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

SYNTHESIS AND EVALUATION OF NOVEL ANTI H.I.V. NUCLEOTIDES.

by

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ABSTRACT

A wide variety of nucleosides have been shown to inhibit human immunodeficiency virus (HIV) which is generally believed to be the causative agent of the disease acquired immunodeficiency syndrome (AIDS). In order to have activity these nucleosides are reported to act as their corresponding 5'-triphosphates, which inhibit reverse-transcriptase (RT), a unique viral enzyme. The phosphorylations are catalysed by cellular kinases.

This thesis describes the synthesis of a number of nucleoside-5'-phosphate triesters along with the results of biological evaluation studies in two different systems. Water, growth medium and plasma stability studies of selected compounds are also reported. It was hoped that these phosphate triesters could act as pro-drugs of the corresponding nucleoside 5'-monophosphates.

A group of 2-hydroxyacetamides was synthesised in a two step procedure from chloroacetyl chloride and the corresponding amine. The 2-hydroxyacetamides, halogenated alcohols, hydroxycarboxylic esters and other unsaturated alcohols were reacted with phosphoryl chlorides to produce a variety of, mainly novel, phosphorochloridates.

Among some prepared nucleosides; 3'-Azido-3'-deoxythymidine. 3'-O-methylthymidine, 3'-O-acetylthymidine, 3'-β-O-methanesulphonylthymidine, also thymidine and 2',3'-dideoxycytidine, were reacted with the phosphorylating reagents to give, in most cases, the required nucleotide products.

Attempts to make 3'-modified nucleoside 5'-phosphonates did not succeed although characterisable products were obtained.

Attempted synthesis of 3'-azido-3'-deoxythymidine-5'-di(S-methyllactyl)phosphite gave an H-phosphonate nucleotide product on isolation, however in situ the product could be converted to the phosphate by dinitrogen tetroxide.

Thymidine-5'-bis(2,2,2-trichloroethyl)phosphate was reacted with a number of acylating reagents to yield 3'-modified nucleotide products.

Biological evaluation of these nucleotides showed some of the compounds to have E.D.50's in the nM region with no toxicity observed at 100 μM. Further testing of the most active compounds by other laboratories using different test systems showed some of the compounds to have therapeutic ratios of over 10,000. Structure activity relationships are discussed.

Some of the biologically active compounds showed instability in growth medium and plasma studies though the initial products of decomposition were not free

nucleosides. The product from one plasma study was purified and identified as the carboxyl de-esterified nucleoside phosphate triester.

The mechanism of action of the compounds is not known though they may act as intracellular sources of nucleotide monophosphate.

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For Myrtle and June.

CONTENTS

ABBREVIATIONS

approx.	approximately
aq.	aqueous
AIDS	acquired immunodeficiency syndrome
ARC	AIDS-related complex
BBB	blood brain barrier
bp	boiling point
CNS	central nervous system
CSF	cerebral spinal fluid
DNA	deoxyribonucleic acid
E.I.M.S.	electron impact mass spectrometry
equivs.	equivalents.
F.A.B.M.S.	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
n.m.r.	nuclear magnetic resonance
PBM	peripheral blood mononuclear
ppm	parts per million
RNA	ribonucleic acid
RT	reverse transcriptase
tlc	thin layer chromatography
TMS	tetramethylsilane
uv	ultraviolet.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) was first identified as a distinct clinical entity in 1981[1]. AIDS has been recognised as a pandemic immuno-suppressive disease[2]. The patient suffers from weight loss and generalised lymphadenopathy, the latter leading to a variety of life-threatening opportunistic infections and malignancies. The most common of these are *pneumocystis carinii* pneumonia (PCP) and Kaposi's sarcoma[3].

A wide variety of other bacterial (eg *Mycobacterium avium-intracellulare*), fungal (eg Candida species) and viral (eg cytomegalovirus, herpes simplex and Epstein-Barr) infections plague AIDS patients. Neurological diseases, including fulminant dementia are also noted[4].

The causative agent of AIDS seems to be a virus. The virus has been referred to as AIDS-associated retrovirus (ARV)[5], lymphadenopathy-associated virus (LAV) or human T-lymphotropic virus type III (HTLV-III)[6] though the term human immunodeficiency virus (HIV)[7] has now been generally accepted.

Two distinct subsets of HIV have emerged termed HIV-1 and HIV-2. It would seem that HIV originated from primates; HIV-2 is closely related to the Simian virus (SIV) found in several old world primates. There also appear to be several different strains of HIV-1 and HIV-2[8].

The major target of HIV is the human immune system, particularly the helper/inducer T-cells. The depletion of these cells is accepted as a measure of disease progression

The virus can be spread by either intimate sexual contact, or the administration of infected blood products or by the maternal-foetal route and possibly by blood aerosol. For an unknown time-span after initial infection the carrier may remain healthy and will not have produced antibodies to the virus, thus the patient is termed antibody-negative. Some patients may then sero-convert and become antibody positive. Some patients will develop subclinical illnesses known as AIDS related complex (ARC) which includes lethargy, skin ailments and general reduction of immune function, while others will go on to develop full blown AIDS. AIDS in almost all cases eventually leads to the death of the patient.

HIV is a retrovirus. Animal retroviruses make up a small subset of the virus pool and are distinct from the more common DNA viruses in that the virus particle contains RNA rather than DNA. In order to replicate, this genetic information must be converted to DNA.

The HIV genome contains elements similar to those found in other viruses (DNA or RNA) type; genes encoding for the core structural proteins (p17, p24, p7 and p6) of the virion are found in a region known as *gag*; genes encoding for the outer envelope glycoprotein are termed *env*, and sequences at both ends of the genome which inform the ribosome replication units that it is the beginning or end of the genome are known as long terminal repeats (*LTR*)[9,10].

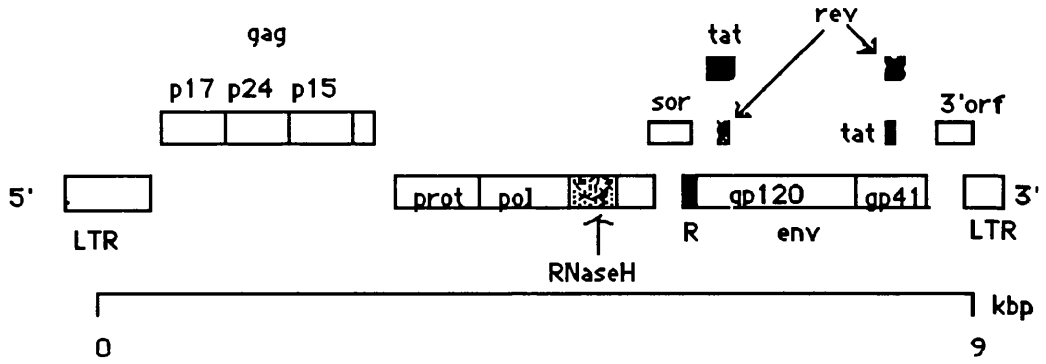


Fig 1 Gene Structure Of HIV-1[9-14]

Unlike DNA viruses, HIV together with other animal retroviruses contains a nucleic acid sequence *pol* which codes for a viral DNA polymerase, otherwise known as reverse transcriptase RT[9]. This is discussed in more detail later in the thesis. Furthermore HIV contains non-structural elements known as *tat*, *rev*, *3'-orf*, *sor* and *R*, which have regulatory functions acting at the viral genome level[11,12] ; *tat* and *rev* also regulate the use of host cell reproduction machinery[11,13]. All are vital for viral replication and infectivity[14].

The lifecycle of HIV and other animal viruses share many similarities. This cycle is in many respects different from the host cell replicative cycle and it is these differences that have been concentrated on in the attempts so far to combat AIDS.

After infection the first stage in the replicative cycle of the virion appears to be binding of the viral particle to a large protein on the surface of T-lymphocytes known as CD4[15]. The glycoprotein which makes up a large proportion of the viral envelope, known as gp120, is thought to be the unit that actually binds (electrostatically, and by Van der Waals forces) to the receptor[16]. Glycoprotein 120 is a resultant product of the *env* gene.

After this initial binding, the host cell with the virion attached can, *in vitro*, form syncitia with other CD4+ cells (any cell that houses CD4 receptors)[7,9]. It has not as yet been proved that this occurs *in vivo*. The process is thought to be mediated by another less common but still prevalent viral envelope glycoprotein gp41.

Interestingly, purified HIV gp120 alone can kill cells that have CD4 receptors. Thus, although infection of the cell has not occurred the glycoprotein must block the usual, as yet not clarified, vital task/s of this receptor[17,18].

After binding to the cell surface HIV enters the cell and the envelope breaks up to release the viral RNA. The actual mechanisms involved that bring about this release of RNA are not known.

A complementary (anti-sense) strand of DNA is then synthesized from the RNA. Again this process does not naturally occur in the host cell, but is mediated by a virally encoded enzyme, reverse transcriptase (RT)[19]. This unique enzyme consists of two polypeptide chains interwound, with molecular masses of 66,000 and 51,000. The enzyme

manufactures a complimentary strand utilising host cell building blocks (eg nucleotides, magnesium chloride and potassium chloride)[20].

RT also has the task of copying a second strand of DNA from the viral DNA/RNA hybrid. This new strand is a 'sense' strand otherwise known as a positive strand.

These two DNA strands, complimentary to one another, are base paired as the sense strand is synthesized. A ribonuclease (RNAaseH), coded for by a region of the RNA follows the RT along the RNA/DNA hybrid degrading the ribonucleotide to individual nucleotides as the double-stranded DNA is synthesised[21]. The degradation of this RNA is vital to the synthesis of the DNA perhaps because of some interaction between the RNAase and RT or the interference of the RNA strand with base pairing of the newly synthesized ribonucleic acids.

The HIV DNA duplex thus formed can then follow two distinct courses. Like many viruses, for instance ϕX174, the DNA duplex can circularize and be repeatedly transcribed into mRNA or become incorporated into part of the host genome, only replicating when host cell division takes place. In order for incorporation to take place an HIV integrase, similar to other viral integrases, is thought to be nec essary[22]. Furthermore, there must be sequences in the incorporated viral genome that inhibit the function of host nucleases. These host nucleases 'check' the authenticity of the nucleic acid sequences in the host genome and excise foreign sequences. The viral genetic information is thus protected from this excision and degradation.

Incorporation into host genome is most probably the reason why viral infection does not automatically lead to mass virion production resulting in full-blown AIDS. That the latency period differs markedly between individuals suggests that the start of mass HIV virion production within a cell is brought about by an external or internal 'trigger' not associated directly with the virus. Mitogens, antigens and cytokines have been found to modify the latency of HIV[23].

Conversion of the viral DNA duplex into mRNA, known as transcription, utilises the biochemical machinery of the host. This process is regulated by the products of at least two domains of the viral genome, *tat* and *rev*.

An example of a host protein binding to the viral genome is the NF-kB protein which associates with a short region of the *LTR* domains, known as *κB* (11-base pairs long). This protein, when bound to the viral genome actually increases proliferation of the virus[23]. Interestingly the *κB* sequence is identical to that found in the host κ-immunoglobulin gene enhancer region and the NF-κB protein is produced in activated T cells[24]. This may suggest that normal defence system T cell activation might lead to induction of latent virus. This gives support to the observation that some virus particles or toxins seem to trigger full blown AIDS.

Polyproteins are synthesized from the viral mRNA by the host translational system. However there are some differences between viral translation and host translation. Ribosomal frameshifting[25,26] is where a ribosome, translating one mRNA chain, will start translating a different chain rather than terminating polypeptide synthesis at some stop codon on the first chain. Presumably this can occur because one mRNA chain is bound to another in certain regions and the ribosome changes onto the 'new' mRNA chain at these quasi duplex regions rather like a "locomotive switching tracks".

The polyproteins thus synthesized are cut-up into proteins by HIV protease, a unique enzyme responsible for splitting the polyproteins in at least seven places to yield viable proteins[27]. The HIV-1 protease, vital to the successful replication of the virion[28-30], is released autocatalytically from the gag-pol polyprotein translated from the *prot* region of the viral genome[31-33]. One fraction of the polyprotein $Pr55^{gag}$[34-38] is further split to yield p17, p24 (the largest capsid protein[39]) and p7 and p6, the essential core elements. The other polyprotein produced from this region yields $Pr160^{gag\text{-}pol}$ on translation. This contains RT, RNAaseH and an endonuclease (function in packaging virion particles)[35,40].

From purified enzyme[41] it has become apparent that HIV protease is an aspartic protease[45], identified as a dimer, each polypeptide component consisting of 99 amino acids[43-46]. The active site of this enzyme consists of a sequence of two lots of 'hydrophobic AA-hydrophobic AA-asp-thr-gly' amino acid chains.

Following nucleic acid and protein synthesis all the components of a potentially infectious virus are assembled at the inner surface of the cell involving host proteins. A process known as viral budding then takes place releasing the virus particles from the host cell wall[47].

There are many steps in the replication of the virus that are not present in the host cell cycle. The viral genome contains domains that code for unique enzymes. Host enzymes are used at other stages, for example the formation of mRNA. It should be possible to inhibit viral reproduction *in vitro* at any of the stages listed above. However if steps that are unique to viral proliferation were to be halted there would be less likelihood of host toxicity.

A wide spectrum of compounds has been found to be effective against HIV out of a myriad of compounds tested. The site and exact mechanism of action of some of these agents have been elucidated. It is extremely difficult to compare activities of compounds unless the anti-HIV agents are tested under identical conditions. This is because activities are dependent on the cell type, culture medium, cell growth rates and the cell growth phase employed[48].

There follows a review of some compounds found to posess anti-HIV activity.

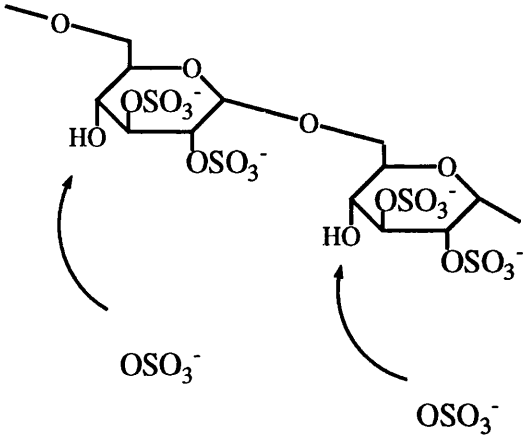
Binding of HIV to $CD4^+$ cells.

Soluble CD4[49], a glycoprotein made up of residues 1-178 of the naturally occurring CD4 receptor has been shown to have substantial anti-HIV activity combined with low

toxicity. The immune system also retains the ability to be stimulated when soluble CD4 is administered. However the polypeptide has a half life in plasma of less than 30 min; hundreds of milligrams per kilo would have to be administered. CD4 glycopolypeptides with longer half lives are being investigated[50]. This compound and other similar $CD4^+$ fragments are proposed to act as antiviral 'sponges' binding to gp120.

ATA,[51] a carboxylic acid based on aurintri-carboxylic acid, selectively alters the CD4 receptor, probably by binding to it. A therapeutic ratio of 300 is observed, activity at 1 μM. ATA may also act at fusion of the virion stage.

α-D- glucose units linked predominantly (1->6), with two or three sulphate groups per glucose moiety[52].



1

Dextran Sulphate **1** and various sulphated polysaccharides including PVAS and PAVAS interfere with viral binding to $CD4^+$ cells[52]. However in clinical trials **1** showed no anti-HIV activity when administered orally.

Al-721, a mixture of neutral lipids extracted from egg yolks, has been found to inhibit HIV *in vitro*, perhaps by disturbing the cell membrane and upsetting binding of the virion[53].

Syncytium formation.

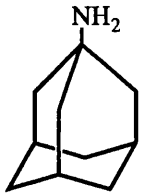
Sulphated polymers and ATA also block formation of syncytia[54]. Generally the higher the density of the sulphate group and the higher the molecular mass the less likely syncytia formation is.

Fusion of the Virion.

ATA may also disturb the major capsid protein p24 by binding to the hydrophobic pocket[51] leading to premature, extracellular uncoating of the virus.

Uncoating of the Virion.

Amantadine **2** is believed to act at this stage[55], although the exact mechanism has yet to be elucidated.



2

Reverse transcriptase inhibition.

The vast majority of anti-HIV agents discovered to date act at this stage. An in-depth discussion of these agents will be left until later in the thesis.

Integration of the viral DNA duplex into the host genome.

Compounds that act by RT inhibition may also act at this stage.

Transcription and translation.

Compounds that act by RT inhibition may also inhibit viral replication at this stage if they affect host replicative machinery.

Ribosomal frame shifting.

Oligodeoxynucleotides, discussed later in the thesis, may also act at this level by interfering with the proposed mRNA quasi duplex formation.

Viral component production and assembly.

The host cell machinery is used for many of the steps involved in viral production and assembly. However some unique viral enzymes are also vital to the successful completion of replication. The inhibition of HIV protease has been concentrated on in efforts to inhibit HIV at this stage.

In general the design of inhibitors of this enzyme follow three principles. Firstly, find and identify a natural oligopeptide (polypeptide) substrate. Secondly, find and identify the scissile region/s. Thirdly change this sequence of amino acids for a sequence that can not be cleaved by the enzyme[56].

This approach has come up with some very active anti-HIV agents *in vitro*. For example Ac-Ser-Leu-Asn-[Phe-HEA-Pro-]-Ile-Val-OMe, shows 50% inhibition of the purified protein at 0.66 nM[57].

This class of inhibitors tend to be linear competitive inhibitors though the HEA (hydroxyethylamine) compounds effectively act as irreversible inhibitors of HIV-protease[58]. The inhibition is measured by the ability of the enzyme to proteolytically process a recombinant form of $Pr55^{gag}$.

In conclusion, investigation into inhibitors of HIV-protease has just begun. Many aspartyl protease inhibitors have been found in the past and this approach holds much hope for the discovery of effective anti-HIV drugs.

Agents that act on RT.

In basic terms RT 'reads' a strand of RNA and synthesizes a complimentary (anti-sense) strand from 2'-deoxynucleotide tri-phosphates (dNTPs) **9**, thus the new strand is a strand of DNA. The enzyme then reads this synthesized strand and synthesizes a complimentary (sense) strand of DNA from more **9**. Compare this with host DNA polymerase a: the polymerase reads both strands of a DNA duplex at the same time synthesizing a new complimentary strand for each old strand from **9**.

At first the two enzymes seem to be involved in different tasks, however on closer inspection there are many similarities; one part of both enzymes action involves the reading of a single strand of nucleic acid and synthesizing a new strand of DNA, both enzymes employing identical building blocks to make DNA.

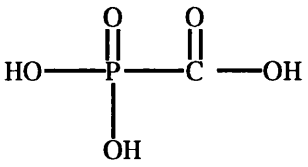
Therefore although RT is a unique enzyme the fact that it does a similar job using identical building blocks to the host polymerase a suggests that unnatural building blocks that could interfere with RT resulting in anti-HIV activity may also interfere with host polymerase resulting in toxicity.

Sumarin was the first compound that was found to inhibit HIV RT[59], however no immunological improvement was observed *in vivo*, indeed the compound showed significant toxicity[60].

Foscarnet **3** inhibits HIV[61] *in vivo* and purified RT *in vitro* at 2 μM[62]. **3** crosses the blood brain barrier at levels capable of combating HIV infection. The compound is thought to act as a mimic of pyrophosphate, specifically acting as a competitive reversible inhibitor at the active site of RT that splits pyrophosphate from **9** in the elongation of the nucleic acid chain. However the beneficial effects of **3** have been found to be transient suggesting that the active site of RT becomes less susceptible to the acid[62].

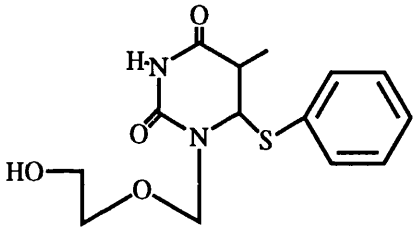
Rifabutin has been shown to inhibit RT and HIV *in vitro*[63], though only limited, dose unrelated activity is observed *in vivo*[60].

Antimoniotungstate (HPA-23), a cryptate material, has been found to inhibit RT *in vitro*[64]. Some clinical trials have been carried out with it, but it leads to only a slight improvement in symptoms.



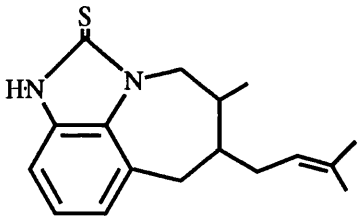
3

HEPT 4 [65,66], inhibits HIV-1 but not HIV-2. This is remarkable, since although many compounds have differing inhibitory effects on HIV-1 and HIV-2 only TIBO 5 (described below) and 4 show such marked preference for HIV-1. The reasons for this are not clear. 4 inhibits T4 cell cultures at 1 μM, with a therapeutic ratio of 500[66]. The compound has only recently been synthesized and so little further data has been collected regarding its pharmacological or pharmacokinetic properties at this time.



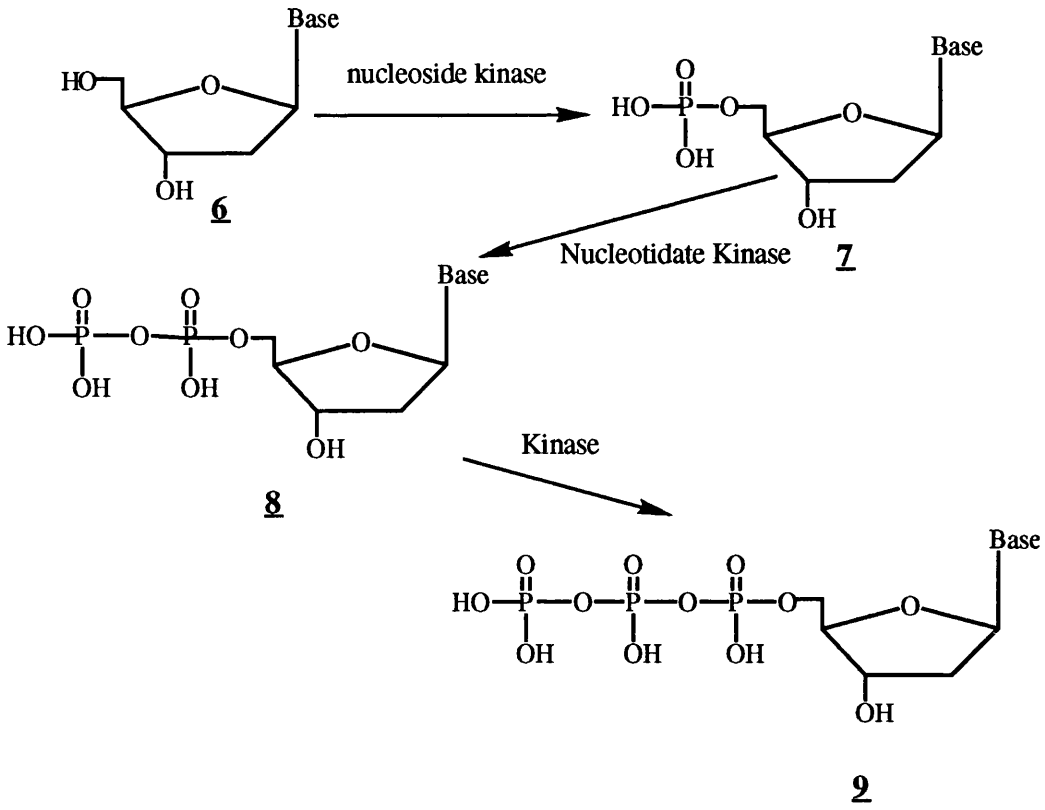
4

5[67], is another very recent discovery. Though not particularly similar to 4 it also acts specifically on HIV-1. Both 4 and 5 act on RT but it is thought that they act in a different manner to most anti-HIV nucleosides. HIV-1 is extremely sensitive to 5. Inhibition, measured in many different ways, occurs within the concentration range of 0.001-0.1 μM.



5

By far the largest group of HIV inhibitors investigated has been the 3'-modified-2',3'-dideoxynucleosides. They seem to act on RT though activity at other stages of viral replication has not been ruled out.

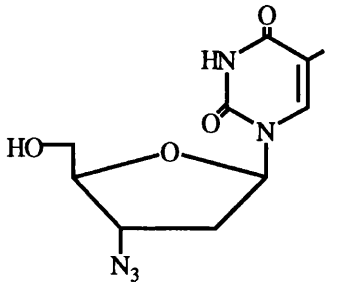


6 7 8 9

These compounds can not be phosphorylated at the 3' position since they lack the necessary hydroxyl group. Therefore the compounds can act as chain terminators if phosphorylated to the triphosphate and recognised by a host or viral polymerase enzyme. Compounds that display anti-HIV activity in this class include 3'-deoxy-3'-azidothymidine (AZT) **10**, 2',3'-dideoxycytidine (DDC) **11**, 2',3'-dideoxy-2',3'-didehydrothymidine (d4T) **12**, 3'-deoxy-3'-fluorothymidine (FdT) **13** and an array of base altered analogues of these compounds.

10[68] is the only drug licensed for clinical use in the treatment of AIDS[69-73]. **10** has the ability to cross the blood brain barrier. This is of importance since a significant number of patients with AIDS and ARC develop neurological complications[74-81], including dementia[82]. HIV can also replicate in the brain[78]. 60% of the plasma level of **10** is achieved in the cerebral spinal fluid (CSF)[83], although it is not known whether the concentration in the CSF is sufficient to effectively suppress viral replication in the central nervous system (CNS).

It has been reported that **10** must be phosphorylated to the triphosphate **16** to give activity[84]. **10** is believed to be phosphorylated to **16** by the same enzymes that phosphorylate thymidine.



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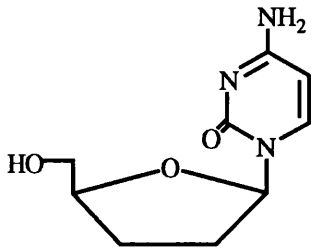

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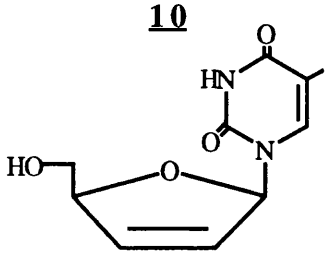

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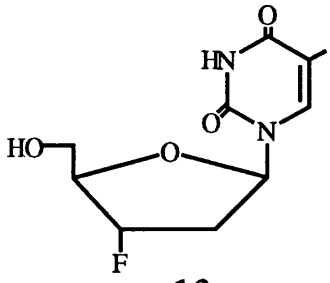


13

Firstly **10** enters the cell (mainly by non-facilitated diffusion)[85] and is phosphorylated by thymidine kinase to the monophosphate (AZTMP) **14**. The kinase rapidly converts **10** to **14**, indeed almost as rapidly as it turns over the natural substrate[86,87]. The monophosphate is then phosphorylated to the diphosphate (AZTDP) **15** by thymidylate kinase. **14** has a K_m of 8 μM, that is 2-fold higher than the K_m of the physiological substrate dTMP **7**. Yet, the V_{max} of **14** for the kinase is only 0.3% of the V_{max} of **7** for this enzyme. Thus **14** is a potent alternative-substrate inhibitor of thymidylate kinase[84,86]. The enzyme that phosphorylates the diphosphate **15** is not known for certain, though it is thought to be thymidine diphosphate kinase[87].

16 has a very high affinity for the RT: k_i =0.002-0.04 μM. **16** can act as a chain terminator since there is no 3'-hydroxyl group to condense with a phosphate, or may act as a competitive inhibitor of RT binding strongly enough to inhibit the enzyme.

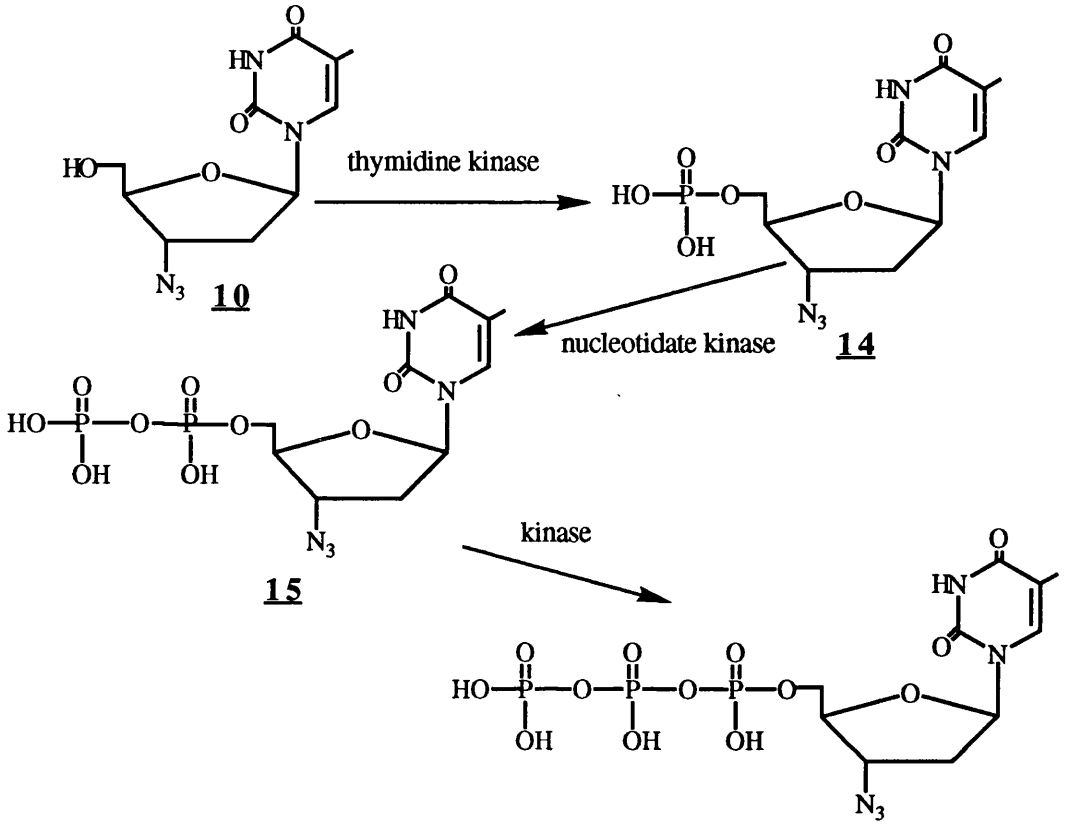
The anti-HIV activity of **10** varies between 0.001 μM and 10 μM depending on the test conditions[87].

10 has many drawbacks that can necessitate a reduction of amount administered to a patient, or indeed to total discontinuation of use[88,89]. One of the most serious side effects[90] of **10** is the susceptibility of bone marrow progenitor cells[91] (the cells that grow bone marrow). Malfunction of these cells can lead to anaemia and in severe cases neutropenia[89].

The cause of toxicity of **10** is not known. Host polymerases a and b are significantly less susceptible than RT to inhibition by **16**[20] although **16** can be incorporated into the host genome thus terminating replication of DNA.

Recent studies show that there is no consistent change in pool size of **9**[92,93]. Since **14** inhibits thymidylate kinase thus presumably inhibiting formation of **8** and **9** there may be

some other mechanism by which **9** is synthesized. It therefore seems doubtful that inhibition of thymidylate kinase by itself is the cause of toxicity of **10**.



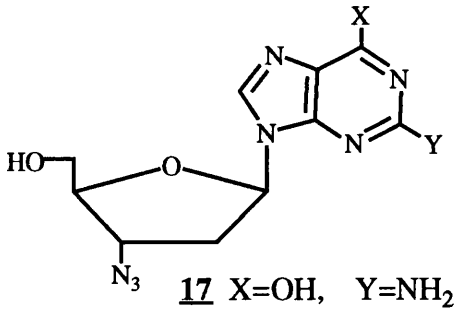
10 **14** **15** **16**

HIV variants resistant to **10** have been isolated from patients on prolonged treatment of **10** [94], which may perhaps go some way to explain evidence that the efficacy of **10** against HIV decreases over time[87]. Generally a sharp fall in efficacy is observed after six months. Reasons for this decreased activity have not been elucidated though it is postulated that RT becomes less susceptible to **16**.

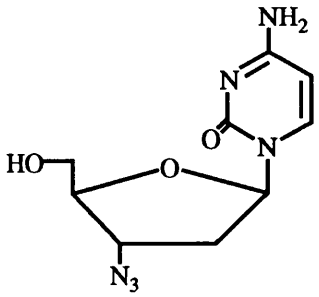
It has also been found that as treatment continues over a long period of time levels of **14** tend to decrease[87]. This may also go some way to explaining why efficacy of **10** diminishes over time.

Many nucleoside analogues have been synthesized containing an azido group at some position in the sugar region. A handful of these compounds have been shown to be active, though none of these display the same levels of efficacy as **10**. 3'-Azido-2',3'-dideoxyguanosine **17** has been shown to be a potent HIV inhibitor *in vitro*[95,96], though the compound does show significant toxicity[97] and so has not proceeded further into clinical trials. 3'-Azido-2,6-diaminopurine-2',3'-dideoxyribose **18** has high activity but low

toxicity[98]. 3'-Azido-2',3'-dideoxycytidine **19** is six times less active than **10** in peripheral blood mononuclear (PBM) cells[99].

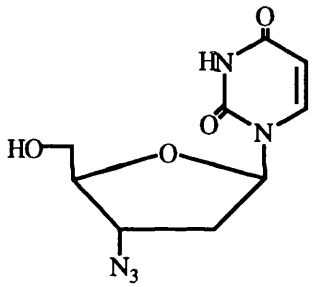


17 X=OH, Y=NH_2
18 X=NH_2, Y=NH_2



19

3'-Azido-2',3'-dideoxyuridine (AZDU) **20**[100] is not as active as **10** in PBM cells[101] but has significantly lower bone marrow toxicity[102]. The lower activity of **20** appears to be due to poor phosphorylation to the monophosphate[103].



20

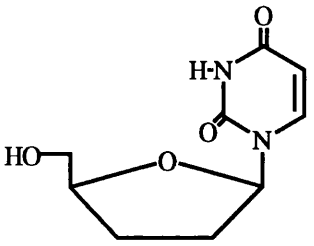
11, currently undergoing active clinical development, seems to inhibit HIV RT[69] in a similar manner to **10**, indeed it displays greater efficacy in most tests. Studies carried out in mice and monkeys showed that the drug's administration was straightforward and its bioavailability good[104].

It has been reported that unlike cytidine, **11** is not deaminated to 2',3'-deoxyuridine **21**[105], however in the presence of pyrimidine nucleoside deaminase inhibitor, the potency of **11** increases 3-4 fold[106]. This does not condone the theory that **11** is resistant to deamination, but it does not necessarily condemn it, since it is known[107] that one nucleoside may inhibit the transport of other nucleosides. Whether pyrimidine nucleoside deaminase inhibitors enhance anti-HIV effects of other nucleoside analogues remains to be studied.

2',3'-Dideoxyuridine (DDU) **21** has low activity against HIV tested in ATH8 and MT-4 cells. **21** is not phosphorylated which is perhaps why the activity is poor. However

the triphosphate of **21** (DDUTP) inhibits HIV RT in vitro at 0.05 μM[108], comparable to the activity of **16**.

2',3'-Dideoxy-2'-β-fluorocytidine **22**, an analogue of **11**, has good activity against HIV *in vitro*; ED_{50} 0.65 μM[109], though few beneficial effects are observed *in vivo*.



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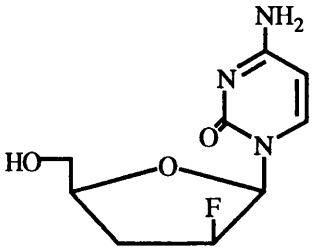
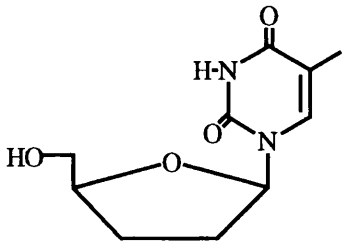


22

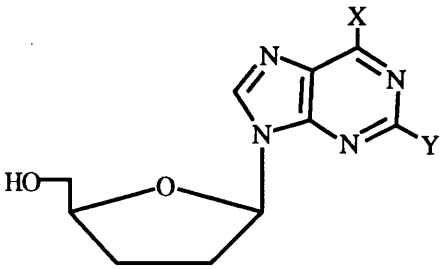
11 does cross the blood brain barrier but not to the same extent as **10**. **11** does exhibit severe side effects: these include cutaneous eruptions, fever, mouth sores, thrombocytopenia and neutropenia[77]. The cause of the toxicity of **11** is not known for certain.

Since the toxicity profiles of **10** and **11** are different the usefulness of administering the two together (combined chemotherapy) has been investigated[73]. It is hoped that the levels of each drug can be reduced, so limiting the toxic side effects whilst retaining the activity required to combat AIDS. Results of combined chemotherapy tests are promising and warrant further investigation.

2',3'-Dideoxythymidine (DDT) **23**, 2',3'-dideoxyadenosine (DDA) **24**, 2',3'-dideoxyinosine (DDI) **25**, and 2',3'-dideoxyguanosine (DDG) **26** also inhibit HIV *in vitro*[70].



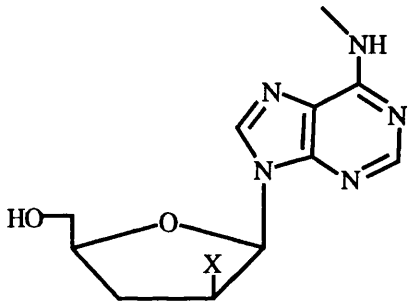
23



24 X=NH_2, Y=H
25 X=OH, Y=H
26 X=OH, Y=NH_2

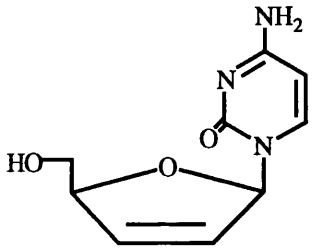
24 Undergoes rapid deamination by adenosine deaminase to **25**[110,111]. **25** is partially phosphorylated to it's monophosphate (DDIMP) which is converted to 2',3'-dideoxydidehydroadenosine diphosphate (DDAMP) which is sequentially phosphorylated up to the triphosphate DDATP[110,111]. Thus it has been proposed that the antiviral activity of **25** is due to DDATP. Both **24** and **25** have recently entered clinical trials[112].

Although **24** and **25** are quite promising anti-HIV agents complete glycosidic bond cleavage occurs in two and one min. respectively at pH 1 at 37 °C[113]. This precludes oral administration of these compounds. With this in mind some 2'-β-fluoro-2',3'-dideoxynucleoside analogues of **24** and **25** have been synthesized[114]. These compounds were found to be indefinitely stable at pH 1 at 37 °C. The analogues were also as active as the parent compounds against HIV-1, though slightly less active against HIV-2. Recently it has been reported that the *N*-6-methyl analogue of DDA **27** is slightly active against HIV, indeed if further substituted with fluorine at the *threo* 2' position the analogue **28** has up to 4% the activity of **24**. The *N*-6-methyl compounds are resistant to adenosine deaminase and the fluoro compound is far more resistant to acid than **24**, making them potential oral anti-HIV agents[115].



27 X=H
28 X=F

12 is another potent anti-HIV agent[114] lacking a 3'-hydroxyl group. Tests show **12** to be active between 0.001 and 0.01 μM[116,117], comparable to **10**. **12** has similar activity to 2',3'-dideoxy-2',3'-didehydrocytidine (D4C) **29**.



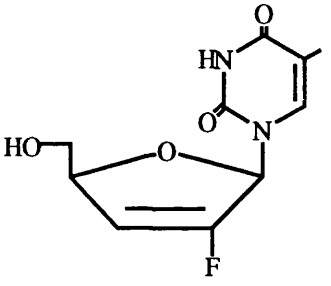
29

12 is thought to act through the triphosphate[118]. The intracellular pharmacology of **12** has been studied and compared to that of **10** in two independent studies[119,120]. Both studies show that 2',3'-dideoxy-2',3'-didehydrothymidine monophosphate (D4TMP), the diphosphate (D4TDP) and the triphosphate (D4TTP) are produced. However unlike **10** which is almost totally metabolized, a significant proportion of **12** remains un-phosphorylated. Furthermore a large proportion of **12** is converted to the triphosphate; whereas most of **10** remains as the monophosphate.

The estimated half life of D4TTP is 3.5 h. in human PBM cells[121], approximately 3.5 times as long as **16** so **12** may not need to be administered as frequently as **10** which must be given every 4 h.

12 shares the toxicological effects of **10** in some tests though it displays different toxicity in others. In general however it shows low toxicity towards human bone marrow cells *in vitro*[115], one of the most serious side effects of **10**. **10** and **12** combination chemotherapy trials are currently in progress.

3'-Deoxy-2',3'-didehydro-2'-fluorothymidine **30** and 2',3'-dideoxy-2',3'-didehydro-2'-fluorocytidine **31** have also been shown to have anti-HIV activity although at 100 μM and 10 μM respectively, at least 100 times less active than **12**[107]



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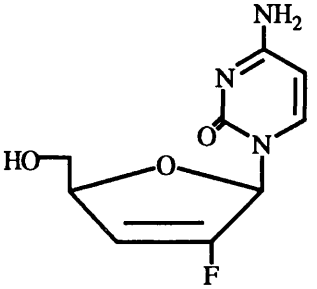
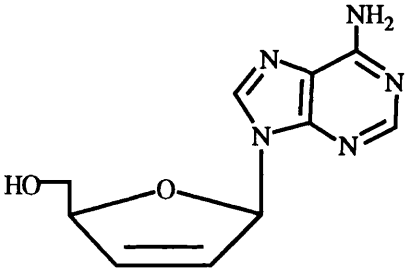


31

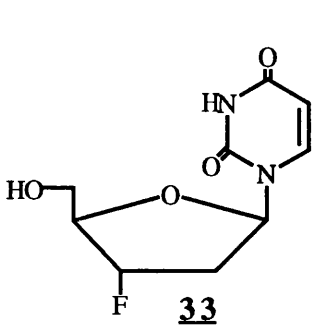
2',3'-Dideoxy-2',3'-didehydroadenosine **32** is also active against HIV though about 10 times less active and slightly more toxic than **12** in ATH8 cells[122].



32

13 has also been shown to be a very active anti-HIV agent in many tests[123-126]. Once again **13** is phosphorylated to the triphosphate (FdTTP). **13** has been shown to be more active than **10** (0.001 μM compared to 0.004 μM) and less toxic to host PBM cells (20 μM compared to 0.2 μM). The therapeutic ratio of **13** is thus 40 times that of **10**[123]. **13** is currently undergoing further clinical trials, and may be able to be used concurrently with or perhaps instead of **10**. 3'-Fluoro-2',3'-dideoxyuridine (FdU) **33**[127] and 3'-fluoro-2',3'-dideoxyadenosine (FdA) **34**[128] have also been found to be active against HIV at low

concentrations with little toxicity. 3'-Fluoro-2'-β-fluoro-2',3'-dideoxycytidine **35** has been shown to have some anti-HIV activity[107].



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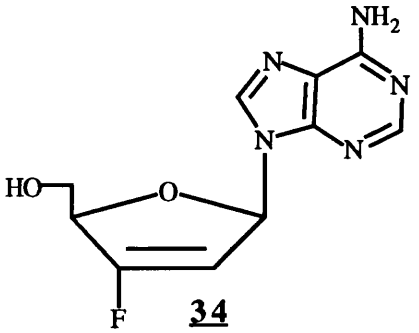

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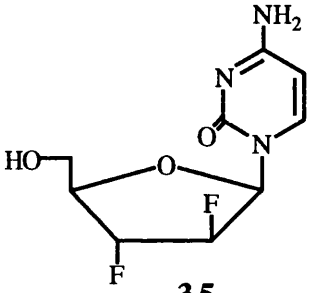
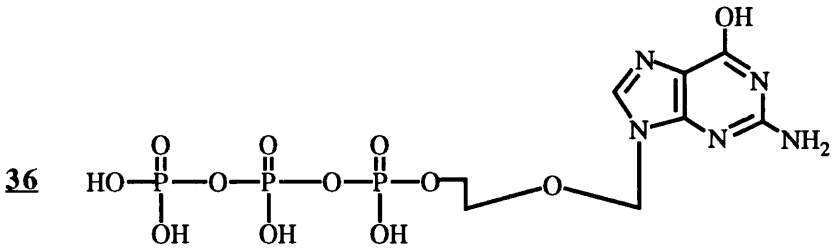


35

All of the aforementioned nucleoside analogues seem to require phosphorylation to their respective triphosphates to be active, indeed there seems to be some correlation between activity and the levels of the nucleoside triphosphate found in the cell[48]. Different enzymes phosphorylate these compounds and may do so at different rates. The toxicity of these compounds appears to be due, at least in part, to the ability of host polymerases to use the nucleotide triphosphate in the host's replicative cycle.

The observation that nucleosides need to be phosphorylated to the nucleotide triphosphate in order to exhibit activity has led some groups to synthesize triphosphates of nucleosides that are themselves inactive. The theory behind this is that the nucleoside itself may not be active because it is not phosphorylated up to the triphosphate in the cell. Therefore administration of the nucleoside as the triphosphate would in theory obviate the need for recognition by the phosphorylating enzyme and the compound may then inhibit RT.

Some success has been achieved by this approach. For example 9-(2-hydroxyethoxymethyl)guanine[129] and 3'-amino-3'-deoxythymidine[99] are inactive against purified RT of HIV *in vitro*. However the tri-phosphates (**36** and **37** respectively) of these nucleosides do inhibit purified reverse transcriptase *in vitro*[20]. Another example of a nucleoside that is inactive[130] but whose triphosphate is active *in vitro* is 3'-O-methylthymidine 5'-triphosphate **38**[131].



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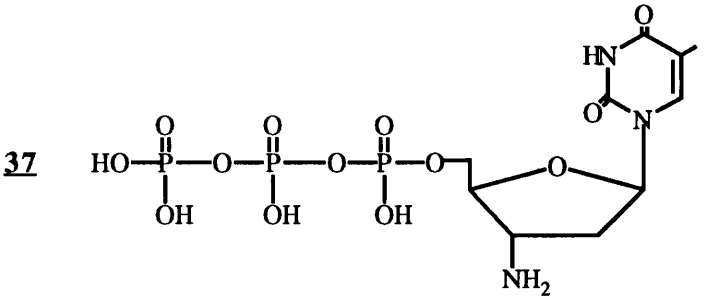

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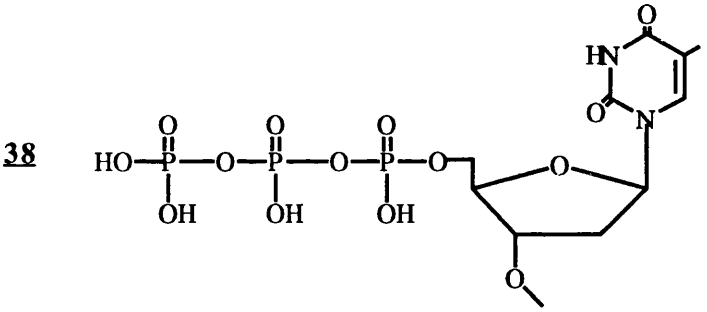


38

Nucleotide triphosphates have shown disappointing results *in vivo*[132]. This is perhaps because the nucleotides are hydrolysed by phosphorodiesterases extracellularly to the nucleoside and also that the highly charged nucleotides permeate the cell membrane with low efficiency.

Since it would appear that nucleosides need to be phosphorylated up to the triphosphate to be active a variety of nucleoside mono-phosphates have been synthesized. The theory behind the synthesis of these compounds is that they may not need to be phosphorylated by the corresponding nucleoside kinase. However as with the triphosphates the membrane permeability of these compounds would be expected to be low and so examples do not tend to have more favourable effects over the corresponding nucleosides[133]. 5'-Phosphates also hydrolyse rapidly to the free nucleoside by the action of phosphatases[134,135].

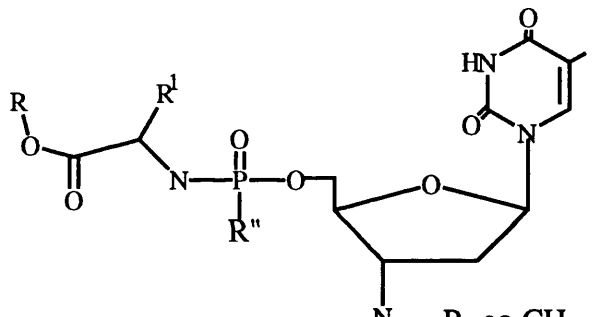
Problems with drug delivery to the desired site of action have been widespread over the years. Apart from the design and synthesis of new compounds that have better bio-availability the main course of action has been to synthesize compounds that are pro-drugs of the compound whose bio-availability needs to be improved.

A prodrug may be defined as a pharmacologically inactive derivative of a parent drug that is converted to the parent compound by virtue of enzymic or chemical lability within the

body system[136-137]. In general prodrugs must have as good if not better solubility and stability in plasma, good bioavailability, clearance and duration of action.

The design and synthesis of pro-drugs of anti-HIV agents is a relatively new field of study, some interesting compounds have been designed and synthesized that exhibit HIV activity.

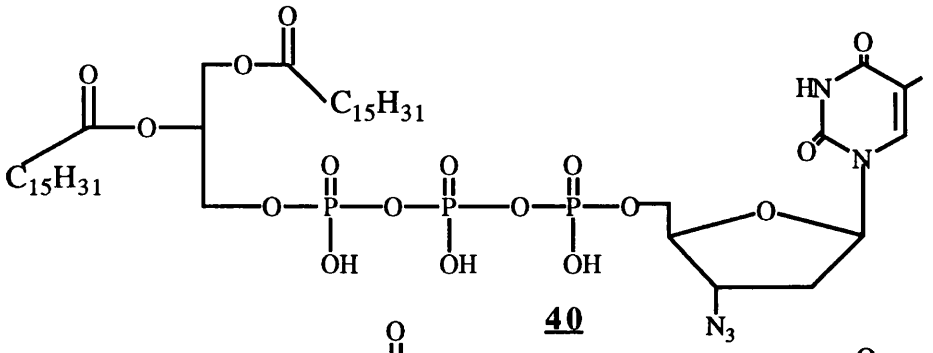
A number of uncharged phosphate triesters of **10** where one group is a simple alkyl group such as ethyl, or a substituted one such as 2,2,2-trichloroethyl, and the other group is a carboxy-protected amino linked amino acid have been reported in the literature **39**[138]. These compounds might be expected to be more stable in plasma than corresponding diesters or nucleoside monophosphates since there is no known phosphorotriesterase. The triesters may also permeate the cell more easily since these compounds are uncharged at cellular pH. Once inside the cell the compounds may breakdown to **10** or **14** or perhaps even act in their own right. These compounds were found to be active in the range 0.1-40 μM. No toxicity was observed at 100 μM. further tests are currently being run on these compounds.



39

R=eg CH_3
R'=eg CH_3
R"=eg alkylO

Phospholipid analogues of **10** (eg **40** and **41**), **11** and **23** have been synthesized[139]. It has been proposed that macrophages infected with HIV may be resistant to dideoxynucleosides due to low levels of nucleoside kinase[140] so the administration of mono, di or triphosphates may overcome this problem. Macrophages take up the bulk of parenterally administered liposomes and this property has been utilised in the design of drugs for diseases involving these cells[141]. By attaching a phospholipid to a bio-active nucleoside site direction of the nucleoside to macrophages may occur.



