The Design, Synthesis and Biological Evaluation of Some Novel Phosphoramidate Prodrugs.

Felice Daverio

A thesis submitted to the faculty for degree of
PHILOSOPHIAE DOCTOR

The Welsh School of Pharmacy
Cardiff University

December 2005

Supervisor: Prof. Christopher McGuigan

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

An introduction to the work presented within this thesis involves a brief overview of nucleosides, nucleotides and nucleic acids. Descriptions of the prodrug concept and pronucleotide approaches are also given.

The work presented within this thesis describes the synthesis and biological evaluation of a number of phosphoramidate derivatives of some nucleoside analogues. This includes phosphoramidates of (E)-5-(2-bromovinyl)-2′-deoxyuridine (BVdU), 2′,3′-dideoxyadenosine (ddA), 9-β-D-arabinofuranosyl-2-fluoroadenine (F-Ara-A, fludarabine), and 2′,2′-difluorodeoxycytidine (Gemzar®, gemcitabine).

Extensive SAR studies of the anticancer lead thymectacin [phenyl-(methoxy-L-alaninyl)-BVdU phosphoramidate] revealed a significant enhancement of potency *in vitro* in colon and prostate cancer cell lines.

A small series of phosphoramidates of the anticancer agents gemcitabine and fludarabine was synthesised and evaluated for their cytotoxic activities but not significant improvement in *in vitro* activity was observed.

Finally, a series of phosphoramidate derivatives of 2′,3′-dideoxyadenosine was synthesised and tested as inhibitors of endothelium-derived hyperpolarizing factor (EDHF). This represents the first example of the application of phosphoramidate derivatives as non antiviral/anticancer agents.

Acknowledgements

A special thanks to my FAMILY: mamma, papa', Roby and Dany for supporting their "beloved and crazy" son and brother. Thank you to Carletto for the lovely chat on the phone interrupted by the evil sister Martina (COROCOROPOLLON).

I would like to thank my supervisor Prof. Christopher McGuigan for his support in the past year and for the opportunities he offered me during the PhD and PostDoc.

Thank you to all my friends and colleagues I worked with over the last few years.

A special thanks to Helen for making me smile and for allowing me to be her "LITTLE XXX".

Also thanks to Ron and Wendy, for their help and support and a nice smile in the morning. Thank you to all the technical staff for their assistance.

Common Abbreviations

3TC	2′,3′-dideoxy-3′-thiacytidine, lamivudine
5′-NT	5′-nucleotidase
5-FU	5-fluorodeoxyuridine
A	Adenine
Å	Angstrom
Ac	Acetyl
Ac_2O	Acetic anhydride
ACh	Acetylcholine
ADA	Adenosine deaminase
ADEPT	Anti-body directed prodrug enzyme
AK	Adenyl kinase
Ala	Alanine
ANP	Acyclic nucleoside phosphonate
ATP	Adenosine-5′-trisphosphate
AZMP	3′-azido-3′-deoxythymidine-5′-monophosphate
AZT	3′-azido-3′-deoxythymidine, azidothymidine
Bn	Benzyl
BnOH	Benzyl alcohol
Bu	Butyl
BVdU	(E)-5-(2-bromovinyl)-2′-deoxyuridine, brivudine
BVdUMP	(E)-5-(2-bromovinyl)-2′-deoxyuridine-5′-monophosphate
C	Cytidine
ca.	Circa
cAMP	Adenosine-3′,5′-cyclic monophosphate
CDA	Cytidine deaminase
CLL	Chronic Lymphocytic Leukemia
COSY	Correlated Spectroscopy
CTP-synthase	Cytidine triphosphate synthase
d4A	2′,3′-dideoxy-2′,3′-didehydroadenosine
d4T	2′,3′-dideoxy-2′,3′-didehydrothymidine, Stavudine
d4TMP	2′,3′-dideoxy-2′,3′-didehydrothymidine-5′-monophosphate
dATP	Deoxyadenosinetriphosphate

dCDA	Deoxycytidine deaminase
dCK	Deoxycytidine kinase
DCM	Dichloromethane
dCMPD	Deoxycytidylate deaminase
dCTP	Deoxycytidinetriphosphate
ddA	2′,3′-dideoxyadenosine
ddATP	2′,3′-dideoxyadenosine-5′-triphosphate
ddI	2′,3′-dideoxyinosine, Didanosine
ddU	2′,3′-dideoxyuridine
ddUTP	2′,3′-dideoxyuridine triphosphate
DEPT	Distortionless Enhancement by Polarization Transfer
dFCTP	Gemcitabine triphosphate
dFdC	2′,2′-difluorodeoxycytidine, Gemzar®, gemcitabine
dFdCDP	gemcitabine disphosphate
dFdCMP	Gemcitabine monophosphate
dFUMP	2′,2′-difluorodeoxyuridine monophosphate
diMeGly	dimethylglycine
DMF	N,N-dimethylformamide
DNA	Deoxyribonucleic acid
dTMP	Deoxythymidine monophosphate
EC	Effective Concentration
EDHF	Endothelium-derived hyperpolarizing factor
Et	Ethyl
F-Ara-A	9-β-D-arabinofuranosyl-2-fluoroadenine
F-ara-A-MP	Fludarabine-5′-monophosphate
F-ara-A-TP	Fludarabine trisphosphate
FDA	Food and Drug Administration
FLT	3′-deoxy-3′-fluorothymidine
G	Guanine
GDEPT	Gene-directed enzyme prodrug therapy
Gemzar®	2′,3′-difluorodeoxycytidine
GJ	Gap junction
HBV	Hepatitis B Virus
hint1	Histidine triad nucleotide-binding protein 1

HIV	Human Immunodeficiency Virus
HMQC	Heteronuclear Multiple-Quantum Coherence Experiment
HSV-1	Herpes Simplex Virus type 1
IBMX	Isobutylmethylxanthine
*i*PrOH	isopropyl alcohol
LD	Lethal Dose
LDA	Lithium diisopropylamide
L-NAME	N^G-nitro-L-arginine methyl ester
L-PhGly	L-phenylglycine
m-	Meta
Me	Methyl
MeOH	Methanol
MTT	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide
NBS	N-bromosuccinimide
NDPK	Nucleoside diphosphate kinase
NHL	non-Hodgkins's Lymphoma
NMI	N-methylimidazole
NMP	Nucleoside-5'-monophosphate
NMR	Nuclear Magnetic Resonance
o-	Ortho
p-	Para
PEG	Polyethylene glycol
Ph	Phenyl
Piv	Pivaloyl
PMEA	9-(2-phosphonylmethoxyethyl) adenine, adefovir
PMPA	(R)-9-(2-phosphonylmethoxypropyl) adenine, tenofovir
Pr	Propyl
Pro	Proline
PTSA	p-toluene sulfonic acid, para-toluene sulfonic acid
Py	Pyridine
Rf	Retention Factor

RNA	Ribonucleic acid
RR	Ribonucleotide reductase
SAR	Structure activity relationship
T	Thymidine
TEA	Triethylamine
*tert*Bu	1,1-dimethylethyl
THF	Tetrahydrofuran
TK	Thymidine kinase
TLC	Thin Layer Chromatography
TS	Thymidylate synthase
Tyr	Tyrosine
U	Uracil
Val	Valine
VZV	Varicella-Zoster Virus

Contents

Chapter One: Introduction

1.1 Nucleotides and Nucleic Acids.[1, 2]

DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid) are macromolecules formed of monomeric units called nucleotides and are responsible for the storage, transmission and expression of genetic information.

Nucleotides are phosphate esters of a pentose (monosaccharide containing 5 carbon atoms) joined covalently through a N-β-glycosidic bond to the nitrogen of a heterocyclic base. The sugar in DNA is D-deoxyribose (the 2-hydroxyl group of ribose is replaced by a hydrogen) whereas in RNA is D-ribose (Figure 1.1).

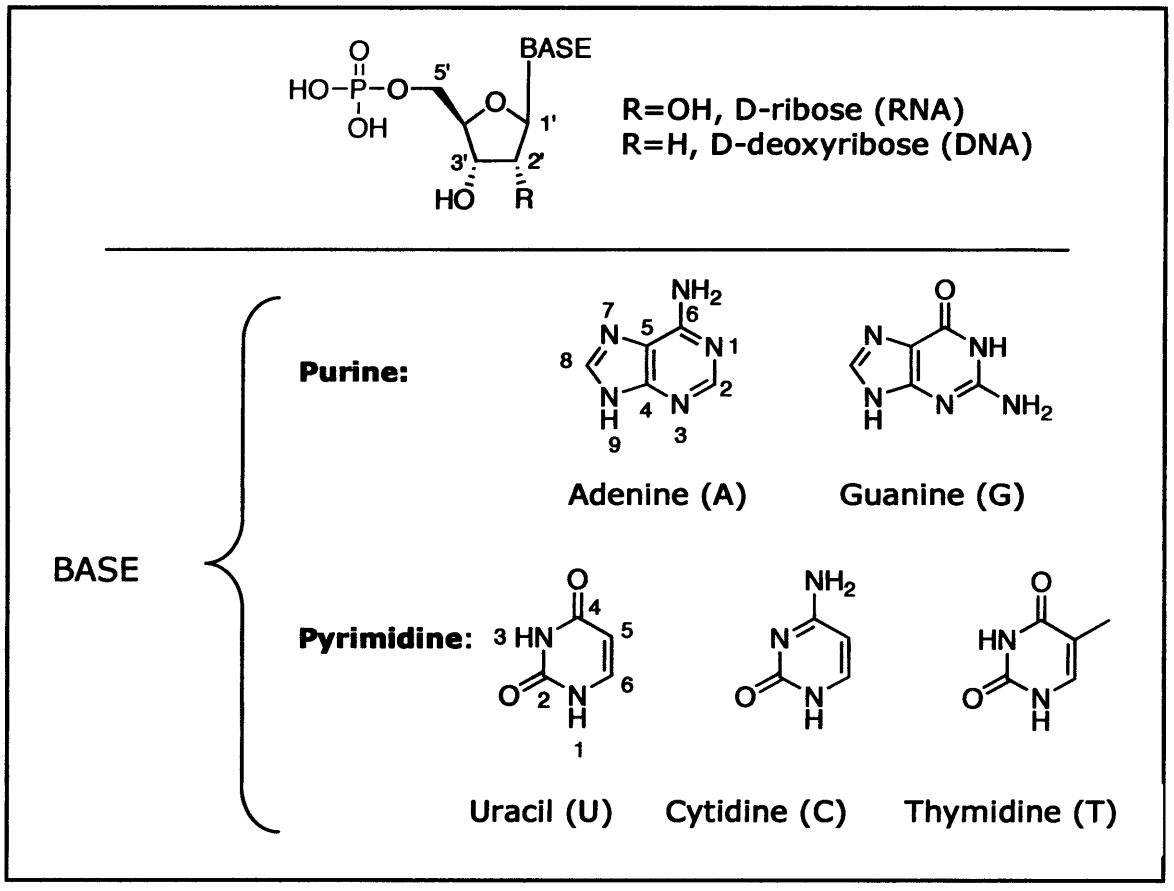


Figure 1.1: *General structure of nucleotides in DNA and RNA and major purine and pyrimidine bases of nucleic acids.*

The bases, commonly present in the structure of DNA and RNA, are derivatives of two parent compounds, purine and pyrimidine. The two purines, Adenine (A) and Guanine (G) are linked at N-9 with the 1′-position of the sugar, while the three pyrimidines, Thymine (T), Cytosine (C) and Uracil (U), form a glycosidic bond with the N-1 and the 1′-anomeric carbon of the pentose. Thymine is commonly only found in DNA, with Uracil in RNA (Figure 1.1). The unit consisting only of the base and the sugar is referred to as a nucleoside.

Nucleosides, in both DNA and RNA, are covalently linked through a phosphodiester linkage, which connects the hydroxyl on the 5′ carbon of one unit and the 3′ hydroxyl of the next. The formation of this phosphodiester linkage is catalysed by the enzyme DNA polymerase (or RNA polymerase for RNA synthesis) during the biosynthesis of DNA. The successive alternating arrangement of phosphate and pentose residues creates the covalent backbone of DNA and RNA where the bases could be considered as side groups linked to the sugar (Figure 1.2).

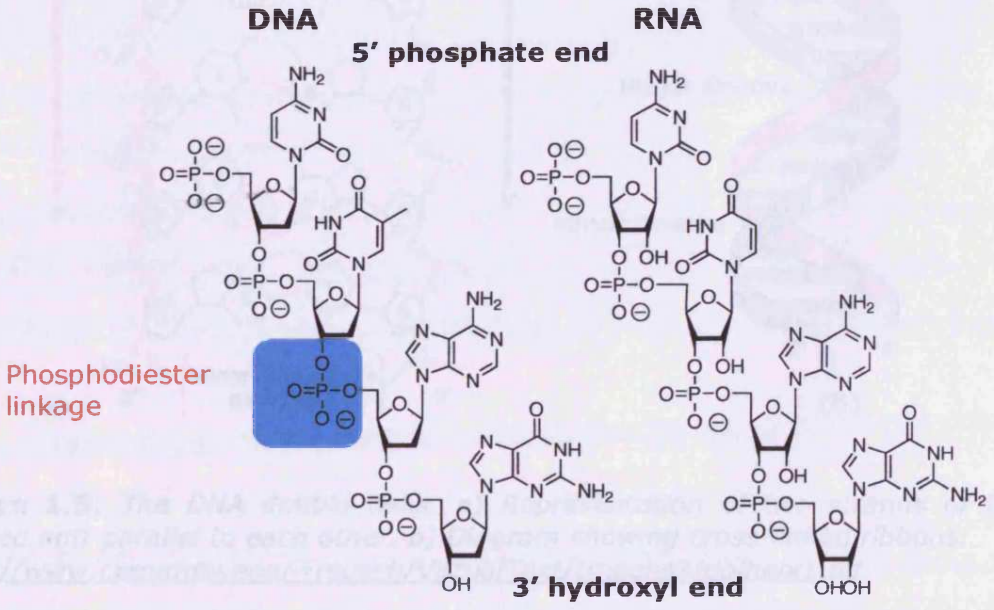


Figure 1.2: *Covalent backbone of DNA and RNA*

In 1953, James Watson and Francis Crick,[3] taking into consideration all the data available (e.g. X-ray diffraction patterns of DNA fibers from R. Franklin and M. Wilkins; Chargaff's rules) proposed a model for the structure of DNA. According to this model,[4] which proved to be essentially correct, DNA consists of two polynucleotide chains coiled together around a common axis to form a right-handed double helix. The two helical chains are aligned in an antiparallel sense, which means that one strand is orientated in the 5′→3′ direction and the other strand in the 3′→5′ direction.

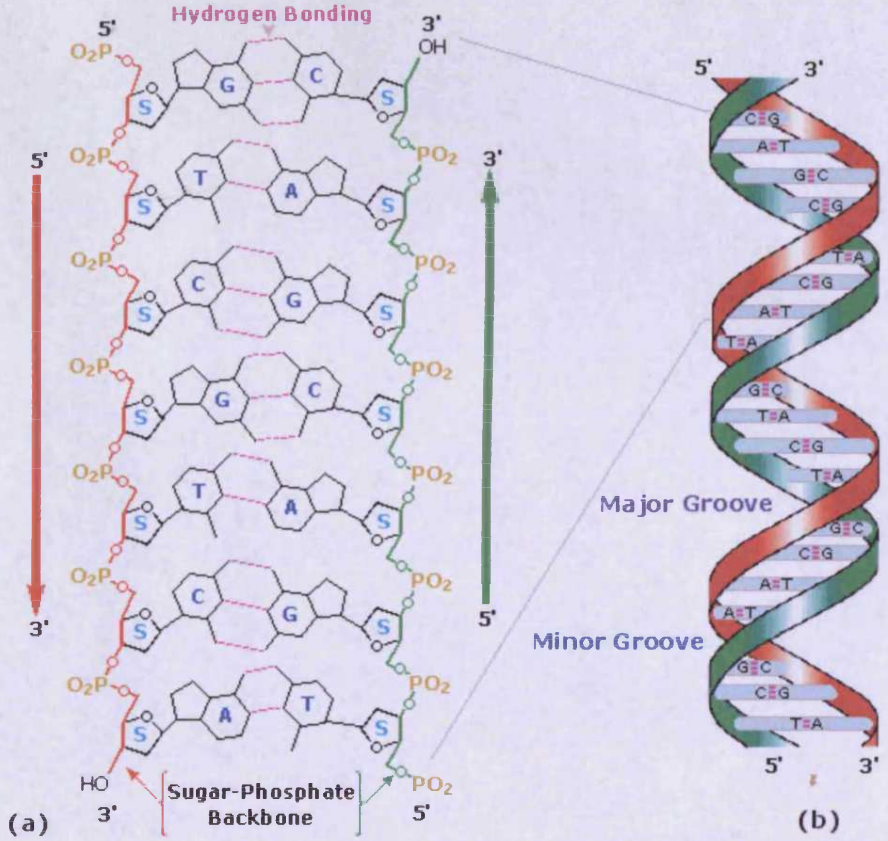


Figure 1.3: *The DNA double helix. a) Representation of two strands of DNA aligned anti-parallel to each other. b) Diagram showing cross-linked ribbons. http://www.cem.msu.edu/~reusch/VirtualText/Images3/dblhelx1.gif*

As shown in figure 1.3, the purine and the pyrimidine bases are stacked inside the double helix with their planes roughly perpendicular to the axis of the helix while the hydrophilic deoxyribose and phosphate groups are on the outside of the double helix. The double helix has a diameter of *ca.* 20Å and each complete turn includes about 10 base pairs.

The most important feature of the double-helical model of DNA structure proposed by Watson and Crick is the specificity of the base pairing which provided the rational for Chargaff's rule. In the late 1940s Chargaff[5] found that in DNAs isolated from different organisms the amount of adenine is equal to the amount of thymine, and the amount of guanine is equal to the amount cytidine. Thus, following the earlier observation of Chargaff, and taking into consideration steric and hydrogen-bonding restrictions, Watson and Crick found that adenine must pair with thymine, and guanine with cytosine. These base pairs (G-C and A-T, purine-pyrimidine) are those that fit best in the space (10.85Å) between the two nucleotide strands. Moreover, the hydrogen bonds and the Van der Waals interactions between the complementary bases, together with the hydrophobic association of the base pairs in the interior of the helix, accounts for the stability of the double helix.

The fundamental role of nucleic acids is the storage and transmission of genetic information. The DNA of cells constitutes the genome which can be replicated or transcribed to allow the expression of the genetic information, in directing the synthesis of RNA and protein molecules.

Replication, the process by which new DNA is synthesised, starts with the unwinding and separation of the two strands of the double helix creating a site called *replication fork*. In this fork, the two strands of parent DNA are used as templates for the synthesis of new daughter strands, which is catalysed by DNA polymerase.[6] The synthesis starts from a short RNA *primer* synthesised by an

RNA polymerase, and the elongation of the DNA chain proceeds in the 5'→3' direction, by addition of deoxynucleoside 5'-triphosphates at the terminal 3'-OH of the growing DNA strand. Because of the antiparallel structure of DNA, one parent strand (leading strand) can be copied continuously down its entire length in a 5' to 3' direction, while the other parent strand (lagging strand) is copied discontinuously in short fragments called Okazaki fragments (Figure 1.4).[7] DNA polymerase III is the enzyme responsible for the synthesis of both strands and has also nuclease activity with error correction capacity. DNA polymerase I removes the RNA primer and fills the gap between the Okazaki fragments. Finally, DNA ligase joins together the fragments.

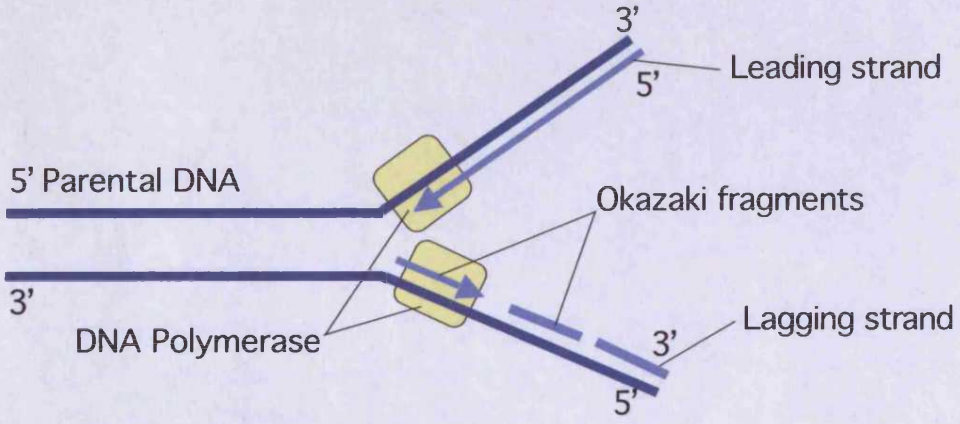


Figure 1.4: *Replication fork.*
http://personal.nbnet.nb.ca/kvhs/biology/fork.html

1.2 The Prodrug concept.

The term prodrug was introduced in 1958 by Adrien Albert[8] to define a pharmacological derivative which "must undergo chemical or enzymatic transformation to the active or parent drug after administration, so that the metabolic product or parent drug can

subsequently exhibit the desired pharmacological response". The prodrug approach aims at modifying the physico-chemical properties of a drug in an effort to improve or overcome pharmaceutical, pharmacokinetic, or pharmacodynamic barriers (i.e. chemical stability, solubility, oral absorption, taste and odor, metabolism, toxicity, duration of action, inadequate blood-brain barrier permeability, formulation problems, etc.)[9, 10] which separate the site of administration of the drug from the site of action (Figure 1.5).

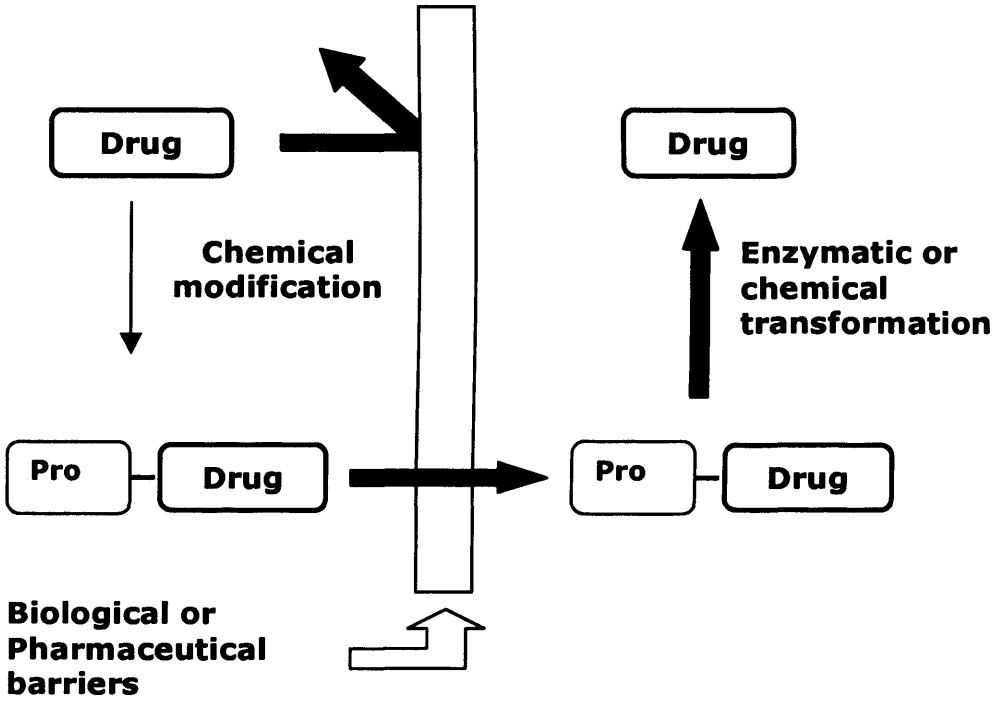


Figure 1.5*: Representation of the prodrug concept.*

Ideally, the prodrug should be readily transported to the site of action, converted to the active drug as soon as the goal is achieved, and the masking group should be rapidly eliminated after release.

A useful classification of prodrug is based on chemical criteria and according to this, prodrugs can be distinguished in four major classes:

- **Carrier-linked prodrugs**: the active drug is linked to a carrier group that can be removed enzymatically (hydrolysis, oxidation, reduction). Carrier-linked prodrugs, according to Wermuth,[11] can be further subdivided into bipartate, tripartate, and mutual prodrugs.

A *bipartate prodrug* is a prodrug with only one "masking group" (carrier), which on cleavage liberates the active drug (Figure 1.6).

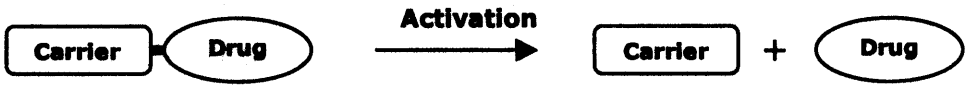


Figure 1.6: *Bipartate concept.*

A *tripartate prodrug* is a drug modified by two component "masking group" (carrier and linker). In these drugs, the carrier is connected to a linker arm, which is connected to the drug itself. The mechanism of liberation involves a first chemical or enzymatic reaction under cleavage of the carrier. The drug still remains inactive, but the first reaction activates the spontaneous cleavage of the linker arm to release the bioactive drug (Figure 1.7).

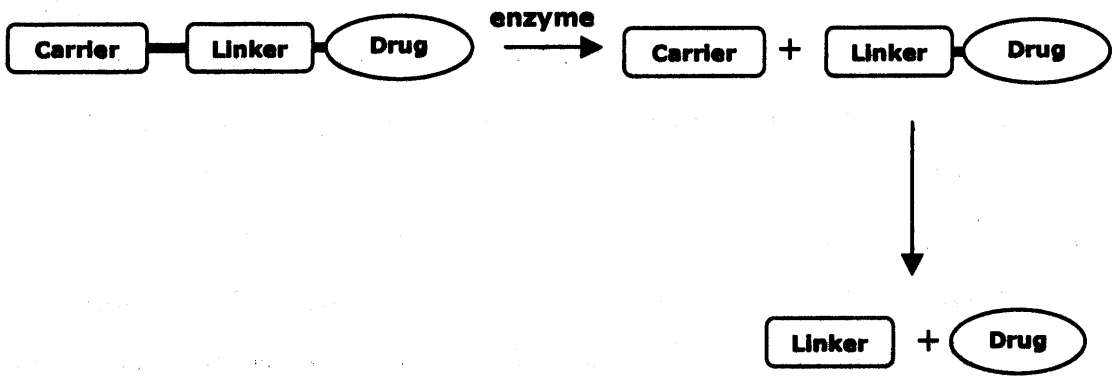


Figure 1.7: *Tripartate prodrugs.*

A *mutual prodrug* consists of two, usually synergistic, drugs attached to each other.

- **Bioprecursor prodrugs:** the compound is metabolised by molecular modification into a new compound which is active or which can be metabolised to the active drug.

- **Macromolecular prodrugs:** the active compound is linked to a macromolecule such as polyethylenglycol (PEG).

- **Drug-antibody conjugate:** the cytotoxic agent is covalently linked to a monoclonal antibody.

Most of the prodrugs in clinical use have been designed in an effort to improve the pharmaceutical, pharmacokinetic and/or pharmacodynamic problems that otherwise would prevent a drug from reaching the market. For this reason, chemical modifications and/or derivatizations of lead candidates or well accepted-drugs aim at improving pharmaceutical (solubility, stability, oganoleptic properties, etc.) and/or pharmacological problems (absorption, organ- or tissue-selective delivery, etc.). Hence, representative

examples of the objectives addressed using the prodrug strategy include:

- **Improved water solubility:** Poor aqueous solubility represents a major problem limiting parenteral, percutaneous, and oral bioavailability. Solubility may be improved by the use of charged pro-moities (e.g. phosphate, hemisuccinate, aminoacyl conjugates) or neutral pro-moieties (e.g. polyethyleneglycols). For example, the sodium salt of the acetylated sulfonamide prodrug afforded a solution to the poor water solubility of the COX-2 inhibitor celecoxib.[12]
- **Minimized toxicity and side effects:** Prodrug design can significantly improve the toxicological profile of a drug lowering its toxicity. For example, side effects of aspirin and other nonsteroidal anti-inflammatory agents, in particularly gastrointestinal effect, can be overcome by esterification.[13]
- **Improved absorption:** In many examples prodrug strategy is used to enhance the lipophilicity and optimise the bioavailability. For carboxylic acid groups, which could play an important role in binding a drug to its active site, increased lipophilicity may be achieved *via* esterification (e.g. pivampicillin, bacampicillin, benazepril, etc.).[14]
- **Improved site-selective delivery:** Site selective delivery represents one of the goals of a prodrug strategy. This favourable pharmacokinetic objective ensures the local activation of the drug reducing toxic side effects and non-specific uptake by other organs or tissues. Prodrug strategies for passive enrichment in the target tissue, targeting specific transporter, targeting tissue- or cell-specific enzymes and targeting surface antigens are currently under investigation[15] and are exemplified below:

Passive enrichment in the target tissue: Innovative drug delivery systems for the treatment of neoplastic diseases have been developed in order to restrict the delivery of the chemotherapeutic

agents to the tumour site. Passive enrichment in target tissue can be achieved as a result of some chemical or physical characteristic of the carrier. Indeed, passive and selective accumulation in tumour cells and improved therapeutic efficacy have been observed for polymer carriers conjugated to cytostatic and cytotoxic agents (e.g. PEG-paclitaxel, PEG-Ala-camptothecin).[16]

Targeting specific transporters:[15, 17] Many types of transporters that are expressed selectively in the liver, kidney and other organs have been identified and they may be promising target for drug delivery. Although the number of prodrugs targeting specific transporters is relative limited, an example of application of this concept is the prodrug Levodopa (L-DOPA) for the treatment of Parkinson's disease. L-DOPA is transported across the blood-brain barrier by amino acid transporters and then decarboxylated by L-amino acid decarboxylase to give dopamine.[18]

Targeting tissue- or cell-specific enzymes:[17, 19] With these approaches, prodrugs are preferentially activated at the response sites. One example is the antiviral agent, Acyclovir. This antimetabolite is phosphorylated only by the kinases coded by the viral genome and expressed in infected cells.

In cancer chemotherapy, promising new strategies have been proposed for the selective delivery of and activation of prodrugs in tumour tissues. These new approaches can be divided into two major classes: a) anti-body directed enzyme prodrug therapy (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT).

ADEPT[20] is a strategy in which a drug-activating enzyme is covalently linked to a tumour-associated monoclonal antibody (mAb) in order to target tumour tissues. After the antibody-enzyme conjugate has localised within the tumour and has been cleared from blood and normal tissues, a prodrug is administered and is converted by the pretargeted enzyme into a toxic drug, resulting in enhanced cytotoxic effect in the region of the tumour cells.

GDEPT,[21] also known as suicide gene therapy, is a two phase therapy in which a gene producing an endogenous enzyme is delivered to tumour cells, followed by administration of a prodrug which is activated after expression of the enzyme.

1.3 Nucleoside analogue prodrugs.

As mentioned earlier, nucleic acids are macromolecules responsible for the storage, transmission and expression of genetic information. Moreover, nucleotides and nucleosides are involved in several important functions in cells (e.g. carriers of chemical energy, structural components of many enzyme cofactors, cellular messengers, etc.). For these reasons, the important and fundamental place occupied by nucleoside and nucleotide analogues in the development of new chemotherapeutic agents is not surprising. Indeed, nucleoside and nucleotide analogues have received significant interest as potential biologically active compounds and are widely used in the treatment of viral or neoplastic diseases.[22, 23]

Nucleoside analogues are structurally different as compared to the corresponding natural DNA or RNA nucleosides with regard to modification of the glycone as well as the aglycone residue. They interfere with processes involved in the proliferation of cancer cells or replication of viral genomes displaying their activity at different stages of the metabolic pathways of nucleosides and nucleotides (e.g. inhibition of thymidylate synthase, ribonucleotide reductase, deoxycytidine monophosphate deaminase) or at the final stage of nucleic acid polymerisation (e.g DNA/RNA polymerase inhibition).

Regardless of the site of action of the nucleoside analogues, kinase- mediated conversion to their 5'-mono, di- or triphosphate is a prerequisite after cell penetration. Unfortunately, due to their

structural modification, nucleoside analogues can be poor substrates for both viral and host kinases. [24, 25]

For example:

- In the case of the anti-HIV active dideoxynucleoside analogue d4T (2′,3′-dideoxy-2′,3′didehydrothymidine, Stavudine, Zerit®), the first phosphorylation to d4T 5′-monophosphate catalysed by thymidine kinase (TK) is the rate-limiting step in human cells.[26]
- The nucleoside 2′,3′-dideoxyuridine (ddU) is virtually ineffective at blocking HIV infection in cultured cells, while the 2′,3′-dideoxyuridine triphosphate (ddUTP) is one of the most powerful and selective inhibitors of HIV reverse transcriptase. Biochemical and pharmacological studies showed that ddU itself was a poor substrate for cellular nucleoside kinases because of the specificity of these enzymes.[27]

In other cases, the efficacy of nucleoside analogues is limited by catabolic enzymatic reaction. For example:

- The nucleoside analogue 2′,3′-dideoxyadenosine (ddA) is rapidly deaminated to 2′,3′-dideoxyinosine (ddI) intracellularly by adenosine deaminase (ADA). ddI has to be converted to the bioactive metabolite 2′,3′-dideoxyadenosine-5′-triphosphate (ddATP), by five enzymatic steps (Figure.[28]

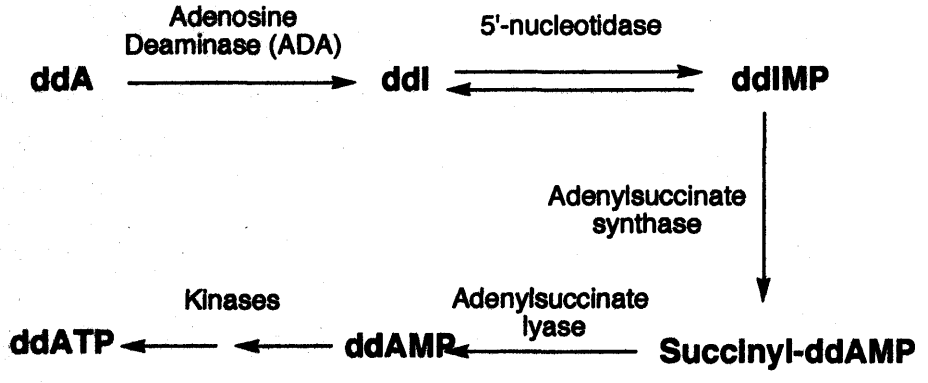


Figure 1.8: *Schematic representation outlining the intracellular metabolism of ddA.*

For these reasons, a possible approach to circumvent these problems could be the administration of preformed nucleoside monophosphates. Unfortunately, highly polar monophosphates have limited passive absorption and would not be able to penetrate cellular membranes or the blood-brain barrier.[29] In addition, blood and cellular surface phosphohydrolases would rapidly convert the phosphates to the corresponding nucleosides via non specific phosphatase action.

A possible approach to potentially overcome these difficulties (poor membrane permeation, poor activation mediated by kinases) is the use of neutral, membrane permeable nucleotide prodrugs.[29-33]

A suitable prodrug should fulfil three requirements: a) it has to be lipophilic enough for passive diffusion into the target cell, b) it should be able to deliver the nucleotides enzymatically/hydrolytically, and c) it should liberate non-toxic masking group(s).

Focusing on nucleoside analogues the prodrug approach is useful for:

1) Adjusting uptake, distribution and elimination of the drug
2) Targeting of the drug (cancer cell, virus etc.)
3) Reaching easily cellular membranes or blood-brain barrier penetration
4) Overcoming the occurrence of drug resistance

In order to overcome the poor cell penetration of the nucleoside 5'-phosphates, various prodrug approaches have been devised and investigated. The following paragraphs will highlight some approaches that have been designed to deliver the nucleotides by a special mechanism (enzyme activity, reducing potential, pH value). The bis(POM)phosphotriester and bis(POC)phosphotriester, Bis(SDTE)-nucleotide and Bis(SATE)-nucleotide, *cyclo*Sal-nucleotide, amino acid phosphoramidate diester and aryloxyphosphoramidate will be taken as examples to demonstrate

the successful intracellular delivery of free nucleotides from lipophilic precursors. The goal of this effort is to improve the passive diffusion through cell membranes and increase the bioavailability of phosphorylated nucleoside.

1.3.1 *Cyclo*Sal-pronucleotides.[33]

The *cyclo*Sal pronucleotides, developed by Meier *et al.*,[34] belong to the class of tripartate prodrugs and differ to other approaches in that the release of the free nucleotide is achieved by a pH-driven process. *cyclo*Sal prodrugs are phosphotriester derivatives with three different types of ester bonds: two ester bonds ($P\text{-}O_{phenyl}$ and $P\text{-}O_{benzyl}$) as a part of a cyclic bifunctional group with the nucleoside attached to the phosphorus through an alkyl ester bond.

The *cyclo*Sal pronucleotide concept was designed with the aim of developing a highly selective delivery mechanism based on controlled and chemically induced hydrolysis involving a successive coupled cleavage (tandem mechanism) of the phenyl- and the benzyl ester of the phosphotriester (Figure 1.9).[35]

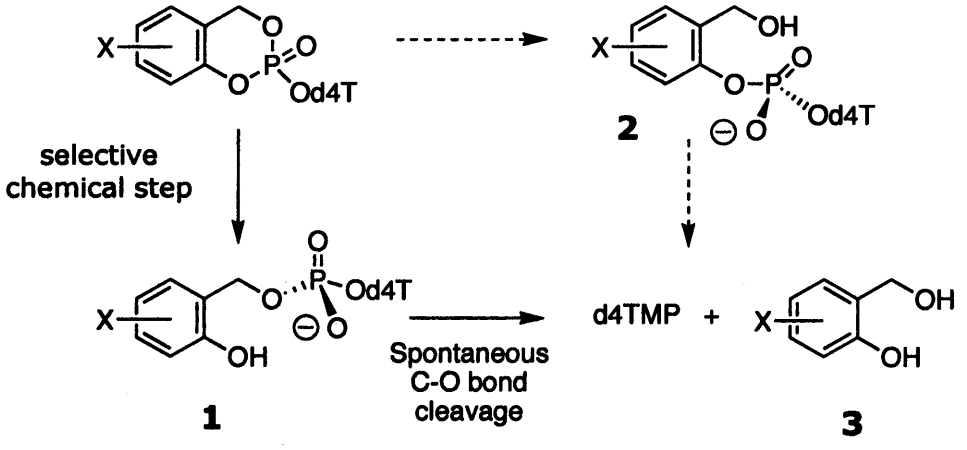


Figure 1.9: *Hydrolysis mechanism of cycloSal-d4T triesters.*

The rationale of these prodrugs relies on the different hydrolysis properties of phenyl- and benzyl- phosphate triesters. Since the negative charge arising from the cleavage of the phenolic moiety can be delocalized on the aromatic ring, the phenyl ester is the most labile and is cleaved selectively to the 2-hydroxybenzylphosphodiester (**1**). In contrast, the cleavage of the benzyl ester to the 2-hydroxymethyl phenylphosphodiester (**2**) is unfavourable. As a consequence of the first step, the remaining masking group ($P\text{-}O_{benzyl}$) is activated by the strongly electron-donating group in the *ortho* position (hydroxyl) resulting in a spontaneous cleavage of the diester (**2**) to yield the nucleotide (**d4TMP**) and salicyl alcohol (**3**).

The *cyclo*Sal approach has been applied to various nucleoside analogues, e.g. d4T,[36] acyclovir,[37] 5-(E)-(2-bromovinyl)-2'-deoxyuridine[38] (BVdU) and ddA.[39]

This pronucleotide concept was successfully introduced with the anti-HIV nucleosides d4T[36] and ddA.[39] While in the case of d4T, *cyclo*Sal-d4TMP was designed to achieve thymidine kinase bypass, the ddA *cyclo*Sal pronucleotides were developed to overcome the susceptibility of ddA to deamination by adenosine deaminase (ADA).[40]

Recently, Meier and co-workers have extended the *cyclo*Sal pronucleotides approach, introducing the second generation of cycloSal-pronucleotides.[33, 41] This second generation differs from the first one by the presence of an enzymatically cleavable carboxylic acid attached to the *cyclo*Sal moiety that should be enzymatically cleaved inside the cells. Preliminary biological results on CEM/O cells infected with HIV-1 and HIV-2 showed a poor increase of activity compared to the parent nucleoside.[33, 41]

1.3.2 Bis(SDTE) and Bis(SATE)-nucleotides.[42]

The bis{S-(2-hydroxyethylsulfidyl)-2-thioethyl}- [Bis(SDTE)] and the bis{S-acyl-2-thioethyl}- [Bis(SATE)] belong to the class of tripartate prodrug and were developed by Imbach and Gosselin.[43, 44]

The Bis(SDTE) was designed to take advantage of the great reducing potential within the cells to liberate the nucleotide into the cytosol.[44] To deliver the nucleotides, in the case of bis(SDTE) derivatives **4**, this approach requires two identical enzymatic activations catalysed by reductase enzymes. The reductase cleavage of the disulfide bond liberates thioethanol and thioethylphosphotriester **6** that eliminates spontaneously episulfide to yield the intermediate monoSDTE phosphodiester **7**. Thus, the second enzyme-catalysed activation step generates the free nucleotide (**d4TMP**, Figure 1.10).

In contrast, the decomposition pathway of bis(SATE) is triggered by a carboxyesterase-mediated hydrolysis of one of the thioester group, to liberate a carboxylic acid and the thioethyl monoSATE phosphate triester **6**. This undergoes fragmentation to episulfide and monoSATE phosphate diester **7**, followed by a second carboxyesterase or phosphodiesterase- mediated hydrolysis to liberate the free nucleotide (**d4TMP**, Figure 1.10).[45] Alternatively, a third decomposition pathway via direct nucleophilic attack of water on the phosphorus atom, has been proposed for bis(SATE)-nucleotides of isoddA.[46]

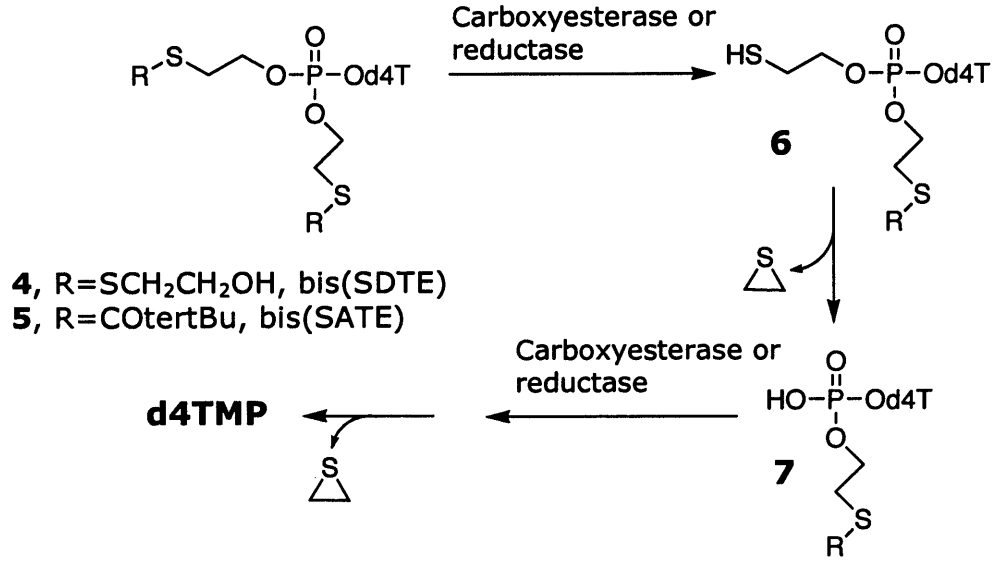


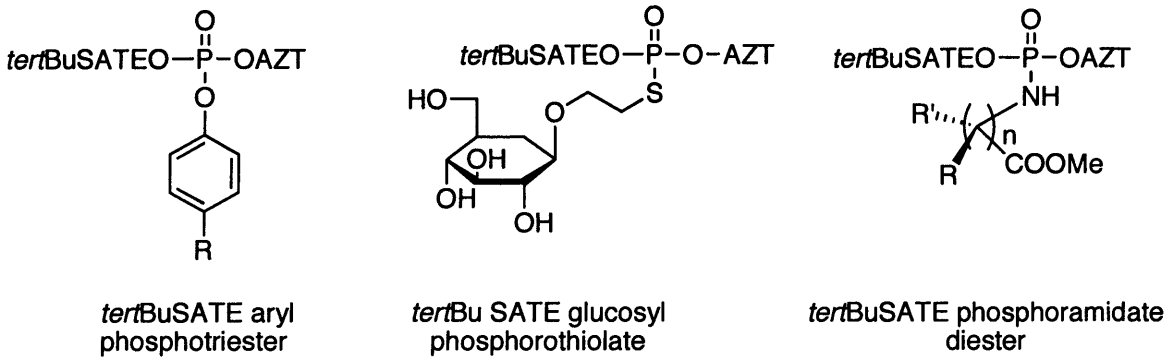
Figure 1.10: *Mechanism of decomposition of bis(SATE) and bis(SDTE) pronucleotides.*

The bis(SDTE) approach has been applied to the nucleoside 5-fluorodeoxyuridine (5-FdU)[47] and to the acyclic nucleoside phosphonate phosphonomethoxyethyladenine (PMEA).[48] While an improved antiviral potency was observed for bis(SDTE)-PMEA derivatives, the bis(SDTE)-5-FdUMP did not show better antitumour activity compared to the free nucleosides.

It should be mentioned that the major limitations to this approach are the restricted chemical stability, the susceptibility to enzymatic-catalysed hydrolysis, and the liberation of toxic episulfide. Indeed, the two activation steps required to free the nucleotide generate two equivalents of episulfide, which has been shown to be toxic in mice and rats. Optimization of bis(SATE) compounds showed that substitution of the acetyl thioester with acyl groups containing longer alkyl chains, increased the stability of these compounds.[46, 48]

However, the bis(SATE) approach has been successfully applied to thymidine kinase bypass for d4T,[49] and to the adenosine deaminase bypass of ddA.[50]

The produg design of bis(SATE) has been extended and three different kinds of mixed pronucleotides containing only one SATE chain and different second groups attached to the phosphorus have been investigated (Figure 1.11).[42, 51] The new pronucleotides were evaluated for their inhibitory effects on the replication of HIV-1 in CEM-SS, MT-4 and thymidine deficient cell line (CEM/TK$^-$), showing EC_{50} values similar to those observed for AZT.



*tert*BuSATE aryl phosphotriester

*tert*Bu SATE glucosyl phosphorothiolate

*tert*BuSATE phosphoramidate diester

Figure 1.11: *Structure of mixed SATE pronucleotide approach. tertBuSATE aryl phosphoester: R=tyrosinyl, tyrosinol, tyrosinamide. tertBuSATE phosphoramidate diester: n=1, 2, 3; R=H, Me; R'=H, Me, CH_2Ph, CH_2PhOH.*

1.3.3 Bis(POM) and Bis(POC)–nucleotides.

Bis(pivaloyloxymethyl)- (POM) and bis(isopropyloxycarbonyloxy methyl)- (POC) nucleotides are a class of nucleotide tripartate prodrug reported by Farquhar *et al.*[52]

The mechanism of activation, depicted in figure 1.12, is triggered by carboxyesterase-catalyzed cleavage of the pivaloyl ester and yields the unstable hydroxymethyl phosphotriester **8** which eliminates formaldehyde to give the mono(POM) phosphodiester **9**. Similarly to the bis(SATE) approach, the free nucleotide (NMP) is liberated after a second activation catalysed by

carboxyesterase or alternatively by phosphodiesterase of the phosphodiester **10** (Figure 1.12).

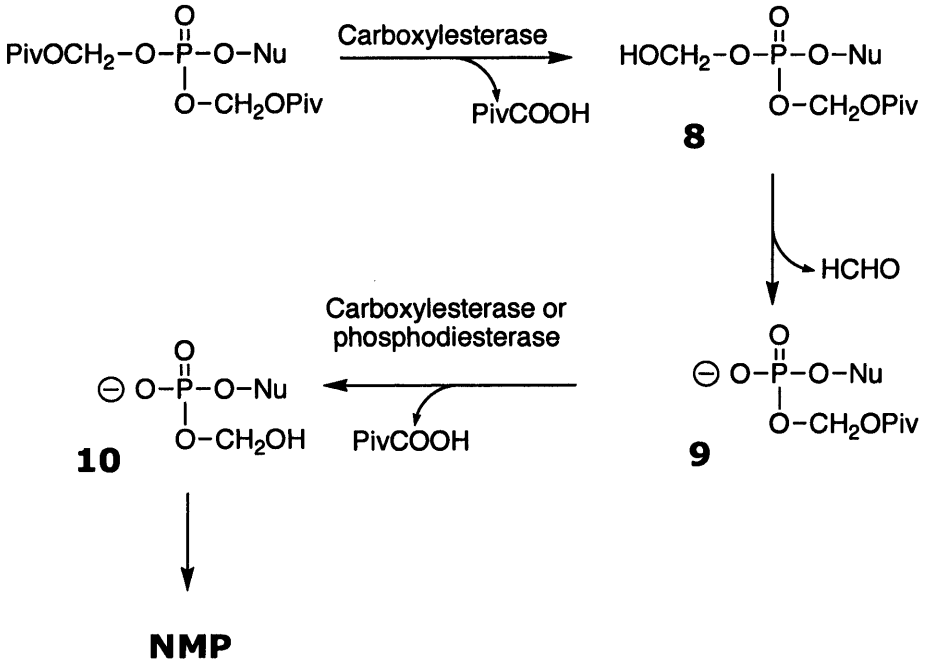


Figure 1.12: *Degradation mechanism of bis(POM)-nucleotides. NMP=nucleoside-5'-monophosphate, Piv=pivaloyl.*

This approach has been applied for the delivery of the anti-HIV AZTMP,[53] the anti HIV and anti-herpes PMEA[54] and the antitumour 5-fluoro-2'-deoxyuridine.[55] As a result of the hydrolysis of bis(POM)-nucleotides, the major limitation of this approach is the liberation of two equivalents of potentially toxic formaldehyde and pivalinic acid. Furthermore, it has been shown that the bis(POM)-phosphotriesters are chemically unstable and highly susceptible to serum-mediated hydrolysis. All these factors taken together could limit the potential utility of this approach for intracellular drug delivery.[53, 55]

The bis(POC)-triesters[56] are a modification of the bis(POM)-approach in which the masking group is

*iso*propyloxycarbonyloxymethyl. The mechanism of activation of bis(POC) derivatives relies again on carboxyesterase-mediated hydrolysis to initiate the release of the nucleoside 5′-monophosphate. Cleavage of the isopropyl ester yields the intermediate phosphonate diester **11** which undergoes fragmention into carbon dioxide to give the highly reactive hydroxymethyl phosphotriester **12** which eliminates spontaneously formaldehyde to give the mono(POC)-ester **13**. Conversion of the mono(POC)-ester **13** to the nucleoside-5′-monophosphate (**NMP**) is then catalysed by a carboxyesterase or phosphodiesterase (Figure 1.13).

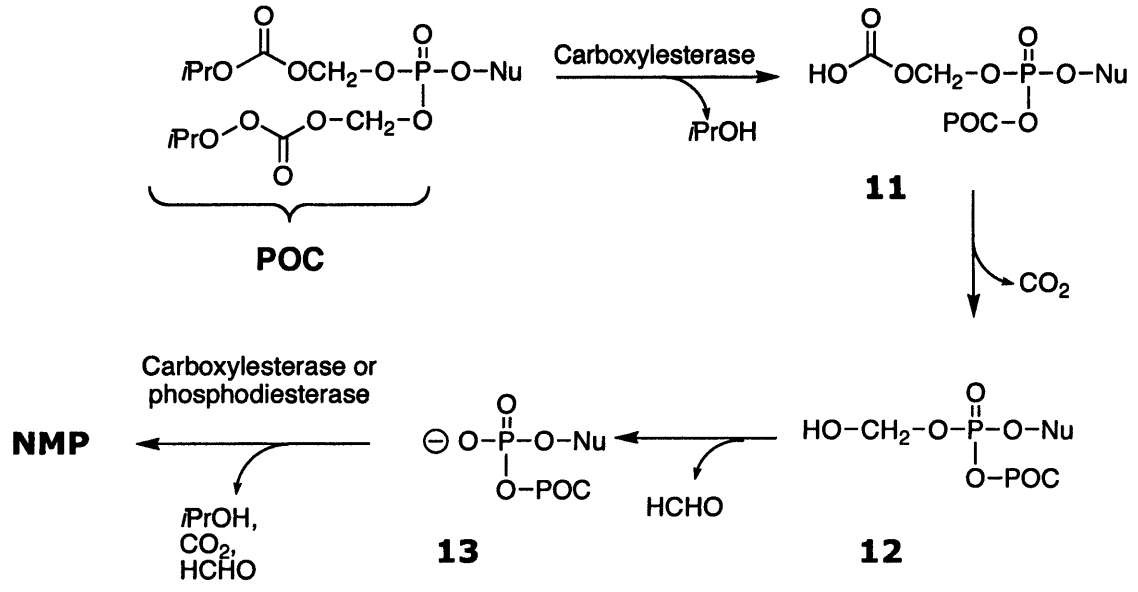


Figure 1.13: *Mechanism of decomposition of bis(POC)-nucleotides.*

In contrast to the bis(POM)-approach, the metabolism of bis(POC)-nucleotides avoids the formation and accumulation of toxic pivalinic acid in the cell.

1.3.4 Amino Acid Phosphoramidate Diester.[30, 31]

Amino acid phosphoramidates diesters are negatively charged bipartate prodrugs in which an aromatic amino acid methyl ester is linked to the phosphorus (Figure 1.14). Adapted from the aryl phosphoramidate nucleotide approach conceived by McGuigan *et al.*,[57] this prodrug design was reported by Wagner *et al.*[58]

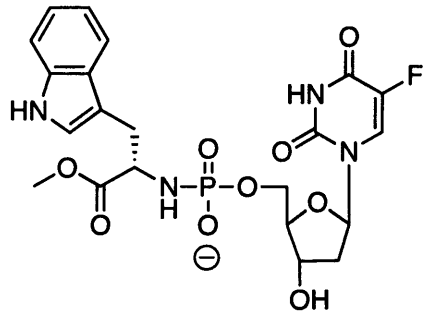


Figure 1.14: *L-tryptophan-5-fluoro-2-deoxyuridine.*

This approach has been applied to d4T,[58] AZT,[59] 5-FdU,[60] and 3'-deoxy-3'-fluorothymidine (FLT).[61]

Due to the presence of the remaining charge, nucleoside amino acid phosphoramidates are highly water soluble and in addition, are indefinitely stable in cell culture medium, water and rat and human plasma.

Several studies on whole cell and cell extract have been performed to characterise the metabolism of amino acid phosphoramidate diesters and support the hypothesis of enzymatic cleavage of the P-N bond by a phosphoramidase.[62] Recently, a protein belonging to the histidine triad superfamily and, identified as a histidine triad nucleotide binding protein (hint1) has been isolated and purified. Studies aiming at understanding whether hint1 is responsible for the hydrolysis of P-N bond, the substrate specificity or the catalytic activity are currently under investigation.[62]

1.3.5 Nucleoside Aryl Phosphoramidates.[63, 30, 31]

Aryl phosphoramidate derivatives are a class of membrane soluble tripartate prodrugs proposed by McGuigan and co-workers. The structure of nucleoside aryl phosphoramidates, shown in figure 1.15 for the anti-HIV d4T, is characterised by a phosphate moiety linked to a nucleoside, a phenyl group, and to an amino acid through a P-N linkage (phosphoramidate linkage).

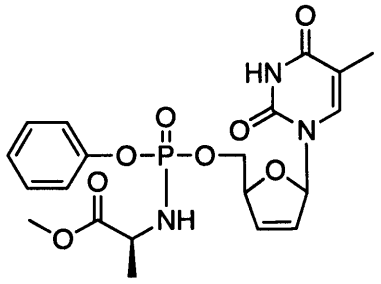


Figure 1.15: *d4T-5'-[phenylmethoxy-L-alaninyl)]-phosphate.*

This approach has been applied to different nucleoside analogues e.g. ddU,[64] AZT,[65] d4T,[66] ddA,[67] PMPA and PMEA,[68] and BVdU.[69]

The mechanism of activation of phosphoramidate derivatives has been the object of extensive studies. In the bioactivation pathway of phosphoramidate prodrugs, the first step is believed to be the esterase-mediated hydrolysis of the carboxylic ester function of the amino acid moiety to yield the phosphoramidate triester **14**.[46] However, Venkatachalam *et al.* have recently reported that the metabolism of 4-bromophenyl(methoxy-L-alaninyl)-d4T phosphoramidate (stampidine), in various tissue microenviroments, occurs through the action of hydrolytic enzymes other than esterase as well. These studies suggested that mammalian proteases, such as cathepsin B and proteinase K, are capable of hydrolysing d4T prodrugs and may be involved in the activation of phosphoramidate derivatives.[70]

The cleavage of the ester moiety is thought to be followed by an intramolecular nucleophilic attack of the phosphorus by the carboxylate, with spontaneous elimination of phenol, and transient formation of a five-membered cyclic anhydride **15**. Hydrolysis of the

cyclic intermediate **15** gives the aminoacyl phosphoramidate diester **16**, which has been described as an important intracellular depot form for the free nucleotide.[71] Compound **16** is then believed to undergo P-N cleavage, mediated by phosphoramidase, to liberate the nucleside-5'-monophosphate (Figure 1.16).[71]

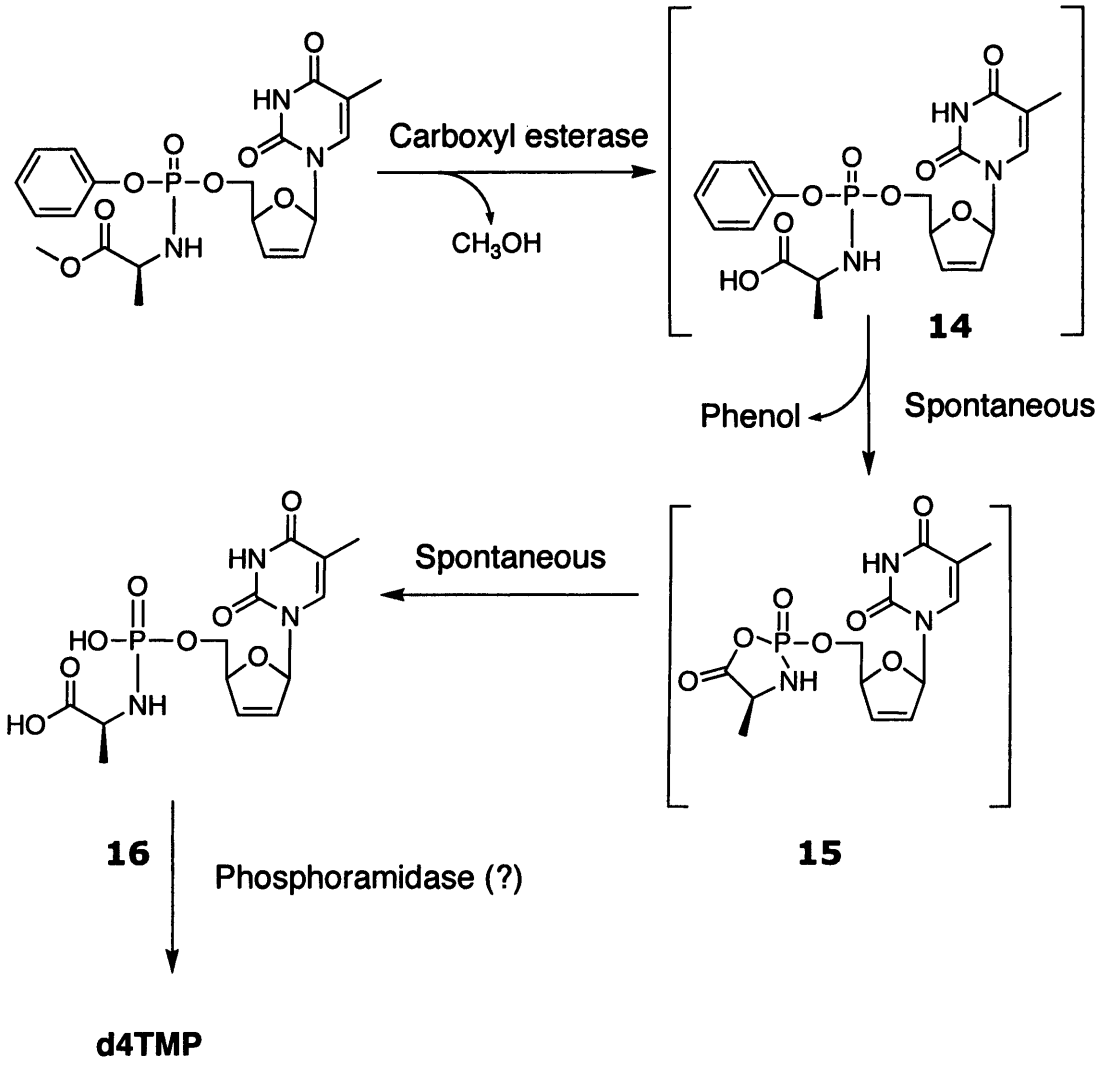


Figure 1.16: *Proposed mechanism of decomposition of aryloxyphosphoramidate of d4T.*

Extensive structure activity relationship studies on d4T have shown that an α-amino acid is essential for the activity of these phosphoramidate derivatives.[72] Indeed, replacement of the amino

acids with alkylamine and β-amino acid derivatives lead to a complete loss of antiviral activity.[73, 74] Furthermore, studies have revealed that changes in the amino acids, such as inversion of the natural stereochemistry, led to a significant reduction in potency in the series.[75] Among the natural amino acids, L-alanine was the most effective whereas L-valine showed a poor antiviral activity.[72] In addition, d4T phosphoramidate derivatives were found to exhibit enhanced activity against HIV-1 and HIV-2 in cell culture compared to the parent nucleoside, with full retention of activity in mutant thymidine kinase-deficient cells (CEM/TK^-).[76]

Application of this approach to ddA and d4A proved to be successful, with a substantial increase in the antiviral potency and selectivity against HIV-1 and HBV.[77]

Despite the successful delivery of the nucleotides of ddA, d4A and d4T, this approach applied to AZT and 3TC showed a decrease of antiviral activity compared to the parent nucleosides. AZT phosphoramidate derivatives showed decreased activity compared to the parent nucleoside against HIV-1 and 2 and furthermore the activity against HIV-2 was not retained in CEM/TK^- cell.[65] In the same way, 3TC-phosphoramidate (phenyl(methoxy-L-alaninyl-phosphoramidate derivative) showed decreased anti-HIV activity compared to the parent nucleoside, but full retention of anti-HBV activity.[78]

1.4 References

1 Stryer, L.; DNA and RNA: Molecules of Heredity. *Biochemistry,* W. H. Freeman and Company, **1988**, pp. 91-116.

2 Blackburn, G.M.; Gait, M.J.; Nucleic acids in chemistry and biochemistry, **1990**, Oxford University Press.

3 Watson, J.D.; Crick, F.H.; Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. *Nature*, **1953**, 171-173.

4 Watson, J.D.; The Double Helix. Weidenfield and Nicholson: London, **1968**.

5 Chargaff, E.; Structure and function of nucleic acids as cell constituents. *Fed. Proc.*, **1951**, 10, 654-659.

6 Focher, F.; Ferrari, E.; Spadari, S.; Hubscher, U.; Do DNA polymerases delta and alpha act coordinately as leading and lagging strand replicates? *FEBS Lett.*, **1988**, 229, 6-10.

7 Voet, D.; Voet, J.G.; DNA replication, Repair, and Recombination. *Biochemistry*, Wiley & Sons, Inc., **1995**, pp.1020-1072.

8 Albert, A.; Chemical aspect of selective toxicity. *Nature*, **1958**, 182, 421-422.

9 Testa, B.; Drug metabolism. *Burger's medicinal chemistry and drug discovery*, **1995**, Wiley-interscience, New York, 129-180.

10 Stella, V.J.; Charman, W.N.A.; Naringrekar, V.H.; Prodrugs. Do they have advantages in clinical practise? Drugs, **1985**, 29, 455-473.

11 Wermuth, C.G; Drug metabolism and drug design: Quo Vadis?. Briot, M.; Cautreel, W.; Roncucci, R.: Montpellier, 1983.

12 Sorbera, L.A.; Leeson, P.A.; Castaner, J.; Castaner, R.M.; Valdecoxib and parecoxib sodium. Analgesic, antiarthritic, cyclooxygenase-2 inhibitors. *Drugs Future*, **2001**, 26, 133-140

13 Nielsen, N.M.; Bundgaard, H.; Evaluation of glycolamide esters and various other esters of aspirin as true aspirin prodrugs. *J. Med. Chem.*, **1989**, 727-734

14 Beaumont, K.; Webster, R.; Gardner, I.; Dack, K.; Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. *Curr. Drug Metab.* **2003**, 4, 461-485.

15 Ettmayer, P.; Amidon, G.L.; Clement, B.; Testa, B.; Lessons learned from marketed and investigational prodrugs. *J. Med. Chem.*, **2004**, 47, 2393-2404.

16 Conover, C.D.; Greenwald, R.B.; Pendri, A.; Shum, K.L.; Camptothecin delivery systems: the utility of the amino acid spacers for the conjugation of camptothecin with poly ethylene gycol to create prodrugs. *Anti-Cancer Drug*, **1999**, 14, 499-506.

17 Han, H.K.; Targeted prodrug design to optimize drug delivery. *AAPS Pharmsci*, **2000**, 2, 2-11.

18 Bradley, D.A.; Prodrugs for improved CNS delivery. *Adv. Drug Delivery Rev.*, **1996**, 19, 171-202.

19 Kearney, A.S.; Prodrugs and targeted drug delivery. *Adv. Drug Delivery Rev.*, **1996**, 19, 225-239.

20 Niculesko-Duvaz, I.; Springer, C.J.; Antibody-directedd enzyme prodrug therapy (ADEPT): a review. *Adv. Drug Delivery Rev.*, **1997**, 151-172.

21 Springer, C.J.; Niculescu-Duvaz, I.; Gene-directed enzyme prodrug therapy (GDEPT): choice of prodrugs. *Adv. Drug Delivery Rev.*, **1996**, 22, 351-364.

22 Chu, C.K.; Baker, D.C.; Nucleosides and nucleotides as antitumor and antiviral agents. **1993**, Plenum Press: New York.

23 Perigaud, C.; Gosselin, G.; Imbach, J. L.; Nucleoside analogues as chemotherapeutic agents: a review. *Nucleosides & Nucleotides,* **1992**, 11, 903-945.

24 Eriksson, S.; Wang, L.; Substrate specificities, expression and primary sequences of deoxynucleoside kinases. *Nucleosides & Nucleotides*, **1997**, 16, 653-659.

25 Furman, P.A.; Fyfe, J.A. St. Clair, M.H.; Weinhold, K.; Rideout, J.L.; Freeman, G.A.; Lehrmann, S.N.; Bolognesi, D.P.; Broder, S.; Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency-virus reverse-transcriptase. *Proc. Natl. Acad. Sci. USA*, **1986**, 83, 8333-8337.

26 Balzarini, J.; Herdewijin, P.; De Clercq, E.; Differential patterns of intracellular metabolism of 2',3'-didehydro- 2',3'-dideoxythymidine and 3'-azido-2',3'-dideoxythymidine, two potent anti-human immunodeficiency virus compounds. *J. Biol. Chem.*, **1989**, 264, 6127-6133.

27 Hao, Z.; Cooney, D. A.; Farquhar, D.: Perno, C. F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N. R.; Balzarini, J.; Johns, D. G.; Potent DNA chain termination activity and selective inhibition of human immunodeficiency virus reverse transcripatase by 2',3'-dideoxyuridine-5'-triphosphate. *Mol. Pharmacol.*, **1989**, 37, 157-163.

28 a) Cooney, D.A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johnson, M.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D.G.; Initial studies on the ccellular pharmacology of 2',3'-dideoxyadenosine, an inhibitor of HTLV-III infectivity. Biochem. Pharmacol. **1987**, 36, 1765-1768. b) Johnson, M.A.; Ahluwalia, G.; Connely, M. C.; Cooney, D.A.; Broder, S.; Johns, D.G. Fridland, A. Metabolic pathways for the activation of the antireatroviral agent 2',3'-dideoxyadenosine in human lymphoid cells. *J. Biol. Chem.*, **1988**, 263, 15354-15357.

29 Jones, R.J.; Bischorfberg, N.; Minireview: Nucleotide prodrugs *Antiviral Res.*, **1995**, 27, 1-17.

30 Meier, C.; Pro-Nucleotides- recent advances in the design of efficient tools for the delivery of biologically active nucleoside monophosphate. *SynLett.*, **1998**, 3, 233-241.

31 Wagner, C.R.; Iyer, V.V.; McIntee, E.J.; Pronucleotides: toward the in vivo delivery of antiviral and anticancer nucleotides. *Med. Res. Rev.*, **2000**, 20, 417-451.

32 Krise, J.P.; Stella, J.V.; Prodrugs of phosphates, phosphonates and phosphinates. *Adv. Drug Delivery Rev.*, **1996**, 19, 287-310.

33 Meier, C.; Ruppel, M.F.H.; Vukadinovic, D.; Balzarini, J.; "Lock-in"- *cyclo*Sal-pronucleotides- A new generation of chemical Trojan horses? *Mini-Rev. Med. Chem.*, **2004**, 4, 383-394.

34 Meier, C.; 2-nucleos-5'-O-yl-4H-1,2,3,-benzodioxaphosphinin-2-oxides. A new concept for lipophilic, potential prodrugs of biologically active nucleoside monophosphates. *Angew. Chem Int. Ed. Eng.*, **1996**, 35, 70-72.

35 Meier, C.; *cyclo*Sal-pronucleotides- Design of chemical Trojan horses. *Mini-Rev. Med Chem.*, **2002**, 2, 219-234.

36 a) Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J.; *Ciclo*Sal-2',3'-didehydrothymidine monophospahte (*cyclo*Sal-d4TMP): synthesis and antiviral evaluation of a new d4TMP delivery system. *J. Med. Chem.*, **1998**, 41, 1417-1427. b) Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J.; Cyclic saligenyl phosphotriesters of 2',3'-didehydrothimidine (d4T)-a new pronucleotide approach. *Biorg. Med. Chem. Lett.*, **1997**, 7, 99-104.

37 Balzarini, J.; Haller-Meier, F.; De Clercq, E.; Meier, C. Antiviral activity of cyclosaligenil prodrugs of acyclovir, carbovir and abacavir. *Antivir. Chem. Chemother.*, **2002**, 12, 301-306.

38 a) Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P.; *Cyclo*Sal-BVDUMP pronucleotides: How to convert an antiviral-inactive nucleoside analogue into a bioactive compound against EBV. *J. Med.*

Chem., **2002**, 45, 5157-5172. b) Meier, C.; Lomp, A.; Meerbach, A.; Wutzler, P.; *Cyclo*Saligenyl-5-[(E)-2-bromovinyl]-2'-deoxyuridine monophosphate (*cyclo*Sal-BVDUMP) pronucleotides active against epstein-barr virus. *ChemBioChem.*, **2001**, 4, 283-285.

39 Meier, C.; Knispel, T.; De Clercq, e.; Balzarini, J.; *Cyclo*Sal-pronucleotides (*cyclo*Sal-NMP) of 2',3'-dideoxyadenosine (ddA) and 2',3'-dideoxy-2',3'-didehydroadenosine (d4A): synthesis and antiviral evaluation of highly efficient nucleotides delivery system. *J. Med. Chem.*, **1999**, 42, 1604-1614.

40 Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J.; ADA-bypass by lipophilic *cyclo*Sal-ddA pronucleotides, a second example of the efficiency of the *cyclo*Sal concept. *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1577-1582.

41 Meier, C.; Ruppel, M.F.H.; Vukadinovic, D.; Balzarini, J.; Second generation of *cyclo*Sal-pronucleotides with esterase-cleavable site: the "lock-in"-concept. *Nucleosides, Nucleotides & Nucleic Acids*, **2004**, 23, 89-115.

42 Peyrottes, S.; Egron, D.; Lefebre, I.; Gosselin, G.; Imbach, J.L; Perigaud, C.; SATE pronucleotide approaches: an overview. *Mini-Rev. Med. Chem.*, **2004**, 4, 395-408.

43 Gosselin, G.; Girardet, J-L.; Perigaud, C.; Benzaria, S.; Lefebvre, I.; Schlienger, N.; Pompon, A.; Imbach, J.L.; New insight regarding the potential of pronucleotides approach in antiviral chemotherapy. *Acta Biochim. Pol.*, **1996**, 43, 196-208.

44 Puech, F.; Gosselin, G.; Lefebvre, I.; Pompon, A.; Aubertin, A.M.; Kirn, A.; Imbach, J.L.; Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process. *Antiviral. Res.*, **1993**, 22, 155-174.

45 Lefebvre, I.; Perigaud, C.; Pompon, A.; Aubertin, A.M.; Girardet, J.L.; Kirn, A.; Gosselin, G.; Imbach, J.L.; Mononucleoside

phosphotriester derivatives with S-acyl—2-thioethyl bioreversible phosphate-protecting groups: intracellular delivery of 3′-azido-2′,3′-dideoxythymidine-5′-monophosphate. *J. Med. Chem.*, **1995**, 38, 3941-3950.

46 Vallette, G.; Pompon, A.; Girardet, J.L.; Cappellacci, L.; Franchetti, P.; Grifantini, M.; La Colla, P.; Loi, A.G.: Perigaud, C.; Gosselin, G.; Imbach, J.L.; Decomposition pathways and *in vitro* HIV inhibitory effects of isoddA pronucleotides: toward a rational approach for intracellular delivery of nucleoside-5′-monophosphates. *J. Med. Chem.*, **1996**, 39, 1981-1990.

47 Girardet, J.L.; Goisselin, G.; Perigaud, C.; Balzarini, J.; De Clercq, E.; Imbach, J.L.; Synthesis and antitumor properties of some neutral triester of 5-fluoro-2′-deoxyuridine-5′-monophosphate and 3′,5′-cyclic monophosphate. *Nucleosides & Nucleotides*, **1995**, 14, 645-647.

48 Benzaria, S.; Pelicano, H.; Johnson, R.; Maury, G.; Imbach, J.L.; Aubertin, A.M.; Obert, G.; Gosselin, G.; Synthesis, *in vitro* antiviral evaluation, and stability studies of bis(S-acyl-2-thioethyl) ester derivatives of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) as potential PMEA prodrugs with improved oral bioavailability. *J. Med. Chem.*, **1996**, 39, 4958-4965.

49 Girardet, J.L.; Perigaud, C.; Aubertin, A.M.; Gosselin, G.; Kirn, A.; Imbach, J.L.; Increase of the anti-HIV activity of d4T in human T-cell culture by the use of the SATE pronucleotide approach. *Bioorg. Med. Chem. Lett.*, **1995**, 5, 2891-2984.

50 Perigaud, C.; Aubertin, A.M.; Benzaria, S.; Pelicano, H.; Girardet, J.L.; Maury, G.; Gosselin, G.; Kirn, A.; Imbach, J.L.; Equal inhibition of the replication of human-immunodeficiency virus in human T-cell culture by ddA bis(SATE) phosphotriester and 3′-azido-2′,3′-dideoxythymidine. *Biochem. Pharmacol.*, **1994**, 48, 11-14.

51 Jochum, A.; Schlienger, N.; Egron, D.; Peyrottes, D.; Perigaud, C.; Biolabile construct for pronucleotide design. *J. Organomet. Chem.*, **2005**, 690, 2614-2625.

52 Farquhar, D.; Srivastva, D.N. Kuttesch, N.J.; Saunders, P.P.; Biologically reversible phosphate-protecting group. *J. Pharm. Sci.*, **1983**, 72, 324-325.

53 Pompon, A.; Lefebvre, I.; Imbach, J.L.; Kahn, S.; Farquhar, D.; Decomposition pathways of the mono-(pivaloyloxymethyl) and bis-(pivaloyloxymethyl) ester of azidothymidine 5'-monophosphate in cell extract and in tissue-culture medium – an application of the online ISP cleaning HPLC technique. *Antiviral Chem. Chemother.*, **1994**, 5-91-98.

