SYNTHESIS AND EVALUATION OF SOME NOVEL NUCLEOTIDE DERIVATIVES AS POTENTIAL ANTI-AIDS DRUGS

by

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ABSTRACT

Many nucleoside analogues are known to inhibit human immunodeficiency virus (HIV), the causative agent of the disease acquired immunodeficiency syndrome (AIDS). In order to act these nucleosides must be phosphorylated to their corresponding 5′-triphosphates, which can then inhibit reverse-transcriptase (RT), a key viral enzyme. These phosphorylations are catalysed by cellular kinases.

The synthesis of a number of nucleoside 5′-dialkyl phosphates is described in this thesis, along with the results of the biological evaluation of some of these derivatives against HIV. It was thought that the 5′-dialkyl phosphates might have been able to act as prodrugs for the corresponding 5′-monophosphates.

Firstly the synthesis of 5′-dialkyl phosphates of 3′-O-mesylthymidine, 3′-O-acetylthymidine and 3′-O-ethylthymidine is described, along with the synthesis of some 5′-dialkyl phosphates of 3′-azido-3′-deoxythymidine, 2′,3′-dideoxycytidine and 2′,3′-dideoxyadenosine. However, when tested, all of the above 5′-dialkyl phosphates were found to be inactive against HIV *in vitro*.

It was thought that this inactivity arose from the metabolic stability of the simple dialkyl esterifying groups present in these compounds. With this in mind some nucleoside 5′-bis(2,2,2-trihaloethyl) phosphate derivatives were synthesised as it was hoped that 2,2,2-trihaloethyl groups would be more labile than simple alkyl ones. Both 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trifluoroethyl) phosphate and 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate were found to be inhibitors of HIV *in vitro*.

In view of these results, the synthesis of some 5′-bis(2,2,2-trihaloethyl) phosphate derivatives of 2′,3′-dideoxycytidine and 3′-fluoro-3′-deoxythymidine was undertaken. The synthesis of some mixed 5′-(alkyl 2,2,2-trihaloethyl) phosphate derivatives of 3′-azido-3′-deoxythymidine was also carried out. The 5′-bis(2,2,2-trichloroethyl) phosphates of 3′-O-mesylthymidine, 3′-O-acetylthymidine, 3′-O-ethylthymidine and 3′-amino-3′-deoxythymidine were also synthesised.

As 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate was able to inhibit HIV *in vitro*, the 5′-bis(2-chloroethyl) phosphate and the 5′-bis(2,2-dichloroethyl) phosphate of 3′-azido-3′-deoxythymidine were prepared, in order to ascertain if these compounds were able to inhibit HIV also. The 5′-bis(2-chloroethyl) phosphate of 3′-azido-3′-deoxythymidine does indeed display activity against HIV *in vitro*.

An attempt was made to synthesise a thymidine 5′-dialkyl phosphonate species, by a Michaelis-Arbuzov type reaction between 5′-bromo-5′-deoxythymidine and diethyl phosphite. However, it was only possible to isolate a 3′-hydrogenphosphate species from this reaction.

As the method by which 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate acts as an inhibitor of HIV probably involves the hydrolysis of the phosphate moiety to either the 5′-monophosphate or the parent nucleoside, some studies on the hydrolysis of 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate at a variety of pH were carried out.

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CONTENTS

ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ARC	AIDS-related complex
bp	boiling point
DNA	deoxyribonucleic acid
EIMS	electron impact mass spectrometry
equivs.	equivalents
FABMS	fast atom bombardment mass spectrometry
h	hour(s)
HIV	human immunodeficiency virus
hplc	high performance liquid chromatography
min	minute(s)
mp	melting point
mRNA	messenger ribonucleic acid
nmr	nuclear magnetic resonance
PBM	peripheral blood mononuclear
ppm	parts per million
RT	reverse-transcriptase
tlc	thin layer chromatography
TMS	tetramethylsilane
uv	ultra violet

INTRODUCTION

It was in 1981 that acquired immunodeficiency syndrome (AIDS) was first identified as a distinct clinical entity[1]. AIDS has since been recognised as a pandemic immuno-suppressive disease. The clinical signs indicative of AIDS[2] include weight loss, generalised lymphadenopathy and the patient developing a number of life-threatening opportunistic infections and malignancies. The most important of these life-threatening opportunistic infections and malignancies include *Pneumocystis carinii* pneumonia (PCP) and Kaposi's sarcoma[3]. A number of other bacterial (eg *Mycobacterium avium-intracellulare*), viral (eg cytomegalovirus, herpes simplex virus, Epstein-Barr virus) and fungal (eg Candida species) infections are also developed by AIDS patients and some may also suffer from neurological diseases which include peripheral neuropathy and fulminant dementia[4]. It became apparent that the causative agent of AIDS is a virus, the human immunodeficiency virus (HIV)[5]. In the past this virus has been referred to as human T-lymphotropic virus type III (HTLV-III) or lymphadenopathy-associated virus (LAV)[6] or occasionally AIDS-associated retrovirus (ARV)[7]. The term HIV is now generally accepted however.

The target of HIV is the human immune system and in particular the helper/inducer T-cells which form an integral part of that system. The virus can be spread by either intimate sexual contact, or the administration of infected blood products or occasionally by the maternal-fetal route. For an unknown time span after initial infection the carrier may remain perfectly healthy and antibody-negative. Some patients may then seroconvert and become antibody-positive. Of these patients, some may then go on to develop full-blown AIDS. Some patients rather than developing full-blown AIDS may develop subclinical illnesses of lesser severity, for which the term AIDS-related complex (ARC) has been coined.

The number of AIDS cases reported to the World Health Organisation up until 30th September 1989 was 182,463, the number of antibody positive people throughout the world is probably many times this figure[8]. There is at present no known cure for AIDS. There are

however a number of strategies which may be of use in the treatment of AIDS. Firstly, therapy may be directed at reconstituting the damaged immune systems of AIDS patients[9]. In an effort to achieve this, transfusions of lymphocytes have been given to patients. Patients have also been given bone marrow transplants and thymic implantations in an effort to supplement the damaged immune system. These attempts have been largely unsuccessful so far, suggesting that HIV is a persistent pathogen throughout the course of the illness. In an effort to enhance what remains of the damaged immune system, various biological response modifiers have been administered to AIDS patients[10]. At present no permanent beneficial effects have been obtained using this technique, however this line of research may prove fruitful in time. Another of the chemotherapeutic strategies aimed at AIDS involves the treatment of the opportunistic infections that AIDS patients suffer from[11]. For example PCP responds well to either sulfamethoxazole, trimethoprim or pentamidine. Also the herpes simplex viral infections common in patients with AIDS may be treated with acyclovir, although treatment may need to be prolonged.

Chemotherapeutic strategy may also be directed at the causative agent of AIDS, that is HIV. Potentially, there are a number of ways in which one or other step in the replicative cycle of HIV may be blocked. If a step in the replicative cycle of HIV were to be blocked, the proliferation of the virus may be inhibited. A lot of attention has recently been given to the replicative cycle of HIV. A discussion of this life cycle, along with some of the ways in which the cycle may be interfered with, is given below.

The first step in the replicative cycle involves the binding of an HIV virion to a suitable receptor of the target cell. It would appear that the T-cell surface glycoprotein CD4 acts as a receptor for HIV's gp120 *env* glycoprotein[12]. Indeed HIV would appear to be only able to replicate in the subset of T-cells which possess a CD4 receptor[13]. It may be expected that patients infected with HIV would develop antibodies which could block the virion's binding to the T-cell. For reasons that are as yet not completely understood, patients infected with HIV do not produce large enough quantities of these antibodies[14]. There has been

great interest in agents which can block the virion's binding to the T-cell. Amongst these agents is a soluble form of the CD4 glycoprotein which can inhibit HIV replication in T-cells[15,16]. Clinical trials with soluble CD4 are currently underway[17]. However, a major problem with soluble CD4 is its low plasma half-life, thus CD4 glycoproteins with longer half-lives are also being investigated[18]. Dextran sulphate (a drug developed in the 1950's as a anti-coagulant) is able to inhibit HIV *in vitro*[19] and this is thought to be due, at least in part, to it having the ability to block the virion's binding to the target cell. In clinical trials, dextran sulphate has not shown an anti-HIV effect when administered orally, intravenous administration is currently being investigated[20]. AL-721, a mixture of neutral lipids extracted from egg yolks, has also been found to inhibit HIV *in vitro*[21]. It is believed that AL-721 acts by "fluidising" the membrane of the host cell, hindering viral binding to the host cell's CD4 receptor. Clinical studies have been instigated using AL-721[10].

After binding to the CD4 receptor, HIV then enters the target cell and the viral RNA is uncoated. It is possible that a drug may be found which can block either the entry of the virus or the uncoating process. The anti-influenza properties of the drug amantadine are believed to arise from this drug's ability to block viral uncoating[22].

The next stage in the replication cycle of HIV involves the uncoated RNA being used as a template in the production of a complementary strand of DNA. This process is catalysed by the viral enzyme reverse-transcriptase (RT). It is at this stage in the replication process and in particular on RT, that many anti-HIV agents act. Any agent that can inhibit the action of RT may have great potential in the treatment of AIDS patients. Once a single-strand piece of DNA has been generated a second complementary copy of DNA is made, the genetic information is therefore encoded in a double strand of DNA. The original viral RNA is then degraded and this is apparently catalysed by ribonuclease H. Any agent able to block this latter step would be quite likely to inhibit the proliferation of HIV. HIV appears to be heavily dependent on RT for its proliferation. A more detailed discussion of RT and the agents known to inhibit it will be left until later in this thesis.

Once HIV DNA has been generated it appears to either circularise or become integrated into the genome of the host cell. This latter process appears to be catalysed by viral integrase[23]. Viral DNA may remain latent for a time before the cell becomes activated by a mechanism which is as yet not fully understood. Viral DNA may then be transcribed into mRNA and genomic RNA and then translation generates the viral proteins. Although these processes use the T-cell's biochemical resources, HIV does specify for two gene products, namely *tat* and *rev*, which play an important regulative role in these stages of the viral replicative cycle[24,25,26]. Like RT, *tat* and *rev* are unique to the virus which makes them promising targets for anti-viral therapy. As more information on their structure and precise function is gained a number of therapies which act at this stage in the replicative cycle will become apparent.

The replication of HIV can be affected by oligodeoxynucleotides; indeed it was known that retroviruses could be inhibited by oligodeoxynucleotides before HIV was discovered[27,28]. It has been found that "anti-sense" oligodeoxynucleotides, that is oligodeoxynucleotides with base pairs complementary to a segment of the viral genome, can inhibit HIV *in vitro*[29,30]. These oligodeoxynucleotides may be short sequences of DNA, or chemically modified DNA (e.g methylphosphonate oligodeoxynucleotides or phosphorothioate oligodeoxynucleotides). Such oligodeoxynucleotides could inhibit HIV by a number of mechanisms, possibly by competing for template-primer at the level of RT, or they may interfere with the binding of the regulatory protein *tat* to its acceptor site[31].

After the viral polyproteins have been formed they undergo a variety of modifications and it may be possible to inhibit one of these. Recently a viral aspartyl protease, which releases structural proteins and enzymes from the viral polyproteins, has had its structure determined by X-ray crystallography. Interestingly, its structure was found to be similar to known aspartyl proteases[32]. It has been shown that HIV is inhibited *in vitro* by pepstatin A, a known aspartyl protease inhibitor and this may well result from inhibition of the viral aspartyl protease[33].

The final stages of the viral replicative cycle involve the assembly of the component parts of the virus at the surface of the target cell, followed by the release of the virus by budding. After budding, the virus is free to infect other target cells. It is thought that interferons act against HIV, at least in part, by interfering with the process of viral budding, although these agents may have other mechanisms of action against HIV as well[34].

A knowledge of the various aspects of the viral replicative cycle of HIV has therefore provided possible approaches for the treatment of patients suffering from the virus. Indeed HIV possesses genes whose exact function is as yet unknown or at least only partially understood. The replicative cycle of HIV is far from being completely understood itself. Yet as more knowledge is amassed on HIV and its replicative cycle a number of new approaches will become apparent.

A key stage in the replicative cycle of HIV (and indeed other retroviruses too) is the production of viral DNA from viral RNA, a process catalysed by RT, the viral DNA polymerase[35]. RT was first discovered in the virions of two RNA tumour viruses, namely Rauscher mouse leukaemia virus and Rous sarcoma virus[36,37]. It became clear that all retroviruses contain RT, an RNA-directed DNA polymerase. RT is dependent on the presence of a divalent cation for activity and Mg^{2+} is preferred for this purpose. RT catalyses the production of viral DNA using the viral genomic RNA as a template and 2′-deoxynucleoside 5′-triphosphates as substrates. RT is a unique viral enzyme and any agent that can inhibit its function is likely to inhibit the proliferation of the virus. The inhibition of HIV-RT has become the target of a number of potential anti-HIV therapies. Moreover it does seem possible that agents do exist which can differentiate between RT and the host's cellular polymerases.

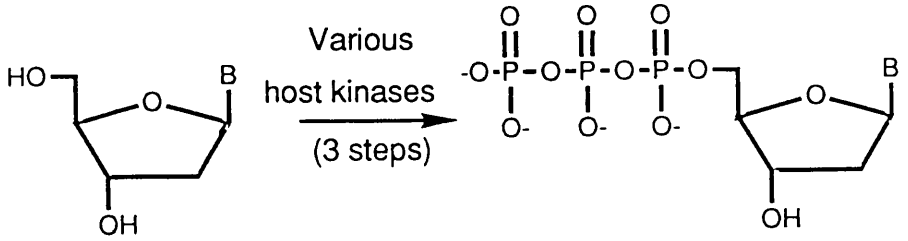
Suramin was known to be an inhibitor of RT before the discovery of HIV and became the first recognised compound that selectively inhibited HIV[38]. Clinical trials were initiated with suramin, however no immunological improvement was found and toxic effects were also noted. It seems that suramin, as currently administered, is not an effective

treatment for AIDS[10]. Phosphonoformate (also known as foscarnet) is known to inhibit HIV replication *in vitro* by inhibiting RT[39,40]. Although phosphonoformate causes a 50% inhibition of the activity of purified HIV-RT at 2 μM, its effect is less marked on inhibiting the replication of HIV in cell cultures, where 50% inhibition of replication occurs at concentrations greater than 100 μM. Phosphonoformate is believed to inhibit RT by interacting with a site where pyrophosphate is split off during the polymerisation process. Clinical trials with phosphonoformate are currently being undertaken[17]. Rifabutin (ansamycin) has been shown to inhibit RT and is also able to inhibit HIV *in vitro*[41]. However no anti-viral response was noted in a dose-escalating clinical trial with rifabutin[10]. This may result from the fact that at the doses used, the drug plasma level was below the *in vitro* effective dose. Antimoniotungstate (HPA-23), a cryptate material, has also been found to inhibit RT *in vitro*[42]. Some clinical trials have been carried out with it but it leads to only a slight improvement in symptoms, despite a dose dependent reduction of RT *in vivo*[10].

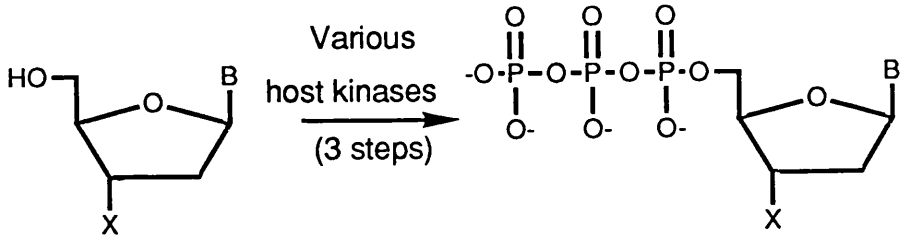
As can be seen a number of compounds have shown the ability to inhibit HIV replication *in vitro* by inhibiting viral RT. Some of these agents have been used clinically. By far the largest group of these HIV inhibitors that have been investigated are the 3′-modified-2′,3′-dideoxynucleosides. There has been extensive reporting of the ability of certain 3′-modified-2′,3′-dideoxynucleosides to inhibit viral replication *in vitro* and these compounds seem to act by inhibiting RT. In explaining the method by which some 3′-modified-2′,3′-dideoxynucleosides can inhibit HIV, it is necessary to consider their similarity to the natural 2′-deoxynucleosides.

3′-Modified-2′,3′-dideoxynucleosides are analogues of 2′-deoxynucleosides which have had their 3′-OH group replaced by another group, which in diagram 1 is depicted by X. The exact nature of this X group will be discussed later. Having entered an infected cell, certain 3′-modified-2′,3′-dideoxynucleosidescan be phosphorylated by host kinases to their 5′-triphosphates. HIV appears not to encode for kinases in its own genome. The 5′-triphosphates of these 3′-modified-2′,3′-dideoxynucleosides can, in turn, become substrates

Diagram 1



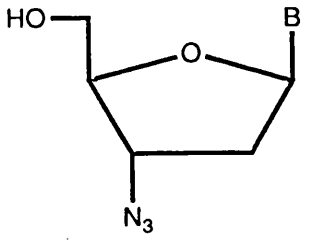
dNTP (natural substrate for RT)



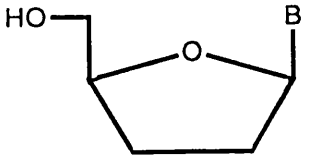
Competitive inhibitors with dNTPs for RT

B= T, A, C, G or modified base

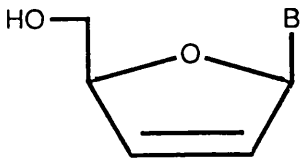
Diagram 2



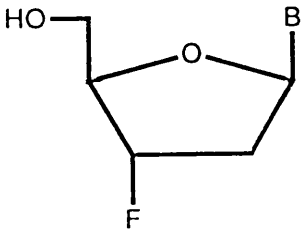
1. 3' -azido-2',3'-dideoxynucleosides



2. 2',3'-dideoxynucleosides



3. 2',3'-olefinic nucleosides



4. 3'-fluoro-2',3'-dideoxynucleosides

for RT and can act as competitive inhibitors with the 5′-triphosphates of the natural 2′-deoxynucleosides for RT. Moreover, if the 5′-triphosphate of a 3′-modified-2′,3′-dideoxynucleoside were to be incorporated into the viral DNA chain by RT it would also act as a chain terminator as it does not possess a 3′-OH group and therefore cannot form a 3′,5′-phosphodiester linkage.

The majority of the nucleosides which have, up to now, been found to be potent inhibitors of HIV fall within four main categories. Those categories are; 1) the 3′-azido-2′,3′-dideoxynucleosides; 2) the 2′,3′-dideoxynucleosides; 3) 2′,3′-olefinic nucleosides and 4) the 3′-fluoro-2′,3′-dideoxynucleosides, (diagram 2).

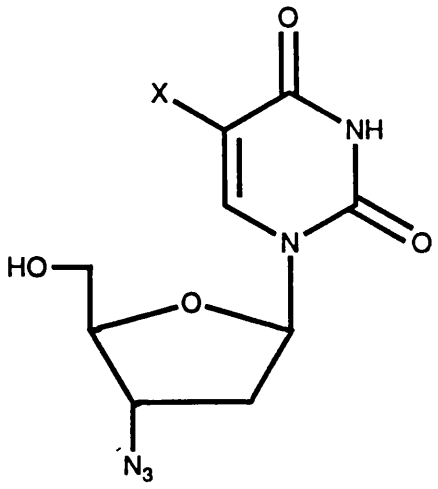
A detailed discussion of the known inhibitors of HIV that belong to these categories is given below.

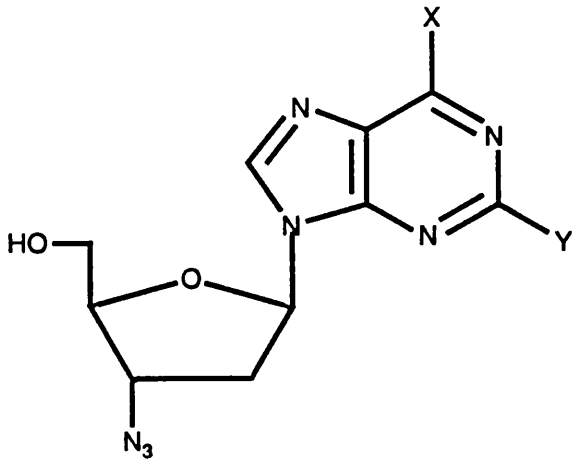
3′-Azido-2′,3′-dideoxynucleosides as inhibitors of HIV (diagram 3).

3′-Azido-3′-deoxythymidine (**1**) was prepared first in 1964[43] and was later shown to inhibit the retrovirus Friend lymphatic leukaemia helper virus[44]. It was Mitsuya *et al* who showed **1** to be a potent inhibitor of HIV[45]. The cellular function of uninfected cells was unaffected even at relatively high concentrations of **1**. A number of studies have been carried out on **1** concerning its inhibition of HIV and its mode of action. Despite **1** being discovered relatively early amongst nucleoside inhibitors of HIV, it remains one of the most potent.

1 acts by first entering the cell (mainly by non-facilitated diffusion)[46]. This is followed by **1** being sequentially phosphorylated, through its 5′-monophosphate and 5′-diphosphate, to its 5′-triphosphate[47] (diagram 4). [^{3}H]-Labelled **1** incubated with HIV-infected cells gave metabolites which were identified as the 5′-mono, 5′-di and 5′-triphosphates of **1**. It is cellular kinases that catalyse the generation of these metabolites[48]. It is the 5′-triphosphate of **1** that is the active anti-HIV agent, the inhibitor of HIV-RT[49,50]. The 5′-triphosphate of **1** competes with the 5′-triphosphate of thymidine for incorporation into the lengthening DNA chain. Amongst the evidence for this is the fact that added thymidine

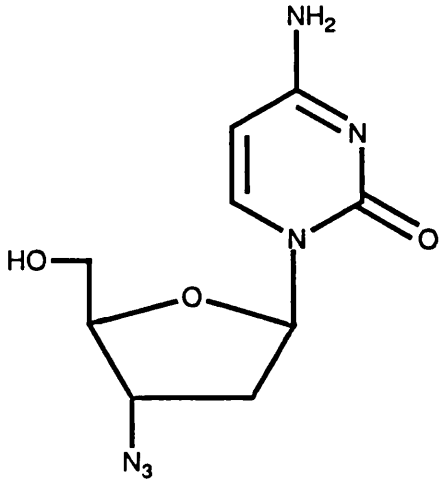
Diagram 3





1, X=CH_3
5, X=H
6, X=C_2H_5
7, X=OH
8, X=F
9, X=SCN

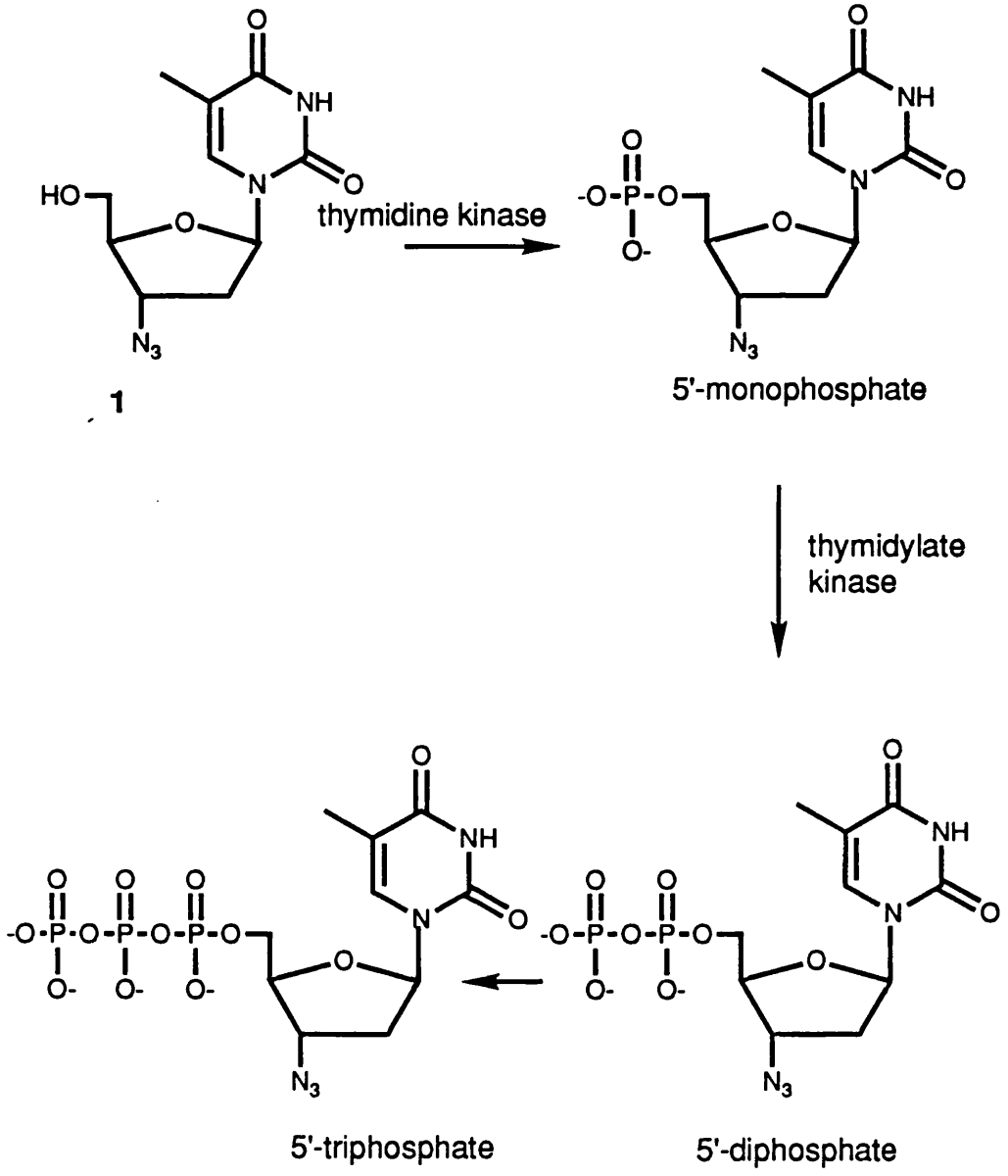
3, X=OH, Y=NH_2
4, X=NH_2 Y=H
11, X=NH_2, Y=NH_2



10

Some 3'-azido-2',3'-dideoxynucleosides able to act as inhibitors of HIV.

Diagram 4



1

5'-monophosphate

5'-triphosphate

5'-diphosphate

The final step from 5'-diphosphate to 5'-triphosphate may be catalysed by nucleoside diphosphate kinase although this is not fully clear at present.

The phosphorylation of **1** to its 5'-triphosphate.

reverses the anti-viral effect of **1**[45]. Once **1** has been incorporated into the DNA chain, further elongation of the chain can not take place. **1** acts as a chain terminator. The 5′-triphosphate of **1** is a poor substrate for cellular DNA polymerase α, although it is a reasonable substrate of DNA polymerase ß and γ. However these polymerases are much less susceptible to inhibition by the 5′-triphosphate of **1** than is RT[51].

In view of its promise, **1** became the first anti-AIDS drug to enter widespread clinical use. Clinical trials first took place in 1986, on patients with AIDS or ARC[52]. Although only a six week trial, the first results were quite promising. **1** was absorbed from the gut and crossed the blood-brain barrier (of interest as HIV can lie dormant in the brain for a time). Some clinical improvement was noted in most patients receiving **1**. A long term, double-blind placebo-controlled trial found that although clinical improvement was maintained, serious toxic side effects induced by **1** were observed[53]. These effects included anaemia, neutropenia and bone-marrow suppression[54]. Many patients required blood transfusions. The therapeutic usefulness of **1** may well be limited by these toxic effects. It has been shown that **1** can inhibit mitochondrial DNA polymerase γ and it has been suggested that this may be a primary cause of the toxic side effects shown by **1**[55]. However this does not explain **1** causing specific toxicity in bone marrow cells.

Recently, it has been reported that HIV isolated from patients receiving **1** for a year or more was less sensitive to the drug than virus isolated from the same patients at the beginning of therapy[56]. This could well indicate that the virus becomes resistant to **1** over a period of time. It is currently thought that the loss of sensitivity to **1** is not a rationale for halting therapy. It has however meant that attempting to treat AIDS with combinations of agents has grown in importance[17]. For example, **1** and 2′,3′-dideoxycytidine (**2**) have been administered together in a clinical trial[57] (details of this trial will be discussed later in this thesis). Interestingly acyclovir, which itself does not inhibit the replication of HIV, has been found to synergistically increase the ability of **1** to inhibit HIV replication *in vitro*[58] and with this in mind a clinical trial using a regimen of **1** and acyclovir has been instigated[59]. **1** has

recently been used in combination with ribavirin[60] in clinical trials and **1** has been shown to act synergistically with phosphonoformate[61] and α-interferon[62] against HIV *in vitro*.

In summary, **1** (marketed under the name Zidovudine) is the only licensed clinical anti-AIDS drug so far. It can improve the quality of life of some AIDS patients, but at the expense of toxic side effects, side effects which may limit its use as a single agent. In view of the anti-HIV properties exhibited by **1**, a number of 3′-azido-2′,3′-dideoxynucleosides have been tested against HIV *in vitro*. Some of these derivatives are themselves potent inhibitors of HIV. 3′-Azido-2′,3′-dideoxyguanosine (**3**) is a potent inhibitor of HIV *in vitro*[63,64], as is 3′-azido-2′,3′-dideoxyadenosine (**4**), however the latter is quite cytotoxic[65]. The main drawback of **1** as a chemotherapeutic agent is its bone marrow toxicity. Indeed an *in vitro* test which evaluates the toxic effects of a drug on bone marrow has been developed using haematopoietic progenitor cells[66]. These cells were inhibited by **1** at a lower concentration than was necessary to inhibit the cytopathic effect of HIV[67]. However two analogues of **1**, 3′-azido-2′,3′-dideoxyuridine (**5**) and 3′-azido-2′,3′-dideoxy-5-ethyluridine (**6**) have much of the activity of **1** but are less cytotoxic to bone marrow cells[68]. A pre-clinical trial of **5** is currently underway[17].

In fact a number of 3′-azido-2′,3′-dideoxy-5-substituted uridine derivatives have been prepared and tested against HIV by Lin *et al*[69]. 5-Hydroxy (**7**), 5-fluoro (**8**) and 5-thiocyano (**9**) substituted 3′-azido-2′,3′-dideoxyuridine derivatives all possess some anti-HIV activity, but there was no clear relationship between anti-viral activity and the electron-withdrawing or the electron-donating properties of the substituent at the 5-position. Lin *et al* also found that 3′-azido-2′,3′-dideoxycytidine (**10**)[70] was an inhibitor of HIV in peripheral blood mononuclear (PBM) cells, but it was around six times less active than **1**. 3′-Azido-2,6-diaminopurine-2′,3′-dideoxyriboside (**11**) has been found to be a potent and selective inhibitor of HIV[71,72]. The activity of **11** is hardly reduced on addition to the assay of the adenosine deaminase inhibitor 2′-deoxycoformycin. This suggests that **11** acts without being substantially converted into **3** by the cell's adenosine deaminase.

2′,3′-Dideoxynucleosides as inhibitors of HIV (diagram 5).

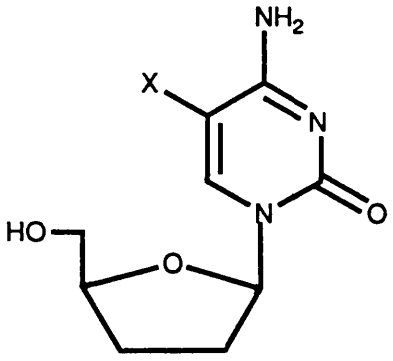
The second type of nucleoside inhibitors of HIV are the 2′,3′-dideoxynucleosides. Of this group **2** has been reported on most extensively in the literature. **2** was, like **1**, known before the discovery of retroviruses[73]. **2** was found to be an inhibitor of HIV replication by Mitsuya *et al*[74]. Indeed in most systems it appears to be a slightly better inhibitor than **1** *in vitro*[10]. A number of pharmacological studies have been carried out on **2**. Mitsuya *et al* found that **2** inhibited HIV replication for long periods *in vitro*[75] without damaging the viability or the immune response of the host cells. This was of great interest with respect to the possible clinical use of **2**.

The cellular metabolism of **2** has been studied. By using [^{3}H]-labelled **2**, it has been shown that **2** is partially converted into its 5′-mono, 5′-di and 5′-triphosphate sequentially in the cell[76,77]. Addition of 2′-deoxycytidine to this assay was found to inhibit the cellular phosphorylation of **2**. It is the 5′-triphosphate of **2** that is the active form of the drug. Once generated, the 5′-triphosphate of **2** can compete with the 5′-triphosphate of 2′-deoxycytidine for incorporation into the viral DNA chain by RT. The 5′-triphosphate of **2** is a poor substrate for cellular DNA polymerases, which accounts for the specificity and lack of toxicity displayed by **2** *in vitro*.

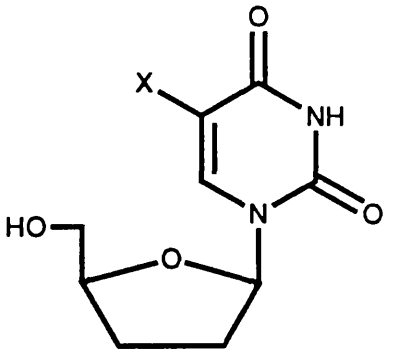
The methods by which both **2** and **1** inhibit RT are very similar. Both are potent and selective anti-HIV agents. Such has been the interest in these agents that **2**[78] and **1**[79] have recently had their structures determined by X-ray crystallography. The solid state structure of **2** contained an unusual furanose ring conformation (a $_3T^4$ pucker), a conformation that is not found in many nucleosides, but which **1** also displays. Whether this is a coincidence or linked to the anti-HIV activity that both compounds display is not clear at present.

Some studies to determine the viability of **2** as an anti-HIV agent *in vivo* have been carried out. Studies carried out in mice and monkeys[80] showed that the drug's administration was straightforward and its bioavalibility was good. **2** was found to be converted to 2′,3′-dideoxyuridine (**12**), presumably by enzymic deamination. **12** was found in the plasma, urine

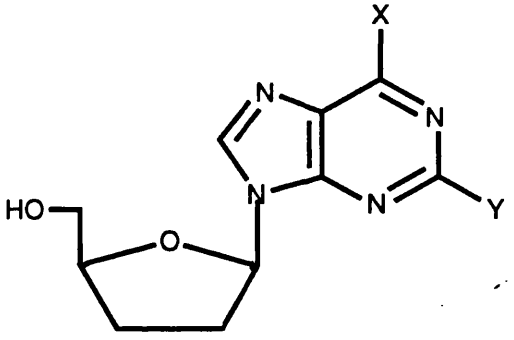
Diagram 5



2, X=H
26, X=F



12, X=H
15, X=CH_3



13, X=NH_2, Y=H
14, X=OH, Y=NH_2
16, X=OH, Y=H
25, X=NH_2, Y=NH_2

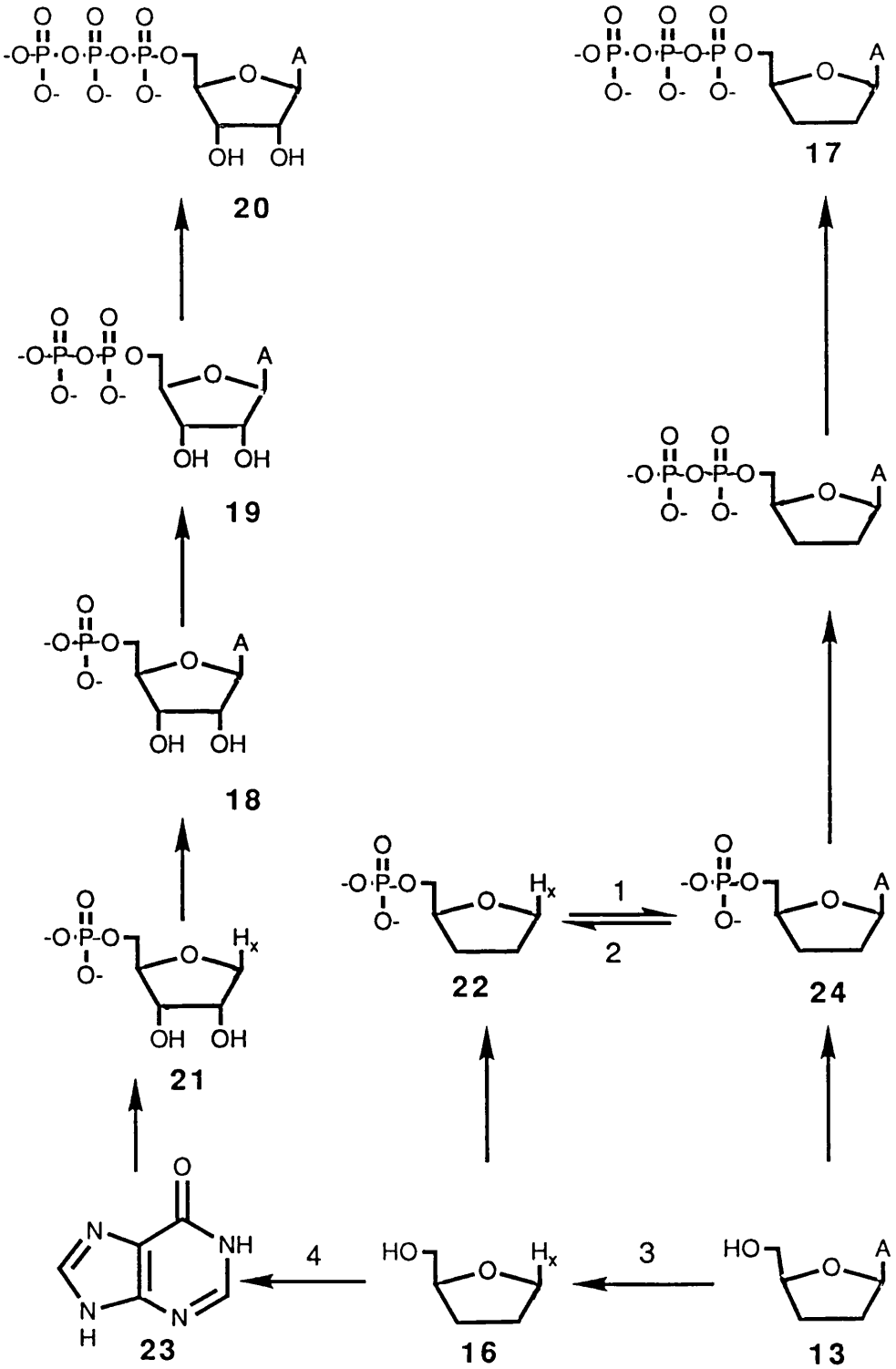
Some 2',3'-dideoxynucleosides able to act as inhibitors of HIV

and cerebrospinal fluid. By administering [^{3}H]-labelled **2** and later examining plasma using hplc it was found that **2** was phosphorylated *in vivo* to its 5′-mono, 5′-di and 5′-triphosphates.

Clinical trials have been carried out on **2**[81]. **2** was found to be tolerated well in the short term and was found to cross the blood-brain barrier. However it gave rise to a number of toxic side effects, principally peripheral neuropathy[82], which may limit its use clinically. In the light of the above, a clinical trial has been instigated involving treatment with an alternate regimen of **1** and **2**[57]. The toxic side effects of these drugs are different so by alternating the administration of the two drugs from week to week, patients may experience fewer toxic side effects overall. Also clinical trials are currently planned whereby **2** is given to patients at lower doses than have previously been administered[17] It is hoped that at these lower doses viral replication would be inhibited and toxic side effects would be less marked, However, it may be these toxic side effects which limit the use of **2** clinically.

As well as **2**, 2′,3′-dideoxyadenosine (**13**), 2′,3′-dideoxyguanosine (**14**), 2′,3′-dideoxythymidine (**15**) and 2′,3′-dideoxyinosine (**16**) also inhibit HIV *in vitro*[74]. Of these compounds **2** is the most potent. **13** and **16** are almost equiactive. The cellular pharmacology of both **13** and **16** has been studied[83,84]. **13** was found to be catabolised extensively. [^{3}H]-labelled **13** was added to infected ATH8 cells. Analysis of the products by hplc, revealed that a small amount of the 5′-triphosphate of **13** (**17**) had been formed; **17** is believed to be the active form of the drug. The major products generated by **13** in ATH8 cells were the ribonucleotides adenosine 5′-monophosphate (**18**), adenosine 5′-diphosphate (**19**), adenosine 5′-triphosphate (**20**) and inosine 5′-monophosphate (**21**). Interestingly when [^{3}H]-labelled **16** was administered to infected ATH8 cells, the same catabolic products generated by **13** were generated by **16**. The explanation for these observations is given in diagram 6 which depicts the rather complex metabolism of **13** and **16**. **13** may be deaminated quickly to **16** by adenosine deaminase[85]. This deamination has been shown to occur *in vivo* in mouse plasma[86].

Diagram 6



1= adenylolyase-succinate
2= adenylate deaminase
3= adenosine deaminase
4= purine nucleoside phosphorylase

A= 9-adenine
H_x=9-hypoxanthine

The metabolic pathways of **13** and **16** ; adapted from Ahluwalia *et al.* [84]

16 can generate its 5′-monophosphate (**22**) or it may generate hypoxanthine (**23**), a process catalysed by purine nucleoside phosphorylase[87]. **23** can be metabolised to **21, 18, 19** and **20**. **22** is able to be aminated to **24** by adenylolyase-succinate[84]. From the metabolic pathways depicted in diagram 6 it can be seen that both **13** and **16** act as precursors of **17**. Both **13** and **16** seem to exert their pharmacological influence through **17**, which empirically at least explains the observation that **13** and **16** are almost equiactive against HIV.

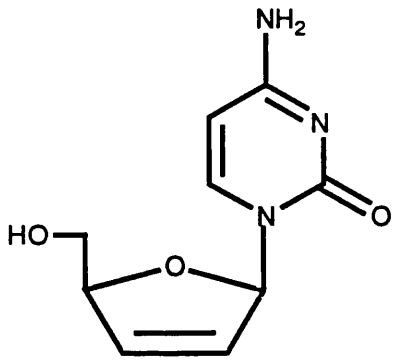
Despite their rather complex metabolism, **13** and **16** are quite potent inhibitors of HIV *in vitro* and have entered clinical trials recently[17]. As **13** and **16** share the same metabolism and act through the same metabolite (ie **17**) they can be considered alternate forms of the same drug. The results obtained from a clinical trial using **16** are promising[88]. In the short term the number of circulating T-cells was increased. Some toxic side effects were noted including mild headaches and insomnia. Further studies are necessary before the role of **13** and **16** in AIDS therapy can be ascertained.

The 2′,3′-dideoxyriboside of 2,6-diaminopurine (**25**) (diagram 5) has been synthesised and tested against HIV *in vitro*[89]. The results of this test showed **25** to be comparable to **13** in its ability to protect MT4 cells against HIV. If deaminated by adenosine deaminase, **25** would yield **14**, a potent inhibitor of HIV itself[74]. It appears that **25** has potential as a chemotherapeutic agent against HIV. Although able to inhibit HIV replication *in vitro*, **15** is considerably less potent than **2**, **13** and **16**[74]. This can be explained by **15** being inefficiently phosphorylated to its 5′-triphosphate (the active form of the drug) by cellular kinases[10]. In view of **2** exhibiting a potent protective quality, various 5-substituted analogs of **2** have been prepared and evaluated against HIV *in vitro*[90]. However, of a variety of derivatives prepared only 5-fluoro-2′,3′-dideoxycytidine (**26**) retained any of the potency and selectivity of **2**.

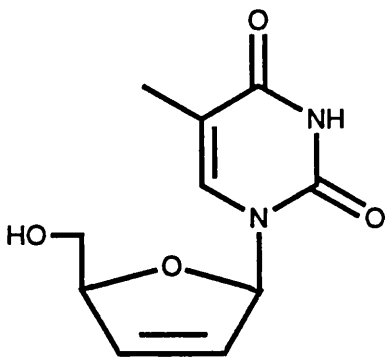
2′,3′-Dideoxy-2′,3′-didehydronucleosides as inhibitors of HIV (diagram 7).

After it was found that 2′,3′-dideoxynucleosides inhibit HIV replication *in vitro*, a number of 2′,3′-dideoxy-2′,3′-dihydronucleosides were found to inhibit HIV replication

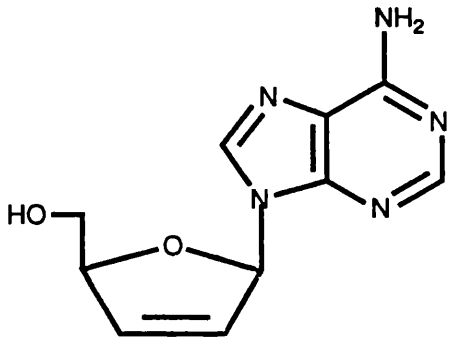
Diagram 7



27



28



29

Some 2', 3'-dideoxy-2',3'-didehydronucleosides able to act as inhibitors of HIV

also. Two comparative studies on the inhibition of HIV by various 2′,3′-dideoxy-2′,3′-dihydronucleosides have been reported[91,92]. In the first study by Chu *et al*[91], 2′,3′-dideoxy-2′,3′-didehydrocytidine (**27**), 2′,3′-dideoxy-2′,3′-didehydrothymidine (**28**) and 2′,3′-dideoxy-2′,3′-didehydroadenosine (**29**) were found to be potent inhibitors of HIV in PBM cells. **27**, **28** and **29** displayed 50% inhibition of the virus at concentrations of 0.01, 0.01 and 0.76 μM and in addition displayed minimal cytotoxicity. Chu *et al* also found that 2′,3′-dideoxy-2′,3′-didehydroguanosine, 2′,3′-dideoxy-2′,3′-didehydrouridine and 2′,3′-dideoxy-2′,3′-didehydroinosine were practically inactive.

In the other comparative study, Balzarini *et al*[92] used infected ATH8 cells and obtained results in general agreement with the first study. **27**, **28** and **29** displayed 50% inhibitory doses at 0.3, 4.1 and 40 μM respectively. In this system **29** was also quite toxic to host cells.

27 was first found to be a potent inhibitor of HIV by Balzarini *et al*[93], in ATH8 cells. This study also showed that **27** is phosphorylated to its 5′-triphosphate by cellular 2′-deoxycytidine kinases, **27** competitively inhibits the phosphorylation of 2′-deoxycytidine. Balzarini *et al*[93] also demonstrated that in a leukaemia cell line lacking 2′-deoxycytidine kinase, **27** had no cytostatic action. Further studies investigating the inhibition of HIV by **27** have shown it to be a potent inhibitor in PBM[94] and MT4 cells[95].

Lin *et al*[96] have studied both **27** and **28** comparing them to their corresponding 2′,3′-dideoxynucleosides (ie **2** and **15** respectively). In a comparative study using a PBM cell line, **27** and **28** had similar anti-HIV activities and were more active than **2**. **15** was active but at a much lower concentration than the other three compounds. The results of Baba *et al*[97] agreed with the finding that **28** is a more active anti-HIV agent than **15**. Indeed Mansuri *et al*[98] have shown **28** to have anti-HIV properties that are similar to **1** and in addition **28** is considerably less toxic to T-cells. Moreover, in a test modelling bone marrow toxicity, **28** was much less toxic to human haematopoietic progenitor cells than **1**[66].

The intracellular pharmacology of **28** has been studied and compared to the

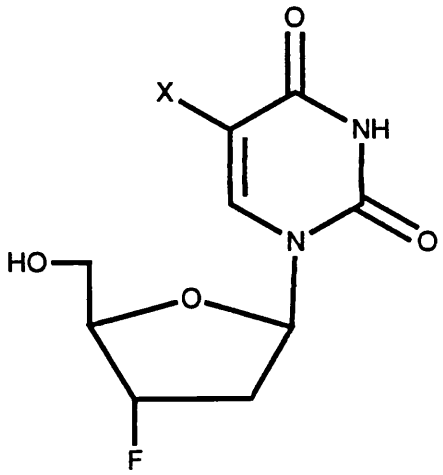
intracellular pharmacology of **1** in two independent studies[99,100]. Both studies indicated that **28** is metabolised to its 5′-monophosphate, 5′-diphosphate and 5′-triphosphate. **1** was metabolised to its 5′-monophosphate, 5′-diphosphate and 5′-triphosphate too. However the relative amounts of each phosphate produced by **28** differed from those produced by **1**. **1** produced relatively large quantities of its 5′-monophosphate, relatively small amounts of its 5′-diphosphate and 5′-triphosphate and hardly any **1** remained unphosphorylated. In contrast, a relatively large amount of **28** remained unphosphorylated but a relatively large quantity of its 5′-triphosphate was produced nevertheless. Therefore there are biochemical differences in cellular metabolism between **28** and **1** which suggests **28** may have a different toxicity potential to **1**. Clinical trials with **28** have been initiated[17].

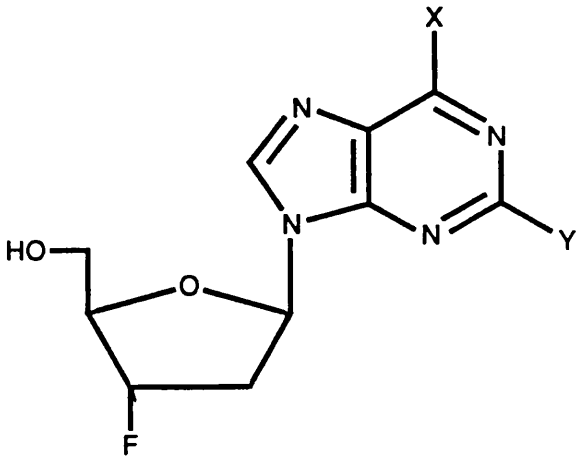
3′-Fluoro-2′,3′-dideoxynucleosides as inhibitors of HIV (diagram 8).

It has been found that some 3′-fluoro-2′,3′-dideoxynucleosides are able to inhibit the replication of HIV. Both 3′-fluoro-3′-deoxythymidine (**30**)[101] and 3′-fluoro-2′,3′-deoxyadenosine (**31**)[65] inhibit HIV replication *in vitro*. In fact the phosphorylation of **30** in cell lines has been studied[102] and it has been shown that **30** can be phosphorylated well by host kinases to its 5′-triphosphate. With HIV-infected MT4 cells, Balzarini *et al*[103] showed that **30** had a lower 50% inhibitory concentration than **1** (0.001 μM compared to 0.004 μM). However the cytotoxic dose of **30** was lower than that of **1** (0.2 μM compared to 20 μM). Nevertheless these results do suggest that **30** merits further study. Balzarini *et al*[103] have also shown that 3′-fluoro-2′,3′-dideoxyuridine (**32**) is a potent and quite selective inhibitor of HIV (this has been confirmed in a further study[104]). Although 3′-fluoro-2′,3′-dideoxycytidine (**33**) inhibited HIV it was very unselective (its therapeutic index was around unity). 3′-Fluoro-2,6-diaminopurine-2′,3′-dideoxyriboside (**34**) and 3′-fluoro-2′,3′-dideoxyguanosine (**35**) have been evaluated for inhibition of HIV and cytotoxicity *in vitro*[71]. The results of these tests show that both compounds have anti-HIV properties comparable with **25** and **14** respectively.