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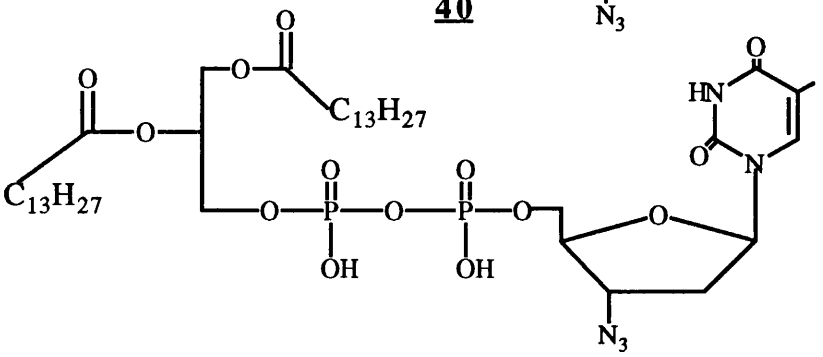
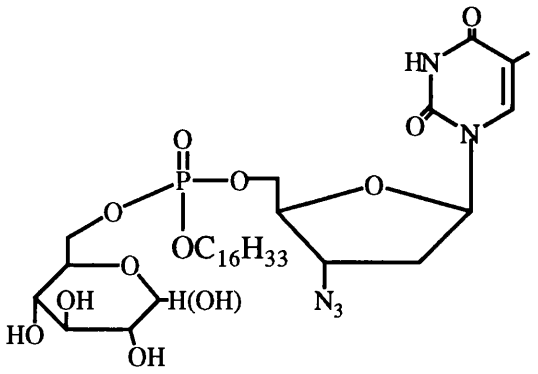


41

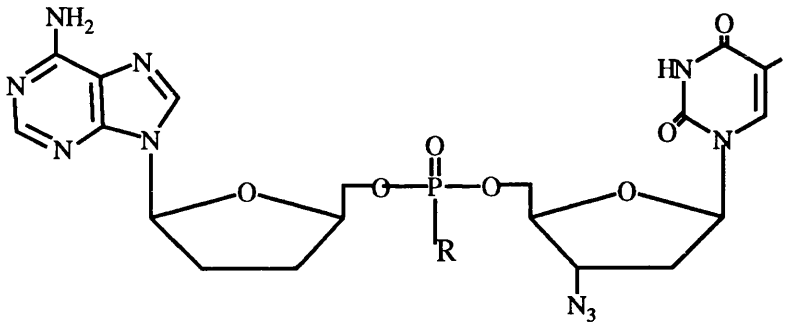
The mono-phosphate compounds are roughly 3-5 times less active than the parent nucleoside *in vitro*. Interestingly a 2',3'-dideoxythymidine-5'-monophosphate analogue displays activity (**23** displays very little activity). Definite reasons are not given for this observation though the activity may be due to the kinase-'bypass' effect where a nucleoside is resistant to phosphorylation by kinase but whose triphosphate does show anti-viral activity. The kinase-'bypass' theory is currently being investigated with mutant CEM cells which lack thymidine kinase. However the fact that these compounds are phosphate diesters means that they may be rapidly degraded *in vivo* to the free nucleoside by phosphodiesterases.

A recently synthesized anti-HIV phosphate triester that may rely on active transport rather than passive diffusion to enter cells has shown anti-HIV activity *in vivo*[142]. The triester contains **10**, a long alkyl chain and glucose. Further investigations are currently in progress.



42

Another class of prodrugs recently reported[143] are hetero and homo dimers of nucleosides with a phosphate linkage. It was found that, on an equimolar basis, greater anti-HIV activity and enhanced therapeutic ratio's are observed for the dimers over the corresponding monomers. Plasma and CSF levels of the dimers were similar to the monomers and *in vitro* stability of the phosphate bonds depended on the attached nucleosides. Triesters were also synthesized, the first two substituents bioactive nucleosides, the third substituent a cyanoethyl group. AZT-P-DDA **43** and AZT-P(Cye)-DDA **44** completely protected MT-2 cells from the formation of syncytia at 0.5 μM, AZT required 1 mM.These results suggest that these dimers are of great interest biologically and again (**39**[138]and **42**[142]) that phosphate triesters can exhibit anti-HIV effects.

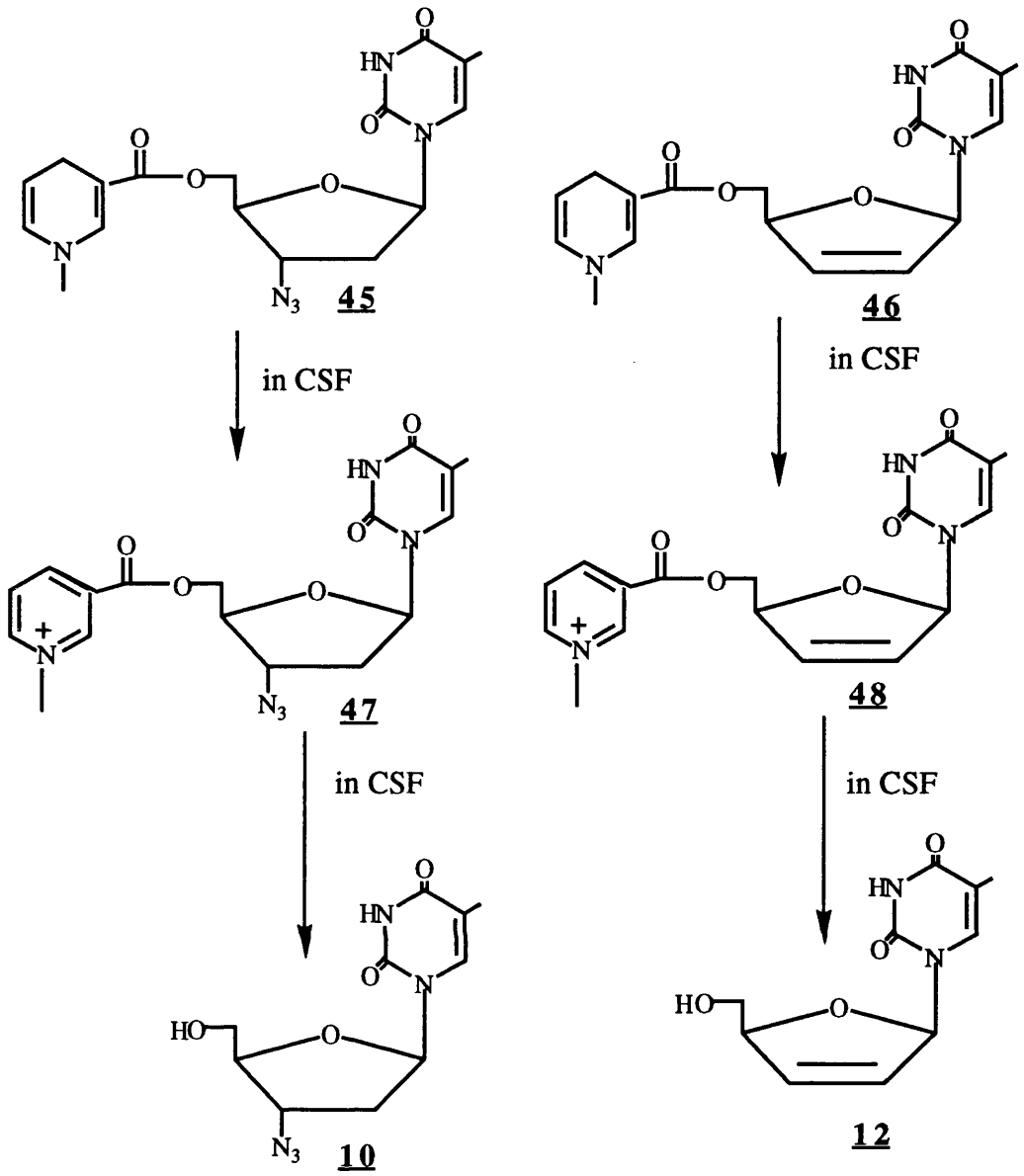


43 R= OH
44 R= OCH_2CH_2CN

Two interesting prodrugs of bioactive nucleosides are 5'-(1,4-dihydro-1-methyl-3-pyridinylcarbonyl)-3'-azido-3'-deoxythymidine **45**[144] 5'-(1,4-dihydro-1-methyl-3-pyridinylcarbonyl)-3'-azido-2',3'-dideoxy-2',3'-didehydrothymidine **46**.

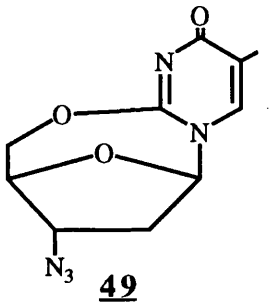
It has been reported[145] that when a biologically active compound is linked to a lipophilic dihydropyridine carrier the resultant prodrug can readily penetrate the blood brain barrier (BBB). Oxidation of the carrier moiety to the hydrophilic quaternary salt results in the pro-drug being locked inside the brain.

45 is active against HIV in MT-4 cells[146], and is significantly less toxic than **10** in murine bone marrow cells[147]. The compound has been shown to obtain a higher concentration than **10** in rat brain and dog CSF[148]. The dihydropyridine derivative of **12**, **46** has also been synthesized and bioavailability studied. The compound was shown to enter the brain, and is oxidised to the quaternary salt. This results in the sustained release of **12**[149].



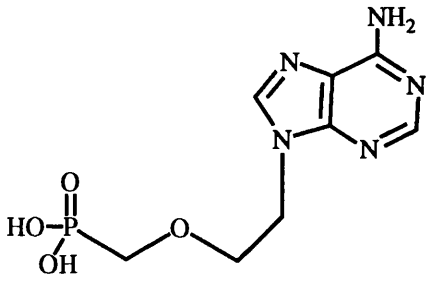
Half lives for the dihydropyridine derivative analogues **20** and **10** in human serum are 4.33 and 7.7 h. respectively[144]. The compounds were also found to achieve much higher CSF/plasma ratios than the corresponding nucleosides in mice. The compounds are currently undergoing further clinical evaluation.

Another HIV inhibitor which may act as a pro-drug of **10** is the 2,5'-anhydro derivative **49**[150]. The compound is slightly less active than **10** and is thought to act by intracellular cleavage of the 2,5'-anhydro linkage to release **10**.



49

2-Phosphonylmethoxyethyladenine (PMEA) **50**[151] is active at 3 μM *in vitro* against HIV. The compound has also been shown to be active against cytomegalovirus, an opportunistic infection that is common among AIDS patients. The pharmacokinetics of this compound are favourable[152] and warrant further study.



50

The replication of retroviruses was known to be inhibited by oligodeoxynucleotides before the discovery of HIV[153,154]. Since HIV has been recognised, oligodeoxynucleotides that are complementary to certain base sequences of the viral genome have been found to exhibit activity *in vitro*[155,156].

Oligodeoxynucleotide phosphorothioates (S-ODNs) are a common class of these anti-HIV agents. S-ODNs are short sequences of nucleic acid and have been found to block the *de novo* infection of susceptible cells by HIV-1[157-159] and inhibit viral expression and proliferation in already infected cells[156-161]. The ability to block *de novo* HIV-1 infection appears to be a common feature of S-ODN's, whereas the ability to inhibit expression in already infected cells is specific to S-ODN sequences that are complimentary (anti-sense) to viral RNA.

The blocking of *de novo* infection by S-ODN's is both composition-dependent and length dependent. For instance S-dA_{28} > S-dC_{28} > S-dC_{14}. The presence of the P(O)S$^-$ functionality is vital for the activity of the S-dC28 analogue[159] as is the presence of the nucleotide bases[162]. Methylphosphonate oligodeoxynucleotides also show anti-HIV activity[155], the same length requirements tend to apply.

The mechanism of activity of these oligomers has yet to be elucidated. Presumably they can act at a number of sites: the compounds could bind to part of the viral RNA chain

which could inhibit RT, or perhaps interfere with ribosome frame shifting or ribonuclease activity. Later on in the cell cycle these compounds could bind to the viral mRNA which stops host enzymes from translating the genetic information. The compounds could also interfere with the binding of a protein, for example the regulatory protein *tat* to its acceptor site.

These segments of unnatural nucleic acid are of interest since specific anti-sense sequences of the viral genome could be designed and synthesized that might bind to the genome thereby inhibiting replication. Toxicity could be minimized by the selection of a sequence that has a low affinity for regions of the host genome. As regards *in vivo* activity, the mechanism of cell entry is questionable since the compounds are extremely large and often carry multiple negative charges.

In summary there are a wide array of compounds that show activity against HIV. Some of the most promising compounds at this time are nucleoside analogues.

The major drawback of all these nucleosides is their toxicity. Pro-drug formulations of a wide variety of very useful drugs have been synthesized over the years. By synthesizing phosphate triesters some of this toxicity may be averted since the triester could be selectively converted to the active drug in infected cells if the mechanism of release relied on the viral replicative machinery. Secondly if the triesters are converted to the free nucleoside monophosphate then the initial kinase dependency will be avoided and nucleotides, could be highly specific, highly active anti-HIV agents.

With this in mind a series of phosphate triesters containing a nucleoside and one alkoxyalkyl group were synthesized. The alkoxyalkyl group is one of the most common pro-units of prodrugs[137]. A series of other phosphate triesters were also synthesized.

RESULTS AND DISCUSSION

The results and discussion is set out as follows:

Preparation of phosphorodichloridates.

Preparation of some compounds containing an OH group.

Preparation of some nucleosides.

Preparation of some phosphorochloridates.

Preparation of some 3'-O-acetylthymidine-5'-phosphates.

Preparation of some 3'-azidothymidine-5'-phosphates.

Preparation of a phosphorochloridite.

Reaction of a phosphorochloridite with 3'-azidothymidine.

Preparation of some 3'-modified -5'-phosphates of thymidine.

Stability of some phosphates in growth medium and plasma.

Anti-HIV activity of phosphates in two different tests.

Attempted preparation of some 5'-phosphonates.

Preparation of some 3'-modified-5'-bis(2,2,2-trichloroethyl) phosphates of thymidine and 2',3'-dideoxycytidine.

It should be noted that compounds that displayed anti-HIV activities at >10 μM are described as inactive in this section.

51-**53**[163] were prepared by the reaction of phosphoryl chloride with the relevant alcohol at low temperature in diethyl ether in the presence of triethylamine as a base. Filtration and concentration of the reaction mixture afforded the products, as oils, in quantitative yield. Employing a large quantity of solvent, addition of the alcohol over a long period of time and the use of a low temperature were required to procure the desired compounds in high purity.

54 could not be prepared in high purity in this solvent owing perhaps to the insolubility of the alcohol at low temperature. **54** could not be prepared using benzene dichloromethane or acetonitrile as the solvent but could be synthesized in near quantitative yield when tetrahydrofuran was used.

55 was prepared pure crude by adding methanol to a large excess of phosphoryl chloride. Concentration under reduced pressure afforded the product in quantitative yield.

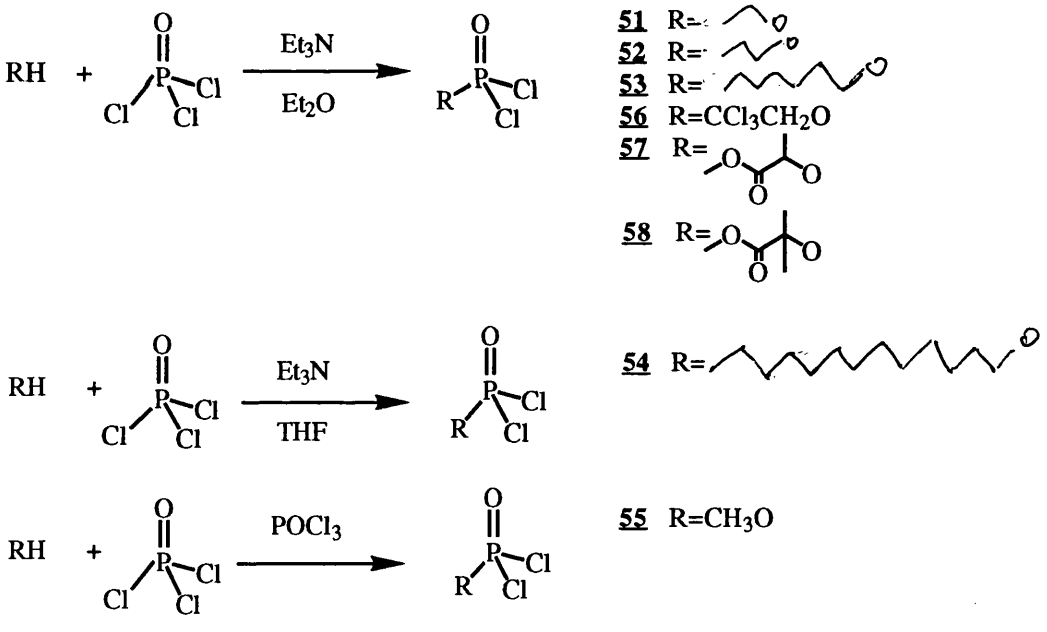
Phosphorus-31 n.m.r.[164], other spectroscopic and analytical data fully support the structures of these products. Phosphorus coupling is observed in carbon-13 n.m.r. spectrum: to the methyl group of **55** (9.2 Hz); and to the two carbons closest to the phosphorus atom in **51**-**54**. Coupling of 6 Hz is observed for the P-O-C carbon atom and 8 Hz for the P-O-C-C carbon atom. The signal furthest downfield in carbon-13 n.m.r. corresponds in each case to

the P-O-C carbon atom. The signal second furthest downfield corresponds to the P-O-C-C carbon atom for **51** and **52** , but this signal corresponds to the P-O-C-C-C carbon atom in **53** and **54**.

56 and **57** were prepared in an analogous fashion to **53**. Both had to be purified by distillation under reduced pressure. The impurities found in the crude material before distillation, analysed by proton and carbon-13 n.m.r. spectroscopy, were found to be **82** and **83** respectively.

58 could be prepared by the addition of the alcohol and base to phosphoryl chloride in diethyl ether, but only if a large excess of phosphoryl chloride was used and the reaction left for a long time. The product was obtained in greater than 95% purity by phosphorus-31 n.m.r. Carbon-13 and proton n.m.r. and phosphorus and chlorine analysis were obtained.

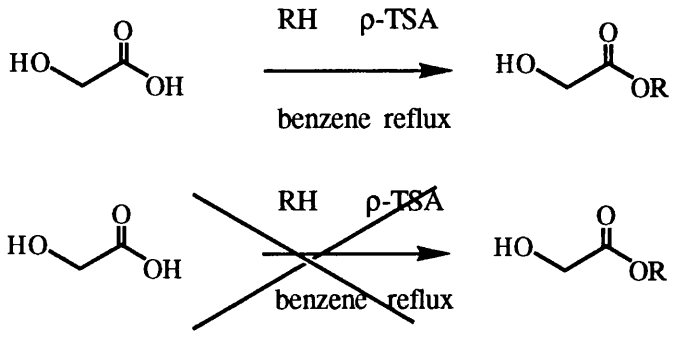
The carbon-13 n.m.r. spectra display phosphorus coupling to the carbonyl carbon in each case; 6 Hz for **57** and 3 Hz for **58**. Phosphorus-31 n.m.r. chemical shifts compare favourably with those found for alkyl derivatives. E.I.M.S. data give an accurate mass for MH^+ for both compounds. Chlorine isotope effects were observed for the two chlorine atoms present. Both compounds show the loss of the $CH_3OC{=}O$ moiety.



In order to investigate the effect on biological activity of different types of carboxylic ester attached to a nucleotide **59** and **60** were synthesized. **59** and **60** were synthesized by standard methodology[165] and distilled directly onto 4 Å activated sieves.

The carbon-13 n.m.r. spectrum is informative, the hydroxymethyl carbon is further downfield than the carbon connected to the carboxyl oxygen by 1 ppm. The assignment was made possible by comparison with literature values for carbon-13 n.m.r. of similar

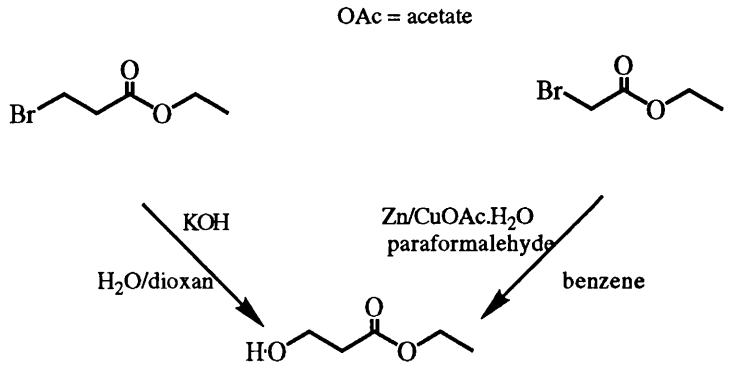
compounds[166]. **61** could not be synthesized by the method used to make **59** and **60** perhaps because of the low boiling point of methanol. The synthesis of **62** was attempted to investigate the effect of a chemically labile group as an ester. The synthesis of **62** was attempted using the method to make **59** and **60** but a polymeric, benzene-insoluble product resulted, presumably some form of glycol polymer.



59 R=C_2H_5O
60 R=C_4H_9O
61 R=CH_3O
62 R=CCl_3CH_2O

To investigate the effect of the number of carbon atoms between the phosphorus and carbonyl centres on biological activity of nucleoside phosphate triesters, the synthesis of ethyl 3-hydroxypropanoate **63** was attempted.

63 was synthesized from ethyl 3-bromopropionate when this was stirred in a mixture of water/dioxan containing potassium hydroxide, but the reaction did not proceed cleanly and low yields of the desired product were obtained after extraction, concentration and distillation under reduced pressure. However, a 40% yield of **63** could be obtained from reaction of paraformaldehyde with ethyl bromoacetate in the presence of a zinc/copper couple[167].



63

Paraformaldehyde and ethyl bromoacetate suspended in benzene were added to a zinc/copper couple prepared from cupric acetate monohydrate and zinc shot. After reflux the mixture was acidified, extracted and the product distilled under reduced pressure.

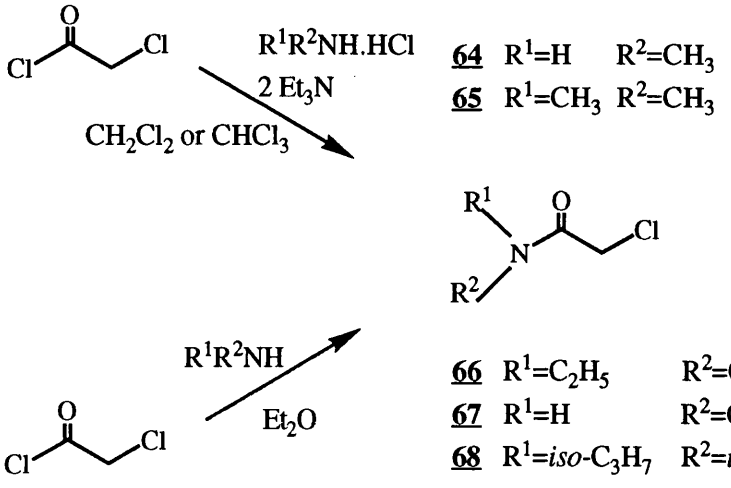
The carbon-13 n.m.r. spectrum of **63** shows the hydroxymethyl carbon further upfield than the carbon connected to the carboxyl oxygen by 2 ppm. The carbon connected to the carbonyl group is observed at 37 ppm which agrees closely with values observed for similar compounds[168]. Proton n.m.r. shows the hydroxymethyl protons are coupled to the neighbouring methylene group with a coupling constant of 6 Hz. The hydroxyl proton is observed as a broad singlet at 3.3 ppm.

N,N-2-hydroxyacetamides have been shown to be more labile than carboxylic esters when the pharmacokinetics of the compounds were tested in biological systems[136]. A series of phosphate triesters with at least one hydroxyacetamide group attached would be of great biological interest since the nucleoside or nucleotide monophosphate could possibly be liberated more easily from this class of compounds than from an alkoxyalkyl series.

The series was synthesized by a two step procedure; firstly *N*-alkyl 2-chloroacetamides were prepared. The chlorine group was then displaced by a hydroxide ion using potassium hydroxide.

Methylamine and dimethylamine are gases at room temperature and so in order to procure **64** and **65** triethylamine was added to acetyl chloride and the appropriate amine salt in chloroform and dichloromethane respectively. After diethyl ether precipitation of triethylamine hydrochloride, filtration and concentration, the required products were distilled as oils.

66-**68** were produced in reasonable yield by the reaction of the amine with chloroacetylchloride in dichloromethane at low temperature in the presence of triethylamine. The resultant materials were distilled under reduced pressure to afford the products as oils in 54-72% yield.



64 R^1=H R^2=CH_3
65 R^1=CH_3 R^2=CH_3

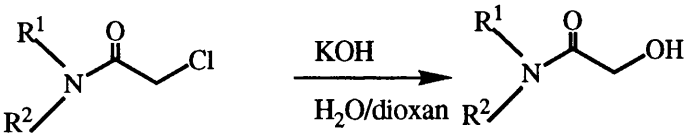
66 R^1=C_2H_5 R^2=C_2H_5
67 R^1=H R^2=C_3H_7
68 R^1=*iso*-C_3H_7 R^2=*iso*-C_3H_7

The carbon-13 n.m.r. spectra of **64**-**68** show that the two alkyl chains attached to the nitrogen atom are in different electronic environments. This would be expected since there is hindered rotation about the amide bond. The chloromethyl carbons are observed at 40-45 ppm and the carbonyls at 167-168 ppm; consistent with the proposed structure[166]. Proton n.m.r. shows the chloromethyl protons at 4.0 ppm. The amido proton is observed where appropriate at 5-6 ppm. Mass spectrometric and analytical data were obtained for these compounds.

64-**68** were converted to the corresponding 2-hydroxyacetamides **69**-**73** by dissolving them in water/dioxan containing potassium hydroxide. In general the reaction proceeded cleanly if left at room temperature for approximately 200 h. Raising the temperature to reflux resulted in some amide bond cleavage. It was found that **69**, **70** and **72** were best synthesized by reaction with hydroxide at reflux, **72** at room temperature and **73** at 55 °C. Purification of **69**-**73** by distillation proved difficult since even under high vacuum the compounds had high boiling points and pyrolysis may have occurred. **69**-**73** also tended to solidify at or just below room temperature and had to be distilled without a water condenser. The yield of **72** was improved significantly by the use of high boiling silicon oil. It was also noted that **69**-**73** were extremely hygroscopic, gaining mass rapidly on a weighing balance.

Carbon-13 n.m.r. data of **69**-**73** showed the carbon of the hydroxymethyl group had shifted downfield by 20 ppm relative to **64**-**68**. The hydroxymethyl carbon atoms had chemical shifts close to that found for **59** and **60**. The carbonyl carbon atoms were observed between 170 and 171 ppm, approximately 2-3 ppm downfield from the corresponding 2-chloroacetamides but 2-3 ppm upfield from the carbonyl carbon atoms in **59** and **60**. Proton n.m.r. shows the hydroxymethyl protons at approximately 4.2 ppm as singlets, about 0.2 ppm further downfield from the 2-chloroacetamide analogues but similar to the shift observed for **59** and **60**. All the other protons are shifted approximately 0.2 ppm upfield. IR shows the O-H stretch at approximately 3100 cm^{-1}, other absorbances were observed, notably the amide stretch at approximately 1660 cm^{-1} was observed as a very strong signal.

Overall yields of **69**-**73** from chloroacetylchloride never exceeded 30%. Although the first step was relatively straightforward, the second step was hard to follow; carbon-13 n.m.r. was found to be the easiest. The temperature of the reaction was crucial to the yield of the required 2-hydroxyacetamide. Isolation was equally complex; a workup procedure for one product could not be successfully employed for another. Distillation was also problematic since the products could quickly solidify in the condenser, even if not water cooled, blocking the system which potentially led to over-heating.

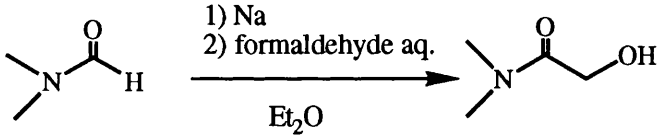


64	R^1=H R^2=CH_3	**69**	R^1=H R^2=CH_3
65	R^1=CH_3 R^2=CH_3	**70**	R^1=CH_3 R^2=CH_3
66	R^1=C_2H_5 R^2=C_2H_5	**71**	R^1=C_2H_5 R^2=C_2H_5
67	R^1=H R^2=C_3H_7	**72**	R^1=H R^2=C_3H_7
68	R^1=*iso*-C_3H_7 R^2=*iso*-C_3H_7	**73**	R^1=*iso*-C_3H_7 R^2=*iso*-C_3H_7

In order to obviate some of these problems a different synthetic approach was investigated.

It has been reported that some 2-hydroxyacetamides have been synthesized in a one step procedure from paraformaldehyde and a dialkylformamide in the presence of sodium, though little experimental and spectroscopic data was given[169]. The preparation of **70** was investigated since dimethylformamide was readily available in large quantities.

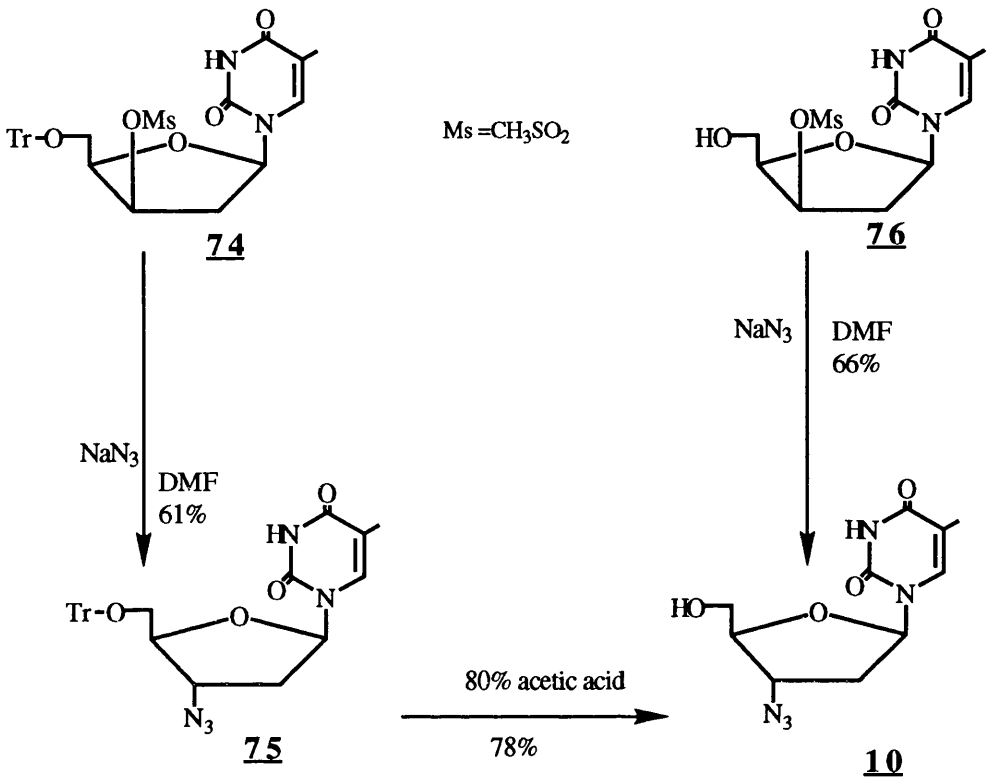
Dimethylformamide was refluxed with sodium in diethyl ether. Aqueous formaldehyde was added and left for an hour. The mixture was neutralised and concentrated under reduced pressure and the crude product extracted with acetonitrile, concentrated and distilled under reduced pressure to afford **70** in 38% yield.



70

The yield of the required product obtained by this method compares favourably with the second step of the first method described for the synthesis of this compound. However it seems dubious that the reaction could be successfully scaled up significantly above 5 g of sodium since the addition of aqueous formaldehyde to the sodium/dimethylformamide suspension resulted in a very vigorous reaction that was difficult to control. It was also difficult to remove all the dimethyl formamide from **70**, which could have detrimental effects at the phosphorylation stage: Though this method has advantages for the synthesis of very short chain analogues (eg **70**), the use of diisopropyl formamide, for instance, to give **73** may lead to considerable difficulties when attempting to separate the formamide from **73**.

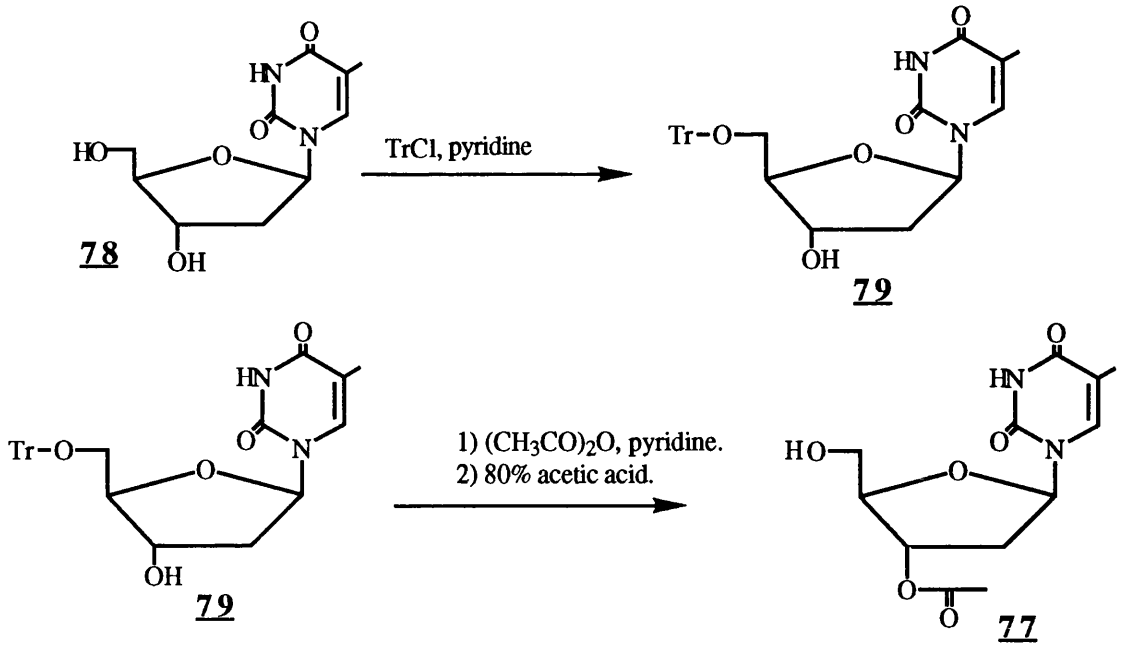
10 was prepared by a slight alteration of standard procedures[170].



75 was prepared by reacting 74 with sodium azide in dimethylformamide. The displacement of the mesyl group operates principally by an S_N2 mechanism and thereby leads to inversion of configuration at C-3'. Detritylation of 75 was achieved with 80% acetic acid to afford 10 in reasonable yield after column chromatography.

10 could be prepared directly from 76 using sodium azide and identical conditions to those employed above. The product was isolated by column chromatography and crystalysed from toluene in reasonable yield.

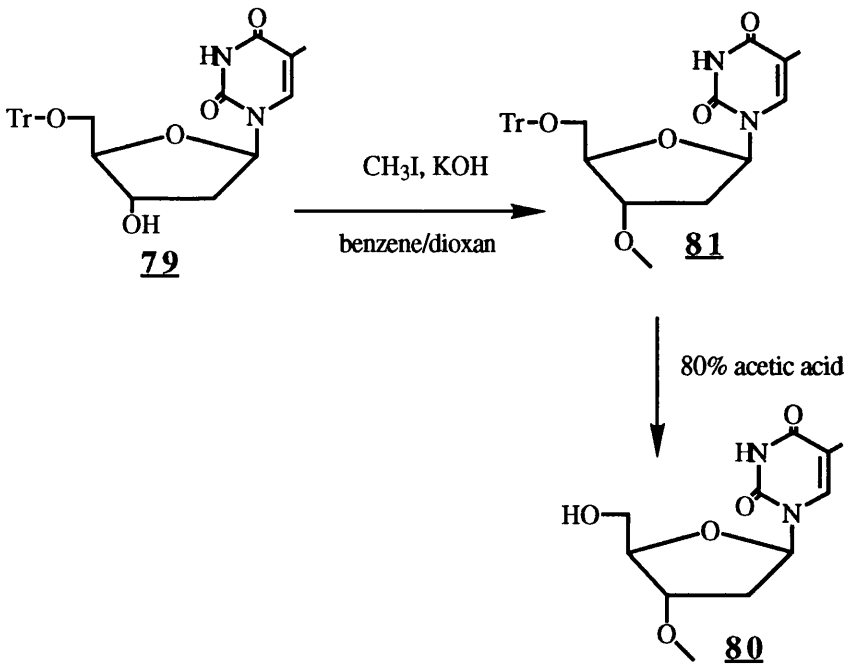
77 was prepared by standard procedures[171]. It seemed unlikely that 77 itself would have any substantial anti-HIV activity, perhaps because the acetyl group is both chemically and enzymatically labile and could yield 78 on hydrolysis or because 77 is not phosphorylated to the monophosphate. 77 was subsequently tested against HIV and found to be inactive at 100 μM However the 5'-phosphate triester derivatives may show activity since if the monophosphate is released it may be further phosphorylated to the triphosphate and then perhaps be recognised by RT thereby inhibiting it.



The preparation of **77** involved the addition of trityl chloride to **78** in pyridine to give **79**. Acetic anhydride was then added *in situ* at room temperature. After detritylation with 80% acetic acid, purification by column chromatography afforded **77** as crystals in overall yield of 64%. Carbon-13 n.m.r. and proton n.m.r. data obtained were fully consistent with structure.

80 has been tested *in vitro* against HIV and has been found to be inactive[172]. This may be a result of the compound being poorly phosphorylated intracellularly. However a similar biological study showed **38** to have significant anti-HIV activity[131]. With this in mind, it was decided to investigate the potential of some 5'-phosphate triesters of **80** in the hope that the 5'-phosphate moiety may help overcome any difficulty involved with cellular phosphorylation of **80**.

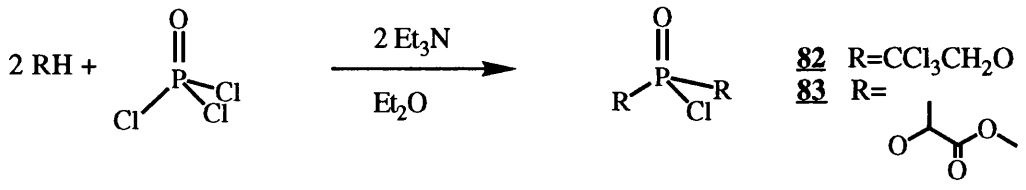
The preparation of **80** was carried out by standard procedures[172]. Methyl iodide and potassium hydroxide were added to a solution of **79** in benzene/dioxan and stirred for 24h. at 50 °C. After concentration to dryness under reduced pressure the gum was dissolved in methanol, poured onto iced water and **81** extracted with chloroform. Detritylation of **81** was accomplished with 80% acetic acid. Purification of the product by column chromatography afforded **80** in 48% overall yield.



The carbon-13 n.m.r. spectrum bears a close resemblance to **78** apart from C-3' which in **80** is shifted downfield, relative to **78**, to 81 ppm. The 3'-O-methyl signal is observed at 57 ppm. the proton n.m.r. spectrum compares closely with literature values[173]. Mass spectrometric and analytical data were obtained. An accurate mass for M^+ was observed.

82 was prepared in an analogous fashion to **56**. The two impurities in the phosphorus-31 n.m.r. spectrum of the crude product were **56** and presumably the tris (2,2,2- trichloroethyl) phosphate. Distillation under reduced pressure gave the required product in reasonable yield as a white solid, mp 51 °C. The phosphorus-31 n.m.r. spectrum of this prepared sample compared favourably with an authentic sample of **82**[174].

83 was prepared in a similar fashion to **57**. the phosphorus-31 n.m.r. spectrum showed that the crude product contained **57** (10%) and another impurity (10%) at -3 ppm. Concentration under reduced pressure gave **83** in 75% purity.



The carbon-13 n.m.r. spectrum of **83** showed that the two methyllactyl chains enjoy slightly different electronic environments; all the carbon signals are doubled up compared with **57**. This is because the chains are diastereotopic. Phosphorus coupling constants for **83** and **57** vary for the CH: (**57**) J=9 Hz, (**83**) J=7 Hz; but are similar for the carbonyl, (**57**) J=6 Hz, (**83**) J=6 Hz. Phosphorus-31 n.m.r. of **83** shows one peak shifted approximately 2 ppm upfield from **57**.

	57	**83**	**84**	**85**	**86**	**87**
C=O	168.41	169.38	169.50	169.16	169.38	169.21
CH	75.31	73.64	73.06	74.20	73.79	73.12
CH_3O	52.74	52.55	52.48	52.78	52.74	52.33
$\underline{C}H_3CH$	18.54	18.72	18.71	18.80	18.67	18.50

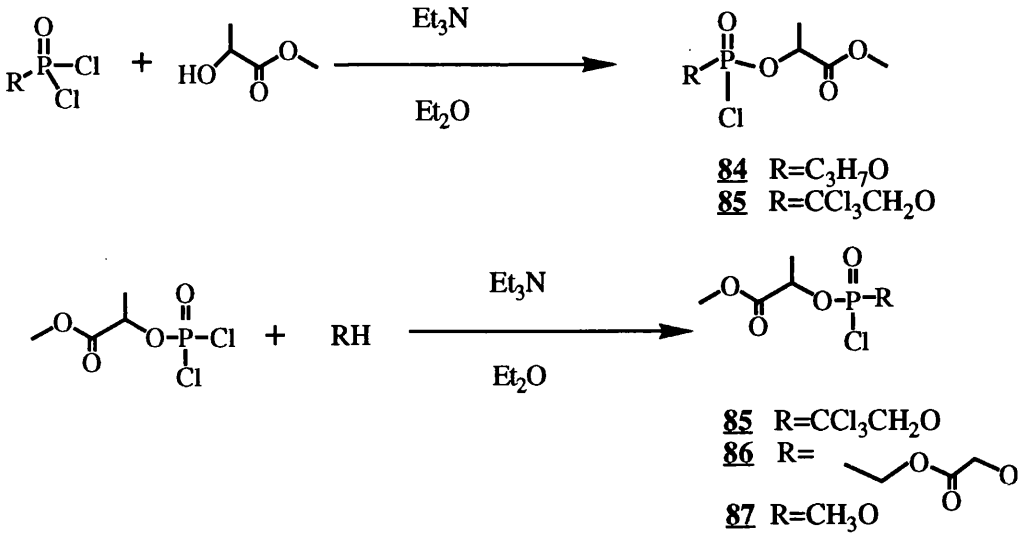
Table showing average values of signals in C-13 n.m.r. spectra of some phosphorylating reagents.

Accurate mass measurement for MH^+ was obtained, the molecular ion signals demonstrated an isotope pattern consistent, with the product containing a single chlorine atom.

84 was prepared from **52** and (S)-(-)-methyl lactate by addition of the alcohol to the phosphorylating reagent in diethyl ether at low temperature with triethylamine present. After work up identical to that used for the synthesis of **52** phosphorus-31 n.m.r. showed the presence of some (8%) unreacted **52** and a further (10%) impurity, presumably propyl bis (methylcarboxy-1-methyl)phosphate. The product was distilled under severely reduced pressure to afford a viscous oil in 62% yield.

85-**87**, were prepared in a similar fashion to **84**. All the products contained some unreacted **57** and presumably some phosphate triester. **57** was removed under reduced pressure and then all were distilled except **86** which decomposed at 150 ^oC at 0.01mm Hg.

As **84**-**87** contain two chiral centres, a mixture of diastereomeric products would be expected. The ratio of these isomers will not necessarily be of parity: In the reaction system employed here the synthesis of one isomer may, because of stereochemical factors, be favoured over the other. Phosphorus-31, carbon-13 and proton n.m.r. would be expected to show these differences.



Carbon-13, and phosphorus n.m.r. spectra were recorded for compounds **84**-**87**. All products existed as diastereomers in the phosphorus-31 n.m.r. The phosphorus-31 n.m.r. spectrum displays diastereomeric differences ranging from 0.8 to 1.4 ppm. The preference for formation of one isomer over another changes across the series from 1:1 (**87**) to 4:1 (**86**).

The carbon-13 n.m.r. spectra showed this diastereomeric effect to most carbons, though phosphorus coupling also further complicated the spectra to give multiplet signals for some atoms.

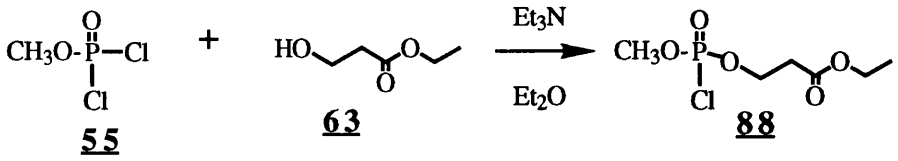
Mass spectrometry data gave accurate mass measurement for **84**, **85** and **87**. Each spectra contained a signal corresponding to the loss of $C_2H_3O_2$ from the molecular ion.

85 was also prepared using **56** and (S)-(-)-methyl lactate under identical conditions to those used for the formation of **85** from **57**. Interestingly, the ratio of diastereomeric abundance found in this instance was the reverse of that found in the first method; 4:3 rather than 3:4.

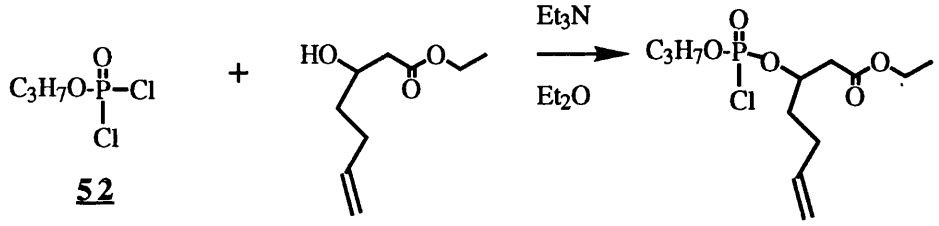
63 was reacted with excess **55** in an analogous fashion to the synthesis of **85** to give the product **88** 85% pure by phosphorus-31 n.m.r. spectroscopy after excess **55** had been removed under reduced pressure. The presence of multiple signals between -6 and -20 ppm which is the region expected for polyphosphates[175] suggested that some water had been present in the reaction mixture, perhaps from **63**. Some of these impurities were successfully removed by their precipitation from hexane.

The phosphorus-31 n.m.r. spectrum of **88** displays a single resonance since diastereomers are not possible as the product lacks a chiral carbon. Carbon-13 n.m.r. showed that the carbonyl and adjacent methylene had shifted upfield from **63** by 3 ppm and 2 ppm respectively. The resonance for the hydroxymethyl shifts 7 ppm downfield demonstrating the considerable change in electronic environment of the alkoxy alkyl group in

88 compared with 63. Phosphorus coupling is observed for the hydroxymethyl carbon of 6.4 Hz and the next methylene of 6.9 Hz. There is no coupling to the carbonyl, which is observed as a singlet 3 ppm downfield from the corresponding carbon atom of 86.



55 63 88



52 89 90

The extension of the chain between carbonyl ester and phosphate had thus been achieved. The effect of unsaturated alkyl substitution on this extended chain was investigated with the use of racemic 89[176].

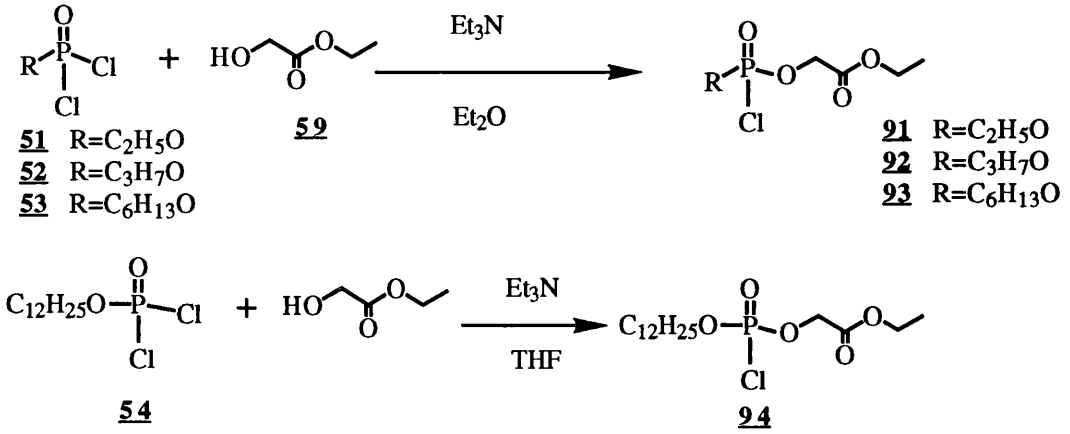
89 was reacted with 52 in an analogous fashion to above. After work-up the crude product 90 was found to be 95% pure according to the phosphorus-13 n.m.r. spectrum, the impurities were suspected to be the product of some phosphorochloridate hydrolysis.

Although it is possible to produce four different isomers from this reaction since a racemic alcohol was used, only two signals, in a ratio of 1:2, were observed for 90 by phosphorus-31 n.m.r.

Carbon-13 n.m.r. of 90 compared with that of the starting alcohol shows the carbonyl carbon and adjacent methylene are shifted downfield 3 ppm and 2 ppm (J=5.4, 5.5 Hz) respectively. The most substituted double bond carbon moves downfield by 2 ppm, the methylene next to this shifts by 1 ppm downfield. The methylene next to this shifted downfield in the product by 2 ppm is phosphorus coupled (J=4.3, 5.5 Hz). As for 88, the carbon of the glycol attached directly to the phosphorus shifts upfield but by 10 ppm in this instance and is observed as a multiplet. The only carbons unaffected by phosphorus coupling and equivalent in each diastereomer are the alkene carbons, the carbonyl and the two methyl groups.

59 was reacted with 51- 54 in an entirely analogues fashion to the formation of 84-88 to give 91-94 respectively with the exception of 94 which was synthesized using

tetrahydrofuran as a solvent owing to poor solubility of **54** in diethyl ether at low temperature.

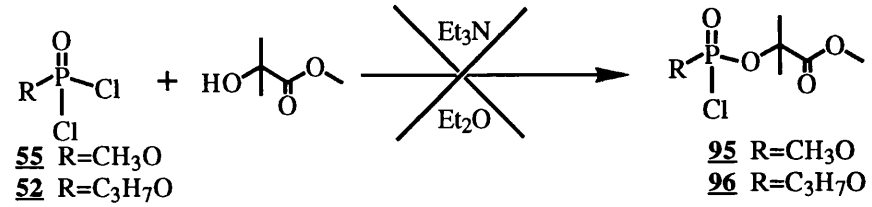


The phosphorus-31 n.m.r. spectra showed that **93** and **94** had been prepared in greater than 95% purity. **51** and **52** were present in the crude oils that resulted from the synthesis of **91** and **92** (*ca.* 10%). These were removed under reduced pressure. Another major impurity, presumably the phosphate triester was present in the reactions to form **91** and **92**.

The phosphorus-31 n.m.r. spectra of **91**-**94** showed signals approximately 3 ppm further upfield than **51**-**54**. Carbon-13 n.m.r. shows the hydroxymethyl carbons are 3 ppm further downfield than in **59**. The carbonyl carbons are shifted 7 ppm upfield for **91**-**94** from the carbonyl shifts for **83**-**87**.

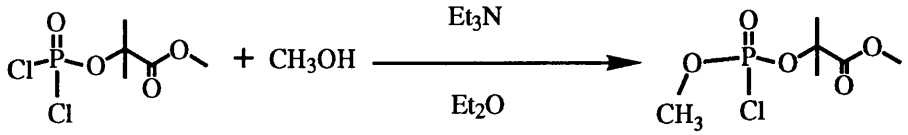
Methyl 2-hydroxyisobutyrate would not react with **55** or **52** to give **95** or **96** respectively under similar conditions to those used to synthesize **87**. In both cases **55** and **52** was retrieved with unreacted alcohol. Presumably this is owing to the considerable steric hindrance associated with the tertiary alcohol.