54 Srinivas, R.V.; Robbins, B.L.; Connelly, M.C.; Gong, Y.F.; Bischofberger, N.; Fridland, A.; Metabolism and in vitro antiretroviral activities of bis(pivaloyloxymethyl) prodrugs of acyclic nucleoside phosphonates. *Antimicrob. Agents Chemother.* **1993**, 37, 2247-2250.

55 Farquhar, D.; Khan, S.; Srivastva, D.N.; Saunders, P.P.; Synthesis and antitumour evaluation of bis[(pivaloyloxy)methyl]-2'-deoxy-5-fluorouridine-5'-monophosphate (FdUMP): A strategy to introduce nucleotides into cells. *J. Med. Chem.*, **1994**, 3902-3909.

56 Armilli, M.N.; Kim, C.U.; Dougherty, J.; Mulato, A.; Oliyai, R.; Shaw, J.P.; Cundy, K.C.; Bischofberger, N.; Synthesis, in vitro biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) prodrugs. *Antiviral Chem. Chemother.*, **1997**, 8, 557-564.

57 McGuigan, C.; Pathirana, R.N.; Balzarini, J.; De Clercq, E.; Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.*, **1993**, 36, 1048-1052.

58 Abraham, T.W.; Wagner, C.R.; A phosphoramidite-based synthesis of phosphoramidate amino-acid diesters of antiviral nucleosides. *Nucleosides & Nucleotides*, **1994**, 13, 1891-1903.

59 Wagner. C.R.; McIntee, E.J.; Schinazi, R.F.; Abraham, T.W.; Aromatic amino-acid phosphoramidate di- and triesters of 3'-azido-3'-deoxythymidine (AZT) are non-toxic inhibitors of HIV-replication. *Bioorg. Med. Chem. Lett.*, **1995**, 5, 1819-1824.

60 Abraham, T.W.; Kalman, T.I.; McIntee, E.J.; Wagner, C.R.; Synthesis and biological evaluation of aromatic amino acid phosphoramidates of 5-fluro-2'-deoxyuridine and 1-beta-D-arabinofuranosylcytosine: evidence of phosphoramidase activity. *J. Med. Chem.*, **1996**, 39, 4569-4575.

61 McIntee, E.J.; Remmel, R.P.; Schinazi, R.F.; Abraham, T.W.; Wagner, C.R.; Probing the mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleoside prodrugs. *J. Med. Chem.*, **1997**, 40, 3323-3331.

62 Drontle, D.P.; Wagner, C.R.; Designing a pronucleotide stratagem: lessons from amino acid phosphoramidates of anticancer and antiviral pyrimidines. *Mini Rev. Med. Chem.*, **2004**, 4, 409-419.

63 Cahard, D.; McGuigan, C.; Balzarini, J.; Aryloxy phosphoramidate trimesters as pro-tides. *Mini Rev. Med. Chem.*, **2004**, 4, 371-381.

64 McGuigan, C.; Bellevergue, P.; Sheeka, H.; Mahmood, N.; Hay, A.J.; Certain phosphoramidate derivatives of dideoxyuridine (ddU) are active against HIV and successfully bypass thymidine kinase. *FEBS Lett.*, **1994**, 351, 11-14.

65 McGuigan, C.; Pathirana, R.N.; De Clercq, E.; Balzarini, J.; Intracellular delivery of bioactive AZT nucleosites by aryl phosphate derivatives of AZT. *J. Med. Chem.*, **1993**, 36, 1048-1052.

66 McGuigan, C.; Cahard, D.; Sheeka, H.M.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of D4T with improved

anti-HIV efficacy retain full activity in thymidine kinase deficient cells. *Bioorg. Med. Chem. Lett.,* **1996**, 6, 1183-1186.

67 McGuigan, C.; Wedgwood, O.M.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of 2',3'-didhydro-2',3'-dideoxyadenosine (d4A) have markedly improved anti-HIV potency and selectivity. *Bioorg. Med. Chem. Lett.*, **1996**, 6, 2359-2362.

68 Ballatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J.; Synthesis and evaluation of novel amidate prodrugs of PMEA and PMPA. *Bioorg. Med. Chem. Lett.*, **2001**, 11, 1053-1056.

69 McGuigan, C.; Harris, S.A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J.; Synthesis and antiviral evaluation of phosphoramidate derivatives of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU). *Antivir. Chem. Chemother.*, **2001**, 12, 293-300.

70 a) Venkatachalam, T.K.; Samuel, P.; Uckun, F.M.; Enzymatic hydrolysis of stampidine and other stavudine phosphormaidates in the presence of mammalian proteases. *Bioorg. Med. Chem. Lett.*, **2005**, 13, 2651-2655. b) Venkatachalam, T.K.; Samuel, P.; Qazi, S.; Uckun, F.M.; Protease-mediated enzymatic hydrolysis and activation of aryl phosphoramidate derivatives of stavudine. *Eur. J. Med. Chem.*, **2005**, 40, 452-466.

71 Saboulard, D.; Naesen, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velasquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J.; Charaterization of the pathway of phosphoramidate triester prodrugs of stavudine and Zivudine. *Mol. Pharmacol.*, **1999**, 56, 693-704.

72 McGuigan, C.; Tsang, H.W.; Cahard, D.; Turner, K.; Velazquez, S. Salgado, A.; Bidois, L.; Naesens, L.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of d4T as inhibitors of HIV: the effect of amino acid variation. *Antiviral Res.*, **1997**, 35, 195-204.

73 McGuigan, C.; Cahard, D.; Salgado, A.; De Clercq, E.; Balzarini, J.; Phosphoramidates as potent pro-drugs of anti-HIV nucleotides: studies in the amino region. *Antivir. Chem. Chemother.*, **1996**, 7, 31-36.

74 McGuigan, C.; Tsang, H.W.; Sutton, P.W.; De Clercq, E.; Balzarini, J.; Synthesis and anti-HIV activity of some novel chain-extended phosphoramidate derivative of d4T (stavudine): esterase hydrolysis as a rapid predicted test for antiviral potency. *Antivir. Chem. Chemother.*, **1998**, 9, 109-115.

75 McGuigan, C.; Salgado, A.; Yarnold, C.; Harries, T.Y.; De Clercq, E.; Balzarini, J.; Novel nucleoside phosphoramidates as inhibitors of HIV; studies on the stereochemical requirements of the phosphoramidate amino acid. *Antivir. Chem. Chemother.*, **1996**, 7, 184-188.

76 Balzarini, J.; Karlsson, A.; Aquaro, S.; Perno, C.F.; Cahard, D.; Naesens, L.; De Clercq, E.; McGuigan, C.; Mechanism of anti-HIV action of masked alaninyl d4T-MP derivatives. *Proc. Natl. Acad. Sci. USA*, **1996**, 7295-7299.

77 a) Balzarini, J.; Kruining, J.; Wedgwood, O.; Pannecouque, C.; Aquaro, S.; Perno, C.F.; Naesens, L.; Witvrouw, M.; Heijtink, R.; De Clercq, E.; McGuigan, C.; Conversion of 2',3'-dideoxyadenosine (ddA) and 2',3'-didehydro-2',3'-dideoxyadenosine (d4A) to their corresponding aryloxyphosphoramidate derivatives markedly potentiates their activity against human immunodeficiency virus and hepatitis B virus. *FEBS Lett.*, **1997**, 410, 324-328.

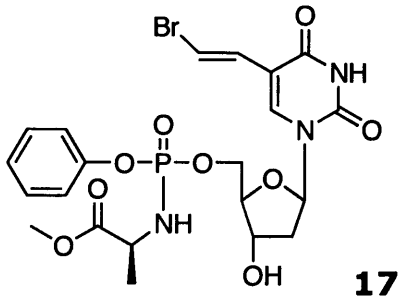
78 Balzarini, J.; Wedgwood, O.; Kruining, J.; Palemans, H.; Heijtink, R.; De Clercq, E.; McGuigan, C.; Anti-HIV and anti-HBV activity and resistance profile of 2',3'-dideoxy-3'-thiacytidine (3TC) and its arylphosphoramidate derivative. *Biochem. Biophys. Res. Commun.*, **1996**, 225, 363-369.

Chapter Two: Synthesis of Phosphoramidates of BVdU Containing Natural Amino Acids.

2.1 Background and aims.

The aryloxy phosphoramidate approach introduced in the early 90s[1] has been applied to a wide range of nucleosides and acyclic nucleoside phosphonates (ANPs) and among these, stavudine (d4T) represents one of the most extensively studied.[2] The subsequent and extensive application of the technology by us and others to 2′,3′-dideoxyadenosine (ddA),[3] 2′,3′-dideoxy-2′,3′-didehydroadenosine (d4A),[4] adenallene,[5] 8-aza-isoddA,[6] PMPA and PMEA phosphonates,[7] revealed a significant enhancement in potency compared to the parent nucleosides, or ANPs.

Despite these remarkable achievements, the application of the phosphoramidate technology was unsuccessful for several nucleoside analogues (e.g. acyclovir,[8] netivudine,[9] and BVdU[10]). Of particular interest was the case of BVdU phosphoramidates that showed an approximate 5- to 25-fold decrease in anti VZV activity in tissue culture[10] and interpreted either as poor activation of the pronucleotides or rapid dephosphorylation of liberated BVdU monophosphate (BVdUMP) to BVdU. On the other hand, the NewBiotics Group, working independently of us and applying our phosphoramidate technology to the antiherpetic agent BVdU, found



17

Figure 2.1: *Structure of 5′-[phenyl-(methoxy-L-alaninyl)]-BVdU phosphoramidate.*

the 5'-[phenyl-(methoxy-L-alaninyl)]-BVdU phosphoramidate (**17,** Figure 2.1) to have a significant and selective *in vivo* antitumour activity versus human colon and breast cancer.[11]

Initial studies conducted by NewBiotics showed that the cytotoxicity of **17** was more pronounced in tumour cells with high levels of thymidylate synthase (TS),[12] a key enzyme involved in the *de novo* synthesis of deoxythymidine monophosphate (dTMP). It was also demonstrated that in cells **17** was converted into the corresponding monophosphate,[11] BVdUMP, which is a competitive TS substrate.[13] Efforts to gain a better understanding of the mechanism of action of **17** using a ^{14}C-labeled analogue of **17** produced unexpected findings. Indeed, no additional bases were detected in DNA or RNA[11] after treatment with **17**, and radiolabeled cellular protein(s) was found to be the eventual target, suggesting a surprising mechanism of action for a nucleoside-based anticancer drug.[14] Compound **17** was approved by FDA to enter clinical trial for patients with advanced colorectal cancer in 2002[15] with only preclinical and phase 1 results, which are available to date, show the compound to be clinically well tolerated at all dose levels tested.[16]

Considering this intriguing discovery and taking into consideration our expertise in the field of the phosphoramidate technology, we sought to improve the anticancer activity of the lead **17** by a parallel tuning of the ester, aryl and amino acid regions.

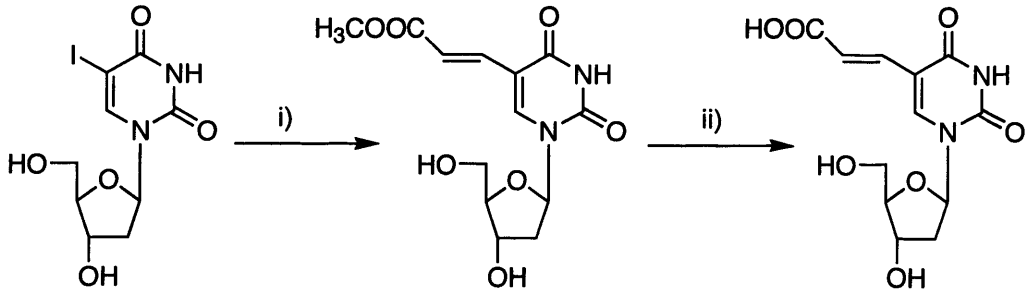
2.2 Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine.

The synthesis and the antiviral activity of BVdU was first briefly reported by Walker *et al.* in 1978[17] and after 25 years, BVdU still remains one of the more active and selective compounds

against HSV-1 and VZV. The method conceived for the preparation of BVdU, involved the condensation of 5-(2-bromovinyl)uracil with the protected ribofuranosyl chloride (3,5-di-O-toluoyl-2-deoxy-α-D-ribofuranosyl chloride) to afford a mixture of α and β deoxynucleoside anomers.[18] As a consequence of the low yields, this procedure was not suitable for the large scale synthesis of BVdU and improved methods were investigated.[19, 20]

The BVdU used for the preparation of the aryloxy phosphoramidates derivatives in this thesis was synthesised following the procedure of Ashwell *et al.*[20]

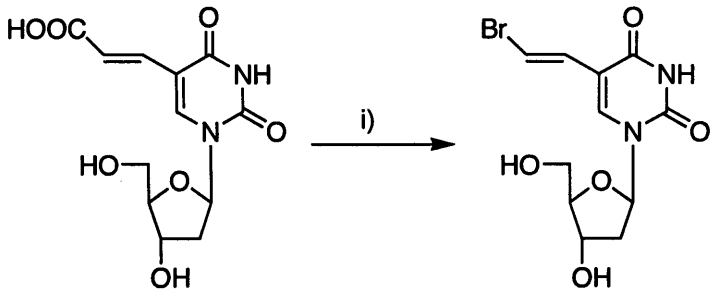
The starting material, 5-iodo-2'-deoxyuridine (**18**), was converted in the presence of methylacrylate, triphenylphosphine, Pd(II) acetate and triethylamine (TEA) into the corresponding (E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine (**19**) in dry 1,4-dioxane at reflux for 1 hour (Scheme 2.1). The hydrolysis of the methyl ester (**19**) with sodium hydroxide in water at room temperature afforded the corresponding acid (**20**, (E)-5-(2-carboxyvinyl)-2'-deoxyuridine) in high yield (Scheme 2.1).



18 **19** **20**

Scheme 2.1: *Synthesis of (E)-5-(carboxyvinyl)-2'-deoxyuridine (**20**).* Reagents and conditions: i) methylacrylate, Pd(II)acetate, $P(Ph)_3$, TEA, 1,4-dioxane, reflux, 1hr; ii) NaOH 1M, water, r.t., 40 mins.

(E)-5-(2-carboxyvinyl)-2'-deoxyuridine (**20**) was finally converted into (E)-5-(2-bromovinyl)-2'-deoxyuridine (**21**) using N-bromosuccinimide (NBS) and potassium carbonate in dry DMF (Scheme 2.2). Beside the main product, the TLC analysis of the reaction showed the formation of by-products running at higher and lower Rf than that of BVdU. These were isolated as crude but not extensively characterised.



20 **21**

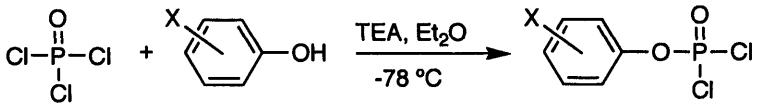
Scheme 2.2: *Synthesis of (E)-5-(2-bromovinyl)-2-deoxyuridine (**21**).* Reagents and conditions: i) NBS, K_2CO_3, DMF, r.t., 1 hr.

Purification by flash column chromatography using chloroform/methanol (gradient elution from 95/5 to 85/15) followed by crystallization from water afforded BVdU (**21**). ^{1}H- and ^{13}C-NMRs of (**21**) are consistent with the reported literature[19, 20] and the assignment of the E configuration is based upon the coupling constants of the vinylic protons which is 13.6 Hz.

2.3 Synthesis of phosphorodichloridates.

Phosphorodichloridate is the term used to refer to the phosphorylating reagent involved in the coupling to generate the phosphorochloridate. According to McGuigan *et al.*[21] the phosphorodichloridates are obtained from the low temperature (-78 ^{o}C) addition of phosphorus oxychloride to the appropriately

substituted phenols in the presence of triethylamine (TEA) in anhydrous ether (Scheme 2.3). The reaction is monitored by ^{31}P-NMR following the disappearance of phosphorus oxychloride (δ_P *ca.* 5.11 ppm). The reaction mixtures were filtered and the ether removed to give crude oils, which were used for the coupling with the amino acid ester hydrochloride salts without further purification. The phosphorodichloridates **22a-22f** were obtained in high yields (77-95 %, Table 2.1) and acceptable purity as judged by ^{31}P-NMR.



22a-22f

Scheme 2.3*: Synthesis of phosphorodichloridates **22a-22f**.*

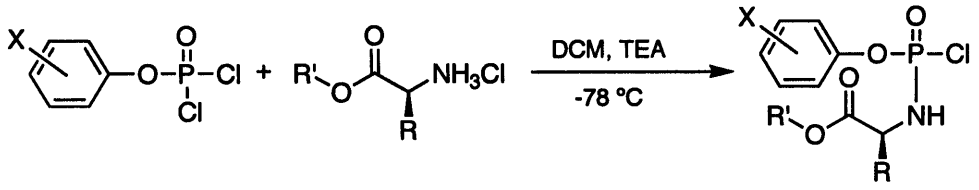
In table 2.1 are listed the yields and the chemical shifts quoted in ppm for the synthesis of the phosphorodichloridates **22a-22f**.

Cmpd	X	Yield	^{31}P-NMR, δ
22a	*p*-F	84 %	5.49
22b	*p*-Cl	77 %	5.18
22c	*p*-CF_3	88 %	4.75
22d	*p*-CH_3O	95 %	5.71
22e	*m*-Cl	85 %	4.92
22f	*o*-Cl	82 %	5.39

Table 2.1*: ^{31}P-NMR chemical shifts and yields for the synthesis of the phosphorodichloridates **22a-22f**.*

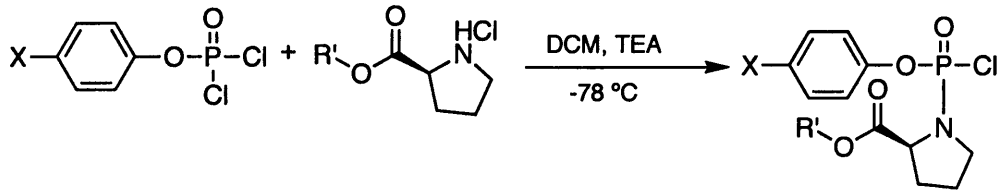
2.4 Synthesis of phosphorylating reagents.

Phosphorochloridate is the term used to refer to the phosphorylating reagents used for the coupling with the nucleosides. According to the procedure of Curley *et al.*,[22] these were obtained from the low temperature (-78 °C) coupling of amino acid ester hydrochloride salts with phenyldichlorophosphodichloridates in the presence of TEA in anhydrous DCM (Scheme 2.4 and 2.5).



22 **23a-23k**

Scheme 2.4: *Synthesis of phosphorochloridates **23a-k**. R'=Me, Et, Bn; R=Me (L-alanine), i-propyl (L-valine).*



22 **23l-23o**

Scheme 2.5: *Synthesis of phosphorochloridates containing L-proline (**23l-o**). R'=Bn; X=H, Cl, F, NO_2.*

The formation of the phosphorochloridate species is followed by ^{31}P-NMR monitoring the disappearance of the phosphorodichloridate which is completed after 2-16 hrs. After removal of DCM, the phosphorochloridates are filtered and washed with anhydrous ether in order to remove the triethylammonium

salt. After evaporation of the ether, the products were obtained as crude oils and generally used for the coupling with the nucleoside without further purification. The phosphorochloridates are obtained in high yields and reasonable purity, as judged by ^{31}P-NMR, which also clearly shows the duplication of the peaks due to the presence of the two diastereoisomers arising from the chiral phosphorus centre (Figure 2.2).

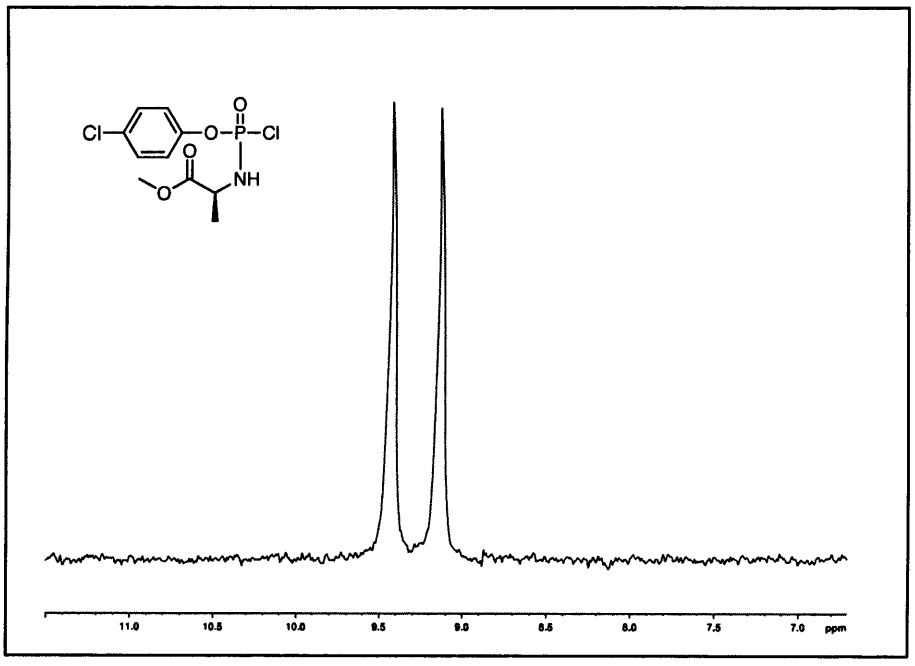


Figure 2.2: *^{31}P-NMR of 4-chlorophenyl-(methoxy-L-alaninyl)-phosphorochloridate (**23a**).*

In table 2.2 are listed the yields and the ^{31}P-NMR chemical shifts quoted in ppm for the synthesis of phosphorochloridates **23a-23o**.

Compd	Amino Acid	R'	X	Yield	^{31}P-NMR, δ
23a	L-alanine	Me	*p*-Cl	73 %	9.36, 9.07
23b	L-alanine	Et	*p*-Cl	85 %	9.54, 9.25
23c	L-alanine	Bn	*p*-Cl	87%	9.43, 9.16
23d	L-alanine	Me	*p*-CF_3	89 %	9.36, 9.22
23e	L-alanine	Et	*p*-CF_3	92 %	9.33, 9.28
23f	L-alanine	Bn	*o*-Cl	97 %	9.96, 9.29
23g	L-alanine	Bn	*m*-Cl	99 %	9.34, 8.90
23h	L-alanine	Bn	*p*-MeO	64 %	10.06, 9.87
23i	L-valine	Bn	H	>100 %	10.90, 10.34
23j	L-valine	Bn	*p*-Cl	95 %	10.31, 10.36
23k	L-valine	Bn	*p*-F	93 %	11.35, 10.81 (2d, $^{6}J_{P-F}$=2.4 Hz)
23l	L-proline	Bn	H	89 %	9.02, 9.09
23m	L-proline	Bn	*p*-Cl	75 %	9.13, 9.22
23n	L-proline	Bn	*p*-F	89 %	9.52, 9.42 (2d, $^{6}J_{P-F}$=2.6 and 2.5 Hz)
23o	L-proline	Bn	*p*-NO_2	91 %	8.75, 8.59

Table 2.2: *^{31}P-NMR chemical shifts and yields for the synthesis of phosphorochloridates **23a-o**.*

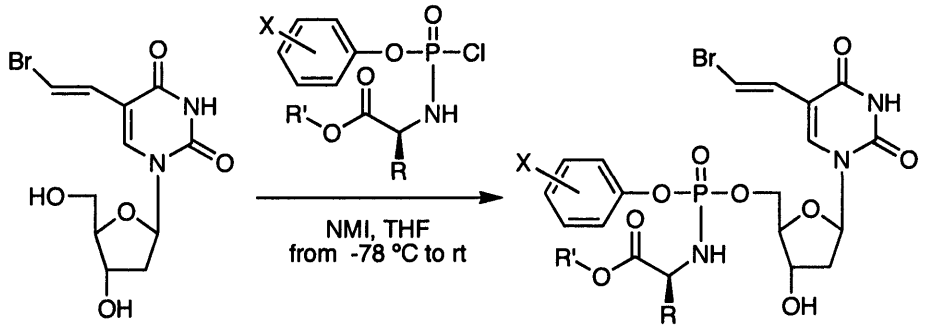
Surprisingly, ^{31}P-NMR data of phosphorochloridates **23k** and **23n** bearing a fluorine atom in the aryl moiety showed the presence of four peaks which were subsequently confirmed to be a splitting of the signals due to an unusually long range P-F coupling (^{6}J). Because of the intrinsic low stability of the phosphorochloridates, further ^{31}P-NMR investigations at different magnetic fields were

subsequently conducted on the BVdU phosphoramidates **24k**, which displayed the same ^{31}P-NMR splitting pattern of **23k**.

2.5 Synthesis of BVdU phosphoramidates.

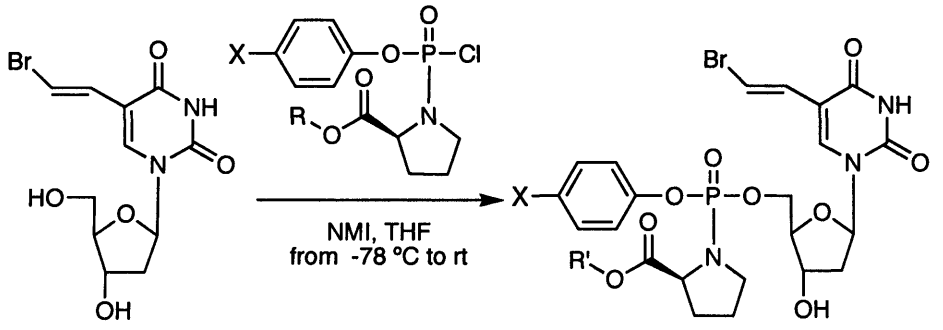
As a part of ongoing studies of SARs of BVdU protides, modifications at the aryl portion and at the carboxylic ester function as well as varying the amino acid were considered. Bearing in mind that the first step in a proposed degradation mechanism of the BVdU prodrugs could be the carboxyl esterase cleavage of the carboxylic ester moiety, structural modification in that specific part of the molecule would presumably affect the enzymatic stability of the phosphoramidates toward esterases. Furthermore, substitution of the aryloxy part of BVdU protides could have the effect of altering not only the rate and/or the nature of the prodrug degradation but also the rate of the membrane transport due to the change in the partition coefficient (LogP).

The synthesis of a series of BVdU-5'-phosphoramidates **24a-24r** containing natural amino acids was attempted following the adapted procedure from Van Boom *et al.*[23] Thus, the phosphorochloridates **23a-23o** were reacted with BVdU in the presence of N-methylimidazole (NMI) in anhydrous tetrahydrofuran (THF) at −78 °C to room temperature following the procedures previously described (Scheme 2.6 and 2.7).[21, 24]



BVdU **24a-k**

Scheme 2.6: *Synthesis of BVdU phosphoramidates* ***24a-k.*** *R'=Me, Et, Bn; R=Me (L-alanine), i-propyl (L-valine); X=H, Cl, CF_3, F, NO_2, MeO.*



BVdU **24l-s**

Scheme 2.7: *Synthesis of benzyl L-proline BVdU phosphoramidates* ***24l-s.*** *R'=Bn; X=H, Cl, F, NO_2.*

TLC analysis of the reaction mixtures showed the formation of the desired products, **24a-s**, after 2-20 hrs. The reactions were quenched with water or methanol, the solvent removed under reduced pressure and the crude washed with 0.1 M HCl. Multiple silica column chromatographies afforded the desired phosphoramidates **24a-s** (Table 2.3) in reasonable yields (30-58%). Besides the phosphoramidates **24a-s**, other products, running at higher Rf than those of the desired compounds were detected. These can be attributed to the formation of the 3',5'-O-diphosphorylated species, and these were isolated as a crude but not extensively characterised. Traces of the 3'-O-

monophosphorylated by-products were also observed by ^{31}P-NMR and these were separated from the desired 5′-O-monophosphorylated compounds by flash column chromatography.

Compd	Amino Acid	R′	X	Yield	^{31}P-NMR, δ
24a	L-alanine	Me	*p*-Cl	38%	4.81, 4.54
24b	L-alanine	Et	*p*-Cl	30%	4.88, 4.65
24c	L-alanine	Bn	*p*-Cl	50%	4.81, 4.51
24d	L-alanine	Bn	*o*-Cl	57 %	4.66, 4.42
24e	L-alanine	Bn	*m*-Cl	48 %	4.71, 4.32
24f	L-alanine	Me	*p*-CF_3	55 %	5.23, 5.07
24g	L-alanine	Et	*p*-CF_3	44 %	4.65, 4.35
24h	L-alanine	Bn	*p*-MeO	63 %	5.91, 5.96
24i	L-valine	Bn	H	55 %	5.91, 5.26
24j	L-valine	Bn	*p*-Cl	58 %	5.95, 5.47
24k	L-valine	Bn	*p*-F	57 %	6.21, 5.63 (2d, $^{6}J_{P\text{-}F}$=1.82 and 1.71 Hz)
24l	L-proline	Bn	H	33 %	3.18
24m	L-proline	Bn	H	4 %	3.32
24n	L-proline	Bn	*p*-Cl	40 %	3.17
24o	L-proline	Bn	*p*-Cl	13 %	3.41
24p	L-proline	Bn	*p*-F	42 %	3.39 (d, $^{6}J_{P\text{-}F}$= 1.88 Hz)
24q	L-proline	Bn	*p*-F	11 %	3.59 (d, $^{6}J_{P\text{-}F}$=2.03 Hz)
24r	L-proline	Bn	*p*-NO_2	40 %	3.50
24s	L-proline	Bn	*p*-NO_2	11 %	3.35

Table 2.3: *^{31}P-NMR chemical shifts and yields for the synthesis of phosphoramidates **24a-s**.*

Phosphoramidates **24a-k** were isolated as a 1:1 mixture of diastereoisomers, as seen by ^{31}P-NMR and ^{1}H-NMR which were found to be consistent with the expected structures. In the case of the *para*-F derivatives **24k**, **24p** and **24q**, ^{31}P-NMRs display four very close peaks (Figure 2.3) due to a long range P-F (^{6}J) coupling and this was proved by the use of ^{31}P-NMR experiments at different magnetic fields.

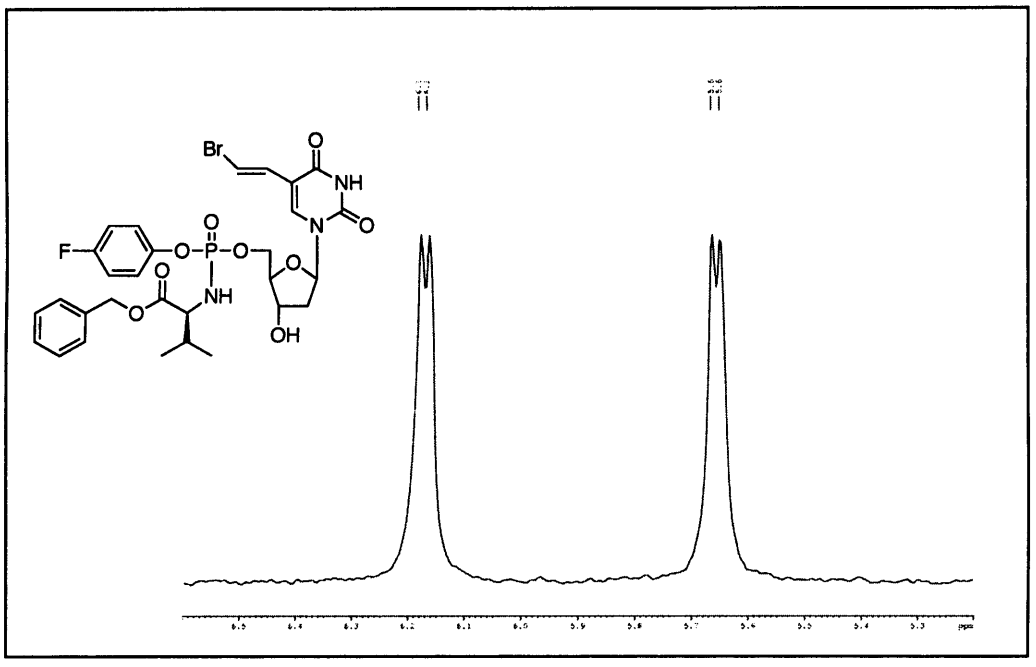


Figure 2.2: *^{31}P-NMR of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-fluorophenyl-(benzoxy-L-valinyl)]-phosphate (**24k**).*

Interestingly, the BVdU protides **24l-s** containing benzyl L-proline were isolated as pure diastereoisomers after purification by standard flash column chromatography. As illustrated in table 2.3, the fastest eluted diastereoisomers (**24l**, **24n**, **24p**, **24r**) were isolated in higher yields compared to the slower diastereoisomers (**24m**, **24o**, **24q**, **24s**).

To the best of our knowledge, the synthesis of all phosphoramidate prodrugs reported in the literature to date is not stereoselective and gives rise to a *ca.* 1:1 mixture of two diastereoisomers. The reason for this unexpected effect is not apparent and a possible explanation for the unusual diastereoselectivity may be attributed to steric hindrance of the five-member ring of L-proline, which could favour the formation of one diastereoisomer.

Target compounds **24l-s** were characterised by ^{31}P-NMR, ^{1}H-NMR and ^{13}C-NMR, and found to be consistent with the expected structures.

Several pieces of evidence support the characterisation of the products to be pure diastereoisomers and not mixtures, and these are detailed as follows on the representative compounds, the (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-L-prolinyl)]-phosphate, **24l** and **24m**:

- The ^{31}P-NMR showed the presence of only one peak in both compounds (Figure 2.4).
- The ^{1}H-NMR was not complex and the characteristic signals H-6, vinylic protons, and H-1' were not "split" for the presence of the two diastereoisomers.
- Full assignment of ^{13}C-NMR signals of **24l** and **24m**, was achieved by comparison with DEPT-135 and HMQC spectra. The ^{13}C-NMR signal of C-5' in both compounds appeared to be downfield as expected for a 5' phosphorylated compound. Furthermore, ^{13}C-NMR and DEPT-135 showed splittings at the C-5' in two signals indicating a C-P coupling. On the other hand, the C-3' did not show any splitting due to the C-P coupling and/or presence of a second diastereoisomer (Figure 2.5 and 2.6).

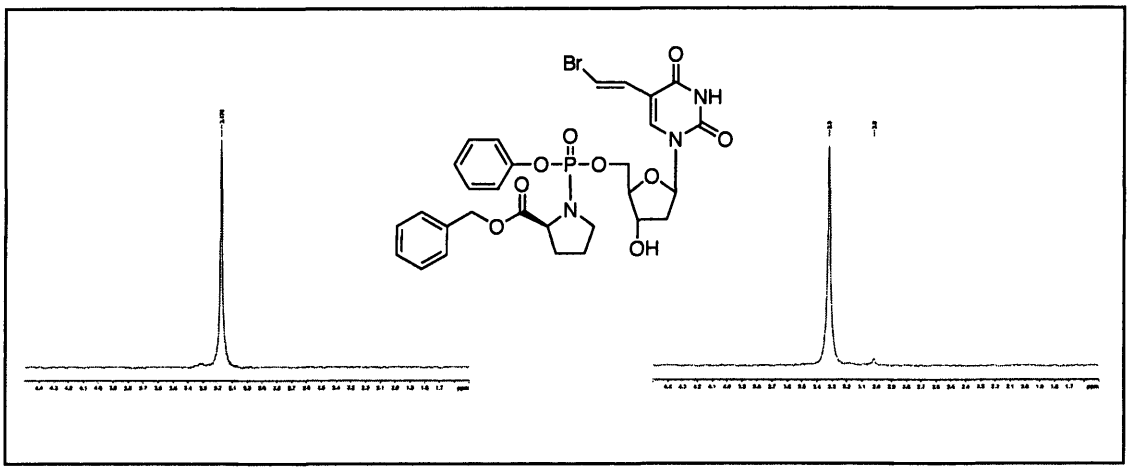


Figure 2.4: *^{31}P-NMR of the isolated diastereoisomers of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-L-prolinyl)]-phosphate (**24l** left and **24m** right).*

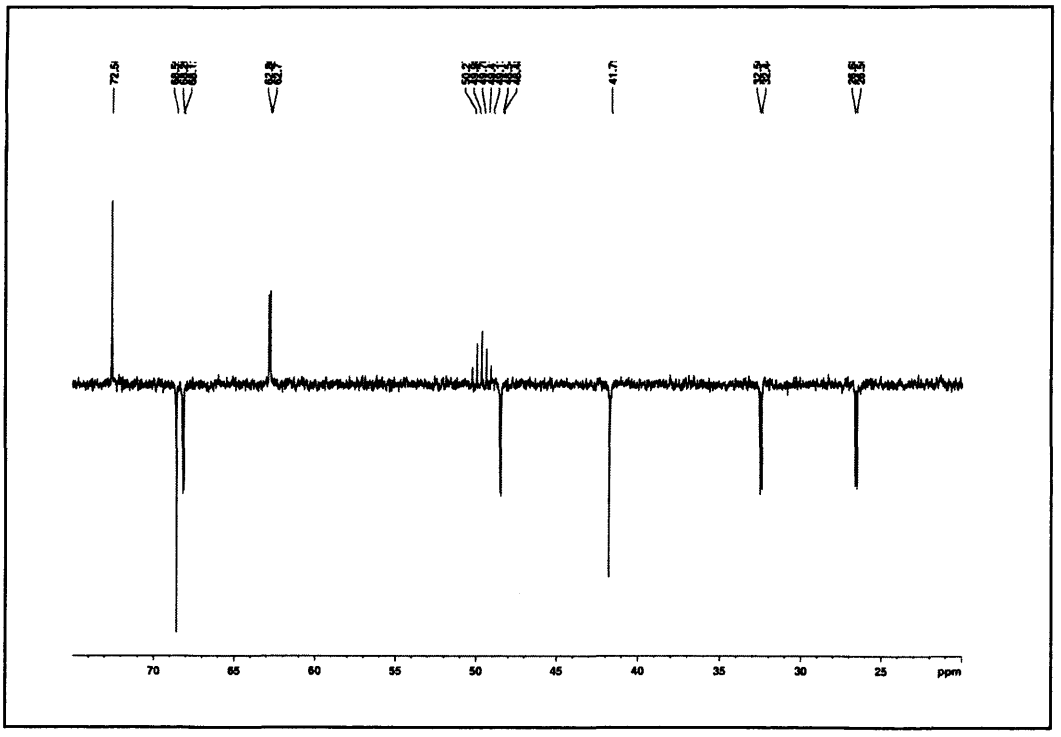


Figure 2.5: *DEPT of the isolated diastereoisomer of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-L-prolinyl)]-phosphate (**24l**).*

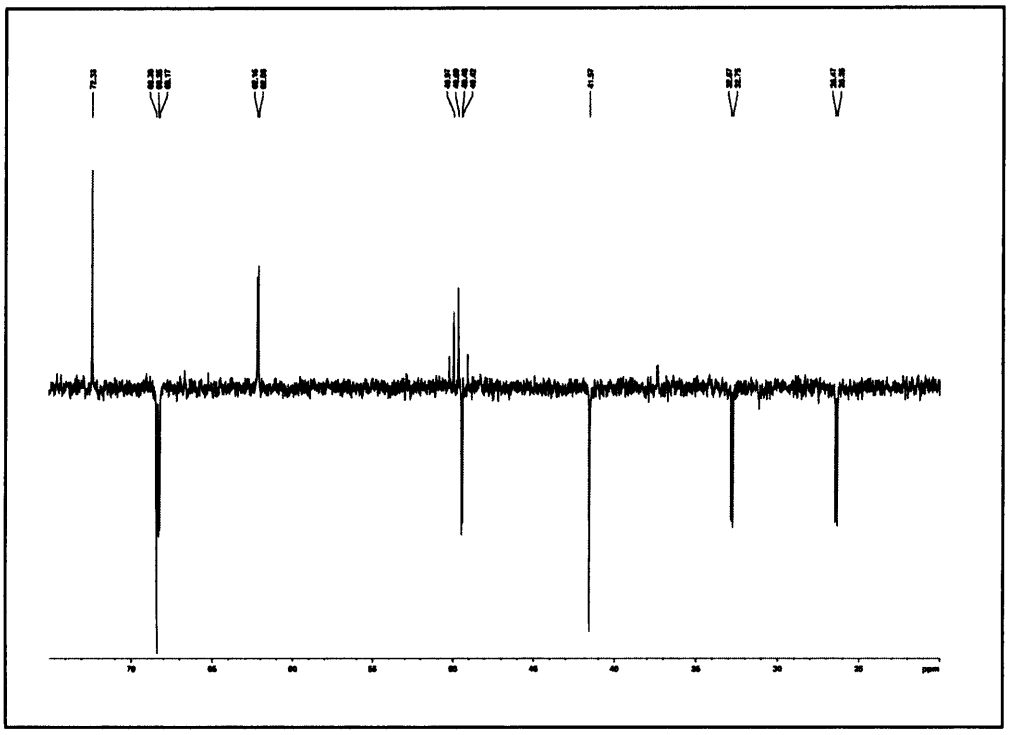
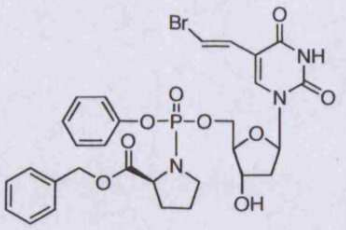


Figure 2.6: *DEPT of the isolated diastereoisomer of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-L-prolinyl)]-phosphate (**24m**).*

In order to provide a precise assignment of the hydrogen and carbon NMR signals (see experimental), a COSY90 experiment and a HMQC experiment were performed on representative compounds of each series. With the aid of these experiments the protons and the corresponding carbons could be unequivocally assigned on the base of the observed cross peaks in the COSY90 and the HMQC (see fig 2.7 and 2.8 for details).

In the following figures (Figure 2.7 and 2.8) are reported as examples COSY90 and HMQS of one of the isolated diastereoisomers of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-L-prolinyl)]-phosphate (**24l**). Similarly, full assignment of mixture of diastereoisomers of BVdU phosphoramidates was performed.



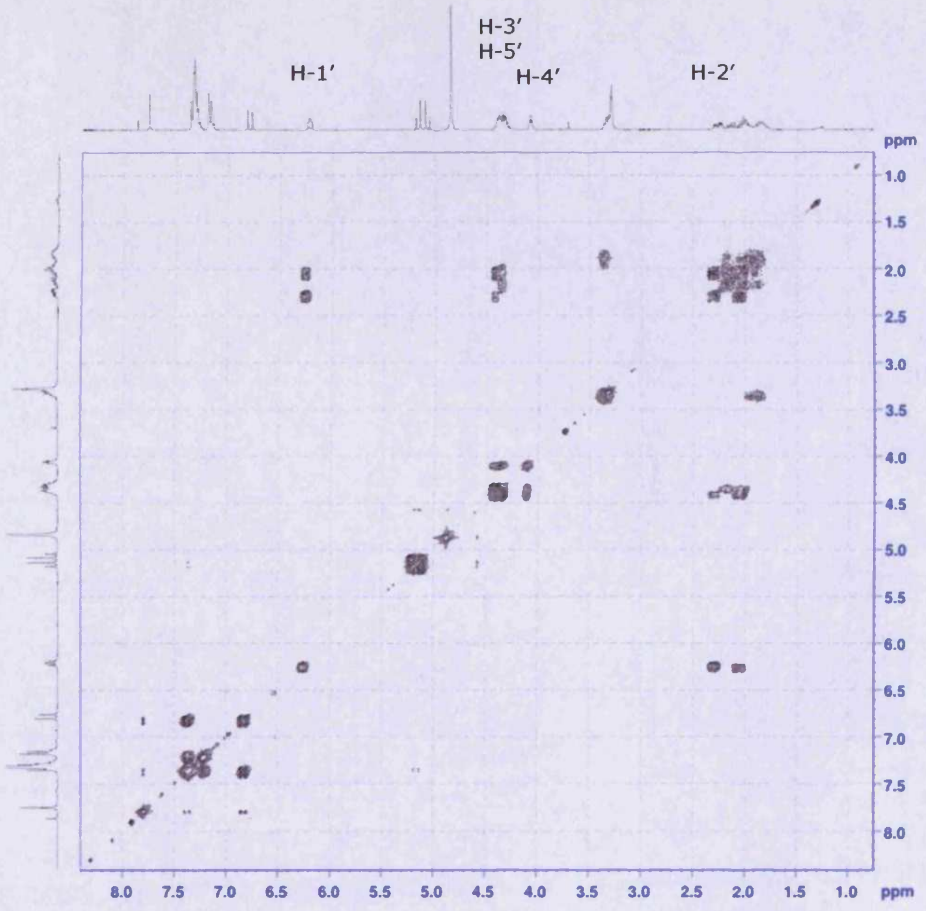
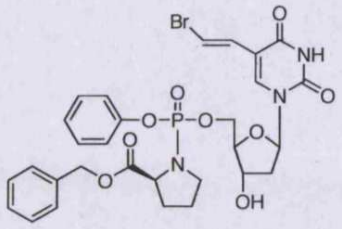


Figure 2.7: *COSY90 experiment (E)-5-(2-bromovinyl)-2′-deoxyuridine-5′-[phenyl-(benzoxy-L-prolinyl)]-phosphate (**24l**).*



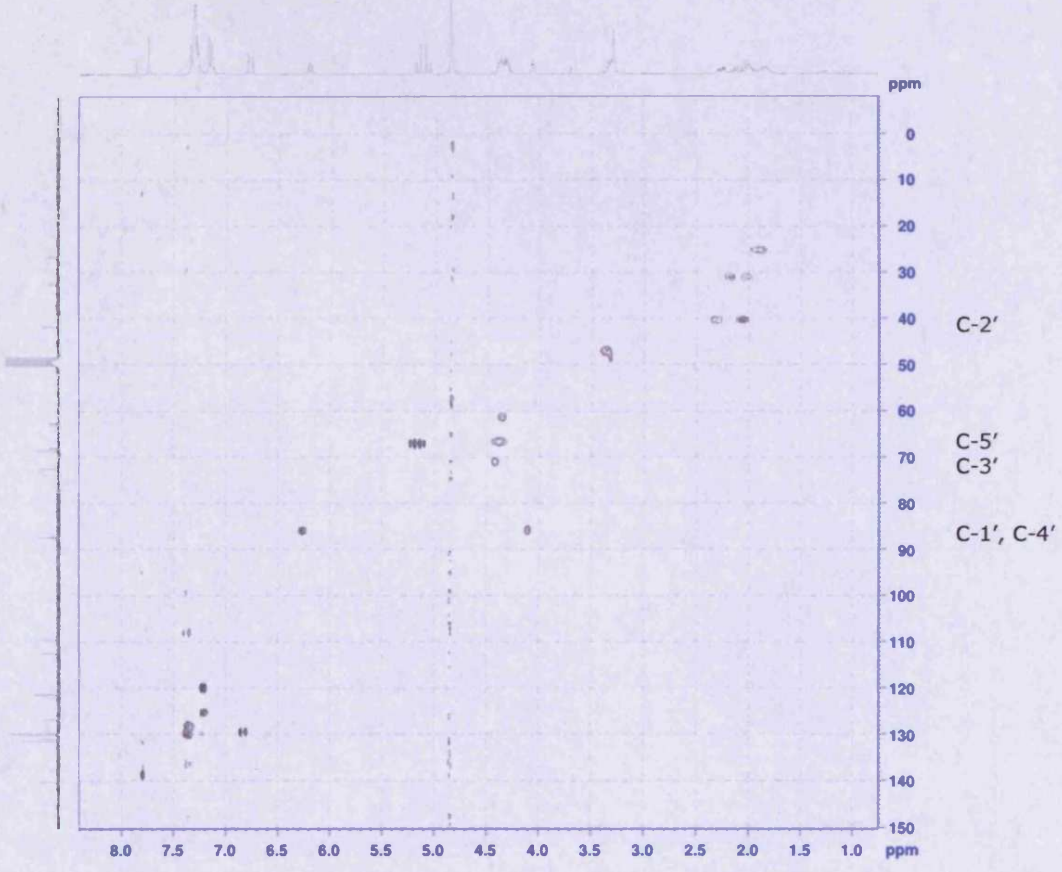


Figure 2.8: *HMQC experiment of (E)-5-(2-bromovinyl)-2′-deoxyuridine-5′-[phenyl-(benzoxy-L-prolinyl)]-phosphate (**24l**).*

2.6 Attempted synthesis of phenyl-[methoxy-L-histidinyl]-BVdU phosphoramidate.

In order to further extend the SAR studies of BVdU protides and in particular provide information on the amino acid SARs, we planned the synthesis of phenyl methoxy L-histidininyl BVdU triester.

Commercially available L-histidine methyl ester dihydrochloride was selected as a starting material in order to explore the reactivity. Thus, as a first attempt, L-histidine methyl ester dihydrochloride was reacted under the usual condition (DCM, TEA, -78 ^{o}C) in an effort to form the phosphorochloridate but unfortunately crude analysis of the mixture by ^{31}P-NMR showed the formation of several products. Furthermore, no improvement was observed when the reaction was repeated and the reaction times varied. A possible explanation for these results may be the insolubility of the amino acid and/or to the fact that the amino acid is available as dihydrochloride salt and it may not be possible to neutralise only the α-amino acid without affecting the hydrochloric salt in the side chain. Therefore, in future attempts to synthesise the corresponding derivative of L-histidine BVdU protide, it may be advantageous to consider the use of suitable protecting groups in order to avoid interference from the side chain.

2.7 Synthesis of BVdU phosphoramidates containing blocked natural amino acids.

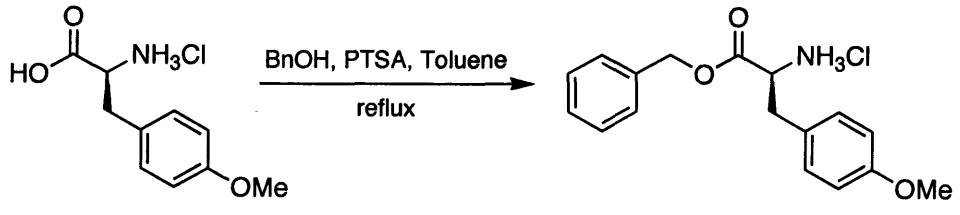
In continuing our studies on the SARs of the BVdU protides, we sought to investigate the effect of other natural amino acids such as L-tyrosine. Preparation of the phosphoramidates containing L-tyrosine would require the protection of the hydroxyl groups of

the amino acids side chain which can compete as a nucleophile in the synthesis of the phosphorochloridate species.

For this reason, commercially available O-methyl-L-tyrosine was chosen for the synthesis of the corresponding phosphoramidates. In addition, the initial SAR studies revealed that replacement of the methyl ester by a benzyl ester leads to an increase in potency for BVdU protides. Thus, as a part of preliminary investigations on the amino acid region of BVdU phosphoramidates, the benzyl ester of L-tyrosine was selected as a candidate for the synthesis of the corresponding protide.

2.7.1 Synthesis of phenyl-[benzoxy-(O-methyl-L-tyrosinyl)]-BVdU phosphoramidate.

The O-methyl-L-tyrosine benzyl ester **25** was synthesised according to the procedure of Yamada *et al.*,[25] starting from O-methyl-L-tyrosine, benzyl alcohol and p-toluene sulfonic acid and refluxing the mixture for 4 hours in a Dean and Stark apparatus (scheme 2.8). The solvent was removed under reduced pressure, the solid filtered and washed with diethyl ether. Exchange of the *p*-toluene sulfonate with chloride ion gave the hydrochloride salt as a white solid in high yield (79 %).

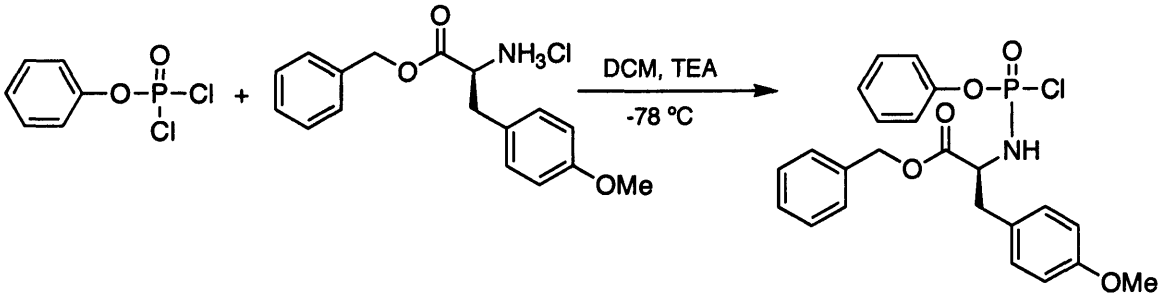


O-methyl-L-tyrosine **25**

Scheme 2.8: *Synthesis of O-methyl-L-tyrosine benzyl ester (**25**).*

The preparation of the phenyl[benzoxy-O-methyl-L-tyrosine]-phosphorochloridate **26**, was made by coupling the phenyl

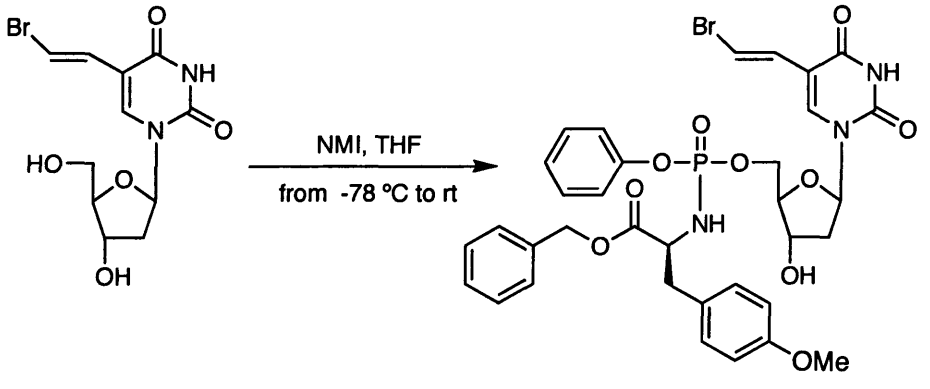
phosphorodichloridate with O-methyl-L-tyrosine benzyl ester hydrochloride salt following the previously described phosphorochloridate chemistry.[22] (Scheme 2.9)



25 **26**

Scheme 2.9: *Synthesis of phenyl-[benzoxy-O-methyl-L-tyrosinyl]-phosphorochloridate (**26**).*

BVdU was then coupled with phenyl-[benzoxy-O-methyl-L-tyrosinyl]-phosphorochloridate **26**, in the presence of NMI and THF at -78 °C (Scheme 2.10).



BVdU **27**

Scheme 2.10: *Synthesis of L-tyrosine BVdU phosphoramidate (**27**).*

Final purification via flash column chromatography gave compound **27**, in 46% yield.

2.8 Biological results.

The synthesised BVdU phosphoramidates (**24a-s** and **27**) were evaluated for their anti-cancer activity against a panel of three tumour cell lines *in vitro*, including human breast cancer cell line MDA MB 231, human prostate cancer cell line PC-3 and human colon cancer cell line HT115. The biological tests were carried out at the College of Medicine, University of Wales, in collaboration with Dr. W. Jiang and Dr. M. Mason. Data are reported in table 2.4.

The cytotoxicity assay was based on MTT assay and on the ability of viable mitochondria to convert MTT into an insoluble formazan precipitate that was dissolved and quantified by spectrometry. Compounds, dissolved in DMSO, were series diluted (1:5) in culture medium, to cover a final concentration range between 0.128 and 2000 μM. The culture plate was incubated for 72 hour at 37 °C. The cells were washed with BSS. A solution of MTT in 0.5 mg/mL in culture medium was added into each well. The culture plate was then incubated at 37 °C for 4 hour and MTT was then removed by aspiration The crystal produced by MTT reagent within the cells were then extracted by the addition of 100 μL of Triton X100 (10% in water). The cells were incubated at 4 °C for 24 hour. The absorbance of the colorimetric products was then measured at a wavelength of 540 nm using a spectrophotometer (Titerteck).

Compound	Aryl	Ester	Amino Acid	$EC_{50}/\mu M$		
				Breast MDAMB231	Colon HT115	Prostate PC-3
17 (thymectacin)	Ph	Me	Ala	79	245	155
CPF2[a]	Ph	Bn	Ala	34	1.4	19
24a	*p*-ClPh	Me	Ala	61	70.2	13
24b	*p*-ClPh	Et	Ala	28.7	14.9	3.4
24c	*p*-ClPh	Bn	Ala	6.2	3.4	2.4
24d	*o*-ClPh	Bn	Ala	5.4	16.2	5.4
24e	*m*-ClPh	Bn	Ala	7.2		20.5
24f	*p*-CF_3Ph	Me	Ala	47	7.92	14
24g	*p*-CF_3Ph	Et	Ala	33.8		4.6
24h	*p*-MeOPh	Bn	Ala	5.2		6.6
24i	Ph	Bn	Val	11.1		31.2
24j	*p*-ClPh	Bn	Val	0.61		0.9
24k	*p*-FPh	Bn	Val	14.5		1.6
24l (F)	Ph	Bn	Pro	2.4		0.76
24m (S)	Ph	Bn	Pro	24.8		46.2
24n (F)	*p*-ClPh	Bn	Pro	7.3		9.2
24o (S)	*p*-ClPh	Bn	Pro	11.2		8.1
24p (F)	*p*-FPh	Bn	Pro	1.8		5.7
24q (S)	*p*-FPh	Bn	Pro	4.8		41.8
24r (F)	*p*-NO_2Ph	Bn	Pro	6.6		5.1
24s (S)	*p*-NO_2Ph	Bn	Pro	3.7		11.0
27	PhO	Bn	Tyr	4.1		1.5

Table 2.4: *Anti-cancer activity for compounds **24a-s** and **27**. [a]: Compound synthesised by J.C. Thiery (Welsh School of Pharmacy, Cardiff University, 2001).*

As seen clearly from the data reported in table 2.4, the synthesised compounds **24a-s** and **27** are characterised by an increase of anticancer activity compared to the lead **17** (thymectacin) which displayed EC_{50} values of 79-245 μM in our assays. In terms of structural activity relationships it is apparent from the data shown in table 2.4 that modification of the structure leads to a significant boost in potency for all the synthesised triester derivatives.

As shown in table 2.4, improvement in the activity was observed by replacing the methyl ester (**17**) by a benzyl ester (**CPF2**) with the benzyl ester being 2-fold more potent versus breast and 8-fold more potent versus prostate cancer cell line. Moreover, the introduction of electron-withdrawing groups within the aryl moiety led to an elevation in activity with the *p*-chloro phenyl derivative **24c** being 13-fold more potent versus breast and 65-fold more potent versus prostate cancer cell line. It is noteworthy that compound **24c** is 50-fold more lipophilic than **17** (estimated ClogP values: **24c** 1.82, **17** 0.11, Chemdraw 7.0) and this may lead to a rapid membrane permeation of **24c**. In the same way, the *meta* and *ortho*-substituted regioisomers (**24d** and **24e**) of **24c** displayed an increase of activity compared to **17,** but no appreciable differences in anticancer activity were observed within these regioisomeric compounds, indicating an apparent tolerance to specific aryl substituent position.

It is interesting to note that, amongst the synthesised BVdU protides, compound **24j** is amongst the most active of the series in both cancer cell lines with an increase of activity of 130-fold versus breast and 177-fold versus prostate cell line. This is in contrast with what was observed with the L-valine d4T prodrug, which showed low antiviral activity, consistent with a low conversion of the prodrug to d4TMP (d4T monophosphate).[26] Whether this is due to a different and higher activity of the carboxyl esterase in the cell lines and/or to a different mechanism of degradation of BVdU prodrugs is currently unclear.

Finally, the separated diastereoisomers **24l-24s** of BVdU triester containing benzyl L-proline displayed an increased activity compared to the lead **17,** indicating that the presence of the blocked amino group and/or the proline ring is not detrimental to activity. From the data shown in table 2.4 it is apparent that the pure diastereoisomers (**24l-24s**, where F refers to the fastest –

more lipophilic- and S the slowest –less lipophilic- isomer) displayed in general similar biological activity, with the fastest diastereoisomers being more active than the slowest. However, in the absence of X-Ray crystal structure studies of the diastereoisomers **24l-24s**, it is impossible to determine any correlating phosphorus configuration-activity.

2.9 References.

1 McGuigan, C.; Pathirana, R.N.; Mahmood, N.; Hay, A.J.; Aryl phosphate derivatives of AZT inhibit HIV replication in cells where the nucleoside is poorly active. *Bioorg. Med. Chem. Lett.*, **1992**, 2, 701-704.

2 McGuigan, C.; Sheeka, H.M.; Mahmood, N.; Hay, A.; Phosphate derivatives of d4T as inhibitors of HIV. *Bioorg. Med. Chem. Lett.*, **1993**, 3, 1203-1206.

3 Balzarini, J.; Kruining, J.; Wedgwood, O.; Pannecouque, C.; Aquaro, S.; Perno, C.F.; Naesens, L.; Witvrouw, M.; Heijtink, R.; De Clercq, E.; McGuigan, C.; Conversion of 2',3'-dideoxyadenosine (ddA) and 2',3'-didehrydro-2',3'-dideoxyadenosine (d4A) to their corresponding aryloxyphosphoramiodate derivatives markedly potentiates their activity against human immunodeficiency virus and hepatitis B virus. *FEBS Lett.*, **1997**, 410, 324-328.

4 McGuigan, C.; Wedgwood, O.M.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of 2',3'-didehydro-2',3'-dideoxyadenosine [d4A] have markedly improved anti-HIV potency and selectivity. *Bioorg. Med Chem. Lett.*, **1996**, 6, 2359-2362.

5 Winter, H; Maeda, Y.; Mitsuya, H.; Zemlika, J.; Phosphodiester amidates of allenic nucleoside analogues: anti-HIV activity and possible mechanism of action. *J. Med. Chem.*, **1996**, 39, 3300-3306.

6 Franchetti, P.; Cappellacci, L.; Grifantini, M.; Messini, L.; Sheika, G.A.; Loi, A.G.; Tromontano, E.; De Montis, A.; Spiga, M.G.; La Colla, P.; Synthesis and Evaluation of the Anti-HIV Activity of Aza and Deaza Analogues of IsoddA and their Phosphates as Prodrugs. *J. Med. Chem.*, **1994**, 37, 3534-3541.

7 Ballatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J.; Synthesis and evaluation of novel amidate prodrugs of PMEA and PMPA. *Bioorg. Med. Chem. Lett.*, **2001**, 11, 1053-1056.

8 McGuigan, C.; Slater, M.J.; Parry, N.R.; Perry, A.; Harris, S.; Synthesis and antiviral activity of acyclovir-5'-(phenyl methoxy alaninyl) phosphate as a possible membrane-soluble nucleotide prodrug. *Bioorg. Med. Chem. Lett.*, **2000**, 10, 645-647.

9 McGuigan, C.; Perry, A.; Yarnold, C.J.; Sutton, P.W.; Lowe, D.; Miller, W.; Rahim, S.G.; Slater, M.J.; Synthesis and evaluation of some marked phosphates of the anti-herpes drug 882C (netivudine) as potential antiviral agents. *Antiviral Chem. Chemother.*, **1998**, 9, 233-243.

10 Harris, S.A.; McGuigan, C.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J.; Synthesis and antiviral evaluation of phosphoramidate derivatives of (E)-5-(2-bromovinyl)-2'-deoxyuridine. *Antiviral Chem. Chemother.*, **2001**, 12, 279-286.

11 Lackey, D.B.; Groziak, M.P.; Sergeeva, M.; Beryt, M.; Boyer, C.; Stroud, R.M.; Sayer, P.; Park, J.W.; Johnston, P.; Slamon, D.; Shepard, H.M., Pegram, M.; Enzyme-catalyzed therapeutic agent (ECTA) design: activation of the antitumor ECTA compound NB1011 by thymidylate synthase. *Biochem. Pharmacol.*, **2001**, 61, 179-189.

12 Li, Q.; Boyer, C.; Lee, J.Y.; Shepard, H.M.; A Novel approach to thymidylate synthase as a target for cancer chemotherapy. *Mol. Pharmacol.*, **2001**, 59, 446-452.

13 Sergeeva, O.A.; Khambatta, H.G.; Cathers, B.E.; Sergeeva, M.V.; Kinetic properties of human thymidylate synthase, an anticancer drug target. *Biochem. Biophys. Res. Comm.*, **2003**, 307, 297-300.

14 Sergeeva, M.V.; Cathers, B.E.; Cellular transformation of the new anticancer drug NB1011, a phosphoramidate of 5-(2-bromovinyl)-2'-deoxyuridine, results in modification of cellular proteins not DNA. *Biochem. Pharm.*, **2003**, 65, 823-831.

15 http://www.newbiotics.com/news_center_press_01072002.html

16 Pegram, M.; Ku, N.; Shepard, M.; Speid, L.; Lenz, H.J.; Enzyme-Catalyzed Therapeutic Activation (ECTA) of NB1011 (Thymectacin™) selectively target thymidylate synthase (TS)-overexpressing tumor cells: preclinical and phase 1 clinical results. *Eur. J. Cancer*, **2002**, 38, 99, Suppl 7.

17 Walker, R.T.; De Clercq, E.; Descamps, A.S.; Jones, A.S.; Serafinowski, P.; *Nucleic Acids Res.*, **1978**, spec. Pub. 4, s103.

18 Barr, J.; Jones, A.S.; Verheist, G.; Walker, R.T.; Synthesis of some 5-halogenovinyl derivatives of uracil and their conversion into 2'-deoxyribonucleosides. *J. Chem. Soc. Perkin Trans. I*, **1981**, 1665-1670.

19 Jones, A.S.; Verheist, G.; Walker, R.T.; The synthesis of the potent anti-herpes virus agent E-5-(2-bromovinyl)-2'-deoxyuridine and related compounds. *Tetrahedron Letts.*, **1979**, 45, 4415-4418.

20 Ashwell, M.; Jones, A.S.; Kumar, A.; Sayers, J.R.; Walker, R.T.; Sakuma, T.; De Clercq, E.; The synthesis and antiviral properties of (E)-5-(2-bromovinyl)-2'-deoxyuridine-related compounds. *Tetrahedron*, **1987**, 43, 4601-4608.

21 McGuigan, C.; Pathirana, R.N.; Balzarini, J.; De Clercq, E.; Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.*, **1993**, 36, 1048-1052.

22 Curley, D.; McGuigan, C.; Devine, K.G.; O'Connor, T.J.; Jeffries, D.J.; Kinchington, D.; Synthesis and anti-HIV evaluation of some phosphoramidate derivatives of AZT: studies on the effect of chain elongation on biological activity. *Antiviral Res.*, **1990**, 14, 345-356.

23 Van Boom, J.H.; Burgers, P.M.J.; Crea, R.; Luyten, W.C.M.M.; Vink, A.B.J.; Phosphorylation of nucleoside derivatives with aryl phosphoramidochloridates. *Tetrahedron*, **1975**, 31, 2953-2959.

24 McGuigan, C.; Cahard, D.; Sheeka, H.M.; De Clercq, E.; Balzarini, J.; Aryl phosphoramidate derivatives of d4T have

improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. *J. Med. Chem.*, **1996**, 39, 1748-1753.

25 Yamada, N.; Okuyama, K.; Serizawa, T.; Kawasaky, M.; Oshima, S.; Periodic change in absorption maxima due to different chain packing between the bilayers amphiphiles possessing even and odd numbers of carbons in the hydrophobic chain. *J. Chem. Soc., Perkin Trans. 2*, **1996**, 12, 2707-2708.

26 Saboulard, D.; Naesens, L.; Cahard, D.; Salgado, A.; Pathirana, R.; Velasquez, S.; McGuigan, C.; De Clercq, E.; Balzarini, J.; Characterization of the activation pathway of phosphoramidate triester prodrugs of stavudine and zidovudine. *Mol. Pharmacol.*, **1999**, 56, 693-704.

Chapter Three: Phosphoramidates of BVdU Containing Unnatural Amino Acids.

3.1 Background and aims.

As part of the extensive SAR studies conducted on the anti-HIV stavudine (d4T) phosphoramidate derivatives, it was observed that the nature of the amino acids is particularly important for the antiviral activity,[1, 2] with L-alanine as the preferred amino acid. Moreover, it was noticed that certain unnatural amino acids such as α-alkyl or α,α-dialkyl glycine could substitute for L-alanine with small or no loss of antiviral activity.[3]

Thus, bearing in mind the notable results that have emerged from our expertise in the antiviral arena, we sought to investigate the effect of unnatural amino acids on BVdU protides. This kind of modification would be of interest in expanding our knowledge on the effect of the amino acid moiety on the lead structure. In addition, the synthesis of BVdU phosphoramidates containing unnatural amino acids such as α-alkyl or α,α-dialkyl glycine, could be useful in elucidating the requirements for activity in terms of side chain length and chirality of the amino acids.

3.2 Synthesis of α,α-dialkyl BVdU phosphoramidates.

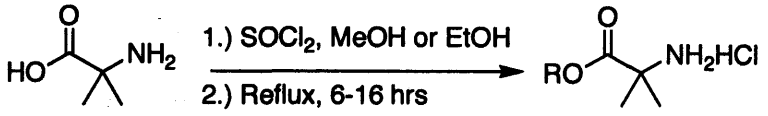
Taking into account the enhanced activity previously noted for aryl-substituted phosphoramidate prodrugs of d4T[4, 5, 6] and the effect that this may have in altering the rate of membrane transport and the prodrug degradation, a limited series of substituted BVdU aryloxy phosphoramidate analogues containing dimethylglycine as

amino acid was synthesised. Furthermore, we were interested in studying the structural modification in the carboxylic ester region and for this reason a small series of BVdU aryl phosphoramidates containg different esters (methyl, ethyl and benzyl) of dimethyl glycine was prepared.

3.2.1 Synthesis of α,α-dialkylglycine esters.

In order to carry out the first series of modifications, it was important to have the desired α,α-dialkylglycine esters to use in the coupling reaction with the phosphorodichloridate, as described above (scheme 2.4, page 40, Chapter Two). However, the desired esters were not commercially available, therefore they were prepared starting from the commercially available amino acids following the schemes reported below (scheme 3.1, 3.2 and 3.3).

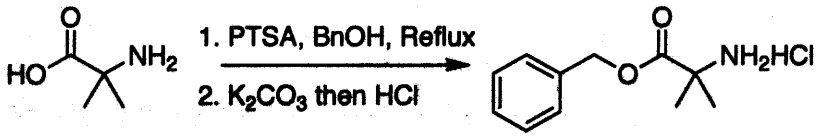
For the synthesis of the α,α-dimethylglycine methyl and ethyl esters (**28** and **29**), the corresponding commercially available α,α-dimethylglycine amino acid (2-aminoisobutyric acid) was added to a solution of the appropriate alcohols (methanol, ethanol), previously treated with thionyl chloride at 0 °C, according to the procedure of Webb *et al.*[7] Thus, the mixtures were refluxed for 6-16 hrs and removal of the solvent afforded the amino acid hydrochloride salts in good yields.



28, R=Me (89%)
29, R=Et (86%)

Scheme 3.1: *Synthesis of dimethylglycine esters* ***28*** *and* ***29***.

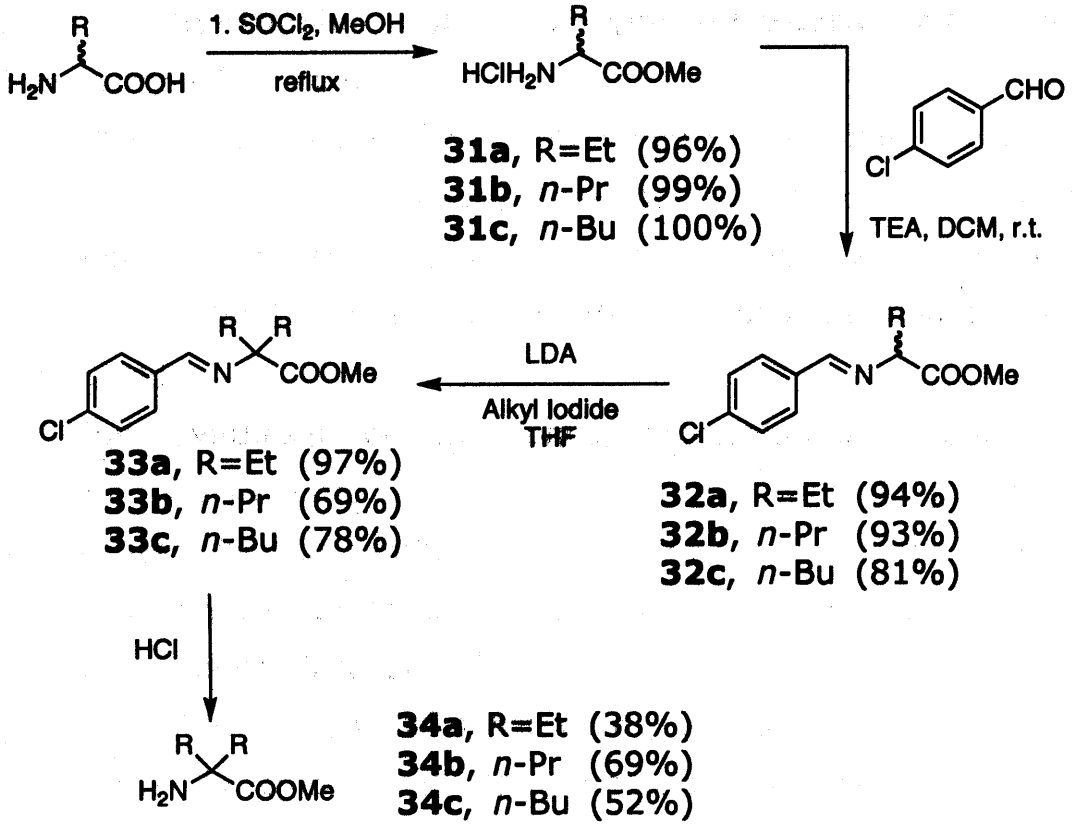
The desired dimethylglycine benzyl ester (**30**) was prepared in good yield (88%) from 2-aminoisobutyric acid, benzyl alcohol and *para*-toluene sulfonic acid in toluene by azeotropic distillation, according to the procedure of Yamada *et al.*[8]



30

Scheme 3.2: *Synthesis of dimethylglycine benzyl ester (**30**).*

Since the required diethyl (**34a**), dipropyl (**34b**) and dibutyl (**34c**) glycine were not commercially available, they were synthesised starting from the corresponding amino acids, following the synthetic route shown in scheme 3.3 (adapted from previous work carried out by Sheila Srinivasan, McGuigan Group, Welsh School of Pharmacy).[9]



Scheme 3.3: *Preparation of α,α-dialkyl amino acid methyl ester hydrochlorides* ***34a-c.***

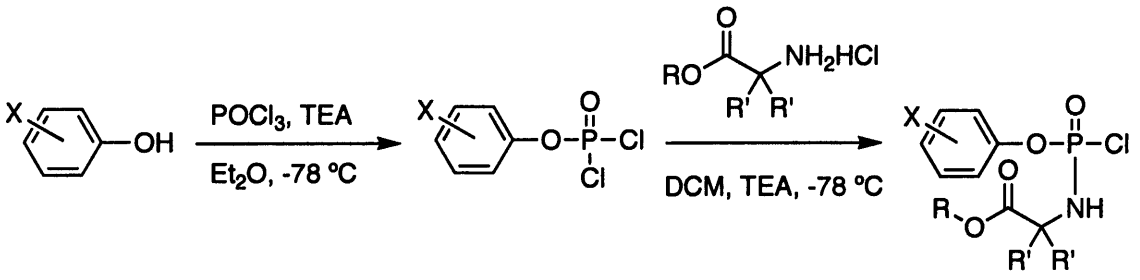
Commercially available DL-alkyl amino acids were converted to the corresponding methyl ester hydrochloric salts (**31a-c**) according to the procedure of Webb *et al.*,[7] and then protected as imines (**32a-c**) in the presence of *para*-chlorobenzaldehyde and TEA in DCM. Deprotonation of the imines **32a-c** using LDA (lithium diisopropylamide) followed by alkylation with the appropriate alkyl iodide afforded compounds **33a-c** in good yields. Deprotection by treatment with aqueous hydrochloric acid gave the desired amino acid hydrochlorides **34a-c**.

