)

$^1$H n.m.r. $\delta$(CDCl$_3$) 7.208-7.627 (9H, m, Ph), 4.788 (2H, m, CH$_2$OP)

E.I.M.S. m/e 404 (M$, 3x^{37}$Cl, 4.71%), 402 (M$, 2x^{37}$Cl, 29.25%), 400 (M$, ^{37}$Cl, 55.44%), 397.915 (M$, C_6H_4Cl_4O_3P$ requires 397.9200, 38.90%), 367 (M$^+$ - Cl, 2x$^{37}$Cl, 1.66%), 365 (M$^+$ - Cl, $^{37}$Cl, 7.43%), 363 (M$^+$ - Cl, 6.82%), 283 (M$^+$ - CCl$_3$, $^{37}$Cl, 3.88%), 281 (M$^+$ - CCl$_3$, 12.93%), 271 (C$_6$H$_5$C$_6$H$_4$OP(OH)$_2$$^{37}$Cl$^+$, 4.86%), 270 (C$_6$H$_5$C$_6$H$_4$OPO(OH)$^{37}$Cl$^+$, 39.87%), 269 (C$_6$H$_5$C$_6$H$_4$OP(OH)$_2$$^{37}$Cl$^+$, 17.08%), 268 (C$_6$H$_5$C$_6$H$_4$OPO(OH)$^{37}$Cl$^+$, 85.10%), 232 (C$_6$H$_5$C$_6$H$_4$OPO$_2$$^+$, 35.32%), 170 (C$_6$H$_5$C$_6$H$_4$OH$^+$, 100%), 169 (C$_6$H$_5$C$_6$H$_4$O$^+$, 63.71%), 153 (C$_{12}$H$_5^+$, 12.43%), 152 (C$_{13}$H$_2^+$, 22.73%), 151 (C$_{13}$H$_2^+$, 9.67%), 141 (C$_{11}$H$_2^+$, 72.95%), 121 (CCl$_5^+$, 2x$^{37}$Cl, 1.14%), 119 (CCl$_5^+$, $^{37}$Cl, 6.59%), 117 (CCl$_3^+$, 8.38%), 115 (C$_9$H$_7^+$, 68.33%)
Microanalysis
Found: C 42.34%; H 3.01%; P 7.36%

C14H11Cl4O3P requires: C 42.04%; H 2.77%; P 7.74%

9-ß-D-Arabino-furanosyladenine-5’-biphenyl (2,2,2-trichloroethyl) phosphate (107)
AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to ambient temperature and biphenyl 2,2,2-trichloroethyl phosphorochloridate (106) (0.75 g, 1.87 mmol, 2 molar equivs.) was added with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 21 h. The reaction mixture was quenched with deionized water (35 µl, 1.94 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting cream solid was purified by successive column chromatography on silica, eluting with 2.5% methanol in ethyl acetate and 10% methanol in chloroform respectively. Pooling and evaporation of appropriate fractions gave a white solid (0.215 g) which was further purified by recrystallization from ethyl acetate, yielding a white solid (107) (0.115 g, 19%).

$^{31}P$ n.m.r. δ(CH$_3$OH/D$_2$O) -6.940, -7.061 (1:1)

$^{13}$C n.m.r. δ(CD$_3$OD) 157.154, 157.119 (2xs, C6), 153.721, 153.671 (2xs, C2), 150.913 (d, ipso-Ph, J=6.7 Hz), 150.465, 150.448 (2xs, C4), 142.789, 142.744 (2xs, C8) 141.144, 141.081 (2xs, ipso’-Ph), 140.320, 140.218 (2xs, para-Ph), 129.928, 129.900 (2xs, meta’-Ph), 129.463, 129.343 (2xs, meta-Ph), 128.559, 128.521 (2xs, para’-Ph), 127.930, 127.869 (2xs, ortho’-Ph), 121.670, 121.619 (2xd, ortho-Ph, J=5.1 Hz, 5.1 Hz), 119.542 (C5’), 91.745 (d, CCl$_3$, J=12.0 Hz), 86.381 (m, C1’), 83.892 (d, C4’, J=5.4 Hz), 78.530 (d, CH$_3$OP, J=4.2 Hz), 77.400, 77.345 (2xs, C2’ or C3’), 76.897, 76.844 (2xs, C2’ or C3’), 70.092 (d, C5’, J=3.6 Hz)
$^1$H n.m.r. δ(CD$_3$OD) 8.349, 8.292 (1H, 2xs, H2), 8.184, 8.166 (1H, 2xs, H8), 7.286-7.596 (9H, m, Ph), 6.473, 6.434 (1H, 2xd, H1', J=3.31 Hz, 3.37 Hz), 4.658-4.783 (3H, m, H2', CH$_2$OP), 4.530 (1H, m, H3'), 4.274 (2H, m, H5'), 4.208 (1H, m, H4')

F.A.B.M.S. m/e 632 (MH$^+$, $^{37}$Cl, < 0.01%), 630 (MH$^+$, < 0.01%), 169 (C$_6$H$_5$C$_3$H$_4$O$^+$, 2.93%), 153 (C$_{12}$H$_7^+$, 24.42%), 152 (C$_{12}$H$_6^+$, 0.99%), 151 (C$_{12}$H$_7^+$ and CCl$_3$CH$_2$, 2x$^{37}$Cl, 8.75%), 149 CCl$_3$CH$_2$,$^{37}$Cl, 0.32%), 147 (CCl$_3$CH$_2^+$, 0.23%), 136 (adenineH$^+$, 3.72%), 135 (adenine$^+$, 0.60%), 97 (C$_3$H$_4$O$_2^+$, 7.44%), 92 (C$_7$H$_8^+$, 33.44%), 91 (C$_{10}$H$_7^+$, 52.55%), 76 (C$_8$H$_4^+$, 74.64%)

Microanalysis

Found: C 45.76%; H 3.97%; N 10.85%; P 4.92%
C$_{24}$H$_{23}$Cl$_3$N$_5$O$_7$P requires: C 45.70%; H 3.68%; N 11.10%; P 4.91%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column
Mobile phase: methanol/water/triethylamine (70/30/0.01), isocratic conditions
(107): retention time 15.51 min

2,2,2-Trichloroethyl p-chlorophenyl phosphorochloridate (108)

2,2,2-Trichloroethyl phosphorodichloridate (101) (2.5 g, 9.39 mmol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of p-chlorophenol (1.21 g, 9.41 mmol) and triethylamine (1.31 ml, 0.95 g, 9.40 mmol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (15 h) and stirred for a further 74 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure, yielding a colourless oil (108) (3.267 g, 97%).
$^{31}$P n.m.r. $\delta$(CDCl$_3$) -2.532

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 147.836 (d, ipso-Ph, J=9.1 Hz), 132.218 (para-Ph), 130.135 (meta-Ph), 121.750 (d, ortho-Ph, J=5.2 Hz), 93.572 (d, CCl$_3$, J=12.7 Hz), 77.674 (d, CH$_2$OP, J=4.1 Hz)

$^1$H n.m.r. $\delta$(CDCl$_3$) 7.156-7.384 (4H, m, Ph), 4.756 (2H, m, CH$_2$OP)

E.I.M.S. m/e 363 (MH$^+$, 3x$^{37}$Cl, 4.44%), 361 (MH$^+$, 2x$^{37}$Cl, 19.71%), 359 (MH$^+$, $^{37}$Cl, 29.52%), 356.8437 (MH$^+$, C$_8$H$_7$Cl$_5$O$_3$P requires 356.8576, 17.92%), 329 (MH$_2^+$ - Cl, 3x$^{37}$Cl, 0.20%), 327 (MH$_2^+$ - Cl, 2x$^{37}$Cl, 7.97%), 325 (MH$_2^+$ - Cl, $^{37}$Cl, 16.16%), 323 (MH$_2^+$ - Cl, 13.21%), 234 (MH$^+$ - ClC$_6$H$_6$O, 2x$^{37}$Cl, 13.53%), 232 (MH$^+$ - ClC$_6$H$_6$O, $^{37}$Cl, 74.53%), 230 (MH$^+$ - ClC$_6$H$_6$O, 100%), 115 (MH$^+$ - ClC$_6$H$_6$O - CCl$_3$, $^{37}$Cl, 11.64%), 113 (MH$^+$ - ClC$_6$H$_6$O - CCl$_3$, 25.69%), 76 (C$_8$H$_4^+$, 30.89%)

Microanalysis

Found: C 27.18%; H 1.98%; P 9.02%

C$_8$H$_7$Cl$_5$O$_3$P requires: C 26.81%; H 1.69%; P 8.64%

9-$\beta$-D-Arabino furanosyl adenine-5'-(2,2,2-trichloroethyl) $p$-chlorophenyl phosphate (109)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and 2,2,2-trichloroethyl $p$-chlorophenyl phosphorochloridate (108) (0.67 g, 1.87 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 27 h. The reaction mixture was quenched with deionized water (35 µl, 1.94 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting white solid was
purified by successive column chromatography on silica, eluting with 5% methanol in chloroform and 2.5% methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions yielded a white solid (109) (0.127 g, 23%).

$^3$P n.m.r. $\delta$(CH$_3$OH/D$_2$O) -7.249, -7.369 (1:1)

$^{13}$C n.m.r. $\delta$(CD$_3$OD) 157.203 (C6), 153.827, 153.785 (2xd, C2, J=4.2 Hz, 4.3 Hz), 150.531, 150.437 (2xs, C4), 150.132 (d, ipso-Ph, J=7.0 Hz), 142.724, 142.677 (2xd, C8, J=4.5 Hz, 5.0 Hz), 132.246, 132.181 (2xs, para-Ph), 130.950, 130.825 (2xs, meta-Ph), 122.970, 122.926 (2xd, ortho-Ph, J=4.3 Hz, 4.6 Hz), 119.518 (C5), 95.313 (bs, CCl$_4$), 86.360 (m, Cl'), 83.872 (d, C4', J=5.2 Hz), 78.504 (d, CH$_2$OP, J=3.2 Hz), 77.343 (bs, C2' or C3'), 76.888, 76.829 (2xs, C2' or C3'), 70.152 (m, C5')

$^1$H n.m.r. $\delta$(CD$_3$OD) 8.337, 8.273 (1H, 2xs, H2), 8.185 (1H, s, H8), 7.212-7.346 (4H, m, Ph), 6.448 (1H, m, H1'), 4.646-4.767 (3H, m, H2', CH$_2$OP), 4.508 (1H, m, H3'), 4.279 (2H, m, H5') 4.186 (1H, m, H4')

F.A.B.M.S. m/e 594 (MH$^+$, 3x$^{37}$Cl, 0.50%), 592 (MH$^+$, 2x$^{37}$Cl, 4.72%), 590 (MH$^+$, $^{37}$Cl, 8.27%), 588 (MH$^+$, 7.17%), 422 (MH$^+$ - C - CCl$_3$CH$_2$, 5.76%), 250 (MH$^+$ - (CCl$_3$H$_2$O)(CCl$_3$CH$_2$O)PO$_2$H, 1.88%), 211 ($^{37}$CCl$_3$H$_2$OP(OH)$_3^-$, 0.84%), 209 (CCl$_3$H$_2$OP(OH)$_3^+$, 3.10%), 164 (adenineCHO$^+$, 8.09%), 156 (adenineH$^+$, 100%), 153 (adenine$^+$, 9.13%), 130 ($^{37}$CCl$_3$H$_2$OH$^+$, 0.54%), 128 (CCl$_3$H$_2$OH$^+$, 1.93%), 115 (C$_6$H$_5$O$_2^+$, 4.23%), 99 (C$_6$H$_5$O$_2^+$, 1.05%), 97 (C$_6$H$_5$O$_2^+$, 5.84%), 81 (C$_6$H$_4$O$^+$, 1.05%), 76 (C$_6$H$_4^+$, 11.16%)

Microanalysis

Found: C 36.80%; H 3.14%; N 11.54%; P 5.48%

C$_{18}$H$_{18}$Cl$_4$N$_5$O$_7$P requires: C 36.70%; H 3.08%; N 11.89%; P 5.26%
H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column

Mobile phase: methanol/water/triethylamine (65/35/0.01), isocratic conditions

(109): retention time 12.37 min

2,2,2-Trichloroethyl p-methoxyphenyl phosphorochloridate (110)

2,2,2-Trichloroethyl phosphorodichloridate (101) (2.51 g, 9.43 mmol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of p-methoxyphenol (1.17 g, 9.43 mmol) and triethylamine (1.315 ml, 0.955 g, 9.43 mmol) dissolved in diethyl ether (100 ml) was added dropwise to the cooled solution over 2 h with vigorous stirring under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (17 h) and stirred for a further 73 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure, yielding a colourless oil (110) (3.344 g, 100%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) -1.809