58 reacted with methanol to give the desired product **95** if the alcohol was added neat to a stirred solution of **58** and triethylamine in a small amount of diethyl ether at room temperature. Indeed the reaction proceeded extremely vigorously to afford the product in greater than 95% purity by phosphorus-31 n.m.r. .



55 R=CH_3O
52 R=C_3H_7O

95 R=CH_3O
96 R=C_3H_7O



58

95

Interestingly, phosphorus-31 n.m.r. showed **95** to have a chemical shift approximately 4 ppm upfield from **87** indicating the significantly different electronic environment of the phosphorus atom in **95**. Carbon-13 n.m.r. shows the carbonyl resonance to have a coupling constant of 4 Hz; markedly smaller than in **87**. The carbon attached to the phosphorus centre is shifted 11 ppm downfield relative to the starting alcohol and is phosphorus coupled by 8 Hz. The methyl carbons connected to this show two signals 0.5 ppm apart and each is coupled to the phosphorus atom by 6.3 Hz and 4.2 Hz respectively. Accurate mass measurement and proton n.m.r. spectra of **95** were obtained. The chlorine isotope effect was observed for MH^+. The product was considered to be of sufficient purity to continue to the next stage.

In order to investigate the effect of unsaturation between carbonyl and phosphorus centres the synthesis of **97** and **99** were investigated.

98 has been prepared in near quantitative yield[177] by the reaction of ethyl acetoacetate and diethylphosphorochloridate, when dissolved in tetrahydrofuran in the presence of sodium hydride. The rational behind this was used to synthesize **97**.

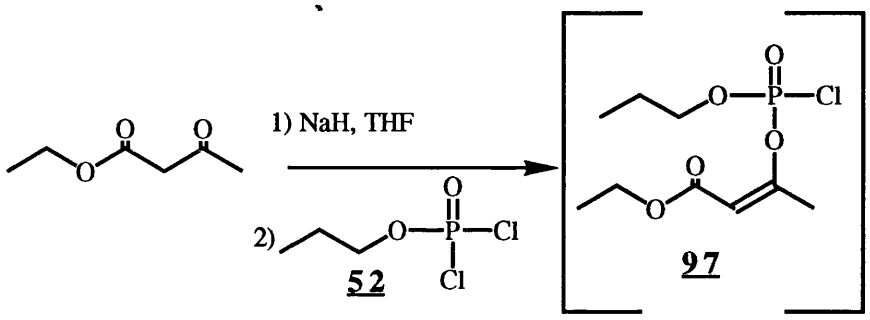
Ethyl acetoacetate was stirred with sodium hydride in tetrahydrofuran. The suspension was then added to **52** with stirring. The suspension had to be reacted immediately with a nucleoside since it was not possible to isolate the intended product from the extremely glutinous reaction mixture.

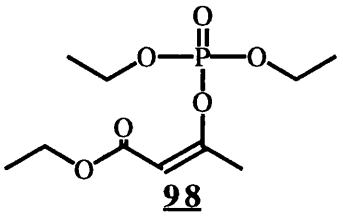
Using sodium hydride in tetrahydrofuran to procure **97** should give the z form exclusively because the sodium anion will associate with the two carbonyl carbon atoms forcing them into this conformation.

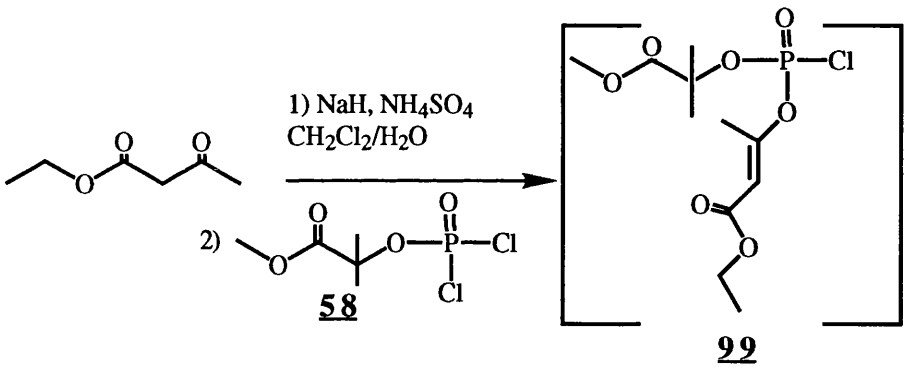
It has also been reported[178] that if tetrabutylammonium hydrogen sulphate is used as a phase transfer catalyst between dichloromethane and water in the presence of ethyl acetoacetate and diethyl phosphorochloridate the E isomer of the product is exclusively

formed. In this case the sodium anion, trapped in the aqueous layer, cannot associate with the ethyl acetoacetate cation and the less sterically hindered E product is produced.

Ethyl acetoacetate was dissolved in dichloromethane and stirred with water and the phase transfer catalyst for 30 min. **58** was added and the reaction stirred for a further 30 min. The dichloromethane layer was taken, dried with magnesium sulphate, concentrated under reduced pressure and stored under nitrogen.



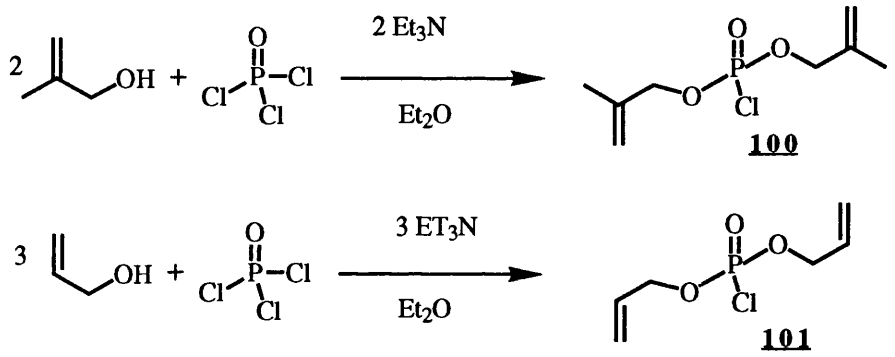




The phosphorus-31 n.m.r. spectrum showed the isolated material consisted of a major component approximately 75% pure by $^{31}P_{NMR}$. This was tentatively assigned to be the desired material **99** and the phosphorochloridate was reacted straight away with a nucleoside. After storage under nitrogen for two months at -20 ^{o}C **99** had decomposed by only 10% and when reacted with **10** still phosphorylated it in extremely high yield.

The effect of unsaturation between the carbonyl and phosphate centres on the anti-HIV activity of some phosphate triesters could thus be investigated.

In order to investigate other types of unsaturation on biological activity 2-methyl-2-propene-1-ol and 2-propene-1-ol were reacted with phosphoryl chloride to give the **100**[179] and **101**[180] respectively. **100** was prepared in high purity however the the reaction to form **101** gave some mono and tri-substituted products which could not be separated from **101** by distillation. To obviate the persistence of the phosphorodichloridate in this reaction an excess of the alcohol was used to give the required product **101** 50% pure and the tri substituted product (50%) by Phosphorus-31 n.m.r. Owing to the unreactivity of the phosphate triester under the reaction conditions employed in the formation of nucleotides the phosphorylating reagent was considered to be of sufficient purity to use in a reaction with a nucleoside.

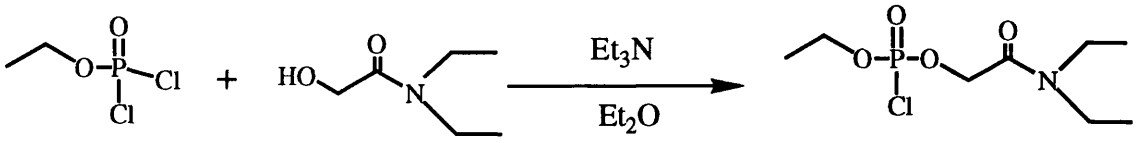


The carbon-13 n.m.r. of **100** shows that the two chains are identical and phosphorus coupling up to 3 bonds away of 8 Hz.

69-**73** were the next compounds to be reacted with phosphorylating reagents.

Reactions of **69**-**73** with phosphorylating reagents, carried out in a variety of solvents, in the presence and absence of different bases and over a range of temperatures produced orange insoluble oils and some unreacted starting phosphorylating reagent. The reactions at low temperature at first appeared to proceed cleanly. However on bringing to ambient temperature the solution would precipitate an orange/brown oil only soluble in methanol. The desired product was obtained from the reaction of **51** and **71** in low yield after unreacted **51** had been removed under reduced pressure.

71 and triethylamine, dissolved in dichloromethane, were added separately but simultaneously to a stirred solution of **51** in a small volume of diethylether at low temperature. After leaving overnight at room temperature, the product was extracted with a large quantity of hexane and concentrated under reduced pressure. **102** was obtained in 29% yield.



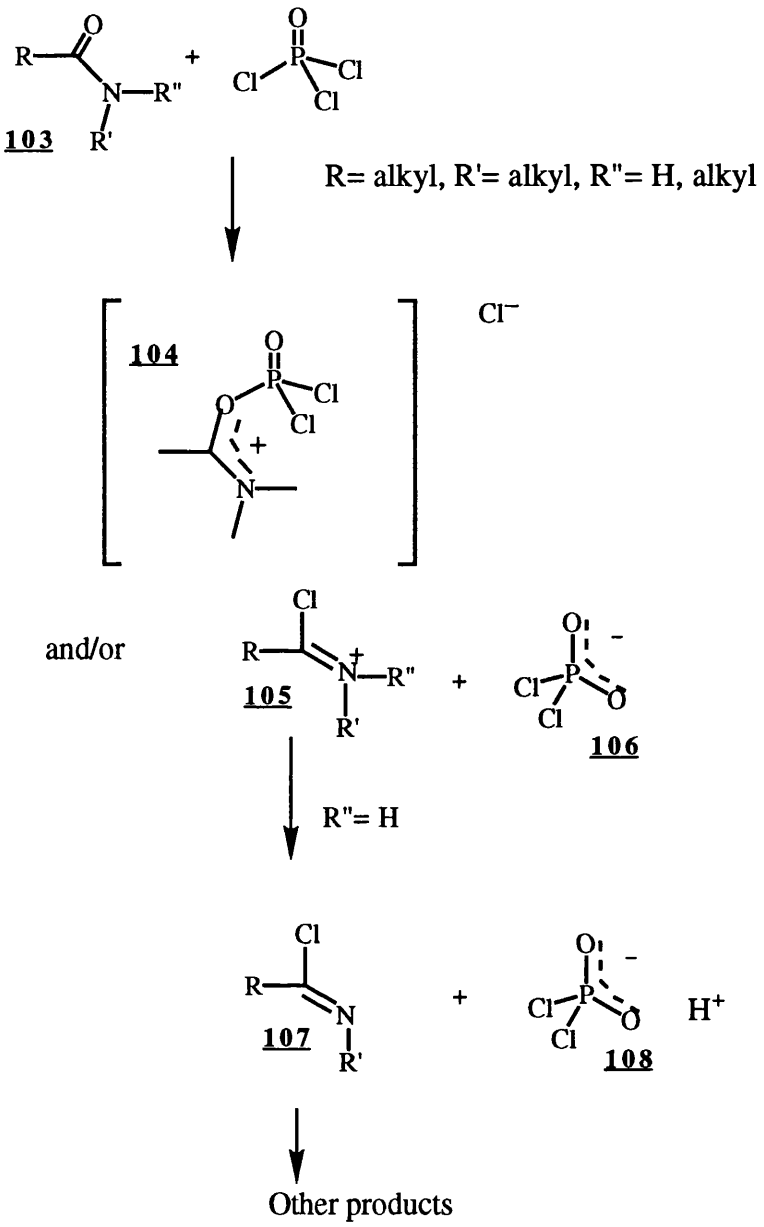
71 **102**

The phosphorus-31 n.m.r. spectrum showed the oil to consist of a major product at -2.8 ppm about 4 ppm further upfield from **91**.

The carbon-13 n.m.r. spectrum of the oil was taken: phosphorus coupling was observed to the carbonyl and the hydroxymethyl of the acetamide of 6 and 7 Hz respectively, approximately what would have been expected from comparison with compound **91**. The carbonyl was shifted 4 ppm upfield and the hydroxymethyl 4 ppm downfield from **71**. Signals for the other carbons were present at expected chemical shifts. Reaction of **102** with **10** in pyridine did not occur, and **10** was retrieved. Unfortunately there was not time to synthesize more **102**. The yield of **102** was very low and it suggested that the desired product was one of many formed under these reaction conditions employed but that the majority of the products, perhaps polymeric in nature were hexane insoluble.

It has been reported that amide containing compounds react with phosphoryl chloride[181-183]: Secondary and tertiary amides **103** both form C=N products **107** with phosphoryl chloride. The hope was that the presence of the hydroxyl group would lead to formation of the desired product which would then be stable enough to isolate and react with a nucleoside. Furthermore alkyl phosphorodichloridates may not be as susceptible to reaction with the amide moiety thus increasing the likelihood of the desired product being synthesized.

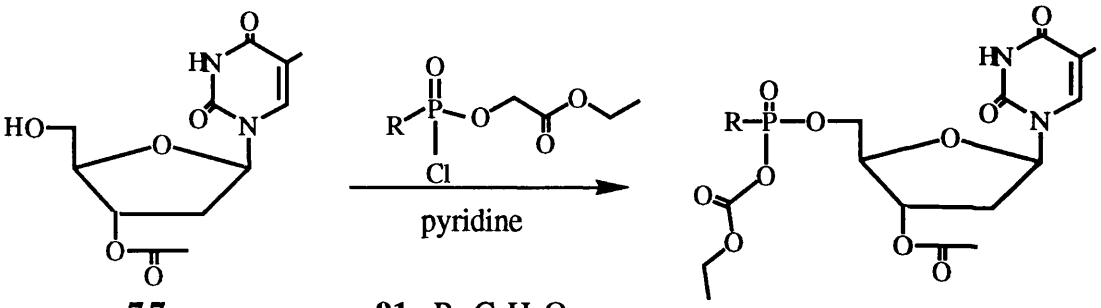
That the reactions appeared to proceed in the desired manner while the reaction was kept at low temperature suggests that the required compounds were formed at this point but were unfortunately unstable at room temperature. It is suggested that there are two possible initial products from reaction of phosphoryl chloride with an amide. One is thought to predominate below 25 °C (**104**) the others (**105** and **106**) above this temperature. The latter, containing polymeric phosphate, would probably be diethyl ether insoluble which could account for the polymeric gum that results from the reaction. That some desired product was obtained in one instance suggests that an amido phosphorylating reagent can be made, however it is only one of a number of products. Perhaps if this had been reacted immediately with a nucleoside in tetrahydrofuran in the presence of *N*-methylimidazole the target nucleoside phosphate triester could have been synthesized.



The phosphorylating reagents synthesised above were reacted with a variety of nucleosides to produce phosphate triesters.

77 was reacted with **91**-**93** in pyridine[184]. The pyridine in this instance acts as both solvent and base. It was expected that phosphorylation would occur only at the 5'-position of **77**. Analysis of the reaction mixture by tlc, after stirring for 3-5 days, showed a component more lipophilic than **77** and a baseline component. At this point the solvent was removed by evaporation under reduced pressure and the residue triturated with toluene to afford an oily gum. Purification was achieved by flash column chromatography, eluting with varying quantities of methanol in chloroform to give the phosphate triesters **109**-**111** as clear gums in 24-58% yields. Phosphorus-31 n.m.r. showed the diastereomeric ratio of products did differ significantly from parity in the case of **109**. **111** was separated into two isomers **111a**

and **111b** by flash column chromatography analysed by multiple development thin layer chromatography. Similarily **110** was separated into the 'fast' **110a** and 'slow' **110b** isomers by normal-phase hplc.



77

91 R=C_2H_5O
92 R=C_3H_7O
93 R=$C_6H_{13}O$

109 R=C_2H_5O
110 R=C_3H_7O
111 R=$C_6H_{13}O$

The carbon-13 n.m.r. spectra of **109**-**111** are informative. The nucleoside carbon signals have chemical shifts that correlate closely with those found for **77** with the exception of C-5' and C-4'. C-5' in each case was observed approximately 5 ppm downfield of the position of the C-5' signal of **77** at approximately 67 ppm and the C-4' signal 1 ppm upfield at 84 ppm. These two carbon signals also displayed phosphorus coupling; of 8 Hz for C-4' and 5 Hz for C-5'. In the spectra of **110a**, **110b**, **111a** and **111b** single peaks were observed for each carbon atom bar C-5' and C-4'. The shift of a particular carbon, for example C-2' in each diastereomer was slightly different, the difference being in the region of 0.1 ppm. With this in mind it would be expected that for **109** doublets would be observed for each carbon of the sugar and base apart from the C-4' and C-5' signals that should be observed as a doublet of doublets in each case. Indeed this is the case, C-5' for instance shows a diastereomeric splitting of 0.7 ppm and a phosphorus coupling of 5 Hz for the least abundant isomer and 6 Hz for the most abundant isomer.

The signals for the glycol and ethyl carbons were also observed, though they were now 'doubled up' in each case, except for the glycol methyl which was still a singlet showing that it occupies an identical electronic environment in each isomer.

The phosphorus-31 n.m.r. of **109**-**111** demonstrated signals between 0 and 2 ppm, for the separated isomers as a single signal and for **110** as two signals. The diastereomeric difference in phosphorus n.m.r. varied between 0.2 and 0.5 ppm.

The proton n.m.r. data are complex for the ethyl mixture of isomers **109**. H-6 is displayed as two doublets, with a diastereomeric difference of 0.02 ppm and proton coupling of 1.22 and 1.17 Hz respectively. More information can be gleaned from the separated diastereomers of **110** and **111**. The second methylene from the phosphorus in **110a** is

observed as a sextet, J=6.77 Hz. As regards the ethylcarboxyethyl chain, the methyl group is observed as a triplet at 1.2 ppm J=7.3 Hz and the signal for the CH_2O is observed at 4.2 ppm as a quartet J=6.56 Hz. The acetyl protons in **109**-**111** are observed as a singlet at approximately δ 2 ppm, integrating for three protons.

The H-5' protons show an extraordinarily complex coupling pattern in the proton n.m.r. spectra for each compound. In the separated isomers the signal at 4.36 ppm seems to be a doublet of doublets of doublets of doublets. Firstly the H-5' protons are non-equivalent. These two signals also couple to each other making four signals. Each H-5' proton is also coupled to H-4' making eight signals. The H-5' signal is also coupled to the phosphorus atom making 16 signals. Owing to the complexity of H-5' it is listed as a multiplet in the experimental section.

The use of pyridine with these phosphorylating reagents always produced low yields of the desired product. An investigation of the stability of these compounds in pyridine was conducted by phosphorus-31 n.m.r. which showed that conversion of the phosphorochloridates to another species did occur over time. It was also difficult to remove all the pyridine before column chromatography and it did tend to 'elute' with the product unless the column was eluted with almost neat chloroform.

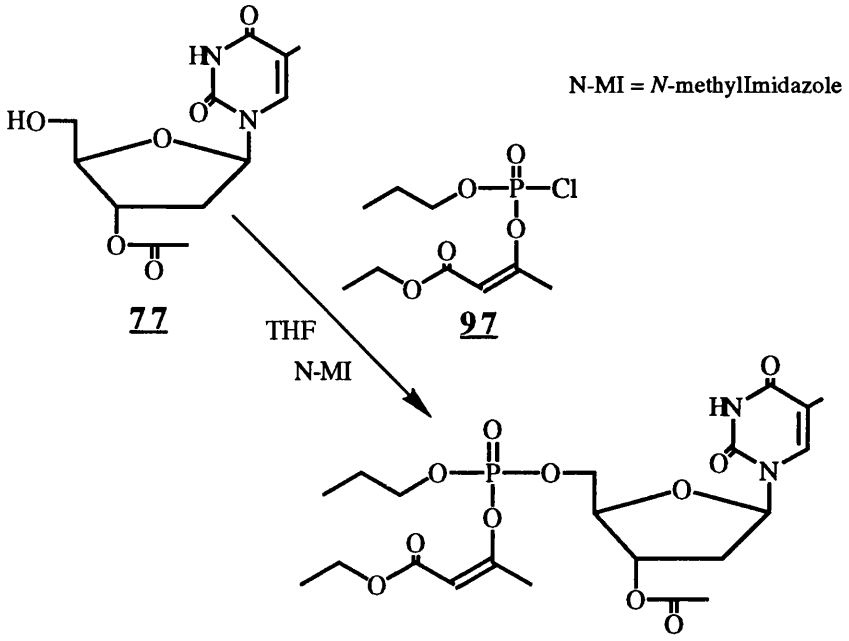
For these reasons another method used in phosphorylations of nucleosides[185] was investigated. Tetrahydrofuran was employed as the solvent and *N*-methylimidazole as the base.

The first compound synthesized by this method was **112**. **97** was added in a large excess to **77** and a 10-fold excess of *N*-methylimidazole dissolved in tetrahydrofuran. A gum was immediately precipitated from the solution. In all the reactions carried out in this solvent a tetrahydrofuran insoluble gum, perhaps *N*-methylimidazole hydrochloride was produced. The reaction was followed by tlc eluted with 5% methanol in chloroform. After 48 h. a faster-running spot than the nucleoside was observed together with a slower running component which was shown to be *N*-methylimidazole hydrochloride.

The reaction was concentrated to dryness, dissolved in chloroform and washed with sodium bicarbonate solution and water. The aqueous layers were extracted with chloroform and the combined organic layers dried, filtered, concentrated and added to petroleum spirit. After cooling overnight the oily precipitate was purified by column chromatography using chloroform as the eluant .

The carbon-13 and proton n.m.r. spectra of the product are very similar to those of **109**-**111** for the sugar and base atoms. The propyl chain also gives signals at similar chemical shift values to those found for **110**. The ethyl acetoacetate chain, however, displays some very interesting resonances in the n.m.r. spectra: The carbon-13 n.m.r. spectra of this chain shows the carbonyl almost 2 ppm upfield from **109**-**111** as two signals The ester chain gives signals similar to those seen for **109**-**111**. Signals are observed at 157 ppm and

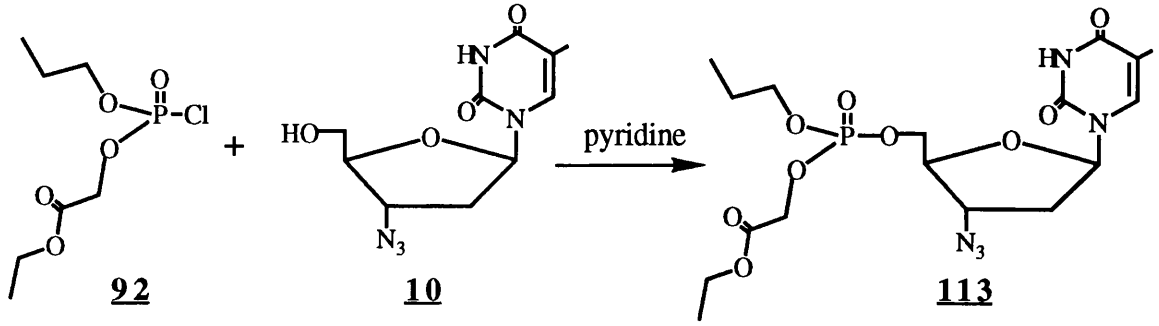
106 ppm, which were assigned to the double bond $CH_3\underline{C}$= and CH= respectively. The $CH_3\underline{C}$= signal was observed at 23.6 ppm. The signals for these three carbons are multiplets.



77 97 112

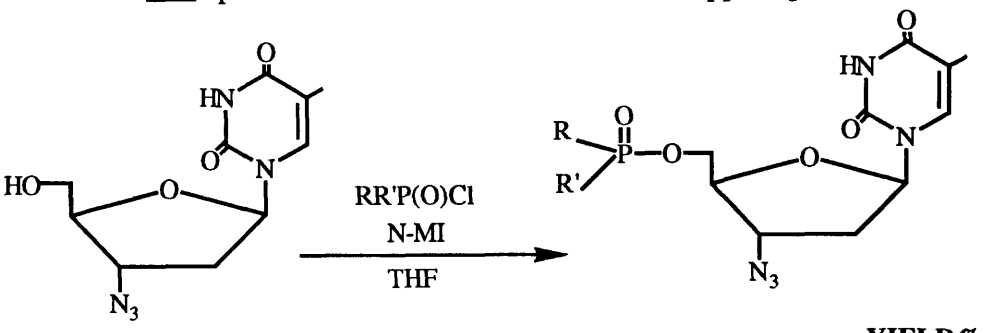
Proton n.m.r. spectra of **112** shows unequivocally that the ester is in a Z configuration since the CH_3C= protons, integrating for 3 protons, are observed as a singlet at 2.1 ppm and the CH= proton integrating for one proton is a doublet at 5.3 ppm. These values correlate closely to data recorded for similar compounds made in the Z form. The E analogue signals are found at approximately 2.3 and 5.8 ppm[178].

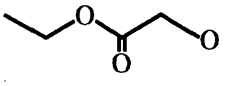
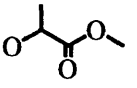
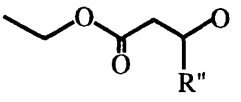
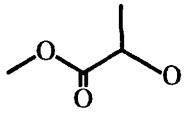
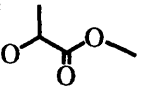
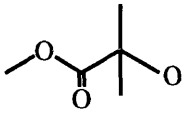
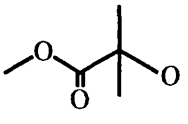
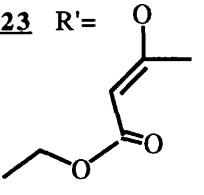
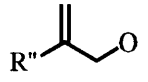
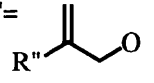
Phosphorus-31 n.m.r. gives two signals, one for each diastereomer, at -8.26 and -8.32 ppm in a 9:10 ratio. This value is 10 ppm further upfield than the signal found for **110**. The value compares favourably with the value of -6 ppm obtained from a prepared sample of **98**[32].



92 10 113

10 was reacted with **92** in an analogous fashion to the formation of **110** to afford **113** as a gum in 37% yield. The Carbon-13, proton and phosphorus-31 n.m.r. spectra were similar to those of **110** apart from the C-3' carbon which is 14 ppm upfield in **10** and **113**.



		YIELD%
R= 	**114** R'= $C_{12}H_{25}O$	79
	115 R'= 	86
R= 	**116** R'=CH_3O R"=H	89
	117 R'=C_3H_7 R"= 	77
R= 	**118** R'=CH_3O	73
	119 R'=C_3H_7O	73
	120 R'=CCl_3CH_2O	79
	121 R'= 	88
R= 	**122** R'=CH_3O	76
R= 	**123** R'= 	91
R= 	R'=  **124** R"=H	97
	125 R"=CH_3	89

114-**125** were synthesized by dissolving **10** in a small amount (1-10 ml) of tetrahydrofuran containing 6-10 equivalents of *N*-methylimidazole. Neat phosphorylating reagent (4-10 equivalents) compounds **94**, **86**, **88**, **90**, **87**, **84**, **85**, **83**, **95**, **99**, **101** and **100** respectively were added in every case except for **99**, which was added as the crude reaction mixture.

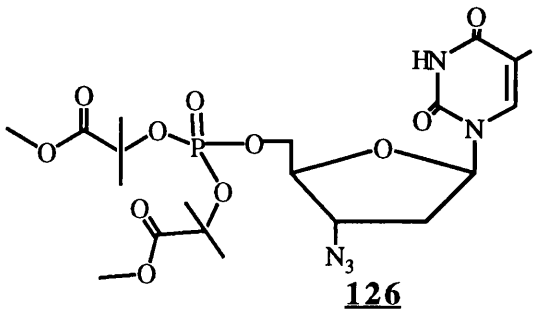
The reaction mixture was generally left for a minimum of 48 h. at ambient temperature with vigorous stirring. In the case of the reaction to give **114** the mixture solidified within 5 minutes of the addition of **94** and a further 10 ml of tetrahydrofuran had to be added to dissolve this jelly. All reactions proceeded cleanly and in each case the product was isolated in a similar way to **112**.

The yields of the desired products varied between 73% and 98%. A slight variation in the work-up procedure had to be used for **114**, since an emulsion formed when saturated sodium bicarbonate solution was added to the concentrated reaction mixture dissolved in chloroform. The emulsion was concentrated to dryness, the residue dissolved in chloroform and partially purified by column chromatography. The eluant containing the desired product was concentrated to small volume and an aqueous bicarbonate wash employed before following the general purification procedure mentioned above.

122 could not be fully purified by flash column chromatography because of a substantial impurity, slightly slower-running than the main product analysed by tlc (5% methanol in chloroform), continually eluted with the desired product. The product was successfully purified by normal phase preparative hplc in 41% yield.

The impurity was analysed by spectroscopic methods, and found to be **126**. Close inspection of the phosphorylating reagents **58** and **95** show that there is an impurity of approximately 4% present in the former and approximately 1% in the latter, that could be bis(2-methyl methyllactyl)phosphorochloridate. 0.016 g of this nucleotide impurity was recovered by hplc.

Either the impurity in the phosphorylating reagent is far more reactive towards **10** than **95**, or **126** was preferentially purified out of the reaction mixture to the detriment of the desired product yield.



126

109-**126** tended to form gums on concentration to dryness. Even under severely reduced pressure for a long time at room temperature, solvents, methanol or 2-propanol, for instance, could still be detected by proton n.m.r. By scratching this gum, dispersing it over a large surface and triturating with diethyl ether, analytical data could, in some cases, be obtained. For the compounds that had been purified by column chromatography with higher levels of methanol used as eluant, the gum had to be dissolved in the minimum volume of 2-propanol and filtered under reduced pressure and concentrated to dryness to rid the compound of small amounts of silica.

To rid the gum of 2-propanol, petroleum spirit 30-40 °C was added to the gum dissolved in the minimum volume of chloroform. The product precipitated as a gum from the liquor, the supernatant was discarded. After repeating a number of times, drying of the gum under reduced pressure afforded analytical data in almost every case.

The carbon-13 n.m.r. spectra of **114**-**126** show that the attachment of the phosphate moiety does affect the C-5' signal, which is shifted 5 ppm downfield, and C-4', which is shifted 2 ppm upfield. Phosphorus coupling is observed for these two carbons of approximately 6 Hz for the former and 8 Hz for the latter. The actual substituents on the phosphate do not affect the base or sugar carbons to a significant degree.

Two diastereomers are possible in the cases where the phosphate is attached to three differing groups and diastereomeric difference is seen for the majority of carbon resonances in the spectra. This difference occurs less often for the sugar carbons furthest from the phosphate and is of a smaller magnitude than for C-5'. The splitting magnitude is largest for the base carbon atoms and occurs most often for C-6 and C-5. This suggests that the base carbons C-6 and C-5 are in closer proximity in space to the alkyl or alkoxyalkyl substituents than C-2 and C-4.

Signals for the alkyl and alkoxyalkyl carbons are also informative. Phosphorus coupling is observed where expected and diastereomeric difference occurs for most carbons in each chain. Exceptions to this tend to be carbon atoms that are furthest from the phosphorus, for example the methyl carbon of the dodecyl chain or the methyl carbon of the ester of the methyl lactyl chain.

Again the ratio of isomers in the series **113**-**120** and **122**-**125** did differ significantly from parity in some instances and thus signals could be assigned to carbon atoms of individual isomers.

The data of **123** is particularly interesting, made more so since the isomers were separated by normal phase hplc; 'fast' **123a** and 'slow' **123b**, thus making the spectra clearer and obviating diastereomeric differences. The $CH_3\underline{C}=$ signal is found at 163 ppm (J=9 Hz), in both isomers 6 ppm further downfield from the analogous carbon atom in **112**. The CH= signal is found at 107 ppm (J=4 Hz), in both isomers 1 ppm downfield from the

analogous carbon atom in **112**. The $\underline{C}H_3C=$ signal is observed at 18.5 ppm, J=4.2 Hz, in both isomers, 5 ppm upfield from the analogous carbon in **112**.

	C-2	C-4	C-6	C-5	C-1'	C-4'	C-5'	C-3'	C-2'	5-CH_3
114	163.8	150.4	135.1	111.4	84.5	82.1	66.5	60.1	37.4	12.3
115	163.3	150.3	135.4	111.4	84.6	82.3	67.0	60.1	37.5	12.4
116	163.4	150.1	135.2	111.5	84.6	82.4	66.3	60.1	37.6	12.4
117	163.9	150.4	135.3	111.6	84.5	82.3	66.2	60.5	37.5	12.5
118	163.8	150.4	135.4	111.5	84.5	82.3	66.4	60.1	37.5	12.4
119	163.9	150.4	135.0	111.4	84.4	82.1	66.4	60.0	37.3	12.4
120	163.4	150.1	135.2	111.6	84.8	82.0	67.2	60.1	37.5	12.5
121	163.8	150.4	135.4	111.5	84.5	82.3	66.4	60.1	37.4	12.4
122	163.5	150.1	135.1	111.5	84.6	82.2	66.5	60.0	37.5	12.4
123a	163.4	150.1	135.1	111.6	84.5	82.1	67.0	59.9	37.5	12.5
123b	163.3	150.0	135.2	111.5	84.8	82.5	66.9	60.0	37.5	12.5
124	163.8	150.2	135.1	111.5	84.6	82.2	66.4	60.2	37.5	12.4
125	163.8	150.3	135.0	111.5	84.7	82.2	66.3	60.1	37.5	12.4
126	163.6	150.1	135.3	111.5	84.6	82.2	66.8	60.2	37.4	12.4
131	163.2	150.0	135.3	111.5	84.9	82.2	**64.0**	60.1	37.5	12.5

Some carbon-13 n.m.r. signals from the spectra of 114-126 and 131

The phosphorus-31 n.m.r. spectra showed that in every case the incorporation of the nucleoside produced a shift upfield relative to the phosphorochloridate. For **113** and **114** this tended to be to 0 ppm; for **115**, **118**, **120** and **121** to -2 ppm, for **122** and **126** to -6 ppm, **112** to -8 ppm; and **123a** and **123b** to -11 ppm.

A difference between resonances for each diastereomer was observed where expected. Shift differences range from 0.04 ppm in **118** to 0.8 ppm in **115**.

Proton n.m.r. spectra of unseparated diastereomers are, as one might predict, highly complex. The base NH is always observed as a broad signal, sometimes as two signals (one for each isomer) but more usually as a singlet and between 8.5 and 10.5 ppm. H-6 is observed at 7.5 ppm as two doublets, one for each diastereomer coupled to H-1', (J=1 Hz). H-1' is found as a multiplet at 6.5 ppm. Irradiation studies of compounds distinguish

between H-3', H-4' and H-5' which all come between 3.8 and 4.8 ppm inevitably as multiplets. The H-2' protons appear as two quite distinct signals, usually at least 0.1 ppm separates them, both are displayed as multiplets.

It is possible to assign certain multiplets to certain diastereomers by integration if the isomers are in different proportions.

When the isomers are separated by flash column chromatography or by hplc the multiplets can be resolved. For instance H-1' now appears as a triplet in most cases, coupling to H-2' protons, and H-5' can appear as 16 signals.

Proton data for the lactyl chains shows some interesting patterns. The methyl of the ester appears as a singlet, integrating for three protons, sometimes diastereomeric shift differences are observed. The other methyl group gives extremely complex signals; diastereomeric differences of 0.02 ppm are observed. The protons are also coupled with the CH proton by 7.0 Hz and the phosphorus by 1 Hz, thus 8 signals of equal intensity are observed. The CH itself appears as two multiplets, one for each diastereomer. Signals for protons in other groups come at expected shifts and usually as multiplets.

The proton n.m.r. data of **123a** and **123b** show unequivocally that the ester is in an E configuration since the CH_3C= signal, integrating for 3 protons, is observed as a singlet at 2.37 ppm and the CH= signal integrating for one proton, is a doublet at 5.81 ppm. These values correlate closely to data recorded for similar compounds made in the E form[177]. The protons of **112** are displayed at approximately 2.1 and 5.3 ppm.

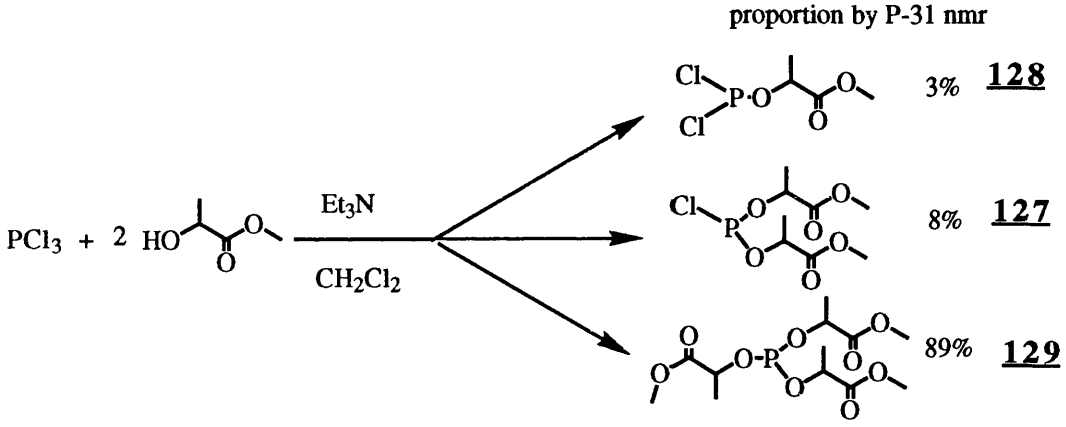
These nucleoside phosphate esters show similar fragments when subjected to E.I.M.S. A common fragment observed is the loss of the carboxylic ester group $CH_3OC(O)^+$ or $C_2H_5OC(O)^+$. Thymine$^+$ and the azido group N_3H^+ are also lost in most cases. The phosphate can be observed as a fragment with one or more of the groups linked to it removed. The sugar as a diene system is always observed at m/e 81, usually as the base peak.

Thiophosphate compounds have been shown to exhibit a wide range of interesting biological properties in a variety of differing organisms and some drugs have been synthesized which contain a thiophosphate, for instance malthiaxon (a potent insecticide[186]) and thiotepa (a potential anticancer agent[187]). Having made protected phosphates of **10** it was decided to investigate the synthesis and biological properties of an equivalent thiophosphate.

Synthesis of thiophosphates can be conducted in a number of ways[188,189]. In theory it would be possible to start with thiophosphoryl chloride, convert it to a thiophosphorochloridate and then react this with nucleoside. However this method was not used because these thiophosphorochloridates tend not to be as reactive towards alcohols as the corresponding phosphates[188]. Phosphites have been used in the successful preparation of thiophosphates as they are easily oxidised with elemental sulphur[188]. Firstly, the phosphite

triester is synthesized from a phosphorodichloridite. The phosphite is then oxidized with sulphur either in dichloromethane or toluene[188]. Another advantage of this method is that it may be possible to isolate and test biologically the phosphite compound. To this end the synthesis of **127** was attempted.

127 was prepared from (S)-(-)-methyl lactate and phosphorus trichloride in dichloromethane in the presence of triethylamine. After concentration and precipitation of triethylamine hydrochloride the product was filtered and concentrated under reduced pressure to afford a clear viscous oil.



Phosphorus-31 n.m.r. data revealed three phosphorus containing moieties as single resonances at 175, (3%) **128**; 162, (8%) **127**; and 136, (89%) **129**. The signal at 162 ppm was assigned to **127** since dialkyl phosphorochloridites are found in this region in phosphorus-31 n.m.r.[189]. The impurities were tentatively assigned to **128** and **129** since mono- and tri-substituted phosphorochloridites are found in these regions[190,191]. Proton coupled phosphorus-31 n.m.r. spectra supported this claim since the signal at 162 ppm becomes a triplet and the one at 136 ppm a quartet, as would be expected from these assignments of structures.

Distillation of the crude oil gave 3 fractions; at 56 °C/0.01 mm Hg **128** was collected in 97% purity (contamination; 3% **127**) by phosphorus-31 n.m.r. At 105 °C/0.01mm Hg **127** in 93% purity, (contamination; **128** 1% and **129** 6%) and at 140 °C/0.01 mm Hg **129** was collected in 94% purity (contamination; phosphate material 4% and **127** 2%).

Since 2 molar equivalents of (S)-(-)-methyl lactate had been allowed to react with one molar equivalent of phosphorus trichloride it would appear that **127** is more reactive towards a further molecule of (S)-(-) methyl lactate than is **128** or phosphorus trichloride. The reason for the increase in reactivity of these substituted phosphorus chlorides could be because of increased nucleophilic attack by the alcohol at the phosphorus centre. This suggests that the phosphorus centre is more electron deficient, as more alkoxy alcohols are substituted at the phosphorus atom. The carboxyl ester centre must have an effect since alkyl alcohols do not show this phenomenon but how this effect comes about has not been investigated.

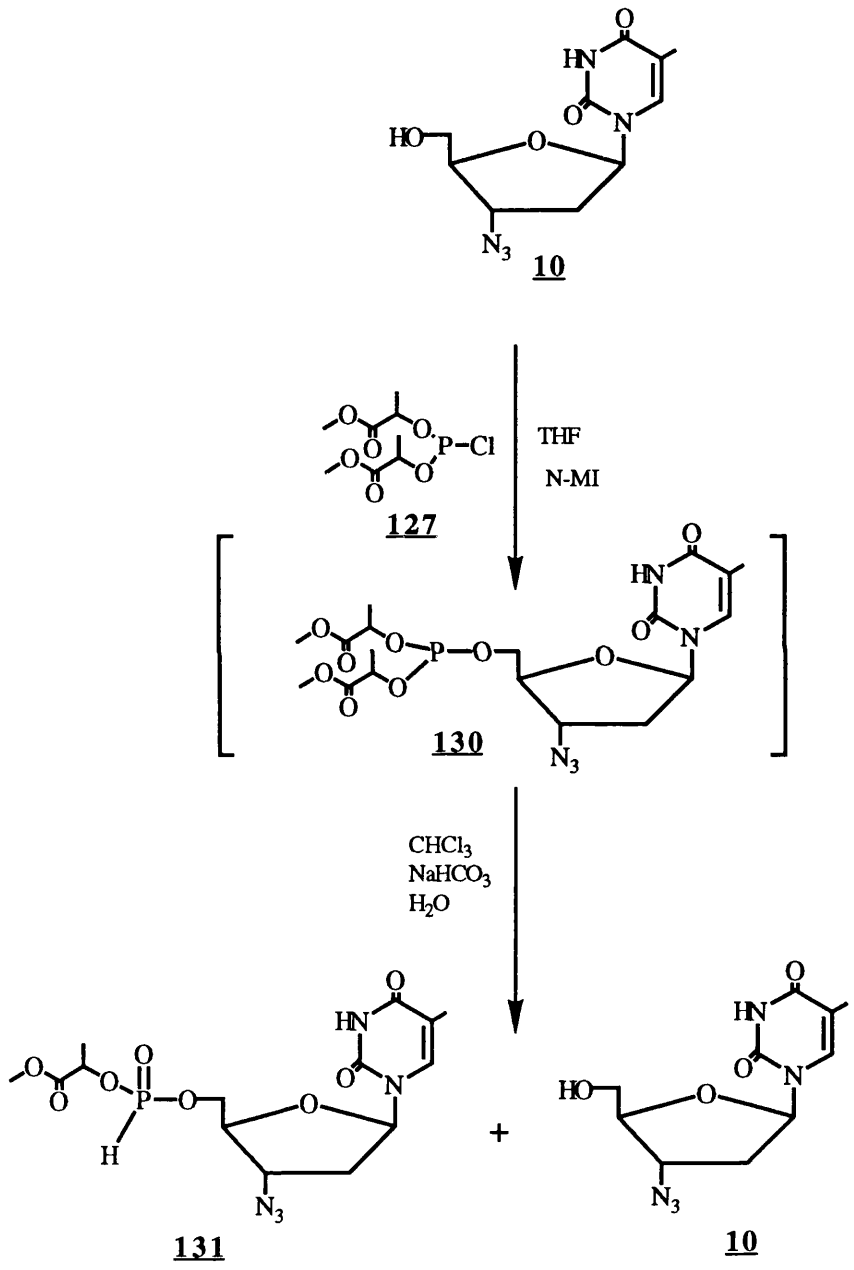
The carbon-13 n.m.r. spectra and the proton n.m.r. spectra of **127**-**129** were fairly similar. The chemical shifts of the carbon and hydrogen atoms were also not particularly altered from those found for **55** and **83** although the phosphorus coupling to each carbon in **128** is half that of **55** in the carbon-13 n.m.r. spectra. The same phenomenon is observed for **83** and **127**.

Reaction of **127** with **10** was performed in THF in the presence of *N*-methylimidazole. The reaction cleanly proceeded to yield a fast running product by tlc eluted with 5% methanol in chloroform. No starting nucleoside remained after 2 h. The tetrahydrofuran was removed under reduced pressure and the gum dissolved in chloroform. The addition of saturated sodium bicarbonate solution gave a violent reaction, the two phase system frothed vigorously. Analysis of the organic and aqueous layers by tlc showed that **10** had been produced in this reaction (approximately 23% by hplc) and the original product had been transformed into another compound with a slightly slower running spot on tlc which ran just above **10**. This product was purified by flash column chromatography using 1% methanol in chloroform as eluant.

The carbon-13 n.m.r. data of the product showed methyl lactyl and 3'-deoxy-3'-azidothymidine carbon atoms were present. Phosphorus coupling to C-5' and C-4' carbons indicated that **10** had been phosphorylated at C-5'. The presence of diastereomers was observed since most carbon signals were 'doubled up'. Indeed a larger diastereomeric split difference than usual was observed indicating that if two isomers were present they were significantly different in spatial arrangement. Diastereomers would not be expected if the desired product had been synthesized.

Phosphorus-31 n.m.r. showed that two phosphorus species were present at approximately 6 ppm. in a ratio of almost 1:1. Although diastereomeric products seemed most likely, a proton coupled spectrum was recorded. Proton-coupled phosphorus-31 n.m.r. gave four signals. Each of these signals was further split to give a quartet. The spectrum showed conclusively that a hydrogen phosphonate product had been synthesized. The P-H coupling of 740 Hz for each diastereomer is close to literature values for similar hydrogen phosphonates[192-5]. The quartets observed were due to coupling to the two H-5' protons and the one CH proton of the lactyl.The proton n.m.r. spectrum showed that only one methyl lactyl group was present per nucleoside which gave further evidence that a hydrogen phosphonate had been produced. The spectrum indicated that diastereomers had been produced, indeed the protons of each isomer had large differences from each other, for example H-1' shows a 0.3 ppm difference. This suggests that the H-5' atoms in the two isomers were in significantly different chemical environments. E.I.M.S. data gave a peak at m/e 418 which corresponds to the hydrogen phosphonate. The loss of thymine as a fragment was observed as was the loss of **10**.

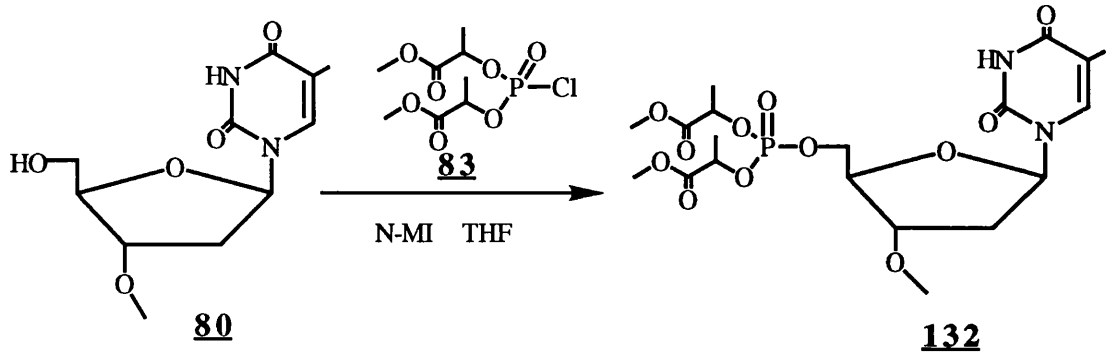
It is suggested that **130** had been produced but that this reacted instantaneously with bicarbonate ion which then rearranged to procure **131**. The fact that a relatively large amount of **10** was recovered suggests that there is no preference as to whether this or a methyl lactyl group is lost during the formation of **131** from **130**.



There was not time to investigate the sulphurization of **130** with sulphur in toluene.

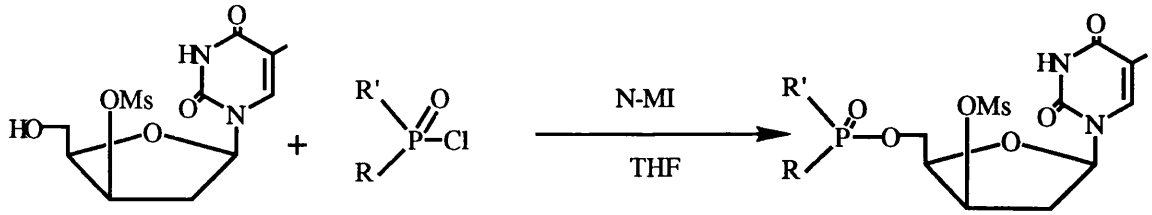
121 was found to display anti-HIV activity (see later). It was therefore thought of interest to investigate the importance of the 3'-azido group by substituting this for a methyl group in one instance and a β-mesyl group in another.

132 was synthesized in an analogous fashion to **121**. Purification of the product by column chromatography afforded a gum in 94% yield.



The carbon-13 and proton n.m.r. data are very similar to those of **121** apart from C-3' which is 20 ppm upfield in **132**. The two methyl CH protons in **132** differ by 0.135 ppm. Each is coupled to the corresponding methyl group (J=6.99 Hz and is observed as a quartet. The quartets are further split by phosphorus coupling (J=1.3 and 0.9 Hz).

In order to investigate the effect on biological activity of changes at C-3' the next compounds to be synthesized were **133** and **134**. **85** and **99** were added to the nucleoside dissolved in tetrahydrofuran in the presence of *N*-methylimidazole. Purification of **133** and **134** was achieved by column chromatography. The products were obtained as froths that could be crushed to white crystalline solids. Analytical data could easily be obtained for these products since solvent was not occluded within a gum as had been the case for the other nucleoside analogues.

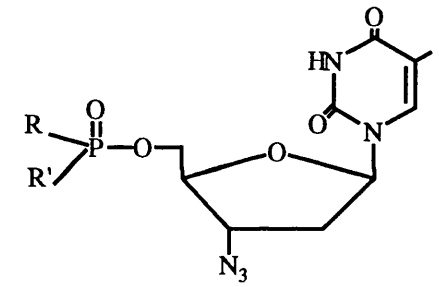


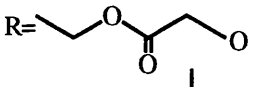
76 **85** **99**

Carbon-13 n.m.r. and proton n.m.r. data of **133** and **134** were similar to **120** and **123** although the C-3' signal was shifted 17 ppm downfield in the case of **133** and **134**. The mesyl methyl was found at 39 ppm and did not show a diastereomeric difference for these analogues. The formation of the E isomer of **134** was observed, no Z isomer was detected. E.I.M.S. data gave MH^+ and fragments corresponding to **76** and thymine. Phosphorus-31 n.m.r. data showed the products displayed signals 1-2 ppm further upfield than **120** and **123**, showing that the β-mesyl group does have a significant effect on the phosphorus centre. The ratio of the diastereomers in the products was also slightly different: 1:3 for **120**; 1:2 **133** for instance.

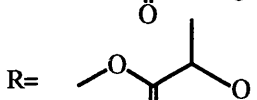
An assessment of the stability of some of the nucleoside analogues in a variety of systems at differing temperatures was investigated by reverse phase hplc and/or phosphorus-31 n.m.r. spectroscopy.

The stability of **119**, **113** and **122** was investigated in growth medium at 37 °C and 5 °C. A control solution of nucleotide in DMSO/water was also made up and kept at 37 °C.