A number of nucleosides are therefore able to act as inhibitors of HIV *in vitro* and

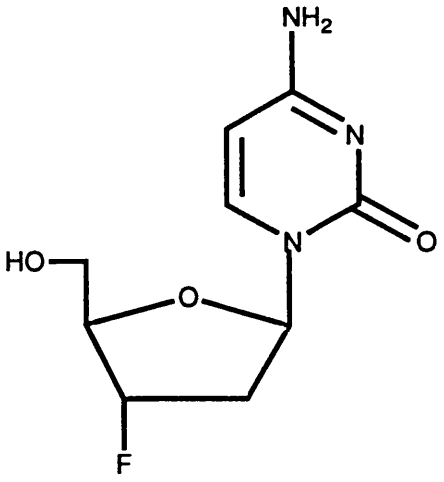
Diagram 8





30, X=CH_3
32, X=H

31, X=NH_2, Y=H
34, X=NH_2,, Y=NH_2
35, X=OH, Y=NH_2



33

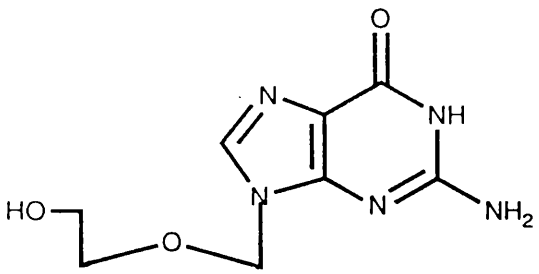
Some 3'-fluoro-2',3'-dideoxynucleosides able to act as inhibitors of HIV

some show promise clinically in the treatment of AIDS. However in order to act, these nucleosides must first be phosphorylated to their bio-active 5′-triphosphate forms. There is therefore a dependence on kinases which bring about activation to these nucleotide forms. Indeed, all nucleosides used as anti-viral and anti-cancer agents act through their nucleotides (diagram 9). For example, acyclovir (9-(2-hydroxyethoxymethyl)guanine) (**36**) has been found to be active against various herpes simplex viruses[105]. **36** is converted to its 5′-monophosphate in infected cells by viral thymidine kinase, yet **36** is phosphorylated to its 5′-monophosphate only to a limited extent in uninfected cells[106]. Unlike HIV, it appears that herpes viruses specify for kinases in their genome. Once formed the 5′-monophosphate of **36** is further phosphorylated by cellular kinases through the 5′-diphosphate to the 5′-triphosphate form. This 5′-triphosphate is able to inhibit herpes viral DNA polymerase, but not cellular DNA polymerases[107]. As a result **36** is far more toxic to herpes viruses in an infected cell than to uninfected cells. **36** is therefore highly selective in its action.

Another nucleoside analogue, 9-ß-D-arabinofuranosyladenine (**37**) has found widespread use in the treatment of a number of herpes infections[108]. **37** has also been shown to demonstrate anti-cancer activity[109]. **37** is capable of being phosphorylated to its 5′-triphosphate by cellular kinases and again it is through the 5′-triphosphate that **37** brings about its biological effect, as the 5′-triphosphate is able to inhibit DNA polymerases[110]. 9-ß-D-Arabinofuranosylcytidine (**38**) has found clinical use in the treatment of a number of cancers, in particular in the treatment of various leukaemias[111]. Here again this nucleoside analogue is dependent on kinase mediated activation to its 5′-triphosphate form, in order to exert its anti-cancer effect[112].

5-Fluoro-2′-deoxyuridine (**39**) demonstrates significant anti-cancer activity[113]. The activity results from **39** being phosphorylated to its 5′-monophosphate and this 5′-monophosphate acting as an inhibitor of the enzyme thymidylate synthetase, the enzyme responsible for the conversion of 2′-deoxyuridylic acid to thymidylic acid[114]. Phosphorylation is again necessary before **39** can exert its biological effect. However **39** is unusual in that

Diagram 9



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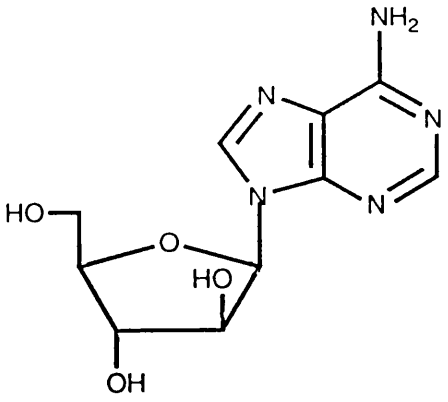
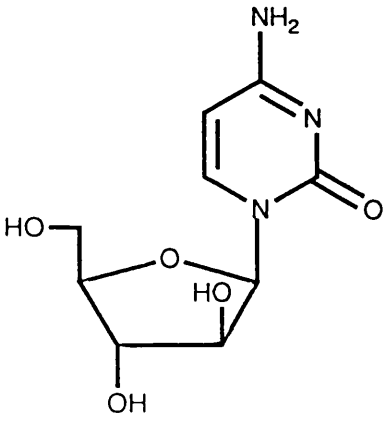


37



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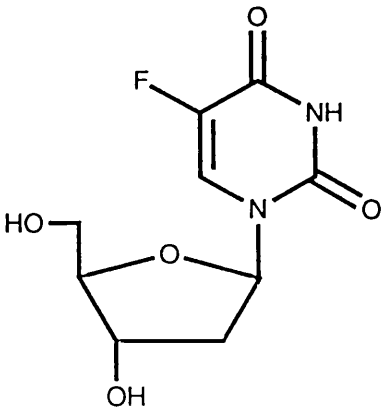


39

Some nucleoside analogues which, in order to act as anti-viral and/or anti-cancer agents, must first be phosphorylated to nucleotide forms.

it exerts that biological effect through its 5′-monophosphate rather than its 5′-triphosphate. The phosphorylation of bio-active nucleosides can be seen to play a key role in the methods by which those nucleosides act. Moreover if the ease with which bio-active nucleosides are converted to their corresponding nucleotides could be increased, then it may be expected that the potency of those nucleosides would be increased also.

Although a number of 3′-modified-2′,3′-dideoxynucleosides are able to inhibit HIV replication, it would appear that these compounds have similar modes of action. These nucleosides appear to be phosphorylated by (host) nucleoside and nucleotide kinases to their respective 5′-triphosphates and it is these 5′-triphosphates that act as substrates for RT, inhibiting the incorporation of the physiological 2′-deoxynucleosides into viral DNA. If incorporated into viral DNA, 3′-modified-2′,3′-dideoxynucleosides can act as chain terminators. A number of 3′-modified-2′,3′-dideoxynucleosides thus show potent anti-HIV activity. It is interesting though that there should be a relatively large difference in the effectiveness of 2′,3′-dideoxynucleosides and related compounds as inhibitors of HIV. As explained earlier in this thesis, **2**, **13**, **14** and **15** are all inhibitors of HIV, yet they differ by several orders of magnitude in their effectiveness as inhibitors[74].

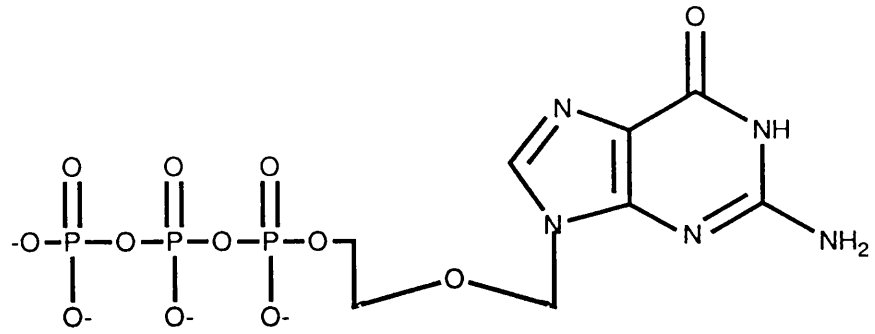
In ATH8 cells, the proliferation of HIV is inhibited by 50% by **2** at a concentration of 0.1 μM. However the proliferation of HIV is inhibited by 50% by **15** at a concentration of 150 μM. The effectiveness of **13** and **14** were intermediate between these two values. A reason for this variation in anti-HIV activity was sought by Hao *et al*[115]. Three possible explanations were explored to account for these differences. The first explanation was that the 2′,3′-dideoxynucleosides may be able to affect the pools of physiological 2′-deoxynucleosides present in the cell to differing extents. The second explanation was that the 2′,3′-dideoxynucleosides may differ in their ability to generate their corresponding 5′-triphosphates. The final explanation was that there may be a difference in the ability of these 5′-triphosphates to inhibit RT. Hao *et al* attempted to find a correlation between the observed activity of some 2′,3′-dideoxynucleosides and related compounds and one of these

three explanations. The compounds investigated were **1**, **2**, **13**, **14** and **15**. No correlation was found between anti-viral activity and the ability of the test compounds to modify the physiological 2′-deoxynucleoside pool sizes. Nor was there a correlation between anti-viral activity and the ability of the 5′-triphosphates of these compounds to inhibit purified RT. Indeed the 5′-triphosphates tested were practically equal in their ability to inhibit RT. However there was a correlation between the anti-viral activity of the compounds tested and their ability to generate their 5′-triphosphates. For example, of the compounds tested **2** was found to generate the greatest amounts of 5′-triphosphate intracellularly and this correlates with **2** displaying the greatest anti-viral activity. **1** also generated a relatively high level of its 5′-triphosphate intracellularly. On the other hand, it was found that the ability of **15** to generate its 5′-triphosphate was relatively poor and this correlates with the relatively low anti-HIV activity displayed by **15**. It may be concluded from these results that the potency displayed by 2′,3′-dideoxynucleosides and related compounds against HIV is strongly dependant on the intracellular levels of the 5′-triphosphate generated by these compounds. It is necessary, of course, that the 5′-triphosphate has the ability to inhibit RT, but the generation of that 5′-triphosphate appears to be an important factor.

Cheng *et al*[116] have recently tested the ability of a number of nucleoside 5′-triphosphates as inhibitors of purified HIV-RT. As may be expected the 5′-triphosphate of **1** was able to strongly inhibit this enzyme. Interestingly though the 5′-triphosphates of both **36**, (**40**), and 3′-amino-3′-deoxythymidine (**41**), (**42**), were also able to inhibit purified RT (diagram 10). However, both **36** and **41** show no activity against HIV *in vitro*[58,69]. A possible explanation for these facts is that neither **36** nor **41** are phosphorylated efficiently (if at all) to their respective 5′-triphosphates by host kinases. Indeed the low toxicity of **36** noted during its use as an anti-herpes agent is believed to result from **36** being a poor substrate for cellular kinases, hence **36** is not phosphorylated to any great extent in an uninfected cell[106].

It has also been reported that 3′-O-methylthymidine 5′-triphosphate (**43**) is a specific

Diagram 10



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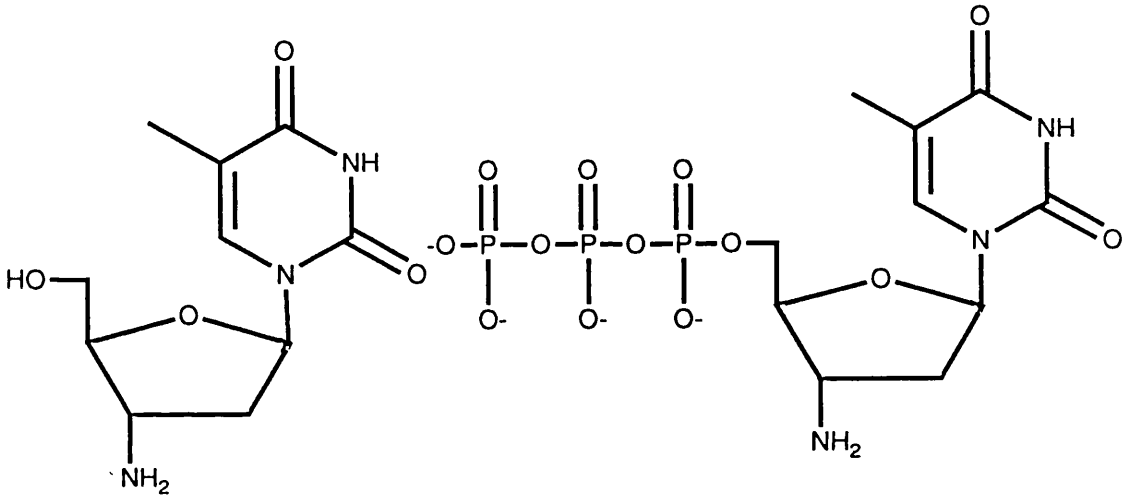

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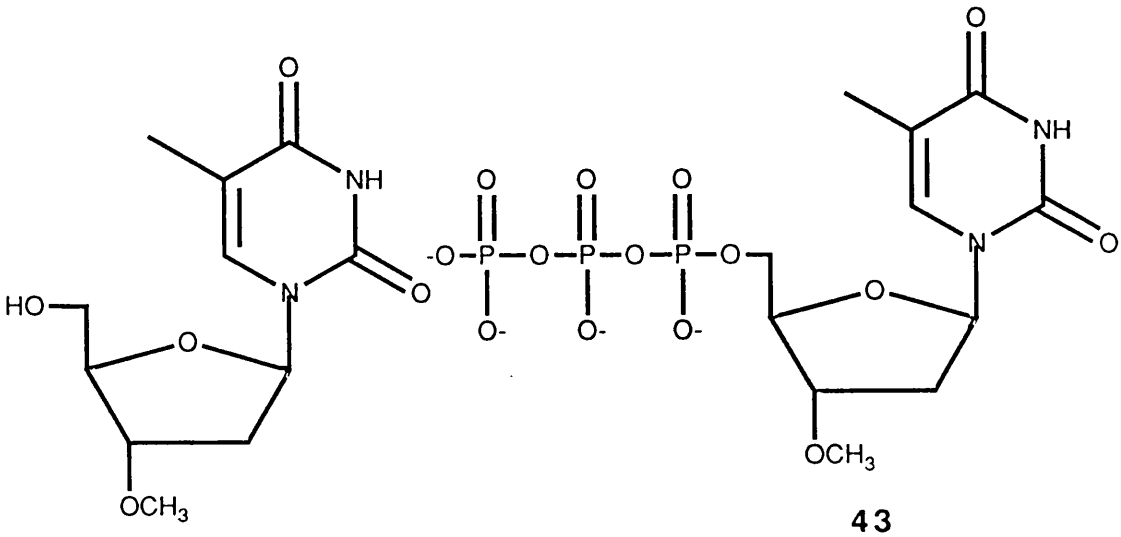

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43

inhibitor of RT[117] even though the parent nucleoside 3′-O-methylthymidine (**44**) is inactive against HIV[101] (diagram 10). Nucleosides active against HIV are able to be phosphorylated to their 5′-triphosphates relatively efficiently. Once formed it is the 5′-triphosphate that directly inhibits RT. It would appear that nucleosides exist which themselves do not inhibit HIV *in vitro* even though their 5′-triphosphates are able to inhibit purified RT. It would seem that a major factor determining the effectiveness of a nucleoside as an inhibitor of HIV is the ability of that nucleoside to be phosphorylated by cellular kinases to its 5′-triphosphate form.

It is clear that bio-active nucleosides act through their 5′-phosphates. As far as nucleoside inhibitors of HIV are concerned they too act through their 5′-phosphates. Therefore it may be thought that it would be beneficial to administer not the bio-active nucleoside but the corresponding nucleotide, that is pre-formed 5′-phosphates. However the work of Heidelberger *et al*[118] has shown that the administration of nucleotides has no advantage over the administration of the corresponding nucleosides.

Heidelberger *et al* investigated the ability of **39** and its 5′-monophosphate and 5-fluorouridine and its 5′-monophosphate against transplanted mouse tumours, thus comparing the biological activity of a nucleoside with its corresponding nucleotide[118]. It was found that although all four compounds were active against the tumours, each nucleotide provided no significant advantage over the corresponding nucleoside. This can be explained by these nucleotides being unable to penetrate the cell membrane as they are charged species. In order to exert a biological effect the nucleotides are dephosphorylated at the membrane surface and enter the cell as the nucleoside before being rephosphorylated in the cell[119]. Nucleotides therefore do not offer any advantages over their corresponding nucleosides because the charge they possess at physiological pH ensures that they are unable to permeate the cell membrane. One method by which this problem could be overcome is to administer an analogue of the nucleotide which, having passed through the cell membrane, could be converted to the nucleotide intracellularly. In other words a prodrug of the nucleotide could

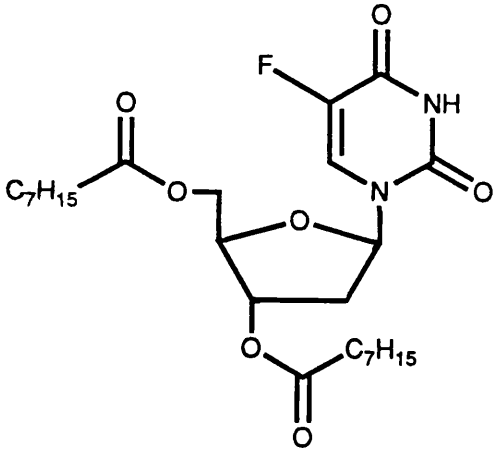
be administered.

A prodrug may be defined as a pharmacologically inactive derivative of a parent drug that requires spontaneous or enzymic transformation within the body in order to release the active drug[120]. The prodrug may offer advantages over the parent drug itself. For example, the prodrug may be delivered more efficiently to the site of action than the parent drug. Once at the site of action the prodrug can then be transformed into the active parent drug. The most common prodrugs are those that require hydrolytic cleavage for activation. For example, if a drug species contains carboxylic acid or hydroxyl groups, a prodrug ester could be synthesised which could yield the active drug in the body by either chemical or enzymic hydrolysis.

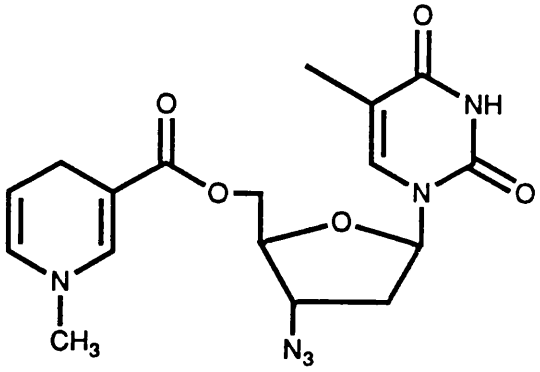
There has been some interest in ester prodrug forms of biologically active nucleosides. For example, diester prodrugs of the anti-cancer agent **39** have been prepared[121]. Of the derivatives prepared, the dioctyl ester **45** (diagram 11) was found to be most active against tumour cell lines, yet it was not as active as the parent nucleoside. **45** acts with hydrolysis to the parent nucleoside. **45** could have some advantages over the parent nucleoside. For example, **45** was found to be more lipophilic than **39** and **45** could therefore penetrate the cell membrane more easily than **39** itself.

Recently an interesting prodrug of **1** has been reported in the literature. This compound is 5′-(1,4-dihydro-1-methyl-3-pyridinylcarbonyl)-3′-azido-3′-deoxythymidine (**46**)[122] (diagram 11). This prodrug was designed to use a more elaborate method of activation than simple hydrolysis. **46** can be oxidised *in vivo* to yield the charged species **47** which itself is then slowly hydrolysed to yield **1**. This dihydropyridine prodrug system can deliver a drug specifically across the blood-brain barrier and this is of particular interest since HIV is known to infect the brain causing neurological disorders[123]. When tested against HIV in PBL cells, **46** was found to be slightly more active than **1**. Moreover the uptake of **46** was significantly greater than **1** in these cells. **46** was also less toxic to bone marrow cells than **1**. The analogue **48** has also been prepared and studied[124] as a potential prodrug

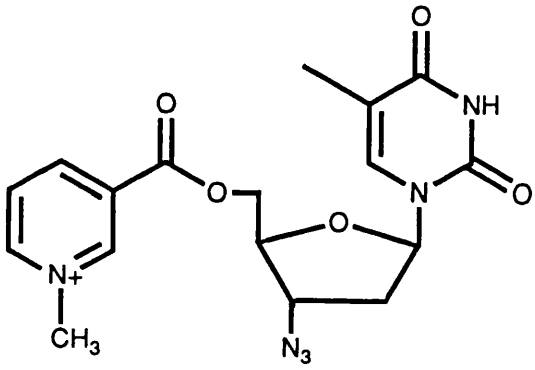
Diagram 11



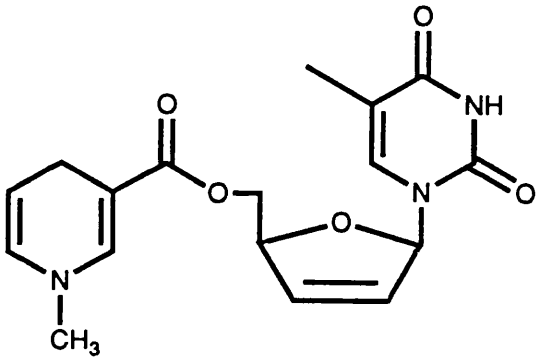
45



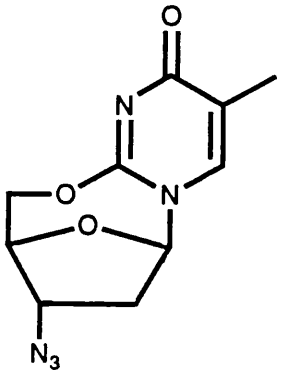
46



47



48



49

of **28** (diagram 11). Indeed studies indicate that **48** can penetrate the mouse blood-brain barrier intact before being oxidised. The prodrugs **46** and **48** may have clinical potential in the treatment of AIDS, particularly in the treatment of the neurological disorders associated with AIDS.

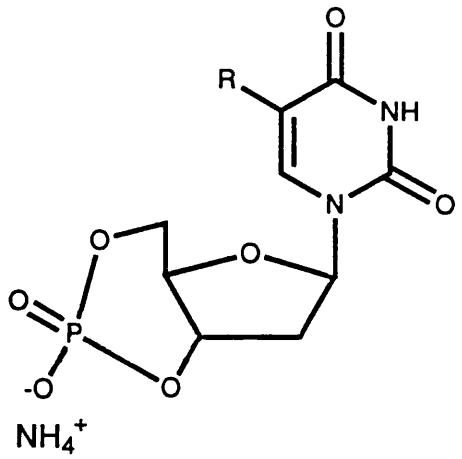
It has been reported that **49** (diagram 11) (a 2,5′-anhydro analogue of **1**) has been found to be an inhibitor of HIV *in vitro*[125]. **49** was slightly less active than **1** itself. It may be that **49** is acting as a prodrug for **1** by undergoing intracellular cleavage of the 2,5′-anhydro linkage, although it has not been ruled out that **49** possesses activity against HIV in its own right.

Thus prodrugs of bio-active nucleosides can offer a number of advantages over the nucleosides themselves. Prodrugs of nucleosides that are active against HIV have been reported to show some promise. However, since chemotherapeutic nucleosides exert their biological effect as their corresponding nucleotides, prodrugs of these nucleotides may offer greater potential as chemotherapeutic agents. Indeed there has been a number of reports of the synthesis and evaluation of potential membrane soluble nucleotide prodrugs. Some of these reports describe the biological evaluation of phosphate diester derivatives.

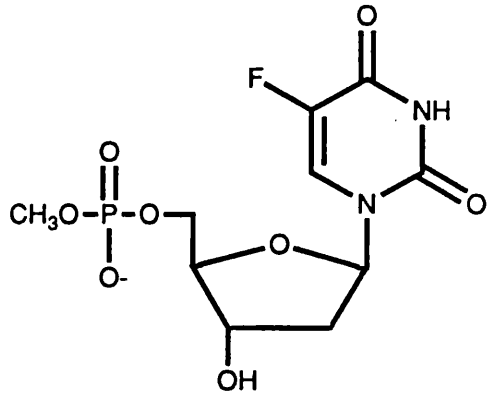
For example, a number of 3′,5′-cyclic monophosphates of some 5-alkyl-2′-deoxyuridine have been prepared[126] (diagram 12). The parent nucleosides of these derivatives, in particular 5-ethyl-2′-deoxyuridine, possess anti-viral activity *in vitro*[127]. It was hoped that the 3′,5′-cyclic monophosphates would act as prodrugs for their corresponding 5′-monophosphates by cleavage of the C3′O-P bond. However all of these 3′,5′-cyclic monophosphates proved to be less potent than their parent nucleosides as either anti-viral or anti-cancer agents.

Remy *et al* have prepared a number of phosphate diester derivatives of **39**, including **50** and **51** (diagram 12), in the hope that they would be able to yield **39** in the cell and that therefore **50** and **51** would have anti-cancer properties[128]. However **50** and **51**, along with other prepared phosphate diester derivatives proved to be less effective as anti-cancer agents

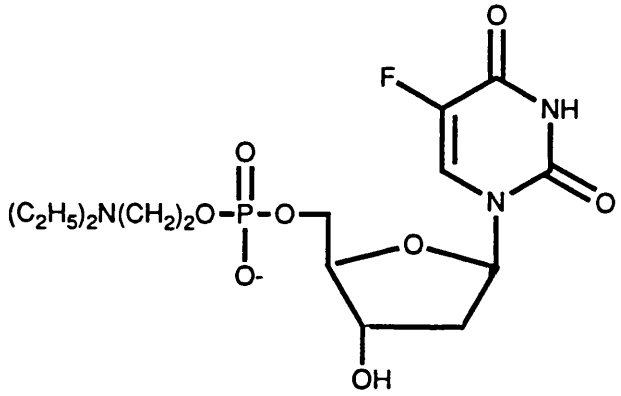
Diagram 12



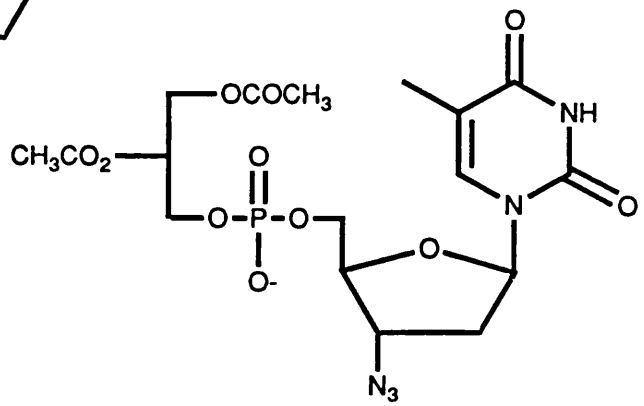
R=Et, i-Pr, n-Bu, n-Pent.



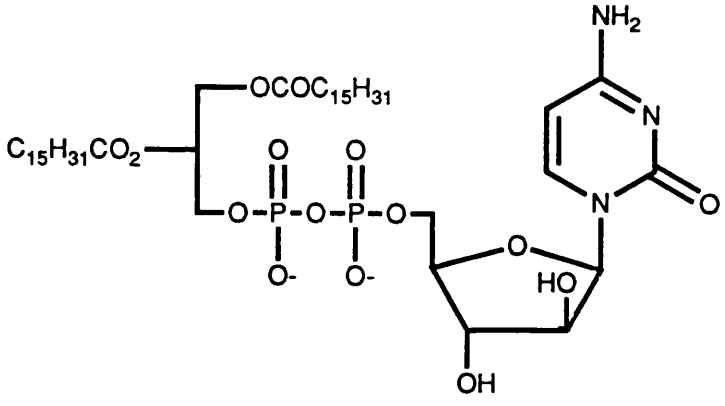
50



51



53



52

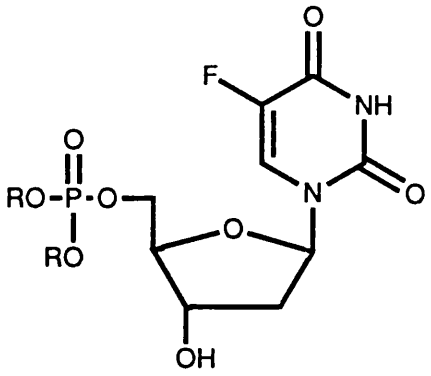
than the parent nucleoside **39**.

Phospholipid-nucleoside conjugate prodrugs of **38** such as **52** (diagram 12) have been found to be effective against some types of cancer in mice[129]. Indeed in studies against L 1210 leukaemia in mice, **52** was shown to be more effective than **38** itself[130]. Nucleoside-phospholipid phosphate diesters may have potential when applied to nucleosides that are able to inhibit HIV *in vitro*[131]. The phosphate diester **53** (diagram 12), a phospholipid derivative of **1** has been reported to inhibit HIV *in vitro*[132]. Phosphate diester derivatives suffer from one disadvantage, they are charged and therefore may not be able to penetrate the cell membrane easily. There are, however, a number of reports on uncharged phosphate triester derivatives being synthesised in the hope that they would penetrate the cell membrane easily and then hydrolyse to yield free phosphate.

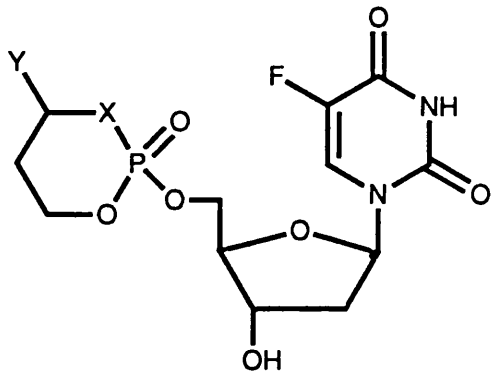
In 1962, a number of 5′-phosphate derivatives of the anti-cancer agent **39** were prepared by Remy *et al*[128]. Amongst these compounds was the phosphate triester **54** (diagram 13), the 5′-diethyl phosphate of **39**. It was hoped that **54** may have been able to penetrate the cell membrane and then be metabolised by intracellular processes to the active anti-metabolite **55**. However it was found that **54** failed to exert an anti-cancer effect as great as **39** or **55**.

It was with a view to generating **55** intracellularly that Farquhar *et al* prepared the cyclic phosphate triesters **56** and **57**[133] (diagram 13). It was hoped that these compounds would be able to yield **55** using the same mechanism that the anti-cancer agent cyclophosphamide[134] uses to exert its biological effect. It was hoped that **56** and **57** would be converted to **58** and **59** by cytochrome mixed function oxidase. **58** and **59** could then penetrate the cell membrane before undergoing ring opening to **60** and **61** respectively. **60** and **61** would subsequently dissociate eliminating acrolein and yielding **55** and the phosphoroamidate **62** respectively (the latter being converted to **55** by hydrolysis). However it was found that **57** was only partially effective against leukaemia P-388 implanted in mice, high dosage being required for optimum activity. **56** was only marginally effective against

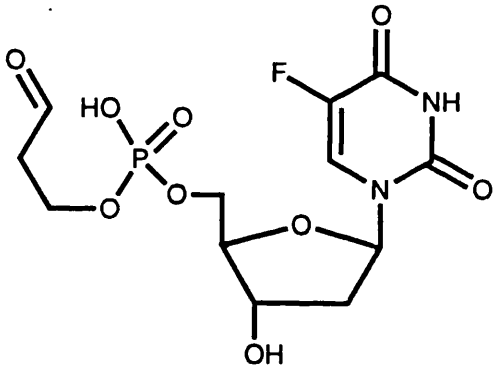
Diagram 13



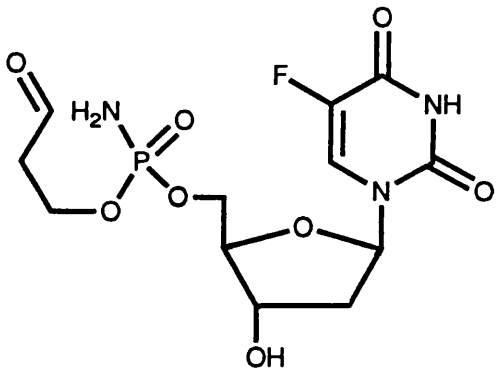
54, R=Et
55, R=H



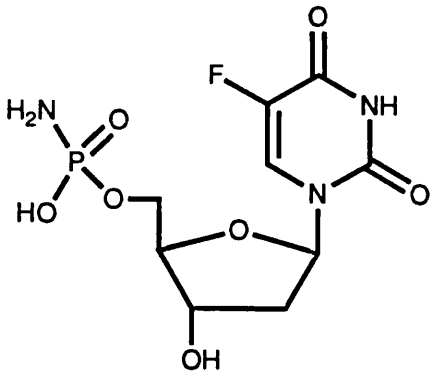
56, X=O, Y=H
57, X=NH, Y=H
58, X=O, Y=OH
59, X=NH, Y=OH



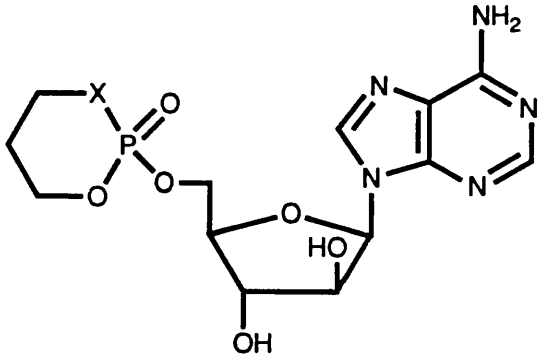
60



61



62



63, X=O
64, X=NH

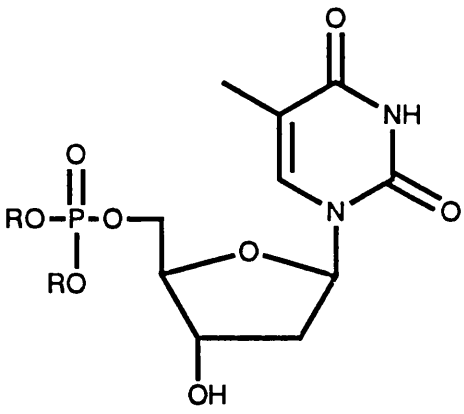
leukaemia P-388. Moreover it was found that **56** and **57** were hardly oxidised to **58** and **59** by hepatic mixed function oxidase, under conditions where cyclophosphamide was extensively degraded. Thus **56** and **57** seem unable to act as effective latent precursors of **55**.

Farquhar *et al* also applied this approach to the anti-viral drug **37**[135] and synthesised **63** and **64** (diagram 13) in the hope that, unlike **56** and **57**, they would be able to be transformed into their 4-hydroxy derivatives by cytochrome mixed function oxidase, penetrate the cell membrane and be converted intracellularly to the 5′-monophosphate of **37**. However again, **63** and **64** were not degraded when incubated with hepatic mixed function oxidase. **63** and **64** were also ineffective against leukaemia P-388 implanted in mice, presumably because they too were not degraded by mixed function oxidases *in vivo*.

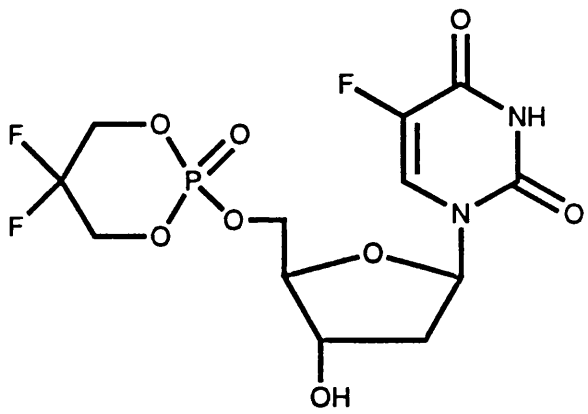
Chawla *et al*[136] prepared **65** and **66** (diagram 14), the bis(m-nitrophenyl) and bis(p-nitrophenyl) esters of thymidine 5′-monophosphate in the hope that they would be able to act as sources of intracellular thymidine 5′-monophosphate. It was thought that **65** and **66** could yield thymidine 5′-monophosphate by either chemical or enzymic hydrolysis. 5′-Amino-5′-deoxythymidine was found to be able to almost completely suppress the function of thymidine kinase in the mouse L fibroblasts, but at the same time only partially inhibit cell division. [^{3}H]-Labelled **65** and **66** were found to be even less effective than [^{3}H]-labelled thymidine at labelling DNA in L 1210 cells grown in the presence of 5′-amino-5′-deoxythymidine. Chawla *et al* concluded from this that these particular aromatic esters of thymidine 5′-monophosphate, **65** and **66**, were not acting as intracellular sources of thymidine 5′-monophosphate by cell penetration and subsequent loss of the phosphate masking groups. Moreover **65** and **66** may have been acting as intracellular sources of thymidine.

Hunston *et al*[137] prepared some cyclic phosphate triester derivatives of **39**. It was hoped that a suitable cyclic phosphate triester could be found which would be able to hydrolyse *in vivo* to give the nucleoside 5′-monophosphate. Studies with murine leukaemia

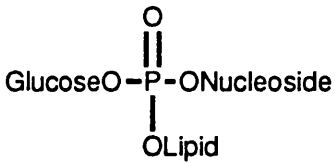
Diagram 14



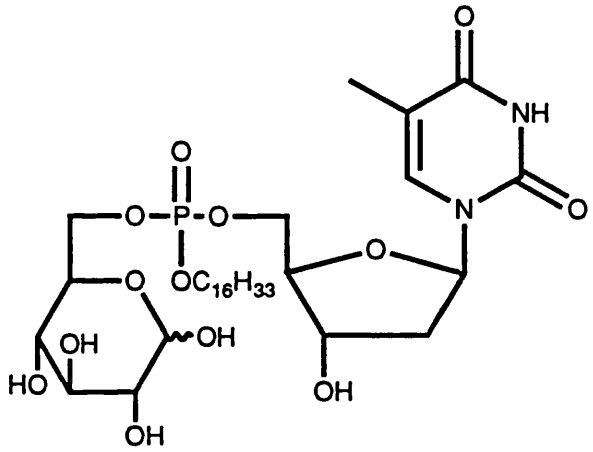
65, R=m$O_2NC_6H_4$
66, R=p$O_2NC_6H_4$



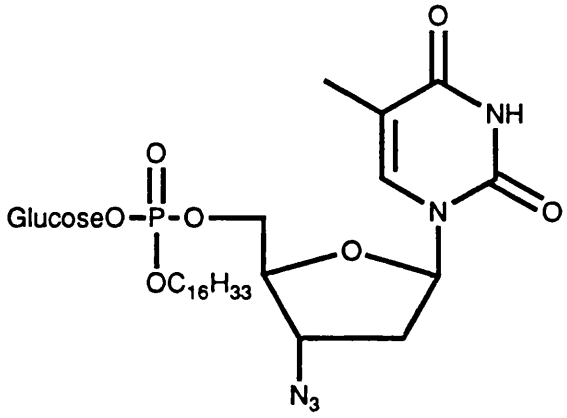
67



68



69



70

L 1210 cells showed **67** (diagram 14) was nearly as potent as **39**, but that **56** (diagram 13) was nearly 300 times less active. It was also found that when tested against a L 1210 cell line deficient in thymidine kinase, **67** and **56**, like the parent nucleoside were far less effective. One explanation for these observations is that **67** and **56** act by yielding the nucleoside **39** intracellularly, which may then be phosphorylated by cellular kinases to **55**. However it is also perfectly possible that the thymidine kinase deficient cell line has properties which it does not share with the parental cell line and that **67** and **56** can act by penetrating the cell membrane and slowly releasing **55** in the cell. **56** may be more stable to hydrolysis than **67** and is therefore unable to yield the 5′-monophosphate as efficiently as **67**.

Inglesias Guerra *et al* have suggested that phosphate triesters such as **68** (diagram 14) may have potential as drug transport and drug targeting prodrugs[138]. It was speculated that these prodrugs would have a number of useful characteristics. They may possess hydrophilic solubility because of their glucose component and hydrophobicity because of their lipid component. It was also speculated that these derivatives may be able to pass through the cell membrane using an active transport mechanism, possibly the glucose-phosphate or dilichol-phosphate transport proteins. It was also speculated that if compounds like **68** were to able to penetrate the cell membrane, hydrolysis could yield the nucleoside 5′-monophosphate. This could have a number of consequences if the parent nucleoside had anti-viral or anti-cancer properties. With all this in mind Inglesias Guerra *et al* prepared **69** (diagram 14) and investigated some of its properties. Studies showed that **69** was more soluble than thymidine in water and that **69** was more lipophilic than thymidine, as measured by partition coefficients in chloroform-water and octanol-water. ^{31}P nmr experiments also showed that **69** was able to pass through the membrane of synthetic large unilamellar vesicles. An analogue of this glucose-phospholipid drug delivery system **70** (diagram 14) has recently been reported to inhibit HIV replication *in vitro*, further investigations into this derivative are currently in progress[139].

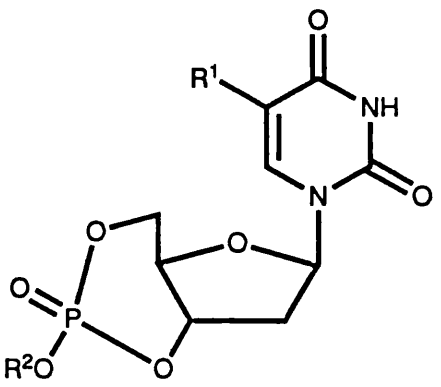
Beres *et al* have prepared a number of cyclic 3′,5′-monophosphate triesters of some 5-alkyl-2′-deoxyuridines (diagram 15)[126]. It was hoped that these derivatives would act as prodrugs for the parental 5-alkyl-2′-deoxyuridine 5′-monophosphate. However these neutral phosphate triesters were inactive against a number of herpes virus strains. These compounds failed to demonstrate significant anti-cancer activity as well. This lack of activity may be because these particular cyclic triester derivatives are not capable of being hydrolysed intracellularly.

A number of dialkyl phosphate triesters of both **38** and **37** have been prepared recently in this department (diagram 16)[140,141]. It was hoped that these compounds would act as membrane soluble prodrugs of either the parent nucleoside or preferably the nucleotide. Both the dialkyl phosphate triesters of **37** and **38** were found to inhibit DNA synthesis *in vitro*. Most interestingly, a link was observed between the inhibition of DNA synthesis by these compounds and their lipophilicity, inhibition increasing with lipophilicity. It is not yet clear however as to whether these compounds act by releasing the nucleoside or nucleotide into the cell, although recent data indicates at least partial release of the nucleotide[142]. The possibility of these triesters acting in their own right has not been ruled out either.

Therefore the synthesis and biological evaluation of a number of phosphate triesters has been reported, phosphate triesters which, it was hoped, would be able to penetrate the cell membrane and hydrolyse intracellularly to bio-active nucleotides. However in some cases, it may be the parent nucleoside that is the product of this intracellular hydrolysis.

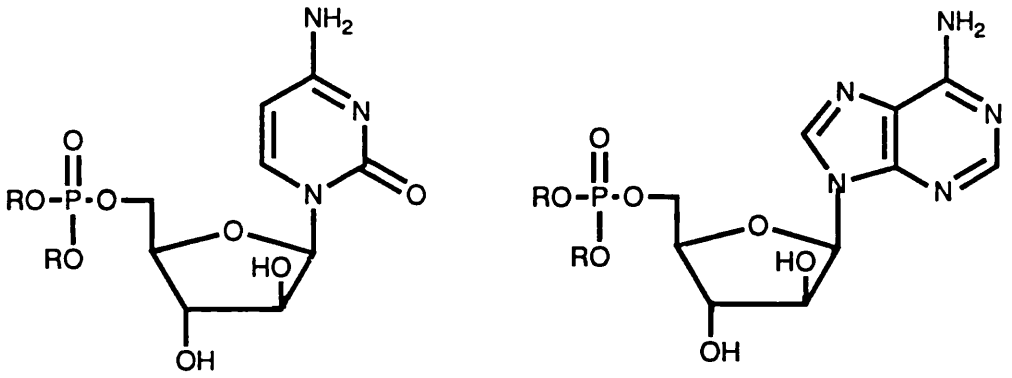
The subject of this thesis is the synthesis of a number of nucleoside 5′-phosphate triester derivatives with a view to these compounds being inhibitors of HIV. It was thought that esterifying groups could be found which would be hydrolysed intracellularly generating the free 5′-phosphate. If a 5′-phosphate triester of a nucleoside could be synthesised, where that nucleoside is itself a potent inhibitor of HIV and the 5′-phosphate triester could generate the corresponding free 5′-phosphate intracellularly, then the nucleosides dependence

Diagram 15



R^1=Et, R^2=Me; R^1=i-Pr, R^2=Me;
R^1=i-Pr, R^2=$PhCH_2$; R^1=n-Bu, R^2=Me

Diagram 16



R=Et, n-Pr, n-Bu, n-Pent.

on kinase may be obviated. Moreover the reason for many 3′-substituted-2′,3′-dideoxynucleoside's lack of anti-HIV activity is that they are incapable of being phosphorylated by cellular kinases. It would be of interest therefore to prepare some phosphate triester derivatives of some inactive 3′-substituted-2′,3′-dideoxynucleosides. If esterifying groups could be found which hydrolyse intracellularly to yield free 5′-monophosphate, then kinase dependence could again be obviated and these 5′-phosphate triesters may act as HIV inhibitors.

5′-Phosphate triesters may have some additional advantages over the parent nucleosides. For example, one of the major limitations to cancer chemotherapy with **39** is rapid degradative deactivation caused by cleavage of the glycosidic bond. However simple phosphate triesters of **39** are entirely resistant to this form of degradative deactivation[143]. It is probable therefore that a similar situation would exist for the phosphate triesters described in this thesis. Also, 5′-phosphate triester derivatives of amino nucleosides may also be resistant to deaminases[144]. Thus 5′-phosphate triesters of **2** may be expected not to degrade to analogues of **12**, this being of interest because **12** itself is not as active against HIV as **2**[92]. It is also probable that phosphate triesters of nucleosides would be more lipophilic than the nucleosides themselves and it may be expected therefore that phosphate triester derivatives would pass through the cell membrane and/or the blood-brain barrier with greater ease. There is also the possibility of these phosphate triesters penetrating the cell membrane by an active transport mechanism.

The first section of this thesis is partly devoted to the synthesis of some simple 5′-dialkyl phosphates of nucleosides which are themselves active against HIV (eg **1**, **2**, and **13**). Some simple 5′-dialkyl phosphate derivatives of inactive nucleosides are also prepared.

RESULTS AND DISCUSSION

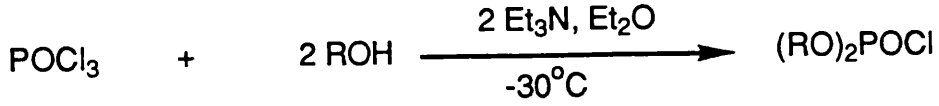
In order to prepare 5′-dialkyl phosphates of various nucleoside analogues, reactions between the relevant dialkyl phosphorochloridate and nucleoside were carried out. Three dialkyl phosphorochloridates were used in synthesis at first, diethyl phosphorochloridate (**71**), dipropyl phosphorochloridate (**72**) and dibutyl phosphorochloridate (**73**). Commercial **71** was used, it being distilled prior to use. **72** and **73** were both prepared the by reaction of slightly over two molar equivalents of both the relevant alcohol and triethylamine with phosphoryl chloride, at -30°C (diagram 17).

There are several alternative methods of preparing dialkyl phosphorochloridates[145]. The above method, using triethylamine as a base, was chosen because the use of the base makes the reaction relatively easy to control. Two impurities were likely from this reaction, the monoalkyl phosphorodichloridate and the trialkyl phosphate. To minimise the formation of these by-products, the reaction was carried out at -30°C and in a large volume of solvent, to increase selectivity. After addition of the relevant alcohol and triethylamine to phosphoryl chloride was complete, the reaction mixture was allowed to warm to ambient temperature.

After stirring overnight, the precipitated triethylamine hydrochloride was filtered off. Removal of the ether solvent and purification by vacuum distillation gave **72** and **73** in 79% and 72% yield respectively. Confirming their identity and purity, both phosphorochloridates gave one peak ^{31}P nmr spectra, with chemical shifts close to literature values[146]. Moreover the ^{13}C nmr and the ^{1}H nmr spectra of both compounds confirmed their formation and purity. Phosphorus coupling was seen in the ^{13}C nmr spectra of both compounds, but only to the two nearest carbon atoms to the phosphorus atom.

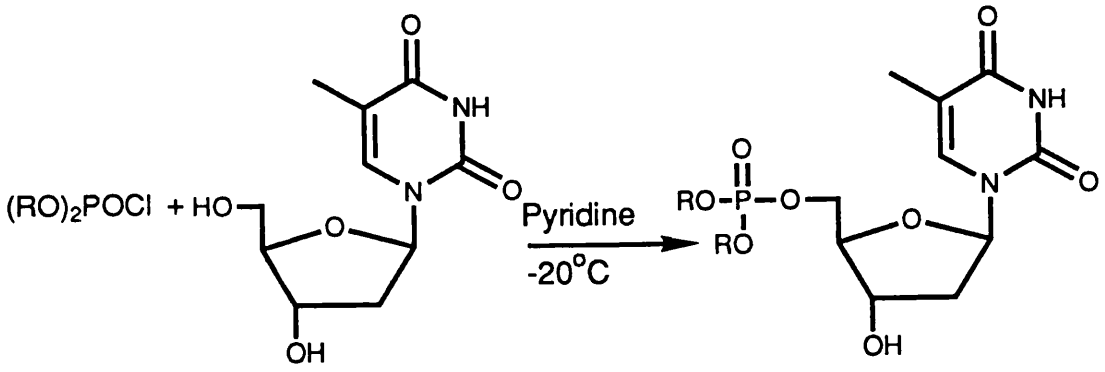
An interesting facet of the ^{1}H nmr spectra of both **72** and **73** was that the R$\underline{CH_2}$OP signals were seen as complicated multiplets, not as the triplets that may have been expected. This results from phosphorus-proton coupling being observed for these signals, in addition to proton-proton coupling. This phosphorus-proton coupling was not seen in any of the other proton resonances.

Diagram 17



72, R=Pr
73, R=Bu

Diagram 18



71, R=Et
72, R=Pr
73, R=Bu

74

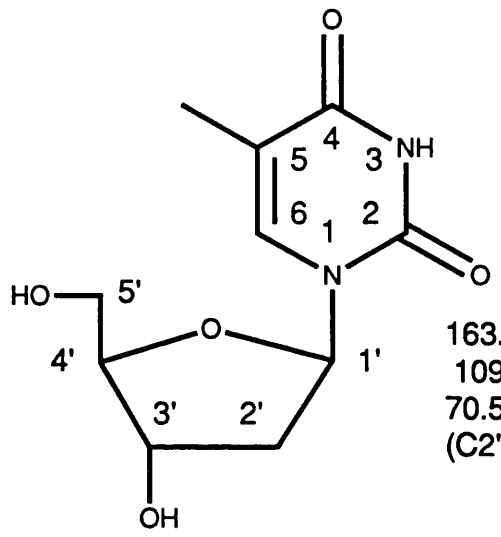
75, R=Et
76, R=Pr
77, R=Bu

A series of thymidine 5′-dialkyl phosphates was prepared by reacting the relevant dialkyl phosphorochloridate with thymidine (**74**) in a solution of pyridine at -20°C (diagram 18). It was hoped that the formation of a series of thymidine 5′-dialkyl phosphates would act as a chemical model for the synthesis of more novel nucleoside phosphate triesters. Moreover once isolated, they would be useful negative biological controls in any subsequent biological testing.

Firstly **71** was added to a solution of **74** in pyridine. The reaction was carried out at -20°C to increase the probability of reaction at the 5′-position rather than the 3′-position. Two equivalents of **71** were reacted with **74** as this had previously been found to drive this type of reaction to completion[140]. After stirring at -20°C for 30 min the reaction mixture was allowed to warm to ambient temperature, where tlc revealed the presence of some unreacted **74** and the formation of a major component, more lipophilic than **74** itself. This was assumed to be the required product, thymidine 5′-diethyl phosphate (**75**). In addition, two minor components were observed with greater R_f values than the major component. Also, a baseline component was observed as it was to be in all other subsequent reactions of nucleosides with phosphorochloridates. After stirring at ambient temperature for 4 h, tlc revealed that no **74** remained in the reaction mixture. The reaction mixture was quenched with water (reacting with any remaining phosphorochloridate) before the pyridine solvent was removed under reduced pressure. Purification of the resulting residue by column chromatography lead to the isolation of **75** in 56% yield. The compound was found to be a colourless hygroscopic oil, which could not be induced to crystallise.