3.2.2 Synthesis of phosphorodichloridates and phosphorylating agents.

After having successfully synthesised the required α,α-dialkyl amino acid esters (**28**, **29**, **30**, **34a-34c**) the standard procedure for coupling the phosphorylating agent and coupling with BVdU was applied and the target α,α-dialkyl BVdU phosphoramidates were synthesised.

The synthesis of the phosphorodichloridates and the phosphorylating agents was carried out by following the procedure already used for the synthesis of the phosphoramidates of BVdU containing natural amino acids (Chapter Two), according to the method previously described.[10, 11]

Thus, the appropriate substituted phenols were coupled with phosphorus oxychloride in the presence of triethylamine in anhydrous diethyl ether at low temperature (-78 °C) to give the corresponding phosphodichloridates (Scheme 3.4). The reaction was checked by ^{31}P-NMR and the crude used without further purification. Phosphorodichloridates (**22a-22f**) were then used in the coupling with the appropriate amino acid ester hydrochlorides in DCM at –78 °C in the presence of triethylamine, yielding the phosphorochloridates **35a-35t** in good yields (Scheme 3.4). In some cases a quick purification, using flash column chromatography and ethyl acetate/petroleum ether (7/3) as eluent, was carried out.



22a-f **35a-u**

Scheme 3.4: *Synthesis of phosphorodichloridates **22a-f** and phosphorochloridates **35a-u**. R'=Me, Et, n-Pr, n-Bu; R=Me, Et, Bn; X=H, Cl, F, CF_3, NO_2, MeO.*

The phosphorochloridates **35a-u** were obtained in reasonable purity, as judged by ^{31}P-NMR, which also clearly shows the presence of a single peak due to the absence of a carbon stereocentre in the amino acid.

The data of the synthesis described above are summarised in Table 3.1. The data relating to the phosphorodichloridates **22a-f** are reported in Table 2.1, Chapter two.

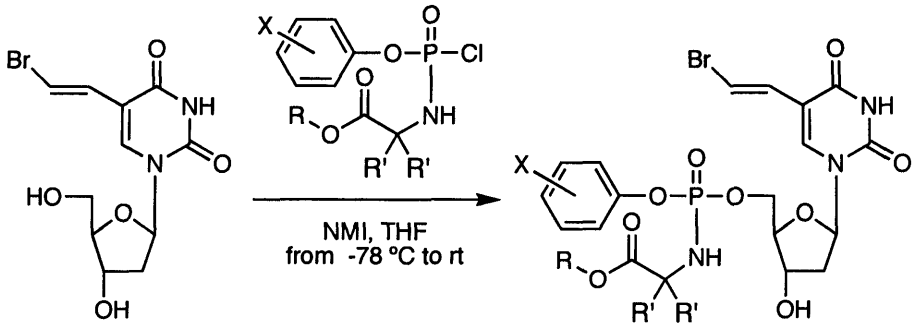
Compound	R	R′	X	Yield	^{31}P-NMR, δ
35a	Me	Me	H	94%	6.99
35b	Et	Me	H	89%	7.02
35c	Bn	Me	H	42%	6.79
35d	Me	Me	*p*-Cl	91%	7.05
35e	Et	Me	*p*-Cl	96%	7.09
35f	Bn	Me	*p*-Cl	94%	7.00
35g	Bn	Me	*o*-Cl	96%	7.13
35h	Bn	Me	*m*-Cl	97%	6.91
35i	Me	Me	*p*-NO_2	76%	6.61
35j	Et	Me	*p*-NO_2	89%	6.64
35k	Bn	Me	*p*-NO_2	90%	6.56
35l	Me	Me	*p*-CF_3	97%	6.80
35m	Et	Me	*p*-CF_3	97%	6.74
35n	Bn	Me	*p*-CF_3	96%	6.74
35o	Me	Me	*p*-F	97%	7.42 (d, $^{6}J_{P-F}$=2.9 Hz)
35p	Et	Me	*p*-F	92%	7.33 (d, $^{6}J_{P-F}$=2.3 Hz)
35q	Bn	Me	*p*-F	91%	7.37 (d, $^{6}J_{P-F}$=3.0 Hz)
35r	Bn	Me	*p*-MeO	91%	7.50
35s	Me	Et	H	55%	5.68
35t	Me	Propyl	H	78%	5.67
35u	Me	Butyl	H	71%	5.73

Table 3.1: *^{31}P-NMR chemical shifts and yields for the synthesis of phosphorochloridates **35a-u**.*

As observed for compound **24k** (Chapter two, page 45), the phosphorochloridates **35o-q** displayed a ^{19}F-^{31}P long range coupling which was confirmed with ^{31}P-NMR experiments at different magnetic fields on the corresponding BVdU protides.

3.2.3 Synthesis of BVdU phosphoramidates containing α,α-dialkyl amino acids.

The method used for the synthesis of BVdU phosphoramidates containing α,α-dialkyl amino acids was based on previously developed phosphochloridate chemistry.[4, 10] Thus, the phosphorochloridates **35a-u** were coupled with BVdU in THF and in the presence NMI at −78 °C (Scheme 3.5).



BVdU **36a-u**

Scheme 3.5: *Synthesis of phosphoramidates **36a-u**. R=Me, Et, Bn; R'=Me, Et, Pr, Bu; X=H, F, Cl, CF_3, NO_2, MeO.*

The formation of the desired phosphoramidates **36a-36u** was followed by TLC and the reaction was quenched after 3-25 hours. The target phosphoramidates **36a-36u** were isolated after multiple column chromatographies in low to high yields (4-67%) and characterised by NMR and found to be consistent with the expected structures.

Synthetic yields and phosphorus shifts for the protides **36a-u** are given in Table 3.2.

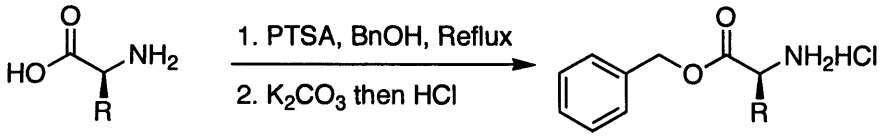
Compound	R	R'	X	Yield	^{31}P-NMR, δ
36a	Me	Me	H	33%	3.36, 3.14
36b	Et	Me	H	29%	3.91, 3.85
36c	Bn	Me	H	27%	3.39, 3.12
36d	Me	Me	*p*-Cl	53%	3.98
36e	Et	Me	*p*-Cl	26%	3.47, 3.33
36f	Bn	Me	*p*-Cl	43%	3.44, 3.26
36g	Bn	Me	*o*-Cl	52%	3.25, 3.23
36h	Bn	Me	*m*-Cl	13%	3.39, 3.15
36i	Me	Me	*p*-NO_2	51%	3.61, 3.56
36j	Et	Me	*p*-NO_2	53%	3.00, 2.96
36k	Bn	Me	*p*-NO_2	50%	2.95, 2.89
36l	Me	Me	*p*-CF_3	33%	3.73, 3.52
36m	Et	Me	*p*-CF_3	44%	3.75
36n	Bn	Me	*p*-CF_3	43%	3.16, 3.01
36o	Me	Me	*p*-F	44%	4.17
36p	Et	Me	*p*-F	36%	4.17
36q	Bn	Me	*p*-F	44%	3.70, 3.52
36r	Bn	Me	*p*-MeO	67%	3.71, 3.56
36s	Me	Et	H	24%	2.49, 2.20
36t	Me	Propyl	H	10%	2.47, 2.29
36u	Me	Butyl	H	4%	2.59, 2.56

Table 3.2: *^{31}P-NMR chemical shifts and yields for the synthesis of phosphoramidates **36a-u**.*

3.3 Synthesis of BVdU phosphoramidates containing L-alkylglycines.

After having successfully synthesised the BVdU protides (**36a-36u**) containing α,α-dialkyl amino acids it was of interest to see how the extension of the hydrophobic side chains on the L-amino acids would affect the anticancer activity.

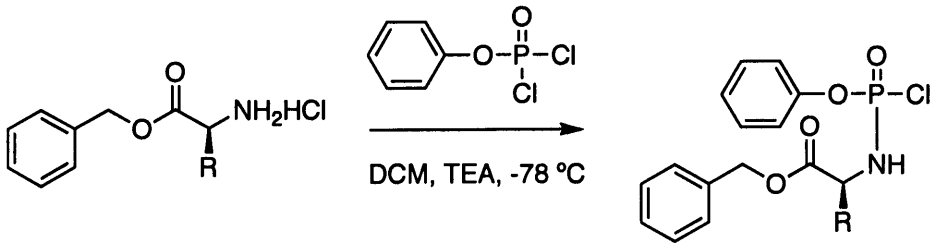
The synthesis of L-alkylglycine benzyl ester hydrochlorides was accomplished in good yields (Scheme 3.6), from the commercially available amino acids, benzyl alcohol and *para*-toluene sulfonic acid in toluene by azeotropic distillation, as described above (Paragraph 3.2: Synthesis of α,α dialkylglycine esters)



37a, R=Et (77%)
37b, R=*n*-Pr (92%)
37c, R=*n*-Bu (89%)

Scheme 3.6: *Preparation of L-alkylglycine benzyl ester hydrochlorides* ***37a-c.***

Each benzyl ester hydrochloride was then treated with commercially available phenyl dichlorophosphate in the presence of TEA in DCM at low temperature (-78 °C), using the previously described phosphorochloridate chemistry,[10, 11] yielding the corresponding phosphorochloridates **38a-c** in good yields (Scheme 3.7).

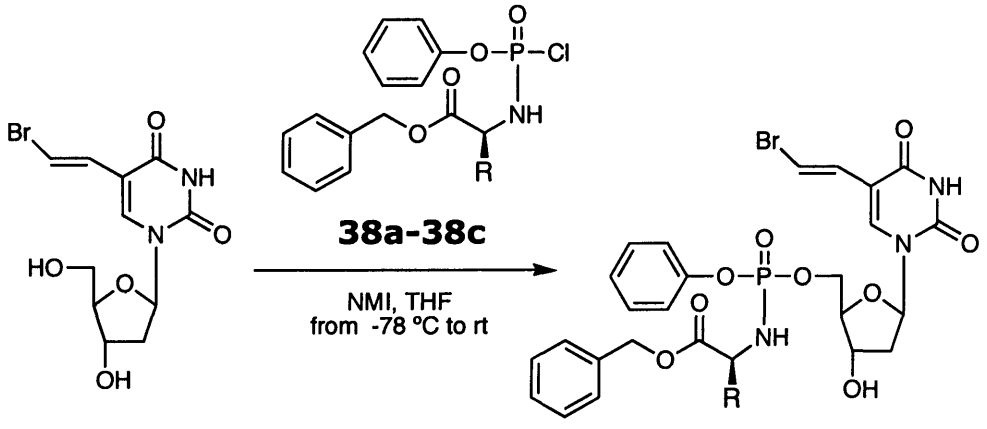


37a-c

38a, R=Et (94%)
38b, R=*n*-Pr (82%)
38c, R=*n*-Bu (90%)

Scheme 3.7: *Synthesis of phosphorochloridates **38a-c.***

Phosphorochloridates **38a-c** were then used in the coupling with BVdU following the conventional phosphoramidate chemistry,[4, 11] yielding target structures **39a-c** in moderate yields (Scheme 3.8).



BVdU

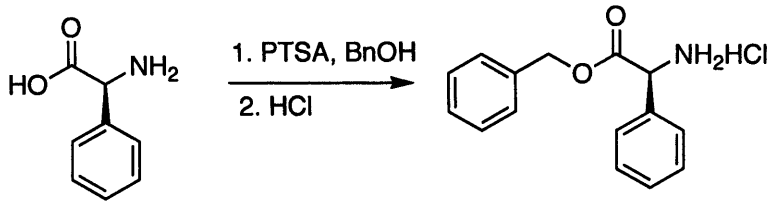
39a, R=Et (56%)
39b, R=*n*-Pr (31%)
39c, R=*n*-Bu (39%)

Scheme 3.8: *Preparation of BVdU protides **39a-c.***

Target compounds **39a-c** were characterised by NMR and found to be pure and consistent with the expected structures.

3.4 Synthesis of BVdU phosphoramidates containing L-phenylglycine.

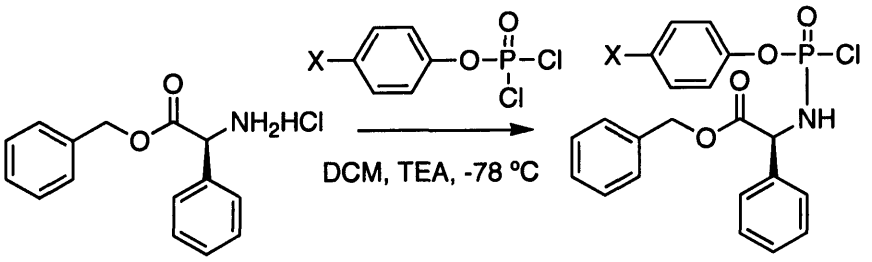
Since the benzyl ester of L-phenylglycine used in the synthesis of phosphorochloridates **41a-d** was not commercially available, it was prepared from (S)-(+)-2-phenylglycine, *p*-toluene sulfonic acid and toluene in a Dean Stark trap;[8] after addition of ether, the solid was filtered and the *p*-toluene sulfonic salt exchange to give the hydrochloride salt of L-phenylglycine benzyl ester (**40**) in good yield (Scheme 3.9).



40 (89%)

Scheme 3.9: *Preparation of L-phenylglycine benzyl ester hydrochloride (**40**).*

Coupling of **40** with the phosphorodichloridates **22a-c** and phenyldichlorophosphate, following the standard procedure for the synthesis of phosphorochloridates, afforded the phosphorochloridates **41a-d**, as depicted in Scheme 3.10.

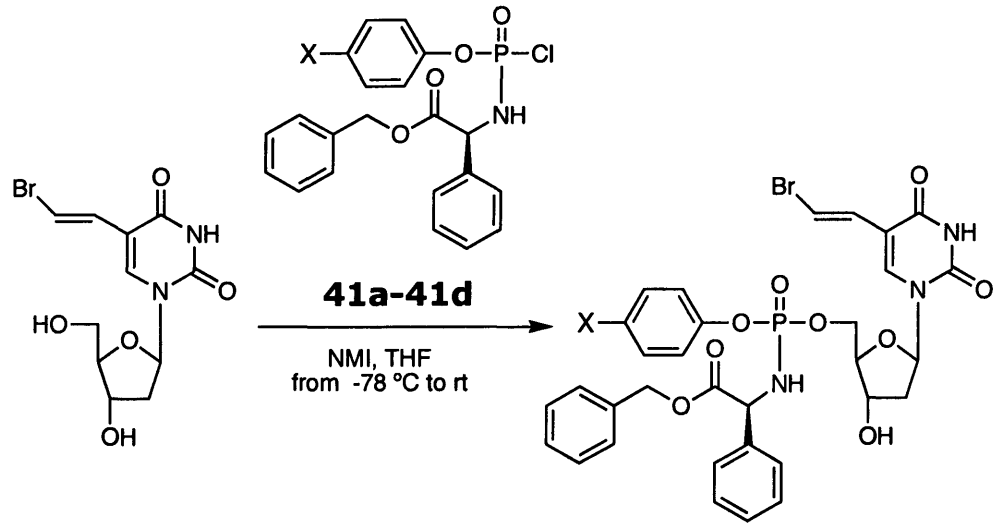


40

41a, X=H (86%)
41b, X=Cl (95%)
41c, X=F (>100%)
41d, X=CF_3 (82%)

Scheme 3.10: *Synthesis of phosphorochloridates **41a-d**.*

BVdU was then reacted with the series of phosphorochlorides **41a-d**, in the presence of NMI in THF at low temperature, to give target protides **42a-d** (Scheme 3.11).



BVdU

42a, X=H (26%)
42b, X=Cl (63%)
42c, X=F (51%)
42d, X=CF_3 (57%)

Scheme 3.11: *Preparation of BVdU protides **42a-d** containing L-phenylglycine.*

3.5 Biological results.

The synthesised BVdU phosphoramidates (**36a-u, 39a-c, 42a-d**) were evaluated for their anti-cancer activity against two tumour cell lines *in vitro*, including human breast cancer cell line MDA MB 231 and human prostate cancer cell line PC-3. The biological tests were carried out at the College of Medicine, University of Wales, in collaboration with Dr. W. Jiang and Dr. M. Mason. Data are reported in table 3.3.

The cytotoxicity assay was based on MTT assay as already described (see Chapter Two, paragraph 2.8) and based on the ability of viable mitochondria to convert MTT into a insoluble formazan precipitate that was dissolved and quantified by spectrometry.

It is evident from the biological data depicted in table 3.3 that, apart from compounds **36b**, **36d**, **36i**, **36o** and **36t**, the synthesised BVdU protides showed in general a significant elevation in activity compared to the lead compound **17** (thymectacin) in both cell lines. In addition, the data reported in table 3.3 suggest that these unnatural amino acids display remarkable biological activities and they may substituted for the natural amino acids retaining or enhancing anticancer activity.

For the α,α-dimethylglycine series, it is apparent that the benzyl ester led to an increase of the activity compared to the methyl and ethyl ester, with the benzyl esters being between 61- and 4-fold more active versus breast and between 260- and 6-fold more active versus prostate cancer cell lines.

Compound	Aryl	Ester	Amino Acid	$EC_{50}/\mu M$	
				Breast MDA MB231	Prostate PC-3
17 (thymectacin)	H	Me	Ala	79	155
36a	H	Me	diMeGly	41.1	1.5
36b	H	Et	diMeGly	217.9	76.1
36c	H	Bn	diMeGly	19	5.1
36d	*p*-Cl	Me	diMeGly	8.7	441.6
36e	*p*-Cl	Et	diMeGly	5.9	1.2
36f	*p*-Cl	Bn	diMeGly	2.3	9.1
36g	*o*-Cl	Bn	diMeGly	5.7	6.6
36h	*m*-Cl	Bn	diMeGly	5.7	6.3
36i	*p*-NO_2	Me	diMeGly	9.4	222.8
36j	*p*-NO_2	Et	diMeGly	2	82.4
36k	*p*-NO_2	Bn	diMeGly	4.5	27.2
36l	*p*-CF_3	Me	diMeGly	62.3	121
36m	*p*-CF_3	Et	diMeGly	23.3	25.1
36n	*p*-CF_3	Bn	diMeGly	1.3	0.6
36o	*p*-F	Me	diMeGly	94	117
36p	*p*-F	Et	diMeGly	61.9	127
36q	*p*-F	Bn	diMeGly	12.2	4.5
36r	*p*-MeO	Bn	diMeGly	4.8	1.1
36s	H	Me	diEtGly	30.5	33.9
36t	H	Me	diPrGly	93.5	73.9
36u	H	Me	diBuGly	8.3	6.7
39a	H	Bn	L-EtGly	7.7	13.2
39b	H	Bn	L-PrGly	5.4	7.7
39c	H	Bn	L-BuGly	8.4	12.5
42a	H	Bn	L-PhGly	0.16	0.75
42b	*p*-Cl	Bn	L-PhGly	14.2	6.5
42c	*p*-F	Bn	L-PhGly	16.8	85
42d	*p*-CF_3	Bn	L-PhGly	2	16

Table 3.3: *Anti-cancer activity for compounds **36a-u, 39a-c** and **42a-d**.*

Moreover, the substitution at the aryloxy part with electron-withdrawing groups enhanced the activity of the majority of the protides containing α,α-dimethylglycine. Although the

mechanism(s) of degradation of BVdU protides may differ from aryloxyphosphoramidates of d4T,[4] the increased activity is substantially in agreement with our prediction that the introduction of electron-withdrawing groups would have boosted the anticancer activities. However, it is interesting to note that, despite compound **36r** being an inferior leaving group compared to any of the phenols bearing electron-withdrawing substituents, there is a significant enhancement of anticancer potency versus breast (16-fold) and prostate (141-fold) cancer cell lines.

The extension of the side chain in the α,α-dialkyl showed an increase of potency compared to the lead thymectacin (**17**) with the BVdU protide **36u** containing α,α-dibutylglycine being 10-fold more active versus breast and 23-fold more active versus prostate cancer cell line. However, the activity of these homologues (**36s**, **36t**, **36u**) did not show regularities through the series C2-C4 with the α,α-dipropylglycine protide **36t** being less active than compound **36s** and **36t**. The reason for this is not apparent and it remains to be investigated.

As it appears from table 3.3, the mono-alkyl series (**39a-39c**) is characterised by an enhanced activity compared to the lead thymectacin (**17**). However, increasing the length of the alkyl side chain of alanine did not lead to a significant difference of activity through the series C2-C4 with compounds **39a-39c** being approximately equipotent.

Finally, for the phenylglycine series, compounds **42a-42d** displayed higher activity than compound **17** with compound **42a** being the most active.

In conclusion, it clearly appears from the data reported in table 3.3 that unnatural amino acid derivatives could substitute for the natural amino acids with no loss of anticancer activity, and with apparent potency boost in same cases.

3.6 References

1 McGuigan, C.; Tsang, H.-W.; Cahard, D .; Turner, K.; Velasquez, S.; Salgado, A.; Bidois, L.; Naesens, L.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of d4T as inhibitors of HIV: the effect of amino acids variation. *Antiviral Res.*, **1997**, 35, 195-204.

2 McGuigan, C.; Salgado, A.; Yarnold, C.; Harries, T.Y; De Clercq, E.; Balzarini, J.; Novel nucleoside phosphoramidates as inhibitors of HIV: studies on the stereochemical requirement of the phosphoramidate amino acid. *Antiviral Chem. Chemother.*, **1996**, 7, 184-188.

3 McGuigan, C.; Bidois, L.; Hiouni, A.; Ballatore, C.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of stavudine as inhibitors of HIV: unnatural amino acids may substitute for alanine. *Antiviral Chem. Chemother.*, **1999**, 11, 111-116.

4 McGuigan, C.; Cahard, D.; Sheeka, H.M.; De Clercq, E.; Balzarini, J.; Aryl phosphoramidate derivatives of d4T have improved anti-HIV efficacy in tissue culture and may act by the generation of a novel intracellular metabolite. *J. Med. Chem.*, **1996**, 39, 1748-1753.

5 Siddiqui, A.; McGuigan, C.; Ballatore, C.; Zuccotto, F.; Gilbert, I.H.; De Clercq, E.; Balzarini, J.; Design and synthesis of lipophilic phosphoramidate d4T-MP prodrugs expressing high potency against HIV in cell culture: structural determinants for in vitro activity and QSAR. *J. Med. Chem.*, **1999**, 42, 4122-4128.

6 Siddiqui, A.; Ballatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J.; The Presence of substituents on the aryl moiety of aryl phosphoramidate derivatives of d4T enhances anti-HIV efficacy in cell culture: a structure activity relationship. *J. Med. Chem.*, **1999**, 42, 393-399.

7 Webb, R.G.; Haskell, M.W.; Stammer, C.H.; A nuclear magnetic resonance method for distinguishing α-amino acids from β and γ isomers. *J. Org. Chem.*, **1969**, 34, 576-580.

8 Yamada, N.; Okuyama, K.; Serizawa, T.; Kawasaky, M.; Oshima, S.; Periodic change in absorption maxima due to different chain packing between the bilayers amphiphiles possessing even and odd numbers of carbons in the hydrophobic chain. *J. Chem. Soc., Perkin Trans. 2*, **1996**, 12, 2707-2708.

9 Srinivasan S., Internal Report, Welsh School of Pharmacy, **2001**.

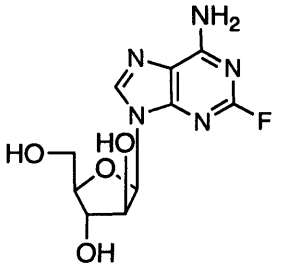
10 McGuigan, C.; Pathirana, R.N.; Balzarini, J.; De Clercq, E.; Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.*, **1993**, 36, 1048-1052.

11 Curley, D.; McGuigan, C.; Devine, K.G.; O'Connor, T.J.; Jeffries, D.J.; Kinchington, D.; Synthesis and anti-HIV evaluation of some phosphoramidate derivatives of AZT: studies on the effect of chain elongation on biological activity. *Antiviral Res.*, **1990**, 14, 345-356.

Chapter Four: Phosphoramidates of 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A).

4.1 Background and aims.

9-β-D-arabinofuranosyl-2-fluoroadenine (fludarabine, F-ara-A, **43**) is a purine nucleoside analogue in which the ribose moiety is replaced with arabinose and the nucleobase is halogenated (Figure 4.1).



43

Figure 4.1: *fludarabine*

Fludarabine has been extensively used to treat various haematological malignancies such as chronic lymphocytic leukemia (CLL) and low grade non-Hodgkins's lymphoma (NHL).[1]

Fludarabine was first synthesised in 1969 by Montgomery *et al.*[2] as an analogue of vidarabine (arabinosyladenine) to circumvent the rapid inactivation by adenosine deaminase.[3, 4] Because of the relatively low solubility, the clinical formulation of fludarabine is as its 5'-monophosphate (fludarabine phosphate, F-ara-A-MP or Fludara® IV).

After intravenous injection, fludarabine monophosphate is rapidly and quantitatively dephosphorylated to F-ara-A by a non selective phosphatase[5] and then transported into cells by nucleoside transport systems.[6] Inside the cells, F-ara-A is subsequently phosphorylated to its mono-, di-, and triphosphates by deoxycytidine kinase (dCK), adenyl kinase (AK) and nucleoside diphosphate kinase (NDPK), respectively (Figure 4.2).[7, 8] Studies on the activity of highly purified dCK showed that fludarabine is a poor substrate for phosphorylation,[9] and the low concentrations of fludarabine monophosphate and diphosphate in cells suggest that

the activity of deoxycytidine kinase is rate-limiting for triphosphate formation.[10]

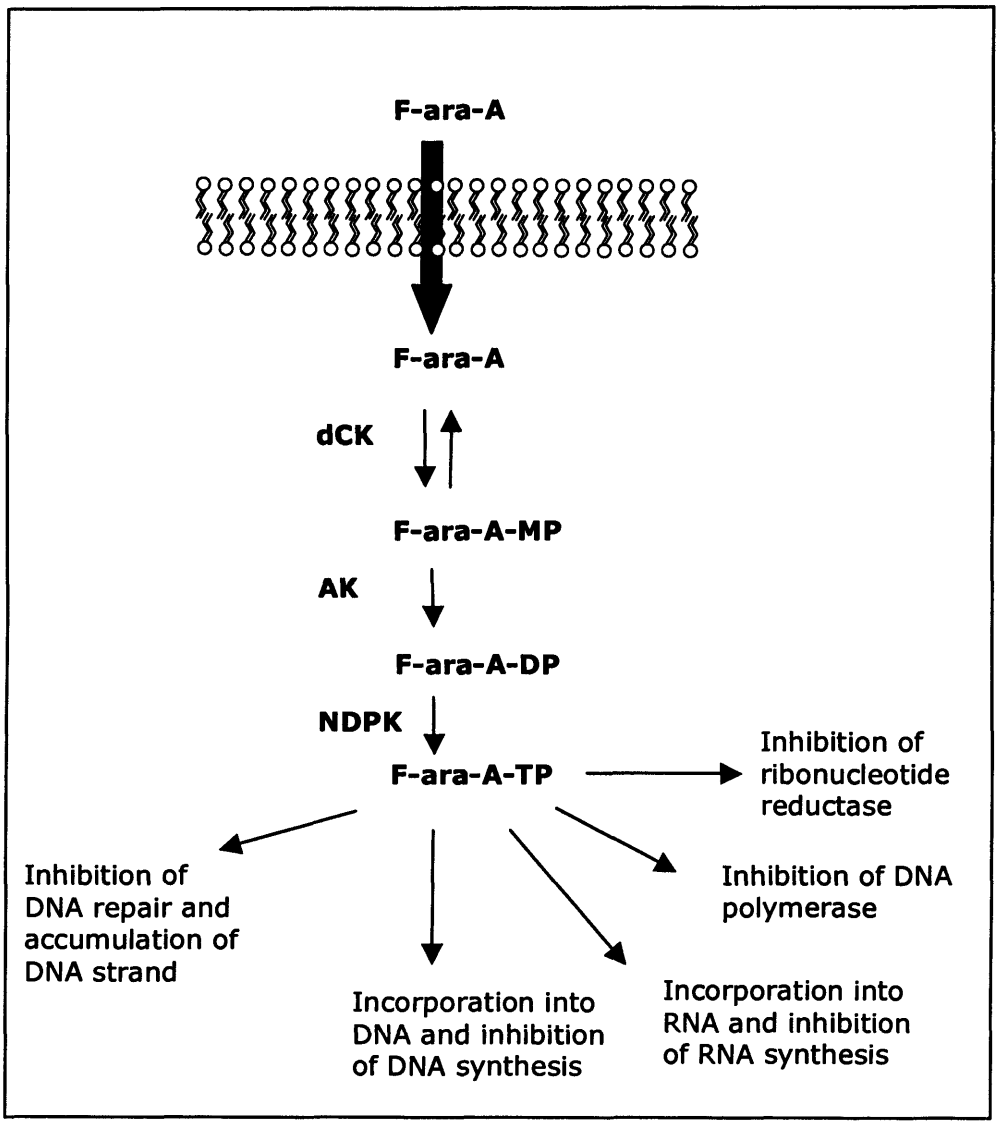


Figure 4.2: *Metabolism and mechanism of action of F-ara-A.*

F-ara-A-TP (fludarabine triphosphate) is the cellular metabolite of fludarabine and the only metabolite known to have pharmacological activity. F-ara-A has cytotoxic activity against both dividing and resting cells.

The major action of F-ara-A-TP is the inhibition of several key enzymes involved in DNA synthesis,[8,11,12,13] which is potentiated by

the decrease of cellular deoxyadenosinetriphosphate (dATP) that results from inhibition of the enzyme ribonucletide reductase (RR).[8] F-ara-A-TP is an inhibitor of human polymerase and competes with the normal substrate, deoxyadenosine triphosphate, for the incorporation into the growing nucleic acid chain.[8, 13, 14] Once incorporated into DNA, chain elongation catalysed by DNA polymerase is terminated, inducing apoptosis in the cells in the S phase of the cell cycle[13] (cell cycle S-phase specific drug).

It has also been demonstrated that F-ara-A-TP can act as an inhibitor of human DNA ligase I, blocking AMP binding and ligation of single strands.[15] In addition, DNA ligase I is not capable of joining adjacent pieces of DNA when F-ara-A-MP is at the 3'-terminus. Moreover, the stability and the resistance of F-ara-A-MP in terminal positions to proof reading activities and accumulation of DNA single-strand breaks are responsible for apoptosis by p53 pathways.[16]

Furthermore, it has been shown that F-ara-A can be incorporated into RNA inducing primer RNA termination and can affect the synthesis of RNA inhibiting RNA polymerase II.[17]

A major problem in the treatment of leukemia is the development of resistance to chemotherapy. Resistance to fludarabine may occur by several means:

- Decreased activity of the activating enzyme dCK.[18]
- Increased activity of RR.[19]
- Increased activity of 5'-nucleotidases.[20]
- Decreased nucleoside transport into the cell.[21]

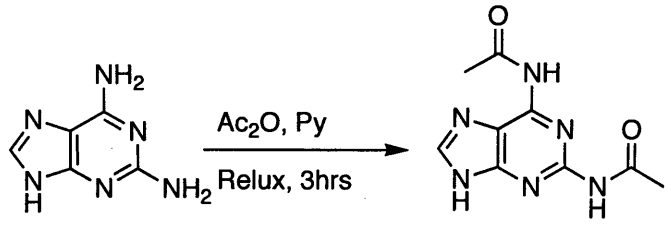
For these reasons, we believed that a phosphoramidate prodrug could be advantageous as it could ideally overcome the problems related with the deficient activity of dCK. Furthermore, the phosphoramidate prodrugs of fludarabine could be more membrane soluble, therefore independent from the nucleoside transporters.

4.2 Synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine.

The first published procedure, developed by Montgomery *et al.*[2] involves five steps starting from 2,6-dichloropurine. The chloro sugar and the 2,6-dichloropurine were coupled in the presence of mercuric cyanide and molecular sieves to give mainly the β-anomer. Substitution of the chloro atoms with azide groups followed by catalytic reduction afforded the 2-amino-9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)adenine. Schiemann reaction on this intermediate followed by treatment with Na and liquid NH_3 gave the 9-β-D-arabinofuranosyl-2-fluoroadenine. The same author reported an improved procedure for the synthesis of fludarabine, based on the preparation of the 2,6-diacetamidopurine and its reaction with 2,3,5-tri-O-benzyl-α-D-arabinofuranosyl chloride.[22]

The procedure followed in this thesis for the preparation of fludarabine is the one investigated by Montgomery *et al.*[22]

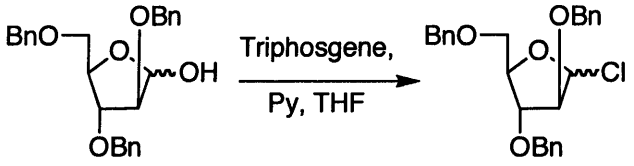
Upon treatment with acetic anhydride in pyridine at reflux, commercially available 2-aminoadenine (**44**) was converted into 2,6-diacetamidopurine (**45**) (Scheme 4.1). No triacetyl purine was detected and the compound was used without further purification for the condensation with the chloro sugar.



44 **45**

Scheme 4.1: *Synthesis of 2,6-diacetamidopurine (**45**).*

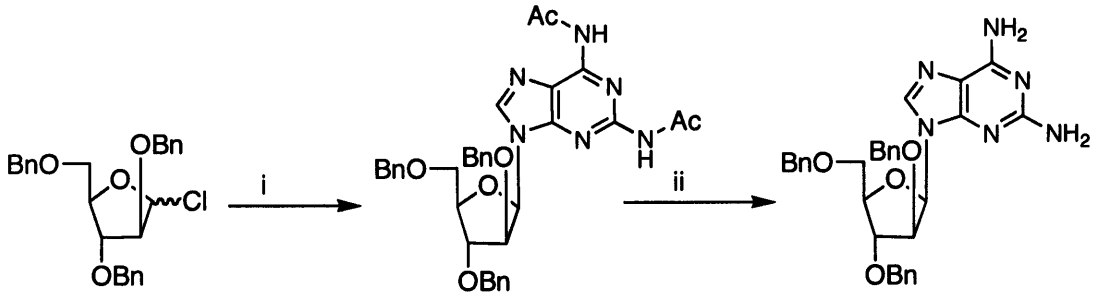
2,3,5-tri-O-benzyl-D-arabinofuranose (**46**) was dissolved in dry THF with 0.4 eq of triphosgene followed by the addition of pyridine.[23] After the second addition of pyridine, pyridinium hydrochloride precipitated as a white solid and CO_2 gas was evolved. Filtration of the solid and evaporation of the solvent afforded a crude product of an α/β mixture of the chloro sugar (**47**, 8:2) (Scheme 4.2). The composition of the anomeric mixture was determined by ^{1}H-NMR which displayed the presence of two peaks relative to H-1′ at 6.31 ppm (H-1′β, d, *J*=3.8 Hz) and 6.26 ppm (H-1′α, s).



46 **47**

Scheme 4.2: *Synthesis of 2,3,5-tri-O-benzyl-α and β arabinofuranosyl chloride.*

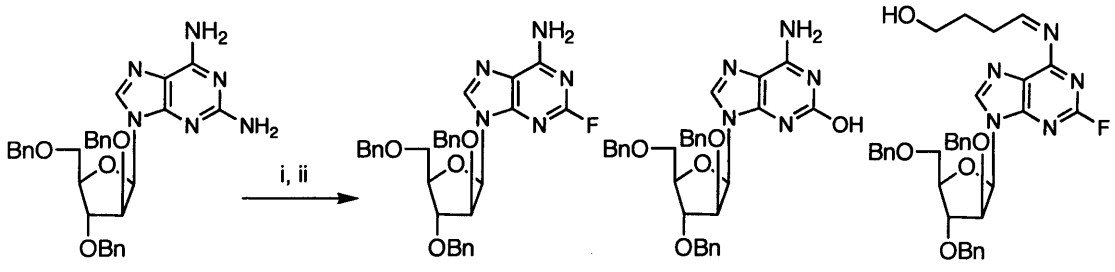
Reaction of **47** with 2,6-diacetamidopurine (**45**) by refluxing in 1,2-dichloroethane in the presence of molecular sieves for 5 days (Scheme 4.3), gave **48** (34%). The presence of other byproducts such as the α-anomer and/or N7 coupled compounds were not detected. Deacetylation of **48**, using a freshly prepared solution of sodium methoxide in methanol (1M) gave **49** in good yield (91%).



47 **48** **49**

Scheme 4.3: *Reagents and Condition*: i) 2,6-diacetamidopurine, 4Å molecular sieves, 1,2-dichloroethane, reflux, 5 days. ii) MeOH/MeONa 1M.

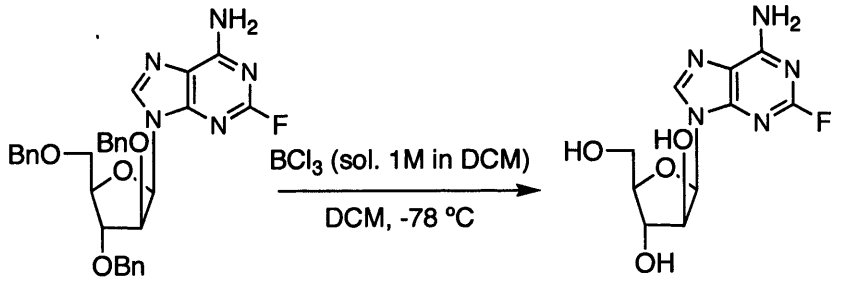
Diazotination of **49** was carried out in a homogenous mixture of tetrahydrofuran and 48% fluoroboric acid in which the nucleoside is soluble. An excess of aqueous $NaNO_2$ (three additions) was used and the intermediate crude was then treated with NH_3 gas (Scheme 4.4). TLC analysis of the crude showed the presence of three products which were isolated and characterised. In agreement with the reported procedure of Montgomery *et al.*,[22] flash column chromatography afforded the desired product (**50a**, Y=35%), compound **50b** [9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-2-hydroxyadenine, Y=25%] and **50c** (9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-N-(4-hydroxybutylidene)adenine.



49 **50a** **50b** **50c**

Scheme 4.4: *Synthesis of **50a-50c**. Reagent and Condition*: i) HBF_4, $NaNO_2$, THF, -10 °C, 1 hr; ii) NH_3, 20 minutes, dimethoxyethane.

Finally, removal of the benzyl group of **50a** by boron trichloride in dichloromethane (Scheme 4.5) gave 9-β-D-arabinofuranosyl-2-fluoroadenine (80%) in which the ^{1}H-NMR analysis was consistent with the literature.[18]



50a **43**

***Scheme 4.5**: Deprotection of 9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (**50a**).*

The synthetic method described above afforded fludarabine in 9% overall yield (based on the starting sugar). A few disadvantages are associated with the process described:

- the coupling reaction and the diazotisation/fluorination suffer from relatively low yields.
- the protected sugar (2,3,5-tri-O-benzyl-D-arabinofuranosylfuranose) is very costly.

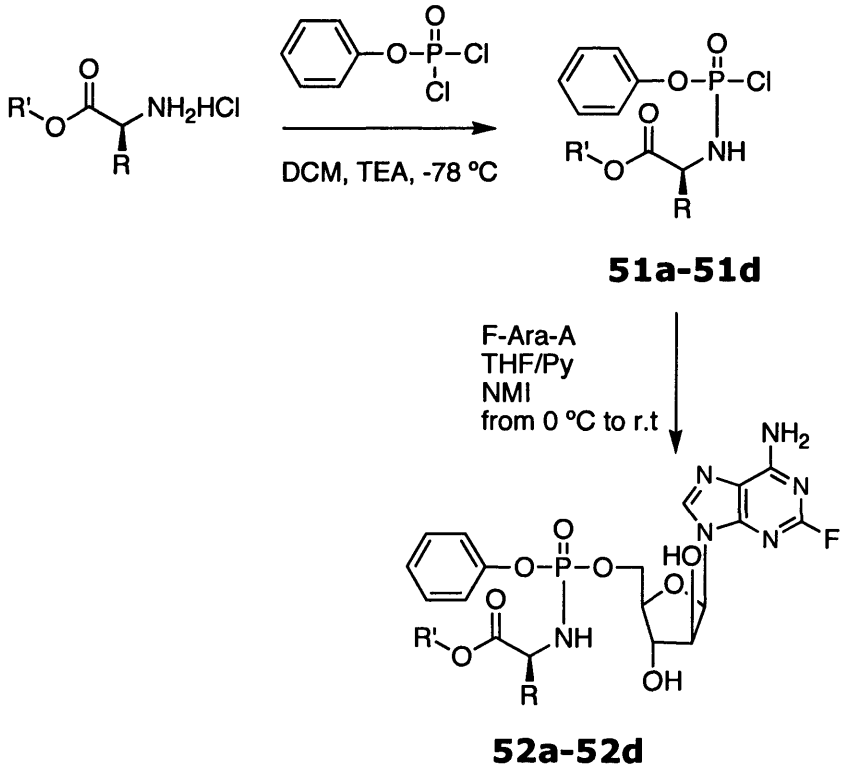
For these reasons, in an effort to better understand and expand the SAR studies of fludarabine phosphoramidates, it would desirable to consider an alternative synthesis for the preparation of fludarabine.

4.3 Synthesis of F-ara-A phosphoramidates.

After having successfully synthesised the starting 9-β-D-arabinofuranosyl-2-fluoroadenine, the conventional strategy of

coupling was applied to the preparation of the target phosphoramidates.

Scheme 4.6 outlines the general synthetic route used to prepare F-ara-A protides bearing amino acids variations (glycine, L-alanine and L-phenylalanine) and ester variations (L-alanine methyl and benzyl ester). Synthetic yields and phosphorus NMR shifts are given in table 4.1 for the phosphorochloridate intermediates (**51a-51d**) and protides (**52a-52d**).



Scheme 4.6: *Synthesis of phosphorochloridates **51a-51d** and phosphoramidates **52a-52d**.*

The phosphorochloridates **51a-51d** were prepared by coupling phenyl phosphorochloridate with the suitable amino acid ester, using previously described phosphorochloridate chemistry (scheme 4.6 and table 4.1).[24]

Compound	R	R'	Yield	^{31}P NMR, δ
51a	CH_3	CH_2Ph	82%	9.32, 9.10
51b	CH_3	CH_3	99%	9.42, 9.20
51c	H	CH_3	79%	10.70
51d	CH_2Ph	CH_3	84%	9.44, 9.20

Table 4.1: *Synthetic yields and phosphorus shifts (^{31}P NMR data) for compounds **51a-51d.***

In order to increase the solubility of F-Ara-A, a mixture of THF/Py (2:1) was chosen as a solvent for the coupling with the phosphorochloridates. Furthermore, bearing in mind that the 5'-OH is less sterically hindered compared to the 2' and 3' hydroxy groups, and should therefore react preferentially with the phosphorochloridate, the synthesis was carried out using the unprotected nucleoside.

In the first attempt at the synthesis of fludarabine protides, the same conditions for the synthesis of BVdU phosphoramidates were followed: a 1M solution in THF of phosphorochloridate **51b** (3 equivalent) was added dropwise to a solution of fludarabine in THF/Pyridine (2/1) containing NMI at -78 °C. Unfortunately, no trace of the desired compound (**52b**) was detected by TLC after overnight reaction. A subsequent attempt, using the same reaction conditions, yielded the desired product in 3% yield (1 mg). A possible reason for the poor yield observed for the coupling might be due to the lack of reactivity of the 5'-OH at low temperature. Thus, in order to improve the course of the reaction, the phosphorochloridate **51b** was reacted with fludarabine at -17 °C. The formation of the desired phosphoramidate was observed on TLC and the purification of the crude afforded the target compound in

10% yield. TLC of the reaction mixture showed the formation of another product, running at a higher Rf than that of the desired product. This could be attributed to the 3′ and/or 2′-phosphorylated species: the supposed regioisomer was isolated crude but not extensively characterised and its yield was not determined.

In order to further improve the yields of the coupling reactions, it was proposed to carry out the condensation between the nucleoside and the phosphorylating agent at 0 °C. For this reason, the synthetic method applied for the synthesis of the phosphoramidates **52a-52d**, involved the addition of the phosphorochloridates **51a-51d** to a solution of fludarabine in THF/pyridine (2:1) at 0 °C.

The protides **52a-52d** were purified by flash column chromatography followed by preparative thin layer chromatography. Synthetic yields and phosphorous shifts for the protides **52a-52d** are given in table 4.2. Interestingly, the ^{31}P of fludarabine phosphoramidate **52b** showed a singlet at 4.99 ppm, presumably from the overlap of the signals arising from the two diastereoisomers. For all the other derivatives, ^{31}P-NMR showed the duplication of the peaks due to the presence of the two diastereoisomers that arise from the chiral phosphorus centre.

Compound	**R**	**R′**	**Yield**	**^{31}P NMR, δ**
52a	CH_3	CH_2Ph	6%	5.02, 4.95
52b	CH_3	CH_3	10%	4.99
52c	H	CH_3	9%	6.28, 6.04
52d	CH_2Ph	CH_3	6%	4.89, 4.46

Table 4.2: *Summary of the synthesis of the phosphoramidate derivatives **52a-52d**.*

4.4 Biological results.

In vitro assessment of the activity of fludarabine phosphoramidates compared to fludarabine were conducted at the Department of Haematology of Llandough Hospital in collaboration with Dr. C. Pepper.

Venous whole blood samples were obtained from patients diagnosed with chronic lymphocytic leukaemia (CLL).

Isolation of mononuclear cells from patients' blood was achieved using standard procedure. Cells were then counted and were aliquoted (1×10^6 cells/ml) into culture tubes prior to the addition of drug.

All cultures were incubated at 37°C in a humidified 5% carbon dioxide atmosphere for 48 h in the presence (1×10^{-7}- 6×10^{-6}M) or absence of fludarabine or the protide analogues. Flow cytometric technique was used to track the changes that occur in cells during the process of apoptosis, allowing the number of apoptotic cells in a sample to be quantified.

Statistical analyses were performed by non-linear regression of the dose-response data. LD_{50} values (the concentration of fludarabine or protide analogue required to kill 50% of cells) were derived from sigmoidal dose-response curves.

Data are given as the concentration required to kill 50% and 90% of the cells (LD_{50} and LD_{90}, respectively) and are reported in table 4.3.

Compound	LD_{50} (μM)	LD_{90} (μM)
Fludarabine (43)	0.96	4.65
52d	1.02	8.70

Table 4.3: *LD_{50} and LD_{90} values derived from fludarabine (**43**) and phenyl-(benzoxy-L-alaninyl)-fludarabine phosphoramidate (**52d**).*

In our assay fludarabine protide **52d** showed a cytotoxicity comparable to fludarabine (**43**), with fludarabine being a slightly more potent than the corresponding phenyl(benzoxy-L-alaninyl)-phosphoramidate (**52d**).

Although the remaining fludarabine phosphoramidate derivatives of this series (**52a-c**) are currently under biological evaluation, these initial results could be useful for future investigations.

Possible explanations for the unsatisfactory activity of **52d** might be attributed to the short time of the assay (48 hrs) and/or low activity of the enzymes involved in the activation pathway of the prodrug. Furthermore, the activity of fludarabine in this assay might be due to higher activity of dCK compared to the enzyme required to liberate the nucleoside analogue from the phosphoramidate **52d**.

For these reasons extension of the incubation time and use of different leukaemia models could be informative for further investigations of this small series of fludarabine prodrugs.

4.5 References

1 Adkins, J.C.; Peters, D.H.; Marklam, A.; Fludarabine: an update of its pharmacology and use in the treatment of haematological malignancies. *Drugs*, **1997**, 53, 1005-1037.

2 Montgomery, J.A.; Hewson, K.; Nucleoside of 2-fluoroadenine. *J. Med. Chem.*, **1969**, 12, 498-504.

3 Bennett, L.L. Jr.; Chang, C.H.; Allan, P.W.; Adamson, D.J.; Rose, L.M.; Brockman, R.W.; Secrist, J.A. III; Shortnacy, A.; Montgomery, J.A.; Metabolism and metabolic effect of halopurine nucleosides in tumor-cell culture. *Nucleosides and Nucleotides*, **1985**, 4, 107.

4 Montgomery, J.A.; Has the Well Gone Dry? The first Cain Memorial Award Lecture. *Cancer Res.*, **1982**, 42, 3911-3917.

5 Danhauser, L.; Plunkett, W.; Keating, M.; et al. 9-β-D-arabinofuranosyl-2-fluoroadenine-5'-monophosphate pharmacokinetics in plasma and tumor cells of patients with relapsed leukemia and lymphoma. *Cancer Chemother. Pharmacol.*, **1996**, 18, 145-152.

6 Molina-Arcas, M; Bellosillo, B.; Cassado, F.J.; Montserrat, E.; Gil, J.; Colomer, D.; Pastor-Anglada, M.; Fludarabine uptake mechanisms in B-cell chronic lymphcytic leukaemia. *Blood*, **2003**, 101, 2328-2334.

7 Gandhi, V.; Plunkett, W; Cellular and clinical pharmacology of fludarabine. *Clin. Pharmacokinet.*, **2002**, 41, 93-103.

8 Plunkett, W.; Chubb, S.; Alexander, L.; Montgomery, J.A.; Comparison of the toxicity and metabolism of 9-β-D-arabinofuranosyl-2-fluoroadenine and 9-β-D-arabinofuranosyladenine in human lymphoblastoid cells. *Cancer Res*, **1980**, 40, 2349-2355.

9 Shewach, D.S.; Reynolds, K.K.; Hertel, L.; Nucleotide specificity of human deoxycytidine kinase. *Mol. Pharmacol.*, **1993**, 42, 518-524.

10 Avramis, V.I.; Plunkett, W.; Metabolism and therapeutic efficacy of 9-β-D-arabinofuranosyl-2-fluoroadenine against murine leukemia P338. *Cancer Res.*, **1982**, 42, 2587-2591.

11 Brokman, R.W.; Schabel, Jr F.M.; Montgomery, J.A.; Biological activity of 9-β-D-arabinofuranosyl-2-fluoroadenine, a metabolically stable analogue of 9-β-D-arabinofuranosyladenine. *Biochem. Pharmacol*, **1977**, 26, 2193-2196.

12 Plunkett, W.; Saunders, P.P.; Metabolism and action of purine nucleoside analogs. *Pharmacol. Ther.*; **1991**, 49, 2339-2368.

13 Huang, P.; Chubb, S.; Plunkett, W.; Termination of DNA synthesis by 9-β-D-arabinofuranosyl-2-fluoroadenine: mechanism for cytotoxicity. *J. Biol. Chem.*, **1990**, 265, 16617-16625.

14 Parker, W.R.; Bapat, A.R.; Shen, J.X.; Interaction of 2-halogenated dATP analogs (F, Cl, and Br) with human DNA polymerases, DNA primase and ribonucleotide reductase. *Mol. Pharmacol.*, **1988**, 34, 485-491.

15 Yang, S.-W.; Huang, P; Plunkett, W.; Becker, F.F.; Chan, J.Y.H.; Dual mode of inhibition of purified DNA ligase I from human cells by 9-β-D-arabinofuranosyl-2-fluoroadenine triphosphate. *J. Biol. Chem.*, **1992**, 267, 2345-2349.

16 Pettitt, A.R.; Sherrington, P.D.; Cawley, J.C.; Role of poly(ADP-ribosyl)ation in the killing of chronic lymphocytic leukaemia cells by purine analogues. *Cancer Res.*, **2000**, 60, 4187-4193.

17 Huang, P.; Plunkett, W.; Action of 9-β-D-arabinofuranosyl-2-fluoroadenine on RNA metabolism. *Mol. Pharmacol.*, **1991**, 39, 449-455.

18 Bai, L.; Yamaguchi, M.; Tatsumi, M.; Kon, K.; Brautigam, M.; Mechanisms responsible for resistance of sublines derived from leukaemia cell lines to an antitumor agent 9-beta-D-arabinofuranosyl-2-fluoroadenine. *J. Cancer Res. Clin. Oncol.*, **1998**, 124, 367-373.

19 Månsson, E.; Spasokoukotskja, T.; Sällström, J.; Eriksson, S.; Albertioni, F.; Molecular and biochemical mechanism of fludarabine and cladribine resistance in a human promyelocytic cell line. *Cancer Res.*, **1999**, 59, 5956-5963.

20 Dumontet, C.; Fabianowska-Majewska, K.; Mantincic, D.; Bauchu, E.C.; Tigaud, I.; Gandhi, V.; Lepoivre, M.; Peters, G.J.; Rolland, M.O.; Wyczechowska, D.; Fang, X.; Gazzo, S.; Voorn, D.A.; Varnier-Viornery, A.; Mackey, J.; Common resistance mechanisms to deoxynucleoside analogues in variants of the human erythroleukaemic line K562; *Br. J. Hematol.*, **1999**, 106, 78-85.

21 Galmarini, C.M.; Makey, J.R.; Dumontet, C.; Nucleosides analogues: mechanisms of drugs resistance and reversal strategies. *Leukemia*, **2001**, 15, 875-890.

22 Montgomery, A.J.; Clayton, S.D.; Shortnacy, A.T.; An improved procedure for the preparation of 9-β-D-arabinofuranosyl-2-fluoroadenine. *J. Heterocycl. Chem.*, **1979**, 16, 157-160.

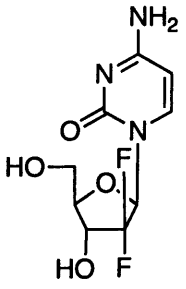
23 Cicchillo, R.M.; Norris, P.; A convenient synthesis of glycosyl chlorides from sugar hemiacetals using triphosgene as chlorine source, *Carbohyd. Res.*, **2000**, 328, 431-434.

24 a) McGuigan, C.; Pathirana, R.N.; Balzarini, J.; De Clercq, E.; Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.*, **1993**, 36, 1048-1052. b) Curley, D.; McGuigan, C.; Devine, K.G.; O'Connor, T.J.; Jeffries, D.J.; Kinchington, D.; Synthesis and anti-HIV evaluation of some phosphoramidate derivatives of AZT: studies on the effect of chain elongation on biological activity. *Antiviral Res.*, **1990**, 14, 345-356.

Chapter Five: Phosphoramidates of 2',2'-difluorodeoxycytidine (Gemcitabine).

5.1 Background and aims.

Gemcitabine (2',2'-difluorodeoxycytidine, dFdC, Gemzar®, **53**) is a deoxycytidine analogue in which the two hydrogens of the 2' position of the carbohydrate (geminal position) are substituted by two fluorine atoms (Figure 5.1).



53

Figure 5.1: *Structure of Gemcitabine*

Gemcitabine was first synthesised in 1986 as an antiviral agent and was subsequently approved by the Food and Drug Administration (FDA) in 1996 for the treatment of patients with locally advanced or metastatic adenocarcinoma of the pancreas.

Gemcitabine has established clinical activity against solid malignancies and shows efficacy against several human cancers, including breast,[1] ovarian,[2] lung,[3] and pancreatic[4] cancers. Gemcitabine is frequently combined with drugs that damage DNA such as cisplatin,[5] topotecan,[2] paclitaxel.[6]

The metabolism and the mechanism(s) of action of gemcitabine are reported in figure 5.2.

As a hydrophilic molecule, dFdC can not permeate the cell membrane by passive diffusion and specialised nucleoside transporter proteins are required for the transport across the cell membrane.[7] After uptake in the cell, gemcitabine is phosphorylated by deoxycytidine kinase (dCK) to gemcitabine monophosphate

(dFdCMP),[8] which is the rate-limiting enzyme in its activation. dFdCMP is then subsequently converted to its diphosphate (dFdCDP) and triphosphate (dFdCTP) derivatives, which are the active metabolites. Gemcitabine can be converted into the inactive metabolite 2′,2′-difluorodeoxyuridine (dFdU) by cytidine deaminase (CDA)[9] while dFdCMP is dephosphorylated by 5′-nucleotidase (5′-NT) and converted into dFdC.

Gemcitabine triphosphate is then incorporated into the growing DNA strand[10] followed by the addition of another nucleotide, which masks gemcitabine and prevents DNA repair by base pair excision ("masked chain termination").

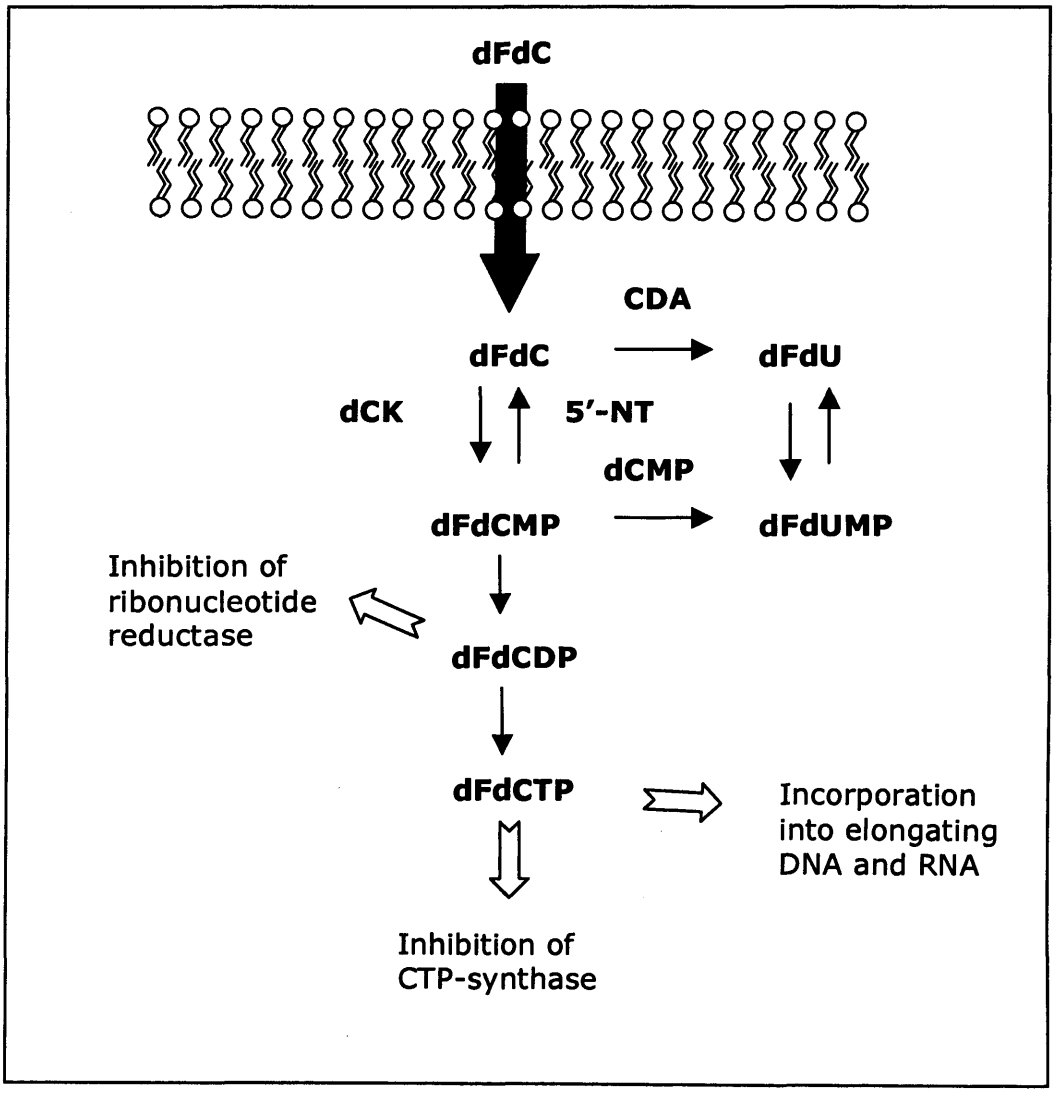


Figure 5.2: *Metabolism and mechanisms of action of gemcitabine.*

Gemcitabine can also be incorporated into RNA,[11] but the effect of its incorporation into RNA remains unclear.

Gemcitabine metabolites can inhibit several enzymes through a number of feed-back effects on metabolism, leading to enhanced activity of dFdCDP itself ("self-potentiation"). dFdDTP can act as an inhibitor of ribonucleotide reductase (RR)[12] blocking the *de novo* DNA synthesis and lowering the deoxyribonucleotide production. dCK is down-regulated by high cellular concentration of dCTP (deoxycytidine triphosphate). Thus, the decreased levels of dCTP increase the incorporation of dFdCTP into elongating DNA and the intracellular concentration of dFdCDP and dFdCTP. Self-potentiation mechanisms include also inhibition of deoxycytidine deaminase (dCDA) and deoxycytidylate deaminase (dCMPD),[8, 13] enzymes responsible for the clearance of dFdC. dCDA is partially regulated by the deoxycytidine triphosphate pool and the reduced concentration of dCTP decreases the catabolic clearance of gemcitabine. dCMPD, the enzyme that catalysed the conversion of dFdCDP into dFdUMP, is inhibited by higher concentration of dFdCTP and requires dCTP as activating cofactor. In addition, inhibition of cytidine triphoshate synthase (CTP-synthase) by dFdCTP, resulting in a depletion of CTP pool has been reported.

As mentioned in chapter four, general mechanisms of resistance to nucleoside analogues are common problems related with chemotherapeutic treatment. Although several enzymes are involved in the metabolism of gemcitabine and are targets for the metabolites of gemcitabine itself, mechanism of resistance could be attributed to nucleoside transport and/or dCK deficiency, overexpression of 5'-NT and/or dCDA. For this reasons the use of phosphoramidate prodrugs, in an effort to improve the therapeutic potential of the nucleoside analogues, might be extremely advantageous. The phosphoramidate prodrugs could circumvent the problems related to the dependence on deoxycytidine kinase.

Furthermore, the increased lipophilicity of the prodrugs could make the derivatives more membrane soluble and therefore independent of nucleoside transporters.

In our assay on the prostate cancer cell line (PC-3), preliminary biological results from phosphoramidates of gemicitabine displayed an increase of activity (*ca.* 14 fold) for the phenyl-L-alanine benzyl ester, compared to dCdF. Thus, in an attempt to investigate and extend the SAR studies, we sought to improve the activity of gemcitabine phosphoramidates by modifying the phosphoramidate moiety.

5.2 Synthesis of dFdC phosphoramidates.

As a part of the SAR studies conducted on BVdU, we observed that the BVdU phosphoramidates containing unnatural amino acids such as dimethylglycine and L-phenylglycine, show remarkable activity against different cell lines (breast cancer, prostate cancer, see chapter three, page 74). Moreover, from the series of BVdU phosphoramidates bearing L-alanine as the amino acid, we recently observed that enhanced activity could be achieved when the ester on the amino acid moiety is the 2-butyl ester (in prostate cancer cell line, PC-3, BVdU phenyl-L-alanine benzyl ester, EC_{50}=34 μM, BVdU phenyl-L-alanine 2-butyl ester, EC_{50}=6.8 μM). In order to further extend the SAR studies on other nucleosides like gemcitabine and, bearing in mind the results obtained from previous experience with BVdU phosphoramidates, we decided to plan the synthesis of a small panel of phosphoramidates of gemcitabine.

Figure 5.3 outlines the proposals for variation of the amino acids and esters of gemcitabine phosphoramidate derivatives.

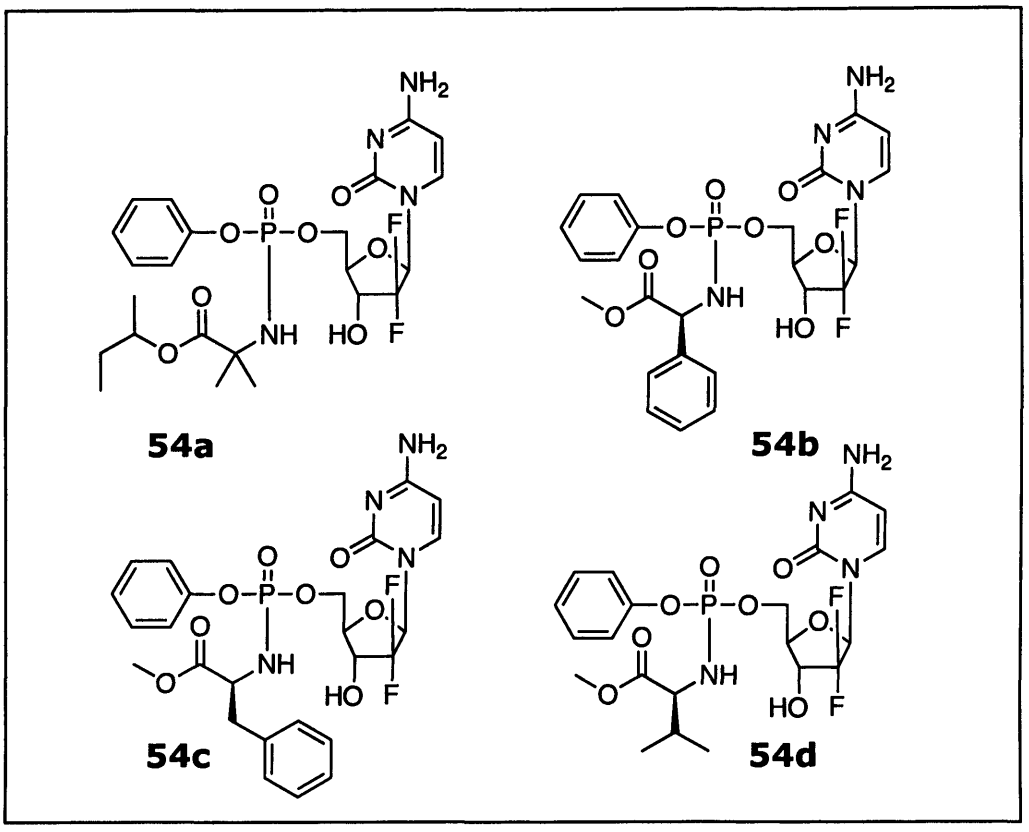


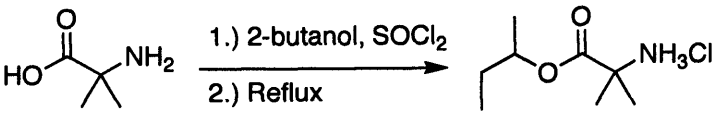
Figure 5.3: *Proposed targets for gemcitabine protides* ***54a-54d****.*

5.2.1 Synthesis of phosphorylating agents.

The phosphorylating agents used for the coupling with gemcitabine were obtained from the low temperature coupling of the corresponding hydrochloric salt with phenyl phosphorodichloridate in the presence of triethylamine (TEA) in anhydrous dichloromethane (DCM) (scheme 5.2), according to the procedure of Curley *et al.*[14]

L-alanine 2-butyl ester (**55**), not commercially available, was prepared according to the procedure of Webb *et al.*,[15] as reported in chapter three. Therefore, L-alanine 2-butyl ester was prepared, in good yield (68%) from the corresponding commercially available 2-

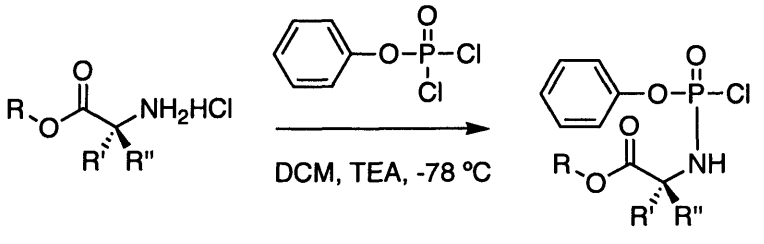
aminoisobutyric acid, thionyl chloride and 2-butanol at reflux for 18 hrs (Scheme 5.1).



55

Scheme 5.1: *Preparation of α,α-dimethylglycine 2-butyl ester hydrochloride (**55**).*

Scheme 5.2 outlines the synthesis of the phosphorochloridates and table 5.1 shows the synthetic yields and phosphorus shifts for **56a-56d**.



56a-56d

Scheme 5.2: *Synthesis of phosphorochloridate **56a-56d**. R=2-butyl, R'=R"=CH_3, **56a**; R=CH_3, R'=H, R"=phenyl, **56b**; R=CH_3, R'=H, R"=i-propyl, **56c**; R=CH_3, R'=H, R"=benzyl, **56d**.*

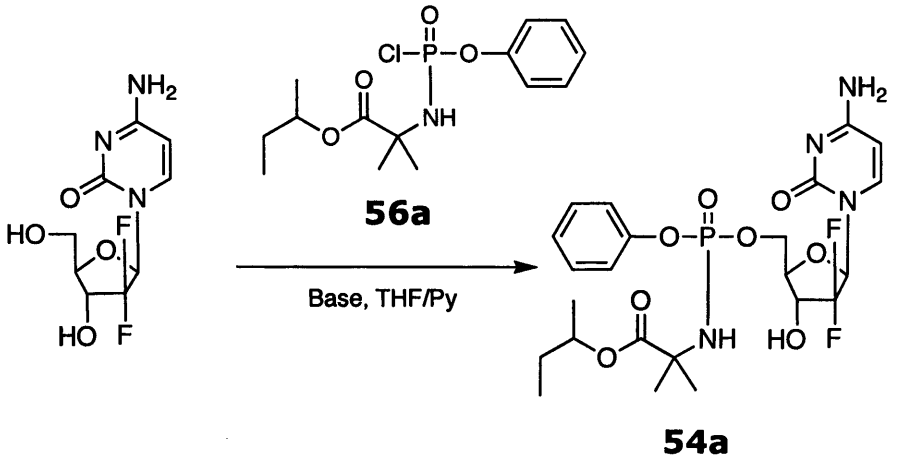
Compound	R	R'	R''	Yield	^{31}P NMR, δ
56a	2-Butyl	CH_3	CH_3	92%	6.90
56b	CH_3	H	Ph	71%	8.80, 8.54
56c	CH_3	H	*i*-propyl	64%	10.69, 10.21
56d	CH_3	H	$PhCH_2$	60%	5.07, 4.59

Table 5.1: *Yields and chemical shifts of phosphorochloridates **56a-56d**.*

5.2.2 Synthesis of gemcitabine phosphoramidates.

In order to apply and optimize the phosphoramidate technology to the synthesis of the target compounds, gemcitabine was reacted using different conditions (Table 5.2). The preparation of such phosphoramidates seems to be markedly more difficult than that of the corresponding phosphoramidates of other nucleosides, such as BVdU. The problems arising from this nucleoside can be principally attributed to solubility and to the fact that the free amino group in position 4 of the cytosine and the 3'-hydroxyl group could compete with the 5'-OH in the reaction with the phosphorochloridate.

Due to the insolubility of the nucleoside in THF, a mixture of THF and pyridine (7:3) was used as the solvent and in an effort to increase the solubility and regioselectivity, different temperatures and methods were considered for the synthesis (Table 5.2 and Scheme 5.3).



Scheme 5.3: *General synthetic scheme for the synthesis of **54a**.*

Entry	Condition	Temperature	Phosphorochloridate	Yield
1	NMI	-78 °C	3 eq	12%
2	NMI	-17 °C	3 eq	4%
3	tBuMgCl	-78 °C	1.1 eq	NR

Table 5.2: *Conditions for the reaction of **56a** with gemcitabine.*

Although the reactions of **56a** at −78 °C with gemcitabine (Table 5.2, entry 1) afforded the phosphoramidates, NMR analysis showed the presence of phosphorylated impurities. Preparative TLCs were performed in an effort to isolate the pure phosphoramidate. However, ^{31}P-NMR spectra displayed two main peaks due to the diastereoisomers (chiral phosphorus center) and two small peaks close to the main phosphoramidate peaks. A further attempt, using the same phosphorylating condition at -17 °C, (entry 2) afforded the phosphorylation of gemcitabine but ^{31}P-NMR analysis displayed the presence of a main broad peak at −1.33 ppm, subsequently characterised as the hydrolysed product of the phosphorochloridate. After several purifications, ^{31}P-NMR showed the disappearance of the broad peak at −1.33 ppm and the appearance of two small peaks near to the main peaks.

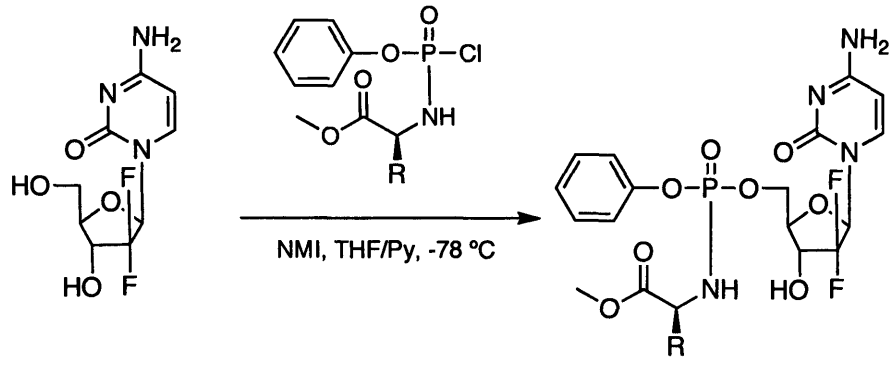
After several purifications by silica column chromatography and preparative TLCs, compound **54a** was isolated in both attempts (entries 1 and 2), as a 5′- and 3′-monophosphorylated mixture, which could not be separated. Furthermore, regioselective improvements were not observed when gemcitabine was reacted with 2 equivalents of phosphorochloridate (**56a**) using NMI at -17 and -78 °C.

This evidence suggests that phosphorylation was not regioselective and that monophosphorylated regioisomers were obtained as an inseparable mixture.

In preliminary studies on gemcitabine, it was also interesting to explore different phosphorylation methods. Uchiyama *et al.*,[16] reported a method based on organometallic reagents that allows O-selective phosphorylation without N-protection in a cytosine based nucleoside analogue. These conditions were applied to the phosphorylation of gemcitabine using a mixture of THF/Py (7:3) as solvent and *tert*-butylmagnesium chloride as base at -78 °C. Surprisingly after several additions of phosphorylating agent (2.2 eq) and Grignard reagent (2.2 eq.) no reaction was observed.

Taking into consideration the inability to improve the reaction under NMI coupling conditions and the more difficult purifications experienced with the use of NMI at -17 °C (Table 5.2, entry 2), the use of NMI at -78 °C (Table 5.2, entry 1) was applied for the synthesis of compounds **54b-54d** (Scheme 5.4).

Table 5.3 outlines yields and phosphorus shifts for target phosphoramidates **54b-54d**.



54b-54d

Scheme 5.4: *Reagents and conditions for the synthesis of gemcitabine protides* ***54b-54d****. R=Ph,* ***54b****; R=Bn,* ***54c****; R=i-Pr,* ***54d****.*

Compound	R′	Yield	^{31}P NMR, δ
54b	Ph	8%	4.77, 4.58
54c	CH_2Ph	4%	4.90, 4.47
54d	*i*-propyl	3%	5.82, 5.79 (main peaks) 5.51, 5.05 (impurities)

Table 5.3: *Yields and ^{31}P-NMR for gemcitabine derivatives **54b-54d**.*

Unfortunately, compounds **54c** and **54d** were isolated in low yields and as mixtures of regioisomers after several purifications by silica column chromatography and preparative TLC. The resulting low yields may be a reflection of steric hindrance of the phosphorochloridates and/or lack of reactivity of the 5′-OH at -78 °C. Thus, in order to improve the yields for the synthesis of protides **54c** and **54d**, the reactions were repeated under the same conditions at -17 °C. After purification by silica column chromatography and further purifications by preparative TLC, compound **54c**, was isolated pure in 5% yield. Repeated purifications afforded compound **54d**, as a mixture of regioisomers in very low yield (2%).

The attempted synthesis of the phosphoramidate prodrugs of gemcitabine resulted to be more difficult compared to other nucleosides (e.g. ddA, BVdU). Low yields and poor regioselectivity observed in the different attempts above mentioned, suggest that the synthetic route may be improved by protections of the 3′-hydroxyl group. However, time precluded such investigations.