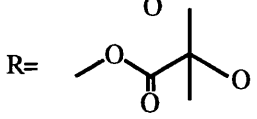




122 R'=C_3H_7O



119 R'=C_3H_7O



122 R'=CH_3O

In each case almost all the nucleotide decomposed within a week at 37 °C in the presence of growth medium. However after a week at 5 °C, 22% to 33% of the nucleotide remained. The proportions of nucleotide in the DMSO/water at 37 °C decreased by approximately 6% over a week for each compound tested.

The main decomposition products of these stability studies had a very short retention time on reverse phase hplc, suggesting that the compounds are extremely hydrophilic,

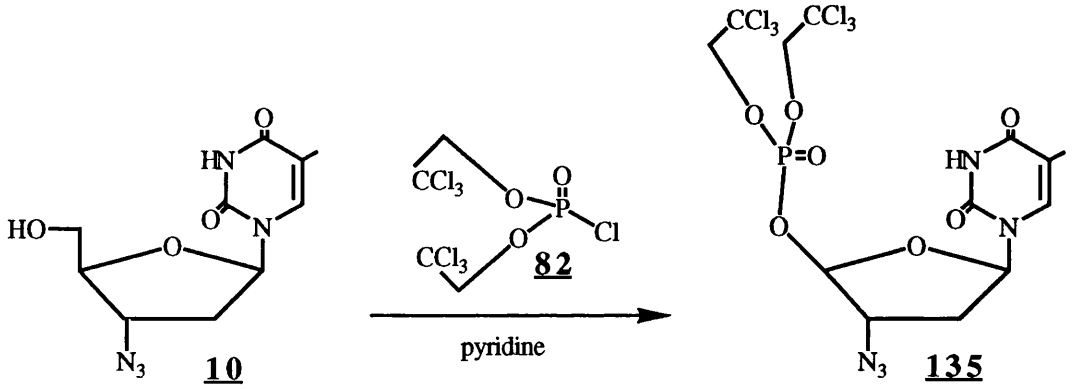
perhaps phosphoric or carboxylic acids. **10** was produced during the course of the stability study but to never more than 0.05% of *uv*-active compounds of each test.

Interestingly, retention of diastereomeric ratio seemed to occur in the investigation into stability of **119**. This compound displayed a diastereomeric abundance of 9:5 (54:30). The two polar products of the breakdown of **119** in growth medium were formed in the ratio of 10:6 (50:30) not significantly different from the initial ratio.

This may be coincidence and two compounds are actually formed. For instance loss of the propyl chain in one product and loss of methyllactate in another would give two distinct products that might have a similar retention time. However, if this is the case then one might expect the loss of **10** to give another compound. No significant quantity of **10** was produced so degradation of the carboxylic ester to the free acid seems more likely.

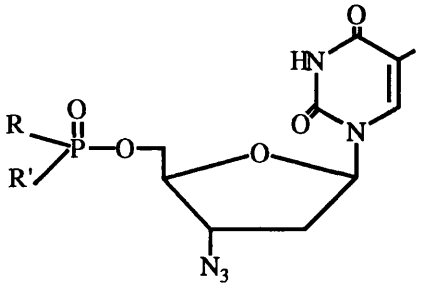
The very slow rate of formation of **10** suggests that **10** is a product of the initial breakdown product of the system. The breakdown of the nucleotides also seems to be affected by the temperature of the system; the reaction being considerably slower for the solution kept at 5 °C. Degradation in DMSO/water by 6% over a week shows that these phosphate triesters are not completely stable to DMSO/water .

The stability of some nucleotide phosphate triesters in human plasma was also investigated at 37 °C over a period of time. For this study **135**, prepared in an analogous fashion to **113**, and the analytical data of which correlated closely to an authentic sample[196], **120** and **121** were chosen.

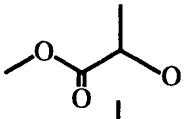


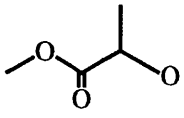
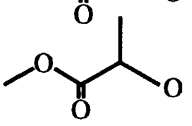
The compounds were dissolved in the minimum volume of methanol and added to human plasma/deuterated water at 37 °C. The breakdown of **135**, **120** and **121** was followed by phosphorus-31 n.m.r. spectroscopy and reverse phase hplc.

The phosphorus-31 n.m.r. data showed that the compounds did break down over time but little other information could be gleaned from this since the concentrations of **135**, **120** and **121** in the plasma were extremely low. The breakdown products also had chemical shifts in the same region as the nucleotide. The spectrum of plasma on its own showed very weak signals by phosphorus-31 n.m.r.



135 R=CCl_3CH_2O R'=CCl_3CH_2O

120 R=CCl_3CH_2O R'= 

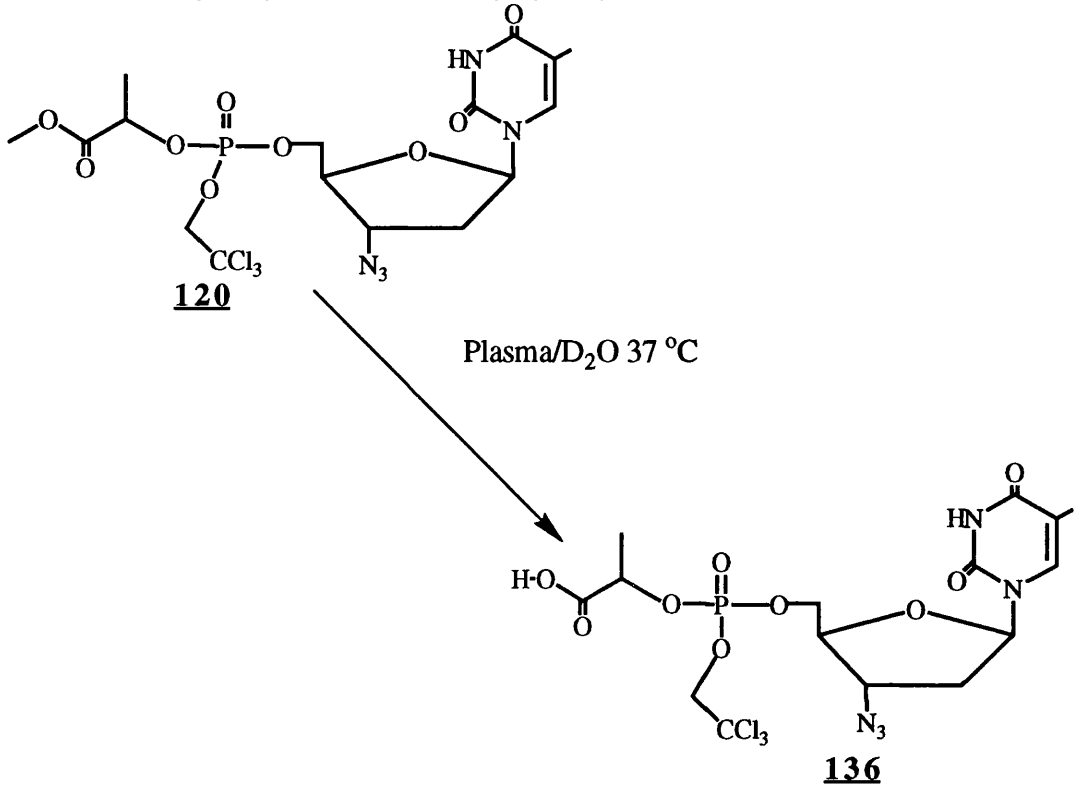
121 R=  R'= 

Reverse-phase hplc was far more informative. **120** (retention time approx. 28 min.) broke down exclusively to one new compound in 20 h. **10** was not formed during this process. Subsequent purification of this product **136** by hplc gave an oily product with a retention time of approx. 12 min. This product had a very low solubility in chloroform, dichloromethane, carbon tetrachloride, diethyl ether, benzene, tetrahydrofuran and dioxan. However, the compound was sufficiently soluble in CD_3OD to obtain proton n.m.r.

Although greater than 99.9% pure by hplc detected by *uv*, the proton n.m.r. spectrum showed that **136** still had impurities in it from the plasma. The trichloroethyl group, **10** and the lactate group were present in a 1:1 ratio. However the methyl of the carboxylic ester was not present and it was concluded that plasma had converted the ester to the carboxylic acid **136**. No further data were obtained owing to the very small (1mg) quantities of nucleotide and the impurity of this sample.

121 was also converted to more polar species. However in this instance two products were observed which displayed a very short retention time indeed (4.5 min.). Total decomposition of the starting nucleotide took 60 h. and no **10** was observed. However after this product was kept at 37 °C for 2 months 95% of the initial breakdown products had been converted to **10**. The total breakdown of **121** took longer than the time found for **120**. If the

breakdown is enzymatic it would appear that either the enzyme involved can recognize **120** more easily or the trichloroethyl moiety somehow makes the ester functionality more labile owing to electronic effects. That the initial breakdown products were converted to **10** over a long period of time shows that the initial products are not plasma stable, they are either broken down by enzymes or chemically hydrolysed.



Decomposition of **135** did occur; after 1 h. the proportion of **135** had decreased by 0.5%, by 2 h. 1%, by 4 h. 2% and after 48 h. by 25%. From this it can be stated that the decomposition is zero order with respect to **135**. Two decomposition products were observed that from retention times could be tentatively assigned as nucleotides having lost one or two trichloroethyl groups respectively. The decomposition products were in a ratio of 1:1. No **10** was produced.

All the nucleotide phosphate triesters synthesized above were evaluated against HIV .

Brief Description of methods used for anti-viral testing.

St. Mary's. Tests carried out by D. Kinchington.

HIV-1_{RF}/C8166:

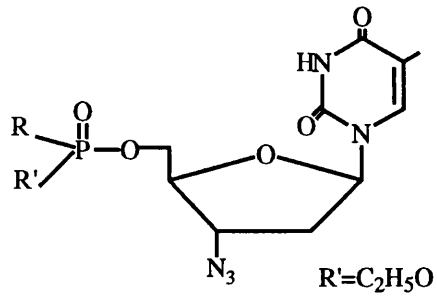
10 TCD50 of HIV-1_{RF} was absorbed to 2 x 10^5 cells for 90 min. at 37 °C. Cells were washed and resuspended in growth medium. The cells (2 x 10^5) were cultured in 6 ml tubes with three concentration of compound (200, 20, 2 mmol/ml) for p24. Dilutions ranging from 200-0.00002 mg/ml were selected for subsequent tests. Cytotoxicity assays were carried out simultaneously with secondary evaluation of compound. Cells (2 x 10^5) were cultured in 6 ml tubes with three concentrations of compound (200, 20, 2 mmol/ml) for 72 h. Cells were washed and resuspended with ^{14}C-protein hydrolysate and after an overnight incubation, ^{14}C incorporation measured.

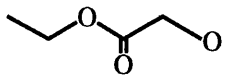
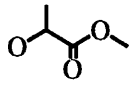
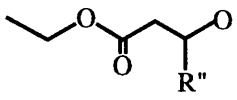
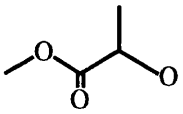
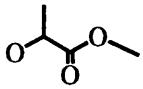
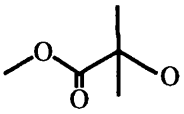
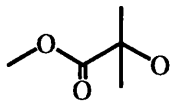
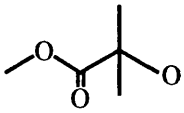
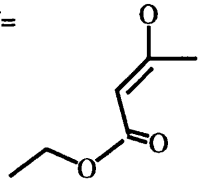
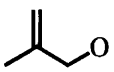
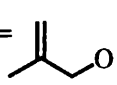
The anti-HIV activity of the compounds synthesized above varied markedly depending on the 3'-substituent and the nature of the phosphate ester.

Firstly the nature of the 3'-substituent is vital to the anti-HIV activity of the nucleotide. In this series of compounds derivatives of **10** are the only ones that display activity in the test. The inactivity of derivatives of **77** may be due to their conversion to their thymidine derivatives by carboxyesterases *in vitro* which would then, if the free phosphates were formed would release **78** or **78**-monophosphate.

132-**134** display no anti-HIV activity in this test. This may be because these nucleotides are stable to degradation or if broken down to the free nucleotide or nucleoside (**80** and **76**) they are not recognized by cellular kinases which procure the di- and tris-nucleotide phosphates.

For the derivatives of **10** there is a 3-fold range of activities depending on the analogue. Firstly there is not much significant difference if one chain is kept the same and the other is altered if the other is an alkyl or substituted alkyl (eg **118**-**120**).



Compound No.			Activity μm
113		$R'=C_2H_5O$	0.5
114		$R'=C_{12}H_{25}O$	3.0
115	R= 	R'= 	0.3
116		$R'=CH_3O$ $R''=H$	0.05
117	R= 	$R'=C_3H_7$ $R''=$ 	5.0
131		R'=H	0.9
118		$R'=CH_3O$	0.8
119	R= 	$R'=C_3H_7O$	7.0
120		$R'=CCl_3CH_2O$	0.4
121		R= 	0.07
122		$R'=CH_3O$	3.0
126	R= 	R'= 	0.06
123a	R= 	R'=  "fast"	0.8
123b		"slow"	0.1
124	R= 	R'=  R"=H	0.2
125		R"=CH_3	0.1

116 shows high activity suggesting that increasing the distance between the carbonyl and phosphoryl centres does increase the activity. If this chain is substituted **117** activity decreases. Further increases of the distance between carbonyl and phosphorus centres has not been investigated in this thesis, but obviously it would be of great interest in future work.

131 displays a 10-fold decrease in activity compared to **121** which may perhaps be due to the stability of the P-H bond in biological systems. The activity of the compound may be due to formation of **10** from the hydrogen phosphonic acid produced from **131** perhaps by loss of the S-(-)-methyllactyl group followed by phosphodiesterase attack on the

phosphodiester produced. The nucleoside **10** produced would then act in its normal manner ie via the triphosphate **16**.

123a and **123b** are less active than would be predicted from the similar highly active compound **116**. this is possibly due to the unsaturation between phosphate and carbonyl centres. Interestingly there seems to be a 8 fold difference in activities of the two diastereomeric products indicating the importance of the three dimensional nature of the phosphate triester.

10 shows toxicity at 100 mM in this test. The only product in these series above, that shows toxicity at this level or greater is **114**. This may be due to the long alkyl chain which could distort membrane structure although this is far from certain.

The most surprising test results are the activities of **124** and **126**. Since simple alkyl chain analogues show no activity at all it is extremely surprising that conversion to the alkyl derivatives gives compounds with considerable anti-HIV activity. There is a possibility that these compounds are metabolised to alcohols by oxidation of the alkene bond. The compounds could then be converted to **10** or **14** by other cellular enzymes perhaps lipases.

Although, as previously mentioned, it is possible that certain 5'-phosphate derivatives of inactive 3'-modified nucleosides may be active, by way of 'kinase by-pass', all the nucleosides other than the derivatives of **10** investigated here were found to be inactive.

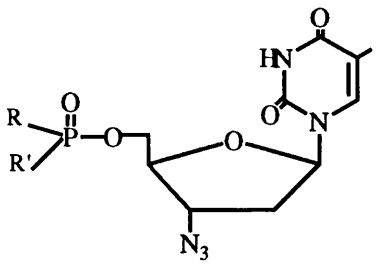
Cambridge: Dr Karpus *et al.*

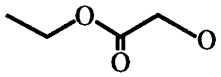
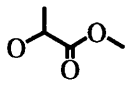
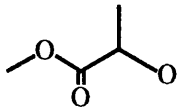
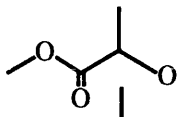
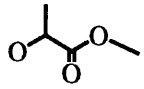
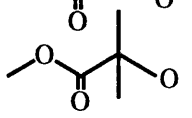
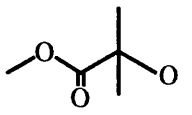
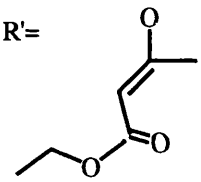
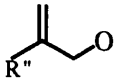
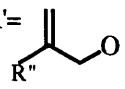
HIV-1_{RF}:C8166:

Cells were infected at room temperature with 10 TCD_{50} of HIV-1_{RF}. After 90 min. cells were washed, resuspended in fresh medium and 100 ml volumes placed in the wells of a microtitre plate containing 10 fold dilutions of compound. After 5 days at 37 °C, syncitia were examined microscopically, p24 release measured by ELISA and virus infectivity assayed. Cell viability was determined by the MTT-formazan method.

Phosphate triesters of **10** were tested by this system. All the compounds show activity though this diminishes in most cases at 0.001mg/ml. **123a** shows no toxicity at 100 mg/ml but total protection against HIV at 0.001 mg/ml.

114 displays toxicity in this test as it does in the St. Mary's one. **124** is far less active than **125** in this test similarly substitution between carbonyl and phosphorus centres increases the activity of the compound. Unfortunately **116** was not tested due to time restrictions.



Compound No				A	B	C
114		$R'=C_{12}H_{25}O$		0.1	50	100
				0.01	70	90
				0.001	90	60
115	R= 	R'= 		0.1	50	100
				0.01	70	100
				0.001	100	95
120	R= 	$R'=CCl_3CH_2O$		0.1	20	100
				0.01	80	100
				0.001	100	90
121		R= 		0.1	40	100
				0.01	80	100
				0.001	100	95
122	R= 	$R'=CH_3O$		0.1	50	100
				0.01	90	100
				0.001	100	95
123a		R'= 	"fast"	0.1	30	100
				0.01	80	100
				0.001	100	100
123b	R=		"slow"	0.1	50	100
				0.01	90	100
				0.001	100	95
124		R'= 	R"=H	0.1	60	100
				0.01	100	50
				0.001	100	0
125	R=		R"=CH_3	0.1	30	100
				0.01	80	100
				0.001	100	95
137	R= C_3H_7O	CCl_3CH_2O		0.1	70	100
				0.01	100	100
				0.001	100	70

Test concentration mg/ml	A
Estimated cell (un-infected) growth	B
Anti-HIV activity %CPE reduction	C

The mechanism by which the derivatives of **10** display their anti-HIV effect is uncertain. However, one explanation consistent with the facts is of membrane penetration, followed by intracellular cleavage of the carboxylic ester bond, which may then further cleave to give a nucleoside phosphoric acid (free nucleotide) which being polar may be trapped

inside the cell, and should then be subject to further phosphorylation to the 5'-triphosphate, which could then inhibit HIV reverse transcriptase.The nucleotides could act, not as **14** or **10** but by another mechanism; perhaps by disturbing the membrane, or inhibiting reverse transcriptase. If they are being converted to **14** or **10** the different therapeutic ratio must be due to the nucleoside phosphate triester not affecting normal cells to the same degree as HIV-infected ones. If this is the case then perhaps there is a viral induced enzyme which recognizes the synthesized nucleotide or one of its breakdown products and thus this infected cell is more susceptible to the compound than the normal cell which lacks the enzyme.

In summary, for activity the nucleoside phosphate triesters could be hydrolysed intracellularly to yield **14**, which would then be sequentially phosphorylated to the bio-active form **16**. An alternative mode of action is that the triester could lose the phosphate moiety entirely to yield **10** which would then be sequentially phosphorylated to **16**.

In conclusion, these active phosphate triesters are of great biological interest. The fact that the compounds never exhibit a higher efficacy than **10** might suggest that they are in the main acting as **10** or **14**. That it is necessary to use this active nucleoside to give phosphate triesters with some efficacy rather than use other 3'-modified nucleosides, which are as themselves inactive, may also suggest that the active protected nucleotides are acting as **10** and/or **14**.

5'-Phosphonate derivatives could give some insight into the mode of action of these triesters since the phosphorus-carbon bond is stable to hydrolysis. Thus **10** should not be produced by hydrolysis of the phosphate protecting groups.

If the mode of action of triesters involves intracellular hydrolysis of the phosphorus-nucleoside bond, it may be expected that the activity of this phosphate triester would be greater than of a phosphonate analogue. If, on the other hand, the mode of action involves the loss of the other substituents then, if the free phosphonate and free phosphate have similar substrate affinities for the kinases to further phosphorylate them, the activity should be fairly similar. Therefore the synthesis of **137** was attempted.

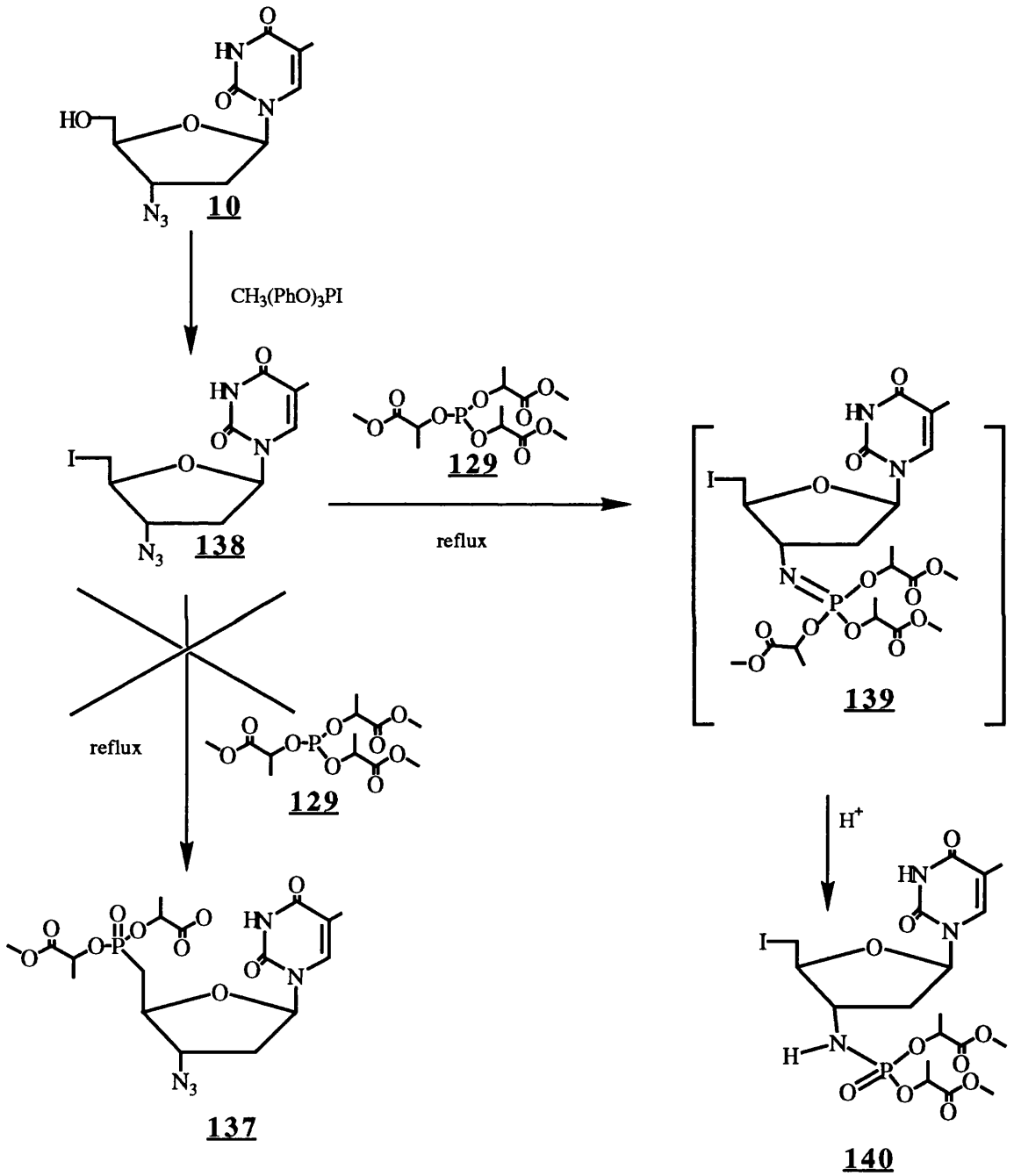
Phosphonates can be synthesized by the reaction of a haloalkane with a trialkyl phosphite in a Michaelis-Arbuzov type reaction[197].

Firstly **138**, was synthesized by dissolving **10** in tetrahydrofuran, adding methyl triphenoxy phosphonium iodide[198] and leaving for at room temperature. The product was isolated by washing a chloroform solution of the reaction mixture with sodium thiosulphate solution and precipitating the product from hexane in 85% yield.

The carbon-13 nmr spectrum was very similar to that obtained for **10** apart from C-5' which shifts from 60 ppm to 7 ppm. Similarly in the proton nmr spectrum the H-5' protons were found at 3.46 ppm, 0.4 ppm upfield from their position in **10**.

138 was dissolved in **129** and heated at 125 °C under reduced pressure for 90 min.. after which time all the nucleoside had been converted to a slower-running product. Reduced

pressure was employed in an effort to drive off any methyl lactyl iodide produced. The product was isolated by pouring the hot solution into diethyl ether and refrigerating this overnight. The precipitate was then purified by column chromatography to remove the last traces of **129**.



The phosphorus-31 nmr spectrum of the product showed a singlet at 7.15 ppm. Phosphate products similar to 5'-phosphonate compounds generally show signals between 20 and 30 ppm[199].

The carbon-13 nmr spectrum of the product clearly showed C-5' at 7 ppm suggesting that the halogen was still present. However C-3' was altered, from 60 ppm to 55 ppm. Of more

importance was C-2', which displayed phosphorus coupling (6.6 Hz) as did C-4' . Although C-3' did not display phosphorus coupling it seemed that the phosphorus was connected somehow through C-3'. The carbon-13 nmr spectrum also showed two methyl lactyl chains were present, each displaying, where appropriate, phosphorus coupling indicating that they were still attached to the phosphate centre.

The proton nmr spectrum showed that two lactyl chains were present per nucleoside and that H-5' was unaltered. Otherwise all other signals were as expected for a 3'-modified 5'-iodo thymidine product.

It has been reported that trialkyl phosphites react vigorously with azido groups at high temperature to give an imide as the product[200,201]. The driving force for this reaction is the loss of nitrogen. In the presence of acid, and in this case the trimethyl lactyl phosphite is acidic since it is slightly impure, this P=N compound is converted to an imido phosphate, the formation of the P=O bond being the driving force here. In this reaction **129** reacts with the azido moiety of **138** to give **139** which in the acidic medium is converted to **140**. Methyl lactate **141** is lost in this process which would be lost under the reduced pressure employed here. Indeed proton nmr of **140** showed a broad singlet at 5.6 ppm which correlates well for H-NR-P[202].

140 was produced in 94% yield overall. No other bi-products or the desired product, had been produced in the reaction. **140** was tested against HIV but was found, not surprisingly owing to its totally unnatural structure, to show no activity or toxicity whatsoever. Since the reaction had proceeded without any production of the desired product it seemed highly unlikely that **137** could be synthesized by this method.

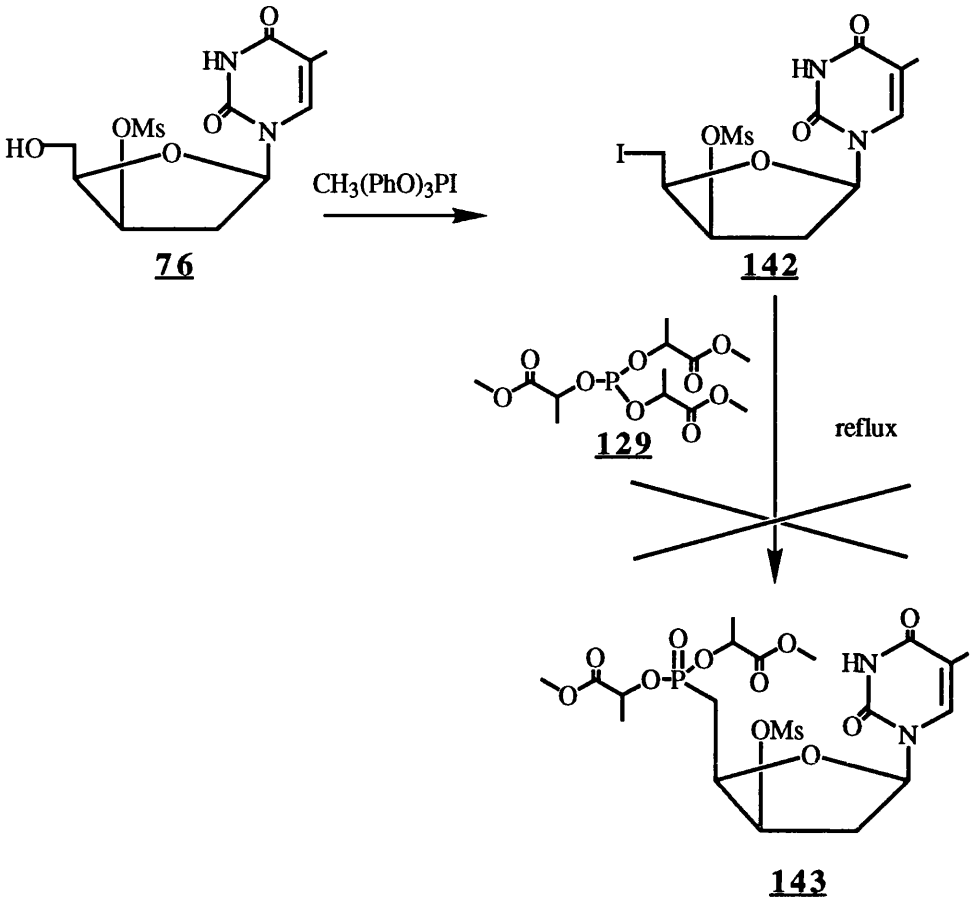
If **142** could be synthesized then perhaps this could be converted to **143** with **129** then to **137** by the use of sodium azide in dimethylformamide at high temperature.

To this end **76** was converted to **142** using the same methodology used in the synthesis of **138** in near quantitative yield.

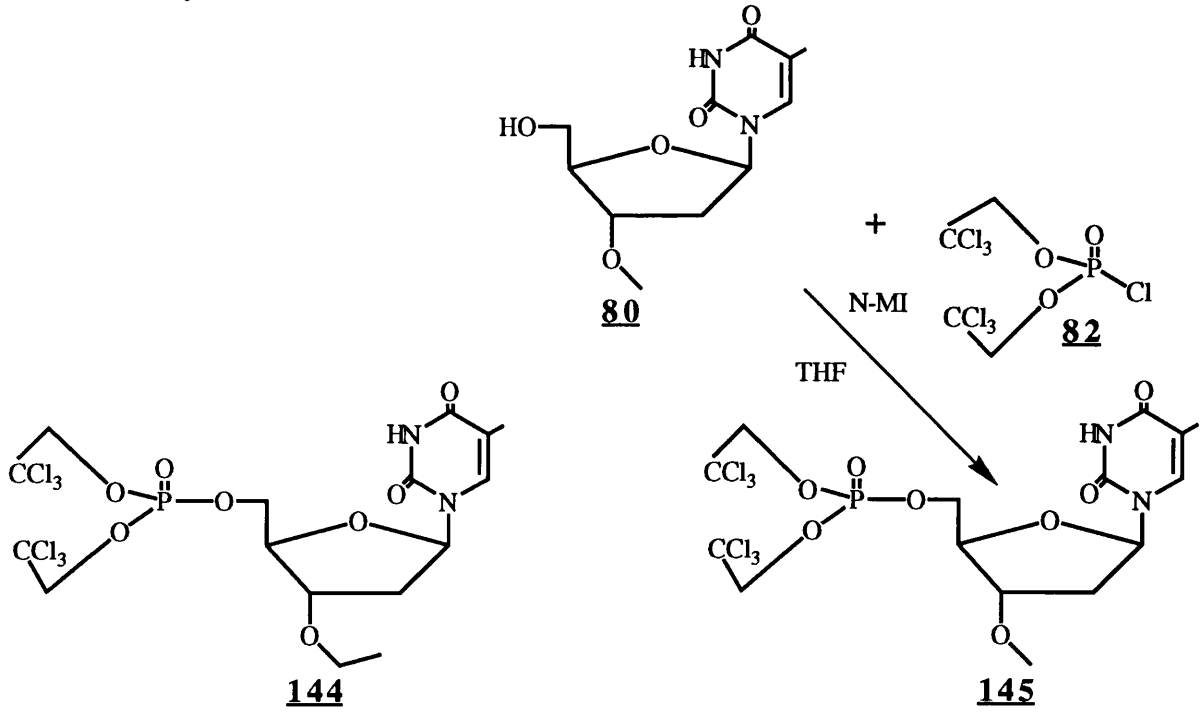
Carbon-13 nmr showed C-5' was approximately 9 ppm upfield from C-5' in **138** at -2.9 ppm. This shows the marked effect of the iodo and O-mesyl groups on the electronic environment of this carbon. The proton nmr spectrum was also recorded which correlated closely to literature values for the 3'-α-analogue[203].

The nucleoside was dissolved in **129** and heated at 180 °C for 96 h. However breakdown products were observed in this reaction followed by tlc (5% methanol in chloroform) although a very small amount of some fast running (R_f 0.45) product was observed. This could not be successfully purified from the reaction mixture. The mesyl group in this reaction seems to be unstable to the high temperatures and solvent/reactant employed in this reaction.

The effect of a 5'-phosphonate could perhaps be investigated if a different 3'-altered nucleoside that showed biological activity, for example **12** or **13**, was used.



144 was shown to display some anti-HIV activity albeit slight[196]. The synthesis of the 3'-O-methyl derivative **145** was therefore considered to be of interest.



145 was synthesized by the addition of **82** to **80** in pyridine. After leaving overnight the reaction mixture was concentrated to dryness and purified by flash column chromatography with chloroform as the eluant to give **145** as a white crystalline solid in 87% yield.

The carbon-13 nmr spectrum of **145** shows phosphorus coupling to C-5' and C-4'. The 3'-O-methyl carbon reasonance is found at 80 ppm and the C-5' at 68 ppm, 2 ppm further downfield from **132**. For the trichloroethyl carbons a discrepancy exists in the literature. Only two independent reports of carbon-13 nmr assignments of 2,2,2-trichloroethoxy species could be located. In each case, two peaks were noted, at *ca.* 74.5 and 94.7 ppm; as we observed. However in one case[204] the former is assigned to the CCl_3 carbon atom, and the latter to the $\underline{C}H_2O$ carbon. In the second case[205] the assignment is reversed. We agree with the latter assignment on the basis of relative phosphorus coupling constants: It has previously been noted[206] a slight increase in coupling constant on proceeding from the POC to the POC$\underline{C}$ atom in simple dialkyl phosphate derivatives of adenosine and other nucleosides.This is noted again here for C-5' and C-4', and for the two trichloroethyl peaks, with the downfield peak (assigned to the CCl_3), displaying the larger coupling. The phosphorus-31 nmr spectrum shows a single peak at -5.5 ppm is 1.5 ppm further downfield from the bis (S)-methyl lactyl analogue and appears as a singlet.

The proton nmr spectrum shows the sugar and base protons at expected shifts and the trichloroethyl protons as a multiplet at 4.65 ppm, close to literature values for these protons[207].

E.I.M.S. data shows MH^+ - thymine and in addition a number of signals in this region. This arises from the fact that chlorine consists of two major isotopes, ^{35}Cl and ^{37}Cl, in the ratio of approximately 3:1. The theoretical pattern of these signals may be readily calculated from the theoretical abundance of isotopes and this may be compared with the pattern observed in the spectrum itself. The isotope pattern observed at around m/e= 478 was quite similar to the pattern calculated for an ion containing six chlorine atoms.

It should be noted that in all the mass spectra data obtained for **145** and other chlorine containing products; '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 peaks could also be due to doubly protonated molecular ion species. Analytical data were obtained for this compound. **145** did not display significant anti-HIV activity (St. Mary's).

It has been reported that **135** shows significant anti-HIV activity *in vitro*[196]. With this in mind **146**-**153** were prepared to see if it was possible to achieve activity without having a 3'-azido moiety.

78 was reacted with **82**[208] in pyridine at low temperature, to give **146** in 66% yield after column chromatography. **146** displays a single resonance in the phosphorus-31 nmr, at approximately -5 ppm, fully consistent with the proposed structure[209].

The carbon-13 nmr spectrum of **146** is particularly informative. Signals for **78** and **146** are very similar for the base carbon atoms, and for C-1', C-2' and C-3' of the sugar. However on phosphorylation at the 5'-position, C-4' shifts 2 ppm upfield (*ca.* 85 ppm) and C-5' shifts 8 ppm downfield (*ca.* 69 ppm). Moreover, as found in the lactyl derivatives, both carbons display phosphorus coupling; of 7 Hz for the former and 6 Hz for the latter. The structure and purity of the compound were confirmed by proton nmr, FAB mass spectrometry, hplc and microanalysis.

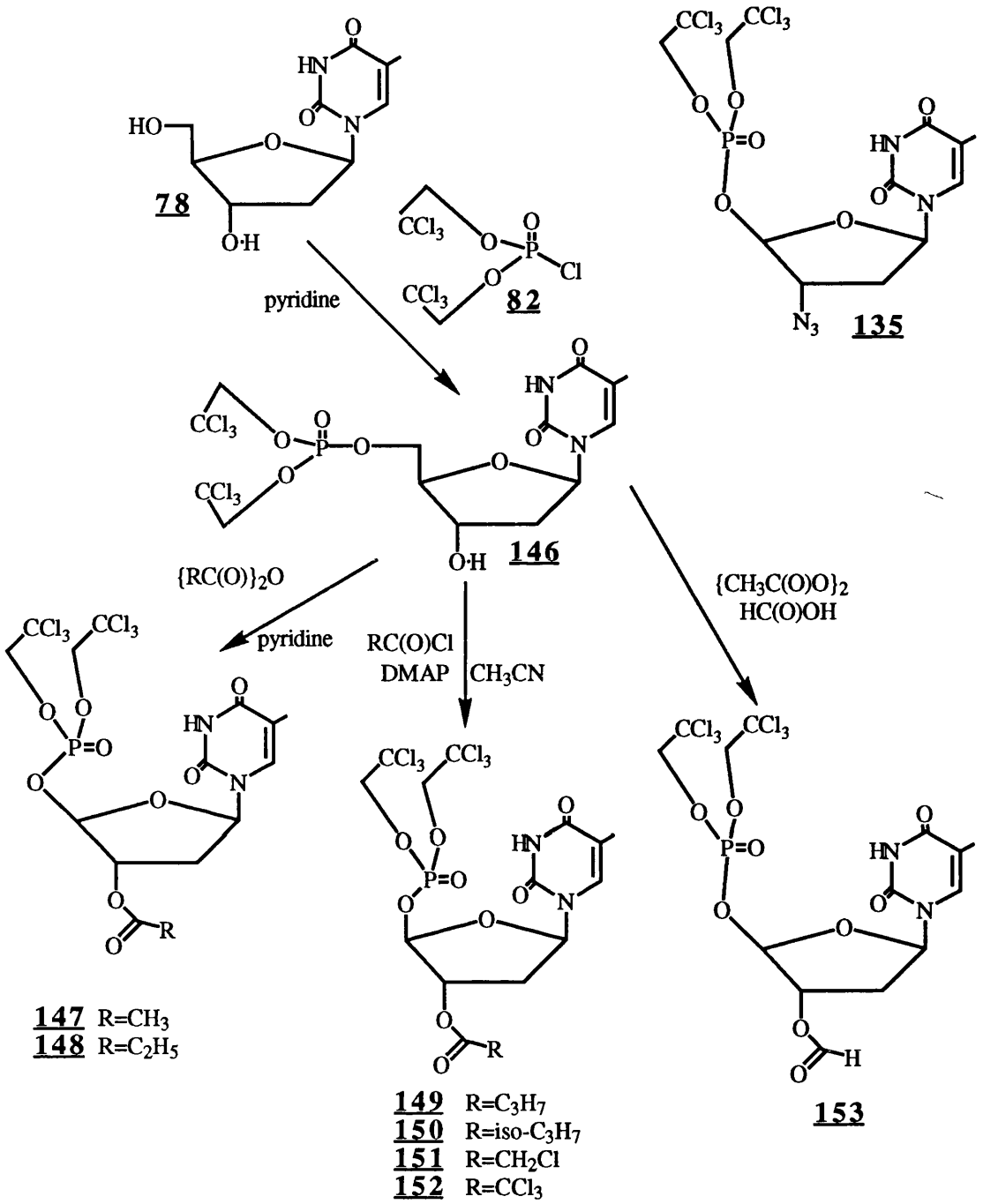
146 was reacted separately with acetic anhydride and propanoic anhydride in pyridine at room temperature. **147** and **148** were purified by flash column chromatography to give the products in 43% and 49% yields respectively. This method of synthesizing 3'-products in pyridine did not proceed in particularly high yield and total purification proved problematic.

4-Dimethylaminopyridine in acetonitrile has been used as the base in the acylation of alcohols[209] and this was used to produce **149**-**152**. **146** was reacted with the corresponding acid chloride in acetonitrile in the presence of 4-dimethylaminopyridine.

152 was found to be unstable to silica eluted with 0-5% methanol/chloroform. Purification was achieved by concentrating the reaction mixture to a gum dissolving this in chloroform and washing with saturated sodium bicarbonate and water. Concentration of the organic layer to dryness afforded **152** analytically pure in 73% yield. **150** was purified this way and crystalised from cyclohexane in 95% yield.

For **149** and **151** the reaction mixture was quenched with water concentrated to dryness, dissolved in chloroform and washed with aqueous sodium bicarbonate solution and water. The organic layer was concentrated to a small volume and purified by column chromatography eluting with methanol in chloroform. Concentration of appropriate fractions afforded **149** and **151** in 86 and 76% respectively.

Formylation of nucleoside hydroxyl groups can be achieved with the use of a formic acid/acetic anhydride mixture. To this end formic acid was added to acetic anhydride and heated then cooled before **146** added. The reaction was quenched with methanol and after concentration to a gum the product was purified by column chromatography to give **153** in 99% yield. The proton nmr spectrum showed that **147** had been produced (0.1% by integration). This was removed by crystallisation of the product from toluene to give a yield of **153** (first crop 67%).



The carbon-13 nmr spectra of **147**-**153** show that on acylation. The C-3' signal is shifted downfield by 2 ppm for all analogues except **151**, by 4-6 ppm and **152**; by 8 ppm when compared with the spectrum of **146**. The base carbon signals were only slightly affected. C-2' and C-4' are generally shifted upfield by 2 ppm (in **152** by 3 ppm).The carbonyl of the acyl varies significantly from 164 to 177 ppm. Other signals for the acylating group are present; the **152** CCl_3 of the trichloroacetyl group carbon is displayed as a singlet at 88.9 ppm.

Phosphorus-31 nmr varies little from compound to compound; from -5 to -6 ppm; which is consistent with the formation of the required products. The proton nmr spectrum is

as expected; in **153** the formyl proton signal comes as a singlet at 8.00 ppm. The isobutryl methyls of **150** are distinct from each other. E.I.M.S of **146**-**153** show MH^+ in all instances except **152**. Chlorine isotope effects were observed for six, seven or nine chlorines where relevant.

Biological testing at St Mary's of these eight compounds showed no activity at better than 10 μM. Toxicity was observed for **151**.

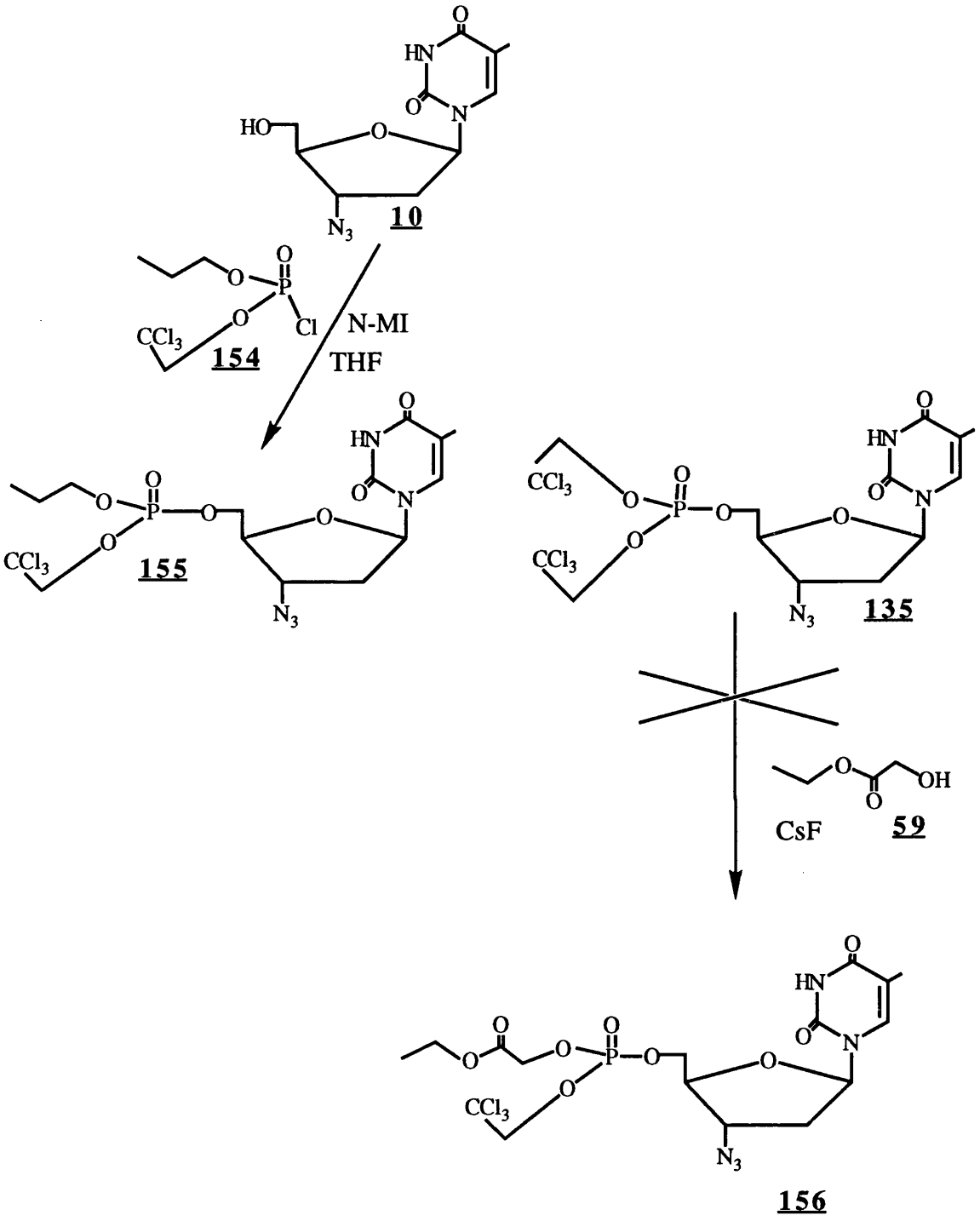
A different synthetic route to alkoxy alkyl nucleoside phosphate triesters was investigated to improve yields of the products and investigate the possibility of synthesizing compounds which could not be synthesized by standard procedures.

It has been reported[210] that a trichloroethyl group, as part of a phosphate triester, can be exchanged for a simple alkyl alcohol if the triester is dissolved in that alcohol in the presence of a large excess of caesium fluoride. If two trichloroethyl groups are present the displacement of one can be achieved at room temperature. If only one 2,2,2-trichloroethyl group is present the reaction has to be heated to reflux for some time.

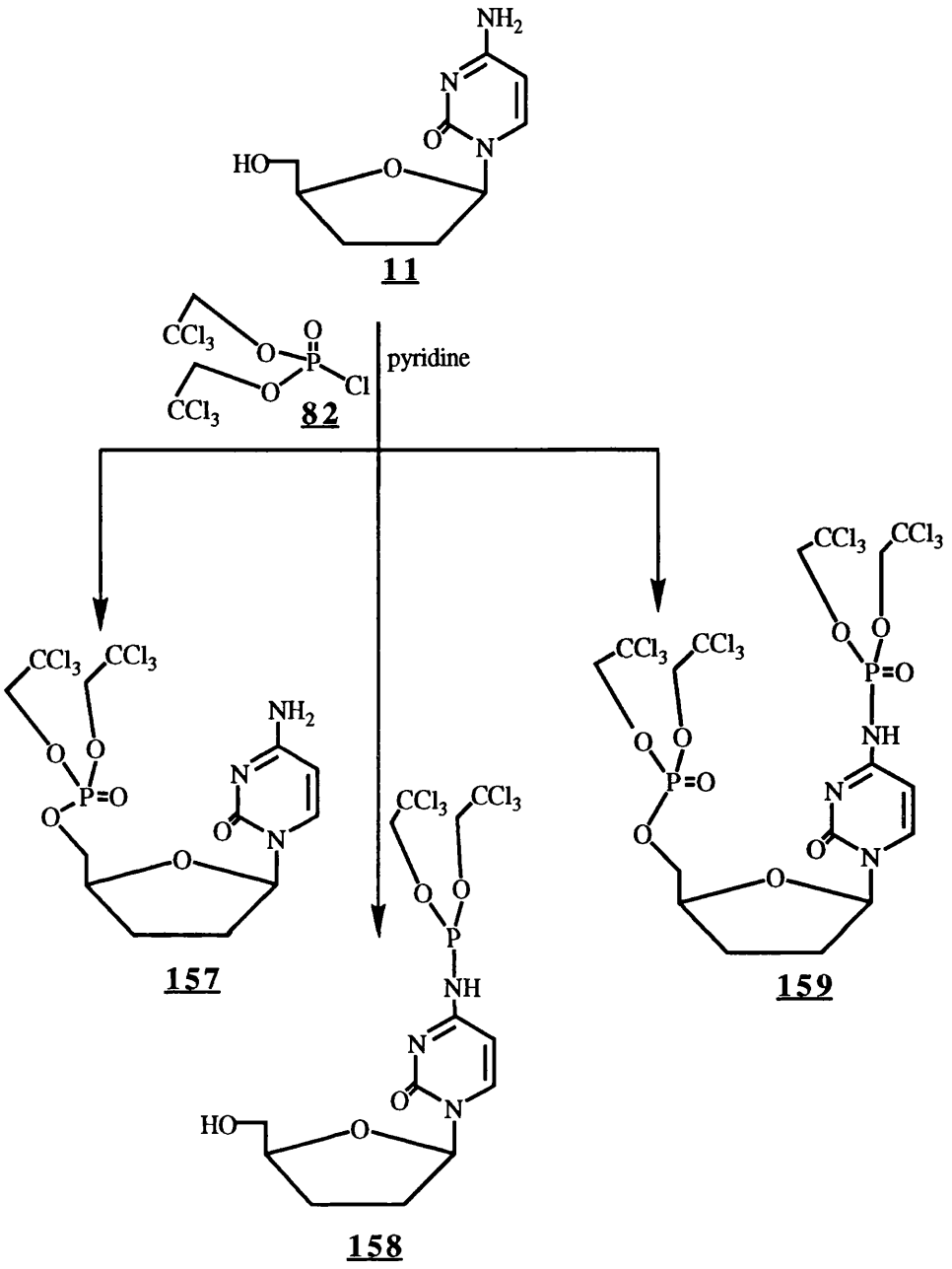
The use of **59** as the displacing alcohol in this system was investigated. **154** was synthesized by standard procedures and purified by distillation under reduced pressure. Phosphorus-31 and carbon-13 nmr of **154** were satisfactory. **10** was reacted with **154** in pyridine. The product 155 was purified by column chromatography to afford a gum in good yield. There was not time to react **155** with **59**.

The carbon-13 nmr spectrum of **155** showed that diastereomers were present and that C-5' and C-4' were phosphorus coupled. The phosphorus-31 nmr spectrum showed the diastereomers had been produced in a 3:2 ratio at -2.3 ppm with a diastereomeric difference of 0.5 ppm. M^+ was observed in E.I.M.S. The compound was tested against HIV (st. Mary's) and found to be active at 3 μM, about as active as **119**.