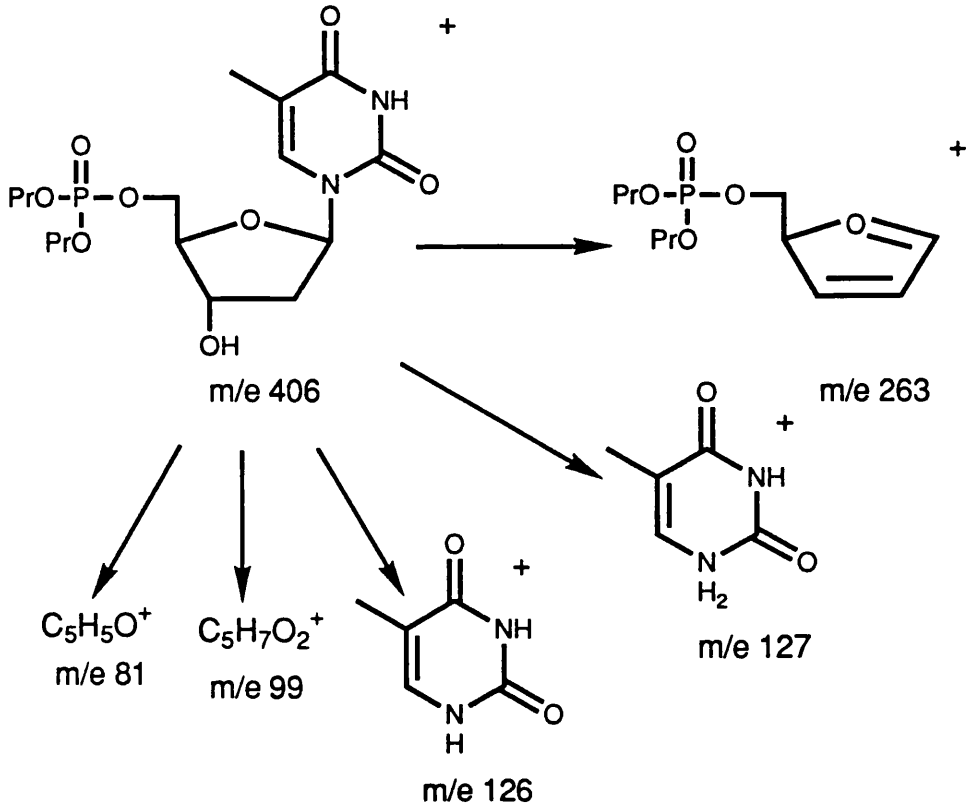
The formation of **75** was confirmed by nmr spectroscopy. A ^{31}P nmr spectrum displayed one signal at δ-2.75. This value is in the region that trialkyl phosphates are known to resonate. For example, triethyl phosphate is reported to resonate at δ-1.0[147]. Furthermore, a ^{13}C nmr spectrum was fully consistent with the formation of **75**. The spectrum was fully assigned by comparison with the known spectrum of **74** itself[148] (diagram 19). Evidence for the fact that **75** was the product of 5′-phosphorylation of **74** and not 3′-

Diagram 19



163.8 (C2), 150.5 (C4), 136.2(C6), 109.5 (C5), 87.3 (C1'), 83.9 (C4'), 70.5 (C3'), 61.4 (C5'), 39.5 (C2'), 12.3 (5-CH_3).

Diagram 20



phosphorylation was obtained from the ^{13}C nmr spectrum. The C5′ resonance of **75** occurred at δ66.84. This compares with the value of δ61.4 for the analogous carbon of **74** itself and represents a marked shift downfield. In contrast the C3′ resonance occurs at δ70.84 and δ70.5 for **75** and **74** respectively. Moreover confirmation of 5′-substitution was given by the phosphorus atom causing the C4′ and C5′ signals to be seen as doublets with coupling constants of 7.6 Hz and 5.3 Hz respectively. These values are within the expected range of these types of phosphorus-carbon coupling[149] and it is of interest that the three-bond coupling constant is of greater magnitude than the two-bond coupling constant. Phosphorus-carbon coupling also results in the $\underline{C}H_3CH_2OP$ and $CH_3\underline{C}H_2OP$ resonances being seen as doublets at chemical shifts of δ16.16 and δ64.46 respectively.

The ^1H nmr spectrum of **75** is also instructive. For example, the H1′ signal is seen as a triplet at δ6.34. The methyl and methylene protons of the ethyl chains are seen at δ1.35 and δ4.15 as a triplet and quintet respectively. The quintet results presumably from the magnitude of the phosphorus-proton coupling and the proton-proton coupling being similar for these protons. A broad signal at δ2.80, integrating for one proton, is consistent with the resonance of the 3′-OH proton.

Thymidine 5′-dipropyl phosphate (**76**) was prepared in entirely analogous way to **75** in 57% yield. Again tlc indicated that, in addition to the required product, two other components, more lipophilic than the required product, were also formed. ^{31}P nmr spectroscopy revealed **76** to have a chemical shift of δ-2.16, very similar to the value observed for **75**. A ^{13}C nmr spectrum of **76** was fully assigned and was very similar to the spectrum obtained for **75**. For example, all the base and sugar carbons displayed chemical shifts similar to those of **75**. The signals of C4′ and C5′ were observed as doublets, again as a result of phosphorus-carbon coupling. However, the $CH_3CH_2\underline{C}H_2OP$ resonance of **76** was seen as two doublets at δ69.82 and δ69.75, in a spectrum obtained at 50 MHz. This results from the non-equivalence of the two alkyl chains. This diastereotopic difference was not seen for the other signals of the alkyl chain, presumably due to coincidence. The

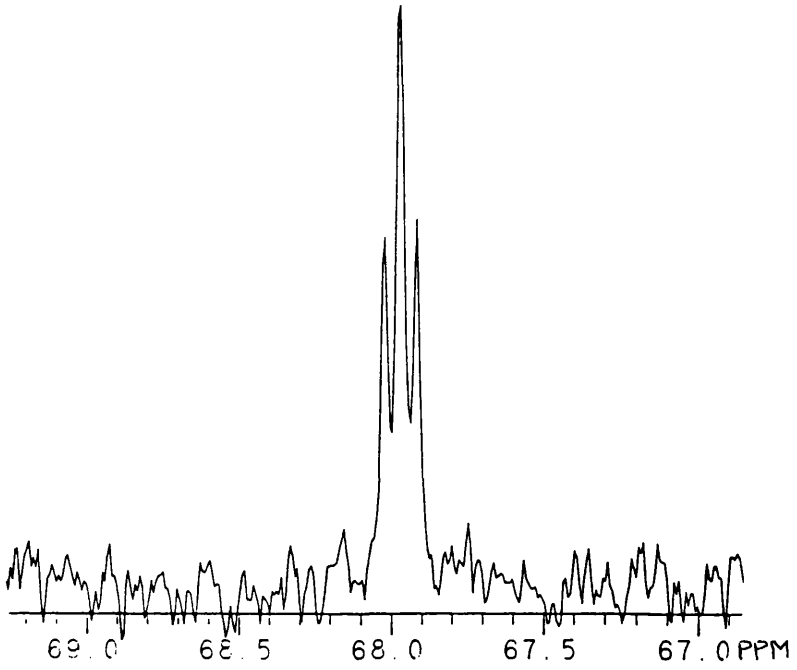
$CH_3\underline{C}H_2CH_2OP$ resonance of **76** was seen as a doublet at δ23.57. The $\underline{C}H_3CH_2CH_2OP$ resonance showed no phosphorus-carbon coupling, the signal was a singlet at δ9.91.

The ^1H nmr spectrum of **76** was very similar to that of **75**, as expected. The non-equivalence of the alkyl chains was further demonstrated in this spectrum. The $C\underline{H}_3CH_2CH_2OP$ signal was resolved as two, albeit closely spaced, triplets. None of the other proton resonances of the propyl chain demonstrated this non-equivalence. Mass spectrometry gave further evidence for the successful isolation of **76**. The molecular ion in the mass spectrum of **76** had an exact mass close to its calculated value. Also the other peaks observed in the mass spectrum were consistent with the fragmentation of the molecular ion in the manner depicted (diagram 20). Peaks observed at m/e 99 and m/e 81 were probably due to ionised fragments of the sugar moiety and were assigned to $C_5H_7O_2^+$ and $C_5H_5O^+$ respectively.

The preparation of thymidine 5′-dibutyl phosphate (**77**) was carried out by the same method as the preparation of **75** and **76** and was isolated in 64% yield. ^{31}P nmr spectroscopy showed that **77** displayed one peak at δ-2.40. The ^{13}C nmr spectrum was entirely consistent with the formation of **77** and was very similar to those of **75** and **76**, as far as the base and sugar resonances were concerned. In addition, signals due to the butyl chains were seen. Interestingly, in a spectrum obtained at 100 MHz, the only alkyl chain signal to display the diastereomeric difference between the two chains was the $CH_3CH_2CH_2\underline{C}H_2OP$ resonance. Moreover the signal was seen as an apparent triplet (see diagram 21), by virtue of two doublets overlapping.

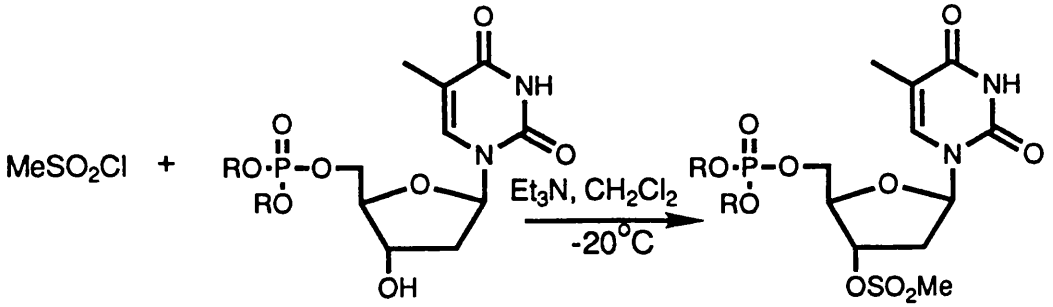
The ^1H nmr spectrum of **77** was assigned by comparison with the ^1H nmr spectra of **75** and **76**. It was found that the $C\underline{H}_3CH_2CH_2CH_2OP$ resonance of **77** was seen as two closely spaced triplets rather like the $\underline{C}H_3CH_2CH_2OP$ resonance of **76**. Mass spectrometry further confirmed the isolation of **77**. The accurate mass measurement of the molecular ion of **77** was close to its theoretical value and the fragmentation of the molecular ion was quite similar to that of the molecular ion of **76** previously discussed.

Diagram 21



The $CH_3CH_2CH_2\underline{C}H_2OP$ resonance of **77**.

Diagram 22



75, R=Et
76, R=Pr
77, R=Bu

78, R=Et
79, R=Pr
80, R=Bu

The first 3′-modification to be investigated was with the methanesulphonyl (mesyl) group. It has been shown that 3′-O-mesylthymidine itself has no anti-HIV activity and this lack of activity was attributed to the poor cellular phosphorylation to the 5′-triphosphate form[101]. It was of interest therefore to ascertain if substitution of a phosphate triester function at the 5′-position of 3′-O-mesylthymidine could overcome these phosphorylation difficulties. Furthermore, the 3′-position is a vital one in the process of DNA polymerisation, a process of crucial importance to the mode of action of many anti-viral agents[150]. By having a chemically labile mesyl group in this position, some very interesting biological properties may be induced.

The preparation of a small series of 3′-O-mesylthymidine 5′-dialkyl phosphates was carried out by mesylating the relevant thymidine 5′-dialkyl phosphate. It was decided that it was preferable to use this method rather than to prepare and then phosphorylate 3′-O-mesylthymidine. This was because the former method involves preparing the target molecule in only two steps from **74**. The latter method would involve preparing the target molecules in four steps from **74**, including the 5′-protection and deprotection steps necessary for the preparation of 3′-O-mesylthymidine.

The mesylation of nucleosides has commonly been carried out in pyridine, which acts as both solvent and base. However the mesylating procedure depicted in diagram 22, adapted from the method of Crossland *et al*[151], involves the addition of mesyl chloride in dichloromethane, using triethylamine as a base.

Thus 1.1 equivalents of mesyl chloride in dichloromethane was added to a solution of both **75** and 1.2 equivalents of triethylamine in dichloromethane at -20°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After stirring for 2 h, tlc indicated that some starting material remained and that one major component had been generated which was more lipophilic than the starting material. Further portions of mesyl chloride and triethylamine were added to the reaction mixture. After stirring the reaction mixture for a further 15 min the reaction had gone to completion and

it was found that the required product could be isolated from the reaction mixture by successive washing with saturated sodium bicarbonate and saturated brine solutions, followed by evaporation of the organic phase. In this way 3′-O-mesylthymidine 5′-diethyl phosphate (**78**) was obtained in 72% yield, without recourse to chromatography.

The ^{31}P nmr spectrum of **78** showed only one resonance, with a chemical shift in the region expected for trialkyl phosphates. The ^{13}C nmr spectrum of **78** proved to be very informative. The C3′ signal was found to occur at δ78.94, around 8 ppm downfield of the analogous signal of **75**. The resonances of C2′ and C4′ of **78** were shifted slightly upfield (by around 2 ppm) relative to the analogous signals of **75**. These observations provide strong evidence for the mesylation occurring at the 3′-position of **75**. The CH_3SO_2 signal was seen at δ37.87. The signals associated with the diethyl phosphate moiety were present and unshifted from their position in the ^{13}C nmr spectrum of **75**. Again the $CH_3\underline{C}H_2OP$ resonance was seen as two doublets, in a spectrum obtained at 50 MHz. This again results from a diastereomeric difference between the two alkyl chains. The $\underline{C}H_3CH_2OP$ signal, however, was seen as a doublet.

The ^1H nmr spectrum of **78** showed some interesting features. The presence of a 3′-O-mesyl group had the effect of shifting the H3′ signal downfield by around 1 ppm relative to its position in **75**. A singlet at δ3.14 was assigned to the CH_3SO_2 protons. The H1′ resonance was seen as a doublet of doublets, the H1′ proton coupling to both the adjacent H2′ protons but with differing magnitudes. This can be accounted for by the Karplus equation[152], which relates the magnitude of coupling between vicinal protons to the dihedral angle between them. The dihedral angle between both H2′ protons and the H1′ proton is different and two coupling constants result, causing the H1′ signal to be seen as a doublet of doublets. Lastly the methyl and methylene protons of the diethyl phosphate group were seen at δ1.33 and δ4.12. The latter signal was a quintet, arising from coupling to both the three adjacent methyl protons and the phosphorus atom. The magnitude of these couplings is similar, about 6 Hz, causing the observed quintet fine structure. It was found

that irradiation of the $C\underline{H}_3CH_2OP$ resonance caused the $CH_3C\underline{H}_2OP$ resonance to collapse to a doublet. The mass spectrum of **78** displayed a molecular ion peak whose accurate mass measurement was close to that calculated for the molecular ion of **78**. The molecular ion appears to be able to fragment with the loss of the mesyl moiety to give a signal at m/e 360 and to fragment with the loss of the mesyl and phosphate moieties to give the signal at m/e 206. The phosphate ion $(EtO)_2P(OH)_2^+$ is seen at m/e 155 and rather like the mass spectra obtained for the thymidine 5′-dialkyl phosphates, signals due to protonated thymine, thymine itself and the sugar fragments $C_5H_7O_2^+$ and $C_5H_5O^+$ were also seen.

The preparations of 3′-O-mesylthymidine 5′-dipropyl phosphate (**79**) and 3′-O-mesylthymidine 5′-dibutyl phosphate (**80**) were carried out using a similar method to the preparation of **78**, in 79% and 72% yield respectively. Again both **79** and **80** were isolated without recourse to chromatography and both displayed one peak ^{31}P nmr spectra. The ^{13}C nmr spectra of these compounds were very similar to the ^{13}C nmr spectrum of **78**. In both C3′ was shifted downfield by around 8 ppm relative to its position in **76** and **77** respectively. The mesyl carbon signal was seen at δ37.80 in **79** and δ37.69 in **80**. The $CH_3CH_2\underline{C}H_2OP$ resonance of **79** and the $CH_3CH_2CH_2\underline{C}H_2OP$ resonance of **80** were both seen as three lines. In each case the two carbon atoms contributing to these signals do not experience the same magnetic environment and resonate at slightly different chemical shifts. Each carbon couples to the adjacent phosphorus and the doublets overlap to give a three-line signal.

The ^1H nmr spectra of **79** and **80** showed the same salient features as **78**. For example, H3′ was seen well downfield, H1′ was seen as a doublet of doublets and the mesyl protons' signal occurred at around δ3 in each compound. The mass spectra of **79** and **80** were consistent with their proposed structures. The accurate mass measurements obtained on the molecular ions, in both cases, were close to those calculated for these ions.

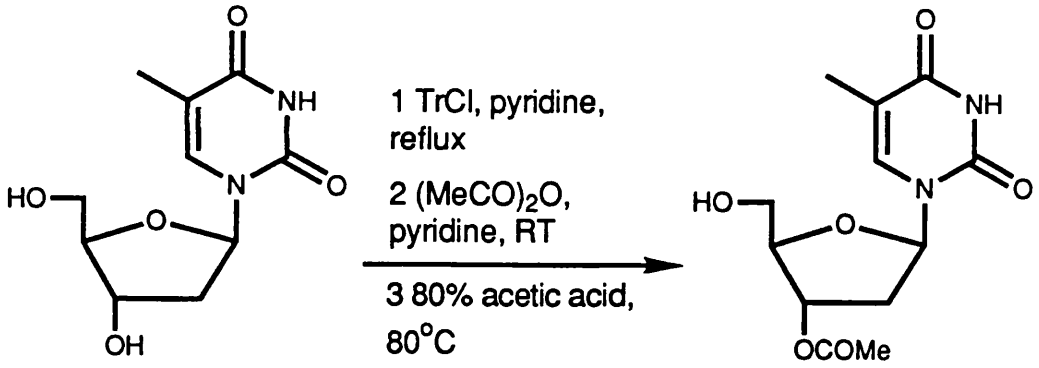
The next series of 3′-substituted thymidine derivatives to be studied was a 3′-O-acetylthymidine series. It seemed unlikely that 3′-O-acetylthymidine (**81**) itself would have

any substantial anti-HIV activity, but 5′-phosphate triester derivatives may. Certain 3′-substituted-2′,3′-dideoxynucleosides can display anti-HIV activity by acting as RT inhibitors. Although 3′-substituted, **81** itself would be unlikely to display anti-HIV activity since the acetyl group is both chemically and enzymically labile and would yield **74** on hydrolysis (**81** was subsequently tested against HIV and was found to be inactive). Moreover **81** may be poorly phosphorylated by host kinases. However this problem of phosphorylation may be overcome by substituting a phosphate triester at the 5′-position. Moreover, if the acetyl group were to be more rapidly metabolised in uninfected cells compared with infected ones, the chemical and/or enzymic cleavage of the acetyl function may be turned into an advantage, by providing a detoxification route. A more rapid breakdown in normal cells than in cancer cells has been used to explain the selective anti-cancer action of fluorinated pyrimidines[153].

The preparation of some 3′-O-acetylthymidine 5′-dialkyl phosphates was attempted, by first preparing **81** and phosphorylating it. **81** was prepared by a modification of the method of Michelson *et al*[154]. This modified procedure involved the addition of triphenylmethyl (trityl) chloride to **74** in pyridine producing 5′-O-tritylthymidine (diagram 23). This was followed by the addition of acetic anhydride *in situ* at room temperature. After detritylation (using 80% acetic acid), purification by column chromatography and recrystallisation, this method yielded analytically pure **81** in 72% yield. ^{13}C nmr and ^{1}H nmr data were obtained on **81** which confirmed its structure and purity. In addition, a mass spectrum was obtained on **81** in which the molecular ion was observed at m/e 284.

Having isolated **81**, three 3′-O-acetylthymidine 5′-dialkyl phosphates were prepared from it (diagram 24). Firstly, an excess of **71** was reacted with **81**. It was expected that phosphorylation would occur only at the 5′-position of **81**. Analysis of the reaction mixture by tlc, after stirring for 2 h, revealed the presence of the starting material, a component more lipophilic than the starting material and a baseline component. The reaction mixture was stirred overnight at room temperature, after which time tlc showed that no starting

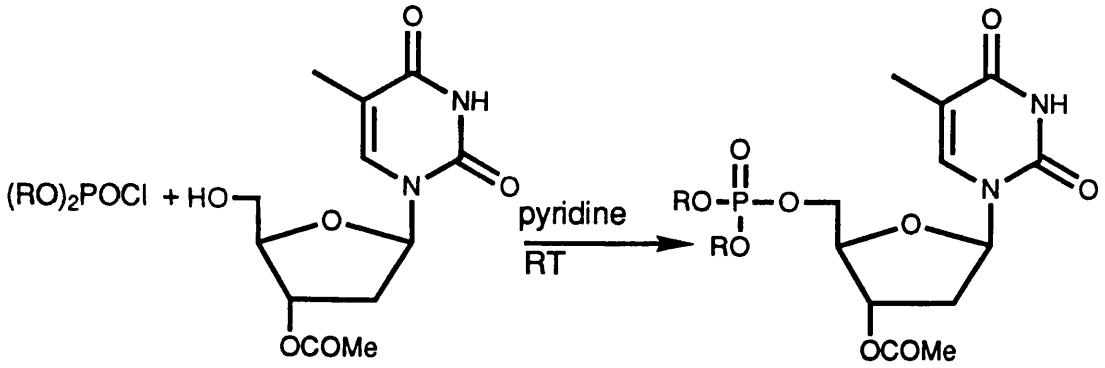
Diagram 23



74 **81**

Tr=Ph_3C-

Diagram 24



71, R=Et
72, R=Pr
73, R=Bu

81

82, R=Et
83, R=Pr
84, R=Bu

material remained. At this point the reaction was quenched with water and the solvent removed by evaporation under reduced pressure yielding a residue.

The residue was purified by column chromatography, although this was initially problematical. By elution with chloroform-methanol mixtures a material was isolated which tlc (uv visualisation) suggested was made up of one component, believed to be 3′-O-acetylthymidine 5′-diethyl phosphate (**82**). However this material was found to be impure by ^{31}P nmr spectroscopy. This technique showed the presence of a major signal at δ-2.75, in the region expected for a trialkyl phosphate. It seemed likely that this signal was due to the required product **82**. However the ^{31}P nmr spectrum also showed three other signals all having a chemical shift of around δ-12. These signals may be due to non-nucleosidic pyrophosphate material. Pyrophosphates have been reported to have chemical shifts of around δ-12[155]. They may have been formed by the reaction of excess **71** with water during the process of quenching. This contaminating pyrophosphate material may have a similar chromatographic mobility as **82**. **82** has a greater chromatographic mobility, in the chloroform-methanol systems used, than the previously prepared thymidine 5′-dialkyl phosphates. This may offer an explanation as to why contamination with pyrophosphate material was not a problem encountered during the isolation of **75**, **76** and **77**. It was found that the required product **82** could be freed of contaminants using column chromatography by initially eluting with chloroform, followed by a chloroform-methanol mixture. In this way **82** was isolated as a colourless oil in 65% yield. The ^{31}P nmr spectrum of **82** isolated in this way showed only one signal at δ-2.71.

The ^{13}C nmr spectrum of **82** was once again informative. The signals associated with both the thymine base and the sugar portion of this molecule have chemical shifts close to the analogous signals of **81**, with the exception of the C5′ signal, which occurs at 4 ppm downfield of the position of the C5′ signal of **81**. This fact is consistent with the successful 5′-phosphorylation of **81**. Further evidence for this occurrence came from the fact that the C4′ and C5′ signals of **82** show phosphorus coupling. Like **78**, the

$CH_3\underline{C}H_2OP$ resonance of **82** was seen as two doublets, again by virtue of the non-equivalence of the two ethyl chains attached to the phosphorus atom. The ^1H nmr spectrum of **82** featured the CH_3CO resonance as a singlet at δ2.12. A mass spectrum was obtained on **82** in which the molecular ion was seen at m/e 420

3′-O-Acetylthymidine 5′-dipropyl phosphate (**83**) and 3′-O-acetylthymidine 5′-dibutyl phosphate (**84**) were prepared by the reaction of **81** with **72** and **73** respectively. These two reactions were carried out in much the same way as the preparation of **82** was. Both **83** and **84** were purified and isolated by column chromatography. As was the case during the isolation of **82**, it was necessary to use chloroform as the eluent first, followed by chloroform-methanol. Having isolated both **83** and **84** in this way, it was found that both compounds displayed a ^{31}P nmr spectrum in which there was a single resonance, at δ-2.79 for **83** and δ-2.58 for **84**. The ^{13}C nmr spectra of both **83** and **84** displayed many of the features that were observed in the ^{13}C nmr spectrum of **82**. The C4′ and C5′ resonances of both compounds were doublets, because of phosphorus-carbon coupling. For both **83** and **84** the $R\underline{C}H_2OP$ resonance was observed as two doublets, this resonance again demonstrating that the two alkyl groups are non-equivalent. Lastly both **83** and **84** displayed a $CH_3\underline{C}O$ resonance (around δ170 for both compounds) and also a $\underline{C}H_3CO$ resonance (around δ21 for both compounds).

The ^1H nmr spectra of both **83** and **84** display the H1′ resonance as a doublet of doublets and both display the singlet of the CH_3CO signal, integrating for three protons and resonating at around δ2.05. The $RC\underline{H}_2OP$ resonance of both **83** and **84** was observed as a quartet and indeed for both compounds this quartet collapsed to a doublet when the adjacent methylene protons were irradiated. For both **83** and **84** a suitable molecular ion was observed in their mass spectra. Indeed a satisfactory accurate mass measurement was obtained on the molecular ions of both compounds. **83** and **84** also display a signal in their mass spectra which corresponds to the loss of both acetic acid and thymine from the appropriate protonated molecular ion. These signals occurred at m/e 263 for **83** and m/e 291

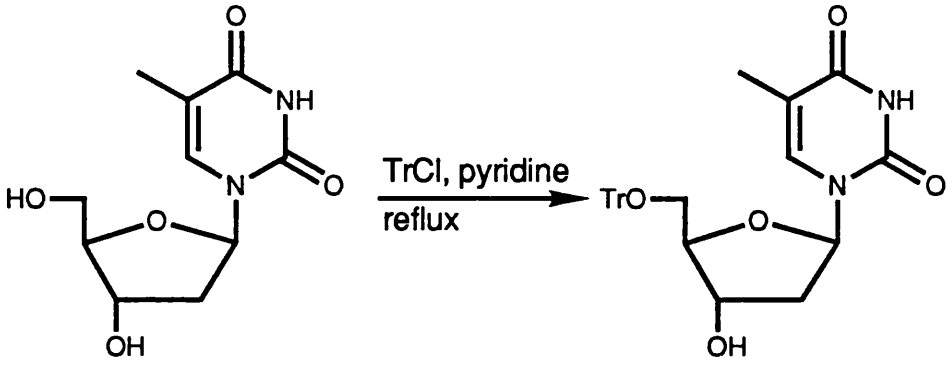
for **84**.

3′-O-Ethylthymidine (**85**) has been tested *in vitro* against HIV and has been found to be inactive[101]. This inactivity may be a result of **85** being poorly phosphorylated intracellularly. With this in mind, it was decided to investigate the potential of some 3′-O-ethylthymidine 5′-dialkyl phosphates as anti-HIV agents, again in the hope that the 5′-dialkyl phosphate moiety may help to overcome the difficulty of cellular phosphorylation. The 3′-O-ethyl group would be relatively stable to chemical and enzymic hydrolysis. This would contrast with the 3′-O-acetyl and 3′-O-mesyl phosphate derivatives discussed earlier. The preparation of some 3′-O-ethylthymidine 5′-dialkyl phosphates was carried out by preparing **85** and then phosphorylating it.

5′-O-Tritylthymidine (**86**) was prepared according to the method of Weimann *et al*[156] and was isolated in 76% yield (diagram 25). The preparation of **85** from **86** was then carried out according to the method of Hampton *et al*[157] (diagram 26). Two equivalents of ethyl iodide and potassium hydroxide were added to a solution of **86** in a mixed benzene-dioxan solution and stirred for 6 h. After this time the solvent was removed under reduced pressure and the residue was suspended in methanol and water which was then extracted with chloroform. Pooling and evaporation of the organic phase gave an oil, which was detritylated with 80% acetic acid. Purification by column chromatography yielded **85** in 71% yield. Purification by column chromatography also lead to the isolation of a minor product more lipophilic than **85** and this was characterised as N3,O3′-diethylthymidine (**87**). Although a minor component was observed using tlc during the preparation of **85** by Hampton *et al*[157], it would appear that it was neither isolated nor identified.

85 was characterised as follows. The melting point was determined and agreed closely with that of Hampton *et al*[157]. Moreover, ^{1}H nmr and ^{13}C nmr spectra of **85** were recorded and they were consistent with its successful isolation. It would appear that they have not been previously reported, although a ^{1}H nmr spectrum of the closely related 3′-O-methylthymidine (**44**) has[101]. The ^{13}C nmr spectrum of **85** bears a close resemblance to

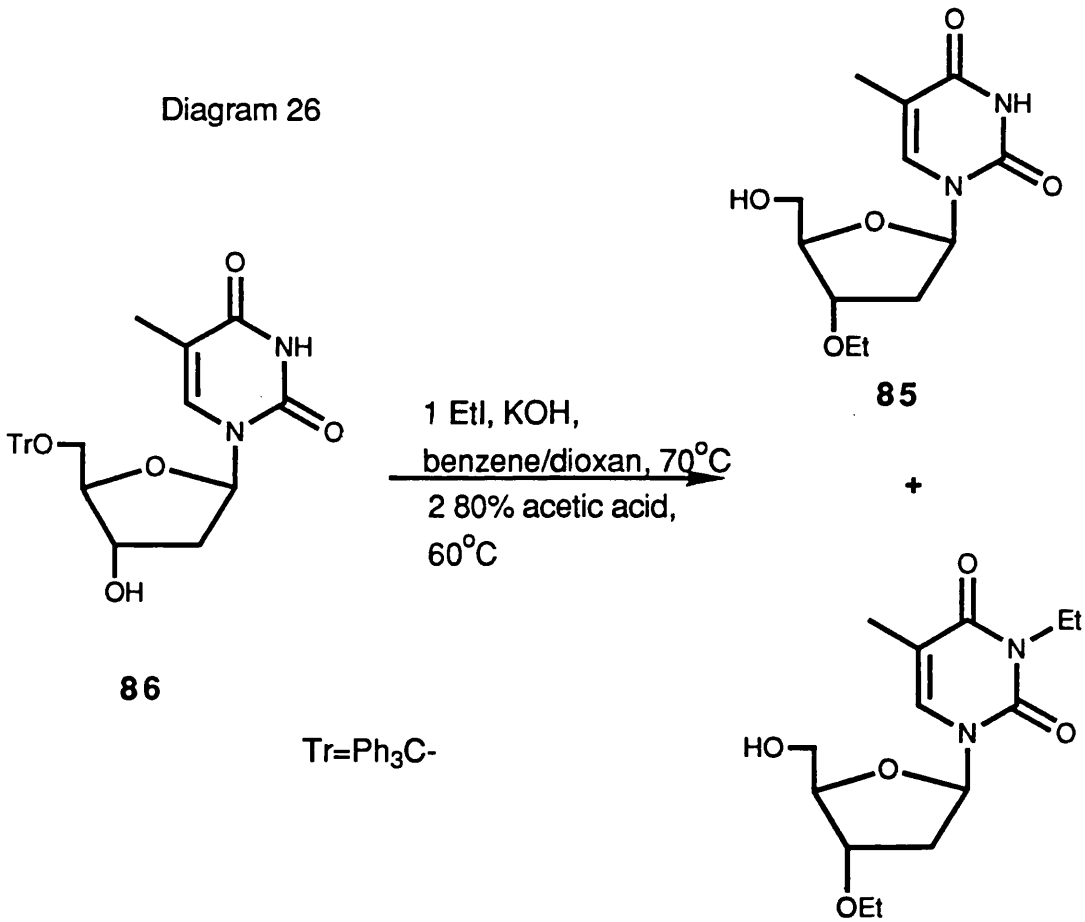
Diagram 25



74 **86**

$Tr=Ph_3C-$

Diagram 26



85

86

$Tr=Ph_3C-$

87

that of **74**, as may be expected. All of the sugar and base carbon resonances of **85** occur at very similar chemical shifts to the analogous resonances of **74** except C3′, which is shifted downfield in **85**, relative to **74**, to δ78.34. Also the $\underline{C}H_3CH_2O$ and $CH_3\underline{C}H_2O$ resonances were seen at δ15.29 and δ64.99.

Further evidence for the successful isolation of **85** came from its ^1H nmr spectrum. For example, resonances due to the $C\underline{H}_3CH_2O$ and $CH_3C\underline{H}_2O$ protons, a triplet and a multiplet, were seen at δ1.21 and δ3.51 respectively. The latter signal is a multiplet because the two protons contributing to this signal are diastereomerically different. The two protons resonate at slightly different chemical shifts. They couple with each other and with the adjacent methyl group, which gives rise to the observed complex multiplet. The H5′ resonance is seen as two relatively widely spaced multiplets and this arises out of the fact that the two protons giving rise to this resonance are diastereomerically different too. The fact that these two protons are in different environments means that they couple to the 5′-OH proton with differing magnitudes. The 5′-OH signal was seen as a doublet of doublets therefore. In the reported ^1H nmr spectrum of **44**[101], the H5′ resonance was also observed as two multiplets.

As discussed earlier, a minor component was isolated during the purification by column chromatography of **85**, which was identified as **87**. This identification was based on the following evidence. A ^{13}C nmr spectrum showed that the carbon resonances arising from the sugar and the base of **87** had similar chemical shifts to the equivalent resonances of **85**. The chemical shifts of the C5′ resonances of both **85** and **87** were almost identical which indicated that no modification had occurred at the 5′-OH. Resonances due to $\underline{C}H_3CH_2OC$ and $CH_3\underline{C}H_2OC$ were also seen in the ^{13}C nmr spectrum of **87**. In addition two signals at δ36.46 and δ13.19 were also observed, which were assigned to the resonances of an ethyl group linked to the N3 atom of the thymine base.

Further evidence for this compound's structure including a N3-ethylated portion came from the ^1H nmr spectrum of **87**. This showed that this molecule contained two

different types of ethyl group. One ethyl group gave rise to two resonances at δ3.43 and δ1.14. These signals bore a close resemblance to the $CH_3C\underline{H}_2OC$ and $C\underline{H}_3CH_2OC$ resonances of **85** and were assigned as being due to a 3′-O-ethyl group. Moreover another set of ethyl group resonances were observed, occurring at δ3.90 and δ1.12. These were assigned to the $CH_3C\underline{H}_2N$ and $C\underline{H}_3CH_2N$ protons of the N3-ethyl group. For the two closely spaced triplets seen at δ1.14 and δ1.12 in the ^1H nmr spectrum of **87** it was only possible to assign each signal fully by irradiating both the $CH_3C\underline{H}_2OC$ and $CH_3C\underline{H}_2N$ resonances. Mass spectrometry data was obtained on **87**. The molecular ion at m/e 298 was observed. Moreover two signals at m/e 154 and m/e 145 can be attributed to the fragmentation represented in diagram 27.

Since **87** contained a modified thymine base, it was of interest to obtain an electronic (uv) spectrum to ascertain if the λ_{max} of **87** was different to that of **85**. It was found that **87** and **85** have almost identical λ_{max} values and that this modification of the thymine base brings about very little change in λ_{max} values.

Having isolated **85**, 3′-O-ethylthymidine 5′-dipropyl phosphate (**88**) and 3′-O-ethylthymidine 5′-dibutyl phosphate (**89**) were prepared from it (diagram 28), using a similar methodology to the preparation and isolation of the 3′-O-acetylthymidine 5′-dialkyl phosphates described earlier.

Isolation of the required products led to **88** and **89** being obtained, analytically pure, in 75% and 81% yield respectively. Unlike the 3′-O-acetylthymidine 5′-dialkyl phosphates discussed earlier, it proved to be possible to isolate **88** and **89** by column chromatography using a chloroform-methanol mixture only as the eluent, rather than chloroform followed by chloroform-methanol.

Both **88** and **89** gave one-peak ^{31}P nmr spectra. Their ^{13}C nmr spectra were consistent with their successful isolation. On phosphorylation of **85**, the C5′ signal shifts downfield by around 4 ppm, to δ66.95 in **88** and δ66.68 in **89**. These shifts in the C5′ resonance mirror those seen for the C5′ resonance on phosphorylation of **74** and **81**, ie around 4 ppm downfield. The ^{13}C nmr spectra of **88** and **89** also reveal the non-equivalence of the two

Diagram 27

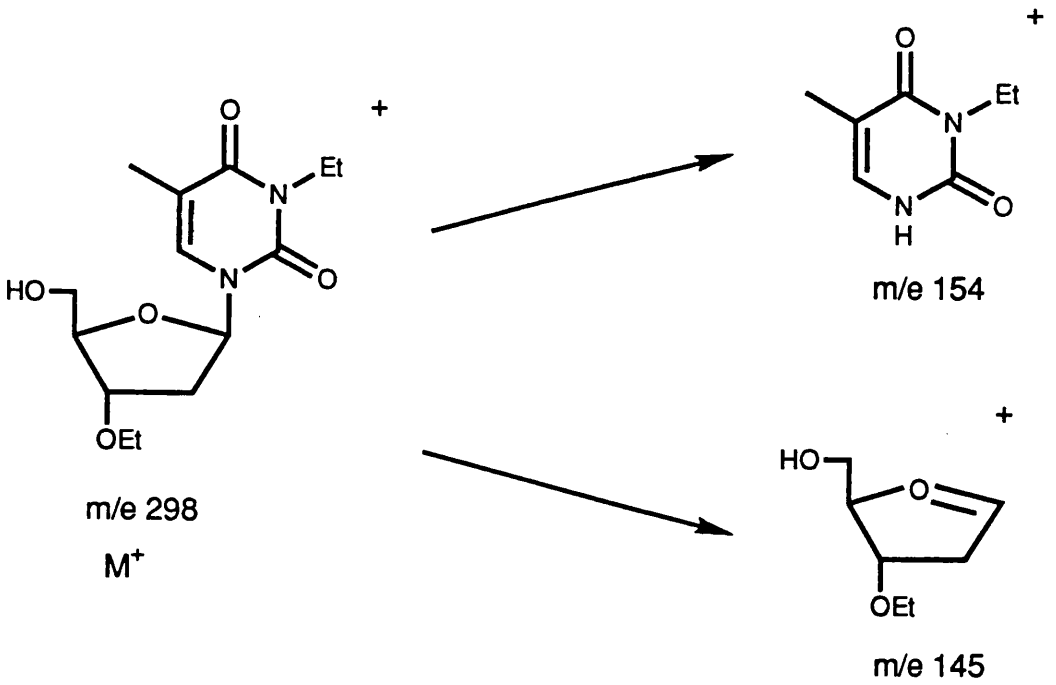
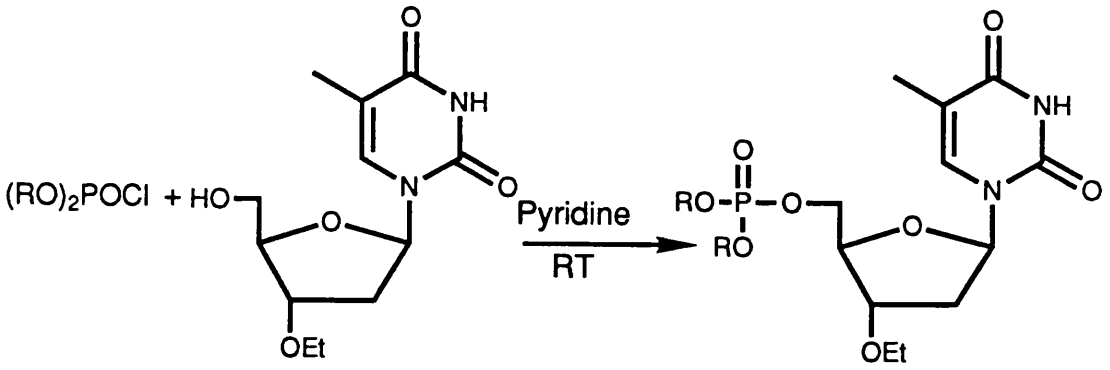


Diagram 28



72, R=Pr
73, R=Bu

85

88, R=Pr
89, R=Bu

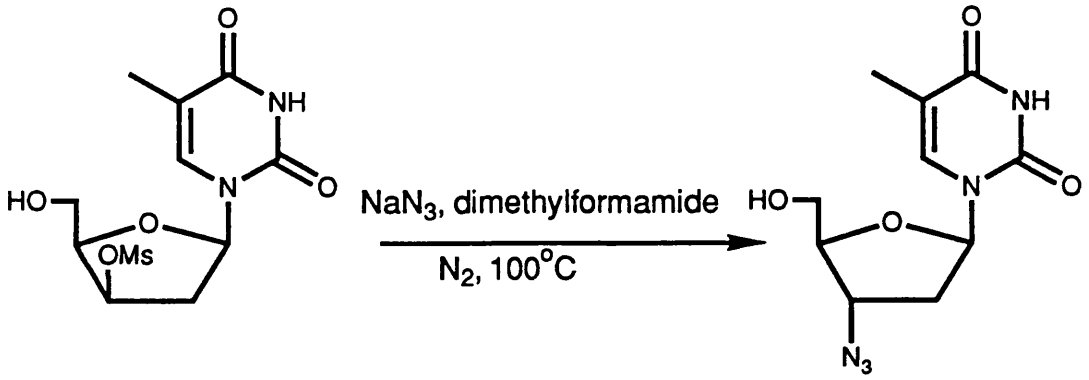
alkyl chains. In both, the RCH_2OP signals are seen as two doublets.

This non-equivalence of the alkyl chains is also seen in the ^1H nmr spectra of **88** and **89**, yet this time it is the $C\underline{H}_3CH_2CH_2OP$ and $C\underline{H}_3CH_2CH_2CH_2OP$ protons that demonstrate it. For both **88** and **89** these resonances are seen as two triplets. Mass spectra were obtained on both **88** and **89** and in both spectra a molecular ion was observed on which a satisfactory accurate mass measurement was obtained. In the mass spectrum of **88**, the prominent signals observed included those at m/e 263 (due to MH^+-EtOH-thymine), at m/e 126 (due to thymine$^+$) and the base peak at m/e 81 (due to $C_5H_5O^+$). The mass spectrum of **89** also displayed signals at m/e 126 and at m/e 81 and in addition a signal at m/e 291 (due to MH^+-EtOH-thymine).

Continuing the studies into a number of 3′-modified thymidine 5′-dialkyl phosphates, some 5′-dialkyl phosphates of the known anti-HIV agent 3′-azido-3′-deoxythymidine (**1**) were made the next targets for synthesis. These derivatives may offer a number of advantages over the parent nucleoside as anti-HIV agents. For example, these dialkyl phosphates may (after intracellular hydrolysis to the monophosphate) be metabolised more easily to the bio-active 5′-triphosphate of **1** than **1** itself. They may also have better membrane penetrative properties than **1**. It is likely that they would be more lipophilic than **1**. It is also possible that they would have different toxicity profiles to **1**.

With these points in mind, three 3′-azido-3′-deoxythymidine 5′-dialkyl phosphates were prepared from **1**. Although free phosphates of **1** are well known[49], 5′-phosphate triesters of **1** are not. **1** itself was prepared by the reaction of 1-(2-deoxy-3-O-mesyl-ß-D-threopentofuranosyl)thymine (**90**) with an excess of sodium azide in dry dimethylformamide, under an atmosphere of nitrogen and at 100°C (diagram 29). This displacement of a mesyl group by azide is similar to that achieved by Horwitz *et al*[43] in the literature preparation of **1**, except for the use of 5′-tritylated nucleoside and lithium azide (diagram 30). The displacement of the mesyl group by an azide ion operates principally by an S_N2 mechanism and thereby leads to inversion of configuration at the C3′ atom. **1** was prepared successfully

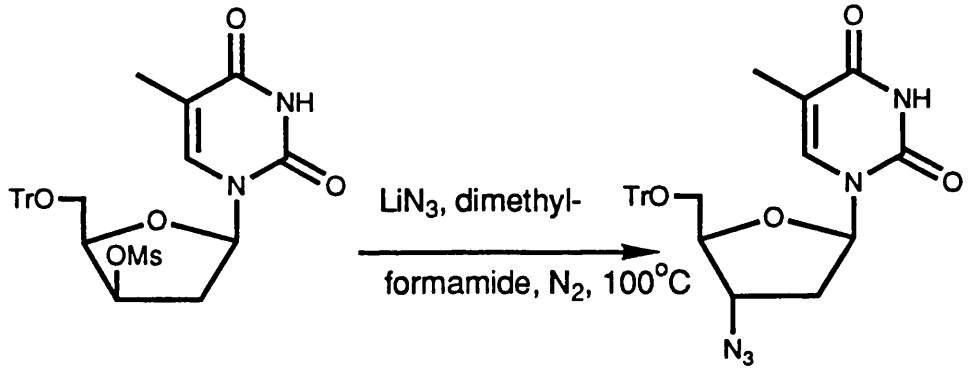
Diagram 29



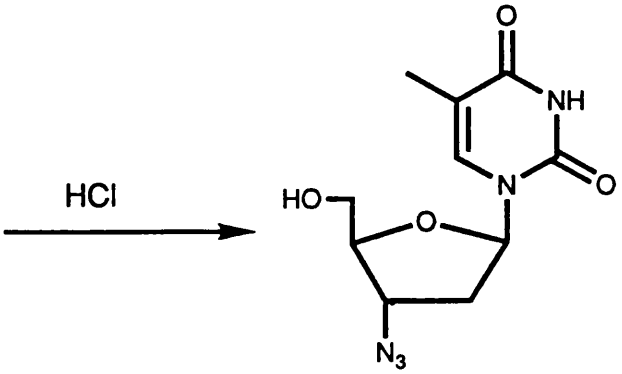
90 **1**

Ms=$MeSO_2$-

Diagram 30



Tr=Ph_3C-
Ms=$MeSO_2$-



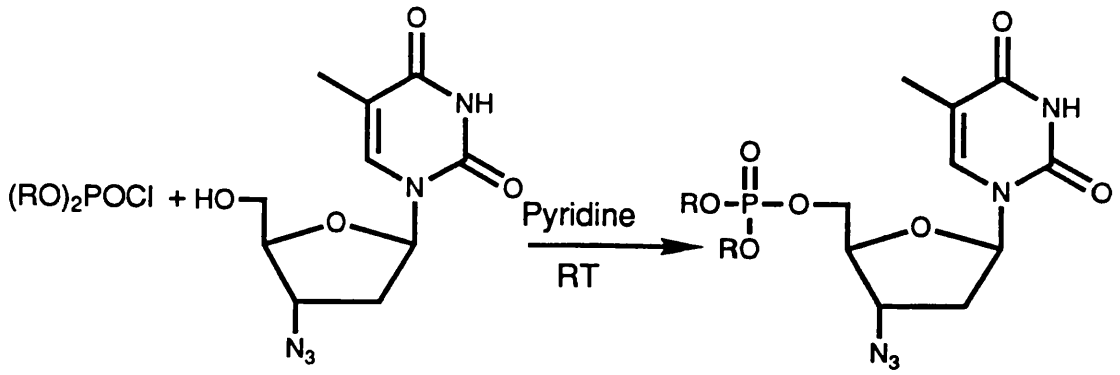
1

by the method depicted in diagram 29, after column chromatography.

1, obtained analytically pure, was found to have a melting point almost identical to that previously reported[43]. The ^{13}C nmr and ^{1}H nmr spectral data of **1** were found to be similar to previously reported data. In the ^{13}C nmr spectrum of **1** the 3′-azide group was seen to have a dramatic effect on the chemical shift of the C3′ resonance. This resonance was shifted upfield relative to its position in **74** by about 10 ppm. The ^{1}H nmr spectrum revealed a number of interesting features. The H3′ resonance was assigned to the signal integrating for one proton at δ4.39. The line-shape of this signal was observed to change on irradiation at δ2.45 - at the H2′ resonance. It is well known that OH protons are able to resonate over a relatively wide range of chemical shifts[158] yet despite this the 5′-OH resonance of **1** was observed at δ2.38 this chemical shift being similar to that of the 5′-OH resonance of **81**. Mass spectrometry bore out the successful isolation of **1**. A satisfactory accurate mass measurement was obtained on the molecular ion. Moreover the two most intense signals at m/e 126 and m/e 142 can be assigned to thymine^{+} and MH^{+}-thymine respectively.

3′-Azido-3′-deoxythymidine 5′-diethyl phosphate (**91**), 3′-azido-3′-deoxythymidine 5′-dipropyl phosphate (**92**) and 3′-azido-3′-deoxythymidine 5′-dibutyl phosphate (**93**) were then prepared from **1** using a similar methodology to that described for the preparation of **82**, **83** and **84** from **81**. Once again two equivalents of the appropriate phosphorochloridate were reacted with **1** in anhydrous pyridine (diagram 31). The reaction was found to be complete after 6 h in the preparation of **91**, but stirring the reaction mixture overnight was necessary to bring about completion for **92** and **93**. It was found that attempting to isolate **91**, **92** and **93** by column chromatography using chloroform-methanol as the eluent, the required product was contaminated with non-nucleosidic phosphorus-containing material, as was found during the preparation and isolation of the 3′-O-acetylthymidine phosphate triester derivatives described earlier. The contaminants had chemical shifts of around δ-12 in the ^{31}P nmr spectrum. Once again it was found possible to obtain **91**, **92** and **93** free of this

Diagram 31

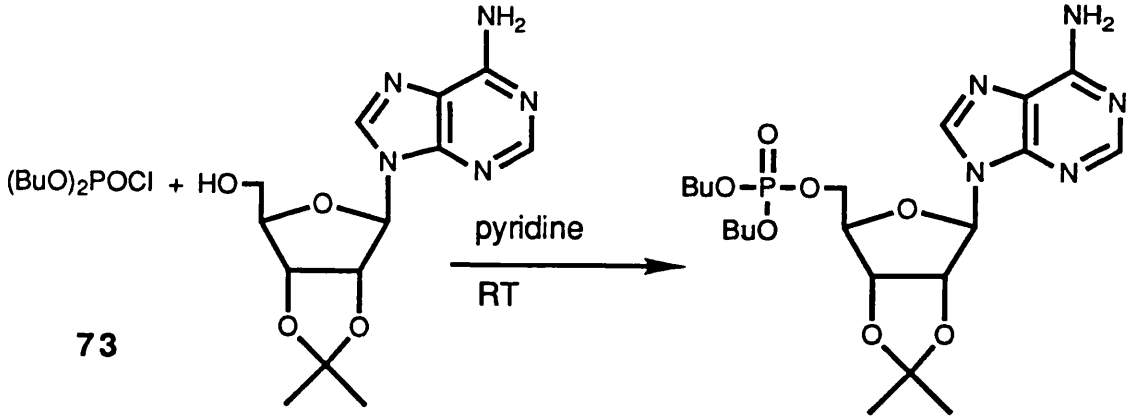


71, R=Et
72, R=Pr
73, R=Bu

1

91, R=Et
92, R=Pr
93, R=Bu

Diagram 32



73

94

95

contaminant by first eluting with chloroform and then with chloroform-methanol.

During the preparations of **91**, **92** and **93**, tlc revealed only one component had been generated which was more lipophilic than **1** itself in each case. These more lipophilic components were assumed to be the required products and were duly isolated. Interestingly, their R_f values in chloroform-methanol were found to be significantly greater than any of the 3′-substituted thymidine 5′-dialkyl phosphates previously discussed.