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 157.734 (para-Ph), 142.927 (d, ipso-Ph, $J$=8.8 Hz), 121.257 (d, ortho-Ph, $J$=4.8 Hz), 114.847 (meta-Ph), 93.698 (d, CCl$_3$, $J$=12.5 Hz), 77.568 (d, CH$_2$OP, $J$=5.5 Hz), 55.569 (CH$_3$O)

$^1$H n.m.r. $\delta$(CDCl$_3$) 7.192 (2H, m, ortho-Ph), 6.871 (2H, d, meta-Ph, $J$=9.14 Hz), 4.751 (2H, m, CH$_2$OP), 3.777 (3H, s, CH$_3$O)

E.I.M.S. m/e 358 (M$^+$, 3x$^{37}$Cl, 1.01%), 356 (M$^+$, 2x$^{37}$Cl, 7.03%), 354 (M$^+$, $^{37}$Cl, 16.01%), 351.8972 (M$^+$, C$_9$H$_5$ClO$_4$P requires 351.8993, 11.72%), 321 (M$^+$ - Cl, 2x$^{37}$Cl, 1.18%), 319 (M$^+$ - Cl, $^{37}$Cl, 5.12%), 317 (M$^+$ - Cl 5.14%), 237 (M$^+$ - CCl$_3$, $^{37}$Cl, 4.48%), 235 (M$^+$ - CCl$_3$, 15.48%), 233 (M$^+$ - CH$_3$OC$_6$H$_4$O, 2x$^{37}$Cl 0.61%), 231 (M$^+$ - CH$_3$OC$_6$H$_4$O, $^{37}$Cl, 1.96%), 229 (M$^+$ - CH$_3$OC$_6$H$_4$O, 1.37%)
224 (MH⁺ - CCl₃CH₂⁻Cl, 33.46%), 222 (MH⁺ - CCl₃CH₂, 100%), 186 (CH₃OC₆H₄PO₄²⁻, 18.85%), 151 (CCl₃CH₂O⁺, 2x⁻Cl, 0.50%), 149 (CCl₃CH₂O⁺, 3¹Cl, 5.60%), 147 (CCl₃CH₂O⁺, 9.13%), 124 (CH₃OC₆H₄OH⁺, 86.19%), 123 (CH₃OC₆H₄O⁺, 57.46%), 121 (CCl₃⁺, 2x⁻Cl, 1.47%), 119 (CCl₃⁺, 3¹Cl, 5.52%), 117 (CCl₃⁺, 7.01%), 77 (C₂H₅⁺, 10.06%)

Microanalysis

Found: C 30.82%; H 2.64%; P 9.25%

C₉H₉Cl₄O₄P requires: C 30.54%; H 2.56%; P 8.75%

9-β-D-Arabinofuranosyladenine-5’-(2,2,2-trichloroethyl) p-methoxyphenyl phosphate (111)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and 2,2,2-trichloroethyl p-methoxyphenyl phosphorochloridate (110) (0.66 g, 1.86 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 19 h. The reaction mixture was quenched with deionized water (35 ml, 1.94 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (5 x 15 ml) and co-evaporation with toluene (3 x 5 ml). The resulting cream solid was purified by successive column chromatography on silica, eluting with 5% methanol in chloroform and 2.5% methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions yielded a white solid (111) (0.187 g, 34%).

³¹P n.m.r. δ(CH₃OH/D₂O) -6.626, -6.793 (1:1)

¹³C n.m.r. δ(CD₃OD) 158.864 (2xs, para-Ph), 157.184 (C6), 153.786, 153.733 (2xd, C2, J=5.3 Hz, 5.3 Hz), 150.538, 150.471 (2xs, C4), 144.938 (d, ipso-Ph, J=5.3 Hz), 142.735, 142.672 (2xd, C8, J=6.1 Hz, 6.6 Hz), 122.166 (d, ortho-Ph, J=4.4 Hz), 119.533 (C5), 115.728 (m, meta-Ph),
95.932 (d, CCl₃, J=12.6 Hz), 86.390, 86.330 (2xd, Cl', J=6.0 Hz, 6.1 Hz), 83.851 (m, C4'),
78.449 (d, CH₂OP, J=4.5 Hz), 77.348 (bs, C2' or C3'), 76.937, 76.852 (2xs, C2' or C3'),
69.972, 69.864 (2xd, C5', J=6.2 Hz, 5.6 Hz), 56.051 (m, CH₃O)

1H n.m.r. δ(CD₃OD) 8.328, 8.261 (1H, 2xs, H2), 8.190 (1H, s, H8), 7.151 (2H, m, ortho-Ph),
6.836 (2H, m, meta-Ph), 6.466, 6.435 (1H, 2xd, H1', J=3.97 Hz, 3.87 Hz), 4.594-4.720 (3H, m,
H2', CH₂OP), 4.486 (1H, m, H3'), 4.275 (2H, m, H5'), 4.179 (1H, m, H4'), 3.747, 3.710 (3H,
2xs, CH₃O)

F.A.B.M.S. (NO₂C₆H₄CH₂OH/Nal) m/e 609 ((M-H)Na⁺, 2x³⁷Cl, 0.15%), 607 ((M-H)Na⁺, ³⁷Cl,
2.05%), 605 ((M-H)Na⁺, 1.92%), 250 (MH⁺ - (CH₃OC₆H₄O)(CCl₃CH₂O)PO₂H, 1.39%), 217
((CH₃OC₆H₄O)(CH₂O)PO₂H⁺, 0.12%), 205 (CH₃OC₆H₄OP(OH)₃⁺, 1.39%), 164 (adenineCHO⁺,
0.99%), 136 (adenineH⁺, 70.53%), 135 (adenine⁺, 4.48%), 124 (CH₃OC₆H₄OH⁺, 2.81%), 123
(CH₂OC₆H₄O⁺, 3.25%), 115 (C₅H₅O⁺, 4.25%), 99 (C₅H₅O₂⁺, 0.15%), 97 (C₅H₅O₂⁺, 3.09%), 81
(C₅H₄O⁺, 5.20%), 77 (C₅H₃⁺, 30.80%)

Microanalysis

Found: C 38.58%; H 4.04%; N 10.29%; P 5.21%

C₁₉H₂₁Cl₂N₃O₅P(H₂O)₁₀ requires: C 37.86%; H 3.85%; N 11.62%; P 5.14%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column
Mobile phase: methanol/water/triethylamine (65/35/0.01), isocratic conditions
(111): retention time 7.73 min, 8.16 min (1:1)

2-O-Benzylethanol (115)

A mixture of 50% w/v sodium hydroxide solution (80 ml, 40 g, 1.0 mol) and monoethylene glycol
(62.10 g, 1.0 mol) was stirred at 100°C for 0.5 h under an atmosphere of nitrogen. Benzyl chloride (31.65 g, 0.25 mol) was added and the reaction mixture was stirred at reflux for 24 h under an atmosphere of nitrogen. After cooling to ambient temperature overnight (18 h), the reaction mixture was diluted with deionized water (400 ml) and extracted with diethyl ether (3 x 250 ml). The combined organic layers were dried over magnesium sulphate, filtered, and solvent was removed from the filtrate under reduced pressure. The resulting red/brown oil was purified by vacuum distillation (bp 64°C, 0.2 mm Hg), yielding a colourless oil (115) (18.632 g, 49%).

**C n.m.r.** δ(CDCl₃) 137.995 (ipso-Ph), 128.454 (ortho-Ph or meta-Ph), 127.825 (ortho-Ph or meta-Ph), 127.783 (para-Ph), 73.259 (PhCH₂), 71.486 (CH₂CH₂OH), 61.775 (CH₂OH)

**H n.m.r.** δ(CDCl₃) 7.343 (5H, s, Ph), 4.548 (2H, s, PhCH₂), 3.728 (2H, m, CH₂CH₂OH), 3.565 (2H, m, CH₂OH), 2.682 (1H, t, OH, J=5.91 Hz)

**E.I.M.S.** m/e 153 (M⁺, 3.01%), 152.0841 (M⁺, C₆H₁₂O₂ requires 152.0837, 34.97%), 121 (C₅H₅CH₂OCH₂⁺, 0.67%), 107 (C₅H₅CH₂O⁺, 62.82%), 106 (C₆H₅CHO⁺, 3.99%), 105 (C₆H₅CO⁺, 9.17%), 92 (C₅H₅⁺, 68.74%), 91 (C₅H₄⁺, 100%)

**Ethyl (2-benzyloxyethyl) phosphorochloridate (116)**

Ethyl phosphorodichloridate (84) (2 g, 0.012 mol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2-O-benzylethanol (115) (1.86 g, 0.012 mol) and triethylamine (1.71 ml, 1.24 g, 0.012 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (16 h) and stirred for a further 50 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy yellow oil was extracted with hexane, yielding a colourless oil (116) (3.005 g, 88%).
\[ ^{31}P \text{n.m.r. } \delta(\text{CDCl}_3) 3.068 \]

\[ ^{13}C \text{n.m.r. } \delta(\text{CDCl}_3) 137.495 \text{ (ipso-Ph)}, 128.329 \text{ (ortho-Ph or meta-Ph)}, 127.592 \text{ (para-Ph),} 127.433 \text{ (ortho-Ph or meta-Ph),} 73.132 \text{ (PhCH})_2, 67.990-68.251 \text{ (m, CH}_3\text{CH}_2\text{OP),} 65.998 \text{ (d, CH}_3\text{CH}_2\text{OP, } J=7.2 \text{ Hz),} 15.707 \text{ (d, CH}_3\text{CH}_2\text{OP, } J=8.0 \text{ Hz)} \]

\[ ^{1}H \text{n.m.r. } \delta(\text{CDCl}_3) 7.329 \text{ (5H, s, Ph),} 4.568 \text{ (2H, s, PhCH})_2, 4.218-4.376 \text{ (4H, m, CH}_3\text{CH}_2\text{OP, CH}_3\text{CH}_2\text{OP),} 3.673 \text{ (2H, m, CH}_3\text{CH}_2\text{OP),} 1.378 \text{ (3H, td, CH}_3\text{CH}_2\text{OP, } J=7.12 \text{ Hz,} 1.21 \text{ Hz)} \]

E.I.M.S. m/e 281 (MH\(^+\), \(^{37}\text{Cl, 4.81}\%\)), 279.0563 (MH\(^+\), \(\text{C}_{11}\text{H}_{17}\text{ClO}_4\text{P requires 279.0553, 19.00}\%\)), 173 (M\(^+\) - \(\text{C}_9\text{H}_7\text{CH}_2\text{O}\), \(^{37}\text{Cl, 7.38}\%\)), 171 (M\(^+\) - \(\text{C}_9\text{H}_7\text{CH}_2\text{O}\), 26.01\%\)), 151 (\(\text{C}_9\text{H}_7\text{CH}_2\text{OCH}_2\text{CH}_2\text{O}^+\), 2.95\%\)), 146 (\(\text{C}_9\text{H}_7\text{ClO}_3\text{P}^+\), 9.24\%\)), 144 (\(\text{C}_9\text{H}_7\text{ClO}_3\text{P}^+\), 34.52\%\)), 135 (\(\text{C}_9\text{H}_7\text{CH}_2\text{OCH}_2\text{CH}_2\text{O}^+\), 5.45\%\)), 129 (\(\text{C}_9\text{H}_7\text{ClO}_3\text{P}^+\), 18.98\%\)), 127 (\(\text{C}_9\text{H}_7\text{ClO}_3\text{P}^+\), 73.26\%\)), 121 (\(\text{C}_9\text{H}_7\text{CH}_2\text{OCH}_2\text{H}^+\), 1.40\%\)), 107 (\(\text{C}_9\text{H}_7\text{CH}_2\text{O}^+\), 63.64\%\)), 106 (\(\text{C}_9\text{H}_7\text{CHO}^+\), 83.15\%\)), 105 (\(\text{C}_9\text{H}_7\text{CO}^+\), 89.19\%\)), 101 (OP(OH)\(^{37}\text{Cl}^+, 10.82\%\)), 99 (OP(OH)\(^{37}\text{Cl}^+, 69.41\%\)), 92 (\(\text{C}_9\text{H}_7^+\), 78.11\%\)), 91 (\(\text{C}_9\text{H}_7^+\), 100\%\))

**Microanalysis**

Found: C 46.79\%; H 5.22\%; Cl 12.30\%; P 11.02\%

\(\text{C}_{11}\text{H}_{17}\text{ClO}_4\text{P requires: C 47.41\%; H 5.79\%; Cl 12.72\%; P 11.11}\%\)

**9-β-D-Arabinofuranosyladenine-5’'-ethyl (2-benzylxyethyl) phosphate (117)**

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and ethyl (2-benzylxyethyl) phosphorochloridate (116) (0.52 g, 1.87 mmol, 2 molar equivs.) was added with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for an 1 h, allowed to warm to ambient temperature and stirred for a further 22 h. The reaction mixture was quenched with deionized water (35 μl, 1.94 mmol) and solvent was removed under reduced pressure. Final traces
of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with
toluene (3 x 5 ml) under reduced pressure. The resulting cream solid was purified by successive
column chromatography, eluting with 10% methanol in chloroform and 5% methanol in ethyl
acetate respectively. Pooling and evaporation of appropriate fractions yielded a white solid (117)
(0.281 g, 59%).