135 was dissolved in **59** and caesium fluoride added in an attempt to synthesize **156**. After leaving the reaction at room temperature three products were observed. These could not be separated by flash column chromatography, and the mixture discarded.



Since **135** was active against HIV it was of interest to prepare **157**. The reaction was conducted under standard pyridine conditions: Two equivalents of **82** were reacted with **11** at room temperature. Three products were isolated; **157**, **158**, and **159**.



The carbon-13 nmr, proton nmr, phosphorus-31 nmr data showed that these compounds were 5'-phosphorylated, NH-base phosphorylated and 5' and NH base phosphorylated respectively FABMS was also recorded.

The carbon-13 nmr spectrum of **159** was interesting. Phosphorus coupling to C-2, C-4 and C-5 were observed of 2, 4 and 18 Hz respectively. Phosphorus coupling to C-5' and C-4' of 7 and 10 Hz were also observed. Chemical shifts of the carbon atoms do not alter significantly from that of **11**. The two sets of trichloroethyl groups were observed at 95 and 94 ppm respectively (J=11 Hz). The proton-1 nmr spectrum shows there to be 8 trichloroethyl protons present confirming diphosphorylation. Phosphorus-31 nmr data showed the presence of two signals at -7 and -14 ppm consistent with literature values. EIMS gave M^+ at 899. Analytical data were also obtained.

The second compound eluted, **158** was shown to be base phosphorylated but not 5' phosphorylated. Again C-2, C-4 and C-6 show coupling; this time of; 3, 6 and 18 Hz respectively. C-5' is found as a single resonance of 62 ppm, C-4' at 82 ppm. The phosphorus-31 nmr spectrum shows a resonance at -13 ppm. The proton-1 nmr spectrum showed 4 trichloroethyl protons were present as a doublet at 4.6 ppm, J=6.2 Hz.Unfortunately this compound rapidly broke down to **11** over time.

The third compound eluted from the column proved to be the 5'-phosphorylated compound **157**. The carbon-13 nmr spectrum showed C-5' resonated at 72 ppm. the phosphorus-31 nmr spectrum displayed a single peak at -6 ppm, proton nmr data was fully consistent with structure.

The compounds were found to contain **11** by reverse phase hplc. Levels of **11** increased over time for the base phosphorylated compounds. **158** and **159** could not be sufficiently purified for biological testing, **157** was found to have slight activity (7 μM).

EXPERIMENTAL SECTION

General methods.

Commercially available Merck Kieselgel F_{254} plates were used for the tlc and the 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. Infra red spectra were recorded on a Perkin Elmer 983 infrared 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. Preparative 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. Analytical hplc was carried out on a Jones 5000 analytical hplc detected at 254 nm.

Phosphorus-31 nmr spectra of phosphorylating reagents were recorded on a Varian XL-200 spectrometer operating at 82 MHz. Phosphorus-31 nmr spectra of phosphate triesters were recorded 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. Carbon-13 nmr spectra were recorded on a Varian XL-200 spectrometer operating at 50 MHz for all compounds except nucleosides and nucleotides which were recorded on a Varian VXR-400 spectrometer operating at 100 MHz and are reported in units of δ relative to $CDCl_3$ at 77.000 ppm unless otherwise stated. Both phosphorus-31 and carbon-13 nmr spectra were proton noise decoupled and all signals were singlets unless otherwise stated. H-1 nmr spectra were recorded on a Varian XL-200 spectrometer operating at 200 MHz for all non nucleoside containing compounds and are reported in units of δ relative to internal $CHCl_3$ at 7.240 ppm unless otherwise stated. H-1 nmr spectra were recorded on a Varian XL-400 spectrometer operating at 400 MHz for all nucleoside containing compounds and are reported in units of δ relative to internal $CHCl_3$ at 7.240 ppm unless otherwise stated. The following abbreviations are used in the assignment of nmr signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dxd (doublet of doublets), dxt (doublet of triplets). 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 4 Å activated molecular sieves. Triethylamine was heated under reflux for several hours and distilled immediately prior to use. Tetrahydofuran was refluxed over potassium for several hours, distilled and stored over activated molecular sieves.

Dimethylformamide was stored over activated molecular sieves. Ethanol, propanol, 2-propanol, hexanol, 2,2,2-trichloroethanol and 2-hydroxacetamides were distilled and stored over activated molecular sieves. Acetonitrile was distilled from phosphorus pentoxide twice, followed by storing over activated molecular sieves. Methanol was dried by heating with magnesium activated with iodine, followed by distillation and storage over activated molecular sieves. Methyl iodide, acetic anhydride, propanoic anhydride, butryl chloride, 2-butryl chloride, trichloroacetyl chloride, chloroacetyl chloride, phosphoryl chloride, phosphorus trichloride and mesyl chloride were distilled prior to use. Compounds **74** and **76** were kindly donated by Pfizer Ltd.

Growth medium studies were conducted using RPM1-1640 growth medium courtesy of Dr D. Kinchington.

The plasma used in stability studies was Human plasma No P-9523 Sigma lot 17F-9412 lyophilised solids from 5 ml plasma containing 1/10 volume 3.8% trisodium citrate.

Ethyl phosphorodichloridate 51.

Ethanol (11.5 g, 14.0 ml, 0.25 mol) and triethylamine (25.3 g, 34.9 ml, 0.25 mol) dissolved in diethyl ether (500 ml) were added dropwise over a period of 6 h. to phosphoryl chloride (38.0 g, 26.8 ml, 0.25 mol) in diethyl ether (200 ml) at -78 °C. After 2 h. at this temperature the reaction mixture was allowed to warm to room temperature. Filtration under reduced pressure and concentration under reduced pressure gave the product as a colourless oil (38.3 g, 97%).

^{13}C n.m.r. δ($CDCl_3$): 68.750 (d, CH_2, J=8.8 Hz), 15.551 (d, CH_3, J=8.8 Hz).

^{31}P n.m.r. δ($CDCl_3$) : 4.609.

Analysis: $C_2H_5O_2PCl_2$ requires: Cl 43.52%. Found: Cl 43.55.

Propyl phosphorodichloridate 52.

Propanol (15.0 g, 19.1 ml, 0.25 mol) and triethylamine (25.3 g, 34.9 ml, 0.25 mol), in diethyl ether (200 ml) were added dropwise over a 2 h. period to a vigorously stirred solution of phosphoryl chloride (38.0 g, 26.8 ml, 0.25 mol) in diethyl ether (200 ml) at -78 °C. After 4 h. at this temperature the reaction mixture was allowed to warm to room temperature. Filtration under reduced pressure and concentration under reduced pressure gave the product as a colourless oil (43.1 g, 98%).

^{13}C n.m.r. δ($CDCl_3$): 73.882 (d, $CH_2\underline{C}H_2O$, J=5.0 Hz), 23.084 (d, $\underline{C}H_2CH_2O$, J=4.7 Hz), 9.876 (CH_3).

^{31}P n.m.r. δ($CDCl_3$) : 4.530.

Hexyl phosphorodichloridate 53.

Hexanol (25.5 g, 31.3 ml, 0.25 mol) and triethylamine (25.0 g, 34.4 ml, 0.25 mol) in diethyl ether (200 ml) were added dropwise over 1 h.to a vigorously stirred solution of phosphoryl chloride (38.0 g, 26.8 ml, 0.25 mol) in diethyl ether (200 ml) at -60 °C. After 2 h. at this temperature the reaction mixture was allowed to warm to room temperature. Filtration and concentration under reduced pressure afforded a colourless oil (51.2 g, 94%).

^{13}C n.m.r. δ($CDCl_3$): 72.537 (d, CH_2O, J=9.7 Hz), 31.125 ($\underline{C}H_2CH_2CH_2O$), 29.521 (d, $CH_2\underline{C}H_2CH_2O$, J=6.9 Hz), 24.925 ($CH_3CH_2\underline{C}H_2$), 22.466 ($CH_3CH_2$), 13.960 ($CH_3$).

^{31}P n.m.r. δ($CDCl_3$) : 4.832.

Dodecyl phosphorodichloridate 54.

Dodecanol (10.0 g, 0.05 mol) and triethylamine (5.43 g, 7.48 ml, 0.05 mol) in tetrahydrofuran (200 ml) were added dropwise to a solution of phosphoryl chloride (8.28 g, 5.03 ml, 0.05 mol) in tetrahydrofuran (200 ml) at -40 °C. and left stirring at this temperature for a further 8 h. The heterogeneous solution was allowed to warm to room temperature and concentrated to *ca.* one third in volume under reduced pressure. Heptane (500 ml) was added and the reaction mixture filtered and concentrated under reduced pressure to afford an oil (15.2 g, 93%).

^{13}C n.m.r. δ($CDCl_3$): 72.46 (d, CH_2O, J=9.8 Hz), 31.879 ($\underline{C}H_2CH_2CH_2O$), 29.572-22.661 (m, $(\underline{C}H_2)_8CH_2\underline{C}H_2CH_2O$), 21.096 ($CH_3$).

^{31}P n.m.r. δ($CDCl_3$) : 5.225.

E.I.M.S. m/e: 139 ($[CH_2]_9CH^+$, 6.75%), 135 ($H_3O_2PCl_2^+$, 48.75%), 125 ($(CH_2)_8CH^+$, 8.27%), 111 ($[CH_2]_7CH^+$, 24.22%), 97 ($[CH_2]_6CH^+$, 54.60%).

F.A.B. m/e: 302 (M^+, 1%), 169 ($C_{12}H_{25}^+$, 100%).

Analysis: $C_{12}H_{25}O_2PCl_2$ requires: P 10.22%. Found: P 9.91.

Methyl phosphorodichloridate 55.

Methanol (8.64 g, 10.6 ml, 0.27 mol), was added dropwise over 1 h. to vigorously stirred phosphoryl chloride (124 g, 75 ml, 0.81 mol), at 5 °C while nitrogen was bubbled through the solution. The reaction mixture was allowed to warm to room temperature and concentrated under reduced pressure (30 °C/12 mm Hg, 3 h.) with vigorous stirring to afford the product as a colourless oil (40 g, 99%).

^{13}C n.m.r. δ($CDCl_3$): 57.296 (d, J=9.2 Hz).

^{31}P n.m.r. δ($CDCl_3$) : 6.646.

E.I.M.S. m/e: 149 (M^+ ^{37}Cl, -H, 0.24%), 146.9165 (M^+ -H; $CH_2O_2PCl_2$ requires 146.9169, 0.32%), 122 (MH^+ 2x^{37}Cl, -CH_3O, 6.83%), 120 (MH^+ -CH_3O, ^{37}Cl, 6.52%), 118 (MH^+, -CH_3O, 100%), 113 (M^+ -Cl, 87.48%), 47 (PO^+, 70.99%).

2,2,2-Trichloroethylphosphorodichloridate 56.

2,2,2-Trichloroethanol (14.9 g, 9.57 ml, 0.10 mol) and triethylamine (10.1 g, 13.9 ml, 0.10 mol) in diethyl ether (250 ml) were added to a vigorously stirred solution of phosphoryl chloride (15.3 g, 9.30 ml, 0.10 mol) in diethyl ether (250 ml) at -78 °C. The reaction was kept at this temperature for a further 6 h. with stirring. After warming to room temperature, filtration and concentration under reduced pressure gave an oil that was distilled (32 °C/1 mm Hg) to give the product as an oil that solidified on standing at room temperature mp 26 °C (19.4 g, 73%).

^{31}P n.m.r. d($CDCl_3$): 3.762.

1-Methylcarboxy-1-methyl phosphorodichloridate 57.

(S)-(-)-methyl lactate (8.72 g, 8.00 ml, 0.08 mol) and triethylamine (8.46 g, 11.6 ml, 0.08 mol) in diethyl ether (200 ml) were added to a vigorously stirred solution of phosphoryl chloride (12.9 g, 7.83 ml, 0.08 mol) in diethyl ether (200 ml) at -78 °C. The reaction was kept at this temperature for a further 12 h. with stirring. After warming to room temperature, filtration and concentration under reduced pressure gave an oil that was distilled (67-70 oC/0.05 mm Hg)\ to afford the product as a colourless oil (12.57 g, 68%).

^{13}C n.m.r. δ($CDCl_3$): 168.405 (d, C(O), J=6.0 Hz), 75.309 (d, CH, J=8.8 Hz), 52.747 (CH_3O), 18.543 ($\underline{C}H_3CH$, J=6.7 Hz).

^{31}P n.m.r. d($CDCl_3$): 6.458.

^{1}H n.m.r. δ($CDCl_3$): 5.12 (1H, m, CH), 3.759 (3H, s, CH_3O), 1.629 (3H, dxd, CH_3C, J_P=1.05 Hz, J_{CH}=6.8 Hz).

E.I.M.S. m/e: 217.0039 (MH^+, $C_5H_{10}OPCl$ requires 217.0033, 0.42%), 216 (M^+, 0.12%), 161 ($C_2H_4O_2PCl_2^+$, 100%), 159 (M^+ -CH_3OCO, 2x^{37}Cl, 100%), 157 (M^+ -CH_3OCO, ^{37}Cl, 5.25%), 133 ($H_2OP(O)ClOCH_3^+$, ^{37}Cl, 10.03%), 131 ($H_2OP(O)ClOCH_2^+$, 89.51%), 113 ($H_2OP(O)ClOCH_2^+$, -H_2O, 4.81%), 99 ($C_5H_7O_2^+$, 22.54%), 87 ($CH_3OC(O)CHCH_3^+$, 6.44%), 59 ($CH_3OC(O)^+$, 55.44%).

Analysis: $C_4H_7OPCl_2$ requires C 21.74%; H 3.19; P 14.02. Found: C 21.66; H 3.19; P 15.01.

1-Methylcarboxy-1,1-dimethyl phosphorodichloridate 58.

Methyl 2-hydroxyisobutyrate (10.2 g, 10.0 ml, 0.09 mol) and triethylamine (8.76 g, 12.1 ml, 0.08 mol) dissolved in diethyl ether (250 ml) were added over 30 min. to a vigorously stirred solution of phosphoryl chloride (16.5 g, 10.0 ml, 0.11 mol) in diethyl ether (250 ml) at -40 °C. The reaction mixture was allowed to warm to room temperature and kept for 14 days with intermittent stirring. Hexane (500 ml) was added and the solution filtered and concentrated under reduced pressure to afford an oil (20.1 g, 98%).

^{13}C n.m.r. $\delta(CDCl_3)$: 171.085 (d, C(O), J=3.0 Hz). 89.021 (d, $CH_3\underline{C}CH_3$, J=5.7 Hz), 53.239 (CH_3O), 26.543 (d, CH_3, J=5.7 Hz).

^{31}P n.m.r. $\delta(CDCl_3)$: 5.558 95%; -3.087 4%; -13.574 1%.

^{1}H n.m.r. $\delta(CDCl_3)$: 3.842 (3H, s, CH_3O), 1.821 (3H, d, CH_3C, J=1.09 Hz).

E.I.M.S. m/e: 237 (MH^+ ^{37}Cl, 0.02%), 234.9698 (MH^+, $C_5H_{10}O_4PCl_2$ requires 234.9694, 0.05%), 219 (M^+, $-CH_3$, 0.05%), 201 (M^+, -Cl, 1.06%), 187 (MH_2^+, $-CH_3$ - Cl, 2.12%), 179 (M^+ $-CH_3CO_2$, 2x^{37}Cl, 6.16%), 177 (M^+ $-CH_3CO_2$, ^{37}Cl, 37.93%), 175 (M^+, $-CH_3CO_2$, 61.96%), 139 ($H_2OPOCl_2^+$ 2x^{37}Cl, 12.61%), 137 ($H_2OPOCl_2^+$ ^{37}Cl, 19.27%), ($H_2OPOCl_2^+$, 100%), 119 ($C_5H_9O_3H_2^+$, 3.35%), 117 ($C_5H_9O_3^+$, 4.21%), 101 ($C_5C_9O_2^+$, 11.46%), 59 ($CH_3CO_2^+$, 11.15%).

Analysis: $C_5H_9O_4PCl_2$ requires: C 25.55%; H 3.86; Cl 30.17; P 13.18.
Found: C 26.56%; H 3.68; Cl 29.48; P 13.06.

1-Ethylcarboxy hydroxymethane <u>59</u>.

Glycolic acid (10.5 g, 0.14 mol) was added to benzene (500 ml) and the solution refluxed for 2 h., water was removed by a Dean Stark apparatus. *p*-Toluene sulphonic acid (0.76 g, 0.004 mol) was added and left to dissolve. Ethanol (14.0 g, 17.8 ml, 0.30 mol) was added dropwise to the refluxing solution and the reaction mixture refluxed for 5 h. The cooled, combined reaction mixture and distillate were washed with saturated sodium bicarbonate solution (50 ml), and water (50 ml). The aqueous layers were back-extracted with benzene (*ca.* 200 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 25 g). Filtration followed by concentration under reduced pressure afforded an oil. The product was distilled (28 °C/12 mm Hg) to afford a colourless oil (6.8 g, 44%).

^{13}C n.m.r. $\delta(CDCl_3)$: 173.365 (C(O)), 61.470 ($C(O)\underline{C}H_2$), 60.568 ($CH_3\underline{C}H_2$), 14.098 (CH_3)

^{1}H n.m.r. $\delta(CDCl_3)$: 4.283 (2H, q, $CH_3C\underline{H}_2$, J=6.44 Hz), 4.132 (2H, s, $C(O)CH_2$), 3.156 (1H, broad s, OH), 1.209 (3H, t, $C\underline{H}_3$).

Analysis: $C_4H_8O_3$ requires: C 43.59%; H 13.01. Found: C 43.09; H 12.89.

1-Butylcarboxy hydroxymethane <u>60</u>.

Glycolic acid (10.5 g, 0.14 mol) dissolved in benzene (500 ml) was refluxed in benzene for 2 h. *p*-Toluene sulphonic acid (0.5 g, 0.003 mol) and butanol (30.7 g, 37.9 ml, 0.41 mol) were added and the reaction mixture was refluxed for a further 5 h. The reaction mixture was concentrated to *ca.* 75 ml under reduced pressure and chloroform (*ca.* 500 ml) added. The solution was washed with saturated sodium bicarbonate solution (100 ml) and water (2 x 60 ml) and the aqueous layers back extracted with chloroform (*ca.* 80 ml). The combined organic layers were separated and dried over magnesium sulphate, (*ca.* 10 g) filtered and

concentrated under reduced pressure. The product was distilled (55-60 °C/0.1 mm Hg) to afford a colourless oil (12.8 g, 70%).

^{13}C n.m.r. δ($CDCl_3$): 173.666 (C(O)), 65.098 ($\underline{C}H_2OC$), 60.634 (CH_2OH), 30.730 ($C\underline{H}_2CH_2O$), 19.163 ($CH_3C\underline{H}_2$), 13.707 (CH_3).

2-Ethylcarboxy hydroxyethane 63.

To a hot nearly refluxing solution of cupric acetate monohydrate (1.2 g) in glacial acetic acid (20 ml) was added zinc shot (10-16 mesh, 12 g). The suspension was stirred at room temperature for 30 min., the supernatant poured off with swirling and the residue washed with diethyl ether (3 x 40 ml) and benzene (40 ml). (The couple was swilled slowly for 30-60 seconds with each wash). Benzene (20 ml) was added to the couple with vigorous stirring. After 2 min. paraformaldehyde (2.4 g, 80 mmol) and ethyl bromoacetate (16.8 g, 11.2 ml, 0.10 mol) suspended in benzene (20 ml) were added to the vigorously stirred couple keeping the reaction mixture just below reflux. After the addition was completed the mixture was refluxed for 1 h., allowed to cool, acidified with 2.5 M sulphuric acid and extracted with diethyl ether (3 x 50 ml). The solution was dried over magnesium sulphate (*ca.* 6 g) and the product distilled (82-90 °C/8 mm Hg) to afford a colourless oil (3.79 g, 40%).

^{13}C n.m.r. δ($CDCl_3$): 172.835 (C(O)), 60.679 ($\underline{C}H_2OC$), 58.110 (CH_2OH), 37.072 ($\underline{C}H_2C(O)$), 14.156 (CH_3).

^{1}H n.m.r. δ($CDCl_3$): 4.176 (2H, q, CH_2OC, J=7.09 Hz), 3.868 (2H, t, $C\underline{H}_2OH$, J_{CH_2}=5.89 Hz), 3.279 (1H, broad s, OH), 2.568 (2H, t, $CH_2C(O)$, J=5.85 Hz), 1.28 (3H, t, CH_3, J=7.27 Hz).

***N*-Methyl-2-chloroacetamide 64.**

Triethylamine (127 g, 175 ml, 1.26 mol) was added dropwise to a stirred solution of methylamine hydrochloride (16.8 g, 0.25 mol) and chloroacetylchloride (28.4 g, 20.0 ml, 0.25 mol) in dichloromethane (600 ml) at -78 °C over a 30 min period. After 1 h. at this temperature and 4 h. at room temperature the reaction mixture was concentrated under reduced pressure and extracted with ethyl acetate (500 ml). Solvent removal under under reduced pressure followed by distillation (57 °C/0.05 mm Hg) gave the product as a colourless oil. (20.1 g, 20%).

^{13}C n.m.r. δ($CDCl_3$): 167.003 (C(O)), 42.185 (CH_2), 26.500 (CH_3).

^{1}H n.m.r. δ($CDCl_3$): 4.032 (2H, s, CH_2), 2.342 (3H, s, CH_3).

Analysis. C_3H_5NOCl requires: C 33.64%; H 5.61; N 13.08. Found: C 33.10; H 5.98; N 13.39.

***N,N*-Dimethyl-2-chloroacetamide 65.**

Triethylamine (127 g, 175 ml, 1.26 mol) was added dropwise to a stirred solution of dimethylamine hydrochloride (20.5 g, 0.25 mol) and chloroacetyl chloride (28.4 g, 20.0 ml, 0.25 mol) in dichloromethane (500 ml) at -78 °C over 1 h. After a further 1 h. at this temperature and 4 h. at room temperature the reaction mixture was concentrated under reduced pressure and extracted with ethyl acetate (500 ml). Solvent removal under reduced pressure followed by distillation (45 °C/0.3 mm Hg) gave the product as an colourless oil (7.17 g, 20%).

^{13}C n.m.r. δ($CDCl_3$): 166.314 (C(O)), 41.262 (C(O)$\underline{C}H_2$), 37.477 ($\underline{C}H_3NCH'_3$), 35.813 ($CH_3N\underline{C}H'_3$).

^{1}H n.m.r. δ($CDCl_3$): 4.007 (2H, s, CH_2), 2.464 (3H, s, $C\underline{H}'_3NCH_3$), 2.356 (3H, s, $CH_3NC\underline{H}'_3$).

Analysis. C_4H_8NOCl requires: C 39.67%; H 6.61; N 11.57. Found C 39.91; H 6.43; N 11.44.

***N,N*-Diethyl-2-chloroacetamide 66.**

Chloroacetyl chloride (11.2 g, 7.90 ml, 0.10 mol) in diethyl ether (200 ml) was added dropwise to diethylamine (14.6 g, 20.3 ml, 0.20 mol) in diethyl ether (100 ml), over 1 h. at -78 °C with vigorous stirring. After 20 min. at this temperature and 1 h. at room temperature the reaction was filtered under reduced pressure and the filtrate concentrated under reduced pressure and distilled (62 °C/0.01 mm Hg) to give the product as a colourless oil (9.54 g, 64%).

^{13}C n.m.r. δ($CDCl_3$): 167.741 (C(O)), 44.769 (C(O)$\underline{C}H_2$), 42.081 ($CH_2N\underline{C}H'_2$), 41.769 ($\underline{C}H_2NCH'_2$), 14.424 ($\underline{C}H_3CH_2NCH'_2CH'_3$), 12.845 ($CH_3CH_2NCH'_2\underline{C}H'_3$).

^{1}H n.m.r. δ($CDCl_3$): 4.002 (2H, s, C(O)CH_2), 3.550 (4H, q, CH_2NCH_2, J=5.87 Hz), 1.23 (6H, m, $C\underline{H}_3CH_2NCH_2C\underline{H}_3$).

E.I.M.S. m/e: 149.0604 (M^+, $C_6H_{12}NOCl$ requires 149.0607)

I.R. cm^{-1} 3632.8 w, 2976.4 s, 2230.9 m, 1642.6 vs, 1461.1 s.

Analysis. $C_6H_{12}NOCl$ requires: C 48.70%; H 8.08; N 9.36. Found: C 48.45; H 8.64; N 9.92.

***N*-Propyl-2-chloroacetamide 67.**

Chloroacetylchloride (11.2 g, 7.90 ml, 0.10 mol) in diethyl ether (200 ml) was added dropwise to propylamine (11.8 g, 16.4 ml, 0.20 mol) in diethyl ether (100 ml) over 2 h. at -78 °C with vigorous stirring. after 1 h. at this temperature and 1 h. at room temperature the reaction was filtered under reduced pressure and the filtrate concentrated under reduced pressure and distilled (56 °C/0.005 mm Hg) to afford the product as a colourless oil (9.72 g, 72%).

^{13}C n.m.r. δ($CDCl_3$): 163.358 (C(O)), 40.006 (C(O)$\underline{C}H_2$), 38.845 ($CH_2\underline{C}H_2N$), 19.877 ($\underline{C}H_2CH_2N$), 8.579 ($\underline{C}H_3$).

^{1}H n.m.r. δ($CDCl_3$): 5.567 (1H, broad s, NH), 4.062 (2H, s, C(O)CH_2), 3.60 (2H, m, $CH_2C\underline{H}_2N$), 1.83 (2H, m, $C\underline{H}_2CH_2N$), 0.957 (3H, t, CH_3).

E.I.M.S. m/e: 135.0439 (M^+ $C_5H_{10}NOCl$ requires 135.0451)

I.R. 3421.4 s, 29965.2 s, 2230.9 m, 1667.6 vs, 1531.3 vs, 1408.9 s.

Analysis. $C_5H_{10}NOCl$ requires: C 44.29%; H 7.43; N 10.33. Found: C 43.51; H 7.58; N 10.51.

***N,N*-Diisopropyl-2-chloroacetamide 68.**

Chloroacetyl chloride (42.5 g, 30.0 ml, 0.38 mol) in diethyl ether (200 ml) was added dropwise to diisopropylamine (76.2 g, 106 ml, 0.73 mol) in diethyl ether (100 ml), over 1 h. at -78 °C with vigorous stirring. After 20 min. at this temperature and 1 h. at room temperature the reaction was filtered under reduced pressure and the filtrate concentrated under reduced pressure and distilled (60 °C/0.01 mm Hg) to afford the product as a solid (36.0 g, 54%).

^{13}C n.m.r. δ($CDCl_3$): 164.512 ($\underline{C}$(O)CH_2), 45.682 ($\underline{C}$HNCH), 45.500 (CHN$\underline{C}$H), 42.491 (C(O)$\underline{C}H_2$), 20.025 ($\underline{C}H_3CH\underline{C}H_3$), 19.382 ($\underline{C}H'_3CH'\underline{C}H'_3$).

^{1}H n.m.r. δ($CDCl_3$): 4.052 (2H, s, C(O)CH_2), 3.920 (1H, m, C$\underline{H}$NCH'), 3.455 (1H, m, CHNC$\underline{H}$'), 1.355 (6H, d, $C\underline{H}_3CHC\underline{H}_3$, J_{CH}=6.11 Hz), 1.258 (6H, d, $C\underline{H}'_3CH'C\underline{H}'_3$, $J_{CH'}$=6.97 Hz).

E.I.M.S. m/e: 177.0906 (M^+ $C_8H_{16}NOCl$ requires 177.092)

I.R. cm^{-1} 3660.6 w, 3420.1 w, 2965.2 vs, 2230.9 s, 1625.9 vs, 1447.8 vs.

Analysis. $C_8H_{16}NOCl$ requires: C 54.08%; H 9.08; N 7.88. Found: C 53.23; H 9.08; N 7.79.

***N*-Methyl-2-hydroxyacetamide 69.**

64 (2.20 g, 0.02 mol) was added to a mixture of dioxan (5.0 ml) and water (5.0 ml), containing sodium hydroxide powder (4.00 g, 0.10 mol). The reaction was left stirring for 96 h. at 80 °C, then extracted with ethyl acetate (*ca.* 10x80 ml), concentrated under reduced pressure and distilled (105 °C/0.01 mm Hg) to give the product as a colourless oil (0.28 g, 18%).

^{13}C n.m.r. δ($CDCl_3$): 171.160 (C(O)), 61.714 (CH_2), 25.928 (CH_3N).

^{1}H n.m.r. δ($CDCl_3$): 4.109 (2H, s, CH_2), 2.148 (3H, s, CH_3N).

1st Preparation of *N,N*-dimethyl-2-hydroxyacetamide 70.

65 (5.00 g, 0.04 mol) was added to a mixture of dioxan (5.0 ml) and water (5.0 ml), containing sodium hydroxide powder (11.5 g, 0.29 mol). The reaction was left stirring for 96 h. at 80 °C, then extracted with ethyl acetate (*ca.* 6x80 ml), concentrated under reduced pressure and distilled (60 °C/0.01 mm Hg) to give the product as a colourless oil (2.06 g, 40%).

^{13}C n.m.r. δ($CDCl_3$): 169.834 (C(O)), 61.879 (CH_2), 36.763 ($\underline{C}H_3NCH'_3$), 35.282 ($CH_3N\underline{C}H'_3$).

^{1}H n.m.r. δ($CDCl_3$): 4.169 (2H, s, CH_2), 2.274 (3H, s, $C\underline{H}_3NCH'_3$), 2.481 (3H, s, $CH_3NC\underline{H}'_3$).

***N,N*-Diethyl-2-hydroxyacetamide 71.**

66 (5.39 g, 5.00 ml, 0.04 mol) was added to a mixture of dioxan (5 ml) and water (5 ml), containing sodium hydroxide powder (6.05 g, 0.15 mol). The reaction was left stirring for 192 h. at room temperature, then extracted with chloroform (*ca.* 3x75 ml), concentrated under reduced pressure and distilled (82 °C/0.01 mm Hg) to afford the product as a colourless oil (2.08 g, 44%).

^{13}C n.m.r. δ($CDCl_3$): 170.516 (C(O)), 59.549 ($C(O)\underline{C}H_2$), 40.292 (CH_2N), 39.887 (NCH_2), 13.682 ($\underline{C}H_3CH_2N$), 12.712 ($\underline{C}H_3CH_2N$).

^{1}H n.m.r. δ($CDCl_3$): 4.345 (1H, broad s, OH), 4.198 (2H, s, $C(O)CH_2$), 3.324 (4H, q, CH_2NCH_2, J=5.87 Hz), 1.20 (6H, m, $C\underline{H}_3CH_2NCH_2C\underline{H}_3$).

Analysis. $C_6H_{13}NO_2$ requires: C 54.94%; H 9.99; N 10.68. Found: C 51.59; H 9.76; N 9.77.

***N*-Propyl-2-hydroxyacetamide 72.**

61 (10.0 g, 0.07 mol) was added to dioxan (10 ml) and water (10 ml) containing potassium hydroxide powder (12.4 g, 0.31 mol). The reaction was left stirring at reflux for 4 days after which the cooled mixture was extracted with ethyl acetate (5x80 ml). The organic layer was separated, dried with magnesium sulphate (40 g), filtered, concentrated under reduced pressure and distilled from silicon oil (bp > 140 °C/0.002 mm Hg, *ca.* 15 ml) to afford the product as a colourless oil (2.51 g, 29%).

^{13}C n.m.r. δ($CDCl_3$): 170.574 (C(O)), 60.601 ($C(O)\underline{C}H_2$), 38.139 ($CH_2\underline{C}H_2N$), 19.844 ($\underline{C}H_2CH_2N$), 8.570 ($\underline{C}H_3CH_2$).

^{1}H n.m.r. δ($CDCl_3$): 6.890 (1H, broad s, NH), 4.066 (2H, s, $C(O)CH_2$), 3.26 (2H, m, $CH_2C\underline{H}_2N$), 1.56 (2H, m, $C\underline{H}_2CH_2N$), 0.949 (3H, t, $\underline{C}H_3CH_2$, J=7.23 Hz).

***N,N*-Diisopropyl-2-hydroxyacetamide 73.**

68 (11.1 g, 0.06 mol) was added to a mixture of dioxan (15 ml) and water (15 ml), containing sodium hydroxide powder (25.6 g, 0.64 mol). The reaction was left stirring for 192 h. at 55 °C. The cooled reaction mixture was extracted with chloroform (*ca.* 10x60 ml) dried with magnesium sulphate (*ca.* 5 g), filtered and concentrated under reduced pressure and distilled (125 °C/0.01 mm Hg) to give the product as a colourless oil that solidified on standing at room temperature, mp 23 °C (5.26 g, 52%).

^{13}C n.m.r. δ($CDCl_3$): 169.847 (C(O)), 60.298 ($\underline{C}H_2$), 46.844 (CHN), 46.088 (CHN), 20.517 ($\underline{C}H_3CH\underline{C}H_3$), 20.416 ($\underline{C}H_3CH\underline{C}H_3$).

^{1}H n.m.r. δ($CDCl_3$): 4.091 (2H, s, CH_2), 3.974 (1H, m, C$\underline{H}$N), 3.705 (1H, m, CHN), 1.419 (6H, d, $C\underline{H}_3CHC\underline{H}_3$, J_{CH}=6.17 Hz), 1.388 (6H, d, $C\underline{H}_3CHC\underline{H}_3$, J_{CH}=7.01 Hz).

2nd Preparation of *N,N*-dimethyl-2-hydroxyacetamide 70.

Sodium (7.62 g, 0.33 g atom) was added to dimethylformamide (130 g, 120 ml, 11.5 mol) in diethyl ether (400 ml) at room temperature with vigorous stirring. After refluxing for 5 h. under a constant stream of nitrogen and cooling to room temperature formaldehyde (57 g, 37%aq., 0.75 mol) was added over 1 h. keeping the reaction mixture cool with the use of an ice bath. After removal of the solvent under reduced pressure, the solution was neutralised with 1M hydrochloric acid and concentrated under reduced pressure to 1/10 of the volume. Acetonitrile (3 x 250 ml) was added and the liquor decanted. After concentration under reduced pressure and dimethylformamide removal (30 °C., 0.1 mm Hg) the product was distilled (60 °C/0.01 mm Hg) to afford a colourless oil which solidified on standing at room temperature, mp 22 °C, (11.7 g, 38%).

^{13}C n.m.r. δ($CDCl_3$)$_{TMS}$: 169.725 (C(O)), 61.878 (CH_2OH), 36.763 (CH_3N), 35.282 (CH_3N).

^{1}H n.m.r. δ($CDCl_3$): 4.187 (2H, s, CH_2), 2.250 (3H, s, $C\underline{H}_3NCH'_3$), 2.480 (3H, s, $CH_3NC\underline{H}'_3$).

5'-O-Trityl-3'-azido-3'-deoxythymidine 75.

Sodium azide (4.00 g, 0.06 mol) was added to a solution of **74** (2.42 g, 0.05 mol) in dimethylformamide (35 ml). The reaction mixture was held at 100 °C for 4 h. under nitrogen. After cooling to room temperature the product was concentrated to dryness under reduced pressure. The resultant gum was triturated with toluene (3x20 ml) and purified by column chromatography to afford an orange gum (1.48 g, 61%).

^{1}H n.m.r. δ($CDCl_3$): 9.564 (1H, broad s, NH), 7.404 - 7.062 (16H, m, phenyl H, H-6), 6.165 (1H, t, H-1', J=6.92 Hz), 5.26 (1H, m, H-3'), 4.05 (1H, m, H-4'), 3.39 (2H, m, H-5'), 2.78 (1H, m, H-2'), 2.16 (1H, m, H-2'), 1.891 (3H, d, base CH_3, J=1.19 Hz).

1st Preparation of 3'-azido-3'-deoxythymidine 10.

A solution of 75 (1.18 g, 0.002 mol) in 80% acetic acid (60 ml) was heated to 100 °C for 1 h. The solution was allowed to cool to room temperature and then concentrated to dryness under reduced pressure. The solid was dissolved in chloroform (*ca.* 80 ml) and washed with aqueous sodium bicarbonate (2 x 15 ml) and water (20 ml). After drying the organic layer over magnesium sulphate (5 g) and removal of the solvent under reduced pressure the gum was dissolved in the minimum of acetone (*ca.* 3 ml) and precipitated slowly by addition of cold petroleum ether (bp 40-60 °C), to afford a white powder mp 120-122 °C (0.42 g, 78%).

2nd Preparation of 3'-azido-3'-deoxythymidine 10.

76 (2.39 g, 0.08 mol) was dissolved in dimethylformamide (20 ml) with stirring. Sodium azide (3.00 g, 0.05 mol) was added and the heterogeneous mixture stirred at 85 °C for 4 h. under nitrogen. After cooling to room temperature the solvent was removed under reduced pressure and the gum triturated with toluene (3 x 20 ml). The solid was dissolved in chloroform (100 ml), washed with water (30 ml), dried over magnesium sulphate (*ca.* 5 g), filtered and concentrated under reduced pressure. The product was purified by flash column chromatography; (silica *ca.* 60 g) eluted with 7% methanol in chloroform. Pooling and evaporation of appropriate fractions afforded a gum that was crystallized from toluene (*ca.* 150 ml) to give the product as white crystals (1.31 g, 66%) mp 121 -122 °C.

^{13}C n.m.r. δ($CDCl_3$): 162.421 (C-2), 150.576 (C-4), 136.458 (C-6), 111.081 (C-5), 85.394 (C-1'), 84.657 (C-4'), 61.413 (C-5'), 59.931 (C-3'), 37.567 (C-2'), 12.20 (base CH_3).

^{1}H n.m.r. δ($CDCl_3$): 8.542 (1H, broad s, NH), 7.349 (1H, d, H-6, J=1.21 Hz), 6.043 (1H, t, H-1', $J_{H\text{-}2'}$=6.62 Hz), 4.39 (1H, m, H-3'), 3.84 (3H, m, H-5', H-4'), 2.62 (1H, broad s, OH), 2.55 (1H, m, H-2'), 2.38 (1H, m, H-2'), 1.904 (3H, d, base CH_3, J=1.21 Hz).

E.I.M.S. m/e: 267.1003 (M^+, $C_{10}H_{13}N_5O_4$ requires 267.0967, 2%), 206 (M^+ - N_3H -H_2O, 1%), 142 (MH^+ -thymine, 96%), 127 (thymine.H^+, 38%), 126 (thymine$^+$, 100%).

Analysis. $C_{10}H_{13}N_5O_4$ requires: C 44.94%; H 4.40; N 26.20. Found: C 44.81; H 4.63; N 25.84.

3'-O-Acetylthymidine 77.

Trityl chloride (6.60 g, 0.02 mol) was added to a solution of 78 (2.00 g, 0.008 mol) in pyridine (30 ml) and the mixture refluxed for 4 h. The solution was then cooled to room temperature and acetic anhydride (8.30 g, 7.67 ml, 0.08 mol) added with stirring and left for

24 h. The mixture was poured onto ice/water (950 ml) with vigorous stirring. The precipitate was filtered, suspended in 80% acetic acid (50 ml) and heated to 80 °C for 90 min. The reaction mixture was concentrated under reduced pressure and the residue purified by flash column chromatography (silica *ca.* 150 g) eluted 3% with methanol in chloroform. Pooling and evaporation of appropriate fractions afforded the product. This was crystallized from toluene/petroleum ether (3:2) (bp 60-80 °C) to give a white crystalline solid, mp 172-174 °C (3.30 g, 69%).

^{13}C n.m.r. δ($CDCl_3$): 170.705 ($CH_3\underline{C}(O)$), 163.720 (C-2), 150.425 (C-4), 136.242 (C-6), 111.396 (C-5), 85.937 (C-1'), 85.056 (C-1'), 74.688 (C-3'), 62.563 (C-5'), 37.163 (C-2'), 21.005 ($\underline{C}H_3C(O)$), 12.575 (base CH_3).

^{1}H n.m.r. δ($CDCl_3$): 8.885 (1H, broad s, NH), 7.445 (1H, s, H-6), 6.194 (1H, t, H-1', $J_{H\text{-}2'}$=7.4Hz) 5.285 (1H, m, H-3'), 4.02 (1H, m, H-4'), 3.86 (2H, m, H-5'), 2.588 (1H, t, OH, J=5.1 Hz), 2.32 (2H, m, H-2'), 2.036 (3H, s, $CH_3C(O)$), 1.854 (3H, s, base CH_3).

E.I.M.S. m/e: 284 (M^+, 0.01%), 126 (thymine$^+$, 27%), 99 ($C_5H_7O_2^+$, 47%), 81 ($C_5H_5O^+$, 8%), 43 (CH_3CO^+, 100%).

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

3'-<u>O</u>-Methylthymidine <u>80</u>.

Methyl iodide (1.17 g, 0.51 ml, 0.008 mol) and dried crushed potassium hydroxide (0.46 g, 0.008 mol) were added to a stirred solution of **<u>79</u>** (2.00 g, 0.005 mol) dissolved in benzene (15 ml) and dioxan (5 ml) and the reaction mixture was stirred at 50 °C for 2 h. The solution was evaporated to dryness and the residue, dissolved in methanol (ca. 1 ml), poured slowly onto stirred ice/water (*ca.* 25 ml) and the resulting white suspension extracted with chloroform (3 x 25 ml). The combined organic layers were evaporated and the residue, dissolved in 80% aqueous acetic acid, heated at 80 °C for 3 h. The acetic acid was removed by co-evaporation with benzene (3x20 ml) under reduced pressure and the product purified by flash column chromatography on silica (*ca.* 60 g) eluted with 4% methanol in chloroform. Pooling and evaporation of appropriate fractions gave a white solid (0.547 g, 48%).

^{13}C n.m.r. δ($CDCl_3$): 163.801 (C-2), 150.416 (C-4), 137.107 (C-6), 111.087 (C-5), 87.262 (C-1'), 84.961 (C-4'), 80.712 (C-3'), 62.904 (C-5'), 57.042 (CH_3O), 36.570 (C-2'), 12.540 (base CH_3).

^{1}H n.m.r. δ($CDCl_3$): 9.2 (1H, broad s, NH), 7.397 (IH, s, H-6), 6.11 (1H, m, H-1'), 4.09 (2H, m, H-3', H-4'), 3.958 (1H, dd, H-5', $J_{H\text{-}4'}$=2.47 Hz, J_{OH}=12.04 Hz), 3.807 (1H, dd, H-5", $J_{H\text{-}4'}$=2.49 Hz, J_{OH}=12.07 Hz), 3.362 (3H, s, CH_3O), 2.962 (1H, broad s, OH), 2.38 (2H, m, H-2'), 1.910 (3H, d, base CH_3, J=0.98 Hz).

E.I.M.S. m/e: 257 (MH^+, 0.06%), 256.1091 (M^+, $C_{11}H_{16}N_2O_5$ requires 256.10592, 1.34%), 131 (M^+ -thymine, 100%), 126 (thymine.H^+, 40.19%).

Analysis. $C_{11}H_{16}N_2O_5$ requires: C 51.56%; H 6.29; N 10.93. Found: C 51.15; H 6.33; N 10.46.

Bis (1-methylcarboxy-1-methyl) phosphorochloridate 83.

S(-)-methyllactate (4.36 g, 4.00 ml, 0.04 mol) and triethylamine (4.24 g, 5.84 ml, 0.04 mol) in diethyl ether (400 ml) were added dropwise to phosphoryl chloride (3.22 g, 1.96 ml, 0.02 mol) in diethyl ether (200 ml) over a period of 1 h. at -50 °C with vigorous stirring and kept at this temperature for a further 3 h. The reaction mixture was allowed to warm to room temperature and stirred for a further 14 h. Hexane (400 ml) was added and the solution filtered under reduced pressure. Concentration under reduced pressure gave an oil. Further concentration under reduced pressure (90 °C/0.1 mm Hg 8 h) afforded an orange oil (6.44 g, 53%).

^{13}C n.m.r. δ($CDCl_3$): 169.440 (d, C'(O), J=6.1 Hz), 169.325 (d, C(O), J=6.1 Hz 73.688 (d, CH' J=6.7 Hz), 73.583 (d, CH J=6.7 Hz), 52.562 (CH'_3O), 52.543 (CH_3O), 18.72 (m, CH_3, CH'_3).

^{31}P n.m.r. δ($CDCl_3$) : 4.104 , 72%; -3.191 23%; -10.3 (m, 5%).

^{1}H n.m.r. δ($CDCl_3$): 4.965 (2H, m, CH, CH'), 3.667 (3H, s, CH_3O), 3.661 (3H, s, CH'_3O), 1.507 (6H, m, $C\underline{H}_3CH$, $C\underline{H}'_3CH'$).

E.I.M.S. m/e: 291 (MH^+, ^{37}Cl, 0.05%), 289.0244 ($C_8H_{15}O_7PCl$ requires 289.0244, 1.35%), 253 (M^+ -Cl , 1.0%), 187 (M^+ -$C_4H_7O_3$, ^{37}Cl, 53.2%), 185 (M^+ -$C_4H_7O_3$, 100%), 87 ($C_4H_7O_2^+$, 42.2%), 59 ($C_2H_3O_2$, 43.1%), 47 (PO^+, 38.2%).

Propyl (1-methylcarboxy-1-methyl) phosphorochloridate 84.

(S)-(-)-Methyl lactate (4.36 g, 4.00 ml, 0.04 mol) and triethylamine (4.24 g, 5.84 ml, 0.04 mol) in diethyl ether (150 ml) were added dropwise to **52** (7.39 g, 6.66 ml, 0.04 mol) in diethyl ether (50 ml) over 1 h. with vigorous stirring at -60 °C. After leaving at this temperature for 4 h. the mixture was allowed to warm to room temperature and left stirring for a further 14 h. Hexane (250 ml) was added and the mixture filtered and concentrated under reduced pressure. The oil was then distilled (128 °C/0.01 mm Hg) to give the product as a colourless oil (6.36 g, 62%).

^{13}C n.m.r. δ($CDCl_3$): 169.590 (d, C(O), J=5.3 Hz), 169.400 (d, C(O), J=6.5 Hz), 73.121 (d, CH, J=7.7 Hz) 72.997 (d, CH, J=7.6 Hz), 71.730 (d, CH_2O, J=7.7 Hz), 52.502 (CH_3O), 52.467 (CH_3O), 22.968 (d, $\underline{C}H_2CH_2O$, J=8.1 Hz), 22.940 (d, $\underline{C}H_2CH_2O$, J=7.8 Hz), 18.846 (d, $\underline{C}H_3CH$, J=5.5Hz), 18.573 (d, $\underline{C}H_3CH$, J=6.0 Hz), 9.629 ($\underline{C}H_3CH_2$).

^{31}P n.m.r. δ($CDCl_3$) : A: 4.553, B: 3.644. (Ratio A:B 9:11).

E.I.M.S. m/e: 247 (MH^+ ^{37}Cl, 0.04%), 245.0345 (MH^+, $C_7H_{15}O_5PCl$ requires 245.0346, 0.43%), 205 (MH^+ -C_3H_6, ^{37}Cl , 2.30%), 203 (MH^+ -C_3H_6, 7.49%), 187 (M^+ -$C_2H_3O_2$, ^{37}Cl, 12.79%), 185 (M^+ -$C_2H_3O_2$, 37.69%), 167 (MH^+ -C_3H_7 -^{37}Cl, 17.32%), 145 (MH^+ -$C_4H_6O_2$, ^{37}Cl, 49.39%), 143 (MH^+- $C_4H_6O_2$, 100%), 87 ($C_4H_7O_2^+$, 35.42%), 59 ($C_2H_3O_2^+$, 22.61%).

Ist Preparation of 2,2,2-trichloroethyl (1-methylcarboxy-1-methyl) phosphorochloridate 85.

S-Methyl lactate (2.18 g, 2.00 ml, 0.02 mol) and triethylamine (2.12 g, 2.72 ml, 0.02 mol) dissolved in diethyl ether (50 ml) were added dropwise to **56** (5.59 g, 0.02 mol) dissolved in diethyl ether (*ca.* 40 ml) over 1 h. at -60 °C with vigorous stirring. After a further 7 h. at this temperature the mixture was allowed to warm to room temperature and left stirring over-night. Hexane (250 ml) was added and the mixture filtered and concentrated under reduced pressure. The product was distilled (120 °C/0.01 mm Hg) to afford a colourless oil (3.93 g, 56%).

^{13}C n.m.r. δ($CDCl_3$): 169.307 (d, C(O), A, J=5.1 Hz), 169.016 (d, C(O), B, J=5.4 Hz), 93.763 (d, CCl_3, J=5.1 Hz), 77.396 (m, $CCl_3\underline{C}H_2$), 74.202 (d, CHO, J=6.9 Hz), 59.784, (CH_3O), 18.801 (d, $\underline{C}H_3CH$, J=6.0 Hz).

^{31}P n.m.r. δ($CDCl_3$) : A: 2.511 B: 1.138, (A:B 3:4) 75%; -3.782 15%; -6.78 2%; -10.98 (m, 8%).

2nd Preparation of 2,2,2-trichloroethyl (1-methylcarboxy-1-methyl) phosphorochloridate 85.

2,2,2-Trichloroethanol (2.16 g, 1.38 ml, 0.015 mol) and triethylamine (1.46 g, 2.01 ml, 0.015 mol) in diethyl ether (150 ml) were added dropwise to a stirred solution of **57** (3.17 g, 2.20 ml, 0.015 mol) in diethyl ether (50 ml) over 1 h. at -70 °C. After leaving at this temperature for 5 h.the mixture was allowed to warm to room temperature and left stirring for another 14 h. Hexane (200 ml) was added and the mixture filtered and concentrated under reduced pressure. The product was distilled (120 °C/0.01 mm Hg) to afford a colourless oil (3.4 g, 54%).

^{31}P n.m.r. δ($CDCl_3$) : A: 2.511 B: 1.169 A:B 4:3.

E.I.M.S. m/e: 339 (MH^+ 3x^{37}Cl, 2.40%), 337 (MH^+ 2x^{37}Cl, 15.63%), 335 (MH^+ ^{37}Cl, 33.52%), 332.9019 (MH^+, $C_6H_9O_5PCl$ requires 332.9020, 26.80%), 301 (MH^+ -HCl, 2x^{37}Cl, 6.25%), 299 (MH^+ -HCl, ^{37}Cl, 19.16%), 297 (MH^+ -HCl, 20.39%), 205 (M^+ -CCl_3CH_2, ^{37}Cl, 9.05%), 203 (M^+ -CCl_3CH_2, 18.45%), 187 (MH^+ -CCl_3CH_2OH, ^{37}Cl, 14.21%), 185 (MH^+ -CCl_3CH_2OH, 48.87%), 87 ($C_4H_7O_2^+$, 100%).

1-Methylcarboxy-1-methyl (1-ethylcarboxy methyl)phosphorochloridate 86.

59 (1.50 g, 1.21 ml, 0.01 mol) and triethylamine (1.46 g, 2.01 ml, 0.14 mol) in diethyl ether (100 ml) were added dropwise to a vigorously stirred solution of 57 (3.17 g, 2.20 ml, 0.14 mol) in diethyl ether (20 ml) over a 2 h. period at -78 °C. After leaving at this temperature for a further 5 h. the reaction mixture was allowed to warm to room temperature and stirred for a further 14 h. Hexane (150 ml) was added and the heterogeneous solution filtered under reduced pressure. Concentration under reduced pressure (60 °C/0.1mm Hg, 8 h), afforded a colourless oil (2.99 g, 72%).

^{31}P n.m.r. δ($CDCl_3$) : A: 5.310, B: 4.694 A:B 4:1, 80%; -2.22 10%; -7.23 (m, 10%).

^{13}C n.m.r. δ($CDCl_3$): A: 169.375 (d, CH<u>C</u>(O), J=5.6 Hz), 166.139 (d, CH_2<u>C</u>(O), J=8.2 Hz), 73.785 (d, CH, J=6.7 Hz), 64.199 (d, CH_2OP, J=6.5 Hz), 61.693 (<u>C</u>H_2OC) 52.746 (CH_3O), 18.670 (d, <u>C</u>H_3CH, J=3.0 Hz), 13.799 (<u>C</u>H_3CH_2).