5.2.3 Scale-up synthesis of CPF31.

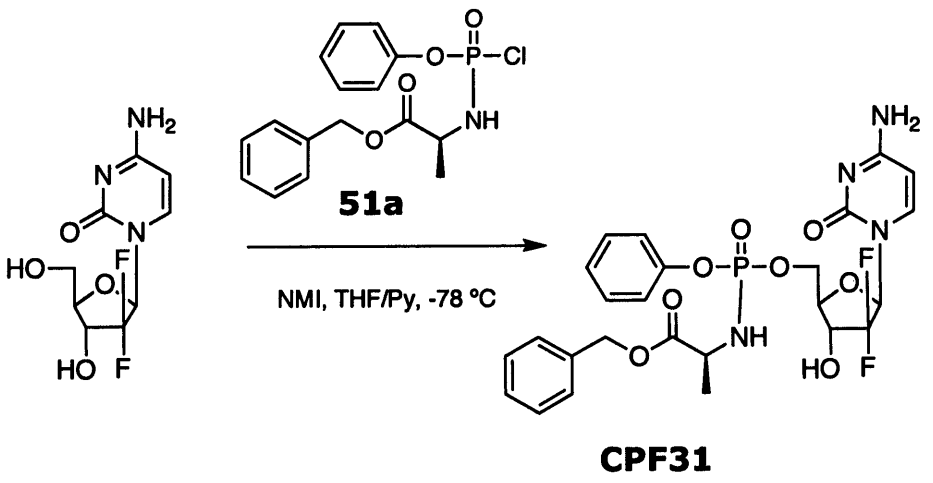
Initial biological results on gemcitabine protides showed an increase of activity compared to the parent nucleoside with the phenyl-(methoxy-L-alaninyl)-gemcitabine phosphoramidate (**CPF31**) being 14-fold more potent than gemcitabine versus prostate cancer cell line (Table 5.4).

Compound	Aryl	Ester	Amino Acid	$EC_{50}/\mu M$	
				Breast MDA MB231	Prostate PC-3
Gemcitabine				2.8	3.12
CPF31[a]	Ph	Bn	L-alanine	42.6	0.22
CPF40[a]	p-ClPh	Bn	L-alanine	9.2	15.4
CPF41[a]	p-ClPh	Bn	diMeGly	3.1	68.8

Table 5.4: *Preliminary in vitro study on gemcitabine protides.* [a]*: compound synthesised by J.C. Thiery.*

The enhanced potency of **CPF31** prompted us to investigate the *in vivo* activity of this protide and for this reason, scale-up for the synthesis of **CPF31** was planned.

Earlier investigation of the phosphorylation of gemcitabine were attempted on a small scale using 2 and 3 equivalents of phosphorochloridate (**51a**), in the presence of NMI in THF/Py (2/1) at -78 °C. In both cases, after several purifications, the phosphoramidate was isolated as a mixture of regioisomers.



Scheme 5.5: *Synthesis of **CPF31**.*

Despite these results, the reaction was tried on a larger scale using 3 equivalents of phosphorochloridate. Surprisingly, after purification by silica column chromatography, **CPF31** was isolated as a pure compound in 8% yield. The reason for this result is not apparent and it remains to be explained.

5.3 Biological results.

The biological tests were carried out at the College of Medicine, University of Wales, in collaboration with Dr. W. Jiang and Dr. M. Mason.

The synthesised gemcitabine phosphoramidates **54b** and **54c** were evaluated for their anti-cancer activity against a panel of two tumour cell lines *in vitro*, including human breast cancer cell line MDA MB 231 and human prostate cancer cell line PC-3, as described for the BVdU derivatives (See Chapter Two, paragraph 2.8). Data are reported in table 5.4.

As it appears from the data reported in table 5.5, the synthesised compounds **54b-54c** are characterised by a reduction of cytotoxic activity compared to the parent nucleoside analogue in both cell lines.

Compound	Aryl	Ester	Amino Acid	$EC_{50}/\mu M$	
				Breast MDA MB231	Prostate PC-3
Gemcitabine				2.8	3.12
CPF31[a]	Ph	Bn	L-alanine	42.6	0.22
54b	Ph	Me	L-PhGly	9.5	5.7
54c	Ph	Me	L-Phe	5.2	4.3

Table 5.5: *Anti-cancer activity for compounds **1b-c**. [a]: compound synthesised by J.C. Thiery (Welsh School of Pharmacy, Cardiff University, 2001).*

In breast cancer cell line (MDA MB231) phosphoramidate derivatives **54b** and **54c** showed an increased activity compared to the lead compound **CPF31**. Substitution of the natural amino acid moiety with non natural amino acid, such as L-phenylglycine, seems to be tolerated with increased activity compared to the lead **CPF31**.

In prostate cancer cell line (PC-3) compounds **54b** and **54c** are less active compared to the lead; being approximately 20-fold less potent then **CPF31**.

5.3.1 *In vivo* evaluation of CPF31.

To determine dose-dependent efficacy of CPF31 in a PC-3 mouse xenograft model, athymic mice bearing s.c. PC-3 tumours were administered i.p. four different doses of CPF31 (0.1, 1, 5 and 10 μM).

In a mouse xenograft model both Gemzar and CPF31 are effective in reducing tumour growth. At day 10 CPF31 at 0.1μM

appears highly effective, with a halving of tumour volume versus control (p=0.027, Figure 5.4).

At day 10, **CPF31** is well tolerated in all mice at concentrations up to 5mM, with 1/5 mice showing weight loss at 10μM. By contrast Gemzar shows weight loss in 2/5 mice at 10 μM and 1/5 mice at 5μM.

In vivo studies were stopped at day 13 as the lost of subjects, at 5 and 10 μM (3 in GMZ and 1 in CPF31 on day 10, collectively 4 in GMZ and 1 in CPF31 on day 11 and 5 in GMZ and 1 in CPF on day 13) preclude the achievement of any statistical difference with the remaining groups.

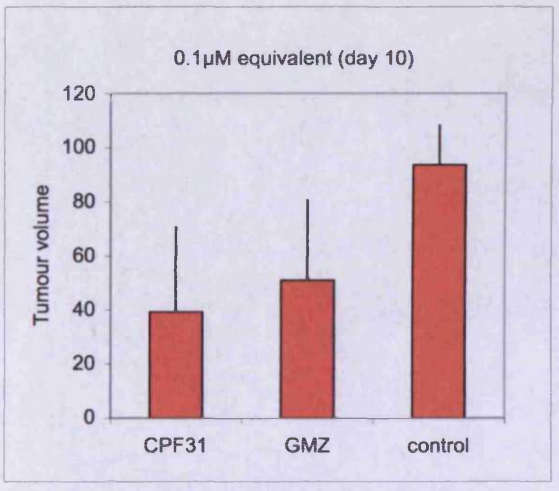


***Figure 5.4**: Antitumour activity of **CPF31**(0.1 μM) in human xenografts at day 10.*

However, at 5 and 10 μM, **CPF31** showed significantly low side effects compared to Gemzar

5.4 References

1 Seidman, A.D.; The evolving role of gemcitabine in the management of breast cancer. *Oncology*, **2001**, 60, 189-198.

2 Greggi, S.; Salerno, M.G.; D'Agostino, G.; Ferrandina, G.; Lorusso, D.; Manzione, L.; Mancuso, S.; Scambia, G.; Topotecan and gemcitabine in platinum/paclitaxel resistant ovarian cancer. *Oncology*, **2001**, 60, 19-23.

3 Abratt, R.P.; Rezwoda, W.; Falkson, G.; Goedhals, L.; Hacking, D.; Efficacy and safety profile of gemcitabine in non-small cell lung cancer. Phase II study. *J. Clin. Oncol.*, **1994**, 12, 1535-1540.

4 Heinemann, V.; Gemcitabine: progress in the treatment of pancreatic cancer. **2001**, *Oncology*, 60, 8-18.

5 Bunn, P.A.; Kelly, K.; New chemotherapeutic agents prolong survival and improve quality of life in non-small cell lung cancer: a review of the literature and future directions. *Clin. Cancer Res.*, **1998**, 5, 1087-1100.

6 Garcia, A.A.; O'Meara, A.; Bahador, A.; Facio, G.; Jeffers, S.; Kim, D.Y.; Roman, L.; A phase II study of gemcitabine and weekly paclitaxel in platinum-resitant ovarian cancer. *Gynecol. Oncol.*, **2004**, 493-498.

7 Mackey, J.R.; Mani, R.S.; Selner, M.; Mowles, D.; Young, J.D.; Belt, J.A,; Crawford, C.R.; Cass, C.E.; Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. *Cancer Res.*, **1998**, 58, 4349-4357.

8 Heinemann, V.; Hertel, L.W.; Grindey, G.B.; Comparison of the cellular pharmacokinetics and toxicity of the 2',2'-difluorodeoxycytidine and 1-β-D-arbinofuranosylcytosine. *Cancer Res.*, **1988**, 48, 4024-4031.

9 a) Bouffard, D.Y.; Laliberte, J.; Momparler, R.L.; Kinetic studies on 2',2'-difluorodeoxicytidine (gemcitabine) with purified

human deoxycytidine kinase and cytidine deaminase. *Biochem. Pharmacol.*, **1993**, 45, 1857-1861. b) Neff, T.; Blau, C.A.; Forced expression of cytidine deaminase confers resistance to cytidine arabinoside and gemcitabine. *Exp. Haematol.*, **1996**, 24, 1340-1346.

10 Huang, P.; Chubb, S.; Hertel, L.W. Grindey, G.B.; Plunkett, W.; Action of 2',2'-difluorodeoxycytidine on DNA synthesis. *Cancer Res.*, **1991**, 6110-6117.

11 Ruiz van Haperen, V.W.T.; Veerman, G.; Vermorken, J.B.; Peters, G.J.; 2',2'-difluorodeoxycytidine (gemcitabine) incorporation into RNA and DNA from tumour cell lines. *Biochem. Pharmacol.*, **1993**, 46, 762-766.

12 Huang, P.; Plunkett, W.; Induction of apoptosis by gemcitabine. *Semin. Oncol.*, **1995**, 22, 19-25.

13 Plunkett, W.; Huang, P.; Xu, Y.Z.; Heinemann, V.; Grnewald, R.; Gandhi, V.; Gemcitabine: metabolism, mechanisms of activation, and self potentiation. *Semin. Oncol.*, **1995**, 22, (4 Suppl. 11), 3-10.

14 Curley, D.; McGuigan, C.; Devine, K. G.; O'Connor, T. J.; Jeffries, D. J.; Kinchington, D.; Synthesis and anti-HIV evaluation of some phosphoramidate derivatives of AZT: studies on the effect of the chain elogation on biological activity. *Antiv. Res.*, **1990**, 14, 345-356.

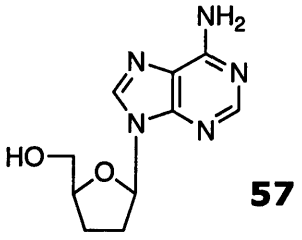
15 Webb, R.G.; Haskell, M.W.; Stammer, C.H.; A nuclear magnetic resonance method for distinguishing α-amino acids from β and γ isomers. *J. Org. Chem.*, **1969**, 34, 576-580.

16 Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y.; Selective phosphorylation of nucleoside without N-protection. *J. Org. Chem.*, **1993**, 58, 373-379.

Chapter Six: Phosphoramidates of 2′,3′-dideoxyadenosine.

6.1 Background and aims.

2′,3′-dideoxyadenosine (ddA, **57**, Figure 6.1) is a nucleoside analogue containing an unsaturated sugar, which has been shown to be active against human immunodeficiency virus (HIV). However, its efficacy is limited due to the catabolic enzymatic conversion into 2′,3′-dideoxyinosine[1, 2, 3] and to the poor affinity for cellular kinases.[1, 4] For these reasons, the use of masked phosphate prodrugs, in an effort to circumvent both nucleoside kinases and adenosine deaminase, has been applied to this nucleoside by us and others in order to improve its therapeutic potential.[5, 6, 7]



57

Figure 6.1: *2′,3′-dideoxyadenosine (ddA).*

Dideoxyadenosine nucleosides, such as 2′,3′-ddA and 2′,5′-ddA, are also P-site inhibitors of adenylyl cyclase,[8] a family of enzymes that catalyse the formation of adenosine-3′,5′-cyclic monophosphate (cAMP) from adenosine-5′-triphosphate (ATP). cAMP is an ubiquitous regulatory molecule involved in the signal transduction for numerous neurotransmitters and hormones.

It has been shown that P-site inhibitors of adenylyl cyclase (2′,3′-ddA and/or 2′,5′-ddA) can attenuate the endothelium-derived hyperpolarizing factor (EDHF),[9] a phenomenon first described in guinea-pig, canine and porcine arteries and involved in the smooth muscle relaxation and hyperpolarization.[10] This phenomenon, which is independent of vasodilators such as nitric oxide (NO) and prostanoids, modulates smooth muscle membrane potential

following its release from the endothelium. The EDHF phenomenon may involve passive electrotonic spread of hyperpolarization from the endothelium to smooth muscle cells by direct cell-to-cell communication via gap junction (GJ), rather than the extracellular space. EDHF-type relaxations are associated with a prostanoid-independent synthesis of cAMP that facilitates electrotonic signalling by increasing permeability/conductance of vascular gap junctions.[11] While increased levels of cAMP evoked by agonists such as acetylcholine (ACh) may enhance mechanical relaxations, addition of inhibitors of adenylyl cyclase, the enzyme that synthesizes cAMP, can attenuate EDHF-type subintimal smooth muscle hyperpolarizations.[9]

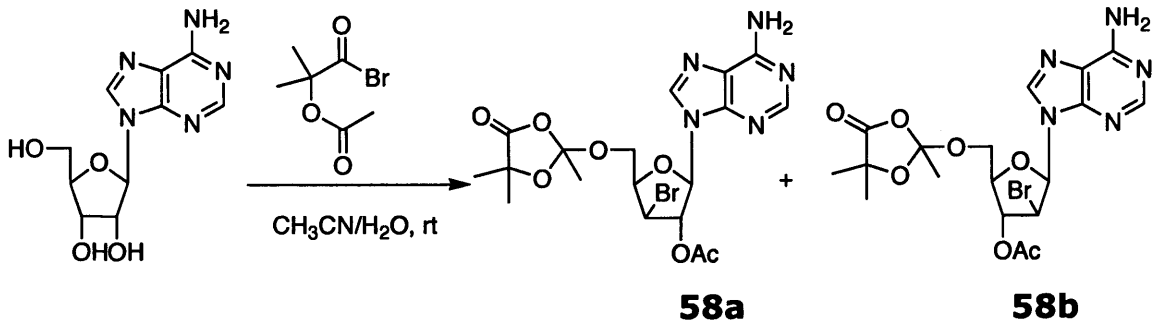
Manipulation of the activity of adenylyl cyclase could therefore represent an interesting strategy for the treatment of diseases that involve patho-physiological changes in vascular reactivity.[2]

Taking into consideration that the addition of phosphates to ddA is known to increase the inhibitory activity against adenylyl cyclase the synthesis of a series of phosphoramidates of 2',3'-ddA was planned in order to evaluate their effect on the inhibition of the EDHF phenomenon.

6.2 Synthesis of 2',3'-dideoxyadenosine.

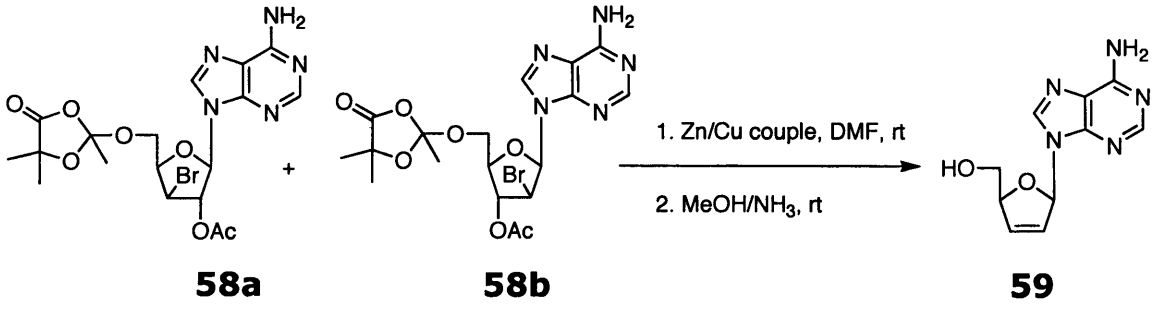
The method described by Robins and *al.*,[12] for the synthesis of ddA, was followed. This method was previously used in our laboratories and found to be an advantageous procedure for the preparation of this nucleoside derivative.[13]

Thus, adenosine was reacted with 2-acetoxyisobutyryl bromide in acetonitrile/water (99:1) at room temperature for 1.5 hrs. Removal of water soluble by-products by extraction with water/ethyl acetate afforded a mixture of **58a** and **58b**, which was used without further purification (Scheme 6.1).



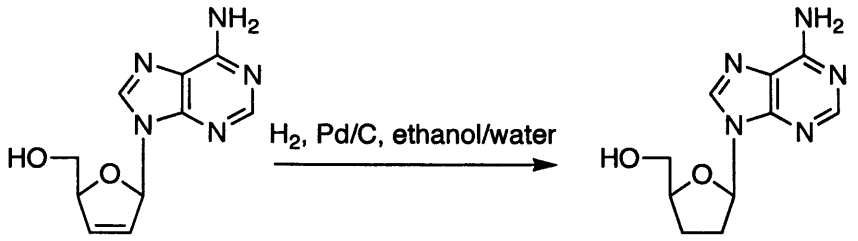
Scheme 6.1: *Synthesis of 9-(2-O-acetyl-3-deoxy-3-bromo-β-D-xylofuranosyl)- and 9-(3-O-acetyl-2-deoxy-2-bromo-β-D-arabinofuranosyl)- adenine* (**58a** and **58b**).

Compounds **58a** and **58b** were dissolved in DMF, treated with freshly prepared zinc/copper couple for 4 hrs at room temperature and then filtered using celite. The solvent was removed under reduced pressure and the crude was extracted with dichloromethane/water. The collected organic layer was then treated with methanolic ammonia for 16 hrs to give compound **59** (2′,3′-dideoxy-2′,3′-didehydroadenosine) in 73% yield (scheme 6.2).



Scheme 6.2: *Synthesis of 2′,3′-dideoxy-2′,3′-didehydroadenosine (**59**).*

Finally, hydrogenation of **59** using Pd/C for 3.5 hrs afforded 2',3'-dideoxyadenosine (**57**) in 61% yield (scheme 6.3).



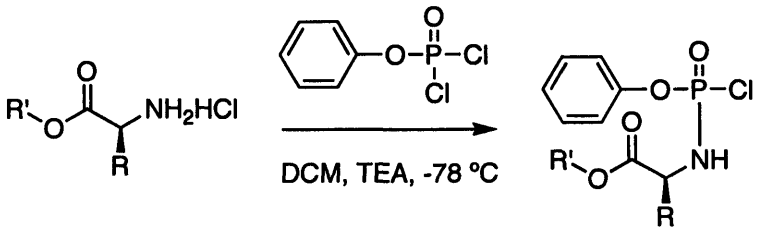
59 **57**

Scheme 6.3: *Hydrogenation of of 2',3'-dideoxy-2',3'-didehydroadenosine (**57**).*

6.3 Synthesis of ddA phosphoramidates.

As a part of preliminary SAR studies conducted on ddA phosphoramidates as inhibitors of the EDHF phenomenon, the synthesis of a small series of phosphoramidate derivatives was undertaken. Thus, it was proposed to investigate the effect of amino acid and ester variations. Natural amino acids bearing an apolar side chains (glycine, L-alanine, L-valine and L-phenylalanine) were considered in first instance. Methyl and benzyl esters were chosen and included in this initial study in order to investigate the effect of the ester variation.

The phosphorochloridate species were synthesised as previously described from the low temperature coupling of the amino acid esters with phenyl dichlorophosphate (Scheme 6.4). After removal of DCM, the phosphorochloridates were quickly purified by flash column chromatography using ethylacetate/petroleum ether (7/3) as eluent (Scheme 6.4).



51c, R=H (glycine), R'=CH_3
60a, R=H (glycine), R'=CH_2Ph
51b, R=CH_3 (L-alanine), R'=CH_3
51a, R=CH_3 (L-alanine), R'=CH_2Ph
60b, R=$CH(CH_3)_2$ (L-valine), R'=CH_3
23i, R=$CH(CH_3)_2$ (L-valine), R'=CH_2Ph
51d, R=CH_2Ph (L-phenylalanine), R'=CH_3
60c, R=CH_2Ph(L-phenylalanine), R'=CH_2Ph

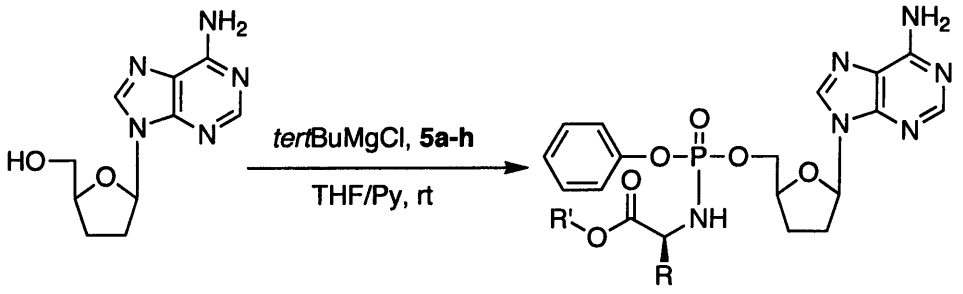
Scheme 6.4: *Preparation of phenyl phosphorochloridates.*

In Table 6.1 are listed yields and ^{31}P-NMR chemical shifts of phosphorochloridates.

Compound	Ester (R')	Amino Acid	Yield	^{31}P-NMR, δ
51c	Me	glycine	51%	10.70
60a	Bn	glycine	67%	10.22
51b	Me	L-alanine	99%	9.42, 9.20
51a	Bn	L-alanine	86%	9.32, 9.10
60b	Me	L-valine	88%	10.69, 10.21
23i	Bn	L-valine	90%	10.84, 10.28
51d	Me	L-phenylalanine	84%	9.44, 9.20
60c	Bn	L-phenylalanine	94%	9.18, 9.12

Table 6.1: *^{31}P-NMR chemical shifts and yields for the synthesis of phosphorochloridates **23i, 51a-51d, 60a-60c.***

Phosphorochloridates **23i**, **51a-51d**, **60a-60c** were then coupled with ddA in the presence of *tert*-butylmagnesium chloride, in agreement with the procedure of Uchiyama *et al.*[14] Due to low solubility of the starting material in THF, a mixture of THF and pyridine (10/1) was used as solvent (Scheme 6.5).



61a-61h

Scheme 6.5: *Preparation of phosphoramidates **61a-61h**; R=H (glycine), R'=Me (**61a**), R'=Bn (**61b**); R=Me (L-alanine), R=Me (**61c**), R=Bn (**61d**); R=i-propyl (L-valine), R'=Me (**61e**), R'=Bn (**61f**); R=PhCH$_2$ (L-phenylalanine), R'=Me (**61g**), R'=Bn (**61h**).*

Formation of the products **61a-61h** was followed by TLC and the reactions were quenched after 1.5-16 hrs. Purification afforded the pure compounds **61a-61h** in low to high yields (29-98%). The yields quoted in table 6.2 are not optimized and are due to incomplete reaction of the starting material and/or multiple purifications by column chromatography. Compounds **61a-61h** displayed two closely spaced signals in the ^{31}P-NMR (*ca.* 1:1), corresponding to the presence of diastereoisomers, resulting from mixed stereochemistry at the phosphate centre.

Compound	Amino Acid	Ester (R')	Yield	^{31}P-NMR, δ
61a	glycine	Me	29%	6.33, 6.17
61b	glycine	Bn	55%	5.89, 5.18
61c	L-alanine	Me	76%	4.11, 4.04
61d	L-alanine	Bn	45%	5.89, 5.18
61e	L-valine	Me	92%	5.95, 5.71
61f	L-valine	Bn	98%	5.94, 5.71
61g	L-phenylalanine	Me	33%	5.07, 4.59
61h	L-phenylalanine	Bn	56%	4.94, 4.63

Table 6.2: *^{31}P-NMR chemical shifts and yields for the synthesis of phosphoramidates **61a-61h**.*

6.4 Biological results.

Electrophysiological tests were conducted at the Welsh Heart Research Institute, Cardiff, Wales, in collaboration with Prof. Tudor M. Griffith.

Arterial strips, obtained from iliac arteries of male New Zealand White (NZW) rabbits, were transferred to cold Holmans buffer containing N^G-nitro-L-arginine methyl ester (L-NAME, NO synthase inhibitor), indomethacin (cyclooxygenase inhibitor) and incubated.

Acetylcholine (ACh) was administered at a concentration (3 μM) that evokes maximal hyperpolarization in the rabbit iliac artery[11, 15] and in some experiments, the phosphoramidate derivative was included in the buffer 30 mins prior the addition of ACh.

The resting membrane potential of subintimal smooth muscle cells was not affected by incubation with 2',3'-ddA (**57**) and 2',3'-ddA phosphoramidate (**61c**) or the combination of

isobutylmethylxanthine (IBMX, cAMP phosphodiesterase inhibitor) with **57** and **61c** (Figure 4.1 A and B).

Compound **61c** showed increased activity as an inhibitor of EDHF-type subintimal smooth muscle hyperpolarizations induced by ACh compared to the parent nucleoside derivative **57** in rabbit iliac artery. The ACh-evoked hyperpolarization was almost abolished when subintimal smooth muscle cells were incubated with **61c** at a concentration of 200 μM, whereas at the same concentration ddA (**57,** 200 μM) showed a reduction of the smooth muscle hyperpolarization of only 50% *ca.* (Figure 6.2 A and B).

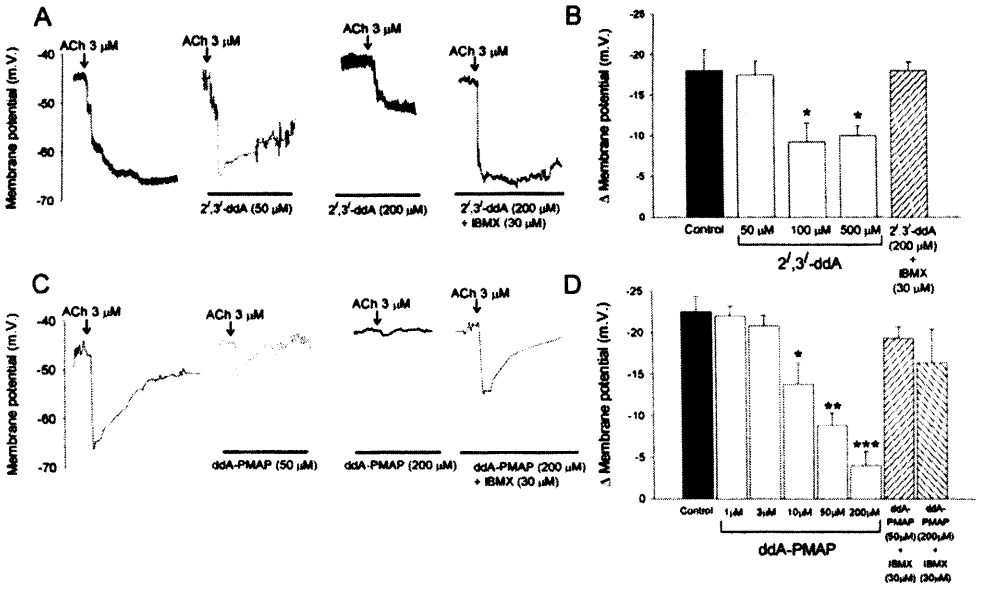


***Figure 6.2**: Effect of ddA (**57**) and its phenyl methoxyalaninyl phosphoramidate (**61c**) on EDHF-type relaxations evoked by acetylcholine. (A, C) Representative traces showing that the cAMP phosphodiesterase inhibitor IBMX prevent the inhibition effects of both compounds. (B, D) Histograms giving peak changes in membrane potential.*

As it appears from figure 6.2 , incubation of ddA and phenyl methylalaninyl phosphoramidate **61c** in the presence of IBMX, an inhibitor of cAMP phosphodiesterase,[11] did not significantly differ from the values of the resting membrane potential of subintimal smooth muscle cells, confirming a link between the EDHF-type

hyperpolarization in iliac artery evoked by ACh and the inhibition of adenylyl cyclase.

IC_{50} values for ddA and its phenyl methoxyalaninyl phosphoramidate were derived as means and 95% confidence intervals using GraphPad Prism software and were respectively estimated as 86.2 μM and 12.6 μM, with the phosphoramidate of ddA being 7 fold more potent than the parent nucleoside.

In conclusion, the phosphoramidate **61c** of ddA showed an increased potency as an inhibitor of EDHF-type smooth muscle hyperpolarizations induced by ACh compared to the parent nucleoside derivative ddA. Taking into consideration the enhanced activity of 2′,5′-dideoxyadenosine-3′-phosphate as an inhibitor of adenynyl cyclase,[8] additional work will be focused on the synthesis of 2′,5′-dideoxyadenosine phosphoramidates.

Phosphoramidates **61a-61h** are currently under biological evaluation.

6.5 References

1 Cooney, D.A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johson, M.A.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D.G.; Initial Studies of the Cellular Pharmacology of 2′,3′-dideoyadenosine, an inhibitor of HTLV-III Infection. *Biochem. Pharmacol.*, **1987**, 36, 1765-1768.

2 Bloch, A.; Robins, M.J.; McCarthy, J.R. Jr.; The role of the 5′-hydroxyl group of adenosine in determining substrate specificity for adenosine deaminase. *J. Med. Chem.*, **1967**, 10, 908-912.

3 Johson, M.A.; Ahluwalia, G.; Connelly, M.C.; Cooney, D.A.; Broder, S.; Johns, D.G.; Metabolic pathway for the activation of the antiretroviral agent 2′,3′-dideoxyadenosine in human lymphoyd cells. *J. Biol. Chem.*, **1988**, 263, 15354-15367.

4 Carson, D.A.; Haertle, T.; Wasson, D.B.; Richman, D.D.; Biochemical genetic analysis of 2′,3′-dideoxyadenosine metabolism in human T lymphocytes. *Biochem. Biophys. Res. Commun.*, **1988**, 151, 788-793.

5 a) Siddiqui, A. Q.; McGuigan, C.; Ballatore, C.; Wedgwood, O.; De Clercq, E.; Balzarini, J.; Simple mono-derivatisation of the aryl moiety of d4A and ddA-based phosphoramidate prodrugs significantly enhances their anti-HIV potency in cell culture. *Bioorg. Med. Chem. Lett.*, **1999**, 9, 2555-2560. b) Balzarini, J.; Kruining, J.; Wedgwood, O.; Pannecouque, C.; Aquaro, S.; Perno, C.F.; Naesens, L.; Witvrouw, M.; Heijtink, R.; De Clercq, E.; McGuigan, C.; Conversion of 2′,3′-dideoxyadenosine (ddA) and 2′,3′-didehydro-2′,3′-dideoxyadenosine (d4A) to their corresponding aryloxyphosphoramidate derivatives markedly potentiates their activity against human immunodeficiency virus and hepatitis B virus. *FEBS Lett.*, **1997**, 410, 324-328.

6 a) Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J.; ADA-bypass by lipophilic cycloSal-ddAMP pro-nucleotides a second

example of the efficiency of cycloSal-concept. *Bioorg. Med. Chem. Lett.*, **1997**, 7, 1577-1582. b) Meier, C.; Knispel, T.; De Clercq, E.; Balzarini, J.; CycloSal-pronucleotides of 2',3'-dideoxyadenosine and 2',3'-dideoxy-2',3'-didehydroadenosine: synthesis and antiviral evaluation of a highly efficient nucleotide delivery system. *J. Med. Chem.*, 1999, 42, 1604-1614. c) Knispel, T.; Meier, C.; N,O-selectivity in the synthesis of 3-Me-cycloSal-ddAMP. *Nucleosides and Nucleotides*, **1999**, 18, 945-946.

7 a) Périgaud, C.; Aubertin, A.-M. Banzaria, S.; Pelicano, H.; Girardet, J.-L.; Maury, G.; Gosselin, G.; Kirn, A.; Imbach, J.-L.; Equal inhibition of the replication of human-immunodeficiency in human T cell culture by ddA bis(SATE)phosphotriester and 3'-azido-2',3'-dideoxythymidine. *Biochem. Pharmacol.*, **1994**, 48, 11-14. b) Thumann-Schweitzer, C.; Gosselin, G.; Périgaud, C.; Benzaria, S.; Girardet, J.-L.; Lefebvre, I.; Imbach, J.-L.; Kirn, A.; Aubertin, A.-M.; Anti-human immunodeficiency virus type 1 activities of dideoxynucleoside phosphotriester derivatives in primary monocytes macrophages. *Res. Vir.*, **1996**, 147, 155-163.

8 Shoshani, I.; Boudous, V.; Pierra, C.; Gosselin, G.; Johnson, R.A.; Enzymatic synthesis of unlabeled and β-^{32}P-labeled β-L-2',3'-dideoxyadenosine-5'-triphosphate as potent inhibitor of adenylyl cyclase and its use as reversible binding ligand. *J. Biol. Chem.*, **1999**, 274, 34735-34741.

9 a) Griffith, T.M.; Taylor, H.J.; Cyclic AMP mediates EDHF-type relaxations of rabbit jugular vein. *Biochem. Biophys. Res. Commun.*, **1999**, 263, 52-57. b) Taylor, H.J.; Chaytor, A.T.; Edwards, D.H.; Griffith, T.M.; Gap junction-dependent increases in smooth muscle camp underpin the EDHF phenomenon in rabbit arteries. *Biochem. Biophys. Res. Commun.*, **2001**, 283, 583-589.

10 a) Bolton, T.B.; Lang, R.J.; Takewaki, T.; Mechanism of action of noradrenaline and carbachol on smooth muscle of guinea-pig

anterior mesenteric artery. *J. Physiol.*, **1994**, 351, 549-572. b) Beny, J.L.; Brunet, P.C.; Neither nitric oxide nor nitroglycerine accounts for all the characteristics of endothelially mediated vasodilatation of pig coronary arteries. *Blood Vessel*, **1988**, 25, 308-311. c) Feletou, M.; Vanhoutte, P.M.; Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br. J. Pharmacol.*, **1988**, 93, 515-524.

11 Griffith, T.M.; Chaytor, A.T.; Taylor, H.J.; Giddings, B.D.; Edwards, D.H.; cAMP facilitates EDHF-type relaxations in conduit arteries by enhancing electrotonic conduction via gap junctions. *Proc. Natl. Acad. Sci. USA*, **2002**, 99, 6392-6397.

12 Robins, M.J.; Hansske, F.; Low, N.H.; In Park, J.; A mild conversion of vicinal diols to alkenes. Efficient transformation of ribonucleosides into 2'-ene and 2', 3'-dideoxynucleosides. *Tetrahedron Lett.*, **1984**, 25, 367-370.

13 Wedgwood, O.M.; Internal Report, **1998**, Welsh School of Pharmacy.

14 Uchiyama, M.; Aso, Y.; Noyori, R.; Hayakawa, Y.; O-Selective phosphorylation of nucleosides without N-protection. *J. Org. Chem.*, **1993**, 58, 373-379.

15 Chaytor, A. T.; Edwards, D. H.; Bakker, L. M.; Griffith, T. M.; Distinct hyperpolarizing and relaxant roles for gap junctions and endothelium-derived H_2O_2 in NO-indipendent relaxations of rabbit arteries. *Proc. Natl. Acad. Sci. U.S.A.*, **2003**, 100, 15212-15217.

Chapter Seven: Conclusions and Future work.

The thesis reports a study on the structure-activity relationships of the phosphoramidate derivatives of some nucleoside analogues (BVdU, Fludarabine, Gemcitabine and ddA). A series of modifications on three sites of the molecules –the esters, the amino acids and the aromatic moieties- have been investigated.

The application of the ProTide methodology has been successfully applied to the preparation of BVdU phosphoramidates and their evaluation in tissue culture versus three different tumour cell lines. These new phosphoramidates of BVdU showed a significantly higher activity compared to the parent nucleotide phosphoramidate, Thymectacin. Modifications on the side chain of the amino acids suggest that, a natural amino acid side chain is not necessary for potent anticancer activity and unnatural amino acids can replace natural amino acids with increased anticancer activity. Whether other unnatural amino acids might be more effective than the naturals should be taken into consideration for further investigations.

A small series of phosphoramidates of the anticancer agents gemcitabine and fludarabine have been synthesised and evaluated for their cytotoxic activities but no significant improvement in *in vitro* activity was observed.

The attempted synthesis of the phosphoramidate prodrugs of gemcitabine was shown to be more difficult compared to other nucleosides (e.g. ddA, BVdU). Low yields and poor regioselectivity suggest that the synthetic route may be improved by protection of the 3'-hydroxyl group. Further structure activity relationship studies and investigations of suitable protecting groups are currently underway in our laboratory.

Several novel fludarabine phosphoramidate derivatives have been successfully synthesised and evaluated for their activity. Preliminary data did not show significantly improvement in *in vitro* activity compared to fludarabine. However, in an effort to better understand and expand the SAR studies of fludarabine phosphoramidates, it would desirable to consider an alternative synthesis for the preparation of fludarabine and to investigate different leukaemia models.

Finally, the first example of the application of phosphoramidate derivatives as non antiviral/anticancer agents has been reported in this thesis. The phosphoramidate **61c** of ddA showed an increased potency as an inhibitor of EDHF-type smooth muscle hyperpolarizations induced by ACh compared to the parent nucleoside derivative ddA. Taking into consideration the enhanced activity of 2′,5′-dideoxyadenosine-3′-phosphate as an inhibitor of adenynyl cyclase further investigations will be focused on the synthesis of 2′,5′-dideoxyadenosine phosphoramidates.

Chapter Eight: Experimental procedures

All the solvents and reagents commercially available were used without further purification. The following anhydrous solvents and reagents were bought from Aldrich with sure sealed stopper: dichloromethane (DCM), diethyl ether, tetrahydrofuran (THF), N,N-dimethylacetamide (DMA), methanol (MeOH), dimethylformamide (DMF), 1,4-dioxane, N-methylimidazole (NMI). Triethylamine was dried on molecular sieves of 4 Å.

The reactions were analyzed by Thin Layer Chromatography (TLC) performed on commercially available Merck Kieselgel 60 F_{254} plates supplied by Merck. Separation of components was visualised using an ultraviolet lamp (254 nm and 366 nm). Preparative TLC was performed on glass backed, PK6F silica gel 60-A plates, (500 µm or 1000 µm thickness), supplied by Whatman.

Glass columns were slurry packed in the appropriate eluent under pressure, with silica gel (C-gel, 60A, 40-60 µm, Phase Sep, UK). Samples were applied as a concentrated solution, in the same eluent, or pre-absorbed onto silica. Fractions containing the product were detected by TLC, "pooled", and concentrated *in vacuo*.

Low resolution mass spectra were run on VG Platform II Fisons instrument (Fisons, Atrincham, UK) (atmosphere pressure ionisation, electronspray mass spectroscopy) in either positive and negative mode. High resolution mass spectroscopy was performed as a service by Birmingham University, using fast atom bombardment (FAB).

^{1}H, ^{13}C, ^{19}F, ^{31}P-NMR spectra were recorded on a Bruker Avance DPX300 spectrometer (300 MHz, 75 MHz, 282 MHz, 121 MHz respectively) and autocalibrated to the deuterated solvent reference peak.

The following abbreviations are used in the assignment of NMR signals: s (singlet), d (doublet), t (triplet), q (quartet), m

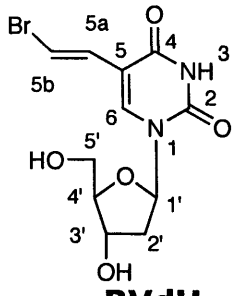
(multiplet), bs (broad singlet), dd (doublet of doublet), dt (doublet of triplet).

* in ^{13}C-NMR, denotes multiple peaks of the same ppm when rounded to one decimal place.

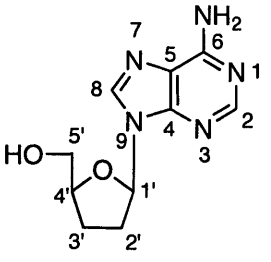
In most of the case, phosphochloridates and phosphoramidates were isolated as a mixture of diastereoisomers corresponding to roughly 50:50 mixed stereochemistry at the phosphate centre. This isomers are readily distinguished by ^{31}P NMR. The presence of phosphate diastereoisomers was also apparent in the ^{1}H and ^{13}C-NMR were several peaks were "split" in 1:1 ratio.

Quantitative purity of the phosphoramidates was estimated by ^{1}H-NMR and found to be higher than 97%.

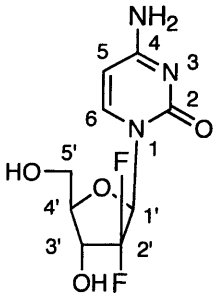
Quasi IUPAC naming is used incorporating standard trivial names for nucleosides and amino acids. Numbering of nucleoside derivatives is based on conventional nucleoside numbering, as shown below:



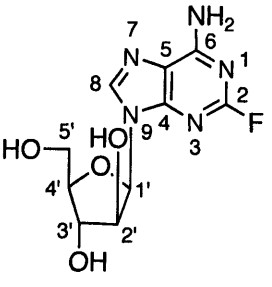
BVdU



ddA



Gemcitabine



Fludarabine

Standard procedures.

For practical purposes, standard procedures are given. Any variations from these procedures are discussed individually.

Procedures that differ from the standard ones are described in full.

Standard procedure 1: Synthesis of amino ester hydrochloride salts.

Thionyl chloride (2.0 mol eq.) was added dropwise to a stirred solution of the appropriate alcohol (10 mol eq.) at 0° C under argon. The mixture was stirred at 0 °C for 1 hr and then slowly allowed to warm to room temperature. The appropriate amino acid (1.0 mol eq) was added and the mixture was heated at reflux for 6-16 hrs. Removal of solvent and recrystallisation from methanol/ether gave the amino ester hydrochloride salts.

Standard procedure 2: Synthesis of amino benzyl ester hydrochloride salts.

The appropriate amino acid (1.0 mol eq.), *p*-toluene sulfonic acid (1.0 mol eq.) and anhydrous benzyl alcohol (4.1 mol eq.) were heated at reflux in toluene (10 mol eq.) with a Dean-Stark trap for 6-16 hrs. On cooling to room temperature, Et_2O was added and the mixture was left in an ice bath for 1hr then filtered and washed with Et_2O. The solid was dissolved in DCM and washed with 10% K_2CO_3 and water. The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give an oil. This was solubilised in acetone and neutralized with 1 M HCl. Et_2O was added

and the solid was filtered and washed with Et_2O to give a white solid.

Standard procedure 3: Synthesis of phosphorodichloridate.

Triethylamine (1.0 mol eq) was added dropwise to a stirred solution of phosphorus oxychloride (1.0 mol eq.) and the appropriate substituted phenol (1.0 mol) in anhydrous diethyl ether (40-60 mL) at -78 °C under argon. Following the addition, the reaction mixture was allowed to slowly warm to room temperature and stirred overnight. The mixture was filtered under nitrogen and the solvent removed under reduced pressure to give the crude product as an oil.

Standard procedure 4: Synthesis of phosphorochloridate.

Triethylamine (2.0 mol eq) was added dropwise to a stirred solution of the appropriate phosphorodichloridate (1.0 mol eq.) and the appropriate amino ester hydrochloric salt (1.0 mol eq.) in anhydrous DCM (40-60 mL), at -78 °C under argon. Following the addition, the reaction mixture was allowed to slowly warm to room temperature and stirred for 2-5 hrs. The formation of phosphorochloridate was monitored by ^{31}P-NMR. The solvent was removed under reduced pressure and the crude residue was re-suspended in anhydrous ether (2x20 ml), filtered, and the filtrate reduced to dryness to give the products as a crude oil.

Standard procedure 5: Synthesis of BVdU phosphoroamidate derivatives.

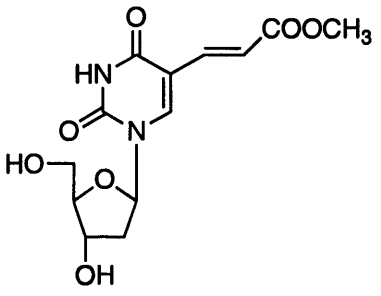
NMI (5.0 mol eq.) was added dropwise to a stirred solution of (E)-5-(2-bromovinyl)-2′-deoxyuridine (1.0 mol eq.) and the appropriate phosphorochloridate (1.5-3.0 mol eq) in anhydrous THF (10-15 mL mol eq.), at −78°C under argon. After 15 min the reaction was allowed to slowly warm to room temperature and stirred at room temperature for 2-19 hrs. The solvent was removed under reduced pressure, the crude residue dissolved in DCM, washed with 0.5 M HCl, and water. The organic layer was dried over $MgSO_4$, filtered, reduced to dryness and purified by flash column chromatography (chloroform/methanol 97/3, or dichloromethane/methanol 97/3).

Standard procedure 6: Synthesis of ddA phosphoroamidate derivatives.

A 1 M solution of tert-butylmagnesium chloride (1.1-1.5 mol eq) in THF was added to a slurry of 2′,3′-dideoxyadenosine (1 mol eq) in THF/Py (10:1). The mixture was allowed to equilibrate for 0.5 hrs under inert atmosphere then a solution 1 M of the appropriate phosphorochloridate (1.5-2.5 mol eq) in anhydrous THF was added dropwise. The reaction was stirred at room temperature for 1.5-19 hrs. and then quenched with sat. solution of NH_4Cl. The solvent was evoparated under reduced pressure, ethyl acetate was added and the organic layer washed with water and dried on $MgSO_4$. The crude was purified by flash column chromatography using chloroform/methanol (95/5) as eluent.

(E)-5-(2-carbomethoxyvinyl)-2'-deoxyuridine.[1] (19)

$C_{13}H_{16}N_2O_7$, MW 312.10



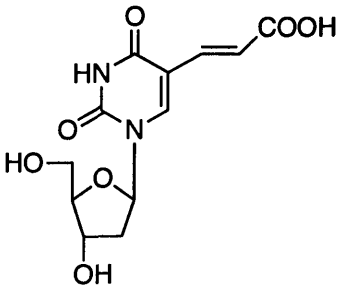
A mixture of $Pd(OAc)_2$ (316 mg, 1.41 mmol), triphenylphosphine (741 mg, 2.82 mmol), and triethylamine (4.9 mL) in 1,4-dioxane (50 mL) was stirred at 70°C until an intense red colour had developed. To this 5-iodo-2'-deoxyuridine (10.00 g, 28.24 mmol) and methylacrylate (4.86 g, 56.48 mmol, 5.1 mL) in 1,4-dioxane (20 mL) were added and the mixture stirred at reflux for 30 mins. The reaction was filtered while still hot and the filtrate cooled over night at 4 °C. The resulting pale yellow precipitate was filtered, washed with DCM and dried *in vacuo* to give the product as white solid (6.20 g, 70%).

^{1}H-NMR (DMSO-d_6; 300 MHz) δ 11.64 (1H, bs, H-3), 8.42 (1H, s, H-6), 7.37 (1H, d, ^3J=15.8 Hz, H vinylic), 6.86 (1H, d, ^3J=15.8 Hz, H vinylic), 6.13 (1H, t, ^3J=6.5 Hz, H-1'), 5.27-5.20 (2H, 2bs, OH-3', OH-5'), 4.27 (1H, m, H-3'), 3.81 (1H, m, H-4'), 3.68 (3H, s, C***H*₃**O), 3.60 (2H, m, H-5'), 2.18 (2H, m, H-2').

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 40.4 (C-2'), 51.6 (***C***H_3O), 66.7 (C-5'), 70.0 (C-3'), 85.2 (C-4'), 88.0 (C-1'), 108.5 (C-5), 116.5 (C-5b), 138.5 (C-5a), 144.4 (C-6), 149.6 (C-4), 162.1 (C-2), 167.6 (***C***$OOCH_3$).

(E)-5-(2-carboxyvinyl)-2′-deoxyuridine.[1] (20)

$C_{12}H_{14}N_2O_7$, MW 298.25



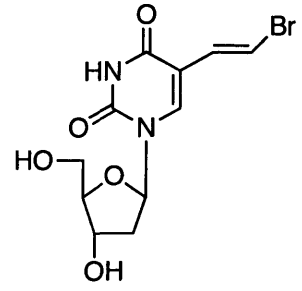
(E)-5-(2-carbomethoxyvinyl)-2′-deoxyuridine (6.00 g, 19.33 mmol) was dissolved in 300 mL of 1 M NaOH and the mixture stirred at room temperature for 3 hrs, filtered and the filtrate adjusted to pH 2 with 1M HCl. On cooling at 4°C a white precipitate formed. This was filtered off and washed with cold water (2x20 mL) and acetone (2x20 mL) and dried to give a white solid (4.44 g, 77%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 12.18 (1H, bs, COO***H***), 11.64 (1H, s, H-3), 8.40 (1H, s, H-6), 7.30 (1H, d, ^3J=15.7 Hz, H vinylic), 6.78 (1H, d, ^3J=15.7 Hz, H vinylic), 6.14 (1H, t, ^3J=6.4 Hz, H-1′), 5.38 - 5.08 (2H, bs, OH-3′, OH-5′), 4.26 (1H, m, H-3′), 3.80 (1H, m, H-4′), 3.64 (2H, m, H-5′), 2.18 (2H, m, H-2′).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 40.1 (C-2′), 61.2 (C-5′), 70.1 (C-3′), 85.1 (C-1′), 88.0 (C-4′), 108.7 (C-5), 118.0 (C-5b), 137.9 (C-6), 143.9 (C-5a), 149.6 (C-4), 162.1 (C-2), 168.4 (***C***OOH).

(E)-5-(2-bromovinyl)-2′-deoxyuridine.[1] (21)

$C_{11}H_{13}BrN_2O_5$, MW 333.14



To a solution of (E)-5-(2-carboxyvinyl)-2′-deoxyuridine (5.78 g, 19.38 mmol) in dimethylformamide (29 mL) was added K_2CO_3 (5.89 g, 42.61 mmol) and the suspension stirred at room temperature for 15 mins. A solution of N-bromosuccinimide (3.66 g, 20.56 mmol) was added dropwise over 30 min at 20 °C. The resulting suspension was filtered and the solid washed with DMF. The

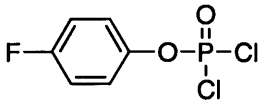
combined filtrates and washings were evaporated to dryness *in vacuo* and the residue dissolved in MeOH. To this silica gel was added and the suspension evaporated to dryness and the solid applied to the top of chromatographic column. The column was eluted with chloroform/methanol 92/8 to give a white solid (5.79 g, 90%). Crystallisation from water gave a white powder.

^{1}H-NMR (DMSO-d_6; 300 MHz) δ 11.59 (1H, bs, H-3), 8.08 (1H, s, H-6), 7.25 (1H, d, ^3J=13.6 Hz, H-5b), 6.85 (1H, d, ^3J=13.6 Hz, H-5a), 6.13 (1H, t, ^3J=6.5 Hz, H-1′), 5.29 (1H, bs, OH-3′), 5.13 (1H, bs, OH-5′), 4.24 (1H, m, H-3′), 3.79 (1H, m, H-4′), 3.66 (2H, m, H-5′), 2.51 (1H, m, H-2′), 2.14 (1H, m, H-2′).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 40.2 (C-2′), 61.3 (C-5′), 70.3 (C-3′), 84.8 (C-1′), 87.8 (C-4′), 106.9 (C-5b), 110.0 (C-5), 130.3 (C-5a), 139.9 (C-6), 149.6 (C-4), 162.1 (C-2).

Synthesis of 4-fluorophenyl-phosphorodichloridate.[2]

$C_6H_4Cl_2FO_2P$, MW=228.97 (22a)



This was synthesised according to *Standard procedure 3*, using phosphorus oxychloride (2.07 g, 13.50 mmol, 1259 μL), 4-fluorophenol (1.52 g, 13.56 mmol, 1884 μL) and TEA (1.37 g, 13.54 mmol, 1692 μL) in diethyl ether (40 mL) to give an oil (2.59 g, 84% yield).

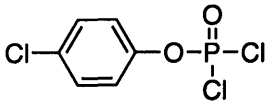
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.49.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.25-7.03 (4H, m, *p*-F***Ph***O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 117.5, 117.6 (*'m'*, ^2J=26 Hz, *p*-F***Ph***O), 122.6, 122.7 (*'o'*, ^3J=9 Hz, *p*-F***Ph***O), 145.7, 145.8, 145.9* (*'ipso'*, *p*-F***Ph***O), 161.3 (*'p'*, *J*=247 Hz, *p*-F***Ph***O).

Synthesis of 4-chlorophenyl-phosphorodichloridate.[3] (22b)

$C_6H_4Cl_3O_2P$, MW=245.43.



This was synthesised according to *Standard procedure 3*, using phosphorus oxychloride (1533 mg, 10.00 mmol, 932 μL), 4-chlorophenol (1.29 g, 10.03 mmol) and TEA (1.01 g, 9.98 mmol, 1394 μL) in diethyl ether (40 mL) to give an oil (1.90 g, 78%).

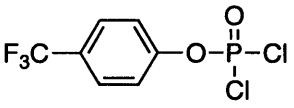
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.18.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.45 (2H, d, ^{3}J=9.0 Hz, *p*-Cl***Ph***O), 7.30 (2H, d, ^{3}J=9.0 Hz, *p*-Cl***Ph***O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 122.5 (*'o'*, *p*-Cl***Ph***O), 130.6 (*'m'*, *p*-Cl***Ph***O), 133.2 (*'p'*, *p*-Cl***Ph***O), 148.5 (*'ipso'*, *p*-Cl***Ph***O).

Sinthesis of 4-trifluoromethylphenyl-phosphorodichloridate.[4] (22c)

$C_7H_4ClF_3O_3P$, MW=278.98



This was synthesised according to *Standard procedure 3*, using phosphorus oxychloride (1.57 g, 10.24 mmol, 955 μL), 4-trifluoromethylphenol (1.66 g, 10.24 mmol) and TEA (1.04 g, 10.28 mmol, 1427 μL) in diethyl ether (40 mL) to give a colourless oil (2.52 g, 88%).

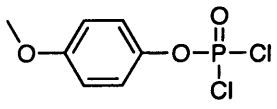
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.75.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.77 (2H, d, ^{3}J=8.4 Hz, *p*-CF_3***Ph***O), 7.49 (2H, d, ^{3}J=8.4 Hz, *p*-CF_3***Ph***O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 121.5, 121.6 (*'o'*, *p*-CF_3***Ph***O), 123.6 (***C***F_3, *J*=271 Hz, *p*-CF_3***Ph***O), 128.2 (*'m'*, *p*-CF_3***Ph***O), 129.7 (*'p'*, *J*=33 Hz, *p*-CF_3***Ph***O), 152.1, 152.3 (*'ipso'*, *p*-CF_3***Ph***O).

Synthesis of 4-methoxyphenyl-phosphorodichloridate.[2] (22d)

$C_7H_7Cl2O_3P$, MW: 241.01.



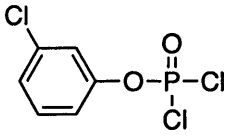
This was synthesised according to *Standard procedure 3*, using phosphorus oxychloride (11,85 g, 12.08 mmol, 1126 μL), 4-methoxyphenol (1500 g, 12.08 mmol) and TEA (1222 mg, 12.08 mmol, 1684 μL) in diethyl ether (40 mL) to give a colourless oil (2768 mg, 95% yield).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.71.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.18 (2H, d, ^{3}J=9.2 Hz, CH_3O***Ph***O), 6.88 (2H, d, ^{3}J=9.2 Hz, ***Ph***O), 3.77 (3H, s, C***H*****$_3$**OPhO)

Synthesis of 3-chlorophenyl-phosphodichloridate.[4] (22e)

$C_6H_4Cl_3O_2P$, MW=245.43



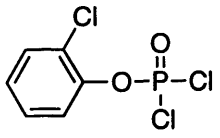
This was synthesised according to *Standard procedure 3*, using phosphorus oxychloride (1.86 g, 12.14 mmol, 1132 μL), 3-chlorophenol (1.53 g, 12.14 mmol, 1232 μL) and TEA (1.228 g, 12.16 mmol, 1692 μL) in diethyl ether (40 mL) to give an oil (2.54 g, 85%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.92.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.45 (4H, m, *m*-Cl***Ph***O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 119.3, 119.4, 121.6, 121.7 (*'o'*, *m*-Cl***Ph***O), 128.0*, 131.3, 131.3, 131.4, 131.5, 135.9, 136.0 (*m*-Cl***Ph***O), 150.1, 150.2 (*'ipso'*, *m*-Cl***Ph***O).

Synthesis of 2-chlorophenyl-phosphorodichloridate.[4] (22f)
$C_6H_4ClO_2P$, MW=278.98.



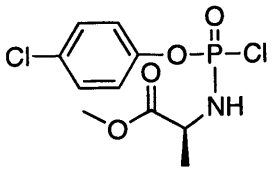
This was synthesised according to *Standard procedure 3*, using phosphorus oxychloride (1.00 g, 6.52 mmol, 608 μL), 2-chlorophenol (850 mg, 6.52 mmol) and TEA (660 mg, 6.52 mmol, 909 μL) in diethyl ether (50 mL) to give an oil (1.23 g, 82%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.39 (s).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.58-7.51 (2H, m, *o*-Cl***Ph***O), 7.41-7.29 (2H, m, *o*-Cl***Ph***O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 122.4, 122.5 (*'o'*, *o*-Cl***Ph***O), 126.4, 126.5, 128.4, 128.5, 128.7, 131.7, 131.8 (*o*-Cl***Ph***O), 146.2, 146.4 (*'ipso'*, *o*-Cl***Ph***O).

Synthesis of 4-chlorophenyl-(methoxy-L-alaninyl)-phosphorochloridate.[4] (23a)
$C_{10}H_{12}Cl_2NO_4P$, MW=312.09



This was synthesised according to *Standard procedure 4,* using L-alanine methyl ester hydrochloride (1.00 g, 7.16 mmol), 4-chlorophenyl-phosphorodichloridate (1.76 g, 7.17 mmol), and TEA (1.45 g, 14.32 mmol, 2.0 mL) in DCM (30 mL). The crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) as eluent to yield 1.62 g (72%) of colourless oil.

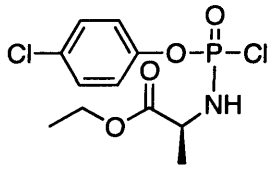
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.36, 9.07.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.35-7.15 (4H, m, *p*-Cl***Ph***O), 4.48-4.36 (1H, bs, N***H***), 4.22-4.04 (1H, m, C***H***CH_3), 3.76-3.74 (3H, 2s, C***H*****$_3$**O), 1.49-1.46 (3H, m, CHC***H*****$_3$**).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 21.0* (***C***H_3CH), 50.8, 51.1 (***C***HCH_3), 53.3, 53.4 (***C***H_3O), 121.9, 122.1, 122.3, 122.4 (*'o'*, *p*-Cl***Ph***O), 130.6, 130.4, 130.2 (*'m'*, *p*-Cl***Ph***O), 132.0 (*'p'*, *p*-Cl***Ph***O), 148.5, 148.6 (*'ipso'*, *p*-Cl***Ph***O), 173.5 (***C***OOCH_3).

Synthesis of 4-chlorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate. (23b)

$C_{11}H_{14}Cl_2NO_4P$, MW=326.11



This was synthesised according to *Standard procedure 4,* using L-alanine ethyl ester hydrochloride (1.00 g, 6.50 mmol), 4-chlorophenyl-phosphorodichloridate (1.60 g, 6.52 mmol), and TEA (1.32 g, 13.04 mmol, 1810 μL) in DCM (20 mL), to yield 1.79 g (85%) of product used without further purification.

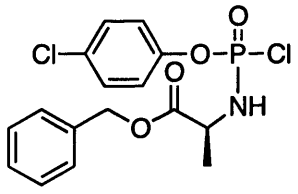
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.54, 9.25.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.44-7.21 (4H, m, *p*-Cl***Ph***O), 4.59 (1H, bs, N***H***), 4.33-4.13 (3H, m, CH_3C***H*****$_2$**O+C***H***CH_3), 1.57-1.56 (3H, m, CHC***H*****$_3$**), 1.43-1.21 (3H, m, C***H*****$_3$**CH_2O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5, 14.6 (***C***H_3CH_2O), 21.0, 21.5 (CH***C***H_3), 50.9, 51.2 (***C***HCH_3), 62.4, 62.5 (CH_3***C***H_2O), 122.3, 122.4 (*'o'*, *p*-Cl***Ph***O), 130.4 (*'m'*, *p*-Cl***Ph***O), 131.9 (*'p'*, *p*-Cl***Ph***O), 148.5, 148.6*, 148.7 (*'ipso'*, *p*-Cl***Ph***O), 173.0*, 173.1 (***C***OOCH_2CH_3).

Synthesis of 4-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate. (23c)

$C_{16}H_{16}Cl_2NO_4P$, MW=388.18



This was synthesised according to *Standard procedure 4,* using L-alanine benzyl ester hydrochloride (1.00 g, 4.63 mmol), 4-chlorophenyl-phosphorodichloridate (1.14 g, 4.63 mmol), and TEA (937 mg, 9.26 mmol, 1290 μL) in DCM (40 mL), to yield 1534 mg (87%) of crude product used without further purification.

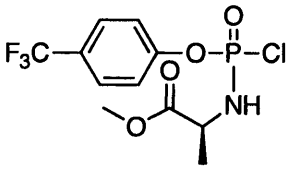
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.43, 9.16.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.08 (9H, m, *p*-Cl***Ph***O+***Ph***CH_2), 5.19 (2H, s, PhC***H_2***), 4.61-4.54 (1H, bs, N***H***), 4.26-4.10 (1H, m, C***H***CH_3), 1.42-1.38 (3H, m, CHC***H_3***).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.9, 21.0 (CH***C***H_3), 51.0, 51.2 (***C***HCH_3), 68.1, 68.2 (Ph***C***H_2), 122.3*, 122.4 (*'o'*, *p*-Cl***Ph***O), 128.6, 128.7, 128.8, 129.1, 129.2, (***Ph***CH_2+*p*-Cl***Ph***O), 131.9 (*'ipso'*, ***Ph***CH_2), 135.3, 135.4 (*'p'*, *p*-Cl***Ph***O), 148.5, 148.6 (*'ipso'*, *p*-Cl***Ph***O), 172.7, 172.8 (***C***$OOCH_2Ph$).

Synthesis of 4-trifluoromethylphenyl-(methoxy-L-alaninyl)-phosphorochloridate.[4] (23d)

$C_{11}H_{12}ClF_3NO_4P$, MW=345.64



This was synthesised according to *Standard procedure 4,* using L-alanine methyl ester hydrochloride (1.00 g, 7.16 mmol), 4-trifluoromethylphenyl-phosphorodichloridate (2.00 g, 7.17 mmol), and TEA (1.45 g, 14.32

mmol, 1916 μL) in DCM (30 mL), to yield 2.20 g (89%) of crude product used without further purification.

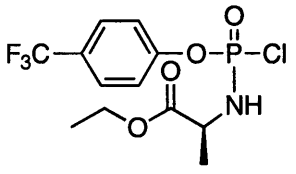
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.36, 9.22.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.66 (2H, d, ^{3}J=8.1 Hz, *p*-CF_3***Ph***O), 7.44-7.33 (2H, m, *p*-CF_3***Ph***O), 5.10 (1H, bs, N***H***), 3.81, 3.78 (3H, 2s, C***H*****$_3$**O), 3.77-3.68 (1H, m, C***H*** CH_3), 1.56-1.52 (3H, m, CHC***H*****$_3$**).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.6, 20.7 (CH***C***H_3), 50.9, 51.1 (***C***HCH_3), 53.2 (***C***H_3O), 121.4, 121.5 (*'o'*, *p*-CF_3***Ph***O), 124.1 (***CF*****$_3$**, *J*=270 Hz), 127.4, 127.5 (*'m'*, *p*-CF_3***Ph***O), 128.6 (*'p'*, *J*=34 Hz, *p*-CF_3***Ph***O), 152.4, 152.5, 152.6 (*'ipso'*, *p*-CF_3***Ph***O), 173.3, 173.4, 173.5 (***C***$OOCH_3$).

Synthesis of 4-trifluoromethylphenyl-(ethoxy-L-alaninyl)-phosphorochloridate. (23e)

$C_{12}H_{14}ClF_3NO_4$, MW=359.67



This was synthesised according to *Standard procedure 4,* using L-alanine ethyl ester hydrochloride (1.00 g, 6.50 mmol), 4-trifluoromethylphenyl-phosphorodichloridate (1.81 g, 6.49 mmol), and TEA (1.32 g, 13.04 mmol, 1740 μL) in DCM (30 mL), to yield 2.15 g (92%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.33, 9.28.

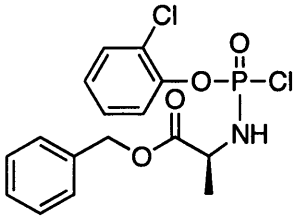
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.70 (2H, d, ^{3}J=8.2 Hz, *p*-CF_3***Ph***O), 7.46-7.39 (2H, m, *p*-CF_3***Ph***O), 4.78 (1H, bs, N***H***), 4.33-4.17 (3H, m, CH_3C***H*****$_2$**O+C***H***CH_3), 1.59-1.55 (1H, m, CHC***H*****$_3$**), 1.56-1.52 (3H, m, CH_2C***H*****$_3$**).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.4 (***C***H_3CH_2O), 20.6, 20.7, 20.8 (CH***C***H_3), 51.0, 51.1 (***C***HCH_3), 62.3, 62.4 (CH_3***C***H_2O), 121.4, 121.5 (*'o'*, *p*-CF_3***Ph***O), 124.1 (***CF*****$_3$**, *J*=270 Hz), 127.7 (*'m'*, *p*-CF_3***Ph***O),

128.7 (*'p'*, *J*=33 Hz, *p*-CF_3***Ph***O), 152.4, 152.5, 152.6 (*'ipso'*, *p*-CF_3***Ph***O), 172.9, 173.0, 173.1 (***C***$OOCH_2CH_3$).

Synthesis of 2-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate. (23f)

$C_{16}H_{16}Cl_2NO_4P$, MW=388.18



This was synthesised according to *Standard procedure 4,* using L-alanine benzyl ester hydrochloride (352 mg, 1.63 mmol), 2-chlorophenyl-phosphorodichloride (400 mg, 1.53 mmol), and TEA (330 mg, 3.26 mmol, 454 μL) in DCM (15 mL), to yield 628 mg (99%) of crude product used without further purification.

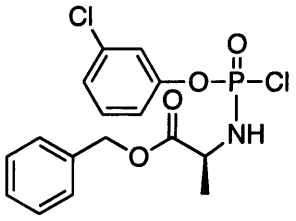
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.34, 8.90.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.68-7.20 (9H, m, *o*-Cl***Ph***O+***Ph***CH_2), 5.27-5.25 (2H, m, PhC***H₂***), 4.77-4.64 (1H, m, N***H***), 4.42-4.30 (1H, m, C***H***CH_3), 1.62, 1.58 (6H, 2d, ^3J=7.1 Hz, CHC***H₃***).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.8, 21.0 (CH***C***H_3), 51.0, 51.2 (***C***HCH_3), 68.0, 68.1 (Ph***C***H_2), 122.6, 122.7 (*'o'*, *o*-Cl***Ph***O), 125.9, 126.0, 128.6, 128.7, 128.8, 128.9, 129.0, 129.1, 131.2 (*o*-Cl***Ph***O+***Ph***CH_2), 135.4, 135.5 (*'ipso'*, ***Ph***CH_2), 146.1, 146.2 (*'ipso'*, *o*-Cl***Ph***O), 172.8, 172.9 (***C***$OOCH_2$Ph).

Synthesis of 3-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate. (23g)

$C_{16}H_{16}Cl_2NO_4P$, MW =388.18



This was synthesised according to *Standard procedure 4,* using L-alanine benzyl ester hydrochloride (379 mg, 1.76 mmol), 3-chlorophenyl-phosphorodichloridate (431 mg, 1.76 mmol), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL) to yield 658 mg (97%) of crude product used without further purification.

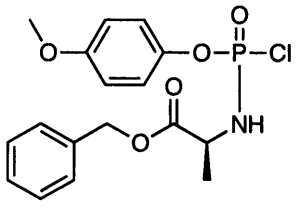
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.96, 9.29.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.40-7.15 (9H, m, *m*-Cl***Ph***O+***Ph***CH_2), 5.25-5.23 (2H, 2s, PhC***H_2***), 5.08-5.05 (1H, 2bs, N***H***), 4.29-4.23 (1H, m, C***H***CH_3), 1.57-1.54 (3H, 2d, ^{3}J=7.1 Hz, C***H_3***CH).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.6, 20.7, 20.8* (CH***C***H_3), 51.0, 51.2 (***C***HCH_3), 67.9, 68.1 (Ph***C***H_2), 119.4*, 121.6, 121.7 (*'o', m*-Cl***Ph***O), 126.7, 126.8* (*'p', m*-Cl***Ph***O), 126.8, 128.7, 128.8, 129.0, 129.1, 131.1 (*m*-Cl***Ph***O+***Ph***CH_2), 135.4, 135.5 (*'m', m*-Cl***Ph***O), 150.4, 150.5 (*'ipso', m*-Cl***Ph***O), 172.7, 172.8, 172.9 (***C***$OOCH_2Ph$).

Synthesis of 4-methoxyphenyl-(benzoxy-L-alaninyl)-phosphorochloridate. (23h)

$C_{17}H_{19}ClNO_5P$, MW=383.76.



This was synthesised according to *Standard procedure 4,* using L-alanine benzyl ester hydrochloride (1000 mg, 4.64 mmol), 4-methoxyphenyl-phosphorodichloridate (1118 mg, 4.64 mmol) and TEA (939 mg, 9.28 mmol, 1293 μL) in DCM (15 mL). After

overnight reaction, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7:3) to yield 1137 mg (yield 64 %) of a colourless oil.

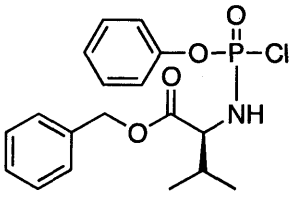
^{31}P-NMR ($CDCl_3$; 121 MHz): δ 10.06, 9.87.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.41-7.32 (5H, m, ***Ph***CH_2O), 7.26-7.17 (2H, m, *p*-CH_3O***Ph***O), 6.93-6.86 (2H, m, *p*-CH_3O***Ph***O), 5.26, 5.25 (2H, 2s, PhC***H$_2$***O), 4.70-4.61 (1H, m, N***H***), 4.33-4.21 (1H, m, C***H***CH_3), 3.83, 3.82 (3H, 2s, *p*-C***H$_3$***OPhO), 1.57, 1.56 (3H, 2d, ^{3}J= 7.1 Hz, C***H$_3$***CH).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 20.8, 20.9, 21.0, 21.5 (CH***C***H_3), 50.1, 51.2 (***C***HCH_3), 56.0 (*p*-***C***H_3OPhO), 67.9, 68.0 (Ph***C***H_2O), 115.2*, 115.3, 121.9*, 128.8, 129.0, 129.1* (*p*-***C***H_3OPhO+***Ph***CH_2O), 135.5* ('*ipso*', ***Ph***CH_2O), 143.6*, 143.7 ('*ipso*', *p*-***C***H_3OPhO), 157.8 ('*p*', *p*-***C***H_3OPhO), 172.8, 173.0, 173.1 (***C***$OOCH_2Ph$).

Synthesis of phenyl-(benzoxy-L-valinyl)-phosphorochloridate. (23i)

$C_{18}H_{21}ClNO_4P$, MW=381.79



This was synthesised according to *Standard procedure 4,* using L-valine benzyl ester hydrochloride (428 mg, 1.76 mmol), phenyl-phosphorodichloridate (370 mg, 1.75 mmol, 262 μL), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL), to yield 711 mg (>100 %) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 10.90, 10.34.

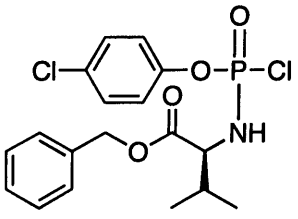
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.34-7.27 (10H, m, ***Ph***O+***Ph***CH_2), 5.27-5.25 (2H, 2s, PhC***H$_2$***), 4.51 (1H, bs, N***H***), 4.12-3.97 (1H, m,

C***H***CH(CH_3)$_2$), 2.28-2.19 (1H, m, CHC***H***(CH_3)$_2$), 0.97-0.96 (6H, 2d, ^3J=6.9 Hz, CHCH(C***H$_3$***)$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 17.6, 17.7, 19.4, 19.5 (CHCH(***C***H_3)$_2$), 32.4, 32.5, 32.6 (CH***C***H(CH_3)$_2$), 60.4, 60.9 (***C***HCH(CH_3)$_2$), 120.9, 121.0, 121.1 (*'o'*, ***Ph***O), 126.4, 128.9, 129.0, 129.1, 130.3, 130.7 (***Ph***O+***Ph***CH_2), 135.4, 135.5 (*'ipso'*, ***Ph***CH_2), 150.1, 150.2, 150.3 (*'ipso'*, ***Ph***O), 172.0*, 172.3* (***C***OOCH_2Ph).

Synthesis of 4-chorophenyl-(benzoxy-L-valinyl)-phosphochloridate. (23j)

$C_{18}H_{20}Cl_2NO_4$, MW=416.24



This was synthesised according to *Standard procedure 4,* using L-valine benzyl ester hydrochloride (467.9 mg, 1.92 mmol), 4-chlorophenyl-phosphorodichloridate (471 mg, 1.92 mmol), and TEA (389 mg, 3.84 mmol, 535 μL) in DCM (15 mL), to yield 757 mg (95%) of crude product used without further purification.

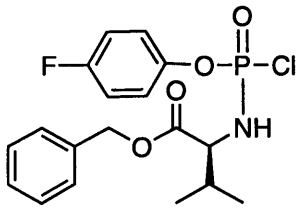
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 10.31, 10.36.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.41-7.14 (9H, m, *p*-Cl***Ph***O+***Ph***CH_2), 5.18, 5.17 (2H, 2s, PhC***H$_2$***), 4.55 (1H, bs, N***H***), 4.09-3.93 (1H, m, C***H***CH(CH_3)$_2$), 2.26-2.11 (1H, m, CHC***H***(CH_3)$_2$), 0.96, 0.95 (6H, 2d, ^3J=6.8 Hz, CHCH(C***H$_3$***)$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 17.7, 19.4, 19.5 (CHCH(***C***H_3)$_2$), 32.4, 32.5, 32.6 (CH***C***H(CH_3)$_2$), 60.4 (***C***HCH(CH_3)$_2$), 67.8, 67.9 (Ph***C***H_2), 122.0, 122.3, 122.4 (*'o'*, *p*-Cl***Ph***O), 128.8, 128.9, 129.0, 129.1, 129.9, 130.3 130.4 (***Ph***O+***Ph***CH_2), 135.4, 135.5 (*'ipso'*, ***Ph***CH_2), 148.6, 148.7 (*'ipso'*, *p*-Cl***Ph***O), 171.8, 171.9, 172.1, 172.2 (***C***OOCH_2Ph).

Synthesis of 4-fluorophenyl-(benzoxy-L-valinyl)-phosphochloridate. (23k)

$C_{18}H_{20}ClFNO_4P$, MW=399.78



This was synthesised according to *Standard procedure 4,* using L-valine benzyl ester hydrochloride (402 mg, 1.76 mmol), 4-fluorophenyl-phosphorodichloridate (428 mg, 1.76 mmol), and TEA (305 mg, 3.51 mmol, 489 μL) in DCM (15 mL), to yield 653 mg (93%) of crude product used without further purification.