$^{31}$P n.m.r. $\delta$(CH$_3$OD) -0.744

$^{13}$C n.m.r. $\delta$(CD$_3$OD) 157.188 (C6), 153.776 (C2), 150.466 (C4), 142.548, 142.520 (2xs, C8),
139.320, 139.294 (2xs, ipso-Ph), 129.379, 129.358 (2xs, ortho-Ph or meta-Ph), 128.890, 128.864
(2xs, ortho-Ph or meta-Ph), 128.736, 128.715 (2xs, para-Ph), 119.575 (C5), 86.428 (C1'), 84.056,
84.004 (2xd, C4', J=7.3 Hz, 7.4 Hz), 77.310, 77.272 (2xs, C2' or C3'), 76.924 (C2' or C3'),
74.030, 74.007 (2xs, PhCH$_2$), 69.921, 69.894 (2xd, CH$_3$CH$_2$OP, J=6.8 Hz, 6.8 Hz), 68.281-68.500
(m, C5', CH$_3$CH$_2$OP) 65.752, 65.698 (2xd, CH$_3$CH$_2$OP, J=5.4 Hz, 5.6 Hz), 16.353 (d, CH$_3$CH$_2$OP,
J=6.6 Hz)

$^1$H n.m.r. $\delta$(CD$_3$OD) 8.317, 8.305 (1H, 2xs, H2), 8.184 (1H, s, H8), 7.226-7.338 (5H, m, Ph),
6.440 (1H, d, H1', J=4.39 Hz), 4.531, 4.510 (2H, 2xs, PhCH$_2$), 4.069-4.463 (9H, m, H2', H3',
H4', H5', CH$_2$CH$_2$OP, CH$_3$CH$_2$OP), 3.657 (2H, m, CH$_2$CH$_2$OP), 1.282, 1.275 (3H, 2xd,
CH$_3$CH$_2$OP, J=7.03 Hz, 1.05 Hz, J=7.12 Hz, 1.05 Hz)

F.A.B.M.S. (NO$_2$C$_7$H$_2$CH$_2$OH/Nal) m/e 532 (MNa*, 21.07%), 510 (MH*, 20.62%), 250 (MH* -
(EtO)(C$_6$H$_2$CH$_2$OCH$_2$CH$_2$O)PO$_2$H, 7.67%), 164 (adenineCHO*, 3.19%), 136 (adenineH*, 100%),
135 (adenine* or C$_6$H$_2$CH$_2$OCH$_2$CH$_2$*, 3.21%), 127 (Et(OH)H$_2$*, 0.38%), 121 (C$_6$H$_5$CH$_2$OCH$_2$*,
2.18%), 115 (C$_6$H$_5$O*, 1.08%), 107 (C$_6$H$_5$CH$_2$O*, 18.56%), 106 (C$_6$H$_5$CHO*, 3.62%), 105
(C$_6$H$_5$CO*, 11.84%), 99 (C$_6$H$_5$O$_2$*, 3.77%), 97 (C$_3$H$_2$O$_2$, 1.55%)
Microanalysis

Found: C 48.02%; H 5.48%; N 12.49%; P 5.63%

C<sub>21</sub>H<sub>28</sub>N<sub>5</sub>O<sub>8</sub>P(H<sub>2</sub>O)<sub>1.0</sub> requires: C 47.82%; H 5.73%; N 13.28%; P 5.87%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column

Mobile phase: methanol/water/triethylamine (55/45/0.01), isocratic conditions

(117): retention time 7.85 min

9-ß-D-Arabinofuranosyladenine-5'-ethyl (2-hydroxyethyl) phosphate (118)

Palladium on charcoal (10%, 0.150 g) was added to a solution of 9-ß-D-arabinofuranosyladenine-5'-ethyl (2-benzyloxyethyl) phosphate (117) (0.166 g, 0.326 mmol) in methanol (33 ml) under an atmosphere of nitrogen. The reaction mixture was flushed with hydrogen and stirred at ambient temperature. Fresh hydrogen was added after stirring for 2.5 h and again after 16 h. After a further 4.5 h, more palladium on charcoal (10%, 0.1 g) was added to the reaction mixture under an atmosphere of nitrogen. The reaction mixture was flushed with fresh hydrogen after a further 3 h and stirred overnight (19 h) at ambient temperature. The reaction mixture was filtered, washing the catalyst with methanol (3 x 25 ml) and solvent was removed from the filtrate under reduced pressure. The resulting grey oil was purified by successive column chromatography on silica, eluting with 5% methanol in chloroform and 10% methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions yielded a white gum (118) (0.062 g, 17%).

<sup>31</sup>P n.m.r. δ(CH<sub>3</sub>OH/D<sub>2</sub>O) -0.301

<sup>13</sup>C n.m.r. δ(CD<sub>3</sub>OD) 157.176 (C6), 153.710, 153.562 (2xs, C2), 150.476 (C4), 142.664, 142.499 (2xs, C8), 119.574 (C5), 86.454, 86.397 (2xs, C1'), 84.013 (d, C4', J=6.3 Hz), 77.247 (C2' or C3'), 76.970, 76.883 (2xs, C2' or C3'), 70.628 (d, CH$_2$CH$_2$OP, J=6.2 Hz), 68.303, 68.258 (2xs,
C5', J=5.5 Hz, 5.6 Hz), 65.775, 65.727 (2xd, CH₂CH₂OP, J=5.9 Hz, 5.8 Hz), 61.933 (d, CH₂CH₂OP, J=7.3 Hz), 16.391, 16.360 (2xd, CH₂CH₂OP, J=6.5 Hz, 6.5 Hz)

¹H n.m.r. δ(CD₃OD) 8.327 (1H, s, H2), 8.194 (1H, s, H8), 6.447 (1H, d, H1', J=3.85 Hz), 4.064-4.448 (9H, m, H₂', H3', H4', H5', CH₂CH₆OP, CH₂CH₆OP), 3.717 (2H, m, CH₂CH₂OP), 1.302 (3H, m, CH₃CH₂OP)

F.A.B.M.S. (NO₃C₂H₄OH/Nal) m/e 442 (MNa+, 0.13%), 420 (MH⁺, 11.49%), 285 (MH⁺ - adenine, 0.63%), 250 (MH⁺ - (EtO)(HOCH₂CH₂O)PO₂H, 0.29%), 171 ((EtO)(HOCH₂H₂O)P(OH)₂⁺, 1.26%), 164 (adenineCHO⁺, 3.20%), 143 (HOCH₂CH₂OP(OH)₂⁺, 0.55%), 136 (adenineH⁺, 100%), 135 (adenine⁺, 7.94%), 127 (EtOP(OH)₂⁺, 2.69%), 115 (C₅H₅O⁺, 5.00%), 99 (C₅H₅O₂⁺, 3.54%), 97 (C₅H₃O₂⁺, 7.25%), 81 (C₅H₂O⁺, 18.23%)

Microanalysis

Found: C 37.64%; H 5.78%; N 12.68%

C₁₄H₁₄N₂O₄P(H₂O)₂ requires: C 36.93%; H 5.76%; N 15.38%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column

Mobile phase: methanol/water (20/80), isocratic conditions

(118): retention time 25.04 min, 27.02 min (1:1)

Ethyl (5-benzyloxy-3-oxapentyl) phosphorochloridate (120)

Ethyl phosphorodichloridate (84) (2 g, 0.012 mol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2-(2-O-benzylethoxy) ethanol (2.41 g, 0.012 mol) and triethylamine (1.71 ml, 1.24 g, 0.012 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the
addition, the reaction mixture was allowed to warm to ambient temperature overnight (17 h) and stirred for a further 50 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy yellow oil was extracted with hexane to give a colourless oil (2.878 g). A minor impurity of unreacted starting material (84) was removed by stirring the crude product under reduced pressure (0.15 mm Hg) at ca. 30°C for 5 h, yielding a colourless oil (120) (2.493 g, 63%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 3.015

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 137.982 (ipso-Ph), 128.229 (ortho-Ph or meta-Ph), 127.558 (ortho-Ph or meta-Ph), 127.487 (para-Ph), 128.229 (ortho-Ph or meta-Ph), 127.364 (PhCH$_2$OCH$_2$), 69.285 (d, CH$_2$OP, J=8.0 Hz), 68.145 (d, CH$_2$OP, J=6.9 Hz), 65.951 (d, CH$_2$OP, J=6.9 Hz), 15.62 (d, CH$_3$OP).

$^1$H n.m.r. $\delta$(CDCl$_3$) 7.300 (5H, s, Ph), 4.531 (2H, s, PhCH$_2$), 4.242 (2H, m, CH$_2$OP), 3.572-3.759 (8H, m, CH$_2$OP), 1.333 (3H, m, CH$_3$OP).

E.I.M.S. m/e 325 (MH$^+$, $^{37}$Cl, 0.13%), 323.0823 (MH$^+$, C$_{13}$H$_{11}$ClO$_3$P requires 323.0815, 0.77%), 218 (MH$^+$ - C$_6$H$_5$CH$_2$O, $^{37}$Cl, 4.30%), 216 (MH$^+$ - C$_6$H$_5$CH$_2$O, 13.88%), 205 (M$^+$ - C$_6$H$_5$CH$_2$OCH$_2$, $^{37}$Cl, 1.92%), 201 (M$^+$ - C$_6$H$_5$CH$_2$OCH$_2$, 6.35%), 195 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$, 7.93%), 190 (MH$^+$ - C$_6$H$_5$CH$_2$OCH$_2$CH$_2$O, $^{37}$Cl, 10.71%), 188 (MH$^+$ - C$_6$H$_5$CH$_2$OCH$_2$H, 31.24%), 179 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$, 0.37%), 175 ((EtO)$_2$P(OH)$^{37}$Cl$^+$ or C$_6$H$_5$ClO$_3$P, 5.58%), 173 ((EtO)$_2$P(OH)Cl$^+$ or C$_6$H$_5$ClO$_2$P$^+$ or (EtO)(CH$_2$CH$_2$O)PO$^{37}$Cl$^+$, 20.40%), 171 ((EtO)(CH$_2$CH$_2$O)POCl$^+$, 9.71%), 165 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$, 0.38%), 151 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$O, 1.76%), 147 (EtO(OPH)$_2$$^{37}$Cl$^+$, 10.91%), 145 (EtO(OPH)$_2$Cl$^+$ or C$_6$H$_5$ClO$_3$P$^+$, 60.81%), 143 (C$_6$H$_5$ClO$_2$P$^+$, 73.39%), 135 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$, 3.00%), 121 (C$_6$H$_5$CH$_2$OCH$_2$O, 1.04%), 119 (P(OH)$_3$$^{37}$Cl$^+$, 34.56%), 117 (P(OH)$_3$Cl$^+$, 64.66%), 107 (C$_6$H$_5$CH$_2$O, 41.79%), 106 (C$_6$H$_5$CHO, 49.90%), 105
(C₆H₂CO⁺, 69.54%), 101 (OP(OH)⁺Cl⁻, 5.83%), 99 (OP(OH)Cl⁺, 21.75), 92 (C₆H₄⁺, 65.90%), 91 (C₅H₅⁺, 100%)

Microanalysis

Found: C 49.22%; H 5.64%; Cl 11.03%

C₁₃H₂₀ClO₅P requires: C 48.38%; H 6.25%; Cl 10.99%

9-β-D-Arabinofuranosyladenine-5′-ethyl (5-benzyloxy-3-oxapentyl) phosphate (121)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and ethyl (5-benzyloxy-3-oxapentyl) phosphorochloridate (120) (0.61 g, 1.89 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 20 h. The reaction mixture was quenched with deionized water (35 µl, 1.94 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting pale yellow oil was purified by successive column chromatography on silica, eluting with 10% methanol in chloroform and 5% methanol in ethyl acetate respectively. Pooling and evaporation of appropriate fractions yielded a white solid (121) (0.327 g, 63%).