Again it was not possible to obtain a crystalline sample of any of these phosphate derivatives. They would appear to be quite hygroscopic and could only be obtained as glass-like oils. The ^{13}C nmr and ^{1}H nmr spectra of **91**, **92** and **93** were entirely consistent with their proposed structures. Like **1**, the ^{13}C nmr spectra of **91**, **92** and **93** each displayed a signal around δ60, corresponding to the C3′ resonance.

The ^{1}H nmr spectra of **91**, **92** and **93** all show a number of signals at around δ4.00. These signals comprise the H3′, H4′, H5′ and the $RC\underline{H}_2OP$ resonances. In order to assign the H3′ signal with certainty, it was necessary to use spin-decoupling. For **91**, irradiation of the H2′ signals caused the multiplet at δ4.32 to collapse to a doublet. This signal must therefore be due to H3′ and collapses to a doublet on irradiation as H3′ is still able to couple to the adjacent H4′ proton. The H3′ signals of both **92** and **93** were assigned by analogy to **91**. The ^{1}H nmr spectra of **91**, **92** and **93** again display the non-equivalence of the alkyl chains. For all these compounds the methyl resonances are seen as two sets of triplets. However, the RCH_2OP proton resonances do not show this effect. These resonances are seen as a quintet for **91** and quartets for both **92** and **93**. The remaining methylene resonances of **92** and **93** are seen as multiplets, again resulting from the non-equivalence of the two alkyl chains. Mass spectra were obtained for **91**, **92** and **93** in which a molecular ion was observed for each compound.

In view of the successful isolation of the phosphate triesters of **1**, it was decided to synthesise some phosphate triesters of another potent anti-HIV agent 2′,3′-dideoxyadenosine (**13**). However, in order to provide a model for the preparation and

isolation of these derivatives of **13**, a phosphorylation of 2′,3′-O-isopropylideneadenosine (**94**) was carried out first (diagram 32). It was feared that phosphorylation at the NH_2 of the adenine base might have taken place quite readily, which would lead to a large reduction in the yield and difficulties in the isolation of the 5′-phosphorylated product. This could be investigated by executing the analogous phosphorylation of **94**.

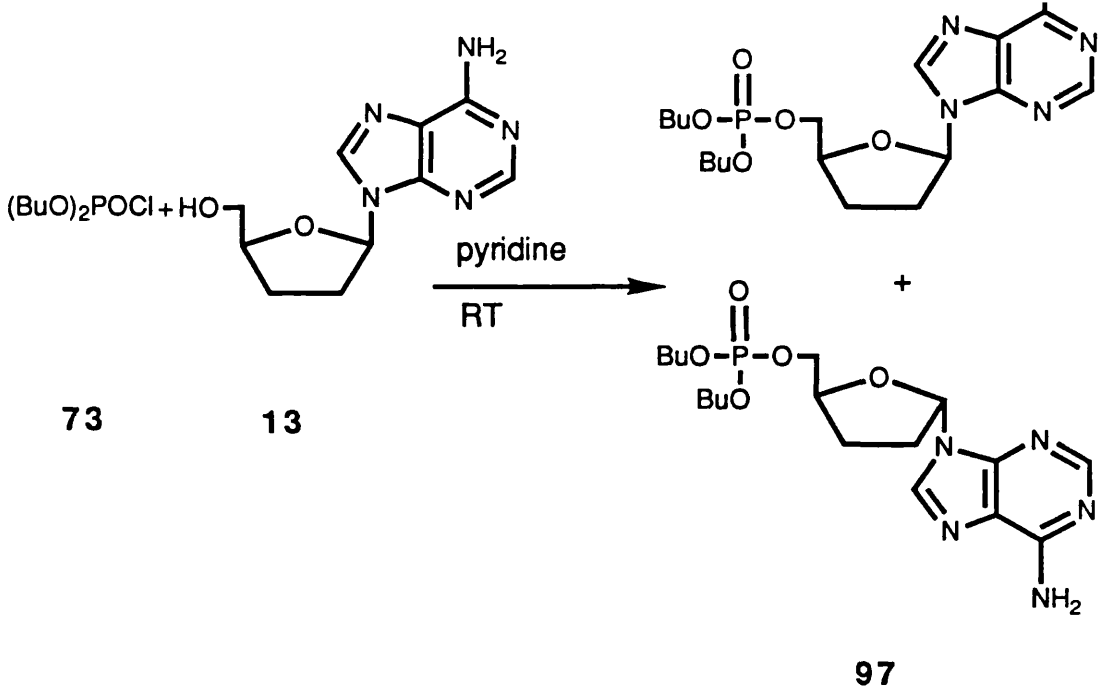
Thus two equivalents of **73** were added to **94** in pyridine at room temperature and the reaction mixture was stirred overnight. After this time, analysis of the reaction mixture by tlc revealed that only one component had been produced which was more lipophilic than the starting material **94**. This suggested that phosphorylation had occurred selectively either at the 5′-OH or at the NH_2 of the adenine base. The reaction appeared to be regioselective. Quenching of the reaction with water, removal of the solvent and purification of the residue by column chromatography yielded a colourless oil, whose ^{31}P nmr spectrum was found to consist of two signals. One signal, the major one, occurred at δ-2.78 -in the region expected for a phosphate triester. The other signal, a minor one, occurred at δ1.04 and was probably due to contaminating non-nucleosidic material.

The colourless oil was therefore further purified using column chromatography and this led to the isolation of a white solid, whose ^{31}P nmr spectrum consisted of a single resonance occurring at δ-2.72. The ^{13}C nmr spectrum of this white solid confirmed it to be the product of phosphorylation at the 5′-OH group of **94**, that it was 2′,3′-O-isopropylideneadenosine 5′-dibutyl phosphate (**95**). This was concluded from the fact that both C4′ and C5′ were observed as doublets as a result of phosphorus-carbon coupling. The rest of the spectrum was consistent with the successful isolation of **95**. Interestingly, a separate carbon resonance was seen for both of the two methyl carbon atoms of the isopropylidene group. One resonance occurred at δ27.10, the other at δ25.28, as these two methyl groups are in two distinct chemical environments. The methyl proton resonances of the isopropylidene group resonated as two separate singlets in the ^{1}H nmr spectrum of **95** too. The ^{1}H nmr spectrum also reveals the presence of a broad singlet integrating for two

protons at δ6.21 due to the NH_2 group of the adenine base. Moreover a doublet, with a relatively small coupling constant of 2.2 Hz is seen at δ6.11, this being the H1′ resonance. Both the H2′ and H3′ resonances of **95** were resolved as doublet of doublets, at δ5.36 and δ5.05 respectively. In order to assign these signals fully it was necessary to carry out a number of spin-decoupling experiments on **95**. Irradiation at the H1′ resonance (δ6.11), led to the signal at δ5.36 collapsing to a doublet. Likewise, irradiation at the H4′ resonance (δ4.23) caused the signal at δ5.05 to collapse to a doublet.

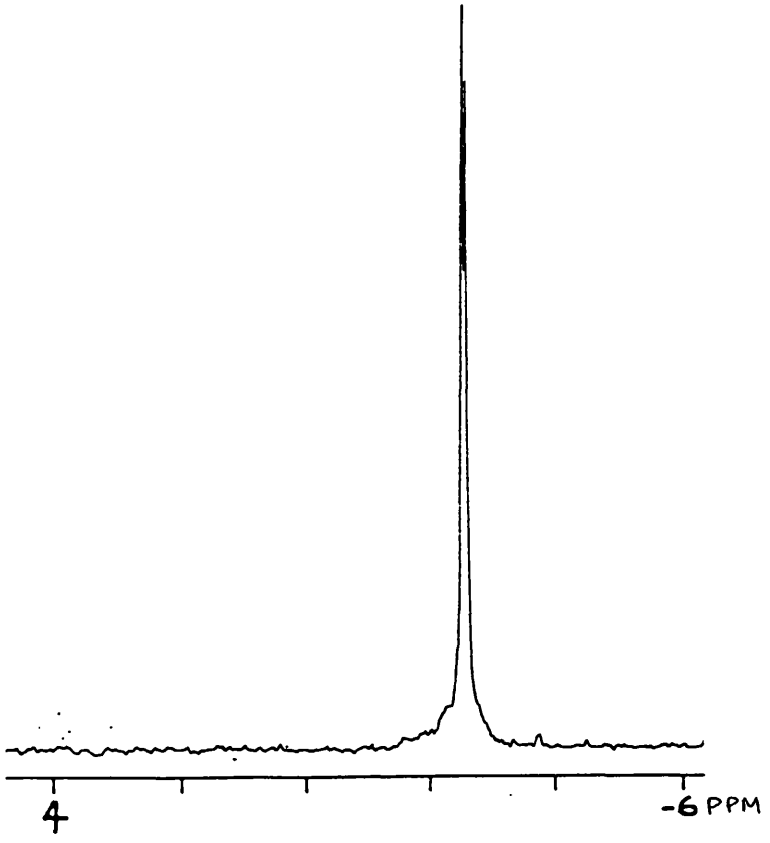
In view of the successful isolation of **95** and the discovery that phosphorylation took place selectively at the 5′-OH group of **94**, the analogous phosphorylation of **13** was attempted. Thus an excess of **73** was reacted with **13** in pyridine, at room temperature (diagram 33). **13** was found to be only sparingly soluble in pyridine at room temperature and before the reaction was commenced **13** could not be completely dissolved in this solvent. Yet as the reaction with **73** proceeded, the undissolved nucleoside went quickly into solution. As was found during the preparation of **95**, analysis of the reaction mixture by tlc revealed that one component more lipophilic than **13** had been generated and by analogy with the preparation of **95**, it seemed likely that this component was due to the product of phosphorylation at the 5′-position. After isolation of the generated product by column chromatography, this material yielded a ^{31}P nmr spectrum which consisted of, not one, but two signals of roughly equal intensity (diagram 34), even though it proved impossible to observe any evidence of two materials using the technique of tlc. The two closely spaced signals in the ^{31}P nmr spectrum were in the expected region for phosphate triesters[147]. One explanation for the spectrum consisting of two signals is that along with the isolation of the expected ß-anomer of 2′,3′-dideoxyadenosine 5′-dibutyl phosphate (**96**), the α-anomer of this compound **97** had also been isolated. Evidence for this conclusion was gained from the ^{13}C nmr and ^{1}H nmr data.

A ^{13}C nmr spectrum displayed resonances in the regions expected for a 2′,3′-dideoxyadenosine 5′-dibutyl phosphate. In the majority of cases, where one signal was



73 13 97

Diagram 34



The ^{31}P nmr spectrum of the isolated mixture of **96** and **97**.

expected, two were actually observed. For example, in the region where the C8 signal resonates (around δ139) two peaks were seen of approximately equal intensity. This situation was repeated for the resonances of the sugar moiety. For example, two signals were observed in the area of the C1′ resonance (around δ86). Moreover, for both the C4′ and the C5′ resonances two sets of doublets were observed, the doublets arising from phosphorus-carbon coupling. The ^{13}C nmr spectrum suggested that the isolated material was comprised of two compounds that were closely related structurally. The observations were consistent with the possibility that a mixture of the α and ß anomers **97** and **96** had been isolated. A ^{1}H nmr spectrum suggests this interpretation too. There were, for example, two H2 resonances, quite widely spaced at δ8.11 and δ7.88. In the region that the H1′ protons resonate, a multiplet was observed, which appeared to be comprised of two different signals. There were also two distinct (broad) NH_2 resonances.

Mass spectrometry of the mixture of **96** and **97** displayed a signal at m/e 427, corresponding to the molecular ion of both species. Moreover the other signals observed included those at m/e 211 and m/e 155, due to $(BuO)_2P(OH)_2^+$ and $BuOP(OH)_3^+$ respectively and at m/e 135 due to adenine$^+$. In summary the nmr and mass spectroscopic data gathered on the product isolated from the reaction of **73** and **13** points to the isolated material being comprised of two, structurally similar compounds. Moreover the data would appear to be consistent with those compounds being the anomers **96** and **97**.

It proved possible to separate these two compounds by preparative high performance liquid chromatography (hplc). A ^{1}H nmr spectrum was recorded for each separated component and not surprisingly both spectra had a number of similarities. Further evidence was gained for the two compounds being **96** and **97**. From a close examination of ^{1}H nmr spectra, Robins *et al*[159] has shown that for purine nucleosides, it is possible to assess the configuration at the C1′ position by a study of the H1′ resonance. In this way ß-anomeric nucleosides are characterised by a triplet being observed for the H1′ protons, whereas their α-anomer's H1′ resonance is seen as a multiplet of four (a doublet of doublets).

After separation of the two components **96** and **97** by hplc, the slightly faster-running component gave a ^{1}H nmr spectrum whose H1′ resonance was seen as a triplet. In the light of the work of Robins *et al*[159], this component may be assigned as the ß-anomer **96**. The slightly slower-running component gave a ^{1}H nmr spectrum whose H1′ signal was seen as a doublet of doublets and this component may be assigned as the α-anomer **97**. As would be expected from two closely related structural isomers the two ^{1}H nmr spectra are otherwise quite similar. However small but significant differences were noted between the two anomers as far as the chemical shifts of their furanose protons were concerned. Most interestingly of all the H4′ resonance of the ß-anomer **96** occurred at δ4.35, yet in the case of the α-anomer the H4′ resonance was observed at δ4.70. This significant difference may be due to the fact that in **96** and **97** there is a differing degree of interaction through space between the adenine base and the H4′ proton, causing each anomer's H4′ proton to be in a slightly different chemical environment. A differing amount of interaction between the adenine base and the furanose sugar protons may account for the observed differences between the chemical shifts of the H2′ and H3′ resonances for each anomer. For example, the ß-anomer displayed two separate multiplets (both integrating for two protons) in the region H2′ and H3′ were expected to resonate. For the α-anomer, three multiplets were seen in this region, one multiplet integrating for two protons and two multiplets integrating for one proton each. The mass spectra of the separated anomers were also very similar and it was possible to obtain a satisfactory accurate mass measurement on each compound's molecular ion. Moreover each molecular ion fragmented in similar ways. Many signals, like that at m/e 135 (due to adenine ions), were common to each compound's mass spectrum.

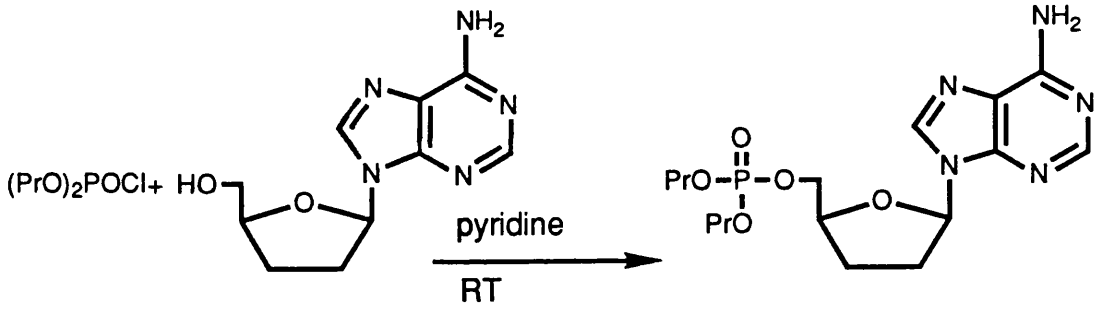
The reason for the two anomers **96** and **97** being isolated from this reaction is far from clear. Whatever the process by which anomerisation takes place, the mechanism presumably involves cleavage of the glycosidic bond. The glycosidic bonds of purine nucleosides are more labile than those of pyrimidine nucleosides in acidic conditions[160]. It is known that the glycosidic bonds of 2′,3′-dideoxynucleosides are quite labile in acidic

media[161]. Therefore the glycosidic bond of 2′,3′-dideoxyadenosine analogues may be capable of being cleaved even under mildly acidic conditions. The silica used during column chromatography may possibly be acidic enough to bring about glycosidic cleavage and it may be during the process of column chromatography that anomerisation took place. However the exact reason for the two anomers **96** and **97** being isolated from this reaction is far from clear.

The preparation of 2′,3′-dideoxyadenosine 5′-dipropyl phosphate (**98**) was carried out by the reaction of **72** with **13** in pyridine at ambient temperature (diagram 35). Analysis of the reaction mixture after 6 h by tlc revealed that no **13** remained in the reaction mixture and that a single product had been generated, more lipophilic than **13** itself. At this point the reaction was quenched and the solvent was removed under reduced pressure. The residue was then purified by column chromatography. The eluent used for chromatography was chloroform-methanol-triethylamine (95:3:2). Triethylamine was added to the eluent in an effort to ensure that the stationary phase used during chromatography would not be acidic. It was hoped that under these conditions anomerisation would not occur. **98** was duly isolated by this technique in 75% yield. Moreover spectroscopic data amassed on **98** would suggest that only the ß-anomer had been isolated.

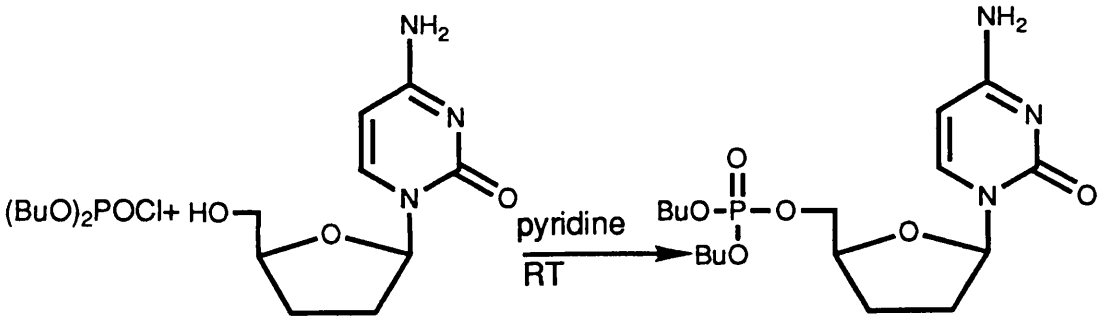
A ^{31}P nmr spectrum of **98** consisted of a single resonance at δ-2.71. The mixture of **96** and **97**, obtained from the reaction of **73** with **13**, gave rise to two peaks in its ^{31}P nmr spectrum. The ^{13}C nmr spectrum of **98** displayed the C4′ and C5′ resonances as doublets. Two other doublets were also observed at δ69.52 and δ23.65, which were assigned to the $CH_3CH_2\underline{C}H_2OP$ and $CH_3\underline{C}H_2CH_2OP$ resonances respectively. The ^1H nmr spectrum of **98** displays the H1′ resonance as a triplet which, in the light of the work of Robins *et al*[159], would suggest that **98** possesses a ß-configuration at the C1′ position. Also observed in the ^1H nmr spectrum of **98** were two multiplets at δ2.17 and δ2.50, integrating for two protons each. These multiplets were caused by the H2′ and H3′ protons. In order to assign these protons fully though, irradiation was carried out at δ6.33 (the chemical shift of the H1′

Diagram 35



72 **13** **98**

Diagram 36



73 **2** **99**

signal). This caused a change in the line-shape of the multiplet at δ2.50 only, which led to the two proton multiplet at δ2.50 being assigned to the H2′ protons and the multiplet at δ2.17 being assigned to the H3′ protons. A mass spectrum was obtained on **98** and this displayed a molecular ion at m/e 399 on which a satisfactory accurate mass measurement was obtained.

The dibutyl phosphate of the highly potent anti-HIV agent 2′,3′-dideoxycytidine (**2**) was the next phosphate triester to be prepared. **2**, like **13**, was relatively insoluble in pyridine. However, it was possible to form a solution of **2** by suspending it in pyridine and heating to 60°C, thus dissolving the nucleoside. This was followed by cooling slowing to room temperature, where it was found that **2** remained in solution. To the solution of **2** in pyridine was added an excess of **73** and the reaction mixture was stirred for 7 h (diagram 36), after which time tlc revealed that no **2** remained in the reaction mixture. In addition, tlc also revealed that two major components had been generated, which were more lipophilic than **2** itself. This appeared to indicate that in addition to phosphorylation occurring at the 5′-OH group of **2**, there may have also been phosphorylation taking place at the NH_2 group of the cytosine base. This contrasts with the previously discussed phosphorylations of **13** where, under similar reaction conditions, phosphorylation of the NH_2 group of the adenine base did not occur.

Once again, the technique of column chromatography was used to isolate the required phosphate triester. Thus 2′,3′-dideoxycytidine 5′-dibutyl phosphate (**99**) was isolated in 49% yield as an analytically pure white solid. However it was not possible to isolate and characterise the other fast-running component observed by tlc. A ^{31}P nmr spectrum of **99** displayed one resonance at δ-2.57, a chemical shift very similar to the phosphate triesters described previously. There was no evidence for the presence of the α-anomer of **99** from the spectral data gathered.

In the ^{13}C nmr spectrum of **99**, the resonances of the cytosine base were similar to the corresponding resonances reported for cytidine nucleosides[162]. The C5′ signal occurred

at a similar chemical shift to the C5′ signal of the 2′,3′-dideoxyadenosine 5′-dialkyl phosphates described earlier. Moreover the C5′ signal along with the C4′ signal appeared as a doublet due to phosphorus-carbon coupling. This data confirmed that the isolated product was indeed **99**, that it was the product of phosphorylation of the 5′-OH and not at the NH_2 group of the cytosine base. Evidence of two and three bond phosphorus-carbon coupling was seen in the respective resonances of $CH_3CH_2CH_2\underline{C}H_2OP$ and $CH_3CH_2\underline{C}H_2CH_2OP$ of **99**. Indeed the former resonance was seen as two doublets, again reflecting the non-equivalence of the two butyl chains. The latter resonance, however, was seen as a doublet.

A ^1H nmr spectrum of **99** was quite consistent with its proposed structure. Three multiplets were observed at δ2.31, δ1.94 and δ1.82, integrating for one, two, and one protons respectively. These resonances seemed likely to be the H2′ and H3′ signals. However in order to assign these signals fully, it was necessary to irradiate at the H1′ resonance (δ6.03). This led to the signals at δ2.31 and δ1.94 changing their line-shape, relative to a fully coupled spectrum, as coupling between the H1′ and the H2′ protons had been eliminated. It was concluded from these observations that the multiplet at δ2.31 was due to one of the H2′ protons, the multiplet at δ1.94 was due to one H2′ and one H3′ proton and the remaining multiplet at δ1.82 was due to one H3′ proton. It was not possible to observe the molecular ion of **99** when using the technique of electron impact mass spectrometry (EIMS). However, using fast atom bombardment mass spectrometry (FABMS) it was possible to observe the protonated molecular ion in the mass spectrum of **99**. The other signals observed included a signal due to the loss of the cytosine base from the protonated molecular ion, at m/e 293 and a signal due to protonated cytosine ions at m/e 112.

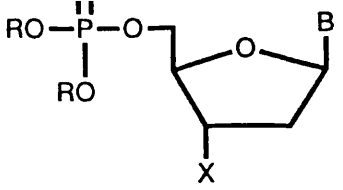
A number of nucleoside phosphate triesters, whose syntheses were described earlier in this thesis, were evaluated against HIV by T.J. O'Connor and D. Kinchington of the Department of Virology at St. Mary's Hospital Medical School London. The compounds

tested are depicted in diagram 37. The phosphate triesters were examined for anti-HIV activity and toxicity on a human lymphoblastoid cell line (C8166) by a procedure that has been reported[163]. However all compounds depicted in diagram 37 were devoid of anti-HIV activity at concentrations up to 100 μM (the highest concentration used). None of these compounds displayed cytotoxicity to uninfected cells at concentrations up to 100 μM either. Under the conditions of this assay, **1** and **2** reduced viral proliferation by 50% at concentrations of 0.03 μM and 0.3 μM respectively. Regarding the phosphate triesters **91**, **92** and **93**, it is clear that the substantial anti-HIV activity of **1** has been eliminated by substitution of a dialkyl phosphate moiety at the 5′-position of **1**, a result that is quite striking. **91**, **92** and **93** were prepared and tested as potential anti-HIV agents because it was thought that they may have been able to hydrolyse to either the 5′-monophosphate or perhaps to the parent nucleoside **1** (diagram 38). Both the 5′-monophosphate and **1** could then have been phosphorylated to the 5′-triphosphate which is known to inhibit HIV-RT[49].

The inactivity of **91**, **92** and **93** when tested against HIV may therefore result from these simple triesters being metabolically stable and being unable to hydrolyse intracellularly (diagram 38). This would also explain why **99** is inactive against HIV even though its parent nucleoside **2** is a potent anti-HIV agent. Similarly **13** too has potent activity yet **98** and **96** were found to be inactive, again possibly as a result of the metabolic stability of the dialkyl phosphate moieties. It is not surprising that **75**, **76** and **77** displayed no anti-HIV activity. These compounds were included only to act as biological controls. Of the remaining compounds depicted in diagram 37, the metabolic stability of the dialkyl phosphate group would preclude any prospect of anti-HIV activity being displayed by these 3′-modified derivatives.

In the light of the negative biological results detailed above, it appeared that a more labile phosphate-esterifying group was required. This could be achieved if electron-withdrawing atoms or groups were present in the esterifying moiety. Illustrating this point Hunston *et al*[137] have noted that whilst the phosphate triester **56** (diagram 39) was quite

Diagram 37

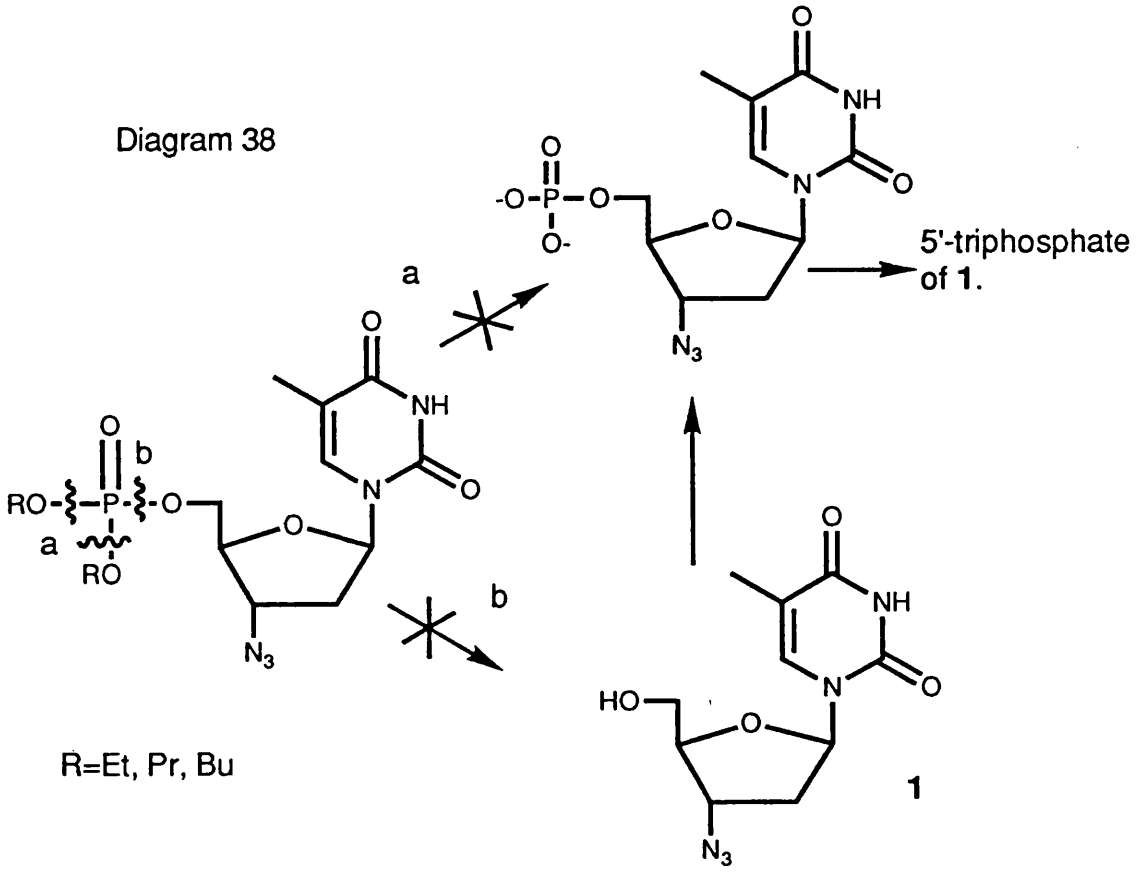


	R	X	B		R	X	B
75	Et	OH	T	**88**	Pr	OEt	T
76	Pr	OH	T	**89**	Bu	OEt	T
77	Bu	OH	T	**91**	Et	N_3	T
78	Et	OSO_2Me	T	**92**	Pr	N_3	T
79	Pr	OSO_2Me	T	**93**	Bu	N_3	T
80	Bu	OSO_2Me	T	**98**	Pr	H	A
82	Et	OCOMe	T	**96***	Bu	H	A
83	Pr	OCOMe	T	**99**	Bu	H	C
84	Bu	OCOMe	T				

* In addition the α-anomer of this compound **97** was tested and proved to have an IC_{50}>100 μM.

T=1-thymine, A=9-adenine, C=1-cytosine.

Diagram 38

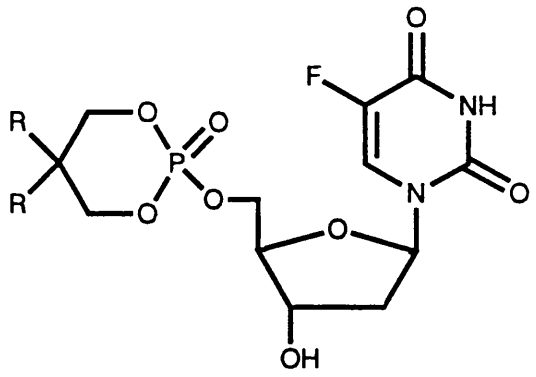


resistant to hydrolysis, the phosphate triester **67** was found to be easily hydrolysed at room temperature forming the diester **100**. The electron-withdrawing fluorine atoms present in **67** would appear to increase the lability of the phosphate moiety.

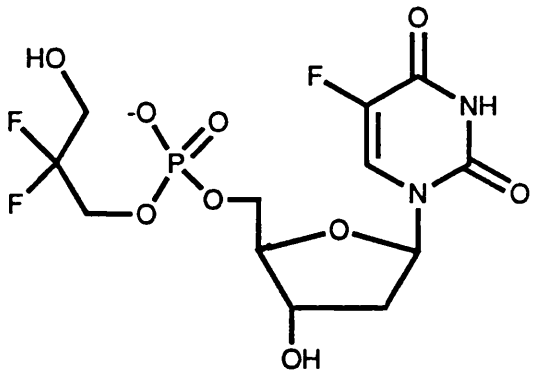
With this in mind, 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trifluoroethyl) phosphate (**101**) and 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**102**) were made the next targets for synthesis, as they may possibly possess anti-HIV activity. **101** and **102** are both uncharged phosphate triesters and would be expected to be able to penetrate the cell membrane and both have electron-withdrawing halogen atoms in their phosphate-esterifying groups. This would hopefully confer lability on the phosphate ester bonds and that therefore these bonds might be metabolically unstable and would hydrolyse intracellularly. If this was to occur both **101** and **102** could directly yield the 5′-monophosphate of **1**, which could then be converted to the 5′-triphosphate of **1** by cellular kinases. Also, cleavage of a single phosphate bond, to yield a diester, followed by phosphodiesterase hydrolysis of that diester, could yield the 5′-monophosphate too. Indeed it would be of interest to prepare some 5′-bis(2,2,2-trihaloethyl) phosphate derivatives of other nucleosides which (like **1**) are known to inhibit HIV.

The methodology used to prepare **101** and **102** was much like that used to prepare **91**, **92** and **93**. For **101**, bis(2,2,2-trifluoroethyl) phosphorochloridate (**103**)[164] was prepared first and this was followed by its reaction with **1**. Rather that being prepared, commercial bis(2,2,2-trichloroethyl) phosphorochloridate (**104**)[165] was reacted with **1**. Both **101** and **102** possess electron-withdrawing halogen atoms at the ß-carbon of the phosphate-esterifying group. Substitution at the α-carbon may have conferred the greatest leaving group ability on the phosphate ester. However this would necessitate, by our standard procedure for the preparation of dialkyl phosphorochloridates, the use of alcohols of the type CX_3OH, which are not stable. It was therefore decided to prepare ß-halo derivatives because of their greater ease of synthesis, despite the fact that the leaving group propensity of the esterifying group may not be as enhanced.

Diagram 39



56, R=H
67, R=F



100

Diagram 40

$$POCl_3 + 2\,CF_3CH_2OH \xrightarrow[-40^{\circ}C]{2\,Et_3N,\ Et_2O} (CF_3CH_2O)_2POCl$$

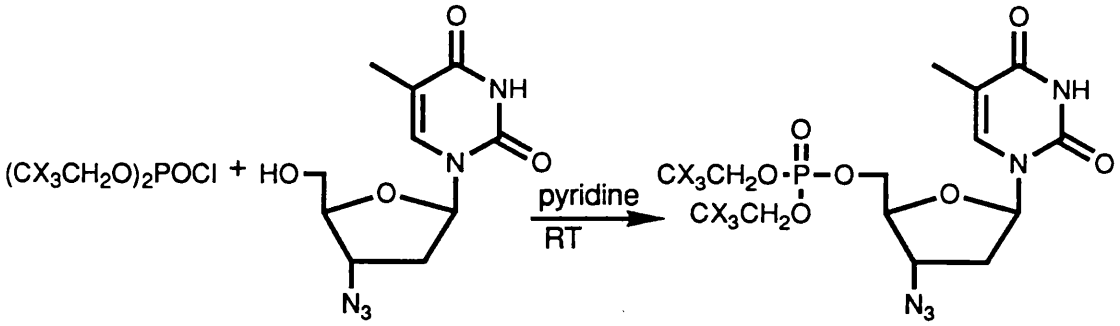
103

The reaction of slightly over two equivalents of both 2,2,2-trifluoroethanol and triethylamine with phosphoryl chloride at -40°C in diethyl ether yielded crude **103**, which was isolated in 65% yield by vacuum distillation (diagram 40). A ^{31}P nmr spectrum of **103** displayed one resonance only, at δ5.61. This resonance occurred within the appropriate region for a dialkyl phosphorochloridate, but interestingly has a slightly higher chemical shift than that reported for diethyl phosphorochloridate **71**. The ^{13}C nmr spectrum of **103** consisted of two quartet of doublets at δ122.05 and δ64.72, the former signal results from $\underline{C}F_3CH_2OP$, the latter from $CF_3\underline{C}H_2OP$. Both these carbons are able to couple to the fluorine atoms and to the phosphorus atom, which accounts for both signals observed multiplicities. For **103** the one bond carbon-fluorine coupling constant was relatively large at 273.7 Hz. The value of the two-bond carbon-fluorine coupling constant measured from the $CF_3\underline{C}H_2OP$ resonance of **103** was smaller at 39.0 Hz. The ^{1}H nmr spectrum of **103** consisted of one resonance, a complex multiplet at δ4.51.

After its successful preparation, **103** was allowed to react with **1** in pyridine at room temperature, in a preparation that was quite analogous to the preparation of **91** (diagram 41). By studying the reaction mixture at intervals by tlc, it became clear that this reaction was proceeding at an appreciably faster rate than did the reaction of **71** with **1**. Indeed, after stirring for 4 h, tlc indicated that the starting material **1** had reacted completely and had yielded a product which was more lipophilic than **1** itself. The greater reactivity between **103** and **1**, relative to that of **71** with **1**, may have been caused by the negative inductive-effect of the two 2,2,2-trifluoroethyl groups on the phosphorus atom causing the phosphorus atom to be made more susceptible to nucleophilic attack by the 5′-OH group of **1**.

After quenching the reaction of **103** with **1** and purification by column chromatography, **1** was obtained a white solid in 63% yield. It was interesting that **101** was obtained as a solid, whereas the simple dialkyl phosphate derivatives **91, 92** and **93** had all been obtained as glassy-oils. The ^{31}P nmr spectrum of **101** revealed a single

Diagram 41

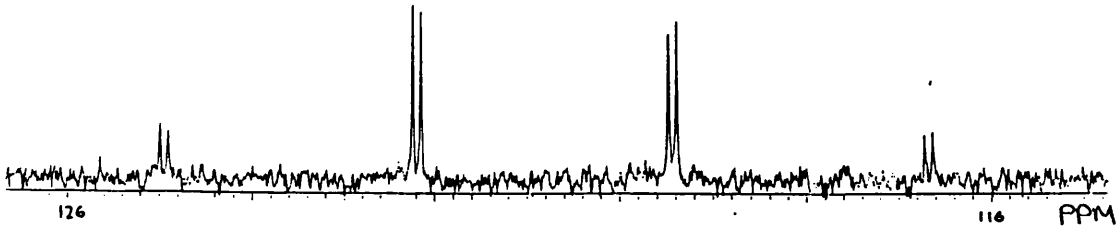


103, X=F
104, X=Cl

1

101, X=F
102, X=Cl

Diagram 42



The $\underline{C}F_3CH_2OP$ resonance of **101**.

resonance of δ-4.85. Although this value is close to the chemical shift of the resonance observed in the ^{31}P nmr spectrum of **101**, it was upfield by around δ2. The ^{13}C nmr of **101** displayed the $\underline{C}F_3CH_2OP$ and $CF_3\underline{C}H_2OP$ resonances as quartet of doublets. The $\underline{C}F_3CH_2OP$ resonance occurred at δ121.10, again displaying coupling to the three equivalent fluorine atoms and to the phosphorus atom (with coupling constants of 277.6 Hz and 8.3 Hz respectively). The $CF_3\underline{C}H_2OP$ resonated at a chemical shift of δ64.14 and also revealed coupling to the three equivalent fluorine atoms and to the phosphorus atom (with coupling constants of 38.2 Hz and 4.1 Hz respectively). The $\underline{C}F_3CH_2OP$ signal of **101** is shown in diagram 42. If one considers the phosphorus-carbon coupling constants of both these signals, it is of interest to note that the three-bond coupling constant is twice the magnitude of the two-bond coupling constant. The C5′ signal of **101** has a chemical shift slightly downfield of the C5′ signal of **91**, this difference being brought about by the 2,2,2-trifluoroethyl groups.

In the ^{1}H nmr spectrum of **101**, the resonance due to the CF_3CH_2OP protons was seen as a quintet. The explanation for this lies in the fact that these methylene protons couple to the three equivalent fluorine atoms and to the phosphorus atom as well. The magnitude of these proton-fluorine and proton-phosphorus couplings is roughly the same, which leads to the observation of a quintet. In addition to the nmr spectral data, a mass spectrum of **101** revealed a molecular ion on which it was possible to obtain a satisfactory accurate mass measurement. **101** was also analysed by hplc, which revealed it to be over 99% pure and also showed there was no trace of any unreacted **1**. If any **1** were present in a sample of **101** tested against HIV, the results of this test would be seriously affected.

The reaction of commercial bis(2,2,2-trichloroethyl) phosphorochloridate (**104**) with **1** was carried out and this too proceeded at an appreciably faster rate than did the reaction of **71** with **1**. After column chromatography a white solid, which was characterised as **102** was obtained in 59% yield (diagram 41). A ^{31}P nmr spectrum of **102** revealed a single resonance and like **101** this resonance occurred at a chemical shift upfield of the position

of the resonance of **91**, presumably this difference is brought about by the influence of the 2,2,2-trichloroethyl groups on the phosphorus atom. A ^{13}C nmr spectrum of **102** in deuterochloroform revealed that the $\underline{C}Cl_3CH_2OP$ resonance, downfield at δ94.38, was observed as a doublet. This was due to phosphorus-carbon coupling, but the magnitude of the coupling constant displayed by this signal (10.7 Hz) was much greater than the coupling constant displayed by the $\underline{C}H_3CH_2OP$ resonance in the ^{13}C nmr spectrum of **91**. The $CCl_3\underline{C}H_2OP$ resonance could not be observed clearly in this spectrum, as it resonated at almost the same chemical shift as the solvent, deuterochloroform. In order to overcome this problem a ^{13}C nmr spectrum of **102** was obtained in d-methanol and in this spectrum the $CCl_3\underline{C}H_2OP$ resonance was displayed as a doublet with a relatively small coupling constant of 4.1 Hz. Otherwise the two spectra of **102** obtained in deuterochloroform and d-methanol were quite similar.

As may be expected, the ^1H nmr spectrum of **102** was very similar to the spectrum of **101**. The CCl_3CH_2OP signal was seen as two doublets almost coincident with each other. This may be explained by this signal being generated by two non-equivalent methylene groups, whose protons resonate at slightly different chemical shifts. In addition, both methylene groups experience proton-phosphorus coupling.

A mass spectrum of **102** was obtained by the technique of FABMS and this showed a number of interesting features. The spectrum itself was obtained by dissolving a sample in nitrobenzyl alcohol and this gave rise to protonated molecular ion species being observed rather than the molecular ion itself. In addition a number of signals were seen in the molecular ion region of **102** and this arises out of the fact that chlorine consists of two major isotopes, ^{35}Cl and ^{37}Cl, in the ratio of approximately 3:1. Molecular ions and indeed fragment ions containing chlorine atoms give rise to a number of signals. Indeed the theoretical pattern of these signals may be readily calculated from the theoretical abundance of isotopes and this may be compared to the pattern observed in the spectrum itself. The isotope pattern observed in the molecular ion region of **102** was quite similar to the pattern

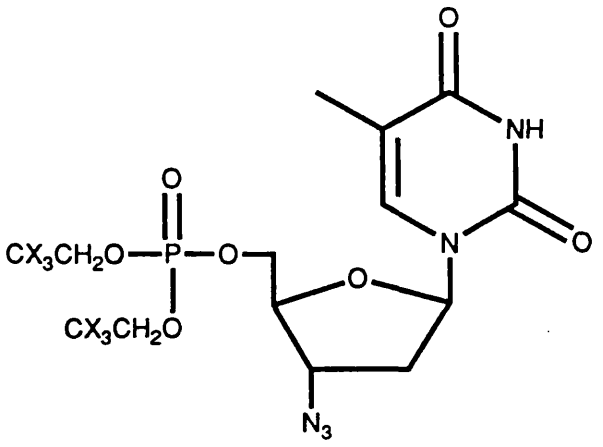
calculated for an ion containing six chlorine atoms.

It should be noted that "satellite-peaks" were observed one mass unit higher than the main peaks. This may be due to the fact that the isotope ^{13}C, which has a natural abundance of about 1%, can give rise to peaks at one mass unit higher than the main peaks in a mass spectrum. These "satellite peaks" may also be due to doubly protonated molecular ion species. The major fragmentation ions included one at m/e 250 arising from the loss of $(CCl_3CH_2O)_2PO_2H$ from the protonated molecular ion and another at m/e 207 arising from the loss of both $(CCl_3CH_2O)_2PO_2H$ and N_3H from the protonated molecular ion. Analysis of a sample of **102** by hplc revealed it to be greater than 99% pure and in addition, like **101**, it contained no unreacted **1**.

101 and **102** were tested against HIV in C8166 cells by T.J. O'Connor and D. Kinchington. The results of these tests are displayed in diagram 43. It can be seen that **101** and **102** are able to inhibit HIV *in vitro*. Under the conditions of this assay **1** inhibited viral proliferation by 50% at a concentration of 0.03 μM. **101** and **102** displayed no cytotoxicity to uninfected cells. The activity against HIV shown by **101** and **102** is in marked contrast to the lack of activity displayed by the simple phosphate triesters **91, 92** and **93**. In order to act both **101** and **102** presumably permeate the cell membrane where both are then capable of being hydrolysed intracellularly. This metabolism may yield the corresponding 5′-monophosphate of **1**, which may then be converted into the corresponding active 5′-triphosphate. It is however possible that intracellular hydrolysis could yield the parent nucleoside **1**, which could also be phosphorylated to the 5′-triphosphate.

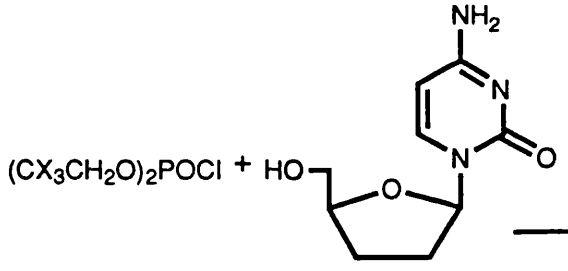
Since **101** and **102** were strong inhibitors of HIV *in vitro*, it was of interest to prepare 2′,3′-dideoxycytidine 5′-bis(2,2,2-trifluoroethyl) phosphate (**105**) and 2′,3′-dideoxycytidine 5′-bis(2,2,2-trichloroethyl) phosphate (**106**). Thus **103** was added to **2** at -20°C (diagram 44). Analysis of the reaction mixture by tlc indicated that two compounds had been generated which were more lipophilic than the starting material **2**. This had also been the case when **73** was reacted with **2** and it had been concluded that phosphorylation

Diagram 43



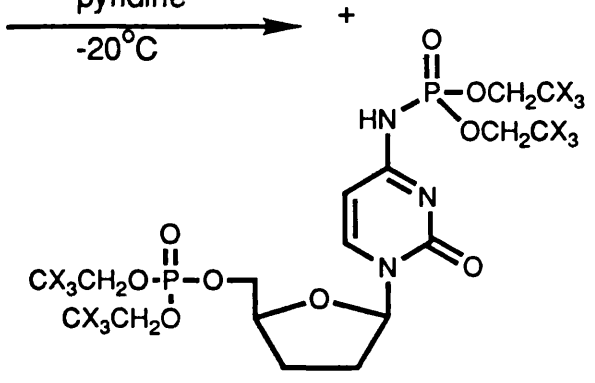
		IC_{50} (μM)
101	X=F	0.5
102	X=Cl	0.8

Diagram 44

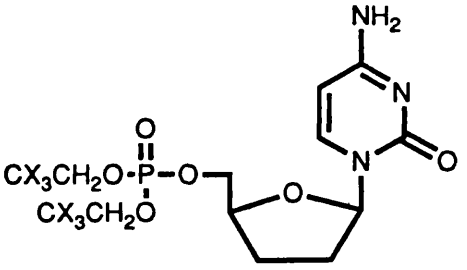


103, X=F
104, X=Cl

2



107, X=F
108, X=Cl



105, X=F
106, X=Cl

of the -NH_2 group of the cytosine base had occurred as well as phosphorylation at the 5′-OH group. However, it was possible to isolate only **99** from this reaction. However in the case of the reaction of **103** with **2**, it was possible to isolate two compounds. The spectral data gathered on both compounds suggests that they are **105** and 2′,3′-dideoxycytidine N4,O5′-di[bis(2,2,2-trifluoroethyl)] phosphate (**107**).

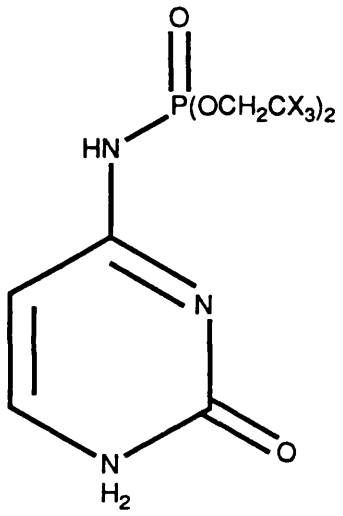
Taking **105** first, its ^{31}P nmr spectrum displayed a single resonance at δ-5.05. In the ^{13}C nmr spectrum of **105** the resonances due to the carbon atoms of the cytosine base had very similar chemical shifts to those of **99**. This suggests that in this molecule there had been no modification of the cytosine base. Both the C4′ and C5′ resonances displayed phosphorus-carbon coupling suggesting that the molecule contained a phosphorus containing moiety at the 5′-position. The $\underline{C}F_3CH_2OP$ and $CF_3\underline{C}H_2OP$ resonances were also seen in the ^{13}C nmr spectrum of **105** and again these resonances were observed as quartet of doublets. The ^1H nmr spectrum of **105** was consistent with its successful isolation. The resonance of the CF_3CH_2OP protons occurred at the same chemical shift as the H4′ resonance and taken together these resonances integrated for a total of five protons. This suggests that **105** contains two 2,2,2-trifluoroethyl groups. The FABMS of **105** displayed a signal at m/e 456, due to its protonated molecular ion.

The assignment of **107** being the second compound isolated from the reaction of **103** with **2** was made in the following way. Its ^{31}P nmr spectrum displayed two signals, with approximately equal integrals at δ2.59 and δ-4.81. This would suggest that this compound contains phosphorus atoms in two different environments. The signal at δ-4.81 may well be due to the phosphorus atom at the 5′-position of **2** as its chemical shift is much like that of **105**. The other resonance at δ2.59 may possibly be due to a phosphorus atom linked to the 4-amino group of the cytosine base. Further to this, it has been reported that $OP(NHC_6H_5)(OCH_2C_6H_5)_2$ gives rise to a resonance at δ3.8 in its ^{31}P nmr spectrum[166]. The ^{13}C nmr spectrum of **107** displays a number of signals due to the carbon atoms of the cytosine base whose chemical shifts are different from the analogous chemical shifts

encountered for **99** or **105**. This may indicate a modification of the cytosine base has occurred and that the NH_2 group of the cytosine base may have been phosphorylated. Further evidence for this occurrence lies in the fact that three of the cytosine base's signals were doublets, due to phosphorus-carbon coupling. There is a marked similarity between the chemical shifts of the resonances of the sugar moiety of **107** with those encountered for **105.** The $\underline{C}F_3CH_2OP$ and $CF_3\underline{C}H_2OP$ resonances are seen in the region of δ122 and δ63 respectively. However two quartet of doublets were in each region. This provides evidence that there are two distinct $(CF_3CH_2O)_2PO$ moieties in a molecule of **107**. The ^1H nmr spectra of **107** provides evidence for it containing a phosphorylated cytosine base. The H6 resonance was seen as a doublet of doublets, due to coupling with the adjacent H5 proton and to the phosphorus atom. Interestingly though the H5 resonance does not show any evidence of phosphorus coupling. The ^1H nmr spectrum of **107** does display a multiplet at δ4.31 which is comprised of the H4′, H5′ and CF_3CH_2OP signals. This multiplet was found to integrate for eleven protons in total which suggests that there are four 2,2,2-trifluoroethyl groups in the molecule, that is that **107** is the product of **2** reacting with two molecules of **103.** Using FABMS, **107** displayed a signal at m/e 700 due to the protonated molecular ion. In addition a signal at m/e 356 was observed and this may be due to the ion represented in diagram 45, which offers further evidence for the presence of a phosphorylated cytosine base being present in the structure of **107**.

Similarly the reaction of **2** with **104** led to the isolation of two products, 2′,3′-dideoxycytidine 5′-bis(2,2,2-trichloroethyl) phosphate (**106**) and 2′,3′-dideoxycytidine N4,O5′-di[bis(2,2,2-trichloroethyl)] phosphate (**108**) (diagram 44). The reaction was carried out in much the same way as the reaction of **2** with **103**, except that a reduction was made in the number of equivalents of phosphorochloridate used. **106** gave rise to one resonance in its ^{31}P nmr spectrum and its ^{13}C nmr spectrum bore a number of similarities to that of **105.** In a ^{13}C nmr spectrum, obtained in d-methanol, the $\underline{C}Cl_3CH_2OP$ signal was seen as a doublet and again displayed a relatively high three-bond coupling constant of 10.1 Hz. The

Diagram 45

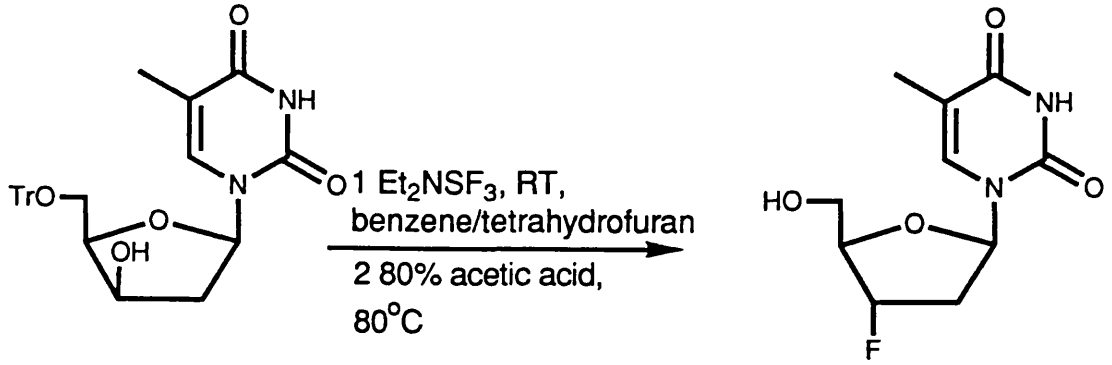


+

In the mass spectrum of **107**, one ion was seen at m/e 356 (X=F).