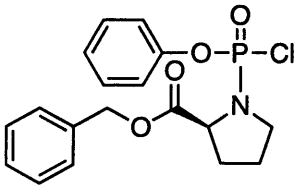
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 11.35, 10.81 (2d, $^6J_{P\text{-}F}$=2.43 Hz).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.04 (9H, m, *p*-F***Ph***O+***Ph***CH_2), 5.26-5.24 (2H, 2s, PhC***H₂***), 4.53 (1H, bs, N***H***), 4.09-3.98 (1H, m, C***H***$CH(CH_3)_2$), 2.19-2.24 (1H, m, CHC***H***$(CH_3)_2$), 0.96, 0.95 (6H, 2d, ^3J=6.8 Hz, $CHCH(C\mathbf{H_3})_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 17.6, 17.7, 19.4, 19.5 ($CHCH(\mathbf{C}H_3)_2$), 32.4, 32.5, 32.6 ($CH\mathbf{C}H(CH_3)_2$), 60.2, 60.4 ($\mathbf{C}HCH(CH_3)_2$), 67.8, 67.9 ($Ph\mathbf{C}H_2$), 117.0 ('*m*', *p*-FPhO, ^2J=24 Hz), 122.3, 122.4, 122.5, 122.6 128.9, 129.0*, 129.1* (*p*-F***Ph***O+***Ph***CH_2), 135.4, 135.5 ('*ipso*', ***Ph***CH_2), 146.0, 146.1 ('*ipso*', *p*-F***Ph***O), 160.7 ('*p*', *p*-F***Ph***O, ^1J=246 Hz), 171.9, 172.0, 172.2 ($\mathbf{C}OOCH_2Ph$).

Synthesis of phenyl-(benzoxy-L-prolinyl)-phosphorochloridate. (23l)

$C_{18}H_{19}ClNO_4P$, MW 379.77



This was synthesised according to *Standard procedure 4,* using L-proline benzyl ester hydrochloride (568 mg, 2.35 mmol), phenyl-phosphorodichloridate (496 mg, 2.35 mmol, 351 μL), and TEA (476 mg, 4.70 mmol, 655 μL) in DCM (50 mL). After overnight reaction the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) as eluent to yield 794 mg (89 %) of a colourless oil.

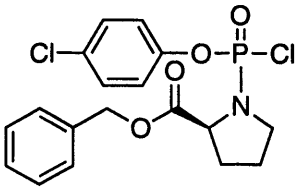
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.02, 9.09.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.43 (10H, m, ***Ph***O+***Ph***CH_2), 5.29-5.18 (2H, m, PhC***H₂***), 4.63-4.57 and 4.53-4.47 (1H, m, C***H***α proline), 3.68-3.41 (2H, m, C***H₂*** proline), 2.37-2.00 (4H, m, C***H₂*** proline).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 25.2, 25.3, 25.4*, 31.6, 31.7*, 31.9, 48.3*, 48.5* (CH_2-proline), 60.8, 60.9, 61.4, 61.5 (Cα-proline), 67.5 (***Ph***CH_2), 120.8, 120.9, 121.0, 126.2*, 128.6, 128.7, 128.8*, 128.9, 129.0, 130.3 (***Ph***CH_2+***Ph***O), 135.9, 136.0 (*'ipso'*, ***Ph***CH_2), 150.2, 150.4, 150.5 (*'ipso'*, ***Ph***O), 172.6 (***C***OOCH_2Ph).

Synthesis of 4-chlorophenyl-(benzoxy-L-prolinyl)-phosphorochloridate. (23m)

$C_{18}H_{18}Cl_2NO_4P$, MW 414.22



This was synthesised according to *Standard procedure 4,* using L-proline benzyl ester hydrochloride (1500 mg, 6.20 mmol), 4-chlorophenylphosphorodichloridate (1523 mg, 6.2 mmol), and TEA (1255 mg, 6.20 mmol, 1728 μL) in DCM (40 mL). After overnight reaction, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) as eluent to yield 1926 mg (75 %) of a colourless oil.

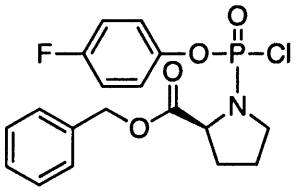
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.13, 9.22.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.40-7.14 (9H, m, ***Ph***O+***Ph***CH_2), 5.24, 5.22 (2H, 2s, PhC***H$_2$***), 4.51-4.45 (1H, m, C***H***α proline), 3.67-3.43 (2H, m, C***H$_2$*** proline), 2.37-1.99 (4H, m, C***H$_2$*** proline).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 25.2, 25.3, 25.5, 31.5, 31.7*, 31.9, 48.3, 48.4, 48.5 (CH_2-proline), 60.8, 60.9, 61.4, 61.5 (Cα-proline), 67.7 (Ph***C***H_2), 122.2, 122.3, 122.4, 128.5, 128.6, 128.8, 128.9, 129.0, 130.3 (***Ph***CH_2+*p*-Cl***Ph***O), 131.6, 131.7 (*'ipso'*, ***Ph***CH_2), 135.8, 135.9 (*'p'*, *p*-Cl***Ph***O), 148.7, 148.8, 148.9 (*'ipso'*, *p*-Cl***Ph***O) 172.5 (***C***OOCH_2Ph).

Synthesis of 4-fluorophenyl-(benzoxy-L-prolinyl)-phosphorochloridate. (23n)

$C_{18}H_{18}Cl_FNO_4P$, MW =397.76



This was synthesised according to *Standard procedure 4,* using L-proline benzyl ester hydrochloride (1000 mg, 4.14 mmol), 4-fluorophenyl-phosphorodichloridate (947 mg, 4.14 mmol), and TEA (838 mg, 8.28 mmol, 1154 μL) in DCM (15 mL). After 4 hrs, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) as eluent to yield 1456 mg (89 %) of a colourless oil.

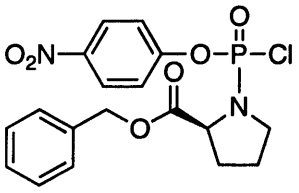
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.52 (d, ^6J=2.6 Hz), 9.42 (d, ^6J=2.5 Hz).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.40-7.00 (9H, m, *p*-F**Ph**O+***Ph***CH_2), 5.27-5.17 (2H, m, PhC***H₂***), 4.61-4.44 (1H, m, C***H***α proline), 3.64-3.42 (2H, m, C***H₂*** proline), 2.34-1.98 (2H, m, C***H₂*** proline).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 25.2, 25.3, 25.4, 25.5, 31.5, 31.7, 31.9 (CH_2-proline), 48.5, 48.6 (CH_2-proline), 61.0, 61.5 (***C***H-proline), 67.5 (Ph***C***H_2), 116.7, 117.0, 122.3, 122.4, 122.5, 128.6, 128.7, 128.8, 129.0 (***Ph***CH_2+*p*-F**Ph**O) 135.8, 135.9 (*'ipso'*, ***Ph***CH_2), 145.9, 146.0, 146.1, 146.2, 146.3 (*'ipso'*, *p*-F**Ph**O), 160.5 (*'p'*, *p*-F**Ph**O, ^1J=245 Hz), 172.5 (***C***OOCH_2Ph).

Synthesis of 4-nitrophenyl-(benzoxy-L-prolinyl)-phosphorochloridate. (23o)

$C_{16}H_{16}Cl_2NO_4P$, MW =388.18



This was synthesised according to *Standard procedure 4,* using L-proline benzyl ester hydrochloride (424 mg, 1.76 mmol), 4-nitrophenyl-phosphorodichloridate (449 mg, 1.76 mmol), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL) to yield 675 mg (91%) of crude product used without further purification .

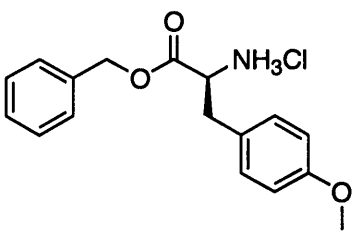
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.75, 8.59.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.15 (2H, d, ^{3}J=9.2 Hz, *p*-NO_2***Ph***), 7.37 (2H, d, ^{3}J=9.2 Hz, *p*-NO_2***Ph***O), 7.39-7.22 (5H, m, ***Ph***CH_2), 5.14-5.06 (2H, m, PhC***H_2***), 4.52-4.36 (1H, m, CHα proline), 3.51-3.38 (2H, m, H-proline), 2.22-2.10 (2H, m, C***H_2*** proline), 2.00-1.93 (2H, m, CH_2 proline).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 25.2, 25.4, 25.5*, 31.5, 31.7, 31.9 48.5, 48.6 (CH_2-proline), 61.0, 61.5 (Cα-proline), 67.7 (Ph***C***H_2), 121.4, 121.5, 121.6, 121.7*, 125.7, 126.1, 128.6, 128.8, 128.9, 129.0 (***Ph***CH_2), 135.7 (*'ipso'*, ***Ph***CH_2O), 145.6 (*'p'*, *p*-NO_2***Ph***O), 154.6, 154.7, 154.8, 154.9 (*'ipso'*, *p*-NO_2***Ph***O), 172.3, 172.4 (***C***$OOCH_2Ph$).

Synthesis of O-methyl-L-tyrosine benzyl ester hydrochloric salt. (25)

$C_{17}H_{20}ClNO_3$, MW 321.80.



This was synthesised according to *standard procedure 2*, using O-methyl-L-tyrosine (950 mg, 4.87 mmol), with p-toluene sulfonic acid monohydrate (1018 mg, 5.35 mmol) and benzyl alcohol (2.0 mL), in toluene (20 mL). The product was isolated as a white solid (1.27 g, 79%).

p*-toluene sulfonate salt.** ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.43 (3H, bs, NH*₃***Tos), 7.51 (2H, d, ^3J=8.2 Hz, *p*-TSA), 7.38-7.25 (5H, m, ***Ph***CH_2), 7.14-7.07 (4H, m, *p*-MeO***Ph***CH_2+*p*-TSA), 7.10 (2H, d, ^3J=8.6 Hz, p-MeO***Ph***CH_2), 6.86 (2H, d, ^3J=8.6 Hz, *p*-MeO***Ph***CH_2), 5.16 (2H, s, PhC***H₂***), 4.33 (1H, m, *p*-MeOPhCH_2C***H***), 3.13-2.96 (2H, m, *p*-MeOPhC***H₂***CH), 2.29 (3H, s, C***H₃*** *p*-TSA),

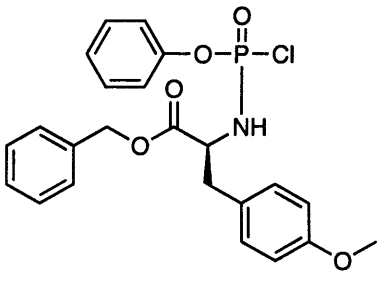
^{13}C-NMR (DMSO-d_6; 75 MHz): 21.1 (***C***H_3 *p*-TSA), 35.6 (*p*-MeOPh***C***H_2CH), 53.7 (*p*-MeOPhCH_2***C***H), 67.5 (Ph***C***H_2), 114.4, 125.9, 126.8, 128.5, 128.7, 128.8, 130.8 (*p*-TSA+***Ph***CH_2+*p*-MeO***Ph***CH_2), 135.2 ('*ipso*', ***Ph***CH_2), 138.1 ('*p*', *p*-TSA), 145.9 ('*ipso*', *p*-TSA), 158.9 (*p*-MeO***Ph***CH_2), 169.3 (***C***$OOCH_2Ph$).

Hydrochloric salt. ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.80 (3H, bs, N***H₃***Cl), 7.37-7.28 (5H, m, ***Ph***CH_2), 7.12 (2H, d, ^3J=8.4 Hz, *p*-MeO***Ph***CH_2), 6.85 (2H, d, ^3J=8.4 Hz, p-MeO***Ph***CH_2), 5.14 (2H, s, PhC***H₂***), 4.25 (1H, m, *p*-MeOPhCH_2C***H***), 3.74 (3H, s, *p*-C***H₃***OPhCH_2), 3.23-3.01 (2H, m, MeOPhC***H₂***CH).

^{13}C-NMR (DMSO-d_6; 75 MHz): 35.4 (*p*-MeOPh***C***H_2CH), 53.7 (*p*-MeOPhCH_2***C***H), 67.3 (Ph***C***H_2), 114.3, 126.7, 128.7, 130.9 (***Ph***CH_2+*p*-MeOPhCH_2), 135.2 ('*ipso*', ***Ph***CH_2), 158.8 (*p*-MeO***Ph***CH_2), 169.3 (***C***$OOCH_2Ph$).

Synthesis of phenyl-(benzoxy-O-methyl-L-tyrosinyl)-phosphorochloridate. (26)

$C_{23}H_{23}ClNO_5P$, MW =459.86



This was synthesised according to *Standard procedure 4,* using O-methyl-L-tyrosine benzyl ester hydrochloride (1050 mg, 3.26 mmol), phenyl-phosphorodichloridate (688 mg, 3.26 mmol), and TEA (660 mg, 6.52 mmol, 909 μL) in DCM (40 mL). After 3 hrs, the crude was quickly purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 1310 mg (87 %) of a colourless oil.

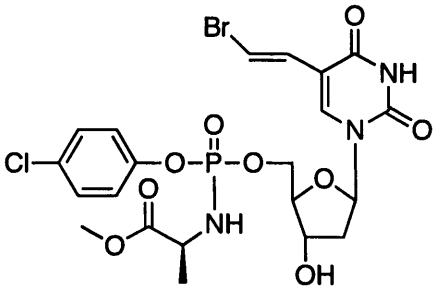
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.22, 9.17.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.48-7.26 (10H, m, ***Ph***CH_2O+***Ph***O), 7.06-6.97 (2H, m, *p*-CH_3O***Ph***CH_2CH), 6.84-6.78 (2H, m, *p*-CH_3O***Ph***CH_2CH), 5.26-5.16 (2H, m, Ph***CH_2***O), 4.57-4.41 (1H, m, *p*-CH_3PhCH_2***C*H**), 4.30-4.14 (1H, m, N***H***), 3.83-3.82 (3H, 2s, *p*-***CH_3***$OPhCH_2CH$), 3.21-3.07 (2H, m, *p*-CH_3OPhC***H_2***CH).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 39.4, 39.5, 39.6 (*p*-CH_3OPh***C***H_2CH), 55.6 (*p*-***C***H_3OPhCH_2CH), 56.0, 56.5 (*p*-***C***H_3OPhCH_2CH), 68.0, 68.1 (Ph***C***H_2), 114.5, 120.9*, 121.0, 126.4, 127.0, 127.1, 129.1*, 129.2*, 130.4, 130.7, 131.1 (***Ph***O, ***Ph***CH_2O, *p*-CH_3O***Ph***CH_2CH), 135.2, 135.3 ('*ipso*', ***Ph***CH_2O), 150.1 ('*ipso*', ***Ph***O), 159.3 ('*p*', *p*-CH_3O***Ph***CH_2CH), 171.4, 171.5, 171.6* (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(methoxy-L-alaninyl)]-phosphate.[5] (24a) (CPF13)

$C_{21}H_{24}BrClN_3O_9P$, MW=608.76



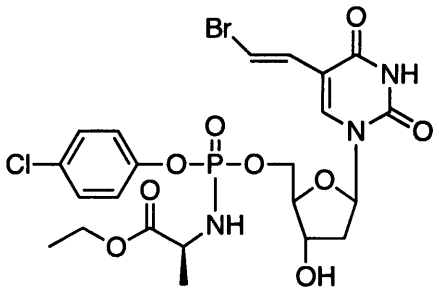
This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), 4-chlorophenyl-(methoxy-L-alaninyl)-phosphorochloridate (375 mg, 1.20 mmol), NMI (246 mg, 3.00 mmol, 239 μL) in THF (8 mL) for 5 hrs. The crude product was purified twice by flash column chromatography, using chloroform/methanol (97/3) to give the pure product as a white foamy solid (139 mg, 38%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.81, 4.54.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.11 (1H, bs, H-3), 7.68 (1H, s, H-6), 7.44, 7.43 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.35-7.20 (4H, m, *p*-Cl***Ph***O), 6.74, 6.69 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.34-6.24 (1H, m, H-1'), 4.58-4.40 (4H, m, H-3'+H-5'+N***H***), 4.36-4.19 (1H, m, H-4'), 4.07-3.99 (1H, m, C***H***CH_3), 3.75 (3H, s, C***H$_3$***O), 2.49-2.48 (1H, m, one of H-2'), 2.17-2.15 (1H, m, one of H-2'), 1.42-1.39 (3H, d, ^{3}J=7.0 Hz, CHC***H$_3$***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.1, 21.2 (CH***C***H_3), 40.7, 40.8 (C-2'), 50.6, 50.8 (***C***HCH_3), 53.2, 53.3 (***C***H_3O), 66.4, 66.7 (C-5'), 70.8, 71.2 (C-3'), 85.4, 85.5, 85.8, 86.2 (C-1'+C-4'), 110.5 (C-5b), 111.9, 112.0 (C-5), 122.0 ('*o*', *p*-Cl***Ph***O), 128.9 (C-5a), 130.3* ('*m*', *p*-Cl***Ph***O), 131.1 ('*p*', *p*-Cl***Ph***O), 138.2 (C-6), 149.1, 149.2* ('*ipso*', *p*-Cl***Ph***O), 149.8 (C-4), 162.1, 162.2 (C-2), 174.5, 174.6* (***C***$OOCH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(ethoxy-L-alaninyl)]-phosphate. (24b) (CPF11)
$C_{22}H_{26}BrN_3O_9P$, MW=622.79



This was synthesised according to *Standard procedure 5*, using BVdU (300 mg, 0.90 mmol), 4-chlorophenyl-(ethoxy-L-alaninyl)-phosphorochloridate (558 mg, 1.71 mmol), NMI (222 mg, 2.70 mmol, 215 μL) in THF (10 mL) for 16 hrs. The crude product was purified by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (168 mg, 30%).

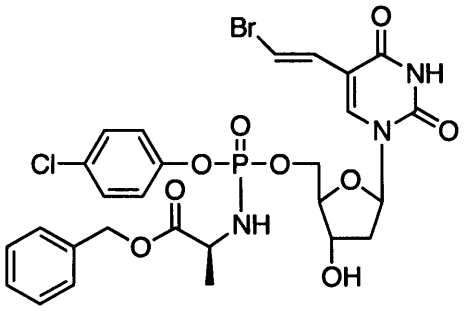
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.88, 4.65.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.51 (1H, bs, H-3), 7.69-7.68 (1H, 2s, H-6), 7.49, 7.43 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.37-7.22 (4H, m, *p*-Cl***Ph***O), 6.77, 6.73 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.33-6.24 (1H, m, H-1'), 4.62-4.34 (3H, m, H-3'+H-5'+N***H***), 4.28-3.89 (5H, m, H-4'+CH_3C***H_2***O+***C*H**CH_3+N***H***), 2.59-2.45 (1H, m, one of H-2'), 2.22-2.14 (1H, m, one of H-2'), 1.43-1.41 (3H, d, ^{3}J=7.0 Hz, CHC***H_3***), 1.31, 1.30 (3H, 2t, ^{3}J=7.2 Hz, C***H_3***CH_2O).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.5 (***C***H_3CH_2O), 21.2, 21.3 (CH***C***H_3), 40.7, 40.8 (C-2'), 50.7, 50.8 (***C***HCH_3), 62.4* (CH_3***C***H_2O), 66.4, 66.8 (C-5'), 70.8, 71.2 (C-3'), 85.4, 85.8, 86.1 (C-1'+C-4'), 110.4 (C-5b), 112.0 (C-5), 122.0, 122.1 (*'o'*, *p*-Cl***Ph***O), 128.9 (C-5a), 130.3* (*'m'*, *p*-Cl***Ph***O), 131.1 (*'p'*, *p*-Cl***Ph***O), 138.2 (C-6), 149.2 (*'ipso'*, *p*-Cl***Ph***O), 150.0 (C-4), 162.2 (C-2), 174.1, 174.2 (***C***$OOCH_2CH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(benzoxy-L-alaninyl)]-phosphate. (24c) (CPF12)

$C_{22}H_{26}BrN_3O_9P$, MW=622.79.



This was synthesised according to *Standard procedure 5*, using BVdU (300 mg, 0.90 mmol), 4-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate (699 mg, 1.80 mmol), NMI (370 mg, 4.51 mmol, 359 μL) in THF (10 mL) for 2 hrs. The crude product was purified by flash column chromatography, using dichloromethane/methanol (95/5) to give the pure product as a white foamy solid (310 mg, yield 50%).

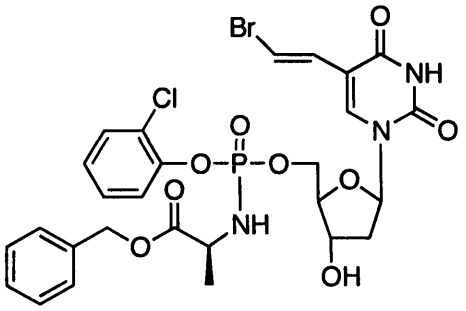
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.81, 4.51.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.10 (1H, bs, H-3), 7.65, 7.63 (1H, 2s, H-6), 7.45, 7.43 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.40-7.17 (9H, m, *p*-Cl***Ph***O+***Ph***CH_2), 6.75 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.33-6.23 (1H, 2t, ^{3}J=6.0 Hz, H-1'), 5.17 (2H, s, PhC***H_2***), 4.60-4.23 (4H, m, H-3'+H-5'+N***H***), 4.20-3.97 (2H, m, H-4'+C***H***CH_3), 2.48-2.44 (1H, m, one of H-2'), 2.15-2.05 (1H, m, one of H-2'), 1.43-1.40 (3H, d, ^{3}J=7.0 Hz, CHC***H_3***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.2, 21.3 (CH***C***H_3), 40.8 (C-2'), 50.8, 50.9 (***C***HCH_3), 66.6, 66.7 (C-5'), 67.9 (Ph***C***H_2), 70.7, 71.1 (C-3'), 85.4, 85.5, 85.8, 86.1 (C-1'+C-4'), 110.5 (C-5b), 111.9, 112.0 (C-5), 122.0* ('*o*', *p*-Cl***Ph***O), 128.7, 129.0, 129.1, 130.3 (*p*-Cl***Ph***O+C-5a), 131.1 ('*ipso*', ***Ph***CH_2), 135.4 ('*p*', *p*-Cl***Ph***O), 138.2 (C-6), 149.1, 149.2 ('*ipso*', *p*-Cl***Ph***O), 150.0 (C-4), 162.1 (C-2), 173.9, 174.0 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[2-chlorophenyl-(benzoxy-L-alaninyl)]-phosphate. (24d) (CPF49)

$C_{27}H_{28}BrClN_3O_9P$, MW=684.86



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 2-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate (524 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 20 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (174 mg, 57%).

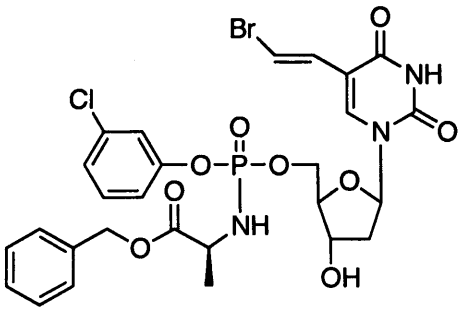
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.66, 4.42.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.92 (1H, bs, H-3), 7.58, 7.56 (1H, 2s, H-6), 7.46-7.03 (10H, m, *o*-Cl***Ph***O+***Ph***CH_2+H-5b), 6.66, 6.65 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.25, 6.18 (1H, 2t, ^{3}J=6.5 Hz, H-1'), 5.16, 5.05 (2H, AB system, ^{2}J=12.4 Hz, PhC***H₂***), 4.52-4.24 (3H, m, H-3'+H-5'), 4.21-4.05 (4H, m, H-4'+C***H***CH_3+N***H***+OH-3'), 2.41-2.22 (1H, m, one of H-2'), 2.01-1.90 (1H, m, one of H-2'), 1.38-1.32 (3H, 2d, ^{3}J=11.5 Hz, CHC***H₃***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.2, 21.3 (CH***C***H_3), 40.8, 40.9 (C-2'), 50.8, 50.9 (***C***HCH_3), 66.8, 66.9 (C-5'), 67.9 (Ph***C***H_2), 71.1, 71.4 (C-3'), 85.5, 85.6, 85.7, 86.0 (C-1'+C-4'), 110.5 (C-5b), 111.9, 112.1 (C-5), 122.0, 122.3 ('*o*', *o*-Cl***Ph***O), 125.7, 125.8, 125.9, 126.7, 128.7, 128.9, 129.0, 129.1, 131.1 (*o*-Cl***Ph***O+***Ph***CH_2+C-5a), 135.5 ('*ipso*', ***Ph***CH_2), 138.0, 138.1 (C-6), 146.6 (C-4), 149.8, 149.9 ('*ipso*', *o*-Cl***Ph***O), 162.1 (C-2), 173.9, 174.0 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[3-chlorophenyl-(benzoxy-L-alaninyl)]-phosphate. (24e) (CPF82)

$C_{22}H_{26}BrN_3O_9P$, MW=622.79



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 3-chlorophenyl-(benzoxy-L-alaninyl)-phosphorochloridate (524 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 20 hrs. The crude product was purified three time by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (148 mg, yield 48%).

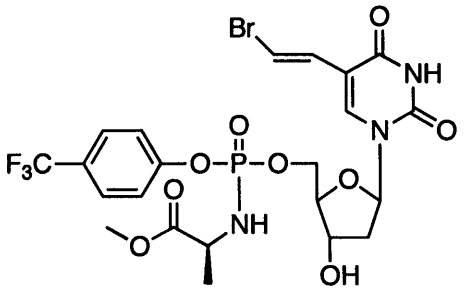
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.71, 4.32.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.76 (1H, bs, H-3), 7.65, 7.62 (1H, 2s, H-6), 7.47-7.15 (10H, m, *m*-Cl***Ph***O+***Ph***CH_2+H-5b), 6.73, 6.68 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.33-6.22 (1H, 2t, ^{3}J=6.3 Hz, H-1'), 5.23-5.17 (2H, 2s, PhC***H_2***), 4.57-4.26 (4H, m, H-3'+H-5'+N***H***), 4.20-4.01 (2H, m, H-4'+C***H***CH_3+OH-3'), 2.48-2.44 (1H, m, one of H-2'), 2.16-2.06 (1H, m, one of H-2'), 1.44 (3H, d, ^{3}J=7.0 Hz, CHC***H_3***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.1, 21.2 (CH***C***H_3), 40.7 (C-2'), 50.8, 50.9 (***C***HCH_3), 66.4, 66.6 (C-5'), 67.9 (Ph***C***H_2), 70.7, 71.1 (C-3'), 85.4, 85.5, 85.8, 86.1 (C-1'+C-4'), 110.5 (C-5b), 111.9, 112.0 (C-5), 118.9, 119.0, 122.0, 121.2 (*'o'*, *m*-Cl***Ph***O), 126.1, 128.7, 128.8, 129.0, 129.1, 129.2, 130.3, 131.1, 135.4 (*m*-Cl***Ph***O+***Ph***CH_2+C-5a), 138.2 (C-6), 149.0, 149.1 (*'ipso'*, *m*-Cl***Ph***O), 150.0 (C-4), 161.9 (C-2), 173.8, 173.9 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-trifluoromethylphenyl-(methoxy-L-alaninyl)]-phosphate. (24f) (CPF15)

$C_{22}H_{24}BrF_3N_3O_9P$, MW=642.31



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), 4-trifluoromethylphenyl-(methoxy-L-alaninyl)-phosphorochloridate (519 mg, 1.50 mmol), NMI (246 mg, 3.00 mmol, 239 μL) in THF (5 mL) for 4 hrs. The crude product was purified by flash column chromatography, using chloroform/methanol (97/3) as eluent to give the pure product as a white foamy solid (211 mg, 55%).

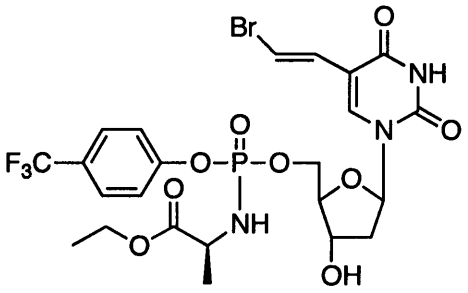
^{31}P-NMR (MeOD, 121 MHz): δ 5.23, 5.07.

^{1}H-NMR (MeOD, 300 MHz): δ 7.80 (1H, s, H-6), 7.70 (2H, d, ^{3}J=8.7 Hz, *p*-CF_3***Ph***O), 7.47-7.42 (2H, m, *p*-CF_3***Ph***O), 7.37 (1H, d, ^{3}J=13.6 Hz, H-5b), 6.80 (1H, d, ^{3}J=13.6 Hz, H-5a), 6.30-6.23 (1H, m, H-1'), 4.52-4.29 (3H, m, H-3'+H-5'), 4.17-4.13 (1H, m, H-4'), 4.05-3.91 (1H, m, C***H***CH_3), 3.67 (3H, s, C***H$_3$***O), 2.35-2.32 (1H, m, one of H-2'), 2.23-2.16 (1H, m, one of H-2'), 1.37-1.34 (3H, d, ^{3}J=7.1 Hz, CH***CH_3***).

^{13}C-NMR (MeOD, 75 MHz): δ 20.6, 20.7, 20.8, 20.9 (CH***C***H_3), 41.5, 41.7 (C-2'), 51.9, 52.0 (***C***HCH_3), 68.2, 68.3* (C-5'), 72.4, 72.5 (C-3'), 87.1, 87.2, 87.4, 87.6 (C-1', C-4'), 109.7 (C-5b), 112.6, 112.7 (C-5), 122.5, 122.6, 122.7 (*'o'*, *p*-CF_3***Ph***O), 125.8 (***C***F_3, J=269 Hz, *p*-CF_3***Ph***O), 128.7 (*'m'*, *p*-CF_3***Ph***O), 128.8 (*'p'*, J=33 Hz, *p*-CF_3***Ph***O), 130.9 (C-5a), 140.2, 140.3 (C-6), 151.4, 151.5 (*'ipso'*, *p*-CF_3***Ph***O), 155.1, 155.2 (C-4), 164.0 (C-2), 151.5, 175.6, 175.8, 175.9, (***C***$OOCH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-trifluoromethylphenyl-(ethoxy-L-alaninyl)]-phosphate. (24g) (CPF25)

$C_{23}H_{26}BrF_3N_3O_9P$, MW=656.34



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), 4-trifluoromethylphenyl-(ethoxy-L-alaninyl)-phosphorochloridate (540 mg, 1.50 mmol), NMI (246 mg, 3.00 mmol, 239 μL) in THF (5 mL) for 20 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (95/5) to give the pure product as a white foamy solid (173 mg, 44%).

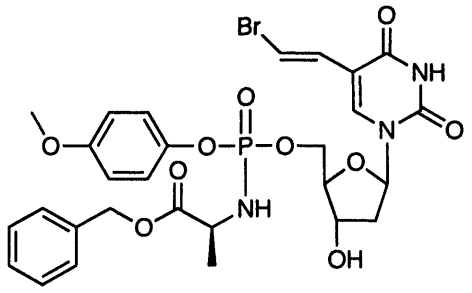
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.65, 4.35.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.05 (1H, s, H-3), 7.69-7.64 (3H, m, H-6+*p*-CF_3***Ph***O), 7.46-7.39 (3H, m, *p*-CF_3***Ph***O+H-5b), 6.74, 6.71 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.34-6.25 (1H, m, H-1'), 4.57-4.35 (4H, m, H-3'+H-5'+N***H***), 4.27-4.13 (4H, m, H-4'+CH_3C***H_2***O+OH-3'), 4.12-3.98 (1H, m, C***H***CH_3), 2.53-2.47 (1H, m, one of H-2'), 2.21-2.12 (1H, m, one of H-2'), 1.43-1.40 (3H, d, ^{3}J=7.0 Hz, CHC***H_3***), 1.28, 1.27 (3H, 2t, ^{3}J=7.0 Hz, C***H_3***CH_2O).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.5 (***C***H_3CH_2O), 21.2, 21.3 (CH***C***H_3), 40.7 (C-2'), 50.8, 50.9 (***C***HCH_3), 62.4 (CH_3***C***H_2O), 66.3, 66.7 (C-5'), 70.7, 71.1 (C-3'), 85.3, 85.4, 85.8, 86.1 (C-1'+C-4'), 110.5 (C-5b), 112.0 (C-5), 120.9, 121.0 ('*o*', *p*-CF_3***Ph***O), 124.2 (***C***F_3, J=271 Hz, *p*-CF_3***Ph***O), 127.7*, 127.8, 128.2 ('*m*', '*p*', *p*-CF_3***Ph***O), 128.8 (C-5a), 138.0 (C6), 149.7 (C-4), 153.2 ('*ipso*', *p*-CF_3***Ph***O), 161.9 (C-2), 174.0, 174.1 (***C***$OOCH_2CH_3$).

Synthesis of (E)-5-(-bromovinyl)-2'-deoxyuridine-5'-[4-methoxyphenyl-(benzoxy-L-alaninyl)]-phosphate. (24h) (CPF162)

$C_{28}H_{31}BrN_3O_{10}P$, MW=680.44



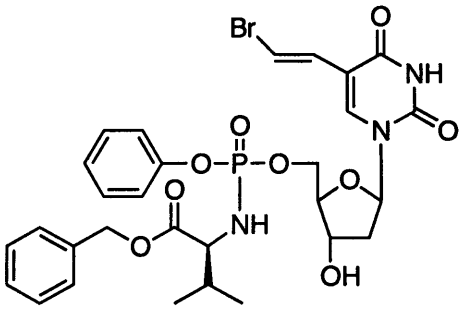
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-methoxyphenyl-(benzoxy-L-alininyl)-phosphorochloridate (518 mg, 1.35 mmol), NMI (184.7 mg, 2.25 mmol, 179.4 μL) in THF (7 mL) for 16 hrs. The crude product was purified by flash column chromatography, using chloroform/methanol (95/5) to give the pure product as a white foamy solid (193 mg, 63%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.91, 5.26.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.72 (1H, bs, H-3), 7.67, 7.65 (1H, 2s, H-6), 7.47, 7.46 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 7.41-7.32 (5H, m, ***Ph***CH_2), 7.18-7.15 (2H, m, *p*-CH_3O***Ph***O), 6.87-6.83 (2H, m, *p*-CH_3O***Ph***O), 6.76, 6.72 (1H, d, ^{3}J=13.6 Hz, H-5b), 6.33-6.23 (1H, m, H-1'), 5.23-5.13 (2H, m, PhC***H$_2$***O), 4.55-4.02 (6H, m, H-3'+H-5'+H-4' +C***H***CH_3), 3.79 (3H, s, *p*-C***H$_3$***OPhO), 2.47-2.42 (1H, m, one of H-2'), 2.13-2.04 (2H, m, one of H-2'), 1.44-1.41 (3H, m, CHC***H$_3$***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 21.2, 21.3 (CH***C***H_3), 40.7, 40.8 (C-2'), 50.8, 51.0 (***C***HCH_3), 56.0 (***C***H_3O), 66.1, 66.2 (C-5'), 67.9 (Ph***C***H_2), 70.7, 71.2 (C-3'), 85.5, 85.7, 86.0 (C-1'+C4'), 110.3 (C-5b), 111.9, 115.2, 121.4, 121.5*, 128.6, 129.0*, 129.1*, 135.5*, (*p*-CH_3O***Ph***O+***Ph***CH_2), 138.1 (C-5a), 144.1, 144.2 ('*ipso*', *p*-CH_3O***Ph***O), 149.7, 149.8 (C-4), 157.3 ('*p*', *p*-CH_3O***Ph***O), 161.9 (C-2), 173.9, 174.0 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(-bromovinyl)-2'-deoxyuridine-5'-[phenyl-benzoxy-L-valinyl)]-phosphate. (24i) (CPF85)
$C_{29}H_{33}BrN3O_9P$, MW=678.46



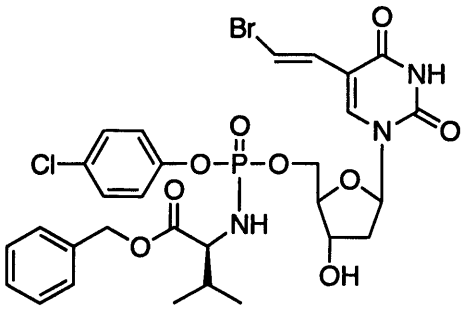
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), phenyl-(benzoxy-L-valinyl)-phosphorochloridate (515.4 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 12 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (169 mg, 55%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.91, 5.26.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.45 (1H, bs, H-3), 7.62 (1H, s, H-6), 7.47, 7.46 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 7.38-7.19 (10H, m, ***Ph***CH_2+***Ph***O), 6.77, 6.73 (1H, d, ^{3}J=13.6 Hz, H-5b), 6.31-6.22 (1H, m, H-1'), 5.22-5.10 (2H, m, PhC***H$_2$***), 4.52-3.70 (7H, m, H-3'+H-5'+H-4'+OH+NH+C***H***$CH(CH_3)_2$), 2.46-2.37 (1H, m, one of H-2'), 2.14-1.93 (2H, m, one of H-2'+CHC***H***$(CH_3)_2$), 0.84-0.85 (6H, m, CHCH(C***H$_3$***)***$_2$***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 17.6, 17.8, 19.4 (CHCH(***C***$H_3)_2$), 32.5 (CH***C***$H(CH_3)_2$), 40.7, 40.8 (C-2'), 60.6, 60.7 (***C***$HCH(CH_3)_2$), 66.0, 66.4 (C-5'), 67.7, 67.8 (Ph***C***H_2), 70.5, 71.2 (C-3'), 85.4, 85.5, 85.9 (C-1'+C-4'), 110.5 (C-5b), 111.9, 112.0 (C-5), 120.4, 120.5 ('*o*', ***Ph***O), 128.8, 128.9, 129.0, 129.1, 130.3 (***Ph***O+***Ph***CH_2+C5-a), 135.5, 135.6 ('*ipso*', ***Ph***CH_2), 137.9, 138.0 (C-6), 149.6 (C-4), 150.7, 150.8 ('*ipso*', ***Ph***O), 163.8 (C-2), 173.1, 173.2 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(benzoxy-L-valinyl)]-phosphate. (24j) (CPF86)
$C_{29}H_{32}BrClN_3O_9P$, MW=712.91



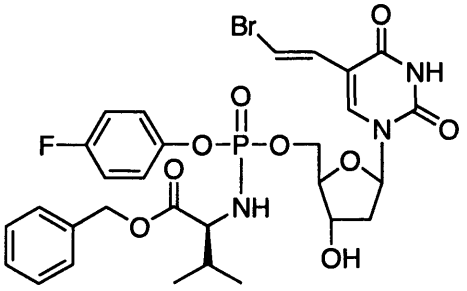
This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(benzoxy-L-valinyl)-phosphorochloridate (562 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 20 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (185 mg, 58%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.95, 5.47.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.78 (1H, bs, H-3), 7.65-7.62 (1H, 2s, H-6), 7.49-7.17 (10H, m, ***Ph***CH_2+*p*-Cl***Ph***O+H-5a), 6.77, 6.68 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.32-6.23 (1H, m, H-1'), 5.22-5.10 (2H, m, PhC***H$_2$***), 4.54-3.89 (6H, m, H-3'+H-5'+H-4'+OH+N***H***), 3.88-3.71 (1H, m, C***H***$CH(CH_3)_2$), 2.52-2.40 (1H, m, one of H-2'), 2.17-2.04 (2H, m, one of H-2'+CHC***H***$(CH_3)_2$), 0.93-0.85 (6H, m, CHCH(C***H$_3$***)$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 17.6, 17.8, 19.4 (CHCH(***C***$H_3)_2$), 32.4, 32.5*, 32.6 (CH***C***$H(CH_3)_2$), 40.8 (C-2'), 60.6, 60.7 (***C***$HCH(CH_3)_2$), 66.3, 66.7 (C-5'), 67.8 (Ph***C***H_2), 70.7, 71.2 (C-3'), 85.3, 85.4, 85.7, 86.1 (C-1'+C-4'), 110.5, 110.6 (C-5b), 111.9, 112.0 (C-5), 121.8, 121.9, 122.0 ('*o*', *p*-Cl***Ph***O), 128.8, 128.9, 129.0, 129.1*, 130.2 (*p*-Cl***Ph***O+***Ph***CH_2+C-5a), 131.0, 131.1 ('*ipso*', ***Ph***CH_2), 135.4, 135.5 ('*p*', *p*-Cl***Ph***O), 138.0* (C-6), 149.2, 149.3*, 149.4 ('*ipso*', *p*-Cl***Ph***O), 149.8 (C-4), 162.0 (C-2), 173.1, 173.2 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(-bromovinyl)-2'-deoxyuridine-5'-[4-fluorophenyl-(benzoxy-L-valinyl)]-phosphate. (24k) (CPF87)
$C_{29}H_{32}BrFN_3O_9P$, MW=695.10



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-fluorophenyl-(benzoxy-L-valinyl)-phosphorochloridate (586 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 15 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (187 mg, 57%).

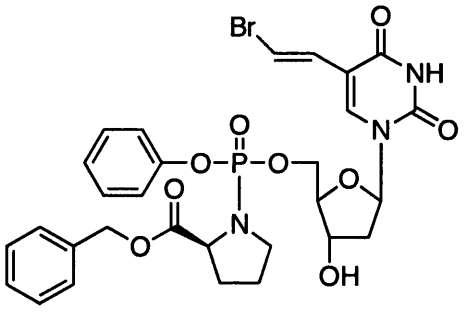
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.21 (d, $^6J_{P\text{-}F}$=1.82 Hz), 5.63 (d, $^6J_{P\text{-}F}$ =1.70 Hz).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.45 (1H, bs, H-3), 7.67, 7.63 (1H, 2s, H-6), 7.47, 7.46 (1H, 2d, ^3J=13.6 Hz, H-5a), 7.42-7.00 (9H, m, ***Ph***CH_2+*p*-F***Ph***O), 6.76, 6.73 (1H, 2d, ^3J=13.6 Hz, H-5a), 6.32-6.22 (1H, m, H-1'), 5.23-5.11 (2H, m, PhC***H$_2$***), 4.52-3.68 (7H, m, H-3'+H-5'+H-4'+OH+NH+C***H***$CH(CH_3)_2$), 2.48-2.41 (1H, m, one of H-2'), 2.23-2.04 (2H, m, one of H-2'+CHC***H***$(CH_3)_2$), 0.94-0.85 (6H, m, CHCH(C***H$_3$***)***$_2$***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 17.5, 17.7, 19.3, 19.4 (CHCH(***C***$H_3)_2$), 32.3, 32.4, 32.5, 32.6 (CH***C***$H(CH_3)_2$), 40.7, 40.8 (C-2'), 60.5, 60.7 (***C***$HCH(CH_3)_2$), 66.1, 66.5 (C-5'), 67.8* (Ph***C***H_2), 70.5, 71.1 (C-3'), 85.3, 85.4, 85.6, 85.9 (C-1'+C-4'), 110.5, 110.6 (C-5b), 111.9, 112.0 (C-5), 116.8 ('*m*', *p*-F***Ph***O, ^2J=24 Hz), 121.8, 121.9*, 122.0, 122.1* ('*o*', *p*-F***Ph***O), 128.8, 128.9*, 129.1* (*p*-F***Ph***O+***Ph***CH_2+C-5a), 135.4, 135.5 ('*ipso*', ***Ph***CH_2), 138.0* (C-6), 146.5, 146.6 ('*ipso*', *p*-F***Ph***O), 149.6 (C-4), 160.2 ('*p*', *p*-F***Ph***O, ^1J=246 Hz), 161.8, 161.9 (C-2), 173.2*, 173.3 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-L-prolinyl)]-phosphate. (24l, CPF160 and 24m, CPF181)

$C_{29}H_{31}BrN_3O_9P$, MW=676.45



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), phenyl-(benzoxy-L-prolinyl)-phosphorochloridate (684 mg, 1.80 mmol), NMI (246 mg, 3.00 mmol, 239 μL) in THF (10 mL) for 16 hrs. The crude product was purified by flash column chromatography, eluting with chloroform/methanol (97/3) to give two products as white foamy solids. (**CPF160**: 133 mg, 33%; **CPF181**: 16 mg, 4%).

CPF160

^{31}P-NMR (MeOD, 121 MHz): δ 3.18 (s).

^{1}H-NMR (MeOD, 300 MHz): δ 7.80 (1H, s, H-6), 7.40-7.18 (11H, m, H-5b+***Ph***CH_2+***Ph***O), 6.83 (1H, d, ^{3}J=13.6 Hz, H-5a), 6.26 (1H, ψt, H-1'), 5.20, 5.13 (2H, AB system, ^{2}J=12.2 Hz, PhC***H$_2$***), 4.45-4.31 (4H, m, H-5'+H-3'+C***H***α proline), 4.11-4.09 (1H, m, H-4'), 3.39-3.32 (2H, m, C***H$_2$*** proline), 2.34-2.26 (1H, m, one of H-2'), 2.20-1.85 (1H, m, one of H-2'), 2.20-1.79 (5H, m, one of H-2'+C***H$_2$*** proline).

^{13}C-NMR (MeOD, 75 MHz): δ 26.6, 26.7, 32.4, 32.6 (CH_2-proline), 41.8 (C-2'), 48.5 (CH_2-proline), 62.8, 62.9 (Cα-proline), 68.1, 68.2 (C-5'), 68.6 (Ph***C***H_2O), 72.6 (C-3'), 87.3, 87.4 (C-4'), 87.5 (C-1'), 109.6 (C-5b), 112.5 (C-5), 121.4, 121.5 (*'o'*, ***Ph***O), 126.8 (*'m'*, ***Ph***O), 129.8, 130.0, 131.0, 131.4 (***Ph***CH_2+C-5a+***Ph***O), 137.5 (*'ipso'*, ***Ph***O), 140.2 (C-6), 151.4 (C-4), 152.2, 152.3 (*'ipso'*, ***Ph***O), 164.0 (C-2), 175.2 (***C***OOCH_2Ph).

CPF181

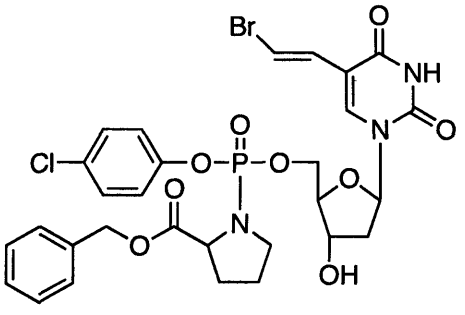
^{31}P-NMR (MeOD, 121 MHz): δ 3.32 (s).

^{1}H-NMR (MeOD, 300 MHz): δ 7.64 (1H, s, H-6), 7.29-7.07 (11H, m, H-5b+***Ph***CH_2+***Ph***O), 6.71 (1H, d, ^{3}J=13.6 Hz, H-5a), 6.10 (1H, t, ^{3}J=6.6 Hz, H-1'), 5.08, 5.02 (2H, AB system, ^{2}J=12.3 Hz, PhC***H₂***), 4.27-4.20 (4H, m, H-5'+ H-3'+C***H***α proline), 4.17-4.10 (1H, m, H-4'), 3.35-3.30 (2H, m, C***H₂*** proline), 2.25-2.14 (1H, m, one of H-2'), 2.12-1.74 (5H, m, one of H-2'+C***H₂*** proline).

^{13}C-NMR (MeOD; 75 MHz): δ 26.4, 26.5, 32.8, 32.9 (CH_2-proline), 41.6 (C-2'), 49.4, 49.5 (CH_2-proline), 62.1, 62.2 (Cα-proline), 68.2*, 68.3 (C-5'), 68.4 (Ph***C***H_2), 72.3 (C-3'), 87.1, 87.2 (C-4'), 87.6 (C-1'), 109.6 (C-5b), 112.6 (C-5), 121.8, 121.9 (*'o'*, ***Ph***O), 126.8 (*'m'*, ***Ph***O), 129.7, 129.8, 130.0, 130.9, 131.3 (***Ph***CH_2+C-5a+***Ph***O), 137.6 (*'ipso'*, ***Ph***O), 140.2 (C-6), 151.4 (C-4), 152.2, 152.3 (*'ipso'*, ***Ph***O), 164.0 (C-2), 174.9 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(benzoxy-L-prolinyl)]-phosphate. (24n, CPF184 and 24o, CPF185)

$C_{29}H_{30}BrClN_3O_9P$, MW=710.89



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), 4-chlorophenyl-(benzoxy-L-prolinyl)-phosphorochloridate (746 mg, 1.80 mmol), NMI (246 mg, 3.00 mmol, 239 μL) in THF (10 mL) for 16 hrs. The crude product was purified by flash column chromatography,

using chloroform/methanol (97/3) to give two products as white foamy solids (**CPF184**: 169 mg, 40%; **CPF185**: 57 mg, 13%).

CPF184

^{31}P-NMR (MeOD, 121 MHz): δ 3.17 (s).

^{1}H-NMR (MeOD, 300 MHz): δ 7.73 (1H, s, H-6), 7.33-7.13 (8H, m, H-5b+***Ph***CH_2+pCl***Ph***O), 7.14 (2H, d, ^{3}J=8.9, p-Cl***Ph***O), 6.75 (1H, d, ^{3}J=13.6 Hz, H-5a), 6.21 (1H, ψt, H-1'), 5.13, 5.05 (2H, AB system, ^{2}J=12.2 Hz, PhC***H$_2$***), 4.40-4.25 (4H, m, H-5'+H-3'+C***H***α proline), 4.06-4.05 (1H, m, H-4'), 3.32-3.26 (2H, m, C***H$_2$*** proline), 2.31-2.23 (1H, m, one of H-2'), 2.17-1.78 (5H, m, one of H-2'+C***H$_2$*** proline).

^{13}C-NMR (MeOD, 75 MHz): δ 26.5, 26.7, 32.4, 32.6 (CH_2-proline), 41.7 (C-2'), 48.5, 48.6 (CH_2-proline), 62.7, 62.8 (Cα-proline), 68.2, 68.3 (C-5'), 68.6 (Ph***C***H_2), 72.4 (C-3'), 87.2, 87.3 (C-4'), 87.5 (C-1'), 109.6 (C-5b), 112.5 (C-5), 123.1, 123.2, 129.8, 130.0, 131.0, 131.4, 132.0 (*p*-Cl***Ph***O+***Ph***CH_2+C-5a), 137.5 ('*p*', *p*-Cl***Ph***O), 140.3 (C-6), 150.9 ('*ipso*', *p*-Cl***Ph***O), 151.4 (C-4), 164.0 (C-2), 175.1 (***C***OOCH_2Ph).

CPF185

^{31}P-NMR (MeOD, 121 MHz): δ 3.41 (s).

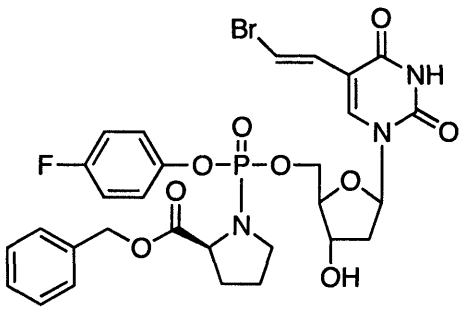
^{1}H-NMR (MeOD, 300 MHz): δ 7.82 (1H, s, H-6), 7.29-7.10 (10H, m, H-5b+***Ph***CH_2+*p*-Cl***Ph***O), 6.69 (1H, d, ^{3}J=13.6 Hz, H-5a), 6.12 (1H, ψt, H-1'), 5.06, 5.03 (2H, AB system, ^{2}J=12.3 Hz, PhC***H$_2$***), 4.29-4.15 (4H, m, H-5'+H-3'+C***H***α proline), 4.13-4.00 (1H, m, H-4'), 3.25-3.24 (2H, m, C***H$_2$*** proline), 2.29-2.19 (1H, m, one of H-2'), 2.16-1.82 (5H, m, one of H-2'+C***H$_2$*** proline).

^{13}C-NMR (MeOD, 75 MHz): δ 26.4, 26.5, 32.7, 32.9 (CH_2-proline), 41.5 (C-2'), 49.4, 49.3 (CH_2-proline), 62.1, 62.2 (Cα-proline), 68.3, 68.4 (C-5'), 68.5 (Ph***C***H_2), 72.3 (C-3'), 87.0, 87.1 (C-4'), 87.7 (C-1'), 109.7 (C-5b), 112.6 (C-5), 123.4, 123.5, 129.7, 129.8, 130.0, 130.8, 131.2, 131.5, 132.0, 132.1 (*p*-Cl***Ph***O+***Ph***CH_2+C-5a), 137.6,

('*p*', *p*-Cl***Ph***O), 140.3 (C-6), 150.9, 151.0 ('*ipso*', *p*-Cl***Ph***O), 151.4 (C-4), 163.9 (C-2), 174.8 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-fluorophenyl-(benzoxy-L-prolinyl)]-phosphate. (24p, CPF182 and 24q, CPF183)

$C_{29}H_{30}BrFN_3O_9P$, MW=694.44



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), 4-fluorophenyl-(benzoxy-L-prolinyl)-phosphorochloridate (716 mg, 1.80 mmol), NMI (246 mg, 3.0 mmol, 232 μL) in THF (10 mL) for 14 hrs. The crude product was purified twice by flash column chromatography, using chloroform/methanol (97/3) as eluent to give two products as white foamy solids (**CPF182**: 176 mg, 42%; **CPF183**: 45 mg, 11%).

CPF182

^{31}P-NMR (MeOD, 121 MHz): δ 3.39 ($^6J_{P-F}$=1.88 Hz).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.72 (1H, bs, H-3), 7.80 (1H, s, H-6), 7.47 (1H, d, ^3J=13.6 Hz, H-5b), 7.43-7.00 (9H, m, ***Ph***CH_2+*p*-F***Ph***O), 6.79 (1H, d, ^3J=13.6 Hz, H-5a), 6.45-6.33 (1H, m, H-1'), 5.19 (2H, AB system, ^2J=12.2 Hz, PhC***H_2***), 4.67-4.11 (6H, m, H-5'+H-4+H-3'+C***H***α proline+NH), 3.43-3.27 (2H, m, C***H_2*** proline), 2.53-2.45 (1H, m, one of H-2'), 2.38-1.32 (5H, m, one of H-2'+C***H_2*** proline).

^{13}C-NMR (MeOD, 75 MHz): δ 26.6, 26.7, 32.5, 32.6 (CH_2-proline), 41.7 (C-2'), 48.5, 48.6 (CH_2-proline), 62.7, 62.8 (Cα-proline), 68.2, 68.3 (C-5'), 68.6 (Ph***C***H_2), 72.4 (C-3'), 87.2, 87.3 (C-4'), 87.5 (C-

1'), 109.7 (C-5b), 112.6 (C-5), 117.8 ('*m*', *J*=23.9 Hz), 123.1, 123.2*, 123.3 ('*o*', *p*-F***Ph***O), 129.7, 129.8, 130.0, 131.0 (*p*-F***Ph***O+***Ph***CH_2+C-5a), 137.5 ('*ipso*', ***Ph***CH_2), 140.3 (C-6), 148.1, 148.2*, 148.3 ('*ipso*', *p*-F***Ph***O), 151.4 (C-4), 161.6 ('*p*', *p*-F***Ph***O, ^1J=244 Hz), 164.0 (C-2), 175.1 (***C***$OOCH_2Ph$).

CPF183

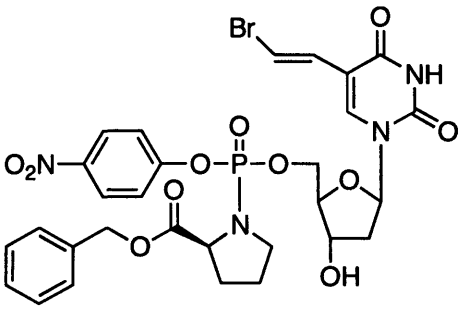
^{31}P-NMR (MeOD, 121 MHz): δ 3.59 ($^6J_{P-F}$=2.03 Hz).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.66 (1H, bs, H-3), 7.69 (2H, s, H-6), 7.48 (1H, d, ^3J=13.6 Hz, H-5b), 7.44-7.00 (9H, m, ***Ph***CH_2+*p*-F***Ph***O), 6.78 (1H, d, ^3J=13.6 Hz, H-5a), 6.44-6.23 (1H, t, ^3J=6.2 Hz, H-1'), 5.18 (2H, s, PhC***H$_2$***), 4.43-3.94 (6H, m, H-5'+H-4+H-3'+C***H***α proline+NH), 3.55-3.42 (2H, m, C***H$_2$*** proline), 2.52-2.44 (1H, m, one of H-2'), 2.24-1.84 (5H, m, one of H-2'+C***H$_2$***-proline).

^{13}C-NMR (MeOD, 75 MHz): δ 26.4, 26.5, 32.7, 32.8 (CH_2-proline), 41.5 (C-2'), 49.3, 49.4 (CH_2-proline), 62.1, 62.2 (Cα-proline), 68.2, 68.3 (C-5'), 68.4 (Ph***C***H_2), 72.3 (C-3'), 87.0, 87.1 (C-4'), 87.7 (C-1'), 109.7 (C-5b), 112.6 (C-5), 117.7 ('*m*', ^2J=24 Hz), 123.4, 123.5*, 123.6 ('*o*', p-F***Ph***O), 129.7, 129.8, 130.0, 130.8 (p-F***Ph***O+***Ph***CH_2+C-5a), 137.6 ('*ipso*', ***Ph***CH_2O), 140.3 (C-6), 148.2*, 148.3* ('*ipso*', *p*-F***Ph***O), 151.4 (C-4), 161.7 ('*p*', *p*-F***Ph***O, ^1J=243 Hz), 163.9 (C-2), 174.9 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(benzoxy-L-prolinyl)]-phosphate. (24r, CPF186 and 24s, CPF187)

$C_{30}H_{30}BrN_4O_{11}P$, MW=721.45



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-nitrophenyl-(benzoxy-L-prolinyl)-phosphorochloridate (561.0 mg, 1.32 mmol), NMI (184.7 mg, 2.25 mmol, 179.3 μL) in THF (5 mL) for 2 hrs. The crude product was purified by flash column chromatography, eluting with chloroform/methanol (97/3) to give two products as white foamy solids. (**CPF186**: 129 mg, 40%; **CPF187**: 37 mg, 11%).

CPF186

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.50 (s).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.57 (1H, bs, H-3), 8.20 (2H, d, ^{3}J=9.1 Hz, *p*-NO_2***Ph***O), 7.71 (1H, s, H-6), 7.41-7.25 (8H, m, H-5b+***Ph***CH_2+*p*-NO_2***Ph***O), 6.68 (1H, d, ^{3}J=13.6 Hz, H-5a), 6.27 (1H, t, ^{3}J=6.0 Hz, H-1'), 5.18, 5.07 (2H, AB system, ^{2}J=12.2 Hz, PhC***H$_2$***), 4.63-4.19 (3H, m, H-5'+H-3'), 4.09-4.06 (1H, m, H-4'), 3.78-3.66 (1H, m, C***H***α proline), 3.39-3.32 (1H, m, C***H$_2$*** proline), 3.25-3.17 (1H, m, C***H$_2$*** proline), 2.48-2.40 (1H, m, one of H-2'), 2.20-1.79 (5H, m, one of H-2'+C***H$_2$*** proline).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 25.6, 25.7, 31.4, 31.5 (CH_2-proline), 40.8 (C-2'), 47.2 (CH_2-proline), 61.7, 61.8 (Cα-proline), 66.3, 66.4 (C-5'), 67.8 (Ph***C***H_2O), 70.4 (C-3'), 85.4, 85.5, 85.6 (C-1'+C-4'), 110.3 (C-5b), 111.8 (C-5), 120.8, 126.3, 128.6, 129.0, 129.1 (***Ph***CH_2+*p*-NO_2***Ph***O+C-5a), 135.6 (*'ipso'*, ***Ph***CH_2), 138.2 (C-6),

145.1 (*'ipso'*, *p*-NO_2***Ph***O), 149.7 (C-4), 155.4, 155.5 (*'p'*, *p*-NO_2***Ph***O), 161.8 (C-2), 173.8 (***C***$OOCH_2Ph$).

CPF187

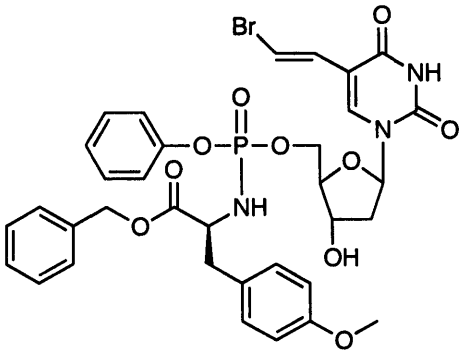
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.35 (s).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.51 (1H, bs, H-3), 8.31 (2H, d, ^{3}J=9.1 Hz, ***Ph***O), 7.72 (1H, s, H-6), 7.56-7.40 (8H, m, H-5b+***Ph***CH_2+*p*-NO_2***Ph***O), 6.79 (1H, d, ^{3}J=13.6 Hz, H-5a), 6.32 (1H, t, ^{3}J=6.2 Hz, H-1'), 5.26 (2H, s, PhC***H₂***), 4.56-4.39 (3H, m, H-5'+H-3'), 4.25-4.18 (1H, m, H-4'), 3.89-3.75 (1H, m, C***H***α proline), 3.64-3.54 (2H, m, C***H₂*** proline), 2.64-2.52 (1H, m, one of H-2'), 2.34-1.94 (5H, m, one of H-2'+C***H₂*** proline).

^{13}C-NMR (DMSO-d_6, 75 MHz): δ 25.1, 25.2, 32.1, 31.5 (CH_2-proline), 39.2 (C-2'), 47.4, 60.4, 60.5 (CH_2-proline), 66.4 (PhC***H₂***), 66.9 (C-5'), 84.9, 85.0 (C-1'+C-4'), 107.3 (C-5b), 110.5 (C-5), 121.3, 121.4, 126.0, 128.0, 128.4, 128.7 (***Ph***CH_2+*p*-NO_2***Ph***O+C-5a), 136.1 (*'ipso'*, ***Ph***CH_2), 139.8 (C-6), 144.3 (*'ipso'*, *p*-NO_2***Ph***O), 149.3 (C-4), 155.7, 155.8 (*'p'*, *p*-NO_2***Ph***O), 162.0 (C-2), 172.9 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-O-methyl-L-tyrosinyl)]-phosphate. (27) (CPF161)

$C_{34}H_{35}BrN_3O_{10}P$, MW=756.53



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), phenyl-(benzoxy-O-methyl-L-tyrosinyl)-phosphorochloridate (621 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (7 mL) for 16 hrs. The crude product was purified twice by flash column chromatography, using chloroform/methanol (97/3) to give 156 mg of white foam (46%).

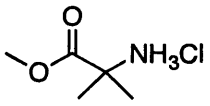
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.22, 9.17.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.81 (1H, bs, H-3), 7.66, 7.63 (1H, 2s H-6), 7.54-7.33 (11H, m, H-5b+***Ph***CH_2), 7.26-7.20 (2H, m, *p*-CH_3O***Ph***CH_2CH), 7.02-6.73 (3H, m, *p*-CH_3O***Ph***CH_2CH+H-5a), 6.34-6.27 (1H, m, H-1'), 5.24-5.13 (2H, m, PhC***H$_2$***), 4.52-4.44 (1H, m, H-3'), 4.39-4.14 (4H, m, H-5'+OH+ *p*-CH_3OPhCH_2C***H***), 4.06-3.99 (1H, m, H-4'), 3.83, 3.82 (3H, 2s, *p*-C***H$_3$***OPhO), 3.03-3.01 (2H, m, *p*-CH_3OPhC***H$_2$***CH), 2.49-2.39 (1H, m, one of H-2'), 2.08-2.00 (2H, m, one of H-2').

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 39.7, 39.8 (*p*-CH_3OPh***C***H_2CH), 40.7, 40.8 (C-2') 55.6, 55.7 (*p*-***C***H_3OPhCH_2CH), 56.3, 56.6 (*p*-CH_3OPhCH_2***C***H), 65.9, 66.3 (C-5'), 70.6, 71.2 (C-3'), 85.4*, 85.5, 85.6, 85.9 (C-4'+C-1'), 110.4, 110.5 (C-5), 111.9*, 114.4, 114.5, 120.4*, 120.5, 120.6, 125.8, 127.5, 127.8, 129.0, 129.1, 130.3, 130.9, 131.0 (***Ph***O+***Ph***CH_2+*p*-CH_3O***Ph***CH_2CH), 135.3, 135.4 ('*ipso*', ***Ph***CH_2O), 138.0 (C-5a), 149.7 (C-4), 150.6 150.7, 150.8 ('*ipso*',

PhO), 159.2 (*'p'*, *p*-CH_3O***Ph***CH_2CH), 161.9 (C-2), 172.8, 172.9 (***C***$OOCH_2Ph$).

Synthesis of dimethylglycine methyl ester hydrochloride salt. $C_5H_{12}ClNO_3$, MW=153.61 (28)

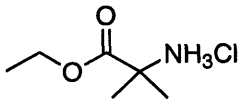


This was synthesised according to *Standard Procedure 1*, using 2-amino-isobutyric acid (5.10 g, 48.46 mmol) with thionyl chloride (11.54 g, 0.1 mol, 7.04 mL) and anhydrous methanol (19.6 mL). The product was isolated as a white solid (6.64 g, 89 %).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.81 (3H, bs, N***H***$_3$Cl), 3.83 (3H, s, C***H***$_3$O), 1.74 (6H, s, C[C***H***$_3$]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.1, 24.3 (C[***C***H_3]$_2$), 53.9 (***C***H_3O) 57.9 (***C***[CH_3]$_2$), 172.4 (***C***$OOCH_3$).

Synthesis of dimethylglycine ethyl ester hydrochloride salt $C_6H_{14}ClNO_2$, MW 167.63. (29)

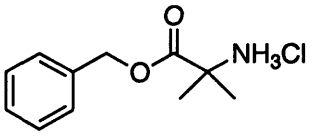


This was synthesised according to *Standard Procedure 1*, using 2-amino-isobutyric acid (5.10 g, 48.46 mmol) with thionyl chloride (11.77 g, 98.93 mmol, 7.2 mL) and anhydrous ethanol (29 mL). The product was isolated as a white solid (7.16 g, 86%).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.93 (3H, bs, N***H***$_3$Cl), 4.29 (2H, q, ^3J=7.1 Hz, CH_3C***H***$_2$O), 1.75 (6H, s, [C***H***$_3$]$_2$C), 1.33 (3H, t, ^3J=7.1 Hz, C***H***$_3CH_2O$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.4 (***C***H_3CH_2O), 24.3 (C[***C***H_3]$_2$), 57.9 (***C***[CH_3]$_2$), 63.1 (CH_3***C***H_2O), 171.6 (***C***$OOCH_2CH_3$).

Synthesis of dimethylglycine benzyl ester hydrochloride salt $C_{11}H_{16}ClNO_2$, MW=229.70 (30)



This was synthesised according to *Standard Procedure 2*, using 2-amino-isobutyric acid (1.96 g, 19.01 mmol) with *p*-toluene sulfonic acid monohydrate (3.75 g, 19.71 mmol) and benzyl alcohol (8.36 g, 77.30 mmol, 8 mL), in toluene (20 mL). The product was isolated as a white solid (2.57 g, 88 %).

p*-toluenesulfonate salt.** ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.40 (3H, bs, NH₃*** *p*-TSA), 7.79 (2H, d, ^3J=8.0 Hz, *'m' p*-TSA), 7.34 (5H, m, ***Ph***CH_2), 7.14 (2H, d, ^3J=8.0 Hz, *'o'* p-TSA), 5.16 (2H, s, PhC***H₂***), 2.38 (3H, s, C***H₃*** *p*-TSA), 1.57 (6H, s, C[C***H₃***]$_2$).

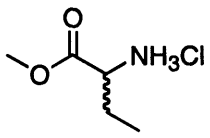
^{13}C-NMR ($CDCl_3$; 75 MHz): δ 21.8 (***C***H_3, *p*-TSA), 23.9 (C[***C***$H_3]_2$), 57.8 (***C***$[CH_3]_2$), 68.3 (Ph***C***H_2), 126.55, 128.5, 128.8, 129.0, 129.3 (***Ph***CH_2+*p*-TSA), 135.4 (*'ipso'*, ***Ph***CH_2), 140.8 (*'p'*, *p*-TSA), 141.9 (*'ipso'*, *p*-TSA), 171.9 (***C***$OOCH_2$Ph).

Hydrochloride salt. ^{1}H-NMR ($CDCl_3$; 300 MHz): δ 9.10 (3H, bs, N***H₃***Cl), 7.41-7.31 (5H, m, ***Ph***CH_2), 5.27 (2H, s, PhC***H₂***), 1.77 (C[C***H₃***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 24.2 (C[***C***$H_3]_2$), 58.0 (***C***$[CH_3]_2$), 68.5 (Ph***C***H_2), 128.6, 129.0, 129.1 (*'o'*, *'m'*, *'p'*, ***Ph***CH_2), 135.2 (*'ipso'*, ***Ph***CH_2), 171.8 (***C***$OOCH_2$Ph).

Synthesis of 2-aminobutanoic acid methyl ester hydrochloride salt. (31a)

$C_5H_{12}ClNO_2$, MW=153.61



This was synthesised according to *standard procedure 1*, using DL-2-aminobutyric acid (20.00 g, 193.95 mmol), thionyl chloride (46.15 g, 387.90 mmol, 28.17 mL) and dry methanol (150 mL).

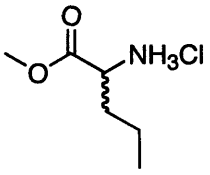
The product was isolated as a white solid (28.62 g, 96%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.80 (bs, 3H, N***H*****$_3$**Cl), 3.93-3.89 (1H, m, C***H***CH_2CH_3), 3.73 (3H, s, C***H*****$_3$**O), 1.87-1.81 (2H, m, CHC***H*****$_2$**CH_3), 0.91 (3H, t, ^3J=7.5 Hz, $CHCH_2$C***H*****$_3$**).

^{13}C-NMR (DMSO-d_6; 75 MHz): 9.5 ($CHCH_2$**C**H_3), 23.6 (CH**C**H_2CH_3), 53.0, 53.3 (**C**HCH_2CH_3+**C**H_3O), 170.1 (**C**$OOCH_3$).

Synthesis of DL-norvaline methyl ester hydrochloride salt.

$C_6H_{14}ClNO_2$, MW=167.63 (31b)



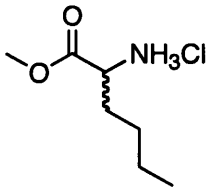
This was synthesised according to *standard procedure 1*, using DL-norvaline (15.00 g, 128.04 mmol), thionyl chloride (30.47 g, 256.08 mmol, 18.60 mL) and dry methanol (80 mL)

The product was isolated as a white solid (21.25 g, 99%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.79 (bs, 3H, N***H*****$_3$**Cl), 3.99-3.95 (1H, m, C***H***$CH_2CH_2CH_3$), 3.76 (1H, s, CH_3O), 1.85-1.78 (2H, m, CHC***H*****$_2$**CH_2CH_3), 1.51-1.26 (2H, m, $CHCH_2$C***H*****$_2$**CH_3), 0.90 (3H, t, ^3J=7.3 Hz, $CHCH_2CH_2$C***H*****$_3$**).

^{13}C-NMR (DMSO-d_6; 75 MHz): 13.8 ($CHCH_2CH_2$**C**H_3), 18.0 ($CHCH_2$**C**H_2CH_3), 32.3 (CH**C**$H_2CH_2CH_3$), 52.0, 53.0 (**C**$HCH_2CH_2CH_3$, **C**H_3O), 170.3 (**C**$OOCH_3$).

Synthesis of DL-norleucine methyl ester hydrochloride salt.
$C_7H_{16}ClNO_2$, MW=181.66 (31c)



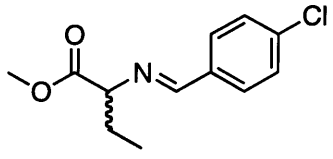
This was synthesised according to *standard procedure 2*, using DL-norleucine (15.00 g, 114.35 mmol), thionyl chloride (27.21 g, 228.70 mmol, 16.6 mL) and dry methanol (80 mL)

The product was isolated as a white solid (20.68 g, 100%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.77 (3H, bs, N***H*****$_3$**Cl), 3.97-3.93 (1H, m, C***H***$CH_2CH_2CH_2CH_3$), 3.74 (1H, s, C***H*****$_3$**O), 1.85-1.78 (2H, m, CHC***H*****$_2$**$CH_2CH_2CH_3$), 1.41-1.22 (4H, m, $CHCH_2C$***H*****$_2$**C***H*****$_2$**CH_3), 0.86 (3H, t, ^3J=7.1 Hz, $CHCH_2CH_2CH_2C$***H*****$_3$**).

^{13}C-NMR (DMSO-d_6; 75 MHz): 13.9 ($CHCH_2CH_2CH_2$***C***H_3), 22.0 ($CHCH_2CH_2$***C***H_2CH_3), 26.6 ($CHCH_2$***C***$H_2CH_2CH_3$), 30.0 (CH***C***$H_2CH_2CH_2CH_3$), 52.1, 53.0 (***C***$HCH_2CH_2CH_2CH_3$, ***C***H_3O), 170.3 (***C***$OOCH_3$).

Synthesis of methyl 2-(4-chlorobenzylideneamino)butanoate.
$C_{12}H_{14}ClNO_2$, MW=239.70 (32a)

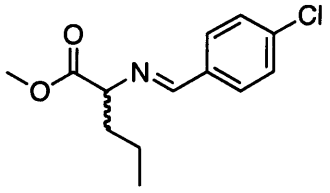


$MgSO_4$ (8.00 g) was added to a suspension of 2-aminobutanoic acid methylester (3.00 g, 19.50 mmol), TEA (1.37 mL, 9.80 mmol), and *p*-chlorobenzaldehyde (1.38 g, 9.80 mmol) in DCM (75 mL) and the mixture stirred at room temperature for 2 hrs. The reaction mixture was left to stand for 2 hrs, then filtered and the solvent removed under reduced pressure. The residue was extracted (3X with Et_2O), dried ($MgSO_4$), filtered again and the solvent removed to leave the crude product as an oil (4.402 g, 94%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.39 (1H, s, C***H***=Ph), 7.80 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 7.52 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 4.01-3.97 (1H, m, C***H***CH_2CH_3), 1.97-1.88 (1H, m, one of CHC***H$_2$***CH_3), 1.81-1.71 (1H, m, one of CHC***H$_2$***CH_3), 0.84 (3H, t, ^3J=7.4 Hz, $CHCH_2$C***H$_3$***).

^{13}C-NMR (DMSO-d_6; 75 MHz): 10.4 ($CHCH_2$***C***H_3), 26.4 (CH***C***H_2CH_3), 52.1 (***C***H_3O), 73.5 (***C***HCH_2CH_3), 129.2, 130.2 (p-Cl***Ph***), 134.8, 136.1 ('*ipso*' p-Cl***Ph***+p-Cl***Ph***), 162.7 (=***C***HPh), 172.1 (***C***$OOCH_3$).

Synthesis of methyl 2-(4-chlorobenzylideneamino)pentanoate.
$C_{13}H_{16}ClNO_2$, MW=253.72 (32b)



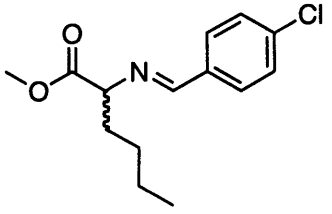
$MgSO_4$ (16.00 g) was added to a suspension of DL-norvaline methyl ester hydrochloric salt (10.00 g, 59.67 mmol), TEA (4.16 mL, 29.84 mmol), and *p*-chlorobenzaldehyde (4.195 g, 29.84 mmol) in DCM (200 mL) and the mixture stirred at room temperature for 2 hrs. The reaction mixture was left to stand for 2 hrs, then filtered and the solvent removed under reduced pressure. The residue was extracted (3X with Et_2O), dried ($MgSO_4$), filtered again and the solvent removed to leave the crude product as an oil (7.04 g, 93%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.41 (1H, s, C***H***=Ph), 7.80 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 7.54 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 4.11-4.06 (1H, m, C***H***$CH_2CH_2CH_3$), 3.66 (3H, s, C***H$_3$***O), 1.90-1.68 (2H, m, CHC***H$_2$***CH_2CH_3), 1.30-1.18 (2H, m, $CHCH_2$C***H$_2$***CH_3), 0.88 (3H, t, ^3J=7.3 Hz, $CHCH_2CH_2$C***H$_3$***).

^{13}C-NMR (DMSO-d_6; 75 MHz): 13.8 ($CHCH_2CH_2$***C***H_3), 18.9 ($CHCH_2$***C***H_2CH_3), 35.2 (CH***C***$H_2CH_2CH_3$), 52.1 (***C***H_3O), 73.5

(***C***$HCH_2CH_2CH_3$), 129.1, 130.1 (p-Cl***Ph***), 134.8, 136.1 ('*ipso*' p-Cl***Ph***+p-Cl***Ph***), 162.6 (=***C***HPh), 172.2 (***C***$OOCH_3$).