³¹P n.m.r. δ(CDCl₃) -3.296

¹³C n.m.r. δ(CDCl₃) 155.220 (C6), 152.396 (bs, C2), 148.681 (C4), 140.160 (bs, C8), 137.970, 137.939 (2xs, ipso-Ph), 128.335, 128.291 (2xs, ortho-Ph or meta-Ph), 127.710, 127.668 (2xs, ortho-Ph or meta-Ph), 127.630, 127.569 (2xs, para-Ph), 118.209 (C5), 84.677 (bs, C1′), 81.434 (bs, C4′), 76.039 (bs, C2′ or C3′), 74.717, 74.554 (2xs, C2′ or C3′), 73.158, 73.101 (2xs, PhCH₂), 70.507, 70.401 (2xs, PhCH₂OCH₂), 69.816, 69.737 (2xd, CH₂CH₂OP, J=8.0 Hz, 8.0 Hz),
$\delta$(CDCl$_3$) 8.182 (1H, bs, H2), 7.939 (1H, bs, H8), 7.217-7.305 (5H, m, Ph), 6.663 (3H, bs, NH$_2$, OH), 6.365 (1H, bs, H1'), 6.040 (1H, bs, OH), 4.514, 4.476 (2H, 2xs, PhCH$_2$), 4.028-4.440 (9H, m, H$_2'$, H$_3'$, H$_4'$, H$_5'$, CH$_2$CH$_2$OP, CH$_2$CH$_2$OP), 3.531-3.690 (6H, m, CH$_2$CH$_2$OCH$_2$CH$_2$OP), 1.277, 1.200 (3H, 2xt, CH$_2$CH$_2$OP, J=7.10 Hz, 7.04 Hz)

F.A.B.M.S. m/e 554 (MH$^+$, 24.69%), 305 ((EtO)(C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$O)P(OH)$_2$i, 0.58%), 250 (MH$^+$ - (EtO)(C$_6$H$_5$CH$_2$OC$_2$H$_4$OC$_2$H$_4$O)PO$_2$H, 4.33%), 195 (C$_6$H$_5$CH$_2$OC$_2$H$_4$OC$_2$H$_4$O+, 0.20%), 179 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$+, 0.79%), 165 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$+, 3.90%), 164 (adenineCHO$^+$, 6.97%), 151 (C$_6$H$_5$CH$_2$OC$_2$H$_4$CH$_2$O$^+$, 2.94%), 136 (adenineH$^+$, 100%), 135 (adenine$^+$ or C$_6$H$_5$CH$_2$OCH$_2$CH$_2$+, 9.28%), 127 (EtOP(OH)$_2$i, 3.28%), 121 (C$_6$H$_5$CH$_2$OCH$_2$H$^+$, 8.80%), 115 (C$_6$H$_5$O$_2^+$, 4.65%), 107 (C$_6$H$_5$CH$_2$O$^+$, 27.73%), 106 (C$_6$H$_5$CHO$^+$, 8.39%), 105 (C$_6$H$_5$CO$^+$, 13.47%), 99 (C$_6$H$_5$O$_2^+$, 3.37%), 97 (C$_6$H$_5$O$_2^+$, 7.15%)

Microanalysis

Found: C 47.97%; H 6.10%; N 11.55%; P 5.65%

C$_{23}$H$_{32}$N$_2$O$_7$P(H$_2$O)$_{1.5}$ requires: C 47.59%; H 6.08%; N 12.06%; P 5.34%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column

Mobile phase: methanol/water/triethylamine (55/45/0.01), isocratic conditions

(121): retention time 8.49 min, 8.75 min (1:1)

9-β-D-Arabinofuranosyladenine-5′-ethyl (5-hydroxy-3-oxapentyl) phosphate (122)

Palladium on charcoal (10%, 0.2 g) was added to a solution of 9-β-D-arabinofuranosyladenine-5′-
ethyl (5-benzyloxy-3-oxapentyl) phosphate (121) (0.189 g, 0.342 mmol) in methanol (35 ml) under an atmosphere of nitrogen. The reaction mixture was flushed with hydrogen and stirred at ambient temperature. Fresh hydrogen was added after stirring for 6 h and again after 16 h. After a further 5 h the reaction mixture was filtered, washing the catalyst with methanol (3 x 25 ml), and solvent was removed from the filtrate under reduced pressure. The resulting grey oil was purified by column chromatography, eluting with 10% methanol in ethyl acetate. Pooling and evaporation of appropriate fractions yielded a white gum (122) (0.095 g, 60%).

$^{31}$P n.m.r. $\delta$(CH$_3$OH/D$_2$O) -0.348

$^{13}$C n.m.r. $\delta$(CD$_3$OD) 157.161 (C6), 153.837, 153.706 (2xs, C4), 150.463 (C2), 142.588, 142.469 (2xs, C8), 119.543 (C5), 86.412, 86.303 (2xs, C1'), 83.869 (d, C4', J=8.0 Hz), 76.857-77.199 (m, C2', C3'), 73.679 (HOCH$_2$CH$_2$), 70.897 (d, CH$_2$CH$_2$OP, J=6.7 Hz), 68.103-68.526 (m, C5', CH$_2$CH$_2$OP), 65.727 (m, CH$_2$CH$_2$OP), 62.134 (HOCH$_2$), 16.351 (m, CH$_3$CH$_2$OP)

$^1$H n.m.r. $\delta$(CD$_3$OD) 8.326 (1H, s, H2), 8.191 (1H, s, H8), 6.449 (1H, d, H1', J=4.49 Hz), 4.106-4.468 (9H, m, H2', H3', H4', H5', CH$_3$CH$_2$OP, CH$_2$CH$_2$OP), 3.638-3.688 (4H, m, CH$_3$OCH$_2$CH$_2$OP), 3.551 (2H, m, HOCH$_2$), 1.306 (3H, m, CH$_3$CH$_2$OP)

F.A.B.M.S. (NO$_2$C$_6$H$_4$CH$_2$OH/NaI) m/e 486 (MNa*, 2.16%), 464 (MH*, 29.40%), 329 (MH* - adenine, 1.10%), 250 (MH* - (EtO)(HOCH$_2$CH$_2$OCH$_2$CH$_2$O)PO$_2$H, 3.43%), 215 ((EtO)(HOCH$_2$CH$_2$OCH$_2$CH$_2$O)P(OH)$_2$*, 2.91%), 164 (adenineCHO*, 6.46%), 127 (EtOP(OH)$_2$*, 6.16%), 136 (adenineH*, 100%), 135 (adenine*, 9.32%), 115 (C$_3$H$_7$O$_3$*, 4.26%), 99 (C$_3$H$_7$O$_2$*, 6.82%), 97 (C$_3$H$_5$O$_2$*, 10.04%), 81 (C$_3$H$_3$O*, 17.65%)
Microanalysis

Found: C 39.99%; H 5.48%; N 13.49%

C_{16}H_{26}N_{2}O_{6}P(H_{2}O)_{1.0} requires: C 39.92%; H 5.86%; N 14.55%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column

Mobile phase: methanol/water (30/70), isocratic conditions

(122): retention time 10.40 min

Ethyl (8-benzylxoy-3,6-dioxaoctyl) phosphorochloridate (124)

Ethyl phosphorodichloridate (84) (2 g, 0.012 mol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2-(2-(2-O-benzylethoxy)ethoxy) ethanol (2.95 g, 0.012 mol) and triethylamine (1.71 ml, 1.24 g, 0.012 mol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (16 h) and stirred for a further 50 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure. The resulting cloudy yellow oil was extracted with hexane, yielding a colourless oil (124) (3.811 g, 85%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 3.008

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 138.094 (ipso-Ph), 128.230 (ortho-Ph or meta-Ph), 127.596 (ortho-Ph or meta-Ph), 127.476 (para-Ph), 73.099 (PhCH$_2$), 70.659 (PhCH$_2$OCH$_2$), 70.571 (PhCH$_2$OCH$_2$CH$_2$), 70.534 (CH$_2$CH$_2$OCH$_2$CH$_2$OP), 69.232-69.390 (m, CH$_3$OCH$_2$CH$_2$OP), 68.162 (d, CH$_3$CH$_2$OP, J=7.2 Hz), 65.953 (d, CH$_3$CH$_2$OP, J=6.9 Hz), 15.595 (d, CH$_3$CH$_2$OP, J=7.8 Hz)
$^1$H n.m.r. $^\delta$(CDCl$_3$) 7.313 (5H, s, Ph), 4.544 (2H, s, PhCH$_3$), 4.268 (2H, m, CH$_2$CH$_2$OP), 3.583-3.770 (12H, m, CH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OP), 1.370 (3H, td, CH$_g$CH$_^\wedge$OP, J=7.06 Hz, 1.29Hz)

E.I.M.S. m/e 369 (MH$^+$, $^{37}$Cl, 0.96%), 367.1046 (MH$^+$, C$_{15}$H$_{25}$Cl$_6$P requires 367.1077, 3.11%), 262 (MH$^+$ - C$_6$H$_3$CH$_2$O, $^{37}$Cl, 0.67%), 260 (MH$^+$ - C$_6$H$_3$CH$_2$O, 2.22%), 247 (M$^+$ - C$_6$H$_3$CH$_2$OCH$_2$,$^{37}$Cl, 0.87%), 245 (M$^+$ - C$_6$H$_3$CH$_2$OCH$_2$, 3.12%), 239 (C$_6$H$_3$CH$_2$O(CH$_2$CH$_2$O)$_2$CH$_2$O$^+$, 1.65%), 223 (C$_6$H$_3$CH$_2$O(CH$_2$CH$_2$O)$_2$CH$_2$CH$_2$O$^+$, 0.56%), 217 (M$^+$ - C$_6$H$_3$CH$_2$OCH$_2$CH$_2$O, $^{37}$Cl, 8.21%), 215 (M$^+$ - C$_6$H$_3$CH$_2$OCH$_2$CH$_2$O, 25.00%), 179 (C$_6$H$_3$CH$_2$OCH$_2$CH$_2$O$^+$, 1.17%), 175 ((EtO)$_2$P(OH)$^{37}$Cl$^+$ or C$_3$H$_7$Cl$_4$P$^+$, 6.72%), 174 (C$_{10}$H$_{19}$Cl$_4$P$^+$ or C$_6$H$_3$Cl$_4$P$^+$, 36.39%), 173 ((EtO)$_2$P(OH)Cl$^+$ or C$_3$H$_7$Cl$_4$P$^+$ or (EtO)(CH$_2$CH$_2$O)PO$^{37}$Cl$^+$, 33.07%), 172 (C$_6$H$_3$Cl$_4$P$^+$ or C$_3$H$_7$Cl$_4$P$^+$, 73.12%), 171 ((EtO)(CH$_2$CH$_2$O)POCl$^+$, 42.69%), 165 (C$_6$H$_3$CH$_2$OCH$_2$CH$_2$O$^+$, 0.48%), 151 (C$_6$H$_3$CH$_2$OCH$_2$CH$_2$O$^+$, 3.72%), 147 (EtOP(OH)$_2$Cl$^+$, 25.17%), 145 (EtOP(OH)$_2$Cl$^+$ or C$_2$H$_3$Cl$_2$O$_2$P$^+$, 82.56%), 143 (C$_2$H$_5$Cl$_2$O$_2$P$^+$, 73.39%), 135 (C$_6$H$_3$CH$_2$OCH$_2$CH$_2$O$^+$, 6.00%), 121 (C$_6$H$_3$CH$_2$OCH$_2$CH$_2$O$^+$, 2.23%), 119 (P(OH)$_3$Cl$^+$, 51.86%), 117 (P(OH)$_3$Cl$^+$, 81.10%), 107 (C$_6$H$_3$CH$_2$O$^+$, 36%), 106 (C$_6$H$_3$CHO$^+$, 61.64%), 105 (C$_6$H$_3$CO$^+$, 74.69%), 101 (OP(OH)$^{37}$Cl$^+$, 14.22%), 99 (OP(OH)Cl$^+$, 32.17%), 92 (C$_4$H$_4$$^+$, 75.00%), 91 (C$_2$H$_4$$^+$, 100%)

Microanalysis
Found: C 49.08%; H 6.35%; Cl 9.43%; P 8.21%

C$_{15}$H$_{24}$Cl$_6$P requires: C 49.12%; H 6.60%; Cl 9.67%; P 8.44%

9-β-D-Arabinofuranosyladenine-5′-ethyl (8-benzyloxy-3,6-dioxaoctyl) phosphate (125)
AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension to reflux at 140°C. The resulting solution was cooled to 0°C and ethyl (8-benzyloxy-3,6-dioxaoctyl) phosphorochloridate (124) (0.69 g, 1.88 mmol, 2 molar equivs.) was added dropwise with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 22 h. More phosphorylating agent
(124) (0.34 g, 0.927 mmol, 1 molar equiv.) was added dropwise at 0°C with vigorous stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to ambient temperature and stirred for a further 18 h. The reaction mixture was quenched with deionized water (50 μl, 2.78 mmol) and solvent was removed under reduced pressure. Final traces of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with toluene (3 x 5 ml) under reduced pressure. The resulting pale yellow oil was purified by successive column chromatography on silica, eluting with 5% methanol in ethyl acetate and 10% methanol in chloroform respectively. Pooling and evaporation of appropriate fractions yielded a white solid (125) (0.321 g, 57%).