Methyl (2-ethylcarboxy ethyl) phosphorochloridate 87.

Methanol (0.32 g, 0.41 ml, 0.01mol) and triethylamine (1.10 g, 1.39 ml, 0.01 mol) in diethyl ether (200 ml) were added dropwise to a solution of 57 (2.2 g, 1.16 ml, 0.01 mol) in diethyl ether (200 ml) at -78 °C with vigorous stirring over a 4 h.period. After leaving at this temperature for a further 8 h.the reaction mixture was kept at -40 °C overnight with stirring. The heterogeneous solution was then allowed to warm to room temperature and filtered under reduced pressure. Concentration under reduced pressure afforded a pale orange oil (2.03 g, 94%).

^{13}C n.m.r. δ($CDCl_3$): 169.325 (d, C(O), J=5.4 Hz), 169.095 (d, C(O), J=6.2 Hz), 73.179 (d, CH, J=2.3 Hz), 73.051 (d, CH, J=2.0 Hz), 55.601 (d, CH_3OP, J=7.1 Hz), 52.366 (CH_3OC), 18.611 (d, CH_3CH, J=5.4 Hz), 18.392 (d, CH_3CH, J=6.2 Hz).

^{31}P n.m.r. δ($CDCl_3$) : A: 4.001, B:3.217, A:B 1:1, 95%, -4.843, 5%.

E.I.M.S. m/e: 219 (MH^+ ^{37}Cl, 0.07%), 217.005 (MH^+, $C_5H_{10}O_5PCl$ requires 217.0034, 0.39%), 185 (MH^+ -CH_3OH, 3.34%), 181 (MH^+ -HCl, 13.72%), 157 (MH^+ - $C_2H_4O_2$, 100%), 133 ($CH_3OPO_2H_2^+$ ^{37}Cl, 21.03%), 131 ($CH_3OPO_2H_2Cl^+$, 67.74%), 115 (CH_3OPOCl^+, 8.60%), 113 (CH_3POCl^+, 25.73%),

Analysis: $C_5H_{10}PO_5Cl$ requires: C 27.75%; H 4.65; P 14.30. Found: C 26.86%; H 4.56; P 14.09.

Methyl (2-ethylcarboxy ethyl) phosphorochloridate 88.

63 (1.18 g, 1.04 ml, 0.01 mol) dissolved in diethyl ether (30 ml) and triethylamine (1.02 g, 1.39 ml, 0.01 mol) dissolved in diethyl ether (30 ml) were added simultaneously but separately to a stirred solution of 55 (4.47 g, 3.00 ml, 0.03 mol) in diethyl ether (30 ml) at -

78 °C over 1 h. The reaction mixture was left at this temperature for a further 6 h. and was then allowed to warm to room temperature overnight. Hexane (*ca.* 100 ml) was added, the mixture filtered and concentrated under reduced pressure (25 °C/0.1 mm Hg) to yield a colourless oil (2.01 g, 87%).

^{13}C n.m.r. δ($CDCl_3$): 169.563 (C(O)), 64.973 (d, CH_2OP, J=6.4 Hz), 61.039 ($\underline{C}H_2OC$), 55.699 (d, CH_3O, J=11.6 Hz), 34.977 (d, $\underline{C}H_2C(O)$, J=8.2 Hz), 14.159 ($\underline{C}H_3CH_2$).

^{31}P n.m.r. δ($CDCl_3$) : 3.875, 90%, -6.619 10%.

Propyl 6-ethylcarboxy-hex-1-ene-5yl phosphorochloridate 90.

89 (0.81 g, 0.76 ml, 0.05 mol) and triethylamine (0.47 g, 0.65 ml, 0.05 mol) dissolved in diethyl ether (25 ml) were added dropwise to **52** (0.82 g, 0.74 ml, 0.06 mol) in diethyl ether (25 ml) over 1 h. at -30 °C. The reaction mixture was stirred for a further 4 h. at this temperature before being allowed to warm to room temperature. After leaving a total of 18 h. at room temperature hexane (200 ml) was added and the heterogeneous solution filtered and the filtrate concentrated under reduced pressure. Further concentration under reduced pressure (0.1mm Hg, 50 °C, 4 h) afforded the product as a colourless oil (0.89 g, 61%).

^{13}C n.m.r. δ($CDCl_3$): 169.097 (C(O)), 136.429 (CH=), 115.400 (CH_2=), 77.45 (m, CHO), 70.978 (d, $CH_2\underline{C}H_2O$, J=7.3 Hz), 60.628 ($CH_3\underline{C}H_2O$, B), 60.580 ($CH_3\underline{C}H_2O$, A), 39.874 (d, $\underline{C}H_2C(O)$, A, J=5.4 Hz), 39.766 (d, $\underline{C}H_2C(O)$, B, J=5.5 Hz), 33.976 (d, $CHCH_2\underline{C}H_2CHO$, B, J=4.3 Hz), 33.779 (d, $CHCH_2\underline{C}H_2CHO$, A, J=5.5 Hz), 28.626 ($\underline{C}H_2CH$=, A), 28.556 ($\underline{C}H_2CH$=, B), 22.987 ($CH_3\underline{C}H_2CH_2O$, B), 22.917 ($CH_3\underline{C}H_2CH_2O$, A), 13.822 ($\underline{C}H_3CH_2O$), 9.62 ($\underline{C}H_3CH_2CH_2O$).

^{31}P n.m.r. δ($CDCl_3$) : A: 2.035, B: 1.805. (A:B 1:2), 95%; -3.87 5%.

Ethyl (1-ethylcarboxy methyl) phosphorochloridate 91.

59 (2.91 g, 2.91 ml, 0.03 mol) and triethylamine (2.64 g, 3.64 ml, 0.03 mol), in diethyl ether (100 ml) were added dropwise over 1 h.to a vigorously stirred solution of **51** (4.68 g, 3.41 ml, 0.03 mol) in diethyl ether at -60 °C. After stirring for 4 h. at this temperature the reaction mixture was allowed to warm to room temperature and left stirring overnight. Filtration and concentration under reduced pressure, followed by hexane (*ca.* 50 ml) extraction afforded an oil. (4.75 g, 79%).

^{31}P n.m.r. δ($CDCl_3$) : 3.343.

Propyl (1-ethylcarboxy methyl) phosphorochloridate 92.

59 (2.91 g, 2.91 ml, 0.03 mol), and triethylamine (1.95 g, 2.64 ml, 0.03 mol) in diethyl ether (100 ml), were added dropwise to a vigorously stirred solution of **52** (4.91 g, 3.55 ml, 0.03 mol) in diethyl ether at -60 °C. After stirring for 4 h. at this temperature and overnight at

room temperature, filtration, concentration of the filtrate and hexane extraction (50 ml) followed by removal of the solvent under reduced pressure, gave the product as a pale yellow oil (4.03 g, 62%).

^{31}P n.m.r. δ($CDCl_3$) : 3.163.

Hexyl (1-ethylcarboxy methyl) phosphorochloridate 93.

59 (0.24 g, 0.18 ml, 0.02 mol) and triethylamine (0.24 g, 0.33 ml, 0.02 mol) in diethyl ether (10 ml) were added dropwise over 1 h. to a vigorously stirred solution of **53** (0.5 g, 0.37 ml, 0.02 mol) in diethyl ether (10 ml) at -60 °C. After stirring for 4 h. at this temperature and room temperature overnight, filtration under reduced pressure and concentration of the filtrate under reduced pressure gave the product as a colourless oil (0.60 g, 92%).

^{31}P n.m.r. δ($CDCl_3$) : 3.841

^{1}H n.m.r. δ($CDCl_3$): 4.725 (2H, d, $C(O)CH_2O$, J=4.1 Hz), 4.27 (4H, m, $CH_2OC(O)$, CH_2CH_2O), 1.754 (2H, quintet, $CH_2CH_2CH_2O$, J=7.25 Hz), 1.32 (9H, m, CH_3CH_2OC, $CH_3(CH_2)_2CH_2CH_2CH_2O$), 0.902 (3H, t, $CH_3CH_2CH_2$, J=7.14 Hz).

Analysis: $C_{10}H_{20}O_5PCl$ requires: C 42.19%; H 6.37; P 10.88%; Cl 12.46. Found: C 42.30; H 7.05; P 10.68; Cl 11.88.

Dodecyl (1-ethylcarboxy methyl) phosphorochloridate 94.

59 (2.00 g, 2.00 ml, 0.02 mol) and triethylamine (1.94 g, 2.64 ml, 0.02 mol) in tetrahydrofuran (50 ml) were added over a 2 h. period to a stirred solution of **54** (5.80 g, 0.02 mol) in tetrahydrofuran (50 ml) at -30 °C and kept at this temperature for a further 6 h. The reaction was then allowed to warm to ambient temperature and left stirring over-night. The heterogeneous solution was reduced to *ca.* 1/10 of the volume under reduced pressure, and heptane (*ca.* 250 ml) added. Filtration and concentration of the filtrate under reduced pressure afforded the product as a pale orange oil (7.11 g, 96%).

^{13}C n.m.r. δ($CDCl_3$): 166.393 (d, C(O), J=8.1Hz), 70.575 (d, CH_2CH_2O, J=7.7Hz), 63.899 (d, $C(O)CH_2OP$, J=5.9 Hz), 61.841 (CH_2OC), 31.806 ($CH_2CH_2CH_2O$), 29.512-22.586 (m, $[CH_2]_8CH_2CH_2CH_2O$), 22.586 ($CH_3CH_2CH_2$), 14.010 (CH_3CH_2O).

^{31}P n.m.r. δ($CDCl_3$) : 3.577.

E.I.M.S. m/e: 373 (MH^+ ^{37}Cl, 1.47%), 371.1754 (MH^+ $C_{16}H_{32}O_5PCl$ requires 371.1754, 5.00%), 335 (M^+, -Cl, 0.92%), 271 (M^+ -$CH_3[CH_2]_6^+$, 8.14%), 205 (MH_2^+ -$CH_3[CH_2]_{11}$, ^{37}Cl, 35.43%), 203 (MH_2^+ -$CH_3[CH_2]_{11}$, 100%), 111 ($[CH_2]_7CH^+$, 4.33%), 83 ($[CH_2]_7CH^+$, 10.14%), 69 ($[CH_2]_3CH^+$, 18.40%), 55 ($[CH_2]_2CH^+$, 33.28%), 41 (CH_2CH^+, 47.13%).

Attempted preparation of methyl (1-methylcarboxy-1,1-dimethyl)

phosphorodichloridate 95.

Methyl 2-hydroxyisobutyrate (10.2 g, 10.0 ml, 0.09 mol) and triethylamine (8.76 g, 12.07 ml, 0.09 mol) dissolved in diethyl ether (250 ml) were added over 2 h. to a vigorously stirred solution of **55** (12.9 g, 8.67 ml, 0.09 mol) in diethyl ether (250 ml) at -78 °C. After leaving for 1 h. the reaction was allowed to warm to room temperature and left stirring for 48 h. Concentrating the solution under reduced pressure afforded a colourless oil (10.5 g).

^{13}C n.m.r. δ($CDCl_3$): 177.795 (C(O)), 72.035 ($CH_3\underline{C}CH_3$), 52.668 (CH_3O), 27.256 ($\underline{C}H_3C\underline{C}H_3$).

Methyl (methyl carboxy-1-methyl-1-ethyl) phosphorochloridate 95.

58 (2.75 g, 2.00 ml, 0.09 mol) was dissolved in diethyl ether (*ca.* 50 ml). Methanol was added (0.38 g, 0.48 ml, 0.01mol), followed by triethylamine (1.19 g, 1.64 ml, 0.01 mol) while the solution was vigorously stirred at room temperature. After leaving the solution overnight diethyl ether (*ca.* 100 ml) was added and the mixture filtered and concentrated under reduced pressure to leave an orange oil (2.15 g, 79%).

^{13}C n.m.r. δ($CDCl_3$): 171.627 (d, C(O), J=4.2 Hz), 84.760 (d, $CH_3\underline{C}O$, J=8.0 Hz), 55.401 (d, CH_3OP, J=7.2 Hz), 53.102 ($\underline{C}H_3OC$), 26.292 ($\underline{C}H_3C$, J=6.3 Hz), 25.782 ($\underline{C}H_3C$, J=4.2Hz).

^{31}P n.m.r. δ($CDCl_3$) : 0.757, 70%; -4.361, 10%; -4.723, 10%, -10 (m, 10%).

^{1}H n.m.r. δ($CDCl_3$): 3.922 (3H, s, J=14.0 Hz, CH_3OP), 3.821 (3H, s, CH_3OC), 1.796 (3H, s, CH_3C, A), 1.742 (3H, s, CH_3C, B).

E.I.M.S. m/e: 233 (MH^+ ^{37}Cl, 3.2%), 231.01889 (MH^+, $C_6H_{13}O_5PCl$ requires 231.01892, 11.3%), 217 (M^+ -CH_3, 49.2%), 199 (M^+ -CH_3O, 77.2%), 195 (M^+ -Cl, 28.9%), 171 (M^+ -$C_2H_3O_2$, 64.7%), 133 ($CH_3OPO_2H_2Cl^+$ ^{37}Cl, 38.1%), 131 ($CH_3OPO_2H_2Cl^+$, 100%), 113 ($CH_3O_2PCl^+$, 11.2%), 101 ($C_5H_9O_2^+$, 65.3%), 59 ($C_2H_3O_2^+$, 33.2%), 47 (PO^+, 46.2%).

Analysis: $C_6H_{12}PC1O_5$ requires: C 31.25%; H 5.25; P 13.43; Cl 15.38. Found: C 32.12; H 5.73; Cl 15.39; P 12.91.

Attempted preparation of propyl (1-Methylcarboxy-1,1-dimethyl) phosphorochloride 96.

Methyl 2-hydroxy isobutyrate (0.75 g, 0.70 ml, 0.06 mol) and triethylamine (0.64 g, 0.90 ml, 0.06 mol) dissolved in diethyl ether (100 ml) were added dropwise to a stirred solution of **52** (1.11 g, 1.00 ml, 0.06 mol) in diethyl ether (50 ml) at -40 °C. After addition the reaction was allowed to warm to room temperature and left stirring for 18 h. Concentration under reduced pressure gave a colourless oil (1.67 g).

^{13}C n.m.r. δ($CDCl_3$): 177.795 (C(O)), 73.950 (d, CH_2O, J=9.7 Hz), 72.035 ($CH_3\underline{C}CH_3$), 52.668 (CH_3O), 27.256 ($\underline{C}H_3C\underline{C}H_3$), 23.093 (d, $\underline{C}H_2CH_2O$, J=8.9 Hz), 9.88 ($\underline{C}H_3CH_2$).

^{31}P n.m.r. δ($CDCl_3$) : 4.865.

Propyl Z-ethylcarboxy prop-2-enyl phosphorochloridate 97.

Sodium hydride (0.88 g, 0.02 mol, 60% dispersion in oil) was washed with tetrahydrofuran (3x3 ml). Tetrahydrofuran (15 ml) was added, followed by ethyl acetoacetate (2.73 g, 2.68 ml, 0.021 mol) over a period of 5 min. at -78 °C. The reaction mixture was left stirring for 1 h. at -78 °C under nitrogen, warmed to room temperature and added dropwise to **52** (3.52 g, 3.14 ml, 0.02 mol) in tetrahydrofuran (10 ml) at -78 °C. The reaction mixture was stirred for 6 h. at this temperature before being allowed to warm to room temperature. The solvent was removed under reduced pressure (50 °C/0.1 mm Hg, 8 h) to afford an extremely viscous suspension.

^{31}P n.m.r. δ($CDCl_3$) : -5.607, 50%; -18.202, 50%.

1-Methylcarboxy-1,1-dimethyl
(t-ethylcarboxy prop-2-enyl) phosphorochloridate 99.

Ethyl acetoacetate (1.30 g, 1.27 ml, 0.01 mol) was dissolved in dichloromethane (25 ml). Tetrabutylammonium hydrogen sulphate (0.34 g, 0.002 mol) was dissolved in de-ionised water (10 ml). The two solutions were combined and stirred vigorously for 30 min. **58** (2.34 g, 0.01 mol) was added dropwise whilst the two-phase mixture was gently stirred and left for 20 min. The organic layer was separated from the aqueous layer and dried over magnesium sulphate (10 g) then concentrated to dryness.

^{31}P n.m.r. δ($CDCl_3$) : -6.98, 75%, -12.02, 12%; -15 -> -30, 13%.

Bis (2-methyl-2-propen-1-yl) phosphorochloridate 100.

2-Methyl-2-propen-1-ol (7.21 g, 8.41 ml, 0.11 mol) and triethylamine (10.1 g, 13.9 ml, 0.11 mol) in diethyl ether (200 ml) were added to a stirred solution of phosphoryl chloride (7.65 g, 4.65 ml, 0.05 mol) in diethyl ether (100 ml) at -78 °C over a period of 1 h. The reaction was kept at this temperature for 3 h. then allowed to warm to room temperature overnight. Filtration and concentration under reduced pressure afforded the product as a colourless oil (22.5 g, 98%).

^{13}C n.m.r. δ($CDCl_3$): 138.237, (d, $CH_3\underline{C}$=, J=8.0 Hz), 114.428 (CH_2=), 72.239 (d, CH_2O, J=7.8 Hz), 18.436 ($\underline{C}H_3C$=).

^{31}P n.m.r. δ($CDCl_3$) : 2.810.

Bis (2-Propen-1-yl) phosphorochloridate 101.

2-Propen-1-ol (5.12 g, 6.00 ml, 0.09 mol) and triethylamine (8.93 g, 12.3 ml, 0.88 mol) in diethyl ether (100 ml) were added to phosphoryl chloride (5.40 g, 3.3 ml, 0.037 mol) in diethyl ether (100 ml) at room temperature and left for 5 h.stirring vigorously. The reaction mixture was filtered and concentrated to a small volume under reduced pressure and hexane (400 ml) added. After standing for 1 h. the solution was decanted, filtered and concentrated under reduced pressure to afford an orange oil. (6.32 g, 86%).

^{31}P n.m.r. δ(CDCl$_3$) : 2.874 50%; -2.566 50%.

Ethyl (*N,N*-diethyl acetamidyl) phosphorochloridate 102.

71 (1.00 g, 0.08 mol) and triethylamine (0.93 g, 1.28 ml, 0.09 mol) in diethyl ether (50 ml) were added dropwise to a stirred solution of **51** (1.49 g, 1.09 ml, 0.09 mol) in diethyl ether (50 ml) over a 3 h. period at -40 °C. The reaction was left at this temperature for a further 6 h. and then allowed to warm to ambient temperature overnight. Filtration under reduced pressure and concentration under reduced pressure yielded a colourless oil. (0.57 g, 29%).

^{31}P n.m.r. δ(CDCl$_3$) : 3.83 (10%), -2.82 (75%), -4.69 (15%). Further concentration under reduced pressure afforded an oil (1.54 g, 85%).

^{31}P n.m.r. δ(CDCl$_3$) : -2.86 (80%), -4.97 (15%), -9.90 (m, 5%).

^{13}C n.m.r. δ(CDCl$_3$): 165.848 (d, C(O), J=6.1 Hz), 64.603 (d, C(O)$\underline{C}H_2$, J=5.9 Hz), 64.592 (d, $CH_3\underline{C}H_2OP$, J=6.0 Hz), 41.114 (CH_2N), 40.591 (CH'_2N), 16.053 (d, CH_3CH_2, J=8.9 Hz). 14.109 ($\underline{C}H_3CH_2N$), 12.837 ($\underline{C}H'_3CH'_2N$).

3'-O-Acetylthymidine-5'-(ethyl 1-ethylcarboxy methyl) phosphate 109.

To a stirred solution of 77 (0.61 g, 0.002 mol), in pyridine (10 ml) was added **91** (1.38 g, 1.27 ml, 0.06 mol). After leaving for 96 h.the reaction mixture was concentrated to dryness and triturated with toluene (*ca.* 3x10 ml). Purification was achieved by flash column chromatography on silica (*ca.* 100 g) eluted with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions afforded a colourless gum (0.22 g, 24%).

^{13}C n.m.r. δ(CDCl$_3$): 170.389 ($CH_3\underline{C}(O)$, A), 170.368 ($CH_3\underline{C}(O)$, B), 167.862 (d, $\underline{C}(O)CH_2$, J=4.3Hz, B), 167.608 (d, $\underline{C}(O)CH_2$, J=4.7 Hz, A), 163.927 (C-2, A), 163.926 (C-2, B) 150.666 (C-4, A), 150.650 (C-4, B), 135.026 (C-6, A), 134.926 (C-6, B), 111.612 (C-5, A), 111.562 (C-5, B), 84.317 (C-1', B), 84.242 (C-1', A), 82.637 (d, C-4', J=8.0 Hz, B), 82.554 (d, C-4', J=8.2 Hz, A), 74.484 (C-3', B), 74.464 (C-3', A), 67.257 (d, C-5', J=5.2 Hz, B), 67.190 (d, C-5', J=5.7 Hz, A), 64.758 (d, C(O)$\underline{C}H_2OP$, J=5.3 Hz, B), 64.699 (d, C(O)$\underline{C}H_2O$, J=6.0 Hz, A), 63.850 (d, $CH_3\underline{C}H_2OP$, J=5.3 Hz, B), 63.660 (d, $CH_3\underline{C}H_2OP$, J=7.0 Hz, A), 61.648 ($CH_3\underline{C}H_2OC$, A), 61.590 ($CH_3\underline{C}H_2OC$, B), 36.993 (C-2', B), 36.931 (C-2', A), 20.776 ($\underline{C}H_3C(O)$), 15.891 (m, $\underline{C}H_3CH_2OP$), 13.929 ($\underline{C}H_3CH_2OC$).

^{31}P n.m.r. δ(CDCl$_3$) : A: 1.723; B: 1.548 A:B, 2:1.

^{1}H n.m.r. δ($CDCl_3$): 9.897 (1H, s, NH), 7.476, 7.458 (1H, d, H-6, J=1.22 Hz, J=1.17 Hz), 6.36 (1H, m, H-1'), 5.27 (1H, m, H-3'), 4.57 (2H, m, $C(O)CH_2O$), 4.36 (2H, m, H-5'), 4.229 - 4.005 (5H, m, CH_3CH_2OC, H-4', CH_3CH_2OP), 2.35 (1H, m, H-2'), 2.16 (1H, m, H-2'), 2.052 (1H, s, $CH_3C(O)$), 1.869 1.872 (3H, d, base CH_3, J=1.09 Hz, J=1.13 Hz), 1.37 (3H, m, CH_3CH_2OP), 1.218 (3H, t, CH_3CH_2OC, J=7.68 Hz).

3'-O-Acetylthymidine-5'-(propyl 1-ethylcarboxy methyl) phosphate 110.

To 77 (0.54 g, 0.002 mol) in pyridine (10 ml) was added 92 (1.46 g, 1.42 ml, 0.006 mol), with stirring at room temperature. After leaving for 70 h.under these conditions the mixture was concentrated under reduced pressure, triturated with toluene (*ca.* 3x10 ml) and purified by flash column chromatography (silica *ca.* 150 g) eluted with 0.5% methanol in chloroform. Pooling and evaporation of appropriate fractions afforded the product as a colourless gum (0.31 g, 32% yield).

The diastereomers were separated by hplc.

'Fast isomer' (0.016 g, 16%).

^{13}C n.m.r. δ($CDCl_3$): 170.389 ($CH_3C(O)$), 167.736 (d, $C(O)CH_2$, J=4.8 Hz), 163.554 (C-2), 150.450 (C-4), 135,154 (C-6), 111.754 (C-5), 84.394 (C-1'), 82.794 (C-4', J=8.4 Hz), 74.628 (C-3'), 70.091 (d, CH_2CH_2O, J=6.1 Hz), 67.321 (d, C-5', J=5.7Hz), 63.666 (d, $C(O)CH_2OP$, J=5.0 Hz), 61.734 (CH_3CH_2OC), 37.089 (C-2'), 23.572 (d, CH_2CH_2OP, J=7.1 Hz), 20.896 ($CH_3C(O)$), 14.073 (CH_3CH_2OC), 12.329 (base CH_3), 9.868 ($CH_3CH_2CH_2O$).

^{31}P n.m.r. δ($CDCl_3$) : 0.776

^{1}H n.m.r. δ($CDCl_3$): 8.836 (1H, s, NH), 7.483 (1H, d, H-6, J=1.26 Hz), 6.358 (CALEB1H, q, H-1'), 5.257 (1H, d, H-3' J=5.98 Hz), 4.56 (2H, m, $C(O)CH_2O$), 4.34 (2H, m, H-5'), 4.170 (2H, q, CH_3CH_2OC, J=6.56 Hz), 4.12 (1H, m, H-4'), 4.05 (2H, m, CH_2CH_2O), 2.34 (1H, m, H-2'), 2.22 (1H, m, H-2'), 2.091 (1H, s, $CH_3C(O)$), 1.877 (3H, d, base CH_3, J=1.01 Hz), 1.674 (2H, sextet, $CH_3CH_2CH_2O$, J=6.77 Hz), 1.218 (3H,t, CH_3CH_2OC, J=7.32 Hz), 0.901 (3H, t, CH_3CH_2OP, J=7.36 Hz).

F.A.B. m/e: 493 (MH^+, 0.05%), 492 (M^+, 0.01%), 102 ($C_4H_6O_3^+$, 100%).

'Slow isomer' (0.015 g 16%).

^{13}C n.m.r. δ($CDCl_3$): 170.409 ($CH_3C(O)$), 167.719 (d, $C(O)CH_2O$, J=4.6 Hz), 163.715 (C-2), 150.378 (C-4), 135.249 (C-6), 111.687 (C-5), 84.561 (C-1'), 82.833 (d, C-4', J=8.3 Hz), 74.589 (C-3'), 70.187 (d, CH_2CH_2O, J=5.9 Hz), 67.374 (d, C-5' J=6.0 Hz), 63.753 (d, $C(O)CH_2$, J=5.4 Hz), 61.753 (CH_2OC), 37.198 (C-2'), 23.540 (d, CH_2CH_2O, J=6.8 Hz), 20.896 ($CH_3C(O)$), 14.076 (CH_3CH_2O), 12.294 (base CH_3), 9.866 ($CH_3CH_2CH_2$).

^{31}P n.m.r. δ($CDCl_3$) : 0.362

^{1}H n.m.r. δ($CDCl_3$): 8.879 (1H, s, NH), 7.463 (1H, d, H-6, J=1.22 Hz), 6.357 (1H, q, H-1', J=6.78 Hz), 5.282 (1H, d, H-3', $J_{H\text{-}4'}$=5.02 Hz), 4.56 (2H, m, $C(O)CH_2O$), 4.34 (2H, m, H-5'), 4.183 (2H, q, $CH_3C\underline{H}_2OC$, J=6.71 Hz), 4.12 (1H, m, H-4'), 4.05 (2H, m, $CH_2C\underline{H}_2OP$), 2.34 (1H, m, H-2'), 2.22(1H, m, H-2'), 2.091 (1H, s, $CH_3C(O)$), 1.878 (3H, d, base CH_3, J=0.98 Hz), 1.668 (2H, sextet, $CH_3C\underline{H}_2CH_2O$, J=6.69 Hz), 1.224 (3H,t, $C\underline{H}_3CH_2OC$, J=7.30 Hz), 0.895 (3H, t, $C\underline{H}_3CH_2OP$, J=7.36 Hz).

F.A.B. m/e: 493 (MH^+, 0.01%), 492 (M^+, 0.01%), 73 ($C_2H_5OCO^+$, 100%).

3'-O-Acetylthymidine-5'-(hexyl 1-ethylcarboxy methyl) phosphate <u>111</u>.

To <u>77</u> (0.67 g, 0.002 mol) in pyridine (10 ml), was added <u>93</u> (3.2 g, 0.012 mol). The reaction was left stirring for 70 h.under nitrogen at room temperature. Concentration under reduced pressure, trituration with toluene (*ca.* 3x10 ml) followed by purification by flash column chromatography (silica *ca.* 60 g) eluted with 0.55% methanol in chloroform separated the product into its 2 diastereomeric forms, envisaged by multiple development tlc. Pooling and evaporation of appropriate fractions gave the isomer with the higher R_f free of the other isomer by reverse phase hplc. The isomer with the lower R_f was contaminated with some of the other isomer analysed by hplc; 3.23% fast isomer. Combined yield. 58%.

'Fast Isomer' 0.36 g.

^{13}C n.m.r. δ($CDCl_3$): 170.470 ($CH_3\underline{C}(O)$), 167.69 (d, $\underline{C}(O)CH_2$, J=4.9 Hz), 163.735 (C-2), 150.564 (C-4), 135.041 (C-6), 111.778 (C-5), 84.495 (C-1'), 82.854 (d, C-4', J=8.2 Hz), 74.629 (C-3'), 68.882 (d, $CH_2\underline{C}H_2OP$, J=6.1 Hz), 67.340 (d, C-5', J=5.2 Hz), 63.702 ($C(O)\underline{C}H_2OP$), 61.731 ($CH_3\underline{C}H_2OC$), 37.175 (C-2'), 30.173 (d, $\underline{C}H_2CH_2OP$ J=6.1 Hz), 29.688 ($\underline{C}H_2CH_2CH_2O$), 25.605 ($CH_3CH_2\underline{C}H_2$), 22.464 ($CH_3\underline{C}H_2CH_2$) , 20.887 ($\underline{C}H_3C(O)$), 14.099 ($\underline{C}H_3CH_2OC$), 13.928 ($\underline{C}H_3CH_2CH_2$), 12.232 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : 1.782

^{1}H n.m.r. δ($CDCl_3$): 9.009 (1H, broad s, NH), 7.502 (1H, d, H-6, J=1.30 Hz), 6.42 (1H, m, H-1'), 5.31 (1H, m, H-3'), 4.62 (2H, m, $C(O)CH_2O$), 4.34 (2H, m, H-5'), 4.23 (2H, m, $CH_3C\underline{H}_2OC$), 4.18 (1H, m, H-4'), 4.15 (2H, m, $CH_2C\underline{H}_2OP$), 2.40 (1H, m, H-2'), 2.38 (1H, m, H-2'), 2.07 ($CH_3C(O)$), 1.932 (3H, s, base CH_3), 1.69 (2H, q, $CH_2C\underline{H}_2CH_2O$, J=6.65 Hz) 1.25 (12H, m, $C\underline{H}_3CH_2OC$, $C\underline{H}_3(C\underline{H}_2)_3$), 0.928 (3H, t, $C\underline{H}_3CH_2CH_2$, J=7.67 Hz).

F.A.B: 536 (MH_2^+, 0.5%), 535 (MH^+, 6%), 433 (MH_2^+ -C_4H_7O, 3%), 267 ($ACT.H^+$ -H_2O 5%), 147 ($C_6H_{12}O_2P^+$, 100%), 81 ($C_5H_5O^+$, 45%).

Analysis: $C_{22}H_{35}N_2O_{11}P$ requires: C 49.44%; H 6.60; N 5.24; P 5.79. Found: C 50.88%; H 7.80; N 3.52; P 3.81.

'Slow isomer' 0.30 g.

^{13}C n.m.r. δ(CDCl$_3$): 170.518 ($CH_3\underline{C}(O)$), 167.708 (d, $\underline{C}(O)CH_2$, J=4.9 Hz), 163.835 (C-2), 150.624 (C-4), 135.081 (C-6), 111.748 (C-5), 84.361 (C-1'), 82.757 (d, C-4', J=8.2 Hz), 74.624 (C-3'), 68.240 (d, $CH_2\underline{C}H_2OP$, J=6.1 Hz), 67.331 (d, C-5', J=5.2 Hz), 63.733 ($C(O)\underline{C}H_2OP$), 61.726 ($CH_3\underline{C}H_2OC$), 37.124 (C-2'), 30.173 (d, $\underline{C}H_2CH_2OP$ J=6.1 Hz), 29.633 ($\underline{C}H_2CH_2CH_2O$), 25.835 ($CH_3CH_2\underline{C}H_2$), 22.962 ($CH_3\underline{C}H_2CH_2$) , 20.826 ($\underline{C}H_3C(O)$), 14.048 ($\underline{C}H_3CH_2OC$), 13.238 ($\underline{C}H_3CH_2CH_2$), 12.312 (base CH_3).

^{31}P n.m.r. δ(CDCl$_3$): 1.625

^{1}H n.m.r. d(CDCl$_3$): 9.282 (1H, broad s, NH), 7.535 (1H, d, H-6, J=1.30 Hz), 6.32 (1H, m, H-1'), 5.323 (1H, m, H-3'), 4.61 (2H, m, $C(O)CH_2O$), 4.34 (2H, m, H-5'), 4.23 (2H, m, $CH_3C\underline{H}_2OC$), 4.17 (1H, m, H-4'), 4.15 (2H, m, $CH_2C\underline{H}_2OP$), 2.38 (1H, m, H-2'), 2.26 (1H, m, H-2'), 2.07 ($CH_3C(O)$), 1.902 (3H, d, base CH_3, J=1.16 Hz), 1.66 (2H, m, $CH_2C\underline{H}_2CH_2O$) 1.24 (12H, m, $C\underline{H}_3CH_2OC$, $C\underline{H}_3(C\underline{H}_2)_3$), 0.902 (3H, t, $C\underline{H}_3CH_2CH_2$, J=7.67 Hz).

F.A.B: 536 (MH_2^+, 1.0%), 535 (MH^+, 8%), 433MH_2^+ -C_4H_7O, 9%), 267 (ACT.H^+ -H_2O 6%), 149 ($C_6H_{14}O_2P^+$, 100%), 81 ($C_5H_5O^+$, 78%).

Analysis: $C_{22}H_{35}N_2O_{11}P$ requires: C 49.44%; H 6.60; N 5.24; P 5.79. Found: C 49.70%; H 6.91; N 4.79; P 5.01.

3'-O-Acetylthymidine-5'-(propyl Z-ethylcarboxy prop-2-enyl) phosphate <u>112</u>.

<u>99</u> was added to <u>77</u> (0.11 g, 0.4 mmol) dissolved in tetrahydrofuran (5 ml) and *N*-methylimidazole (0.33 g, 0.32 ml, 0.004 mol). The reaction mixture was allowed to stir for 48 h. at room temperature before concentrating to dryness under reduced pressure. The resultant oily solid was suspended in chloroform (25 ml), washed with saturated sodium bicarbonate (50 ml) then water (50 ml). The aqueous layers were back-extracted with chloroform (*ca.* 20 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 5 g). The solution was concentrated under reduced pressure to small volume (*ca.* 1 ml) and added over a minute to stirred petroleum ether (bp 30-40 °C) the resultant suspension left overnight at -20 °C. The precipitate was then dissolved in the minimum of chloroform and purified by flash column chromatography (silica *ca.* 40 g) eluted with chloroform. Pooling and evaporation of appropriate fractions afforded the product as a colourless gum (0.13 g, 63%).

^{13}C n.m.r. δ(CDCl$_3$): 170.472 ($CH_3\underline{C}(O)$), 170.420 ($CH_3\underline{C}(O)$), 166.132 ($\underline{C}(O)CH$), 166.002 ($\underline{C}(O)CH$), 163.371 (C-2), 157.32 (m, $CH_3\underline{C}$=), 150.325 (C-4), 135.295 (C-6), 135.176 (C-6), 111.725 (C-5), 111.683 (C-5), 106.10 (m, CH=), 84.569 (C-1'), 84.426 (C-1'), 82.751 (d, C-4', J=8.5 Hz.), 82.720 (d, C-4', J=8.7 Hz), 74.656 (C-3'), 74.563 (C-3'), 70.753 (d, $CH_3CH_2\underline{C}H_2O$, J=6.6 Hz), 70.654 (d, $CH_3CH_2\underline{C}H_2O$,

J=6.3 Hz), 67.779 (d, C-5', J=5.5 Hz.), 59.922 ($CH_3\underline{C}H_2OC(O)$), 37.127 (C-2'), 37.082 (C-2'), 23.594 (d, $\underline{C}H_3C$=, J=7.1 Hz), 21.864 (d, $CH_3\underline{C}H_2CH_2OP$, J=7.1 Hz), 21.826 ($\underline{C}H_3C(O)$), 20.948 ($\underline{C}H_3C(O)$), 14.214 ($\underline{C}H_3CH_2OC(O)$), 12.361 (base CH_3), 9.935 ($\underline{C}H_3CH_2CH_2O$).

^{31}P n.m.r. δ($CDCl_3$) : A: -8.261, B: -8.319, A:B 1:1.

^{1}H n.m.r. δ($CDCl_3$): 8.544 (1H, s, NH base), 7.478 (1H, m, H-6), 1H. 6.34 (1H, m, H-1'), 5.313 (1H, d, CH=, J=5.55 Hz), 5.27 (1H, m, H-3'), 4.44 (2H, m, H-5'), 4.416-4.005 (5H, m, H-4', $CH_3CH_2C\underline{H}_2O$, $CH_3C\underline{H}_2OC$), 2.33 (1H, m, H-2'), 2.19 (1H, m, H-2'), 2.113 (3H, s, CH_3C=). 2.045 (3H, s, CH_3), 1.863 (3H, s, base CH_3), 1.69 (2H, m, $CH_3CH_2CH_2OP$), 1.21 (3H, m, $C\underline{H}_3CH_2OC$) 0.94 (3H, m, $C\underline{H}_3CH_2CH_2O$).

E.I.M.S. m/e: 519 (MH^+, 4.52%), 267 (ACT.H^+ -H2O, 6.67%), 193 ($C_6H_9O_3O_2H^+$, 3.01%), 127 (thymine.H_2^+, 1.14%), 99 ($C_5H_7O_2^+$, 0.89%), 81 ($C_5H_5O^+$, 100%).

3'-Azido-3'-deoxythymidine-5'-(propyl-1-ethylcarboxymethyl)phosphate, <u>113</u>.

<u>92</u> (1.10 g, 0.004 mol) was added to **<u>10</u>** (0.2 g, 0.75 mmol) in pyridine (10 ml) with stirring. After leaving for 14 days water (*ca.* 2 ml) was added and the reaction mixture concentrated to dryness and triturated with toluene (3 x 20 ml). Purification by flash column chromatography (silica *ca.* 60 g) eluted with 3% methanol in chloroform gave a colourless gum (0.13 g, 37%).

^{13}C n.m.r. δ($CDCl_3$): 167.785 (d, $\underline{C}(O)CH_2$, J=4.4 Hz), 167.716 (d, $\underline{C}(O)CH_2$, J=4.8 Hz), 163.913 (C-2), 150.392 (C-4), 135.191 (C-6), 135.073 (C-6), 111.395 (C-5) 111.352 (C-5), 84.516 (C-1'), 84.469 (C-1'), 82.144 (d, C-4', J=8.3 Hz), 70.128 (d, $CH_2\underline{C}H_2O$, J=6.6 Hz), 70.062 (d, $CH_2\underline{C}H_2O$, J=6.5 Hz), 66.573 (d, C-5' J=5.8 Hz), 66.440 (d, C-5', J=5.3 Hz), 63.717 (d, $C(O)\underline{C}H_2$, J=4.3 Hz), 63.639 (d, $C(O)\underline{C}H_2$, J=5.1 Hz), 61.683 ($\underline{C}H_2OC$), 60.110 (C-3'), 60.051 (C-3'), 37.395 (C-2'), 37.326 (C-2'), 23.415 (d, $CH_3\underline{C}H_2CH_2$, J=7.0 Hz), 13.971 ($\underline{C}H_3CH_2O$), 12.290 (base CH_3), 12.263 (base CH_3), 9.767 ($\underline{C}H_3CH_2CH_2$).

^{31}P n.m.r. δ($CDCl_3$) : A: 0.766, B: 0.362 A:B, 1:1.

^{1}H n.m.r. δ($CDCl_3$): 9.732 (1H, s, NH), 7.394, 7.379 (1H, d, H-6, J=1.26 Hz, J=1.19 Hz), 6.21 (1H, m, H-1'), 4.58 (2H, m, $C(O)CH_2$) 4.39 (1H, m, H-3'), 4.34 (2H, m, H-5'), 4.18 (2H, m, $CH_2C\underline{H}_2O$), 4.04 (2H, m, $CH_2OC(O)$), 3.98 (1H, m, H-4'), 2.43 (2H, m, H-2'), 1.863, 1.855 (3H, d, base CH_3, J=1.07 Hz, J=1.07 Hz), 1.65 (2H, m, $CH_3C\underline{H}_2CH_2$), 1.226 (3H, t, $\underline{C}H_3CH_2O$, J=7.17 Hz) 0.899 (3H, t, $C\underline{H}_3CH_2CH_2$, J=7.36 Hz).

E.I.M.S. m/e: 476 (MH^+ 5.91%), 373 (MH^+ - $C_4H_7O_2$, 1.01%), 350 (MH^+-thymine, 1.85%), 329 ($AZTPO_2^+$, 0.98%), 307 (MH_2^+-thymine -N_3H, 36.90%), 250 (thymidine.H^+

-H_2O, 17.19%), 209 (M^+-AZT, 4.36%), 185 ($C_4H_7O_3PO_2H_3^+$, 18.41%) 167 ($C_4H_7O_3PO_2H^+$, 14.56%), 139 ($C_3H_7OPO_2H^+$, 54.71%), 127 (thymineH^+, 23.29%), 126 (thymine$^+$, 30.36%), 123 ($C_3H_7OPO_2H^+$, 18.71%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{17}H_{26}O_9N_5P$ requires: C 42.95%; H 5.51; N 14.73; P 6.52. Found: C 42.80%; H 5.39; N 14.25; P 6.45.

3'-Azido-3'-deoxythymidine-5'-(dodecyl 1-ethylcarboxy methyl) phosphate 114.

54 (1.66 g, 0.004 mol), was added to **10** (0.2 g, 0.007 mol) and *N*-methylimidazole (0.49 g, 0.48 ml, 0.006 mol) in tetrahydrofuran (2.5 ml) at room temperature. After five min. a further 10 ml of tetrahydrofuran was added to the solidified mixture. After leaving for two days the reaction mixture was concentrated to dryness under reduced pressure, dissolved in chloroform (35 ml) and saturated sodium bicarbonate solution (15 ml) was added. The emulsion was concentrated to dryness and dissolved in chloroform (*ca.* 4 ml). This was added portionwise to a stirred solution of petroleum ether (bp 30-40 °C) (*ca.* 500 ml) and the mixture refrigerated overnight at -20 °C. Further purification of the product was achieved by flash column chromatography (silica *ca.* 60 g) eluted with 4% methanol in chloroform, pooling and evaporating the appropriate fractions. Final purification was achieved by flash chromatography (silica *ca.* 60 g) eluted with chloroform. (0.36 g, 79%).

^{13}C n.m.r. δ($CDCl_3$): 167.793 (d, $\underline{C}(O)CH_2$, A, J=4.3 Hz), 167.717 (d, $\underline{C}(O)CH_2$, B, J=4.6 Hz), 163.855 (C-2, A), 163.838 (C-2, B), 150.391 (C-4), 135.155 (C-6, A), 135.033 (C-6, B), 111.425 (C-5, A) 111.382 (C-5, B), 84.509 (C-1', A), 84.453 (C-1', B), 82.166 (C-4', J=8.1 Hz), 68.755 (d, $CH_2CH_2\underline{C}H_2O$, A, J=6.1 Hz), 68.678 (d, $CH_2CH_2\underline{C}H_2O$, B, J=5.5 Hz), 66.564 (C-5', A, J=5.9 Hz), 66.444 (d, C-5', B, J= 5.3 Hz), 63.723 (d, $C(O)\underline{C}H_2$, A, J=5.2 Hz), 63.643 (d, $C(O)\underline{C}H_2$, B, J=5.1 Hz), 61.682 ($\underline{C}H_2OC$), 60.124 (C-3', A), 60.066 (C-3', B), 37.435 (C-2', A), 37.356 (C-2', B), 31.771 ($\underline{C}H_2CH_2CH_2O$), 30.059 (d, $CH_2\underline{C}H_2CH_2O$, J=6.9 Hz), 29.486 - 25.220 (m, $(\underline{C}H_2)_8CH_2\underline{C}H_2CH_2O$), 22.555 ($\underline{C}H_3CH_2CH_2$), 13.999 ($\underline{C}H_3CH_2O$, A), 13.985 ($\underline{C}H_3CH_2O$, B), 12.320 (base CH_3, A), 12.293 (base CH_3, B).

^{31}P n.m.r. δ($CDCl_3$) : A: 0.510 B: 0.137; A:B 7:3.

^{1}H n.m.r. δ($CDCl_3$): 9.7 (1H, s, NH), 7.428, 7.411 (d, H-6, A, B, J=1.22 Hz, J=1.19 Hz), 6.253, 6.250 (1H, t, H-1', A, B, J=7.31 Hz, J=6.95 Hz), 4.6 (2H, m, $C(O)CH_2$), 4.42 (1H, m, H-3'), 4.36 (2H, m, H-5'), 4.22 (2H, m, $CH_2CH_2C\underline{H}_2O$), 4.102 (2H, q, CH_2OC, J=6.9 Hz), 4.01 (1H, m, H-4'), 2.38 (1H, m, H-2'), 2.31 (1H, m, H-2'), 1.895, 1.888 (3H, d, base CH_3, B, A, J=1.01 Hz, J=0.95 Hz), 1.661 (2H, m, $C\underline{H}_2CH_2CH_2O$), 1.26 (3H, m, $C\underline{H}_3CH_2O$), 1.215 (18H, m, $(C\underline{H}_2)_8CH_2O$), 0.838 (3H, t, $C\underline{H}_3CH_2CH_2$, J=6.68 Hz).

E.I.M.S. m/e: 433 ($MH^+ - C_{12}H_{25}$, 26.79%), 250 ($C_{10}H_{12}N_5O_3^+$, 18.25%), 186 ($C_{12}H_{25}OH^+$, 6.87%), 185 ($C_{12}H_{25}O^+$, or $C_4H_7O_3PO_3H_3^+$, 78.68%), 167 ($C_4H_7O_3PO_2H^+$, 13.34%), 141 (167 $-CH_3CH_2$), 127 (141 $-CH_2$, 30.11%), 126 (thymine.H^+, 49.14%), 113 (127 $-CH_2$, 18.63%), 99 (113 $-CH_2$, 22.85%), 85 (99 $-CH_2$, 11.73%),81 ($C_5H_5O^+$, 100%), 71 (85 $-CH_2$, 27.54%), 57 (71 $-CH_2$, 64.23%), 43 (57 - CH_2, 85.41%), 29 ($C_2H_5^+$, 80.26%).

F.A.B. m/e: 603 (MH_2^+, 2%), 602 (MH^+, 8%), 434 ($MH_2^+ - C_{12}H_{25}$, 0.5%), 433 ($MH^+ - C_{12}H_{25}$, 1%), 353 ($MH_2^+ - C_{10}H_{12}N_5O_3$, 1%), 250 ($C_{10}H_{12}N_5O_3^+$, 8%), 185 ($C_{12}H_{12}O^+$, 11%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{26}H_{44}N_5O_9P.0.3\%aq.$ requires: C 51.95%; H 7.37; N 11.64; P 5.15. Found: C 51.49; H 7.41; N 11.54; P 5.19.

3'-Azido-3'-deoxythymidine-5'-(1-methylcarboxy-1-methyl) (1-ethylcarboxy methyl) phosphate 115.

86 (1.62 g, 1.5 ml, 0.006 mol) was added to **10** (0.25 g, 0.94 mmol) and *N*-methylimidazole (0.61 g, 0.55 ml, 0.007 mol) in tetrahydrofuran (5 ml) with stirring at room temperature. After stirring at room temperature for two days the mixture was concentrated to dryness, dissolved in chloroform (30 ml) and washed with saturated sodium bicarbonate solution (15 ml) and water (10 ml). The aqueous layers were back extracted with chloroform (15 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 5 g). Filtration and concentration to a small volume (*ca.* 3 ml) followed by precipitation from petroleum ether (bp 30-40 °C) (500 ml) overnight at -20 °C gave an amber gum. Flash column chromatography, (silica *ca.* 60 g) eluted with 0.5% methanol in chloroform gave the product as a colourless gum. This was dissolved in 2-propanol, filtered and concentrated under reduced pressure to yield the product as a glass. (0.42 g 86%).

^{13}C n.m.r. δ($CDCl_3$): 170.1 (m, C(O)CH), 167.9 (m, C(O)CH_2), 163.607 (C-2), 150.246 (C-4), 135.492 (C-6, A), 135.207 (C-6, B), 111.470 (C-5, A), 111.6159 (C-5, B), 84.587 (C-1'), 82.318 (d, C-4', A, J=8.4 Hz), 82.200 (d, C4', B, J=8.3 Hz), 72.753 (d, CHO, A, J=5.4 Hz), 72.698 (d, CHO, B, J=5.8 Hz), 67.023 (d, C-5', A , J=5.8 Hz), 66.901 (d, C-5', B, J=5.3 Hz), 64.066 (d, C(O)CH_2, B, J=5.1 Hz), 63.813 (d, C(O)CH_2, A, J=5.0 Hz), 61.907 (CH_2OC, A), 61.826 (CH_2OC, B), 60.236 (C-3', A), 60.150 (C-3', B), 52.727 (CH_3O), 37.529 (C-2', A), 37.492 (C-2', B), 19.067 (d, CH_3CH, B, J=6.6 Hz), 18.960 (d, CH_3CH, A, J=6.9 Hz), 14.117 (CH_3CH_2), 12.410 (base CH_3, B), 12.365 (base CH_3, A).

^{31}P n.m.r. δ($CDCl_3$) : A: -1.605, B: -2.369 A:B 3:1

^{1}H n.m.r. δ($CDCl_3$): 8.811, 8.830 (1H,s, NH, B, A), 7.486, 7.457 (1H, d, H6 A, B, J=1.30 Hz, J=1.22 Hz), 6.30 (1H, m, H-1'), 5.04 (1H, m, CHO), 4.72 (1H, m, H-3'), 4.53 (4H, m, H-5', C(O)CH_2), 4.249 (2H, q, CH_2OC, J=7.12 Hz), 4.07 (1H, m, H-4'),

3.871, 3.790 (3H, s, CH_3O, A, B), 2.49 (2H, m, H-2'), 1.943, 1.931 (3H, d, base CH_3, A, B, J=1.17 Hz, J=1.19 Hz), 1.60 (m, $C\underline{H}_3CH$, B, A), 1.30 (3H, m, $C\underline{H}_3CH_2$).

E.I.M.S. m/e: 519 (M^+, 2.35%), 435 (MH_3^+ -$C_4H_7O_2$, 0.1%), 416 (M^+ -$C_4H_7O_3$, 1.41%), 393 (M^+ thymine -H, 0.41%), 386 (MH^+ -$C_4H_7O_3$ -CH_3O, 30.68%), 373 (MH^+ -$C_4H_7O_3$ -N_3H, 5.33%), 351 (MH^+ -thymine -N_3H, 30.68%), 313 (M^+ -$C_4H_7O_3$ -$C_4H_7O_3$, 0.9%), 271 (MH_3O^+ -AZT, 6.76%), 250 (AZT.H^+ -H_2O, 18.33%), 185 ($C_4H_7O_3PO_3H^+$, 3.43%), 167 ($C_4H_7O_3PO_2H^+$, 45.61%), 149 (167$^+$ -H_2O, 16.46%), 126 (thymine.H^+, 25.76%), 87 ($C_4H_7O_2^+$, 9.72%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{18}H_{26}N_5O_{11}P$ requires: C 41.12%; H 5.05; N 13.48; P 5.96. Found: C 41.45; H 5.03; N 12.86; P 6.25.

3'-Azido-3'-deoxythymidine-5'-(propyl 2-ethylcarboxy-ethyl) phosphate <u>116</u>.