Synthesis of methyl-2-(4-chlorobenzylideneamino)hexanoate.
$C_{14}H_{18}ClNO_2$, MW=267.75 (32c)



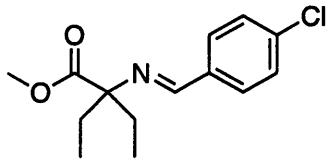
$MgSO_4$ (16.00 g) was added to a suspension of DL-norleucine methyl ester hydrochloric salt (7.00 g, 38.53 mmol), TEA (2.69 mL, 19.27 mmol), and *p*-chlorobenzaldehyde (2.709 g, 19.27 mmol) in DCM (150 mL) and the mixture stirred at room temperature for 2 hrs. The reaction mixture was left to stand for 2 hrs, then filtered and the solvent removed under reduced pressure. The residue was extracted (3x with Et_2O), dried ($MgSO_4$), filtered again and the solvent removed to leave the crude product as a white solid. ^{1}H-NMR showed the presence of triethylammonium salt. The solid was suspended in petroleum ether (50 mL), stirred for 10 mins and filtered on celite to give a yellow oil (4.19 g, 81%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.42 (1H, s, C***H***=Ph), 7.81 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 7.55 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 4.09-4.05 (1H, m, C***H***$CH_2CH_2CH_2CH_3$), 3.66 (3H, s, C***H$_3$***O), 1.90-1.71 (2H, m, CHC***H$_2$***$CH_2CH_2CH_3$), 1.37-1.18 (4H, m, $CHCH_2C$***H$_2$***C***H$_2$***CH_3), 0.85 (3H, t, ^3J=7.2 Hz, $CHCH_2CH_2CH_2C$***H$_3$***).

^{13}C-NMR (DMSO-d_6; 75 MHz): 14.1 ($CHCH_2CH_2CH_2$***C***H_3), 22.2, 27.8 ($CHCH_2$***C***H_2***C***H_2CH_3), 32.8 (CH***C***$H_2CH_2CH_2CH_3$), 52.1 (***C***H_3O), 73.5 (***C***$HCH_2CH_2CH_2CH_3$), 129.2, 130.1 (p-Cl***Ph***), 134.8, 136.1 ('*ipso*' p-Cl***Ph***+p-Cl***Ph***), 162.6 (=***C***HPh), 172.2 (***C***$OOCH_3$).

Synthesis of methyl 2-(4-chlorobenzylideneamino)-2-ethylbutanoate. (33a)

$C_{14}H_{18}ClNO_2$, MW=267.75



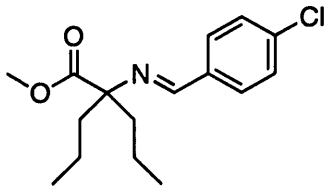
To a solution of methyl 2-(4-chlorobenzylideneamino)butanoate (1.667 g, 6.96 mmol) in THF (20 mL), 1.8 M solution of LDA (22.99 mL, 41.39 mmol) was added dropwise and the mixture stirred for 30 mins at –50 °C. The mixture was then cooled to –78 °C and iodoethane (5.1159 g, 30.35 mmol, 2.9 mL) was added dropwise and the mixture was then allowed to slowly warm to room temperature o.n. The solvent was removed, ether was added and the mixture was washed with 10 % $NaHCO_3$ solution and water. The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the crude product as a dark oil. The crude product was purified by flash column chromatography, using ethyl acetate/petroleum ether (2/98) containing 0.2% of TEA, to give a yellow oil (7.926 g, 97%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.34 (1H, s, C***H***=Ph), 7.84 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 7.53 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 3.70 (3H, s, C***H***$_3$O), 2.04-1.88 (4H, m, C[C***H***$_2$CH$_3$]$_2$), 0.82 (6H, t, ^3J=7.4 Hz, C[CH$_2$C***H***$_3$]).

^{13}C-NMR (DMSO-d_6; 75 MHz): 10.4 (C[CH$_2$***C***H$_3$]$_2$), 26.4 (C[***C***H$_2$CH$_3$]$_2$), 52.1 (***C***H$_3$O), 73.5 (***C***[CH$_2$CH$_3$]$_2$), 129.2, 130.2 (p-Cl***Ph***), 134.8, 136.1 ('*ipso*' p-Cl***Ph***+p-Cl***Ph***), 162.7 (=***C***HPh), 172.1 (***C***OOCH$_3$).

Synthesis of methyl 2-(4-chlorobenzylideneamino)-2-propylpentanoate. (33b)

$C_{16}H_{22}ClNO_2$, MW=295.80



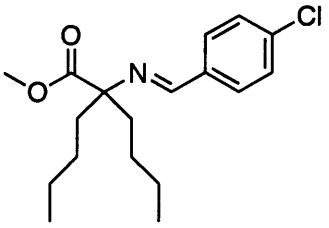
To a solution of methyl 2-(4-chlorobenzylideneamino)pentanoate (7.00 g, 27.59 mmol) in THF (50 mL), 1.8 M solution of LDA (5.8 mL, 10.44 mmol) was added dropwise and the mixture stirred for 30 mins at −50 °C. The mixture was then cooled to −78 °C and iodopropano (1.195 g, 7.66 mmol, 613 µL) was added dropwise and the mixture was then allowed to slowly warm to room temperature o.n. The solvent was removed, ether was added and the mixture was washed with 10 % $NaHCO_3$ solution and water. The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the crude product as a dark oil. The crude product was purified by flash column chromatography, using ethyl acetate/petroleum ether/TEA (2/98) containing 0.2% of TEA to give a yellow oil (5.63 g, 69%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.35 (1H, s, C***H***=Ph), 7.81 (2H, d, ^3J=8.4 Hz, p-Cl***Ph***), 7.50 (2H, d, ^3J=8.4 Hz, p-Cl***Ph***), 3.68 (3H, s, C***H***$_3$O), 1.99-1.72 (4H, m, C[C***H***$_2$CH$_2$CH$_3$]$_2$), 1.27-1.15 (4H, m, C[CH$_2$C***H***$_2$CH$_3$]$_2$), 0.86 (6H, t, ^3J=7.2 Hz, C[CH$_2$CH$_2$C***H***$_3$]$_2$).

^{13}C-NMR (DMSO-d_6; 75 MHz): 14.6 (C[CH$_2$CH$_2$***C***H$_3$]$_2$), 17.2 (C[CH$_2$***C***H$_2$CH$_3$]$_2$), 39.5 (C[***C***H$_2$CH$_2$CH$_3$]$_2$), 52.1 (***C***H$_3$O), 71.3 (***C***[CH$_2$CH$_2$CH$_3$]$_2$), 129.0, 129.8 (p-Cl***Ph***), 135.5, 135.8 (*'ipso'* p-Cl***Ph***+p-Cl***Ph***), 158.6 (=***C***HPh), 173.8 (***C***OOCH$_3$).

Synthesis of methyl 2-(4-chlorobenzylideneamino)-2-butylhexanoate. (33c)

$C_{18}H_{26}ClNO_2$, MW=323.86



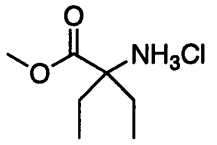
To a solution of methyl 2-(4-chlorobenzylideneamino)hexanoate (3.80 g, 14.19 mmol) in THF (50 mL), 1.8 M solution of LDA (1.78 mL, 21.29 mmol) was added dropwise and the mixture stirred for 30 mins at –50 °C. The mixture was then cooled to –78 °C and iodobutane (2.873 g, 15.61 mmol, 1.78 mL) was added dropwise and the mixture was then allowed to slowly warm to room temperature o.n. The solvent was removed, ether was added and the mixture was washed with 10 % $NaHCO_3$ solution and water. The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give the crude product as a dark oil. The crude product was purified by flash column chromatography, using ethyl acetate/petroleum ether (2/98) containing 0.2% of TEA, to give a yellow oil (3.58 g, 78%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.35 (1H, s, C***H***=Ph), 7.81 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 7.49 (2H, d, ^3J=8.5 Hz, p-Cl***Ph***), 3.67 (3H, s, C***H***$_3$O), 1.92-1.75 (4H, m, C[C***H***$_2$CH$_2$CH$_2$CH$_3$]$_2$), 1.29-1.15 (8H, m, C[CH$_2$C***H***$_2$C***H***$_2$CH$_3$]$_2$), 0.83 (6H, t, ^3J=7.0 Hz, C[CH$_2$CH$_2$CH$_2$C***H***$_3$]$_2$).

^{13}C-NMR (DMSO-d$_6$; 75 MHz): 14.1 (C[CH$_2$CH$_2$CH$_2$***C***H$_3$]$_2$), 22.9, 26.0 (C[CH$_2$***C***H$_2$***C***H$_2$CH$_3$]$_2$), 36.9 (C[***C***H$_2$CH$_2$CH$_2$CH$_3$]$_2$), 52.0 (***C***H$_3$O), 71.3 (***C***[CH$_2$CH$_2$CH$_2$CH$_3$]$_2$), 129.0, 129.6 (p-Cl***Ph***), 135.5, 135.8 (*'ipso'* p-Cl***Ph***+p-Cl***Ph***), 158.6 (=***C***HPh), 173.8 (***C***OOCH$_3$).

Synthesis of diethylglycine methyl ester hydrochloride salt. (34a)

$C_7H_{16}ClNO_2$, MW=181.66



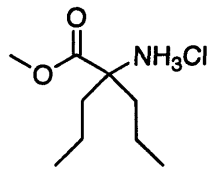
Methyl 2-(4-chlorobenzylideneamino)-2-ethylbutanoate (12.20 g, 45.56 mmol) was dissolved in ether (100 mL) and washed with 2M aq. HCl (4x 50 mL). The solvent was evaporated to give an oil, the residue dissolved in DCM and extracted with 10% K_2CO_3. The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give an oil. The oil was purified by flash column chromatography using an eluent of dichloromethane/methanol (9/1+0.1% TEA). The appropriate fractions were combined and the solvent was removed *in vacuo* to give a colourless oil which was dissolved in acetone and neutralized with 1 M HCl. The solvent was removed, ether was added and the solid was filtered and washed with ether to give a white solid (3.14 g, 38%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.79 (3H, bs, N***H*****$_3$**Cl), 3.75 (3H, s, C***H*****$_3$**O), 1.93-1.79 (4H, m, C[C***H*****$_2$**$CH_3]_2$), 0.88 (6H, t, ^3J=7.4 Hz, C[CH_2C***H*****$_3$**]).

^{13}C-NMR (DMSO-d_6; 75 MHz): 8.2 (C[CH_2**C**$H_3]_2$), 28.8 (C[**C**$H_2CH_3]_2$), 53.3 (**C**H_3O), 64.1 (**C**[$CH_2CH_3]_2$), 171.3 (**C**$OOCH_3$).

Synthesis of di-n-propylglycine methyl ester hydrochloride salt. (34b)

$C_9H_{20}ClNO_2$, MW=209.71



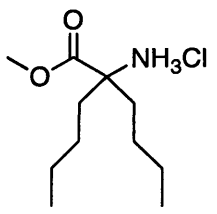
Methyl 2-(4-chlorobenzylideneamino)-2-propylpentanoate (7.93 g, 26.80 mmol) was dissolved in ether (100 mL) and washed with 2M aq. HCl (4x 50 mL). The aqueous layer was evaporated to give a solid which was washed with hexane and filtered to give an off-white solid (3.87 g, 69%).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.75 (3H, bs, N***H₃***Cl), 3.75 (3H, s, C***H₃***O), 1.87-1.71 (4H, m, C[C***H₂***$CH_2CH_3]_2$), 1.54-1.42 (2H, m, $C[CH_2C$***H₂***$CH_3]_2$), 1.19-1.06 (2H, m, $C[CH_2C$***H₂***$CH_3]_2$), 0.85 (6H, t, ^3J=7.1 Hz, $C[CH_2CH_2C$***H₃***$]_2$).

^{13}C-NMR (DMSO-d_6; 75 MHz): 14.2 ($C[CH_2CH_2$***C***$H_3]_2$), 16.6 ($C[CH_2$***C***$H_2CH_3]_2$), 38.2 (C[***C***$H_2CH_2CH_3]_2$), 53.3 (***C***H_3O), 63.0 (***C***$[CH_2CH_2CH_3]_2$), 171.5 (***C***$OOCH_3$).

Synthesis of di-n-butylglycine methyl ester hydrochloride salt. (34c)

$C_{11}H_{24}ClNO_2$, MW=237.77



Methyl 2-(4-chlorobenzylideneamino)-2-butylhexanoate (5.62 g, 17.34 mmol) was dissolved in ether (100 mL) and washed with 2M aq. HCl (4x 50 mL). The aqueous layer was evaporated to give a solid which was washed with hexane and filtered to give an white solid (2.14 g, 52%).

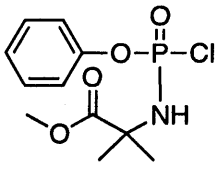
^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.76 (3H, bs, N***H₃***Cl), 3.75 (3H, s, C***H₃***O), 1.90-1.74 (4H, m, C[C***H₂***$CH_2CH_2CH_3]_2$), 1.50-1.39 (2H, m, C[C***H₂***$CH_2CH_2CH_3]_2$), 1.30-1.19 (4H, m, $C[CH_2C$***H₂***$CH_2CH_3]_2$), 1.14-

1.03 (4H, m, C[$CH_2CH_2C\underline{\boldsymbol{H_2}}CH_3$]$_2$), 0.86 (6H, t, ^3J=7.2 Hz, C[$CH_2CH_2CH_2C\underline{\boldsymbol{H_3}}$]$_2$),).

^{13}C-NMR (DMSO-d_6; 75 MHz): 14.0 (C[$CH_2CH_2CH_2\underline{\boldsymbol{C}}H_3$]$_2$), 22.8, 22.9 (C[$CH_2CH_2\underline{\boldsymbol{C}}H_2CH_3$]$_2$), 26.6., 26.8 (C[$CH_2\underline{\boldsymbol{C}}H_2CH_2CH_3$]$_2$), 38.8, 38.9 (C[$\underline{\boldsymbol{C}}H_2CH_2CH_2CH_3$]$_2$), 53.5 ($\underline{\boldsymbol{C}}H_3O$), 67.3, 67.4 ($\underline{\boldsymbol{C}}$[$CH_2CH_2CH_2CH_3$]$_2$), 175.1 ($\underline{\boldsymbol{C}}OOCH_3$).

Synthesis of phenyl-(methoxy-dimethylglycinyl)-phosphorochloridate.[5] (35a)

$C_{11}H_{15}ClNO_4P$, MW=291.67



This was synthesised according to *Standard procedure 4,* using dimethylglycine methyl ester hydrochloride (584 mg, 3.80 mmol), phenyl phosphorodichloridate (791 mg, 3.75 mmol, 560 μL), and TEA (760 mg, 7.50 mmol, 1045 μL) in DCM (20 mL), to yield 1.04 g (94%) of crude product used without further purification.

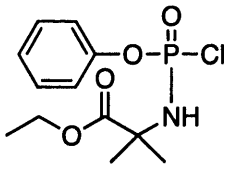
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.99 (s).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.41-7.17 (5H, m, $\underline{\boldsymbol{Ph}}O$), 4.98 (1H, bs, N$\underline{\boldsymbol{H}}$), 3.80 (3H, s, C$\underline{\boldsymbol{H_3}}O$), 1.71-1.69 (6H, 2s, C[C$\underline{\boldsymbol{H_3}}$]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.2, 27.3 (C[$\underline{\boldsymbol{C}}H_3$]$_2$), 53.6 ($\underline{\boldsymbol{C}}H_3O$), 58.8 ($\underline{\boldsymbol{C}}$[$CH_3$]$_2$), 121.0, 121.1 (*'o'*, $\underline{\boldsymbol{Ph}}O$), 126.2, 126.3 (*'p'*, $\underline{\boldsymbol{Ph}}O$), 130.3 (*'m'*, $\underline{\boldsymbol{Ph}}O$), 150.2, 150.3 (*'ipso'*, $\underline{\boldsymbol{Ph}}O$), 175.6, 175.7 ($\underline{\boldsymbol{C}}OOCH_3$).

Synthesis of phenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate. (35b)

$C_{12}H_{17}ClNO_4P$, MW=305.69



This was synthesised according to *Standard procedure 4,* using dimethylglycine ethyl ester hydrochloride (629 mg, 3.75 mmol), phenyl phosphodichloridate (791 mg, 3.75 mmol, 560 μL), and TEA (759 mg, 7.50 mmol, 1045 μL) in DCM (20 mL), to yield 1.02 g (89%) of crude product used without further purification.

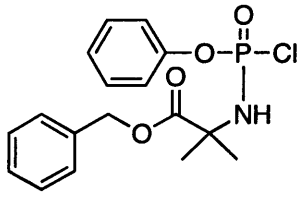
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.02 (s).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.23-7.37 (5H, m, ***Ph***O), 4.98 (1H, bs, N***H***), 4.24 (2H, q, ^{3}J=7.1 Hz, $CH_3C\mathbf{H_2}O$), 1.70, 1.68 (6H, 2s, $C[C\mathbf{H_3}]_2$), 1.30 (3H, t, ^{3}J=7.1 Hz, $C\mathbf{H_3}CH_2O$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 ($\mathbf{C}H_3CH_2O$), 26.9, 27.0, 27.2, 27.5 ($C[\underline{C}H_3]_2$), 58.7 ($\underline{C}[CH_3]_2$), 62.7 ($CH_3\mathbf{C}H_2O$), 121.1, 121.0 (*'o'*, ***Ph***O), 126.2, 126.3 (*'p'*, ***Ph***O), 130.2, 130.3 (*'m'*, ***Ph***O), 150.2, 150.3 (*'ipso'*, ***Ph***O), 175.1, 175.2 ($\mathbf{C}OOCH_2CH_3$).

Synthesis of phenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35c)

$C_{17}H_{19}ClNO_4P$, MW= 367.76



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (861 mg, 3.75 mmol), phenyl phosphorodichloridate (791 mg, 3.75 mmol, 560 μL), and TEA (759 mg, 7.50 mmol, 1045 μL) in DCM (30 mL). The crude was purified by flash chromatography (ethyl

acetate/petroleum ether 6:4) affording 580 mg (42%) of colourless oil.

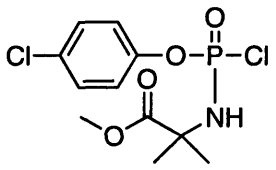
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.79 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.45-7.27 (10H, m, ***Ph***O+***Ph***CH_2), 5.28 (2H, s, Ph***CH_2***), 4.81, 4.78 (1H, 2bs, N***H***), 1.78, 1.75 (6H, 2s, C[C***H_3***]).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 26.9, 27.0, 27.2, 27.3 (C[***C***H_3]), 58.9 (***C***$[CH_3]_2$), 68.4 (Ph***C***H_2), 121.0, 121.1, 126.3, 126.4, 128.6, 129.0, 129.1, 130.3, (***Ph***O+***Ph***CH_2), 135.5 (*'ipso'*, ***Ph***CH_2), 150.3, 150.2 (*'ipso'*, ***Ph***O), 175.0, 175.2 (***C***$OOCH_2$Ph).

Synthesis of 4-chlorophenyl-(methoxy-dimethylglycinyl)-phosphorochloridate. (35d)

$C_{11}H_{14}Cl_2NO_4P$, MW=326.11



This was synthesised according to *Standard procedure 4,* dimethylglycine methyl ester hydrochloride (280 mg, 1.82 mmol), 4-chlorophenylphosphorodichloridate (447 mg, 1.82 mmol), and TEA (368 mg, 3.64 mmol, 507 μL) in DCM (20 mL) to yield 554 mg (91%) of crude product used without further purification.

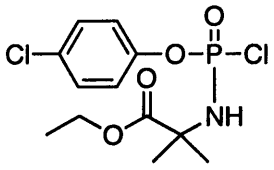
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.05 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.38 (2H, d, ^{3}J=9.0 Hz, *p*-Cl***Ph***O), 7.28, 7.24 (2H, 2d, ^{3}J=9.0 Hz, *p*-Cl***Ph***O), 4.87-4.83 (1H, 2bs, N***H***), 3.84 (3H, s, C***H_3***O), 1.73, 1.71 (6H, 2s, C[C***H_3***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0*, 27.3* (C[***C***$H_3]_2$), 53.7, 53.8 (***C***H_3O), 58.9, 59.0 (***C***$[CH_3]_2$), 122.5, 122.6 (*'o'*, *p*-Cl***Ph***O), 130.3, 130.4 (*'m'*, *p*-Cl***Ph***O), 131.8 (*'p'*, *p*-Cl***Ph***O) 148.7, 148.9 (*'ipso'*, *p*-Cl***Ph***O), 175.5, 175.7 (***C***$OOCH_3$).

Synthesis of 4-chlorophenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate. (35e)

$C_{12}H_{16}Cl_2NO_4P$, MW=340.14.



This was synthesised according to *Standard procedure 4,* using dimethylglycine ethyl ester hydrochloride (293 mg, 1.75 mmol), 4-chlorophenyl-phosphorodichloridate (430 mg, 1.75 mmol), and TEA (354 mg, 3.50 mmol, 488 μL) in DCM (15 mL), to yield 572 mg (yield 96%) of crude product used without further purification.

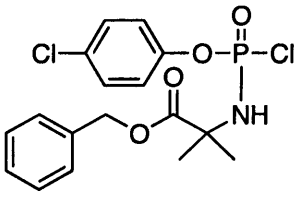
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.09 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.38 (2H, d, ^{3}J=9.1 Hz, *p*-Cl***Ph***O), 7.26 (2H, d, ^{3}J=9.1 Hz, *p*-Cl***Ph***O), 4.88-4.84 (1H, 2bs, N***H***), 4.29 (2H, q, ^{3}J=7.1 Hz, $CH_3C\boldsymbol{H_2}O$), 1.74-1.70 (6H, 2s, C[$C\boldsymbol{H_3}$]), 1.35 (3H, t, ^{3}J=7.1 Hz, $C\boldsymbol{H_3}CH_2O$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.4, 14.5 ($\boldsymbol{C}H_3CH_2O$), 27.0*, 27.3* (C[$\boldsymbol{C}H_3$]$_2$), 58.9* (C[CH_3]$_2$), 62.8 ($CH_3\boldsymbol{C}H_2O$), 122.5, 122.6 (*'o', p*-Cl***Ph***O), 130.3, 130.4 (*'m',* O*Ph*), 131.8* (*'p', p*-Cl***Ph***O), 148.7, 148.8 (*'ipso', p*-Cl***Ph***O), 175.1, 175.3 ($\boldsymbol{C}OOCH_2CH_3$).

Synthesis of 4-chlorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35f)

$C_{17}H_{18}Cl_2NO_4P$, MW=402.21



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (402 mg, 1.75 mmol), 4-chlorophenyl-phosphorodichloridate (430 mg, 1.75 mmol), and TEA (354 mg, 3.50 mmol, 488 μL) in DCM (15 mL), to yield 658 mg (yield 94%) of crude product used

without further purification.

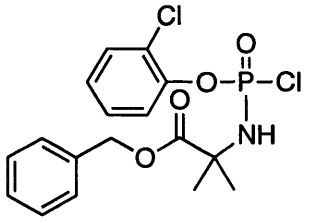
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.00 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.39-7.12 (9H, m, ***Ph***CH_2+*p*-Cl***Ph***O), 5.18 (2H, s, PhC***H₂***), 4.75-4.72 (1H, 2bs, N***H***), 1.68-1.65 (6H, 2s, C[C***H₃***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0*, 27.3*, (C[***C***H_3]$_2$), 59.0* (***C***[CH_3]$_2$), 68.4 (Ph***C***H_2), 122.4, 122.5*, 128.6*, 129.1, 130.7 (***Ph***CH_2+*p*-Cl***Ph***O), 131.8 (*'ipso'*, ***Ph***CH_2), 135.4 (*'p'*, *p*-Cl***Ph***O), 148.6, 148.7 (*'ipso'*, *p*-Cl***Ph***O), 174.9, 175.1 (***C***OOCH_2Ph).

Synthesis of 2-chlorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35g)

$C_{17}H_{18}Cl_2NO_4P$, MW=402.21



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (374 mg, 1.63 mmol), 2-chlorophenyl-phosphodichloride (400 mg, 1.63 mmol), and TEA (330 mg, 3.26 mmol, 454 μL) in DCM (15 mL), to yield 631 mg (96%) of crude product used without further purification.

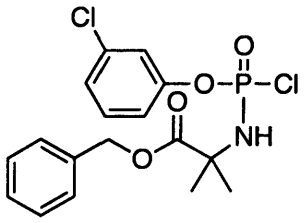
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.13 (s).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.63-7.22 (9H, m, *o*-Cl***Ph***O+***Ph***CH_2), 5.27 (2H, s, PhC***H₂***), 5.20-5.19 (1H, 2bs, N***H***), 1.80-1.74 (6H, 2s, C[C***H₃***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 26.9, 27.0, 27.2, 27.3 (C[***C***H_3]$_2$), 59.0, 59.1 (***C***[CH_3]$_2$), 68.4 (Ph***C***H_2), 122.8, 122.9 (*'o'*, *o*-Cl***Ph***O), 125.9, 126.0, 127.1, 127.2, 128.3, 128.5*, 128.6, 128.9, 129.0, 129.1*, 131.2 (*o*-Cl***Ph***O+***Ph***CH_2), 135.3, 135.5 (*'ipso'*, ***Ph***CH_2), 146.3, 146.4 (*'ipso'*, *o*-Cl***Ph***O), 174.9, 175.1 (***C***OOCH_2Ph).

Synthesis of 3-chlorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35h)

$C_{17}H_{18}Cl_2NO_4P$, MW=402.21



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (403 mg, 1.76 mmol), 3-chlorophenyl-phosphorodichloridate (431 mg, 1.76 mmol), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL) to yield 684 mg (97%) of crude product used without further purification.

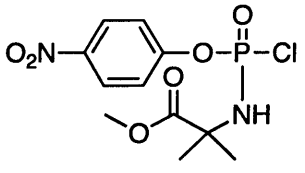
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.91.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.43-7.21 (9H, m, *m*-Cl***Ph***O+***Ph***CH_2), 5.27 (2H, s, PhC***H$_2$***), 4.97-4.93 (1H, 2bs, N***H***), 1.76-1.74 (6H, 2s, C[C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 26.9, 27.0, 27.3* (C[***C***H_3]$_2$), 59.0 (***C***[CH_3]$_2$), 68.4 (Ph***C***H_2), 119.3, 119.4, 121.6, 121.7 (*'o', m-*Cl***Ph***O), 126.7, 128.4, 128.5, 128.6, 129.0, 129.1* (*m-*Cl***Ph***O+***Ph***CH_2), 131.0 (*'m', m-*Cl***Ph***O), 135.2, 135.4, 135.5 (*m-*Cl***Ph***O+PhCH_2), 150.5, 150.6 (*'ipso',* O*Ph*), 174.9, 175.1 (***C***OOCH_2Ph).

Synthesis of 4-nitrophenyl-(methoxy-dimethylglycinyl)-phosphorochloridate. (35i)

$C_{11}H_{14}ClN_2O_6P$, MW=336.67



This was synthesised according to *Standard procedure 4,* using dimethylglycine methyl ester hydrochloride (290 mg, 1.89 mmol), 4-nitrophenyl-phosphorodichloridate (483 mg, 1.89 mmol), and TEA (383 mg, 3.79 mmol,

527 μL) in DCM (15 mL), to yield 486 mg (yield 76%) of crude product used without further purification.

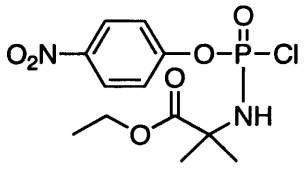
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.61 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.25 (2H, d , ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 7.43 (2H, d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 4.91-4.87 (1H, 2bs, N***H***), 3.79 (3H, s, C***H$_3$***O), 1.69-1.66 (6H, 2s, C[C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.1, 27.3* (C[***C***H_3]$_2$), 53.8 (O***C***H_3), 59.1, 59.2 (***C***[CH_3]$_2$), 121.7, 121.8 (*'o'*, *p*-NO_2***Ph***O), 126.2 (*'m'*, *p*-NO_2***Ph***O), 145.7 (*'p'*, *p*-NO_2***Ph***O), 154.7, 154.8 (*'ipso'*, *p*-NO_2***Ph***O), 175.4, 175.5 (***C***$OOCH_3$).

Synthesis of 4-nitrophenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate. (35j)

$C_{12}H_{16}ClN_2O_6P$, MW=350.69



This was synthesised according to *Standard procedure 4,* using dimethylglycine ethyl ester hydrochloride (270 mg, 1.61 mmol), 4-nitrophenylphosphorodichloridate (412 mg, 1.61 mmol), and TEA (326 mg, 3.22 mmol, 449 μL) in DCM (15 mL), to yield 500 mg (yield 89%) of crude product used without further purification.

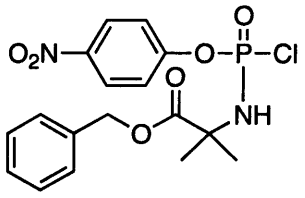
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.64 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.35 (2H, d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 7.53 (2H, d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 4.99-4.96 (1H, 2bs, N***H***), 4.34 (2H, q, ^{3}J=7.1 Hz, CH_3C***H$_2$***O), 1.79-1.76 (6H, 2s, C[C***H$_3$***]$_2$), 1.40 (3H, t, ^{3}J=7.1 Hz, C***H$_3$***CH_2O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (***C***H_3CH_2O), 27.0*, 27.3* (C[***C***H_3]$_2$), 59.1, 59.2 (***C***[CH_3]$_2$), 62.9, 63.0 (CH_3***C***H_2O), 121.7, 121.8 (*'o'*, ***Ph***O), 126.2 (*'m'*, ***Ph***O), 145.7 (*'p'*, *p*-NO_2***Ph***O), 154.7, 154.8 (*'ipso'*, *p*-NO_2***Ph***O), 175.0, 175.1 (***C***$OOCH_2CH_3$).

Synthesis of 4-nitrophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35k)

$C_{17}H_{18}ClN_2O_6P$, MW=412.76



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (578 mg, 2.52 mmol), 4-nitrophenyl-phosphorodichloride (645 mg, 2.52 mmol), and TEA (510 mg, 5.04 mmol, 703 μL) in DCM (20 mL), to yield 936 mg (90%) of crude product used without further purification.

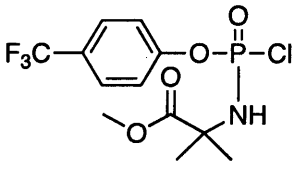
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.56 (s).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.29 (2H, d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 7.47 (2H, d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 7.40-7.37 (5H, m, ***Ph***CH_2), 5.27 (2H, s, PhC***H$_2$***), 5.04-5.01 (1H, 2bs, N***H***), 1.77, 1.74 (6H, 2s, C[C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0*, 27.3*, (C[***C***H_3]$_2$), 59.2* (***C***[CH_3]$_2$), 68.5 (Ph***C***H_2), 121.7, 121.8, 126.2, 128.3, 128.4, 128.6, 129.1* (***Ph***CH_2+*p*-NO_2***Ph***O), 135.4 (*'ipso'*, ***Ph***CH_2), 145.7 (*'p'*, *p*-NO_2***Ph***O), 154.7, 154.8 (*'ipso'*, *p*-NO_2***Ph***O), 174.7, 174.8 (***C***$OOCH_2$Ph).

Synthesis of 4-trifluoromethylphenyl-(methoxy-dimethylglycinyl)-phosphorochloridate. (35l)

$C_{12}H_{14}ClF_3NO_4P$, MW=359.67



This was synthesised according to *Standard procedure 4,* using dimethylglycine methyl ester hydrochloride (270 mg, 1.76 mmol), 4-trifluoromethylphenyl-phosphorodichloridate (490 mg, 1.76 mmol), and TEA (355 mg,

3.51 mmol, 489 μL) in DCM (40 mL), to yield 611 mg (97%) of crude product used without further purification.

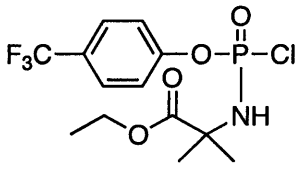
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.80 (s).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.68 (2H, d, ^{3}J=8.5 Hz, *p*-CF_3***Ph***O), 7.43 (2H, d, ^{3}J=8.5 Hz, *p*-CF_3***Ph***O), 5.00-4.97 (1H, 2bs, N***H***), 3.83 (3H, s, C***H$_3$***O), .1.73-1.70 (6H, 2s, C[C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0*, 27.2, 27.3 (C[***C***H_3]$_2$), 53.7 (***C***H_3O), 58.9, 59.0 (***C***[CH_3]$_2$), 121.0, 121.2, 121.4, 121.5 (*'o'*, *p*-CF_3***Ph***O), 124.1 (***C***F_3, *J*=265 Hz), 127.6, 127.7* (*'m'*, *p*-CF_3***Ph***O), 152.6, 152.7 (*'ipso'*, *p*-CF_3***Ph***O), 175.5, 175.6 (***C***$OOCH_3$).

Synthesis of 4-trifluoromethylphenyl-(methoxy-dimethylglycinyl)-phosphorochloridate. (35m)

$C_{13}H_{16}ClF_3NO_4P$, MW=373.69



This was synthesised according to *Standard procedure 4,* using dimethylglycine ethyl ester hydrochloride (294 mg, 1.75 mmol), 4-trifluoromethylphenyl-phosphorodichloridate (490 mg, 1.76 mmol), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (40 mL), to yield 636 mg (97%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.74, 6.83.

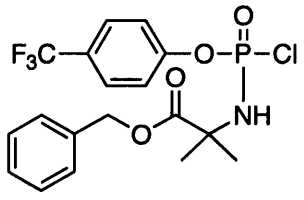
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.70 (2H, d, ^{3}J=8.6 Hz, *p*-CF_3***Ph***O), 7.45 (2H, d, *p*-CF_3***Ph***O), 4.93-4.89 (2H, 2bs, N***H***), 4.30 (2H, q, ^{3}J=7.1 Hz, CH_3C***H$_2$***O), 1.75-1.72 (6H, 2s, C[C***H$_3$***]$_2$), 1.36 (3H, t, ^{3}J=7.1 Hz, C***H$_3$***CH_2O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.5 (***C***H_3CH_2O), 26.9, 27.0, 27.2, 27.3 (C[***C***H_3]$_2$), 59.0* (***C***[CH_3]$_2$), 62.9 (CH_3***C***H_2O), 121.4, 121.5 (*'o'*, *p*-CF_3***Ph***O), 124.3 (***C***F_3, *J*=264 Hz) 127.7, 127.8 (*'m'*, *p*-

CF_3***Ph***O), 152.6, 152.7 (*'ipso'*, *p*-CF_3***Ph***O), 175.0, 175.2 (***C***$OOCH_2CH_3$).

Synthesis of 4-trifluoromethylphenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35n)

$C_{18}H_{18}ClF_3NO_4P$, MW=435.76



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (341 mg, 1.49 mmol), 4-trifluoromethylphenyl-phosphorodichloridate (414 mg, 1.49 mmol), and TEA (301 mg, 2.97 mmol, 414 μL) in DCM (15 mL), to yield 624 mg (96%) of crude product used without further purification.

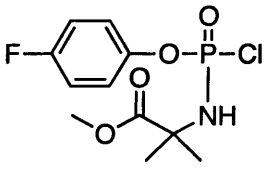
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 6.74 (s)

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.66 (2H, d, ^{3}J=8.8 Hz, *p*-CF_3***Ph***O), 7.42-7.30 (7H, m, *p*-CF_3***Ph***O+***Ph***CH_2), 5.25 (2H, s, PhC***H₂***), 4.95-4.91 (1H, 2bs, N***H***), 1.75-1.72 (6H, 2s, C[C***H₃***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 26.9, 27.0, 27.3* (C[***C***H_3]$_2$), 59.0, 59.1 (***C***[CH_3]$_2$), 68.4 (Ph***C***H_2), 121.1, 121.4, 121.5, 127.7, 128.4, 128.5*, 128.6, 128.9 (***Ph***CH_2+*p*-CF_3***Ph***O), 124.2 (**C**F_3, *J*=265 Hz), 135.4 (*'ipso'*, ***Ph***CH_2), 152.6, 152.7 (*'ipso'*, *p*-CF_3***Ph***O), 174.9, 175.0 (***C***$OOCH_2$Ph).

Synthesis of 4-fluorophenyl-(methoxy-dimethylglycinyl)-phosphorochloridate. (35o)

$C_{11}H_{14}ClFNO_4P$, MW=309.66



This was synthesised according to *Standard procedure 4,* dimethylglycine methyl ester hydrochloride (270 mg, 1.76 mmol), 4-fluorophenyl-phosphodichloridate (402 mg, 1.76 mmol), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL), to yield 527 mg (97%) of crude product used without further purification.

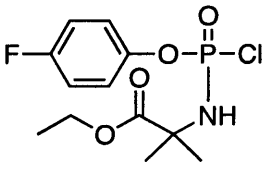
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.42 (^6J=2.9 Hz).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.30-7.05 (4H, m, *p*-F***Ph***O), 4.81-4.78 (1H, bs, N***H***), 3.83 (3H, s, C***H₃***O), 1.69-1.72 (6H, 2s, C[C***H₃***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 25.6*, 25.8* (C[***C***H$_3$]$_2$), 52.2 (***C***H$_3$O), 57.4 (***C***[CH$_3$]$_2$), 115.5* (*'m'*, ^3J=25 Hz, *p*-F***Ph***O), 121.1, 121.2 (*'o'*, ^4J=14 Hz, *p*-F***Ph***O), 144.5, 144.6*, 144.7 (*'ipso'*, *p*-F***Ph***O), 159.2 (*'p'*, ^6J=245 Hz, *p*-F***Ph***O), 174.1, 174.3 (***C***OOCH$_3$).

Synthesis of 4-fluorophenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate. (35p)

$C_{12}H_{16}ClFNO_4P$, MW=323.68



This was synthesised according to *Standard procedure 4,* using dimethylglycine ethyl ester hydrochloride (294 mg, 1.75 mmol), 4-fluorophenyl-phosphorodichloridate (402 mg, 1.76 mmol), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL), to yield 525 mg (92%) of crude product used without further purification.

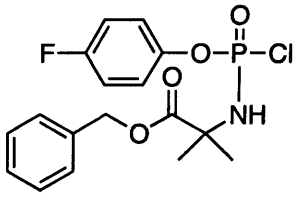
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.33 (^6J=2.31 Hz).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.32-7.26 (2H, m, *p*-F***Ph***O), 7.12-7.06 (2H, m, *p*-F***Ph***O), 4.87-4.83 (1H, bs, N***H***), 4.29 (2H, q, ^{3}J=7.14 Hz, $CH_3C\mathbf{H_2}O$), 1.73-1.70 (6H, 2s, $C[C\mathbf{H_3}]_2$), 1.35 (3H, t, ^{3}J=7.14 Hz, $C\mathbf{H_3}CH_2O$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 13.1 ($\mathbf{C}H_3CH_2O$), 25.4, 25.5, 25.7, 25.8 ($C[\mathbf{C}H_3]_2$), 57.4* ($\mathbf{C}[CH_3]_2$), 61.4 ($CH_3\mathbf{C}H_2O$), 115.5* (*'m'*, ^{3}J=24 Hz, *p*-F***Ph***O), 121.1, 121.2 (*'o'*, ^{4}J=9 Hz, *p*-F***Ph***O), 144.5, 144.6, 144.7 (*'ipso'*, *p*-F***Ph***O), 159.2 (*'p'*, ^{6}J=245 Hz, *p*-F***Ph***O), 173.6, 173.8 ($\mathbf{C}OOCH_2CH_3$).

Synthesis of 4-fluorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35q)

$C_{17}H_{18}Cl_2NO_4P$, MW=388.75



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (403 mg, 1.75 mmol), 4-fluorophenyl-phosphorodichloride (401 mg, 1.75 mmol), and TEA (354 mg, 3.50 mmol, 487μL) in DCM (15 mL), to yield 615 mg (yield 91%) of crude product used without further purification.

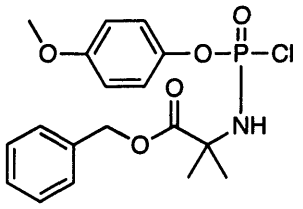
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 7.37 (^{6}J=3 Hz).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.05 (9H, m, ***Ph***CH_2+*p*-F***Ph***O), 5.26 (2H, s, PhC$\mathbf{H_2}$), 4.89-4.86 (1H, 2bs, N***H***), 1.76, 1.73 (6H, 2s, $C[C\mathbf{H_3}]_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 26.9, 27.0, 27.2, 27.3 ($C[\mathbf{C}H_3]_2$), 58.9* ($\mathbf{C}[CH_3]_2$), 68.4 (Ph$\mathbf{C}H_2$), 116.9, 117.0 (*'m'*, ^{3}J=25 Hz, *p*-F***Ph***O), 122.5, 122.6 (*'o'*, *J*=13 Hz, *p*-F***Ph***O), 128.6, 129.0, 129.1 (***Ph***CH_2), 135.5 (*'ipso'*, ***Ph***CH_2), 145.9, 146.0*, 146.1 (*'ipso'*, *p*-F***Ph***O), 160.6 (*'p'*, *J*=246 Hz, *p*-F***Ph***O), 174.9, 175.1 ($\mathbf{C}OOCH_2Ph$).

Synthesis of 4-methoxyphenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate. (35r)

$C_{18}H_{21}ClNO_5P$, MW=397.79.



This was synthesised according to *Standard procedure 4,* using dimethylglycine benzyl ester hydrochloride (1000 mg, 4.35 mmol), 4-methoxyphenyl-phosphorodichloridate (1048 mg, 4.35 mmol) and TEA (880 mg, 8.70 mmol, 1213 μL) in DCM (15 mL). After overnight reaction, the crude was quickly purified by silica column chromatography using as eluent ethyl acetate/petroleum ether (7/3) to yield 1570 mg (yield 91 %) of a colourless oil.

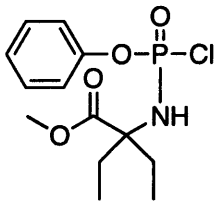
^{31}P-NMR ($CDCl_3$; 121 MHz): δ 7.50.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42.7.39 (5H, m, ***Ph***CH_2O), 7.25-7.21 (2H, m, *p*-CH_3O***Ph***O), 6.92-6.90 (2H, m, *p*-CH_3O***Ph***O), 5.27 (2H, 2s, PhC***H***$_2$O), 4.19-4.16 (1H, m, N***H***), 3.84 (3H, s, *p*-C***H***$_3$OPhO), 1.76-1.74 (3H, 2s, (C***H***$_3$)$_2$CH).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 26.9, 27.0, 27.3* (C(***C***H_3)$_2$), 56.0 (*p*-***C***H_3OPhO), 58.8, 58.9 (***C***$(CH_3)_2$), 68.3 (Ph***C***H_2), 115.1, 115.2, 121.9, 122.0, 128.5, 129.0, 129.1 (*p*-***C***H_3OPhO+***Ph***CH_2O), 135.6 ('*ipso*', ***Ph***CH_2), 143.7, 143.8 ('*ipso*', *p*-***C***H_3OPhO), 157.7, 157.8 ('*p*', *p*-***C***H_3OPhO), 175.0, 175.1 (***C***$OOCH_2$Ph).

Synthesis of phenyl-(methoxy-diethylglycinyl)-phosphorochloridate. (35s)

$C_{13}H_{19}ClNO_4P$, MW=319.72



This was synthesised according to *Standard procedure 4,* using diethylglycine methyl ester hydrochloride (800 mg, 4.40 mmol), phenyl phosphorodichloridate (928 mg, 4.40 mmol, 657 μL), and TEA (891 mg, 8.80 mmol, 1227 μL) in DCM (15 mL). After 16 hrs, the crude was quickly purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 776 mg (55 %) of a colourless oil.

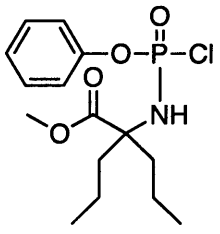
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.68.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.45-7.25 (5H, m, ***Ph***O), 4.88-4.84 (1H, m, NH), 2.38-2.23 (2H, m, C[C***H***$_2$$CH_3$]$_2$), 0.98, 0.86 (6H, t, ^{3}J=7.4 Hz, C[CH_2C***H***$_3$]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 8.9, 9.3 (C[CH_2***C***H_3]$_2$), 31.9, 32.0 (C[***C***H_2CH_3]$_2$), 53.6 (***C***H_3O), 68.7, 68.8 (***C***[CH_2CH_3]$_2$), 121.0*, 121.1, 121.2, 126.2, 126.3*, 130.2, 130.3 (***Ph***O) 150.4, 150.5 (*'ipso'*, ***Ph***O), 174.5, 174.8 (***C***OOCH_3).

Synthesis of phenyl-(methoxy-di-n-propylglycinyl)-phosphorochloridate. (35t)

$C_{15}H_{23}ClNO_4P$, MW=347.77



This was synthesised according to *Standard procedure 4,* using di-n-propylglycine methyl ester hydrochloride (600 mg, 2.86 mmol), phenyl phosphorodichloridate (603 mg, 2.86 mmol, 427 μL), and TEA (579 mg, 5.72 mmol, 797 μL) in DCM (15 mL). After 16 hrs, the crude was purified

by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 771 mg (78%) of a colourless oil.

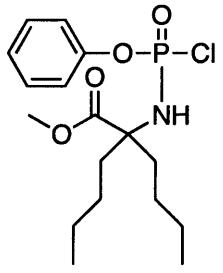
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.67.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.18 (5H, m, ***Ph***O), 4.92-4.83 (1H, m, NH), 3.82 (1H, s, C***H$_3$***O), 2.29-2.15 (2H, m, C[$CH_2CH_2CH_3$]$_2$), 1.90-1.76 (2H, m, C[$CH_2CH_2CH_3$]$_2$), 1.74-1.57 (2H, m, C[$CH_2CH_2CH_3$]$_2$), 1.56-1.42 (2H, m, C[$CH_2CH_2CH_3$]$_2$), 0.95, 0.88 (6H, 2t, ^{3}J=7.0 Hz, C[CH_2CH_2C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.2 (C[CH_2CH_2***C***H_3]$_2$), 17.8, 18.0 (C[CH_2***C***H_2CH_3]$_2$), 41.2, 41.3 (C[***C***$H_2CH_2CH_3$]$_2$), 53.5 (***C***H_3O), 67.2, 67.3 (***C***[$CH_2CH_2CH_3$]$_2$), 121.0, 121.1, 126.2, 126.3, 130.2, 130.3 (***Ph***O) 150.4, 150.5 (*'ipso'*, ***Ph***O), 174.8, 175.1 (***C***OOCH_3).

Synthesis of phenyl-(methoxy-di-propylglycinyl)-phosphorochloridate. (35u)

$C_{17}H_{27}ClNO_4P$, MW=375.83



This was synthesised according to *Standard procedure 4,* using di-n-butylglycine methyl ester hydrochloride (700 mg, 2.94 mmol), phenyl phosphorodichloridate (620 mg, 2.94 mmol, 439 μL), and TEA (595 mg, 5.88 mmol, 820 μL) in DCM (15 mL). After 16 hrs, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (1/1) to yield 783 mg (71%) of a colourless oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.73.

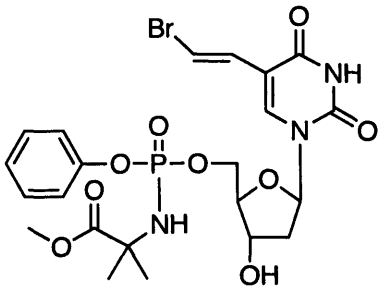
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.38-7.14 (5H, m, ***Ph***O), 4.81-4.77 (1H, m, NH), 3.77 (1H, s, C***H$_3$***O), 2.26-2.12 (2H, m, C[$CH_2CH_2CH_2CH_3$]$_2$), 1.87-1.73 (2H, m, C[$CH_2CH_2CH_2CH_3$]$_2$), 1.63-1.51 (2H, m, C[$CH_2CH_2CH_3$]$_2$), 1.42-1.13 (2H, m,

$C[CH_2CH_2CH_2CH_3]_2$), 0.87, 0.81 (6H, 2t, ^3J=7.3 Hz, $C[CH_2CH_2CH_2C\underline{\boldsymbol{H_3}}]_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.3 ($C[CH_2CH_2CH_2\underline{\boldsymbol{C}}H_3]_2$), 22.8, 22.9 ($C[CH_2CH_2\underline{\boldsymbol{C}}H_2CH_3]_2$), 26.6, 26.8 ($C[CH_2\underline{\boldsymbol{C}}H_2CH_2CH_3]_2$), 38.8, 38.9 ($C[\underline{\boldsymbol{C}}H_2CH_2CH_2CH_3]_2$), 53.5 ($\underline{\boldsymbol{C}}H_3O$), 67.3, 67.4 ($\underline{\boldsymbol{C}}[CH_2CH_2CH_2CH_3]_2$), 121.1, 121.2, 126.2, 126.3, 130.2, 130.3* (***Ph***O) 150.4, 150.5 (*'ipso'*, ***Ph***O), 174.8, 175.1 ($\underline{\boldsymbol{C}}OOCH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(methoxy-dimethylglycinyl)]-phosphate.[5] (36a) (CPF26)

$C_{22}H_{27}BrN_3O_9P$, MW=588.34



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), phenyl-(methoxy-dimethylglycinyl)-phosphorochloridate (438 mg, 1.5 mmol), NMI (246 mg, 3.00 mmol, 239 μL) in THF (5 mL) for 4 hrs. The crude product was purified by flash column chromatography using an eluent of chloroform/methanol (97/3) to give the pure product as a white foamy solid (117 mg, 33%).

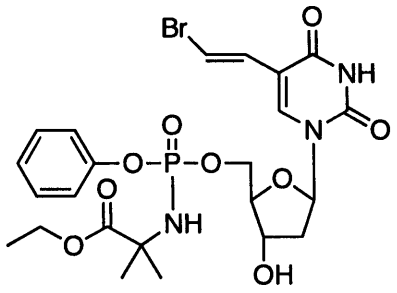
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.36, 3.14.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 9.91 (1H, bs, H-3), 7.73, 7.65 (1H, 2s, H-6), 7.48, 7.46 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.41-7.02 (5H, m, ***Ph***O), 6.79, 6.73 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.34-6.28 (1H, m, H1'), 4.55-4.17 (6H, m, H-5'+H-4'+H-3', NH, OH-3'), 3.78 (3H, s, C***H$_3$***O), 2.53-2.39 (1H, m, one of H-2'), 2.25-1.99 (1H, m, one of H-2'), 1.60 (6H, s, C[C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.1, 27.2. 27.3, 27.4, 27.5* (C[***C***H$_3$]$_2$), 40.7, 40.6 (C-2'), 53.5 (***C***H$_3$O), 57.6* (***C***[CH$_3$]$_2$), 66.2, 66.4, 66.5 (C-5'), 70.7, 71.1 (C-3'), 85.4, 85.5, 85.6*, 85.9 (C-1', C-4'), 110.4 (C-5b), 111.9, 112.0 (C-5), 120.4, 120.5, 120.6, 120.7 ('*o*', ***Ph***O), 125.7 ('*p*', ***Ph***O), 128.9 (C-5a), 130.3* ('*m*', ***Ph***O), 138.0, 138.3 (C-6), 149.8* (C-4), 150.8*, 150.9* ('*ipso*', ***Ph***O), 162.0, 162.1 (C-2), 176.1, 176.3*, 176.4 (***C***OOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(ethoxy-dimethylglycinyl)]-phosphate. (36b) (CPF27)

$C_{23}H_{29}BrN_3O_9P$, MW=602.37



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), phenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate (458 mg, 1.50 mmol), NMI (246 mg, 3.00 mmol, 239 μL) in THF (5 mL) for 5 hrs. The crude product was purified by flash column chromatography, using chloroform/methanol (97/3) as eluent to give the pure product as a white foamy solid (106 mg, 29%).

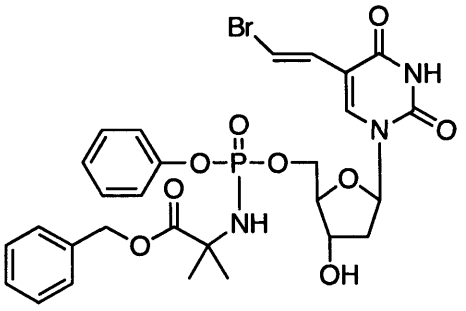
^{31}P-NMR (MeOD, 121 MHz): δ 3.91, 3.85.

^{1}H-NMR (MeOD, 300 MHz): δ 9.92 (1H, bs, H-3), 7.84, 7.81 (1H, 2s, H-6), 7.44-7.20 (6H, m, ***Ph***O+H-5b), 6.86, 6.84 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.34-6.28 (1H, m, H-1'), 4.50-4.34 (3H, m, H-5'+H-3'), 4.23-4.15 (3H, m, H-4'+$CH_3C\mathbf{H_2}O$), 2.38-2.28 (1H, m, one of H-2'), 2.22-2.09 (1H, m, one of H-2'), 1.51 (6H, s, $C[C\mathbf{H_3}]_2$), 1.29 (3H, t, ^{3}J=7.0 Hz, $C\mathbf{H_3}CH_2O$).

^{13}C-NMR (MeOD, 75 MHz): δ 14.9 ($\mathbf{C}H_3CH_2O$), 27.8, 27.9*, 28.2, 28.3, 28.4 ($C[\mathbf{C}H_3]_2$), 41.5 (C-2'), 58.5 ($\mathbf{C}[CH_3]_2$), 63.0, 63.1 ($CH_3\mathbf{C}H_2O$), 68.1, 68.2 (C-5'), 72.6* (C-3'), 87.1, 87.2, 87.3, 87.4 (C-1'+C-4'), 109.6* (C-5b), 112.7* (C-5b), 122.0*, 122.1, 122.2, ('*o*', ***Ph***O), 126.7 ('*p*', O***Ph***), 131.0, 131.2 (C-5a, '*m*' ***Ph***O), 140.4, 140.5 (C-6), 151.4 (C-4), 152.4, 152.5*, 152.6 (C-4), 164.0 (C-2), 177.1, 177.2, 177.3 ($\mathbf{C}OOCH_2CH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36c) (CPF14)

$C_{28}H_{31}BrN_3O_9P$, MW=664.44



This was synthesised according to *Standard procedure 5*, using BVdU (242 mg, 0.73 mmol), phenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate (533 mg, 2.00 mmol), NMI (298 mg, 3.63 mmol, 289 μL) in THF (5 mL) for 4 hrs. The crude product was purified by flash column chromatography using chloroform/methanol (97/3) to give the pure product as a white foamy solid (129 mg, 27%).

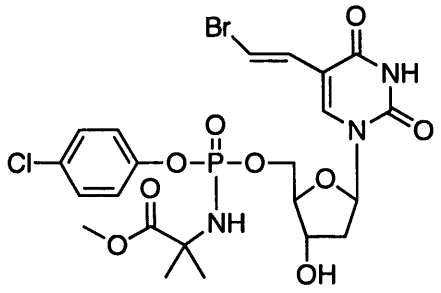
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.39, 3.12.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.92 (1H, bs, H-3), 7.67-7.60 (1H, 2s, H-6), 7.46, 7.44 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.40-7.16 (10H, m, ***Ph***O+***Ph***CH_2), 6.76, 6.70 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.31-6.25 (1H, m, H-1'), 5.18 (1H, s, PhC***H$_2$***), 4.50-4.09 (6H, m, H-3'+H-5'+H-4', N***H***, OH-3'), 2.48-2.25 (1H, m, one of H-2'), 2.16-1.82 (1H, m, one of H-2'), 1.60 (6H, s, C[C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.0, 27.1, 27.3*, 27.4, 27.5* (C[***C***H_3]$_2$), 40.6, 40.7 (C-2'), 57.6, 57.7* (***C***[CH_3]$_2$), 66.2, 66.5, 66.6 (C-5'), 68.1 (Ph***C***H_2), 70.6, 71.1 (C-3'), 85.4, 85.5, 85.6*, 85.8 (C-1'+C-4'), 110.4* (C-5b), 112.0* (C-5), 120.4, 120.5, 120.6, 120.7, 125.7, 128.4, 128.5, 128.8, 128.9*, 129.1, 130.3* (***Ph***O+***Ph***CH_2 C-5a), 135.7 (*'ipso'*, ***Ph***CH_2) 138.1, 138.3 (C-6), 149.8 (C-4) 150.8*, 150.9* (*'ipso'*, ***Ph***O), 162.0, 162.1 (C-2), 175.5, 175.6*, 175.7 (***C***OOCH_2Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(methoxy-dimethylglycinyl)]-phosphate. (36d) (CPF42)

$C_{22}H_{26}BrClN_3O_9P$, MW=622.79



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(methoxy-dimethylglycinyl)-phosphorochloridate (440 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 6 hrs. The crude product was purified by twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (147 mg, 53%).

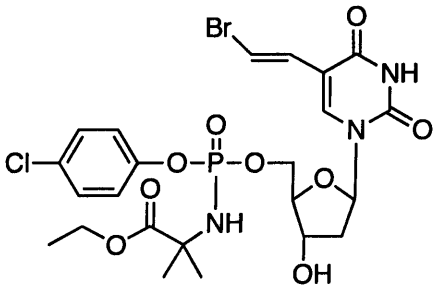
^{31}P-NMR (MeOD, 121 MHz): δ 3.98 (s).

^{1}H-NMR (MeOD, 300 MHz): δ), 7.71-7.69 (1H, 2s, H-6), 7.31-7.13 (5H, m, *p*-Cl***Ph***O+H-5b), 6.71, 6.68 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.23-6.16 (1H, m, H-1'), 4.39-4.22 (3H, m, H-3'+H-5'), 4.05-4.03 (1H, m, H-4'), 3.61 (3H, s, C***H***$_3$O), 2.29-2.19 (1H, m, one of H-2'), 2.15-2.05 (1H, m, one of H-2'), 1.38 (6H, s, C[C***H***$_3$]$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 28.0*, 28.2, 28.3, 28.4 ([***C***H$_3$]$_2$C), 41.5, 41.6 (C-2'), 53.5, 53.6 (***C***H$_3$O), 58.6 (***C***[CH$_3$]$_2$), 68.2, 68.3 (C-5'), 72.4, 72.5 (C-3'), 87.1, 87.2, 87.3, 87.4 (C-1'+C-4'), 109.7 (C-5b), 112.7 (C-5), 123.7*, 123.8 (*'o'*, *p*-Cl***Ph***O), 130.9, 131.1 (*'m'*, *p*-Cl***Ph***O+C-5a), 131.8 (*'p'*, *p*-Cl***Ph***O), 140.4 (C-6), 151.1, 151.2, 151.4 (*'ipso'*, *p*-Cl***Ph***O+C-4), 164.0 (C-2), 177.6*, 177.7 (***C***OOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(ethoxy-dimethylglycinyl)]-phosphate. (36e) (CPF43)

$C_{23}H_{28}BrClN_3O_9P$, MW=636.81



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate (413 mg, 1.22 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 16 hrs. The crude product was purified by flash column chromatography, eluting with dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (74 mg, 26 %).

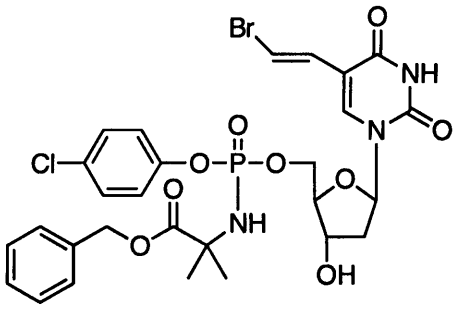
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.47, 3.33.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.03-9.99 (1H, 2bs, H-3), 7.70, 7.67 (1H, 2s, H-6), 7.45, 7.43 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.35-7.20 (4H, m, *p*-Cl***Ph***O), 6.77-6.68 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.33-6.27 (1H, m, H-1'), 4.55-4.29 (5H, m, H-3'+H-5'+OH-3'+N***H***), 4.22-4.17 (3H, q+m, ^{3}J=7.1 Hz, CH_3C***H_2***O+H-4'), 2.53-2.42 (1H, m, one of H-2'), 2.22-2.08 (1H, m, one of H-2'), 1.57-1.54 (6H, 2s, C[C***H_3***]$_2$), 1.31-1.30 (3H, 2t, ^{3}J=7.1 Hz, CH_3C***H_2***O).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.5 (***C***H_3CH_2O), 27.1, 27.2, 27.3, 27.4 (C[***C***H_3]$_2$), 40.7 (C-2'), 57.6 (***C***[CH_3]$_2$), 62.6 (CH_3***C***H_2O), 66.5, 66.6 (C-5'), 70.8, 71.1 (C-3'), 85.4, 85.5, 85.7, 86.0 (C-1'+C-4'), 110.4 (C-5b), 112.0* (C-5), 121.9*, 122.0, 122.1 ('*o*', *p*-Cl***Ph***O), 128.9, 130.2, 130.9 (*p*-Cl***Ph***O+C-5a), 138.2, 138.3 (C-6), 149.4 ('*ipso*', *p*-Cl***Ph***O), 149.9 (C-4), 162.1, 162.2 (C-2), 175.7, 175.8, 175.9 (***C***$OOCH_2CH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36f) (CPF44)

$C_{28}H_{30}BrClN_3O_9P$, MW=698.88



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate (505 mg, 1.25 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 16 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (135 mg, 43%).

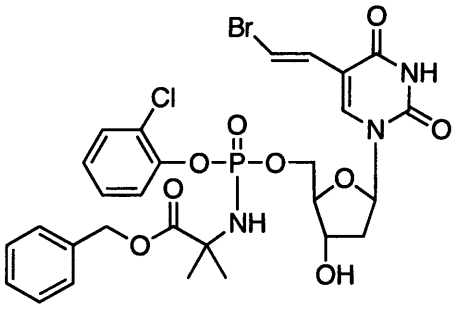
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.44, 3.26.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.96-9.93 (1H, 2bs, H-3), 7.66, 7.65 (1H, 2s, H-6), 7.45, 7.43 (1H, 2d, ^{3}J=13.5, H-5b), 7.39-7.18 (9H, m, *p*-Cl***Ph***O+***Ph***CH_2) 6.75, 6.70 (1H, 2d, ^{3}J=13.5 Hz, H-5a), 6.31-6.25 (1H, m, H-1'), 5.19 (2H, s, PhC***H₂***), 4.51-4.29 (4H, m, H-3'+H-5'+N***H***), 4.15-4.12 (2H, m, H-4'+OH-3'), 2.48-2.40 (1H, m, one of H-2'), 2.18-2.05 (1H, m, one of H-2'), 1.60-1.59 (6H, 2s, C[C***H₃***]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.1, 27.4, 27.5 (C[***C***H_3]$_2$), 40.7 (C-2'), 57.7 (***C***[CH_3]$_2$), 66.4, 66.6 (C-5'), 68.2 (Ph***C***H_2), 70.7, 71.1 (C-3'), 85.4, 85.5, 85.7, 86.0 (C-1'+C-4'), 110.4, 110.5 (C-5b), 111.9, 112.0 (C-5), 121.8, 121.9, 122.0*, 128.4, 128.5, 128.9, 129.0, 129.1 (***Ph***CH_2+*p*-Cl***Ph***O+C-5a), 131.0 (*'ipso'*, ***Ph***CH_2), 135.6 (*'p'*, *p*-Cl***Ph***O), 138.1, 138.2 (C-6), 149.3, 149.8 (*'ipso'*, p-Cl***Ph***O+C-4), 162.0, 162.1 (C-2), 175.6 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[2-chlorophenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36g) (CPF50)

$C_{28}H_{30}BrClN_3O_9P$, MW=698.88.



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 2-chlorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate (543 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 20 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (172 mg, 52%).

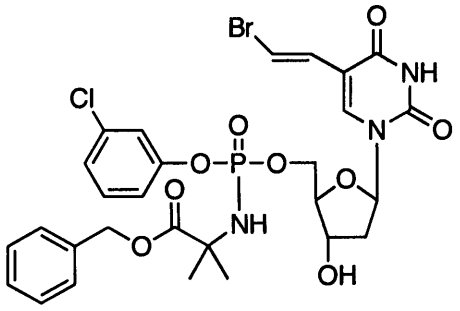
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.25, 3.23.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.92 (1H, bs, H-3), 7.71, 7.75 (1H, 2s, H-6), 7.57-7.11 (10H, m, *o*-Cl***Ph***O+***Ph***CH_2+H-5b), 6.75, 6.72 (1H, 2d, ^{3}J=13.6, H-5a), 6.35-6.28 (1H, m, H-1'), 5.18 (2H, s, PhC***H_2***), 4.53-4.40 (4H, m, H-3'+H-5'+N***H***), 4.17-4.14 (2H, m, H-4'+OH-3'), 2.46-2.43 (1H, m, one of H-2'), 2.13-2.07 (1H, m, one of H-2'), 1.61-1.56 (6H, 2s, C[C***H_3***]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.3, 27.4, 27.6 (C[***C***H_3]$_2$), 40.8 (C-2'), 57.9 (***C***[CH_3]$_2$), 66.8 (C-5'), 68.1 (Ph***C***H_2), 71.2, 71.3 (C-3'), 85.4, 85.5, 85.6, 85.9 (C-1'+C-4'), 110.4, 110.5 (C-5b), 112.0, 112.1 (C-5), 122.2, 122.4 ('*o*', *o*-Cl***Ph***O), 125.6, 126.5, 128.4, 128.5, 128.8, 128.9, 129.1, 131.0 (*o*-Cl***Ph***O+***Ph***CH_2+C-5a), 135.7 ('*ipso*', ***Ph***CH_2), 138.1, 138.3 (C-6), 146.7, 146.8 ('*ipso*', *o*-Cl***Ph***O), 149.9 (C-4), 162.1 (C-2), 175.5 (***C***OOCH_2Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[3-chlorophenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36h) (CPF83)

$C_{28}H_{30}BrClN_3O_9P$, MW=698.88.



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 3-chlorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate (543 mg, 1.71 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 19 hrs. The crude product was purified by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (44 mg, 13 %).

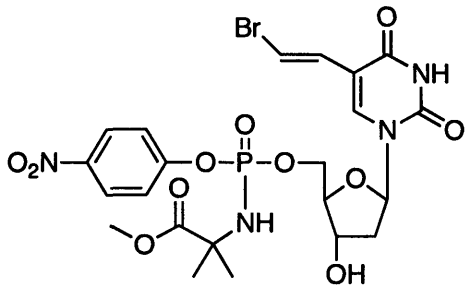
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.39, 3.15.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.35 (1H, bs, H-3), 7.59, 7.56 (1H, 2s, H-6), 7.41-7.09 (10H, m, H-5b+*m*-Cl***Ph***O+***Ph***CH_2), 6.70, 6.59 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.25-6.18 (1H, m, H-1'), 5.14-5.13 (2H, 2s, PhC***H*****$_2$**), 4.46-4.18 (4H, m, H-3'+H-5'+N***H***), 4.16-4.04 (2H, m, H-4'+OH-3'), 2.44-2.32 (1H, m, one of H-2'), 2.09-1.95 (1H, m, one of H-2'), 1.54, 1.52 (6H, 2s, C[C***H*****$_3$**]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.1, 27.5 (C[***C***H_3]$_2$), 40.7 (C-2'), 57.7 (***C***[CH_3]$_2$), 66.3, 66.6 (C-5'), 68.2 (Ph***C***H_2), 70.6, 71.0 (C-3'), 85.4, 85.6, 85.9 (C-1'+C-4'), 110.5 (C-5b), 112.0 (C-5), 118.8, 118.9, 121.1, 121.2 ('*o*', *m*-Cl***Ph***O), 126.0, 128.4, 128.5, 128.6, 128.8, 128.9, 129.0, 129.1, 131.0 (*m*-Cl***Ph***O+***Ph***CH_2+C-5a), 135.4, 135.6 (*m*-Cl***Ph***O+***Ph***CH_2), 138.0, 138.2 (C-6), 149.7 (C-4), 151.3, 151.4 ('*ipso*', *m*-Cl***Ph***O), 149.8 (C-4), 162.0 (C-2), 175.6, 175.7 (***C***OOCH_2Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(methoxy-dimethylglycinyl)]-phosphate. (36i) (CPF45)

$C_{22}H_{26}BrN_4O_{11}P$, MW=633.34



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-nitrophenyl-(methoxy-dimethylglycinyl)-phosphorochloridate (379 mg, 1.13 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 3 hrs. The crude product was purified by flash column chromatography using dichloromethane/methanol (97/3) as eluent to give the pure product as a white foamy solid (146 mg, 51%).

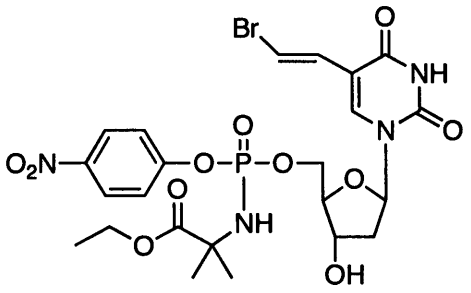
^{31}P-NMR (MeOD, 121 MHz): δ 3.61, 3.56.

^{1}H-NMR (MeOD, 300 MHz): δ 8.30-8.25 (2H, 2d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 7.79, 7.78 (1H, 2s, H-6), 7.48 (2H, d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 7.37, 7.32 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 6.79, 6.72 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.32-6.25 (1H, m, H-1'), 4.48-4.35 (3H, m, H-3'+H-5'), 4.15-4.14 (1H, m, H-4'), 3.71 (3H, s, C***H***$_3$O), 2.41-2.17 (2H, m, H-2'), 1.51 (6H, s, C[C***H***$_3$]$_2$).

^{3}C-NMR ($CDCl_3$, 75 MHz): δ 28.0, 28.1, 28.2, 28.3 (C[***C***H_3]$_2$), 41.4, 41.5 (C-2'), 53.6* (***C***H_3O), 58.7 (***C***[CH_3]$_2$), 68.4, 68.5, 68.6, 68.7 (C-5'), 72.3, 72.4 (C-3'), 86.9, 87.0, 87.4, 87.5 (C-1'+C-4'), 109.7 (C-5b), 112.6 (C-5), 122.8, 122.9 ('*o*', *p*-NO_2***Ph***O), 127.0* ('*m*', *p*-NO_2***Ph***O), 130.9 (C-5a), 140.5 (C-6), 146.5 ('*p*', *p*-NO_2***Ph***O), 151.4, 151.5 (C-4), 157.3, 157.4 ('*ipso*', *p*-NO_2***Ph***O), 163.9 (C-2), 177.5* (***C***$OOCH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(ethoxy-dimethylglycinyl)]-phosphate. (36j) (CPF46)

$C_{23}H_{28}BrN_4O_{11}P$, MW=647.37



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-nitrophenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate (442 mg, 1.26 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 4 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (153 mg, 53%).

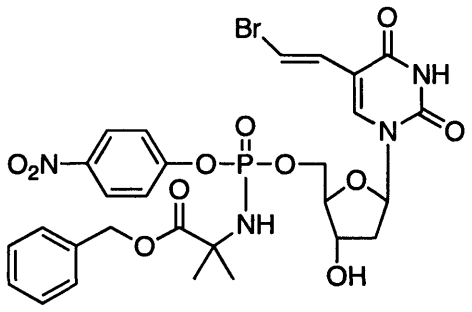
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.00, 2.96.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.28 (1H, bs, H-3), 8.19 (2H, 2d, ^{3}J=9.0 Hz, *p*-NO_2***Ph***O), 7.68, 7.67 (1H, 2s, H-6), 7.46-7.32 (3H, m, *p*-NO_2***Ph***O+H-5b), 6.69, 6.67 (1H, 2d, ^{3}J=13.5 Hz, H-5a), 6.32-6.26 (1H, m, H-1'), 4.75-4.36 (5H, m, H-3'+H-5'+OH-3'+N***H***), 4.25-4.17 (3H, m, CH_3C***H_2***O+H-4'), 2.60-2.98 (1H, m, one of H-2'), 2.31-2.10 (1H, m, one of H-2'), 1.58 (6H, s, C[C***H_3***]$_2$), 1.30-1.28 (3H, 2t, ^{3}J=7.1 Hz, C***H_3***CH_2O).

^{13}C-NMR (MeOD, 75 MHz): δ 14.9 (***C***H_3CH_2O), 27.9, 28.0*, 28.1, 28.2, 28.3 (C[***C***H_3]$_2$), 41.4, 41.5 (C-2'), 58.7 (***C***[CH_3]$_2$), 63.2 (CH_3***C***H_2O), 68.4, 68.5, 68.6 (C-5'), 72.3, 72.4 (C-3'), 86.9, 87.0, 87.4, 87.5 (C-1'+C-4'), 109.7 (C-5b), 112.6* (C-5), 122.7, 122.8 ('*o*', *p*-NO_2***Ph***O), 126.6, 126.7 ('*m*', *p*-NO_2***Ph***O), 130.9 (C-5a), 140.5 (C-6), 146.5 ('*p*', *p*-NO_2***Ph***O), 151.4, 151.5 (C-4), 157.3, 157.4*, 157.5 ('*ipso*', *p*-NO_2***Ph***O), 162.2 (C-2), 177.0*, 177.1 (***C***$OOCH_2CH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-nitrophenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36k) (CPF47)

$C_{28}H_{30}BrN_4O_{11}P$, MW=709.44



This was synthesised according to *Standard procedure 5*, using BVdU (100 mg, 0.30 mmol), 4-nitrophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate (310 mg, 0.75 mmol), NMI (124 mg, 1.51 mmol, 120 μL) in THF (5 mL) for 5 hrs. The crude product was purified by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (160 mg, 50%).

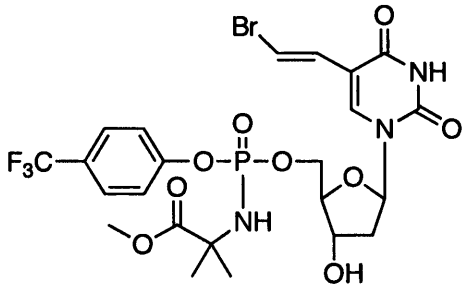
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 2.95, 2.89.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.16 (1H, bs, H-3), 8.25 (2H, 2d, ^{3}J=9.1 Hz, p-NO_2***Ph***O), 7.71, 7.69 (1H, 2s, H-6), 7.48-7.37 (8H, m, *p*-NO_2***Ph***O+***Ph***CH_2+H-5b), 6.75, 6.72 (1H, 2d, ^{3}J=13.5 Hz, H-5a), 6.36-6.29 (1H, m, H-1'), 5.24 (2H, s, PhC***H₂***), 4.81-4.40 (5H, m, H-3'+H-5'+OH-3'+N***H***), 4.22-4.21 (1H, m, H-4'), 2.57-2.36 (1H, m, one of H-2'), 2.27-2.22 (1H, m, one of H-2'), 1.64 (6H, s, C[C***H₃***]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.3*, 27.4*, 27.5, 27.6 (C[***C***H_3]$_2$), 40.7, 40.8 (C-2'), 58.0, 58.1 (***C***[CH_3]$_2$), 67.6, 67.7, 67.8, 67.9 (C-5'), 68.2 (Ph***C***H_2), 71.6, 71.7 (C-3'), 86.2, 86.3, 86.7, 86.8 (C-1'+C-4'), 109.1 (C-5b), 111.9, 112.0 (C-5), 122.0, 122.1, 126.2, 126.3, 128.9, 129.0*, 129.1, 129.3, 130.2 (***Ph***CH_2+***Ph***O+C-5a), 136.9 (*'ipso'*, ***Ph***CH_2), 139.8 (C-6), 145.7 (*'p'*, ***Ph***O), 150.7, 150.8 (C-4), 156.6*, 156.7* (*'ipso'*, ***Ph***O), 163.3 (C-2), 176.0, 176.1* (***C***OOCH_2Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-trifluoromethylphenyl-(methoxy-dimethylglycinyl)]-phosphate. (36l) (CPF58)

$C_{23}H_{26}BrF_3N_3O_9P$, MW=656.34



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-trifluoromethylphenyl-(methoxy-dimethylglycinyl)-phosphorochloridate (486 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179.4 μL) in THF (5 mL) for 25 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) as eluent to give the pure product as a white foamy solid (98 mg, 33%).