\[ ^{31}P \text{n.m.r.} \delta(\text{CDCl}_3) -3.356 \]

\[ ^{13}C \text{n.m.r.} \delta(\text{CDCl}_3) 155.206 (C6), 152.259 (bs, C2), 148.612 (C4), 140.176 (bs, C8), 137.950 (ipso-Ph), 128.198, 128.183 (2xs, ortho-Ph or meta-Ph), 127.586 (ortho-Ph or meta-Ph), 127.468, 127.438 (2xs, para-Ph), 118.147 (C5), 84.671 (bs, C1'), 81.538 (bs, C4'), 75.879 (bs, C2' or C3'), 74.876 (bs, C2' or C3'), 73.048, 72.988 (2xs, PhCH₂), 70.354 (bs, PhCH₂OCH₂CH₂OCH₂), 69.708, 69.636 (2xd, CH₂CH₂OP, J=7.3 Hz, 7.3 Hz), 69.165 (CH₂OCH₂CH₂OP), 66.844 (bs, CH₂CH₂OP), 66.574 (bs, C5'), 64.409, 64.349 (2xd, CH₂CH₂OP, J=6.1 Hz, 6.1 Hz), 15.903, 15.829 (2xd, CH₃CH₂OP, J=7.2 Hz, 7.8 Hz)

\[ ^{1}H \text{n.m.r.} \delta(\text{CDCl}_3) 8.140 (1H, bs, H2), 7.896 (1H, bs, H8), 7.163-7.242 (5H, m, Ph), 6.656-6.732 (3H, bs, NH₂, OH), 6.333 (1H, bs, H1'), 6.040 (1H, bs, OH), 4.457, 4.467 (2H, 2xs, PhCH₂), 3.983-4.445 (9H, m, H2', H3', H4', H5', CH₃CH₂OP, CH₂CH₂OP), 3.506-3.608 (10H, m, CH₂CH₂OCH₂CH₂OCH₂CH₂OP), 1.180 (3H, m, CH₃CH₂OP)

F.A.B.M.S. m/e 598 (MH⁺, 26.47%), 349 ((EtO)(C₅H₆O₄)P(OH)₂⁺, 1.17%), 250 (MH⁺ - (EtO)(C₅H₆O₄)PO₂H, 5.90%), 239 (C₅H₆OCH₂CH₂OCH₂CH₂OCH₂CH₂O⁺, 0.03%), 223
(C₆H₅CH₂OCH₂CH₂OCH₂CH₂CH₂⁺, 0.2%), 209 (C₆H₅CH₂OCH₂CH₂OCH₂CH₂OCH₂⁺, 0.03%), 195 (C₆H₅CH₂OCH₂CH₂OCH₂CH₂O⁻, 0.18%), 179 (C₆H₅CH₂OCH₂CH₂OCH₂CH₂⁺, 0.69%), 165 (C₆H₅CH₂OCH₂CH₂OCH₂⁺, 3.87%), 164 (adenineCHO⁺, 8.67%), 151 (C₆H₅CH₂OCH₂CH₂O⁺, 2.68%), 136 (adenineH⁺, 100%), 135 (adenine⁺ or C₆H₅CH₂OCH₂CH₂⁺, 9.66%), 127 (EtOP(OH)₂⁺, 6.42%), 121 (C₆H₅CH₂OCH₂⁺, 6.86%), 115 (C₆H₅O₂⁺, 4.41%), 107 (C₆H₅CH₂O⁺, 24.92%), 106 (C₆H₅CHO⁺, 6.97%), 105 (C₆H₅CO⁺, 10.97%), 99 (C₆H₅O₂⁺, 4.00%), 97 (C₆H₅O₂⁺, 4.51%)

Microanalysis

Found: C 49.92%; H 6.18%; N 10.98%; P 5.20%

C₂₅H₃₆O₃N₂O₁₀P(H₂O)₀.₅ requires: C 49.50%; H 6.15%; N 11.55%; P 5.11%

H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 5 μM column

Mobile phase: methanol/water/triethylamine (55/45/0.01)

(125): retention time 8.99 min

9-β-D-Arabinofuranosyladenine-5’-ethyl (8-hydroxy-3,6-dioxaoctyl) phosphate (126)

Palladium on charcoal (10%, 0.2 g) was added to a solution of 9-β-D-arabinofuranosyladenine-5’-ethyl (8-benzyloxy-3,6-dioxaoctyl) phosphate (125) (0.225 g, 0.377 mmol) in methanol (38 ml) under an atmosphere of nitrogen. The reaction mixture was flushed with hydrogen and stirred at ambient temperature. Fresh hydrogen was added after stirring for 3 h and again after 16 h. After a further 8 h, the reaction mixture was flushed with fresh hydrogen and stirred overnight (23 h). The reaction mixture was filtered, washing the catalyst with methanol (3 x 25 ml) and solvent was removed under reduced pressure. The resulting grey oil was purified by column chromatography on silica, eluting with 10% methanol in ethyl acetate. Pooling and evaporation of appropriate fractions yielded a white gum (126) (0.15 g, 79%).
\textsuperscript{31}P n.m.r. $\delta$(CH$_3$OH/D$_2$O) -0.362

\textsuperscript{13}C n.m.r. $\delta$(CD$_3$OD) 157.200 (C6), 153.828 (2xs, C2), 150.489 (C4), 142.590, 142.530 (2xd, C8, J= 6.4 Hz, 5.7 Hz), 119.563 (C5), 86.396 (C1'), 84.018 (d, C4', J=7.3 Hz), 77.265 (C2' or C3'), 76.939 (C2' or C3'), 73.663 (HOCH$_2$CH$_3$), 71.557, 71.536 (2xs, HOCH$_2$CH$_2$OCH$_3$), 71.442, 71.397 (2xs, CH$_2$OCH$_2$CH$_2$O), 70.942 (d, CH$_2$CH$_2$O, J=7.0 Hz), 68.290-68.452 (m, C5', CH$_2$CH$_2$O), 65.773, 65.718 (2xd, CH$_3$CH$_2$OP, J=5.3 Hz, 5.7 Hz), 62.190 (HOCH$_2$), 16.429, 16.360 (2xd, CH$_3$CH$_2$O, J=6.8 Hz, 7.0 Hz)

\textsuperscript{1}H n.m.r. $\delta$(CD$_3$OD) 8.331, 8.322 (1H, 2xs, H2), 8.192 (1H, s, H8), 6.450, 6.447 (1H, 2xd, H1', J=3.97 Hz, 4.16 Hz), 4.093-4.473 (9H, m, H2', H3', H4', H5', CH$_3$CH$_2$OP, CH$_3$CH$_2$OP), 3.580-3.697 (8H, m, CH$_3$OCH$_2$CH$_2$OCH$_2$CH$_2$OP), 3.512-3.548 (2H, m, HOCH$_2$), 1.312 (3H, td, CH$_3$CH$_2$OP, J=7.07 Hz, 1.01 Hz)

F.A.B.M.S. (NO$_2$C$_6$H$_4$CH$_2$OH/NaI) m/e 530 (MNa$, 9.20\%$), 508 (MH$, 36.51\%$), 373 (MH$^+$ - adenine, 0.17\%), 259 ((EtO)(HOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$O)P(OH)$_2^+$, 2.75\%), 250 (MH$^+$ - (EtO)(HOCH$_2$CH$_2$OCH$_2$CH$_2$CH$_2$O)P(OH)$_2^+$, 12.04\%), 231 (HOCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OP(OH)$_2^+$, 0.10\%) 164 (adenineCHO$^+$, 12.20\%), 136 (adenineH$^+$, 100\%), 135 (adenine$^+$, 5.90\%), 127 (EtOP(OH)$_2^+$, 3.84\%), 115 (C$_2$H$_3$O$_2^+$, 2.74\%), 99 (C$_2$H$_3$O$_2^+$, 8.22\%), 97 (C$_2$H$_3$O$_2^+$, 9.72\%), 91 (C$_5$H$_7$O$^+$, 15.28\%)

Microanalysis

Found: C 41.42%; H 6.25%; N 12.65%; P 6.06%

C$_{18}$H$_{36}$N$_2$O$_{12}$P(H$_2$O)$_{1.1}$ requires: C 41.14%; H 6.14%; N 13.33%; P 5.89%
H.P.L.C. (analytical)
Stationary phase: 250 x 4.6 mm Kromasil C18 5 µM column
Mobile phase: methanol/0.01% triethylamine in water (30/70), isocratic conditions
(126): retention time 11.66 min, 12.74 min (1:1)

2,2,2-Trichloroethyl (8-benzyloxy-3,6-dioxaoctyl) phosphorochloridate (127)
2,2,2-Trichloroethyl phosphorodichloridate (101) (2.55 g, 9.58 mmol) was dissolved in diethyl ether (100 ml) and cooled to -78°C. A solution of 2-(2-(2-0-benzylethoxy)ethoxy) ethanol (2.30 g, 9.57 mmol) and triethylamine (1.335 ml, 0.969 g, 9.58 mmol) dissolved in diethyl ether (100 ml) was added dropwise over 2 h with vigorous stirring to the cooled solution under an atmosphere of nitrogen. On completion of the addition, the reaction mixture was allowed to warm to ambient temperature overnight (17 h) and stirred for a further 71 h. The reaction mixture was filtered and solvent removed from the filtrate under reduced pressure, yielding a colourless oil (127) (4.538 g, 100%).

$^{31}$P n.m.r. $\delta$(CDCl$_3$) 2.412

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 138.047 (ipso-Ph), 128.229 (ortho-Ph or meta-Ph), 127.593 (ortho-Ph or meta-Ph), 127.485 (para-Ph), 93.816 (d, CCl$_3$, J=13.2 Hz), 77.187 (d, CCl$_3$CH$_2$OP, J=5.0 Hz), 73.085 (PhCH$_2$), 70.520-70.673 (m, PhCH$_2$OCH$_2$CH$_2$OCH$_2$), 69.056-69.361 (m, CH$_2$OCH$_2$CH$_2$OP)

$^1$H n.m.r. $\delta$(CDCl$_3$) 7.312 (5H, s, Ph), 4.657 (2H, m, CCl$_3$CH$_2$OP), 4.542 (2H, s, PhCH$_2$), 4.379 (2H, m, CH$_2$CH$_2$OP), 3.588-3.801 (10H, m, CH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OP)

E.I.M.S. m/e 472 (M$^+$, 2x$^{37}$Cl, 1.88%), 470 (M$^+$, $^{37}$Cl, 2.09%), 467.9769 (M$^+$, C$_{15}$H$_2$Cl$_4$O$_8$P requires 467.9830, 0.48%), 307 ((CCl$_3$CH$_2$O)(CH$_2$OCH$_2$CH$_2$O)POCl$^+$, 2x$^{37}$Cl, 3.67%), 305 ((CCl$_3$CH$_2$O)(CH$_2$OCH$_2$CH$_2$O)POCl$^+$, $^{37}$Cl, 6.52%), 303 ((CCl$_3$CH$_2$O)(CH$_2$OCH$_2$CH$_2$O)POCl$^+$,
4.09%), 253 ((CCl₃CH₂O)P(OH)₂Cl⁺, 3X³⁷Cl, 0.11%), 251 ((CCl₃CH₂O)P(OH)₂Cl⁺, 2X³⁷Cl, 1.11%),
249 ((CCl₃CH₂O)P(OH)₂Cl⁺, ³⁷Cl, 3.38%), 247 ((CCl₃CH₂O)P(OH)₂Cl⁺, 2.04%), 239
(C₆H₅CH₂O(CH₂CH₂O)₃⁺, 0.06%), 233 (CCl₃CH₂OPOCP, 2X³¹Cl, 0.68%), 231 (CCl₃CH₂OPOCl⁺,
³⁷Cl, 2.62%), 229 (CCl₃CH₂OPOCl⁺, 1.48%), 223 (C₆H₅CH₂O(CH₂CH₂O)₂CH₂CH₂⁺, 0.57%), 179
(C₆H₅CH₂OCH₂CH₂OCH₂CH₂⁺, 0.33%), 175 (C₆H₅³⁷ClO₄P⁺, 5.38%), 173 (C₆H₅ClO₄P⁺, 14.79%),
151 (C₆H₅CH₂OCH₂CH₂O⁺, 3.33%), 145 (C₆H₅³⁷ClO₃P⁺, 18.95%), 143 (C₆H₅ClO₃P⁺, 55.98%), 135
(C₆H₅CH₂OCH₂CH₂⁺, 9.23%), 121 (C₆H₅CH₂OCH₂⁺, 1.48%), 119 (P(OH)₃³⁷Cl⁺, 16.08%), 117
(P(OH)₃Cl⁺, 28.72%), 107 (C₆H₅CH₂O⁺, 21.66%), 106 (C₆H₅CHO⁺, 40.19%), 105 (C₆H₅CO⁺,
18.39%), 92 (C₆H₅⁺, 19.26%), 91 (C₆H₅⁺, 100%)