In the mass spectrum of **108**, a number of ions were seen (as a result of chlorine consisting of two isotopes) at around m/e 454 (X=Cl).

Diagram 46



111

110

Tr=Ph_3C-

$CCl_3\underline{C}H_2OP$ signal by contrast displayed a coupling constant of 4.8 Hz. The ^1H nmr spectrum of **106** showed many of the features of the ^1H nmr spectrum of **105**. The CCl_3CH_2OP resonance was observed as two doublets integrating for four protons in total.

Further evidence for the isolation of **106** came from the technique of FABMS. Again the protonated molecular ions of **106** were observed. Moreover these protonated molecular ions gave an isotope pattern which was consistent with the ions forming them containing six chlorine atoms. A group of signals were observed in the mass spectrum around m/e 443, which were due to the loss of cytosine from the protonated molecular ions. As would be expected, these ions gave rise to a pattern of signals which was consistent with the ions forming them containing six chlorine atoms too. The base peak of the mass spectrum of **106** occurred at m/e 112 and was due to protonated cytosine ions.

The identification of **108** being the second of the isolated compounds from the reaction of **2** with **104** was made in the following way. Its ^{31}P nmr spectrum, like **107**, consisted of two peaks with roughly equal integrals. As may be expected, the ^{13}C nmr and ^1H nmr spectra of both **108** and **107** possessed a number of similarities too. Evidence was found from the ^{13}C nmr spectrum that **108** contained trichloroethyl groups in two different environments. There were two $\underline{C}Cl_3CH_2OP$ resonances observed, both doublets, at δ95.12 and δ94.43. The coupling constants shown by these two doublets were relatively high (11.3 Hz and 10.9 Hz respectively). The ^1H nmr spectrum of **108** confirmed the presence of two types of CCl_3CH_2OP resonance. Two such resonances were observed at δ4.60 and δ4.55 and both integrated for four protons each. The former resonance was a multiplet and the latter a doublet. The mass spectrum of **108** contained a group of molecular ion signals displaying an isotopic pattern which was consistent with these molecular ions containing twelve chlorine atoms. Also observed in the mass spectrum of **108** were a group of signals around m/e 454 whose isotopic pattern was consistent with the ions forming them containing six chlorine atoms. The ions that may give rise to these latter signals are shown in diagram 45.

The next phosphate triester to be synthesised was 3′-fluoro-3′-deoxythymidine 5′-

bis(2,2,2-trichloroethyl) phosphate (**109**), a derivative of 3′-fluoro-3′-deoxythymidine (**110**). **110** itself is known to have a significant inhibitory effect against HIV *in vitro*[101]. The method chosen to obtain **109** was to first synthesise **110** and to follow this by the reaction of **110** with **104**. The synthesis of **110** followed the method of Herdewijn *et al*[101] with some minor modifications and utilised the known fluorinating agent diethylaminosulphur trifluoride[167]. Thus nearly four equivalents of diethylaminosulphur trifluoride were added to 1-(2-deoxy-5-O-trityl-ß-D-threopentofuranosyl)thymine (**111**) in benzene-tetrahydrofuran solution at ambient temperature (diagram 46). A slightly higher proportion of tetrahydrofuran was used in the solvent mixture than by Herdewijn *et al.* This was in order to allow the nucleoside to dissolve completely. After stirring at this temperature for 1.5 h, analysis of the reaction mixture by tlc revealed that no starting material remained and that one major component had been generated. The reaction mixture was then poured into sodium bicarbonate solution and extracted with ethyl acetate. The organic phase was then dried and the solvent was removed under reduced pressure.

The resulting residue was detritylated. Herdewijn *et al* noted some decomposition (possibly glycosidic cleavage) during their detritylation procedure which involved heating to 100°C for 15 min in 80% acetic acid. For this reason detritylation was carried out here at 80°C for 15 min in 80% acetic acid. Even so, after detritylation and removal of the solvent under reduced pressure, analysis of the resulting residue by tlc revealed the presence of two major components, one of which was thymine. A significant amount of glycosidic bond cleavage seemed to occur during detritylation. Purification of the resulting residue by column chromatography did yield **110**, but in low yield.

It would appear that the conditions used for detritylation were still too harsh. Unfortunately, this reaction could not be repeated through lack of time. Had it been, milder detritylation conditions would have been employed in order to minimise glycosidic cleavage and maximise the yield of the required product. Also, a monomethoxytrityl protecting group could be used, as milder acidic conditions are needed for this group's removal[168]. A small

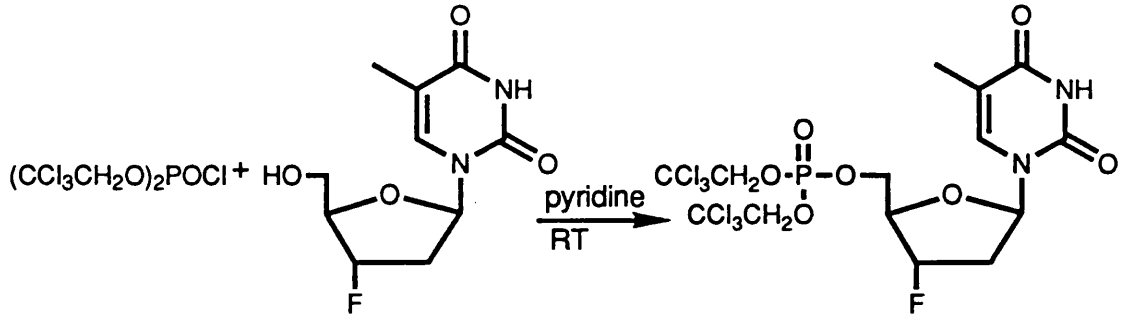
amount of **110** was isolated though for further synthesis.

The melting point and spectroscopic data obtained on **110** was almost identical to that reported in the literature[101]. The ^{13}C nmr spectrum of **110** displays the C3′ resonance well downfield at δ95.06. This chemical shift is approximately 25 ppm downfield of the chemical shift of the C3′ resonance of **74**. The C3′ resonance of **110** was a doublet, due to carbon-fluorine coupling and had a coupling constant of 175.8 Hz. Indeed carbon-fluorine coupling caused the C2′, C3′, C4′ and C5′ resonances (but not C1′) to be displayed as doublets in the ^{13}C nmr spectrum of **110**. The ^{1}H nmr spectrum of **110** revealed the H3′ resonance, downfield at δ5.28, as a doublet of multiplets. The H4′ resonance was a doublet of multiplets too. From the mass spectrum of **110** (which is hitherto unreported in the literature) it was possible to obtain a satisfactory accurate mass measurement on the molecular ion.

Having isolated a small amount of **110**, its reaction with **104** was investigated. From this reaction the intended product **109** was obtained in 59% yield (diagram 47). ^{31}P nmr spectroscopy revealed that **109** gave rise to one signal in its spectrum, at a chemical shift of δ-5.65. **109** gave a ^{13}C nmr spectrum in which the carbon-fluorine coupling constants of the C2′, C3′, C4′ and C5′ resonances were almost identical to those displayed by the analogous resonances of **110**. Both the C4′ and C5′ resonances were actually observed as doublet of doublets, both are able to couple to the phosphorus atom as well. The ^{1}H nmr spectrum of **109** showed the H3′ resonance as a doublet of multiplets. The CCl_3CH_2OP resonance, integrating for four protons, was seen as a multiplet. FABMS revealed that the isotope pattern of the molecular ions of **109** was very close to that calculated for an ion containing six chlorine atoms. FABMS also revealed fragment ions in the mass spectrum at m/e 227 (due to MH^+-$(CCl_3CH_2O)_2PO_2H$), m/e 127 (due to thymineH^+) and at m/e 81 (due to $C_5H_5O^+$).

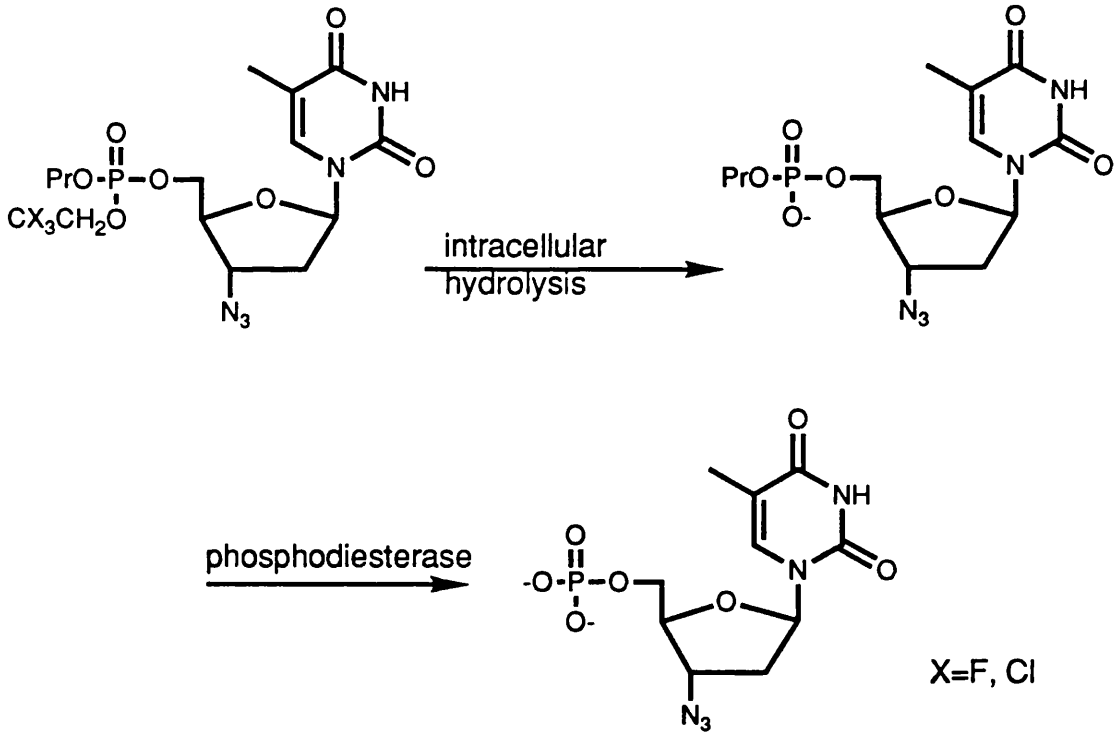
Simple 5′-dialkyl phosphates of **1**, for example 3′-azido-3′-deoxythymidine 5′-dipropyl phosphate (**92**), have been found in this study to be inactive against HIV *in vitro*.

Diagram 47



104 **110** **109**

Diagram 48



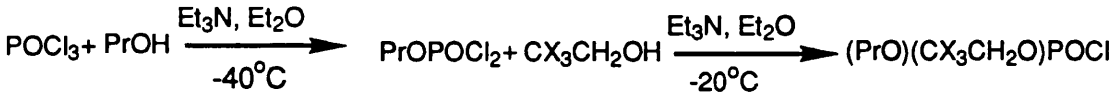
However, some 5′-bis(2,2,2-trihaloethyl) phosphate derivatives of **1** are active against HIV. This may be because 2,2,2-trihaloethyl moieties are more labile than the simple alkyl ones. With this in mind some mixed 5′-(alkyl 2,2,2-trihaloethyl) phosphate derivatives of **1** were prepared. If such derivatives were able to penetrate the cell membrane and the 2,2,2-trihaloethyl moiety hydrolyse, the resulting diester could be a substrate for phosphodiesterase enzymes and could be metabolised to the 5′-monophosphate of **1** (diagram 48). Thus it was decided to synthesise 3′-azido-3′-deoxythymidine 5′-(propyl 2,2,2-trifluoroethyl) phosphate (**112**) and 3′-azido-3′-deoxythymidine 5′-(propyl 2,2,2-trichloroethyl) phosphate (**113**).

The strategy used to synthesise **112** and **113** was as follows. Firstly propyl phosphorodichloridate[169] (**114**) was prepared and this was reacted separately with 2,2,2-trifluoroethanol and 2,2,2-trichloroethanol to yield propyl 2,2,2-trifluoroethyl phosphorochloridate (**115**) and propyl 2,2,2-trichloroethyl phosphorochloridate (**116**) respectively. Both **115** and **116** were reacted separately with **1** to yield the required compounds, **112** and **113** respectively.

114 was prepared by the addition of 1-propanol and the base triethylamine to phosphoryl chloride in diethyl ether (diagram 49). This procedure was much the same as that used to prepare dipropyl phosphorochloridate (**72**) earlier, except that only one equivalent of both 1-propanol and triethylamine were added. In order to maximise the formation of **114** at the expense of any possible formation of **72** the reaction was carried out at -40°C. After addition of 1-propanol and triethylamine to phosphoryl chloride, the reaction mixture was allowed to warm to ambient temperature, where it was stirred for a further five hours. Once again, as the reaction proceeded the by-product triethylamine hydrochloride precipitated out. **114** was isolated after vacuum distillation in 86% yield. A ^{31}P nmr spectrum of **114**, which contained a single resonance at δ5.82, was obtained. This chemical shift is close to values reported for other alkyl phosphorodichloridates[170].

A solution of 2,2,2-trifluoroethanol and triethylamine was then added to a solution of **114**, again using diethyl ether as the solvent (diagram 49). This time the reaction was

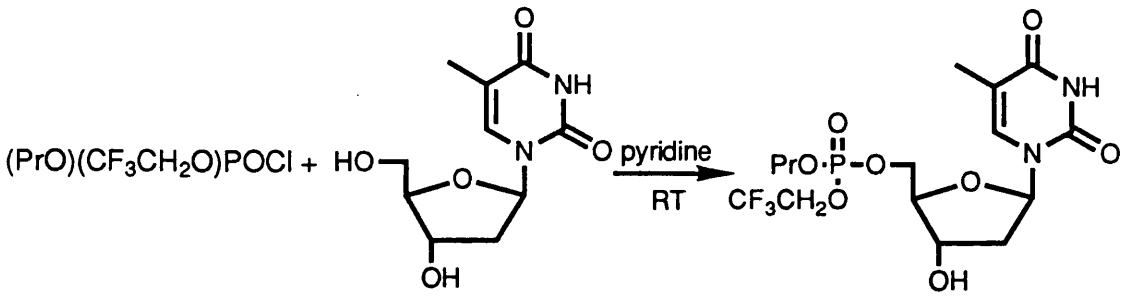
Diagram 49



114

115, X=F
116, X=Cl

Diagram 50



115 **74** **117**

carried out at -20°C. After allowing the reaction mixture to warm to ambient temperature and stirring for 17 hours, the reaction mixture was filtered, the solvent removed under reduced pressure and the residue was purified by vacuum distillation. **115** was obtained as a colourless liquid in 61% yield.

The ^{31}P nmr spectrum of **115** consisted of one peak at δ3.10. This value is close to the chemical shift of the resonance displayed by **72** in its ^{31}P nmr spectrum. The ^{13}C nmr spectrum of **115** displayed two quartet of doublets at δ121.73 and δ63.47 which were the $\underline{C}F_3CH_2OP$ and $CF_3\underline{C}H_2OP$ resonances respectively. This spectrum also displayed the three resonances of the propyl group at δ72.17, δ23.04 and δ9.56. The ^1H nmr spectrum confirmed the successful isolation of **115**. Two multiplets were observed in this spectrum at δ4.46 and δ4.16, both signals integrating for two protons each. It seemed likely that these multiplets were due to the CF_3CH_2OP and $CH_3CH_2\underline{C}H_2OP$ resonances respectively. However in order to assign each multiplet correctly, the spectrum was irradiated at δ1.73 (the chemical shift of the $CH_3\underline{C}H_2CH_2OP$ protons). This led to the multiplet at δ4.16 collapsing to a doublet, from which it was concluded that this signal was the resonance of the $CH_3CH_2\underline{C}H_2OP$ protons and that therefore the multiplet at δ4.46 was the resonance of the CF_3CH_2OP protons.

Having isolated **115**, thymidine 5′-(propyl 2,2,2-trifluoroethyl) phosphate (**117**) was prepared from it. **117** itself would not be expected to possess any anti-HIV properties, but it would be a useful negative control in any future biological testing. Thus two equivalents of **115** were added to a solution of **74** in pyridine (diagram 50). Like the other reactions of the dialkyl phosphorochloridates with **74**, tlc revealed that one major component and two minor ones had been formed, all of which were more lipophilic than **74** itself. The major component was isolated by column chromatography and this was identified as **117**.

The ^{31}P nmr spectrum of **117** consisted of two closely spaced signals, at δ-2.94 and δ-3.04, of almost equal intensity. The reason for two signals being observed arises from the fact that **117** was isolated as a mixture of two diastereomers, which are depicted in diagram

Diagram 51

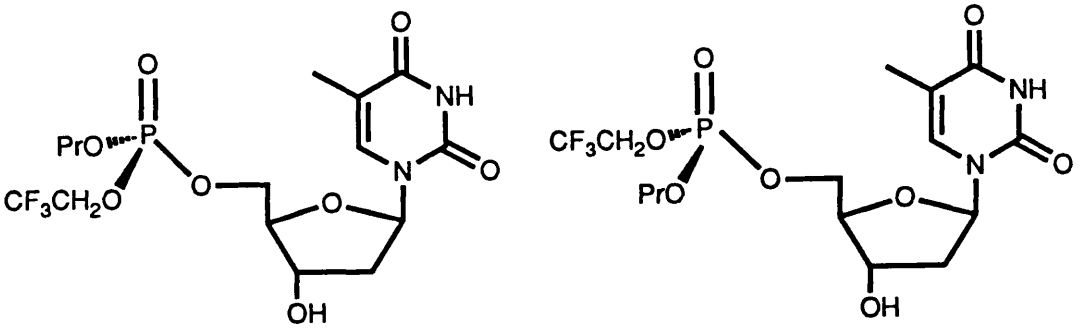
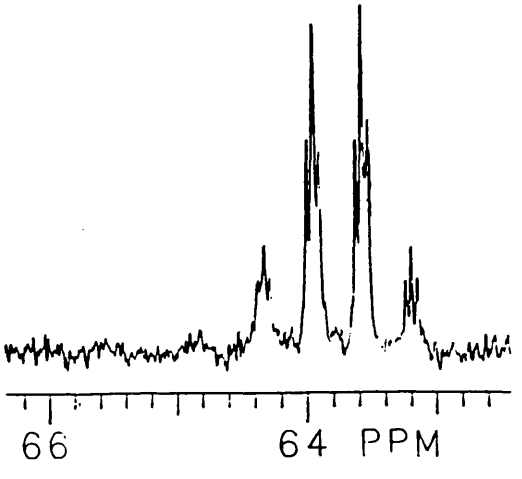


Diagram 52



The $CF_3\underline{C}H_2OP$ resonance of **117**.

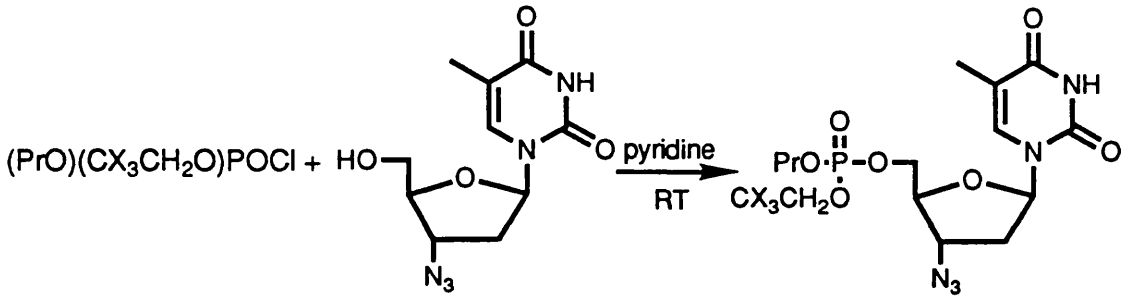
51. It would be expected that in a ^{31}P nmr spectrum, the diastereomers depicted in diagram 51 would resonate at slightly differing chemical shifts.

The ^{13}C nmr and ^{1}H nmr spectra of **117** confirmed its successful isolation and also showed that two diastereomers had been isolated. For example, in the ^{13}C nmr spectrum of **117** two C5 resonances were observed at δ111.47 and δ111.45. This was not necessarily repeated for all the resonances of **117**. Coincidence of some resonances (eg the C2 resonances) did occur. The $CF_3\underline{C}H_2OP$ resonance of **117**, in a spectrum obtained at 100 MHz, is shown in diagram 52. At first sight, this seems quite complex, an apparent quartet of triplets. It actually results from two overlapping quartet of doublets. Each diastereomer of **117** gave rise to one quartet of doublets. The ^{1}H nmr spectrum of **117** also revealed that a mixture of two diastereomers had been isolated. Two H6 resonances were observed and two sets of triplets were seen at δ0.96 and δ0.95, these were assigned to the $C\underline{H}_3CH_2CH_2OP$ resonances. EIMS of **117** gave a mass spectrum in which the molecular ion was observed at m/e 446 and a satisfactory accurate mass measurement was obtained on this ion. Interestingly an ion was observed at m/e 223 and this may be assigned to $(CF_3CH_2O)(PrO)P(OH)_2^+$.

The preparation of **112** was carried out using much the same methodology as that used to prepare **117**. Thus **1** was reacted with **115** in pyridine at ambient temperature (diagram 53). **112** was isolated from this reaction mixture, using column chromatography, in 47% yield. A ^{31}P nmr spectrum of **112** consisted of two signals, whose chemical shifts were very similar to the chemical shifts of the two signals observed in the ^{31}P nmr spectrum of **117**. Again the observation of two signals in the ^{31}P nmr spectrum of **112** suggests that **112** had been isolated as two diastereomers.

The ^{13}C nmr spectrum of **112** featured resonances due to the carbon atoms of the propyl and 2,2,2-trifluoroethyl moieties. The ^{1}H nmr spectrum featured the CF_3CH_2OP resonance (a multiplet) at δ4.35 and the $CH_3CH_2C\underline{H}_2OP$ resonance (a quartet) at δ4.03. The identity of the latter resonance was confirmed by carrying out irradiation at δ1.68 (the

Diagram 53

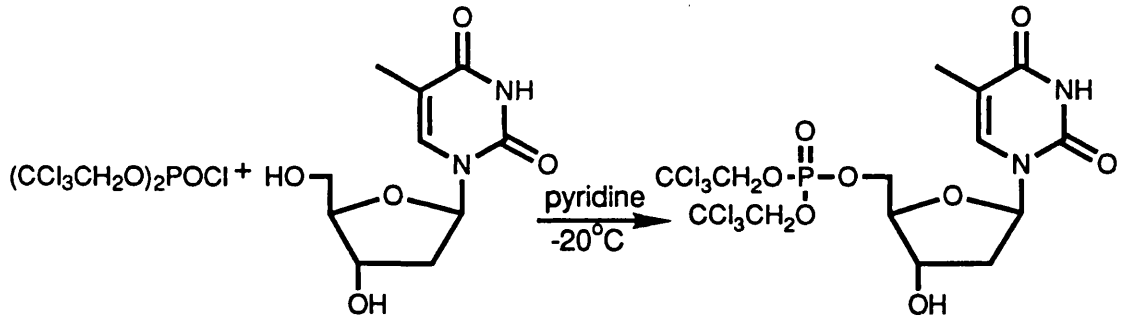


115, X=F
116, X=Cl

1

112, X=F
113, X=Cl

Diagram 54



104

74

122

chemical shift of the $CH_3C\underline{H}_2CH_2OP$ resonance). This caused the $CH_3CH_2C\underline{H}_2OP$ resonance to collapse to a doublet. A mass spectrum was obtained on **112** using the technique of EIMS. In this spectrum the molecular ion was not observed, but the protonated molecular ion was, at m/e 472. In addition, a signal was observed at m/e 223 (as there was in the mass spectrum obtained on **117**) and this signal was assigned to $(CF_3CH_2O)(PrO)P(OH)_2^+$.

Having successfully prepared **117** and **112**, **113** was prepared. In order to achieve this **116** was prepared first, by the addition of 2,2,2-trichloroethanol and triethylamine to **114** in diethyl ether, at -20°C (diagram 49). After addition, the reaction mixture was allowed to warm to ambient temperature and was stirred overnight. The desired product **116** was isolated from the reaction mixture by a completely analogous method to the isolation of **115**. The spectroscopic data amassed on **116** proved to be quite consistent with its proposed structure.

Two equivalents of **116** were then reacted with **1** in pyridine at room temperature (diagram 53). From this was isolated **113** by column chromatography. Like **117** and **112**, **113** was isolated as a mixture of two diastereomers. Evidence for this was gained from the ^{31}P nmr spectrum of **113** which displayed two signals, whose chemical shifts were slightly upfield of the chemical shifts of **112**. Many of the signals observed in the ^{13}C nmr spectrum of **113** indicated that **113** consisted of two diastereomers. For example, the C2, C6 and C3′ resonances all appeared as two distinct signals. The C5′ resonance of **113** was observed as a doublet at δ66.80. It is of interest to compare this value with the chemical shift of the C5′ resonance of **1**, which occurred at δ61.41. Once again, 5′-phosphorylation of a nucleoside results in the C5′ resonance being shifted downfield, this time by around 5 ppm.

The ^1H nmr spectrum of **113** was consistent with its proposed structure and clearly displayed the presence of both trichloroethyl and propyl moieties. The CCl_3CH_2OP resonance was observed as two closely spaced doublets. FABMS of **113** gave a mass spectrum which confirmed the structural assignment. The isotopic pattern of the molecular ions observed in this mass spectrum was consistent with **113** containing three chlorine atoms.

The preparations of some simple 5′-dialkyl phosphates of 3′-O-mesylthymidine, 3′-O-acetylthymidine and 3′-O-ethylthymidine were described earlier in this thesis. The parent nucleosides of these compounds have been found to be inactive against HIV[101]. Some simple 5′-dialkyl phosphates of 3′-azido-3′-deoxythymidine were inactive against HIV too, yet the 5′-bis(2,2,2-trichloroethyl) phosphate of 3′-azido-3′-deoxythymidine was found to be active. This may be explained by the trichloroethyl groups being more labile than simple alkyl ones and that the 5′-bis(2,2,2-trichloroethyl) phosphate of 3′-azido-3′-deoxythymidine may be able to yield the 5′-monophosphate intracellularly. It was of interest therefore to prepare some 5′-bis(2,2,2-trihaloethyl) phosphate derivatives of 3′-O-mesylthymidine, 3′-O-acetylthymidine and 3′-O-ethylthymidine. It was hoped that these compounds having penetrated the cell membrane would be able to yield their corresponding 5′-monophosphates and that these 5′-monophosphates would prove to be substrates for cellular kinases and could generate the corresponding 5′-triphosphates. The 5′-triphosphates which could then inhibit the viral enzyme RT. With this in mind the syntheses of 3′-O-mesylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**118**), 3′-O-acetylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**119**) and 3′-O-ethylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**120**) were undertaken. Also, it is known that 3′-amino-3′-deoxythymidine (**41**) is unable to inhibit retroviruses even though its 5′-triphosphate can inhibit purified RT[116]. This is presumably because of poor cellular phosphorylation of the parent nucleoside. With this in mind, the synthesis of 3′-amino-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**121**) was also attempted.

The preparation of 3′-O-mesylthymidine 5′-diethyl phosphate (**78**) which was described earlier in this thesis involved the preparation of thymidine 5′-diethyl phosphate (**75**) first, followed by the mesylation of **75** to give **78**. Similarly **118** was synthesised by preparing thymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**122**)[165] first, which was then mesylated to give **118**. The methodology used to prepare **122** here involved the reaction of **104** with **74** in pyridine at -20°C (diagram 54). After the stirred reaction mixture had been kept at this temperature for 1 hour, it was allowed to warm to ambient temperature

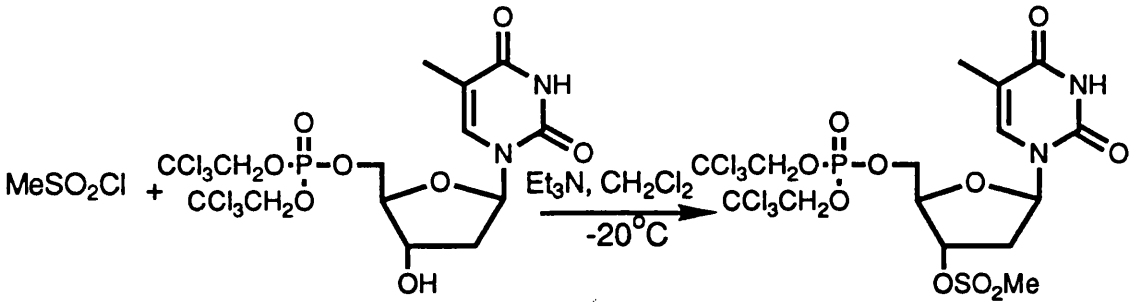
where it was stirred for a further 5 hours. Analysis of the reaction mixture by tlc revealed a major product and two minor products had been generated in the reaction mixture. The major product was isolated by column chromatography and was identified as **122**, the desired product.

The ^{31}P nmr spectrum of **122** consisted of one signal, with a chemical shift of δ-5.08. The ^{13}C nmr spectrum of **122** was instructive, especially in comparison with the known ^{13}C nmr spectrum of **74**[148]. It must be borne in mind though that these spectra were obtained in different solvents (**122** in d-methanol and **74** in deuterium oxide). Even so, the chemical shifts of the resonances of both the base and the sugar carbons of **122** were quite similar to the chemical shifts of the corresponding resonances of **74**, with one exception. That exception was the C5′ resonance. For **122**, the C5′ resonance occurred at δ69.21, for **74** the C5′ resonance is reported to occur at δ61.4. This provides strong evidence that **122** is the product of phosphorylation at the 5′-position of **74**. The ^{13}C nmr spectrum of **122** also displayed the $\underline{C}Cl_3CH_2OP$ and the $CCl_3\underline{C}H_2OP$ resonances, at δ95.10 and δ77.61 respectively. Both of these resonances were doublets.

The salient features of the ^{1}H nmr spectrum of **122** included the CCl_3CH_2OP resonance, a doublet and integrating for four protons, at δ4.58. Also a broad singlet integrating for one proton was observed at δ3.91 which was assigned to the 3′-OH proton. The technique of FABMS was used to obtain a mass spectrum of **122**. It was found that in order to obtain a mass spectrum, the sample had to be mixed with nitrobenzyl alcohol and sodium iodide before a spectrum was taken. This meant that instead of protonated molecular ion species being observed, MNa^+ ions were observed instead. In this way the observed isotope pattern of the molecular ions was found to be consistent with **122** containing six chlorine atoms.

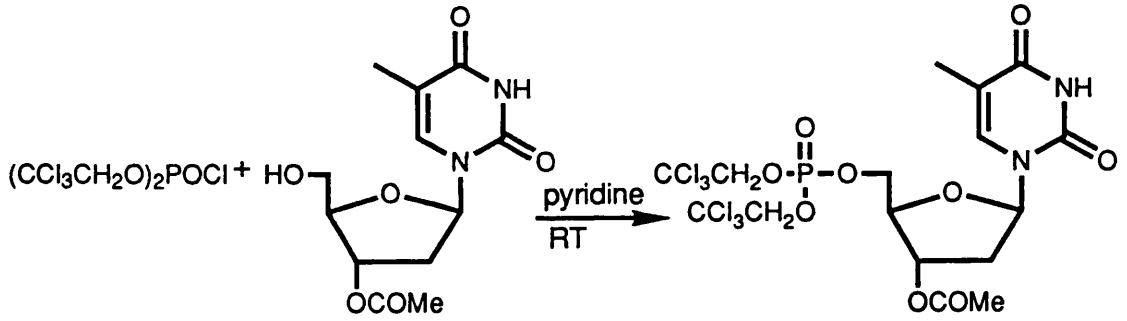
Having prepared **122**, it was reacted with mesyl chloride and the base triethylamine in dichloromethane at -20°C (diagram 55). The methodology used followed that of Crossland *et al*[151] and was used successfully in the mesylation of some thymidine 5′-dialkyl phosphates

Diagram 55



122 118

Diagram 56



104 81 119

discussed earlier in this thesis. A solution of mesyl chloride in dichloromethane was added to a solution of **122** and triethylamine also in dichloromethane. This addition was carried out at -20°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After the reaction mixture had been at ambient temperature for 30 min, analysis of the mixture by tlc revealed that one product had been generated and that no **122** remained in the reaction mixture. **118** was obtained as a white solid after aqueous-organic extraction. The bis(2,2,2-trichloroethyl) phosphate moiety was found to be stable to the conditions used during this mesylation procedure. Moreover **118** was isolated without recourse to chromatography.

The ^{31}P nmr spectrum of **118** consisted of one signal resonating at δ-4.80. Regarding the ^{13}C nmr spectrum of **118**, C3′ resonated at δ79.29. For **122**, the C3′ resonance occurred at δ70.89, thus mesylation of the 3′-OH of **122** has brought about a shift downfield of around 8 ppm in the chemical shift of the C3′ resonance. In the ^{1}H nmr spectrum of **118**, the H3′ resonance was observed downfield at δ5.36. The H1′ resonance of **118** was a doublet of doublets. The H1′ resonance of **122** was a triplet. This difference may reflect a variation in the conformation of the sugar moiety between **118** and **122**. Other features observed in the ^{1}H nmr spectrum of **118** include the CCl_3CH_2OP resonance, a doublet at δ4.65 and also a singlet at δ3.10, which was assigned to the mesyl group. The technique of FABMS was used to obtain a mass spectrum of **118** in which a number of protonated molecular ions could be seen. The isotope pattern of these ions was consistent with **118** containing six chlorine atoms. Also prominent in the mass spectrum of **118** were signals at m/e 303 (assigned to MH^+-$(CCl_3CH_2O)_2PO_2H$), m/e 207 (assigned to MH^+-$MeSO_3H$-$(CCl_3CH_2O)_2PO_2H$) and the base peak at m/e 81 (assigned to $C_5H_5O^+$). Finally, hplc showed that **118** had been obtained in a satisfactorily pure state.

3′-O-Acetylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**119**) was prepared by the reaction of **104** with 3′-O-acetylthymidine (**81**) in pyridine[171] (diagram 56). Analysis of the reaction mixture by tlc after 3 h indicated that no **81** remained and that only one

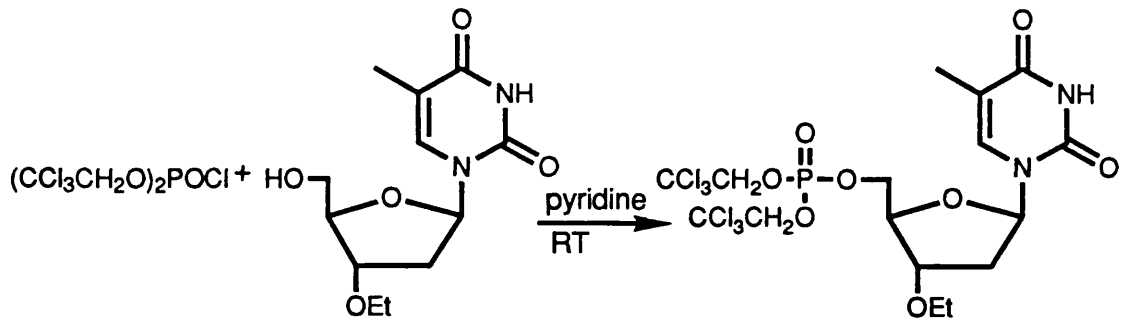
product had been generated, more lipophilic than **81**. This product was isolated as a white solid by column chromatography, using chloroform as an eluent. The white solid proved to be **119** and displayed a single resonance at δ-4.64 in its ^{31}P nmr spectrum. This chemical shift is very similar to the chemical shifts of the resonances that **122** and **118** displayed in their ^{31}P nmr spectra.

A ^{13}C nmr spectrum of **119** was obtained and not surprisingly there were a number of similarities between it and the ^{13}C nmr spectrum of the parent nucleoside **81**. The thymine base and the sugar resonances of **119** had very similar chemical shifts to the corresponding resonances of **81**, with the exception of the C5′ resonance which occurred at δ68.48 for **119** and δ62.56 for **81**. In addition to the thymine base and sugar resonances of **119**, a doublet was observed at δ94.38, which was assigned to the $\underline{C}Cl_3CH_2OP$ resonance. The $CCl_3\underline{C}H_2OP$ resonance was not observed in the ^{13}C nmr spectrum of **119** as this resonance coincided with the resonances of the solvent deuterochloroform.

The ^{1}H nmr spectrum of **119** displayed the H3′ resonance at δ5.36, much like the chemical shift of the H3′ resonance of **81**. Also observed in the ^{1}H nmr spectrum was a singlet integrating for three protons at δ2.08, which was assigned to the acetyl protons. A doublet integrating for four protons was observed at δ4.64 and this was assigned to the CCl_3CH_2OP resonance. Indeed **122**, **118** and **119** all display CCl_3CH_2OP resonances as doublets around δ4.6 in their ^{1}H nmr spectra. Once again a satisfactory mass spectrum of **119** (using the technique of FABMS) could only be obtained by dissolving a sample of **119** in nitrobenzyl alcohol with sodium iodide before obtaining the spectrum. This meant that (as described for the mass spectrum of **122**) the mass spectrum of **119** displayed MNa^+ molecular ion species rather than MH^+. Nevertheless the isotope pattern of these molecular ion species was consistent with **119** containing six chlorine atoms.

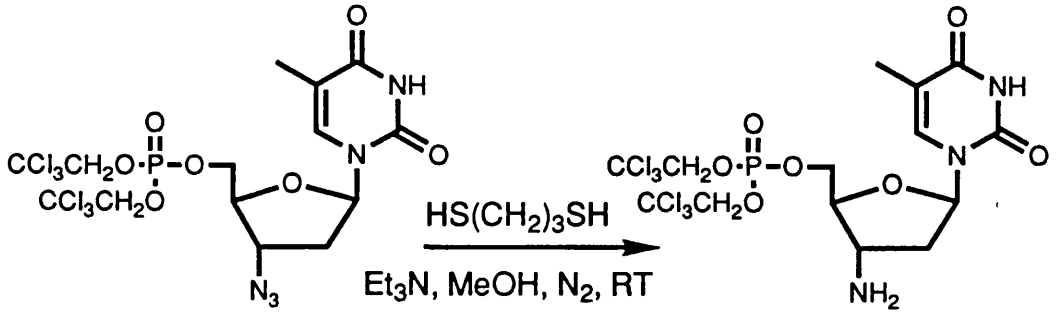
3′-O-Ethylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**120**) was prepared by the reaction of **104** with a solution of 3′-O-ethylthymidine (**85**) in pyridine at ambient temperature (diagram 57). After its isolation using column chromatography, **120** was obtained

Diagram 57



104 **85** **120**

Diagram 58



102 **121**

as a white solid in 76% yield. **120** gave rise to one resonance in its ^{31}P nmr spectrum at δ-4.82. The ^{13}C nmr spectrum obtained on **120** was consistent with its successful isolation. The C5′ resonance was identified at δ68.58, again downfield of the C5′ resonance of the parent nucleoside **85**. This spectrum was obtained in deuterochloroform and this resulted in the $CCl_3\underline{C}H_2OP$ resonance being obscured by the resonances of the solvent. The ^1H nmr spectrum of **120** was completely assigned and displayed the CCl_3CH_2OP resonance (a doublet at δ4.58) and the resonances of the ethyl group (a multiplet at δ3.44 and a triplet at δ1.18). In obtaining a mass spectrum of **120** it was necessary once again to dissolve the sample of **120** in nitrobenzyl alcohol with sodium iodide. The resulting spectrum was nonetheless satisfactory, featuring an isotopic pattern of the MNa^+ ions which was consistent with **120** containing six chlorine atoms. Again hplc showed that **120** had been isolated in a satisfactorily pure state.

3′-Amino-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**121**) was the next target molecule to be synthesised. **121** was prepared by the reduction of 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**102**). A number of agents are known to reduce azido functions to the corresponding amine. These reducing agents include Cr(II)/H^+[172] and H_2/Lindlar's catalyst[173]. It has been demonstrated that dithiols bring about the rapid reduction of azides to amines[174]. Dithiols have been found to reduce the azido group in the presence of a variety of other functionalities, including double bonds and phosphoramidates. With this in mind the reduction with dithiol of **102** to **121** was attempted using the methodology of Bayley *et al*[174], in the hope that the 5′-bis(2,2,2-trichloroethyl) phosphate group and the thymine base would be unaffected by these reductive conditions.

Thus five equivalents of both 1,3-propanedithiol and triethylamine were added to a solution of **102** in dry methanol at ambient temperature (diagram 58). The reaction was carried out in an inert atmosphere. Analysis of the reaction mixture by tlc after 17 hours, revealed that no **102** remained and that a component, less lipophilic than **102**, had been generated. The solvent was then removed under reduced pressure yielding a residue. The

residue contained unreacted 1,3-propanedithiol which was removed by dissolving the residue in chloroform, adding hexane and collecting the precipitate. The precipitate was purified by column chromatography. Elution was carried out with a chloroform-methanol-triethylamine mixture, the triethylamine being added in an attempt to eliminate the possibility of the amine becoming irreversibly bound to the silica during column chromatography. The product **121** was isolated but in disappointing yield. The reason for this is not entirely clear but it may be that the amine did become irreversibly bound to the silica. Unfortunately it was not possible to repeat this reaction through lack of time. Had it been possible, the isolation of **121** would have been attempted without recourse to column chromatography.

Although obtained in low yield, the compound isolated from the reduction of **102** was the desired product **121**. The ^{31}P nmr spectrum of **121** consisted of one resonance at δ-4.51. The ^{13}C nmr spectrum of **121** revealed that the C3′ resonance occurred at δ50.86. The C3′ resonance occurred at δ60.05 in the ^{13}C nmr spectrum of **102**, therefore reduction of the 3′-azido group to the 3′-amino group has led to the position of the C3′ resonance being shifted upfield by around 9 ppm. The C4′ and C5′ resonances of **121** were observed as doublets, due to phosphorus-carbon coupling. The ^{1}H nmr spectrum of **121** includes a broad singlet, integrating for two protons, at δ1.62. This was assigned to the 3′-amino protons. The CCl_3CH_2OP resonance was also observed, again as a doublet integrating for four protons, at δ4.64. Two multiplets, both integrating for one proton each, were observed at δ3.84 and δ3.66, which were assigned to the H4′ and H3′ resonances respectively. In order to make this assignment, irradiation was carried out at the chemical shift of the H2′ resonance (at δ2.66). The line-shape of the H3′ resonance at δ3.66 was affected by this. In the mass spectrum of **121** the isotope pattern of the observed protonated molecular ions was consistent with **121** containing six chlorine atoms.

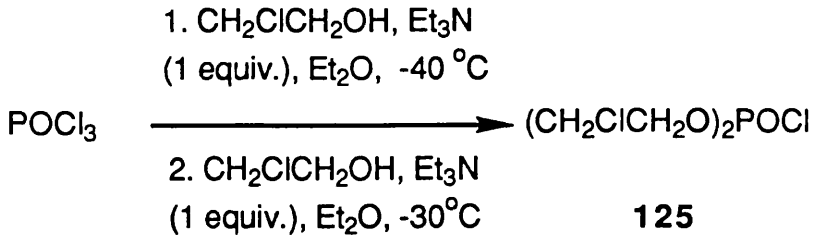
In view of the anti-HIV activity displayed by **102**, it was of interest to discover if anti-HIV activity would be maintained in analogous 2-chloroethyl and 2,2-dichloroethyl derivatives of 3′-azido-3′-deoxythymidine (**1**). With this in mind, 3′-azido-3′-deoxythymidine

5′-bis(2-chloroethyl) phosphate (**123**) and 3′-azido-3′-deoxythymidine 5′-bis(2,2-dichloroethyl) phosphate (**124**) were made the next targets for synthesis. The synthesis of these compounds was attempted by the reaction of the relevant phosphorochloridate with **1**. Bis(2-chloroethyl) phosphorochloridate (**125**) has been prepared by the reaction of chlorine with bis(2-chloroethyl) hydrogenphosphate[175]. Bis(2,2-dichloroethyl) phosphorochloridate (**126**) is not reported.

The preparation of **125** was far from straightforward. At first this preparation was undertaken using the same methodology that had been used to prepare the other dialkyl phosphorochloridates described in this thesis. Thus two equivalents of both 2-chloroethanol and triethylamine were added to a solution of phosphoryl chloride in diethyl ether. The isolation of **125** by vacuum distillation was attempted, as prior to distillation ^{31}P nmr spectroscopy indicated that in addition to **125**, the reaction mixture contained two impurities. However the isolation of **125** by vacuum distillation proved impossible. This may well be due to the boiling point of **125** being so high that it decomposed before it could be distilled over. The distillation of **125** proved impossible even when a high vacuum (0.05 mmHg) was used. Distillation was also impossible when a short-path distillation (Kugelrohr) apparatus was employed.

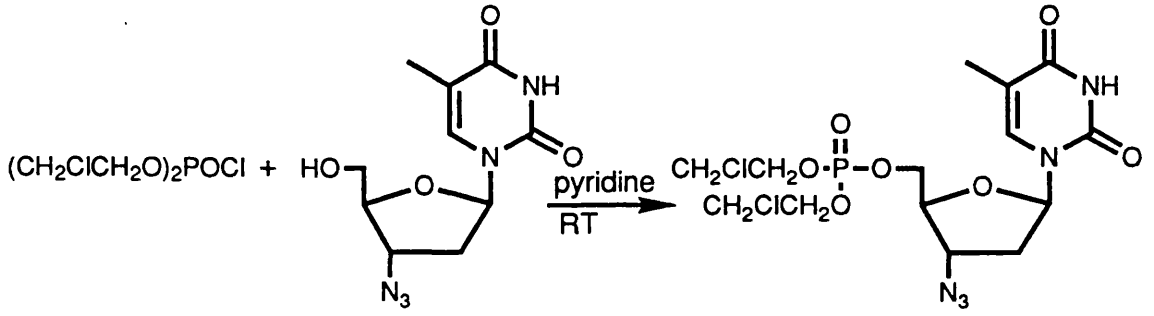
Thus in view of these difficulties, an attempt was made to prepare **125** without recourse to distillation, that is to carry out a preparation in which **125** would be prepared selectively (diagram 59). Thus one equivalent of both 2-chloroethanol and triethylamine were added to phosphoryl chloride in diethyl ether at -40°C. After this addition was complete the reaction mixture was allowed to warm slowly to ambient temperature over the course of 5 hours. Under these conditions, it was hoped that the starting material, phosphoryl chloride, would be completely and selectively converted into 2-chloroethyl phosphorodichloridate. Without isolating this compound from the reaction mixture, a further equivalent of both 2-chloroethanol and triethylamine were added *in situ*, this time at -30°C. The reaction mixture was then allowed to warm to 0°C and was stirred overnight at this

Diagram 59



125

Diagram 60



125 **1** **123**

temperature. After stirring overnight the reaction mixture was allowed to warm to ambient temperature where it was filtered and the solvent removed from the filtrate under reduced pressure to yield a pale-yellow liquid. A ^{31}P nmr spectrum of this pale-yellow liquid displayed a major signal at δ2.45 which seemed likely to be the resonance of **125**. Two minor phosphorus-containing impurities were also present as two other signals were observed at δ5.53 (possibly 2-chloroethyl phosphorodichloridate) and δ-4.05 (possibly tris(2-chloroethyl) phosphate). Despite the precautions taken in an attempt to prepare **125** selectively, it would appear that impurities were present in small amounts. The mixture was then subjected to a high vacuum for 4 hours after which time ^{31}P nmr spectroscopy revealed that the 2-chloroethyl phosphorodichloridate had been removed from the mixture as the resonance at δ2.45 was no longer present (it is known that 2-chloroethyl phosphorodichloridate has a much lower boiling point than **125** and tris(2-chloroethyl) phosphate[176]). The other resonance due to impurity at δ-4.05 remained. As tris(2-chloroethyl) phosphate would not be expected to react with **1**, this crude mixture could be used in further synthesis. Had time allowed it may have been possible to perfect the selective preparation of **125**.

Although not entirely pure, ^{13}C nmr and ^{1}H nmr data was obtained on the crude sample of **125**. The ^{13}C nmr spectrum consisted of two doublets at δ68.71 and δ41.67. The ^{1}H nmr spectrum of **125** consisted of a multiplet at δ4.27 (the $CH_2ClC\underline{H}_2OP$ resonance) and a triplet at δ3.62 (the $C\underline{H}_2ClCH_2OP$ resonance), along with some minor impurity signals.

A sample of **125** had been obtained, although it was apparently contaminated with tris(2-chloroethyl) phosphate. This crude sample of **125** was added to a solution of **1** in pyridine with stirring at ambient temperature (diagram 60). After quenching the reaction with water, removing the pyridine solvent under reduced pressure and purifying the resulting residue by column chromatography, a sample of **123** was obtained. A ^{31}P nmr spectrum of **123** consisted of one resonance at δ-3.34. The ^{13}C nmr spectrum of **123** was also recorded. The base and sugar resonances of **123** occurred at chemical shifts almost identical to the

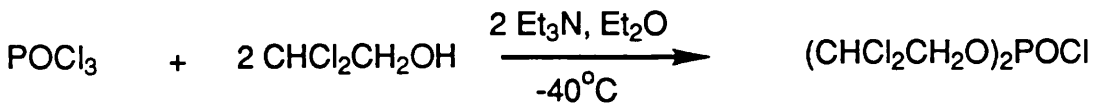
corresponding resonances of **102**. In addition, the resonances of the 2-chloroethyl moiety were observed. The CH_2ClCH_2OP resonance was seen as a doublet at δ67.76 and the CH_2ClCH_2OP resonance, also seen as a doublet, at δ42.53. The ^1H nmr spectrum of **123** also displayed the resonances of the 2-chloroethyl group. The CH_2ClCH_2OP resonance was a triplet at δ3.67 and the CH_2ClCH_2OP resonance was observed at δ4.29, in a multiplet integrating for seven protons, along with the H3′ and H5′ resonances.

Using the technique of FABMS, a satisfactory mass spectrum of **123** was obtained. In this mass spectrum, the observed isotopic pattern of the protonated molecular ions was consistent with **123** containing two chlorine atoms. Unlike the mass spectra described earlier that had been obtained using FABMS, the mass spectrum of **123** also displayed an unprotonated molecular ion signal of low intensity at m/e 471. Although satisfactory elemental analysis was not obtained on **123**, the bulk purity of **123** was assessed by the technique of analytical hplc. This technique revealed that **123** had been prepared in a satisfactorily pure state and that it did not contain any contaminant **1**. Thus a sample of **123** could be tested against HIV *in vitro*.

With the successful isolation of **123**, the preparation of 3′-azido-3′-deoxythymidine 5′-bis(2,2-dichloroethyl) phosphate (**124**) was carried out. First, bis(2,2-dichloroethyl) phosphorochloridate (**126**) was prepared by the addition of just over two equivalents of both 2,2-dichloroethanol and triethylamine to phosphoryl chloride in diethyl ether at -40°C, followed by stirring the reaction mixture at 0°C overnight (diagram 61).