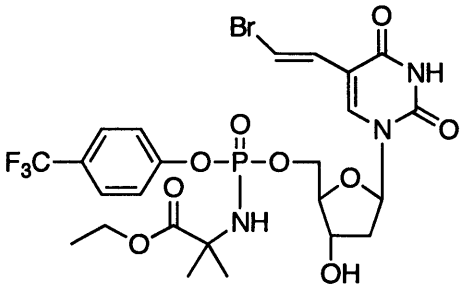
^{31}P-NMR (MeOD, 121 MHz): δ 3.73, 3.52.

^{1}H-NMR (MeOD, 300 MHz): δ 7.85-7.83 (1H, 2s, H-6), 7.75-7.72 (2H, m, *p*-CF_3***Ph***O), 7.49-7.46 (2H, m, *p*-CF_3***Ph***O), 7.41, 7.40 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 6.84, 6.82 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.35-6.29 (1H, m, H-1'), 4.52-4.37 (3H, m, H-3'+H-5'), 4.18-4.16 (1H, m, H-4'), 3.72 (1H, s, C***H***$_3$O), 2.42-2.32 (1H, m, one of H-2'), 2.29-2.20 (1H, m, one of H-2'), 1.53-1.52 (6H, 2s, C[C***H***$_3$]$_2$).

^{13}C-NMR (MeOD, 75 MHz): δ 27.9, 28.0*, 28.2, 28.3, 28.4 (C[***C***H$_3$]$_2$), 41.4, 41.5 (C-2'), 53.5, 53.6 (***C***H$_3$O), 58.7 (***C***[CH$_3$]$_2$), 68.3, 68.4 (C-5'), 72.4, 72.5 (C-3'), 87.0*, 87.1, 87.4, 87.5 (C-1'+C-4'), 109.7 (C-5b), 112.7 (C-5), 122.7 ('*o*', *J*=4 Hz, *p*-CF_3***Ph***O), 125.8 (***C***F$_3$, *J*=271 Hz), 128.6, 128.8 ('*m*', *J*=24 Hz, *p*-CF_3***Ph***O), 128.7 ('*p*', *p*-CF_3***Ph***O, *J*=32.5 Hz), 129.3 (C-5a) 140.4, 140.5 (C-6), 151.4 (C-4), 155.2, 155.3 ('*ipso*', *p*-CF_3***Ph***O), 164.0 (C-2), 177.5, 177.6* (***C***OOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-trifluoromethylphenyl-(ethoxy-dimethylglycinyl)]-phosphate. (36m) (CPF59)

$C_{24}H_{28}BrF_3N_3O_9P$, MW=670.37



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-trifluoromethylphenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate (505 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 25 hrs. The crude product was purified by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (132 mg, 44%).

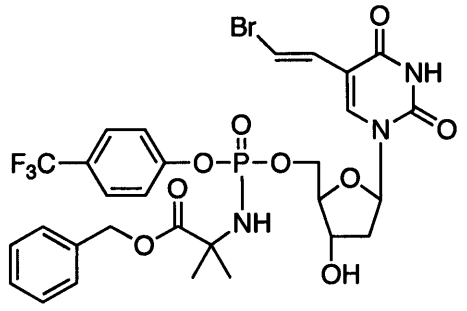
^{31}P-NMR (MeOD, 121 MHz): δ 3.75 (s).

^{1}H-NMR (MeOD, 300 MHz): δ 7.88-7.86 (1H, 2s, H-6), 7.78-7.73 (2H, m, *p*-CF_3***Ph***O), 7.52-7.50 (2H, m, *p*-CF_3***Ph***O), 7.44, 7.43 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 6.87, 6.85 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.38-6.32 (1H, m, H-1'), 4.55-4.40 (3H, m, H-3'+H-5'), 4.29-4.18 (3H, m, CH_3C***H_2***O+H-4'), 2.45-2.35 (1H, m, one of H-2'), 2.32-2.22 (1H, m, one of H-2'), 1.57, 1.55 (6H, 2s, C[C***H_3***]$_2$), 1.33-1.29 (3H, m, C***H_3***CH_2O).

^{13}C-NMR (MeOD, 75 MHz): δ 14.9 (***C***H_3CH_2O), 27.9*, 28.1, 28.2, 28.3 (C[***C***H_3]$_2$), 41.5 (C-2'), 58.6 (***C***[CH_3]$_2$), 63.1 (CH_3***C***H_2O), 68.3, 68.4 (C-5'), 72.5 (C-3'), 87.0, 87.1, 87.4, 87.5 (C-1'+C-4'), 109.7 (C-5b), 112.7 (C-5), 122.7, 122.8 (*'o'*, *p*-CF_3***Ph***O), 125.8 (***C***F_3, J=271 Hz), 128.6 (*'m'*, *p*-CF_3***Ph***O), 128.7 (*'p'*, *p*-CF_3***Ph***O, J=32.5 Hz), 130.9 (C-5a) 140.5* (C-6), 151.4 (C-4), 155.4, 155.5 (*'ipso'*, *p*-CF_3***Ph***O), 164.0 (C-2), 177.1* (***C***$OOCH_2CH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-trifluoromethylphenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36n) (CPF48)

$C_{29}H_{30}BrF_3N_3O_9P$, MW=732.44



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-trifluoromethylphenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate (529 mg, 1.22 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 4 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (142 mg, 43%).

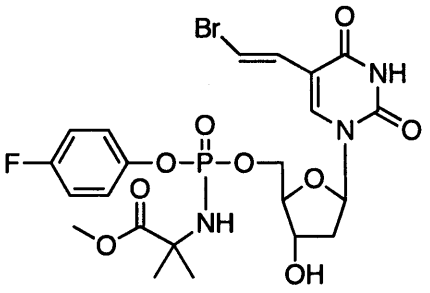
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.16, 3.01.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.06-10.02 (1H, 2bs, H-3), 7.67, 7.66 (1H, s, H-6), 7.63, 7.62 (2H, 2d, ^{3}J=8.8 Hz, *p*-CF_3***Ph***O), 7.46-7.32 (8H, m, *p*-CF_3***Ph***O+***Ph***CH_2+H-5b), 6.75, 6.71 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.31-6.26 (1H, m, H-1'), 5.18 (2H, s, PhC***H*****$_2$**), 4.61-4.32 (4H, m, H-3'+H-5'+N***H***), 4.16-4.15 (2H, m, H-4'+OH-3'), 2.48-2.41 (1H, m, one of H-2'), 2.23-2.09 (1H, m, one of H-2'), 1.60-1.58 (6H, 2s, C[C***H*****$_3$**]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.0, 27.4, 27.5 (***C***[*C*H_3]$_2$), 40.6 (C-2'), 57.7, 57.8 (***C***[CH_3]$_2$), 66.5, 66.8 (C-5'), 68.2 (Ph***C***H_2), 70.8, 71.1 (C-3'), 85.4, 85.7, 86.0 (C-1'+C-4'), 110.4 (C-5b), 111.9, 112.0 (C-5), 120.8*, 120.9, 121.0, 127.6, 127.7, 128.0, 128.4, 128.5, 128.8, 129.0* (*p*-CF_3***Ph***O+***Ph***CH_2+C-5a), 124.2 (***C***F_3, *J*=267 Hz), 135.6 (*'ipso'*, ***Ph***CH_2), 138.2 (C-6), 149.9 (C-4), 153.3, 153.4 (*'ipso'*, *p*-CF_3***Ph***O), 162.0, 162.1 (C-2), 175.4 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-fluorophenyl-(methoxy-dimethylglycinyl)]-phosphate. (36o) (CPF65)

$C_{22}H_{26}BrFN_3O_9P$, MW 606.33



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-fluorophenyl-(methoxy-dimethylglycinyl)-phosphorochloridate (488 mg, 1.58 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 4 hrs. The crude product was purified by flash column chromatography, using with chloroform/methanol (97/3) to give the pure product as a white foamy solid (119 mg, 44%).

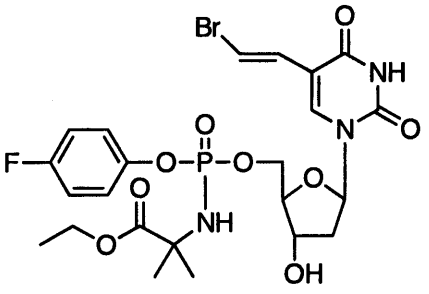
^{31}P-NMR (MeOD, 121 MHz): δ 4.17.

^{1}H-NMR (MeOD, 300 MHz): δ 7.73-7.71 (1H, 2s, H-6), 7.29, 7.28 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.20-6.97 (4H, m, *p*-F***Ph***O), 6.72, 6.70 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.23-6.17 (1H, m, H1'), 4.38-4.26 (3H, m, H-5'+H-3'), 4.06-4.03 (1H, m, H-4'), 3.61 (3H, s, C***H*****$_3$**O), 2.28-2.19 (1H, m, one of H-2'), 2.16-2.08 (1H, m, one of H-2'), 1.40-1.37 (6H, s, C[C***H*****$_3$**]$_2$).

^{13}C-NMR (MeOD, 75 MHz): δ 28.0, 28.2, 28.3, 28.4 (C[***C***H$_3$]$_2$), 41.4, 41.5 (C-2'), 53.5* (***C***H$_3$O), 58.6 (***C***[CH$_3$]$_2$), 68.1, 68.2 (C-5'), 72.5* (C-3'), 87.1, 87.2, 87.3, 87.4 (C-1'+C-4'), 109.7 (C-5b), 112.7 (C-5), 117.6 (*'m'*, ^{3}J=23 Hz, *p*-F***Ph***O), 123.7, 123.8 (*'o'*, J=14 Hz, *p*-F***Ph***O), 130.9 (C-5a), 140.4 (C-6), 148.4, 148.5 (*'ipso'*, *p*-F***Ph***O), 151.5 (C-4), 161.6 (*'p'*, ^{6}J=243 Hz, *p*-F***Ph***O), 164.0 (C-2), 177.6 (***C***OOCH$_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-fluorophenyl-(ethoxy-dimethylglycinyl)]-phosphate. (36p) (CPF60)

$C_{23}H_{28}BrFN_3O_9P$, MW 620.36



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-fluorophenyl-(ethoxy-dimethylglycinyl)-phosphorochloridate (436.9 mg, 1.35 mmol), NMI (184.7 mg, 2.25 mmol, 179.4 μL) in THF (5 mL) for 24 hrs. The crude product was purified by flash column chromatography, eluting with chloroform/methanol (97/3) to give the pure product as a white foamy solid (101 mg, yield 36%).

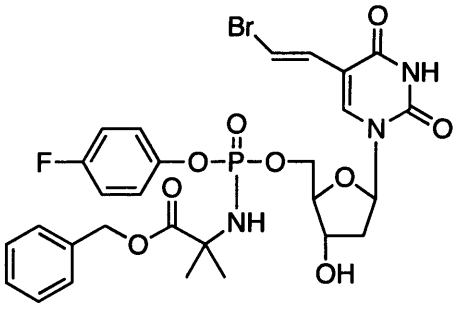
^{31}P-NMR (MeOD, 121 MHz): δ 4.17 (s).

^{1}H-NMR (MeOD, 300 MHz): δ 7.82-7.80 (1H, 2s, H-6), 7.39, 7.38 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.29-7.23 (2H, m, *p*-F***Ph***O), 7.13-7.06 (2H, m, *p*-F***Ph***O), 6.82, 6.80 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.32-6.26 (1H, m, H-1'), 4.47-4.41 (3H, m, H-5'+H-3'), 4.24-4.11 (3H, m, H-4'+$CH_3C\underline{\boldsymbol{H_2}}O$), 2.37-2.29 (1H, m, one of H-2'), 2.28-2.15 (1H, m, one of H-2'), 1.49-1.47 (6H, s, $C[C\underline{\boldsymbol{H_3}}]_2$), 1.26 (3H, t, ^{3}J=7.1 Hz, $C\underline{\boldsymbol{H_3}}CH_2O$).

^{13}C-NMR (MeOD, 75 MHz): δ 14.8 ($\underline{\boldsymbol{C}}H_3CH_2O$), 27.8, 27.9*, 28.2, 28.3, 28.4 ($C[\underline{\boldsymbol{C}}H_3]_2$), 41.5* (C-2'), 58.6 ($\underline{\boldsymbol{C}}[CH_3]_2$), 63.1, 63.2 (C-5'), 68.1, 68.2 ($CH_3\underline{\boldsymbol{C}}H_2O$), 72.5* (C-3'), 87.1*, 87.2*, 87.3, 87.4 (C-1'+C-4'), 109.6 (C-5b), 112.7 (C-5), 117.6 ('*m*', ^{3}J=24 Hz, *p*-F***Ph***O), 123.7, 123.8 ('*o*',^{4}J=10 Hz, *p*-F***Ph***O), 130.9 (C-5a), 140.4, 140.5 (C-6), 148.4, 148.5 ('*ipso*', *p*-F***Ph***O) 151.5 (C-4), 161.6 ('*p*', ^{2}J=243 Hz, *p*-F***Ph***O), 164.0 (C-2), 177.1, 177.2* ($\underline{\boldsymbol{C}}OOCH_2CH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-flurophenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36q) (CPF65)

$C_{28}H_{30}BrFN_3O_9P$, MW 682.43



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-fluorophenyl-(benzoxy-dimethylglycinyl)-phosphorochloridate (520 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 24 hrs. The crude product was purified twice by flash column chromatography, using chloroform/methanol (97/3) to give the pure product as a white foamy solid (135 mg, yield 44%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.70, 3.52.

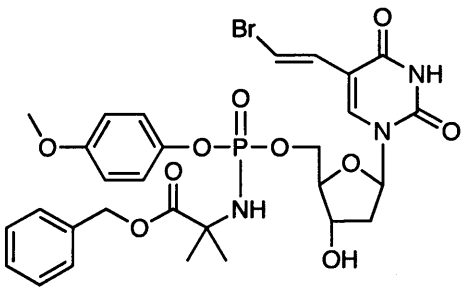
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.68, 9.65 (1H, 2bs, H-3), 7.67, 7.66 (1H, 2s, H-6), 7.46, 7.44 (1H, 2d, ^{3}J=13.7 Hz, H-5b), 7.40-7.37 (5H, m, ***Ph***CH_2), 7.35-7.00 (4H, m, *p*-F***Ph***O), 6.76, 6.71 (1H, 2d, ^{3}J=13.7 Hz, H-5a), 6.31-6.25 (1H, m, H-1'), 5.18 (2H, s, PhC***H$_2$***), 4.51-4.43 (1H, m, H-3'), 4.40-4.33 (3H, m, H-5'+ N***H***), 4.30-3.95 (2H, m, H-4'+OH-3'), 2.51-2.40 (1H, m, one of H-2'), 2.18-2.06 (1H, m, one of H-2'), 1.60-1.57 (6H, s, C[C***H$_3$***]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.1*, 27.3*, 27.4, 27.5 (C[***C***H_3]$_2$), 40.6, 40.7 (C-2'), 57.6, 57.7 (***C***[CH_3]$_2$), 66.2, 66.6 (C-5'), 68.1, 68.4 (Ph***C***H_2), 70.6, 71.1 (C-3'), 85.3, 85.4, 85.5, 85.6, 85.9 (C-1'+C-4'), 110.4, 110.5 (C-5b), 111.9, 112.0 (C-5), 116.8 ('*m*', ^{3}J=24 Hz, *p*-F***Ph***O), 121.8, 121.9*, 122.0*, 122.1, 122.2 ('*o*', *Ph*O), 128.4, 128.5, 128.9*, 129.0, 129.1, 135.6 (***Ph***CH_2+C-5a), 138.1, 138.2 (C-6), 146.6, 146.7 ('*ipso*', *p*-F***Ph***O), 149.8 (C-4), 161.1 ('*p*',

J=244 Hz, *p*-F***Ph***O), 162.0 (C-2), 175.5, 175.6*, 175.7 (***C***OOCH$_2$Ph).

Synthesis of (E)-5-(-bromovinyl)-2'-deoxyuridine-5'-[4-methoxyphenyl-(benzoxy-dimethylglycinyl)]-phosphate. (36r) (CPF163)

$C_{29}H_{33}BrN_3O_{10}P$, MW=694.46.



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-methoxyphenyl-(benzoxy-dimethylglycinyl)phosphorochloridate (5.37 mg, 1.35 mmol), NMI (184.7 mg, 2.25 mmol, 179.4 μL) in THF (7 mL) for 16 hrs. The crude product was purified by flash column chromatography, using chloroform/methanol (95/5) to give the pure product as a white foamy solid (209 mg, yield 67%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 3.71, 3.56.

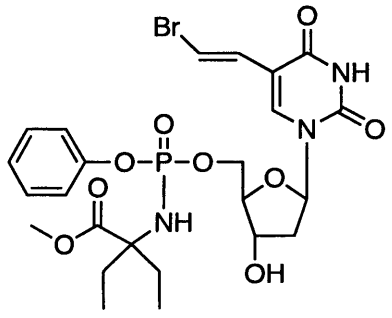
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 10.14, 10.10 (1H, 2bs, H-3), 7.68-7.63 (1H, 2s, H-6), 7.46-7.43 (1H, 2d, ^3J=13.6 Hz, H-5a), 7.40-7.29 (5H, m, ***Ph***CH$_2$O), 7.19-7.14 (2H, m, *p*-CH$_3$O***Ph***O), 6.86-6.82 (2H, m, *p*-CH$_3$O***Ph***O), 6.76, 6.72 (1H, d, ^3J=13.6 Hz, H-5a), 6.32-6.25 (1H, m, H-1'), 5.18 (2H, s, PhC***H$_2$***O), 4.51-4.02 (5H, m, H-3'+H-5'+H-4' +NH), 3.78, 3.77 (3H, 2s, *p*-C***H$_3$***OPhO), 2.49-2.39 (1H, m, one of H-2'), 2.13-1.99 (2H, m, one of H-2'), 1.60, 1.57 (6H, 2s, C(C***H$_3$***)$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 27.1, 27.2, 27.3*, 27.5 (C(***C***H$_3$)$_2$), 40.6, 40.7 (C-2'), 56.0 (***C***H$_3$O), 57.6, 57.7* (***C***(CH$_3$)$_2$), 66.1, 66.4 (C-5'), 68.1 (Ph***C***H$_2$), 70.5, 71.1 (C-3'), 85.4, 85.5, 85.6, 85.9 (C-1'+C4'), 110.3* (C-5b), 111.9* (C-5), 115.1, 121.3, 121.4, 121.5,

121.6, 128.4, 128.5, 128.9*, 129.0*, 129.1*, 135.7 (*p*-CH_3O***Ph***O+***Ph***CH_2), 138.1, 138.2 (C-5a), 144.3, 144.4 (*'ipso'*, *p*-CH_3O***Ph***O), 149.7 (C-4), 157.2 (*'p'*, *p*-CH_3O***Ph***O), 161.9, 162.0 (C-2), 175.5, 175.6, 175.7, 175.8 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-methoxy-diethylglycinyl)]-phosphate. (36s) (CPF180)

$C_{24}H_{31}BrN_3O_9P$, MW=616.40



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), phenyl (methoxy-diethylglycinyl)-phosphorochloridate (384 mg, 1.2 mmol), NMI (239 mg, 3.00 mmol, 232 μL) in THF (10 mL) for 16 hrs. The crude product was purified twice by flash column chromatography using chloroform/methanol 97/3 as eluent to give the pure product as a white foamy solid (88 mg, 24%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 2.49, 2.20.

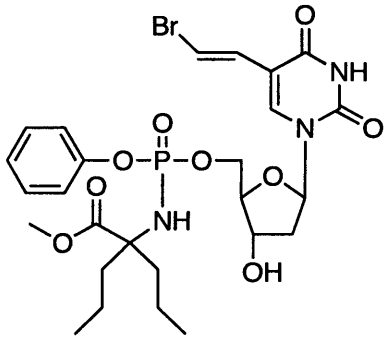
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.48, 9.43 (1H, 2bs, H-3), 7.67, 7.60 (1H, 2s, H-6), 7.48, 7.47 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.40-7.10 (5H, m, ***Ph***O), 6.78, 6.74 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.31-6.27 (1H, m, H-1'), 4.49-4.25 (4H, m, H-3'+H-5'+NH), 4.17-4.14 (1H, m, H-4'), 3.83, 3.81 (3H, 2d, C***H***$_3$O), 2.50-2.31 (1H, m, one of H-2'), 2.21-1.81 (5H, m, one of H-2'+C[C***H***$_2$$CH_3$]$_2$), 0.92-0.85 (3H, m, C[$CH_2$C***H***$_3$]$_2$), 0.77, 0.73 (3H, 2t, ^{3}J=7.3 Hz, C[CH_2C***H***$_3$]$_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 8.6, 8.7, 8.9* (C[CH_2***C***H_3]$_2$), 31.6, 31.7, 31.8 (C[***C***H_2CH_3]$_2$), 40.6, 40.8 (C-2'), 53.5 (***C***[CH_2CH_3]$_2$), 66.4, 66.7, 66.9* (C-5'), 70.9, 71.3 (C-3'), 85.5, 85.6, 85.7, 85.9

(C-1'+C-4'), 110.6 (C-5b), 112.0, 112.1 (C-5), 120.4, 120.5, 120.7, 120.8, 125.7, 125.8 (***Ph***O), 128.8 (C-5a), 130.3 (***Ph***O), 137.9, 138.0 (C-6), 149.6* (C-4), 151.0, 151.1, 151.2* (*'ipso'*, ***Ph***O), 161.7, 161.8 (C-2), 174.9, 175.1*, 175.3 (***C***$OOCH_3$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-methoxy-di-n-propylglycinyl)]-phosphate. (36t) (CPF188)

$C_{26}H_{35}BrN_3O_9P$, MW=644.45



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), phenyl (methoxy-di-n-propylglycinyl)-phosphorochloridate (470 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (7 mL) for 16 hrs. The crude product was purified by flash column chromatography and with preparative TLC using chloroform/methanol (97/3) as eluent to give the pure product as a white foamy solid (28 mg, 10%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 2.47, 2.29.

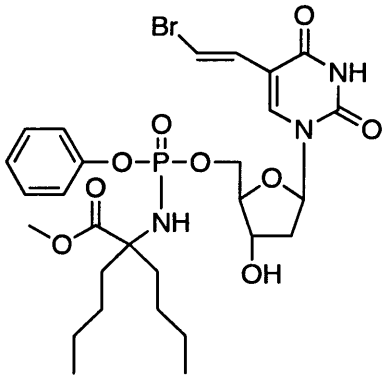
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.42 (1H, bs, H-3), 7.69, 7.60 (1H, 2s, H-6), 7.50, 7.49 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.40-7.03 (5H, m, ***Ph***O), 6.77 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.32-6.26 (1H, m, H-1'), 4.49-4.26 (4H, m, H-3'+H-5'+NH), 3.81-3.79 (1H, m, H-4'), 3.81, 3.79 (3H, 2d, C***H***$_3$O), 2.53-2.24 (1H, m, one of H-2'), 2.17-1.95 (3H, m, one of H-2'+C$[CH_2CH_2CH_3]_2$), 1.86-1.69 (2H, m, C$[CH_2CH_2CH_3]_2$), 1.59-1.49 (1H, m, C$[CH_2CH_2CH_3]_2$), 1.36-1.23 (1H, m, C$[CH_2CH_2CH_3]_2$), 1.05-0.80 (8H, m, C[C***H***$_2$$CH_2CH_3]_2$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 12.8, 12.9 (C$[CH_2CH_2$***C***$H_3]_2$), 16.1*, 16.4 (C$[CH_2$***C***$H_2CH_3]_2$), 39.1, 39.4, 39.5, 39.6, 39.9 (C-2'+

C[***C***$H_2CH_2CH_3]_2$), 52.0 (***C***H_3O), 64.2*, 64.3 (***C***$[CH_2CH_2CH_3]_2$), 64.9, 65.1, 65.2 (C-5'), 69.3, 69.7 (C-3'), 84.0, 84.1, 84.5 (C-1'+C-4'), 109.2 (C-5b), 110.6 (C-5), 118.9, 119.0, 119.2, 119.3, 124.2 (***Ph***O), 127.4 (C-5a), 128.8 (***Ph***O), 136.5, 136.6 (C-6), 148.2 (C-4), 149.6, 149.7, 149.8 ('*ipso*', ***Ph***O), 160.3, 160.4 (C-2), 173.7, 173.8, 173.9, 174.1, (***C***$OOCH_3$).

Synthesis of (E)-5-(-bromovinyl)-2'-deoxyuridine-5'-[phenyl-methoxy-di-n-butylglycinyl)]-phosphate. (36u) (CPF189)

$C_{28}H_{39}BrN_3O_9P$, MW=672.50



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), phenyl (methoxy-di-n-butylglycinyl)-phosphorochloridate (507 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (7 mL) for 16 hrs. The crude product was purified twice by flash column chromatography using chloroform/methanol (97/3) as eluent to give the pure product as a white foamy solid (13 mg, 4%).

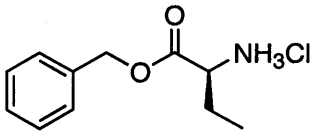
^{31}P-NMR (MeOD, 121 MHz): δ 2.59, 2.56.

^{1}H-NMR (MeOD, 300 MHz): 7.71, 7.76 (1H, 2s, H-6), 7.33-7.08 (6H, m, ***Ph***O+H-5b), 6.77, 6.73 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.22-6.11 (1H, m, H-1'), 4.37-4.10 (3H, m, H-3'+H-5'), 4.10-4.02 (1H, m, H-4'), 3.63, 3.62 (3H, 2d, C***H***$_3$O), 2.26-2.04 (2H, m, one of H-2'+C[C***H***$_2CH_2CH_2CH_3$]), 1.98-1.82 (2H, m, one of H-2'+C[C***H***$_2CH_2CH_2CH_3$]), 1.80-1.44 (2H, m, $C[CH_2CH_2CH_2CH_3]$), 1.33-1.05 (6H, m, $C[CH_2CH_2CH_2CH_3]$), 0.93-0.69 (8H, m, $C[CH_2CH_2CH_2CH_3]$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.7 C[$CH_2CH_2CH_2$**C**H_3]), 24.1 C[CH_2CH_2**C**H_2CH_3]), 27.5 (C[CH_2**C**$H_2CH_2CH_3$]), 39.2, 39.6 C[**C**$H_2CH_2CH_2CH_3$]), 41.2, 41.4 (C-2'), 53.5 (**C**H_3O), 66.3 (**C**[$CH_2CH_2CH_2CH_3$]), 68.2, 68.3, 68.4, 68.5 (C-5'), 72.2, 72.4 (C-3'), 87.0, 87.1, 87.2, 87.3 (C-1'+C-4'), 109.7 (C-5b), 112.8 (C-5), 121.9*, 122.0, 122.1, 126.7 (**Ph**O), 130.9, 131.0, 131.2 (**Ph**O+C-5b), 140.5, 140.6 (C-6), 151.4 (C-4), 152.6, 152.7, 152.8 ('*ipso*', **Ph**O), 164.0 (C-2), 176.3, 176.4 (**C**$OOCH_3$).

Synthesis of L-ethylglycine benzyl ester hydrochloride salt. (37a)

$C_{11}H_{16}ClNO_2$, MW 229.70.



This was synthesised according to *standard procedure 2*, using (S)-(+)-2-amino butyric acid (2.50 g, 24.24 mmol), with p-toluene sulfonic acid monohydrate (4.80 g, 25.21 mmol) and benzyl alcohol (10.1 mL), in toluene (40 mL). The product was isolated as a white solid (4.31 g, yield 77 %).

***p*-toluene sulfonate salt.** ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.35 (3H, bs, N**H**$_3$Tos), 7.50 (2H, d, ^3J=9.0 Hz, '*m*' p-TSA), 7.44-7.36 (5H, m, CH_2**Ph**), 7.13 (2H, d, ^3J=9.0 Hz, '*o*' p-TSA), 5.26 (2H, s, C**H**$_2$Ph), 4.10 (1H, t, ^3J=6.0 Hz, CH_3CH_2C**H**), 2.30 (3H, s, CH_3 *p*-TSA), 1.88-1.78 (2H, m, CH_3C**H**$_2$CH), 0.90 (3H, t, ^3J=6.0 Hz, C**H**$_3CH_2CH$).

^{13}C-NMR (DMSO-d_6; 75 MHz): 9.4 (**C**H_3CH_2CH), 21.2 (CH_3 pTSA), 23.8 (CH_3**C**H_2CH), 53.4 (CH_3CH_2**C**H), 67.5 (Ph**C**H_2), 125.8, 128.5, 128.7, 128.8, 128.9 (pTSA+$PhCH_2$), 135.5 ('*ipso*', $PhCH_2$), 138.1 ('*p*', p-TSA), 145.9 ('*ipso*' pTSA), 169.8 ($COOCH_2Ph$).

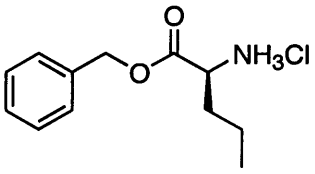
Hydrochloride salt. ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.79 (bs, 3H, N**H**$_3$Cl), 7.45-7.34 (5H, m, **Ph**CH_2), 5.29-5.24 (2H, m, PhC**H**$_2$),

4.01-3.99 (1H, m, $CH_3CH_2C\underline{\boldsymbol{H}}$), 1.93-1.83 (2H, m, $CH_3C\underline{\boldsymbol{H_2}}CH$), 0.89 (3H, t, ^3J=7.5 Hz, $C\underline{\boldsymbol{H_3}}CH_2CH$).

^{13}C-NMR (DMSO-d_6; 75 MHz): 9.4 ($\underline{\boldsymbol{C}}H_3CH_2CH$), 23.7 ($CH_3\underline{\boldsymbol{C}}H_2CH$), 53.3 ($CH_3CH_2\underline{\boldsymbol{C}}H$), 67.3 ($Ph\underline{\boldsymbol{C}}H_2$), 128.6, 128.7, 128.8 ($\underline{\boldsymbol{Ph}}CH_2$), 135.6 ('*ipso*', CH_2Ph), 169.7 ($COOCH_2Ph$).

Synthesis of L-norvaline benzyl ester hydrochloride salt. (37b)

$C_{12}H_{18}ClNO_2$, MW 243.73



This was synthesised according to *standard procedure 2*, using L-norvaline (5.00 g, 42.68 mmol), with p-toluene sulfonic acid monohydrate (8.93 g, 46.95 mmol) and benzyl alcohol (17.8 mL), in toluene (80 mL). The product was isolated as a white solid (8.75 g, yield 92 %).

***p*-toluene sulfonate salt.** ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.37 (3H, bs, $N\underline{\boldsymbol{H_3}}$Tos), 7.51 (2H, d, ^3J=8.0 Hz, '*m*' p-TSA), 7.44-7.36 (5H, m, $\underline{\boldsymbol{Ph}}CH_2$), 7.15 (2H, d, ^3J=9.0 Hz, 'o' p-TSA), 5.26 (2H, s, $PhC\underline{\boldsymbol{H_2}}$), 4.13 (1H, t, ^3J=6.3 Hz, $CH_3(CH_2)_2C\underline{\boldsymbol{H}}$), 2.31 (3H, s, CH_3 *p*-TSA), 1.81-1.70 (2H, m, $CH_3CH_2C\underline{\boldsymbol{H_2}}CH$), 1.44-121 (2H, m, $CH_3C\underline{\boldsymbol{H_2}}CH_2CH$), 0.87 (3H, t, ^3J=7.3 Hz, $C\underline{\boldsymbol{H_3}}(CH_2)_2CH$).

^{13}C-NMR (DMSO-d_6; 75 MHz): 13.8 ($\underline{\boldsymbol{C}}H_3(CH_2)_2CH$), 17.9 ($CH_3\underline{\boldsymbol{C}}H_2CH_2CH$) 21.2 ($CH_3$ pTSA), 32.4 ($CH_3CH_2\underline{\boldsymbol{C}}H_2CH$), 52.2 ($CH_3(CH_2)_2\underline{\boldsymbol{C}}H$), 67.5 ($Ph\underline{\boldsymbol{C}}H_2$), 125.8, 128.5, 128.7, 128.8, 128.9 (pTSA+CH_2Ph), 135.5 ('*ipso*', $PhCH_2$), 138.1 ('*p*', p-TSA), 145.9 ('*ipso*' pTSA), 169.9 ($COOCH_2Ph$).

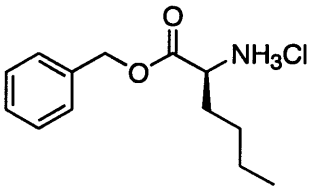
Hydrochloride salt. ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.73 (3H, bs, $N\underline{\boldsymbol{H_3}}Cl$), 7.43-7.34 (5H, m, $\underline{\boldsymbol{Ph}}CH_2$), 5.25-5.24 (2H, m, $PhC\underline{\boldsymbol{H_2}}$), 4.08-4.02 (1H, m, CH_3 $C\underline{\boldsymbol{H}}(CH_2)_2CH_3$), 1.84-1.77 (2H, m,

$CH_3CH_2C\underline{\boldsymbol{H_2}}CH$), 1.48-1.20 (2H, m, $CH_3C\underline{\boldsymbol{H_2}}CH_2CH$), 0.86 (3H, t, ^3J=7.3 Hz, $C\underline{\boldsymbol{H_3}}(CH_2)_2CH$).

^{13}C-NMR (DMSO-d_6; 75 MHz): 13.8 ($\underline{\boldsymbol{C}}H_3(CH_2)_2CH$), 17.9 ($CH_3\underline{\boldsymbol{C}}H_2CH_2CH$), 32.4 ($CH_3CH_2\underline{\boldsymbol{C}}H_2CH$), 52.1 ($CH_3CH_2CH_2\underline{\boldsymbol{C}}H$), 67.4 ($Ph\underline{\boldsymbol{C}}H_2$), 128.6, 128.7, 128.8 ($\underline{\boldsymbol{Ph}}CH_2$), 135.6 ('*ipso*', $PhCH_2$), 169.8 ($COOCH_2Ph$).

Synthesis of L-norleucine benzyl ester hydrochloride salt. (37c)

$C_{13}H_{20}ClNO_2$, MW 257.76



This was synthesised according to *standard procedure 2*, using L-norleucine (2.500 g, 19.06 mmol), with p-toluene sulfonic acid monohydrate (3.988 g, 20.97 mmol) and benzyl alcohol (7.9 mL), in toluene (40 mL). The product was isolated as a white solid (4.509 g, yield 89.2 %).

***p*-toluene sulfonate salt.** ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.34 (3H, bs, $N\underline{\boldsymbol{H_3}}Tos$), 7.50 (2H, d, ^3J=8.0 Hz, '*m*' p-TSA), 7.43-7.37 (5H, m, $CH_2\underline{\boldsymbol{Ph}}$), 7.13 (2H, d, ^3J=8.0 Hz, '*o*' p-TSA), 5.23, 5.28 (2H, AB system, ^2J= 12.3 Hz, $C\underline{\boldsymbol{H_2}}Ph$), 4.11 (1H, t, ^3J=6.1 Hz, $CH_3(CH_2)_3C\underline{\boldsymbol{H}}$), 2.29 (3H, s, CH_3 *p*-TSA), 1.81-1.74 (2H, m, $CH_3(CH_2)_2C\underline{\boldsymbol{H_2}}CH$), 1.36-1.19 (4H, m, $CH_3(C\underline{\boldsymbol{H_2}})_2CH_2CH$), 0.82 (3H, t, ^3J=7.1 Hz, $C\underline{\boldsymbol{H_3}}(CH_2)_3CH$).

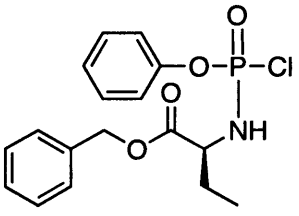
^{13}C-NMR (DMSO-d_6; 75 MHz): 13.8 ($\underline{\boldsymbol{C}}H_3(CH_2)_2CH$), 17.9 ($CH_3\underline{\boldsymbol{C}}H_2CH_2CH$) 21.2 ($CH_3$ pTSA), 22.0 ($CH_3\underline{\boldsymbol{C}}H_2(CH_2)_2CH$), 26.5 ($CH_3CH_2\underline{\boldsymbol{C}}H_2CH_2CH$), 30.1 ($CH_3(CH_2)_2\underline{\boldsymbol{C}}H_2CH$), 52.3 ($CH_3(CH_2)_3\underline{\boldsymbol{C}}H$), 67.5 ($\underline{\boldsymbol{C}}H_2Ph$), 125.8, 128.7, 128.8, 128.9 (pTSA+CH_2Ph), 135.5 ('*ipso*', CH_2Ph), 138.1 ('*p*', p-TSA), 145.9 ('*ipso*' pTSA), 169.9 ($COOCH_2Ph$).

Hydrochloride salt. ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.77 (3H, bs, N***H₃***Cl), 7.42-7.36 (5H, m, ***Ph***CH_2), 5.25, 5.21 (2H, AB system, ^{2}J= 12.3 Hz, PhC***H₂***), 4.02 (1H, m, $CH_3(CH_2)_3C$***H***), 1.86-1.79 (2H, m, $CH_3(CH_2)_2C$***H₂***CH), 1.36-1.15 (4H, m, $CH_3(C$***H₂***$)_2CH_2CH$), 0.81 (3H, t, ^{3}J=7.0 Hz, C***H₃***$(CH_2)_3CH$).

^{13}C-NMR (DMSO-d_6; 75 MHz): 13.9 (***C***$H_3(CH_2)_3CH$), 22.0 (CH_3***C***$H_2(CH_2)_2CH$), 26.5 (CH_3CH_2***C***H_2CH_2CH), 30.0 ($CH_3(CH_2)_2$***C***H_2CH), 52.2 ($CH_3(CH_2)_3$***C***H), 67.3 (Ph***C***H_2), 128.6, 128.7, 128.8 (***Ph***CH_2), 135.6 (*'ipso'*, $PhCH_2$), 169.9 ($COOCH_2Ph$).

Synthesis of phenyl-(benzoxy-L-ethylglycinyl)-phosphorochloridate. (38a)

$C_{17}H_{19}NO_4P$, MW=367.76



This was synthesised according to *Standard procedure 4,* using L-ethylglycine benzyl ester hydrochloride (403 mg, 1.76 mmol), phenyl-phosphorochloridate (370 mg, 1.76 mmol, 262 μL), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL), to yield 606 mg (94%) of crude product used without further purification.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.74, 9.67.

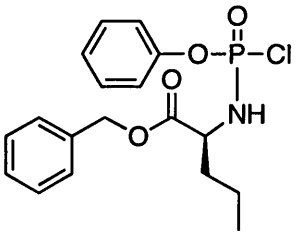
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.27 (10H, m, ***Ph***O+***Ph***CH_2), 5.27-5.25 (2H, m, PhC***H₂***), 4.59-4.49 (1H, bs, N***H***), 4.24-4.14 (1H, m, C***H***CH_2CH_3), 2.04-1.80 (2H, m, CHC***H₂***CH_3), 1.00, 0.99 (3H, 2t, ^{3}J=7.4 Hz, $CHCH_2C$***H₃***).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 9.5, 9.6 ($CHCH_2$***C***H_3), 27.6, 27.7 (CH***C***H_2CH_3), 56.2, 56.5 (***C***HCH_2CH_3), 67.9, 68.0 (Ph***C***H_2), 120.7, 120.9*, 121.0, 126.4, 128.8, 128.9, 129.0, 129.1*, 130.1, 130.3

(***Ph***O+***Ph***CH_2), 135.4, 135.5 (*'ipso'*, ***Ph***CH_2), 150.1, 150.2* (*'ipso'*, ***Ph***O), 172.2, 172.3, 172.5* (***C***$OOCH_2Ph$).

Synthesis of phenyl-(benzoxy-L-propylglycinyl)-phosphorochloridate. (38b)

$C_{18}H_{21}NO_4P$, MW=381.79



This was synthesised according to *Standard procedure 4,* using L-norvaline benzyl ester hydrochloride (929 mg, 2.35 mmol), phenyl-phosphorodichloridate (496 mg, 2.35 mmol, 351 μL), and TEA (476 mg, 4.70 mmol, 655 μL) in DCM (20 mL). After 16 hrs, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7:3) as eluent to yield 744 mg (82%) of a colourless oil.

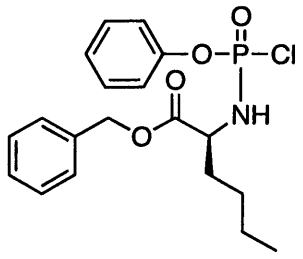
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.74, 9.67.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.34-7.19 (10H, m, ***Ph***O+***Ph***CH_2), 5.19, 5.17 (2H, m, PhC***H***$_2$), 4.43-4.31 (1H, bs, NH), 4.21-4.06 (1H, m, C***H***$CH_2CH_2CH_3$), 1.87-1.63 (2H, m, CHC***H***$_2CH_2CH_3$), 1.46-1.28 (2H, m, $CHCH_2$C***H***$_2CH_3$), 0.89 (3H, t, ^{3}J=7.3 Hz, $CHCH_2CH_2$C***H***$_3$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.0 ($CHCH_2CH_2$***C***H_3), 18.5, 18.6 ($CHCH_2$***C***H_2CH_3), 36.4, 36.5 (CH***C***$H_2CH_2CH_3$), 54.9, 55.4 (***C***$HCH_2CH_2CH_3$), 67.9, 68.0 (Ph***C***H_2), 120.9*, 121.0*, 126.4, 128.8, 128.9*, 129.0, 129.1*, 130.1, 130.3* (***Ph***O+***Ph***CH_2), 135.4, 135.5 (*'ipso'*, ***Ph***CH_2), 150.1, 150.3 (*'ipso'*, ***Ph***O), 172.5, 172.6, 172.7* (***C***$OOCH_2Ph$).

Synthesis of phenyl-(benzoxy-L-butylglycinyl)-phosphorochloridate. (38c)

$C_{19}H_{23}NO_4P$, MW=395.82



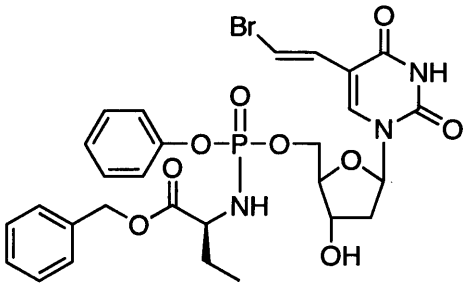
This was synthesised according to *Standard procedure 4,* using L-norleucine benzyl ester hydrochloride (1000 mg, 3.88 mmol), phenyl phosphorodichloridate (819 mg, 3.88 mmol, 580 μL), and TEA (785 mg, 7.76 mmol, 1082 μL) in DCM (40 mL). After 16 hrs, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/'3) to yield 1382 mg (90 %) of a colourless oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.74, 9.57.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.27 (10H, m, ***Ph***O+***Ph***CH_2), 5.28-5.23 (2H, m, PhC***H$_2$***), 4.49-4.37 (1H, m, NH), 4.27-4.15 (1H, m, C***H***$CH_2CH_2CH_2CH_3$), 1.97-1.73 (2H, m, CHC***H$_2$***$CH_2CH_2CH_3$), 1.50-1.29 (4H, m, $CHCH_2C$***H$_2$***C***H$_2$***CH_3), 0.93-0.89 (3H, m, $CHCH_2CH_2CH_2C$***H$_3$***).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 14.2 ($CHCH_2CH_2CH_2$***C***H_3), 22.6 ($CHCH_2CH_2$***C***H_2CH_3), 27.2* ($CHCH_2$***C***$H_2CH_2CH_3$), 34.1, 34.2 (CH***C***$H_2CH_2CH_2CH_3$), 55.1, 55.5 (***C***$HCH_2CH_2CH_2CH_3$), 67.8, 68.0 (Ph***C***H_2), 120.9, 121.0*, 126.4, 128.8, 128.9, 129.0, 129.1*, 130.3*, 130.7 (***Ph***O+***Ph***CH_2), 135.5, 135.6 (*'ipso'*, ***Ph***CH_2), 150.1, 150.2*, 150.3 (*'ipso'*, ***Ph***O), 172.5*, 172.6, 172.7 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-benzoxy-L-ethylglycinyl)]-phosphate. (39a) (CPF93)
$C_{28}H_{31}BrN_3O_9P$, MW=664.44



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), phenyl-(benzoxy-L-ethylglycinyl)-phosphorochloridate (497 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179.4 μL) in THF (5 mL) for 12 hrs. The crude product was purified by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (168 mg, 56%).

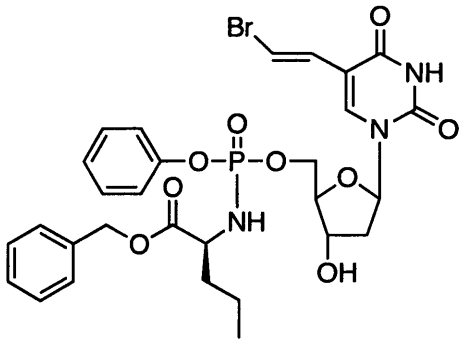
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.24, 4.73.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.78 (1H, bs, H-3), 7.64, 7.63 (1H, 2s, H-6), 7.47, 7.46 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.38-7.17 (10H, m, ***Ph***CH_2+***Ph***O), 6.76, 6.71 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.32-6.23 (1H, m, H-1'), 5.23-5.11 (2H, m, PhC***H$_2$***), 4.53-3.89 (7H, m, H-3'+H-5'+H-4'+OH+NH+C***H***CH_2CH_3), 2.46-2.38 (1H, m, one of H-2'), 2.09-1.86 (2H, m, one of H-2'), 1.79-1.69 (2H, m, CHC***H$_2$***CH_3), 0.87 (3H, t, ^{3}J=7.3 Hz, $CHCH_2C$***H$_3$***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 9.7, 9.8 ($CHCH_2$***C***H_3), 27.9, 28.0, 28.1 (CH***C***H_2CH_3), 40.7, 40.8 (C-2'), 56.2, 56.3 (***C***HCH_2CH_3), 66.1, 66.4 (C-5'), 67.8 (Ph***C***H_2), 70.7, 71.1 (C-3'), 85.5, 85.6, 85.9 (C-1', C-4'), 110.5 (C-5b), 111.9 (C-5), 120.5, 120.6*, 125.7, 125.8*, 128.8, 128.9, 129.0*, 129.1, 130.3 (***Ph***O+***Ph***CH_2+C-5a), 135.4, 135.6 ('*ipso*', ***Ph***CH_2), 138.0 (C-6), 149.8, 150.7* (C-4+'*ipso*' ***Ph***O), 162.0 (C-2), 173.5* (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-benzoxy-L-propylglycinyl)]-phosphate. (39b) (CPF178)

$C_{29}H_{33}BrN_3O_9P$, MW=678.46



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), phenyl-(benzoxy-L-propylglycinyl)-phosphorochloridate (515 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (5 mL) for 16 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (93 mg, 31%).

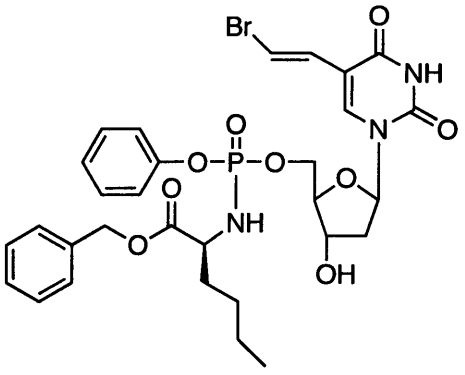
^{31}P-NMR (MeOD, 121 MHz): δ 5.94, 5.33.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.45 (1H, bs, H-3), 7.64 (1H, s, H-6), 7.47, 7.46 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.39-7.18 (10H, m, ***Ph***CH_2+***Ph***O), 6.77, 6.73 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.30, 6.25 (1H, 2t, ^{3}J=6.4 Hz, H-1'), 5.21-5.12 (2H, m, ***Ph***CH_2), 4.55-3.74 (6H, m, H-3'+H-5'+H-4'+OH+NH+C***H***$CH_2CH_2CH_3$), 2.47-2.38 (1H, m, one of H-2'), 2.10-1.98 (1H, m, one of H-2'), 1.80-1.58 (2H, m, CHC***H$_2$***CH_2CH_3), 1.37-1.23 (2H, m, $CHCH_2C$***H$_2$***CH_3), 0.86 (3H, t, ^{3}J=7.3 Hz, $CHCH_2CH_2C$***H$_3$***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 13.9, 14.0 ($CHCH_2CH_2$***C***H_3), 18.6, 18.7 ($CHCH_2$***C***H_2CH_3), 36.7, 36.6, 36.9 (CH***C***$H_2CH_2CH_3$), 40.7, 40.8 (C-2'), 55.0, 55.1 (***C***$HCH_2CH_2CH_3$), 66.1*, 66.4, 66.5 (C-5'), 67.8 (Ph***C***H_2), 70.7, 71.1 (C-3'), 85.4, 85.5, 85.6, 85.9 (C-1'+C-4'), 110.5 (C-5b), 111.9 (C-5), 120.4, 120.5*, 120.6, 125.7, 125.8*, 128.8, 128.9, 129.0*, 129.1*, 130.3 (***Ph***O+***Ph***CH_2+C-5a), 135.5, 135.6 ('*ipso*', ***Ph***CH_2), 138.0, 138.1 (C-6), 149.7* (C-4), 150.7*, 150.8 ('*ipso*', ***Ph***O), 162.0 (C-2), 173.7*, 173.8 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-benzoxy-L-butylglycinyl)]-phosphate. (39c) (CPF179)

$C_{30}H_{35}BrN_3O_9P$, MW=692.49



This was synthesised according to *Standard procedure 5*, using BVdU (200 mg, 0.60 mmol), phenyl-(benzoxy-L-butylglycinyl)-phosphorochloridate (356 mg, 0.90 mmol), NMI (246 mg, 3.00 mmol, 232 μL) in THF (10 mL) for 4 hrs. The crude product was purified twice by silica column chromatography, eluting with dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (161 mg, 39%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 5.18, 4.56.

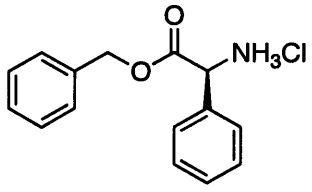
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.76 (1H, bs, H-3), 7.64 (1H, s, H-6), 7.47, 7.46 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.42-7.17 (10H, m, ***Ph***CH_2+***Ph***O), 6.77, 6.73 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.31, 6.25 (1H, 2t, ^{3}J=6.4 Hz, H-1'), 5.23-5.11 (2H, m, ***Ph***CH_2), 4.55-4.23 (3H, m, H-3'+H-5'), 4.12-3.93 (3H, m, H-4'+C***H***$CH_2CH_2CH_2CH_3$, NH), 2.48-2.38 (1H, m, one of H-2'), 2.11-1.97 (1H, m, one of H-2'), 1.73-1.67 (2H, m, CHC***H$_2$***$CH_2CH_2CH_3$), 1.36-1.21 (4H, m, $CHCH_2C$***H$_2$***C***H$_2$***CH_3), 0.86-0.70 (3H, m, $CHCH_2CH_2C$***H$_3$***).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 14.2 ($CHCH_2CH_2CH_2$***C***H_3), 22.5* ($CHCH_2CH_2$***C***H_2CH_3), 27.3, 27.4 ($CHCH_2$***C***$H_2CH_2CH_3$), 34.3, 34.5, 34.6 (CH***C***$H_2CH_2CH_2CH_3$), 40.7, 40.8 (C-2'), 55.1, 55.3 (***C***$HCH_2CH_2CH_2CH_3$), 66.1, 66.3, 66.4 (C-5'), 67.8 (Ph***C***H_2), 70.6, 71.1 (C-3'), 85.4, 85.5, 85.6, 85.9 (C-1'+C-4'), 110.5 (C-5b), 111.9 (C-5), 120.4, 120.5, 120.6 ('*o*', ***Ph***O), 125.7, 125.8, 128.8, 128.9, 129.0*, 129.1, 130.3 (***Ph***O+***Ph***CH_2+C-5a), 135.5, 135.6

('*ipso*', ***Ph***CH_2), 138.0, 138.1 (C-6), 149.7* (C-4), 150.7, 150.8 ('*ipso*', ***Ph***O), 161.9 (C-2), 173.6, 173.7* (***C***$OOCH_2Ph$).

Synthesis of L-phenylglycine benzyl ester hydrochloride salt. (40)

$C_{15}H_{16}ClNO_2$, MW= 277.75



This was synthesised according to *Standard Procedure 2*, (S)-(+)-2-phenyl glycine (10.10 g, 66.15) with *p*-toluene sulfonic acid monohydrate (13.09 g, 68.82 mmol) and benzyl alcohol (29.05 g, 268.6 mmol, 27.8 mL), in toluene (120 mL). The product was isolated as a white solid (6.90 g, 89%).

p*-toluenesulfonate salt.** ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.90 (3H, bs, NH***$_3$ *p*-TSA), 7.52-7.11 (10H, m, *p*-TSA+***Ph***CH_2+***Ph***O), 5.41 (1H, s, C***H***Ph), 5.27, 5.22 (2H, AB system, ^2J= 12.4 Hz, PhC***H***$_2$), 2.30 (3H, s, C***H***$_3$ *p*-TSA)

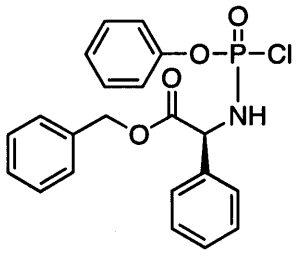
^{13}C-NMR (DMSO-d_6; 75 MHz): δ 21.1 (***C***H_3, *p*-TSA), 55.7 (***C***HPh), 67.7 (Ph***C***H_2), 125.6, 128.2, 128.4, 128.6, 128.7, 128.8, 129.4, 130.0 (***Ph***CH_2+*p*-TSA+CH***Ph***), 132.8, 135.4, 138.0 ('*ipso*', p-TSA+CH***Ph***+ ***Ph***CH_2), 146.0 ('*ipso*', *p*-TSA), 168.7 (***C***$OOCH_2Ph$).

Hydrochloride salt. ^{1}H-NMR (DMSO-d_6; 300 MHz): δ 9.22 (3H, bs, N***H***$_3$Cl), 7.52-7.19 (10H, m, CH***Ph***+***Ph***CH_2), 5.30 (1H, s, C***H***Ph), 5.22, 5.18 (2H, AB system, ^2J=12.6 Hz, Ph***CH***$_2$), 1.77 (C[C***H***$_3$]$_2$).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 55.7 (***C***$[CH_3]_2$), 67.5 (Ph***C***H_2), 128.1, 128.6, 128.7, 129.3, 129.9 (CH***Ph***+***Ph***CH_2), 132.9, 135.9 ('*ipso*', CH***Ph***+***Ph***CH_2), 168.6 (***C***$OOCH_2Ph$).

Synthesis of phenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate. (41a)

$C_{21}H_{19}ClNO_4P$, MW=415.81



This was synthesised according to *Standard procedure 4,* using L-phenylglycine benzyl ester hydrochloride (488 mg, 1.76 mmol), phenyl-phosphorodichloridate (370 mg, 1.76 mmol, 262 μL), and TEA (356 mg, 3.52 mmol, 489 μL) in DCM (15 mL), to yield 857 mg (86%) of crude product used without further purification.

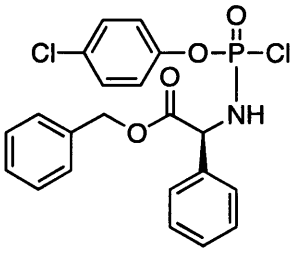
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.49, 8.10.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.50-7.11 (15H, m, ***Ph***O+***Ph***CH_2+CH***Ph***), 5.36-5.06 (3H, m, PhC***H$_2$***+C***H***Ph).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 59.1* (***C***HPh), 68.3, 68.4 (Ph***C***H_2), 120.7, 120.8, 127.5, 127.6, 127.7, 128.5*, 128.9*, 129.0, 129.2, 129.3*, 129.4, 130.3*, 130.4 (***Ph***O+***Ph***CH_2+CH***Ph***), 135.2, 135.3, 137.4 (*'ipso'*, CH_2***Ph***+CH***Ph***), 150.0, 150.1*, 150.2 (*'ipso'*, ***Ph***O), 170.7, 170.9, 171.0 (***C***OOCH_2Ph).

Synthesis of 4-chlorophenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate. (41b)

$C_{21}H_{19}Cl_2NO_4P$, MW=450.25



This was synthesised according to *Standard procedure 4,* using L-phenylglycine benzyl ester hydrochloride (533 mg, 1.92 mmol), 4-chlorophenyl-phosphorodichloridate (471 mg, 1.76 mmol), and TEA (389 mg, 3.84 mmol, 535 μL) in DCM (15 mL), to yield 821 mg (95 %) of crude product used without

further purification.

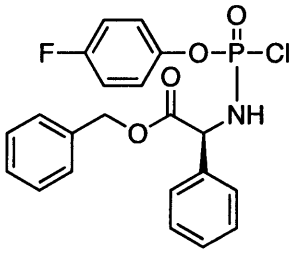
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.85, 8.51.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.46-7.10 (14H, m, *p*-Cl***Ph***O+***Ph***CH_2+CH***Ph***), 5.32-5.02 (3H, m, C***H₂***Ph+C***H***Ph).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 58.7, 58.8, 59.0, 59.1 (***C***HPh), 68.4, 68.5 (Ph***C***H_2), 122.0, 122.1, 122.3 ('*o*', *p*-Cl***Ph***O), 127.5, 127.6, 128.3, 128.4, 128.5, 129.0, 129.2, 129.3, 129.4, 130.3, 130.4 (*p*-Cl***Ph***O+***Ph***CH_2+CH***Ph***), 135.1, 136.5 ('*ipso*', ***Ph***CH_2+CH***Ph***), 148.6, 148.7 ('*ipso*', *p*-Cl***Ph***O), 172.4, 172.6 (***C***$OOCH_2$Ph).

Synthesis of 4-fluorophenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate. (41c)

$C_{21}H_{18}ClFNO_4P$, MW=433.80



This was synthesised according to *Standard procedure 4,* using L-phenylglycine benzyl ester hydrochloride (488 mg, 1.76 mmol), 4-fluorophenyl-phosphorodichloridate (402 mg, 1.76 mmol), and TEA (355 mg, 3.51 mmol, 489 μL) in DCM (15 mL), to yield 771 mg (>100%) of crude product used without further purification.

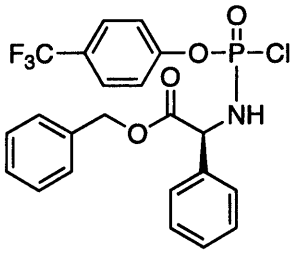
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.12 (d, $^{6}J_{P-F}$ =2.67 Hz), 8.74 (d, $^{6}J_{P-F}$ =2.80 Hz).

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.33-6.91 (14H, m, *p*-F***Ph***O+***Ph***CH_2+CH***Ph***), 5.27-4.91 (3H, m, PhC***H₂***+C***H***Ph).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 59.1, 59.2 (***C***HPh), 68.4, 68.5 (Ph***C***H_2), 117.0 ('*m*', ^{3}J=25 Hz, *p*-F***Ph***O), 122.3, 122.4, 122.6, 127.6, 129.2, 129.4 (*p*-F***Ph***O+***Ph***CH_2+CH***Ph***), 136.6, 135.1 ('*ipso*', ***Ph***CH_2+CH***Ph***), 145.3, 145.9 ('*ipso*', *p*-F***Ph***O), 162.3 ('*p*', ^{1}J=244 Hz, *p*-F***Ph***O), 170.6 (***C***$OOCH_2$Ph).

Synthesis of 4-trifluoromethylphenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate. (41d)

$C_{22}H_{18}ClF_3NO_4P$, MW=483.80



This was synthesised according to *Standard procedure 4,* using L-phenylglycine benzyl ester hydrochloride (489 mg, 1.76 mmol), *p*-trifluoromethylphenyl-phosphorochloridate (491 mg, 1.76 mmol), and TEA (356 mg, 3.52 mmol, 491 μL) in DCM (15 mL), to yield 700 mg (yield 82%) of crude product used without further purification.

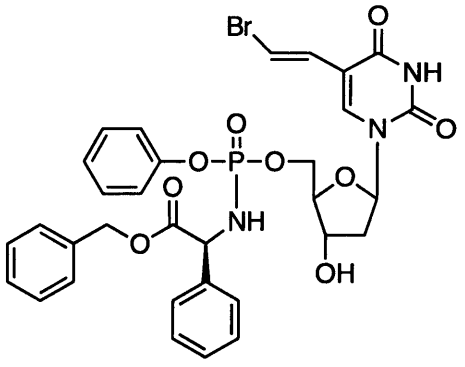
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 8.51, 8.21.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.69-7.09 (14H, m, *p*-CF_3***Ph***O+***Ph***CH_2+ C***H***Ph), 5.29-5.11 (3H, m, PhC***H$_2$***+C***H***Ph).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 58.8, 59.1 (***C***HPh), 68.5, 68.6 (Ph***C***H_2), 121.0, 121.1, 121.4 (*'o'*, *p*-CF_3***Ph***O), 125.3 (***C***F_3, *J*=265 Hz), 127.4, 127.5, 127.6, 127.7, 128.3, 128.4, 128.5, 128.8, 128.9, 129.0, 129.2, 129.3, 129.4, 129.5 (*p*-CF_3***Ph***O+***Ph***CH_2+CH***Ph***), 135.0, 137.2 (*'ipso'*, CH_2***Ph***+CH***Ph***), 156.1 (*'ipso'*, *p*-CF_3***Ph***O), 171.3, 171.4 (***C***$OOCH_2$Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[phenyl-(benzoxy-L-phenylglycinyl)]-phosphate. (42a) (CPF88)

$C_{32}H_{31}BrN_3O_9P$, MW=712.48



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), phenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate (561 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (7 mL) for 15 hrs. The crude product was purified by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (83 mg, 26%).

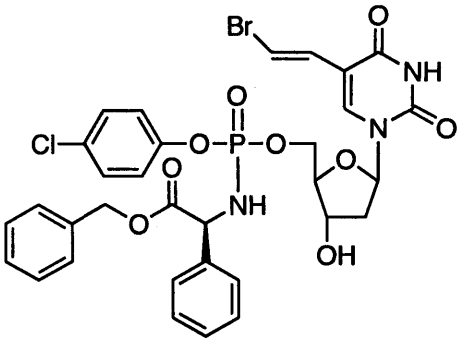
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.29, 4.17.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.60-9.53 (1H, 2bs, H-3), 7.56, 7.54 (1H, 2s, H-6), 7.47, 7.46 (1H, 2d, ^{3}J=13.6 Hz, H-5b), 7.37-7.12 (15H, m, ***Ph***CH_2+***Ph***O+CH***Ph***), 6.73, 6.72 (1H, 2d, ^{3}J=13.6 Hz, H-5a), 6.24-6.18 (1H, m, H-1'), 5.21-5.09 (2H, m, PhC***H_2***), 4.94-4.82 (1H, m, C***H***Ph), 4.45-3.61 (5H, m, H-3'+H-5'+H-4'+N***H***), 2.38-2.34 (1H, m, one of H-2'), 1.98-1.89 (2H, m, one of H-2').

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 40.6, 40.8 (C-2'), 58.8 (***C***HPh), 66.5 (C-5'), 68.2 (Ph***C***H_2), 70.4, 71.0 (C-3'), 85.3, 85.4, 85.6, 86.0 (C-1'+C-4'), 110.4, 110.5 (C-5b), 111.9 (C-5), 120.4, 120.5, 120.6 ('*o*', ***Ph***O), 125.8, 127.3, 127.4, 128.4, 128.9, 129.0, 129.1, 129.1, 129.2, 129.4, 129.5, 130.2, 130.3 (CH***Ph***CH+***Ph***O+***Ph***CH_2+C-5a), 135.3, 137.7, 137.8 ('*ipso*', ***Ph***CH_2+CH***Ph***), 137.9 (C-6), 149.5 (C-4), 150.4, 150.5 ('*ipso*', ***Ph***O), 161.7 (C-2), 171.6, 171.7 (***C***OOCH_2Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-chlorophenyl-(benzoxy-L-phenylglycinyl)]-phosphate. (42b) (CPF89)

$C_{32}H_{30}BrClN_3O_9P$, MW=746.93



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-chlorophenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate (608 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (7 mL) for 20 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (210 mg, 63%).

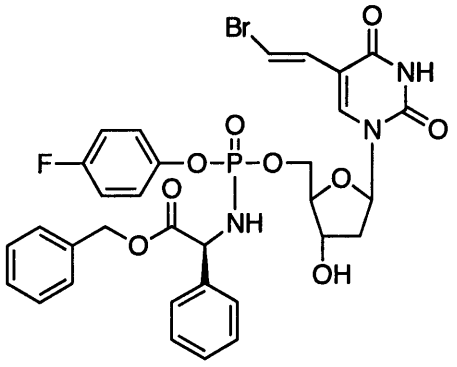
^{31}P-NMR (DMSO-d_6, 121 MHz): δ 5.24.

^{1}H-NMR (DMSO-d_6, 300 MHz): δ 11.62 (1H, s, H-3), 7.82-7.78 (1H, 2s, H-6), 7.82-7.12 (14H, m, CH***Ph***+***Ph***CH_2+*p*-Cl***Ph***O+H-5b), 6.84-6.91 (2H, m, H-5a+NH), 6.15 (1H, t, ^{3}J=6.8 Hz, H-1'), 5.43 (1H, d, ^{3}J=4.3 Hz, OH-3'), 5.10-5.09 (2H, 2s, PhC***H₂***), 5.05-5.02 (1H, m, C***H***Ph), 4.21-4.11 (3H, m, H-3'+H-5'), 4.09-3.95 (1H, m, H-4'), 2.13-2.06 (2H, m, H-2').

^{13}C-NMR (DMSO-d_6, 75 MHz): δ 39.5, 39.6 (C-2'), 58.4 (***C***HPh), 66.7 (C-5'), 70.4 (***C***H_2Ph), 70.4 (C-3'), 85.0, 85.1, 85.2 (C-1'+C-4'), 107.3 (C-5b), 110.4 (C-5), 122.3, 122.4 ('*o*', *p*-Cl***Ph***O), 127.4, 127.5, 127.9, 128.0, 128.4, 128.7, 128.8, 129.1, 129.7, 130.2, (CH***Ph***+*p*-Cl***Ph***O+***Ph***CH_2+C-5a), 135.9, 138.2, 138.3 ('*ipso*', ***Ph***CH_2+CH***Ph***), 139.5 (C-6), 149.5, 149.6 ('*ipso*', *p*-Cl***Ph***O+C-4), 162.0 (C-2), 171.1, 171.2 (***C***OOCH_2Ph).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-fluorophenyl-(benzoxy-L-phenylglycinyl)]-phosphate. (42c) (CPF90)

$C_{32}H_{30}BrClN_3O_9P$, MW=730.47



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-fluorophenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate (586 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (6 mL) for 15 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (187 mg, 57%).

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 4.62 (d, $^6J_{P\text{-}F}$=1.82 Hz), 4.49 (d, $^6J_{P\text{-}F}$ =1.88 Hz).

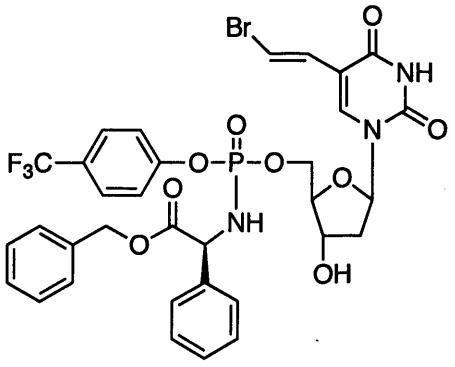
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.61 (1H, s, H-3), 7.61, 7.58 (1H, 2s, H-6), 7.46, 7.45 (1H, 2d, ^3J=13.6 Hz, H-5b). 7.35-6.90 (14H, m, CH***Ph***+***Ph***CH_2+*p*-F***Ph***O), 6.74 (1H, d, ^3J=13.6 Hz, H-5a), 6.26-6.19 (1H, m, H-1'), 5.21-5.06 (3H, m, PhC***H$_2$***+C***H***Ph), 4.94-4.82 (1H, m, N***H***), 4.56-4.20 (3H, m, H-3'+H-5'), 4.12-4.01 (1H, m, H-4'), 3.79-3.59 (1H, bs, OH-3'), 2.45-2.37 (1H, m, one of H-2'), 2.06-1.96 (1H, m, one of H-2').

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 40.7, 40.8 (C-2'), 58.8 (***C***HPh), 66.0, 66.5, 66.6 (C-5'), 68.2 (***C***H_2Ph), 70.5, 71.1 (C-3'), 85.3, 85.4, 85.7, 86.1 (C-1', C-4'), 110.4, 110.5 (C-5b), 111.9 (C-5), 116.8 ('*m*', *p*-F***Ph***O, ^2J=24 Hz), 122.0, 122.1 ('*o*', *p*-F***Ph***O, ^3J=5 Hz)), 127.3, 127.4, 128.4, 128.8, 128.9, 129.0, 129.1, 129.2, 129.4, 129.5 (CH***Ph***+*p*-F***Ph***O+***Ph***CH_2+C-5a), 135.2, 137.6, 137.7 ('*ipso*', ***Ph***CH_2+CH***Ph***), 138.0 (C-6), 146.3, 146.4 ('*ipso*', *p*-F***Ph***O) 149.6

(C-4), 160.2 ('*p*', ^1J=244 Hz, *p*-F***Ph***O), 161.8 (C-2), 171.4, 171.5*, 171.6 (***C***$OOCH_2Ph$).

Synthesis of (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-[4-trifluoromethylphenyl-(benzoxy-L-phenylglycinyl)]-phosphate. (42d) (CPF91)

$C_{33}H_{30}BrF_3N_3O_9P$, MW=746.93



This was synthesised according to *Standard procedure 5*, using BVdU (150 mg, 0.45 mmol), 4-trifluoromethylphenyl-(benzoxy-L-phenylglycinyl)-phosphorochloridate (653 mg, 1.35 mmol), NMI (185 mg, 2.25 mmol, 179 μL) in THF (7 mL) for 15 hrs. The crude product was purified twice by flash column chromatography, using dichloromethane/methanol (97/3) to give the pure product as a white foamy solid (151 mg, 51%).

^{31}P-NMR (DMSO-d_6, 121 MHz): δ 5.24.

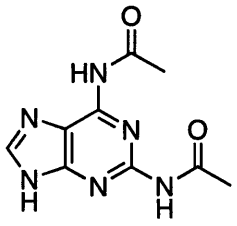
^{1}H-NMR (DMSO-d_6, 300 MHz): δ 11.62 (1H, s, H-3), 7.82-7.79 (1H, 2s, H-6), 7.67-7.64 (1H, d, ^3J=8.6 Hz, *p*-CF_3***Ph***O), 7.40-7.19 (13H, m, CH***Ph***+CH_2***Ph***+O***Ph***), 6.93 (1H, t, J=11.5 Hz, NH), 6.86 (1H, d, ^3J=13.6 Hz, H-5a), 6.18-6.14 (1H, m, H-1'), 5.43 (1H, d, OH-3'), 5.09 (2H, s, C***H*****$_2$**Ph), 5.08-5.04 (1H, m, C***H***Ph), 4.24-4.15 (3H, m, H-3'+H-5'), 4.13-3.97 (1H, m, H-4'), 2.14-2.07 (2H, m, H-2').

^{13}C-NMR (DMSO-d_6, 75 MHz): δ 39.5 (C-2'), 58.4, 58.5 (***C***HPh), 66.7 (C-5'+***C***H_2Ph), 70.4 (C-3'), 84.9, 85.0 (C-1'+C-4'), 107.3 (C-5b), 110.4 (C-5), 121.2, 121.3, 121.4 ('*o*', *p*-CF_3***Ph***O), 123.9 (***C***F_3, J=240, *p*-CF_3***Ph***O), 126.0 ('*p*', J=32.2 Hz, *p*-CF_3***Ph***O), 127.3, 127.4, 127.5, 127.9, 128.0, 128.4, 128.7* (CH***Ph***+*p*-

CF_3***Ph***O+***Ph***CH_2), 130.2 (C-5a), 135.9, 138.1, 138.2 (*'ipso'*, ***Ph***CH_2+CH***Ph***), 139.5 (C-6), 149.5, 153.7 (*'ipso'*, *p*-CF_3***Ph***O+C-4), 162.0 (C-2), 171.1, 171.2 (***C***$OOCH_2Ph$).

Synthesis of 2,6-diacetoamidopurine.[6] (45)

$C_9H_{10}N_6O_2$, MW=234.21



A solution of 2,6-diaminopurine (5,00 g, 33.3 mmol) in a mixture of pyridine (74 mL) and acetic anhydride (10.80 g, 9.9 mL, 105.5 mmol) was refluxed for 3 hrs. On cooling overnight, the solution deposited a solid which was collected by filtration and washed with pyridine, ethanol and then ether before it was dried overnight. The solid was stirred with 25 mL of saturated solution of $NaHCO_3$ for 0.5 hrs, diluted with water (25 mL) and stirred for 10 mins, collected by filtration and washed with water until all bicarbonate was removed. The solid was dried for 2 days over phosphorus pentoxide *in vacuo* at 60 °C to give 5.50 g of white powder (71%).

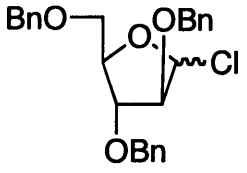
^{1}H-NMR (DMSO-d_6; 300 MHz): δ 10.20 (1H, bs, H-9), 8.34 (1H, s, H-8), 2.29, 2.22 (6H, 2xs, C***H***$_3$CO).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 24.1, 24.7 (***C***H_3CO), 112.5 (C-adenine), 145.3 (C-adenine), 146.0 (C-8), 152.1 (C-adenine), 169.5, 170.7 (2x***C***OCH_3).

MS [ES (+ve)] found 257.7 ($[MNa]^+$), required $C_9H_{10}N_6NaO_2$ 257.20.

Synthesis of 2,3,5-tri-O-benzyl-α- and β-D-arabinofuranosyl chlorides.[7] (47)

$C_{26}H_{27}ClO_4$, MW=438.94

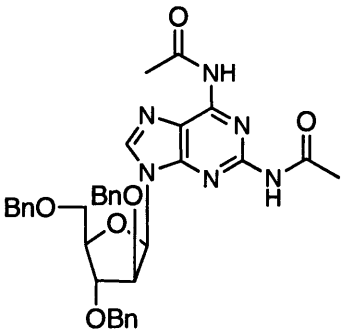


2,3,5-tri-O-benzyl-D-arabinofuranosylfuranose (1.0 g, 2.38 mmol) was co-evaporated twice with toluene (20 mL) and dissolved in THF (15 mL). Triphosgene (282.5 mg, 0.95mmol) was added and the mixture stirred at room temperature with exclusion of moisture. The solution was cooled at 0 °C in ice-water bath and pyridine (238 µL) was added dropwise in three portions. The mixture was left to rise at room temperature and stirred for 4.5 hrs. The pyridinium hydrochloride was filtered, the solid washed with THF, and the filtrate evaporated under reduced pressure below 40 °C. The crude (1.098 g, Y>100%) was used without further purification.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.44-7.26 (15H, m, ***Ph***CH_2), 6.31 (1H, d, ^3J=3.8 Hz, H-1β), 6.26 (1H, s, H-1α), 4.70-4.46 (9H, m, H-2′, H-5′, PhC***H$_2$***O), 4.07 (1H, m, H-4′), 3.74 (1H, m, H-5′).

Synthesis of 2,6-acetyl-[9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-2-aminoadenine.[6] (48)

$C_{35}H_{36}N_6O_6$, MW=636.70



To a solution of 2,3,5-tri-O-benzyl-α- and β-D-arabinofuranosyl chlorides (1000 mg, 2.28 mmol) in 1,2-dichloroethane (30 mL) were added 2,6-diacetamidopurine (533.9 mg, 2.28 mmol) and molecular sieves powder (12.6 g, 4 Å). The mixture was refluxed for 5 days then stirred with celite and filtered. The solid was washed with chloroform and the combined filtrates were evaporated to dryness. The crude product was purified by flash column chromatography, using petroleum ether/ethyl acetate (6/2) as eluent. The appropriate fractions were combined and the solvent removed under reduced pressure to yield a white foam (418 mg, 29%).