**Microanalysis**

Found: C 39.09%; H 4.89%; Cl 30.30%

C₁₅H₂₁Cl₄O₆P requires: C 38.32%; H 4.50%; Cl 30.17%

9-β-D-Arabinofuranosyladenine-5'-O-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl)
phosphate (128)

AraA (0.25 g, 0.935 mmol) was dissolved in pyridine (20 ml) by heating the stirred suspension
to reflux at 140°C. The resulting solution was cooled to 0°C and 2,2,2-trichloroethyl (8-benzyloxy-
3,6-dioxaoctyl) phosphorochloridate (127) (0.88 g, 1.87 mmol) was added dropwise with vigorous
stirring. The reaction mixture was stirred in a dry atmosphere at 0°C for 1 h, allowed to warm to
ambient temperature and stirred for a further 46 h. The reaction mixture was quenched with
deionized water (35 μl, 1.94 mmol) and solvent was removed under reduced pressure. Final traces
of pyridine were removed by trituration with diethyl ether (3 x 15 ml) and co-evaporation with
toluene (3 x 5 ml) under reduced pressure. The resulting white solid was purified by successive
column chromatography on silica, eluting with 5% methanol in chloroform and 2.5% methanol
in ethyl acetate respectively. Pooling and evaporation of appropriate fractions yielded a white solid
(128) (0.340 g, 52%).
$^{31}$P n.m.r. $\delta$(CDCl$_3$) -2.184, -2.358 (1:1)

$^{13}$C n.m.r. $\delta$(CDCl$_3$) 155.195 (C6), 152.729 (bs, C2), 148.692 (bs, C4), 140.402 (bs, C8), 137.967 (ipso-Ph), 128.358 (ortho-Ph or meta-Ph), 127.769 (ortho-Ph or meta-Ph), 127.652 (para-Ph), 118.337, 118.289 (2xs, C5), 94.867 (d, Cl, J=11.1 Hz), 84.898 (bs, Cl''), 81.515 (bs, C4''), 76.879 (d, CCI$_3$CH$_2$OP, J=4.0 Hz), 76.021 (bs, C2'' or C3''), 75.196 (bs, C2'' or C3''), 73.178, 74.163 (2xs, PhCH$_2$), 70.435 (bs, PhCH$_2$OCH$_2$CH$_2$OCH$_2$), 69.690, 69.632 (2xd, CH$_2$CH$_2$OP, J=5.8 Hz, 5.8 Hz), 69.254 (CH$_2$OCH$_2$CH$_2$OP), 67.851 (bs, CH$_2$CH$_2$OP), 67.431 (bs, C5'')

$^1$H n.m.r. $\delta$(CDCl$_3$) 8.125 (1H, bs, H2), 7.910 (1H, bs, H8), 7.223-7.298 (5H, m, Ph), 6.531 (3H, bs, NH$_2$, OH), 6.335 (1H, bs, H1''), 5.780 (1H, bs, OH), 4.584 (1H, d, H2'', J=6.41 Hz), 4.511, 4.495 (2H, 2xs, PhCH$_2$), 4.143-4.474 (8H, m, H3', H4', H5', CCI$_3$CH$_2$OP, CH$_2$CH$_2$OP), 3.568-3.694 (10H, m, CH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OP)

F.A.B.M.S. (thioglycerol/glycerol/2,2,2-trifluoroacetic acid) m/e 704 (MH$^+$, 2x$^{37}$Cl, 3.47%), 702 (MH$^+$, $^{35}$Cl, 7.51%), 700 (MH$^+$, 6.94%), 453 ((CCI$_3$CH$_2$O)(C$_{13}$H$_{19}$O$_2$)PO$_2$)$^+$, $^{37}$Cl, 0.29%), 451 ((CCI$_3$CH$_2$O)(C$_{13}$H$_{19}$O$_4$)PO$_2$)$^+$, 0.29%), 250 (MH$^+$ - (CCI$_3$CH$_2$O)(C$_{13}$H$_{19}$O$_2$)PO$_2$H, 8.09%), 179 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$H$^+$, 7.51%), 165 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$OCH$_2$H$^+$, 2.6%), 164 (adenineCHO$^+$, 12.72%), 151 (C$_6$H$_5$CH$_2$OCH$_2$CH$_2$O$^+$, 3.18%), 136 (adenineH$^+$, 100%), 135 (adenine$^+$ or C$_6$H$_5$CH$_2$OCH$_2$CH$_2$H$^+$, 7.51%), 115 (C$_3$H$_5$O$_2$$^+$, 2.31%), 107 (C$_3$H$_5$CO$^+$, 2.31%), 106 (C$_3$H$_5$CHO$^+$, 1.45%), 105 (C$_3$H$_5$CO$^+$, 4.62%), 99 (C$_3$H$_5$O$_2$$^+$, 5.78%), 97 (C$_3$H$_5$O$_2$$^+$, 4.62%), 92 (C$_3$H$_5$H$^+$, 6.36%), 91 (C$_3$H$_7$H$^+$, 72.25%)

Microanalysis

Found: C 42.78%; H 4.88%; N 9.40%; P 4.18%

C$_{23}$H$_{30}$Cl$_3$N$_4$O$_{10}$P(H$_2$O)$_{0.5}$ requires: C 42.30%; H 4.83%; N 9.87%; P 4.36%
H.P.L.C. (analytical)

Stationary phase: 250 x 4.6 mm Kromasil C18 7 μM column

Mobile phase: methanol/water/triethylamine (60/40/0.01), isocratic conditions

(128): retention time 17.55 min

**Attempted hydrogenolysis of 9-β-D-arabinofuranosyladenine-5’-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate (128)**

Palladium on charcoal (10%, 0.2 g) was added to a solution of 9-β-D-arabinofuranosyladenine-5’-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxaoctyl) phosphate (128) (0.256 g, 0.365 mmol) in methanol (37 ml) under an atmosphere of nitrogen. The reaction mixture was flushed with hydrogen and stirred at ambient temperature. Fresh hydrogen was added after stirring for 5 h and again after 16 h. After a further 5 h the reaction mixture was filtered, washing the catalyst with methanol (3 x 25 ml), and solvent was removed from the filtrate under reduced pressure. The resulting clear grey oil was further concentrated under reduced pressure to give a more viscous, opaque white oil. Attempted purification of the crude product (0.201 g) by column chromatography on silica, eluting with 5% methanol in ethyl acetate, proved unsuccessful.
**BIOLOGICAL TESTING**

**General Methods**

(i) General cleaning and sterilizing procedures

All glassware and plastic containers used for tissue culture were immersed in cleaning liquid (Decon) overnight, rinsed with tap water, left in 2% HCl overnight, washed with tap water, rinsed three times with distilled water and dried. Graduated pipettes were left in 2% bleach solution (Chloros) overnight, washed three times with tap water, rinsed three times with distilled water and dried. Bottle caps were rinsed in tap water, boiled in distilled water containing detergent (Fairy liquid) for 5 min, rinsed with tap water then with distilled water, and dried. General cleaning of the laboratory was carried out using 1% Hycolin and the work area was swabbed down with Precept (containing 1000 ppm available chlorine) prior to sterile work.

Non-plastic and non-rubber materials were sterilized by hot air oven at 160°C for 2 h. Plastics (e.g. bottle caps and disposable pipette tips) were sterilized by auto-claving for 15 min, while falcons were sterilized by γ-irradiation. Pipettes were plugged with non-absorbent cotton wool, placed in pipette cannisters and sterilized by hot air oven.

(ii) Thawing and freezing of cells

Cells taken from liquid nitrogen storage were reconstituted by rapid thawing in warm water and then added to culture flasks (falcons, Falcon Scientific Supplies Ltd.) containing growth medium. The latter was replaced the following day and after the first sub-culture, some cells were sub-cultured further for experiments, some screened for *Mycoplasma* contamination by autoradiography, and some re-frozen for storage. On freezing, cells were protected from low temperature damage by the addition of sterile dimethylsulphoxide (DMSO) to the medium. The cells were spun down and the resulting pellet resuspended in growth medium containing sterile 7.5% DMSO to give a cell concentration of ca. 2x10⁶ cells/ml. The suspensions were transferred in 1-2 ml aliquots to sterile 2 ml polypropylene ampoules and placed in a polystyrene container.
Stepwise freezing was carried out for 3 h or overnight at 4°C, followed by 30 min at -18°C, then overnight at -70°C. The ampoules were subsequently transferred to liquid nitrogen for long term storage.

(iii) Sub-culturing cells

The cells employed were from a mammalian epithelial cell line CNCM I.211 (Collection Nationale de Cultures de Micro-organisms Institute Pasteur, Paris) established on March 22nd 1972. The cells were routinely cultivated in falcons in growth medium consisting of minimum essential medium (Eagle with Earle’s salts, Flow Labs Ltd.), supplemented with foetal bovine serum (10%, Flow Labs Ltd.), L-glutamine (2 mM), sodium bicarbonate (0.03%), penicillin (Glaxo, 100 units/ml) and streptomycin (Glaxo, 100 μg/ml), and buffered to pH 7.4 with HEPES (20 mM). Cells were grown to confluence in a 37°C incubator with a 2% carbon dioxide atmosphere and routinely sub-cultured at weekly intervals, after detachment using 5% trypsin (trypsin powder, Hopkin and Williams 883600) from a 10% stock solution (in 60 mM glucose and 1 mM phosphate buffer) which was diluted with serum free medium (SFM).

Considering one large falcon (30 ml) of confluent cells, the spent medium was poured off and 5% trypsin (pH adjusted, 1 ml) and SFM (1 ml) were added. The falcon was laid flat at ambient temperature until the cells began to detach from the growth surface when agitated (after 2-4 min). The cells were then transferred to a centrifuge tube and washed in with growth medium (1 ml). The tube was spun (800 rpm) for 4 min, the supernatant poured off and the cells resuspended in growth medium (1 ml). An aliquot of the suspension (20 μl) was diluted with saline solution (10 ml) and the number of cells determined by taking the mean of three counts recorded using a coulter counter (model ZBI) calibrated with ragweed pollen. The cells were diluted with growth medium as required and seeded into large clean falcons with growth medium (30 ml) at a density of $2 \times 10^3$ cells/ml and left in the incubator for growth to confluence by the following week.
(iv) *Mycoplasma* screening by autoradiography

A microscope slide in a close-fitting plastic case inside a square petri dish was seeded at a density of $5 \times 10^4$ cells/ml by placing growth medium (5 ml) on the slide and carefully running a suspension containing $10^5$ cells/ml (0.25 ml) down the centre of the slide. The slide was incubated at 37°C in a 2% carbon dioxide atmosphere for 48 h, before labelling with (methyl-^H)thymidine in phosphate buffered saline solution (50 μl of a 100 μCi/ml solution, giving a final concentration of 1.0 μCi/ml). After 5 h, the slide was washed five times with non-sterile phosphate buffered saline solution (composition: NaCl, 8.0 g; KCl, 0.2 g; CaCl$_2$·2H$_2$O, 0.132 g; MgCl$_2$·H$_2$O, 0.1 g; Na$_2$HPO$_4$·2H$_2$O, 1.15 g; KH$_2$PO$_4$, 0.2 g; made up to 990 ml with distilled water, pH adjusted to 7.4, and made up to 1000 ml with distilled water) and fixed with 4% glutaraldehyde for 10 min. The glutaraldehyde was prepared by diluting 4 ml of a 25% stock solution (TAAB Laboratory Equipment Ltd.) with 25 ml of Sorensen's buffer, pH 7.4 (19.6 ml of $6.7 \times 10^{-2}$ M KH$_2$PO$_4$ and 80.4 ml of $6.7 \times 10^{-4}$ M Na$_2$HPO$_4$). The slide was then sequentially washed with distilled water, 70% alcohol, 95% alcohol and absolute alcohol.

The slide was coated and developed by Mr. L. Bowler of the Chemical Pathology Department, University College and Middlesex School of Medicine, London. Coating of the slide took place in a dark room. A heaped spatula of emulsion (Emulsion K2 in gel form, Ilford Ltd.) was mixed with an equal volume of distilled water (previously heated to 37°C in a water bath) to form a thickish slurry and kept warm. The emulsion was poured down the slide, holding the latter almost vertically, to form an even coat. The edges of the slide were wiped, before placing it in a rack in a drying cabinet for 10 min. The slide was subsequently transferred to a light-proof box which was sealed, taped and left at 4°C for 7 days. Developing of the slide took place in a dark room. Developer (15 ml, Contrast FF developer, Ilford Ltd.) was added to distilled water (100 ml) at ambient temperature, poured into the slide box and mixed with the lid on. The box was left for 15 min with mixing every 5 min, and then washed with distilled water. Hypam fixative (Ilford Ltd.) was added and the slide left for 5 min with mixing. The slide was removed from the dark room and thoroughly washed with tap water and rinsed with distilled water. The dry slide
was stained with freshly filtered Giemsa R66 (BDH) for 20 min and then sequentially washed and dehydrated with distilled water, 70% alcohol, 95% alcohol, absolute alcohol (for 1 min), absolute alcohol (for 2 min), and xylene (for 2x1 min) to clear. The slide was air-dried, mounted in Deepex and examined under a microscope. All the cells employed in the biological testing of 5'-phosphate derivatives of araA were found to be free from *Mycoplasma* contamination.