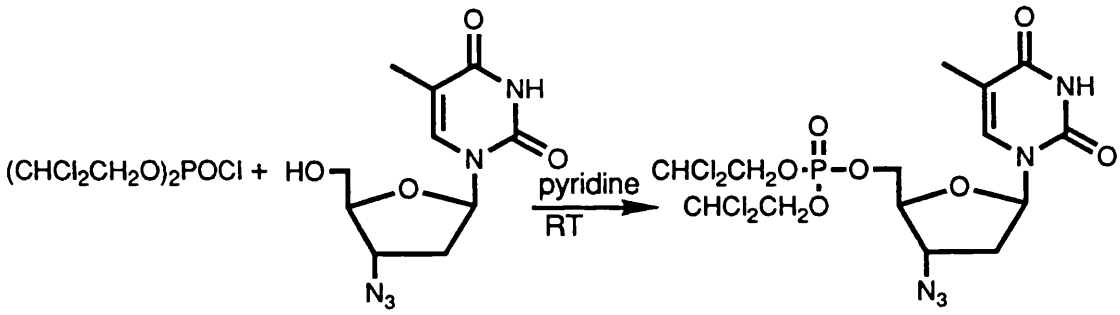
126 was isolated by vacuum distillation. Although it was not possible to carry this out using the conventional distillation apparatus, it was possible to distill crude **126** using a short-path (Kugelrohr) distillation apparatus. This yielded pure **126** as a colourless liquid, whose ^{31}P nmr spectrum consisted of a single resonance at δ2.22. The ^{13}C nmr spectrum of **126** consisted of two doublets, at δ72.12 and δ68.07. The ^1H nmr spectrum consisted of a triplet, integrating for two protons, at δ5.86 and a multiplet, integrating for four protons, at δ4.38.

Diagram 61



126

Diagram 62

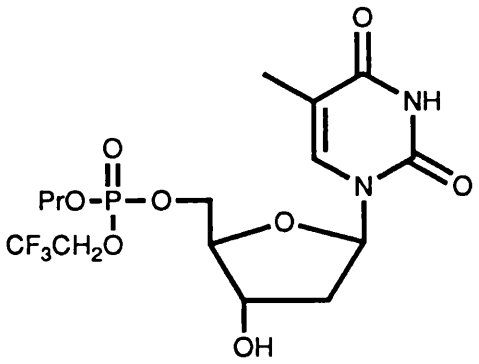


126 **1** **124**

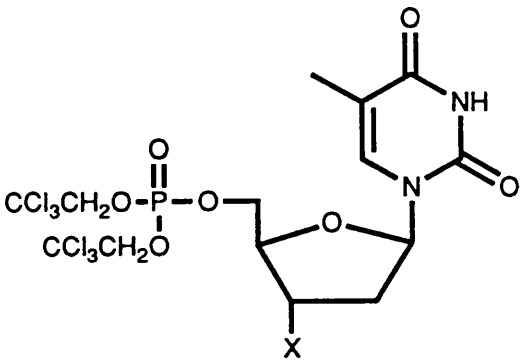
Having prepared **126**, it was reacted with **1** and the desired product **124** was isolated by column chromatography (diagram 62). A ^{31}P nmr spectrum of **124** consisted of one resonance at δ-4.32. Regarding the ^{13}C nmr spectrum of **124**, the base resonances occurred at chemical shifts that were quite similar to the chemical shifts of the corresponding resonances of **102**. Regarding the resonances of the 2,2-dichloroethyl groups, the $\underline{C}HCl_2CH_2OP$ resonance occurred at δ68.44 as a doublet. The $CHCl_2\underline{C}H_2OP$ resonance was observed as two almost coincident doublets, the non-equivalence of the two 2,2-dichloroethyl groups explaining this occurrence.

In the ^1H nmr spectrum of **124**, the $CHCl_2C\underline{H}_2OP$ resonance was seen along with the H3′ and H5′ resonances as a complex multiplet at δ4.38. The $C\underline{H}Cl_2CH_2OP$ resonance was seen as two closely spaced triplets integrating for two protons in total. Again the non-equivalence of the two 2,2-dichloroethyl groups provides the explanation for two sets of triplets being observed for this resonance. FABMS provided a mass spectrum of **124** in which the isotopic pattern of the protonated molecular ions was consistent with **124** containing four chlorine atoms.

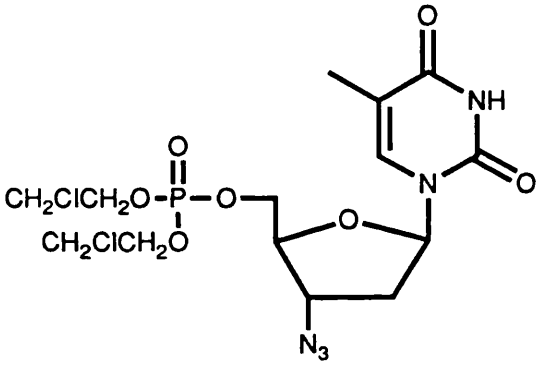
Further to the anti-viral testing described earlier in this thesis, five other compounds have been tested against HIV in C8166 cells by T.J. O'Connor and D. Kinchington. The compounds tested were thymidine 5′-(propyl 2,2,2-trifluoroethyl) phosphate (**117**), 3′-O-mesylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**118**), 3′-O-ethylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**120**), 3′-O-acetylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**119**) and 3′-azido-3′-deoxythymidine 5′-bis(2-chloroethyl) phosphate (**123**). The results of these tests are depicted in diagram 63. The inactivity of **117** was not surprising given that it is a derivative of the natural nucleoside **74**. It would obviously have been more illuminating to have tested the analogous 3′-azido-3′-deoxythymidine compounds whose preparations were described earlier in this thesis. It was not possible to test these derivatives because a sample completely free of contaminant 3′-azido-3′-deoxythymidine (**1**) could not be obtained. The anti-HIV activity displayed by **118**, **120** and **119**, although marginal, is of



117



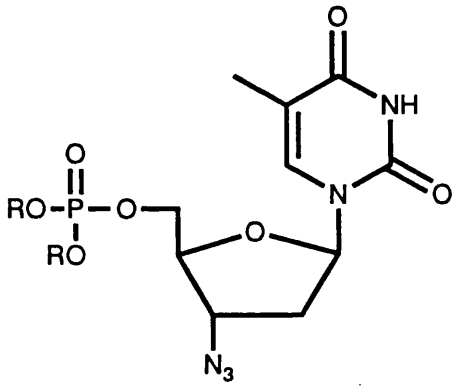
118, X=OSO_2Me
119, X=OCOMe
120, X=OEt



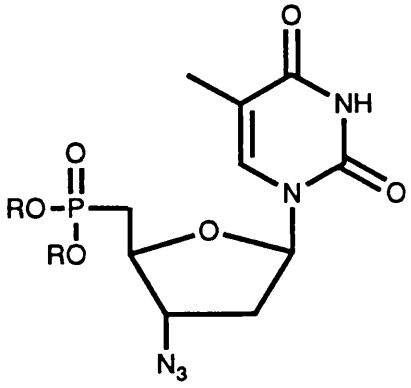
123

	IC_{50} (μM)
117	>100
118	10
119	15
120	10
123	<1

Diagram 64



102, R=CCl_3CH_2
127, R=H



128, R=CCl_3CH_2
129, H

particular interest. These results are consistent with these compounds undergoing intracellular cleavage to the corresponding 5′-monophosphate, followed by conversion to the 5′-triphosphate which may then inhibit HIV-RT. These derivatives may, of course, owe their activity to a process other than intracellular cleavage to a nucleotide. As the parent nucleosides of these compounds (ie 3′-O-mesylthymidine, 3′-O-ethylthymidine and 3′-O-acetylthymidine) display no anti-HIV activity[101] it seems unlikely that **118, 120** and **119** would undergo intracellular cleavage to their parent nucleosides. The activity of these compounds may well result from the intracellular production of bio-active nucleotides. These results would appear to suggest the need for further testing on **118, 120** and **119** and also that other 3′-substituted thymidine phosphates be prepared and tested in the hope that the activity displayed by **118, 120** and **119** could be improved upon.

Although it is not possible as yet to give an exact figure, initial studies have shown that **123** possesses an $IC_{50}< 1$. **123** has comparable activity with **102** at least and may possibly be more active. This result is slightly surprising as it might have been expected that the 2-chloroethyl group would not be as labile as the 2,2,2-trichloroethyl group and that therefore **123** would undergo intracellular hydrolysis with greater difficulty than **102**. The activity of **123** relative to **102** does suggest that nucleoside dialkyl phosphates with other phosphate esterifying groups should be investigated as potential anti-HIV agents.

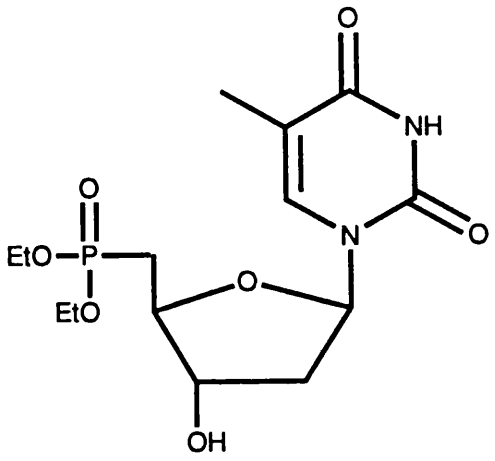
The synthesis of a number of nucleoside 5′-dialkyl phosphates have been described in this thesis. Some of these have been shown to possess anti-HIV activity. It would be of great interest to assess their exact mode of action. For example, with 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**102**) it is possible that the desired aim was achieved, that is that this triester is hydrolysed intracellularly to yield the 5′-monophosphate **127**, which is then sequentially phosphorylated to the bio-active form, the 5′-triphosphate. An alternative mode of action is that **102** may lose the phosphate moiety entirely and yield 3′-azido-3′-deoxythymidine (**1**) before being phosphorylated to the bio-active form.

It is obviously of interest to attempt to establish the mode of action of 5′-phosphate triester derivatives like **102**. It was thought that this might be achieved by preparing 5′-phosphonate derivatives, like **128** (diagram 64) and to evaluate their biological activity against HIV. Phosphorus-carbon bonds are very stable to hydrolysis[177]. If the mode of action of triesters like **102** involves intracellular hydrolysis of the phosphorus-nucleoside bond, it may be expected that the activity of **102** would be very much greater than **128** against HIV. If, on the other hand, the mode of action of these phosphate triesters involves hydrolysis of the phosphorus-alkyl bond it is possible that there would be no great difference in the activity of **102** and **128**. These arguments are based on the assumptions that **102** and **128** have similar substrate affinities for any enzymes involved in the hydrolysis to the respective free phosphate **127** and the phosphonate **129** (diagram 64) and that once formed the phosphate **127** and the phosphonate **129** have similar substrate affinities for the kinases necessary for their conversion into bio-active forms.

With all this in mind, some preliminary studies were made into the synthesis of some 5′-phosphonate derivatives. Unfortunately studies could only be made on the attempted synthesis of thymidine 5′-diethyl phosphonate (**130**) (diagram 65), through lack of time. This compound was to act as model for the synthesis of other 5′-phosphonates and to act as a negative biological control. It was decided to attempt the preparation of **130** by the reaction of 5′-bromo-5′-deoxythymidine (**131**) with triethyl phosphite in a Michaelis-Arbuzov type reaction[178].

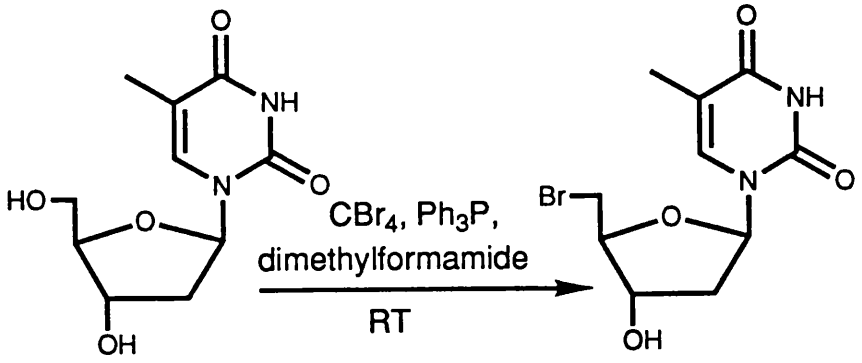
A number of attempts were made to synthesise **131** by apparently well known methods[179], but without success. 5′-O-p-Toluenesulphonylthymidine was heated with sodium bromide in both acetone and acetonitrile, these solvents being dried rigorously beforehand[180]. However, 5′-O-p-toluenesulphonylthymidine failed to react during these attempted brominations. Why these brominations should fail is far from obvious. It was decided therefore to attempt the preparation of **131** by the method of Verheyden *et al*[181].

Diagram 65



130

Diagram 66



74 **131**

Verheyden *et al* have carried out the preparation of **131** direct from **74**[181]. This was accomplished by stirring of **74** with triphenylphosphine and carbon tetrabromide in dimethylformamide solution at ambient temperature. In this way **131** was obtained by these workers in 60% yield. It was reported that bromination at the 5′-position of **74** was much favoured over bromination at the 3′-position. Thus largely following the procedure of Verheyden *et al*, 1.75 equivalents of both triphenylphosphine and carbon tetrabromide were added to a solution of **74** in dimethylformamide and stirred for a total of 45 hours (diagram 66). After this time, analysis of the reaction mixture by tlc revealed that no starting material remained and that one major component had been generated along with some minor components. The reaction was then quenched with methanol and the solvent removed under reduced pressure. The residue was purified by column chromatography. In order to isolate **131**, Verheyden *et al* used column chromatography successively eluting with ethyl acetate, ethyl acetate-chloroform and acetone. It was found that **131** could be isolated by column chromatography, using a chloroform-methanol mixture as eluent. In this way **131** was obtained as an analytically pure white solid in 55% yield.

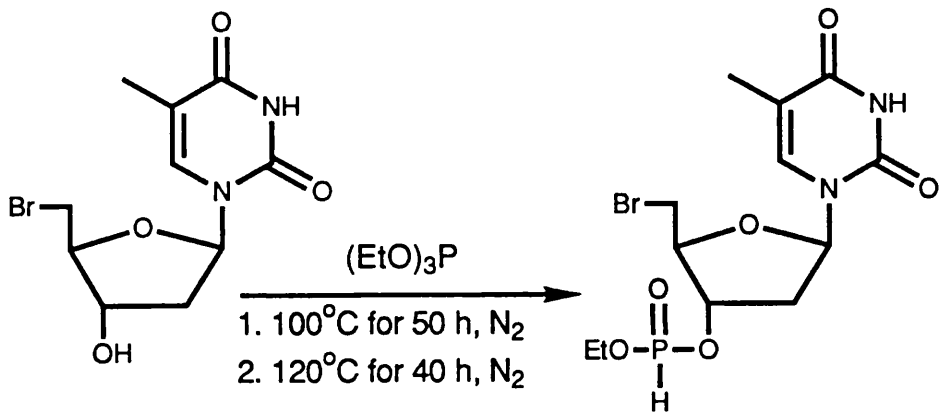
A ^{13}C nmr spectrum of **131** was obtained. This displayed the C5′ resonance upfield of its position in **74** at δ32.07. The chemical shift of the C5′ resonance of **131** is close to the chemical shifts generally displayed by carbon resonances of this type[182]. The chemical shifts of the remaining resonances were similar to the chemical shifts displayed by the corresponding resonances of **74**. The ^{1}H nmr spectrum of **131** was obtained in d_6-dimethylsulphoxide. Coupling was observed between the 3′-OH proton and the H3′ resonance, the 3′-OH resonance being observed as a doublet. The ^{1}H nmr spectrum of **131** also displayed the H1′ resonance as a doublet of doublets and the H3′, H4′ and H5′ resonances as multiplets at δ4.19, δ3.90 and δ3.66. The mass spectrum of **131** revealed two molecular ion signals, of equal intensity, at m/e 304 and m/e 306. The fact that bromine has two major isotopes (^{79}Br and ^{81}Br) which are almost equally abundant explains this observation. Satisfactory accurate mass measurements were obtained on both molecular ions

at m/e 304 and m/e 306. Fragment ions due to the loss of thymine from these molecular ions were also observed at m/e 181 and m/e 179. Having isolated a sample of **131**, its reaction with triethyl phosphite was investigated.

131 was heated in triethyl phosphite at 100°C with stirring (diagram 67). The reaction was carried out under a stream of nitrogen to drive off any ethyl bromide that may have been generated during the reaction. After stirring for 50 hours, it was necessary to add a further portion of triethyl phosphite. The reaction mixture was then stirred for a further 40 hours at 120°C. As the reaction proceeded, the reaction mixture turned a deep violet colour. After stirring for a total of 90 hours, analysis of the reaction mixture by tlc seemed to indicate that no unreacted **131** remained. In addition, tlc indicated that a number of components had been generated in the reaction mixture, including a major component more lipophilic than the starting material **131**. The excess triethyl phosphite was removed by evaporation under high vacuum to yield a residue. However, analysis of this residue by tlc indicated that the starting material **131** was present and in a significant amount. The residue was purified by column chromatography. In this way a product (more lipophilic than **131**) was isolated. This product was not the required 5′-phosphonate product **130**, instead evidence suggested that the isolated material was the 3′-hydrogenphosphonate species **132**.

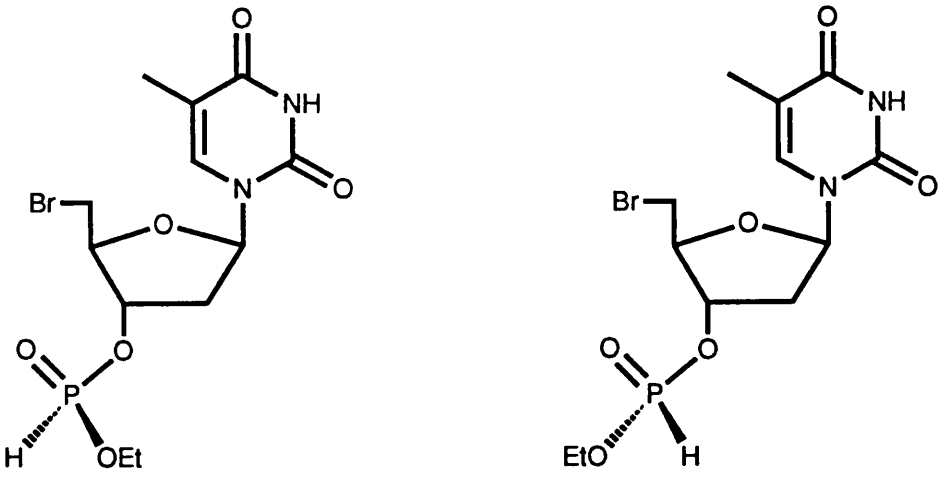
The proton-decoupled ^{31}P nmr spectrum of **132** consisted of two signals, in the region expected for hydrogenphosphonate species[183]. There are two signals because **132** is comprised of two diastereomers (diagram 68). A proton-coupled ^{31}P nmr spectrum displayed two doublet of quartets. This rather complicated spectrum may be explained in the following way. Each diastereomer's signal was made up of a widely-spaced doublet due to a one bond phosphorus-proton coupling. This coupling constant was of the order of 700 Hz and is similar to those reported in the literature. For example $(EtO)_2POH$ displays a one bond coupling constant of 690 Hz[183]. Also two bond phosphorus-proton coupling is observed between the phosphorus atom and both H3′ and $CH_3C\underline{H}_2OP$. These two bond couplings are of roughly equal magnitude, which accounts for the observed quartet fine structure.

Diagram 67



131 **132**

Diagram 68



In the ^{13}C nmr spectrum of **132**, the chemical shift of the C5′ signal occurred at δ31.67, a value similar to the chemical shift of the C5′ signal of **131**. This would appear to suggest that **132** contains a bromine atom at the 5′-position. The C3′ resonance was observed at δ77.64, phosphorus-carbon coupling caused this resonance to be observed as a doublet. The C2′ and C4′ resonances were both observed as two sets of doublets. For these signals each of the diastereomers resonate at slightly different chemical shifts and each signal exhibits coupling to the adjacent phosphorus atom, accounting for the observed four-line pattern.

The ^{1}H nmr spectrum of **132** shares a number of similarities with the ^{1}H nmr spectrum of **131**. However the H3′ resonance occurs at δ5.01, downfield of the chemical shift of the H3′ resonance of **131**. Also two signals resonating at δ4.08 and δ1.29, a multiplet and a triplet, were observed. These signals were the $CH_3C\underline{H}_2OP$ and $C\underline{H}_3CH_2OP$ resonances respectively. The mass spectrum of **132** displayed two signals of almost equal intensity at m/e 396 and m/e 398. These signals were the molecular ion species of **132**.

The mechanism by which **132** was formed during the reaction of **131** with triethyl phosphite is not entirely clear. The 5′-phosphonate species **130** would appear not to have been generated, at least not in a significant amount. Reaction at the 3′-OH group by triethyl phosphite would appear to occur more readily than does the Michaelis-Arbuzov type reaction at the 5′-bromo group. In order to prepare the 5′-phosphonate species **130** it seems necessary to protect the 3′-OH group before carrying out the Michaelis-Arbuzov reaction. Unfortunately, it was not possible to carry out a Michaelis-Arbuzov reaction on a suitably 3′-protected species through lack of time.

Some of the phosphate triester derivatives described in this thesis demonstrated anti-HIV activity. It is likely that intracellular hydrolysis plays an important role in the method by which compounds like 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (**102**) act. It was decided therefore to undertake some studies into the hydrolysis of **102**, at various pH values, using the techniques of ^{31}P nmr spectroscopy and tlc. It was found that

102 was quite insoluble in water. However water-methanol mixtures (mixed in the ratio 30:70) could dissolve **102** in sufficient quantities for study. Therefore the hydrolysis of **102** was studied at a number of pH values, in mixtures of buffer solution and d-methanol. It was found that when a prepared buffer solution of known pH was mixed with d-methanol, the pH of the resulting mixture was lower than that of the original buffer solution. A 2,4,6-trimethylpyridine/hydrochloric acid buffer solution of pH 7.0 was prepared and this buffer was mixed with d-methanol in the ratio 30:70. This buffer/d-methanol mixture was found to be pH 6.1. Similarly a 2,4,6-trimethylpyridine/hydrochloric acid buffer solution of pH 8.0 was prepared and mixed with d-methanol. This buffer/d-methanol mixture was found to be pH 7.0. Lastly, a 2-amino-2-methyl-1,3-propanediol/hydrochloric acid buffer of pH 9.3 was prepared and mixed with d-methanol. This buffer/d-methanol mixture was found to be pH 8.4. A sample of **102** was dissolved in each of these three buffer/d-methanol mixtures at pH 6.1, pH 7.0 and pH 8.4 and kept at 37°C. Periodically the mixtures were analysed using ^{31}P nmr spectroscopy and tlc. It was found that the pH of each mixture was unchanged over the course of the experiment.

102 dissolved in the buffer/d-methanol mixture of pH 7.0 appeared stable at this pH. Both ^{31}P nmr spectroscopy and tlc indicated that **102** remained unchanged even after a period of 95 days under these conditions. This was in contrast to **102** in the buffer/d-methanol mixtures at pH 6.1 and pH 8.4. **102** dissolved in buffer/d-methanol mixture at pH 6.1 showed signs of decomposition after a period of 30 days. After a period of 86 days, a ^{31}P nmr spectrum of the mixture displayed the resonance of **102** at δ-4.06 (40% of the mixture) and in addition four other resonances at δ-1.11, δ-1.19, δ-1.36 and δ-3.21 could be identified. It would seem that decomposition of **102** was occurring and that a number of phosphorus containing species were being formed. Analysis of the solution of **102** at pH 6.1 after 86 days by tlc confirmed that decomposition was occurring. In addition to **102** (at R_f 0.51 using chloroform-methanol (95:5) as the eluent) two other minor components were observed by tlc (at R_f 0.38 and R_f 0.00 in the same eluent), which also

indicated that some 3′-azido-3′-deoxythymidine (**1**) had been liberated by **102**, but only in trace amounts.

Examination of the solution of **102** in the buffer/d-methanol mixture at pH 8.4 revealed that **102** decomposed at this pH too. Indeed under these conditions **102** appeared to decompose at an appreciably faster rate than that noted for the acidic solution. Decomposition of **102** was apparent after 1 day at pH 8.4. After 35 days at pH 8.4, ^{31}P nmr spectroscopy indicated that **102** had almost completely decomposed (it represented 15% of the mixture). Apart from the resonance of **102**, four other resonances could be seen at δ-1.10, δ-1.19, δ-1.35 and δ-3.19. The chemical shifts of these resonances are almost identical to the resonances seen in the ^{31}P nmr spectrum of the buffer/d-methanol mixture at pH 6.1. **102** decomposes at pH 6.1 and pH 8.4 to yield the same products. The ^{31}P nmr spectrum of buffer/d-methanol mixture at pH 8.4 after 35 days also showed that the resonance at δ-1.35 was considerably more intense than the other resonances observed in the spectrum. Analysis of the buffer/d-methanol mixture at pH 8.4 after 35 days by tlc indicated the presence of two major components (R_f 0.38 and R_f 0.00 using chloroform-methanol (95:5) as the eluent). These same components were observed by tlc in the buffer/d-methanol mixture at pH 6.1. Analysis by tlc also indicated that a trace amount of **1** had been generated in the buffer/d-methanol mixture at pH 8.4. A ^{31}P nmr spectrum of this mixture after a total of 77 days, revealed that **102** had completely decomposed. Moreover the spectrum contained only one resonance at δ-1.33. Analysis of the buffer/d-methanol mixture after this time revealed the presence of a major component (R_f 0.00), the presence of a minor component (R_f 0.38) and the presence of **1** in a trace amount.

The results of these kinetic studies do show that **102** decomposes in both acidic and basic media but not in a neutral medium. Although the decomposition of **102** produces the same products in acidic and basic conditions, **102** would appear to decompose more readily under basic conditions. The identity of these decomposition products is not clear. One of these products of decomposition may be a diester resulting from the loss of a single

2,2,2-trichloroethyl group from **102**. This diester may be responsible for the resonances observed at around δ-1.33 in the various ^{31}P nmr spectra. This diester may give rise to the single resonance observed in the ^{31}P nmr spectrum of the basic medium after 77 days. Diesters are known to resonate in this region[184]. This diester may also be responsible for the component with R_f 0.00 observed by tlc in both the acidic and basic media. Interestingly **102** only yielded trace amounts of **1** on hydrolysis under acidic and basic conditions. This may suggest that **102** is more likely to undergo intracellular cleavage to a nucleotide form rather than to the parent nucleoside.

SUMMARY AND CONCLUSIONS

The synthesis of a number of nucleoside 5′-dialkyl phosphates has been described in this thesis. It was thought that these derivatives would have potential as anti-HIV agents.

Firstly diethyl, dipropyl and dibutyl phosphates of some 3′-modified thymidines were prepared. 3′-O-Mesylthymidine 5′-dialkyl phosphates were prepared by the mesylation of the relevant thymidine 5′-dialkyl phosphate. 5′-Dialkyl phosphates of 3′-O-acetylthymidine, 3′-O-ethylthymidine and 3′-azido-3′-deoxythymidine were prepared by the reaction of a dialkyl phosphorochloridate with the relevant 3′-modified thymidine, in pyridine. An attempt to prepare 2′,3′-dideoxyadenosine 5′-dibutyl phosphate apparently resulted in the isolation of the α and ß anomers of that compound, although it was possible to isolate 2′,3′-dideoxyadenosine 5′-dipropyl phosphate as the ß anomer only. 2′,3′-Dideoxycytidine 5′-dibutyl phosphate was also prepared.

In biological testing, it was found that none of the above nucleoside 5′-dialkyl phosphates were able to inhibit the proliferation of HIV *in vitro*, even at a concentration of 100 μM. This lack of activity suggested that these simple dialkyl phosphates were unable to be hydrolysed to the corresponding 5′-monophosphate intracellularly and that more labile phosphate esterifying groups were required.

With this in mind, the 5′-bis(2,2,2-trifluoroethyl) phosphate and 5′-bis(2,2,2-trichloroethyl) phosphate of 3′-azido-3′-deoxythymidine were prepared and tested as anti-HIV agents. Both compounds were able to inhibit HIV *in vitro*, which was in marked contrast to the lack of activity of the diethyl, dipropyl and dibutyl phosphates of 3′-azido-3′-deoxythymidine. In view of this success, some 5′-bis(2,2,2-trihaloethyl) phosphates of 2′,3′-dideoxycytidine and 3′-fluoro-3′-deoxythymidine were also prepared.

Some mixed 5′-(propyl 2,2,2-trihaloethyl) phosphates of 3′-azido-3′-deoxythymidine were also prepared, as it was thought that these derivatives might have had anti-HIV properties too.

The 5′-bis(2,2,2-trichloroethyl) phosphates of 3′-O-mesylthymidine, 3′-O-

acetylthymidine and 3′-O-ethylthymidine were then prepared. 3′-Amino-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate was also prepared (though in low yield) from the reduction of 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate with 1,3-propanedithiol.

As the 5′-bis(2,2,2-trichloroethyl) phosphate of 3′-azido-3′-deoxythymidine displayed anti-HIV activity, the 5′-bis(2-chloroethyl) phosphate and the 5′-bis(2,2-dichloroethyl) phosphate of 3′-azido-3′-deoxythymidine were prepared in order to ascertain if these compounds would also display anti-HIV activity. Indeed the 5′-bis(2-chloroethyl) phosphate of 3′-azido-3′-deoxythymidine did display anti-HIV activity.

An attempt was made on the preparation of a thymidine 5′-dialkyl phosphonate species. However, from a reaction between 5′-bromo-5′-deoxythymidine and triethyl phosphite, only a 3′-hydrogenphosphate species could be isolated.

As the method by which 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate acts against HIV may involve intracellular hydrolysis, the hydrolytic stability of this compound was investigated at a variety of pH. This triester was stable at pH 7.0, although decomposition was noted at pH 6.1 and pH 8.4.

EXPERIMENTAL

General methods.

Commercially available Merck Kieselgel 60 F_{254} plates were used for tlc and the separated components were visualised by uv light. Column chromatography was carried out using Woelm silica (32-63 μm) as the stationary phase. The ratio of silica-compound varied between 50:1 and 100:1 (w/w). Melting points were determined on a Riechert hot stage melting point apparatus and are uncorrected. Electronic (uv) spectra were recorded on a Perkin-Elmer 554 spectrophotometer. EIMS was carried out by Dr. M. Mruzek on a VG 7070H mass spectrometer fitted with a Finnigan Incos II data system. FABMS was carried out by the University of London mass spectrometry service on a VG Zab1F spectrometer and hplc was carried out by Mr. S. Corker on a Gilson Binary Gradient HPLC system, fitted with a Gilson 115 uv detector (detection at 254 nm) and Rheodyne injector. Where appropriate, the anti-HIV starting materials 3′-azido-3′-deoxythymidine, 2′,3′-dideoxycytidine, 2′,3′-dideoxyadenosine or 3′-fluoro-3′-deoxythymidine were not detected in the samples analysed or isolated using hplc.

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

All experiments involving water sensitive reagents were carried out under

scrupulously dry conditions. Where needed, anhydrous solvents and reagents were obtained in the following ways: benzene, dichloromethane, diethyl ether, dioxan, and pyridine were heated under reflux over calcium hydride for several hours, distilled and stored over activated molecular sieves. Tetrahydrofuran was heated under reflux over lithium aluminium hydride for several hours, distilled and stored over activated molecular sieves. Triethylamine was heated under reflux over calcium hydride for several hours and distilled immediately prior to use. Dimethylformamide was placed over activated molecular sieves overnight, distilled under reduced pressure and stored over activated molecular sieves. 1-Butanol, 2,2,2-trichloroethanol, 2,2,2-trifluoroethanol and 1-propanol were stored over activated molecular sieves. Acetonitrile was distilled from phosphorus pentoxide twice, followed by storing over activated molecular sieves. Anhydrous methanol was obtained by heating with magnesium activated with iodine, followed by distillation and storage over activated molecular sieves. Acetic anhydride, ethyl iodide, 1,3-propanedithiol, phosphoryl chloride, triethyl phosphite and mesyl chloride were distilled prior to use. 1-(2-Deoxy-3-O-mesyl-ß-D-threopentofuranosyl)thymine and 1-(2-deoxy-5-O-trityl-ß-D-threopentofuranosyl)thymine were kindly donated by Pfizer Ltd.

Dipropyl phosphorochloridate (72).

A solution of 1-propanol (12.95 g, 216 mmol, 2.02 equivs.) and triethylamine (21.78 g, 216 mmol, 2.02 equivs.) in diethyl ether (50 ml) was added dropwise to a solution of phosphoryl chloride (16.46 g, 107 mmol) in diethyl ether (150 ml), with vigorous stirring at -30°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After a further 17 h, the reaction mixture was filtered and the solvent removed from the filtrate under reduced pressure. The residue was purified by vacuum distillation to give the product as a colourless liquid, (16.95 g, 79%), bp 71-72°C at 1 mmHg.

^{31}P nmr $\delta(CDCl_3)$ 3.12.

^{13}C nmr $\delta(CDCl_3)$ 71.13 (d, $CH_3CH_2\underline{C}H_2OP$, J=7.0 Hz), 23.31 (d, $CH_3\underline{C}H_2CH_2OP$, J=7.8 Hz), 9.88 ($\underline{C}H_3CH_2CH_2OP$).

^1H nmr $\delta(CDCl_3)$ 4.06 (4H, m, $CH_3CH_2C\underline{H}_2OP$), 1.66 (4H, sextet, $CH_3C\underline{H}_2CH_2OP$, J=6.3 Hz), 0.91 (6H, t, $C\underline{H}_3CH_2CH_2OP$, J=7.3 Hz).

Dibutyl phosphorochloridate (73).

A solution of 1-butanol (15.98 g, 216 mmol, 2.02 equivs.) and triethylamine (21.78 g, 216 mmol, 2.02 equivs.) in diethyl ether (75 ml) was added dropwise to a solution of phosphoryl chloride (16.46 g, 107 mmol) in diethyl ether (150 ml), with vigorous stirring at -30°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After a further 17 h, the reaction mixture was filtered and the solvent removed from the filtrate under reduced pressure. The residue was purified by vacuum distillation to give the product as a colourless liquid, (17.60 g, 72%), bp 89-91°C at 0.5 mmHg.

^{31}P nmr $\delta(CDCl_3)$ 2.76.

^{13}C nmr $\delta(CDCl_3)$ 69.22 (d, $CH_3CH_2CH_2\underline{C}H_2OP$, J=7.3 Hz), 31.55 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=7.7 Hz), 18.36 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.23 ($\underline{C}H_3CH_2CH_2CH_2OP$).

^1H nmr $\delta(CDCl_3)$ 4.19 (4H, m, $CH_3CH_2CH_2C\underline{H}_2OP$), 1.73 (4H, quintet, $CH_3CH_2C\underline{H}_2CH_2OP$, J=7.6 Hz), 1.43 (4H, sextet, $CH_3C\underline{H}_2CH_2CH_2OP$, J=7.3 Hz), 0.91 (6H, t, $C\underline{H}_3CH_2CH_2CH_2OP$, J=7.3 Hz).

Thymidine 5′-diethyl phosphate (75).

Diethyl phosphorochloridate (2.85 g, 16.5 mmol, 2 equivs.) was added to a solution of thymidine (2.00 g, 8.25 mmol) in pyridine (100 ml) with stirring at -20°C. After stirring at

this temperature for 30 min, the reaction mixture was allowed to warm to ambient temperature. After a further 2 h, water (0.3 ml, 16.5 mmol) was added and the solvent removed under reduced pressure. Analysis of the resulting residue by tlc revealed one major component and two minor faster running components. The residue was purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of the appropriate fractions gave the product as a colourless oil, (1.74 g, 56%).

^{31}P nmr δ($CDCl_3$) -2.75.

^{13}C nmr δ($CDCl_3$) 164.41 (C2), 150.80 (C4), 135.67 (C6), 112.71 (C5), 84.90 (C1′), 84.81 (d, C4′, J=7.6 Hz), 70.84 (C3′), 66.84 (d, C5′, J=5.3 Hz), 64.46 (d, $CH_3\underline{C}H_2OP$, J=6.1 Hz), 40.10 (C2′), 16.16 (d, $\underline{C}H_3CH_2OP$, J=6.7 Hz), 12.42 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 9.54 (1H, s, NH), 7.45 (1H, s, H6), 6.34 (1H, t, H1′, J=6.8 Hz), 4.40 (4H, m, H3′, H4′, H5′), 4.15 (4H, quintet, $CH_3\underline{CH_2}OP$, J=6.2 Hz), 2.80 (1H, bs, 3′-OH), 2.41 (1H, m, H2′), 2.10 (1H, m, H2′), 1.93 (3H, s, 5-CH_3), 1.35 (6H, t, $\underline{CH_3}CH_2OP$, J=6.9 Hz).

Thymidine 5′-dipropyl phosphate (76).

Dipropyl phosphorochloridate (1.04 g, 5.19 mmol, 2.5 equivs.) was added to a solution of thymidine (0.50 g, 2.07 mmol) in pyridine (75 ml), with stirring at -20°C. After stirring at this temperature for 30 min, the reaction mixture was allowed to warm to ambient temperature. After a further 4 h water (92 µl, 5.11 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.46 g, 57%).

^{31}P nmr δ($CDCl_3$) -2.16.

^{13}C nmr δ($CDCl_3$) 164.21 (C2), 150.82 (C4), 135.62 (C6), 111.39 (C5), 85.19 (C1′), 83.13 (d, C4′, J=7.7 Hz), 71.10 (C3′), 69.82, 69.75 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=6.7 Hz, J=6.6 Hz), 66.81 (d, C5′, J=5.6 Hz), 40.92 (C2′), 23.57 (d, $CH_3\underline{C}H_2CH_2OP$, J=7.1 Hz), 12.40 (5-$CH_3$), 9.91 ($\underline{C}H_3CH_2CH_2OP$).

^1H nmr δ($CDCl_3$) 10.16 (1H, s, NH), 7.48 (1H, s, H6), 6.40 (1H, t, H1′, J=6.6 Hz), 4.35 (5H, m, H3′, H4′, H5′, 3′-OH), 4.00 (4H, q, $CH_3CH_2C\underline{H}_2OP$, J=6.1 Hz), 2.25 (2H, m, H2′), 1.90 (3H, s, 5-CH_3), 1.82 (4H, m, $CH_3C\underline{H}_2CH_2OP$), 0.94, 0.93 (6H, 2xt, $C\underline{H}_3CH_2CH_2OP$, J=6.1 Hz, J=6.1 Hz).

EIMS m/e 406.1476 (M^+, 0.5%, calc. for $C_{16}H_{27}N_2O_8P$ 406.1447), 263 (MH^+-H_2O-thymine, 6), 127 (thymineH^+, 11), 126 (thymine$^+$, 11), 99 ($C_5H_7O_2^+$, 58), 81 ($C_5H_5O^+$, base peak).

Found C 45.52%, H 7.07, N 6.30, $C_{16}H_{27}N_2O_8P.H_2O$ requires C 45.28, H 6.89, N 6.60.

Thymidine 5′-dibutyl phosphate (77).

Dibutyl phosphorochloridate (1.81 g, 7.92 mmol, 1.9 equivs.) was added to a solution of thymidine (1.00 g, 4.13 mmol) in pyridine (75 ml), with stirring at -20°C. After stirring at this temperature for 30 min, the reaction mixture was allowed to warm to ambient temperature. After a further 17 h water (140 µl, 7.77 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (1.09 g, 64%).

^{31}P nmr δ($CDCl_3$) -2.40.

^{13}C nmr δ($CDCl_3$) 164.10 (C2), 150.74 (C4), 135.45 (C6), 111.28 (C5), 84.94 (d, C4′, J=7.2 Hz), 84.79 (C1′), 70.99 (C3′), 67.98, 67.92 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=5.3 Hz, J=5.2 Hz), 66.75 (d, C5′, J=5.8 Hz), 40.16 (C2′), 32.14 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=6.6 Hz), 18.53 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.45 ($\underline{C}H_3CH_2CH_2CH_2OP$), 12.36 (5-$CH_3$).

^{1}H nmr δ($CDCl_3$) 9.95 (1H, s, NH), 7.41 (1H, s, H6), 6.32 (1H, t, H1′, J=6.7 Hz), 4.43 (2H, m, H3′, 3′-OH), 4.21 (2H, m, H5′), 4.02 (5H, m, $CH_3CH_2CH_2\underline{CH_2}OP$, H4′), 2.35 (1H, m, H2′), 2.08 (1H, m, H2′), 1.87 (3H, s, 5-CH_3), 1.59 (4H, m, $CH_3CH_2\underline{CH_2}CH_2OP$), 1.34 (4H, m, $CH_3\underline{CH_2}CH_2CH_2OP$), 0.91, 0.90 (6H, 2xt, $\underline{CH_3}CH_2CH_2CH_2OP$, J=7.0 Hz, J=6.9 Hz).

EIMS m/e 434.1830 (M^+, 0.3%, calc. for $C_{18}H_{31}N_2O_8P$ 434.1818), 309 (MH^+-thymine, 1), 127 (thymineH^+, 5), 126 (thymine$^+$, 10), 99 ($C_5H_7O_2^+$, 52), 81 ($C_5H_5O^+$, base peak).

3′-O-Mesylthymidine 5′-diethyl phosphate (78).

A solution of mesyl chloride (0.19 g, 1.65 mmol, 1.1 equivs.) in dichloromethane (15 ml) was added to a solution of thymidine 5′-diethyl phosphate (0.57 g, 1.51 mmol) and triethylamine (0.18 g, 1.81 mmol, 1.2 equivs.) in dichloromethane (30 ml), with stirring at -20°C. The reaction mixture was then allowed to warm to ambient temperature. After 2 h at this temperature, further portions of mesyl chloride (0.050 g, 0.44 mmol) and triethylamine (0.045 g, 0.44 mmol) were added to the reaction mixture. After stirring for a further 15 min, the reaction mixture was washed with saturated sodium bicarbonate solution (5x40 ml) and saturated brine (2x50 ml). The organic phase was dried with magnesium sulphate and the solvent removed under reduced pressure to yield the product as a colourless oil, (0.50 g, 72%).

^{31}P nmr δ($CDCl_3$) -3.15.

^{13}C nmr $\delta(CDCl_3)$ 163.63 (C2), 150.43 (C4), 134.90 (C6), 111.94 (C5), 83.55 (C1'), 82.71 (d, C4', J=7.4 Hz), 78.94 (C3'), 66.13 (d, C5', J=5.8 Hz), 63.46, 63.41 (2xd, $CH_3\underline{C}H_2OP$, J=5.4 Hz, J=5.4 Hz), 38.61 (C2'), 37.87 (CH_3SO_2), 16.15 (d, $\underline{C}H_3CH_2OP$, J=6.5 Hz), 12.41 (5-CH_3).

^1H nmr $\delta(CDCl_3)$ 9.92 (1H, s, NH), 7.44 (1H, s, H6), 6.31 (1H, dd, H1', J=8.0 Hz, J=5.6 Hz), 5.35 (1H, m, H3')*, 4.31 (1H, m, H4'), 4.22 (2H, m, H5'), 4.12 (4H, quintet, $CH_3C\underline{H}_2OP$, J=6.2 Hz), 3.14 (3H, s, CH_3SO_2), 2.36 (1H, m, H2')**, 2.25 (1H, m, H2')**, 1.95 (3H, s, 5-CH_3), 1.33 (6H, t, $C\underline{H}_3CH_2OP$, J=6.5 Hz).

*Affected by irradiation at δ2.30. **Affected by irradiation at δ6.31.

EIMS m/e 456.0999 (M^+, 5%, calc. for $C_{15}H_{25}N_2O_{10}PS$ 456.0968), 360 (M^+-$MeSO_3H$, 5), 206 (M^+-$MeSO_3H$-$(EtO)_2PO_2H$, 12), 155 ($(EtO)_2P(OH)_2^+$, 13), 127 (thymineH^+, 36), 126 (thymine$^+$, 37), 99 ($C_5H_7O_2^+$, 37), 81 ($C_5H_5O^+$, base peak).

Found C 39.38%, H 5.72, N 5.75, P 6.56, $C_{15}H_{25}N_2O_{10}PS$ requires C 39.47, H 5.52, N 6.14, P 6.79.

3'-O-Mesylthymidine 5'-dipropyl phosphate (79).

A solution of mesyl chloride (0.12 g, 1.08 mmol, 1.3 equivs.) in dichloromethane (5 ml) was added to a solution of thymidine 5'-dipropyl phosphate (0.35 g, 0.84 mmol) and triethylamine (0.12 g, 1.17 mmol, 1.4 equivs.) in dichloromethane (20 ml), with stirring at -20°C. The reaction mixture was then allowed to warm to ambient temperature. After stirring for a further 30 min, the reaction mixture was washed with saturated sodium bicarbonate solution (4x50 ml) and saturated brine (2x50 ml). The organic phase was dried with magnesium sulphate and the solvent removed under reduced pressure to yield the product as a colourless oil, (0.36 g, 79%).

^{31}P nmr $\delta(CDCl_3)$ -3.10.

^{13}C nmr $\delta(CDCl_3)$ 163.68 (C2), 150.45 (C4), 134.82 (C6), 111.88 (C5), 84.45 (C1'), 82.87 (d, C4', J=7.8 Hz), 78.94 (C3'), 69.83, 69.77 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=6.2 Hz, J=6.1 Hz), 66.10 (d, C5', J=6.5 Hz), 38.58 (C2'), 37.80 (CH_3SO_2), 23.58 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.7 Hz), 12.38 (5-$CH_3$), 9.98 ($\underline{C}H_3CH_2CH_2OP$).

^1H nmr $\delta(CDCl_3)$ 10.01 (1H, bs, NH), 7.39 (1H, s, H6), 6.33 (1H, dd, H1', J=7.9 Hz, J=5.7 Hz), 5.31 (1H, m, H3'), 4.34 (1H, m, H4'), 4.24 (2H, m, H5'), 3.99 (4H, q, $CH_3CH_2\underline{C}H_2OP$, J=7.1 Hz), 3.08 (3H, s, CH_3SO_2), 2.48 (1H, m, H2'), 2.22 (1H, m, H2'), 1.87 (3H, s, 5-CH_3), 1.65 (4H, sextet, $CH_3\underline{C}H_2CH_2OP$, J=6.8 Hz), 0.89, 0.88 (6H, 2xt, $\underline{C}H_3CH_2CH_2OP$, J=6.6 Hz, J=6.7 Hz).

EIMS m/e 484.1267 (M^+, 0.1%, calc. for $C_{17}H_{29}N_2O_{10}PS$ 484.1279), 388 (M^+-$MeSO_3H$, 1), 263 (MH^+-$MeSO_3H$-thymine, 1), 206 (M^+-$MeSO_3H$-$(PrO)_2PO_2H$, 3), 183 ($(PrO)_2P(OH)_2^+$, 1), 141 ($PrOP(OH)_3^+$, 3), 127 (thymineH^+, 2), 126 (thymine$^+$, 8), 99 ($C_5H_7O_2^+$, 37), 81 ($C_5H_5O^+$, base peak).

Found C 42.43%, H 6.10, N 5.50, P 6.01, $C_{17}H_{29}N_2O_{10}PS$ requires C 42.15, H 6.03, N 5.78, P 6.39.

3'-O-Mesylthymidine 5'-dibutyl phosphate (80).

A solution of mesyl chloride (0.19 g, 1.66 mmol, 1.1 equivs.) in dichloromethane (10 ml) was added to a solution of thymidine 5'-dibutyl phosphate (0.65 g, 1.50 mmol) and triethylamine (0.18 g, 1.81 mmol, 1.2 equivs.) in dichloromethane (40 ml), with stirring at -20°C. The reaction mixture was then allowed to warm to ambient temperature. After stirring for a further 30 min, the reaction mixture was washed with saturated sodium bicarbonate

solution (4x50 ml) and saturated brine (2x50 ml). The organic phase was dried with magnesium sulphate and the solvent removed under reduced pressure to yield the product as a colourless oil, (0.76 g, 72%).

^{31}P nmr δ($CDCl_3$) -2.74.

^{13}C nmr δ($CDCl_3$) 163.80 (C2), 150.51 (C4), 134.81 (C6), 111.71 (C5), 84.40 (C1'), 82.25 (d, C4', 7.8 Hz), 79.01 (C3'), 68.11, 68.05 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=6.2 Hz, J=6.2 Hz), 66.04 (d, C5', J=5.1 Hz), 38.39 (C2'), 37.69 (CH_3SO_2), 32.10 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=6.8 Hz), 18.46 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.38 ($\underline{C}H_3CH_2CH_2CH_2OP$), 12.30 (5-$CH_3$).

^{1}H nmr δ($CDCl_3$) 9.51 (1H, bs, NH), 7.43 (1H, d, H6, J=1.1 Hz), 6.38 (1H, dd, H1', J=8.7 Hz, J=5.6 Hz), 5.34 (1H, m, H3'), 4.40 (1H, m, H4'), 4.27 (2H, m, H5'), 4.07 (4H, q, $CH_3CH_2CH_2\underline{CH_2}OP$, J=6.8 Hz), 3.11 (3H, s, CH_3SO_2), 2.58 (1H, m, H2'), 2.28 (1H, m, H2'), 1.92 (3H, d, 5-CH_3, J=1.1 Hz), 1.64 (4H, m, $CH_3CH_2\underline{CH_2}CH_2OP$), 1.40 (4H, m, $CH_3\underline{CH_2}CH_2CH_2OP$), 0.90, 0.88 (6H, 2xt, $\underline{CH_3}CH_2CH_2CH_2OP$, J=7.4 Hz, J=7.4 Hz).

EIMS m/e 512.1562 (M^+, 0.3%, calc. for $C_{19}H_{33}N_2O_{10}PS$ 512.1588), 416 (M^+-$MeSO_3H$, 3), 211 ($(BuO)_2P(OH)_2^+$, 1), 206 (M^+-$MeSO_3H$-$(BuO)_2PO_2H$, 4), 155 ($BuOP(OH)_3^+$, 2), 127 (thymineH^+, 3), 126 (thymine$^+$, 6), 99 ($C_5H_7O_2^+$, 28), 81 ($C_5H_5O^+$, base peak).

Found C 44.34%, H 6.45, N 5.64, P 6.32, $C_{19}H_{33}N_2O_{10}PS$ requires C 44.53, H 6.49, N 5.47, P 6.04.

3'-O-Acetylthymidine (81).

Trityl chloride (3.25 g, 11.67 mmol, 1.4 equivs.) was added to a solution of thymidine (2.00 g, 8.26 mmol) in pyridine (30 ml) and the mixture was refluxed for 3 h. The solution

was then cooled to ambient temperature and acetic anhydride (4.21 g, 41.27 mmol, 5 equivs.) was added. The mixture was stirred for 17 h and then poured into iced water (500 ml) with vigorous stirring. The precipitate was filtered, suspended in 80% acetic acid (30 ml) and heated to 80°C for 1 h. The solvent was then removed under reduced pressure and the residue purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a pale yellow solid, which was further purified by recrystallisation from acetone-petroleum ether (bp 40-60°C), (1.69 g, 72%), mp 175-177°C.

^{13}C nmr δ($CDCl_3$) 170.70 ($CH_3\underline{C}O$), 163.63 (C2), 150.42 (C4), 136.24 (C6), 111.39 (C5), 85.93 (C1′ or C4′), 85.05 (C4′ or C1′), 74.68 (C3′), 62.56 (C5′), 37.16 (C2′), 21.00 ($\underline{C}H_3CO$), 12.57 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 8.88 (1H, bs, NH), 7.44 (1H, s, H6), 6.19 (1H, t, H1′, J=7.4 Hz), 5.28 (1H, m, H3′), 4.02 (1H, m, H4′), 3.86 (2H, m, H5′), 2.58 (1H, t, 5′-OH, J=5.1 Hz), 2.32 (2H, m, H2′), 2.03 (3H, s, CH_3CO), 1.85 (3H, s, 5-CH_3).