<u>88</u> (0.47 g, 0.42 ml, 0.002 mol) was added to **<u>10</u>** (0.09 g, 0.34 mmol) and *N*-methylimidazole (0.27 g, 0.27 ml, 0.004 mol) in tetrahydrofuran (2.5 ml) with stirring at room temperature. The heterogeneous solution was stirred for 24 h. The reaction mixture was concentrated under reduced pressure, dissolved in chloroform (30 ml), washed with saturated sodium bicarbonate solution (15 ml) and water (10 ml) .The aqueous layers were back extracted with chloroform (10 ml) and the combined organic layers dried over magnesium sulphate (5 g). Filtration and concentration to a small volume (*ca.* 3 ml) under reduced pressure followed by precipitation of the product from cold petroleum ether (bp 30-40 °C) (500 ml) refrigerated overnight afforded an amber gum. The gum was dissolved in 1% methanol in chloroform (*ca.* 4 ml) and purified by flash column chromatography (silica *ca.* 75 g) employing 1% methanol in chloroform as the eluant. Pooling and evaporation of appropriate fractions afforded a gum (0.13 g, 89%).

^{13}C n.m.r. δ($CDCl_3$): 170.136 (C(O)), 170.112 (C(O)) 163.994 (C-2), 150.468 (C-4), 135.252 (C-6), 111.552, (C-5), 111.486 (C-5). 84.811, (C-1'), 84.778, (C-1'), 82.17 (m, C-4'), 66.437 (C-5', J=5.5 Hz), 63.615 (m, CH_2OP), 61.038 ($\underline{C}H_2OC$), 60.154 (H-3'), 60.091 (H-3'), 54.71, (m, CH_3OP), 37.454 (C-2'). 35.210 (C(O)$\underline{C}H_2$ J=7.8 Hz.), 14.158 ($\underline{C}H_3CH_2O$) 12.413 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A:-0.503 B:0.353; A:B; 1:1.

^{1}H n.m.r. δ($CDCl_3$): 9.708 (1H, s, NH), 7.371 (1H, s, H-6, J=1.23 Hz), 6.212 (1H, t, H-1', J=6.46 Hz), 4.358-4.193 (5H, H-5', H-4', CH_2OP), 4.106 (2H, m, $CH_2OC(O)$), 3.99 (1H, m, H-3'), 3.80 (3H, m, CH_3OP), 2.67 (2H, m, C(O)$C\underline{H}_2$) , 2.39 (1H, m, H-2'), 2.26 (1H, m, H-2'), 1.942, 1.849 (3H, d, base CH_3, J=1.22 Hz, J=1.22 Hz), 1.28 (3H, m, $C\underline{H}_3CH_2O$).

3'-Azido-3'-deoxythymidine-5'-(Propyl 6-ethylcarboxy-hex-1-ene-5yl) phosphate 117.

90 (0.89 g, 0.78 ml, 0.02 mol) was added to 10 (0.2 g, 0.75 mmol) and *N*-methylimidazole (0.49 g, 0.48 ml, 0.005 mol) in tetrahydrofuran (3 ml) with stirring at room temperature. The heterogeneous solution was stirred overnight. The reaction mixture was concentrated under reduced pressure, dissolved in chloroform (30 ml), washed with saturated sodium bicarbonate solution (15 ml), and water (10 ml). The aqueous layers were back extracted with chloroform (10 ml) and the combined organic layers dried over magnesium sulphate (5 g). Filtration and concentration to a small volume (*ca.* 3 ml) under reduced pressure followed by precipitation of the product from cold petroleum ether (bp 30-40 °C) (500 ml) and refrigeration over-night afforded an amber gum. Purification was achieved by flash column chromatography on silica (*ca.* 60 g,) eluted with chloroform. Pooling and evaporation of appropriate fractions gave the product as a colourless gum (0.31 g, 77%).

^{13}C n.m.r. δ($CDCl_3$): 169.921 ($\underline{C}(O)CH_2$), 169.821 ($\underline{C}(O)CH_2$), 163.896 (C-2), 150.453 (C-4), 150.409 (C-4), 136.847 (CH=), 136.722 (CH=), 135.409 (C-6), 135.262 (C-6), 115.687 (CH_2=), 111.619 (C-5), 111.584 (C-5), 84.673 (C-1'), 84.262 (C-1'), 82.322 (d, C-4', J=8.4 Hz), 82.204 (C-4'), 75.808 (m, CHOP), 69.891 (d, $CH_3CH_2\underline{C}H_2O$, J=6.1 Hz), 69.764 (d, $CH_3CH_2\underline{C}H_2O$, J=6.3 Hz), 66.242 (m, C-5'), 60.932 ($CH_3\underline{C}H_2O$), 60.894 ($CH_3\underline{C}H_2O$), 60.5 (m, C-3'), 40.185 (d, $C(O)\underline{C}H_2$, J=4.7 Hz), 40.083 ($C(O)\underline{C}H_2$), 37.510 (C-2'), 37.479 (C-2'), 37.452 (C-2'), 34.408 (d, $CH_2\underline{C}H_2CHO$, J=5.3 Hz), 34.296 ($CH_2\underline{C}H_2CHO$), 28.935 ($\underline{C}H_2CH$=), 23.644 (d, $CH_3\underline{C}H_2CH_2O$, J=6.9 Hz), 14.175 ($\underline{C}H_3CH_2O$), 12.504 (base CH_3), 9.978 ($CH_3\underline{C}H_2CH_2O$).

^{31}P n.m.r. δ($CDCl_3$) : A: -0.074 B: -0.305; C:-0.465; A:B:C 1:1:2.

^{1}H n.m.r. δ($CDCl_3$): 9.51 (1H, s, NH), 7.41 (1H, m, H-6), 6.22 (1H, m, H-1'), 5.75 (1H, m, CH=), 4.98 (2H, m, CH_2=), 4.82 (1H, m, CHOP), 4.38 (1H, m, H-3'), 4.23 (2H, m, H-5'), 4.1 (2H, m, $CH_3C\underline{H}_2O$), 4.02 (3H, m, H-4' , $CH_3CH_2C\underline{H}_2O$), 2.74 (1H, m, $C(O)CH_2$), 2.59 (1H, m, $C(O)CH_2$), 2.41 (1H, m, H-2'), 2.29 (1H, m, H-2'), 2.13 (2H, m, $CH_2C\underline{H}_2CHO$), 1.893 (3H, d, base CH_3, J=1.41 Hz), 1.78 (2H, m, $C\underline{H}_2CH$=), 1.68 (2H, m, $CH_3C\underline{H}_2CH_2O$), 1.21 (3H, m, $C\underline{H}_3CH_2O$), 0.92 (3H, m, $C\underline{H}_3CH_2CH_2O$).

E.I.M.S. m/e: 390 (MH_2^+ -$C_9H_{15}O_2$, 0.04%), 375 (390 -CH_3, 1.80%), 295 (MH_3O^+ -AZT), 250 (AZT.H^+ -H_2O, 1.84%), 155 ($C_9H_{15}O_2^+$, 5.35%), 154 (155 -H, 6.94%), 141 ($C_3H_7OPO_3H^+$, 0.85%), 126 (thymine.H^+, 32.14%), 99 ($C_5H_7O_2^+$, 51.73%), 81 ($C_5H_5O^+$, 100%).

F.A.B. m/e: 545 (MH_2^+, 2%), 544 (MH^+, 6%), 518 (MH_2^+ -C_2H_3, 5%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{22}H_{34}N_5O_9P$ requires: C 48.62%; H 6.31; N 12.89; P 5.70. Found: C 48.41; H 6.01; N 12.67; P 5.95.

3'-Azido-3'-deoxythymidine-5'-(methyl 1-methylcarboxy-1-methyl) phosphate 118.

87 (0.24 g, 0.17 ml, 0.001 mol) was added to a stirred solution of **10** (0.08 g, 0.28 mmol) and *N*-methylimidazole (0.14 g, 0.13 ml, 0.002 mol) in tetrahydrofuran at room temperature with stirring. After leaving for 48 h.the reaction mixture was concentrated to dryness under reduced pressure, dissolved in chloroform (40 ml) and washed with saturated sodium bicarbonate solution (15 ml) and water (15 ml).The aqueous layers were back extracted with chloroform (*ca.* 20 ml) and the combined organic layers concentrated to small volume (*ca.* 2 ml). This solution was pipetted into stirred petroleum ether (bp 40-60 °C) (400 ml) and refrigerated overnight. The supernatant was decanted off and the pale crystals dissolved in chloroform (*ca.* 2 ml). The product was purified by flash column chromatography (silica *ca.* 80 g), pooling and evaporation of the appropriate fractions yielding a gum (0.13 g, 73%).

^{13}C n.m.r. δ($CDCl_3$): 170.604 (m, C(O)CH), 163.593 (C-2, A), 163.546 (C-2, B), 150.134 (C-4, A), 150.120 (C-4, B), 135.179 (C-6, A), 135.136 (C-6, B), 111.607 (C-5, B), 111.484 (C-5, A), 84.621 (C-1'), 82.321 (C-4', A, J=8.3 Hz), 81.971 (d, C4', B, J=7.8 Hz), 72.530 (d, CHO, A, J=5.3 Hz), 72.370 (d, CHO, B, J=5.1 Hz), 66.738 (d, C-5', A, J=5.8 Hz), 66.331 (d, C-5', B, J=5.6 Hz), 60.141 (C-3', A), 59.902 (C-3', B), 55.045 (d, CH_3OP, B, J=11.8 Hz), 54.409 (d, CH_3OP, A, J=11.9 Hz), 52.719 (CH_3OC, A), 52 .665 (CH_3OC, B), 37.605 (C-2', A), 37.489 (C-2', B), 19.082 (m, CH_3CH), 12.369 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A: -2.442, B: -2.487 A:B 2:3

^{1}H n.m.r. δ($CDCl_3$): 8.95 (1H, s, NH), 7.426, 7.399 (1H, d, H-6, A, B, J=1.25 Hz, J=1.22 Hz), 6.24 (1H, m, H-1'), 4.97, 4.91 (2H, m, CHO, A, B), 4.523 - 4.245 (3H, m, H-3', H-5'), 4.02 (1H, m, H-4'), 3.877 - 3.731 (6H, m, CH_3OP, CH_3OC), 2.42 (1H, m, H-2'), 2.31 (1H, m, H-2'), 1.922, 1.916 (3H, d, base CH_3, B, A, J=1.18 Hz, J=1.22 Hz), 1.56 (3H, m, CH_3CH).

3'-Azido-3'-deoxythymidine-5'-propyl(1-methylcarboxy-1-methyl)phosphate 119.

84 (0.69 g, 0.003 mol), was added to **10** (0.13 g, 0.50 mmol) and *N*-methylimidazole (0.61 g, 0.60 ml, 0.007 mol) in tetrahydrofuran (5 ml), at room temperature with stirring. After stirring for 48 h.the mixture was concentrated to dryness, dissolved in chloroform (30 ml), washed with saturated sodium bicarbonate solution (15 ml), and water (10 ml). The aqueous layers were back-extracted with chloroform (*ca.* 15 ml), and the combined organic layers dried over magnesium sulphate (*ca.* 3 g). Filtration and concentration under reduced pressure

to small volume (*ca.* 3 ml) followed by precipitation from petroleum ether (bp 30-40 °C) (500 ml) overnight at - 20 °C gave an amber gum. Purification was achieved by flash column chromatography (silica *ca.* 60 g), eluted with 1% methanol in chloroform. The gum produced from pooling and evaporation of appropriate fractions was dissolved in 2-propanol (*ca.* 2.5 ml), filtered and concentrated under reduced pressure to afford a colourless gum. (0.33 g, 73%).

^{13}C n.m.r. δ($CDCl_3$): 170.866 (d, C(O)CH, A, J=3.7 Hz), 170.511 (d, C(O)CH, B, J=4.6 Hz), 163.953 (C-2, A), 163.909 (C-2, B), 150.416 (C-4, A), 150.382 (C-4, B), 135.029 (C-6, A), 134.995 (C-6, B), 111.464 (C-5, B), 111.329 (C-5, A), 84.440 (C-1'), 82.176 (d, C-4', A, J=8.2 Hz), 81.971 (d, C4', B, J=8.0 Hz), 72.205 (d, CHO, A, J=5.2 Hz), 72.052 (d, CHO, B, J=5.2 Hz), 70.160 (d, CH_2CH_2O, B, J=6.1 Hz), 69.659 (d, CH_2CH_2O, A, J=5.2 Hz), 66.566 (d, C-5',A, J=6.1 Hz), 66.185 (d, C-5', B, J=5.6 Hz), 60.160 (C-3', A), 59.909 (C-3', B), 52.514 (CH_3O, A), 52 .441 (CH_3O, B), 37.356 (C-2', A), 37.238 (C-2', B), 23.361 (m, CH_2CH_2O), 19.972 (d, CH_3CH, B, J=5.4 Hz), 18.918 (d, CH_3CH, A, J=5.6 Hz), 12.259 (base CH_3), 9.765 (CH_3CH_2, A), 9.732 (CH_3CH_2, B).

^{31}P n.m.r. δ($CDCl_3$) : A: -2.168, B: -2.312 A:B 4:5

^{1}H n.m.r. δ($CDCl_3$): 10.078, 10.030 (1H, s, NH, B, A), 7.43 (1H, M, H-6), 6.25 (1H, m, H-1'), 4.94 (1H, m, CHO) , 4.36 (1H, m, H-3'), 4.32 (2H, m, H-5'), 4.103 (1H, q, H-4', J=6.88 Hz), 4.02 (2H, m, CH_2CH_2O), 3.739 (3H, s, CH_3O), 2.42 (1H, m, H-2'), 2.34 (1H, m, H-2'), 1.903, 1.896 (3H, d, base CH_3, B, A, J=1.23 Hz, J=1.19 Hz), 1.689 (2H, septet, CH_2CH_2O, $J_{CH2,CH3}$=7.01 Hz), 1.53 (3H, m, CH_3CH), 0.94 (3H, m, CH_3CH_2).

E.I.M.S. m/e: 476 (MH^+, 0.40%), 416 (MH^+ -$C_2H_3O_2$, 0.01%), 373 (MH^+ -$C_4H_7O_3$, 0.11%), 350 (M^+ -thymine, 0.12%), 329 (M^+ -$C_4H_7O_3$ -C_3H_7O, 1.19%) , 3O7 (MH_2^+ -$C_4H_7O_2$ -C_3H_7 -N_3H, 9.20%), 250 (AZT.H^+ -H_2O, 2.48%) , 227 (MH_3O^+ -AZT, 1.64%). 209 (MH^+ -AZT), 185 ($C_4H_7O_3PO_3H_3^+$, 3.44%), 167 ($C_4H_7O_3PO_2H^+$, 11.46%), 126 (thymine.H^+, 7.99%),125 (thymine$^+$, 16.13%), 87 ($C_4H_7O_2^+$, 3.52%), 99 ($C_5H_7O_2^+$, 11.42%), 81 ($C_5H_5O^+$,100%).

Analysis: $C_{17}H_{26}N_5O_9P$ requires: C 42.95%; H 5.51; N 14.73; P 6.52.

3'-Azido-3'-deoxythymidine-5'-2,2,2,-trichloroethyl (1-methylcarboxy-1-methyl) phosphate 120.

85 (1.16 g, 0.88 ml, 0.003 mol) was added to **10** (0.25 g, 0.94 mmol) and *N*-methylimidazole (0.61 g, 0.60 ml, 0.007 mol) in tetrahydrofuran (5 ml) with stirring at room temperature. After stirring at room temperature for two days the reaction mixture was concentrated to dryness, dissolved in chloroform (30 ml) and washed with saturated sodium bicarbonate solution (15 ml) and water (10 ml). The aqueous layers were back extracted with

chloroform (15 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 3 g). Filtration and concentration to small volume (*ca.* 3 ml) followed by precipitation from petroleum ether (500 ml) overnight at -20 °C gave an amber gum. Flash column chromatography (silica *ca.* 70 g). eluted with methanol 0.5% in chloroform afforded the product as a glass on pooling and evaporation of the appropriate fractions. This was dissolved in 2-propanol and filtered under reduced pressure. Concentration to dryness yielded the product as a glass. (0.38 g, 79%).

^{13}C n.m.r. δ($CDCl_3$): 170.529 (d, C(O)CH, A, J=3.9 Hz), 170.211 (d, C(O)CH, B, J=4.2 Hz), 163.450 (C-2, A), 163.421 (C-2, B), 150.108 (C-4, A), 150.076 (C-4, B), 135.168 (C-6), 111.677 (C-5, B), 111.545 (C-5, A), 94.80 (m, CCl_3), 84.835 (C-1', B), 84.788 (C-1', A), 82.108 (d, C-4', A, J=8.4 Hz), 81.965 (d, C4', B, J=7.9 Hz), 77 ($CCl_3\underline{C}H_2$), 73.139 (CHO, B, J=5.5 Hz), 72.112 (d, CHO, A, J=5.2 Hz), 67.353 (d, C-5',A, J=6.1 Hz), 67.119 (d, C-5', B, J=6.0 Hz), 60.163 (C-3', A), 60.032 (C-3', B), 52.862 (CH_3O), 37.446 (C-2', A), 37.344 (C-2', B), 18.94 (m, $\underline{C}H_3CH$), 12.476 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A: -1.118, B: -1.462 A:B 1:3

^{1}H n.m.r. δ($CDCl_3$): 8.789, 8.718 (1H, broad s, NH, B, A), 7.379, 7.360 (1H, d, H-6, A, B, J=1.26 Hz, J=1.22 Hz), 6.21 (1H, m, H-1'), 5.01 (1H, m, CHO), 4.699, 4.570 (2H, m, CCl_3CH_2, B, A), 4.487 - 4.314 (3H, M, H-3', H-5'), 4.027 (1H, m, H-4'), 3.773, 3.770 (3H, s, CH_3O, B, A), 2.417 (1H, m, H-2'), 2.344 (1H, m, H-2'), 1.922, 1.914 (3H, d, base CH_3, B, A, J=1.25 Hz, J=1.23Hz), 1.596, 1.570 (3H, dd, $C\underline{H}_3CH$, J_P=1.18 Hz, J_{CH}=7.00 Hz, J_P=1.17 Hz, J_{CH}=6.97 Hz).

3'-Azido-3'-deoxythymidine-5'-bis (1-methylcarboxy-1-methyl) phosphate 121.

83 (0.65 g, 0.002 mol) was added to **10** (0.10 g 0.31 mmol) and *N*-methylimidazole (0.25 g, 0.24 ml, 0.003 mol) in tetrahydrofuran (3 ml) with stirring at room temperature. After stirring for a further 48 h. the mixture was concentrated to dryness under reduced pressure, dissolved in chloroform (30 ml) and washed with saturated sodium bicarbonate solution (15 ml) and then water (10 ml). The aqueous layers were back extracted with chloroform (*ca.*15 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 3 g). Filtration and concentration to small volume (*ca.* 3 ml) under reduced pressure followed by precipitation of the product from petroleum ether (bp 30-40 °C) (500 ml) overnight at -20 °C gave an amber gum. Further purification was achieved with flash column chromatography, (silica *ca.* 60 g) eluted with chloroform containing 0.5% methanol. Pooling and evaporation of the appropriate fractions gave a gum. (0.17 g, 88%).

^{13}C n.m.r. δ($CDCl_3$): 171.085 (d, C(O)CH, J=3.3 Hz), 170.676 (d, C(O)CH, J=3.9 Hz), 163.907 (C-2), 150.456 (C-4), 135.228 (C-6), 84.578 (C-1'), 82.267 (C-4',

J=8.4 Hz), 72.726 (d, CHO J=5.2 Hz), 72.530 (d, CHO J=5.1 Hz), 66.593 (d, C-5', J=5.9 Hz), 60.278 (C-3'), 52.687 (CH_3O), 37.511 (C-2'), 19.065 (d, $\underline{C}H_3CH$, J=6.9 Hz), 18.868 (d, $\underline{C}H_3CH$, J=7.1 Hz).

^{31}P n.m.r. δ($CDCl_3$) : -2.709

^{1}H n.m.r. δ($CDCl_3$): 9.456 (1H, s, NH), 7.482 (1H, s, H-6), 6.305 (1H, t, H-1', J=6.74 Hz), 5.08 (1H, m, CHO), 4.93 (1H, m, CHO), 4.52 (2H, m, H-5'), 4.45 (1H, m, H-3'), 4.08 (1H, m, H-4'), 3.785 (3H, s, CH_3O), 3.774 (3H, s, CH_3O), 2.44 (1H, m, H-2'), 2.37 (1H, m, H-2'), 1.942 (3H, d, base CH_3), J=1.23 Hz), 1.601 (3H, dxd, $C\underline{H}_3CH$, J_{CH}=7.0 Hz, J_P=1.01 Hz), 1.580 (3H, dxd, $C\underline{H}_3CH$, J_{CH}=6.91 Hz, J_P=0.95 Hz).

E.I.M.S. m/e: 519 (M^+, 0.88%), 518 (M^+ -H, 0.37%), 460 (M^+ -$C_2H_3O_2$, 0.06%), 416 (M^+ -$C_4H_7O_3$, 5.03%), 394 (M^+ -thymine, 0.25%), 373 (M^+ -$C_4H_7O_3$ -N_3H, 9.05%), 351 (MH^+ -thymine -N_3H, 31.00%), 313 (M^+ -$C_4H_7O_3$, 0.95%), 271 (MH_3O^+ -AZT, 1.74%), 250 ($AZTH^+$ -H_2O, 13.55%), 185 ($C_4H_7O_3PO_3H^+$, 3.15%), 167 ($C_4H_7OPO_2H^+$, 79.20%), 126 (thymine.H^+, 11.84%), 99 ($C_5H_7O_2^+$, 16.97%), 87 ($C_4H_7O^+$, 24.67%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{18}H_{26}N_5O_{11}P$ requires: C 41.12%; H 5.05; N 13.48; P 5.96. Found: C 41.12; H 5.10; N 13.14; P 6.00.

3'-Azido-3'-deoxythymidine-5'-methyl (1-methylcarboxy-1-1-dimethyl) phosphate <u>122</u>.

<u>95</u> (0.69 g, 0.58 ml, 0.03 mol) was added to **<u>10</u>** (0.2 g 0.75 mmol), and *N*-methylimidazole (0.37 g, 0.36 ml, 0.04 mol) in tetrahydrofuran (4 ml) with vigorous stirring. After leaving for a further 24 h. a further 6 equivalents of *N*-methylimidazole and a further 4 equivalents of phosphorylating reagent were added and the reaction left for 2 days. Another 6 equivalents of *N*-methylimidazole and 4 equivalents of phosphorylating reagent were added and the reaction left stirring for a further 8 days at room temperature. After concentrating the heterogeneous mixture to dryness the gum was dissolved in chloroform (*ca.* 25 ml) and washed with saturated sodium bicarbonate solution (15 ml), and water (10 ml). The aqueous material was back extracted with chloroform (*ca.* 20 ml), and the combined organic fractions dried over magnesium sulphate, filtered and evaporated.

The residue dissolved in chloroform (*ca.* 1 ml) was added dropwise to stirred petroleum ether (bp 30-40 °C) (500 ml) and the suspension refrigerated overnight. The supernatant was decanted from the oily precipitate which was purified by flash column chromatography (silica *ca.* 60g) eluted chloroform. The resulting gum (0.270 g) was found to consist of two closely running products when examined by multiple development tlc. These were separated by preparative normal phase hplc.

Fast running product (yield: 0.047 g).

^{13}C n.m.r. δ($CDCl_3$): 172.446 (d, C(O)CH, J=4.2 Hz), 172.340 (d, C(O)CH, J=5.8 Hz), 163.839 (C-2), 150.405 (C-4), 135.393 (C-6), 111.481 (C-3), 84.452 (C-1'), 82.446 - 82.268 (d, m, C-4', CH_3CCH_3, CH_3CCH_3), 66.350 (d, C-5', J=6.1 Hz), 60.155 (C-3'), 52.804 (CH_3O), 52.783 (CH_3O), 37.477 (C-2'), 26.567 (d, CH_3CH, J=5.9 Hz), 26.391 (d, CH_3CH, J=5.1 Hz), 26.138 (d, CH_3CH, J=4.1 Hz), 26.022 (d, CH_3CH, J=3.8 Hz), 12.430 (CH_3base).

^{31}P n.m.r. δ($CDCl_3$) : -11.452.

^{1}H n.m.r. δ($CDCl_3$): 9.161 (1H, s, NH), 7.514 (1H, s, H-6), 6.307 (1H, t, H-1', J=6.40 Hz), 4.497 (1H, dxt, H-3', $J_{H-2'}$=4.58 Hz, $J_{H-4'}$=6.98 Hz), 4.40 (2H, m, H-5'), 4.06 (1H, m, H-4'), 3.776 (3H, s, CH_3O), 3.764 (3H, s, CH_3O), 2.43 (2H, m, H-2'), 1.947 (3H, s, base CH_3), 1.740 (3H, s, CH_3CH), 1.723 (3H, s, CH_3CH), 1.683 (3H, s, CH_3CH), 1.674 (3H, s, CH_3CH).

F.A.B. m/e: 548 (MH^+, 3.8%), 299 ($C_{10}H_{12}O_8P^+$, 7%), 250 ($C_{10}H_{12}N_5O_3^+$, 21%), 181 ($C_5H_9OPO_2H_2^+$, 45%), 127 (thymineH_2^+, 63%).

slower running product (yield: 0.016 g)

^{13}C n.m.r. δ($CDCl_3$): 172.636 (d, C(O), J=5.2 Hz), 172.604 (d, C(O), J=5.3 Hz), 163.429 (C-2), 150.113 (C-4), 135.264 (C-6), 135.229 (C-6), 111.521 (C-3), 111.433 (C-3), 84.614 (C-1'), 82.440 - 82.061 (m, C-4', CH_3CCH_3), 66.336 (d, C-5', J=6.0 Hz), 66.257 (d, C-5', J=5.5 Hz), 60.085 (C-3'), 54.383 (m, CH_3OP), 52.946 (CH_3O), 37.627 (C-2'), 26.803 -26.149 (m, CH_3CH), 12.430 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A: -5.44. B: -5.69. A:B; 1:1.

^{1}H n.m.r. δ($CDCl_3$): 8.543 (1H, broad s, NH), 7.482, 7.468 (1H, d, H-6, J=1.23 Hz, J=1.22 Hz), 6.28 (1H, m, H-1'), 4.44 (1H, m, H-3'), 4.35 (2H, m, H-5'), 4.06 (1H, m, H-4'), 3.821, 3814 (3H, d, CH_3OP, J=11.56 Hz, J=11.55 Hz), 3.797 (3H, s, CH_3OC), 3.790 (3H, s, CH_3OC), 2.46 (1H, m, H-2'), 2.38 (1H, m, H-2'), 1.949, 1.942 (3H, d, base CH_3, J=1.22 Hz, J=1.22 Hz), 1.730, 1.721 (3H, s, CH_3C, s, CH_3C), 1.669 (3H, s, CH_3C, CH_3C).

F.A.B. m/e: 462 (MH^+, 4%), 461 (M^+, 4%), 250 ($C_{10}H_{12}N_5O_3$, 6%), 181 ($C_5H_9OPO_2H_2^+$, 2%), 127 (thymineH_2^+, 5%).

3'-Azido-3'-deoxythymidine-5'-(1-methylcarboxy-1-1-dimethyl) (t-ethylcarboxy prop-2-enyl) phosphate 123.

99 (0.33 g, 0.1 mmol) was added to a stirred solution of **10** (0.03 g, 0.09 mmol) and *N*-methylimidazole (0.08 g, 0.08 ml, 0.001 mol) in tetrahydrofuran (1 ml) with stirring. After 36 h. at ambient temperature with vigorous stirring the mixture was concentrated to dryness, suspended in dichloromethane (*ca.* 10 ml) and washed successively with saturated sodium bicarbonate solution (*ca.* 30 ml) and water (*ca.* 10 ml). The aqueous extracts were back-

extracted with chloroform (*ca.* 10 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 3 g). Purification of the product was achieved by flash column chromatography (silica *ca.* 20 g) eluted with 2% methanol in chloroform. Pooling and evaporation afforded the product aa a gum (0.05 g, 91%).

^{31}P n.m.r. d($CDCl_3$) : A; -10.768, B; -10.984, A:B 3:4.

The diastereomers were separated by normal phase hplc Separation of the compounds was almost completely achieved (detected by hplc **123a** 100% A; **123b** 98% A, 2% B.).

123a

^{13}C n.m.r. δ($CDCl_3$): 172.198 (d, C(O)C(CH_3)$_2$, J=3.5 Hz), 165.936 (C(O)CH=), 163.322 (C-2), 162.676 (d, CH_3C=, J=9.0 Hz), 149.980 (C-4), 135.236 (C-6), 111.475 (C-5), 107.127 (d, CH=, J=4.4 Hz), 83.707 (C-1'), 83.493 (d, C-4', J=6.0 Hz), 82.149 (d, CH_3CCH_3, J=8.2 Hz), 66.900 (C-5', J=5.3 Hz), 60.367 (CH_3CH_2OC(O)), 59.989 (C-3'), 53.091 (CH_3OC(O)), 37.526 (C-2'), 26.891 (d, CH_3CCH_3, J=6.6 Hz), 25.883 (d, CH_3CCH_3, J=6.6 Hz), 18.584 (d, CH_3C=, J=4.2 Hz), 14.203 (CH_3CH_2OC(O)), 12.445 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A; -10.664

^{1}H n.m.r. δ($CDCl_3$): 8.460 (1H, s, NH), 7.401 (1H, m, H-6), 6.240 (1H, t, H-1', J=6.46 Hz), 5.814 (1H, d, CH=, J_P=0.90 Hz), 4.502 -4.352 (3H, m, H-3', H-5'), 4.140 (2H, q, CH_3CH_2OC, J=7.11 Hz), 4.07 (1H, m, H-4'), 3.767 (3H, s, CH_3O), 2.498 - 2.301 (5H, m, H-2', CH_3C=), 1.902 (3H, d, base CH_3, J=1.12 Hz), 1.732 (3H, s, CH_3CCH_3), 1.647 (3H, s, CH_3CCH_3), 1.243 (3H, t, CH_3CH_2OC J=7.14 Hz).

E.I.M.S. m/e: 500 (M^+ -C_2H_5O, 0.05%), 430 (MH_2^+ - $C_5H_9O_3$, 2.65%), 250 (AZT.H^+ -H_2O 3.89%), 127 (thymine.H_2^+, 17.21%), 126 (thymine.H^+, 59.13%), 81 ($C_5H_5O^+$, 100%).

123b

^{13}C n.m.r. δ($CDCl_3$): 172.155 (d, C(O)C(CH_3)$_2$, J=3.8 Hz), 165.918 (C(O)CH=), 163.379 (C-2), 162.669 (d, CH_3C=, J=9.0 Hz), 150.057 (C-4), 135.153 (C-6), 111.589 (C-5), 107.117 (d, CH=. J=4.2 Hz), 84.649 (C-1'), 83.480 (d, C-4', J=6.9 Hz), 82.145 (d, CH_3CCH_3, J=8.2 Hz), 66.961 (d, C-5', J=6.4 Hz), 60.369 (CH_3CH_2OC(O)), 59.987 (C-3'), 53.092 (CH_3OC(O)), 37.528 (C-2'), 26.971 (d, CH_3CCH_3, J=6.3 Hz), 25.831 (d, CH_3CCH_3, J=6.8 Hz), 18.565 (d, CH_3C=, J=4.2 Hz), 14.184 (CH_3CH_2OC(O)), 12.449 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : B; -10.849

^{1}H n.m.r. δ($CDCl_3$): 8.495 (1H, s, NH), 7.388 (1H, m, H-6), 6.240 (1H, t, H-1', J=6.46 Hz), 5.814 (1H, d, CH=, J_P=0.84 Hz), 4.502 -4.356 (3H, m, H-3', H-5'), 4.125 (2H, q, CH_3CH_2OC, J=7.14 Hz), 4.04 (1H, m, H-4'), 3.776 (3H, s, CH_3O), 2.42 (1H, m, H-2'), 2.373 (3H, s, CH_3C=), 2.34 (1H, m, H-2'), 1.908 (3H, d, base CH_3, J=1.02

Hz), 1.740 (3H, s, $C\underline{H}_3CCH_3$), 1.648 (3H, s, $CH_3CC\underline{H}_3$), 1.232 (3H, t, $C\underline{H}_3CH_2OC$ J=7.14 Hz).

E.I.M.S. m/e: 500 ($M^+ - C_2H_5O$, 0.06%), 430 ($MH_2^+ - C_5H_9O_3$, 1.44%), 250 (AZT.$H^+ - H_2O$ 1.64%), 127 (thymine.H_2^+, 12.58%), 126 (thymine.H^+, 64.87%), 81 ($C_5H_5O^+$, 100%).

3'-Azido-3'-deoxythymidine-5'-bis (2-propen-1-yl) phosphate 124.

101 (0.59 g, 0.57 ml, 0.003 mol) was added to **10** (0.1 g, 0.30 mmol) and *N*-methylimidazole (0.24 g, 0.24 ml, 0.003 mol) in tetrahydrofuran (5 ml) with stirring at room temperature. After stirring for a further 48 h.the mixture was concentrated to dryness under reduced pressure, dissolved in chloroform (40 ml) and washed with saturated sodium bicarbonate solution (20 ml) and then water (2x20 ml).

The aqueous layers were back extracted with chloroform (*ca.* 25 ml) and the combined organic layers dried over magnesium sulphate. (*ca.* 5 g). Filtration and concentration to a small volume (*ca.* 2 ml) under reduced pressure followed by precipitation of the product from petroleum ether (bp 30-40 °C) (500 ml) overnight at -20 °C gave an amber gum. Further purification was achieved with flash column chromatography, (silica *ca.* 80 g) eluted with chloroform containing 2% methanol. Pooling and evaporation of the appropriate fractions gave a gum (0.069 g, 54%).

^{13}C n.m.r. δ($CDCl_3$): 163.165 (C-2), 149.893 (C-4), 135.106 (C-6), 131.947 (d, $CH_2\underline{C}H=$, J=6.7 Hz), 131.914 (d, $CH_2\underline{C}H=$, J=6.9 Hz), 119.132 ($CH_2=$), 111.531 (C-5), 84.701 (C-1'), 82.252 (d, C-4', J=8.0 Hz), 68.736 (d, $CH\underline{C}H_2O$, J=5.8 Hz), 68.645 (d, $CH\underline{C}H_2O$, J=6.1 Hz), 66.244 (d, C-5', J=5.4 Hz), 60.048 (C-3'), 37.605 (C-2'), 12.488 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : 1.338

^{1}H n.m.r. δ($CDCl_3$): 9.465 (1H, NH), 7.354 (1H, d, H-6, J=1.19 Hz), 6.280 (1H, t, H-1' J=6.72 Hz), 5.85 (1H, m, CH=), 5.74 (1H, m, CH=), 5.09 (2H, m, $CH_2=$), 4.95 (2H, m, $CH_2=$), 4.61 (4H, m, CH_2O), 4.356 - 4.269 (3H, m, H-5', H-3'), 4.08 (1H, m, H-4'), 2.46 (1H, m, H-2'), 2.19 (1H, m, H-2'), 1.968 (3H, d, base CH_3, J=1.16 Hz).

3'-Azido-3'-deoxythymidine-5'-bis
(2-methyl-2-propen-1-yl) phosphate 125.

100 (0.22 g, 0.21 ml, 0.001 mol) was added to **10** (0.10 g 0.30 mmol) and *N*-methylimidazole (0.12 g, 0.12 ml, 0.002 mol) in tetrahydrofuran (3 ml) with stirring at room temperature. After stirring for a further 12 h.the mixture was concentrated to dryness under reduced pressure, dissolved in chloroform (20 ml) and washed with saturated sodium bicarbonate solution (10 ml) and then water (10 ml). The aqueous layers were back extracted with chloroform (*ca.* 15 ml) and the combined organic layers dried over magnesium sulphate.

(*ca.* 3 g). Filtration and concentration to a small volume (*ca.* 3 ml) under reduced pressure followed by precipitation of the product from petroleum ether (bp 30-40 °C) (500 ml) overnight at -20 °C gave an amber gum. Further purification was achieved with flash column chromatography, (silica *ca.* 40g) eluted with chloroform containing 2%% methanol. Pooling and evaporation of the appropriate fractions afforded a gum. (0.12 g, 93%).

^{13}C n.m.r. δ(CDCl$_3$): 163.762 (C-2), 150.288 (C-4), 139.513 (d, $CH_3\underline{C}$=, J=6.8 Hz), 139.481 (d, $CH_3\underline{C}$=, J=6.9 Hz), 135.033 (C-6), 113.887 (CH_2=), 111.539 (C-5), 84.662 (C-1'), 82.170 (d, C-4', J=7.9 Hz), 71.340 (d, $C\underline{C}H_2O$, J=7.7 Hz), 71.287 (d, $C\underline{C}H_2O$, J=7.6 Hz), 66.252 (d, C-5', J=5.5 Hz), 60.077 (C-3'), 37.467 (C-2'), 18.897 ($\underline{C}H_3C$=), 12.412 (base CH_3).

^{31}P n.m.r. δ(CDCl$_3$) : 0.286

^{1}H n.m.r. δ(CDCl$_3$): 9.477 (1H, NH), 7.377 (1H, d, H-6, J=1.26 Hz), 6.212 (1H, t, H-1' J=6.56 Hz), 5.01 (2H, m, CH_2=), 4.93 (2H, m, CH_2=), 4.45 (4H, m, CCH_2O), 4.357 - 4.271 (3H, m, H-5', H-3'), 4.00 (1H, m, H-4'), 2.41 (1H, m, H-2'), 2.27 (1H, m, H-2'), 1.988 (3H, d, base CH_3, J=1.20 Hz), 1.739 (3H, s, CH_3C=), 1.728 (3H, s, CH_3C=).

F.A.B. m/e: 460 (MH_5^+, 0.22%), 459 (MH_4^+, 4.95%), 250 ($AZT.H^+ - H_2O$, 3.9%), 207 ($C_8H_{14}O_3POH_2^+$, 0.21%), 56 ($CH_3CC_2H_4^+$, 100%).

Analysis: $C_{18}H_{26}N_5O_7P$ requires: C 45.47%; H 5.75; N 15.38. Found: C 45.68; H 5.45; N 14.35.

Bis (1-methylcarboxy-1-methyl) phosphorochloridite <u>127</u>.

S-methyl lactate (7.58 g, 6.95 ml, 0.07 mol) and triethylamine (7.37 g, 10.2 ml, 0.07 mol) in diethyl ether (80 ml) was added to phosphorus trichloride (5.00 g, 3.66 ml, 0.036 mol) in diethyl ether (20 ml) at -70 °C over 15 min with stirring. The reaction mixture was kept at this temperature for 30 min. then allowed to warm to room temperature and stirred for a further 30 min. Diethyl ether (200 ml) was added and the reaction filtered and concentrated under reduced pressure (11.2 g).

^{31}P n.m.r. δ(CDCl$_3$) : A: 176.444, 3%; B: 162.367, 8%; C: 136.95, 89%.

^{31}P n.m.r. δ(CDCl$_3$) : (^{1}H coupled spectra). A: 176.4 (d); B: 162.50 (t); C: 136.4 (q).

The oil was distilled under reduced pressure; 3 fractions were collected.

56 °C/0.01 mm Hg <u>128</u> (0.39 g, 5%).

^{13}C n.m.r. δ(CDCl$_3$): 170.576 (d, C(O), J=3.3 Hz), 72.644 (d, $CH_3\underline{C}H$, J= 4.8 Hz), 52.623 ($\underline{C}H_3OC$), 19.387 (d, $\underline{C}H_3CH$, J=3.7 Hz).

^{31}P n.m.r. δ(CDCl$_3$) : 176.729, 97%; 162.305, 3%.

^{1}H n.m.r. δ(CDCl$_3$): 5.06 (1H, m, CH), 3.669 (3H, s, CH_3OC), 1.480 (3H, d, $C\underline{H}_3CH$, J_{CH}=6.91 Hz).

105 °C/0.01 mm Hg 127. (2.10 g, 21%).

^{13}C n.m.r. δ($CDCl_3$): 171.196 (d, C(O), J=2.8 Hz), 70.612 (d, $CH_3\underline{C}H$, J= 4.0 Hz), 52.420 ($\underline{C}H_3OC$), 19.387 (d, $\underline{C}H_3CH$, J=3.7 Hz).

^{31}P n.m.r. δ($CDCl_3$) : 176.702 1%; 162.313 93%; 136.570 6%.

^{1}H n.m.r. δ($CDCl_3$): 4.88 (1H, m, CH), 4.79 (1H, m, CH), 3.684 (6H, s, CH_3OC), 1.46 (6H, m, $C\underline{H}_3CH$).

140 °C/0.01 mm Hg 129. (16.3 g, 66%).

^{13}C n.m.r. δ($CDCl_3$): 172.252 (d, C(O), J=2.9 Hz), 67.694 (d, $CH_3\underline{C}H$, J= 4.4 Hz), 51.939 ($\underline{C}H_3OC$), 19.611 (d, $\underline{C}H_3CH$, J=3.3 Hz).

^{31}P n.m.r. δ($CDCl_3$) : 162.627, 2%; 136.627, 94%; 5.232, 2%, -5.98, 2%.

^{1}H n.m.r. δ($CDCl_3$): 5.06 (3H, m, CH), 3.669 (9H, s, CH_3OC), 1.480 (9H, d, $C\underline{H}_3CH$, J_{CH}=6.91 Hz).

Attempted synthesis of 3'-Azido-3'-deoxythymidine-5'-bis (1-methylcarboxy-1-methyl) phosphite 130.

127 (0.33 g, 0.23 ml, 0.001 mol) was added to a vigorously stirred solution of **10** (0.16 g, 0.59 mmol) and triethylamine (1.21 g, 0.17 ml, 0.001 mol) in tetrahydrofuran (4 ml) at room temperature under nitrogen. After stirring for 2 h.the reaction was concentrated to an oil under reduced pressure and dissolved in dichloromethane (25 ml). The solution was washed with saturated sodium bicarbonate solution (10 ml), then water (10 ml), dried over magnesium sulphate (*ca.* 5 g) filtered and concentrated under reduced pressure. The product was purified by flash column chromatography (silica *ca.* 90 g) eluted with 1% methanol in chloroform. Pooling and evaporation of appropriate fraction afforded an oily gum (0.19 g).

^{13}C n.m.r. δ($CDCl_3$): 170.036 ($\underline{C}$(O)CH), 169.849 ($\underline{C}$(O)CH), 163.244 (C-2), 149.969 (C-4), 135.290 (C-6), 135.117 (C-6), 111.552 (C-5), 111.512 (C-5), 84.882 (C-1'), 84.845 (C-1'), 82.181 (d, C-4', J=7.5 Hz), 82.025 (d, C4', J=7.0 Hz), 71.914 (Cd, HO, J=5.5 Hz), 71.262 (d, CHO, J=6.7 Hz), 64.329 (d, C-5', J=6.2 Hz), 63.738 (d, C-5', J=6.2 Hz), 60.129 (C-3'), 59.829 (C-3'), 52.988 ($\underline{C}H_3OC$), 52.866 ($\underline{C}H_3OC$), 37.470 (C-2'), 37.375 (C-2'), 19.161 ($\underline{C}H_3CH$, J=6.9 Hz), 19.161 ($\underline{C}H_3CH$, J=7.0 Hz), 12.468 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A: 6.465, B: 5.996 A:B 1.1:1

^{31}P n.m.r. δ($CDCl_3$) : ^{1}H coupled: (J_H= 738.4 Hz, 728.3 Hz, $J_{CH,\ H\text{-}5'}$=7.5 Hz, 8.2 Hz).

^{1}H n.m.r. δ($CDCl_3$): 8.942, 8.935 (1H, s, NH), 7.390, 7.357 (1H, d, H-6, A, B, J=1.22 Hz, J=1.17 Hz), 6.174, 6.076 (1H, t, H-1', J=6.52 Hz, J=6.53 Hz, B, A), 5.17 (1H, m, CHO, A, B), 4.392 - 4.225 (3H, m, H-3', H-5'), 4.05 (1H, m, H-4'), 3.785,

3.774 (3H, m, CH_3OC), 2.40 (1H, m, H-2'), 2.32 (1H, m, H-2'), 1.916 (3H, d, base CH_3, J=0.78Hz), 1.597, 1.572 (3H, d, $C\underline{H}_3CH$, J_{CH}=3.09Hz, J_{CH}=3.81 Hz).

F.A.B. m/e: 418 (MH+, 0.05%), 292 (M+ -thymine, 0.01%), 181($C_4H_6O_3PO_3^+$, 100%), 167 ($C_4H_7O_3PO_2H_2^+$, 36.85%), 127 (thymine.H_2^+, 9.02%).

Analysis: $C_{14}H_{20}N_5O_8P$ requires: C 40.29%; H 4.83; N 16.79. Found: C 41.92; H 5.03; N 14.33.

3'-O-Methylthymidine-5'-bis (1-methylcarboxy-1-methyl) phosphate 132.

83 (1.13 g, 0.004 mol), was added to a stirred solution of **80** (0.16 g, 0.66 mmol), in tetrahydrofuran (5 ml) in the presence of *N*-methylimidazole (0.53 g, 0.52 ml, 0.007 mol). The reaction was left stirring at room temperature for five days, concentrated to a gum and water (*ca.* 20 ml) added. The resultant heterogeneous mixture was extracted with chloroform (*ca.* 2x50 ml) which was washed with saturated sodium bicarbonate solution (*ca.* 25 ml) and water (*ca.* 20 ml). The aqueous layers were combined and back extracted with chloroform (*ca.* 50 ml). The organic layers were dried over magnesium sulphate, filtered and concentrated to small volume (*ca.* 1 ml). The resultant solution was added slowly to a stirred solution of petroleum ether (bp 30-40 °C) (500 ml) and refrigerated at -25 °C overnight. The precipitate formed was dissolved in the minimum of 1% methanol in chloroform and the product purified by flash column chromatography. Pooling and evaporation of the appropriate fractions yielded a colourless gum. (0.31 g, 94%).

^{13}C n.m.r. d($CDCl_3$): 170.855 (d, $\underline{C}$(O)CH, J=3.8 Hz), 170.581 (d, $\underline{C}$(O)CH, J=4.2 Hz), 163.90 (C-2), 150.446 (C-4), 135.285 (C-6), 111.283 (C-5), 84.784 (C-1'), 82.446 (d, C-4', J=8.5 Hz), 80.625 (C-3'),72.421 (d, CHO, J=4.6 Hz), 72.288 (d, CHO, J=3.8 Hz), 67.746 (d, C-5', J=5.8 Hz), 56.833 ($\underline{C}H_3O$, C-3'), 52.545 ($\underline{C}H_3OC(O)$), 52.463 ($\underline{C}H_3OC(O)$), 36.641 (C-2'), 18.963 (d, $\underline{C}H_3CH$, J=6.5 Hz), 18.808 (d, $\underline{C}H_3CH$, J=6.8 Hz), 12.294 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -4.135.

^{1}H n.m.r. δ($CDCl_3$): 9.492 (1H, s, NH), 7.492 (1H, d, H-6, J=1.22 Hz), 6.34 (1H, m, H-1'), 5.07 (1H, dq, CHO, J_P=1.34 Hz, J_{CH3}=6.99 Hz), 4.935 (1H, dq, CHO, J_P=O.91 Hz, J_{CH3}=7.02 Hz), 4.405 (2H, m, H-5'), 4.204 (1H, quintet, H-4', $J_{H-5', H-3'}$=2.81Hz), 4.126(1H, m, H-4'), 3.781 (3H, s, $CH_3OC(O)$), 3.771 (3H, s, $CH_3OC(O)$), 3.367 (3H, s, CH_3O C-3'), 2.423 (1H, m, H-2'), 2.04 (1H, m, H-2'), 1.938 (3H, d, base CH_3, J=1.15 Hz), 1.608 (3H, dd, J_{CH}=7.83 Hz, J_P=0.84 Hz), 1.564 (3H, dd, J_{CH}=7.80 Hz, J_P=0.84 Hz).

Analysis: $C_{19}H_{29}N_2O_{12}P$ requires: C 44.25%; H 5.75; N 5.51. Found: C 44.65; H 5.23; N 5.77.

3'-O-mesylthymidine-5'-2,2,2,-trichloroethyl (methyl carboxy-1-methyl-1-methyl) phosphate 133.

85 (0.57 g, 0.43 ml, 0.002 mol) was added to 76 (0.10 g, 0.31 mmol) and *N*-methyl imidazole (0.23 g, 0.23 ml, 0.003 mol) in tetrahydrofuran (2.5 ml) with stirring at room temperature. The reaction mixture was left overnight with stirring then concentrated to dryness, dissolved in chloroform (*ca.* 30 ml) washed with saturated sodium bicarbonate solution (15 ml) and water (10 ml). The aqueous organic layers were back extracted with chloroform (15 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 3 g). Filtration and concentration to a small volume (*ca.* 3 ml) followed by precipitation from petroleum ether (bp 30-40 °C) (500 ml) overnight at -20 °C afforded an amber gum. Flash column chromatography, silica (*ca* 50 g) eluted with 2% methanol in chloroform followed by pooling and evaporation of appropriate fractions yielded the product as a glass that could be crushed to a powder (0.10 g 52%).

^{13}C n.m.r. δ($CDCl_3$): 170.563 (d, CH<u>C</u>(O), J=4.2 Hz, A). 170.498 (d, CH<u>C</u>(O), J=3.7 Hz, B), 163.617 (C-2), 150.530 (C-4), 135.021 (C-6, A), 134.934 (C-6, B), 111.734 (C-5, B) 111.681 (C-5, A), 94.766 (m, CCl_3), 83.591 (C-1', B), 83.559 (C-1', A) 79.455 (d, C-4', J=8.4 Hz, B) 79.342 (d, C-4', J=8.8 Hz, A), 77 (m, C-3', $CCl_3\underline{C}H_2O$), 73.065, (d, $CH_3\underline{C}H$, J=5.6 Hz, B), 72.986 (d, $CH_3\underline{C}H$, J=5.4 Hz, A), 64.794 (d, C-5', J=5.1 Hz, B), 64.603 (d, C-5', J=5.1 Hz, A), 52.793 ($\underline{C}H_3OC$) , 39.188 (CH_3S, B), 39.100 (CH_3S, A), 38.513 (C-2', B), 38.460 (C-2', A), 18.987 (d, $C\underline{H}_3CH$, J=6.3 Hz, A), 18.954 (d, $\underline{C}H_3CH$, J=6.8 Hz, B), 12.611 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A:-2.807, B:-3.108, A:B; 1:2.

^{1}H n.m.r. δ($CDCl_3$): 9.504 (1H, s, NH, B) 9.495 (1H, s, NH, A) 7.410, 7.340 (1H, d, H-6, J=1.26 Hz, B, J=1.25 Hz, A), 6.34 (1H, m, H-1'), 5.62 (1H, m, H-3'), 5.00 (1H, m, $CH_3C\underline{H}$), 4.693, 4.593 (2H, dd, CCl_3CH_2O, J_P=1.49 Hz, J_H=6.26 Hz, B, J_P=1.65 Hz, J_H=6.42 Hz, A), 4.50 (2H, m, H-5'), 4.350 (1H, m, H-4'), 3.778 (3H, s, $\underline{C}H_3OC$), 3.122, 3.119 (3H, s, CH_3S, B, A), 2.87 (1H, m, H-2'), 2.47 (1H, m, H-2'), 1.938 (3H, d, base CH_3, J=0.95 Hz), 1.592 (3H, dd, $C\underline{H}_3CH$, J_{CH}=6.93 Hz, J_P=0.98 Hz).

F.A.B. m/e: 620 (MH^+ 2x^{37}Cl, 0.29%), 619 (MH^+ ^{37}Cl, 1.14%), 618 (M^+ ^{37}Cl, 0.12%), 616 (M^+, 0.87%), 303 (MH^+ -$CCl_3CH_2OPO_2H$ -$C_4H_7O_3H$, 1.84%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{17}H_{24}N_2O_{12}PSCl_3$ requires: C 33.06%; H 2.80; N 3.92; Cl 17.22. Found: C 33.03; H 3.61; N 4.10; Cl 18.41.