**Tritiated thymidine incorporation assay**

**Day 1:** Cells were sub-cultured (as previously described) at a seeding density of $2.3 \times 10^8$ cells/ml in a large falcon (30ml) for use the following week. Experiments were performed in multiwell trays, with a growth area of 2.1 cm$^2$/ml. Only the inner eight wells of each tray were used, being seeded with cells at a density of $5 \times 10^4$ cells/ml in growth medium (1ml/well) in a "snake fashion", while the outer sixteen wells were filled with SFM (1 ml). The trays were placed unstacked in a 37°C incubator with a 2% carbon dioxide atmosphere for 48 h.

**Day 2:** Solutions of the compounds to be tested were prepared in non-sterile distilled water at the highest concentration, filter sterilized and diluted with sterile distilled water to the other required concentrations.

**Day 3:** All solutions for addition to the eight centre wells of each tray were allowed to warm to 37°C in the warm room prior to use in the experiment. Solutions of the compounds to be tested, and the sterile distilled water control, were added (100 μl/well) to a set of four replicate wells and mixed by gentle rocking. The cells were incubated at 37°C in a 2% carbon dioxide atmosphere for 30 min. Tritiated thymidine (i.e. (methyl-$^3$H)thymidine, specific activity 925 GBq/mmol, Amersham International P.L.C.) from a stock solution (1000 μCi/ml) was diluted in sterile phosphate buffered saline solution prior to use (giving a concentration of 20 μCi/ml), added to each well (50 μl/well, giving a final concentration of 1 μCi/ml) and mixed by gentle rocking. The cells were incubated at 37°C in a 2% carbon dioxide atmosphere for a further 30 min. After this incubation period, the cells were washed carefully five times with phosphate buffered saline solution to remove any unincorporated isotope, drained and fixed by incubation at 4°C for 30 min.
with 2,2,2-trichloroacetic acid solution (5%, 1ml/well). The cells were subsequently washed twice with phosphate buffered saline solution, drained and dried carefully in a stream of warm air. The acid insoluble cell contents were dissolved in sodium hydroxide solution (1M, 250 μl/well) by overnight digestion, placing the trays in sealed plastic boxes (to minimize evaporation) at 37°C in a 2% carbon dioxide atmosphere.

**Day 4:** Hydrochloric acid (1 M, 100 μl) and Ecoscint A (4 ml) were added to an aliquot (100 μl) of the digest from each well, mixed, and the radioactivity of the digest was measured using a scintillation counter (Intertechnique ABAC SL/40). The remaining digest from each set of four replicate wells was pooled (i.e. 4 x 150 μl), added to a cuvette containing sodium hydroxide solution (1 M, 2400 μl), mixed, and the absorbance at λ=280 nm was measured using a uv/vis spectrophotometer (Unicam SP500 series 2). The latter had been zeroed using two cuvettes each containing sodium hydroxide solution (1 M, 3000 μl). The absorbance was measured as an estimate of the total cellular protein and hence cell numbers for each set of four replicate wells.

This gives an indication of the regularity of cell seeding and whether cells have been lost during the washings.

The results of the assay were recorded in counts/min of β-emission from the tritiated thymidine incorporated into the DNA of the cells. Since thymidine incorporation into cellular DNA may be regarded as a measure of DNA synthesis, these results may be used to calculate the mean % inhibition of DNA synthesis, relative to the distilled water control, and standard error of the mean (SEM) for each set of replicate wells. A two-tail student’s t-test was used to determine whether the difference between the mean % inhibition values for two particular compounds at a given concentration was statistically significant (i.e. P < 5%).
Assay Results

(i) Chapter 2

Table 2: Inhibition of the incorporation of tritiated thymidine into cellular DNA in the presence of araA, (39), (60), (61) and (62) at final concentrations of 0.03 mM and 0.003 mM, relative to the distilled water control

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Inhibition&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>77.7</td>
<td>1.1</td>
<td>31.3</td>
<td>1.3</td>
</tr>
<tr>
<td>(39)</td>
<td>45.4</td>
<td>5.1</td>
<td>23.2</td>
<td>3.5</td>
</tr>
<tr>
<td>(60)</td>
<td>23.9</td>
<td>3.6</td>
<td>6.3</td>
<td>3.2</td>
</tr>
<tr>
<td>(61)</td>
<td>79.1</td>
<td>1.1</td>
<td>20.5</td>
<td>5.1</td>
</tr>
<tr>
<td>(62)</td>
<td>52.0</td>
<td>2.0</td>
<td>15.4</td>
<td>8.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> and <sup>b</sup> = final concentrations of 0.03 mM and 0.003 mM respectively

Table 2: Two-tail student’s t-test data for araA, (39), (60), (61) and (62)

<table>
<thead>
<tr>
<th>Compound</th>
<th>P(%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P(%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(39) vs. araA</td>
<td>0.28</td>
<td>15.6 (NS)</td>
</tr>
<tr>
<td>(60) vs. araA</td>
<td>5.82x10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.129</td>
</tr>
<tr>
<td>(61) vs. araA</td>
<td>55.8 (NS)</td>
<td>17.3 (NS)</td>
</tr>
<tr>
<td>(62) vs. araA</td>
<td>0.164</td>
<td>19.6 (NS)</td>
</tr>
<tr>
<td>(39) vs. (60)</td>
<td>1.16</td>
<td>0.865</td>
</tr>
<tr>
<td>(61) vs. (62)</td>
<td>5.38x10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>62.8 (NS)</td>
</tr>
</tbody>
</table>

<sup>a</sup> and <sup>b</sup> = final concentrations of 0.03 mM and 0.003 mM respectively

NS = not significant (i.e. P > 5%) in a two-tail student’s t-test

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(ii) Chapter 3

Table 3: Inhibition of the incorporation of tritiated thymidine into cellular DNA in the presence of araA, (86), (83), (80), (38), (39) and (40) at final concentrations of 0.03 mM and 0.003 mM, relative to the distilled water control

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition</th>
<th>SEM</th>
<th>% Inhibition</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>63.7</td>
<td>2.1</td>
<td>27.1</td>
<td>3.4</td>
</tr>
<tr>
<td>(86)</td>
<td>59.5</td>
<td>1.7</td>
<td>11.5</td>
<td>2.3</td>
</tr>
<tr>
<td>(83)</td>
<td>67.4</td>
<td>1.7</td>
<td>13.7</td>
<td>4.6</td>
</tr>
<tr>
<td>(80)</td>
<td>77.6</td>
<td>1.4</td>
<td>27.6</td>
<td>2.1</td>
</tr>
<tr>
<td>(38)</td>
<td>25.8</td>
<td>1.7</td>
<td>11.9</td>
<td>1.9</td>
</tr>
<tr>
<td>(39)</td>
<td>35.6</td>
<td>2.7</td>
<td>12.9</td>
<td>2.7</td>
</tr>
<tr>
<td>(40)</td>
<td>67.3</td>
<td>3.1</td>
<td>31.2</td>
<td>3.9</td>
</tr>
</tbody>
</table>

a and b = final concentrations of 0.03 mM and 0.003 mM respectively

Table 4: Two-tail student's t-test data for araA, (86), (83), (80), (38), (39) and (40)

<table>
<thead>
<tr>
<th>Compound</th>
<th>P(%)</th>
<th>P(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(86) vs. araA</td>
<td>16.6 (NS)</td>
<td>0.35</td>
</tr>
<tr>
<td>(83) vs. araA</td>
<td>22.0 (NS)</td>
<td>5.13 (NS)</td>
</tr>
<tr>
<td>(80) vs. araA</td>
<td>0.0303</td>
<td>59.3 (NS)</td>
</tr>
<tr>
<td>(38) vs. araA</td>
<td>1.49x10^-4</td>
<td>0.601</td>
</tr>
<tr>
<td>(39) vs. araA</td>
<td>2.17x10^-3</td>
<td>0.898</td>
</tr>
<tr>
<td>(40) vs. araA</td>
<td>38.3 (NS)</td>
<td>48.4 (NS)</td>
</tr>
<tr>
<td>(86) vs. (38)</td>
<td>1.6x10^-4</td>
<td>59.3 (NS)</td>
</tr>
<tr>
<td>(83) vs. (39)</td>
<td>7.51x10^-4</td>
<td>61.9 (NS)</td>
</tr>
<tr>
<td>(80) vs. (40)</td>
<td>1.26</td>
<td>45.2 (NS)</td>
</tr>
</tbody>
</table>

a and b = final concentrations of 0.03 mM and 0.003 mM respectively

NS = not significant (i.e. P > 5%) in a two-tail student's t-test
Table 5: Inhibition of the incorporation of tritiated thymidine into cellular DNA in the presence of araA, (93), (80) and (43) at final concentrations of 0.03 mM and 0.003 mM, relative to the distilled water control

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition</th>
<th>SEM</th>
<th>% Inhibition</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>71.3</td>
<td>0.6</td>
<td>27.0</td>
<td>3.5</td>
</tr>
<tr>
<td>(93)</td>
<td>77.4</td>
<td>0.5</td>
<td>29.0</td>
<td>2.2</td>
</tr>
<tr>
<td>(80)</td>
<td>77.0</td>
<td>0.8</td>
<td>31.9</td>
<td>1.4</td>
</tr>
<tr>
<td>(43)</td>
<td>57.6</td>
<td>2.7</td>
<td>26.3</td>
<td>4.7</td>
</tr>
</tbody>
</table>

a and b = final concentrations of 0.03 mM and 0.003 mM respectively

Table 6: Two-tail student's t-test data for araA, (93), (80) and (43)

<table>
<thead>
<tr>
<th>Compound</th>
<th>P(%)</th>
<th>P(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(93) vs. araA</td>
<td>3.76x10^-3</td>
<td>64.6 (NS)</td>
</tr>
<tr>
<td>(80) vs. araA</td>
<td>0.0217</td>
<td>24.9 (NS)</td>
</tr>
<tr>
<td>(43) vs. araA</td>
<td>0.0529</td>
<td>58.4 (NS)</td>
</tr>
<tr>
<td>(93) vs. (80)</td>
<td>66.7 (NS)</td>
<td>32.6 (NS)</td>
</tr>
<tr>
<td>(93) vs. (43)</td>
<td>5.0x10^-3</td>
<td>62.7 (NS)</td>
</tr>
</tbody>
</table>

a and b = final concentrations of 0.03 mM and 0.003 mM respectively

NS = not significant (i.e. P > 5%) in a two-tail student’s t-test
### Table 7: Inhibition of the incorporation of tritiated thymidine into cellular DNA in the presence of araA, (94), (97), (105), (109), (111) and (61) at final concentrations of 0.03 mM and 0.003 mM, relative to the distilled water control

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Inhibition&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SEM&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>63.7</td>
<td>2.1</td>
<td>27.1</td>
<td>3.4</td>
</tr>
<tr>
<td>(94)</td>
<td>56.4</td>
<td>5.7</td>
<td>23.0</td>
<td>6.3</td>
</tr>
<tr>
<td>(97)</td>
<td>77.5</td>
<td>1.3</td>
<td>24.2</td>
<td>2.5</td>
</tr>
<tr>
<td>(105)</td>
<td>63.1</td>
<td>1.6</td>
<td>17.3</td>
<td>2.5</td>
</tr>
<tr>
<td>(109)</td>
<td>79.2</td>
<td>2.0</td>
<td>25.7</td>
<td>3.0</td>
</tr>
<tr>
<td>(111)</td>
<td>76.9</td>
<td>1.1</td>
<td>16.7</td>
<td>2.6</td>
</tr>
<tr>
<td>(61)</td>
<td>79.6</td>
<td>1.2</td>
<td>24.6</td>
<td>5.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> and <sup>b</sup> = final concentrations of 0.03 mM and 0.003 mM respectively