EIMS m/e 284 (M^+, 0.01%), 126 (thymine$^+$, 27), 99 ($C_5H_7O_2^+$, 47), 81 ($C_5H_5O^+$, 8), 43 (CH_3CO^+, base peak).

Found C 51.01%, H 5.69, N 9.73, $C_{12}H_{16}N_2O_6$ requires C 50.70, H 5.67, N 9.85.

3′-O-Acetylthymidine 5′-diethyl phosphate (82).

Diethyl phosphorochloridate (0.36 g, 2.10 mmol, 2 equivs.) was added to a solution of 3′-O-acetylthymidine (0.30 g, 1.05 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 17 h water (38 µl, 2.10 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by

chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave a colourless oil, (0.31 g).

^{31}P nmr δ($CDCl_3$) -2.75 (major), -12.65 (minor), -12.72 (minor), -12.83 (minor).

This product was further purified by chromatography on silica, with elution by chloroform (400 ml), followed by chloroform-methanol (95:5). Pooling and evaporation of the appropriate fractions of the latter eluent gave the product as a colourless oil, (0.28 g, 65%).

^{31}P nmr δ($CDCl_3$) -2.71.

^{13}C nmr δ($CDCl_3$) 170.52 ($CH_3\underline{C}O$), 163.92 (C2), 150.71 (C4), 135.09 (C6), 111.67 (C5), 84.40 (C1'), 82.90 (d, C4', J=8.1 Hz), 74.71 (C3'), 66.95 (d, C5', J=5.7 Hz), 64.30, 64.25 (2xd, $CH_3\underline{C}H_2OP$, J=5.6 Hz, J=5.4 Hz), 37.21 (C2'), 20.92 ($\underline{C}H_3CO$), 16.19 (d, $\underline{C}H_3CH_2OP$, J=6.5 Hz), 12.41 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 9.70 (1H, s, NH), 7.50 (1H, d, H6, J=1.2 Hz), 6.45 (1H, dd, H1', J=7.8 Hz, J=6.8 Hz), 5.33 (1H, m, H3'), 4.21 (3H, m, H4', H5'), 4.15 (4H, quintet, $CH_3C\underline{H}_2OP$, J=7.0 Hz), 2.30 (2H, m, H2'), 2.12 (3H, s, CH_3CO), 1.97 (3H, d, 5-CH_3, J=1.2 Hz), 1.37, 1.36 (6H, 2xt, $C\underline{H}_3CH_2OP$, J=6.1 Hz, J=6.1 Hz).

EIMS m/e 421 (MH^+, 2%), 420.1390 (M^+, 4, calc. for $C_{16}H_{25}N_2O_9P$ 420.1297), 235 (MH^+-AcOH-thymine, 50), 155 ($(EtO)_2P(OH)_2^+$, 44), 127 (thymineH^+, 50), 126 (thymine$^+$, 33), 99 ($C_5H_7O_2^+$, 61), 81 ($C_5H_5O^+$, base peak).

Found C 44.60%, H 6.21, N 5.95, P 7.37, $C_{16}H_{25}N_2O_9P(H_2O)_{0.5}$ requires C 44.76, H 6.10, N 6.52, P 7.21.

3′-O-Acetylthymidine 5′-dipropyl phosphate (83).

Dipropyl phosphorochloridate (0.31 g, 1.55 mmol, 2 equivs.) was added to a solution of 3′-O-acetylthymidine (0.22 g, 0.77 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 17 h water (27 µl, 1.55 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform (1000 ml), followed by chloroform-methanol (95:5). Pooling and evaporation of the appropriate fractions of the latter eluent gave the product as a colourless oil, (0.25 g, 69%).

^{31}P nmr δ($CDCl_3$) -2.79.

^{13}C nmr δ($CDCl_3$) 170.45 ($CH_3\underline{C}O$), 163.76 (C2), 150.67 (C4), 134.89 (C6), 111.80 (C5), 84.37 (C1′), 82.86 (d, C4′, J=8.1 Hz), 74.59 (C3′), 68.71, 68.65 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=6.0 Hz, J=5.9 Hz), 66.86 (d, C5′, J=5.5 Hz), 37.18 (C2′), 23.59 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.6 Hz), 20.84 ($\underline{C}H_3CO$), 12.33 (5-$CH_3$), 9.87 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 9.56 (1H, s, NH), 7.50 (1H, s, H6), 6.41 (1H, dd, H1′, J=7.6 Hz, J=6.9 Hz), 5.27 (1H, m, H3′), 4.26 (2H, m, H5′), 4.14 (1H, m, H4′), 4.01 (4H, q, $CH_3CH_2C\underline{H}_2OP$, J=6.9 Hz)*, 2.37 (1H, m, H2′), 2.13 (1H, m, H2′), 2.08 (3H, s, CH_3CO), 1.92 (3H, s, 5-CH_3), 1.68 (4H, sextet, $CH_3C\underline{H}_2CH_2OP$, J=7.0 Hz), 0.92 (6H, t, $C\underline{H}_3CH_2CH_2OP$, J=7.3 Hz).

*Collapsed to a doublet on irradiation at δ1.68.

EIMS m/e 448.1692 (M^+, 0.3%, calc. for $C_{18}H_{29}N_2O_9P$ 448.1611), 263 (MH^+-AcOH-thymine, 5), 183 ($(PrO)_2P(OH)_2^+$, 1), 141 ($PrOP(OH)_3^+$, 2), 127 (thymineH^+, 1), 126 (thymine$^+$, 1), 99 ($C_5H_7O_2^+$, 7), 81 ($C_5H_5O^+$, base peak).

Found C 46.75%, H 6.57, N 5.69, P 6.71, $C_{18}H_{29}N_2O_9P.H_2O$ requires C 46.35, H 6.70, N 6.01, P 6.64.

3′-O-Acetylthymidine 5′-dibutyl phosphate (84).

Dibutyl phosphorochloridate (0.38 g, 1.69 mmol, 2 equivs.) was added to a solution of 3′-O-acetylthymidine (0.24 g, 0.85 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 17 h water (30 µl, 1.66 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform (400 ml), followed by chloroform-methanol (95:5). Pooling and evaporation of the appropriate fractions of the latter eluent gave the product as a colourless oil, (0.24 g, 61%).

^{31}P nmr δ($CDCl_3$) -2.58.

^{13}C nmr δ($CDCl_3$) 170.43 ($CH_3\underline{C}O$), 163.73 (C2), 150.61 (C4), 134.89 (C6), 111.82 (C5), 84.37 (C1′), 82.86 (d, C4′, J=8.3 Hz), 74.60 (C3′), 68.02, 67.92 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=5.6 Hz, J=5.6 Hz), 66.87 (d, C5′, J=5.4 Hz), 37.23 (C2′), 32.20 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=6.9 Hz), 20.85 ($\underline{C}H_3CO$), 18.56 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.51 ($C\underline{H}_3CH_2CH_2CH_2OP$), 12.35 (5-$CH_3$).

^{1}H nmr δ($CDCl_3$) 9.95 (1H, s, NH), 7.45 (1H, s, H6), 6.30 (1H, dd, H1′, J=7.6 Hz, J=6.8 Hz), 5.40 (1H, m, H3′), 4.31 (2H, m, H5′), 4.10 (1H, m, H4′), 4.00 (4H, q, $CH_3CH_2CH_2C\underline{H}_2OP$, J=6.8 Hz)*, 2.40 (1H, m, H2′), 2.14 (1H, m, H2′), 2.05 (3H, s, CH_3CO), 1.90 (3H, s, 5-CH_3), 1.60 (4H, m, $CH_3CH_2C\underline{H}_2CH_2OP$), 1.35 (4H, m, $CH_3C\underline{H}_2CH_2CH_2OP$), 0.92, 0.90 (6H, 2xt, $C\underline{H}_3CH_2CH_2CH_2OP$, J=7.2 Hz, J=7.1 Hz).

*Collapsed to a doublet on irradiation at δ1.60.

EIMS m/e 476.1885 (M^+, 0.4%, calc. for $C_{20}H_{33}N_2O_9P$ 476.1924), 291 (MH^+-AcOH-thymine, 11), 211 ($(BuO)_2P(OH)_2^+$, 3), 155 ($BuOP(OH)_3^+$, 3), 127 (thymineH^+, 3), 126 (thymine$^+$, 3), 99 ($C_5H_7O_2^+$, 32), 81 ($C_5H_5O^+$, base peak).

Found C 46.92%, H 7.02, N 5.66, P 6.41, $C_{20}H_{33}N_2O_9P(H_2O)_2$ requires C 46.87, H 7.28, N 5.47, P 6.04.

5′-O-Tritylthymidine (86).

Trityl chloride (1.27 g, 4.54 mmol, 1.1 equivs.) was added to a solution of thymidine (1.00 g, 4.13 mmol) in pyridine (30 ml) and the mixture was refluxed with stirring for 2 h. The solution was then cooled to ambient temperature, poured into iced-water (300 ml) and vigorously stirred for a further 1 h. The resulting suspension was extracted with chloroform (3x50 ml). The organic phase was dried with magnesium sulphate and the solvent removed under reduced pressure to give the product as a yellow solid. The yellow solid was recrystallised twice from benzene, (1.55 g, 76%), mp 159-160°C.

^{13}C nmr δ($CDCl_3$) 164.81 (C2), 150.97 (C4), 143.81 (Ph), 135.61 (C6), 128.51 (Ph), 128.09 (Ph), 127.40 (Ph), 112.21 (C5), 87.00 ($Ph_3\underline{C}$), 86.07 (C4′), 84.64 (C1′), 71.21 (C3′), 64.07 (C5′), 40.61 (C2′), 12.23 (5-CH_3).

^1H nmr δ($CDCl_3$) 9.88 (1H, bs, NH), 7.62 (1H, s, H6), 7.21 (15H, m, Ph), 6.48 (1H, t, H1′, J=6.3 Hz), 4.51 (1H, bs, 3′-OH), 4.13 (1H, m, H4′), 3.64 (1H, m, H3′), 3.42 (2H, m, H5′), 2.40 (1H, m, H2′), 2.25 (1H, m, H2′), 1.41 (3H, s, 5-CH_3).

3′-O-Ethylthymidine (85).

Ethyl iodide (1.13 g, 7.24 mmol, 2 equivs.) and potassium hydroxide powder (0.40 g, 7.14 mmol, 2 equivs.) were added to a solution of 5′-O-tritylthymidine (1.75 g, 3.62 mmol) in

benzene (17 ml) and dioxan (5 ml) and the suspension stirred at 70°C for 6 h. The solvent was then removed under reduced pressure. The residue was dissolved in methanol (3 ml) and water (6 ml) was added. The resulting suspension was extracted with chloroform (3x5 ml). Pooling and evaporation of the organic extracts gave an oil, which was heated to 60°C for 1 h in 80% acetic acid (15 ml). The solvent was then removed under reduced pressure and the residue purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a white solid, (0.69 g, 71%), mp 181-182°C. Analytical data were obtained on a small sample recrystallised from methanol.

uv (MeOH) λ_{max}=264 nm, λ_{min}=229 nm.

^{13}C nmr δ($CDCl_3$) 163.47 (C2), 150.19 (C4), 137.05 (C6), 111.04 (C5), 87.38 (C1′), 85.14 (C4′), 78.34 (C3′), 64.99 ($CH_3\underline{C}H_2O$), 62.86 (C5′), 37.09 (C2′), 15.29 ($\underline{C}H_3CH_2O$), 12.54 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 8.47 (1H, bs, NH), 7.34 (1H, d, H6, J=1.2 Hz), 6.10 (1H, t, H1′, J=6.8 Hz), 4.18 (1H, m, H3′)*, 4.05 (1H, m, H4′) 3.90, 3.75 (2H, 2xm, H5′), 3.51 (2H, m, $CH_3\underline{CH_2}O$)**, 2.53 (1H, dd, 5′-OH, J=6.9 Hz, J=5.0 Hz), 2.34 (2H, m, H2′), 1.90 (3H, d, 5-CH_3, J=1.2 Hz), 1.21 (3H, t, $\underline{C}H_3CH_2O$, J=7.0 Hz).

*Affected by irradiation at δ2.34. **Affected by irradiation at δ1.21.

Found C 53.26%, H 6.64, N 10.32, $C_{12}H_{18}N_2O_5$ requires C 53.33, H 6.71, N 10.36.

Pooling and evaporation of appropriate fractions corresponding to a faster running by-product gave a colourless oil, (0.11 g, 11%).

uv (MeOH) λ_{max}=265 nm, λ_{min}=233 nm.

^{13}C nmr δ($CDCl_3$) 163.18 (C2), 150.67 (C4), 134.88 (C6), 110.24 (C5), 87.93 (C1′), 85.14 (C4′), 78.75 (C3′), 64.95 ($CH_3\underline{C}H_2OC$), 62.83 (C5′), 37.18 (C2′), 36.46 ($CH_3\underline{C}H_2N$), 15.27 ($\underline{C}H_3CH_2OC$), 13.19 ($\underline{C}H_3CH_2N$), 12.75 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 7.31 (1H, d, H6, J=1.0 Hz), 6.07 (1H, t, H1′, J=7.2 Hz), 3.90 (7H, m, H3′, H4′, H5′, 5′-OH, $CH_3C\underline{H}_2N$), 3.43 (2H, m, $CH_3C\underline{H}_2OC$), 2.27 (2H, m, H2′), 1.85 (3H, d, 5-CH_3, J=1.0 Hz), 1.14 (3H, t, $C\underline{H}_3CH_2OC$, J=7.2 Hz)*, 1.12 (3H, t, $C\underline{H}_3CH_2N$, J=7.2 Hz)**.

*Collapsed to a singlet on irradiation at δ3.43. **Collapsed to a singlet on irradiation at δ3.90.

EIMS m/e 298 (M^+, 2%), 155 ($C_7H_{11}N_2O_2^+$, 31), 154 ($C_7H_{10}N_2O_2^+$, 37), 145 (MH^+-$C_7H_{10}N_2O_2$, 48), 126 (thymine$^+$, 33), 101 ($C_5H_9O_2^+$, base peak), 99 ($C_5H_7O_2^+$, 35).

3′-O-Ethylthymidine 5′-dipropyl phosphate (88).

Dipropyl phosphorochloridate (0.23 g, 1.14 mmol, 2 equivs.) was added to a solution of 3′-O-ethylthymidine (0.15 g, 0.55 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 17 h water (21 µl, 1.14 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.20 g, 81%).

^{31}P nmr δ($CDCl_3$) -2.59.

^{13}C nmr δ($CDCl_3$) 163.93 (C2), 150.47 (C4), 135.33 (C6), 111.31 (C5), 84.94 (C1′), 82.90

(d, C4′, J=7.7 Hz), 78.68 (C3′), 69.61, 69.60 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=5.5 Hz, J=5.4 Hz), 66.95 (d, C5′, J=5.4 Hz), 65.03 ($CH_3\underline{C}H_2OC$), 37.48 (C2′), 23.64 (d, $CH_3\underline{C}H_2CH_2OP$, J=7.0 Hz), 15.22 ($\underline{C}H_3CH_2OC$), 12.43 (5-$CH_3$), 9.95 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 9.41 (1H, s, NH), 7.38 (1H, d, H6, J=1.2 Hz), 6.25 (1H, dd, H1′, J=8.0 Hz, J=5.9 Hz), 4.10 (4H, m, H3′, H4′, H5′), 3.95 (4H, q, $CH_3CH_2C\underline{H}_2OP$, J=6.6 Hz), 3.41 (2H, m, $CH_3C\underline{H}_2OC$), 2.30 (1H, m, H2′), 1.96 (1H, m, H2′), 1.85 (3H, d, 5-CH_3, J=1.2 Hz), 1.60 (4H, sextet, $CH_3C\underline{H}_2CH_2OP$, J=6.4 Hz), 1.11 (3H, t, $C\underline{H}_3CH_2OC$, J=7.0 Hz), 0.87, 0.85 (6H, 2xt, $C\underline{H}_3CH_2CH_2OP$, J=7.0 Hz, J=7.3 Hz).

EIMS m/e 434.1787 (M^+, 0.6%, calc. for $C_{18}H_{31}N_2O_8P$ 434.1818), 309 (MH^+-thymine, 1), 263 (MH^+-EtOH-thymine, 39) 183 ($(PrO)_2P(OH)_2^+$, 6), 126 (thymine$^+$, 25), 99 ($C_5H_7O_2^+$, 71), 81 ($C_5H_5O^+$, base peak).

Found C 48.85%, H 6.88, N 6.10, P 6.89, $C_{18}H_{31}N_2O_8P(H_2O)_{0.35}$ requires C 49.05, H 7.25, N 6.36, P 7.03.

3′-O-Ethylthymidine 5′-dibutyl phosphate (89).

Dibutyl phosphorochloridate (0.26 g, 1.14 mmol, 2 equivs.) was added to a solution of 3′-O-ethylthymidine (0.15 g, 0.56 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 17 h water (20 µl, 1.14 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.19 g, 75%).

^{31}P nmr δ($CDCl_3$) -2.63.

^{13}C nmr δ($CDCl_3$) 163.92 (C2), 150.45 (C4), 135.32 (C6), 111.31 (C5), 84.91 (C1′), 82.89 (d, C4′, J=8.1 Hz), 78.66 (C3′), 67.93, 67.97 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=5.6 Hz, J=5.6 Hz), 66.88 (d, C5′, J=5.4 Hz), 65.01 ($CH_3\underline{C}H_2OC$), 37.46 (C2′), 32.23 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=6.9 Hz), 18.61 ($CH_3\underline{C}H_2CH_2CH_2OP$), 15.21 ($\underline{C}H_3CH_2OC$), 13.52 ($\underline{C}H_3CH_2CH_2CH_2OP$), 12.43 (5-$CH_3$).

^{1}H nmr δ($CDCl_3$) 9.40 (1H, bs, NH), 7.43 (1H, d, H6, J=1.2 Hz), 6.29 (1H, dd, H1′, J=8.0 Hz, J=6.1 Hz), 4.20 (4H, m, H3′, H4′, H5′), 4.03 (4H, q, $CH_3CH_2CH_2\underline{CH_2}OP$, J=6.9 Hz), 3.41 (2H, m, $CH_3\underline{CH_2}OC$), 2.35 (1H, m, H2′), 2.10 (1H, m, H2′), 1.89 (3H, d, 5-CH_3, J=1.2 Hz), 1.62 (4H, m, $CH_3CH_2\underline{CH_2}CH_2OP$), 1.40 (4H, m, $CH_3\underline{CH_2}CH_2CH_2OP$), 1.16 (3H, t, $\underline{CH_3}CH_2OC$, J=6.9 Hz), 0.88, 0.87 (6H, 2xt, $\underline{CH_3}CH_2CH_2CH_2OP$, J=7.1 Hz, J=7.2 Hz).

EIMS m/e 462 (M^+, 0.3%), 291 (MH^+-EtOH-thymine, 19), 126 (thymine$^+$, 7), 99 ($C_5H_7O_2^+$, 30), 81 ($C_5H_5O^+$, base peak).

Found C 51.42%, H 7.49, N 6.05, P 6.83, $C_{20}H_{35}N_2O_8P(H_2O)_{0.2}$ requires C 51.54, H 7.66, N 6.01, P 6.65.

3′-Azido-3′-deoxythymidine (1).

Sodium azide (1.61 g, 24.8 mmol, 6 equivs.) was added to a solution of 1-(2-deoxy-3-O-mesyl-ß-D-threopentofuranosyl)thymine (1.00 g, 4.13 mmol) in dimethylformamide (20 ml), under an atmosphere of nitrogen, with stirring. After heating at 100°C for 4 h, the suspension was cooled and the solvent removed under reduced pressure. The residue was dissolved in chloroform (40 ml) and washed with water (5x20 ml). The organic phase was dried with magnesium sulphate and the solvent removed under reduced pressure. The resultant oil was purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a white solid,

(0.65 g, 59%), mp 119-120°C.

^{13}C nmr δ($CDCl_3$) 164.42 (C2), 150.58 (C4), 136.45 (C6), 111.08 (C5), 85.39 (C1'), 84.58 (C4'), 61.41 (C5'), 59.91 (C3'), 37.56 (C2'), 12.29 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 8.54 (1H, bs, NH), 7.35 (1H, d, H6, J=1.2 Hz), 6.04 (1H, t, H1', J=6.6 Hz), 4.39 (1H, m, H3')*, 3.85 (3H, m, H4', H5'), 2.55 (1H, m, H2'), 2.38 (2H, m, H2', 5'-OH), 1.90 (3H, d, 5-CH_3, J=1.2 Hz).

*Affected by irradiation at δ2.42.

EIMS m/e 267.1003 (M^+, 2%, calc. for $C_{10}H_{13}N_5O_4$ 267.0967), 206 (M^+-N_3H-H_2O, 1), 142 (MH^+-thymine, 96), 127 (thymineH^+, 38), 126 (thymine$^+$, base peak).

Found C 44.81%, H 4.63, N 25.84, $C_{10}H_{13}N_5O_4$ requires C 44.94, H 4.90, N 26.20.

3'-Azido-3'-deoxythymidine 5'-diethyl phosphate (91).

Diethyl phosphorochloridate (0.28 g, 1.61 mmol, 2 equivs.) was added to a solution of 3'-azido-3'-deoxythymidine (0.21 g, 0.80 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 6 h water (29 µl, 1.61 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform (500 ml), followed by chloroform-methanol (97:3). Pooling and evaporation of appropriate fractions of the latter eluent gave the product as a colourless oil, (0.24 g, 76%).

^{31}P nmr δ($CDCl_3$) -2.82.

^{13}C nmr δ($CDCl_3$) 163.97 (C2), 150.40 (C4), 135.05 (C6), 111.36 (C5), 84.55 (C1'), 82.14 (d, C4', J=7.8 Hz), 65.93 (d, C5', J=5.5 Hz), 64.25 (d, $CH_3\underline{C}H_2OP$, J=5.2 Hz), 59.97 (C3'),

37.39 (C2′), 16.03 (d, $\underline{C}H_3CH_2OP$, J=6.6 Hz), 12.30 (5-CH_3).

^1H nmr δ($CDCl_3$) 9.98 (1H, bs, NH), 7.39 (1H, d, H6, J=1.1 Hz) 6.22 (1H, t, H1′, J=6.5 Hz), 4.32 (1H, m, H3′)*, 4.23 (2H, m, H5′), 4.12 (4H, quintet, $CH_3C\underline{H}_2OP$, J=7.4 Hz), 3.99 (1H, m, H4′), 2.40 (1H, m, H2′), 2.28 (1H, m, H2′), 1.89 (3H, d, 5-CH_3), 1.30, 1.29 (6H, 2xt, $C\underline{H}_3CH_2OP$, J=7.0 Hz, J=7.0 Hz).

*Collapsed to a doublet on irradiation at δ2.35.

EIMS m/e 403.1226 (M^+, 0.6%, calc. for $C_{14}H_{22}N_5O_7P$ 403.1256), 235 (MH^+-N_3H-thymine, 5), 155 ($(EtO)_2P(OH)_2^+$, 2), 127 (thymineH^+, 5), 126 (thymine$^+$, 4), 99 ($C_5H_7O_2^+$, 9), 81 ($C_5H_5O^+$, base peak).

Found C 39.53%, H 5.53, N 16.96, P 7.30, $C_{14}H_{22}N_5O_7P.H_2O$ requires C 39.91, H 5.74, N 16.62, P 7.35.

3′-Azido-3′-deoxythymidine 5′-dipropyl phosphate (92).

Dipropyl phosphorochloridate (0.27 g, 1.35 mmol, 2 equivs.) was added to a solution of 3′-azido-3′-deoxythymidine (0.18 g, 0.67 mmol) in pyridine (10 ml), with stirring at ambient temperature. After 17 h water (24 μl, 1.35 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform (500 ml), followed by chloroform-methanol (97:3). Pooling and evaporation of appropriate fractions of the latter eluent gave the product as a colourless oil, (0.23 g, 72%).

^{31}P nmr δ($CDCl_3$) -2.90.

^{13}C nmr δ($CDCl_3$) 163.94 (C2), 150.39 (C4), 135.03 (C6), 111.40 (C5), 84.56 (C1′), 82.17

(d, C4′, J=7.9 Hz), 67.80, 67.74 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=5.7 Hz, J=5.7 Hz), 65.99 (d, C5′, J=5.6 Hz), 60.03 (C3′), 37.40 (C2′), 23.50 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.7 Hz), 12.32 (5-$CH_3$), 9.82 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 9.90 (1H, bs, NH), 7.39 (1H, s, H6), 6.20 (1H, t, H1′, J=6.7 Hz), 4.31 (1H, m, H3′), 4.21 (2H, m, H5′), 3.98 (5H, m, H4′, $CH_3CH_2C\underline{H}_2OP$), 2.39 (1H, m, H2′), 2.26 (1H, m, H2′), 1.88 (3H, s, 5-CH_3), 1.64 (4H, sextet, $CH_3C\underline{H}_2CH_2OP$, J=6.5 Hz), 0.89, 0.88 (6H, 2xt, $C\underline{H}_3CH_2CH_2OP$, J=6.8 Hz, J=6.8 Hz).

EIMS m/e 431.1591 (M^+, 0.4%, calc. for $C_{16}H_{26}N_5O_7P$ 431.1570), 263 (MH^+-N_3H-thymine, 3), 183 ($(PrO)_2P(OH)_2^+$, 1), 141 ($PrOP(OH)_3^+$, 3), 127 (thymineH^+, 1), 126 (thymine$^+$, 4), 99 ($C_5H_7O_2^+$, 18), 81 ($C_5H_5O^+$, base peak).

Found C 41.80%, H 6.05, N 15.27, $C_{16}H_{26}N_5O_7P(H_2O)_{1.5}$ requires C 41.92, H 6.38, N 15.28.

3′-Azido-3′-deoxythymidine 5′-dibutyl phosphate (93).

Dibutyl phosphorochloridate (0.44 g, 1.93 mmol, 2 equivs.) was added to a solution of 3′-azido-3′-deoxythymidine (0.26 g, 0.96 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 17 h water (35 μl, 1.93 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform (300 ml), followed by chloroform-methanol (97:3). Pooling and evaporation of appropriate fractions of the latter eluent gave the product as a colourless oil, (0.29 g, 64%).

^{31}P nmr δ($CDCl_3$) -2.67.

^{13}C nmr δ($CDCl_3$) 163.94 (C2), 150.37 (C4), 134.91 (C6), 111.20 (C5), 84.40 (C1′), 82.01 (d, C4′, J=7.9 Hz), 67.78, 67.76 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=5.8 Hz, J=5.8 Hz), 65.89 (d,

C5′, J=5.5 Hz), 59.93 (C3′), 37.20 (C2′), 31.94 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=7.5 Hz), 18.34 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.25 ($\underline{C}H_3CH_2CH_2CH_2OP$), 12.17 (5-$CH_3$).

^{1}H nmr δ($CDCl_3$) 10.14 (1H, s, NH), 7.35 (1H, d, H6, J=1.1 Hz), 6.18 (1H, t, H1′, J=6.5 Hz), 4.29 (1H, m, H3′), 4.19 (2H, m, H5′), 4.01 (4H, q, $CH_3CH_2CH_2\underline{C}H_2OP$, J=6.9 Hz), 3.96 (1H, m, H4′), 2.35 (1H, m, H2′), 2.25 (1H, m, H2′), 1.84 (3H, d, 5-CH_3, J=1.1 Hz), 1.62 (4H, m, $CH_3CH_2\underline{C}H_2CH_2OP$), 1.32 (4H, m, $CH_3\underline{C}H_2CH_2CH_2OP$), 0.85, 0.83 (6H, 2xt, $\underline{C}H_3CH_2CH_2CH_2OP$, J=7.0 Hz, J=7.0 Hz).

EIMS m/e 459.1805 (M^+, 4%, calc. for $C_{18}H_{30}N_5O_7P$ 459.1875), 291 (MH^+-N_3H-thymine, 7), 211 ($(BuO)_2P(OH)_2^+$, 2), 155 ($BuOP(OH)_3^+$, 4), 127 (thymineH^+, 4), 126 (thymine$^+$, 8), 99 ($C_5H_7O_2^+$, 44), 81 ($C_5H_5O^+$, base peak).

Found C 46.85%, H 6.27, N 14.59, P 6.34, $C_{18}H_{30}N_5O_7P(H_2O)_{0.35}$ requires C 46.42, H 6.64, N 15.04, P 6.65.

2′,3′-O-Isopropylideneadenosine 5′-dibutyl phosphate (95).

Dibutyl phosphorochloridate (0.44 g, 1.93 mmol, 2 equivs.) was added to a solution of 2′,3′-O-isopropylideneadenosine (0.30 g, 0.98 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 17 h water (34 μl, 1.93 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave a colourless oil, (0.24 g).

^{31}P nmr δ($CDCl_3$) 1.04 (minor), -2.78 (major).

This product was further purified by chromatography on silica, with elution by chloroform-

methanol (98:2). Pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.19 g, 57%).

^{31}P nmr $\delta(CDCl_3)$ -2.72.

^{13}C nmr $\delta(CDCl_3)$ 155.71 (C6), 153.20 (C2), 149.24 (C4), 139.40 (C8), 120.03 (C5), 112.72 ($(CH_3)_2\underline{C}$), 90.87 (C1′), 85.30 (d, C4′, J=8.1 Hz), 84.23 (C2′ or C3′), 81.89 (C3′ or C2′), 67.72 (d, $CH_3CH_2CH_2\underline{C}H_2OP$, J=6.1 Hz), 66.67 (d, C5′, J=6.2 Hz), 32.12 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=6.7 Hz), 27.10, 25.28 (2xs, $(\underline{C}H_3)_2C$), 18.55 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.50 ($\underline{C}H_3CH_2CH_2CH_2OP$).

^{1}H nmr $\delta(CDCl_3)$ 8.30 (1H, s, H8), 7.94 (1H, s, H2), 6.21 (2H, bs, NH_2), 6.11 (1H, d, H1′, J=2.2 Hz), 5.36 (1H, dd, H2′, J=6.2 Hz, J=2.2 Hz)*, 5.05 (1H, dd, H3′, J=6.2 Hz, J=2.9 Hz)**, 4.23 (1H, m, H4′), 4.17 (2H, m, H5′), 3.91 (4H, q, $CH_3CH_2CH_2\underline{CH_2}OP$, J=7.0 Hz), 1.57 (3H, s, $(CH_3)_2C$), 1.51 (4H, m, $CH_3CH_2\underline{CH_2}CH_2OP$), 1.34 (3H, s, $(CH_3)_2C$), 1.30 (4H, m, $CH_3\underline{CH_2}CH_2CH_2OP$), 0.84, 0.82 (6H, 2xt, $\underline{CH_3}CH_2CH_2CH_2OP$, J=6.9 Hz, J=6.9 Hz).
*Collapsed to a doublet on irradiation at δ6.11. **Collapsed to a doublet on irradiation at δ4.23.

Found C 49.03%, H 6.66, N 13.53, P 5.88, $C_{21}H_{34}N_5O_7P.H_2O$ requires C 48.74, H 7.01, N 13.53, P 5.99.

Attempted synthesis of 2′,3′-dideoxyadenosine 5′-dibutyl phosphate.

Dibutyl phosphorochloridate (0.38 g, 1.66 mmol, 1.9 equivs.) was added to a suspension of 2′,3′-dideoxyadenosine (0.21 g, 0.89 mmol) in pyridine (30 ml), with stirring at ambient temperature. After stirring the resulting solution for a further 7 h water (30 µl, 1.66 mmol) was added and the solvent removed under reduced pressure. The residue was purified by

chromatography on silica, with elution by chloroform (500 ml), followed by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions of the latter eluent gave a colourless oil, (0.22 g, 58%).

^{31}P nmr $\delta(CDCl_3)$ -2.32, -2.37.

^{13}C nmr $\delta(CDCl_3)$ 155.33 (C6), 152.91, 152.84 (C2), 149.22, 149.21 (C4), 138.77, 138.72 (C8), 120.25, 120.05 (C5), 86.36, 85.53 (C1'), 79.59, 79.12 (2xd, C4', J=7.6 Hz, J=7.6 Hz), 68.47, 67.92 (2xd, C5', J=5.9 Hz, J=5.7 Hz), 67.77, 67.70 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=6.0 Hz, J=5.2 Hz), 32.47 (C2'), 32.21, 32.19 (2xd, $CH_3CH_2\underline{C}H_2CH_2OP$, J=6.9 Hz, J=6.9 Hz), 31.80 (C2'), 26.28, 25.82 (C3'), 18.63, 18.59 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.55 ($\underline{C}H_3CH_2CH_2CH_2OP$).

^{1}H nmr $\delta(CDCl_3)$ 8.32 (2H, s, H8), 8.11 (1H, s, H2), 7.88 (1H, s, H2), 6.30 (2H, m, H1'), 5.84 (2H, bs, NH_2), 5.74 (2H, bs, NH_2), 4.66 (1H, m, H4'), 4.37 (1H, m, H4'), 4.18 (2H, m, H5'), 4.14 (2H, m, H5'), 4.02 (8H, m, $CH_3CH_2CH_2\underline{CH_2}OP$), 2.58 (4H, m, H2'), 2.33 (1H, m, H3'), 2.17 (2H, m, H3'), 2.03 (1H, m, H3'), 1.62 (8H, m, $CH_3CH_2\underline{CH_2}CH_2OP$), 1.37 (8H, m, $CH_3\underline{CH_2}CH_2CH_2OP$), 0.89 (12H, m, $\underline{CH_3}CH_2CH_2CH_2OP$).

EIMS m/e 427 (M^+, 0.2%), 293 (MH^+-adenine, 10), 211 ($(BuO)_2P(OH)_2^+$, 17), 155 ($BuOP(OH)_3^+$, 28), 135 (adenine$^+$, 59), 99 ($C_5H_7O_2^+$, base peak), 81 ($C_5H_5O^+$, 36).

The two components of this colourless oil were separated by preparative hplc.

Preparative hplc stationary phase 50+250 mm x 4.6 mm Lichrosob column, mobile phase chloroform-methanol-triethylamine (97.4:2.5:0.1), isocratic conditions, flow rate 4.0 ml/min. Retention times 10.34 min, 10.47 min.

Product 1, retention time 10.34 min.

^{1}H nmr δ($CDCl_3$) 8.34 (1H, s, H8), 8.11 (1H, s, H2), 6.29 (1H, t, H1′, J=5.1 Hz), 5.60 (2H, bs, NH_2), 4.35 (1H, m, H4′), 4.18 (2H, m, H5′), 4.00, 3.99 (4H, 2xq, $CH_3CH_2CH_2C\underline{H}_2OP$, J=6.5 Hz, J=6.5 Hz), 2.54 (2H, m, H2′), 2.16 (2H, m, H3′), 1.66 (4H, m, $CH_3CH_2C\underline{H}_2CH_2OP$), 1.33 (4H, m, $CH_3C\underline{H}_2CH_2CH_2OP$), 0.89, 0.87 (6H, 2xt, $C\underline{H}_3CH_2CH_2CH_2OP$, J=7.0 Hz, J=7.1 Hz).

EIMS m/e 428 (MH^+, 2%), 427.1970 (M^+, 5, calc. for $C_{18}H_{30}N_5O_5P$ 427.1983), 293 (MH^+-adenine, 47), 211 ($(BuO)_2P(OH)_2^+$, 10), 136 (adenineH^+, 25), 135 (adenine$^+$, 60), 99 ($C_5H_7O_2^+$, 91), 84 (base peak).

Product 2, retention time 10.47 min.

^{1}H nmr δ($CDCl_3$) 8.34 (1H, s, H8), 7.89 (1H, s, H2), 6.32 (1H, dd, H1′, J=7.5 Hz, J=4.9 Hz), 5.58 (2H, bs, NH_2), 4.70 (1H, m, H4′), 4.15 (2H, m, H5′), 4.04, 4.03 (4H, 2xq, $CH_3CH_2CH_2C\underline{H}_2OP$, J=7.5 Hz, J=7.7 Hz), 2.60 (2H, m, H2′), 2.34 (1H, m, H3′), 2.08 (1H, m, H3′), 1.65 (4H, m, $CH_3CH_2C\underline{H}_2CH_2OP$), 1.36 (4H, m, $CH_3C\underline{H}_2CH_2CH_2OP$), 0.91 (6H, t, $C\underline{H}_3CH_2CH_2CH_2OP$, J=7.0 Hz).

EIMS m/e 428 (MH^+, 0.1%), 427.2028 (M^+, 0.1, calc. for $C_{18}H_{30}N_5O_5P$ 427.1983), 293 (MH^+-adenine, 85), 211 ($(BuO)_2P(OH)_2^+$, 3), 136 (adenineH^+, 40), 135 (adenine$^+$, 32), 99 ($C_5H_7O_2^+$, 73), 83 (base peak).

2′,3′-Dideoxyadenosine 5′-dipropyl phosphate (98).

Dipropyl phosphorochloridate (0.25 g, 1.27 mmol, 2 equivs.) was added to a solution of 2′,3′-dideoxyadenosine (0.15 g, 0.64 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 6 h water (22 μl, 1.22 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by

chloroform-methanol-triethylamine (95:3:2). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.19 g, 75%).

^{31}P nmr δ($CDCl_3$) -2.71.

^{13}C nmr δ($CDCl_3$) 155.63 (C6), 152.84 (C2), 148.51 (C4), 138.61 (C8), 119.69 (C5), 85.50 (C1′), 79.46 (d, C4′, J=8.0 Hz), 69.52 (d, $CH_3CH_2\underline{C}H_2OP$, J=5.8 Hz), 67.91 (d, C5′, J=5.3 Hz), 32.57 (C2′), 25.70 (C3′), 23.65 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.8 Hz), 9.94 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 8.31 (1H, s, H8), 8.19 (1H, s, H2), 6.40 (2H, bs, NH_2), 6.33 (1H, t, H1′, J=7.1 Hz), 4.39 (1H, m, H4′), 4.21 (2H, m, H5′), 3.93, 3.91 (4H, 2xq, $CH_3CH_2C\underline{H}_2OP$, J=7.0 Hz, J=7.0 Hz), 2.50 (2H, m, H2′)*, 2.17 (2H, m, H3′), 1.78 (4H, m, $CH_3C\underline{H}_2CH_2OP$), 0.90 (6H, m, $C\underline{H}_3CH_2CH_2OP$).

*Affected by irradiation at δ6.33.

EIMS m/e 399.1761 (M^+, 0.1%, calc. for $C_{16}H_{26}N_5O_5P$ 399.1671), 265 (MH^+-adenine, 19), 183 ($(PrO)_2P(OH)_2^+$, 4), 141 ($PrOP(OH)_3^+$, 13), 136 (adenineH^+, 25), 135 (adenine$^+$, 27), 99 ($C_5H_7O_2^+$, base peak).

Found C 44.94%, H 6.81, P 7.05, $C_{16}H_{26}N_5O_5P(H_2O)_{1.5}$ requires C 45.07, H 6.86, P 7.26.

2′,3′-Dideoxycytidine 5′-dibutyl phosphate (99).

Dibutyl phosphorochloridate (0.43 g, 1.89 mmol, 2 equivs.) was added to a solution of 2′,3′-dideoxycytidine (0.20 g, 0.95 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 7 h water (34 µl, 1.89 mmol) was added and the solvent removed under reduced pressure. Analysis of the resulting residue by tlc revealed two major components. The residue was purified by chromatography on silica, with elution by chloroform-methanol

(90:10). Pooling and evaporation of appropriate fractions gave the product as a white solid, (0.18 g, 49%), mp 102-104°C.

^{31}P nmr $\delta(CDCl_3)$ -2.57.

^{13}C nmr $\delta(CDCl_3)$ 165.74 (C4), 155.86 (C2), 140.34 (C6), 94.25 (C5), 87.06 (C1'), 79.01 (d, C4', J=7.7 Hz), 67.76 (d, C5', J=6.9 Hz), 67.74, 67.69 (2xd, $CH_3CH_2CH_2\underline{C}H_2OP$, J=6.2 Hz, J=6.1 Hz), 32.69 (C2'), 32.10 (d, $CH_3CH_2\underline{C}H_2CH_2OP$, J=6.6 Hz), 25.08 (C3'), 18.51 ($CH_3\underline{C}H_2CH_2CH_2OP$), 13.44 ($\underline{C}H_3CH_2CH_2CH_2OP$).

^{1}H nmr $\delta(CDCl_3)$ 7.70 (1H, d, H6, J=7.5 Hz), 6.03 (1H, dd, H1', J=6.5 Hz, J=3.5 Hz), 5.79 (1H, d, H5, J=7.5 Hz), 4.21 (2H, m, H5'), 4.12 (1H, m, H4'), 4.00 (4H, q, $CH_3CH_2CH_2\underline{C}H_2OP$, J=7.0 Hz), 2.31 (1H, m, H2')*, 1.94 (2H, m, H2', H3')*, 1.82 (1H, m, H3'), 1.60 (4H, quintet, $CH_3CH_2\underline{C}H_2CH_2OP$, J=7.2 Hz), 1.36 (4H, sextet, $CH_3\underline{C}H_2CH_2CH_2OP$, J=7.0 Hz), 0.87, 0.86 (6H, 2xt, $\underline{C}H_3CH_2CH_2CH_2OP$, J=7.1 Hz, J=7.2 Hz).

*Affected by irradiation at δ6.03.

FABMS m/e 404 (MH^+, 13%), 293 (MH^+-cytosine, base peak), 112 (cytosineH^+, 86), 99 ($C_5H_7O_2^+$, 43).

Found C 49.64%, H 7.72, N 9.77, P 7.33, $C_{17}H_{30}N_3O_6P(H_2O)_{0.5}$ requires C 49.51, H 7.58, N 10.19, P 7.51.

Analytical hplc stationary phase 50+250 mm x 4.6 mm spherisorb CN 5 μM column, mobile phase A=water B=acetonitrile-water (95:5), gradient conditions 18% B 0 min, 18% B 10 min, 82% B 30 min, flow rate 2 ml/min. Retention time 15.42 min.

Bis(2,2,2-trifluoroethyl) phosphorochloridate (103).

A solution of 2,2,2-trifluoroethanol (5.44 g, 53 mmol, 2.05 equivs.) and triethylamine (5.30 g, 53 mmol, 2.05 equivs.) in diethyl ether (50 ml) was added dropwise to a solution of phosphoryl chloride (4.11 g, 26 mmol) in diethyl ether (200 ml), with vigorous stirring at -40°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After a further 17 h, the reaction mixture was filtered and the solvent removed from the filtrate under reduced pressure. The residue was purified by vacuum distillation to give the product as a colourless liquid, (4.88 g, 65%), bp 40-42°C at 1 mmHg.

^{31}P nmr $\delta(CDCl_3)$ 5.61.

^{13}C nmr $\delta(CDCl_3)$ 122.05 (qd, $\underline{C}F_3CH_2OP$, J=273.7 Hz, J=11.0 Hz), 64.72 (qd, $CF_3\underline{C}H_2OP$, J=39.0 Hz, J=5.5 Hz).

^{1}H nmr $\delta(CDCl_3)$ 4.51 (4H, m, CF_3CH_2OP).

3′-Azido-3′-deoxythymidine 5′-bis(2,2,2-trifluoroethyl) phosphate (101).

Bis(2,2,2-trifluoroethyl) phosphorochloridate (0.42 g, 1.49 mmol, 2 equivs.) was added to a solution of 3′-azido-3′-deoxythymidine (0.20 g, 0.75 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 4 h water (27 µl, 1.49 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (97:3). Pooling and evaporation of the appropriate fractions of the latter eluent gave the product as a white solid, (0.24 g, 63%), mp 62-65°C.

^{31}P nmr $\delta(CDCl_3)$ -4.85.

^{13}C nmr δ($CDCl_3$) 163.99 (C2), 150.37 (C4), 135.53 (C6), 121.10 (qd, $\underline{C}F_3CH_2OP$, J=277.6 Hz, J=8.9 Hz), 111.61 (C5), 85.60 (C1'), 81.70 (d, C4', J=7.9 Hz), 67.54 (d, C5', J=5.8 Hz), 64.14 (qd, $CF_3\underline{C}H_2OP$, J=38.2 Hz, J=4.1 Hz), 59.86 (C3'), 37.03 (C2'), 12.16 (5-CH_3).

^1H nmr δ($CDCl_3$) 9.90 (1H, bs, NH), 7.22 (1H, s, H6), 6.12 (1H, t, H1', J=6.3 Hz), 4.41 (4H, quintet, CF_3CH_2OP, J=7.8 Hz), 4.36 (3H, m, H3', H5'), 4.01 (1H, m, H4'), 2.41 (2H, m, H2'), 1.82 (3H, s, 5-CH_3).

EIMS m/e 511.0629 (M^+, 1%, calc. for $C_{14}H_{16}F_6N_5O_7P$ 511.0691), 263 ($(CF_3CH_2O)_2P(OH)_2^+$, 2), 126 (thymine$^+$, 43), 81 ($C_5H_5O^+$, base peak).

Found C 32.33%, H 3.07, N 13.18, P 6.22, $C_{14}H_{16}F_6N_5O_7P(H_2O)_{0.6}$ requires C 32.21, H 3.22, N 13.41, P 5.93.

Analytical hplc stationary phase 50+250 mm x 4.6 mm partisil 5 μM column, mobile phase ethyl acetate-petroleum spirit (60-80) (4:1), isocratic conditions, flow rate 2 ml/min. Retention time 4.66 min.

3'-Azido-3'-deoxythymidine 5'-bis(2,2,2-trichloroethyl) phosphate (102).

Bis(2,2,2-trichloroethyl) phosphorochloridate (0.57 g, 1.49 mmol, 2 equivs.) was added to a solution of 3'-azido-3'-deoxythymidine (0.20 g, 0.75 mmol) in pyridine (30 ml), with stirring at ambient temperature. After 5 h water (27 μl, 1.49 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform. Pooling and evaporation of appropriate fractions gave the product as a white solid, (0.27g, 59%), mp 55-57°C.

^{31}P nmr δ($CDCl_3$) -4.01.

^{13}C nmr δ($CDCl_3$) 163.81 (C2), 150.31 (C4), 135.48 (C6), 111.70 (C5), 94.38 (d, $\underline{C}Cl_3CH_2OP$, J=10.7 Hz), 85.50 (C1'), 81.77 (d, C4', J=8.5 Hz), 67.76 (d, C5', J=5.1 Hz), 60.05 (C3'), 37.20 (C2'), 12.57 (5-CH_3).

^{13}C nmr δ(CH_3OD) 165.19 (C2), 151.11 (C4), 137.04 (C6), 111.14 (C5), 95.00 (d, $\underline{C}Cl_3CH_2OP$, J=10.9 Hz), 85.83 (C1'), 82.27 (d, C4', J=7.5 Hz), 77.52 (d, $CCl_3\underline{C}H_2OP$, J=4.1 Hz), 68.56 (d, C5', J=5.9 Hz), 60.59 (C3'), 36.58 (C2'), 11.82 (5-CH_3).

^1H nmr δ($CDCl_3$) 9.67 (1H, bs, NH), 7.28 (1H, s, H6), 6.15 (1H, t, H1', J=6.7 Hz), 4.45 (2H, m, H5'), 4.38 (1H, m, H3'), 4.04 (1H, m, H4'), 2.49 (2H, m, H2'), 1.91 (3H, s, 5-CH_3).

FABMS m/e 614 (MH^+, $3x^{37}Cl$, 2%), 612 (MH^+, $2x^{37}Cl$, 4), 611 (MH_2^+, $1x^{37}Cl$, 1), 610 (MH^+, $1x^{37}Cl$, 6), 609 (MH_2^+, 2), 608 (MH^+, 3), 250 (MH^+-$(CCl_3CH_2O)_2PO_2H$, 7), 207 (MH^+-N_3H-$(CCl_3CH_2O)_2PO_2H$, 13), 127 (thymineH^+, 28), 81 ($C_5H_5O^+$, base peak).

Found C 27.28%, H 2.61, P 4.95, $C_{14}H_{16}Cl_6N_5O_7P$ requires C 27.57, H 2.64, P 5.07.

Analytical hplc stationary phase 50+250 mm x 4.6 mm partisil 5 μM column, mobile phase ethyl acetate-petroleum spirit (60-80) (4:1), isocratic conditions, flow rate 2 ml/min. Retention time 4.32 min.

The reaction of 2',3'-dideoxycytidine (2) with bis(2,2,2-trifluoroethyl) phosphorochloridate (103).

Bis(2,2,2-trifluoroethyl) phosphorochloridate (0.53 g, 1.88 mmol, 2 equivs.) was added to a solution of 2',3'-dideoxycytidine (0.20 g, 0.94 mmol) in pyridine (25 ml), with stirring at -20°C. After stirring at this temperature for 4 h, the reaction mixture was allowed to

warm to ambient temperature. After a further 2 h water (34 µl, 1.89 mmol) was added and the solvent removed under reduced pressure. Analysis of the resulting residue by tlc revealed two major components. The residue was purified by chromatography on silica, with elution by chloroform-methanol (90:10). Pooling and evaporation of appropriate fractions corresponding to the slower running component gave a white solid, (0.14 g, 33%), mp 72-74°C.

^{31}P nmr δ($CDCl_3$) -5.05.

^{13}C nmr δ($CDCl_3$) 165.48 (C4), 155.66 (C2), 140.63 (C6), 122.16 (qd, $\underline{C}F_3CH_2OP$, J=270.2 Hz, J=8.1 Hz), 93.91 (C5), 87.41 (C1'), 78.60 (d, C4', J=7.4 Hz), 69.52 (d, C5', J=5.8 Hz), 64.05 (qd, $CF_3\underline{C}H_2OP$, J=38.1 Hz, J=3.8 Hz), 32.60 (C2'), 25.00 (C3').

^{1}H nmr δ($CDCl_3$) 7.69 (1H, d, H6, J=7.4 Hz), 6.08 (1H, dd, H1', J=6.6 Hz, J=3.8 Hz), 5.78 (1H, d, H5, J=7.5 Hz), 4.43 (5H, m, H4', CF_3CH_2OP), 4.30 (2H, m, H5'), 2.50 (1H, m, H2'), 2.06 (2H, m, H2', H3'), 1.80 (1H, m, H3').

FABMS m/e 456 (MH^+, 18), 345 (MH^+-cytosine, 7), 263 ($(CF_3CH_2O)_2P(OH)_2^+$, 5), 112 (cytosineH^+, base peak).

Found C 34.54%, H 3.64, N 8.83, P 6.84, $C_{13}H_{16}F_6N_3O_6P$ requires C 34.30, H 3.54, N 9.23, P 6.80.

Pooling and evaporation of appropriate fractions corresponding to the faster running component gave a white solid, (0.11 g, 26%), mp 61-62°C.

^{31}P nmr δ($CDCl_3$) 2.59, -4.81.

^{13}C nmr δ($CDCl_3$) 160.65 (d, C4, J=6.1 Hz), 148.25 (d, C2, J=2.8 Hz), 140.24 (C6), 122.44, 122.41 (2xqd, $\underline{C}F_3CH_2OPO_2$, $\underline{C}F_3CH_2OPONH$, J=278.0 Hz, J=10.0 Hz, J=276.2 Hz, J=8.9 Hz), 101.32 (d, C5, J=7.9 Hz), 87.04 (C1'), 78.97 (d, C4', J=7.6 Hz), 69.10 (d, C5', J=5.7 Hz), 64.10, 63.89 (2xqd, $CF_3\underline{C}H_2OPO_2$, $CF_3\underline{C}H_2OPONH$, J=37.9 Hz, J=4.4 Hz, J=37.6 Hz, J=4.8 Hz), 32.28 (C2'), 24.90 (C3').