3'-O-β-Mesylthymidine-5'-(1-methylcarboxy-1-1-dimethyl) (t-ethylcarboxy prop-2-enyl) phosphate 134.

99 (1.86 g, 0.006 mol) was added to a stirred solution of 76 (0.20 g, 0.62 mmol) and *N*-methylimidazole (0.47 g, 0.45 ml, 0.006 mol) in tetrahydrofuran (5 ml) with stirring. After 48 h. at ambient temperature with vigorous stirring the mixture was concentrated to dryness, suspended in dichloromethane (*ca.* 40 ml) and washed successively with saturated sodium bicarbonate solution (*ca.* 30 ml) and water (*ca.* 25 ml). The aqueous extracts were back-extracted with chloroform (*ca.* 20 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 4 g). Purification of the product was achieved by flash column chromatography (silica *ca.* 30 g) eluted with 2% methanol in chloroform. Pooling and evaporation afforded the product as a glass that could be crushed to a fine white powder (0.35 g, 93%).

^{13}C n.m.r. δ($CDCl_3$): 172.215 (d, $\underline{C}(O)C(CH_3)_2$, J=3.5 Hz), 165.945 ($\underline{C}(O)CH=$), 163.753 (C-2), 162.744 (m, $CH_3\underline{C}=$), 150.549 (C-4), 135.061 (C-6), 111.599 (C-5), 111.562 (C-5), 106.935 (m, CH=), 83.525 (C-1'), 83.287 (d, C-4', J=7.0 Hz), 83.241 (d, C-4', J=6.8 Hz), 79.481 (d, $CH_3\underline{C}CH_3$, J=8.1 Hz), 79.442 (d, $CH_3\underline{C}CH_3$, J=8.5 Hz), 77.828 (C-3'), 64.707 (d, C-5', J=5.3 Hz), 64.557 (d, C-5', J=5.4 Hz), 60.259 ($CH_3\underline{C}H_2OC(O)$), 52.969 ($\underline{C}H_3OC(O)$), 39.125 ($CH_3S$), 38.374 (C-2'), 26.675 (d, $\underline{C}H_3CCH_3$, J=6.7 Hz), 26.654 (d, $CH_3C\underline{C}H_3$, J=6.7Hz), 25.736 (d, $\underline{C}H_3C=$, J=3.2 Hz), 18.456 (d, $\underline{C}H_3C=$, J=4.8 Hz), 14.120 ($\underline{C}H_3CH_2OC(O)$), 12.507 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : A; -11.411, B; -11.443, A:B 9:10

^{1}H n.m.r. δ($CDCl_3$): 8.582 (1H, s, NH), 7.369 (1H, m, H-6), 6.25 (1H, m, H-1'), 5.76 ($2/_3$H, m, CH=), 5.24 (1H, m, H-3'),4.43 (2H, m, H-5'), 4.29 (1H, m, H-4'), 4.04 (2H, m, $CH_3C\underline{H}_2OC$), 3.723, 3.720 (3H, s, CH_3O), 3.055, 3.050 (3H, s, CH_3S), 2.80 (1H, m, H-2'), 2.43 (1H, m, H-2'), 2.32 (3H, m, $CH_3C=$), 1.884 (3H, d, base CH_3 J=1.24 Hz), 1.673 (3H, s, $C\underline{H}_3CCH_3$), 1.596 (3H, s, $CH_3CC\underline{H}_3$), 1.201, 1.191 (3H, t, $C\underline{H}_3CH_2OC$ J=7.16 Hz, J=7.16 Hz).

F.A.B. m/e: 634 (MNa^+, 12.28%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{22}H_{33}N_2O_{14}PS$ requires: C 43.13%; H 5.43; N 4.60. found: C 42.70; H 5.29; N 4.33.

3'-Azidothymidine-5'-bis (2,2,2-trichloroethyl) phosphate 135.

82 (0.57 g, 0.001 mol) was added to 10 (0.2 g, 0.75 mmol) in pyridine (20 ml) at room temperature. After 5 h. water (*ca.* 1 ml) was added and the reaction mixture left stirring for 20 min. The reaction was then concentrated to dryness under reduced pressure. Purification was achieved by flash column chromatography on silica (*ca.* 60 g), eluted with chloroform. Pooling and evaporation of appropriate fractions yielded a glass, mp 55-57 °C (0.40 g, 84%).

^{13}C n.m.r. $\delta(CDCl_3)$: 163.598 (C-2), 150.174 (C-4), 135.423 (C-6), 111.683 (C-5), 94.367 (d, CCl_3, J=13.4 Hz), 85.472 (C-1'), 81.861 (d, C-4', J=8.0 Hz), 77 ($CCl_3\underline{C}H_2$), 67.658 (d, C-5', J=5.9 Hz), 60.026 (C-3'), 37.121 (C-2'), 12.538 (base CH_3).

^{31}P n.m.r. $\delta(CDCl_3)$: - 3.933.

^{1}H n.m.r. $\delta(CDCl_3)$: 9.665 (1H, broad s, NH), 7.279 (1H, s, H-6), 6.145 (1H, t, H-1', J=6.78 Hz), 4.61 (4H, m, CCl_3CH_2O), 4.46 (2H, m, H-5'), 4.38 (1H, m, H-3'), 4.04 (1H, m, H-4'), 2.49 (2H, m, H-2'), 1.924 (3H, d, CH_3, J=1.32 Hz).

F.A.B. m/e: 614 (MH^+, $3x^{37}Cl$, 2%), 612 (MH^+, $2x^{37}Cl$, 4%), 611 (MH_2^+ ^{37}Cl, 2%), 610 (MH^+, ^{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 (thymine.H^+, 28%), 81 ($C_5H_5O^+$, 100%).

5'-Iodo-3'-azidothymidine <u>139</u>.

Methyl triphenoxyphosphonium iodide (0.34 g, 0.82 mmol) was added to **<u>10</u>** (0.15 g, 0.16 mmol) in tetrahydrofuran (3 ml) with stirring at room temperature. After 5 h. the reaction mixture was concentrated to dryness, dissolved in chloroform (50 ml), washed briefly with saturated sodium thiosulphate solution and then water (30 ml) and back extracted with chloroform (30 ml). The combined organic layers were dried over magnesium sulphate (*ca.* 5 g), filtered and concentrated under reduced pressure to small volume (*ca.* 2 ml). The product was crystallized from hexane (180 ml) to afford the product (0.22 g, 94%).

^{13}C n.m.r. $\delta(CDCl_3)$: 163.831 (C-2), 150.189 (C-4) , 135.879 (C-6), 111.555 (C-5), 84.939 (C-1'), 81.818 (C-4'), 63.928 (C-3'), 37.165 (C-2'), 12.597 (base CH_3), 6.664 (C-5').

^{1}H n.m.r. $\delta(CDCl_3)$: 9.604 (1H, s, NH), 7.378 (1H, d, H-6, J=1.22 Hz), 6.138 (1H, t, H-1', J=6.71 Hz), 4.14 (1H, m, H-4'), 3.71 (1H, m, H-3'), 3.46 (2H, m, H-5'), 2.42 (2H, m, H-2'), 1.917 (3H, d, base CH_3, J=1.22 Hz).

E.I.M.S. m/e: 379 (MH_2^+, 0.45%), 378 (MH^+, 15.93%), 377 (M^+, 9.12%), 252 (MH^+ -thymine, 5.95%), 209 (MH^+ -thymine -N_3H, 2.13%), 154 ($C_2H_2I^+$, 100%).

5'-Iodothymidine-3'-bis (1-methylcarboxy-1-methyl) phosphoroamidate <u>141</u>.

<u>139</u> (0.08 g, 0.21 mmol) was added to **<u>129</u>** (5 ml) at 125 °C. The mixture was kept at this temperature under reduced pressure (1mm Hg) with stirring. After 90 min. under reduced pressure nitrogen was allowed to enter the system and the hot solution poured into stirred diethyl ether (200 ml). Hexane was added (*ca.* 30 ml) and the mixture kept at -20 °C overnight. The oily precipitate was dissolved in 1% methanol in chloroform and purified by

flash column chromatography (silica *ca.* 120 g). Pooling and evaporation of the appropriate fractions afforded a gum (0.11 g).

^{13}C n.m.r. δ($CDCl_3$): 171.249 (m, CH<u>C</u>(O)), 163.631 (C-2), 151.221 (C-4), 135.247 (C-6), 112.102 (C-5), 83.519 (d, C-4', J=2.5 Hz), 83.021 (C-1'), 71.426 (d, CH_3<u>C</u>H, J=5.2Hz), 71.216 (d, CH, J=5.6 Hz), 55.551 (C-3'), 52.606 (<u>C</u>H_3OC), 52.576 (<u>C</u>H_3OC), 39.227 (d, C-2', J=6.6 Hz), 19.325 (<u>C</u>H_3CH, J=6.0 Hz), 19.141 (d, <u>C</u>H_3CH J=5.4 Hz), 12.715 (base CH_3), 9.790 (C-5').

^{31}P n.m.r. δ($CDCl_3$) : 7.152

^{1}H n.m.r. δ($CDCl_3$): 10.208 (1H, s, base NH), 7.453 (1H, d, H-6, J=1.22 Hz), 6.53 (1H, m, H-1'), 5.65 (1H, broad s, NHP), 4.968 (1H, dxq, CH, Jp=8.70 Hz, J_{CH3}=6.99 Hz), 4.823 (1H, dxq, CH, J_P=8.39 Hz, J_{CH3}=6.87 Hz), 3.770 (3H, s, CH_3O), 3.76 (1H, m, H-4' or H-3'), 3.736 (3H, s, CH_3O), 3.73 (1H, m, H-3' or H-4'), 3.59 (2H, m, H-5'), 2.259 (2H, m, H-2'), 1.924 (3H, d, base CH_3, J=1.22 Hz), 1.555 (6H, dxd <u>C</u>H_3CH, J=7.09 Hz).

5'-Iodo-3'-β-<u>O</u>-mesylthymidine <u>142</u>.

Methyltriphenoxyphosphonium iodide (0.34 g, 0.82 mmol) was added to **<u>76</u>** (0.32 g, 0.56 mmol) in tetrahydrofuran (4 ml) with stirring at room temperature. After 2 h. the reaction mixture was concentrated to dryness, dissolved in chloroform (60 ml), washed briefly with saturated sodium thiosulphate solution and then water (30 ml) and back extracted with chloroform (40 ml). The combined organic layers were dried over magnesium sulphate (*ca.* 5 g), filtered and concentrated under reduced pressure to small volume (*ca.* 3 ml). The product was crystallized from diethyl ether/hexane (*ca.* 100 ml, 1:2) to afford the product (0.24 g, 99%).

^{13}C n.m.r. δ($CDCl_3$): 163.840 (C-2), 150.651 (C-4) , 135.139 (C-6), 111.740 (C-5), 83.969 (C-1'), 82.116 (C-4'), 79.051 (C-3'), 39.507 (CH_3S), 38.984 (C-2'), 12.602 (base CH_3), -2.963 (C-5').

^{1}H n.m.r. δ($CDCl_3$): 9.720 (1H, s, NH), 7.348 (1H, d, H-6, J=1.23 Hz), 6.341 (1H, dd, H-1', $J_{H-2'}$=3.09 Hz, $J_{H-2'}$=8.4 Hz), 5.257 (1H, dd, H-3', $J_{H-4'}$=3.2 Hz, $J_{H-2'}$=5.07 Hz), 4.22 (1H, m, H-4'), 3.35 (2H, m, H-5'), 3.140 (CH_3S), 2.84 (1H, m, H-2'), 2.335 (1H, dd, H-2', $J_{H-3'}$=3.08 Hz, $J_{H-1'}$= 6.33 Hz), 1.909 (3H, d, base CH_3, J=1.23 Hz).

E.I.M.S. m/e: 432 (MH_2^+, 2.37%), 431 (MH^+, 1.14%), 306 (MH^+ -thymine 8.64%), 154 ($C_2H_3I^+$, 100%).

Analysis. $C_{11}H_{15}N_2O_6IS$ requires: C 30.71%; H 3.51; N 6.51. Found: C 31.94; H 3.29; N 5.70.

Attempted synthesis of 3'-O-β-mesylthymidine-5'-bis (1-methylcarboxy-1-methyl) phosphonate 143.

142 (0.10 g, 0.23 mmol) was added to 129 (5 ml) at 125 °C. The mixture was kept at this temperature under reduced pressure (1 mm Hg) with stirring. After 12 h. under these conditions the reaction mixture was heated to almost reflux (180 °C/1 mm Hg), and kept under these conditions for a further 48 h. Nitrogen was then allowed to enter the system and the hot solution poured into stirred diethyl ether (200 ml) . Hexane (*ca.* 30 ml) was added and the mixture kept at -20 °C overnight. The oily precipitate was dissolved in 1% methanol in chloroform. Flash column chromatography (silica *ca.* 120 g) failed to afford the product.

3'-O-Methylthymidine-5'-bis (2,2,2-trichloroethyl) phosphate 145.

82 (0.35 g, 0.93 mmol) was added to a stirred solution of 80 (0.15 g, 0.62 mmol) in pyridine (8 ml) at room temperature and left stirring overnight. The reaction was concentrated to dryness under reduced pressure and the product purified by flash column chromatography (silica *ca.* 40 g) eluted with chloroform. (0.32 g, 87%).

^{13}C n.m.r. δ($CDCl_3$): 163.409 (C-2), 150.043 (C-4), 135.012 (C-6), 111.234 (C-5), 94.151 (d, CCl_3, J=10.4 Hz), 85.022 (C-1'), 81.922 (d, C-4', J=7.8 Hz), 80.009 (C-3'), 77 ($CCl_3\underline{C}H_2$), 68.312 (d, C-5', J=6.2 Hz), 56.888 (CH_3O), 36.315 (C-2'), 12.268 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -5.459.

^{1}H n.m.r. δ($CDCl_3$): 9.158 (1H, s, NH), 7.313 (1H, s, H-6), 6.221 (1H, dt, H-1', $J_{H\text{-}2'}$=6.32 Hz, $J_{H\text{-}6}$=1.29Hz), 4.65 (4H, m, CCl_3CH_2), 4.41 (2H, m, H-5'), 4.16 (CALEB1H, m, H-4'), 4.02 (1H, m, H-3'), 3.330 (3H, s, CH_3O), 2.40 (1H, m, H-2'), 2.13 (1H, m, H-2'), 1.909 (3H, s, base CH_3).

E.I.M.S. m/e: 481 (MH^+ 5x^{37}Cl -thymine.H^+, 0.04%), 479 (MH^+ 4x^{37}Cl -thymine.H^+, 0.05%), 477 (MH^+ 3x^{37}Cl -thymine.H^+, 0.02%), 475 (MH^+ 2x^{37}Cl -thymine.H^+, 0.13%), 473 (MH^+ ^{37}Cl -thymine.H^+, 0.2%), 471 (MH^+ -thymine.H^+, 0.06%), 449 (MH^+ 5x^{37}Cl -thymine.H^+ -CH_3OH, 0.03%), 447 (MH^+ 4x^{37}Cl -thymine.H^+ -CH_3OH, 0.06%), 445 (MH^+ 3x^{37}Cl -thymine.H^+ -CH_3OH, 0.11%), 443 (MH^+ 2x^{37}Cl -thymine.H^+ -CH_3OH, 0.51%), 441 (MH^+ ^{37}Cl -thymine.H^+ -CH_3OH, 0.61%), 439 (MH^+ -thymine.H^+ -CH_3OH, 0.24%), 421 (MH^+ 2x^{37}Cl -CH_3OH -CCl_3CH_2OH, 0.25%), 419 (MH^+ ^{37}Cl -CH_3OH -CCl_3CH_2OH, 1.10%), 417 (MH^+ -CH_3OH -CCl_3CH_2OH, 1.21%), 255 (methylthymidine -H, 0.07%), 239 (methylthymidine.H^+ -H_2O, 2.43%), 82 ($C_5H_5OH^+$, 20.54%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{15}H_{19}N_2O_8PCl_6$ requires: C 30.08%; H 3.20; N 4.68; Cl 35.51. Found: C 30.66; H 3.10; N 4.47; Cl 35.60.

Thymidine-5'-bis (2,2,2-trichloroethyl) phosphate 146.

82 (3.44 g, 0.009 mol) was added to a stirred solution of 78 (2.00 g, 0.008 mol) in pyridine (20 ml) at -20 °C. The reaction was left stirring at this temperature for 5 h. and refrigerated at -40 °C for 2 days. The reaction was quenched with water (*ca.* 1 ml) and the mixture concentrated to dryness azeotroping with toluene (*ca.* 3x10 ml). The gum so produced was purified by flash column chromatography (silica *ca.* 60 g) eluted with 2% methanol in chloroform. Pooling and evaporation of the appropriate fractions gave a glass that could be crushed to a fine white powder mp 139 - 141 °C (3.42 g, 66%).

^{13}C n.m.r. δ(CD_3OD)CD_3OD: 165.364 (C-2), 151.304 (C-4), 136.955 (C-6), 111.090 (C-5), 95.071 (d, CCl_3, J=10.7 Hz), 85.737 (C-1'), 84.970 (d, C-4', J=7.0 Hz), 77.596 (d, $CCl_3\underline{C}H_2$, J=4.2 Hz), 70.858 (C-3'), 69.184 (d, C-5',J=6.2 Hz), 39.511 (C-2'), 11.771 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -4.970.

Analysis: $C_{14}H_{17}N_2O_8PCl_6$ requires: C 28.74%; H 2.93; N 4.79; P 5.29; Cl 36.36. Found: C 29.01; H 2.99; N 4.53; P 5.48; Cl 36.32.

3'-Acetylthymidine-5'-bis (2,2,2-trichloroethyl) phosphate 147.

146 (0.20 g, 0.34 mmol) was dissolved in pyridine (10 ml), freshly distilled Acetic anhydride (0.18 g, 0.12 ml, 0.002 mol) was added and the reaction mixture left stirring overnight. The pyridine was azeotropically removed under reduced pressure with toluene (*ca.* 3x15 ml) and the gum dissolved in water (*ca.* 10 ml) was left stirring at room temperature for 20 min. The resultant suspension was washed with chloroform (3x30 ml) and the organic fractions combined and evaporated. The product was purified by flash column chromatography (silica *ca.* 60 g) eluted with chloroform, pooling and evaporating the appropriate fractions afforded a gum (0.11 g, 51%).

^{13}C n.m.r. δ($CDCl_3$): 174.546 ($CH_3\underline{C}(O)$), 163.642 (C-2), 150.646 (C-4), 134.863 (C-6), 111.948 (C-5), 94.332 (d, CCl_3, J=12.3 Hz), 84.643 (C-1'), 82.563 (d, C-4', J=7.8 Hz), 77 ($CCl_3\underline{C}H_2$), 74.104 (C-3'), 68.481 (d, C-5', J=6.2 Hz), 37.120 (C-2'), 20.967 ($\underline{C}H_3C(O)$), 12.559 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -5.569.

^{1}H n.m.r. δ($CDCl_3$): 9.976 (1H, s, NH), 7.495 (1H, d, H-6, J=1.18 Hz), 6.34 (1H, m, H-1'), 5.271 (1H, m, H-3'), 4.67 (4H, m, CCl_3CH_2), 4.57 (2H, m, H-5'), 4.10 (1H, m, H-4'), 2.40 (1H, m, H-2'), 2.28 (1H, m, H-2'), 1.931 (3H, s, base CH_3), 2.062 (3H, s, $CH_3C(O)$).

F.A.B. m/e: ($NO_2C_6H_4CH_2OH$/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, 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^+ -CH_3CO_2H -$(CCl_3CH_2O)_2PO_2H$, 8%), 127 (thymine.H^+, 10%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{16}H_{19}N_2O_9PCl_6$ requires: C 29.86%; H 3.05; N 4.35; P 4.81; Cl 33.93. Found: C 30.28; H 2.98; N 3.85; P 4.76; Cl 34.25.

3'- Propanoylthymidine-5'-bis (2,2,2-trichloroethyl) phosphate 148.

146 (0.20 g, 0.34 mmol) was dissolved in pyridine (10 ml), freshly distilled propanoic anhydride (0.22 g, 0.22 ml, 0.002 mol) was added and the reaction mixture left stirring overnight. The pyridine was azeotropically removed under reduced pressure with toluene (*ca.* 3x15 ml) and the gum dissolved in water (*ca.* 10 ml) was left stirring at room temperature for 20 min. The resultant suspension was washed with chloroform (3x30 ml) and the organic fractions combined and evaporated. The product was purified by flash column chromatography (silica *ca.* 60 g) eluted with chloroform, pooling and evaporating the appropriate fractions afforded a gum (0.11 g, 56%).

^{13}C n.m.r. δ($CDCl_3$): 174.054 ($CH_2\underline{C}(O)$), 163.532 (C-2), 150.461 (C-4), 134.762 (C-6), 111.984 (C-5), 95.43 (m, CCl_3), 84.547 (C-1'), 82.563 (d, C-4', J=7.8 Hz), 77 (m, $CCl_3\underline{C}H_2$), 73.913 (C-3'), 68.431 (d, C-5', J=6.2 Hz), 36.952 (C-2'), 27.329 ($\underline{C}H_2C(O)$), 12.552 (base CH_3), 8.839 ($\underline{C}H_3CH_2$).

^{31}P n.m.r. δ($CDCl_3$) : -5.583.

^{1}H n.m.r. δ($CDCl_3$): 9.206 (1H, s, NH), 7.345 (1H, d, H-6, J=1.02 Hz), 6.32 (1H, m, H-1'), 5.24 (1H, m, H-3'), 4.62 (4H, m, CCl_3CH_2), 4.56 (2H, m, H-5'), 4.12 (1H, m, H-4'), 2.40 (1H, m, H-2'), 2.322 (2H, q, $CH_2C(O)$, J=6.99 Hz), 2.24 (1H, m, H-2'), 1.899 (3H, s, base CH_3), 1.090 (3H, t, $C\underline{H}_3CH_2$, J=7.49 Hz).

Analysis: $C_{17}H_{21}N_2O_9PCl_6$ requires: C 31.85%; H 3.30; N 4.37; P4.83. Found: C 32.38; H 3.19; N 3.75; P 4.73.

3'-Butylthymidine-5'-bis (2,2,2-trichloroethyl) phosphate 149.

Freshly distilled butryl chloride (0.04 g, 0.04 ml, 0.34 mmol) was added to a stirred solution of **146** (0.20 g, 0.34 mmol) and 4-dimethylaminopyridine, (0.01 g, 0.03 mmol) in acetonitrile at 0 °C and kept at this temperature for 1 h, after which time hardly any appreciable conversion had taken place. The reaction mixture was allowed to warm to room temperature and one equivalent. of acylating reagent added. After 48 h.four more equivalents were added and after another 24 h. a further 10% equivalent. of 4-dimethylaminopyridine was added. After leaving a further 60 h.methanol was added to the reaction which was left stirring for 1 h. The mixture was concentrated to dryness under reduced pressure and the gum dissolved up in chloroform .(*ca.* 40 ml). This solution was washed with water (*ca.* 25 ml), and saturated sodium bicarbonate solution (*ca.* 20 ml). The organic layer was dried over magnesium sulphate (*ca.* 5 g) and concentrated to small volume. Purification was achieved

by flash column chromatography (silica *ca.* 60 g) eluted with 1% methanol in chloroform. Pooling and evaporation of the appropriate fractions yielded a glass that could be crushed to a powder (0.17 g, 76%).

^{13}C n.m.r. δ($CDCl_3$): 173.227 ($CH_2\underline{C}(O)$), 163.657 (C-2), 150.528 (C-4), 134.73 (C-6), 111.946 (C-5), 94.363 (CCl_3 J=10.7 Hz), 84.511 (C-1'), 82.556 (C-4', J=7.9 Hz), 77 ($CCl_3\underline{C}H_2$), 73.805 (C-3'), 68.340 (C-5'), 36.906 (C-2'), 35.761 ($\underline{C}H_2C(O)$), 18.179 ($CH_3\underline{C}H_2$), 13.555 ($\underline{C}H_3CH_2$), 12.325 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -5.394.

^{1}H n.m.r. δ($CDCl_3$): 9.591 (1H, broad s, NH), 7.431 (1H, s, H-6), 6.406 (1H, m, H-1'), 5.316 (1H, d, H-3', $J_{H\text{-}4'}$=6.74 Hz), 4.65 (4H, m, CCl_3CH_2), 4.50 (2H, m, H-5'), 4.193 (1H, m, H-4'), 2.394 (2H, t, $CH_2C(O)$, J=7.5 Hz), 2.390 (1H, m, H-2'), 2.203 (1H, m, H-2'), 1.961 (3H, s, base CH_3), 1.672 (2H, m, $CH_3C\underline{H}_2CH_2$), 0.971 (3H, t, $C\underline{H}_3CH_2CH_2$, J=7.36 Hz).

F.A.B. m/e: 663 (MH^+ 5x^{37}Cl, 18%), 662 (M^+ 5x^{37}Cl, 10%), 661 (MH^+ 4x^{37}Cl, 15%), 660 (M^+ 4x^{37}Cl, 4%), 659 (MH^+ 3x^{37}Cl, 40%), 658 (M^+ 3x^{37}Cl, 21%), 657 (M^+ 2x^{37}Cl, 78%), 656 (M^+ 2x^{37}Cl, 28%), 655 (MH^+ ^{37}Cl, 100%), 654 (M^+ ^{37}Cl, 20%), 653 (MH^+ , 54%).

Analysis: $C_{18}H_{23}N_2O_9PCl_6$ requires: C 33.00%; H 3.54; N 4.28; P 4.73; Cl 32.47. Found: C 33.45; H 3.42; N 4.49; P 4.91; Cl 32.66.

3'-Isobutylthymidine-5'-bis (2,2,2-trichloroethyl) phosphate <u>150</u>.

Isobutryl chloride (0.18 g, 0.18 ml, 0.002 mol) was added to a stirred solution of **<u>146</u>** (0.20 g, 0.34 mmol) and 4-dimethylaminopyridine, (0.03 g, 0.07 mmol), in acetonitrile at room temperature and left stirring for 42 h. Methanol (10 ml) was added to the reaction mixture which was then left stirring for 1 h. The solution was concentrated to dryness under reduced pressure and the gum dissolved up in chloroform (*ca.* 40 ml). Water was added (*ca.* 25 ml), and saturated sodium bicarbonate solution (*ca.* 20 ml) added to the washed organic layer. The organic layer was separated and dried over magnesium sulphate (*ca.* 5 g) and concentrated to dryness. Purification was achieved by crystallisation from cyclohexane (200 ml) to give the product as a white powder (0.22 g, 96%).

^{13}C n.m.r. δ($CDCl_3$): 176.810 ($CH\underline{C}(O)$), 163.485 (C-2), 150.402 (C-4), 134.813 (C-6), 112.012 (C-5), 94.405 (d, CCl_3 J=10.6 Hz), 84.589 (C-1'), 82.707 (d, C-4', J=7.7 Hz), 77 ($CCl_3\underline{C}H_2$), 73.896 (C-3'), 68.490 (d, C-5', J=6.2 Hz), 36.994 (C-2'), 33.701 ($CH_3\underline{C}HCH_3$), 18.790 ($\underline{C}H_3CHCH_3$), 18.728 ($CH_3CH\underline{C}H_3$), 12.575 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -6.207.

^{1}H n.m.r. δ($CDCl_3$): 9.136 (1H, broad s, NH), 7.434 (1H, s, H-6), 6.40 (1H, m, H-1'), 5.300 (1H, d, H-3', $J_{H\text{-}4'}$=6.59 Hz), 4.66 (4H, m, CCl_3CH_2), 4.50 (2H, m, H-5'),

4.16 (1H, m, H-4'), 2.600 (1H, septet, $CH_3C\underline{H}CH_3$, $J_{CH3,CH3}$=7.02 Hz), 1.964 (3H, s, base CH_3), 1.193 (6H, d, $C\underline{H}_3CHC\underline{H}_3$, J=6.99 Hz).

F.A.B. m/e: 663 (MH^+ 5x^{37}Cl, 18%), 662 (M^+ 5x^{37}Cl, 8%), 661 (MH^+ 4x^{37}Cl, 19%), 660 (M^+ 4x^{37}Cl, 2%), 659 (MH^+ 3x^{37}Cl, 42%), 658 (M^+ 3x^{37}Cl, 19%), 657 (MH^+ 2x^{37}Cl, 76%), 656 (M^+ 2x^{37}Cl, 28%), 655 (MH^+ ^{37}Cl, 100%), 654 (M^+ ^{37}Cl, 20%), 653 (MH^+, 56%).

Analysis: $C_{18}H_{23}N_2O_9PCl_6$ requires: C 33.00%; H 3.54; N 4.28; P 4.73; Cl 32.47. Found: C 33.52; H 3.69; N 4.16; P 4.79; Cl 32.10.

3'-Chloroacetylthymidine-5'-bis (2,2,2-trichloroethyl) phosphate <u>151</u>.

Chloroacetyl chloride (0.19 g, 0.14 ml, 0.002 mol) was added to a stirred solution of **<u>146</u>** (0.20 g, 0.34 mmol) and 4-dimethylaminopyridine (0.03 g, 0.07 mmol) in acetonitrile (5 ml). After leaving at room temperature with stirring for 60 h., methanol (*ca.* 5 ml) was added and the reaction stirred for 20 min before concentrating to dryness. The gum was dissolved in the minimum of chloroform and the product purified by flash column chromatography (silica *ca.* 30 g) eluted with 2% methanol in chloroform. Pooling and evaporation of appropriate fractions gave a gum. This was dissolved in dichloromethane (*ca.* 20 ml), washed with saturated sodium bicarbonate solution (*ca.* 15 ml), water (*ca.* 20 ml) and dissolved in isopropanol (*ca.* 2 ml). Filtration and concentration under reduced pressure afforded a glass that could be crushed into a fine white powder (0.20 g, 86.3%).

^{13}C n.m.r. δ($CDCl_3$): 167.029 ($ClCH_2\underline{C}(O)$), 163.403 (C-2), 150.407 (C-4), 134.783 (C-6), 112.108 (C-5), 94.467 (d, CCl_3, J=10.6 Hz), 84.869 (C-1'), 82.292 (d, C-4', J=7.9 Hz), 77 ($CCl_3\underline{C}H_2$), 75.905 (C-3'), 68.284 (d, C-5', J=6.1 Hz), 40.458 (CH_2Cl), 36.901 (C-2'), 12.537 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -5.183

^{1}H n.m.r. δ($CDCl_3$): 9.239 (s, 1H, NH), 7.338 (1H, d, H-6, J=1.25 Hz), 6.32 (1H, m, H-1'), 5.36 (1H, m, H-3'), 4.65 (4H, m, CCl_3CH_2), 4.47 (2H, m, H-5'), 4.22 (1H, m, H-4'), 4.061 (2H, s, CH_2Cl), 2.48 (1H, m, H-2'), 2.25 (1H, m, H-2'), 1.890 (3H, d, base CH_3, J=1.18 Hz).

F.A.B. m/e: 661 (MH^+, 1%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{19}H_{29}N_2O_{12}P$ requires: C 29.05%; H 2.74; N 4.24; Cl 37.52. Found: C 34.01; H 4.21; N 4.15; Cl 33.26.

3'-Trichloroacetylthymidine-5'-bis (2,2,2-trichloroethyl) phosphate <u>152</u>.

Trichloroacetyl chloride (0.31 g, 0.20 ml, 0.002 mol), was added to a stirred solution of **<u>146</u>** (0.20 g, 0.34 mmol) and 4-dimethylaminopyridine (0.26 g, 0.07 mmol) in acetonitrile (5 ml) at room temperature. After leaving at ambient temperature for 84 h.saturated sodium bicarbonate solution (*ca.* 5 ml) was added and the reaction concentrated under reduced

pressure. The oil was dissolved up in chloroform (*ca.* 200 ml) and washed with water (2x200 ml). The organic layer was concentrated to a foam (0.20 g, 73%).

^{13}C n.m.r. δ($CDCl_3$): 163.661 (C-2), 161.463 ($Cl_3C\underline{C}(O)$), 150.319 (C-4), 134.683 (C-6), 112.008 (C-5), 94.265 (d, CCl_3, J=10.6 Hz), 88.849 (CCl_3), 85.059 (C-1'), 81.665 (d, C-4', J=7.9 Hz), 77.911 (C-3'), 77 ($CCl_3\underline{C}H_2$), 68.085 (d, C-5', J=5.9 Hz), 36.457 (C-2'), 12.482 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -5.227.

^{1}H n.m.r. δ($CDCl_3$): 9.660 (1H, s, NH), 7.331 (1H, d, H-6, J=O.87 Hz), 6.32 (1H, m, H-1'), 5.473 (1H, d, H-3', $J_{H\text{-}4'}$=6.6 Hz), 4.64 (4H, m, CCl_3CH_2), 4.62 (2H, m, H-5'), 4.276 (1H, t, H-4', $J_{H\text{-}5'}$=2.52 Hz), 2.53 (1H, m, H-2'), 2.40 (1H, m, H-2'), 1.890 (3H, s, base CH_3).

F.A.B. m/e: 611 (MH^+ ^{37}Cl, -$CHCl_3$, 3%), 609 (MH^+ -$CHCl_3$, 6%).

Analysis: $C_{16}H_{16}N_2O_9PCl_9$ requires: C 26.31%; H 2.21; N 3.84; P 4.24; Cl 43.69. Found: C 26.29; H 2.02; N 3.41; P 3.94; Cl 43.28.

3'-Formylthymidine-5-bis (2,2,2-trichloroethyl) phosphate <u>153</u>.

Formic acid (5.75 g, 4.71 ml, 0.13 mol) was added to vigorously stirred, distilled acetic anhydride (12.76 g, 11.8 ml, 0.13 mol) over a 10 minute period. The solution was kept in an oil bath at 65 °C for 1 h. and then kept in ice/water (*ca.* 5 °C). After the mixture had cooled to this temperature, <u>146</u> (0.2 g, 0.34 mmol) was added and the reaction stirred at 0-5 °C for 3 h. and at room temperature for 24 h. Methanol (*ca.* 90 ml) was added while the reaction mixture was held below 20 °C. The solution was then stirred below this temperature for a further hour. The solution was concentrated to a gum and purified by column chromatography (silica *ca.* 60 g) eluted with 3% methanol in chloroform. Further purification by chromatography (silica *ca.* 60 g) eluted with 1% methanol in chloroform afforded the product. (0.207 g, 99%). This was dissolved in warm toluene (*ca.* 3 ml). After cooling to room temperature the product was allowed to crystalise out of the solution (0.14 g, 67%).

^{13}C n.m.r. δ($CDCl_3$): 163.645 (C-2), 160.162 (HC(O)), 150.469 (C-4), 134.634 (C-6), 112.139 (C-5), 94.399 (d, CCl_3, J=10.4 Hz), 84.737 (C-1'), 82.257 (d, C-4', J=8.8 Hz), 77 ($CCl_3\underline{C}H_2$), 73.722 (C-3'), 68.185 (d, C-5', J=6.1Hz), 36.902 (C-2'), 12.597 (base CH_3).

^{31}P n.m.r. δ($CDCl_3$) : -5.112.

^{1}H n.m.r. δ($CDCl_3$): 8.885 (1H, broad s, NH), 8.003 (1H, s, HC(O)), 7.338 (1H, d, H-6 $J_{H\text{-}1'}$=1.16 Hz), 6.31 (1H, m, H-1'), 5.399 (1H, d, H3', $J_{H\text{-}4'}$=6.74 Hz), 4.70 (4H, m, CCl_3CH_2), 4.55 (2H, m, H-5'), 4.184 (1H, t, H-4', $J_{H\text{-}5'}$=2.73 Hz), 2.48 (1H, m, H-2'), 2.28 (1H, m, H-2'), 1.894 (3H, d, CH_3, J=1.12 Hz).

F.A.B. m/e: 619 (MH^+ $4x^{37}Cl$, 17%), 618 (M^+ $4x^{37}Cl$, 2%), 617 (MH^+ $3x^{37}Cl$, 53%), 616 (M^+ $3x^{37}Cl$, 19%), 615 (MH^+ $2x^{37}Cl$, 98%), 614 (M^+ $2x^{37}Cl$, 34%), 613 (MH^+ ^{37}Cl, 100%), 612 (M^+ ^{37}Cl, 22%), 611 (MH^+, 78%).
Analysis: $C_{15}H_{17}N_2O_9PCl_6$ requires: C 29.39%; H 2.80; N 4.57; P 5.05; Cl 34.70. Found: C 30.22; H 2.75; N 4.26; P 4.90; Cl 34.49.

Propyl 2,2,2-trichloroethyl phosphorochloridate 154.
2,2,2-trichloroethanol (0.31 g, 0.20 ml, 0.02 mol) and triethylamine (2.12 g, 2.93 ml, 0.02 mol) dissolved in diethyl ether (50 ml) were added dropwise over a 4 h.period to **53** (3.70 g, 3.33 ml, 0.02 mol), in diethyl ether (50 ml) at -55 °C. After leaving at this temperature for a further 4 h. and overnight at room temperature hexane (200 ml) was added and the reaction mixture filtered and concentrated under reduced pressure. The product was distilled (80 °C/0.07 mm Hg) to afford a colourless oil. (5.50 g, 91%).
^{13}C n.m.r. δ($CDCl_3$): 93.832 (d, CCl_3 J=13.2 Hz), 77.136 (d, $CCl_3\underline{C}H_2$, J=9.2 Hz), 72.325 ($CH_3CH_2\underline{C}H_2$, J=6.3 Hz), 23.080 ($CH_3\underline{C}H_2CH_2$,J=7.3 Hz), 9.170 (CH_3).
^{31}P n.m.r. δ($CDCl_3$) : 1.943.
^{1}H n.m.r. δ($CDCl_3$): 4.50 (2H, m, CCl_3CH_2), 4.11 (2H, m, $CH_3CH_2C\underline{H}_2$), 1.69 (2H, sextet, $CH_3C\underline{H}_2CH_2$, J=6.1 Hz), 0.92, 0.91 (3H, t, $C\underline{H}_3CH_2CH_2$, J=5.7 Hz, J=5.9 Hz).

3'-Azidothymidine-5'-propyl 2,2,2-trichloroethyl phosphate 155.
154 (0.81 g, 0.81 ml, 0.003 mol) was added to **10** (0.25 g, 0.94 mmol) and *N*-methylimidazole (0.61 g, 0.55 ml, 0.007 mol) in tetrahydrofuran (5 ml) with stirring. After 2 days the mixture was concentrated to dryness, dissolved in chloroform (30 ml) and washed with saturated sodium bicarbonate solution (15 ml) and water (10 ml). The aqueous layers were back extracted with chloroform (15 ml) and the combined organic layers dried over magnesium sulphate (*ca.* 5 g). Filtration and concentration under reduced pressure to a small volume (*ca.* 3 ml) followed by precipitation from petroleum ether (bp 30-40 °C) (500 ml) overnight at -20 °C gave an amber gum. Flash column chromatography (silica *ca.* 60 g), eluted with 0.5% methanol in chloroform followed by pooling and evaporation of the appropriate fractions afforded a gum. This was dissolved in isopropanol, (*ca.* 3 ml) filtered and concentrated under reduced pressure to afford a glass (0.23 g, 48%).
^{13}C n.m.r. δ($CDCl_3$): 163.706 (C-2, A), 163.683 (C-2, B), 150.259 (C-4), 135.211 (C-6), 111.644 (C-5, A), 111.663 (C-5, B), 94.534 (d, $\underline{C}Cl_3CH_2$, J=12.9 Hz), 94.142 (d, $\underline{C}Cl_3CH_2$, J=12.2 Hz), 85.034 (C-1', A), 84.946 (C-1', B), 82.084 (d, C-4', J=7.9 Hz), 77.216 (d, $CCl_3\underline{C}H_2$, A, J=4.3 Hz), 77.145 (d $CCl_3\underline{C}H_2$, B, J=4.3 Hz), 70.745 (d, $CH_3CH_2\underline{C}H_2$, A, J=6.1 Hz), 70.708 (d, $CH_3CH_2\underline{C}H_2$, B, J=5.9 Hz), 66.877

(d, C-5', J=5.8 Hz), 60.076 (C-3'), 37.443 (C-2', A), 37.406 (C-2', B), 23.574 (d, $CH_3\underline{C}H_2CH_2$, J=6.9 Hz), 12.534 (base CH_3), 9.914 ($\underline{C}H_3CH_2$).

^{31}P n.m.r. δ($CDCl_3$) : A: -2.638 B: -2.178. A:B; 3:2.

^{1}H n.m.r. δ($CDCl_3$): 9.320 (1H, s, NH) , 7.325, 7.277 (1H, d, H-6, A, B, J=1.23 Hz, J=1.21 Hz), 6.23 (1H, m, H-1'), 4.62 (2H, m, CCl_3CH_2), 4.38 (3H, m, H-3', H-5'), 4.15 (2H, m, $CH_3CH_2C\underline{H}_2$), 4.06 (1H, m, H-4'), 2.46 (1H, m, H-2'), 2.36 (1H, m, H-2'), 1.95 (3H, m, base CH_3), 1.76 (2H, m, $CH_3C\underline{H}_2CH2$), 0.981 (3H, m, $C\underline{H}_3CH_2$).

E.I.M.S. m/e: 521 (M^+ ^{37}Cl, 1.08%), 519 (M^+, 1.30%), 476 (M^+ -C_3H_7, 4.2%), 396 (M^+ ^{37}Cl, -thymine, 0.70%), 394 (M^+ -thymine, 0.77%), 372 (M^+ -CCl_3CH_2O, 0.1%), 353 (396 -N_3H, 2.11%), 351 (394 -N_3H, 2.74%), 329 (372 -N_3H, 2.41%), 271 (MH_3O^+ -AZT, 5.62%), 263 (M^+ -CCl_3CH_2 -thymine, 6.24%), 250 ($C_{10}H_{12}N_5O_3^+$, 11.45%), 211 ($CCl_3CH_2OPO_2H^+$, 6.7%), 195 ($CCl_3CH_2OPOH^+$, 9.8%), 126 (thymine.H^+, 35.4%), 99 ($C_5H_7O_2^+$, , 100%), 81 ($C_5H_5O^+$).

F.A.B. 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^+ ^{37}Cl, 2%), 522 (MH^+ ^{37}Cl, 9%), 521 (MH_2^+, 3%), 520 (MH^+, 9%), 250 (MH^+ -$CCl_3CH_2O(C_3H_7O)PO_2H^+$, 5%), 127 (thymine H^+, 9%), 81 ($C_5H_5O^+$, 100%).

Analysis: $C_{15}H_{21}N_5O_7PCl_3$ requires: C 34.60%; H 4.07; N 13.45; P 5.95; Cl 20.43. Found: C 36.17; H 4.51; N 12.29; P 5.72; Cl 19.88.

Attempted synthesis of 3'-azido-3'-deoxythymidine-5'-2,2,2,-trichloroethyl (ethyl carboxy-1-methyl) phosphate <u>156</u>.

<u>135</u> (0.10 g, 0.17 mmol) and caesium fluoride (0.75 g, 0.005 mol) were dissolved in **<u>59</u>** (2.00 g, 2.00 ml, 0.02 mol) at room temperature with stirring. After leaving for 256 h. the reaction mixture was evaporated to dryness under reduced pressure, dissolved in chloroform, filtered and partially purified by column chromatography. Pooling and evaporation of the appropriate fractions yielded an orange oil.

^{31}P n.m.r. δ($CDCl_3$) : -4.023, 60%; -2.377, 20% -2.563, 20%.

2',3'-Dideoxycytidine-5'-bis (2,2,2-trichloroethyl phosphate) <u>157</u>.

<u>82</u> (0.38 g, 0.002 mol) was added to a stirred solution of <u>11</u> (0.20 g, 0.001 mol) in pyridine (10 ml) at ambient temperature. After leaving for 5 h.water (*ca.* 1 ml) was added and the mixture stirred for 20 min. before concentrating to dryness (triturated with toluene 3x10 ml). Purification was achieved by flash column chromatography (silica *ca.* 80 g). Three products were eluted with R_f of 0.7 (0.038 g), 0.5 (0.034 g), 0.25 (0.052 g) respectively.

R_f 0.25: <u>157</u>.

^{13}C n.m.r. δ(CDCl$_3$): 166.423 (C-4), 156.454 (C-2), 141.587 (C-6), 95.173 (C-5), 95.102 (d, $\underline{C}Cl_3CH_2O$, J=10.8 Hz), 87.684 (C-1'), 79.534 (d, C-4', J=7.9 Hz), 77 ($CCl_3\underline{C}H_2O$), 70.272 (d, C-5', J=6.34 Hz), 32.186 (C-2'), 25.373 (C-3').

^{31}P n.m.r. δ(CDCl$_3$): -5.749.

^{1}H n.m.r. δ(CDCl$_3$): 7.698 (1H, d, H-6, J_{H-5}=7.52 Hz), 6.047 (1H, dd, H-1', $J_{H-2'}$=6.52, $J_{H-2''}$=3.65 Hz), 5.998 (1H, d, H-5, J_{H-6}=7.58 Hz), 4.62 (4H, m, CCl_3CH_2O), 4.44 (1H, m, H-4'), 4.34 (2H, m, H-5'), 2.44 (1H, m, H-2'), 2.193 -1.987 (2H, m, H-3', H-2'), 1.89 (1H, m, H-3').

F.A.B. m/e: 558 (MH^+ 3x^{37}Cl, 4%), 557 (MH_2^+, 2x^{37}Cl, 1%), 556 (MH^+, 2x^{37}Cl, 8%), 555 (MH_2^+ ^{37}Cl, 2%), 554 MH^+ ^{37}Cl, 12%), 553 (MH_2^+, 1%), 552 (MH^+, 4%), 447 (MH^+ 3x^{37}Cl, -cytosine, 2%), 445 (MH^+ 2x^{37}Cl, -cytosine, 5%), 444 (MH_2^+ ^{37}Cl, -cytosine, 1%), 443 (MH^+ ^{37}Cl, -cytosine, 6%), 441 (MH^+ -cytosine, 3%), 112 (cytosine.H^+, 100%).

Analysis. $C_{13}H_{16}N_3O_6PCl_6$ requires: C 28.19%; H 2.91; N 7.59. Found: C 30.98; H 3.49; N 5.85.

R_f 0.5: 2',3'-Dideoxycytidine-C-5 bis (2,2,2-trichloroethyl phosphoramidate) <u>158</u>.

^{13}C n.m.r. δ(CDCL3): 160.445 (d, C-2, J=6.5 Hz), 148.212 (d, C-4 J=2.7 Hz), 141.526 (C-6), 101.041 (d, C-5, J=18.4 Hz), 95.208 (d, $\underline{C}Cl_3CH_2O$, J=11.4 Hz), 87.109 (C-1'), 82.282 (C-4'), 77 ($CCl_3\underline{C}H_2O$), 62.760 (C-5'), 33.200 (C-2'), 24.328 (C-3').

^{31}P n.m.r. δ(CDCl$_3$): -2.452 5%, -12.599 95%.

^{1}H n.m.r. δ(CDCl$_3$): 8.070 (1H, dd, H-6, J_{H-5}=8.01 Hz, $J_{H-1'}$=1.68 Hz), 6.142 (1H, H-5, J_{H-6}=8.08 Hz), 6.008 (1H, dd, H-1', $J_{H-2'}$=6.74 Hz $J_{H-2''}$=3.99 Hz,), 4.593 (4H, d, CCl_3CH_2O, J_P=6.2 Hz), 4.19 (2H, m, H-5'), 4.031 (1H, dd, H-4', J=2.25 Hz, J=12.01 Hz), 3.733 (1H, dd, OH, $J_{H-5'}$=3.31 Hz), 2.43 (1H, m, H-2'), 2.10 (1H, m, H-3'), 2.056 - 1.872 (m, 2H, H-3', H-2').

Analysis. $C_{13}H_{16}N_3O_6PCl_6$ requires: C 28.19%; H 2.91; N 7.59. Found: C 24.79; H 2.80; N 6.24.

R_f 0.70: . 2',3'-Dideoxycytidine-5'-bis (2,2,2-trichloroethyl phosphate) -C-5 bis (2,2,2-trichloroethyl phosphoramidate) <u>159</u>

^{13}C n.m.r. δ(CDCL3): 159.675 (d, C-4, J=5.9 Hz), 147.653 (d, C-2, J=1.9 Hz), 140.227 (C-6), 101.676 (d, C-5, J=18.9 Hz), 95.120 (d, $(\underline{C}Cl_3CH_2O)_2OPNH$, J=11.3 Hz), 94.443 (d, $\underline{C}Cl_3CH_2O)_2OPO$, J=10.9 Hz), 86.986 (C-1'), 78.922 (d, C-4', J=7.6 Hz), 77 ($CCl_3\underline{C}H_2O$), 69.109 (d, C-5', J=6.04 Hz), 32.624 (C-2'), 25.043 (C-3').

^{31}P n.m.r. δ(CDCl$_3$): -6.443 (C-5'OP), -13.539 (NH-P).

^{1}H n.m.r. δ($CDCl_3$): 7.686 (1H, dd, H-6, J_{H-5}=8.05 Hz, $J_{H-1'}$=2.03 Hz), 6.12 (1H, m, H-5), 6.014 (1H, dd, H-1', $J_{H-2'}$=6.52 Hz, $J_{H-2''}$=4.00 Hz), 4.64 (4H, m, $(CCl_3CH_2O)_2OPO$), 4.592 (4H, d, $(CCl_3CH_2O)_2OPN$ Jp=6.27 Hz), 4.48 (1H, m, H-4'), 4.38 (2H, m, H-5'), 2.49 (1H, m, H-2'), 2.297 -2.155 (2H, m, H-2', H-3'), 1.98 (1H, m, J-3').

F.A.B. m/e: 899 (M^+ 4x^{37}Cl, 37%), 897 (M^+ 3x^{37}Cl, 76%), 895 (M^+ 2x^{37}Cl 92%), 893 (M^+ ^{37}Cl, 32%), 891 (M+, 14%), 459 $C_8H_{10}N_3O_4PCl_6$+ 3x^{37}Cl, 4%), 458 ($C_8H_9N_3O_4PCl_6^+$ ^{3x37}Cl, 31%), 457 ($C_8H_{10}N_3O_4PCl_6^+$ ^{2x37}Cl, 8%), 456 ($C_8H_9N_3O_4PCl_6^+$ ^{2x37}Cl, 79%), 455 ($C_8H_{10}N_3O_4PCl_6^+$ ^{37}Cl, 10%), 454 ($C_8H_9N_3O_4PCl_6^+$ ^{37}Cl, 100%), 453 ($C_8H_{10}N_3O_4PCl_6^+$, 8%), 452 ($C_8H_9N_3O_4PCl_6^+$, 53%),

Analysis. $C_{17}H_{19}N_3O_9P_2Cl_{12}$ requires: C 22.17%; H 2.14; N 4.69; P 6.91; Cl 47.44. Found: C 22.86; H 2.18; N 4.17; P 6.20; Cl 47.47.

Growth medium study.

The nucleotide was dissolved in DMSO at a concentration of 40 μM. This solution was diluted to 4 μM with de-ionised water for the control. Growth medium was added to the solution to give a concentration of nucleoside of 0.4 μM and solutions kept at 5 °C and 37 °C.

Stability studies: plasma

Deuterated water (1 ml) was added to lyophilized plasma solids from 5 ml of plasma. Distilled water was added to make the solution up to 5 ml. Sufficient nucleoside was added, dissolved in the minimum of methanol, to make a 40 μM solution. The solution was analysed by phosphorus-31 nmr and reverse phase hplc using the following gradient system.

A= water, B= acetonitrile

0 min.	95% A	5% B
10 min.	95% A	5% B
30 min.	50% A	50% B
45 min.	5% A	95% B

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