### Table 8: Two-tail student’s t-test data for araA, (94), (97), (105), (109), (111) and (61)

<table>
<thead>
<tr>
<th>Compound</th>
<th>P(%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P(%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(94) vs. araA</td>
<td>27.8 (NS)</td>
<td>60.5 (NS)</td>
</tr>
<tr>
<td>(97) vs. araA</td>
<td>0.0272</td>
<td>54.6 (NS)</td>
</tr>
<tr>
<td>(105) vs. araA</td>
<td>67.2 (NS)</td>
<td>4.54</td>
</tr>
<tr>
<td>(109) vs. araA</td>
<td>0.0374</td>
<td>68.6 (NS)</td>
</tr>
<tr>
<td>(111) vs. araA</td>
<td>0.0435</td>
<td>3.91</td>
</tr>
<tr>
<td>(61) vs. araA</td>
<td>0.0149</td>
<td>68.6 (NS)</td>
</tr>
<tr>
<td>(94) vs. (61)</td>
<td>0.425</td>
<td>64.7 (NS)</td>
</tr>
<tr>
<td>(97) vs. (61)</td>
<td>29.7 (NS)</td>
<td>48.7 (NS)</td>
</tr>
<tr>
<td>(97) vs. (94)</td>
<td>0.472</td>
<td>64.0 (NS)</td>
</tr>
<tr>
<td>(105) vs. (97)</td>
<td>6.98x10⁻³</td>
<td>9.09 (NS)</td>
</tr>
<tr>
<td>(109) vs. (97)</td>
<td>52.4 (NS)</td>
<td>68.0 (NS)</td>
</tr>
<tr>
<td>(111) vs. (97)</td>
<td>68.6 (NS)</td>
<td>7.65 (NS)</td>
</tr>
</tbody>
</table>

<sup>a</sup> and <sup>b</sup> = final concentrations of 0.03 mM and 0.003 mM respectively

NS = not significant (i.e. P > 5%) in a two-tail student’s t-test
Table 9: Inhibition of the incorporation of tritiated thymidine into cellular DNA in the presence of araA, (117), (121), (125), (128), (118), (122), (126) and (38) at a final concentration of 0.03 mM, relative to the distilled water control

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>71.7</td>
<td>2.1</td>
</tr>
<tr>
<td>(117)</td>
<td>70.0</td>
<td>1.9</td>
</tr>
<tr>
<td>(121)</td>
<td>59.8</td>
<td>2.1</td>
</tr>
<tr>
<td>(125)</td>
<td>56.3</td>
<td>4.9</td>
</tr>
<tr>
<td>(128)</td>
<td>86.9</td>
<td>1.5</td>
</tr>
<tr>
<td>(118)</td>
<td>56.6</td>
<td>3.8</td>
</tr>
<tr>
<td>(122)</td>
<td>46.3</td>
<td>3.1</td>
</tr>
<tr>
<td>(126)</td>
<td>21.3</td>
<td>2.3</td>
</tr>
<tr>
<td>(38)</td>
<td>24.9</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table 10: Two-tail student’s t-test data for araA, (117), (121), (125), (128), (118), (122), (126) and (38)

<table>
<thead>
<tr>
<th>Compound vs. Compound</th>
<th>P(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(117) vs. araA</td>
<td>61.6  (NS)</td>
</tr>
<tr>
<td>(121) vs. araA</td>
<td>1.34</td>
</tr>
<tr>
<td>(125) vs. araA</td>
<td>4.55</td>
</tr>
<tr>
<td>(128) vs. araA</td>
<td>0.0575</td>
</tr>
<tr>
<td>(118) vs. araA</td>
<td>2.53</td>
</tr>
<tr>
<td>(122) vs. araA</td>
<td>0.151</td>
</tr>
<tr>
<td>(126) vs. araA</td>
<td>2.28x10^-3</td>
</tr>
<tr>
<td>(38) vs. araA</td>
<td>0.013</td>
</tr>
<tr>
<td>(117) vs. (38)</td>
<td>1.5x10^-3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound vs. Compound</th>
<th>P(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(121) vs. (38)</td>
<td>0.03</td>
</tr>
<tr>
<td>(125) vs. (38)</td>
<td>0.311</td>
</tr>
<tr>
<td>(118) vs. (38)</td>
<td>0.0809</td>
</tr>
<tr>
<td>(122) vs. (38)</td>
<td>0.377</td>
</tr>
<tr>
<td>(126) vs. (38)</td>
<td>37.9  (NS)</td>
</tr>
<tr>
<td>(117) vs. (118)</td>
<td>1.15</td>
</tr>
<tr>
<td>(121) vs. (122)</td>
<td>1.95</td>
</tr>
<tr>
<td>(125) vs. (126)</td>
<td>0.0424</td>
</tr>
<tr>
<td>(125) vs. (128)</td>
<td>0.0143</td>
</tr>
</tbody>
</table>

a = final concentration of 0.03 mM

NS = not significant (i.e. P > 5%) in a two-tail student’s t-test
Evaluation of some 5′-phosphate triesters of araA against the herpes simplex-1 virus in Vero cells

Following the encouraging results of the tritiated thymidine incorporation assay determining the ability of some 5′-phosphate derivatives of araA to inhibit the synthesis of DNA in vitro, a number of these compounds were submitted for further biological testing. Since araA is of clinical use in the treatment of various herpesvirus infections, it was of interest to evaluate these derivatives against the herpes simplex-1 virus (HSV-1) in vitro. The present anti-viral assay involved the testing of some of the mixed, unsymmetrical 5′-phosphate triesters of araA and araA-5′-bis(2,2,2-trichloroethyl) phosphate (43), synthesized during the course of this research, along with the previously prepared araA-5′-diphenyl phosphate (61), for anti-HSV-1 activity and toxicity in Vero cells. The biological evaluation was carried out by Dr. N. Mahmood at the Medical Research Council Collaborative Centre, Mill Hill, London. The compounds were tested in two groups at concentrations ranging from 8 to 500 μM, with araA present in each experiment as a positive control.

The results of the anti-viral assay are reported as EC50 and TC50 values, where the former represents the concentration of compound which reduces antigen production by 50% in HSV-1 infected Vero cells (i.e. a measure of anti-HSV-1 activity) and the latter represents the concentration of compound which reduces cell growth by 50% in uninfected Vero cells (i.e. a measure of toxicity). This data is tabulated on the following two pages (tables 11, 12). Several interesting features are evident from the results of the anti-viral assay. Each of the compounds tested shows an inhibitory effect against HSV-1, with the exception of araA-5′-ethyl (2,2,2-trichloroethyl) phosphate (86), and these effects are dose-dependent. None of the 5′-phosphate triesters of araA display a greater biological activity than araA, with the latter reducing antigen production by 50% at a concentration of 20-40 μM. A separation between
Table 11: Anti-HSV-1 activities and toxicities of araA, (86), (80), (43), (61) and (128) in Vero cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. (µM)</th>
<th>Envelope Antigen (%)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>TC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>200</td>
<td>24</td>
<td>40</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(86)</td>
<td>500</td>
<td>83</td>
<td>inactive</td>
<td>&gt; 500</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(80)</td>
<td>500</td>
<td>50</td>
<td>500</td>
<td>&gt; 500</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(43)</td>
<td>500</td>
<td>29</td>
<td>100</td>
<td>&gt; 500</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(61)</td>
<td>500</td>
<td>57</td>
<td>100</td>
<td>&gt; 500</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>78</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(128)</td>
<td>500</td>
<td>62</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a = percentage of control

EC<sub>50</sub> represents the concentration of compound which reduces the antigen production by 50% in HSV-1 infected Vero cells

TC<sub>50</sub> represents the concentration of compound which reduces cell growth by 50% in uninfected Vero cells
Table 12: Anti-HSV-1 activities and toxicities of araA, (97), (105), (109) and (111) in Vero cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. (µM)</th>
<th>Envelope Antigen (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>TC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>araA</td>
<td>200</td>
<td>30</td>
<td>20</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(97)</td>
<td>500</td>
<td>53</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>123</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(105)</td>
<td>200</td>
<td>3.5</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(109)</td>
<td>200</td>
<td>18</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>104</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(111)</td>
<td>200</td>
<td>51</td>
<td>200</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>111</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> = percentage of control

EC<sub>50</sub> represents the concentration of compound which reduces the antigen production by 50% in HSV-1 infected Vero cells.

TC<sub>50</sub> represents the concentration of compound which reduces cell growth by 50% in uninfected Vero cells.
anti-viral activity and toxicity is also clearly apparent for araA, but less so for the other compounds tested.

Considering the 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA, araA-5'-ethyl (2,2,2-trichloroethyl) phosphate (86) is essentially inactive up to the highest concentration tested (500 μM), while araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80) reduces antigen production by 50% at this concentration. Thus, it would appear that the 5'-alkyl (2,2,2-trichloroethyl) phosphate triesters of araA display an increasing biological activity with increasing chain length of their constituent alkyl group. A similar correlation has recently been observed for these derivatives in their inhibitory effect against the synthesis of DNA in vitro (chapter 3). It is also of interest to note that araA-5'-bis(2,2,2-trichloroethyl) phosphate (43) is more efficacious against HSV-1 than its 5'-alkyl (2,2,2-trichloroethyl) analogues (86) and (80), suggesting that the presence of two 2,2,2-trichloroethyl moieties in a 5'-phosphate triester of araA enhances biological activity. Additionally, a separation is observed between effective and toxic doses for araA-5'-bis(2,2,2-trichloroethyl) phosphate (43), while it is possible that the anti-viral activity of araA-5'-butyl (2,2,2-trichloroethyl) phosphate (80) may be due to the toxicity of this compound.

A comparison of the biological activity of araA-5'-((2,2,2-trichloroethyl)phenyl phosphate (97) with araA-5'-diphenyl phosphate (61), reveals that these compounds are equally efficacious against HSV-1. However, the greater toxicity of (61) towards uninfected cells may indicate that the anti-viral activity of this compound is, to some degree, due to toxic effects. Considering the 5'-(2,2,2-trichloroethyl) para-substituted phenyl phosphate triesters of araA (105), (109) and (111), each of these derivatives displays a greater ability to reduce antigen production than araA-5'-(2,2,2-trichloroethyl) phenyl phosphate (97). A slight increase in anti-viral activity is observed for these 5'-(2,2,2-trichloroethyl) para-substituted phenyl phosphate triesters of araA on passing from the p-methoxy (111) to the p-chloro (109) to the p-isopropyl (105) analogue, with EC50 values of 200 μM, 100 μM and 50 μM respectively. Thus, it would appear that different para-substituents confer changes in the biological activity of these 5'-aryl (2,2,2-trichloroethyl) phosphate triesters of araA in this assay system.
Finally, a comparison of the biological activity of araA-5'-ethyl (2,2,2-trichloroethyl) phosphate (86) with araA-5'-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxo-8-ethyl) phosphate (128), reveals that replacing an ethyl group with a benzyl-protected glycolyl moiety enhances biological activity. The former compound (86) is essentially devoid of anti-HSV-1 activity up to the maximum concentration tested (500 µM), while the latter compound (128) reduces antigen production by 62% at this concentration. However, there appeared to be no separation between anti-viral activity and toxicity for araA-5'-(2,2,2-trichloroethyl) (8-benzyloxy-3,6-dioxo-8-ethyl) phosphate (128).

Thus, some interesting relationships were observed between the structures of the compounds tested and their biological activity under the conditions of this assay. Unfortunately, araA was found to display the greatest inhibitory effect against HSV-1, although the results for araA-5'-bis(2,2,2-trichloroethyl) phosphate (43), araA-5'-(2,2,2-trichloroethyl) p-isopropylphenyl phosphate (105) and araA-5'-(2,2,2-trichloroethyl) p-chlorophenyl phosphate (109) may be considered encouraging. However, it must be noted that this data is obtained from a preliminary in vitro assay and as such may poorly reflect the in vivo or clinical properties of these 5'-phosphate triesters of araA. This is particularly noteworthy in view of the fact that under in vivo conditions araA would be subject to deamination, while the 5'-phosphate triesters of araA should be resistant to this mode of deactivation.

Anti-viral assay

Anti-viral activity against herpes simplex-1 virus (HSV-1) was determined by measuring viral antigen production in infected cells by ELISA. Vero cells were grown to confluence in 96 well plates, and five-fold dilutions of compounds were added to duplicate wells just prior to adding virus at a multiplicity of infection of 0.01 plaque-forming units/cell. After incubation at 37°C for 16-18 h, the cells were fixed with 3% formalin for 1-2 h. The plates were washed three times with distilled water, before adding rabbit anti-herpes antibodies (50 µl, Dakopatts, Denmark) diluted 1/500 in medium containing 10% calf serum and 0.1% Tween 20. Incubation of the plates at 37°C
for a further 1-2 h followed. The unbound antibody was removed by washing three times with distilled water. An aliquot (50 μl) of anti-rabbit Ig conjugated to horseradish peroxidase, diluted 1/1000 in medium containing 10% calf serum and 0.1% Tween, was added and incubation at 37°C was continued for 90 min. The plates were washed again with distilled water and developed with o-phenylenediamine (1 mg/ml) and 0.03% hydrogen peroxide in citrate buffer (0.1 M, pH 5). The reaction was stopped after 5-10 min at 25°C by the addition of H₂SO₄ (1 M) and optical densities were read at 492 nm against uninfected cell controls.
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