^{1}H nmr δ($CDCl_3$) 7.60 (1H, dd, H6, J=8.0 Hz, J=1.5 Hz), 6.07 (1H, d, H5, J=8.4 Hz), 5.95 (1H, dd, H1', J=6.6 Hz, J=3.8 Hz), 4.31 (11H, m, H4', H5', $CF_3CH_2OPO_2$, $CF_3C\underline{H}_2OPONH$), 2.40 (1H, m, H2'), 2.03 (2H, m, H2', H3'), 1.87 (1H, m, H3').

FABMS m/e 700 (MH^+, 4%), 456 (MH_2^+-$(CF_3CH_2O)_2PO$, 17), 356 ($C_8H_9F_6N_3O_4P^+$, 32), 345 (MH^+-$C_8H_8F_6N_3O_4P$, 10), 263 ($(CF_3CH_2O)_2P(OH)_2^+$, 5), 112 (cytosineH^+, base peak).

The reaction of 2',3'-dideoxycytidine (2) with bis(2,2,2-trichloroethyl) phosphorochloridate (104).

Bis(2,2,2-trichloroethyl) phosphorochloridate (0.62 g, 1.63 mmol, 1.75 equivs.) was added to a solution of 2',3'-dideoxycytidine (0.20 g, 0.94 mmol) in pyridine (25 ml), with stirring at -20°C. After stirring at this temperature for 30 min, the reaction mixture was allowed to warm to ambient temperature. After a further 3 h water (29 µl, 1.63 mmol) was added and the solvent removed under reduced pressure. Analysis of the resulting residue by tlc revealed two major components. The residue was purified by chromatography on silica, with elution by chloroform-methanol (90:10). Pooling and evaporation of appropriate fractions corresponding to the slower running component gave a white solid, (0.18 g, 35%), mp 81-83°C.

^{31}P nmr δ($CDCl_3$) -4.21.

^{13}C nmr δ($CDCl_3$) 165.79 (C4), 155.84 (C2), 140.34, 140.29 (C6), 94.66 (C5), 94.50 (d, $\underline{C}Cl_3CH_2OP$, J=11.3 Hz), 87.24, 87.18 (C1'), 78.53 (d, C4', J=7.4 Hz), 69.58 (d, C5', J=6.0 Hz), 32.53 (C2'), 25.27 (C3').

^{13}C nmr δ(CH_3OD) 166.42 (C4), 156.45 (C2), 141.59 (C6), 95.17 (C5), 95.07 (d, $\underline{C}Cl_3CH_2OP$, J=10.1 Hz), 87.68 (C1'), 79.42 (d, C4', J=7.4 Hz), 77.57 (d, $CCl_3\underline{C}H_2OP$, J=4.8 Hz), 70.27 (d, C5', J=6.2 Hz), 32.18 (C2'), 25.37 (C3').

^{1}H nmr δ($CDCl_3$) 7.61 (1H, d, H6, J=7.4 Hz), 6.04 (1H, dd, H1', J=6.8 Hz, J=3.9 Hz), 5.83 (1H, d, H5, J=7.5 Hz), 4.61, 4.59 (4H, 2xd, CCl_3CH_2OP, J=4.1 Hz, J=4.2 Hz), 4.41 (1H, m, H4'), 4.30 (2H, m, H5'), 2.41 (1H, m, H2'), 2.04 (2H, m, H2', H3'), 1.82 (1H, m, H3').

FABMS m/e 558 (MH^+, 3x^{37}Cl, 4%), 557 (MH_2^+, 2x^{37}Cl, 1), 556 (MH^+, 2x^{37}Cl, 8), 555 (MH_2^+, 1x^{37}Cl, 2), 554 (MH^+, 1x^{37}Cl, 12), 553 (MH_2^+, 1), 552 (MH^+, 4), 447 (MH^+-cytosine, 3x^{37}Cl, 2), 445 (MH^+-cytosine, 2x^{37}Cl, 5), 444 (MH_2^+-cytosine, 1x^{37}Cl, 1), 443 (MH^+-cytosine, 1x^{37}Cl, 6), 441 (MH^+-cytosine, 3), 112 (cytosineH^+, base peak).

Pooling and evaporation of appropriate fractions corresponding to the faster running component gave a white solid, (0.16 g, 31%), mp 51-53°C.

^{31}P nmr δ($CDCl_3$) 2.72, -4.51.

^{13}C nmr δ($CDCl_3$) 159.88 (d, C4, J=5.9 Hz), 147.95 (d, C2, J=1.9 Hz), 140.07 (C6), 102.06 (d, C5, J=8.9 Hz), 95.12 (d, $\underline{C}Cl_3CH_2OPONH$, J=11.3 Hz), 94.43 (d, $\underline{C}Cl_3CH_2OPO_2$, J=10.9 Hz), 86.90 (C1'), 78.95 (d, C4', J=7.6 Hz), 69.10 (d, C5', J=6.0 Hz), 32.36 (C2'), 25.04 (C3').

^{1}H nmr δ($CDCl_3$) 7.64 (1H, dd, H6, J=7.9 Hz, J=1.9 Hz), 6.07 (1H, d, H5, J=8.0 Hz), 5.97 (1H, dd, H1′, J=6.6 Hz, J=4.0 Hz), 4.60 (4H, m, $CCl_3CH_2OPO_2$), 4.55 (4H, d, $CCl_3C\underline{H_2}OPONH$, J=6.3 Hz), 4.44 (1H, m, H4′), 4.30 (2H, m, H5′), 2.41 (1H, m, H2′), 2.06 (2H, m, H2′, H3′), 1.94 (1H, m, H3′).

FABMS m/e 899 (M^+, $4x^{37}Cl$, 37%), 897 (M^+, $3x^{37}Cl$, 58), 895 (M^+, $2x^{37}Cl$, 57), 893 (M^+, $1x^{37}Cl$, 32), 891 (M^+, 14), 459 ($C_8H_{10}Cl_6N_3O_4P^+$, $3x^{37}Cl$, 4), 458 ($C_8H_9Cl_6N_3O_4P^+$, $3x^{37}Cl$, 34), 457 ($C_8H_{10}Cl_6N_3O_4P^+$, 2x37Cl, 8), 456 ($C_8H_9Cl_6N_3O_4P^+$, $2x^{37}Cl$, 79), 455 ($C_8H_{10}Cl_6N_3O_4P^+$, $1x^{37}Cl$, 10), 454 ($C_8H_9Cl_6N_3O_4P^+$, $1x^{37}Cl$, base peak), 453 ($C_8H_{10}Cl_6N_3O_4P^+$, 7), 452 ($C_8H_9Cl_6N_3O_4P^+$, 53).

3′-Fluoro-3′-deoxythymidine (110).

Diethylaminosulphur trifluoride (0.61 g, 3.79 mmol, 3.9 equivs.) was added to a solution of 1-(2-deoxy-5-O-trityl-ß-D-threopentofuranosyl)thymine (0.47 g, 0.97 mmol) in benzene (16 ml) and tetrahydrofuran (4 ml), with stirring at ambient temperature. After 1.5 h analysis of the reaction mixture by tlc revealed it to be comprised of one major component. The reaction mixture was then poured into a 5% solution of sodium bicarbonate (20 ml) and extracted with ethyl acetate (2x20 ml). The organic phase was dried with magnesium sulphate and the solvent removed under reduced pressure. The residue was heated to 80°C for 15 min in 80% acetic acid (15 ml). The solvent was removed under reduced pressure and analysis of the resulting residue by tlc revealed the presence of two major components, one of which had the same R_f as thymine. The residue was purified by chromatography on silica, with elution by chloroform (250 ml), followed by chloroform-methanol (99:1). Pooling and evaporation of appropriate fractions of the latter eluent gave the product as an off white solid, (40 mg, 19%), mp 176-177°C.

^{13}C nmr δ(CH_3OD) 165.41 (C2), 151.50 (C4), 137.01 (C6), 110.99 (C5), 95.06 (d, C3′,

J=175.8 Hz), 86.01 (d, C4′, J=24.0 Hz), 85.39 (C1′), 62.84 (d, C5′, J=11.0 Hz), 38.23 (d, C2′, J=20.7 Hz), 11.61 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 8.70 (1H, bs, NH), 7.34 (1H, d, H6, J=1.2 Hz), 6.08 (1H, dd, H1′, J=8.2 Hz, J=6.6 Hz), 5.28 (1H, dm, H3′, J=56.3 Hz), 4.26 (1H, dm, H4′, J=27.6 Hz), 3.81 (2H, m, H5′), 2.47 (2H, m, H2′), 1.85 (3H, d, 5-CH_3, J=1.1 Hz).

EIMS m/e 244.0877 (M^+, 3%, calc. for $C_{10}H_{13}FN_2O_4$ 244.0859), 127 (thymineH^+, 25), 126 (thymine$^+$, 76), 99 ($C_5H_7O_2^+$, 55), 81 ($C_5H_5O^+$, base peak).

3′-Fluoro-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (109).

Bis(2,2,2-trichloroethyl) phosphorochloridate (100 mg, 0.28 mmol, 2 equivs.) was added to a solution of 3′-fluoro-3′-deoxythymidine (35 mg, 0.14 mmol) in pyridine (2 ml), with stirring at ambient temperature. After 3 h water (5 µl, 0.28 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (99:1). Pooling and evaporation of appropriate fractions gave the product as a white solid, (60 mg, 59%), mp 67-69°C.

^{31}P nmr δ($CDCl_3$) -5.65.

^{13}C nmr δ($CDCl_3$) 163.51 (C2), 150.28 (C4), 135.02 (C6), 111.89 (C5), 94.40 (d, $\underline{C}Cl_3CH_2OP$, J=10.7 Hz), 93.11 (d, C3′, J=180.0 Hz), 85.24 (C1′), 82.41 (dd, C4′, J=27.4 Hz, J=7.7 Hz), 67.95 (dd, C5′, J=11.0 Hz, J=6.1 Hz), 37.80 (d, C2′, J=21.0 Hz), 12.58 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 9.06 (1H, bs, NH), 7.28 (1H, s, H6), 6.32 (1H, dd, H1′, J=9.1 Hz, J=5.3 Hz), 5.26 (1H, dm, H3′, J=53.4 Hz), 4.61 (4H, m, CCl_3CH_2OP), 4.40 (2H, m, H5′), 4.38

(1H, m, H4'), 2.55 (1H, m, H2'), 2.14 (1H, m, H2'), 1.88 (3H, s, 5-CH_3).

FABMS m/e 591 (MH^+, $3x^{37}Cl$, 6%), 590 (MH_2^+, $2x^{37}Cl$, 3), 589 (MH^+, $2x^{37}Cl$, 13), 588 (MH_2^+, $1x^{37}Cl$, 4), 587 (MH^+, $1x^{37}Cl$, 16), 586 (MH_2^+, 3), 585 (MH^+, 9), 227 (MH^+-$(CCl_3CH_2O)_2PO_2H$, 9), 127 (thymineH^+, 24), 81 ($C_5H_5O^+$, base peak).

Propyl phosphorodichloridate (114).

A solution of 1-propanol (5.10 g, 85 mmol, 1.05 equivs.) and triethylamine (8.59 g, 85 mmol, 1.05 equivs.) in diethyl ether (50 ml) was added dropwise to a solution of phosphoryl chloride (12.43 g, 81 mmol) in diethyl ether (100 ml), with vigorous stirring at -40°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After a further 5 h, the reaction mixture was filtered and the solvent removed from the filtrate under reduced pressure. The residue was purified by vacuum distillation to give the product as a colourless liquid, (12.33 g, 86%), bp 41-43°C at 1 mmHg.

^{31}P nmr $\delta(CDCl_3)$ 5.82.

Propyl 2,2,2-trifluoroethyl phosphorochloridate (115).

A solution of 2,2,2-trifluoroethanol (2.97 g, 29.7 mmol, 1.05 equivs.) and triethylamine (3.00 g, 29.7 mmol, 1.05 equivs.) in diethyl ether (50 ml) was added dropwise to a solution of propyl phosphorodichloridate (5.00 g, 28.2 mmol) in diethyl ether (100 ml), with vigorous stirring at -20°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After a further 17 h, the reaction mixture was filtered and the solvent removed from the filtrate under reduced pressure. The residue was purified by vacuum distillation to give the product as a colourless liquid, (4.14 g, 61%), bp 84-86°C at 1.5 mmHg.

^{31}P nmr $\delta(CDCl_3)$ 3.10.

^{13}C nmr $\delta(CDCl_3)$ 121.73 (qd, $\underline{C}F_3CH_2OP$, J=271.7 Hz, J=8.7 Hz), 72.17 (d, $CH_3CH_2\underline{C}H_2OP$, J=6.5 Hz), 63.47 (qd, $CF_3\underline{C}H_2OP$, J=35.1 Hz, J=4.2 Hz), 23.04 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.9 Hz), 9.56 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr $\delta(CDCl_3)$ 4.46 (2H, m, CF_3CH_2OP), 4.16 (2H, m, $CH_3CH_2C\underline{H}_2OP$)*, 1.73 (2H, sextet, $CH_3C\underline{H}_2CH_2OP$, J=7.4 Hz), 0.89, 0.88 (3H, 2xt, $C\underline{H}_3CH_2CH_2OP$, J=6.4 Hz, J=6.5 Hz).

*Collapsed to a doublet on irradiation at δ1.73.

Thymidine 5′-(propyl 2,2,2-trifluoroethyl) phosphate (117).

Propyl 2,2,2-trifluoroethyl phosphorochloridate (0.62 g, 2.57 mmol, 2 equivs.) was added to a solution of thymidine (0.31 g, 1.28 mmol) in pyridine (25 ml), with stirring at ambient temperature. After 24 h water (46 µl, 2.57 mmol) was added and the solvent removed under reduced pressure. Analysis of the resulting residue by tlc revealed one major component and two faster running minor components. The residue was purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of the appropriate fractions gave the product as a white solid, (0.27 g, 49%), mp 140-142°C.

^{31}P nmr $\delta(CDCl_3)$ -2.94, -3.04.

^{13}C nmr $\delta(CDCl_3)$ 164.22 (C2), 150.83 (C4), 135.44 (C6), 122.44 (qd, $\underline{C}F_3CH_2OP$, J=277.5 Hz, J=9.3 Hz), 111.47, 111.45 (C5), 84.96 (C1′), 84.63 (d, C4′, J=7.0 Hz), 71.00 (C3′), 70.67, 70.63 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=6.1 Hz, J=5.9 Hz), 67.61 (d, C5′, J=5.4 Hz), 63.78, 63.73 (2xqd, $CF_3\underline{C}H_2OP$, J=37.6 Hz, J=5.0 Hz, J=37.5 Hz, J=4.9 Hz), 40.04, 40.00 (C2′), 23.43 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.9 Hz), 12.30, 12.26 (5-CH_3), 9.75 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 9.98 (1H, bs, NH), 7.40, 7.39 (1H, 2xs, H6), 6.35 (1H, t, H1′, J=6.2 Hz), 4.42 (5H, m, H3′, H5′, CF_3CH_2OP), 4.11 (2H, m, $CH_3CH_2\underline{CH_2}OP$), 2.46 (1H, m, H2″), 2.10 (1H, m, H2″), 1.91 (3H, s, 5-CH_3), 1.72 (2H, sextet, $CH_3\underline{CH_2}CH_2OP$, J=7.3 Hz), 0.96, 0.95 (3H, 2xt, $\underline{CH_3}CH_2CH_2OP$, J=7.3 Hz, J=7.4 Hz).

EIMS m/e 446.1077 (M^+, 0.3%, calc. for $C_{15}H_{22}F_3N_2O_8P$ 446.1066), 321 (MH^+-thymine, 2), 223 ($(CF_3CH_2O)(PrO)P(OH)_2^+$, 6), 181 ($CF_3CH_2OP(OH)_3^+$, 25), 127 (thymine$H^+$, 8), 126 (thymine$^+$, 9), 99 ($C_5H_7O_2^+$, 4), 81 ($C_5H_5O^+$, base peak).

Found C 40.30%, H 4.96, N 6.19, P 7.10, $C_{15}H_{22}F_3N_2O_8P$ requires C 40.37, H 4.97, N 6.28, P 6.94.

3′-Azido-3′-deoxythymidine 5′-(propyl 2,2,2-trifluoroethyl) phosphate (112).

Propyl 2,2,2-trifluoroethyl phosphorochloridate (0.32 g, 1.33 mmol, 2 equivs.) was added to a solution of 3′-azido-3′-deoxythymidine (0.18 g, 0.67 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 17 h water (24 μl, 1.33 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (97:3). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.15 g, 47%).

^{31}P nmr δ($CDCl_3$) -3.10, -3.21.

^{13}C nmr δ($CDCl_3$) 163.58 (C2), 150.16 (C4), 135.14, 135.09 (C6), 121.31 (qd, $\underline{C}F_3CH_2OP$, J=269.2 Hz, J=8.2 Hz), 84.96 (C1″), 81.97 (d, C4′, J=7.9 Hz), 70.72, 70.67 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=5.4 Hz, J=5.2 Hz), 66.75, 66.69 (2xd, C5′, J=6.3 Hz, J=6.4 Hz), 63.88, 63.84 (2xqd, $CF_3\underline{C}H_2OP$, J=37.6 Hz, J=4.2 Hz, J=37.5 Hz, J=4.4 Hz), 59.95, 59.93 (C3″), 37.38 (C2″), 23.48 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.8 Hz), 12.35 (5-$CH_3$), 9.80 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 9.08 (1H, s, NH), 7.26 (1H, d, H6, J=1.2 Hz), 6.15 (1H, t, H1′, J=6.5 Hz), 4.35 (2H, m, CF_3CH_2OP), 4.27 (3H, m, H3′, H5′), 4.03 (2H, q, $CH_3CH_2\underline{CH_2}OP$, J=6.6 Hz)*, 3.96 (1H, m, H4′), 2.37 (1H, m, H2′), 2.27 (1H, m, H2′), 1.87 (3H, d, 5-CH_3, J=1.2 Hz), 1.68 (2H, m, $CH_3\underline{CH_2}CH_2OP$), 0.91, 0.90 (3H, t, $\underline{CH_3}CH_2CH_2OP$, J=7.4 Hz, J=7.4 Hz)**. *Collapsed to a doublet on irradiation at δ1.68. **Collapsed to two singlets on irradiation at δ1.68.

EIMS m/e 472.1357 (MH^+, 0.3%, calc. for $C_{15}H_{22}F_3N_5O_7P$ 472.1209), 346 (MH^+-thymine, 20), 223 ($(CF_3CH_2O)(PrO)P(OH)_2^+$, 2), 181 ($CF_3CH_2OP(OH)_3^+$, 27), 126 (thymine$^+$, 16), 81 ($C_5H_5O^+$, base peak).

Propyl 2,2,2-trichloroethyl phosphorochloridate (116).

A solution of 2,2,2-trichloroethanol (4.43 g, 29.7 mmol, 1.05 equivs.) and triethylamine (3.00 g, 29.7 mmol, 1.05 equivs.) in diethyl ether (50 ml) was added dropwise to a solution of propyl phosphorodichloridate (5.00 g, 28.2 mmol) in diethyl ether (100 ml), with vigorous stirring at -20°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature. After a further 17 h, the reaction mixture was filtered and the solvent removed from the filtrate under reduced pressure. The residue was purified by vacuum distillation to give the product as a colourless liquid, (6.63 g, 81%), bp 105-108°C at 1 mmHg.

^{31}P nmr δ($CDCl_3$) 2.82.

^{13}C nmr δ($CDCl_3$) 93.90 (d, $\underline{C}Cl_3CH_2OP$, J=12.9 Hz), 72.37 (d, $CH_3CH_2\underline{C}H_2OP$, J=7.9 Hz), 23.10 (d, $CH_3\underline{C}H_2CH_2OP$, J=7.7 Hz), 9.79 ($\underline{C}H_3CH_2CH_2OP$).

^{1}H nmr δ($CDCl_3$) 4.50 (2H, m, CCl_3CH_2OP), 4.11 (2H, m, $CH_3CH_2\underline{CH_2}OP$), 1.69 (2H,

sextet, $CH_3\underline{CH_2}CH_2OP$, J=6.1 Hz), 0.92, 0.91 (3H, 2xt, $\underline{CH_3}CH_2CH_2OP$, J=5.7 Hz, J=5.9 Hz).

3′-Azido-3′-deoxythymidine 5′-(propyl 2,2,2-trichloroethyl) phosphate (113).

Propyl 2,2,2-trichloroethyl phosphorochloridate (0.39 g, 1.34 mmol, 2 equivs.) was added to a solution of 3′-azido-3′-deoxythymidine (0.18 g, 0.67 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 7 h water (24 µl, 1.34 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (98:2). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.16 g, 46%).

^{31}P nmr δ($CDCl_3$) -2.33, -2.41.

^{13}C nmr δ($CDCl_3$) 163.78, 163.75 (C2), 150.28 (C4), 135.21, 135.17 (C6), 111.59, 111.57 (C5), 94.31 (d, $\underline{C}Cl_3CH_2OP$, J=10.1 Hz), 84.98, 84.90 (C1′), 82.01 (d, C4′, J=7.9 Hz), 70.68, 70.64 (2xd, $CH_3CH_2\underline{C}H_2OP$, J=6.2 Hz, J=6.0 Hz), 66.80 (d, C5′, J=5.9 Hz), 60.02, 59.95 (C3′), 37.36, 37.32 (C2′), 23.51 (d, $CH_3\underline{C}H_2CH_2OP$, J=6.7 Hz), 12.47 (5-$CH_3$), 9.85 ($\underline{C}H_3CH_2CH_2OP$).

^1H nmr δ($CDCl_3$) 9.05 (1H, bs, NH), 7.35 (1H, s, H6), 6.21 (1H, t, H1′, J=6.5 Hz), 4.58, 4.57 (2H, 2xd, CCl_3CH_2OP, J=6.8 Hz, J=6.7 Hz), 4.34 (3H, m, H3′, H5′), 4.12 (2H, q, $CH_3CH_2\underline{CH_2}OP$, J=6.9 Hz), 4.02 (1H, m, H4′), 2.35 (2H, m, H2′), 1.92 (3H, s, 5-CH_3), 1.73 (2H, sextet, $CH_3\underline{CH_2}CH_2OP$, J=6.9 Hz), 0.95, 0.94 (3H, 2xt, $\underline{CH_3}CH_2CH_2OP$, J=7.3 Hz, J=7.1 Hz).

FABMS m/e 526 (MH^+, $3x^{37}Cl$, 0.5%), 525 (MH_2^+, $2x^{37}Cl$, 1), 524 (MH^+, $2x^{37}Cl$, 3), 523 (MH_2^+, $1x^{37}Cl$, 2), 522 (MH^+, $1x^{37}Cl$, 9), 521 (MH_2^+, 3), 520 (MH^+, 9), 250 (MH^+-

$(CCl_3CH_2O)(PrO)PO_2H$, 5), 127 (thymineH^+, 9), 81 ($C_5H_5O^+$, base peak).

Thymidine 5´-bis(2,2,2-trichloroethyl) phosphate (122).

Bis(2,2,2-trichloroethyl) phosphorochloridate (1.25 g, 3.30 mmol, 2 equivs.) was added to a solution of thymidine (0.40 g, 1.65 mmol) in pyridine (20 ml), with stirring at -20°C. After stirring at this temperature for 1 h, the reaction mixture was allowed to warm to ambient temperature. After a further 5 h water (30µl, 1.65 mmol) was added and the solvent removed under reduced pressure. Analysis of the resulting residue by tlc revealed one major component and two minor faster running components. The residue was purified by chromatography on silica, with elution by chloroform-methanol (95:5). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.58 g, 61%).

^{31}P nmr δ(CH_3OD) -5.08.

^{13}C nmr δ(CH_3OD) 165.41 (C2), 151.33 (C4), 137.00 (C6), 111.09 (C5), 95.10 (d, $\underline{C}Cl_3CH_2OP$, J=11.0 Hz), 85.74 (C1´), 85.01 (d, C4´, J=7.0 Hz), 77.61 (d, $CCl_3\underline{C}H_2OP$, J=4.1 Hz), 70.89 (C3´), 69.21 (d, C5´, J=6.1 Hz), 39.52 (C2´), 11.78 (5-CH_3).

^1H nmr δ($CDCl_3$) 10.12 (1H, bs, NH), 7.33 (1H, s, H6), 6.24 (1H, t, H1´, J=6.1 Hz), 4.58 (4H, d, CCl_3CH_2OP, J=6.4 Hz), 4.48 (1H, m, H3´), 4.41 (2H, m, H5´), 4.08 (1H, m, H4´), 3.91 (1H, bs, OH), 2.40 (1H, m, H2´), 2.11 (1H, m, H2´), 1.91 (3H, s, 5-CH_3).

FABMS ($NO_2C_6H_4CH_2OH$/NaI) m/e 612 ($MHNa^+$, $3x^{37}Cl$, 2), 611 (MNa^+, $3x^{37}Cl$, 8), 610 ($MHNa^+$, $2x^{37}Cl$, 4), 609 (MNa^+, $2x^{37}Cl$, 18), 608 ($MHNa^+$, $1x^{37}Cl$, 4), 607 (MNa^+, $1x^{37}Cl$, 24) 606 ($MHNa^+$, 2), 605 (MNa^+, 12), 207 (MH^+-H_2O-$(CCl_3CH_2O)_2PO_2H$, 11), 154 (thymineCHO^+, 6), 81 ($C_5H_5O^+$, 61), 73 (base peak).

Found C 28.73%, H 2.93, N 4.42, P 5.39, $C_{14}H_{17}Cl_6N_2O_8P$ requires C 28.75, H 2.93, N 4.79, P 5.30.

3′-O-Mesylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (118).

A solution of mesyl chloride (0.048 g, 0.42 mmol, 1.30 equivs) in dichloromethane (5 ml) was added to a solution of thymidine 5′-bis(2,2,2-trichloroethyl) phosphate (0.19 g, 0.32 mmol) and triethylamine (0.045 g, 0.45 mmol, 1.4 equivs) in dichloromethane (20 ml) with stirring at -20°C. The reaction mixture was allowed to warm to ambient temperature. After 30 min at this temperature the reaction mixture was washed with saturated sodium bicarbonate solution (4x50 ml) and saturated brine (2x50 ml). The organic phase was dried with magnesium sulphate and the solvent removed under reduced pressure to yield the product as a white solid, (0.11 g, 52%), mp 72-74°C.

^{31}P nmr δ($CDCl_3$) -4.80.

^{13}C nmr δ(CH_3OD) 165.30 (C2), 151.18 (C4), 137.18 (C6), 111.26 (C5), 95.05 (d, $\underline{C}Cl_3CH_2OP$, J=11.2 Hz), 86.27 (C1′), 82.73 (d, C4′, J=7.6 Hz), 79.29 (C3′), 77.65 (d, $CCl_3\underline{C}H_2OP$, J=4.3 Hz), 68.16 (d, C5′, J=6.0 Hz), 37.49 (CH_3SO_2), 37.10 (C2′), 11.77 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 8.92 (1H, bs, NH), 7.30 (1H, d, H6, J=1.1 Hz), 6.29 (1H, dd, H1′, J=7.2 Hz, J=5.6 Hz), 5.36 (1H, m, H3′), 4.65 (4H, d, CCl_3CH_2OP, J=6.4 Hz), 4.42 (3H, m, H4′, H5′), 3.10 (3H, s, CH_3SO_2), 2.56 (1H, m, H2′), 2.36 (1H, m, H2′), 1.91 (3H, d, 5-CH_3, J=1.1 Hz).

FABMS m/e 667 (MH^+, $3x^{37}Cl$, 3), 666 (MH_2^+, $2x^{37}Cl$, 2), 665 (MH^+, $2x^{37}Cl$, 7), 664 (MH_2^+, $1x^{37}Cl$, 3), 663 (MH^+, $1x^{37}Cl$, 8), 662 (MH_2^+, 2), 661 (MH^+, 4), 303 (MH^+-

$(CCl_3CH_2O)_2PO_2H$, 8), 207 (MH^+-$MeSO_3H$-$(CCl_3CH_2O)_2PO_2H$, 6), 127 (thymineH^+, 11), 81 ($C_5H_5O^+$, base peak).

Found C 27.11%, H 2.92, N 3.79, P 4.29, $C_{15}H_{19}Cl_6N_2O_{10}PS(H_2O)_{0.25}$ requires C 26.99, H 2.87, N 4.19, P 4.64.

Analytical hplc stationary phase 50+250 mm x 4.6 mm spherisorb OD52 5 μM column, mobile phase A=water-acetonitrile (95:5) B=acetonitrile-water (95:5), gradient conditions 20% B 0 min, 20% B 10 min, 80% B 30 min, flow rate 1 ml/min. Retention time 30.54 min.

3′-O-Acetylthymidine 5′-bis(2,2,2-trichloroethyl) phosphate (119).

Bis(2,2,2-trichloroethyl) phosphorochloridate (0.53 g, 1.40 mmol, 2 equivs.) was added to a solution of 3′-O-acetylthymidine (0.20 g, 0.70 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 3 h water (25 μl, 1.40 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform. Pooling and evaporation of appropriate fractions gave the product as a white solid, (0.32 g, 72%), mp 63-65°C.

^{31}P nmr $\delta(CDCl_3)$ -4.64.

^{13}C nmr $\delta(CDCl_3)$ 170.57 ($CH_3\underline{C}O$), 163.64 (C2), 150.64 (C4), 134.86 (C6), 111.94 (C5), 94.38 (d, $\underline{C}Cl_3CH_2OP$, J=10.0 Hz), 84.64 (C1′), 82.56 (d, C4′, J=7.2 Hz), 74.10 (C3′), 68.48 (d, C5′, J=5.9 Hz), 37.06 (C2′), 20.96 ($\underline{C}H_3CO$), 12.60 (5-CH_3).

^1H nmr $\delta(CDCl_3)$ 8.99 (1H, s, NH), 7.40 (1H, d, H6, J=1.0 Hz), 6.34 (1H, dd, H1′, J=8.4 Hz, J=5.5 Hz), 5.36 (1H, m, H3′), 4.64 (4H, d, CCl_3CH_2OP, J=7.2 Hz), 4.51 (2H, m, H5′),

4.18 (1H, m, H4'), 2.40 (1H, m, H2'), 2.18 (1H, m, H2'), 2.08 (3H, s, CH_3CO), 1.90 (3H, d, 5-CH_3, J=1.0 Hz).

FABMS ($NO_2C_6H_4CH_2OH$/NaI) m/e 655 (MNa^+, $4x^{37}Cl$, 4%), 654 ($MHNa^+$, $3x^{37}Cl$, 4), 653 (MNa^+, $3x^{37}Cl$, 14), 652 ($MHNa^+$, $2x^{37}Cl$, 6), 651 (MNa^+, $2x^{37}Cl$, 28), 650 ($MHNa^+$, $1x^{37}Cl$, 8), 649 (MNa^+, $1x^{37}Cl$, 36), 648 ($MHNa^+$, 5), 647 (MNa^+, 18), 289 (MNa^+-$(CCl_3CH_2O)_2PO_2H$, 5), 267 (MH^+-$(CCl_3CH_2O)_2PO_2H$, 8), 207 (MH^+-AcOH-$(CCl_3CH_2O)_2PO_2H$, 8), 127 (thymineH^+, 10), 81 ($C_5H_5O^+$, base peak).

Found C 30.20%, H 2.98, N 3.85, P 4.76, $C_{16}H_{19}Cl_6N_2O_9P$ requires C 29.86, H 3.05, N 4.35, P 4.81.

Analytical hplc stationary phase 50+250 mm x 4.6 mm spherisorb OD52 5 μM column, mobile phase A=water B=acetonitrile-water (95:5), gradient conditions 50% B 0 min, 50% B 1 min, 80% B 30 min, flow rate 1 ml/min. Retention time 13.25 min.

3'-O-Ethylthymidine 5'-bis(2,2,2-trichloroethyl) phosphate (120).

Bis(2,2,2-trichloroethyl) phosphorochloridate (0.42 g, 1.10 mmol, 2 equivs.) was added to a solution of 3'-O-ethylthymidine (0.15 g, 0.55 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 3 h water (20 μl, 1.10 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (98:2). Pooling and evaporation of appropriate fractions gave the product as a white solid, (0.27 g, 76%), mp 53-55°C.

^{31}P nmr $\delta(CDCl_3)$ -4.82.

^{13}C nmr $\delta(CDCl_3)$ 163.72 (C2), 150.38 (C4), 135.39 (C6), 111.47 (C5), 94.51 (d,

$\underline{C}Cl_3CH_2OP$, J=10.8 Hz), 85.47 (C1´), 82.49 (d, C4´, J=7.5 Hz), 78.41 (C3´), 68.58 (d, C5´, J=6.0 Hz), 65.25 ($CH_3\underline{C}H_2OC$), 37.25 (C2´), 15.21 ($\underline{C}H_3CH_2OC$), 12.49 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 9.11 (1H, bs, NH), 7.29 (1H, s, H6), 6.22 (1H, t, H1´, J=7.0 Hz), 4.59 (4H, d, $CCl_3\underline{CH_2}OP$, J=7.4 Hz), 4.37 (2H, m, H5´), 4.10 (2H, m, H3´, H4´), 3.44 (2H, m, $CH_3\underline{CH_2}OC$), 2.36 (1H, m, H2´), 2.04 (1H, m, H2´), 1.88 (3H, s, 5-CH_3), 1.18 (3H, t, $\underline{CH_3}CH_2OC$, J=7.0 Hz).

FABMS ($NO_2C_6H_4CH_2OH$/NaI) m/e 641 (MNa^+, 4x^{37}Cl, 7%), 640 ($MHNa^+$, 3x^{37}Cl, 5), 639 (MNa^+, 3x^{37}Cl, 28), 638 ($MHNa^+$, 2x^{37}Cl, 14), 637 (MNa^+, 2x^{37}Cl, 68), 636 ($MHNa^+$, 1x^{37}Cl, 16), 635 (MNa^+, 1x^{37}Cl, 78), 634 ($MHNa^+$, 9), 633 (MNa^+, 42), 275 (MNa^+-$(CCl_3CH_2O)_2PO_2H$, 5), 253 (MH^+-$(CCl_3CH_2O)_2PO_2H$, 2), 207 (MH^+-EtOH-$(CCl_3CH_2O)_2PO_2H$, 8), 154 (thymineCHO$^+$, 27), 148 (thymineNa$^+$, 15), 127 (thymineH$^+$, 10), 81 ($C_5H_5O^+$, base peak).

Found C 31.01%, H 3.62, P 5.09, $C_{16}H_{21}Cl_6N_2O_8P$ requires C 31.35, H 3.45, P 5.05.

Analytical hplc stationary phase 50+250 mm x 4.6 mm spherisorb OD52 5 μM column, mobile phase A=water B=acetonitrile-water (95:5), gradient conditions 50% B 0 min, 50% B 1 min, 80% B 30 min, flow rate 1 ml/min. Retention time 15.41 min.

3´-Amino-3´-deoxythymidine 5´-bis(2,2,2-trichloroethyl) phosphate (121).

1,3-Propanedithiol (0.119 g, 1.102 mmol, 5 equivs.) and triethylamine (0,111 g, 1.098 mmol, 5 equivs.) were added to a solution of 3´-azido-3´-deoxythymidine 5´-bis(2,2,2-trichloroethyl) phosphate (0.134 g, 0.219 mmol) in methanol (2 ml), with stirring at ambient temperature under an atmosphere of nitrogen. After 17 h the solvent was removed under reduced pressure. The residue was dissolved in chloroform (5 ml) and hexane (100 ml) was added.

The solvent was decanted and the precipitate purified by chromatography on silica, with elution by chloroform-methanol-triethylamine (93:5:2). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (18 mg, 14%).

^{31}P nmr $\delta(CDCl_3)$ -4.51.

^{13}C nmr $\delta(CDCl_3)$ 164.04 (C2), 150.35 (C4), 135.39 (C6), 111.18 (C5), 94.29 (d, $\underline{C}Cl_3CH_2OP$, J=10.2 Hz), 84.66 (C1'), 84.55 (d, C4', J=7.5 Hz), 67.74 (d, C5', J=6.1 Hz), 50.86 (C3'), 41.03 (C2'), 12.42 (5-CH_3).

^1H nmr 7.36 (1H, s, H6), 6.22 (1H, t, H1', J=6.2 Hz), 4.64 (4H, d, CCl_3CH_2OP, J=7.0 Hz), 4.44 (2H, m, H5'), 3.84 (1H, m, H4'), 3.66 (1H, m, H3')*, 2.26 (2H, m, H2'), 1.88 (3H, s, 5-CH_3), 1.62 (2H, bs, NH_2).

*Affected by irradiation at δ2.26.

FABMS m/e 590 (MH^+, $4x^{37}Cl$, 2%), 589 (MH_2^+, $3x^{37}Cl$, 1), 588 (MH^+, $3x^{37}Cl$, 4), 587 (MH_2^+, $2x^{37}Cl$, 2), 586 (MH^+, $2x^{37}Cl$, 9), 585 (MH_2^+, $1x^{37}Cl$, 3), 584 (MH^+, $1x^{37}Cl$, 12), 583 (MH_2^+, 1), 582 (MH^+, 7), 127 (thymineH^+, 14), 81 ($C_5H_5O^+$, base peak).

Bis(2-chloroethyl) phosphorochloridate (125).

A solution of 2-chloroethanol (4.31 g, 54 mmol) and triethylamine (5.41 g, 54 mmol) in diethyl ether (50 ml) was added dropwise to a solution of phosphoryl chloride (8.23 g, 54 mmol) in diethyl ether (150 ml), with vigorous stirring at -40°C. After addition was complete, the reaction mixture was allowed to warm to ambient temperature, over the course of 5 h. A further portion of 2-chloroethanol (4.31 g, 54 mmol) and triethylamine (5.41 g, 54 mmol) in diethyl ether (50 ml) was then added at -30°C. After addition was complete, the reaction mixture was allowed to warm to 0°C and stirred at this temperature for a

further 17 h. The reaction mixture was allowed to warm to ambient temperature, filtered and the solvent removed from the filtrate under reduced pressure to give the product as a yellow liquid.

^{31}P nmr δ($CDCl_3$) 5.53 (minor), 2.45 (major), -4.05 (minor).

The product was then subjected to a high vacuum (*ca.* 0.5 mmHg) for 4 h, (10.48 g, 81%).

^{31}P nmr δ($CDCl_3$) 2.50 (major), -4.12 (minor).

^{13}C nmr δ($CDCl_3$) 68.71 (d, $CH_2Cl\underline{C}H_2OP$, J=6.4 Hz), 41.67 (d, $\underline{C}H_2ClCH_2OP$, J=9.1 Hz).

^{1}H nmr δ($CDCl_3$) 4.27 (4H, m, $CH_2ClC\underline{H}_2OP$), 3.62 (4H, t, $C\underline{H}_2ClCH_2OP$, J=7.0 Hz).

3′-Azido-3′-deoxythymidine 5′-bis(2-chloroethyl) phosphate (123).

Crude bis(2-chloroethyl) phosphorochloridate (162 mg, 0.68 mmol, 2 equivs.) was added to solution of 3′-azido-3′-deoxythymidine (90 mg, 0.34 mmol) in pyridine (10 ml), with stirring at ambient temperature. After 17 h water (12 µl, 0.68 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (98:2). Pooling and evaporation of the appropriate fractions gave the product as a colourless oil, (78 mg, 49%).

^{31}P nmr δ($CDCl_3$) -3.34.

^{13}C nmr δ($CDCl_3$) 163.38 (C2), 150.04 (C4), 135.28 (C6), 111.60 (C5), 85.04 (C1′), 82.11 (d, C4′, J=7.9 Hz), 67.76 (d, $CH_2Cl\underline{C}H_2OP$, J=5.4 Hz), 66.79 (d, C5′, J=5.6 Hz), 60.07 (C3′), 42.53 (d, $\underline{C}H_2ClCH_2OP$, J=5.5 Hz), 37.41 (C2′), 12.50 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 9.29 (1H, bs, NH), 7.30 (1H, d, H6, J=1.2 Hz), 6.15 (1H, t, H1′, J=6.4 Hz), 4.29 (7H, m, H3′, H5′, $CH_2ClC\underline{H}_2OP$), 3.98 (1H, m, H4′), 3.67 (4H, t, $C\underline{H}_2ClCH_2OP$, J=6.4 Hz), 2.36 (2H, m, H2′), 1.88 (3H, d, 5-CH_3, J=1.2 Hz).

FABMS m/e 476 (MH^+, $2x^{37}Cl$, 0.5%), 475 (MH_2^+, $1x^{37}Cl$, 1), 474 (MH^+, $1x^{37}Cl$, 2), 473 (MH_2^+, 1), 472 (MH^+, 5), 471 (M^+, 1), 207 (MH^+-N_3H-$(CH_2ClCH_2O)_2PO_2H$, 5), 127 (thymineH^+, 75), 81 ($C_5H_5O^+$, base peak).

Analytical hplc stationary phase 50+250 mm x 4.6 mm spherisorb OD52 5μM column, mobile phase A=water B=acetonitrile-water (95:5), gradient conditions 18% B 0 min, 18% B 10 min, 82% B 30 min, flow rate 1 ml/min. Retention time 27.34 min.

Bis(2,2-dichloroethyl) phosphorochloridate (126).

A solution of 2,2-dichloroethanol (12.63 g, 110 mmol, 2.05 equivs.) and triethylamine (11.10 g, 110 mmol, 2.05 equivs.) in diethyl ether (50 ml) was added dropwise to a solution of phosphoryl chloride (8.23 g, 54 mmol) in diethyl ether (200 ml), with vigorous stirring at -40°C. After addition was complete, the reaction mixture was allowed to warm to 0°C and stirred at this temperature for 17 h. The reaction mixture was allowed to warm to ambient temperature, filtered and the solvent removed from the filtrate under reduced pressure. The residue was purified by vacuum distillation, using a Kugelrohr apparatus, to give the product as a colourless liquid, (5.49 g, 33%).

^{31}P nmr δ($CDCl_3$) 2.22.

^{13}C nmr δ($CDCl_3$) 72.12 (d, $CHCl_2\underline{C}H_2OP$, J=5.5 Hz), 68.07 (d, $\underline{C}HCl_2CH_2OP$, J=8.9 Hz).

^{1}H nmr δ($CDCl_3$) 5.86 (2H, t, $C\underline{H}Cl_2CH_2OP$, J=6.4 Hz), 4.38 (4H, m, $CHCl_2C\underline{H}_2OP$).

3′-Azido-3′-deoxythymidine 5′-bis(2,2-dichloroethyl) phosphate (124).

Bis(2,2-dichloroethyl) phosphorochloridate (0.46 g, 1.49 mmol, 2 equivs.) was added to a solution of 3′-azido-3′-deoxythymidine (0.20 g, 0.75 mmol) in pyridine (20 ml), with stirring at ambient temperature. After 17 h water (27 µl, 1.50 mmol) was added and the solvent removed under reduced pressure. The residue was purified by chromatography on silica, with elution by chloroform-methanol (98:2). Pooling and evaporation of appropriate fractions gave the product as a colourless oil, (0.25 g, 62%).

^{31}P nmr δ($CDCl_3$) -4.32.

^{13}C nmr δ($CDCl_3$) 163.76 (C2), 150.26 (C4), 135.55 (C6), 111.66 (C5), 85.54 (C1′), 81.87 (d, C4′, J=7.6 Hz), 71.38, 71.37 (2xd, $CHCl_2\underline{C}H_2OP$, J=5.2 Hz, J=5.1 Hz), 68.44 (d, $\underline{C}HCl_2CH_2OP$, J=8.0 Hz), 67.28 (d, C5′, J=5.8 Hz), 59.95 (C3′), 37.12 (C2′), 12.49 (5-CH_3).

^{1}H nmr δ($CDCl_3$) 9.58 (1H, s, NH), 7.28 (1H, d, H6, J=1.1 Hz), 6.12 (1H, t, H1′, J=6.4 Hz), 5.84, 5.83 (2H, 2xt, $C\underline{H}Cl_2CH_2OP$, J=6.1 Hz, J=6.0 Hz), 4.38 (7H, m, H3′, H5′, $CHCl_2C\underline{H_2}OP$), 4.00 (1H, m, H4′), 2.40 (2H, m, H2′), 1.89 (3H, d, 5-CH_3, J=1.1 Hz).

FABMS m/e 546 (MH^+, 3x^{37}Cl, 1%), 545 (MH_2^+, 2x^{37}Cl, 1), 544 (MH^+, 2x^{37}Cl, 6), 543 (MH_2^+, 1x^{37}Cl, 2), 542 (MH^+, 1x^{37}Cl, 10), 541 (MH_2^+, 2), 540 (MH^+, 8), 250 (MH^+-$(CHCl_2CH_2O)_2PO_2H$, 5), 127 (thymineH^+, 25), 81 ($C_5H_5O^+$, base peak).

Found C 31.39%, H 2.90, N 12.52, P 5.55, $C_{14}H_{18}Cl_4N_5O_7P$ requires C 31.08, H 3.35, N 12.94, P 5.72.

Analytical hplc stationary phase 50+250 mm x 4.6 mm spherisorb OD52 5µM column,

mobile phase A=water B=acetonitrile-water (95:5), gradient conditions 18% B 0 min, 18% B 10 min, 80% B 30 min, flow rate 1 ml/min. Retention time 28.09 min.

5′-Bromo-5′-deoxythymidine (131).

Triphenylphosphine (1.31 g, 5.00 mmol, 1.75 equivs.) and carbon tetrabromide (1.66 g, 5.00 mmol, 1.75 equivs.) were added to a solution of thymidine (0.69 g, 2.85 mmol) in dimethylformamide (5 ml), with stirring at ambient temperature. After 45 h methanol (10 ml) was added and the solvent removed under reduced pressure. Analysis of the residue by tlc revealed one major component along with a number of minor components. The residue was purified by column chromatography on silica with elution by chloroform-methanol (98:2). Pooling and evaporation of appropriate fractions gave the product as a white solid, (0.48 g, 55%), mp 157-158°C.

^{13}C nmr δ(CH_3OD) 165.69 (C2), 151.26 (C4), 137.10 (C6), 111.18 (C5), 86.30 (C1′ or C4′), 85.40 (C4′ or C1′), 72.56 (C3′), 39.63 (C2′), 32.07 (C5′), 11.63 (5-CH_3).

^{1}H nmr δ($(CD_3)_2SO$) 11.32 (1H, s, NH)*, 7.49 (1H, s, H6), 6.20 (1H, dd, H1′, J=7.8 Hz, J=6.5 Hz), 5.47 (1H, d, 3′-OH, J=4.3 Hz)*, 4.19 (1H, m, H3′), 3.90 (1H, m, H4′), 3.66 (2H, m, H5′), 2.21 (1H, m, H2′), 2.11 (1H, m, H2′), 1.76 (3H, s, 5-CH_3).

*Exchangeable with D_2O.

EIMS m/e 305.9978 (M^+, 1x^{81}Br, 4%, calc. for $C_{10}H_{13}{}^{81}BrN_2O_4$ 306.0039), 304.0079 (M^+, 1x^{79}Br, 4, calc. for $C_{10}H_{13}{}^{79}BrN_2O_4$ 304.0059), 181 (MH^+-thymine, 1x^{81}Br, base peak), 179 (MH^+-thymine, 1x^{79}Br, 94), 163 (MH^+-thymine-H_2O, 1x^{81}Br, 10), 161 (MH^+-thymine-H_2O, 1x^{79}Br, 10), 127 (thymineH^+, 89), 126 (thymine$^+$, 67).

Found C 39.17%, H 4.16, N 9.13, $C_{10}H_{13}BrN_2O_4$ requires C 39.36, H 4.29, N 9.18.

The reaction of 5′-bromo-5′-deoxythymidine (131) with triethyl phosphite.

A mixture of 5′-bromo-5′-deoxythymidine (0.15 g, 0.49 mmol) and triethyl phosphite (1.44 g, 8.67 mmol) was heated, with stirring and in a stream of nitrogen, to 100°C. After 50 h, a further portion of triethyl phosphite (1.44 g, 8.67 mmol) was added. The reaction mixture was stirred at 120°C for a further 40 h. The solvent was removed under high vacuum (*ca* 1 mmHg) at 30°C. The residue was purified by chromatography on silica, with elution by chloroform-methanol (97:3). Pooling and evaporation of the appropriate fractions gave a product as a colourless oil, (0.07 g, 36%).

^{31}P nmr $\delta(CDCl_3)$ 7.37, 7.08, (proton coupled 2xdq, J=707.7 Hz, J=5.5 Hz, J=707.1 Hz, J=5.4 Hz).

^{13}C nmr $\delta(CH_3OD)$ 165.35 (C2), 151.27 (C4), 136.91 (C6), 111.19 (C5), 85.67 (C1′), 84.31, 84.20 (2xd, C4′, J=5.5 Hz, J=6.2 Hz), 77.64 (d, C3′, J=5.5 Hz), 63.14 (d, $CH_3\underline{C}H_2OP$, J=5.2 Hz), 37.98, 37.74 (2xd, C2′, J=3.5 Hz, J=3.9 Hz), 31.67 (C5′), 15.77 (d, $\underline{C}H_3CH_2OP$, J=6.0 Hz), 11.62 (5-CH_3).

^{1}H nmr $\delta(CDCl_3)$ 8.84 (1H, bs, NH), 7.36 (1H, s, H6), 6.28 (1H, t, H1′, J=6.1 Hz), 5.01 (1H, m, H3′), 4.32 (1H, m, H4′), 4.08 (2H, m, $CH_3C\underline{H}_2OP$), 3.66 (2H, m, H5′), 2.32 (2H, m, H2′), 1.76 (3H, s, 5-CH_3), 1.29 (3H, t, $C\underline{H}_3CH_2OP$, J=6.4 Hz).

EIMS m/e 398.0066 (M^+, 1x^{81}Br, 0.2%, calc. for $C_{12}H_{18}{}^{81}BrN_2O_6P$ 398.0066), 396.0049 (M^+, 1x^{79}Br, 0.2, calc. for $C_{12}H_{18}{}^{79}BrN_2O_6P$ 396.0086), 207 (MH^+-Br-$EtOPH(OH)_2$, 19), 111 ($EtOPH(OH)_2^+$, base peak), 81 ($C_5H_5O^+$, 94).

Studies into the hydrolysis of 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (102).

A buffer solution of pH 7.0 was prepared by mixing 0.2 M 2,4,6-trimethyl pyridine (25 ml) with 0.2 M hydrochloric acid (17.5 ml) and diluting to a total volume of 100 ml with water. A mixture of this buffer solution and d-methanol (30:70) was prepared and the resulting solution was found to be pH 6.1. 3′-Azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (28.5 mg) was dissolved in this buffer/d-methanol mixture (3 ml) and kept at 37°C.

A buffer solution of pH 8.0 was prepared by mixing 0.2 M 2,4,6-trimethyl pyridine (25 ml) with 0.2 M hydrochloric acid (5 ml) and diluting to a total volume of 100 ml with water. A mixture of this buffer solution and d-methanol (30:70) was prepared and the resulting solution was found to be pH 7.0. 3′-Azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (26.1 mg) was dissolved in this buffer/d-methanol mixture (3 ml) and kept at 37°C.

A buffer solution of pH 9.3 was prepared by mixing 0.2 M 2-amino-2-methyl-1,3-propanediol (25 ml) with 0.2 M hydrochloric acid (6.25 ml) and diluting to a total volume of 100 ml with water. A mixture of this buffer solution and d-methanol (30:70) was prepared and the resulting solution was found to be pH 8.4. 3′-Azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate (27.4 mg) was dissolved in this buffer/d-methanol mixture (3 ml) and kept at 37°C.

The three solutions of 3′-azido-3′-deoxythymidine 5′-bis(2,2,2-trichloroethyl) phosphate in the various buffer/d-methanol mixtures were studied by ^{31}P nmr spectroscopy (162 MHz) and by tlc at suitable intervals.

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