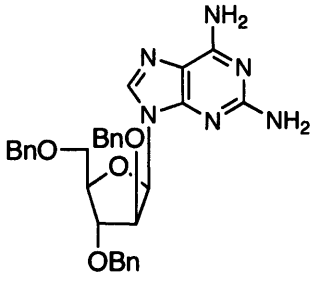
^{1}H-NMR (DMSO-d_6; 300 MHz): δ 10.60, 10.39 (2H, 2xbs, N***H***Ac) 8.31 (1H, s, H-8), 7.41-7.19 (13H, m, ***Ph***CH_2), 6.97-6.94 (2H, m, ***Ph***CH_2), 6.43 (1H, d, ^3J=5.4 Hz, H-1′), 4.73-4.27 (8H, m, H-2′ +H-3′+PhC***H$_2$***), 4.17 (1H, m, H-4′), 3.77 (2H, ψd, H-5′), 2.34, 2.25 (6H, 2xs, C***H$_3$***CO).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 25.0 (***C***H_3CO), 69.9 (C-5′), 71.8, 72.2, 72.6 (Ph***C***H_2), 80.4 (C-4′), 81.1, 82.0 (C-2′, C-3′), 82.7 (C-1′), 119.5 (adenosine-C), 127.9, 128.0, 128.5, 128.6, 128.7 (***Ph***CH_2), 137.4, 138.3, 138.4 (*'ipso'*, ***Ph***CH_2), 142.6 (C-8), 149.9, 152.6, 152.8 (adenosine-C), 169.6, 169.8 (***C***$OOCH_3$).

MS [ES (+ve)] found 659.9 ($[MNa]^+$), required $C_{35}H_{36}N_6O_6Na$, 659.3

Synthesis of 2-amino-9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)adenine.[6] (49)

$C_{31}H_{32}N_6O_4$, MW=552.62



Compound **48** (1500 mg, 2.36 mmol) was dissolved in a solution of methanolic sodium methoxide (20 mL, 1M) and the solution stirred at reflux for 3 hrs. After 3 hrs the solution was cooled, neutralized with acetic acid and the solvent removed under reduced pressure. The crude was purified by silica column chromatography using petroleum ether/ethyl acetate as eluent (1:9) to give 1190 mg (91%) of a white foam.

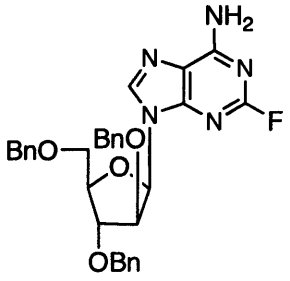
^{1}H-NMR (DMSO-d_6; 300 MHz): δ 7.74 (1H, s, H-8), 7.38-7.01 (13H, m ***Ph***CH_2), 7.03-7.01 (3H, m, ***Ph***CH_2), 6.75 (2H, bs, NH_2), 6.26 (1H, d, ^3J=4.9 Hz, H-1′), 5.88 (2H, bs, NH_2), 4.70-4.32 (8H, m, H-2′+H-3′ +PhC***H$_2$***O), 4.13 (1H, m, H-4′), 3.71 (2H, ψd, H-5′).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 69.9 (C-5′), 71.5, 72.0, 72.6 (Ph***C***H_2O), 80.1, 81.4, 81.7, 82.1 (C-1′, C-2′, C-3′, C-4′), 112.7 (adenosine-C), 127.8, 127.9, 128.0, 128.6, 128.7 (***Ph***CH_2O), 136.7 (C-8), 137.6, 138.2, 138.5 (*'ipso'*, ***Ph***CH_2O), 152.0, 156.5, 160.8 (adenosine-C).

MS [ES (+ve)] found 575.0 ($[MNa]^+$), required $C_{31}H_{32}N_6O_4Na$, 575.2.

Synthesis of 9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine.[6] (50a)

$C_{31}H_{30}FN_5O_4$, MW=555.6.



To a solution of 2-amino-9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)adenine (1100 mg, 1.99 mmol) in THF (15 mL) was added a mixture of fluoroboric acid (48% wt, 70 mL) at −10 °C (ice/salt bath). A solution of aqueous $NaNO_2$ (117 mg, 2.57 mmol, 1 mL water) was added dropwise over 20 mins. The addition of $NaNO_2$ was repeated 3 times. After the third addition, the ice/salt bath was removed, the mixture cooled at −40 °C (acetonitrile/liquid nitrogen) and the tetrafluoroboric acid neutralized to pH=6 with 50% NaOH. After warming the mixture at room temperature, chloroform was added and the solution extracted four times. The collected organic layers were washed with a saturated solution of sodium chloride then dried over magnesium sulfate before they were evaporated to dryness *in vacuo*. The crude oil was co-evaporated with toluene twice, dissolved in dry 1,2-dimethoxyethane (15mL) and the solution was stirred while ammonia was bubbled through for 20 mins.

The solution was evaporated and the crude purified by flash column chromatography using an eluent of dichloromethane/methanol (95/5).

^{19}F-NMR ($CDCl_3$, 282 MHz): δ -52.22.

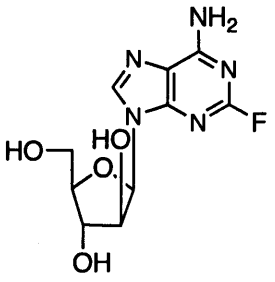
^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.24 (1H, s, H-8), 7.48-7.28 (13H, m, ***Ph***CH_2), 7.07-7.04 ((2H, m, ***Ph***CH_2), 6.50 (1H, d, ^3J=3.6 Hz, H-1′), 6.29 (2H, bs, N***H₂***), 4.76-4.65 (4H, m, PhC***H₂***), 4.46-4.30 (5H, m, H-3′+H-4′+H-2′+PhC***H₂***), 3.81 (2H, m, H-5′).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 69.4 (C-5′), 72.7, 73.3, 73.9 (Ph***C***H_2O), 81.2, 81.5, 82.3 (C-3′, C-2′, C-4′, 83.7 (C-1′), 117.5

(adenosine-C), 128.2, 128.3, 128.4, 128.5, 128.6, 128.9, 129.0 (***Ph***CH_2), 136.8, 137.8, 138.1 (*'ipso'*, ***Ph***CH_2), 151.5 (adenosine-C, ^3J=19.6 Hz), 157.6 (adenosine-C, ^3J=20.8 Hz), 159.6 (C-2, ^3J=210.0 Hz).

Synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine.[6] (43) (CPF108)

$C_{10}H_{12}FN_5O_4$, MW=285.23.



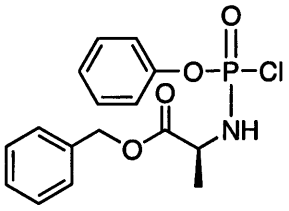
To a solution of 9-(2,3,5-tri-O-benzyl-β-D-arabinofuranosyl)-2-fluoroadenine (126 mg, 0.228 mmol), in DCM (10 mL), at –78 °C and under inert atmosphere, was added dropwise a solution of BCl_3 in DCM (5.02 mmol, 5.02 mL, 1M). The solution was stirred for 3 hrs, the solvent removed under reduced pressure and the crude dissolved in dichloromethane and reduced to dryness (4 times). The crude was purified by flash column chromatography using dichloromethane/methanol (9/1) as eluent to give a white powder (52 mg, 80%).

^{19}F-NMR ($CDCl_3$, 282 MHz): δ -52.91.

^{1}H-NMR (DMSO; 300 MHz): δ 8.16 (1H, s, H-8), 7.78 (2H, bs, N***H₂***), 6.09 (1H, d, ^3J=4.9 Hz, H-1'), 5.63 (1H, d, ^3J=5.3 Hz OH-2'), 5.52 (1H, d, ^3J=4.6 Hz, OH-3'), 5.06 (1H, t, ^3J=5.4 Hz, OH-5'), 4.15-4.09 (2H, m, H-2' + H-3'), 3.76-3.73 (1H, m, H-4'), 3.67-3.61 (2H, m, H-5').

Synthesis of phenyl-(benzoxy-L-alaninyl)-phosphorochloridate. (51a)

$C_{16}H_{17}ClNO_4P$, MW=353.74



This was synthesised according to *Standard procedure 4,* using L-alanine benzyl ester hydrochloride (1000 mg, 4.64 mmol), phenyl phosphorochloridate (1030 mg, 4.64 mmol, 729.8 μL), and TEA (939 mg, 9.28 mmol, 1293 μL) in DCM (40 mL). After overnight reaction, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 1405 mg (86%) of a colourless oil.

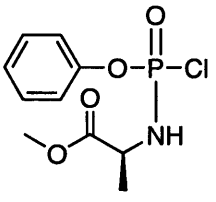
^{31}P-NMR ($CDCl_3$; 121 MHz): δ 9.32, 9.10.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.44-7.25 (10H, m, ***Ph***O+***Ph***CH_2), 5.27, 5.25 (2H, 2s, PhC***H*****$_2$**), 4.76-4.70 (1H, m, N***H***), 4.32-4.21 (1H, m, C***H***CH_3), 1.58, 1.57 (3H, 2d, ^3J= 7.1 Hz, C***H*****$_3$**CH).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.7, 20.8, 20.9 (***C***H_3CH), 51.0, 51.2 (***C***HCH3), 67.9, 68.0 (Ph***C***H_2), 120.9, 121.0, 126.3, 126.4, 128.7, 128.8, 129.0, 129.1, 130.3, 130.4, 130.7 (***Ph***O+***Ph***CH_2), 135.4, 135.5 (*'ipso', **Ph***CH_2), 150.1, 150.2, 150.3, 150.3 (*'ipso', **Ph***O), 171.6, 172.8, 173.0, 173.1 (***C***$OOCH_2$Ph).

Synthesis of phenyl-(methoxy-L-alaninyl)-phosphochloridate.[2] (51b)

$C_{10}H_{13}ClNO_4$, MW=267.64



This was synthesised according to *Standard procedure 4,* using L-alanine methyl ester hydrochloride (2150 mg, 15.40 mmol), phenyl phosphochloridate (3249 mg, 15.40 mmol, 4293 μL), and TEA (3116 mg, 30.80 mmol, 489 μL) in

DCM (50 mL). After overnight reaction the crude was purified by silica column chromatography using ethyl acetate/petroleum ether (7/3) to yield 4085 mg (99 %) of a colourless oil.

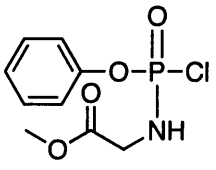
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.42, 9.20.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.24 (5H, m, ***Ph***O), 4.82-4.68 (1H, bs, NH), 4.23-4.21 (1H, m, C***H***CH_3), 3.83-3.80 (3H, s, C***H$_3$***O), 1.55-1.54 (3H, t, ^{3}J=7.1 Hz, CHC***H$_3$***).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 20.8, 20.98* (***C***H_3CH), 50.8, 51.1 (CH_3***C***H), 53.2, 53.3 (***C***H_3O), 120.9, 121.0, 126.3*, 126.4, 130.3, 130.4 (***Ph***O), 150.1, 150.2 (*'ipso'*, ***Ph***O), 173.4, 173.6 (***C***$OOCH_3$).

Synthesis of phenyl-(methoxy-glycinyl)-phosphorochloridate.[8] (51c)

$C_9H_{11}ClNO_4P$, MW=263.61



This was synthesised according to *Standard procedure 4*, using glycine methyl ester hydrochloride (2.00 g, 15.9 mmol), phenyl phosphorodichloridate (2.37 mL, 15.9 mmol), and TEA (4.43 mL, 31.8 mmol) in DCM (40 mL). After overnight reaction, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 3310 mg (79%) of a colourless oil.

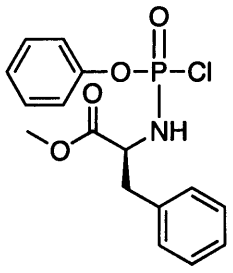
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 10.70.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.40-7.22 (5H, m, ***Ph***O), 4.97-4.89 (1H, m, N***H***), 3.96, 3.90 (2H, 2d, ^{1}J= 6.1 Hz, C***H$_2$***$COOCH_3$), 3.80 (3H, s, COOC***H$_3$***).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 43.3, 43.4 (***C***H_2NH), 53.1 (***C***H_3O), 120.9, 121.0 (*'o'*, ***Ph***O), 126.4, 126.5 (*'p'*, ***Ph***O), 130.3, 130.4 (*'m'*, ***Ph***O) 150.1, 150.2 (*'ipso'*, ***Ph***O), 170.4, 170.5 (***C***$OOCH_3$).

Synthesis of phenyl-(methoxy-L-phenylalaninyl)-phosphorochloridate.[8] (51d)

$C_{16}H_{17}ClNO_4P$, MW=353.74



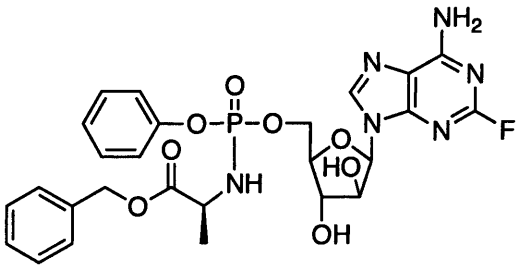
This was synthesised according to *Standard procedure 4*, using L-phenylalanine methyl ester hydrochloride (1.00 g, 4.60 mmol), phenyl phosphodichloridate (971 mg, 687 µL, 4.60 mmol), and TEA (931 mg, 1282 µL, 9.20 mmol) in DCM (15 mL). After overnight reaction, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 1358 mg (84%) of a colourless oil.

^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.44, 9.26.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.43-7.18 (10H, m, ***Ph***O+***Ph***CH_2CH), 4.59-4.14 (2H, m, $PhCH_2C$***H***+N***H***), 3.78-3.76 (3H, 2s, C***H***$_3O$), 3.19 (2H, d, ^{3}J=5.5 Hz, PhC***H***$_2CH$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 40.3, 40.4*, 40.5 ($PhCH_2CH$), 52.9, 53.0 ($PhCH_2CH$), 56.0, 56.4 (CH_3O), 120.8, 120.9, 121.0, 126.4, 127.4, 127.5, 127.7, 127.8, 128.9, 129.0, 129.1, 129.2, 129.9 130.0, 130.2, 130.3*, 131.3 (***Ph***O+***Ph***CH_2CH), 135.4, 135.5 (*'ipso'*, ***Ph***CH_2), 150.1, 150.2, 150.3 (*'ipso'*, ***Ph***O), 171.9, 172.0, 172.1, 172.2 ($COOCH_3$).

Synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine-5'-[phenyl-(benzoxy-L-alaninyl)]-phosphate. (52a) (CPF173) $C_{26}H_{28}FN_6O_8P$, MW 602.51.



9-β-D-arabinofuranosyl-2-fluoroadenine (80 mg, 0.28 mmol) was co-evaporeted twice with toluene, dissolved in 6 mL of THF/Pyridine (mixture 2:1 respectively) and NMI (115 mg, 1.40 mmol, 112 μL) was added. The mixture was cooled at 0 °C and under inert atmosphere a 1 M solution of phenyl-(benzoxy-L-alaninyl)phosphorochloridate in THF (297 mg, 0.84 mmol, 840 μL) was added dropwise over 10 mins. After 1 hr the reaction was left to rise to room temperature, stirred for 16 hr then quenched with methanol. The solvent was removed under reduce pressure and the crude purified by flash column chromatography using chloroform/methanol (gradient elution from 95/5 to 85/5). The isolated compound was further purified by preparative thin layer chromatography using as solvent chloroform/methanol (94/6) to give the product as a clear, colourless oil, which solidified to a white foam after trituration and co-evaporation with diethyl ether (10 mg, 6%).

^{19}F-NMR (MeOD; 282 MHz): δ -54.0, -54.03.

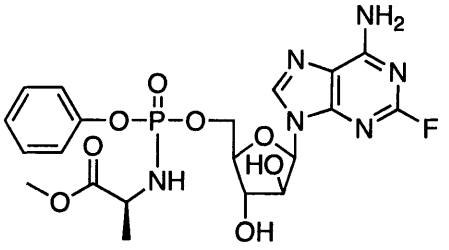
^{31}P-NMR (MeOD; 121 MHz): δ 5.02, 4.95.

^{1}H-NMR (MeOD; 300 MHz): δ 8.22, 8.20 (1H, 2xs, H-8), 7.35-7.15 (10H, m, ***Ph***O+***Ph***CH_2), 6.32-6.30 (1H, m, H-1'), 5.13-5.09 (2H, m, $PhCH_2O$), 4.48-4.26 (4H, m, H-2' +H-5' +H-4'), 4.14-4.01 (1H, m, H-3'), 3.99-3.82 (1H, m, C***H***CH_3), 1.35, 1.31 (3H, ^{3}J=7.1 Hz, CHC***H$_3$***).

^{13}C-NMR (MeOD; 75 MHz): δ 20.0, 20.1*, 20.2 (CH***C***H$_3$), 51.3, 51.4 (CH$_3$***C***H), 67.4, 67.5, 68.0, 68.1 (C-5'), 76.7, 76.8, 77.1 (C-4'+C-2'), 83.4, 83.5, 83.6, 83.7 (C-3'), 85.9, 86.1 (C-1'), 121.2*, 121.3, 125.9, 128.9, 129.0*, 129.3*, 130.5 (***Ph***O+***Ph***CH$_2$), 136.9, 137.0 ('*ipso*', ***Ph***CH$_2$), 142.2, 142.3 (C-8), 151.9, 152.0 ('*ipso*', ***Ph***O), 158.9 (C-adenosine, *J*=13.7 Hz), 160.1 (C-2, *J*=245.8 Hz), 174.4, 174.5*, 174.6 (***C***OOCH$_2$Ph).

Accurate mass: $C_{26}H_{28}FN_6O_8PNa$, requires 625.1588; found 625.1586

Synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine-5'-[phenyl-(methoxy-L-alaninyl)]-phosphate. (52b) (CPF109) $C_{20}H_{24}FN_6O_8P$, MW 526.41.



9-β-D-arabinofuranosyl-2-fluoroadenine (50 mg, 0.18 mmol) was co-evaporated twice with toluene, dissolved in 6 mL of THF/Pyridine (mixture 2:1 respectively) and NMI (72 mg, 0.88 mmol, 70 µL) was added. The mixture was cooled at −17 °C in ice/salt bath and under inert atmosphere a 1 M solution of phenyl-(methoxy-L-alaninyl)-phosphorochloridate in THF (146 mg, 0.53 mmol, 525 µL) was added dropwise over 1 hr. After 1 hr the reaction was left to rise to room temperature, stirred for 16 hr then quenched with methanol. The solvent was removed under reduce pressure and the crude purified by flash column chromatography using dichloromethane/methanol (gradient elution from 95:5 to 85/5). The isolated compound was further purified by preparative thin layer chromatography using chloroform/methanol (94/6) to give the product as a clear, colourless oil, which solidified to a white

foam after trituration and co-evaporation with diethyl ether (10 mg, 10%).

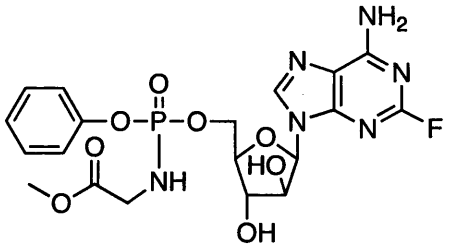
^{19}F-NMR (MeOD; 282 MHz): δ -54.04.

^{31}P-NMR (MeOD; 121 MHz): δ 4.99.

^{1}H-NMR (MeOD; 300 MHz): δ 8.22, 8.20 (1H, 2xs, H-8), 7.38-7.19 (5H, m, ***Ph***O), 6.33, 6.32 (1H, 2xd, ^{3}J=3.4 Hz, H-1'), 4.89-4.27 (4H, m, H-2' +H-5' +H-4'), 4.17-4.03 (1H, m, H-3'), 4.00-3.85 (1H, m, C***H***CH_3), 3.66-3.65 ((3H, 2xs, C***H***$_3$O), 1.34, 1.29 (3H, 2xd, ^{3}J=7.1 Hz, C***H***$_3$CH).

^{13}C-NMR (MeOD; 75 MHz): δ 20.7, 20.8*, 20.9 (***C***H_3CH), 51.9 (CH_3***C***H), 53.1, 53.2 (***C***H_3O), 67.4, 67.5, 67.8, 67.9 (C-5'), 77.3, 77.4, 77.6 (C-4'+C-2'), 84.0, 84.1, 84.2, 84.3 (C-3'), 86.5, 86.7 (C-1'), 118.2 (adenosine-C), 121.8, 121.9 ('*o*', ***Ph***O), 126.5 ('*p*', ***Ph***O), 131.1 ('*m*', ***Ph***O), 142.9, 143.0 (^{5}J=2.9 Hz, C-8), 142.8, 142.9 (C-8), 152.4, 152.5, 152.6 (adenosine-C+'*ipso*' ***Ph***O), 159.2, 159.4, 159.6, 162.4 (adenosine-C), 175.7, 175.8*, 175.9 (***C***OOMe).

Synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine-5'-[phenyl-(methoxyglycinyl)]-phosphate. (52c) (CPF175)
$C_{19}H_{22}FN_6O_8P$, MW 512.39.



9-β-D-arabinofuranosyl-2-fluoroadenine (85 mg, 0.26 mmol) was co-evaporated twice with toluene, dissolved in 7 mL of THF/Pyridine (mixture 2:1 respectively) and NMI (105 mg, 1.28 mmol, 102 µL) was added. The mixture was cooled at 0 °C and under inert atmosphere a 1 M solution of phenyl-(methoxyglycinyl)-phosphorochloridate in THF (203 mg, 0.768 mmol, 768 µL) was

added dropwise over 10 mins. After 1 hr the reaction was left to rise to room temperature, stirred for 16 hr then quenched with methanol. The solvent was removed under reduced pressure and the crude purified by flash column chromatography using chloroform/methanol (gradient elution from 90/10 to 85/5). The isolated compound was further purified by preparative thin layer chromatography using as solvent chloroform/methanol (95/5) to give the product as a clear, colourless oil, which solidified to a white foam after trituration and co-evaporation with diethyl ether (12 mg, 9%).

^{19}F-NMR (MeOD; 282 MHz): δ -54.04, -54.05.

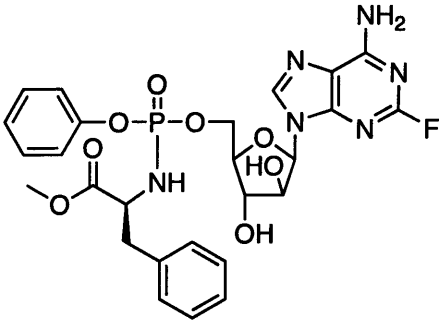
^{31}P-NMR (MeOD; 121 MHz): δ 6.28, 6.04.

^{1}H-NMR (MeOD; 300 MHz): δ 8.15, 8.10 (1H, 2xs, H-8), 7.29-7.07 (5H, m, ***Ph***O), 6.23-6.21 (1H, m, H-1′), 4.46-4.19 (4H, m, H-2′+H-5′ +H-4′), 4.05-4.02 (1H, m, H-3′), 3.65, 3.58 (2H, 2d, ^{2}J=13.4 Hz, C***H*****$_2$**N), 3.59, 3.58 (3H, 2xs, C***H*****$_3$**O).

Accurate mass: $C_{19}H_{22}FN_6O_8PNa$ requires 535.1118; found 535.1121.

Synthesis of 9-β-D-arabinofuranosyl-2-fluoroadenine-5'-[phenyl-(methoxy-L-phenylalaninyl)]-phosphate. (52d) (CPF174).

$C_{26}H_{28}FN_6O_8P$, MW 602.51.



β-D-arabinofuranosyl-2-fluoroadenine (100 mg, 0.35 mmol) was co-evaporated twice with toluene, dissolved in 10 mL of THF/Pyridine (mixture 9:1 respectively) and NMI (144 mg, 1.75 mmol, 140 μL) was added. The mixture was cooled at 0 °C and under inert atmosphere a 1 M solution of phenyl-(methoxy-L-phenylnylalaninyl)-phosphorochloridate in THF (372 mg, 1.05 mmol, 1.05 μL) was added dropwise over 10 mins. After 1 hr the reaction was left to rise to room temperature, stirred for 16 hr then quenched with methanol. The solvent was removed under reduced pressure and the crude purified by flash column chromatography using chloroform/methanol (gradient elution from 90/10 to 85/5). The isolated compound was further purified by preparative thin layer chromatography using as solvent chloroform/methanol (95/5) to give the product as a clear, colourless oil, which solidified to a white foam after trituration and co-evaporation with diethyl ether (13 mg, 6%).

^{19}F-NMR (MeOD; 282 MHz): δ -54.03.

^{31}P-NMR (MeOD; 121 MHz): δ 4.89, 4.46.

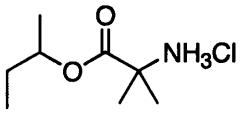
^{1}H-NMR (MeOD; 300 MHz): δ 8.18, 8.15 (1H, 2xs, H-8), 7.33-7.10 (10H, m, ***Ph***O+ CHCH$_2$***Ph***), 6.33-6.29 (1H, m, H-1'), 4.33-3.93 (6H, m, H-2'+H-5'+H-4'+H-3'+C***H***CH$_2$Ph), 3.61, 3.60 (1H, m,

C***H₃***O), 3.10-3.00 (1H, m, one of CHC***H₂***Ph), 2.92-2.83 (1H, m, one of CHC***H₂***Ph).

Accurate mass: $C_{26}H_{28}FN_6O_8PNa$ requires 625.1588; found 625.1607.

Synthesis of dimethylglycine 2-butyl ester hydrochloride salt. (55)

$C_8H_{18}ClNO_2$, MW=195.7



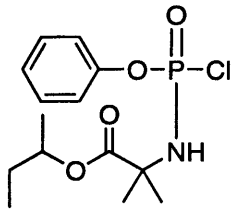
This was synthesised according to *Standard Procedure 1*, 2-amino-isobutyric acid (4900 mg, 47.5 mmol), thionyl chloride (11.3 g, 95.0 mmol, 6.9 mL) and 2-butanol (35.2 g, 43.6 mL). The product was isolated as a white solid (6.35 g, yield 68%)

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.83 (3H, bs, N***H₃***Cl), 4.84 (1H, m, $CH_3C\underline{H}CH_2CH_3$), 1.64-1.51 (2H, m, $CH_3CHC\underline{H_2}CH_3$), 1.22 (3H, d, ^3J=6.2 Hz, C***H₃***$CHCH_2CH_3$), 0.88 (3H, t, ^3J=7.5 Hz, $CH_3CHCH_2C\underline{H_3}$).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 9.8 ($\underline{C}H_3CHCH_2CH_3$), 19.4 ($CH_3CHCH_2\underline{C}H_3$), 23.5, 23.6 ($[\underline{C}H_3]_2C$), 28.4 ($CH_3CH\underline{C}H_2CH_3$), 56.2 ($[CH_3]_2\underline{C}$), 74.4 ($CH_3\underline{C}HCH_2CH_3$), 171.6 ($\underline{C}$OOBu).

Synthesis of phenyl-(2-butoxy-dimethylglycine)-phosphorochloridate (56a)

$C_{14}H_{21}ClNO_4P$, MW=333.75



This was synthesised accoding to *Standard Procedure*, dimethylglycine 2-butyl ester hydrochloric salt (1500 mg, 7.67 mmol), phenyldichlorophosphate (1703 mg, 7.67 mmol, 1.146 mL), TEA (1552 mg, 15.35 mmol, 2.138 mL) in DCM (50 mL). The solvent was removed under reduced pressure and the crude purified by flash column chromatography using as eluent ethyl acetate/petroleum ether 7:3. The appropriate fractions were combined and the solvent was removed *in vacuo* to yield the product (2.350 g, yield 92%) as an colourless oil.

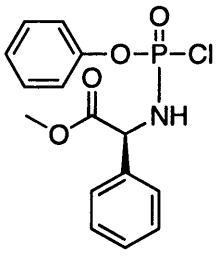
^{31}P-NMR ($CDCl_3$; 175 MHz): δ 6.90.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.34-7.15 (5H, m, ***Ph***O), 4.89-4.79 (2H, m, $CH_3C\boldsymbol{H}CH_2CH_3$+N***H***), 1.65, 1.64, 1.62, 1.61 (6H, 4s, ([***C***H_3]$_2$C), 1.65-1.53 (2H, m, $CH_3CHC\boldsymbol{H_2}CH_3$), 0.86 (3H, t, ^{3}J=7.4 Hz, $CH_3CHCH_2C\boldsymbol{H_3}$).

^{13}C-NMR (CDCl3; 75 MHz): δ 10.0 ($CH_3CHCH_2\boldsymbol{C}H_3$), 19.7 ($\boldsymbol{C}H_3CHCH_2\boldsymbol{C}H_3$), 26.8, 26.9, 27.0, 27.2, 27.3 ([***C***H_3]$_2$C), 29.1 ($CH_3CH\boldsymbol{C}H_2CH_3$), 58.8, 58.9 ([$CH_3$]$_2$***C***), 75.0 ($CH_3\boldsymbol{C}HCH_2CH_3$), 121.0, 121.1 ('*o*', ***Ph***O), 126.2, 126.3 ('***m***', ***Ph***O), 130.3, 130.4 ('*p*', ***Ph***O), 150.3, 150.4 ('***ipso***', ***Ph***O), 174.8, 174.9 (***C***OOBut).

Synthesis of phenyl-(methoxy-L-phenylglycinyl)-phosphorochloridate.[9] (56b)

$C_{15}H_{15}ClNO_4P$, MW=339.71.



This is synthesised according to standard procedure 4, using (S)-(+)-2-L-phenylglycine methyl ester hydrochloride (842 mg, 3.99 mmol), phenyl-phosphorodichloridate (800 mg, 3.99 mmol, 596 µL), and TEA (808 mg, 7.98 mmol, 1112 µL) in DCM (15 mL), to give 962 mg (yield 71%), of crude product used without further purification.

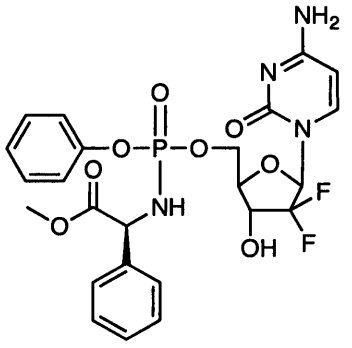
^{31}P-NMR ($CDCl_3$; 121 MHz): δ 8.80, 8.54

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.54-7.07 (10H, m, ***Ph***O +***Ph***), 5.50-5.24 (1H, m, C***H***Ph), 3.83, 3.82 (3H, 2d, CH_3O).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 51.7*, 52.1, 52.2 (***C***HPh), 56.8 (***C***H_3O), 57.2, 57.3, 57.5, 57.6 (***C***HPh), 119.3*, 119.5, 119.6, 124.8, 124.9, 125.9, 126.0, 126.1, 126.2, 127.7*, 127.9*, 128.5, 128.7, 128.8*, 128.9 (***Ph***O+CH***Ph***), 135.3, 135.4* ('*ipso*', CH***Ph***), 148.6, 148.7, 148.8 ('*ipso*', ***Ph***O), 169.9, 170.0, 170.1 (***C***OOMe).

Synthesis of gemcitabine-5′-[phenyl-(methoxy-L-phenylglycinyl)]phosphate. (54b) (CPF176)

$C_{24}H_{25}F_2N_4O_8P$, MW=566.45.



Under inert atmosphere to a stirring suspension of gemcitabine (200 mg, 0.667 mmol) in THF/Pyridine (10 mL, 7/3) and NMI (274 mg, 3.34 mmol, 266 µL) at –17 °C (ice/salt bath), a solution 1M in THF of phenyl(methoxy-L-phenylglycinyl)-phosphorodichloridate (2 mmol, 2 mL), was added dropwise over 30 minutes. After 30 minutes the reaction was left to rise room temperature and stirred for 14 hrs. Methanol was added, the solvent evaporated under reduced pressure and the crude purified by silica column chromatography using an eluent of dichloromethane/methanol (gradient elution from 93/7 to 85/15), followed by preparative TLC (dichloromethane/methanol 93/7) to give a white foam (30 mg, 8%)

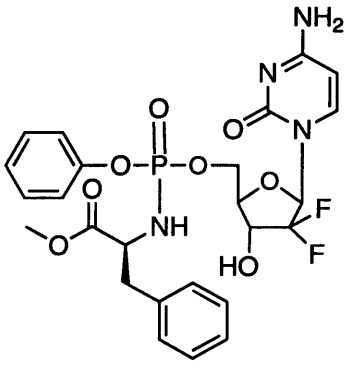
^{31}P-NMR (MeOD; 121 MHz): δ 4.78, 4.54.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.41, 7.37 (1H, 2d, ^{3}J=7.59 Hz, H-6), 7.28-6.98 (10H, m, ***Ph***O+CH***Ph***), 6.21-6.12 (1H, m, H-1′), 5.80, 5.74 (1H, 2d, ^{3}J=7.59 Hz, H-5), 5.07-5.02 (1H, m, C***H***Ph), 4.45-4.32 (2H, m, H-5′), 4.31-4.27 (1H, m, H-4′), 4.20-4.14 (1H, m, H-3′), 4.69, 4.67 (3H, 2s, C***H$_3$***O).

^{13}C-NMR (MeOD; 75 MHz): δ 53.1 (***C***H$_3$O), 59.8*, 59.9*, 60.0 (***C***HPh), 65.6*, 65.7*(C-5′), 70.9, 71.0, 71.2, 71.4 (C-3′), 80.2* (C-4′), 85.7, 86.0, 86.2 (C-1′), 96.7* (C-5), 121.3, 121.4*, 121.5* (***Ph***O), 124.5 (C-2′, ^{1}J=259 Hz), 128.2, 126.3, 128.4, 129.5*, 129.8, 129.9*, 130.7, 130.8 (CH***Ph***+***Ph***O), 139.2*, 139.4* (*'ipso'*, CH***Ph***), 142.3 (C-6), 152.0* (*'ipso'*, ***Ph***O), 157.7, 157.8 (C-4), 167.7 (C-2), 173.1, 173.2, 173.4* (***C***OOCH$_3$).

Synthesis of gemcitabine-5'-[phenyl-(methoxy-L-phenylalaninyl)]-phosphate. (54c)(CPF177)

$C_{25}H_{27}F_2N_4O_8P$, MW=580.15



Under inert atmosphere to a stirring suspension of gemcitabine (200 mg, 0.667 mmol) in THF/Pyridine (10 mL, 7/3) and NMI (274 mg, 3.34 mmol, 266 µL) at −17 °C (ice/salt bath), a solution 1M in THF of phenyl(methoxy-L-phenylglycinyl)-phosphorochloridate (2 mmol, 2 mL), was added dropwise over 30 minutes. After 30 minutes the reaction was left to rise room temperature and stirred for 14 hrs. Methanol was added, the solvent evaporated under reduced pressure and the crude purified by flash column chromatography using an eluent of dichloromethane/methanol (gradient elution from 93/7 to 85/15), followed by preparative TLC (dichloromethane/methanol 93/7) to give a white foam (21 mg, 5%).

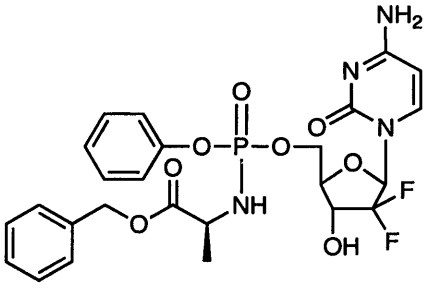
^{31}P-NMR (MeOD; 121 MHz): δ 4.89, 4.47.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 7.42-7.35 (1H, m, H-6), 7.28-6.97 (10H, m, ***Ph***O+CHCH$_2$***Ph***), 6.20-6.11 (1H, m, H-1'), 6.20-6.11 (1H, m, H-5), 4.17-3.82 (5H, m, H-5'+H-4'+H-3'+C***H***CH$_2$Ph), 3.24, 3.23 (3H, 2s, C***H$_3$***O), 3.06-2.97 (1H, m, one of CHC***H$_2$***Ph), 2.83-2.76 (1H, m, one of CHC***H$_2$***Ph).

^{13}C-NMR (MeOD; 75 MHz): δ 41.1, 41.2, 41.3 (CH***C***H$_2$Ph), 53.1 (***C***H$_3$O), 59.2, 59.4 (***C***HCH$_2$Ph), 65.5, 65.6, 65.7, 65.86 (C-5'), 71.1, 71.3, 71.7 (C-3'), 80.6, 80.7 (C-4'), 85.8, 86.1, 86.6 (C-1'), 97.2 (C-5), 120.4, 121.5*, 121.8*, 121.9, 126.5, 126.6, 127.3, 128.4, 130.0*, 130.8, 130.9, 131.2, 138.5, 138.6 (CHCH$_2$***Ph***+***Ph***O+C-2'), 142.6, 142.8 (C-6), 152.3, 152.4, 152.5

('*ipso*', ***Ph***O), 158.1 (C-4), 168.0 (C-2), 174.7, 174.8, 174.9* (***C***$OOCH_3$).

Synthesis of gemcitabine-5'-[phenyl-(benzoxy-L-alaninyl)]phosphate. (CPF31).
$C_{26}H_{27}F_2N_4O_8P$, MW=580.47.



Under inert atmosphere at -78 °C, to a solution of gemcitabine (1820 mg, 6.94 mmol) and NMI (2266 µL, 34.7 mmol) a solution 1 M of phenyl(benzoxy-L-alaninyl)-phosphorochloridate (20.82 mL, 20.82 mmol) was added dropwise over 15 mins. After 0.5 hrs the mixture was left to rise to room temperature and stirred for 16 hrs. The reaction was quenched with MeOH and the solvent removed under reduced pressure. The crude product, after absorption on silica, was purified twice by flash column chromatography, using an eluent of chloroform/methanol (gradient elution from 95/5 to 85/5). The product was obtained as a white foam (520 mg, 13%).

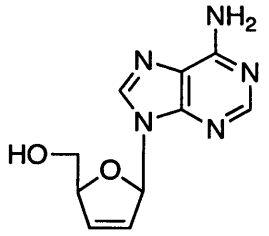
^{31}P-NMR (MeOD; 121 MHz): δ 4.89, 5.06.

^{1}H-NMR (MeOD; 300 MHz): δ 7.57, 7.52 (1H, 2d, ^{3}J=7.7 Hz and ^{3}J=7.6 Hz, H-6), 7.39-7.11 (10H, m, ***Ph***O+***Ph***CH_2), 6.29-6.23 (1H, m, H-1'), 5.89, 5.85 (1H, 2d, ^{3}J=7.7 Hz and ^{3}J=7.6 Hz, H-5), 5.17-5.15 (2H, m, PhC***H_2***), 4.47-4.15 (3H, m, H-5'+H-3'), 4.06-3.96 (2H, m, C***H***CH_3+H-4'), 1.40-1.28 (3H, m, CHC***H_3***).

^{13}C-NMR (MeOD; 75 MHz): δ 20.6, 20.7*, 20.8 (CH***C***H_3), 52.0, 52.2 (***C***HCH_3), 65.9, 66.0, 66.1, 66.2 (C-5'), 68.4 (Ph***C***H_2), 71.1, 71.4, 71.7, 72.0 (C-3'), 80.68 (C-4'), 85.9, 86.4, 86.7 (C-1'), 97.1* (C-5), 121.8*, 121.9, 122.0* (***Ph***O+***Ph***CH_2), 124.1 (C-2', ^{1}J=227 Hz), 126.7, 127.3, 129.6, 129.7*, 129.9, 130.0, 130.4, 131.3

(***Ph***O+***Ph***CH_2), 137.6*, 137.8 (*'ipso'*, ***Ph***CH_2), 142.7, 142.8 (C-6), 152.4, 152.5 (*'ipso'*, ***Ph***O), 158.1, 158.2 (C-4), 168.0 (C-2), 174.9, 175.0, 175.2, 175.3 (***C***$OOCH_2Ph$).

Synthesis of 2',3'-dideoxy-2',3'-didehydroadenosine.[10] (59)
$C_{10}H_{11}N_5O_2$, MW=233.23



A solution of 2-acetoxyisobutyryl bromide (3.131 mg, 14.98 mmol, 2.205 mL) in acetonitrile/water (7.42 mL acetonitrile, 68 µL water) was added to as slurry of (-)-adenosine (1.00 g, 3.74 mmol) in 75 mL of dry acetonitrile. The mixture was allowed to stir at room temperature for 1 hr, during which time the adenosine was consumed and a clear solution formed after 45 mins. The reaction was quenched with $NaHCO_3$, allowed to stir for 5 mins., and extracted with ethyl acetate (3x100 mL). The combined organic layers were dried on $MgSO_4$ and the solvent removed under reduced pressure to give a white solid foam. The residue was dissolved in DMF dry (15 mL) and added to a freshly prepared Zn/Cu couple (see preparation below) at 0 °C. After 4 hrs the mixture was filtered through a celite pad, the pad washed with DCM and the solvent removed under reduced pressure. The residue was dissolved in DCM and washed with a saturated solution $NaHCO_3$ and the aqueous layer was re-extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated under *vacuo*. The residue was dissolved in 50 mL of solution 7N of methanolic ammonia and the mixture stirred at room temperature for 16 hrs. The solvent was removed, the crude absorbed on silica and purified by flash column chromatography using dichloromethane/methanol (9/1) as eluent to give 639 mg (73%) of a white solid.

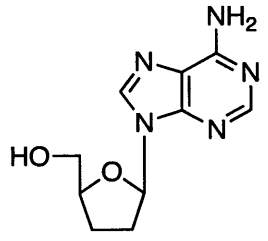
Preparation of Zn/Cu couple: the couple was prepared immediately before use. Zinc powder (1.047 g) was added to a cold solution of copper (II) acetate monohydrate (30 mg) in acetic acid (7.5 mL). The mixture was stirred until the blue colour disappeared and the zinc took on a pink hue. The couple was filtered, washed with acetic acid (2x10mL), ethanol (2x10mL), and ether (2x10mL). The solid was dried under high vacuum for 2 hrs and the couple covered with dry DMF (6mL).

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.19 (1H, s, H-8), 8.17 (1H, s, H-2), 7.32 (1H, bs, -N***H₂***), 6.96 (1H, s, H-1′), 6.49 (1H, d, ^3J=5.8 Hz, H-3′), 6.15 (1H, d, ^3J=5.8 Hz, H-2′), 5.07 (1H, t, ^3J=5.8 Hz, OH-5′), 4.90 (1H, m, H-4′), 3.60 (2H, m, H-5′).

^{13}C-NMR (DMSO-d_6; 75 MHz): δ 63.1 (C-5′), 88.2 (C-4′), 88.4 (C-1′), 119.1 (C-5), 125.9 (C-3′), 134.7 (C-2′), 139.5 (C-8), 149.5 (C-4), 153.0 (C-6), 156.4 (C-2).

Synthesis of 2′,3′-dideoxyadenosine.[10] (57)

$C_{10}H_{13}N_5O_2$, MW=235.20

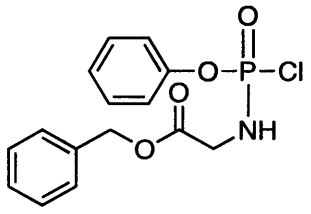


To a solution of 2′,3′-dideoxy-2′,3′-didehydroadenosine (600 mg, 2.57 mmol) in EtOH/water (9:1, 40 mL), Pd 5% wt on activated carbon (310 mg) was added and the reaction stirred under hydrogen atmosphere for 3.5 hrs. The mixture was filtered through a celite pad, washed with hot ethanol and the combined filtrate was evaporated to dryness. The crude was purified by flash column chromatography using dichloromethane/methanol (9:1). The appropriate fractions were combined and the solvent was removed in *vacuo* to yield the product (374 mg, 61%) as a white solid.

^{1}H-NMR (DMSO-d_6; 300 MHz): δ 8.32 (1H, s, H-8), 8.11 (1H, s, H-2), 7.25 (1H, bs, -N***H₂***), 6.19 (1H, t, ^3J=5.2 Hz, H-1′), 5.04 (1H, t, ^3J=5.4 Hz, OH-5′), 4.08 (1H, m, H-4′), 3.60 (1H, m, 1 of H-5′), 3.47 (1H, m, 1 of H-5′), 2.38 (2H, m, H-3′), 2.02 (2H, m, H-2′).
^{13}C-NMR (DMSO-d_6; 75 MHz): δ 26.0 (C-3′), 32.1 (C-2′), 63.3 (C-5′), 82.0 (C-4′), 84.8 (C-1′), 119.4 (C-5), 139.4 (C-8), 149.2 (C-4), 152.8 (C-6), 156.4 (C-2).

Synthesis of phenyl-(benzoxy-glycinyl)-phosphorochloridate. (60a)

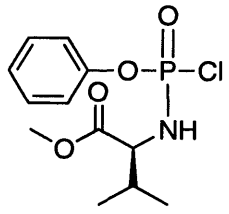
$C_{15}H_{15}ClNO_4P$, MW=339.71



This was synthesised according to *Standard procedure 4*, using glycine benzyl ester hydrochloride (1.00 g, 4.96 mmol), phenyl phosphorochloridate (1.05 g, 741 µL, 4.96 mmol), and TEA (4.43 mL, 31.8 mmol) in DCM (15 mL). After overnight reaction, the crude was purified by flash column chromatography using acetate/petroleum ether (7/3) to yield 1136 mg (67%) of a colourless oil.
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 10.22.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.45-7.27 (10H, m, ***Ph***O+***Ph***CH_2), 5.29 (2H, s, PhC***H₂***), 4.54-4.22 (1H, m, N***H***), 4.04-3.98 (2H, m, C***H₂***$COOCH_2Ph$).
^{13}C-NMR ($CDCl_3$; 75 MHz): δ 43.6* (***C***H_2NH), 68.1 (Ph***C***H_2), 120.9, 121.0, 126.5*, 129.0, 129.2, 130.4* (***Ph***O+***Ph***CH_2), 135.3 (*'ipso'*, ***Ph***CH_2), 150.1, 150.2 (*'ipso'*, ***Ph***O), 169.7, 169.9 (***C***$OOCH_2Ph$).

Synthesis of phenyl-(methoxy-L-valinyl)-phosphorochloridate. (60b)

$C_{12}H_{17}ClNO_4P$, MW=305.69



This was synthesised according to *Standard procedure 4*, using L-valine methyl ester hydrochloride (1.00 g, 5.97 mmol), phenyl phosphorodichloridate (892 µL, 5.97 mmol), and TEA (1664 µL, 11.94 mmol) in DCM (15 mL). After overnight reaction, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 1604 mg (88%) of a colourless oil.

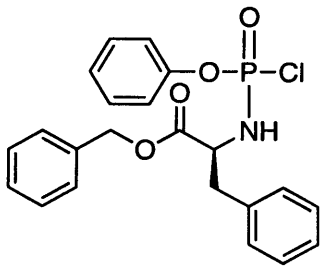
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 10.69, 10.21.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.43-7.26 (5H, m, ***Ph***O), 4.30-4.16 (1H, m, N***H***), 4.05-3.93 (1H, m, C***H***$CH(CH_3)_2$), 3.84, 3.83 (3H, 2s, COOC***H₃***), 2.28-2.12 (1H, m, CHC***H***$(CH_3)_2$), 1.10-0.97 (6H, m, CHCH(C***H₃***)$_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 17.8, 17.9, 19.3, 19.4 (CHCH(***C***$H_3)_2$), 32.3, 32.4, 32.5 (CH***C***$H(CH_3)_2$), 52.8, 52.9 (***C***$HCH(CH_3)_2$), 60.8, 61.0 (COO***C***H_3), 115.9, 120.9, 121.0*, 126.3, 130.0, 130.3 (***Ph***O), 150.1, 150.2, 150.3, 150.4 (*'ipso'*, ***Ph***O), 172.5, 172.6, 172.8, 172.9 (***C***$OOCH_3$).

Synthesis of phenyl-(benzoxy-L-phenylalaninyl)-phosphorochloridate. (60c)

$C_{22}H_{21}ClNO_4P$, MW=429.83



This was synthesised according to *Standard procedure 4*, using L-phenylalanine benzyl ester hydrochloride (1.00 g, 3.43 mmol), phenyl phosphorochloridate (513 mL, 3.43 mmol), and TEA (694 mg, 956 µL, 6.86 mmol) in DCM (15 mL). After overnight reaction, the crude was purified by flash column chromatography using ethyl acetate/petroleum ether (7/3) to yield 1388 mg (94%) of a colourless oil.

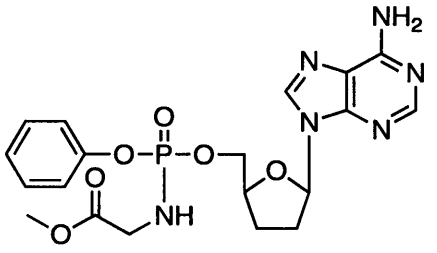
^{31}P-NMR ($CDCl_3$, 121 MHz): δ 9.12, 9.18.

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.45-7.07 (15H, m, ***Ph***O+***Ph***CH_2+***Ph***CH_2CH), 5.22-5.21 (2H, m, PhC***H_2***), 4.60-4.45 (1H, m, $PhCH_2C$***H***), 4.31-4.14 (1H, m, N***H***), 3.27-3.13 (2H, m, PhC***H_2***CH).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ 40.3, 40.4 (CH***C***H_2Ph), 55.7, 55.8, 56.3 (***C***HCH_2Ph), 60.8 (Ph***C***H_2), 120.7, 120.8, 120.9, 121.0, 125.0, 126.4, 127.4, 127.8, 129.0, 129.1, 129.2, 130.0, 130.4 (***Ph***O+***Ph***CH_2+***Ph***CH_2CH), 135.1, 135.2 (*'ipso'*, ***Ph***CH_2), 150.0 (*'ipso'*, ***Ph***O), 171.5 (***C***$OOCH_2Ph$).

Synthesis of 2′,3′-dideoxyadenosine-5′-[phenyl-(methoxy-glycinyl)]-phosphate.[11] (61a) (CPF135)

$C_{19}H_{23}N_6O_6P$, MW=462.40



This was synthesised according to *standard procedure 6*, using 2′,3′-dideoxyadenosine (50 mg, 0.21 mmol), *tert*-butylmagnesium chloride (294 µL, 0.29 mmol), phenyl-(methoxy-glycinyl)-phosphorochloridate (420 µL, 111 mg, 0.42 mmol) in THF/Pyr (10:1, 5 mL) for 19 hrs.

After 2 hrs the reaction was monitored by TLC (eluent chloroform/methanol 9:1) and *tert*-butylmagnesium chloride (126 µL, 0.13 mmol, 15 mg) and phenyl-(methoxy-glycinyl)-phosphorochloridate (210 µL, 0.21 mmol, 55 mg) were added dropwise. The reaction was left to stir overnight. The crude was purified twice by flash column chromatography using chloroform/methanol (95/5) to give 28 mg (29%) of a white foam.

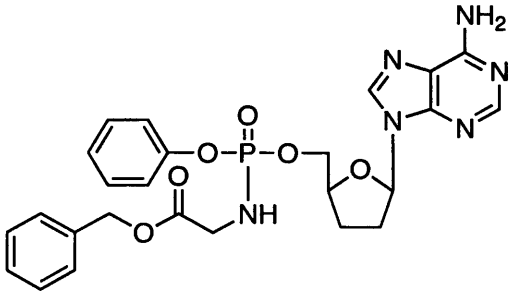
^{31}P-NMR (MeOD; 121 MHz): δ 6.33, 6.17.

^{1}H-NMR (MeOD; 300 MHz): δ 8.28 (1H, s, H-8), 8.25, 8.16 (1H, s, H-2), 7.29-7.06 (5H, m, ***Ph***O), 6.29-6.24 (1H, m, H-1′), 4.40-4.34 (1H, m, H-4′), 4.32-4.17 (2H, m, H-5′), 3.66-3.56 (2H, m, C***H$_2$***NH), 3.60, 3.59 (3H, 2s, C***H$_3$***O), 2.58-2.43 (2H, m, H-2′), 2.26-2.08 (2H, m, H-3′).

^{13}C-NMR (MeOD; 75 MHz): δ 26.9, 27.2 (C-3′), 33.4 (C-2′), 43.9, 44.0 (***C***H$_2$NH), 53.0 (***C***H$_3$O), 68.9, 69.0, 69.4, 69.5 (C-5′), 81.4, 81.5 (C-4′), 87.1, 87.2 (C-1′), 120.8 (C-5), 121.7, 121.8* (′*o*′, ***Ph***O), 126.5 (′*p*′, ***Ph***O), 131.1 (′*m*′, ***Ph***O), 141.0* (C-8), 150.5 (C-4), 152.4, 152.5 (′*ipso*′, ***Ph***O), 154.1 (C-2), 157.6 (C-6), 173.2*, 173.3 (***C***OOCH$_3$).

Synthesis of 2',3'-dideoxyadenosine-5'-[phenyl-(benzoxy-glycinyl)]-phosphate. (61b) (CPF136)

$C_{25}H_{27}N_6O_6P$, MW=538.49



This was synthesised according to *standard procedure 6*, using 2',3'-dideoxyadenosine (75 mg, 0.32 mmol), *tert*-butylmagnesium chloride (480 µL, 0.48 mmol), phenyl-(benzoxy-glycinyl)-phosphorochloridate (800 µL, 272 mg, 0.80 mmol) in THF/Pyr (10:1, 15 mL) for 19 hrs.

The reaction was left to stir overnight. The crude was purified three times by flash column chromatography using chloroform/methanol (95/5) to give 94 mg (55%) of a white foam.

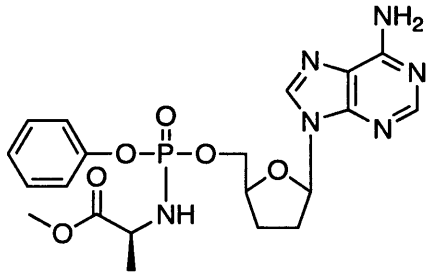
^{31}P-NMR ($CDCl_3$; 121 MHz): δ 6.31, 6.12.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.31, 8.28 (1H, s, H-8), 8.21, 8.20 (1H, s, H-2), 7.33-7.12 (10H, m, ***Ph***O+***Ph***CH_2), 6.31-6.27 (1H, m, H-1'), 5.13, 5.12 (2H, 2s, PhC***H_2***), 4.44-4.20 (3H, m, H-5'+H-4'), 3.80-3.32 (2H, m, C***H_2***NH), 2.58-2.43 (2H, m, H-2'), 2.20-2.10 (2H, m, H-3').

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 25.5, 25.7 (C-3'), 32.1* (C-2'), 42.8* (***C***H_2NH), 66.9 (Ph***C***H_2), 67.5, 67.6, 67.9, 68.0 (C-5'), 80.0*, 80.1, 80.2 (C-4'), 85.7 (C-1'), 119.4 (C-5), 120.3, 120.4*, 125.1, 128.3*, 128.5, 129.7 (***Ph***CH_2+***Ph***O), 136.2 ('*ipso*', ***Ph***CH_2), 139.5, 139.6 (C-8), 149.1 (C-4), 151.0, 151.1 ('*ipso*', ***Ph***O), 152.7 (C-2), 156.2 (C-6), 171.2* (***C***$OOCH_2Ph$).

Synthesis of 2′,3′-dideoxyadenosine-5′-[phenyl-(methoxy-L-alaninyl)]-phosphate.[12] (61c) (CPF135)

$C_{20}H_{25}N_6O_6P$, MW=476.42.



This was synthesised according to *standard procedure 6*, using 2′,3′-dideoxyadenosine (25 mg, 0.106 mmol), *tert*-butylmagnesium chloride (117 µL, 0.12 mmol), phenyl-(methoxy-L-alaninyl)-phosphorochloridate (117 µL, 33 mg, 0.12 mmol) in THF/Pyr (10:1, 5 mL) for 3 hrs. After 2 hrs the reaction was monitored by TLC (eluent chloroform/methanol 9/1) and *tert*-butylmagnesium chloride (23 µL) and phosphorochloridate (23 µL) were added dropwise.

The crude was purified twice by flash column chromatography using chloroform/methanol (95/5) to give 40 mg (76%) of a white foam.

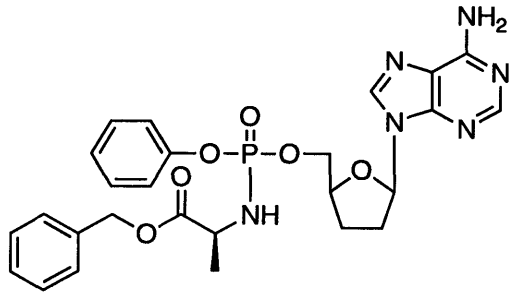
^{31}P-NMR ($CDCl_3$; 121 MHz): δ 4.11, 4.04.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.38 (1H, s, H-2), 8.13 (1H, s, H-8), 7.38-7.15 (5H, m, ***Ph***O), 6.36-6.32 (1H, m, H-1′), 6.00 (1H, bs, NH_2), 4.46-4.02 (5H, m, H-5′+H-4′+C***H***CH_3+N***H***), 3.72, 3.70 (3H, 2s, C***H***$_3$O), 2.66-2.52 (2H, m, H-2′), 2.24-2.15 (2H, m, H-3′), 1.37, 1.33 (3H, 2d, ^{3}J=6.3 Hz, C***H***$_3$CH).

^{13}C-NMR (MeOD; 75 MHz): δ 19.3, 19.4, 19.5 (***C***H_3CH), 25.6, 25.8 (C-3′), 32.0 (C-2′), 51.8 (CH_3***C***H), 53.2 (***C***H_3O) 68.8, 68.9, 69.3, 69.4 (C-5′), 81.5, 81.6 (C-4′), 87.1, 87.2 (C-1′), 119.5 (C-5), 120.4, 120.5 (′*o*′, ***Ph***O), 124.1 (′*p*′, ***Ph***O), 129.7 (′*m*′, ***Ph***O), 139.5, 139.6 (C-8), 149.2 (C-4), 151.1, 151.2 (′*ipso*′, ***Ph***O), 152.8 (C-2), 156.2, 156.3 (C-6), 174.2, 174.3, 174.4, 174.5 (***C***OOCH_2Ph).

Synthesis of 2′,3′-dideoxyadenosine-5′-[phenyl-(benzoxy-L-alaninyl)]-phosphate. (61d) (CPF134)

$C_{26}H_{29}N_6O_6P$, MW=552.52



This was synthesised according to *standard procedure 6*, using 2′,3′-dideoxyadenosine (50 mg, 0.21 mmol), *tert*-butylmagnesium chloride (231 µL, 0.23 mmol), phenyl-(benzoxy-L-alaninyl)-phosphorochloridate (231 µL, 82 mg, 0.23 mmol) in THF/Pyr (10:1, 10 mL) for 19 hrs. After 2 hrs the reaction was monitored by TLC (eluent chloroform/methanol, 9/1) and *tert*-butylmagnesium chloride (60 µL) and phosphorochloridate (60 µL) were added dropwise. The crude was purified twice by flash column chromatography using chloroform/methanol (95/5) to give 52 mg (45%) of a white foam.

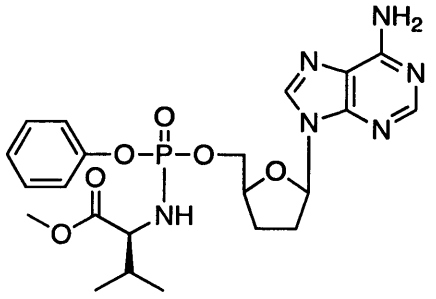
^{31}P-NMR (MeOD; 121 MHz): δ 5.89, 5.18.

^{1}H-NMR (MeOD; 300 MHz): δ 8.35, 8.32 (1H, s, H-8), 8.27, 8.26 (1H, s, H-2), 7.98-7.19 (10H, m, ***Ph***O+***Ph***CH_2O), 6.38-6.32 (1H, m, H-1′), 5.17, 5.14 (2H, 2s, PhC***H_2***), 4.43-4.21 (3H, m, H-5′+C***H***CH_3), 4.08-3.91 (1H, m, H-4′), 2.64-2.52 (2H, m, H-2′), 2.25-2.15 (2H, m, H-3′), 1.33, 1.32 (3H, 2d, ^{3}J=7.2 Hz, CHC***H_3***).

^{13}C-NMR (MeOD; 75 MHz): δ 20.6, 20.7, 20.8 (CH***C***H_3), 27.0, 27.1 (C-3′), 33.4 (C-2′), 51.9, 52.0 (***C***HCH_3), 68.3 (Ph***C***H_2) 68.9, 69.0, 69.2, 69.3 (C-5′), 81.4, 81.5 (C-4′), 87.1 (C-1′), 120.8 (C-5), 121.7, 121.8, 121.9, 126.5, 129.7, 129.9, 131.1 (***Ph***CH_2+***Ph***O), 137.6, 137.7 (*'ipso'*, ***Ph***CH_2), 141.0 (C-8), 150.5 (C-4), 152.4, 152.5 (*'ipso'*, ***Ph***O), 154.1 (C-2), 157.6 (C-6), 175.0, 175.1, 175.2 (***C***$OOCH_2Ph$).

Synthesis of 2′,3′-dideoxyadenosine-5′-[phenyl-(methoxy-L-valinyl)]-phosphate.[11] (61e) (CPF137)

$C_{22}H_{29}ClN_6O_6P$, MW=504.48



This was synthesised according to *standard procedure 6*, using 2′,3′-dideoxyadenosine (75 mg, 0.32 mmol), *tert*-butylmagnesium chloride (480 µL, 0.48 mmol), phenyl-(methoxy-L-valinyl)-phosphorochloridate (800 µL, 245 mg, 0.80 mmol) in THF/Pyr (10:1, 15 mL) for 1.5 hrs.

The crude was purified by flash column chromatography using chloroform/methanol (95/5) to give 149 mg (93%) of a white foam.

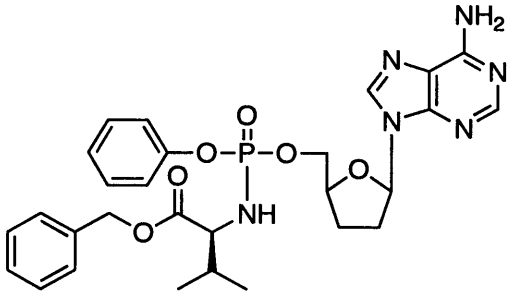
^{31}P-NMR (MeOD; 121 MHz): δ 5.71, 5.95.

^{1}H-NMR (MeOD; 300 MHz): δ 8.31, 8.29 (1H, s, H-8), 8.21 (1H, s, H-2), 7.34-7.13 (5H, m, ***Ph***O), 6.34-6.30 (1H, m, H-1′), 4.46-4.33 (2H, m, H-5′), 4.29-4.20 (1H, m, H-4′), 3.65-3.57 (1H, m, C***H***CH$(CH_3)_2$), 3.65, 3.61 (3H, 2s, COOC***H$_3$***), 2.48-2.04 (2H, m, H-2′), 2.27-2.01 (2H, m, H-3′), 0.79-0.89 (CHCH(C***H$_3$***$)_2$).

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 18.5, 18.7, 19.8, 19.9 (CHCH(***C***$H_3)_2$), 27.0, 27.2 (C-3′), 33.3, 33.4 (C-2′), 33.5, 33.6 (CH***C***H$(CH_3)_2$), 52.8* (***C***HCH$(CH_3)_2$), 62.2* (***C***H_3O), 69.0, 69.1, 69.5, 69.6 (C-5′), 81.4, 81.5 (C-4′), 87.1, 87.2 (C-1′), 120.9 (C-5), 121.7, 121.8*, 126.4, 126.5, 131.1 (***Ph***O), 140.9, 141.1 (C-8), 150.5 (C-4), 152.5, 152.6, 152.7 (*'ipso'*, ***Ph***O), 154.1 (C-2), 157.7 (C-6), 174.8, 174.9, 175.1, 175.2 (***C***OOCH_3).

Synthesis of 2',3'-dideoxyadenosine-5'-[phenyl-(benzoxy-L-valinyl)]-phosphate. (61f) (CPF138)

$C_{22}H_{29}ClN_6O_6P$, MW=504.48



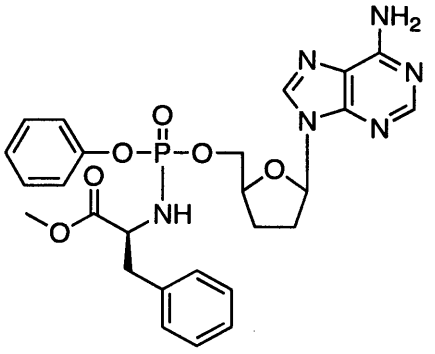
This was synthesised according to *standard procedure 6*, using 2',3'-dideoxyadenosine (75 mg, 0.32 mmol), *tert*-butylmagnesium chloride (480 µL, 0.48 mmol), phenyl-(benzoxy-L-valinyl)-phosphorochloridate (800 µL, 305 mg, 0.80 mmol) in THF/Pyr (10:1, 15 mL) for 1.5 hrs. The crude was purified by flash column chromatography using chloroform/methanol (95/5) to give 183 mg (98%) of a white foam.

^{31}P-NMR (MeOD; 121 MHz): δ 5.71, 5.94.

^{1}H-NMR (MeOD; 300 MHz): δ 8.28, 8.27 (1H, s, H-8), 8.21, 8.20 (1H, s, H-2), 7.37-7.12 (10H, m, ***Ph***O+***Ph***CH_2), 6.31-6.27 (1H, m, H-1'), 5.16-5.02 (2H, 2m, PhC***H_2***), 4.82-4.27 (2H, m, H-5'), 4.23-4.15 (1H, m, H-4'), 3.70-3.65 (1H, m, C***H***$CH(CH_3)_2$), 2.62-2.42 (2H, m, H-2'), 2.25-2.09 (2H, m, H-3'), 2.05-1.92 (CHC***H***$(CH_3)_2$), 0.76-0.68 (CHCH(C***H_3***$)_2$).

^{13}C-NMR (MeOD; 75 MHz): δ 18.5, 18.7, 19.9 (CHCH(***C***$H_3)_2$), 27.0, 27.2 (C-3'), 33.3, 33.4 (C-2'), 33.6, 33.7 (CH***C***$H(CH_3)_2$), 62.3 (CH***C***$H(CH_3)_2$), 68.1, 68.2 (Ph***C***H_2), 69.1, 69.2, 69.5, 69.6 (C-5'), 81.4, 81.5 (C-4'), 87.1 (C-1'), 120.9 (C-5), 121.7*, 121.8, 121.9, 126.4, 126.5, 129.7, 129.9, 130.0, 131.1 (***Ph***CH_2O+***Ph***O), 137.5, 137.6 ('*ipso*', ***Ph***CH_2), 140.9, 141.0 (C-8), 150.5 (C-4), 152.4, 152.5, 152.7 ('*ipso*', ***Ph***O), 154.2 (C-2), 157.7 (C-6), 174.2*, 174.4 (***C***$OOCH_2$Ph).

Synthesis of phenyl-(methoxy-L-phenylalaninyl)-phosphorochloridate.[11] (61g) (CPF139)
$C_{26}H_{29}ClN_6O_6P$, MW=552.52



This was synthesised according to *standard procedure 6*, using 2',3'-dideoxyadenosine (50 mg, 0.21 mmol), *tert*-butylmagnesium chloride (294 µL, 0.29 mmol), phenyl-(methoxy-L-phenylalaninyl)-phosphorochloridate (525 µL, 186 mg, 0.53 mmol) in THF/Pyr (10:1, 10 mL) for 19 hrs.

The reaction was left to stir overnight. The crude was purified twice by flash column chromatography using chloroform/methanol (95/5) to give 38 mg (33%) of a white foam.

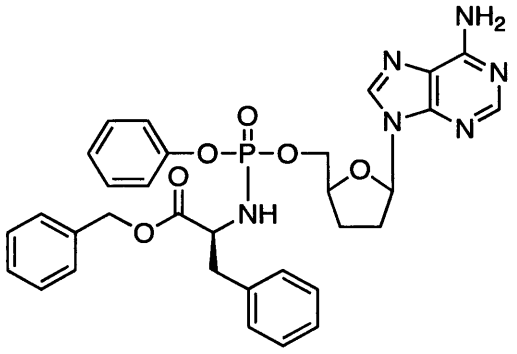
^{31}P-NMR (MeOD; 121 MHz): δ 5.07, 4.59.

^{1}H-NMR (MeOD; 300 MHz): δ 8.24, 8.23 (1H, s, H-8), 8.21, 8.20 (1H, s, H-2), 7.30-7.03 (10H, m, ***Ph***O+ ***Ph***CH_2CH), 6.30-6.25 (1H, m, H-1'), 4.31-3.77 (4H, m, H-4'+H-5'+$PhCH_2C$***H***), 3.61-3.58 (3H, 2s, C***H***$_3O$), 3.10-2.98 (1H, m, 1 of PhC***H***$_2CH$), 2.82-2.78 (1H, m, 1 of PhC***H***$_2CH$), 2.56-2.39 (2H, m, H-2'), 2.17-1.98 (2H, m, H-3').

^{13}C-NMR (MeOD; 75 MHz): δ 27.0, 27.1 (C-3'), 33.4* (C-2'), 41.2*, 41.3 ($PhCH_2CH$), 53.0* ($PhCH_2CH$), 58.1* (CH_3O), 68.8, 68.9, 69.0, 69.1 (C-5'), 81.3*, 81.4* (C-4'), 87.1 (C-1'), 120.9 (C-5), 121.5, 121.6, 121.7, 121.8, 121.9, 126.4, 126.3, 128.3, 129.9, 131.0, 131.1* (***Ph***O+***Ph***CH_2CH), 138.5, 138.6 ('*ipso*', ***Ph***CH_2), 140.9, 141.0 (C-8), 150.5 (C-4), 152.3, 152.4, 152.5 ('*ipso*', ***Ph***O), 154.2 (C-2), 157.7 (C-6), 174.7, 174.8, 174.9 ($COOCH_3$).

Synthesis of 2',3'-dideoxyadenosine-5'-[phenyl-(benzoxy-L-phenylalaninyl)]-phosphate. (61h) (CPF140)

$C_{32}H_{33}N_6O_6P$, MW=628.61



This was synthesised according to *standard procedure 6*, using 2',3'-dideoxyadenosine (75 mg, 0.32 mmol), *tert*-butylmagnesium chloride (480 µL, 0.48 mmol, 56 mg), phenyl-(benzoxy-L-phenylalaninyl)-phosphochloridate (800 µL, 344 mg, 0.80 mmol) in THF/Pyr (10:1, 13 mL) for 19 hrs.

The reaction was left to stir overnight. The crude was purified twice by flash column chromatography using chloroform/methanol (95/5) to give 113 mg (56%) of a white foam.

^{31}P-NMR ($CDCl_3$; 121 MHz): δ 4.94, 4.63.

^{1}H-NMR ($CDCl_3$; 300 MHz): δ 8.22, 8.21 (1H, s, H-8), 8.20, 8.19 (1H, s, H-2), 7.33-7.02 (15H, m, ***Ph***O+***Ph***CH_2CH+***Ph***CH_2), 6.28-6.23 (1H, m, H-1'), 5.06-5.02 (2H, 2s, PhC***H_2***), 4.63-4.15 (1H, m, H-4'), 4.13-3.82 ($PhCH_2C$***H***+H-5'), 3.09-2.98 (1H, m, 1 of PhC***H_2***CH), 2.88-2.80 (1H, m, 1 of PhC***H_2***CH), 2.57-2.36 (2H, m, H-2'), 2.14-1.95 (2H, m, H-3').

^{13}C-NMR ($CDCl_3$; 75 MHz): δ 27.0, 27.1 (C-3'), 33.4* (C-2'), 41.2, 41.3, 41.4 (Ph***C***H_2CH), 58.2, 58.3 ($PhCH_2$***C***H), 68.3* (Ph***C***H_2), 68.9, 69.0, 69.1, 69.2 (C-5'), 81.2, 81.3*, 81.4 (C-4'), 87.1, 87.2 (C-1'), 120.9 (C-5), 121.6*, 121.7, 121.8, 126.4*, 128.3, 129.7, 129.8, 129.9, 131.0, 131.1 (***Ph***O+***Ph***CH_2CH+***Ph***CH_2), 137.4*, 138.4, 138.5 ('*ipso*', ***Ph***CH_2CH+***Ph***CH_2), 140.9* (C-8), 150.5 (C-4), 152.4, 152.5 ('*ipso*', ***Ph***O), 154.1, 154.2 (C-2), 157.7 (C-6), 174.1, 174.2* (***C***$OOCH_2Ph$).

References.

1 Ashwell, M.; Jones, A.S.; Kumar, A.; Sayers, J.R.; Walker, R.T.; Sakuma, T.; De Clercq, E.; The synthesis and antiviral properties of (E)-5-(2-bromovinyl)-2'-deoxyuridine-related compounds. *Tetrahedron*, **1987**, 43, 4601-4608.

2 McGuigan, C.; Pathirana, R.N.; Balzarini, J.; De Clercq, E.; Intracellular delivery of bioactive AZT nucleotides by aryl phosphate derivatives of AZT. *J. Med. Chem.*, **1993**, 36, 1048-1052.

3 Siddiqui, A.; Ballatore, C.; McGuigan, C.; De Clercq, E.; Balzarini, J.; The Presence of substituents on the aryl moiety of aryl phosphoramidate derivatives of d4T enhances anti-HIV efficacy in cell culture: a structure activity relationship. *J. Med. Chem.*, **1999**, 42, 393-399.

4 Siddiqui, A.; McGuigan, C.; Ballatore, C.; Zuccotto, F.; Gilbert, I.H.; De Clercq, E.; Balzarini, J.; Design and synthesis of lipophilic phosphoramidate d4T-MP prodrugs expressing high potency against HIV in cell culture: structural determinants for in vitro activity and QSAR. *J. Med. Chem.*, **1999**, 42, 4122-4128.

5 Harris, S.A.; McGuigan, C.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J.; Synthesis and antiviral evaluation of phosphoramidate derivatives of (E)-5-(2-bromovinyl)-2'-deoxyuridine. *Antiviral Chem. Chemother.*, **2001**, 12, 279-286.

6 Montgomery, A.J.; Clayton, S.D.; Shortnacy, A.T.; An improved procedure for the preparation of 9-β-D-arabinofuranosyl-2-fluoroadenine. *J. Heterocycl. Chem.*, **1979**, 16, 157-160.

7 Cicchillo, R.M.; Norris, P.; A convenient synthesis of glycosyl chlorides from sugar hemiacetals using triphosgene as chlorine source, *Carbohyd. Res.*, **2000**, 328, 431-434.

8 McGuigan, C.; Tsang, H.-W.; Cahard, D .; Turner, K.; Velasquez, S.; Salgado, A.; Bidois, L.; Naesens, L.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of d4T as inhibitors of HIV: the effect of amino acids variation. *Antiviral Res.*, **1997**, 35, 195-204.

9 McGuigan, C.; Bidois, L.; Hiouni, A.; Ballatore, C.; De Clercq, E.; Balzarini, J.; Phosphoramidate derivatives of stavudine as inhibitors of HIV: unnatural amino acids may substitute for alanine. *Antiviral Chem. Chemother.*, **1999**, 11, 111-116.

10 Robins, M.J.; Hansske, F.; Low, N.H.; In Park, J.; A mild conversion of vicinal diols to alkenes. Efficient transformation of ribonucleosides into 2'-ene and 2', 3'-dideoxynucleosides. *Tetrahedron Lett.*, **1984**, 25, 367-370.

11 Wedgwood, O.M.; Internal Report, **1998**, Welsh School of Pharmacy.

12 Siddiqui, A. Q.; McGuigan, C.; Ballatore, C.; Wedgwood, O.; De Clercq, E.; Balzarini, J.; Simple mono-derivatisation of the aryl moiety of d4A and ddA-based phosphoramidate prodrugs significantly enhances their anti-HIV potency in cell culture. *Bioorg. Med. Chem. Lett.*, **1999**, 9, 2555-2560